The Adverse Effects Of Contaminants On Predator-Prey Interactions: Implications For Ecological Risk Assessment

Amy Chantal Brooks

"Success is not measured by what you accomplish, but by the opposition you have encountered, and the courage with which you have maintained the struggle against overwhelming odds"

O.S. Marden

Acknowledgements

Summary

Predator-prey interactions play an important role in maintaining community structure and thus ecosystem function. However, these interactions can be disrupted by exposure of prey to contaminants released into the environment. Depending on the exposure concentration, prey can experience lethal or sublethal toxic effects. They may also take up contaminants into their tissues. This may affect prey availability or the transfer of contaminants to, and potentially toxic effects in, predators. The aim of this thesis was to examine whether changes in predator-prey interactions could exacerbate the negative impacts of contaminants via such effects.

An experimental approach was taken, using a macroinvertebrate food web consisting of two predator (*Notonecta glauca* and *Ischnura elegans*) and three prey species (*Asellus aquaticus*, *Chironomus riparius* and *Cloëon dipterum*), and two contaminants, a non-essential metal (cadmium) and a hydrophobic organic (benzophenone). Three main conclusions were drawn from this work. Firstly, the extent of trophic transfer depends on prey exposure pathways and predator feeding behaviours, with subsequent secondary poisoning depending on predator critical body residues, contaminant modes of action, and species traits. Secondly, contaminants with low acute to chronic toxicity ratios can act by reducing prey survival, resulting in increased predation or altered prey choice by predators, thereby reducing prey populations that are unable to recover. Thirdly, contaminants with high acute to chronic ratios can have sublethal effects on prey, affecting prey susceptibility to predation or reducing prey populations. The impact of the resulting increased predation depends on the type of sublethal effect, predator traits, and the potential for recovery.

This thesis indicates that the current risk assessment approach of focusing on direct exposures of single species to contaminants may be under protective. Instead, a scenario-based approach may be more appropriate, taking into account the interactions between contaminant properties, species traits, landscape profiles and exposure durations to identify situations where changes in predator-prey interactions may be particularly detrimental.

Contents

5. The importance of prey handling and contaminant packaging for dietary exposure and secondary poisoning of predators

6. The adverse effects of contaminants on predator-prey interactions: implications for ecological risk assessment

Appendices

Chapter 1

The potential adverse effects of contaminants with different chemical properties on predator-prey interactions and the implications for ecological risk assessment

---

1.1 INTRODUCTION

Natural assemblages consist of collections of species interacting with one another, forming complex communities and ecosystems (Campbell, 1993). Interactions occurring between species, including competition (Connell, 1961) and predation (Paine, 1966), are important in shaping these communities. Therefore any disruptions to these interactions will have consequences for ecosystem structure and function (Paine, 1966; Williamson *et al.*, 1989). Exposure of organisms to stressors can perform such a role, from both natural (e.g. changes in temperature) and anthropogenic (e.g. contaminants) sources. The impact of stressors on communities will depend on the sensitivity of species to exposure, resulting in either lethal or sublethal effects, affecting population densities (Helgen *et al.*, 1988; Whaley *et al.*, 1989) or behaviours (Clements *et al.*, 1989; Dodson *et al.*, 1995; Carlson *et al.*, 1998; Hanazato, 2001), respectively. Due to the connections between species within communities, the effects occurring at one trophic level may also be perceived and passed on to subsequent trophic levels (Clements *et al.*, 1989; Wallace *et al.*, 2000; Elliott, 2004). Thus there are not only initial direct impacts of stressors on particular species, but also numerous indirect effects that may arise due to their interactions with other components of the assemblage (deNoyelles Jr. *et al.*, 1994; Boyle *et al.*, 1996; Peither *et al.*, 1996; Preston, 2002).

The interactions between predators and their prey are important in structuring communities (Paine, 1966; Williamson *et al.*, 1989). The most famous examples of this are the rocky shore experiments carried out in the 1960s, in which the removal of the predatory starfish (*Pisaster ochraceus*) from communities resulted in a reduction in species diversity, from a 15 to an eight species system (Paine, 1966). The relative importance of these types of biotic interactions compared to abiotic factors is

1

dependent on the harshness of the environment, the so-called harsh-benign hypothesis (Connell, 1975; Menge, 1976; Peckarsky et al., 1990). This hypothesis predicts that the impact of predators on a community will be greater in physically benign environments, whereas competition between prey will be more important in harsher environments (Connell, 1975; Menge, 1976; Peckarsky et al., 1990), with abiotic factors becoming more important than biotic interactions in extreme environments (Menge and Sutherland, 1987). Subsequent research has highlighted that the indirect effects of both environmental stressors (e.g. increased use of refuges by prey during flood events) and predators (e.g. predator avoidance by prey) are also important in determining the relative influence of abiotic factors and predation (Walde, 1986; Lancaster, 1996; Thomson et al., 2002).

Anthropogenic activities are an important source of environmental stress, due to the widespread release of contaminants into the environment (Anon, 2007). These contaminants originate from agricultural, industrial and domestic uses, and include both metals and organics (Rand and Petrocelli, 1985). Many animals living in the natural environment are therefore being repeatedly exposed to a range of different substances (Walker et al., 1996). Predator-prey interactions can be disrupted by exposure to such stressors (Hanazato, 1991; Peither et al., 1996; Hanazato, 2001; Chang et al., 2005); for example, predacious copepods (*Mesocyclops pehpeiensis*) responded to a contaminant reduced abundance in cladoceran prey by feeding more on alternative rotifer prey (Chang et al., 2005). The prey capture ability of grass shrimp predators (*Palaemonetes pugio*) fed cadmium-contaminated prey (*Limnodrilus hoffmeisteri*) was significantly impaired compared to those fed control prey, resulting in reduced predation (Wallace et al., 2000).

The type of effect of contaminant exposure on predator-prey interactions will depend on the exposure concentration and the properties of the contaminant (Figure 1.1). Exposure to lethal concentrations will result in reduced prey survival, and consequently reduced prey population densities (Helgen et al., 1988; Whaley et al., 1989). Sublethal concentrations have less extreme effects, resulting in a wide array of prey responses including behavioural, morphological and reproductive effects (Clements et al., 1989; Dodson et al., 1995; Carlson et al., 1998; Hanazato, 2001). In
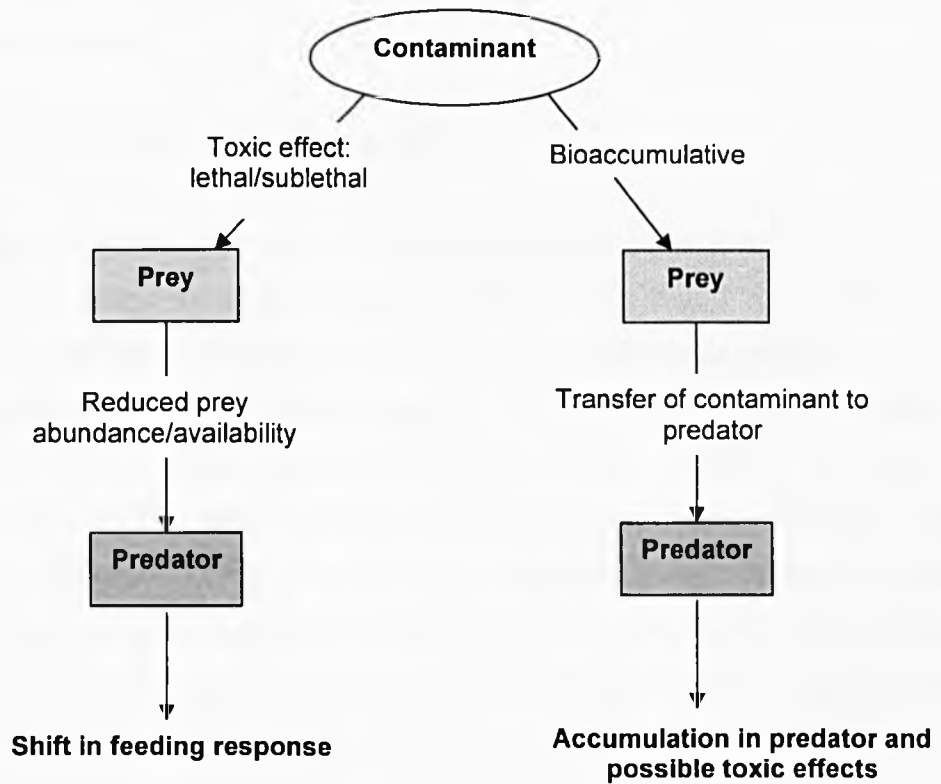
Figure 1.1. The possible effects of contaminant exposure on predator-prey interactions according to the exposure concentration and the properties of the contaminant.

addition to affecting the survival or physiology of organisms, some contaminants also have bioaccumulative properties, with exposure resulting in accumulation within prey (Kaag et al., 1997; Wang and Fisher, 1999; Gaskell et al., 2007). The potential impacts of these lethal (Section 1.2), sublethal (Section 1.3) and bioaccumulative (Section 1.4) contaminant exposures for predator-prey interactions are discussed in the following sections.

1.2 LETHAL EFFECTS OF CONTAMINANTS

Contaminants are not equally toxic to all species, with some species being much more sensitive to exposure than others (e.g. Slooff, 1983; Slooff et al., 1983). For example, the concentration required to kill 50% of the individuals exposed (LC_{50}) to 15 different metal and organic contaminants for 48-h differed by over two orders of magnitude for 13 macroinvertebrate species (Slooff, 1983). Therefore the extent of change in density of a species following contaminant exposure is driven by their sensitivity to exposure. In some cases, the contaminant-induced reduction in density of sensitive species can be mirrored by an increase in the density of a more resistant species, such that, although the relative availabilities of prey species change, the total abundance of prey may remain high (Hanazato, 1991; Chang et al., 2005). For example, carbaryl exposure resulted in the direct reduction of sensitive cladoceran prey but subsequently allowed resistant rotifer prey to increase, due to release from competition (Chang et al., 2005). The effects of exposure can also depend on the mode of action of the contaminant; for example, diflubenzuron inhibits the formation of chitin and therefore is only directly toxic to organisms with a chitinous exoskeleton (Boyle et al., 1996).

In natural systems, sensitive and resistant prey species are embedded in complex food webs. Therefore, any predators of prey populations reduced by contaminant exposure would experience reduced food availability (Boyle et al., 1996; Hamers and Krogh, 1997). Predators respond to such changes in prey availability in terms of their feeding behaviour (Holling, 1959; Elliott, 2004). The type of change by the predator will depend on whether they feed on only a select type of prey (specialist) or on a broad range of species (generalist).

4

The response of specialist predators to changes in the density of a single prey species can be described by the functional response of the predator, which can be assigned to one of three main functional response types: Type I, II and III (Holling, 1959). The proportion of prey eaten by predators can either remain the same (Type I), increase (Type II), or decrease (Type III) following a reduction in prey density (Juliano, 2001). Most predators have Type II functional responses (Begon et al., 1996), including leeches (Bronmark, 1992), flatworms (Beier et al., 2004), stoneflies (Elliott, 2003), odonates (Akre and Johnson, 1979; Colton, 1987), notonectids (Fox and Murdoch, 1978) and midge larvae (Spitze, 1985). Prey populations that have been reduced by contaminant exposure may experience further reductions in population size if predated by Type II predators. This link between the functional response type of predators and their additional impact on prey populations following contaminant-induced reductions in density has not yet been made in the current literature.

The response of generalist predators to changes in the density of sensitive prey is more complex as they have the option of feeding on alternative, more resistant prey (e.g. Elliott, 2004). Predators that have no preference for a particular prey species will feed according to the ratios of prey species present in the environment (Murdoch, 1969). Such predators would not increase the impact of contaminant exposure and instead would feed less on sensitive prey as they become less abundant in the environment. If predators have a preference for either the sensitive or resistant prey species, they will maintain the same intake of prey independent of prey densities (Murdoch, 1969; Elliott, 2004). Predators with a preference for sensitive prey species would, therefore, compound the effect of contaminant-induced population reductions. There are also predators that have a density-dependent prey preference, feeding disproportionately more on the more abundant prey species (Murdoch, 1969; Akre and Johnson, 1979). In this scenario it is the resistant prey species that experiences intensified predation from such predators, as they will be the more abundant prey species. For example, predacious copepods (*Mesocyclops pehpeiensis*) responded to a contaminant reduced abundance in cladoceran prey by feeding more on the alternative rotifer prey (Chang et al., 2005). It is therefore apparent that not only can sensitive prey species suffer further population reductions from increased predation pressures, but resistant prey species can also be affected.

The complex responses of generalist predators to changes in prey density can potentially be addressed by using a mathematical modelling approach. Various models are in existence, including those that account for inherent preferences of predators (Murdoch, 1969; Cock, 1978; Chesson, 1983), and models based on functional response parameters from single-prey-single-predator interactions (Colton, 1987). The latter makes the assumption that multiple predator and prey species systems are a sum of their parts, with the interactions between multiple species being predictable from single-predator-single-prey interactions. This will not always be the case, with interactions between prey species resulting in changes in their behaviour and therefore susceptibility to predation (Huang and Sih, 1990; Johansson, 1995). One problem that currently exists is deciding *a priori* which scenario will occur and therefore which model will be the most suitable for a particular combination of predator and prey species.

1.3 SUBLETHAL EFFECTS OF CONTAMINANTS

Although prey populations can obviously be reduced by lethal exposures to contaminants, sublethal exposures may also reduce prey availability to predators. Sublethal responses to contaminant exposure can result in a wide array of effects, including morphological, reproductive and behavioural effects (e.g. Hanazato, 2001). Behavioural changes in prey animals include changes in locomotory activity (Gerhardt, 1990; Zhou and Weis, 1998; Schulz and Dabrowski, 2001), escape response (Dodson *et al.*, 1995; Gerhardt, 1995), use of refuges (Zhou and Weis, 1998; Schulz and Dabrowski, 2001), involuntary muscle spasms and paralysis (Carlson *et al.*, 1998), swimming speed (Preston *et al.*, 1999a), and feeding behaviour (Riddell *et al.*, 2005a). Such behavioural changes have been demonstrated to affect prey encounters with predators and therefore susceptibility to predation (e.g. Carlson *et al.*, 1998; Preston *et al.*, 1999b; Riddell *et al.*, 2005b). For example, juvenile medaka (*Oryzias latipes*) were more susceptible to predation by bluegill (*Lepomis maccrochirus*) following exposure to chlorpyrifos, carbaryl, fenvalerate, endosulfan, phenol, 1-octanol and 2,4-dinitrophenol (DNP) (Carlson *et al.*, 1998). It is therefore possible for prey populations to be reduced following sublethal contaminant exposure due to intensified predation.

6

As with acute toxicity, variation exists between species in their sensitivity to sublethal exposures (Koivisto *et al.*, 1992; Preston *et al.*, 1999b). Therefore generalist predators may also experience shifts in prey availability according to prey sensitivity to sublethal exposures. The effect of sublethal exposures on prey choice by predators has only been addressed in a limited number of studies (e.g. Clements *et al.*, 1989; Riddell *et al.*, 2005b). The predictability of the predator response is therefore more challenging. As prey availability does not change in terms of actual prey abundance it is not possible to use the mathematical models proposed for predator responses to prey density. Instead it may be more relevant to consider the traits of the prey and predators of concern, particularly how changes in their behaviour would affect the frequency of encounters between predator and prey.

Effects on the susceptibility of prey to predation can result from changes in both prey activity (Spitze, 1985) and responses to attack (Peckarsky and Penton, 1989a). However, the perception of such changes by predators may depend on their hunting strategy. Predators can be ambush hunters (e.g. odonate larvae, flounder fish (*Platichthys flesus*)), sitting and waiting for their prey to come to them, or active, "cruising" hunters (e.g. waterboatman (*Notonecta glauca*), rainbow trout (*Oncorhynchus mykiss*)) that actively seek out their prey (Peckarsky, 1982; Greene, 1986). Both types of predators would be expected to respond to changes in prey activity; for example, Spitze (1985) found that the attack rate of the ambush predator, *Chaoborus americanus*, decreased as the swimming speed, and thus encounter rates, of its *Daphnia* prey decreased. However, only predation by active predators would be expected to change due to effects on prey responsiveness; for example, increased responsiveness in mayfly nymphs reduced predation by active predatory stoneflies (Peckarsky and Penton, 1989a).

1.4 EFFECTS OF BIOACCUMULATIVE CONTAMINANTS

The accumulation of contaminants within organisms has been widely documented for both vertebrates (Kidd *et al.*, 1998; Egeler *et al.*, 2001; Simon and Boudou, 2001; Wang and Wong, 2003; Heiden *et al.*, 2005; Pääkkonen *et al.*, 2005; Maul *et al.*, 2006) and invertebrates (Hickey *et al.*, 1995; Meador *et al.*, 1995; Meador *et al.*,

1997; Munger and Hare, 1997; Munger *et al.*, 1998; Penry and Weston, 1998; Goodyear and McNeill, 1999; Christensen *et al.*, 2002; Gaskell *et al.*, 2007; Dumas and Hare, 2008). Accumulation can occur directly from aqueous exposure, by both adsorption to external surfaces and uptake via respiratory surfaces, e.g. gills (Rand and Petrocelli, 1985). In addition to aqueous exposure, there is the potential for bioaccumulative contaminants to be transferred through the food chain. Such dietary exposure has been demonstrated for a range of different exposure media, with both metals (Table 1.1) and organic contaminants (Table 1.2). Organisms, including algae, plants and animals, can therefore act as an important exposure pathway for the animals feeding on them (Timmermans *et al.*, 1992; Egeler *et al.*, 2001; Hook and Fisher, 2001a, 2002).

Transfer of contaminants through food chains can result in them becoming more widely distributed in the environment in a number of different ways. Firstly, contaminants can be redistributed within the same habitat; for example, hexachlorobenzene can be transferred from sediment to pelagic fish (*Gasterosteus aculeatus*) via accumulation in their sediment-dwelling prey (*Tubifex tubifex*) (Egeler *et al.*, 2001). Contaminants can also be transferred along great distances; for example, migrating sockeye salmon returning from the Arctic to freshwater lakes in order to spawn are thought to transfer chlorinated fatty acids to resident Arctic grayling (Mu *et al.*, 2004). It is also possible for contaminants to be transferred between different types of habitat, such as from aquatic to terrestrial habitats; for example, diving ducks can accumulate organochlorines from their diet of zebra mussels (Custer and Custer, 2000). Recent research has also demonstrated that adult insects emerging from streams contaminated with polychlorinated biphenyls (PCBs) provide an exposure pathway to their riparian predators (Walters *et al.*, 2008). These examples demonstrate that contaminants can be transferred beyond the initial site of contamination, mainly through interactions between prey and predators. As a result, predators that were not directly exposed to the original source of contamination can still be exposed to the contaminant through their diet.

Dietary exposure may be particularly important for contaminants that have a low solubility i.e. hydrophobic substances (Qiao *et al.*, 2000; Lu *et al.*, 2004). As a result,

Table 1.1. Examples from the literature of aquatic animals that have demonstrated dietary uptake following exposure to **metals** in various exposure media.

Exposure media	Species exposed	Taxa	Substance	Reference
Sediment	*Leptocheirus plumulosus*	Arthropoda	mercury, methylmercury	(Lawrence and Mason, 2001)
	Lumbriculus variegatus	Annelida	silver	(Hirsch, 1998)
Algae/plant	*Leptocheirus plumulosus*	Arthropoda	mercury, methylmercury	(Lawrence and Mason, 2001)
	Acartia hudsonica and A. tonsa	Arthropoda	cadmium, mercury	(Hook and Fisher, 2001a)
	Acartia hudsonica, A. tonsa, Ceriodaphnia dubia, Ceriodaphnia sp. and Simocephalus sp.	Arthropoda	Silver	(Hook and Fisher, 2001b)
	Acartia hudsonica and A. tonsa	Arthropoda	silver, zinc	(Hook and Fisher, 2002)
	Eliminius modestus	Arthropoda	cadmium, chromium, selenium, zinc	(Rainbow and Wang, 2001)
	Siganus canaliculatus	Pisces	cadmium, chromium, zinc	(Chan *et al.*, 2003)
	Gammarus pulex	Arthropoda	zinc	(Wilding, 2004)
Prey	*Eliminius modestus*	Arthropoda	cadmium, chromium, selenium, zinc	(Rainbow and Wang, 2001)
	Mysis relicta	Arthropoda	cadium, copper	(Smokorowski *et al.*, 1998)
	Chaoborus punctipennis	Arthropoda	cadmium	(Munger and Hare, 1997)
	Mystacides spp., Limnesia maculata	Arthropoda	cadmium, zinc	(Timmermans *et al.*, 1992)
	Plectorhinchus gibbosus	Pisces	mercury	(Wang and Wong, 2003)
	Lutjanus argentimaculatus	Pisces	cadmium, selenium, zinc	(Xu and Wang, 2002)
	Danio reiro	Pisces	chromium, cadmium, zinc	(Liu *et al.*, 2002)
Artificial fish food	*Pimephales promelas*	Pisces	methylmercury	(Hammerschmidt *et al.*, 2002)

Table 1.2. Examples from the literature of aquatic animals that have demonstrated dietary uptake following exposure to **organic chemicals** in various exposure media.

Exposure media	Species exposed	Taxa	Substance	Reference
Sediment	*Mercenaria mercenaria*	Mollusca	chlorpyrifos	(Bejarano *et al.*, 2003)
Algae/plant	*Mercenaria mercenaria*	Mollusca	chlorpyrifos	(Bejarano *et al.*, 2003)
	Perna viridis	Mollusca	benzo[a]pyrene	(Wang and Chow, 2002)
Prey	*Gasterosteus aculeatus*	Pisces	hexachlorobenzene (HCB)	(Egeler *et al.*, 2001)
	Lota lota	Pisces	polychlorinated biphenyl congeners (PCBs)	(Pääkkonen *et al.*, 2005)
	Lutjanus argentimaculatus	Pisces	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT)	(Wang and Wang, 2005)
	Plectorhinchus gibbosus	Pisces	methylmercury	(Wang and Wong, 2003)
Artificial fish food	*Chironomus tentans*	Arthropoda	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)	(West *et al.*, 1997)
	Lumbriculus variegatus	Annelida	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)	(West *et al.*, 1997)
	Salmo salar	Pisces	4-nonylphenol	(Arukwe *et al.*, 2000)
	Oncorhynchus mykiss, Coregonus clupeaformis	Pisces	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)	(Fisk *et al.*, 1997)
	Pimephales promelas	Pisces	4-nonylphenol	(Pickford *et al.*, 2003)
	Oncorhynchus mykiss	Pisces	1,2,4-trichlorobenzene (1,2,4-TCB); 1,2,3,4,5-pentachlorobenzene (PcCB); 2,2',4,4',6,6'-hexachlorobiphenyl (HCBP)	(Qiao *et al.*, 2000)
	Danio reiro	Pisces	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)	(Heiden *et al.*, 2005)

the aqueous exposure concentration will be very low, and the contaminant will instead partition out of the dissolved phase and become associated with particulate matter (Kukkonen and Landrum, 1996). In natural aquatic systems hydrophobic contaminants would tend to accumulate in particulate matter (Kukkonen and Landrum, 1996). As a result, animals that inhabit sediments may be at risk of exposure to, and therefore accumulation of, hydrophobic contaminants. However, animals that do not come into direct contact with the sediment may still be exposed by predating on benthic prey.

Studies have demonstrated that variation exists between benthic species in their ability to bioaccumulate contaminants under the same exposure conditions (Meador *et al.*, 1995; Gaskell *et al.*, 2007). The accumulation of hydrophobic chlorinated hydrocarbons by a non-selective deposit feeding polychaete (*Armandia brevis*) was up to 3-10 times higher than that of a non-deposit feeding amphipod (*Rhepoxynius abronius*) (Meador *et al.*, 1997). Therefore, predators of these animals would potentially be exposed to very different doses depending on the prey species they feed on.

Variation in the body burden of prey species may be due to a number of factors. One such factor may be the exposure pathway by which they accumulated the contaminant. For example, animals that inhabit contaminated sediments may achieve higher body loadings if they ingest particulate matter in addition to being exposed to contaminants in the pore water between particles (Kaag *et al.*, 1997; Meador *et al.*, 1997). In addition to exposure pathway, there are a number of other mechanisms that may contribute to variation in prey body burden. These mechanisms include species differences in ingestion rate (Kukkonen and Landrum, 1995; Means and McElroy, 1997; Penry and Weston, 1998), digestive physiology (Ahrens *et al.*, 2001; Wang and Chow, 2002; Gaskell *et al.*, 2007), and elimination rate (Kane Driscoll and McElroy, 1997; Verrengia Guerrero *et al.*, 2002).

The feeding behaviour of sediment dwellers may not only affect their total body burden but may also influence the internal distribution of the accumulated contaminant. Dietary exposure, such as through sediment ingestion, results in accumulation in mainly internal tissues and aqueous exposure being mainly

externally accumulated (Fisk *et al.*, 1997; Arukwe *et al.*, 2000; Liu *et al.*, 2002; Xu and Wang, 2002; Wang and Wong, 2003; Heiden *et al.*, 2005). Differences in the distribution of contaminants within tissues may have implications for food chain transfer. A recent study using cadmium demonstrated that the proportion of accumulated contaminant transferred from prey (*Chironomus riparius*) to predator (*Danio rerio*) was higher for prey exposed via sediment compared to water exposure (Béchard *et al.*, 2008). The extent of transfer to predators may also depend upon the feeding behaviour of their predators.

Aquatic predators differ in the way in which they handle their food (Peckarsky, 1982). Some predators, such as the leech *Erpobdella octoculata* and fish such as *Gasterostreus aculeatus*, feed on the whole prey item ("engulfers"). Engulfer predators will therefore be exposed to contaminants that have been both internally and externally accumulated by the prey. However, other predators, such as the water boatman *Notonecta glauca* and the water scorpion *Nepa cinerea*, do not consume the whole prey item and instead suck out their internal tissues ("piercers") (Peckarsky, 1982). Therefore, these predators will only be exposed to contaminants that have been internally accumulated by the prey into their internal tissues. Few studies exist that have directly investigated the effect of predator feeding behaviour on the transfer of contaminants through food chains. However, one study demonstrated that incomplete consumption of chironomid prey by caddisfly predators resulted in lower body burdens compared to water mite predators (Timmermans *et al.*, 1992).

The extent of transfer to, and thus accumulation in, predators has important implications for the toxic effects that may result in predators, termed secondary poisoning (Feijtel *et al.*, 1997). Toxic effects resulting from dietary exposure have been documented for a wide range of predators, including mammals (Heaton *et al.*, 1995), fish (Berntssen *et al.*, 1999) and invertebrates (Wallace *et al.*, 2000). Some contaminants are not toxic to prey and can therefore be accumulated at high concentrations; for example, dietary exposure of *Lumbriculus variegatus* to tetrachlorodibenzo-*p*-dioxin (TCDD) resulted in a maximum residue of 9,533 ng [^{3}H]TCDD/g lipid without exhibiting toxic effects (West *et al.*, 1997). This is hypothesized to be due to the lack of aryl hydrocarbon (Ah) receptors in invertebrates through which toxicity is expressed (West *et al.*, 1997). However,

vertebrates possess Ah receptors and are much more sensitive to exposure (Elonen *et al.*, 1998). Therefore secondary poisoning of vertebrate predators may arise from feeding on contaminated invertebrate prey (West *et al.*, 1997). If the magnitude of toxic response in predators is related to the exposure dose they receive, then the feeding behaviour of predator and prey may determine the extent of secondary poisoning exhibited (Walker *et al.*, 1996).

The type of secondary poisoning effects that occur in predators will partly depend on the mode of action of the contaminant. Dietary exposure to predators can result in effects on reproduction (Heaton *et al.*, 1995), growth (Friedmann *et al.*, 1996) and feeding rates (Wallace *et al.*, 2000). Negative effects of contaminants on predator feeding rate are important for two reasons: firstly, a reduction in food intake may result in less energy available for growth and reproduction for predators, which over the long term may reduce population sizes. Secondly, reduced predation on prey may have consequences for ecosystem structure and function, releasing prey from predation and allowing an increase in their population size (Relyea and Hoverman, 2008). Therefore, there may be indirect effects on ecosystems from secondary poisoning of predators, due to changes in both prey and predator populations.

1.5 IMPLICATIONS FOR ECOLOGICAL RISK ASSESSMENT

The preceding sections have demonstrated that predator-prey interactions can be affected by both lethal and sublethal effects, and by the accumulation and transfer of contaminants through the food chain. Although the occurrence of such indirect effects is known, current approaches to ecological risk assessment are mainly focused on single species effects (Preston, 2002). These assessments are conducted to protect natural systems from the potential impacts of anthropogenic activities, including exposure to contaminants (Suter II, 1993). A tiered approach is usually taken to risk assessment (Forbes and Forbes, 1994). The lower-tier is based on acute exposures to a small number of species (i.e. alga, daphnid and fish), usually measured as the lethal concentration required to kill 50% of the organisms exposed (LC_{50}) (Maltby, 2006). Contaminants that do not pass the lower-tier assessment trigger the need for higher-tier assessment which requires the collection of more complex data (EC, 2003). Higher tier data can include further single-species toxicity

testing, indoor multispecies tests and field/mesocosm studies (Campbell *et al.*, 1998; Boxall *et al.*, 2001; EC, 2003). As multispecies tests can be more complex to conduct and subsequently interpret, preference is often given to collecting more single species data in order to pass the risk assessment (Forbes and Forbes, 1994; Preston, 2002). This can include the generation of species sensitivity distributions (SSDs) or population studies using different life stages (Campbell *et al.*, 1998).

By focusing on single species toxicity tests, the ecosystem is treated as a collection of species exposed to a single compound under constant conditions (Cairns, 1983). This issue of scaling up to whole ecosystems is currently addressed by the application of various assessment or 'extrapolation' factors (Forbes and Calow, 2002). These factors range from between 1-1000 for freshwater systems depending on the complexity of the data set available (EC, 2003). If the adjusted toxicity endpoints are within acceptable ranges of the predicted environmental concentration (PEC) for that contaminant following application of the appropriate assessment factor, then the contaminant passes the effects assessment.

In order for assessment factors to truly represent the worst-case scenario they must account for differences in sensitivity between species, differences between acute and chronic effect concentrations, and the difference between simple laboratory and complex field systems (Forbes and Calow, 2002). However, achieving the latter is problematic as toxicity tests using single species cannot predict the indirect effects that occur due to changes in biotic interactions (deNoyelles Jr. *et al.*, 1994; Boyle *et al.*, 1996; Peither *et al.*, 1996; Preston, 2002). A group of contaminants for which effects on predator-prey interactions may be particularly important are the persistent, bioaccumulative and toxic (PBT) contaminants, highlighted as a group of high risk contaminants in the new EU chemical strategy, REACH (EC, 2006). Being bioaccumulative, there is a risk of transfer from prey to predators, and, being toxic, they can have either lethal or sublethal effects on prey availability. Therefore single-species tests may under-represent the effects of PBT contaminant exposure, as there may be additional impacts occurring due to changes in predator-prey interactions (Hanazato, 1991; Peither *et al.*, 1996; Hanazato, 2001; Preston, 2002; Fleeger *et al.*, 2003; Chang *et al.*, 2005; Relyea and Hoverman, 2008).

1.6 MAIN THESIS AIMS AND OBJECTIVES

The review presented in this chapter has demonstrated that changes in predator-prey interactions following contaminant exposure can potentially have consequences for both prey and predators. Therefore the current trend in risk assessment for using single-species data may result in risk being underestimated. Predators can exacerbate contaminant-induced reductions in prey populations due to additional predation pressures and shifts in prey choice. Population reductions of prey can also result from sublethal changes in prey characteristics that cause an increased susceptibility to predation. Prey may also act as a source of exposure to predators for bioaccumulative contaminants. Therefore toxic effects may result in predators that would not arise from single species exposures.

The overall aim of this thesis is to address the hypothesis that changes in predator-prey interactions from contaminant exposure result in additional impacts, via toxic effects either at the prey or predator level. Accordingly the thesis is divided into two main sections: the first two data chapters address the impact of toxic effects on prey on predation response, the second two chapters address contaminant accumulation by prey and the subsequent impacts on predators. There were five main objectives, which are outlined below.

The first objective was to explore the changes in predator-prey interactions arising from toxic effects in prey and, in particular, to investigate the potential additional impacts of predators on single and multiple prey species populations reduced by contaminant exposure **(Chapter 2)**. In order to distinguish between the effects on predation of changes in prey density and possible sublethal effects of exposure, prey density was manipulated directly in the absence of contaminant exposure. When fed single prey species, predators were expected to feed on greater proportions of prey as their density decreased (i.e. Type II functional response). In the presence of alternative prey species, predators were expected to respond according to either their inherent prey preferences or according to the relative abundances of prey (i.e. exhibit prey switching).

The second objective was to examine the feeding response of predators to sublethal changes in their prey **(Chapter 3)**. Contaminant-induced changes in prey susceptibility were used to investigate whether reduced prey populations could result from increases in predation. This was tested in single and multiple prey systems, using predators with different hunting strategies (i.e. active and ambush predators). Predators were expected to feed less on less susceptible prey, resulting in shifts in preference when alternative prey species were available.

The third objective was to assess the potential for benthic prey to act as an exposure pathway for sediment-associated contaminants **(Chapter 4)**. This objective focused on the importance of prey feeding behaviour (sediment ingestion vs non-ingestion) in determining the overall accumulation and internal distribution of contaminants. Benthic species that ingested sediment were expected to have higher body burdens and a higher proportion internally accumulated than non-ingester species.

The forth objective was to examine the subsequent transfer of contaminants from prey to predators **(Chapter 5)**. The importance of feeding behaviour of predators (engulfers vs piercers) combined with prey feeding behaviour (ingester vs non-ingester) in determining extent of transfer and subsequent toxic effects in predators was investigated. Engulfer predators were expected to achieve higher body burdens than piercer predators, with both predators achieving higher body burdens from consuming ingester compared to non-ingester prey.

The fifth objective was to synthesize the findings from Chapters 2 to 5 and identify scenarios in which current practices in ecological risk assessment may underestimate the negative impacts of contaminants **(Chapter 6)**. A scenario-based approach is discussed, using contaminant properties, species traits, landscape profiles and exposure durations to identify situations where changes in predator-prey interactions may be particularly detrimental.

1.7 EXPERIMENTAL SYSTEM

In order to address the objectives described in Section 1.6, a suitable experimental system of test species and contaminants was required. The reasoning for their selection is described in the following sections.

1.7.1 TEST SPECIES

A system of five species of freshwater macroinvertebrates was chosen that would allow all of the objectives to be addressed. They consisted of two predator species (*Ischnura elegans* Vander Linden (Insecta, Odonata) and *Notonecta glauca* Linnaeus (Insecta, Heteroptera)) and three prey species (*Asellus aquaticus* Linnaeus (Crustacea, Isopoda), *Chironomus riparius* Meigen (Insecta, Diptera) and *Cloëon dipterum* Linnaeus (Insecta, Ephemeroptera)). All five species are common inhabitants of ponds in the local Sheffield area (Zasada and Smith, 1981), allowing the collection of large numbers of animals required for experiments.

The two predator species are both known to be generalist predators, feeding on a variety of different prey types (Thompson, 1978; Warren, 1989). However, the two predators differ in their hunting strategies. *Ischnura elegans* is a sit and wait predator, ambushing its prey when encountered (Heads, 1985). *Notonecta glauca* is a more active visual predator, hunting their prey by responding to visual cues (Savage, 1989). They also differ in the way in which they handle their prey. *Ischnura elegans* is classified as an engulfer predator, consuming their prey whole, whereas *N. glauca* is a piercer predator, using piercing mouthparts to feed only on the internal tissues of prey (Peckarsky, 1982).

The three prey species chosen are benthic macroinvertebrates, living in close proximity to the sediment. The relative exposures of the three species to sediment differed according to their feeding behaviour. *Chironomus riparius* is a deposit feeder, therefore feeding on sediment and other organic matter (Oliver, 1971; Andersen and Sedell, 1979). *Asellus aquaticus* feeds on a varied diet, including detritus and sediment (Marcus *et al.*, 1978). *Cloëon dipterum* is an algal grazer (Brown, 1960). The three prey species also differ in their behaviours: *C. dipterum* is

a fast moving mayfly nymph whereas *C. riparius* and *A. aquaticus* are more sedentary.

1.7.2 CONTAMINANTS

Two contaminants were used to address the objectives described in Section 1.6. Cadmium was chosen to investigate the influence of sublethal changes in prey on predator feeding response (Chapter 3). Cadmium occurs naturally in the environment, but its concentration is increased due to mining operations, waste incineration, combustion of coal and oil, and the application of sludge-based and phosphate fertilizers to agricultural land (Robards and Worsfold, 1991). It is consequently found in concentrations of 1 to 1000 µg/L in freshwater (Robards and Worsfold, 1991). Previous studies have demonstrated that cadmium can affect macroinvertebrate behaviour, including reduced response to attack (Gerhardt, 1995), reduced locomotory activity (Gerhardt, 1990), and prey capture success (Ham *et al.*, 1995; Riddell *et al.*, 2005a, b).

Identifying a clearly defined PBT that met the criteria for the project was difficult, therefore a chemical was chosen that had some PBT-like properties, i.e. sufficiently toxic and with the potential to accumulate (but not necessarily persistent in the environment). Benzophenone was thus selected for use in Chapters 4 and 5. Benzophenone has a variety of uses, including being incorporated into agricultural chemicals, pharmaceuticals and paints (EC, 2000; Hayashi *et al.*, 2006). It has been reported in the environment at concentrations of <2.6 to 1040 ng/L in water (Oros *et al.*, 2003; Pojana *et al.*, 2004; Pojana *et al.*, 2007) and 14 to 200 µg/kg in sediment (Burkhardt *et al.*, 2005; Pojana *et al.*, 2007). Benzophenone has a Log K_{ow} of 3.2 (Cichna *et al.*, 1995) and is therefore within the Log K_{ow} range subject to sediment effects assessment (EC, 2003). Although no sediment toxicity data could be identified, aqueous exposure endpoints of 280 µg/L (*Daphnia* 48-h EC_{50} (Tosato *et al.*, 1991)) and 10,890-14,200 µg/L (*Pimephales promelas* 96-h LC_{50} (Marchini *et al.*, 1992)) have been reported. Therefore a low concentration of benzophenone in sediment was selected (30 µg/g) to maximise bioaccumulation by prey but allowing for potential secondary poisoning within predators.

Chapter 2

Effect of changes in prey abundance on predator-prey interactions

---

2.1 INTRODUCTION

Ecological risk assessments (ERAs) are conducted to protect ecosystems from anthropogenic activities, such as the release of chemicals into the environment (Suter II, 1993). Standard laboratory toxicity tests with a limited number of individual species provide the basic data for setting acceptable environmental concentrations of chemicals (Forbes and Forbes, 1994; Maltby, 2006). For certain groups of chemicals, e.g. pesticides, information from model ecosystems containing assemblages of species may be used, but these 'higher-tier' studies are not required for most chemicals (Forbes and Forbes, 1994; Campbell *et al.*, 1998; Preston, 2002).

Single-species toxicity tests have the advantage of being reproducible and easy to interpret, using standardized methods and conducted in controlled laboratory conditions (Rand and Petrocelli, 1985; Graney *et al.*, 1994). However, testing of single species in isolation gives no consideration to the many interactions that occur between species, such as competition and predation (Wootton, 1994; Menge, 1995; Wootton, 2002). Predator-prey interactions play a key role in the structure and functioning of ecosystems (Paine, 1966; Addicott, 1974) and have been shown to be sensitive to environmental contaminants (Clements *et al.*, 1989; Riddell *et al.*, 2005b). Contaminants can alter prey density (Chang *et al.*, 2005) or behaviour (Preston *et al.*, 1999a; Riddell *et al.*, 2005a), both of which will alter the strength and pattern of predator-prey interactions (Schulz and Dabrowski, 2001; Elliott, 2004). Because the relative sensitivity of species to contaminants is chemical specific (Slooff, 1983), predators will experience shifts in prey availability that are dependent on the prey species present and the specific chemical to which they are exposed. For instance, dippers feeding along acid streams consume proportionally more stoneflies and fewer mayflies than those along circumneutral streams (Ormerod and Tyler,

19

1991). Unlike the pH-tolerant stoneflies, mayflies are only found in rivers at pH>6, with dippers preferentially feeding on them when they are available (Ormerod and Tyler, 1991).

Most predators show a Type II functional response (Holling, 1959) to changing prey density, with predators consuming a greater proportion of prey as prey density decreases (Juliano, 2001), i.e. predation is inversely density-dependent. Consequently, if exposure to a contaminant reduces the population of a prey species, increased predation pressure may exacerbate the negative effects of the contaminant. However, non-specialist predators (i.e. generalists) feed on multiple prey and can thus change their diet as the relative abundance of prey changes (Ormerod and Tyler, 1991; Chang et al., 2005). In some cases this can act as a stabilising mechanism for communities, as the predation pressure on the rarer prey species is reduced (Murdoch, 1969). Whether predator-prey interactions mitigate or exacerbate the direct effects of chemicals on prey species depends on the feeding preferences of the predator and also how the prey species interact.

Predators with no inherent prey preference will feed in proportion to the abundances of prey in the environment. Predators can respond to contaminant-induced reductions in density of sensitive prey species by feeding disproportionately more on the more abundant, resistant prey species, traditionally termed 'prey switching' (Murdoch, 1969). Predators with a consistent inherent preference for a particular prey species will maintain their prey intake ratio, independent of prey densities (Tschanz et al., 2007), with proportionally more sensitive prey consumed as their density decreases. Other predators may have an apparent preference by passively responding to changes in the relative availability of prey, due to either increased vulnerability of lower-density (sensitive) prey (Huang and Sih, 1990) or reduced vulnerability of higher-density (resistant) prey (Abrams and Matsuda, 1993). Thus predators can either increase predation on the more abundant resistant prey species or the contaminant-reduced sensitive prey population, depending on their prey preference.

Thus, it is clear that single species toxicity tests can not indicate the possible positive and negative indirect effects of a contaminant on prey species, as these only occur in the presence of a more complex food web. However, the complex responses of

generalist predators to changes in prey density can potentially be addressed by using existing ecological theory regarding species interactions. Various models are in existence, accounting for inherent preferences of predators (Murdoch, 1969; Cock, 1978; Chesson, 1983), and using functional response parameters from single-prey-single-predator interactions (Colton, 1987). The latter assumes that multiple predator and prey species systems are a sum of their parts, with the interactions between multiple species being predictable from single-predator-single-prey interactions. Therefore it may be possible to use these models to predict the outcomes of contaminant-induced effects in food webs by collecting data on the interactions of predators with single prey species.

The results of a series of experiments, guided by this ecological theory, are presented that illustrate the potential impacts of contaminants within a series of simple one-predator-two-prey species communities. The impacts of contaminants are mimicked by manipulating prey abundance, thereby removing any potentially confounding sublethal responses. First examine the response of predators to reduced densities of single 'sensitive' prey species is examined by generating functional response curves, representing the case of specialist predators with no prey choice. Estimates of handling time and attack rate from these models are then used in conjunction with three models of prey choice to predict the outcome of prey choice for generalist predators experiencing shifts in prey availability.

2.2 METHODS

2.2.1 TEST ORGANISMS

Four of the five test species were collected from field populations in Sheffield, South Yorkshire, UK. *Asellus aquaticus* was collected from Havelock Damn in the Rivelin Valley (National Grid Reference (NGR) SK 324 887) or the River Don (NGR SK 316 921), *Cloëon dipterum* was collected from Lower Crabtree Pond (NGR SK 361 899), *Ischnura elegans* from Arbourthorne Pond (NGR SK 371 850) and *Notonecta glauca* from both Lower Crabtree Pond (NGR SK 332 828) and Millhouses Boating Pond (NGR SK 361 899). All species were maintained in aquaria filled with artificial pond water (APW; (HSE, 1982)) at 15°C with a light:dark period of 16:8

hours. *Asellus aquaticus* were fed with detritus (predominantly alder leaves, *Alnus* sp.) and *C. dipterum* were fed with detritus and fresh plant material (predominantly *Elodea* sp. and *Ceratophyllum* sp.), all collected from the source ponds. *Ischnura elegans* and *Notonecta glauca* were fed *ad libitum* with a mixture of *Asellus aquaticus*, *Cloëon dipterum* and *Chironomus riparius*.

The fifth prey species, *Chironomus riparius*, was cultured at The University of Sheffield, UK according to a method adapted from Credland (1973). Briefly, small plastic containers (approx. 10 cm diameter) with play pit sand (1 cm deep) and overlying APW (2 cm deep) were used. Larvae were fed three times a week on powdered Tetramin® tropical fish food flakes. The main cultures were maintained at 20°C but larvae used in experiments were moved to 15°C at least two days prior to experimental use. All animals were subject to a photoperiod of 16 h light to 8 h dark.

2.2.2 SINGLE PREY SYSTEM

Following standard functional response experimental design, the number of prey eaten at different starting prey densities was used to generate functional response curves for each of the six predator-prey combinations. Forty predators were placed into individual 10-cm diameter plastic containers with a 3-cm depth of APW. A thin layer of small glass beads (1.00-1.25 mm diameter: Jencons-PLS, Leighton Buzzard, UK) was added to each feeding arena to act as a sediment substitute to minimise clustering of prey and encourage more natural behaviour. A piece of plastic mesh (approximately 2 cm wide x 6 cm long with 1-cm mesh holes) was also added to function as a 'fishing platform' for the predators (Akre and Johnson, 1979). Predators were fed *ad libitum* for 24 hours before being starved for four days to reduce variation in their hunger levels. Individual predators were then fed with either 2, 4, 6, 10, 15, 20, 25, or 50 individual prey animals, with five replicates of each prey abundance. The number of prey eaten per predator in eight hours was then recorded.

Logistic regression was used to determine which functional response type was most appropriate by analysing the proportion of prey eaten at each prey density (Trexler *et al.*, 1988; Trexler and Travis, 1993). Due to prey depletion over time, estimates of

the handling time and attack rate for each functional response curve were then generated by fitting the Type II Random Predator equation (2.1) (Rogers, 1972):

$$N = N_0(1 - e^{a(Nh - PT)})$$

(2.1)

where N is the number of prey eaten, N_0 is the starting prey abundance, a and h are baseline attack rate and handling time, P is the number of predators and T is the total exposure time. The parameter estimates of a and h were calculated using non-linear least squares regression and the *Lambert W* function (Bolker, 2006).

2.2.3 TWO PREY SYSTEM

The animal species and apparatus set-up was the same as that used in the single prey species functional response experiments (Section 2.2.2), except that predators were offered two prey species instead of one. Thirty predators were placed in individual feeding arenas and fed ten individuals of each prey species to prevent 'training' for a particular prey species (Bergelson, 1985). Prey were then removed after 24 hours and the predators starved for four days. Each predator was then fed with 20 prey, consisting of two prey species in one of three different ratios (10:10, 4:16, 16:4) with ten replicates of each ratio. The total prey density remained equal across the three different ratios to separate the response of predators to changes in total prey availability from changes in the relative availability of prey species (Elliott, 2004). In this way, only the effect of contaminant-induced density changes on predator prey preference was investigated. The number of prey eaten of each species was monitored every two hours for the next eight hours, replacing any eaten prey. Using this method, the ratios of the two prey species remained approximately constant over the eight hour period.

Mean observed percentages of prey species in the diet were visually compared with the predictions from three different models of prey choice: 1) the proportion of a prey species in the diet equals the proportion present in the environment, 2) the proportion Y of a prey species in the diet equals the proportion present in the diet weighted for any inherent preference. Y was calculated as (Murdoch, 1969):

$$Y = \frac{100cX}{(100 - X + cX)} \qquad (2.2)$$

where X is the percentage of prey species 1 in the environment, Y is the percentage of prey species 1 in the diet, and c is a proportionality constant. The proportionality constant was calculated using the following (Murdoch, 1969):

$$c = \frac{p_1}{p_2} \qquad (2.3)$$

where p_1 is the proportion of prey species 1 in the diet, and p_2 is the proportion of prey species 2 in the diet, when they are at a 1:1 ratio in the environment. Finally, 3) the proportion of a prey species expected in the diet was calculated using the two-prey random predator model (Colton, 1987):

$$E_1 = N_1\left(1 - e^{-a_1(T - h_1 E_1 - h_2 E_2)}\right)$$
$$E_2 = N_2\left(1 - e^{-a_2(T - h_1 E_1 - h_2 E_2)}\right) \qquad (2.4)$$

where E_i is the number of prey eaten by a single predator, N_i is the starting prey density, T is the total exposure time (hours), a_i is the attack rate (no. prey attacked hour^{-1}) and h_i is the handling time (hours), with subscripts referring to the two prey types. Attack rate and handling time parameters were generated by fitting equation (2.1) to the functional response data. Equation (2.4) was solved by optimization using a quasi-Newton method and the "gr" and "$optim$" functions in R (R, 2007), developed by Bolker (pers. comm.). This allowed predictions to be made of the numbers of each prey species eaten at different starting densities during the feeding period.

A sensitivity analysis was performed on the two prey random predator model to determine the influence of the error associated with parameter estimates of handling time (h) and attack rate (a) on the model predictions. The model predictions for $N.$ glauca feeding on $C.$ dipterum and $C.$ riparius were selected as parameter estimates were not statistically significant for h and a, respectively. The proportion of $C.$

24

riparius in the diet was predicted using the mean parameter estimates ± the standard error associated with the mean. The proportions predicted using these four models (maximum *C. riparius* attack rate, minimum *C. riparius* attack rate, maximum *C. dipterum* handling time, minimum *C. dipterum* handling time) were compared to the observed proportion of *C. riparius* in the diet.

All data analyses were performed using the statistical software package, *R* (R, 2007).

2.3 RESULTS

2.3.1 SINGLE PREY SYSTEM

Five of the six predator-prey combinations were best described by Type II functional response models based on logistic regression (Figure 2.1 and 2.2). The exception was *N. glauca* feeding on *C. dipterum*, which was not described by either Type I, II or III models (Figure 2.2c). However, in all cases, there is a trend for predators to feed on a greater proportion of the prey when at lower starting densities (Figure 2.1 and 2.2). Following the selection of appropriate functional response models, handling times (T_h) and attack rates (a) were generated for each predator-prey combination using the Random Predator Equation (Table 2.1). The highest attack rate for *I. elegans* was when feeding on *C. riparius* (0.13 h^{-1}), whereas for *N. glauca* it was when fed with *A. aquaticus* (0.47 h^{-1}). The longest handling time for both predators was when feeding on *C. riparius*, with *I. elegans* needing 1.75 hours to handle a single *C. riparius*, whereas *N. glauca* needed slightly longer (2.15 hours). Interestingly, the attack rate of *N. glauca* on *C. riparius* is not significantly different to zero, evidenced in the rapidly flattening out shape of the curve (Figure 2.2bii). The handling time parameter was not significantly different to zero for both predators feeding on *C. dipterum*, indicating that handling time was negligible.

2.3.2 TWO PREY SYSTEM

The total intake of prey by predators did not vary between the three different ratios of prey species for four of the six predator-prey combinations tested (ANOVA: $F \leq 2.7$, df=2,29, p>0.05). The exceptions were predators feeding on *C. dipterum* and *C.*
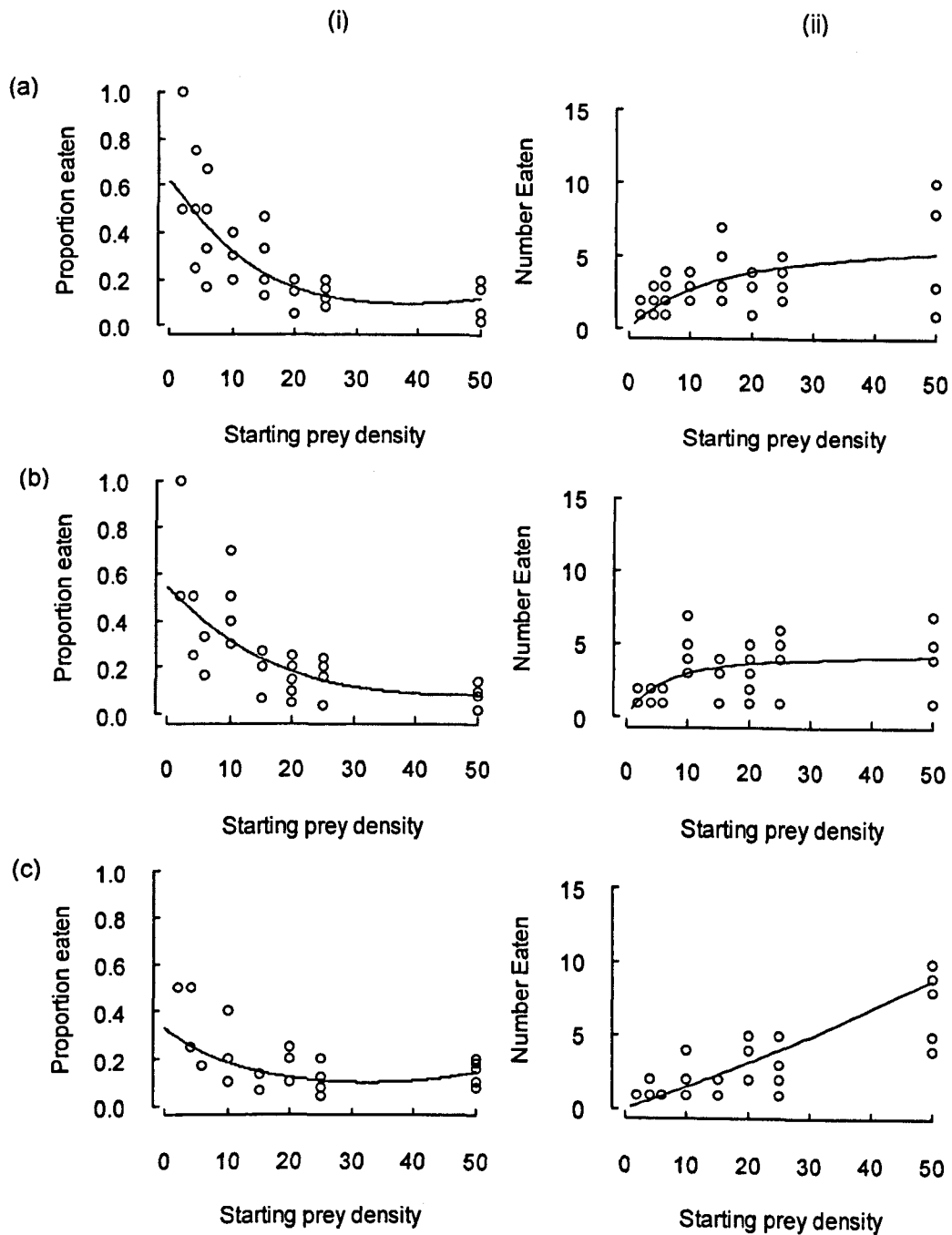
Figure 2.1. Functional response curves for *Ischnura elegans* feeding on: a) *Asellus aquaticus*; b) *Chironomus riparius* c) *Cloëon dipterum*. For each predator-prey combination i) shows the Type II model for proportion of prey eaten at each starting density fitted using logistic regression, and ii) shows the Type II functional response curve fitted using the Random Predator Equation (Rogers, 1972).

26
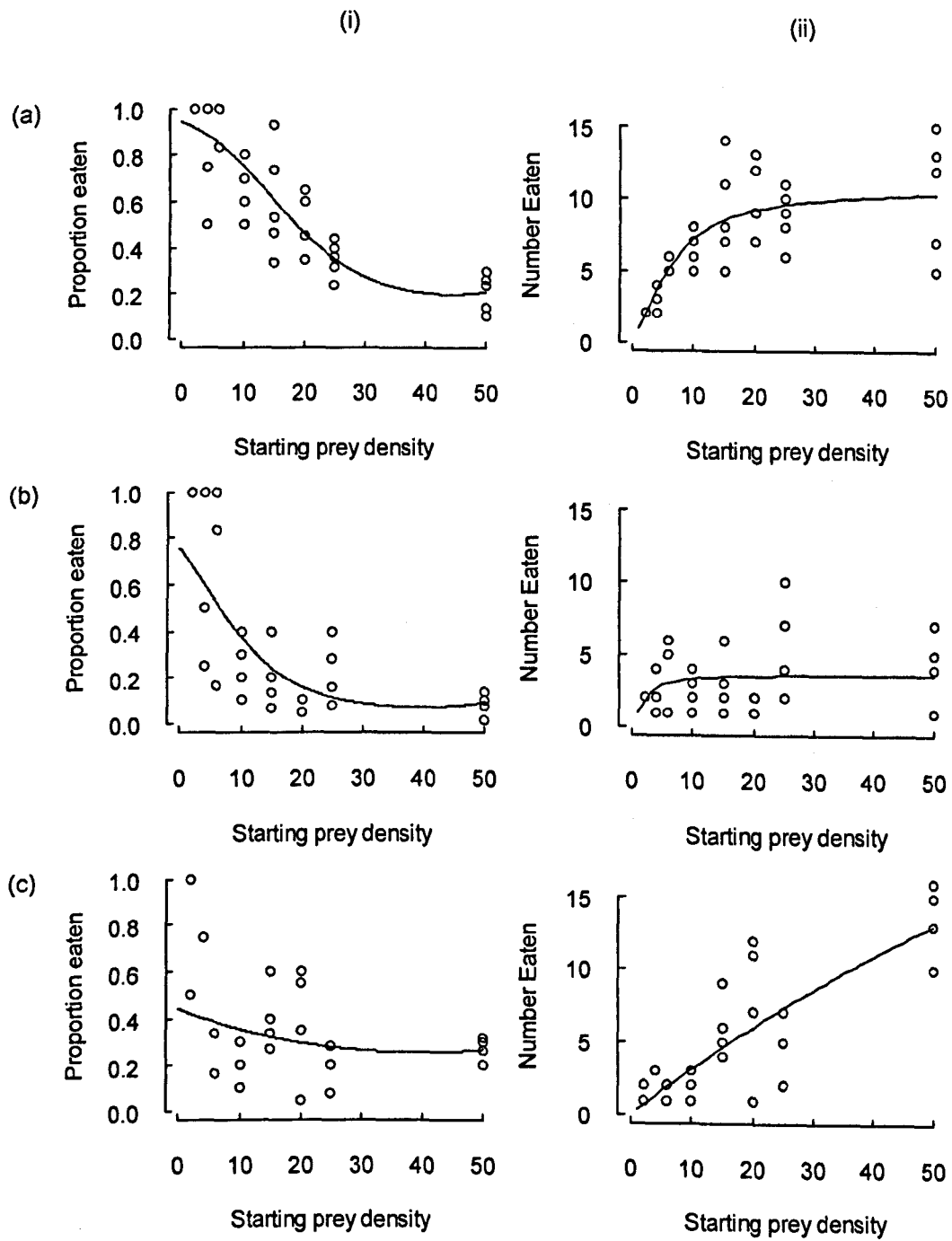
Figure 2.2. Functional response curves for *Notonecta glauca* feeding on: a) *Asellus aquaticus*; b) *Chironomus riparius* c) *Cloëon dipterum*. For each predator-prey combination i) shows the Type II model for proportion of prey eaten at each starting density fitted using logistic regression, and ii) shows the Type II functional response curve fitted using the Random Predator Equation (Rogers, 1972).

Table 2.1. Type II functional response parameter estimates for six predator-prey combinations generated using the Random Predator Equation (Rogers, 1972).

Predator	Prey	Attack rate (no. prey attacked hour^{-1}; a)	Handling time (hours; h)
Ischnura elegans	*Asellus aquaticus*	0.07 (0.03)	1.24 (0.28)
	Chironomus riparius	0.13 (0.06)	1.75 (0.27)
	Cloëon dipterum	0.02 (0.003)	-0.19[*] (0.34)
Notonecta glauca	*Asellus aquaticus*	0.47 (0.23)	0.73 (0.07)
	Chironomus riparius	0.49[*] (0.70)	2.15 (0.37)
	Cloëon dipterum[†]	0.05 (0.01)	0.15[*] (0.14)

[*] denotes parameter estimates not significantly different to zero;

[†] denotes where Type II functional response model was not a significant fit to the data using logistic regression method; numbers in parentheses are standard errors for parameter estimates

riparius, in which both predators ate more prey when *C. riparius* comprised 80% of the prey available compared to at 20% and 50% (ANOVA: F≥4.3, df=2,29, p<0.05).

The feeding preferences of the predators in the six different predator-prey combinations are shown in Figure 2.3. *Notonecta glauca* had a preference for a particular prey species in all three prey combinations, either for *Chironomus riparius* (Figure 2.3a and c) or *A. aquaticus* (Figure 2.3b). The preference for *C. riparius* was particularly strong when comprising 20% of the available prey in combination with *C. dipterum*, as *C. riparius* forms 60% of the diet (Figure 2.3c). *Ischnura elegans* preferred *C. riparius* when in combination with both *A. aquaticus* (Figure 2.3d) and *C. dipterum* (Figure 2.3f). Again, this preference was particularly notable when *C. riparius* comprised 20% of the available prey, forming at least 60% of the diet in both cases (Figure 2.3d and f). The prey preference of *I. elegans* when offered *A. aquaticus* and *C. dipterum* was not consistent across the three prey availability treatments (Figure 2.3e). There was a preference for *C. dipterum* when *A. aquaticus* formed 50% and 80% of the available prey. However, *A. aquaticus* became the preferred prey when comprising 20% of the available prey.

For all six predator-prey combinations, the proportion of a prey species in the diet was not equal to the proportion of that same prey species in the environment, indicating prey preference by the predator. Only one predator-prey combination showed any similarity with the two-prey random predator model predictions (*I. elegans* feeding on *C. dipterum* and *C. riparius* (Figure 2.3f)). The prey preference model was a good predictor of the data for only two of the predator-prey combinations: *N. glauca* feeding on either *A. aquaticus* and *C. riparius* (Figure 2.3a) or *A. aquaticus* and *C. dipterum* (Figure 2.3b). The prey preference model overlapped with two-thirds of the data for *N. glauca* (Figure 2.3c) and *I. elegans* (Figure 2.3f) feeding on *C. dipterum* and *C. riparius*. However, a higher proportion of *C. riparius* were consumed by *N. glauca* than predicted by the model when forming 20% of the prey available (Figure 2.3c) and a lower proportion of *C. riparius* were consumed by *I. elegans* when forming 80% of the prey available (Figure 2.3f). The prey choices of the remaining two predator-prey combinations, *I. elegans* feeding on *A. aquaticus* and *C. riparius* (Figure 2.3d) or *A. aquaticus* and *C. dipterum* (Figure 2.3e), were not predicted by any of the models used.

29
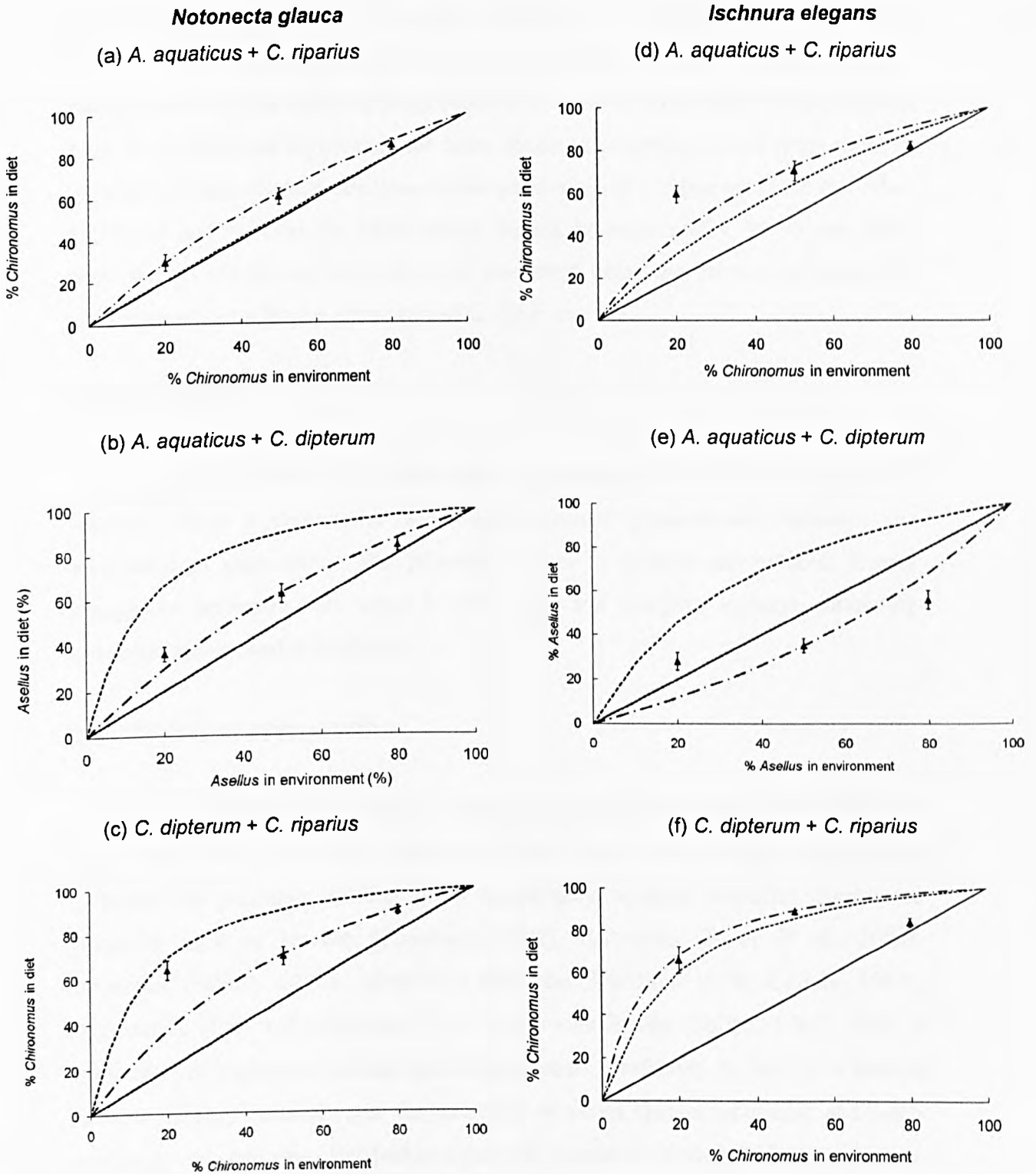
Figure 2.3. Relationship between the mean (± 1 SE) percentage of a prey species eaten by a predator and the percentage of that prey species present in the environment out of a total of 20 prey. Data generated for six combinations of a single predator and two prey species: a) *Notonecta glauca* feeding on *Asellus aquaticus* and *Chironomus riparius*, b) *N. glauca* feeding on *A. aquaticus* and *Cloëon dipterum*, c) *N. glauca* feeding on *C. dipterum* and *C. riparius*, d) *Ischnura elegans* feeding on *A. aquaticus* and *C. riparius*, e) *I. elegans* feeding on *A. aquaticus* and *C. dipterum*, f) *I. elegans* feeding on *C. dipterum* and *C. riparius*. Data fitted with predictions from the proportions of prey in the environment (—), prey preference model (Equation 2.2) (— - —), and the two-prey random predator model (Equation 2.4) (---).

The sensitivity analysis for *N. glauca* feeding on *C. dipterum* and *C. riparius* indicated little improvement for matching predictions from the two prey random predator model to the observed proportions of *C. riparius* in the diet. The predictions from all models were higher than the mean observed proportions at all proportions in the environment. Model predictions for the percentage of *C. riparius* in the diet when at 20, 50 and 80% in the environment ranged between 66-71, 90-93 and 98%, respectively. The lowest estimates were generated using the mimimum (mean-SE) parameter estimate for the attack rate of *C. riparius*.

2.4 DISCUSSION

This series of experiments investigated how populations of predators and prey may respond within a community when environmental contaminants influence the abundance of prey items. The potential effects of contaminant-induced density changes on predation were tested in both single and two prey systems, simulating specialist and generalist predators.

2.4.1 SPECIALIST PREDATORS

When offered a single prey species, predators were found to consume proportionally more prey as the prey density decreased. Such Type II functional responses are common for predators feeding on a single prey species, including freshwater predators such as leeches (Bronmark, 1992), flatworms (Beier *et al.*, 2004), stoneflies (Elliott, 2003), odonates (Akre and Johnson, 1979; Colton, 1987), notonectids (Fox and Murdoch, 1978) and midge larvae (Spitze, 1985). This is important for predators that are specialised feeders, preferring to feed on a limited number of prey species/types; for example, of seven species of triclad and leech predators, only one was classified as a generalist predator (Young, 1981).

The presence of Type II predators in natural assemblages has important implications for the impact of contaminants. If the populations of prey that are consumed by specialised predators are reduced by exposure to contaminants, increased predation pressure will exacerbate the negative effects of the contaminants on the prey. Therefore the impact of a contaminant may be higher in systems containing predators

compared to in single species laboratory tests. Such differences were observed for copepods and rotifers exposed to an organophosphate insecticide (diazinon), experiencing higher mortality in pond microcosm exposures compared to single species laboratory exposures (Giddings *et al.*, 1996). Similarly, *Daphnia longispina* was an order of magnitude more sensitive to 3,4-dichloroaniline (industrial intermediate) when exposed within pond assemblages compared to in laboratory conditions (Crossland and Hillaby, 1985). Therefore the current approach in risk assessment of generating toxicity end points for single species in isolation actually represents the minimum effect that a contaminant will have on a population. Contaminants, when combined with increased predation pressure, could potentially halve the abundance of a prey species at a far lower toxicant concentration than the conventionally-predicted lethal concentration (LC_{50}).

2.4.2 GENERALIST PREDATORS

In natural systems most predators are generalists, and therefore can potentially compensate for any changes in abundance of sensitive prey species by feeding disproportionately more on more abundant, resistant species. However, none of the six predator-prey combinations examined demonstrated such prey switching behaviour. An absence of prey switching has also been observed in other studies of aquatic predators, including damselflies (Lawton *et al.*, 1974), perlid stoneflies (Elliott, 2004), and predatory snails (Murdoch, 1969). However, prey switching has been demonstrated in other predator-prey systems, including fish (Murdoch *et al.*, 1975), notonectid (Lawton *et al.*, 1974), larval dragonfly (Akre and Johnson, 1979), and perlodid stonefly (Elliott, 2004) predators. Various mechanisms have been suggested to account for the absence or occurrence of prey switching, including strength of prey preference (Murdoch, 1969; Oaten and Murdoch, 1975), learning behaviour (Murdoch, 1969; Lawton *et al.*, 1974; Oaten and Murdoch, 1975), switching between patches (Murdoch *et al.*, 1975) and hunting strategies of predators (Elliott, 2004).

In predator-prey systems in which prey switching does occur, the implications of predation for sensitive prey may depend on the exposure scenario. Continuous exposure causes a direct reduction in populations of sensitive prey species (Ward *et*

al., 1995), potentially leading to increased predation on the resistant prey species. Pulsed or short-term exposure allows the sensitive prey population to recover from the acute effects of exposure (Kallander *et al.*, 1997; Naddy and Klaine, 2001; Reynaldi and Liess, 2005), and reduced predation pressures from prey switching may allow an increase in their population size. However, as their population size increases, they may become the favoured prey species and become predated on more. As a result of density-dependent prey preference in switching predators, the community may be more stable (Oaten and Murdoch, 1975), potentially allowing recovery of sensitive prey species populations following contaminant exposure.

Rather than demonstrating prey switching, predators in the current study fed preferentially on one of the available prey species. In two of the six predator-prey combinations the predators demonstrated a consistent preference for a single prey species across all relative densities (*N. glauca* feeding on *A. aquaticus* and either *C. riparius* or *C. dipterum*), with data fitting well to the prey preference model. Consistent preferences in predators have been previously documented; for example, two perlid stonefly predator species consistently preferred mayfly over chironomid prey over a range of prey ratios (Elliott, 2004). The ratio of prey species in the diet of such predators will therefore remain unchanged following contaminant-induced reductions in the density of sensitive prey. Therefore, preference for the sensitive prey species will result in an increase in predation pressure because of their reduced density, further reducing their populations.

The predators in the remaining four predator-prey combinations also demonstrated preference for one prey species over the other, but their preference became stronger as the relative abundance of the favoured prey species decreased. This has been demonstrated in other studies in which the presence of the alternative prey species enhances predation on the preferred species (Tschanz *et al.*, 2007). The prey choice by predators in the current study could not be predicted by either the prey preference model or the two-prey random predator model, which accounts for the different functional responses for the component prey species (Colton, 1987). One explanation for the observed patterns in prey choice by predators is that the presence of alternative prey species may be affecting their relative availability to predators. For example, female isopods (*Lirceus fontinalis*) became more vulnerable to predation by

33

green sunfish (*Lepomis cyanellus*) in the presence of salamander larvae (*Ambystoma texanum*) due to competition for refuges (Huang and Sih, 1990). In such cases where sensitive prey species experience a much greater predation pressure, their already reduced populations may become more vulnerable to local extinctions.

As with standard LC_{50} tests, the predator-prey experiments performed in the current study were not performed on populations and did not involve prey replacement. Therefore the experiments did not allow for the possibility of recovery, via reproduction or recolonisation. However, recovery is possible in natural assemblages (van den Brink *et al.*, 1996; Beketov *et al.*, 2008), which may prevent local extinctions of sensitive prey as a result of additional predation. The extent and rapidity of recovery may depend on the life history traits of prey species (Wallace, 1990; Beketov *et al.*, 2008). Aquatic invertebrates vary extensively in their reproductive and dispersal characteristics, with some species reproducing continually throughout the year and others reproducing only once (Sherratt *et al.*, 1999). Species that have low intrinsic rates of increase (Sherratt *et al.*, 1999; Stark *et al.*, 2004; Beketov *et al.*, 2008), minimal ability to recolonise by migration (Liess and Von der Ohe, 2005), and are present in the aquatic phase of their lifecycle during peak exposure (Liess and Von der Ohe, 2005) are likely to have a slow recovery following an impact on their population. This was demonstrated in mesocosms dosed with esfenvalerate, in which rapidly reproducing cladoceran species showed recovery whereas relatively slow reproduction in copepods resulted in little recovery (Lozano *et al.*, 1992). Consequently if species with these "slow recovery" traits are preferred by predators, they may be more susceptible to becoming locally extinct, having a limited ability to increase their population size following contaminant-induced reductions.

This study has highlighted the potential additional impacts that predators can have on prey populations reduced by contaminant exposures. However, if the current risk assessment approach of protecting the 'most sensitive species' is adequately protective, such contaminant-induced reductions should not occur in natural assemblages (Cairns, 1986). Although the standard toxicity species (e.g. *Daphnia magna*) have higher relative sensitivities than most other macroinvertebrates, there will be some species that are more sensitive (Wogram and Liess, 2001). Detecting

the most sensitive species may also depend on the level of organisation tested, with populations being more sensitive than individuals (Beketov and Liess, 2005; Stark, 2005). The sensitivity of a species can also depend on the exposure scenario, with chronic exposures inducing toxic effects at concentrations much lower than the LC_{50} (Schultz and Liess, 1995; van den Brink et al., 1995; Liess and Schultz, 1996). The combination of these factors may explain why reductions in abundance have been detected in monitoring studies, even though pesticides are present at concentrations two to three orders of magnitude lower than the LC_{50} of the most sensitive species (Liess and Von der Ohe, 2005; Schäfer et al., 2007). Thus, predators in natural systems will be experiencing shifts in availability of prey, depending on prey sensitivity to exposure. Ecological risk assessments therefore need to incorporate the effects not only of anthropogenic stressors (contaminants) but also natural stressors (predators) when assessing the extent of the risk of exposure.

Chapter 3

Sublethal effects and predator-prey interactions

3.1 INTRODUCTION

Ecological risk assessments (ERAs) are conducted to protect ecosystems from anthropogenic activities, including the release of chemicals into the environment (Suter II, 1993). The basic data for establishing acceptable environmental concentrations of chemicals is generated using standard laboratory toxicity tests with a limited number of individual species (Forbes and Forbes, 1994; Maltby, 2006). More natural assemblages of species are used for certain groups of chemicals, e.g. pesticides, but most chemicals do not require such 'higher-tier' studies (Forbes and Forbes, 1994; Campbell *et al.*, 1998; Preston, 2002).

Single-species toxicity tests are favoured as they use standardized methods in controlled laboratory conditions, and are therefore easy to reproduce and interpret (Rand and Petrocelli, 1985; Graney *et al.*, 1994). However, such single-species tests do not account for the many interactions that occur between species in natural assemblages, such as competition and predation (Wootton, 1994; Menge, 1995; Wootton, 2002). Contaminants can alter prey density (Chang *et al.*, 2005) and behaviour (Preston *et al.*, 1999a; Riddell *et al.*, 2005a), both of which can affect predator-prey interactions (Clements *et al.*, 1989; Riddell *et al.*, 2005b). Behavioural responses are more sensitive endpoints than survival, with changes in locomotory behaviour in fish occurring as low as 0.7-5% of the lethal effect concentration (LC_{50}) (Little and Finger, 1990). A number of behavioural endpoints exist for ecotoxicological studies (Boyd *et al.*, 2002), but human-perceived changes in such behaviours may differ to those detected by predators. The ecological relevance of different endpoints may depend on the response of the prey to exposure, as well as the hunting strategies of the predators feeding upon them.

Contaminants have been shown to affect 'virtually every aspect of behaviour of terrestrial and aquatic organisms' (Little, 1990). The responses of prey to contaminants that can influence predation can be divided into two types: general activity and response to external stimuli, such as predator attacks. The effects of contaminants on these behaviours can be broadly categorised into three behavioural toxicity syndromes (Drummond and Russom, 1990; Russom et al., 1997). Hypoactivity is categorised by reduced activity and reduced response to external stimuli, generally caused by exposure to narcotics (e.g. phenol, 1-octanol) and cholinesterase inhibitors (e.g. carbaryl, chlorpyrifos) (Carlson et al., 1998). Hyperactivity is categorised by increased activity and increased response to external stimuli, resulting from exposure to excitatory agents (e.g. pentachlorophenol) (Preston et al., 1999a). Finally, physical deformity syndrome is categorised mainly by the occurrence of convulsions and incoordination, but can also result in reduced activity and increased response to external stimuli, induced by exposure to reactive chemicals (e.g. acrylamide, benzaldehyde) (Russom et al., 1997), organometals (e.g. methylmercury) (Zhou and Weis, 1998), and central nervous system agents (e.g. endosulfan, fenvalerate) (Carlson et al., 1998). Some contaminants, such as metals, are more difficult to categorise, as, although metals elicit behavioural effects, each metal results in a different response (Barron, 2002). The outcome of behavioural changes in prey for their susceptibility to predation may depend on the hunting strategy of the predator that they encounter.

Predators can be ambush hunters (e.g. odonate larvae, flounder fish (*Platichthys flesus*)) that sit and wait for their prey to come to them, or active, "cruising" hunters (e.g. waterboatman (*Notonecta glauca*), rainbow trout (*Oncorhynchus mykiss*)) that actively seek out their prey (Peckarsky, 1982; Greene, 1986). Both types of predator would be expected to have a reduced feeding rate if prey were to become generally less active due to hypoactivity. For example, Spitze (1985) found that the attack rate of the ambush predator, *Chaoborus americanus*, decreased as the swimming speed, and thus encounter rates, of its *Daphnia* prey decreased. Reduced activity would also provide less visual cues for active hunters to locate their prey, important for eliciting a predator attack (Greene, 1986). The effect of reduced responses to attacks in hypoactive prey may depend on predator hunting strategy. Active predators may experience fewer visual cues from less responsive hypoactive prey, thereby

stimulating fewer attacks (Peckarsky and Penton, 1989a). Ambush predators attempt to strike at prey prior to an escape response being triggered, and will therefore be less sensitive to changes in attack response compared to encounter rates. Any change in predation pressure will have implications for prey population dynamics. Increased predation on caddisfly (*Hydropsyche morosa*) by active stonefly predators (*Paragnetina media*) associated with copper exposure resulted in reduced abundance of *H. morosa* (Clements *et al.*, 1989). In contrast, lindane-exposed *Daphnia magna* experienced reduced predation by their ambush predators (*Hydra oligactis*), allowing higher recruitment of *D. magna* (Taylor *et al.*, 1995).

Some predators are generalists, able to compensate for a reduction in the relative availability of one prey species by feeding on an alternative. For example, four predatory stonefly species responded to shifts in midge larvae (*Chironomus* sp.) prey abundance by feeding on mayfly nymphs (*Baetis rhodani*) (Elliott, 2004). Following sublethal contaminant exposures, prey species may be equally abundant in terms of absolute numbers present, but may differ in their relative availabilities due to changes in susceptibility to predation (Preston *et al.*, 1999b). Therefore predators may alter their prey choice as a result of sublethal exposures of prey. There is currently a paucity of data available on the sublethal effects of chemicals in multiple predator-prey systems. Riddell *et al* (2005b) examined the impact of sublethal cadmium exposure on prey choice in salmon feeding on mayfly and midge larvae. Their results suggest that these active hunters shift from having no prey preference in the controls to preferring mayfly in cadmium-exposed systems, though this was not statistically significant due to large variation in the data (Riddell *et al.*, 2005b). Clements *et al* (1989) used an experimental stream approach to examine the effect of copper exposure on the feeding behaviour of an active stonefly predator (*Paragnetina media*). A greater proportion of net-spinning caddisfly larvae (Hydropsychidae) were present in the guts of predators following copper exposure (Clements *et al.*, 1989). This was possibly due to disrupted silk-spinning in the caddisfly prey, thereby requiring them to spend more time maintaining their nets, and subsequently becoming more vulnerable to predation (Clements *et al.*, 1989).

The aim of this study was to investigate whether feeding responses of predators were influenced by behavioural changes in prey from sublethal exposure, and the relative

importance of predator hunting strategy (ambush vs. active predators) on their response. A freshwater macroinvertebrate system was used, consisting of one ambush (*Ischnura elegans* Vander Linden (Insecta, Odonata)) and one active (*Notonecta glauca* Linnaeus (Insecta, Heteroptera)) predator species. Predators were fed with three naturally co-occurring prey species (*Asellus aquaticus* Linnaeus (Crustacea, Isopoda), *Cloëon dipterum* Linnaeus (Insecta, Ephemeroptera), and *Chironomus riparius* Meigen (Insecta, Diptera)), using cadmium as a model contaminant. Cadmium is known to affect macroinvertebrate behaviour (e.g. Gerhardt, 1990; Ham *et al.*, 1995; Riddell *et al.*, 2005a, b), with the prey species exhibiting different sensitivities to acute cadmium exposure (Table 3.1). Firstly the responses of ambush and active predators to extreme changes in prey behaviour were tested, using immobile (dead) and mobile (live) prey. The influence of more subtle changes in prey behaviour (general activity and response to simulated predator attack) on active and ambush predators was then investigated by exposing single prey species to cadmium. Prey choices by predators were predicted according to changes in prey behaviour (Table 3.2), and tested by offering predators pairs of prey exposed to cadmium solution or control conditions.

3.2 METHODS

3.2.1 TEST SPECIES

Four of the five test species were collected from field populations in Sheffield, South Yorkshire, UK. *Asellus aquaticus* was collected from Havelock Dam in the Rivelin Valley (National Grid Reference (NGR) SK 324 887) or the River Don (NGR SK 316 921), *Cloëon dipterum* was collected from Lower Crabtree Pond (NGR SK 361 899), *Ischnura elegans* from Arbourthorne Pond (NGR SK 371 850) and *Notonecta glauca* from both Lower Crabtree Pond (NGR SK 332 828) and Millhouses Boating Pond (NGR SK 361 899). All species were maintained in aquaria filled with artificial pond water (APW; (HSE, 1982)) at 15°C with a light:dark period of 16:8 hours. *Asellus aquaticus* were fed with detritus (predominantly alder leaves, *Alnus* sp.) and *C. dipterum* were fed with detritus and fresh plant material (predominantly *Elodea* sp. and *Ceratophyllum* sp.), all collected from the source ponds. *Ischnura*

Table 3.1. Toxicity data (96 hour LC_{50} values) for study species exposed to cadmium.

Species	LC_{50} (µg/L)	Reference
Asellus aquaticus	160[a]	(Ham *et al.*, 1995)
	150-450[b]	(Green *et al.*, 1986)
Chironomus riparius	1760	(Watts and Pascoe, 2000)
Baetis tricaudatus[c]	1611	(Irving *et al.*, 2003)

[a] - Used maximum concentration of 450 µg Cd/L and individuals measuring 4-6 mm in length

[b] - Used maximum concentration of 1750 µg Cd/L individuals measuring 2-6 mm in length

[c] - No 96-hr LC_{50} value could be identified for *Cloëon dipterum* so a closely related species was selected instead

Table 3.2. Predicted change in prey choice of ambush and active predators following behavioural changes in prey species from sublethal exposure to a contaminant

Prey behaviour	Effect of exposure to sensitive prey species[*]	Effect on prey choice for	
		Ambush predator	Active predator
General activity	Increase	Increase feeding on sensitive prey	Increase feeding on sensitive prey
	Decrease	Decrease feeding on sensitive prey	Decrease feeding on sensitive prey
Response to attack	Increase	No effect	Increase feeding on sensitive prey
	Decrease	No effect	Decrease feeding on sensitive prey

[*] Predator has the choice of two prey species: one that is affected by exposure to the contaminant (sensitive) and one that is not (resistant). Therefore the behaviour of the resistant prey species remains the same, but its relative availability to predators is affected by the behavioural changes in the sensitive prey species.

elegans and *Notonecta glauca* were fed a mixture of *Asellus aquaticus*, *Cloëon dipterum* and *Chironomus riparius ad libitum*.

Chironomus riparius was cultured according to a method adapted from Credland (1973). Briefly, small plastic containers (approx. 10 cm diameter) with play pit sand (1 cm deep) and overlying APW (2 cm deep) were used. Larvae were fed three times a week on powdered Tetramin® tropical fish food flakes. The main cultures were maintained at 20°C but larvae used in experiments were moved to 15°C at least two days prior to experimental use. All animals were subject to a photoperiod of 16 h light to 8 h dark.

3.2.2 PREDATOR RESPONSE TO PREY BEHAVIOUR

3.2.2.1 SINGLE PREY SPECIES

Twenty predators were separated into individual polypropylene plastic cups (7 cm diameter) containing a 3-cm depth of APW. Half were fed ten prey that had been kept at 15°C (live prey) and half were fed prey that had been frozen at -30°C for 30 minutes (dead prey). Frozen prey were defrosted immediately prior to use. Predators were left to feed for two hours after which the numbers of live and dead prey eaten were recorded. This experiment was performed for all six predator-prey combinations.

3.2.2.2 TWO PREY SPECIES

Forty *Notonecta glauca* were separated into individual plastic cups containing a 3-cm depth of APW. Each predator was fed with ten prey, consisting of five *Cloëon dipterum* and five *Chironomus riparius* in a fully factorial design of each prey species being either dead or alive. Prey were prepared as detailed previously in Section 3.2.2.1. Predators were left to feed for two hours after which the numbers of live and dead prey eaten were recorded in each treatment. This experiment was repeated for *Ischnura elegans* feeding on *Cloëon dipterum* and *Chironomus riparius*.

3.2.3 BEHAVIOURAL EFFECTS OF CADMIUM ON PREY

3.2.3.1 PREY ACTIVITY

Four of each prey species were exposed to APW and four to 336 μg Cd/L. This cadmium concentration was chosen following a range-finding experiment, aiming to maximise sublethal effects but minimise changes in survival. Individual prey animals were exposed to 50 ml of the appropriate solution in a 60-ml glass jar. Artificial pond water (APW) was used to prepare the cadmium solution and was used as the control treatment (0 μg Cd/L). Animals were exposed for four days, after which time any mortality was recorded. Following this exposure period, surviving prey were moved to clean jars containing APW and their activity monitored after a 15-minute acclimation period. Individual prey animals were observed for one minute and the amount of time spent active (i.e. visible to predators) within this minute was recorded. The experiment was repeated in four blocks so that each treatment for each species comprised a total of 16 independent replicates.

3.2.3.2 RESPONSE TO SIMULATED PREDATOR ATTACK

Prey animals were exposed to APW or cadmium, as detailed in the prey activity experiment. Following this exposure period, surviving prey were moved to individual polypropylene plastic pots (10 cm diameter) containing a 3-cm depth of APW. After fifteen minutes the response to attack was measured by prodding an inactive individual prey animal using a plastic pipette. The duration of the response (i.e. amount of time spent active after prodding) was recorded. This experiment was repeated in four blocks resulting in 16 independent replicates for each treatment and prey species.

3.2.4 EFFECT OF CADMIUM ON PREDATION OF A SINGLE PREY SPECIES

Twenty prey animals were added to a polypropylene plastic cup (7 cm diameter) containing 100 ml of either APW or 336 μg Cd/L solution, with ten replicates per treatment. Twenty predators were placed in individual 10-cm diameter plastic pots containing a 5-cm depth of APW, a thin layer of small glass beads as a substrate, and

43

were fed *ad libitum* on the prey species being tested for 24 hours before being starved for four days to reduce variation in their hunger levels. After this starvation period, predators were given twenty prey that had been exposed to either APW or cadmium solution for 96 h. Predator feeding rate was measured by counting how many prey remained alive after two hours. This experiment was repeated with each of the six predator-prey combinations.

3.2.5 EFFECT OF CADMIUM ON PREY CHOICE

This experiment was repeated for all six single-predator-two-prey species combinations. Pairs of prey species were exposed to two different treatments, either APW or 336 µg Cd/L for 96 h, before being offered to a predator. Groups of 20 individuals of a single prey species were added to a plastic cup containing 100 ml of either APW or cadmium solution, with ten replicates per treatment. Twenty predators were placed in individual 10-cm diameter plastic pots containing a 5-cm depth of APW, a thin layer of small glass beads as a substrate, and a piece of plastic mesh (approximately 6 cm long) to act as a fishing platform. They were fed *ad libitum* on the two prey species being used for 24 hours before being starved for four days to reduce variation in their hunger levels. After this starvation period, predators were given ten individuals per prey species, all of which had been previously exposed either to cadmium or APW. The proportion of each prey species in the diet was then calculated to determine the effect of cadmium exposure on prey choice.

3.3 RESULTS

3.3.1 PREDATOR RESPONSE TO PREY BEHAVIOUR

3.3.1.1 SINGLE PREY SPECIES

Notonecta glauca consumed significantly more mobile (live) than immobile (dead) *C. dipterum* and *C. riparius* (Figure 3.1 a, b; One-tailed Mann-Whitney U Test: $U \leq 45$, $n_1 = 10$, $n_2 = 10$, $p < 0.05$), but, there was no effect of prey activity on the number of *A. aquaticus* consumed (Figure 3.1c; $U = 57$, $n_1 = 10$, $n_2 = 10$, $p > 0.05$). *Ischnura*

44
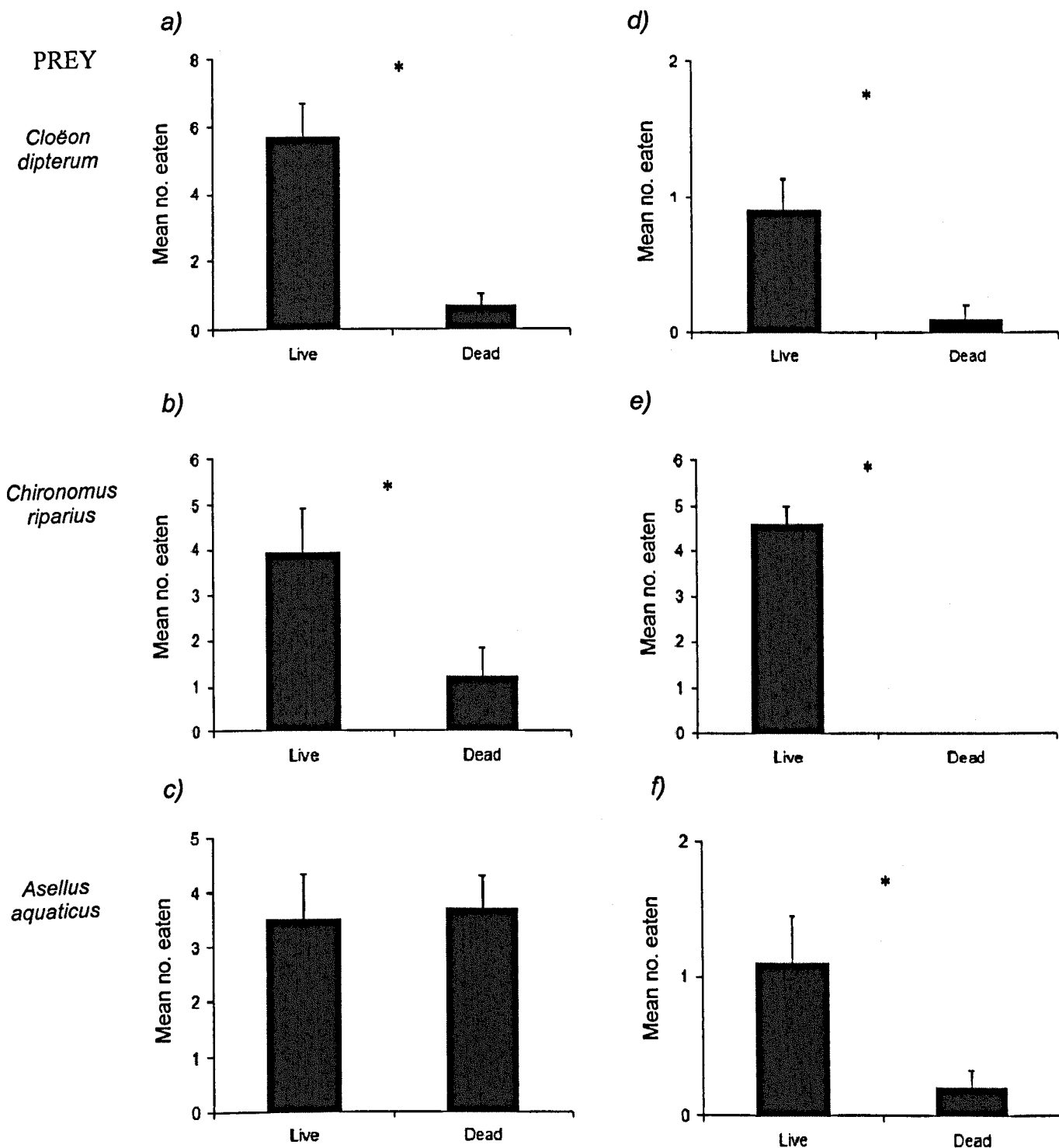
Figure 3.1. The mean (+ 1 SE) number of immobile (dead) or mobile (live) prey items eaten in two hours by *Notonecta glauca* feeding on a) *Cloëon dipterum*, b) *Chironomus riparius* or c) *Asellus aquaticus*; or *Ischnura elegans* feeding on d) *C. dipterum*, e) *C. riparius*, or f) *Asellus aquaticus*. Predators initially given 10 live or dead prey animals. Asterisk denotes significant difference between means (p<0.05). Note that *Ischnura elegans* ate no dead *C. riparius* e).

elegans consumed significantly more live than dead *C. dipterum* (Figure 3.1d; U=19, n_1=10, n_2=10, p<0.05) and *A. aquaticus* (Figure 3.1f; U=26, n_1=10, n_2=10, p<0.05). They also only ate live *C. riparius* (Figure 3.1e).

3.3.1.2 TWO PREY SPECIES

Ten replicates were established per prey choice treatment, but only replicates where the predator fed during the prey choice experiment were included in the analysis, resulting in *n* < 10 for some combinations. There was a significant effect of prey mobility (i.e. alive or dead) on the feeding behaviour of both *Notonecta glauca* and *Ischnura elegans* (Figure 3.2; Kruskal-Wallis test: H≥12.5, df=3, p<0.05). Both *N. glauca* and *I. elegans* ate a smaller proportion of *C. dipterum* in the live *C. riparius*:dead *C. dipterum* treatment compared to the remaining three treatments, which did not significantly differ from each other.

3.3.2 BEHAVIOURAL EFFECTS OF CADMIUM IN PREY

The effect of cadmium on general activity was determined for each prey species (Figure 3.3a). There was no significant effect of cadmium exposure on the activity of either *Chironomus riparius* (U=45, n_1=8, n_2=13, p>0.05) or *Asellus aquaticus* (U=89, n_1=13, n_2=6, p>0.05). There was a 63% reduction in activity of *Cloëon dipterum* following cadmium exposure, but this was not statistically significant (U=85, n_1=16, n_2=16, p>0.05). There was no effect of cadmium exposure on the response of either *Asellus aquaticus* (U=71, n_1=16, n_2=15, p>0.05) or *Cloëon dipterum* (U=122, n_1=16, n_2=16, p>0.05) to simulated predator attack, but there was for *Chironomus riparius* (Figure 3.3b). Cadmium exposure resulted in a significant reduction in the duration of the response of *Chironomus riparius* to simulated predator attack (U=67, n_1=16, n_2=15, p<0.05).

3.3.3 EFFECT OF CADMIUM ON PREDATION FOR SINGLE PREY SPECIES

The effect of cadmium exposure on the proportion of each prey species eaten by each predator species is summarised by Figure 3.4. There was a 36% reduction in the proportion of *C. dipterum* eaten by *N. glauca* following cadmium exposure, but this
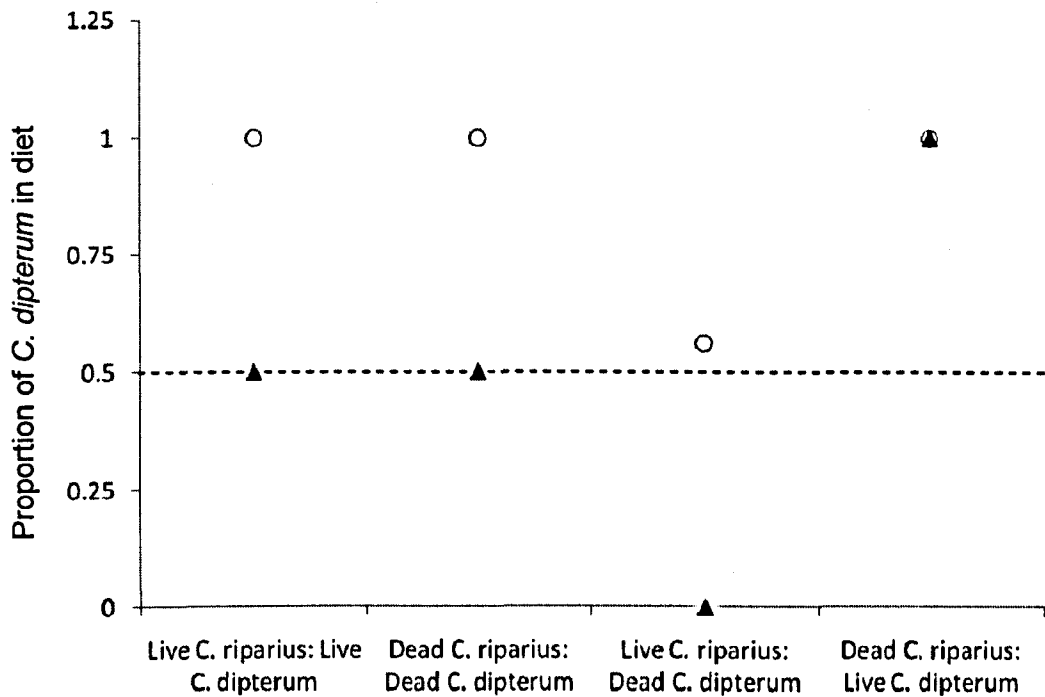
Figure 3.2. Effect of prey being offered dead or alive on prey choice in *Notonecta glauca* (○) or *Ischnura elegans* (▲). Predators were fed five *Chironomus riparius* and five *Cloëon dipterum* for two hours, with the median proportion of *C. dipterum* in the diet indicating prey choice. Dashed line indicates equal preference for both prey species.

a)
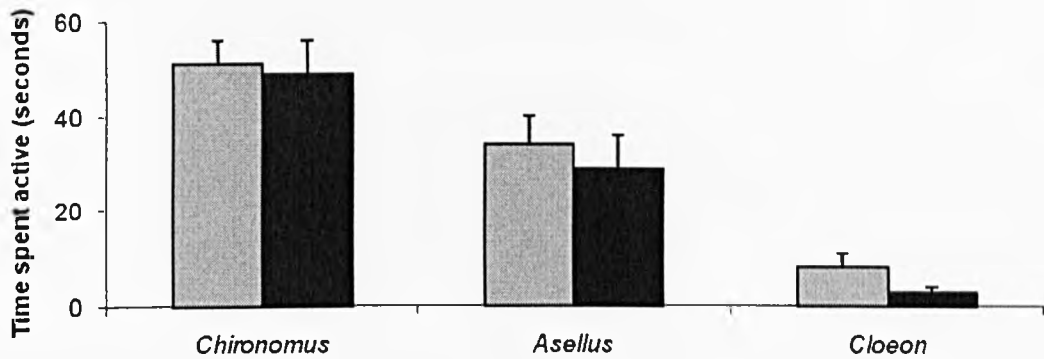
b)
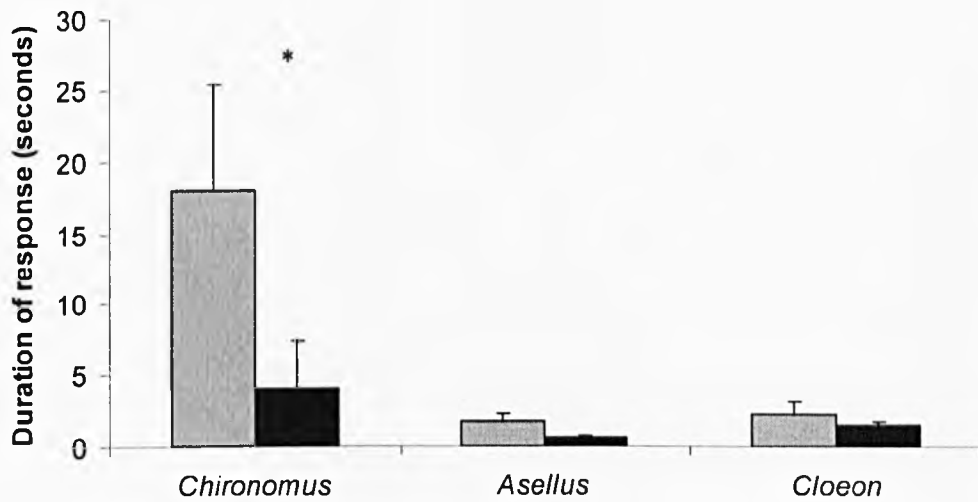
Figure 3.3. Effect of cadmium exposure on the mean (+ 1 SE) a) time spent active within one minute (seconds), and b) response duration (seconds) following a simulated predator attack for *Chironomus riparius*, *Asellus aquaticus* and *Cloëon dipterum* exposed to 336 μg/L cadmium (black bars) or APW only (grey bars). Asterisk denotes significant difference between bars (p<0.05).
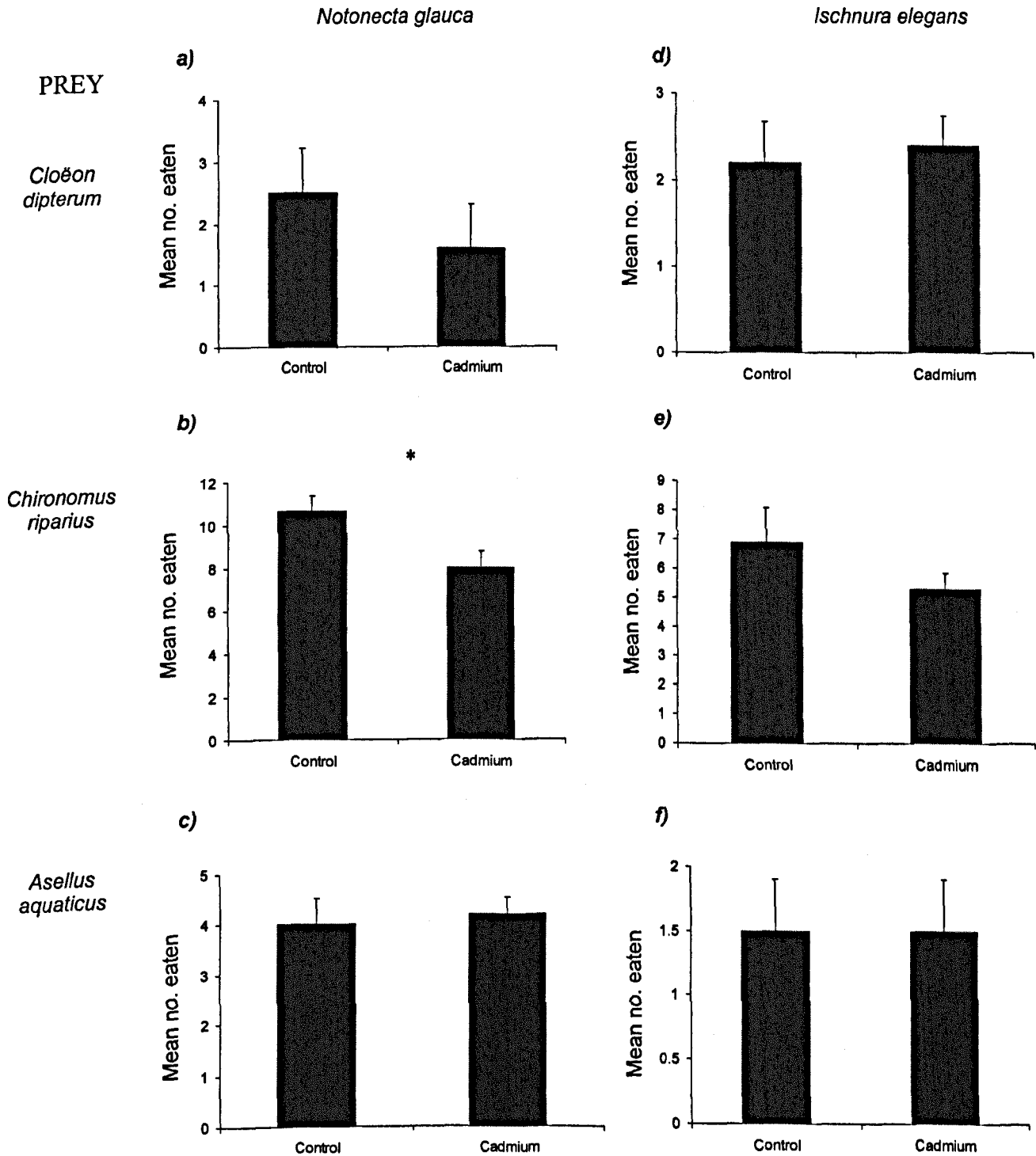
Figure 3.4. The mean number of prey eaten (+ 1 SE) in two hours by *Notonecta glauca* feeding on a) *Cloëon dipterum*, b) *Chironomus riparius*, or c) *Asellus aquaticus*; and *Ischnura elegans* feeding on d) *C. dipterum*, e) *C. riparius* or f) *A. aquaticus*. Prey were previously exposed for 96 hours to either artificial pond water (control) or 336 µg Cd/L (cadmium). Asterisk denotes significant difference between means (p<0.05).

was not statistically significant (Figure 3.4a; One-tailed two sample T-test: $t=0.9$, $df=17$, $p>0.05$). There was a significant reduction (25%) in the proportion of *C. riparius* eaten by *N. glauca* following cadmium exposure (Figure 3.4b; $t=2.7$, $df=17$, $p<0.01$), but there was no effect of cadmium exposure on the proportion of *A. aquaticus* eaten by *N. glauca* (Figure 3.4c; $t=0.3$, $df=15$, $p>0.05$). There was also no effect of cadmium exposure on the proportion of any of the prey species eaten by *I. elegans* ($t\leq0.1$, $df\leq13$, $p>0.05$; Figure 3.4d-f).

3.3.4 EFFECT OF CADMIUM ON PREY CHOICE

There was no effect of cadmium exposure of prey on the proportions of prey eaten by *N. glauca* when feeding on any combination of prey (Figure 3.5a-c; Wilcoxon test: $W\leq29.5$, $n_1\leq9$, $n_2<8$, $p>0.05$). Similarly, there was no change in the proportions of prey in the diet of *I. elegans* feeding either on *C. dipterum* and *C. riparius* (Figure 3.5d; $W=36.5$, $n_1=10$, $n_2=9$, $p>0.05$) or *C. riparius* and *A. aquaticus* (Figure 3.5e; $t=1.1$, $df=17$, $p>0.05$). There was, however, an increase in the proportion of *A. aquaticus* in the diet of *I. elegans* when fed in combination with *C. dipterum* following cadmium exposure (Figure 3.5f; $t=2.3$, $df=14$, $p<0.05$). The total number of prey items eaten in each predator-prey combination was comparable between the APW and cadmium exposed treatments.

3.4 DISCUSSION

This study investigated the consequences of sublethal contaminant effects in prey on predator-prey interactions, with a particular focus on how changes in prey behaviour influence the consumption rate and food choice of predators that exhibit different hunting strategies. Predators can be classified as 'ambush' or 'active' predators (Greene, 1986). In this study, the damselfly *Ischnura elegans* was used as an example of the former (Heads, 1985) and the bug, *Notonecta glauca*, as an example of the latter (Savage, 1989). Both predators consume a range of macroinvertebrates (Thompson, 1978; Warren, 1989), including *Asellus aquaticus*, *Chironomus riparius* and *Cloëon dipterum* used in this study.
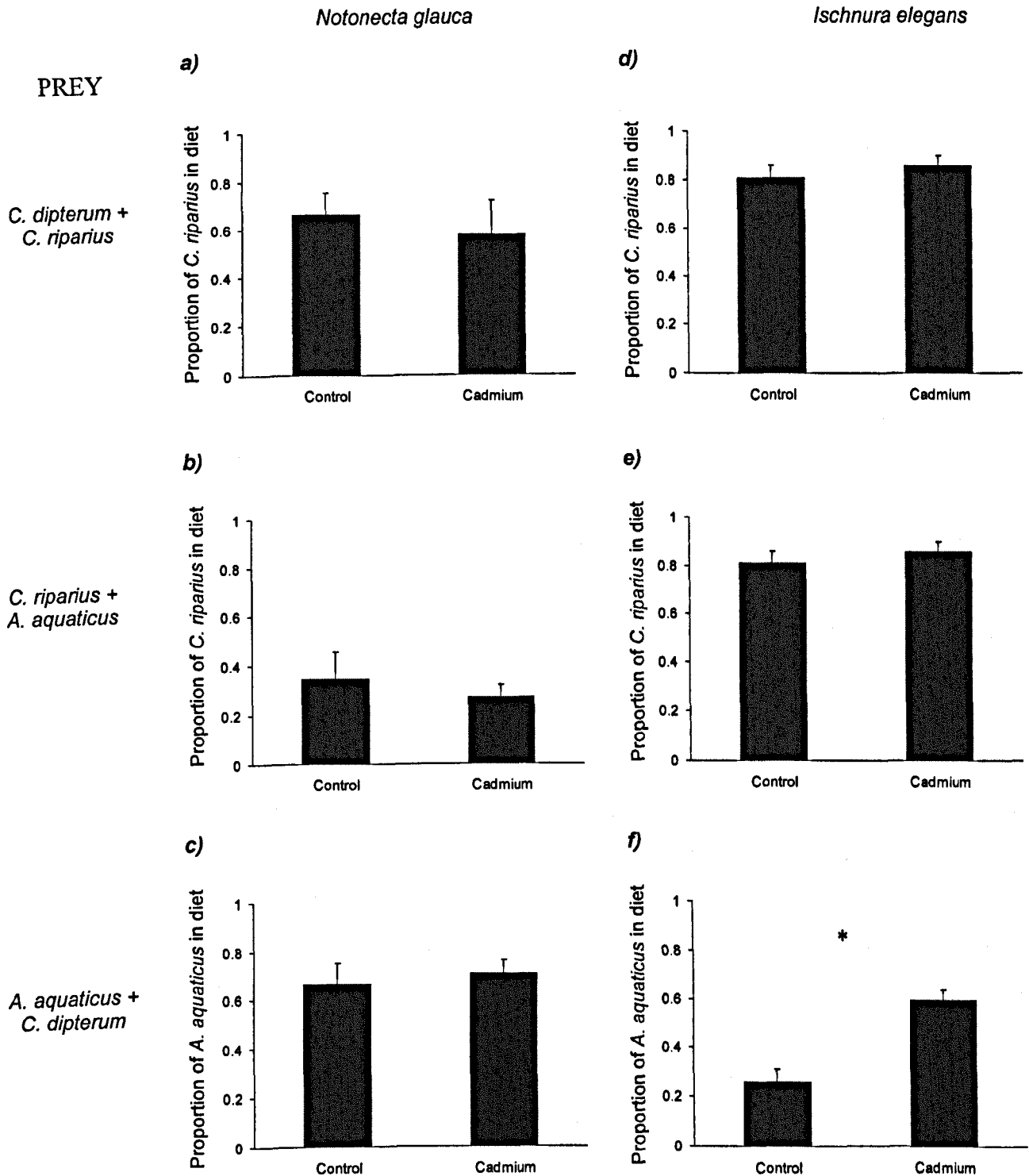
Figure 3.5. The effect of exposure of prey to cadmium on the prey choice of *Notonecta glauca* feeding on a) *Cloëon dipterum* and *Chironomus riparius*, b) *C. riparius* and *Asellus aquaticus* or c) *A. aquaticus* and *C. dipterum*; and *Ischnura elegans* feeding on d) *C. dipterum* and *C. riparius*, e) *C. riparius* and *A. aquaticus* or f) *A. aquaticus* and *C. dipterum*. Prey were previously exposed for 96 hours to either artificial pond water (control) or 336 μg Cd/L (cadmium). Mean proportion of prey species in diet (*y*-axis) indicates prey choice by predator (+ 1 SE). Asterisk denotes significant difference between means (p<0.05).

Active and ambush predators respond to changes in prey activity, affecting visual cues (Greene, 1986) and encounter rates (Spitze, 1985), respectively. As expected, both predator types fed on more mobile (live) than immobile (dead) prey when offered either *C. riparius* or *C. dipterum*, but only *I. elegans* consumed more mobile than immobile *A. aquaticus*. The effect of prey species on predator response may be attributable to their different behaviours. *Cloëon dipterum* is able to rapidly 'dart' through the water column at high speed (*pers. obs.*). *Chironomus riparius* either wriggles vigorously on the substrate or swims into the water column for extended periods (*pers. obs.*). Unlike *C. riparius* and *C. dipterum*, *A. aquaticus* is a slow moving species, unable to swim rapidly in the water column, instead crawling slowly along available surfaces (*pers. obs.*). Therefore, activity of *A. aquaticus* may be less significant for prey detection by active predators, with mechanical (Oakley and Palka, 1967), olfactory (Bronmark and Hansson, 2000), or hydrodynamic (Peckarsky and Penton, 1989b) cues being more important. From these results, it would be expected that changes in behaviour of *C. riparius* and *C. dipterum* following cadmium exposure would affect predation rates of both predators, but only *I. elegans* would be affected by changes in *A. aquaticus* behaviour.

Generalist predators are potentially able to compensate for reduced prey availability by switching to feed on the more available prey species (Elliott, 2004). Therefore, given a prey choice, both ambush and active predators would be expected to feed on whichever prey species was most active (mobile). This was observed for *N. glauca*, with predators feeding on the same proportion of the more active prey (*C. dipterum*) in all treatments except when *C. dipterum* was immobilised and *C. riparius* was mobile, in which case a greater proportion of *C. riparius* was consumed. In contrast, *I. elegans* had no prey preference unless one prey species was immobilised, in which case the mobile species was preferred. A previous study on predatory stoneflies (*Hesperoperla pacifica*), an active hunting species, also demonstrated that prey choice was influenced by immobilisation of prey, with a preference for *Baetis tricaudatus* when dead prey were offered and preference for *Ephemerella altana* when live prey were offered (Molles and Pietruszka, 1983). These results demonstrate that changes in prey activity arising from sublethal chemical exposure are potentially able to affect prey choice by both active and ambush predators. In addition to responding to prey activity, prey choice of active predators would also be

expected to shift due to sublethal effects on the escape responses of prey, affecting the visual cues required to elicit further attacks (Peckarsky and Penton, 1989a). Therefore the feeding behaviours of active predators may be more sensitive to changes in prey behaviour than ambush predators.

The results thus far have demonstrated the potential for both ambush and active predators to respond to changes in prey behaviour, but sublethal exposures to contaminants are unlikely to cause such extreme effects as complete immobilisation of prey. There was no significant effect of cadmium exposure on general activity levels in the three prey species used in this study, though there was the suggestion of a reduction in *C. dipterum* activity. Previous studies have demonstrated the inhibitory effects of cadmium (0.5-20 µg/L) on mayfly nymphs, such as general activity (Gerhardt, 1990) and drift behaviour (Riddell *et al.*, 2005a). *Chironomus riparius* larvae demonstrated a reduction in their response to a simulated predator attack following cadmium exposure. Similar effects were observed in the mayfly *Leptophlebia marginata* when exposed to ≥ 1 mg Cd/L (Gerhardt, 1995). Therefore exposure of *C. riparius* and *C. dipterum* to cadmium may affect their susceptibility to predation, with no such effects for *A. aquaticus* due to an absence of cadmium-induced changes in activity or responsiveness.

Feeding rates of predators were expected to vary according to the shifts in availability of prey resulting from cadmium exposure. Although both the ambush and active predator species reduced their feeding rate as a response to immobilisation of prey, neither responded to the cadmium-induced reduction in *C. dipterum* activity. Reductions in activity have been previously linked with reduced predation rates (Spitze, 1985), thus it is possible that the reduction in activity of *C. dipterum* observed in this study was insufficient to affect the visual cues required by active predators, and encounter rates with ambush predators. As expected, only the active (*N. glauca*) not the ambush (*I. elegans*) predator species had a reduced feeding rate on *C. riparius* following the cadmium-induced reduction in attack response. Such reductions have been previously attributed to reduced visual cues, with active predatory stoneflies (*Kogotus modestus*) having reduced predation on mayfly prey (*Baetis bicaudatus*) with inhibited attack responses (Peckarsky and Penton, 1989a).

53

The predators used in this study are generalist feeders, and therefore any change in the availability of a particular prey species following sublethal exposure to contaminants can potentially be compensated for by a shift in their prey choice (Clements *et al.*, 1989; Riddell *et al.*, 2005b). A number of predictions were made for the effect of behavioural changes on prey choice according to the hunting strategy of the predator (Table 3.2). The active predator species (*N. glauca*) was thus expected to compensate for a reduction in availability of *C. riparius*, due to a cadmium-induced reduction in escape response, by feeding more on the alternative prey species offered. However, no such shift in prey choice was observed for either *C. riparius* fed in combination with *C. dipterum* or *A. aquaticus*. Shifts in prey choice of active predators have been observed in other studies involving sublethal exposures of prey, with both invertebrate (Clements *et al.*, 1989) and, though not statistically significant, vertebrate (Riddell *et al.*, 2005b) predators. Therefore, although the behavioural shift in *C. riparius* caused by cadmium exposure was insufficient to affect prey choice in the active predator species used in this study, such effects have been demonstrated in other systems.

No shifts in prey choice were expected for any of the prey combinations involving the ambush predator species, as no effects on predation were detected in the single prey species experiments. However, there was a significant shift in prey choice of *I. elegans* when offered *A. aquaticus* and *C. dipterum*, preferring *A. aquaticus* following cadmium exposure. One explanation for this is that the reduction in general activity of *C. dipterum* following cadmium exposure, although not statistically significant, may have been sufficient to affect encounter rates with ambush predators. To compensate for reduced availability of *C. dipterum*, *I. elegans* subsequently switched to feeding on more *A. aquaticus*. Although examples exist for contaminant-induced shifts in prey choice for active predators (Clements *et al.*, 1989; Riddell *et al.*, 2005b), no such examples previously existed for ambush predators, such as *I. elegans*.

The shift in prey choice of the ambush predator species highlights one of the limitations of current ecological risk assessment practice; using a single species approach would result in the conclusion that *C. dipterum* is not significantly affected by cadmium exposure, as activity was not significantly reduced. However, this subtle

change in behaviour was detected by predators, and only in the more complex two-prey species system. The resultant shift in predation caused a negative impact on the otherwise unaffected *A. aquaticus* prey. Thus, if the main rationale for risk assessment is to minimise effects on populations and communities (Suter II, 1993), it is critical that the most appropriate endpoints are being measured, and hence the full extent of the impact is detected. Natural detectors, such as predators, may be more sensitive measures of subtle changes in behaviour than human experimenters.

This study has focused on the effect of changing aspects of prey behaviour on predator feeding response. Suppressed behaviours, such as reduced escape responses, can occur with contaminants other than cadmium that also cause hypoactivity, including narcotics and cholinesterase inhibitors (Carlson *et al.*, 1998). However, other contaminants act as stimulants, resulting in hyperactivity and therefore providing a stronger visual cue to predators or increasing encounter rates (Preston *et al.*, 1999a). Contaminants that result in a physical deformity syndrome, increasing prey response to external stimuli (Drummond and Russom, 1990), may also result in prey becoming more susceptible to predation by active predators. Such increased activity or responsiveness in prey would be expected to result in predators feeding more on the more active, 'stimulated' prey species. Therefore sublethal effects can indirectly result in lethal effects, with prey populations being reduced due to increased predation pressures rather than direct toxic effects of the contaminant on survival. In terms of ecological risk assessment, sublethal effects can therefore both increase and decrease prey populations via changes in predator-prey interactions, depending upon the mode of action of the contaminant. The implications of such results are that shifts in prey populations that occur as a result of behavioural effects may be misinterpreted as direct responses of the prey to contaminant exposure, rather than due to indirect effects via trophic interaction changes (Fleeger *et al.*, 2003). There is generally a need for more research into the impact of such behavioural changes on populations and communities, either due to reduced survival or reproductive success (Grue *et al.*, 2002).

The relative importance of sublethal effects for contaminants will depend on their dose-response profile. Contaminants, like cadmium in this study, can have low acute to chronic ratios (ACRs) for their toxicity endpoints. Using the sublethal

concentration used in the current study (336 µg/L) and lethal concentrations from the literature (Table 1), cadmium has an ACR of 1-5. Länge *et al* used the ECETOC database to calculate ACRs for 71 substances, with ACRs ranging from 0.13-1290 and the 90th percentile calculated as 72.9 (Länge *et al.*, 1998). Generally, organics, excluding pesticide active ingredients, had relatively low ACRs, ranging from 0.13-27.5 (Länge *et al.*, 1998). For these contaminants, sublethal effects will be relatively unimportant as by the time prey exhibit extreme enough sublethal effects to affect prey choice in predators, they will be on the verge of death. However, other contaminants have much higher ACRs, with metals and organometals having ACRs ranging from 0.3-1290 (Länge *et al.*, 1998). There is therefore more scope for prey to experience sublethal effects before any changes in survival occur. In addition, as sublethal effects occur over a wider range of concentrations, prey may experience an increasing magnitude of effect, potentially resulting in more extreme responses. Thus sublethal effects for contaminants with high ACRs may be more important as more extreme shifts in prey behaviour can occur, resulting in more exaggerated shifts in prey choice by predators, as demonstrated by using immobilised prey animals.

3.5 CONCLUSIONS

The interactions between predators and their prey can be altered by sublethal exposures. The outcome of sublethal exposure is influenced by the sensitivity and response of prey to exposure and the hunting strategy of the predator. The importance of sublethally-induced changes in predator-prey interactions will also depend on the contaminant under investigation, with mode of action and dose-response profile requiring consideration. The results of this study highlight that the full effects of contaminants cannot be predicted by single species acute toxicity tests. Ecological risk assessments therefore need to incorporate population reductions that can occur as an indirect result of sublethal exposures within natural assemblages.

Chapter 4

Importance of feeding behaviour for the accumulation and internal distribution of a hydrophobic contaminant by benthic macroinvertebrates

4.1 INTRODUCTION

Thus far the impacts of lethal (Chapter 2) and sublethal (Chapter 3) toxicant exposure to prey on predator feeding response and the subsequent indirect effects in multiple prey systems have been considered. However, predator-prey interactions can also be affected by prey accumulating these contaminants and passing them on to their predators, where they may cause toxic effects (Feijtel *et al.*, 1997). Dietary uptake and food chain transfer may be particularly important for hydrophobic contaminants as aqueous exposure to predators will be minimal (Qiu and Davis, 2004). One such group of hydrophobic contaminants are the persistent, bioaccumulative and toxic (PBT) chemicals, a group of concern in the new EU strategy REACH (EC, 2006). Chemicals that are PBT tend to associate with sediment when in aquatic systems and therefore benthic species will be exposed to them. Any predators that subsequently feed on these animals may be exposed to PBTs even though they may have limited direct contact with the contaminated sediment themselves (Knezovich *et al.*, 1987).

There is a growing body of evidence that sediment-associated contaminants are bioavailable to animals exposed to them (Knezovich *et al.*, 1987; Landrum, 1989; Wang and Fisher, 1999). However, animals exposed to the same conditions can accumulate contaminants to different extents (Kaag *et al.*, 1997; Gaskell *et al.*, 2007). For example, the polychaete *Armandia brevis* accumulated up to 3-10 times more chlorinated hydrocarbons (Meador *et al.*, 1997) and 3-20 times more high molecular weight polycyclic aromatic hydrocarbons (Meador *et al.*, 1995) than the amphipod *Rhepoxynius abronius*. As bioaccumulation occurs when uptake rate of a compound by an organism exceeds its elimination rate, any differences between

species in uptake and loss processes may result in differences in the amount of compound accumulated.

The mechanisms determining the accumulation of sediment-associated contaminants by invertebrates can be divided into two main categories: those that affect food intake and those that affect uptake from the gut. The importance of gut physiology in determining the absorption of contaminants from gut contents has been demonstrated, with gut passage time and gut surfactancy playing an important role in determining uptake (Penry and Weston, 1998; Weston and Mayer, 1998; Voparil and Mayer, 2000; Ahrens *et al.*, 2001; Wang and Chow, 2002; Gaskell *et al.*, 2007). However, the amount of contaminant that the organism is initially exposed to will be dependent on their feeding behaviour. Previous studies have demonstrated that animals living in sediment but not feeding on it (non-ingesters; e.g. *Cloëon dipterum*, Tanypodiae spp.) have a lower exposure to contaminants in sediment than those that also ingest it (ingesters; e.g. *Chironomus riparius, Lumbriculus variegatus*) (Kaag *et al.*, 1997; Meador *et al.*, 1997). It has also been found that species processing large volumes of sediment are able to achieve higher body burdens than those that process smaller volumes (Hickey *et al.*, 1995; Kaag *et al.*, 1997; Christensen *et al.*, 2002). For example, the bioaccumulation factors of a deposit-feeding polychaete species (*Arenicola marina*) were 5 to 10 times higher than those of a non-deposit feeding species (*Nereis diversicolor*) (Christensen *et al.*, 2002).

The final body burden within benthic organisms will not only be driven by their accumulation of contaminants from sediment, but also their ability to eliminate contaminants from their body. Previous studies have demonstrated that the extent of elimination can be influenced by the capacity of the organism to metabolise or biotransform the contaminant to more polar, easily excretable metabolites (Kane Driscoll and McElroy, 1997; van Leeuwen and Vermeire, 2007). The type of biotransformation reaction (e.g. oxidation, reduction, conjugation) to occur depends on the structure of the contaminant (van Leeuwen and Vermeire, 2007). The biotransformation ability of organisms varies between both compounds (Penry and Weston, 1998; Selck *et al.*, 2003) and species (Kane Driscoll and McElroy, 1997; Bott and Standley, 2000; McElroy *et al.*, 2000; Verrengia Guerrero *et al.*, 2002; Schuler *et al.*, 2003), depending on the suite of enzymes present in the organism

(Livingstone, 1998; van Leeuwen and Vermeire, 2007). The subsequent elimination of metabolites is an important stage in determining the final body burden of the organism (Verrengia Guerrero et al., 2002). For example, both Chironomus riparius and Lumbriculus variegatus are able to biotransform 2,4,5-trichlorophenol, but only C. riparius is able to excrete the metabolites produced, resulting in a lower body burden than L. variegatus (Verrengia Guerrero et al., 2002). Hence organisms that can extensively metabolize and subsequently excrete accumulated contaminants would be expected to have lower body burdens than those that have either lesser biotransformation capabilities or slower elimination rates.

Species that achieve the same body burden may still differ in the distribution of that contaminant within their tissues. Dietary exposure, such as from sediment ingestion, results in mainly internal accumulation, compared to mainly external accumulation from aqueous exposure (Fisk et al., 1997; Arukwe et al., 2000; Liu et al., 2002; Xu and Wang, 2002; Wang and Wong, 2003; Heiden et al., 2005). Hence sediment ingesters would be expected to have both internal and external accumulation, whereas non-ingesters would be expected to have only external accumulation in their tissues. Investigations of the distribution of metals within the tissues of invertebrates have shown that the exposure pathway can affect the relative proportions associated with particular tissues, including the exoskeleton, gut wall, and haemolymph (Inza et al., 2001). Differences in the distribution of contaminants within prey, in terms of the internal vs. external accumulation and also the specific structures with which it is associated, may have implications for the trophic transfer of contaminants to their predators.

The distribution of contaminants within prey may be important in determining the extent of trophic transfer due to the feeding behaviour of predators. Aquatic predators can be categorised as either engulfers or piercers (Peckarsky, 1982). Piercer predators, such as the water scorpion Nepa cinerea, feed using piercing mouthparts and suck out the internal tissues of their prey. Engulfer predators, such as the leech Erpobdella octoculata, feed on their prey whole. Therefore, the extent of exposure to piercer and engulfer predators to hydrophobic contaminants may depend on whether they are feeding on prey that accumulate mainly internally (ingesters) or

externally (non-ingesters). This hypothesis has yet to be addressed and is subsequently the focus of Chapter 5.

The aim of this study was to assess the potential for benthic prey to act as an exposure pathway for sediment-associated contaminants. This objective focused on the importance of prey feeding behaviour (sediment ingestion vs non-ingestion) in determining the overall accumulation and internal distribution of contaminants. Two ingester (*Asellus aquaticus* Linnaeus (Crustacea, Isopoda) and *Chironomus riparius* Meigen (Insecta, Diptera)) and one non-ingester (*Cloëon dipterum* Linnaeus (Insecta, Ephemeroptera)) species of macroinvertebrate were used. Animals were exposed to artificial sediment dosed with [14C] benzophenone and their total body burden was determined. The internal distribution of the accumulated [14C] was determined using a microautoradiography (e.g. Craig *et al.*, 1998; Munger *et al.*, 1998; Inza *et al.*, 2001) and a manual separation approach. The elimination and metabolism of benzophenone was also determined for each species to indicate the extent of loss, using high performance liquid chromatography (HPLC). The implications for differences in accumulation and distribution of contaminants by benthic macroinvertebrates are discussed with regard to trophic transfer.

4.2 METHODS

4.2.1 MODEL COMPOUND

The [14C] benzophenone (>99% purity) used in this study was purchased from ARC (American Radiolabelled Chemicals, St. Louis, USA) and had a specific activity of 11.2 MBq/mg.

4.2.2 TEST SPECIES

Collection and maintenance of test species as described in Section 2.2.1.

4.2.3 BIOACCUMULATION AND ELIMINATION

Artificial rather than field collected sediment was used for these experiments to allow more consistent replication of exposure conditions. The composition of the

sediment was sand (75%), kaolin (20%) and cellulose (5%), according to mass. Sixty-millilitre glass test vessels were used for exposures, each containing 2.5 g dry weight of artificial sediment. The LC_{50} for benzophenone was taken to be 280 µg/L (Tosato *et al.*, 1991), and therefore a nominal concentration of approximately 10% of LC_{50} (30 µg/g) was used to minimise mortality but maximise bioaccumulation in prey. Half a millilitre of a 150 mg/L stock solution of benzophenone dissolved in HPLC-grade acetone was added to thirty test vessels and mixed thoroughly. Benzophenone stock solution was produced by diluting radiolabelled benzophenone with unlabelled benzophenone (Sigma-Aldrich, Gillingham, United Kingdom), using a dilution ratio of 1 : 132 (labelled : unlabelled). The acetone was allowed to evaporate for at least two hours. One millilitre of APW was added to sediment in each vessel and mixed to form a sediment paste. Overlying APW (25 ml) was then added to each vessel, placing a plastic disc over the sediment to minimise sediment disturbance. Thirty control test vessels were prepared in a similar way to those containing benzophenone, except that 0.5 ml of acetone was added instead of 0.5 ml benzophenone stock solution.

Three animals of a single prey species (either *A. aquaticus*, *C. dipterum* or *C. riparius*) were added to 20 exposure vessels. This resulted in 10 control and 10 dosed replicates per species. Animals were exposed for five days at 15°C in a light:dark regime of 16:8 hours. Following exposure, two animals were removed from each vessel and rinsed in distilled water, with the third animal being discarded if spare. One animal was blotted dry, weighed and frozen at -30°C until required for body burden analysis. The second animal was transferred to a clean vessel containing 25 ml of APW to begin a 24-hr elimination phase. At the end of this 24-hr period, the animal was blotted dry, weighed and frozen.

Individual animals were added to rigid Combusto-cones® (PerkinElmer, The Netherlands), topped with approximately 2-mm layer of cellulose powder to aid complete combustion and capped with compacted tissue paper. Animals were then combusted in a sample oxidizer (model 307, Packard, Meriden, USA) until complete combustion was observed and collected in a scintillation cocktail consisting of 10 ml Permafluor® and 10 ml Carbosorb®. The disintegrations per minute (dpm) in the resulting samples were counted for 10 minutes per sample using a liquid scintillation

analyzer (Tri-carb 3100TR, Packard, Meriden, USA). Combusto-cones containing only cellulose powder and tissue paper were also oxidized and analyzed to obtain a background level of activity. The total µg benzophenone residues/g fresh weight was calculated using equation 4.1:

$$C = \frac{D(A - B)}{MZ} \qquad (4.1)$$

Where C is the total µg benzophenone residues/g fresh weight, A is the activity of the sample (dpm), B is the background activity (dpm), M is the mass of the sample (g), Z is the activity per microgram of benzophenone (674586 dpm/µg), and D is the dilution factor of unlabelled to radiolabelled benzophenone.

4.2.4 METABOLITE PROFILES

Twenty-four 60 ml glass jars containing artificial sediment and APW were set up as described in Section 4.2.3. Half were dosed with 0.5 ml acetone (controls) and half were dosed with 0.5 ml benzophenone stock solution. Benzophenone stock solution (150 mg/L) was produced by diluting radiolabelled benzophenone with unlabelled benzophenone, using a dilution ratio of 1 : 2.7 (labelled : unlabelled). A higher dilution factor was used in order for the resultant samples to contain >10,000 dpm/100 µl, as required for subsequent metabolite analysis (David Sanders (Unilever), personal communication).

Eighteen of the twenty-four exposure vessels had groups of a single species added to them, either 6 *A. aquaticus*, 45 *C. dipterum* or 35 *C. riparius*. This gave a total of three dosed and three control replicates for each species. The remaining six vessels (three dosed and three control) did not have animals added to them to enable the metabolite profile of the sediment to be determined. All vessels were exposed for five days at 15°C in a light:dark regime of 16:8 hours.

At the end of the exposure period, groups of animals (5 *A. aquaticus*, 44 *C. dipterum* and 34 *C. riparius*) were removed from the vessels and rinsed with distilled water, with spare animals being discarded if necessary. Groups were then macerated using a

glass rod in glass test-tubes. Half a millilitre of HPLC-grade acetonitrile was added to each sample and samples were then sonicated at 30°C for 60 minutes (Ultrasonic Bath, Grant Instruments (Cambridge) Limited, Cambridgeshire, United Kingdom). The solvent was then transferred into 1.5-ml Eppendorf tubes and centrifuged at 13,000 rpm for 2.5 minutes (Micro Centaur, MSE (UK) Limited, Kent, United Kingdom). Comparison with samples analysed using the sample oxidation approach (Section 4.2.3) indicated extraction efficiencies of 26, 47, 81 and 54% for *A. aquaticus*, *C. dipterum*, *C. riparius* and sediment, respectively. The 0.5-ml of supernatant was transferred to 4-ml glass screw-top vials and stored at 4°C until shipped on ice in an insulated container to Unilever (Unilever SEAC, Colworth, Bedfordshire, United Kingdom) for high performance liquid chromatography (HPLC) analysis by David Sanders. The HPLC method used by D. Sanders is described in Appendix 4.1.

4.2.5 DISTRIBUTION OF [14C] IN TISSUES

4.2.5.1 PROPORTION OF [14C] IN TISSUES

Seventy-five 60 ml glass jars were set up as described in Section 4.2.3. Half were dosed with 0.5 ml acetone (controls) and half were dosed with 0.5 ml benzophenone stock solution. Benzophenone stock solution (150 mg/L) was produced by diluting radiolabelled benzophenone with unlabelled benzophenone, using a dilution ratio of 1 : 66.5 (labelled : unlabelled).

Individual animals were added to each vessel, giving 25 replicates for *C. dipterum*, *A. aquaticus* and *C. riparius*. Following five days of exposure at 15°C in a light:dark regime of 16:8 hours, five animals of each species were removed, blotted dry and weighed for total body burden analysis. The remaining twenty of each species were then prepared to allow the separate analysis of external and internal tissue loadings. Each animal was laid on the lid of a glass Petri dish and tightly covered with a layer of cling film. The body of the animal was then pierced with a syringe needle so that the internal fluids of the animal were squeezed onto the surface of the cling film. These fluids were then absorbed onto a small piece of filter paper (approximately 1 cm²). The remaining external tissues were then weighed. All apparatus was cleaned

with acetone between samples to minimise cross contamination. All samples were analysed using sample oxidation and liquid scintillation counting (see Section 4.2.3). The percentage of [14C] in the internal and external tissues of each species was calculated on both a whole body and weight adjusted ([14C]/mg) basis (see Section 4.2.3).

The proportion of activity within internal and external tissues was also normalised for any losses occurring during the manual separation process. These losses were likely due to the loss of internal fluids rather than the loss of easily visible external structures. Therefore the mean proportion of activity lost was added to the proportion internally accumulated.

4.2.5.2 LOCATION OF [14C] IN TISSUES

Six 60-ml glass jars containing artificial sediment and APW were set up as described in Section 4.2.3. Half were dosed with 0.5 ml acetone (controls) and half were dosed with 0.5 ml benzophenone stock solution. Benzophenone stock solution (150 mg/L) was produced by diluting radiolabelled benzophenone with unlabelled benzophenone, using a dilution ratio of 1 : 4.5 (labelled : unlabelled).

Groups of a single species were added to each vessel, either 15 *A. aquaticus*, 15 *C. dipterum* or 30 *C. riparius*, with one dosed and one control group for each species. At the end of the five-day exposure period at 15°C in a light:dark regime of 16:8 hours, animals were removed and rinsed with distilled water to remove sediment particles. Animals were then transferred to clean 60-ml glass jars containing 50 ml of APW and then shipped on ice in an insulated container to Unilever for microautoradiography by Helen Minter. The microautoradiography techniques used by H. Minter are described in Appendix 4.2.

4.3 RESULTS

4.3.1 BIOACCUMULATION AND ELIMINATION

Compared to controls, all three prey species exhibited a significant accumulation of benzophenone after five days of exposure (One tailed T-test: $t \geq 3.6$, $df \geq 9$, $p < 0.01$). There was significant interspecific variation in accumulation (Figure 4.1; ANOVA: $F = 30.2$, $df = 2,29$, $p < 0.001$), in the order *A. aquaticus* > *C. riparius* > *C. dipterum* (Tukey multiple comparison test: $p < 0.05$).

Significant elimination of benzophenone occurred during the 24-hr elimination phase in all three species (Figure 4.1; $t \geq 2.3$, $df \geq 9$, $p < 0.05$). The highest proportion of body burden loss (63%) occurred in *C. riparius*, followed by 41% in *C. dipterum* and 16% in *A. aquaticus*.

4.3.2 METABOLITE PROFILES

Six main compounds were identified within the samples, defined as composing more than 10% of the total radioactivity detected within samples (Appendix 4.1, Table A4.1.1). It was not possible to identify each compound; instead, letters A-F were assigned to each metabolite according to its retention time during HPLC. Comparison with standards suggests that metabolite D is benzohydrol and F is the residual parent benzophenone. It is assumed that metabolites A-E are more polar than the parent (F) according to the retention times.

The proportions of metabolites and parent compound in each sample type are presented in Figure 4.2. The majority of benzophenone in the sediment remained in the parent form, but a small proportion was converted to benzohydrol. *Asellus aquaticus* retained one fifth of the benzophenone in its parent form, with the remainder being converted to benzohydrol and metabolite A. *Chironomus riparius* retained a quarter as parent benzophenone, with the remainder being converted to benzohydrol and metabolite E. *Cloëon dipterum* retained only a small proportion of the parent compound, with the remainder being converted into benzohydrol and
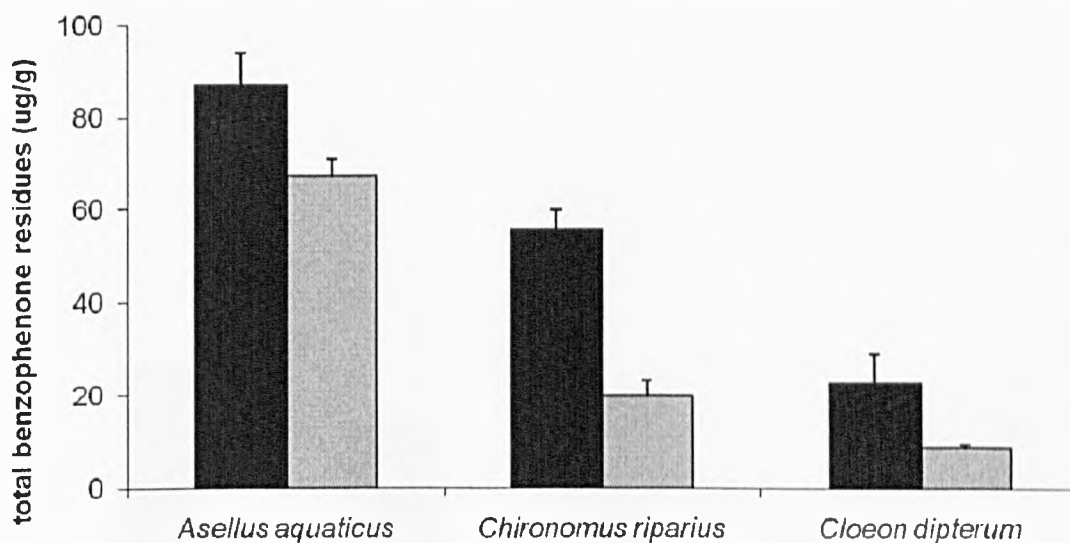
Figure 4.1. The mean (+ 1 SE) accumulation and elimination of total [14C] benzophenone residues (µg/g) in sediment by three benthic macroinvertebrate species with different feeding habits. *Asellus aquaticus* and *Chironomus riparius* are both sediment ingesters, whereas *Cloëon dipterum* is a non-ingester. Accumulation (black bars) occurred over a five-day exposure period, elimination (grey bars) occurred over a 24-hr period. Significant accumulation and elimination occurred within each species (p<0.05).
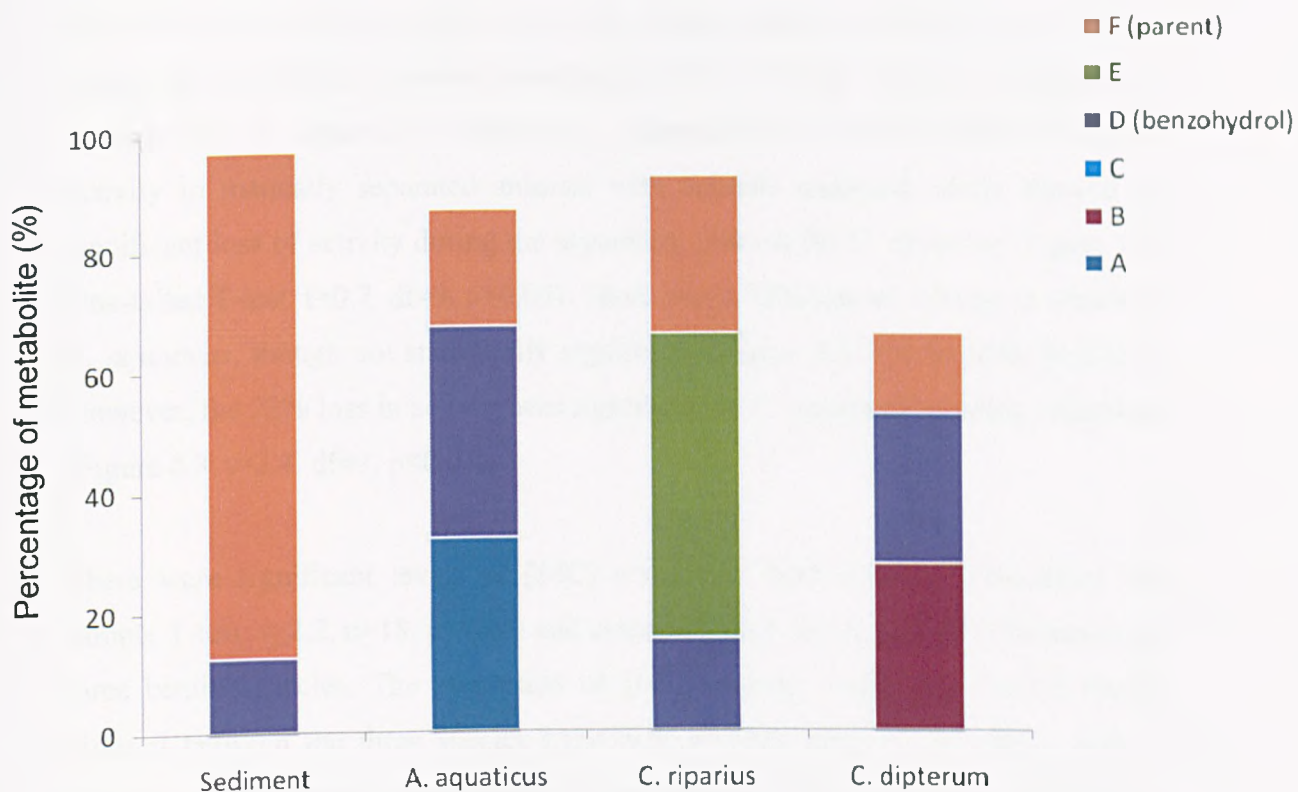
Figure 4.2. Mean percentages of metabolites (A-E) and parent compound (F) found in extracts of sediment, *Asellus aquaticus*, *Chironomus riparius* and *Cloëon dipterum* after five days exposure to [14C] benzophenone. Only metabolites contributing > 10% of the total activity have been included.

metabolite B. Metabolite C was also detected in one of the *C. dipterum* extracts, and metabolite E in another.

4.3.3 DISTRIBUTION OF [14C] IN TISSUES

4.3.3.1 PROPORTION OF [14C] IN TISSUES

One individual of each species was lost during sample preparation, and one *A. aquaticus* died during exposure, resulting in n=19, 19 and 18 for *C. dipterum*, *C. riparius* and *A. aquaticus*, respectively. Comparison of the total level of [14C] activity in manually separated animals with animals analysed whole showed no significant loss of activity during the separation process for *C. dipterum* (Figure 4.3; One-tailed T-test: t=0.7, df=9, p>0.05). There was a 32% loss of activity in separated *A. aquaticus*, though not statistically significant (Figure 4.3; t=1.9, df=6, p=0.057). However, the 22% loss in activity was significant in *C. riparius* following separation (Figure 4.3; t=2.4, df=7, p<0.05).

There were significant levels of [14C] activity in both internal (One-tailed one sample T-test: t≥2.2, n≥18, p<0.05) and external (t≥6.4, n≥18, p<0.001) tissues of all three benthic species. The proportion of [14C] activity within the internal tissues differed between the three species (ANOVA: F=15.6, df=2,56, p<0.001), with *A. aquaticus* (29%) having significantly higher levels of internal activity compared to *C. riparius* (12%) and *C. dipterum* (5%) (Figure 4.4a). Following correction for losses in activity during the manual separation process (see Figure 4.3), the mean percentage of activity associated with internal tissues increased for *C. riparius* and *A. aquaticus* (Figure 4.4b).

4.3.3.2 LOCATION OF [14C] IN TISSUES

The untreated controls contained very low background levels of radioactivity for *C. dipterum* (Figure 4.5), *C. riparius* (Figure 4.6) and *A. aquaticus* (Figure 4.7). There was some auto fluorescence associated with the gut contents of these animals, but this was due to components of the sediment used rather than radioactivity (Helen Minter, personal communication). Dosed *C. dipterum* individuals contained low
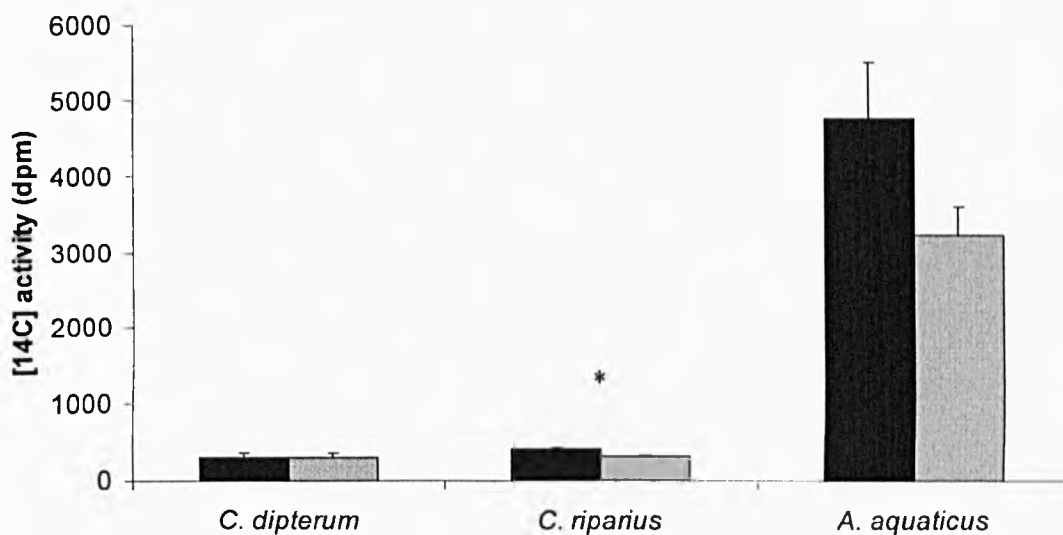
Figure 4.3. The amount of [14C] activity (disintegrations per minute (dpm) + 1 SE) in *Cloëon dipterum*, *Chironomus riparius* and *Asellus aquaticus* either analysed as whole organisms (black bars) or the combined activity in internal and external tissues obtained from manual separation (grey bars). Asterisk denotes significant loss of activity following manual separation (p<0.05)
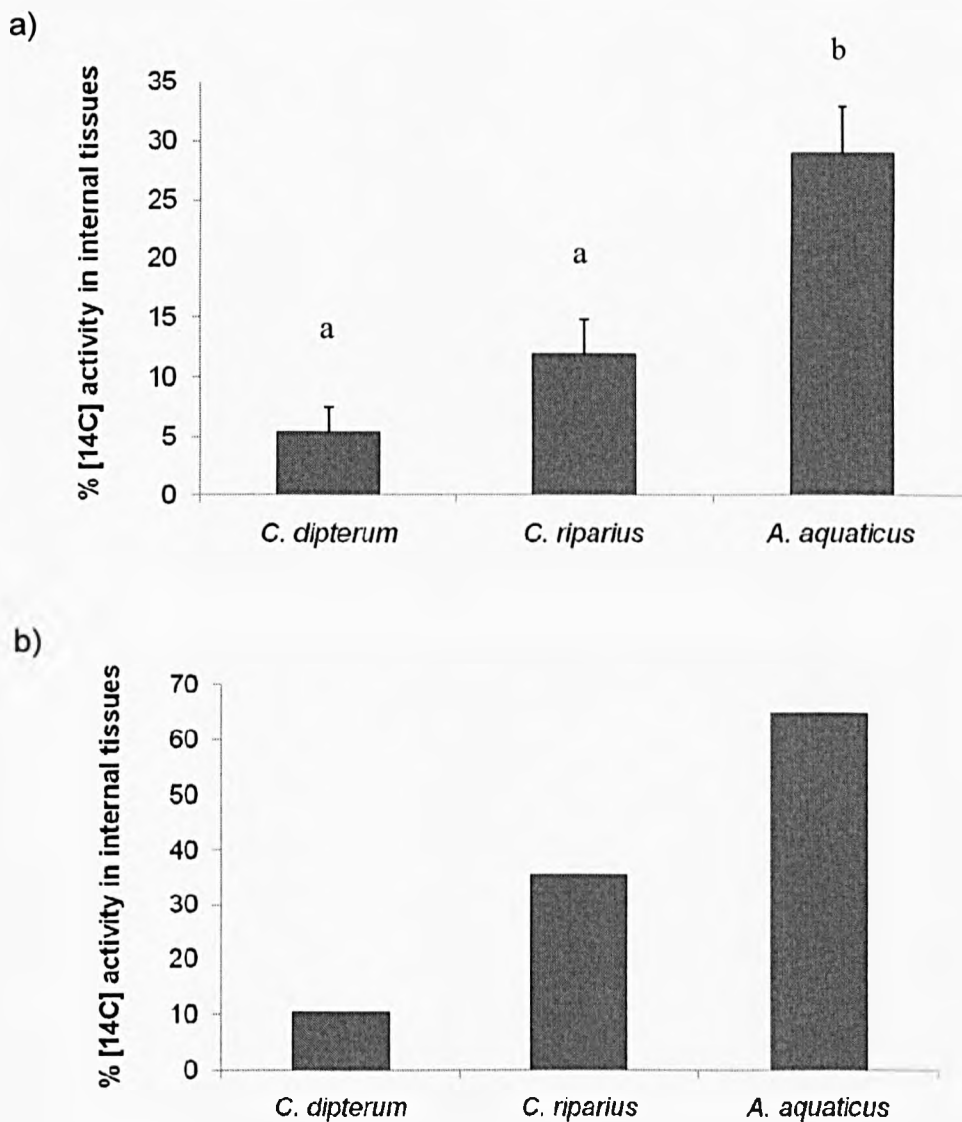
Figure 4.4. a) The mean percentage of [14C] activity (disintegrations per minute) (+ 1 SE) located in the internal tissues of two sediment ingesters (*Chironomus riparius* and *Asellus aquaticus*) and one non-ingester (*Cloëon dipterum*) species following five days of exposure to sediment dosed with [14C] benzophenone. b) Percentage internally accumulated following correction for losses in activity during manual separation process. The amount of [14C] activity was determined separately for internal and external tissues of individual animals, and then the proportion internally accumulated was calculated. Letters above the bars in (a) indicate significant differences between means (Tukey multiple comparison test: p<0.05).
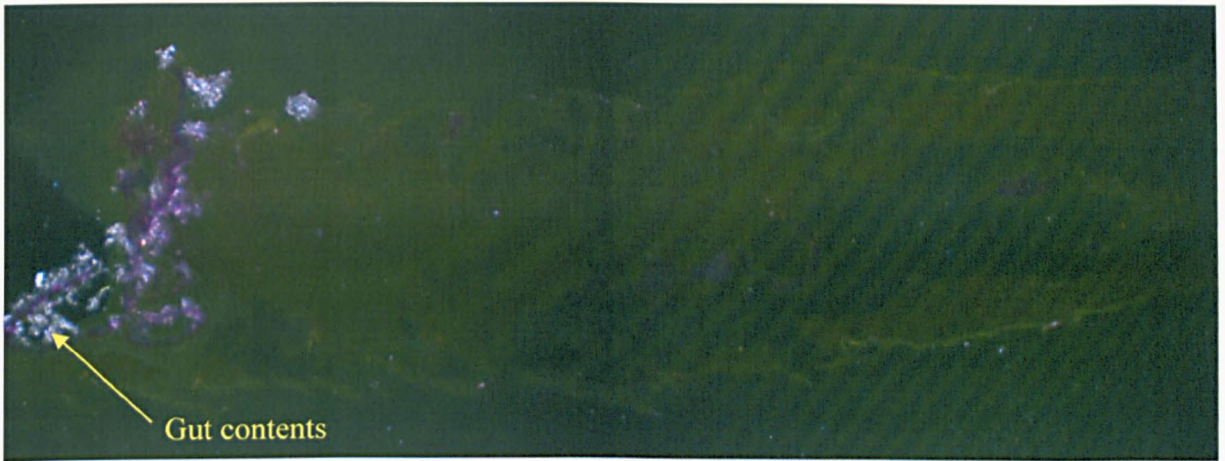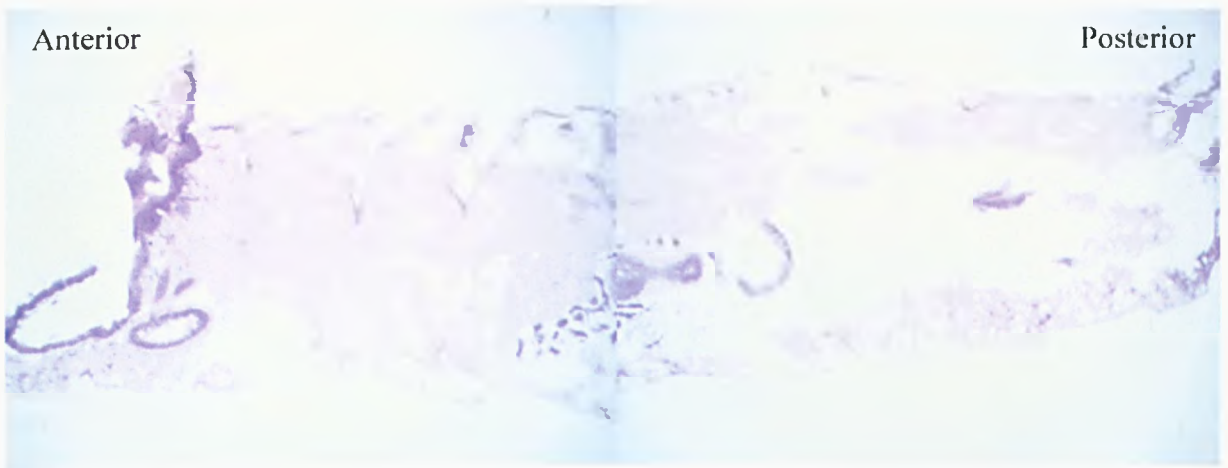
Figure 4.5. *Cloëon dipterum* untreated control viewed under a) dark field and b) bright field illumination. Silver grained areas represent radioactive material.

a)

b)

Gut contents
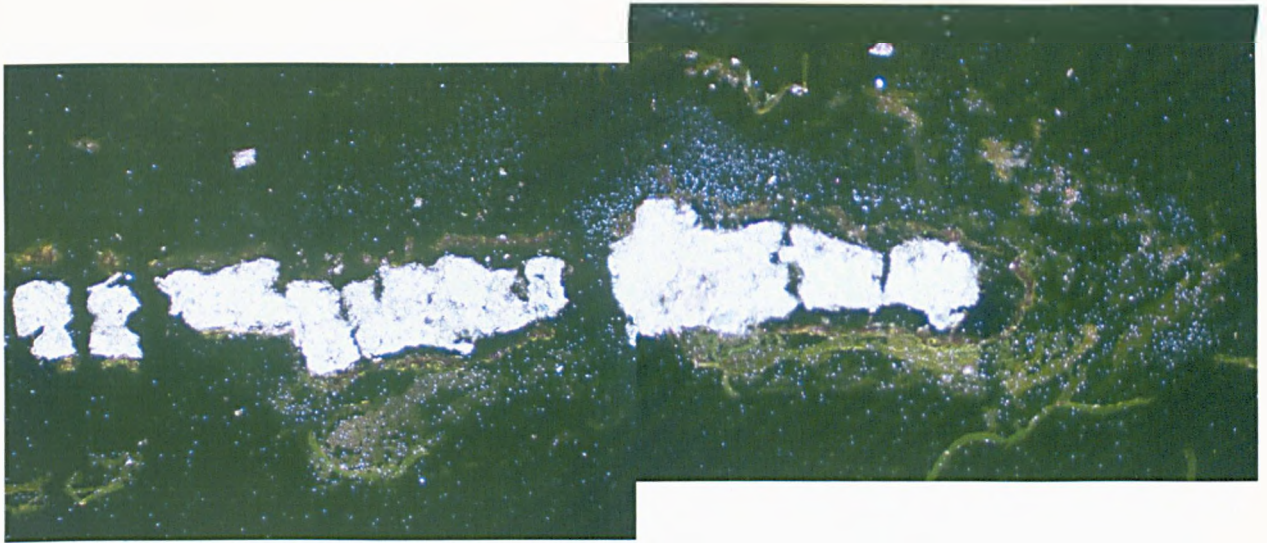
Figure 4.6. *Chironomus riparius* untreated control viewed under a) dark field and b) bright field illumination. Silver grained areas represent radioactive material.

a)

Gut contents

b) Anterior

Gut

Posterior

Appendages
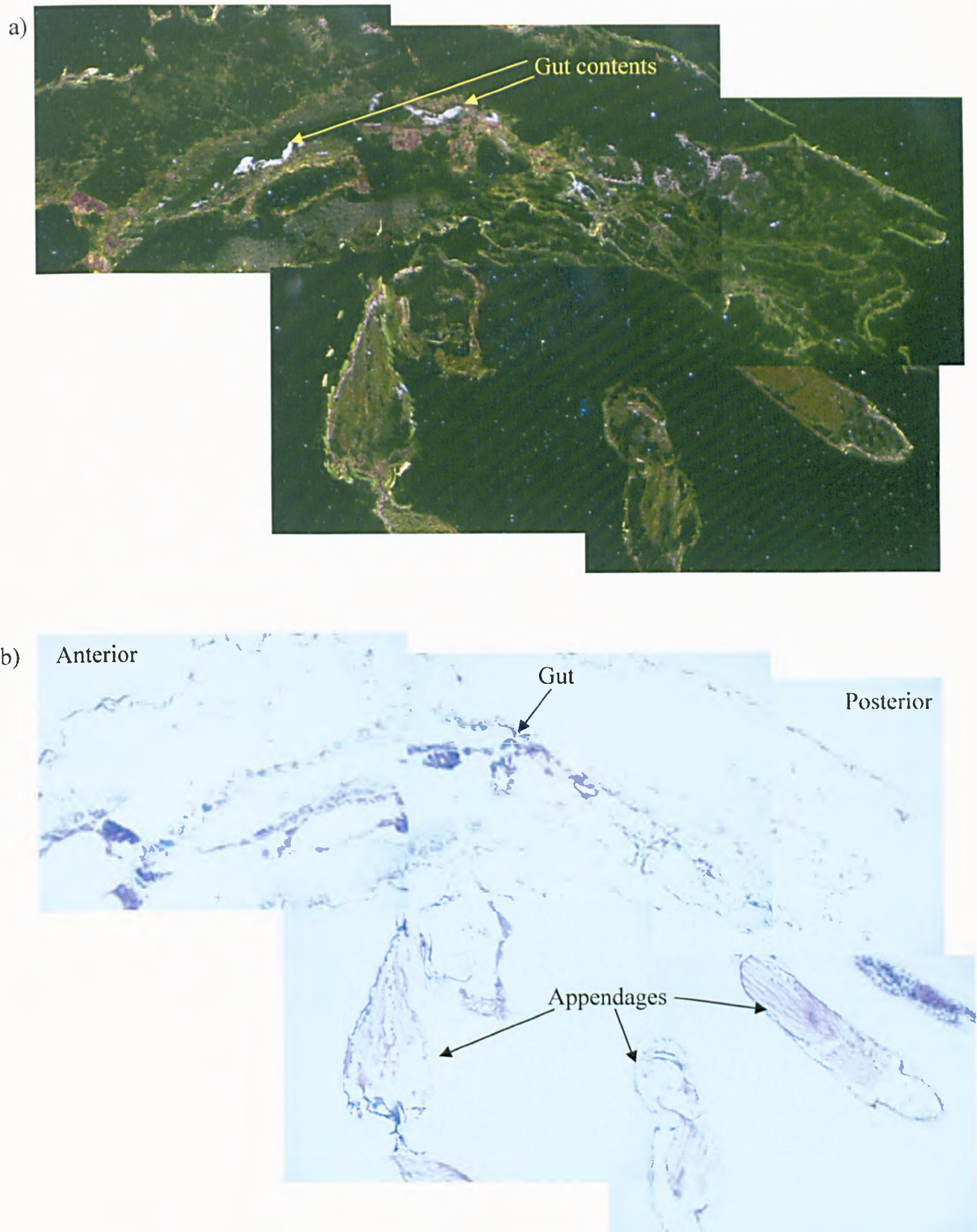
Figure 4.7. *Asellus aquaticus* untreated control viewed under a) dark field and b) bright field illumination. Silver grained areas represent radioactive material.

levels of radioactivity, mainly associated with the exoskeleton (Figure 4.8) and mouthparts (Figure 4.9). Low levels of activity were associated with gut contents and internal tissues (Figure 4.10). Higher levels of activity were detected in *C. riparius*, located throughout the internal tissues, particularly the gut wall and contents (Figure 4.11). Relatively low levels of activity were detected in the external tissues of *C. riparius* (Figure 4.11). There were high levels of activity associated with the gut contents of *A. aquaticus*, and also throughout the internal tissues (Figures 4.12 and 4.13). Lower levels of activity were associated with the exoskeleton and appendages of *A. aquaticus* (Figures 4.12 and 4.13).

DISCUSSION

All three benthic species were able to accumulate benzophenone from contact with, or ingestion of, contaminated sediment. These species therefore provide a potential exposure pathway to hydrophobic contaminants for predators feeding on them (Egeler *et al.*, 2001). However, the extent of dietary exposure to predators will depend on the prey species consumed. In agreement with existing studies, interspecific differences in accumulation of a hydrophobic contaminant were demonstrated in the three benthic species (Kaag *et al.*, 1997; Gaskell *et al.*, 2007). The three test species differed in their feeding behaviours, with *Chironomus riparius* and *Asellus aquaticus* ingesting sediment whereas *Cloëon dipterum* did not. Although the two ingester species both accumulated more than the non-ingester species, feeding behaviour alone did not explain the pattern in accumulation achieved, being in the order *A. aquaticus* > *C. riparius* > *C. dipterum*. Therefore, as accumulation is the result of uptake exceeding loss, the interspecific differences observed may be due to the influence of other mechanisms that affect these.

Hydrophobic contaminants such as benzophenone are often assumed to accumulate mainly within the lipids of organisms (Mackay, 1982; Di Toro *et al.*, 1991). Therefore the differences in body burden observed between the benthic species may be due to differences in their relative lipid contents. Lipid contents reported in the literature vary between the three species used, with the percentage of lipid on a fresh

Figure 4.8. *Cloëon dipterum* following exposure to sediment dosed with [14C] benzophenone, viewed under a) dark field and b) bright field illumination. Silver grained areas represent radioactive material.

a)

¹⁴C material

b)

Mouthparts

Head
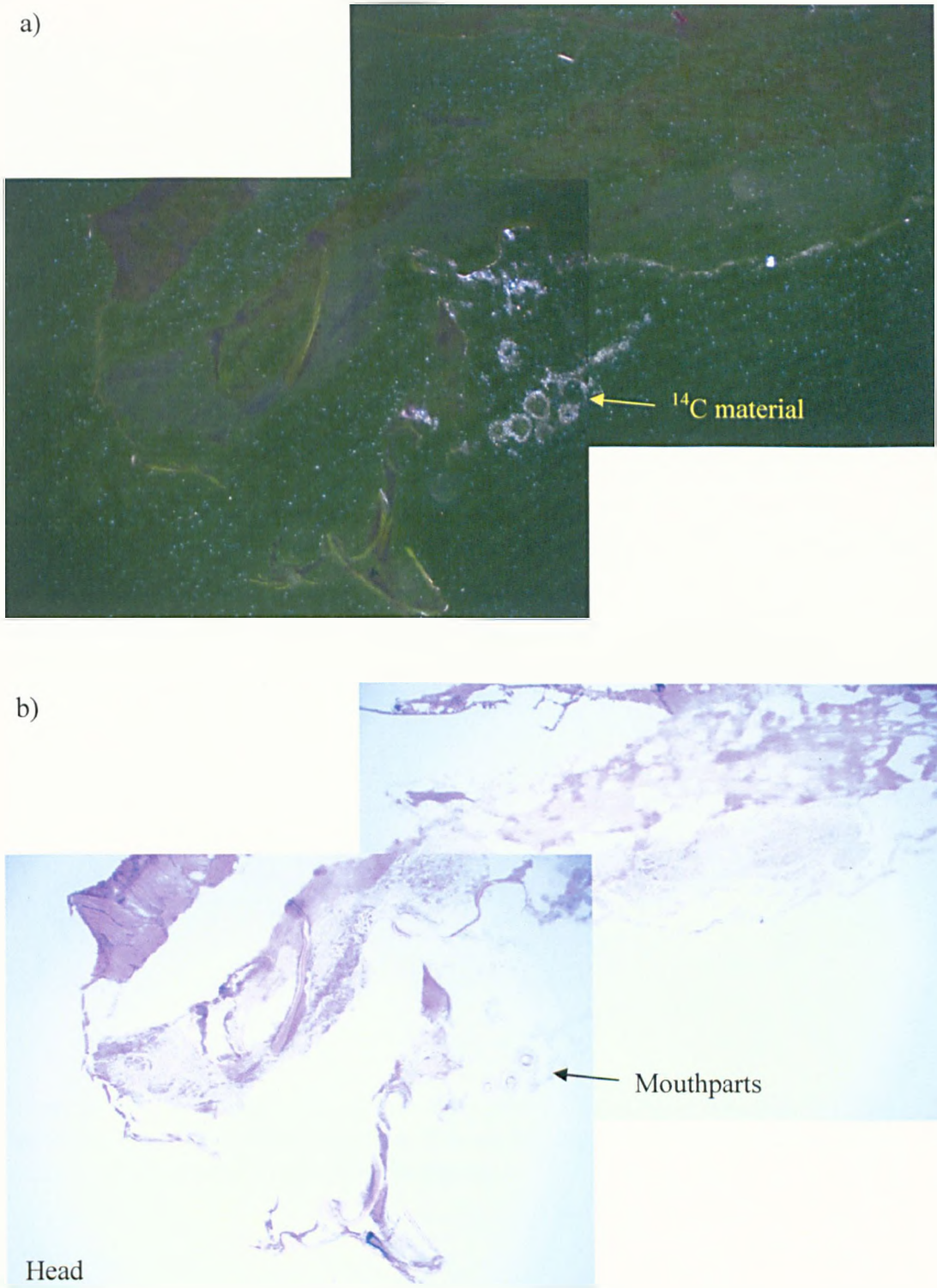
Figure 4.9. *Cloëon dipterum* following exposure to sediment dosed with [14C] benzophenone, viewed under a) dark field and b) bright field illumination. Silver grained areas represent radioactive material.
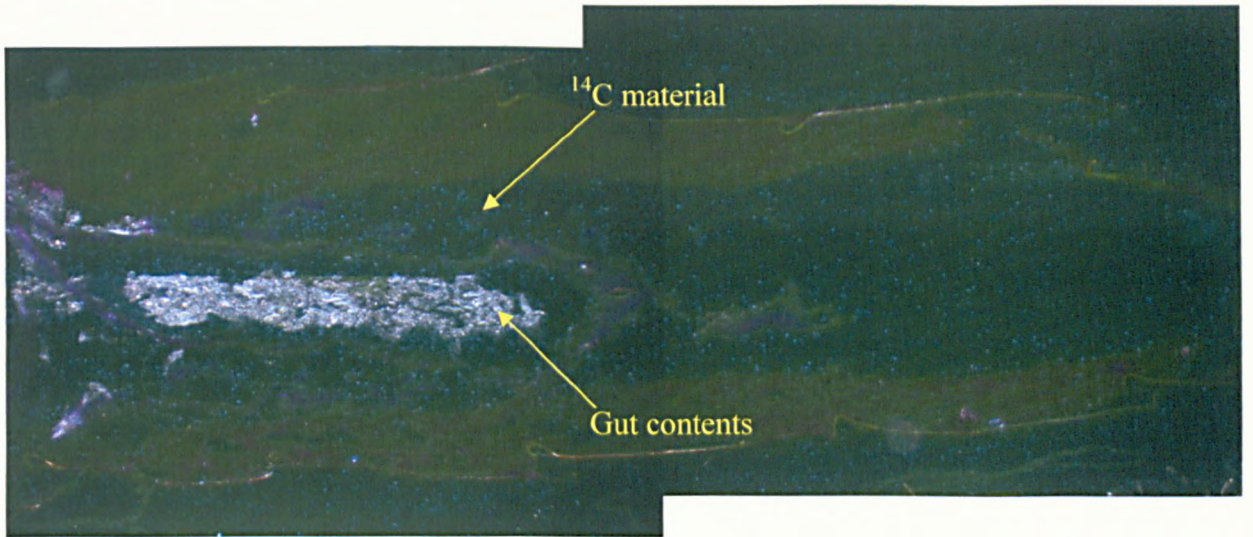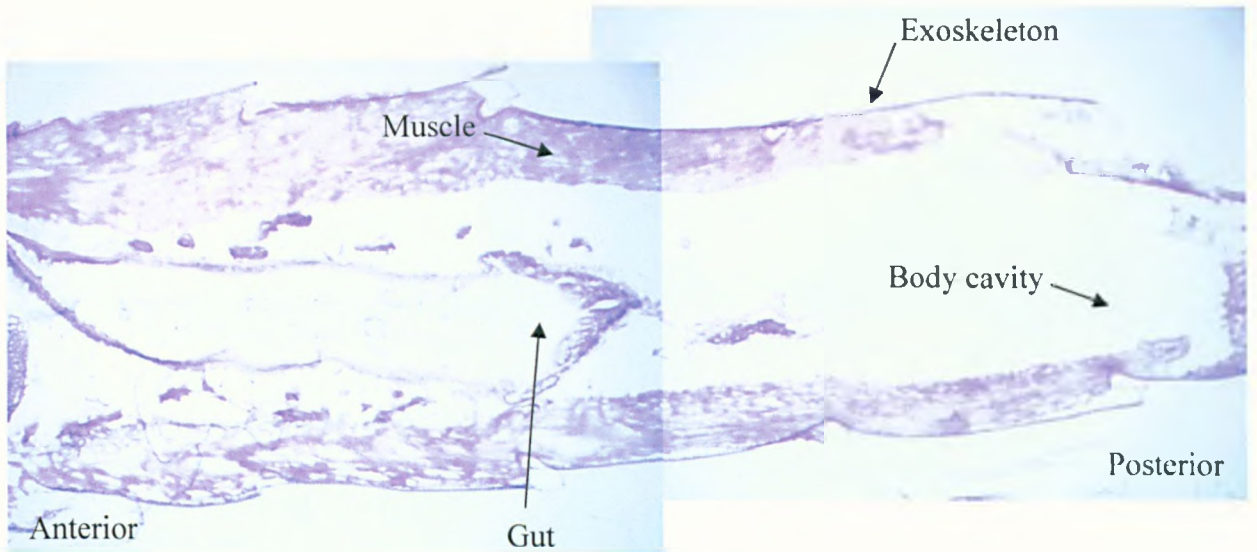
Figure 4.10. *Cloëon dipterum* following exposure to sediment dosed with [14C] benzophenone, viewed under a) dark field and b) bright field illumination. Silver grained areas represent radioactive material.

a)
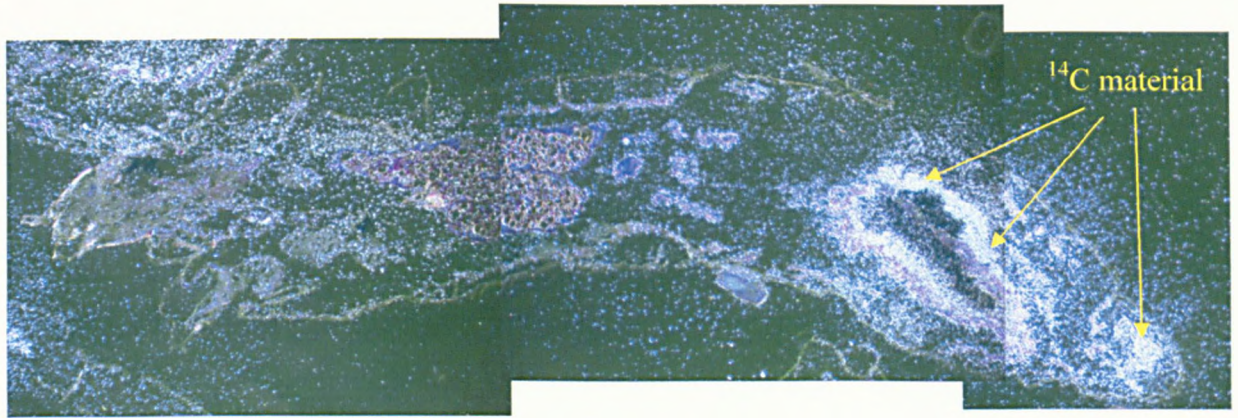
b)

Figure 4.11. *Chironomus riparius* following exposure to sediment dosed with [14C] benzophenone, viewed under a) dark field and b) bright field illumination. Silver grained areas represent radioactive material.
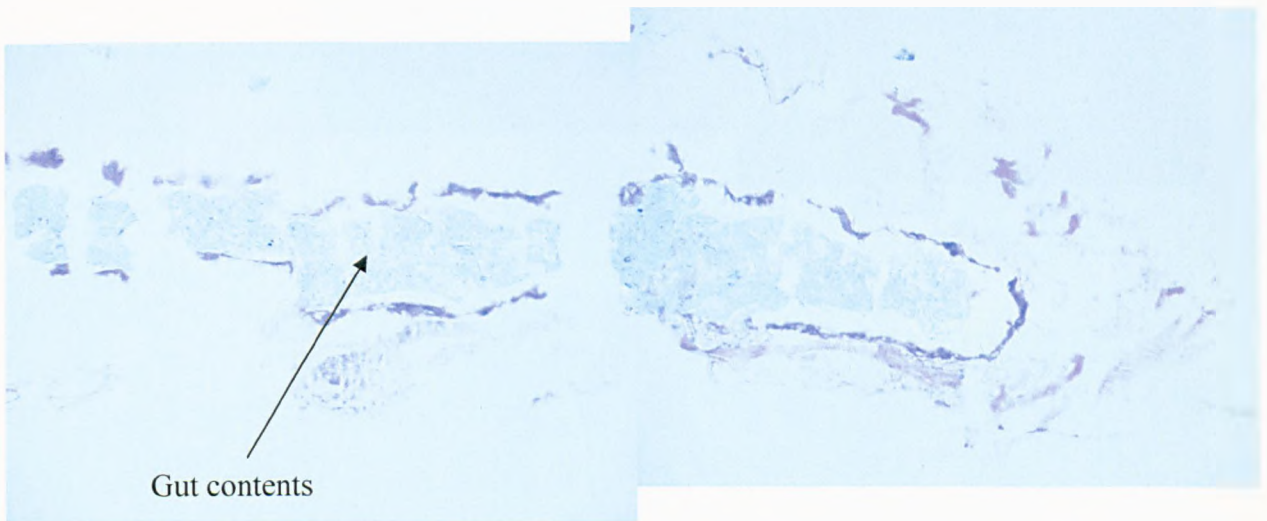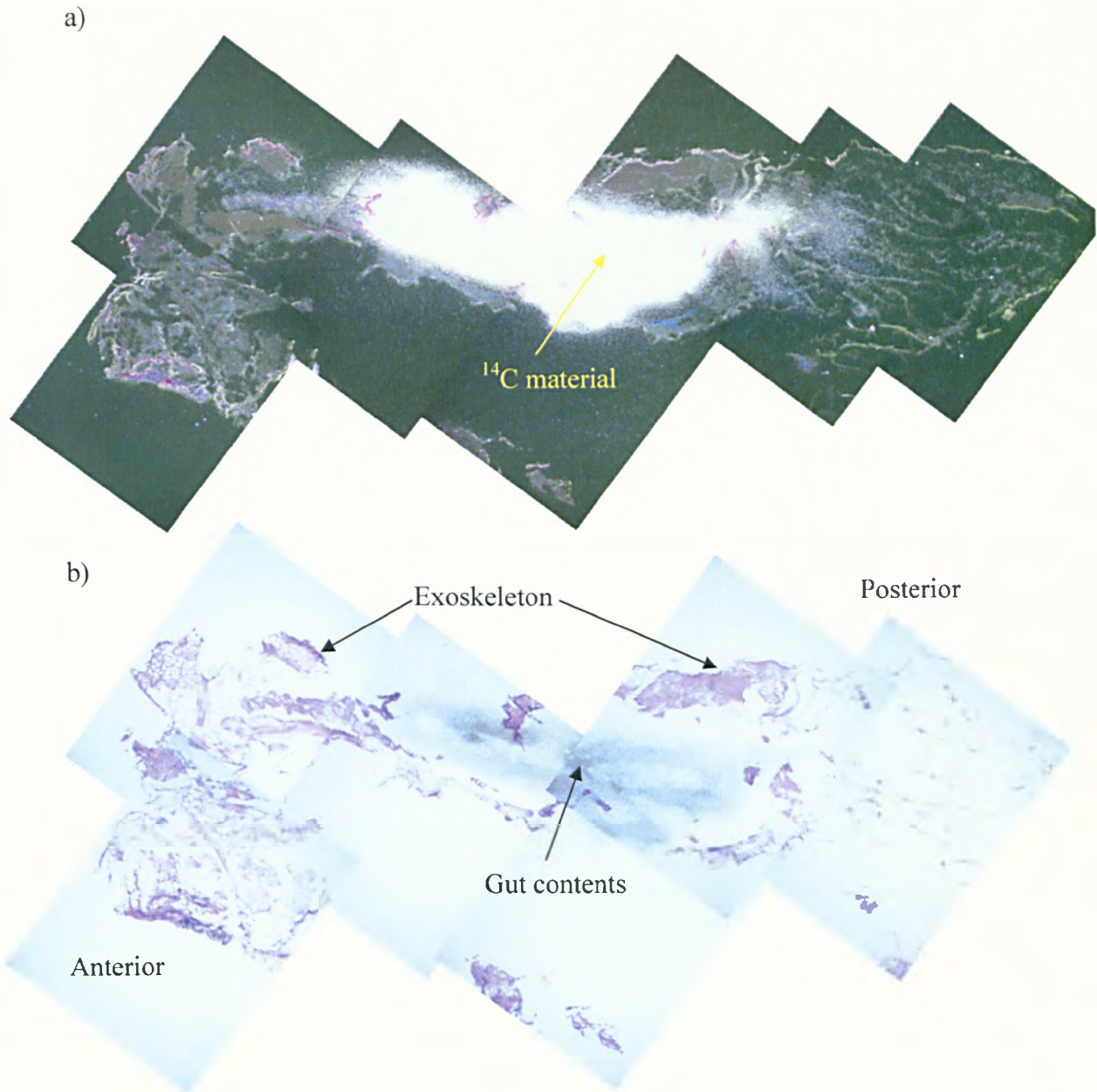
Figure 4.12. *Asellus aquaticus* following exposure to sediment dosed with [14C] benzophenone, viewed under a) dark field and b) bright field illumination. Silver grained areas represent radioactive material.

a)   b)   Anterior

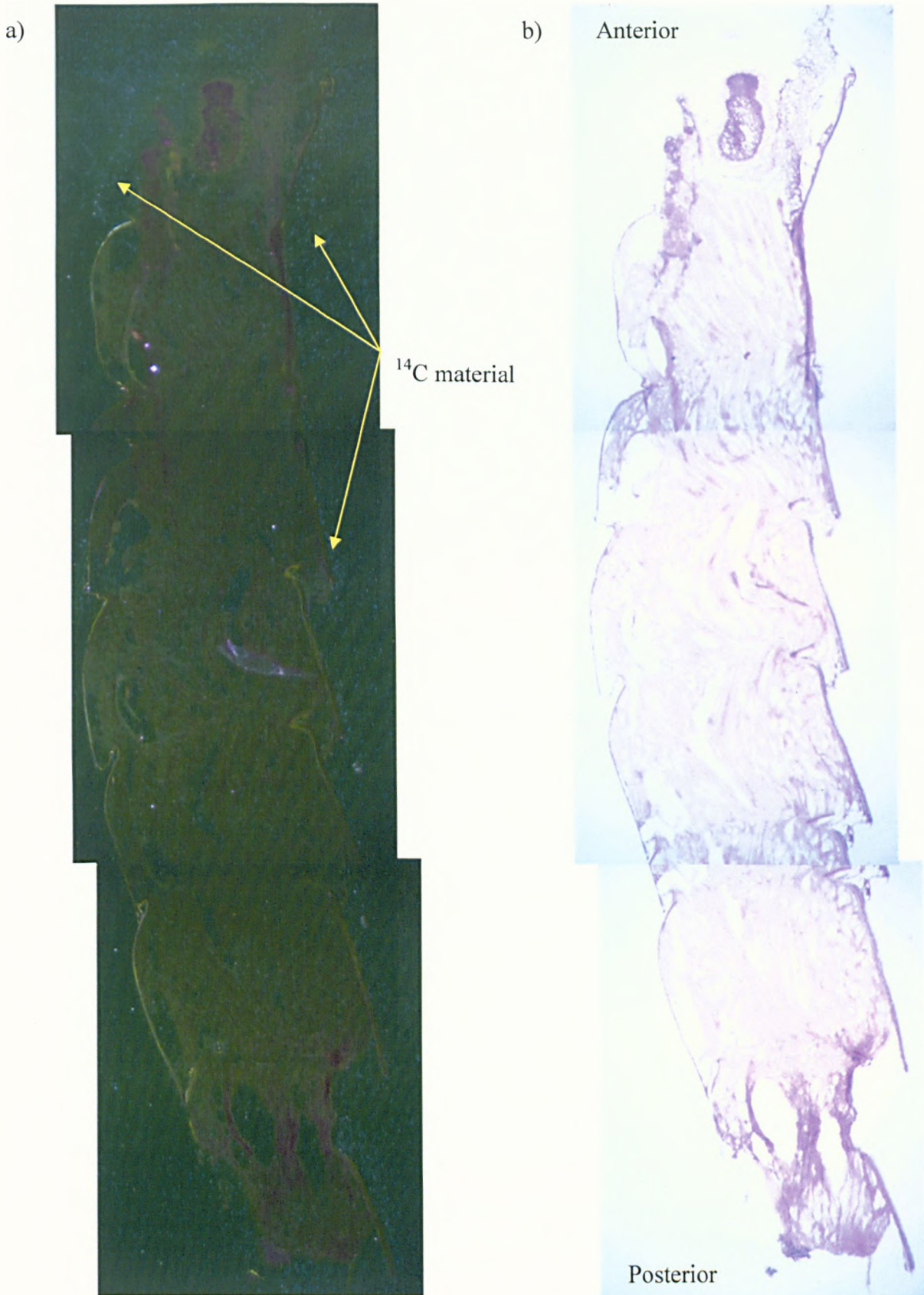Appendages

Gut contents

^{14}C material

Posterior
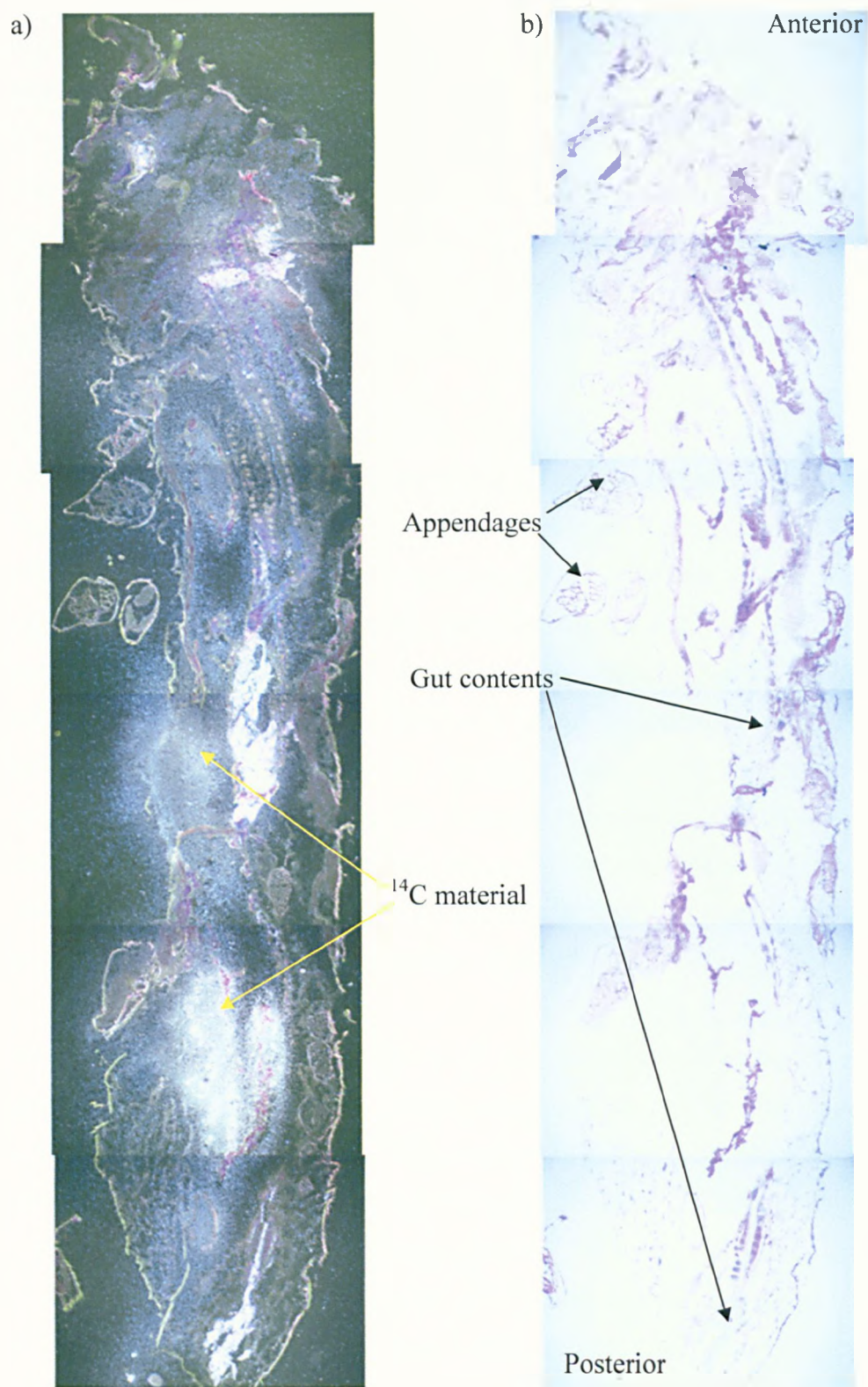
Figure 4.13. *Asellus aquaticus* following exposure to sediment dosed with [14C] benzophenone, viewed under a) dark field and b) bright field illumination. Silver grained areas represent radioactive material.

weight basis being 0.69, 0.82-1.08 and 2.1% for *A. aquaticus* (Cid Montañés *et al.*, 1995), *Chironomus* sp. (West *et al.*, 1997), and mayfly nymphs (Sushchik *et al.*, 2003), respectively. Therefore, the predicted order for body burden based on lipid content would be *C. dipterum* > *C. riparius* > *A. aquaticus*. However, the observed pattern in body burden was *A. aquaticus* > *C. riparius* > *C. dipterum*. Hence factors other than lipid content are important in determining overall body burden, as found in other studies (Russell *et al.*, 1999; Fisk *et al.*, 2001; Gaskell *et al.*, 2007).

Uptake of contaminants can also be influenced by the feeding rate and gut physiology of the organism. Previous studies have demonstrated that the greater the volume of sediment processed, the greater the body burden achieved (Hickey *et al.*, 1995; Kaag *et al.*, 1997; Christensen *et al.*, 2002). Although *A. aquaticus* and *C. riparius* are both ingester species, they ingest sediment at different rates. The feeding rates of *A. aquaticus* and *C. riparius* have been reported as <6 µg dry weight food/mg dry weight/h (Adcock, 1982) and 14 µg dry sediment/mg dry weight/h (Bervoets *et al.*, 2003), respectively. Therefore, based on the volume of sediment processed during the exposure period, it would expected for *C. riparius* to have a higher body burden than *A. aquaticus*, but in fact the opposite was observed. Other studies have also found feeding rate to be a poor predictor of body burden, with gut physiology being cited as a better predictor (Gaskell *et al.*, 2007).

Gut physiology can affect the uptake of contaminants by influencing the extraction efficiency from the gut contents and the subsequent absorption into tissues (Penry and Weston, 1998; Ahrens *et al.*, 2001). *Asellus aquaticus* and *Chironomus riparius* have different strategies for achieving absorption, with *A. aquaticus* having a short gut passage time but high surfactancy, whereas *C. riparius* has a long gut passage time and low surfactancy (Gaskell *et al.*, 2007). Although the strategy of *C. riparius* was previously thought to more effective than that of *A. aquaticus* (Gaskell *et al.*, 2007), it may depend on the contaminant involved. Studies have demonstrated differences in accumulation within a species for different contaminants (Clements *et al.*, 1994; Means and McElroy, 1997). Exposure of *C. riparius* for 72-h to 4,040 µg fluoanthene/kg sediment or 2,740 µg benzo[*a*]pyrene/kg sediment resulted in average body burdens of 181,000 µg/kg and 6,030 µg/kg, respectively (Clements *et al.*,

1994). Exposure of a bivalve (*Yoldia limatula*) to 1-2 µg/g of hexachlorobiphenyl or tetrachlorobiphenyl resulted in average body burdens of 500 µg/g and <5µg/g, respectively (Means and McElroy, 1997). Therefore accumulation of different contaminants within a species can vary over several of orders of magnitude.

The observed pattern in bioaccumulation may also be driven by the ability of the three species to metabolize and subsequently eliminate accumulated benzophenone. The extent of metabolite production differed between species, in the order *C. dipterum* > *A. aquaticus* > *C. riparius*. The biotransformation capabilities of *Chironomus* sp. have been demonstrated for a range of different organic compounds, including surfactants (McKinnell, 1994), organophosphate insecticides (Ankley and Collyard, 1995) and polycyclic aromatic hydrocarbons (PAHs) (Leversee *et al.*, 1982; Borchert *et al.*, 1997). As observed in the current study, the extent of metabolism of the parent compound in *C. riparius* is often extensive, with 72% metabolism of benzo[*a*]pyrene after 8 hours exposure (Leversee *et al.*, 1982) and >90% metabolism of the surfactant dodecyl tetra(oxyethylene) ether (C12 EO4) within 210 minutes exposure (McKinnell, 1994). Although some studies have stated that biotransformation has limited importance for *A. aquaticus* (van Hattum and Montanes, 1999), production of metabolites has been observed, with 26% of terbutryn and 74% benzo[*a*]pyrene metabolized after 48 hours of dietary exposure (Richter and Nagel, 2007). No data on the biotransformation capabilities of *C. dipterum* could be identified in the literature. However, adults of the stream mayfly *Centroptilum triangulifer* contained 13% metabolites following 4-8 weeks dietary exposure of nymphs to the technical form of the pesticide chlordane (Standley *et al.*, 1994).

The ability of organisms to eliminate accumulated contaminants has previously been linked with their capacity to metabolize parent compounds into more polar, and therefore more easily excretable, metabolites (Kane Driscoll and McElroy, 1997). There were differences between all three species in their metabolite profiles for benzophenone. The polarity of the metabolites produced, and therefore the expected order for the extent of elimination, was in the order *A. aquaticus* > *C. dipterum* > *C. riparius*. However, the observed order of elimination was *C. riparius* > *C. dipterum* > *A. aquaticus*. *Chironomus riparius* eliminated nearly four times more

benzophenone residues than *A. aquaticus* in the 24-h elimination period, which may help explain why *C. riparius* had a lower body burden than the other ingester species. Therefore, although the extent of elimination may help to explain why the accumulation in *C. riparius* was lower than expected for an ingester, it does not seem to be attributable to either the extent of biotransformation or the polarity of the metabolites produced. It is possible that *A. aquaticus* is able to produce metabolites, but is unable to subsequently excrete them (e.g. Verrengia Guerrero *et al.*, 2002).

Not only did the overall body burden of the three test species differ, but the distribution of that accumulated contaminant within the organisms also differed. Two methods were used in order to assess this: a qualitative microautoradiography approach and a quantitative manual separation approach. The microautoradiography results demonstrated more internal accumulation within the two ingester species compared to the non-ingester. This was expected due to the additional dietary exposure experienced by the ingesters, resulting in internal accumulation (Fisk *et al.*, 1997; Arukwe *et al.*, 2000; Liu *et al.*, 2002; Xu and Wang, 2002; Wang and Wong, 2003; Heiden *et al.*, 2005). The results from the manual separation approach also demonstrated internal accumulation for *A. aquaticus*, but the majority of accumulation was external for all three species. However, once losses in activity during the manual separation procedure were taken into account, the percentages internally accumulated increased for *C. riparius* and *A. aquaticus*. Hence, although the extent of internal accumulation is not extensively greater than that externally accumulated, the proportions internally accumulated within ingesters (35-65%) are much greater than that of the non-ingester (5%).

The three test species therefore represent very different packages of accumulated contaminant. Not only do they differ in the overall body burden achieved, but also in the identity of the compounds being stored and whether internally or externally accumulated by organisms. Recent work using cadmium has demonstrated that the transfer of contaminants from prey to predator can be affected by the exposure pathway of the prey (Béchard *et al.*, 2008). Béchard *et al* found that a higher proportion of cadmium was transferred to a fish predator (*Danio rerio*) from *C. riparius* exposed via sediment compared to water only exposure (Béchard *et al.*, 2008). Predators that feed on their prey whole (engulfers) will be exposed to higher

doses in the order *A. aquaticus* > *C. riparius* > *C. dipterum* due to their total body burdens. Predators that feed on only the internal tissues of their prey (piercers) will also be exposed to higher doses in the order *A. aquaticus* > *C. riparius* > *C. dipterum*, but due to the extent of internal accumulation rather than total body burden. The high proportion of contaminant being externally accumulated by all three species means that all benthic species can potentially act as vectors for exposure to engulfer predators, even those that do not ingest sediment e.g. *C. dipterum*.

Chapter 5

The importance of prey handling and contaminant packaging for dietary exposure and secondary poisoning of predators

5.1 INTRODUCTION

Uptake of contaminants through the diet has been found to be an important exposure pathway in both aquatic (Wang and Fisher, 1999) and terrestrial (Smith *et al.*, 2007) systems and can contribute to the long range transport of contaminants (Mu *et al.*, 2004). Dietary exposure may be a particular issue for contaminants that are persistent, bioaccumulative and toxic (PBT), highlighted as a high risk group in the new EU chemicals strategy REACH (EC, 2006). Although bioaccumulation in itself is not a toxic effect, it does represent an exposure pathway for subsequent trophic levels and may result in secondary poisoning in predators (Feijtel *et al.*, 1997). Risk assessment assumes that the body burden achieved by a prey animal represents the exposure dose to their predators (Feijtel *et al.*, 1997). However, the extent of predator exposure may depend on not only how the contaminant is distributed within the tissues of their prey, but also which parts of the prey the predator eats.

A scenario in which dietary exposure to PBTs may be particularly important is sediment-associated aquatic food chains. Due to their hydrophobic nature, PBTs will move out of the water phase and become associated with sediments (Qiu and Davis, 2004). Animals that reside in or on these sediments are at risk of bioaccumulating hydrophobic contaminants, acting as vectors for transfer to predators that may have limited direct contact with contaminated sediments (Rubinstein *et al.*, 1984; Clements *et al.*, 1994; Egeler *et al.*, 2001). For example, the consumption of *Tubifex tubifex* worms previously exposed to contaminated sediment resulted in significantly higher accumulation of hexachlorobenzene in three-spined sticklebacks (*Gasterosteus aculeatus*) compared to sediment-exposure alone (Egeler *et al.*, 2001). Similarly, bluegill (*Lepomis macrochirus*) accumulated c. 20 µg benzo[*a*]pyrene/kg when exposed to contaminated sediment, but this increased to c. 150 µg/kg with the

addition of dietary exposure from *Chironomus riparius* prey (Clements *et al.*, 1994). Dietary exposure from feeding on contaminated polychaetes (*Nereis virens*) also accounted for at least 53% of the total polychlorinated biphenyl (PCB) body burden in a demersal fish (*Leiostomus xanthurus*) exposed to contaminated sediments (Rubinstein *et al.*, 1984).

Sediment-dwellers vary in their ability to bioaccumulate contaminants from sediment (Clements *et al.*, 1994; Kaag *et al.*, 1997; Gaskell *et al.*, 2007) and mechanisms proposed to explain this variation include differences in feeding rates, gut physiology, biotransformation and excretion rates (Gaskell *et al.*, 2007). Species that process large volumes of sediment achieve higher body burdens than those that process smaller volumes (Hickey *et al.*, 1995; Kaag *et al.*, 1997; Christensen *et al.*, 2002). Therefore those that inhabit sediment but do not ingest it (non-ingesters) would generally have lower body burdens than those that live in and feed on sediment (ingesters). Differences in feeding behaviour between ingesters and non-ingesters may also affect the distribution of the accumulated contaminant within the tissues. Dietary exposure, such as through sediment ingestion, results in accumulation mainly in internal tissues whereas aqueous exposure is mainly externally accumulated (Fisk *et al.*, 1997; Arukwe *et al.*, 2000; Hook and Fisher, 2001b; Liu *et al.*, 2002; Xu and Wang, 2002; Wang and Wong, 2003; Heiden *et al.*, 2005). Therefore, non-ingesters would be expected to have mainly external accumulation compared to internal and external accumulation in ingesters. Consequently the distribution of accumulated contaminants may not be uniform throughout the body of the prey, which may have implications for transfer to predators that feed on them. Although the influence of exposure pathway and internal distribution in prey on trophic transfer has been addressed for metals (Wallace and Lopez, 1996; Wallace and Luoma, 2003; Béchard *et al.*, 2008; Dumas and Hare, 2008), no such studies exist for organic contaminants. The transfer of contaminants through a sediment-prey-predator food chain may therefore depend on which prey type the predator feeds on and how it handles that prey package.

Aquatic predators differ in their feeding habits and can be broadly categorised into two groups: piercers and engulfers (Peckarsky, 1982). Piercers have stabbing mouthparts adapted for sucking out the internal tissues and fluids from their prey.

Engulfers consume their prey whole or eat pieces of their prey. Therefore the exposure of predators to contaminants will partly depend on which parts of their prey they consume. Piercers will only be exposed to contaminants that are accumulated in the internal tissues of their prey whereas engulfers will be exposed to both internally and externally accumulated contaminants. The combination of predator type (engulfer or piercer) and prey type (ingester or non-ingester) may therefore influence the extent of predator exposure, and hence their dietary uptake. Engulfers would be expected to achieve a higher body burden than piercers when feeding on either prey type, due to their additional exposure to the externally accumulated contaminants in prey. This may be particularly evident when feeding on non-ingester prey, as there would be very low internal tissue residues for the piercer to be exposed to. Both predators would be expected to achieve higher body burdens when feeding on ingester compared to non-ingester prey, due to higher body burdens in ingester prey. The influence of packaging and handling of prey on predator exposure has not been studied previously, but may be important in determining the extent of secondary poisoning in predators.

Dietary exposure to contaminants has been found to result in toxic effects in various predators, including mammals (Heaton *et al.*, 1995), fish (Berntssen *et al.*, 1999) and invertebrates (Wallace *et al.*, 2000). These toxic effects have included reduced reproductive fitness (Heaton *et al.*, 1995), impaired growth (Friedmann *et al.*, 1996) and reduced food intake (Wallace *et al.*, 2000). It is hypothesized in the literature that dietary exposure results in toxic effects when a critical body residue (CBR) is reached in the predator (Landrum *et al.*, 1992; McCarty and Mackay, 1993). Therefore if the combination of predator and prey type affects exposure to predators, it may also determine the magnitude of toxic response (Walker *et al.*, 1996).

The aim of this study was to investigate whether differences in the packaging and handling of prey determines the transfer of contaminants to predators, and thus the extent of secondary poisoning. The model hydrophobic contaminant used was [14C] benzophenone, with a Log K_{ow} of 3.2 (Cichna *et al.*, 1995). Benzophenone has a variety of uses, including being incorporated into agricultural chemicals, pharmaceuticals and paints (EC, 2000; Hayashi *et al.*, 2006). It has been reported in the environment at concentrations of <2.6-1040 ng/L in water (Oros *et al.*, 2003;

Pojana *et al.*, 2004; Pojana *et al.*, 2007) and 14-200 µg/kg in sediment (Burkhardt *et al.*, 2005; Pojana *et al.*, 2007). A radiolabelled isotope was used to enable detection of small quantities of the contaminant being transferred through the food chain. Two ingester (*Asellus aquaticus* Linnaeus (Crustacea, Isopoda); *Chironomus riparius* Meigen (Insecta, Diptera)) and one non-ingester (*Cloëon dipterum* Linnaeus (Insecta, Ephemeroptera)) prey species were exposed to sediment spiked with [14C] benzophenone. Prey were then fed to an engulfer (*Ischnura elegans* Vander Linden (Insecta, Odonata)) and a piercer (*Notonecta glauca* Linnaeus (Insecta, Heteroptera)) predator species. The uptake and depuration of benzophenone by predators was determined for all six predator-prey species combinations. The percentage of benzophenone transferred from prey to predator was calculated for each predator-prey combination to determine the influence of prey packaging and handling. The effect of dietary benzophenone exposure on the feeding rates of predators was also investigated.

5.2 METHODS

5.2.1 MODEL COMPOUND

The [14C] benzophenone (>99% purity) used in this study was purchased from ARC (American Radiolabelled Chemicals, St. Louis, USA) and had a specific activity of 11.2 MBq/mg, verified using high performance liquid chromatography (HPLC) at Unilever Research (Unilever, Bedfordshire, UK).

5.2.2 TEST SPECIES

Four of the five test species were collected from field populations in Sheffield, South Yorkshire, UK. *Asellus aquaticus* (5 mm in length) were collected from Havelock Dam in the Rivelin Valley (National Grid Reference (NGR) SK 324 887) or the River Don (NGR SK 316 921), *Cloëon dipterum* (10 mm in length) were collected from Lower Crabtree Pond (NGR SK 361 899), *Ischnura elegans* (20-25 mm in length) from Arbourthorne Pond (NGR SK 371 850) and adult *Notonecta glauca* (15-20 mm in length) from both Lower Crabtree Pond (NGR SK 332 828) and Millhouses Boating Pond (NGR SK 361 899). All species were maintained in

aquaria filled with artificial pond water (APW; (HSE, 1982)) at 15°C with a light:dark period of 16:8 hours. *Asellus aquaticus* were fed with detritus (predominantly alder leaves, *Alnus* sp.) and *C. dipterum* were fed with detritus and fresh plant material (predominantly *Elodea* sp. and *Ceratophyllum* sp.), all collected from the source ponds. *Ischnura elegans* and *Notonecta glauca* were fed a mixture of *Asellus aquaticus*, *Cloëon dipterum* and *Chironomus riparius* (5 mm in length, third/fourth instar) *ad libitum*.

Chironomus riparius was cultured according to a method adapted from Credland (1973). Briefly, small plastic containers (approx. 10 cm diameter) with play pit sand (1 cm deep) and overlying APW (2 cm deep) were used. Larvae were fed three times a week on powdered Tetramin® tropical fish food flakes and were maintained at 20°C. All animals were subject to a photoperiod of 16 h light to 8 h dark.

5.2.3 SEDIMENT DOSING

Artificial rather than field collected sediment was used for these experiments to allow more consistent replication of exposure conditions. The composition of the sediment was sand (75%), kaolin (20%) and cellulose (5%), according to dry mass. Sixty-millilitre glass test vessels were used for prey exposures, each containing 2.5 g dry weight of artificial sediment. The LC_{50} for benzophenone was taken to be 280 µg/L (Tosato *et al.*, 1991), and therefore a nominal concentration of approximately 10% of LC_{50} (30 µg/g) was used for prey exposures to minimise mortality but maximise bioaccumulation in prey. Half a millilitre of a 150 mg/L stock solution of benzophenone dissolved in HPLC-grade acetone was added to each test vessel and mixed thoroughly. Benzophenone stock solution was produced by diluting radiolabelled benzophenone with unlabelled benzophenone (Sigma-Aldrich, Gillingham, United Kingdom), using a dilution ratio of 1 : 266 (labelled : unlabelled) for *N. glauca* and 1 : 133 for *I. elegans*, to account for lower feeding rates, and hence exposures, in *I. elegans*. The acetone was allowed to evaporate for at least two hours. One millilitre of APW was added to sediment in each vessel and mixed to form a sediment paste. Overlying APW (25 ml) was then added to each vessel, placing a plastic disc over the sediment to minimise sediment disturbance. Control test vessels

89

were prepared in a similar way to those containing benzophenone, except that 0.5 ml of acetone was added instead of 0.5 ml benzophenone stock solution.

5.2.4 UPTAKE AND DEPURATION OF BENZOPHENONE BY PREDATORS

Pairs of predators (either *I. elegans* (engulfer) or *N. glauca* (piercer)) were placed into plastic cups containing 100 ml APW and a plastic rod to act as a perch. For each predator, groups of twelve *A. aquaticus* (ingester) were added to each of 135 sediment test vessels, 90 of which contained benzophenone-dosed sediment and 45 were control vessels. In order to continually feed 42 pairs of predators dosed or control prey over a five day period, the exposure of prey was staggered over the preceding five days. This allowed each predator to be fed daily with a group of ten prey that had been previously exposed to dosed or control sediment for five days. On Day 0, three pairs of predators were sampled to measure the background [14C] in animals. Each pair was blotted dry, weighed and frozen at -30°C until required for analysis. The remaining pairs of predators were each fed ten *A. aquaticus* from a single dosed or control sediment exposure vessel. Prey were rinsed with distilled water before being offered to predators to minimise the transfer of sediment particles into predator feeding vessels. On Days 1-5, three pairs of predators fed dosed prey and three pairs fed control prey were removed, blotted dry, weighed and frozen as before. Remaining predators were again fed ten *A. aquaticus* from a single dosed or control sediment exposure vessel. All prey from the previous day's feeding were removed, and the number remaining alive were counted. Feeding was terminated on Day 5 and the remaining nine pairs of predators were transferred to clean plastic cups containing 100 ml APW. During this depuration phase, three pairs of predators were removed, blotted dry, weighed and frozen on Day 6, 7 and 10. This allowed the generation of a 5-day uptake curve and a 5-day depuration curve for each predator species.

A random sample of five control prey and five spiked prey was taken on each feeding day to determine whether predators were being exposed to similar levels of benzophenone in their prey over the five-day feeding period. Prey were removed from the sediment exposure vessels, rinsed in distilled water, blotted dry, weighed and frozen until required for analysis.

At the end of the experiment, animal samples were defrosted and added to individual rigid Combusto-Cones (PerkinElmer, The Netherlands), topped with approximately 2-mm layer of cellulose powder to aid complete combustion and capped with compacted tissue paper. Animals were then combusted in a sample oxidizer (model 307, Packard, Meriden, USA) until complete combustion was observed and collected in a scintillation cocktail consisting of 10 ml Permafluor® and 10 ml Carbosorb®. The disintegrations per minute (dpm) in the resulting samples were counted for 10 minutes per sample using a liquid scintillation analyzer (Tri-carb 3100TR, Packard, Meriden, USA). Combusto-cones containing only cellulose powder and tissue paper were also oxidized and analyzed to obtain a background level of activity. The total μg benzophenone/g fresh mass was calculated using equation 5.1:

$$C = \frac{D(A-B)}{MZ} \qquad (5.1)$$

Where C is the total μg benzophenone/g fresh mass, A is the activity of the sample (dpm), B is the background activity (dpm), M is the mass of the sample (g), Z is the activity per microgram of benzophenone (674586 dpm/μg), and D is the dilution factor of cold to radiolabelled benzophenone (266 for *N. glauca* and 133 for *I. elegans*).

This experiment was repeated for both predators using *C. dipterum* (non-ingester) and *C. riparius* (ingester) as prey, but there was no depuration phase for these two prey species. Therefore only 33 pairs of predators were required and 90 sediment test vessels prepared per predator-prey combination. The experiment involving *I. elegans* and *C. riparius* was run for four days rather than five, with six predators instead of three being sampled for each treatment on Day 4.

5.2.5 KINETIC MODELS

First-order two-compartment bioaccumulation models were fitted to the uptake and depuration data generated for each predator-prey combination. Dietary uptake over time can be modeled by equation 5.2 (simplified from Arnot and Gobas, 2006)

$$\frac{dC_f}{dt} = k_1 C_1 - k_2 C_2 \qquad (5.2)$$

Where C_1 is the concentration of benzophenone in the prey (µg/prey animal), t is time (d), k_1 is the uptake rate constant for benzophenone from the prey (µg/g/d), C_2 is the concentration in the predator (µg/g), and k_2 is the rate constant for chemical elimination (µg/d). Uptake from water has been removed from the model as predators are fed contaminated prey in clean APW. The integrated form of equation 5.2 was used to fit the uptake curves to the data and estimate k_1 (Equation 5.3) (Rand and Petrocelli, 1985):

$$C_f = \frac{k_1}{k_2} C_1 \left[1 - e^{-k_2 t} \right] \qquad (5.3)$$

The elimination rate constant (k_2) was estimated using equation 5.4 on the 5-day depuration phase data (Rand and Petrocelli, 1985)

$$\frac{dC_f}{dt} = -k_2 C_0 \qquad (5.4)$$

Where C_0 is the body burden in the predator at the start of the depuration phase (µg/g). All parameter estimates were generated using the statistical software package R (R, 2007).

5.2.6 TRANSFER EFFICIENCY FROM PREY TO PREDATOR

The transfer efficiency (TE) for each predator-prey combination was calculated as the percentage of benzophenone transferred from prey to predator (Equation 5.5):

$$TE = \frac{q}{rb} \times 100 \qquad (5.5)$$

Where q is the average body burden of a pair of predators on Day 1 (µg), r is the average number of prey animals eaten by Day 1 per pair of predators, and b is the average body burden of a single prey animal (µg).

5.2.7 FEEDING RATES OF PREDATORS

Forty-three individual *I. elegans* or *N. glauca* were fed dosed or control prey using the method previously described for the uptake and depuration experiment. However, prey were exposed in groups of six rather than twelve, to allow predators to be fed five prey per day for five days. Three predators were sampled on Day 0 to measure the background [14C] in animals. The remaining predators were fed control or dosed prey for the whole five-day exposure period. The proportion of prey eaten each day was calculated by dividing the number of prey animals remaining alive by five. Proportional data were transformed using the arcsine square root transformation to comply with the assumptions of parametric statistics. A random sample of five control prey and five spiked prey was taken on each feeding day as described in the previous experiment.

At the end of the five-day exposure period, five control-fed predators and five benzophenone-fed predators were sampled to determine the body burden of predators at the start of a feeding trial, either for two hours with *N. glauca* or four hours with *I. elegans* to allow for their slower feeding rate. Ten clean *A. aquaticus* not previously exposed to dosed or control sediment were offered to each predator. The number of prey remaining alive after the 2 or 4 hour feeding period was recorded. Predators were then blotted dry, weighed and frozen to be stored for analysis. Animal samples were defrosted, oxidized and the activity within them counted using liquid scintillation counting as in the previous experiment. This experiment was repeated for both predators using *C. dipterum* (non-ingester) as prey and with *C. riparius* (ingester) for *N. glauca*.

5.3 RESULTS

5.3.1 UPTAKE AND DEPURATION OF BENZOPHENONE BY PREDATORS

Both predator species accumulated benzophenone from their prey (Figure 5.1). There was significant accumulation of benzophenone in *N. glauca* (ANOVA: $F \geq 35.0$, df=1,24, p<0.05) and *I. elegans* (ANOVA: $F \geq 25.3$, df$\geq$1,24, p<0.05) compared to the controls for all three prey species. Uptake of benzophenone by the piercer predator (*N. glauca*) depended on the prey species consumed. The consumption of *A. aquaticus* (Figure 5.1a) resulted in the highest body burden by Day 5 of 5.2 µg/g whereas consumption of *C. dipterum* (Figure 5.1c) resulted in the lowest body burden by Day 5 of 0.2 µg/g. Uptake by the engulfer predator (*I. elegans*) was approximately equal for all three prey species by Day 5, reaching a maximum of between 2.2 and 3.5 µg/g (Figure 5.1 d-f).

Uptake by predators depended on the prey species being consumed and the predator species consuming them (Figure 5.1). The engulfer predator (*I. elegans*) achieved a higher body burden by Day 5 than the piercer predator (*N. glauca*) for both *C. riparius* (ingester: Figure 5.1b and e) and *C. dipterum* (non-ingester: Figure 5.1c and f). However, the piercer predator achieved a higher body burden than the engulfer when feeding on *A. aquaticus* (ingester: Figure 5.1a and d).

Both predator species were able to eliminate benzophenone during the five-day depuration phase (Figure 5.1a and d). Benzophenone concentrations decreased rapidly in *Notonecta glauca*, from 5 µg/g on Day 5 to background levels (0.2 µg/g) by Day 10 (Figure 5.1a). *Ischnura elegans* had a slight reduction in benzophenone concentration on Day 5, before the depuration phase had been initiated (Figure 5.1d). By Day 10, benzophenone concentrations were still 1 µg/g above background levels.

5.3.2 KINETIC MODELS

Uptake rate constants ranged between 0.005 and 0.043 µg benzophenone residues/g/d (Table 5.1). Consumption of *C. dipterum* resulted in the highest (0.043 µg/g/d in *I. elegans*) and lowest (0.005 µg/g/d in *N. glauca*) uptake rate constants.
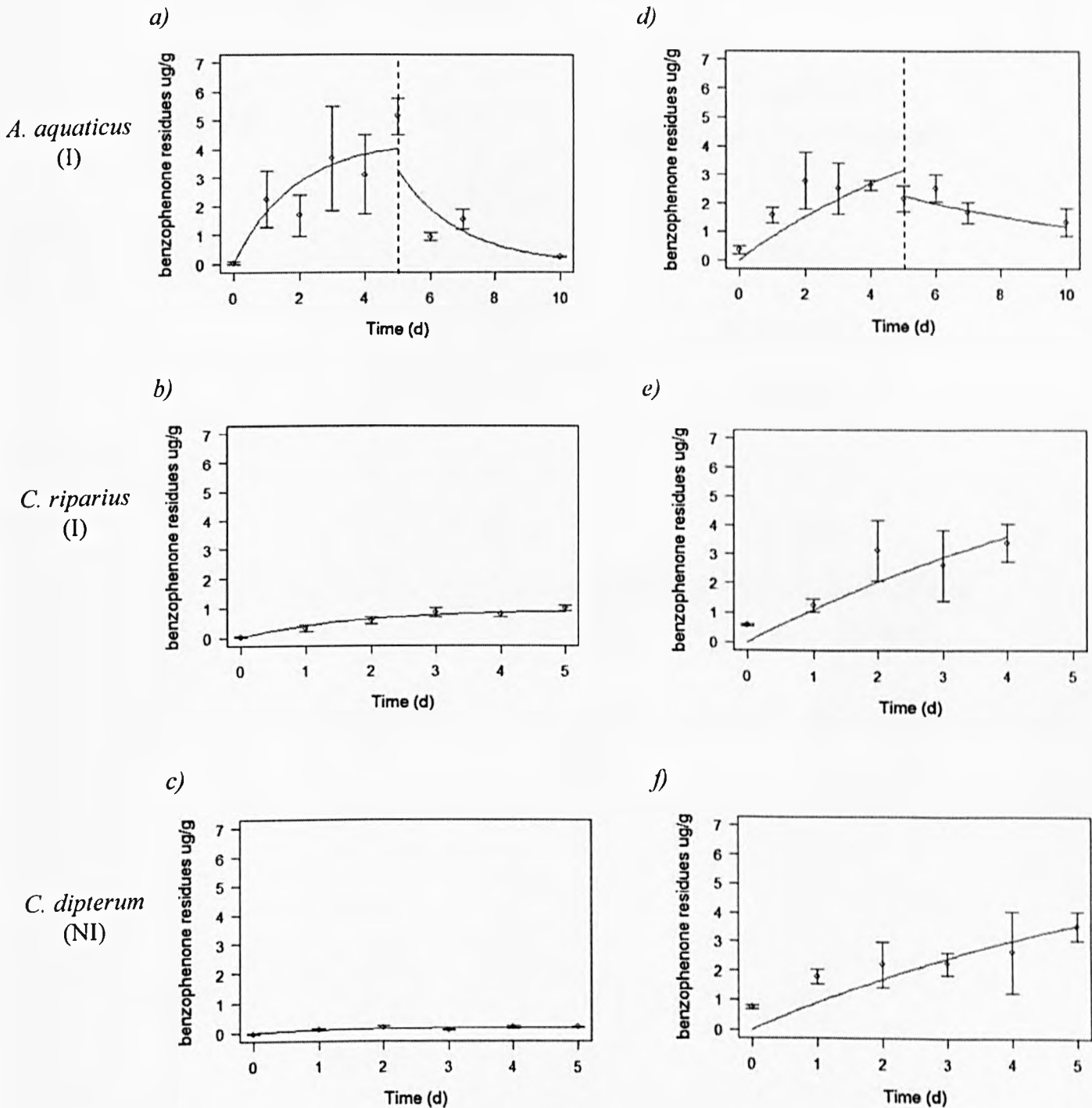
Figure 5.1. The mean uptake of benzophenone (± 1 SE) over five days by a piercer predator (*Notonecta glauca*) feeding on a) *Asellus aquaticus*, b) *Chironomus riparius* or c) *Cloëon dipterum*; or on engulfer predator (*Ischnura elegans*) feeding on d) *A. aquaticus*, e) *C. riparius* or f) *C. dipterum*. Depuration over five days is shown for a) *N. glauca* and d) *I. elegans* when previously fed *A. aquaticus* (dotted vertical line indicates start of depuration phase). Solid curves are two compartment bioaccumulation models fitted using equation 5.3 (uptake) and equation 5.4 (depuration). Prey were classified as either ingesters (I) or non-ingesters (NI).

Table 5.1. Uptake constants generated for each predator-prey combination during the five-day uptake phase for benzophenone.

Predator	Prey	Uptake constant, k_1 (μg benzophenone residues/g/d)
Notonecta glauca	*Asellus aquaticus*	0.027 (0.004)
	Chironomus riparius	0.009 (0.001)
	Cloëon dipterum	0.005 (0.001)
Ischnura elegans	*Asellus aquaticus*	0.010 (0.001)
	Chironomus riparius	0.021 (0.003)
	Cloëon dipterum	0.043 (0.005)

Numbers in parentheses denote standard errors associated with parameter estimates. All parameter estimates were significantly different to zero (T-test: t≥7.2, df=17, p<0.001).

The order of uptake rate constants therefore varied between the two predator species, being *A. aquaticus>C. riparius>C. dipterum* in *N. glauca* and *C. dipterum>C. riparius>A. aquaticus* in *I. elegans*.

Elimination rate constants were generated for the depuration phases of experiments involving *N. glauca* (Figure 5.1a) and *I. elegans* (Figure 5.1d) feeding on *A. aquaticus*. *Notonecta glauca* had a higher elimination rate constant (0.54 ± 0.085 µg/d) compared to *I. elegans* (0.13 ± 0.065 µg/d; t=2.7, df=16, p<0.05), though the estimate for *I. elegans* was not significantly different to zero (t=2.0, df=10, p>0.05).

5.3.3 TRANSFER EFFICIENCY FROM PREY TO PREDATOR

The transfer efficiencies (TEs) calculated for each predator-prey combination are presented in Table 5.2. The TEs for the piercer predator feeding on ingesters (14-16%) were much higher than for the non-ingester prey (2%). The engulfer predator had TEs ranging from 4-25%. All three prey species resulted in very comparable body burdens in the engulfer, ranging from 0.10-0.13 µg/g. The lowest TE in the engulfer (4%) was calculated for *Asellus aquaticus*, which had the highest total µg benzophenone in prey. The remaining two prey species both had 0.5 total µg benzophenone and very similar TEs (21-25%).

5.3.4 FEEDING RATES OF PREDATORS

There was no significant effect of benzophenone exposure on the proportion of prey animals eaten each day by *Notonecta glauca* (ANOVA: $F \leq 0.1$, df=1,190, p>0.05) or *Ischnura elegans* ($F \leq 0.6$, $df \leq 1,182$, p>0.05) over the five day feeding period for any of the prey species. The variability in feeding rates differed between the predator prey combinations. The coefficients of variation (C_v) for *Notonecta glauca* feeding on *C. riparius*, *C. dipterum* and *A. aquaticus* were 0.05, 0.10 and 0.41, respectively. The C_v values for *I. elegans* feeding on *C. riparius*, *C. dipterum* and *A. aquaticus* were 0.53, 0.37 and 0.61, respectively.

There was no significant reduction in the proportion of prey animals eaten by dosed-fed compared to control-fed *N. glauca* within the two hour feeding period for *A.*

Table 5.2. The transfer efficiency (%) of benzophenone residues from prey to predator on Day One of exposure.

Predator	Prey*	Mean no. prey eaten	Body burden (µg) per prey animal	µg in predators	Transfer efficiency %
Notonecta glauca (Piercer)	*Asellus aquaticus (I)*	5	0.80	0.65	16
	Chironomus riparius (I)	10	0.07	0.10	14
	Cloëon dipterum (NI)	9	0.17	0.03	2
Ischnura elegans (Engulfer)	*Asellus aquaticus (I)*	3	0.80	0.10	4
	Chironomus riparius (I)	8	0.06	0.10	21
	Cloëon dipterum (NI)	3	0.17	0.13	25

* I = ingester prey species; NI = non-ingester prey species

aquaticus (Figure 5.2a), *C. dipterum* (Figure 5.2b) or *C. riparius* (Figure 5.2c) (one-tailed T-test: $t \leq 0.4$, $df \leq 27$, $p > 0.05$). Neither was there a significant reduction in the proportion of *A. aquaticus* eaten by *I. elegans* within two or four hours (Figure 5.2d) ($t \geq 0.3$, $df \geq 18$, $p > 0.05$). However there was a significant reduction in the proportion of *C. dipterum* eaten by *I. elegans* within two hours ($t = 2.3$, $df = 20$, $p = 0.02$), and more so within four hours ($t = 3.1$, $df = 13$, $p = 0.004$) (Figure 5.2e).

There was no significant depuration in *N. glauca* during the two hour period feeding on uncontaminated *C. dipterum*, *A. aquaticus* or *C. riparius* (Figure 5.3a-c: one tailed T-test: $t \leq 1.2$, $df \leq 27$, $p > 0.05$). However there were significant reductions in benzophenone body burden of *I. elegans* by the end of the four hour feeding period, when feeding on uncontaminated *C. dipterum* (Figure 5.3d: ANOVA: $F = 17.0$, $df = 2,17$, $p < 0.05$) or *A. aquaticus* (Figure 5.3e: ANOVA: $F = 3.8$, $df = 2,16$, $p < 0.05$).

5.4 DISCUSSION

In agreement with existing studies, the current data demonstrates that feeding on contaminated prey can result in predator exposure to hydrophobic contaminants associated with sediments (Rubinstein *et al.*, 1984; Clements *et al.*, 1994; Egeler *et al.*, 2001). As previously demonstrated with metals (Béchard *et al.*, 2008), the exposure pathway of prey to organics can influence the transfer of contaminants to predators. This study has shown for the first time that the extent of exposure is dependent not only on the feeding behaviour of the prey, but also that of their predators. The piercer predator (*Notonecta glauca*) achieved higher body burdens of benzophenone when feeding on ingester compared to non-ingester prey. The engulfer predator (*Ischnura elegans*) reached approximately the same body burden for all three prey species of between 2.2 and 3.5 µg/g by Day 5. There was a large amount of variation within the body burdens achieved by predators in all of the predator-prey combinations except for *N. glauca* feeding on *C. riparius* or *C. dipterum*. As the body burden accumulated by predators is partly a result of the number of prey eaten, variation in feeding rates of predators may be able to account for variation in body burden. Indeed, the two predator-prey combinations with the least variable predator body burdens (*N. glauca* feeding on *C. riparius* or *C. dipterum*) were associated with the lowest coefficients of variation for feeding rate over the five-day uptake phase.
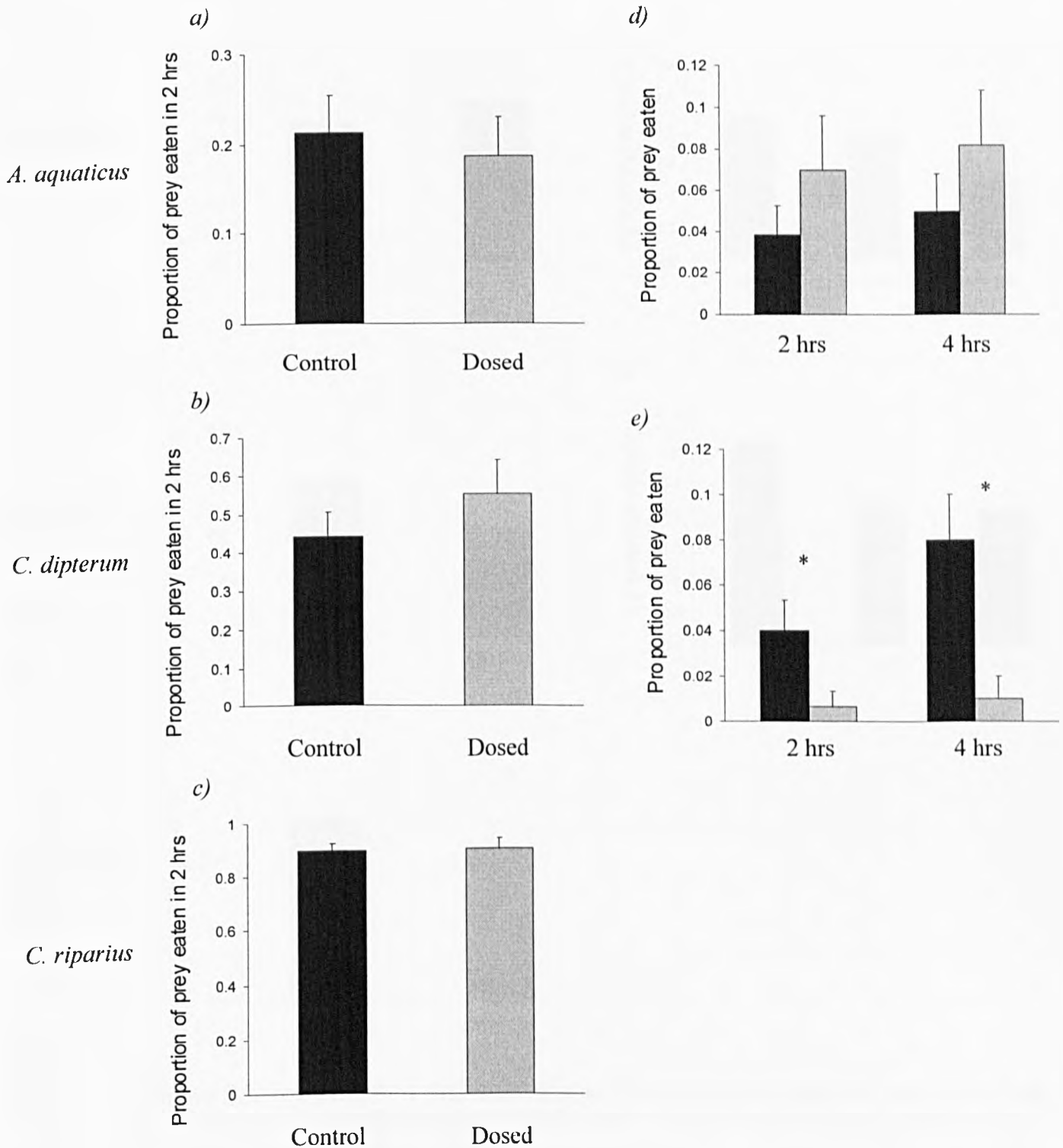
Figure 5.2. The effect of dietary uptake of benzophenone by predators on the mean (+ 1 SE) proportion of prey animals eaten over a 2 or 4 hr feeding period by dosed-fed (grey bars) and control-fed (black bars) predators. Data shown for a piercer predator (*Notonecta glauca*) feeding on a) *Asellus aquaticus*, b) *Cloëon dipterum*, or c) *Chironomus riparius*, and an engulfer predator (*Ischnura elegans*) feeding on d) *A. aquaticus* and e) *C. dipterum*. Asterisk indicates a significant difference in the proportion of prey eaten by dosed compared to control predators (p<0.05).

PIERCER   ENGULFER

a)

C. dipterum

d)

b)

A. aquaticus

e)

c)

C. riparius
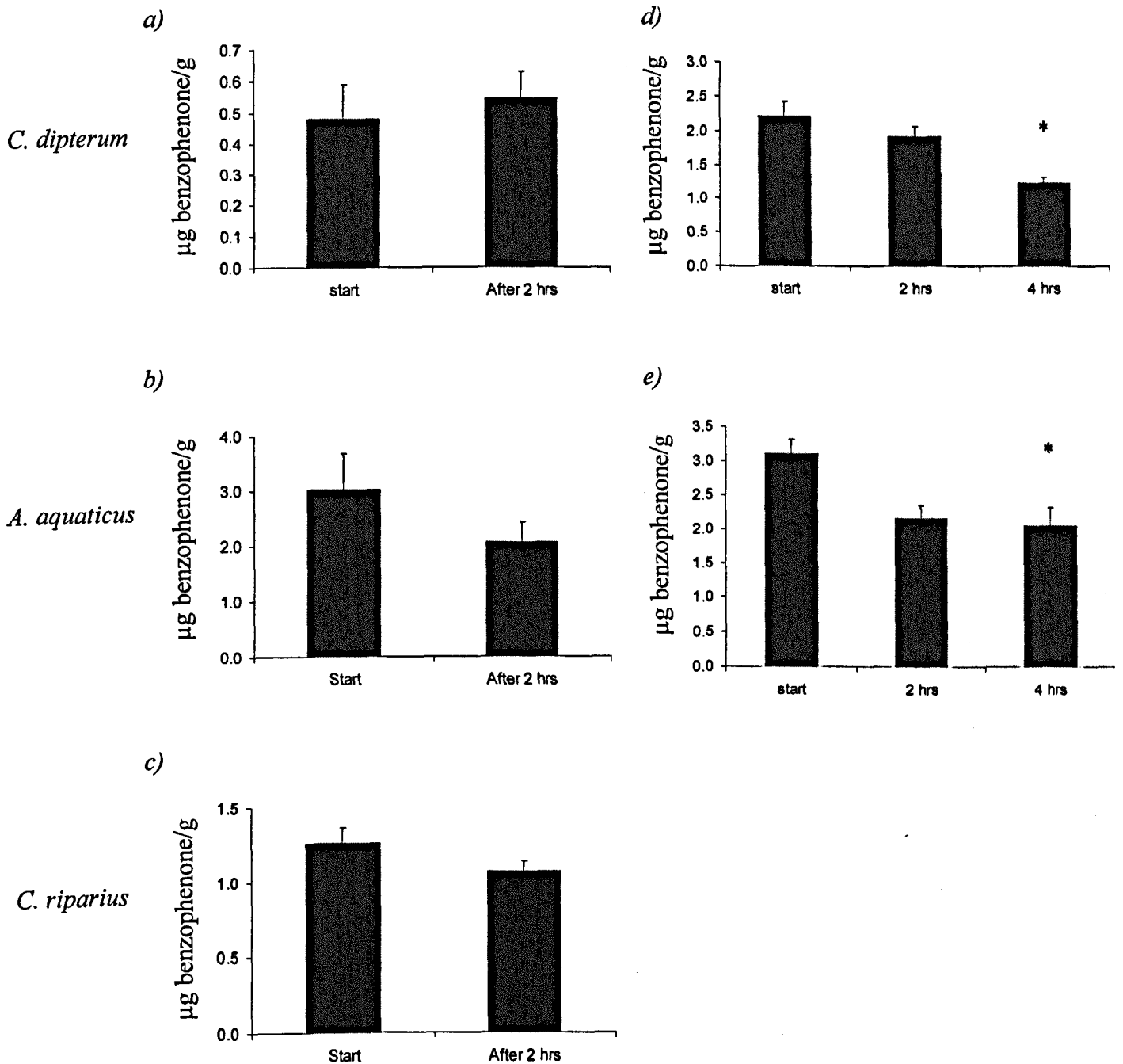
Figure 5.3. The mean (+ 1 SE) concentration of benzophenone (μg total benzophenone/g of predator tissue) at the start and end of a two to four hour feeding period for a piercer predator (*Notonecta glauca*) feeding on uncontaminated a) *Cloëon dipterum*, b) *Asellus aquaticus* and c) *Chironomus riparius*; and an engulfer predator (*Ischnura elegans*) feeding on d) *C. dipterum* and e) *A. aquaticus*. Asterisk indicates where there has been a significant reduction in body burden compared to that at the start ($p<0.05$). Experiment not conducted for *I. elegans* feeding on *C. riparius*.

The importance of feeding behaviour of prey and predators was supported by the transfer efficiencies calculated. In the piercer predator, the transfer efficiencies for the two ingester prey (14-16%) were much higher than for the non-ingester prey (2%). This suggests that contaminant residues in ingester prey were more available to piercers than those in non-ingesters as they would be mainly internally accumulated and therefore consumed by piercing feeding behaviour. Recent work using cadmium also demonstrated a higher trophic transfer efficiency from prey (*C. riparius*) to predator (*Danio rerio*) when prey had been exposed via sediment-ingestion compared to aqueous exposure (Béchard *et al.*, 2008). In the engulfer predator, the transfer efficiencies did not follow the same pattern, with the highest transfer being for the non-ingester (25%). However, the lack of agreement with the prediction that ingesters would result in a higher transfer than non-ingester prey may be explained by the predator achieving a very similar body burden for all three prey species. Therefore, lower transfer efficiencies result from feeding on prey with high body burdens and vice versa. Although examples of predators reaching approximately equal body burdens when fed different prey species have been documented previously (Munger and Hare, 2000), other studies have also demonstrated that engulfer predators can indeed achieve different body burdens depending on the prey species (Blackmore and Morton, 2002; Wang and Wong, 2003). Therefore although the engulfer used in this study did not demonstrate the expected patterns in uptake for ingester and non-ingester prey, this may be specific to the species used. Further experiments using different species are required to establish how general the pattern found in this study is amongst engulfers.

Hydrophobic contaminants such as benzophenone are often assumed to accumulate mainly within the lipids of organisms (Mackay, 1982; Di Toro *et al.*, 1991). Therefore the differences in body burden observed between the predator-prey combinations may be due to the relative lipid contents of the species involved. Lipid contents reported for similar predator species to those used in this study indicate little difference between them, being 11.2% for an *Ischnura* species and 9.4-14.2% for Notonectidae, on a dry weight basis (Hanson *et al.*, 1985). Therefore the higher maximum body burden in *N. glauca* cannot be attributed to a higher lipid content. The prey species used differ in their lipid contents, with the percentage of lipid on a fresh weight basis being 0.69, 0.82-1.08 and 2.1% for *A. aquaticus* (Cid Montañés *et*

al., 1995), *Chironomus* sp. (West *et al.*, 1997), and mayfly nymphs (Sushchik *et al.*, 2003), respectively. It might be expected therefore for the transfer efficiency of benzophenone residues to be highest for prey with the highest lipid content, in this case *C. dipterum*. Although this was the case for *I. elegans*, *A. aquaticus* resulted in the highest trophic transfer efficiency in *N. glauca*. Therefore, although lipid content plays a role in the accumulation of contaminants, it does not always solely explain the patterns in accumulation (Russell *et al.*, 1999; Fisk *et al.*, 2001).

Both predator species were able to eliminate benzophenone, to different extents, during the five-day depuration phase. The calculated elimination constants for benzophenone were higher for *N. glauca* than *I. elegans*, though not statistically significant in *I. elegans* due to variable data. This may explain why the bioaccumulation model underestimated the extent of accumulation in *I. elegans* when feeding on *A. aquaticus*. Previous studies have demonstrated a link between the ability of animals to eliminate accumulated contaminants and the polarity of metabolites produced during biotransformation (Kane Driscoll and McElroy, 1997). Though biotransformation in predators was not tested in this study, benzophenone is known to be metabolized by rats (Nakagawa *et al.*, 2000) and to differing extents by all three prey species used in this study (Chapter 4, Section 4.3.2). Therefore, the rapid elimination of benzophenone by *N. glauca* may be due to more polar metabolites being produced and subsequently excreted than in *I. elegans*. No existing studies on the biotransformation capabilities for organic compounds by either notonectids or odonates could be identified in the literature.

As both predator species were able to accumulate benzophenone from their prey there was potential for secondary poisoning effects to occur. The reported critical body residue for chronic effects of narcotic organic contaminants is 200-400 µmol/kg (McCarty, 1986), which converts to 36.4-72.9 µg/g for benzophenone (molecular weight of 182.22 g/mol). Although none of the five predator-prey combinations achieved this body burden, secondary poisoning effects were observed at a body residue an order of magnitude lower (2-3 µg/g) for *I. elegans* feeding on *C. dipterum*. The toxic response to benzophenone exposure is not solely dependent on total body burden, as no toxic effects were observed in *N. glauca* with a higher body

burden (5 µg/g) or in *I. elegans* with the same body burden (2-3 µg/g) but feeding on ingester prey.

The observed differences in toxicity of benzophenone between the predator-prey combinations may be due to an interaction between contaminant mode of action and species traits. Exposure to narcotic organic contaminants, such as benzophenone, results in hypoactivity (Barron, 2002). Such behavioural changes may alter the ability of predators to successfully capture prey, thereby reducing their feeding rates (Perez and Wallace, 2004; Relyea and Hoverman, 2008). However, the sensitivity of predators may depend on their hunting strategy. *Ischnura elegans* is an ambush hunter species that sits and waits for prey to come to them, whereas *N. glauca* is an active, "cruising" hunter species that actively seeks out prey (Greene, 1986). A more pronounced response would be expected for ambush predators feeding on fast moving prey, as rapid predator response to prey encounters would be more important for their successful capture. This contention is supported by the fact that feeding rate was significantly inhibited when *I. elegans* was feeding on *C. dipterum* (a fast-moving mayfly species) but not when feeding on *A. aquaticus* (a slow-moving isopod). As *N. glauca* is an active hunter, if they are unsuccessful in capturing prey on the first attempt they may chase and capture another. More detailed observations on the ratios of attacks and successful captures by the two predator species are required to fully understand the mechanisms of toxicity with benzophenone.

The feeding rate of predators was used here as a short term measure of toxicity, but there may be further impacts in natural systems. If predators have inhibited feeding due to reduced capture success they may need to spend more time hunting for prey, and therefore expending energy. This can have implications for growth/development and reproductive output (Lawton *et al.*, 1980; Fritz and Morse, 1985). Some predators are generalists, able to feed on more than one prey species (Strauss, 1991). In these cases, predators may compensate for reduced capture success of one prey species by feeding more on an alternative prey species (Riddell *et al.*, 2005b), which may be easier to catch. Therefore prey species can experience indirect toxic effects from these changes in predator feeding behaviour, with prey populations being influenced by the ease with which individuals can be successfully captured by predators. Consequently it is possible that community composition may be altered,

for example to more fast moving prey species that can more successfully avoid predators. No studies investigating the occurrence of such shifts in community composition due to secondary poisoning of predators could be identified in the literature.

The mode of action of a contaminant may also determine the predator hunting strategy that is sensitive to toxic effects. In this study, exposure of predators to a narcotic contaminant resulted in inhibited feeding by an ambush predator, *Ischnura elegans*. Contaminants causing uncoordinated movement or convulsions may impair the swimming behaviour of active predators, thereby reducing their feeding response (Weis and Weis, 1998). Other contaminants may cause toxic effects in both active and ambush predators, with exposure resulting in reduced attempts to capture prey (Smith and Weis, 1997). Therefore the predator-prey combination that will demonstrate the greatest toxic response may vary depending on the mode of action of the contaminant. Hence, the extent of dietary exposure is influenced by the feeding behaviour of prey and predators, and the subsequent occurrence and nature of toxic effects will depend on the hunting strategy of the predator and chemical mode of action.

Chapter 6

The adverse effects of contaminants on predator-prey interactions: implications for ecological risk assessment

6.1 Aim of thesis

Organisms living in the natural environment are being repeatedly exposed to a variety of different contaminants (Walker *et al.*, 1996). These originate from anthropogenic activities within agriculture, industry and domestic households (Rand and Petrocelli, 1985). Depending on the exposure concentration, organisms can experience lethal (reduced abundance) (Helgen *et al.*, 1988; Whaley *et al.*, 1989) or sublethal (e.g. changes in behaviour) (Clements *et al.*, 1989; Dodson *et al.*, 1995; Carlson *et al.*, 1998; Hanazato, 2001) toxic effects. Organisms may also take up contaminants into their tissues (Kaag *et al.*, 1997; Wang and Fisher, 1999; Gaskell *et al.*, 2007). Such changes may affect either availability of prey to predators or result in the transfer of contaminants to, and potentially toxic effects in, predators. The disruption of predator-prey interactions has implications for both community structure and function (Paine, 1966; Williamson *et al.*, 1989). However, the current risk assessment approach for contaminants mainly focuses on direct exposures of single species to contaminants (Preston, 2002). The aim of this thesis was to examine whether changes in predator-prey interactions could exacerbate the negative effects of contaminants in ways that would not be identified by studying single species in isolation. This was proposed to either occur via either changes in prey availability (Section 1.1.2) or transfer of contaminants to predators leading to toxic effects (Section 1.1.3). This aim was addressed in the experimental work conducted in Chapters 2 to 5, the main findings of which are described in the following section (Section 6.2). The implications of these findings for ecological risk assessment are then discussed (Section 6.3), and the main conclusions are then summarised (Section 6.4).

106

6.2 Main thesis findings

Five species of freshwater macroinvertebrates were used to test the hypothesis that ecological risk assessments based on single species toxicity data do not represent the full extent of risk, due to the additional impacts of disrupted predator-prey interactions. These consisted of two predator species (*Ischnura elegans* and *Notonecta glauca*) and three prey species (*Asellus aquaticus*, *Chironomus riparius* and *Cloëon dipterum*). Both predator species are generalist predators but differ in their hunting strategies; *I. elegans* is an ambush predator whereas *N. glauca* is a more active predator. They also differ in the way in which they handle their prey; *I. elegans* consumes its prey whole (engulfer), whereas *N. glauca* feeds on only the internal tissues of its prey (piercer). The three prey species are benthic macroinvertebrates, living in close proximity to the sediment but differ in their feeding behaviour; *C. riparius* is a deposit feeder, *A. aquaticus* feeds on detritus and sediment and *C. dipterum* is an algal grazer. The three prey species also differ in their behaviours: *C. dipterum* is a fast moving mayfly nymph whereas *C. riparius* and *A. aquaticus* are slower moving species.

Changes in predator-prey interactions that may arise from toxic effects in prey were addressed. Predators experiencing reduced prey availability due to lethal exposures of prey were expected to compensate by increasing predation on the remaining prey or feeding more on alternative prey species. When offered a single prey species (*A. aquaticus*, *C. dipterum* or *C. riparius*), predators (*N. glauca* or *I. elegans*) consumed proportionally more prey as prey density decreased. However, the predator responses to the presence of alternative prey were not predictable from the feeding parameters generated by predation on single prey species. Instead, two of the predator-prey combinations (*N. glauca* feeding on *A. aquaticus* and either *C. riparius* or *C. dipterum*) followed a consistent prey preference model, with *N. glauca* consistently preferring one prey species (*A. aquaticus*) over the other at all relative abundances. The responses of predators in the remaining four predator-prey combinations were possibly explained by interactions between the prey species affecting their relative availabilities to predators. In three of those four predator-prey combinations, the

species accounting for 20% of the prey available (i.e. sensitive species) comprised more than 60% of the predator's diet.

It was also hypothesized that predators may experience reduced prey availability due to sublethal changes in prey that affect their susceptibility to predation, thereby compensating by predating more intensely on more susceptible prey species. Both predator species consumed more mobile than immobile prey, indicating that prey behaviour was important for initiating a feeding response by both active (*N. glauca*) and ambush (*I. elegans*) predators. The three prey species varied in their sensitivity to sublethal exposure to contaminants, with only *C. riparius* exhibiting reduced mobility in response to tactile stimuli following cadmium exposure. There was also some reduced activity in *C. dipterum*, though not statistically significant. The hunting strategy of predators determined the impact of behavioural changes in prey on predation response, with only the active predator having a reduced feeding rate on cadmium-exposed *C. riparius*. No shifts in prey preference were observed for the active predator when offered alternative prey, possibly due to the effects of cadmium on *C. riparius* behaviour being insufficient to affect their relative availabilities. However, a shift in prey preference towards *A. aquaticus* was observed for the ambush predator, possibly due to cadmium-reduced activity lowering predator encounter rates with the alternative prey (*C. dipterum*). Thus, a prey species can become more susceptible following contaminant exposure simply because the toxin immobilises the alternative prey.

Changes in predator-prey interactions may also occur as a result of toxic effects in predators. It was expected that benthic prey species could act as vectors of exposure to predators for hydrophobic contaminants, accumulating contaminants from sediment according to their feeding behaviour. The three benthic prey species (*A. aquaticus*, *C. dipterum* and *C. riparius*) were all able to accumulate a hydrophobic contaminant (benzophenone) from sediment. The extent of accumulation differed between the two sediment-ingester (*A. aquaticus* and *C. riparius*) and non-ingester species (*C. dipterum*), in the order *A. aquaticus* > *C. riparius* > *C. dipterum*. All three species were able to biotransform benzophenone, with the extent of metabolite production being in the order *C. dipterum* > *A. aquaticus* > *C. riparius*. Differences in accumulation by the two ingesters may be attributable to differences in their

ability to eliminate benzophenone residues, though this could not be linked to the extent of biotransformation or the polarity of the metabolites that were produced. The distribution of accumulated benzophenone within the tissues also varied between the three species, with more internal accumulation being detected in the two ingester species compared to the non-ingester species, using both microautoradiography and manual separation techniques.

The magnitude of the subsequent transfer of contaminant from prey to predator, and hence the resultant toxic effects, was hypothesized to depend on the exposure pathway to the prey (ingester vs. non-ingester) and the feeding behaviour of the predator (piercer vs. engulfer). Both piercer (*N. glauca*) and engulfer (*I. elegans*) predators accumulated benzophenone from their benthic prey. The magnitude of accumulation in piercers depended upon the exposure pathway to the prey consumed, being higher when feeding on ingester compared to non-ingester prey. This was reflected in the trophic transfer efficiencies calculated for piercers, being 14-16% for ingester prey and only 2% for non-ingester prey. The level of accumulation within the engulfer predator was similar across all three prey species, resulting in a lower transfer efficiency (2%) for prey with high body burdens (*A. aquaticus*). Although there was significant accumulation in predators from all predator-prey combinations, toxic effects were only detected for the engulfer feeding on the non-ingester, in terms of a reduced feeding rate. Therefore, the body burden achieved by predators did not predict the occurrence of toxic effects, possibly due to the narcotic mode of action of benzophenone.

6.3 Implications of main thesis findings for ecological risk assessment

The results from Chapters 2 to 5 have demonstrated that single species toxicity tests can underestimate the extent of a contaminant impact due to exclusion of predator-prey interactions. This may be particularly important for persistent, bioaccumulative and/or toxic (PBT) substances, a group of hazardous contaminants highlighted in the new EU chemical strategy, REACH (EC, 2006). These contaminants can not only bioaccumulate and/or cause toxic effects in exposed organisms, but, being persistent, they remain within systems for long periods of time. Therefore, any negative effects will potentially be occurring during long term exposure.

Both bioaccumulative and toxic effects on predator-prey interactions were investigated within this thesis. Using the findings for Chapters 2 to 5, a number of scenarios for which negative impacts may be exacerbated by predator-prey interactions can be identified (Figure 6.1). This can occur either due to secondary poisoning of predators due to dietary exposure, or increased predation pressures on prey populations from lethal/sublethal exposures (Figure 6.1). The occurrence of these scenarios is discussed in the following sections, in terms of chemical properties, exposure durations, landscape profiles and species traits that may be particularly important.

6.3.1 Bioaccumulative contaminants

The accumulation of contaminants within organisms is usually measured directly as the bioconcentration factor (BCF), normally in fish (OECD, 1996). However, these experiments are costly to run and thus the BCF is often estimated from chemical properties (Rand and Petrocelli, 1985). The criterion commonly used for organic contaminants is the 1-octanol/water partition coefficient (Log K_{ow}), with a higher Log K_{ow} indicating a higher affinity for lipids and thus a higher bioaccumulation potential (Feijtel et al., 1997). The 'cut off' value for a contaminant being classified as 'bioaccumulative' is usually a Log $K_{ow} \geq 3$ (EC, 2002, 2003), with the new REACH legislation using a Log $K_{ow} > 4.5$ to indicate hazardous PBT status (ECHA, 2008). The bioaccumulative potential of metals is more difficult to predict, varying widely between species and metals (Luoma and Rainbow, 2005). However, there is some evidence that the covalent index of a metal and the ventilation rate of a species can account for much of the variation in bioaccumulation of a range of metals (Veltman et al., 2008).

Bioaccumulative contaminants entering a water body can accumulate in sediments, plants/algae and animals (Figure 6.2). The accumulation of contaminants within animals directly from aqueous exposure can occur by both adsorption to external surfaces and uptake via respiratory surfaces, e.g. gills (Rand and Petrocelli, 1985). Bioaccumulation via aqueous exposure pathways results in accumulation in mainly external tissues
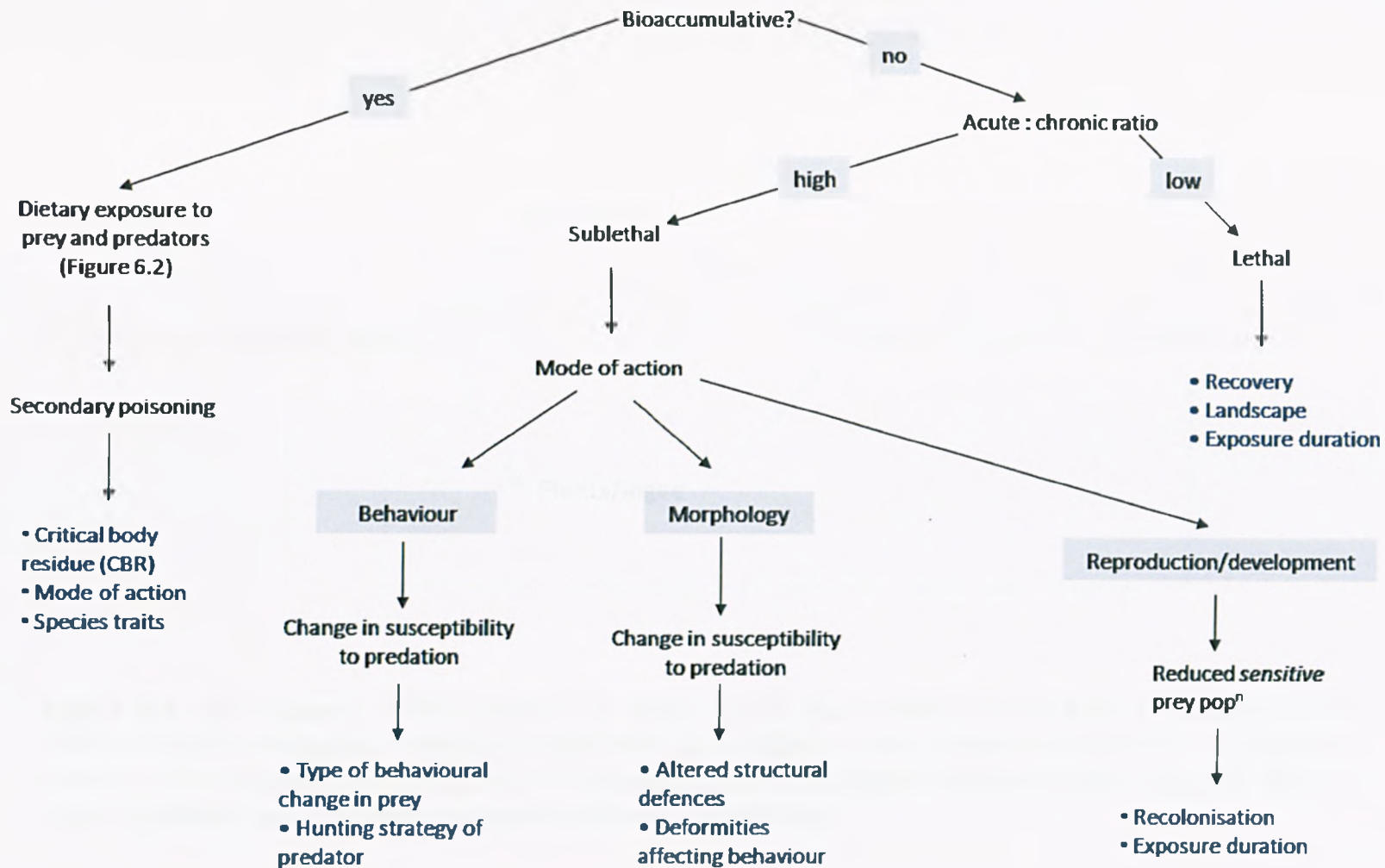
Figure 6.1. A conceptual model illustrating the potential effects of bioaccumulative or toxic contaminants on predator-prey interactions, with the outcomes dependent on chemical properties, exposure durations, surrounding landscape profiles and species traits. See Section 6.3 for more detailed descriptions of scenarios requiring consideration in ecological risk assessment.
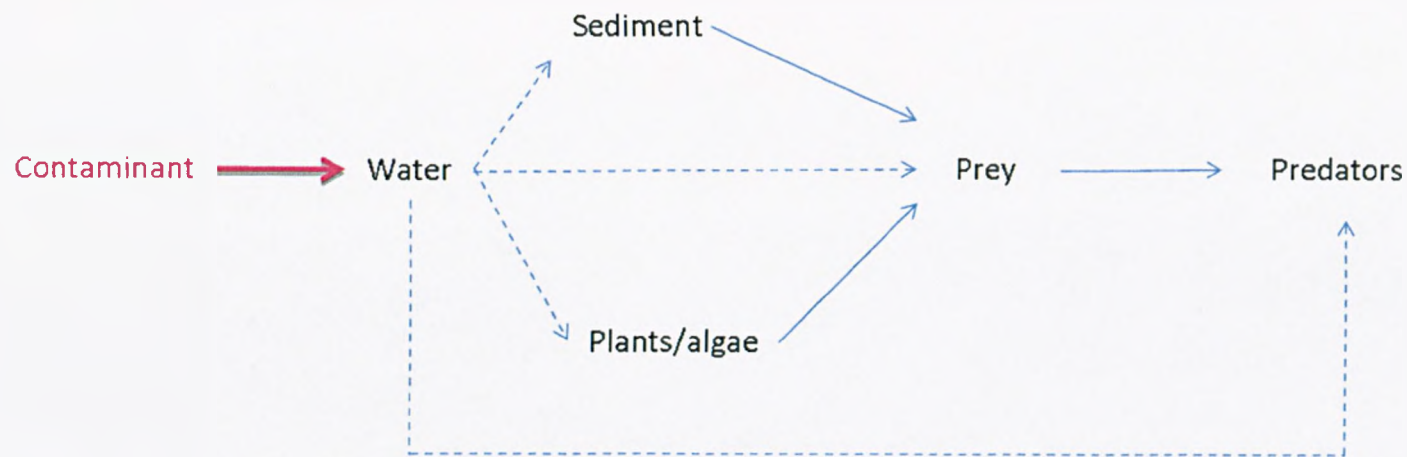
Figure 6.2. The aqueous (dashed lines) and dietary (solid lines) routes of exposure to predators of a bioaccumulative contaminant entering a water body. In addition to water exposure, prey can be exposed via contact with or ingestion of sediment, or consumption of plants/algae. Predators can be exposed via the aqueous pathway and also from consumption of contaminated prey.

(Fisk *et al.*, 1997; Arukwe *et al.*, 2000; Liu *et al.*, 2002; Xu and Wang, 2002; Wang and Wong, 2003; Heiden *et al.*, 2005). The relationship between distribution of contaminants in tissues and transfer to predators will be discussed in due course. In addition to aqueous exposure, there is the potential for bioaccumulative contaminants to be transferred through the food chain. The pathway via which this occurs will depend on the diet of the prey and their predators (Figure 6.2). The focus of Chapter 5 was the accumulation of contaminants associated with sediments by epifauna and subsequent transfer to their pelagic predators. Such sediment-based food chain transfer has been demonstrated with other organic contaminants, using fish as predators (Rubinstein *et al.*, 1984; Clements *et al.*, 1994; Egeler *et al.*, 2001). Although accumulation within sediments is likely for contaminants that have a Log $K_{ow} \geq 3$ (EC, 2003), not all prey are sediment-ingesters, or indeed benthic, and may thus be exposed via other routes. Accumulation of contaminants has also been demonstrated from consumption of contaminated algae (Gossiaux *et al.*, 1998; Hook and Fisher, 2001a; Lawrence and Mason, 2001; Bejarano *et al.*, 2003) and aquatic plants (Simon and Boudou, 2001; Chan *et al.*, 2003). Therefore the predators of herbivorous prey may also experience dietary exposure, as demonstrated with the transfer of heavy metals, first from algae (*Chlorella vulgaris*) to *Daphnia magna*, and then to their predators (*Danio reiro*) (Liu *et al.*, 2002).

The extent of transfer of contaminants from prey to predators will not only depend on the feeding behaviour of the prey (sediment ingesters, herbivores), but also that of the predators. As discussed in Chapter 5, predators can feed on their prey whole (engulfers) or consume only the internal tissues of the prey animal (piercers). The combination of predator and prey feeding behaviour was demonstrated to affect the extent of food chain transfer. Although only tested with benthic invertebrate prey, it is likely that this would also be the case for pelagic prey ingesting algae or macrophytes. In both cases, ingestion of contaminants would result in internal accumulation, with any aqueous exposure resulting in mainly external accumulation. Therefore engulfer predators would be expected to experience higher exposure from herbivorous prey compared to piercer predators, due to consumption of both internally and externally accumulated contaminants. This is a particularly interesting area for future research as *Daphnia magna*, an algivore, is the standard toxicity test species in ecological risk assessments.

The relative importance of predator feeding behaviour may depend on the adsorptive properties of the contaminant. The extent to which contaminants associate with organic matter is estimated using the 1-octanol/water partition coefficient (Log K_{ow}), with a higher Log K_{ow} indicating an increased association with organic matter (Walker et al., 1996). Previous research suggests that the importance of exposure route varies with Log K_{ow} (Qiao et al., 2000; Lu et al., 2004), with dietary exposure being significant for Log K_{ow} >5 (Qiao et al., 2000; Lu et al., 2004). Therefore external accumulation from sediment desorption or aqueous exposure would be expected to decrease with increasing Log K_{ow}. As a result, the main dietary exposure route for both piercer and engulfer predators would be from the internally accumulated contaminants within dietary-exposed prey (ingesters). This being the case, food chain transfer should be assessed using ingester prey and either piercer or engulfer predators for Log K_{ow} >5.

The distribution of predator species classified as piercers or engulfers is not uniform. All hemipteran predators are classified as piercers, along with some coleopteran and dipteran predators (Peckarsky, 1982). However, all odonate, plectopteran, and tricopteran predators are engulfers, along with some dipteran predators (Peckarsky, 1982), and perhaps most importantly, all vertebrate predators, including fish. Therefore, in general terms, most aquatic predators are engulfers. As such, the potential for food chain transfer to occur should be tested with the worst case scenario likely to occur in natural assemblages, being engulfer predators feeding on ingester prey.

The main implication of food chain transfer is that risk assessments based on aqueous exposure (BCF) alone may underestimate the extent of accumulation that would occur in natural systems. Studies using sediment- (Rubinstein et al., 1984; Clements et al., 1994; Egeler et al., 2001) and algal- (Wang and Wang, 2006) based exposure systems have demonstrated that predators can accumulate higher body burdens when a dietary exposure route is included in addition to aqueous exposure. Such food chain transfer can also result in terrestrial predators being exposed to contaminants within the water body; for example, diving ducks (Custer and Custer, 2000), tree swallows (Maul et al., 2006) and riparian predators (Walters et al., 2008)

can accumulate sediment-associated contaminants from their aquatic prey. The extent of exposure is important as the critical body residue (CBR), at which toxic effects are induced, may be achieved at lower concentrations than predicted by aqueous exposure alone (Feijtel *et al.*, 1997).

In order to determine the critical body residue of predators it is also important to consider the mode of action of the contaminant. This was demonstrated in Chapter 5, with *N. glauca* able to achieve higher body burdens than *I. elegans*, but only *I. elegans* exhibited toxic effects from dietary exposure. The discrepancy between these two predatory species was hypothesised to be due to the narcotic mode of action of benzophenone affecting the ability of the ambush predator (*I. elegans*) to successfully capture its prey. The more pronounced response observed in the ambush predator feeding on fast moving prey (*C. dipterum*) would be expected, as rapid predator response to prey encounters would be required for their successful capture. Conversely, the active predator (*N. glauca*) would be able to chase and capture another prey animal if unsuccessful on the first attempt. Contaminants that cause uncoordinated movement or convulsions, thus impairing swimming behaviour, may reduce the feeding response of active predators (Weis and Weis, 1998). Other contaminants may cause toxic effects in both active and ambush predators, with exposure resulting in reduced attempts to capture prey (Smith and Weis, 1997). Therefore the most sensitive predator-prey combination for determining a CBR may vary depending on the mode of action of the contaminant.

6.3.2 Toxic contaminants

Contaminants that are not bioaccumulative may affect predator-prey interactions by inducing toxic effects in prey. This was addressed in Chapters 2 and 3, in which lethal and sublethal effects, respectively, were demonstrated to affect prey availability, resulting in shifts in predator feeding rates and prey choice. The probability of such effects occurring in the environment will depend on the dose-response profile of the contaminant, represented as the acute to chronic ratio (ACR) of toxicity. Contaminants with a low ACR cause sublethal effects over a very narrow range of concentrations. For such contaminants, sublethal effects will be relatively unimportant, with a low probability that the environmental concentration will be

within the narrow effects range, and when it does occur, prey will be verging on lethal effects. A review of 71 substances from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) database found that organics, excluding pesticide active ingredients, generally had relatively low ACRs, ranging from 0.13-27.5 (Länge et al., 1998). In the same review, other contaminants had much higher ACRs, with metals and organometals having ACRs ranging from 0.3-1290 (Länge et al., 1998). For contaminants with high ACRs there is more scope for prey to experience sublethal effects in the environment before any changes in survival occur, as sublethal effects occur over a wider range of concentrations. A high ACR may also result in prey experiencing an increasing magnitude of effect, potentially resulting in more extreme responses. The scenarios for which the negative impacts of contaminants may be exacerbated by changes in predator-prey interactions are discussed separately for low and high ACR contaminants in the following sections.

6.3.2.1 Contaminants with low acute to chronic ratio

The results obtained in Chapter 2 illustrated the potential for prey densities reduced by lethal exposures to contaminants to be further reduced by increased predation pressures. This occurred in both single and multiple prey systems, implying that both specialist and generalist predators can exacerbate the negative effects of contaminants. Therefore the current approach in risk assessment of generating toxicity end points (LC_{50}, NOEC) for single species in isolation actually represents the minimum effect that a contaminant will have on a population. Contaminants, when combined with increased predation pressure, could potentially halve the abundance of a prey species at a far lower toxicant concentration than the conventionally-predicted LC_{50}.

Cases where the negative impacts of contaminants have been underestimated from single species toxicity tests have been described in previous studies (Crossland and Hillaby, 1985; Giddings et al., 1996). Laboratory-generated LC_{50}s for copepods and rotifers exposed to an organophosphate insecticide (diazinon) were higher than those from pond microcosm exposures (Giddings et al., 1996). Daphnia longispina was found to have an EC_{50} of 440 µg/L when exposed to 3,4-dichloroaniline (industrial

intermediate) in laboratory conditions, but had significantly reduced populations following exposure to 45 µg/L in pond mesocosms (Crossland and Hillaby, 1985). A potential, but untested, mechanism for increased chemical potency in these mesocosms may have been elevated predation on chemically-impacted prey by the predators present within the mesocosms.

If the current risk assessment approach of protecting the 'most sensitive species' is adequately protective then contaminant-induced reductions should not occur in natural assemblages (Cairns, 1986). Although standard toxicity species (e.g. *Daphnia magna*) generally have higher sensitivities than most other macroinvertebrates, there will still be some species that are more sensitive (Wogram and Liess, 2001). Detecting the most sensitive species may also depend on the level of organisation tested, with populations being potentially more sensitive than individuals (Beketov and Liess, 2005; Stark, 2005). Monitoring studies have provided evidence of such effects occurring in the environment, detecting reductions in abundance at pesticide concentrations two to three orders of magnitude lower than the LC_{50} of the most sensitive species (Liess and Von der Ohe, 2005; Schäfer *et al.*, 2007). Thus, predators in natural systems can experience shifts in availability of prey, depending on prey sensitivity to exposure.

Using the data generated in Chapter 2 and from existing studies it is not possible to identify the causal mechanisms that determine how predators will respond to changes in prey density. Therefore, although the negative effects of contaminants are not always enhanced by predators, the direction of predation on sensitive prey species cannot be predicted *a priori* with certainty. As such, a precautionary approach could be taken, in which the most vulnerable scenarios for which changes in prey abundance and subsequent predation would be most detrimental are identified. These are now discussed in terms of the inherent ability of sensitive species' populations to recover following contaminant exposure, and the duration of exposure, thereby affecting the success of such recovery strategies.

Sensitive prey species possessing traits that allow their populations to recover following contaminant exposure may be able to compensate for any increased predation that they experience. Indeed, populations of sensitive prey species have

been shown to recover in natural assemblages (van den Brink *et al.*, 1996; Beketov *et al.*, 2008). Species traits affecting recovery include the capacity of a species to reproduce, avoid exposure, or recolonise from unimpacted sites. These concepts form the basis of the recent SPEcies At Risk (SPEAR) classification system (Liess and Von der Ohe, 2005). Sensitive prey species that have high intrinsic rates of increase (Lozano *et al.*, 1992; Sherratt *et al.*, 1999; Stark *et al.*, 2004; Beketov *et al.*, 2008), or produce multiple generations per year (Sherratt *et al.*, 1999; Kowalik and Ormerod, 2006; MacCausland and McTammany, 2007), may be able to recover more rapidly than those with lower reproductive outputs. Sensitive prey species that are able to avoid exposure, by emerging as terrestrial adults, may also be better able to recover following exposure compared to those that have a fully aquatic lifecycle (Liess and Von der Ohe, 2005). Terrestrial adults also allow for wider dispersal, and thus greater potential for recolonisation from other populations (Liess and Von der Ohe, 2005). Sensitive crustacean prey species may therefore be more vulnerable to local extinctions from increased predation pressures compared to sensitive insect prey species, due to the fully aquatic lifecycles of crustaceans preventing terrestrial avoidance or recolonisation (e.g. van den Brink *et al.*, 1996).

The ability of a population to recolonise may not only depend on the traits of that species, but also the profile of the landscape in which it resides. Previous research has demonstrated that recovery in streams is greater where unimpacted reaches exist upstream, allowing downstream drift and recolonisation (Liess and Von der Ohe, 2005; Schriever *et al.*, 2007). There is also evidence that connectivity and proximity are important in determining the occurrence of species within ponds (Sanderson *et al.*, 2005). Therefore, reductions in sensitive prey populations due to additional predation pressures may be more significant within isolated ponds or streams without unimpacted upstream reaches from which new individuals can migrate.

If a sensitive prey species has the potential to recover, either by reproducing or recolonising, the success of that recovery will depend on the exposure duration of the contaminant. Long term, chronic exposures can generally result in longer lasting impacts compared with short term, acute exposures (Schultz and Liess, 1995; van den Brink *et al.*, 1995; Liess and Schultz, 1996). This is because, unlike chronic exposure, pulsed or short-term exposure allows the sensitive prey population to

recover (Kallander *et al.*, 1997; Naddy and Klaine, 2001; Reynaldi and Liess, 2005). Chronic exposure can be the result of continual release of contaminants into the environment, such as effluents from water treatment plants and agricultural run-off (Kolpin *et al.*, 2002). It can also occur if contaminants are very persistent, thus remaining within the system for prolonged periods even if released relatively infrequently (Tanabe *et al.*, 1994). Therefore, recovery of the sensitive prey species may not be possible if the exposure concentration of contaminant remains above their toxicity threshold.

6.3.2.2 Contaminants with high acute to chronic ratio

The likelihood of contaminants occurring in the environment at concentrations that will result in sublethal effects is greater for contaminants with high compared to low ACRs. Sublethal exposures can result in changes in prey behaviour (Gerhardt, 1990; Riddell *et al.*, 2005a), morphology (Hanazato and Dodson, 1993; Kiesecker, 2002), reproduction or development (Wollenberger *et al.*, 2000; Krang, 2007), depending on the mode of action of the contaminant (Table 6.1). Such changes in prey can affect their availability to predators, by changing their susceptibility to predation or by reducing their population size. As demonstrated in Chapter 3, the perception of predators to sublethal changes in prey may not be generic, instead being dependent on particular predator traits. The importance of the combination of sublethal effect type and predator traits is discussed in the following section.

The susceptibility of prey to predation can be affected by changes in prey behaviour, as demonstrated in Chapter 3. Cadmium acted by reducing either the escape response or activity of prey, as demonstrated in other studies (Gerhardt, 1990, 1995; Riddell *et al.*, 2005a). However, other contaminants can affect prey behaviour in different ways. Drummond and Russom (1990) classified the effects of over 300 organic chemicals on behaviour into three syndromes: hypoactivity, hyperactivity and physical deformity syndrome. Hypoactivity results in generally depressed activity and responsiveness, whereas hyperactivity causes the opposite, resulting in heightened responsiveness and activity (Drummond and Russom, 1990). Physical deformity syndrome indicates neurological dysfunction, with symptoms including

Table 6.1. A summary of the main modes of action that can result in behavioural, morphological, or reproductive/developmental effects in prey following contaminant exposure.

Type of effect on prey	Mode of action*	Toxic effects	Examples of chemicals and studies where toxic effects demonstrated
Behavioural	*Narcotics (polar and non-polar)*	Progressive lethargy, unconsciousness and eventually death, without any sustained symptoms such as hyperventilation, erratic or convulsive swimming or haemorrhage; polar narcotics slightly more toxic than non-polar narcotics (baseline toxicity)	Polycyclic aromatic hydrocarbons (e.g. fluoranthene (Lotufo, 1998)), polychlorinated n-alkanes (Fisk *et al.*, 1999)
	Neurotoxins: a) direct action on receptors/pores in nerve membrane	a) uncoordinated movement and convulsions	Organochlorines (e.g. endosulfan (Carlson *et al.*, 1998)), pyrethroid insecticides (e.g. esfenvalerate (Floyd *et al.*, 2008)), copper (O'Gara *et al.*, 2004)
	b) Inhibitors of acetylcholinesterase (AChE)	b) results in synaptic block, causing reduced activity/paralysis	Organophosphates (e.g. azamethiphos (Canty *et al.*, 2007), chlorpyrifos (Carlson *et al.*, 1998)), carbamates (e.g. TATTU (Falfushinska *et al.*, 2008))

	Mitochondrial poisons	Uncouplers of oxidative phosphorylation, results in impaired general metabolism	Chlorophenols (e.g. pentachlorophenol, 2,4-dichlorophenol (Borgmann and Ralph, 1986)), Roundup (Peixoto, 2005)
	Reactives with sulphydryl groups	Sulphydryl groups on enzymes and other proteins have important functional roles e.g. formation of disulphide bridges	Methylmercury (Zhou and Weis, 1998), mercury (Boening, 2000)
Morphological	*Genotoxins*	Damage to DNA, resulting in mutations	Polycyclic aromatic hydrocarbons (e.g. fluoranthene (Palmqvist *et al.*, 2003), benzo[a]pyrene (Wessel *et al.*, 2007), phenanthrene (Francioni *et al.*, 2007))
Reproductive/ developmental	*Endocrine disruptors*	Mimic or block the actions of true hormones, disrupting growth, development and reproduction	Nonylphenols (Rivero *et al.*, 2008), tetrachlorodibenzodioxin (Heiden *et al.*, 2005)
	Genotoxins (affecting reproductive structures)	Damage to DNA, resulting in mutations	Polycyclic aromatic hydrocarbons (e.g. fluoranthene (Palmqvist *et al.*, 2003), benzo[a]pyrene (Wessel *et al.*, 2007),

		phenanthrene (Francioni *et al.*, 2007))
Vitamin K antagonists	Compete with vitamin K in the vitamin K cycle, preventing the formation of blood clotting proteins; results in haemorrhaging	Brodifacoum (Primus *et al.*, 2005)
Thyroxine antagonists	Compete with thyroxine, resulting in displacement of thyroxine and retinol complexes from the blood	Polychlorinated biphenyls (e.g. Arochlor (LeRoy *et al.*, 2006))
Inhibitors of adenosine triphosphatases (ATPase)	Inhibition of ATPase enzymes involved in ion transport, including osmoregulation; can disrupt calcium ion transport and result in egg shell thinning	Organochlorines (e.g. DDE (metabolite of DDT) (Blus *et al.*, 1979; Buet *et al.*, 2006))

* Categories adapted from (McCarty and Mackay, 1993; Walker *et al.*, 1996; Escher and Hermens, 2002)

frequent convulsions and tetany (Drummond and Russom, 1990). It was found that nearly half (49%) of the chemicals tested caused hypoactivity, with the rest causing either hyperactivity (28%) or physical deformity (23%) (Drummond and Russom, 1990). Additionally, 61% of the chemical classes tested caused more than one type of behavioural syndrome, with 35% causing all three (Drummond and Russom, 1990).

The effect of prey behavioural changes on predator-prey interactions will depend on the type of behavioural change induced in prey combined with the hunting strategy of the predator (ambush or active). Effects on prey activity would affect predation by both active and ambush predators, with hypoactivity resulting in reduced predation (Spitze, 1985) and hyperactivity resulting in increased predation (Schulz and Dabrowski, 2001) of the sensitive prey species. Changes in prey responsiveness would only affect predation by active predators, with hypoactivity resulting in reduced predation (Peckarsky and Penton, 1989a), and, though not tested, hyperactivity resulting in increased predation. Physical deformity syndrome in prey may result in convulsing prey (Drummond and Russom, 1990), and thus stronger visual cues for active hunters and increased predation, or reduced activity due to tetany symptoms (Drummond and Russom, 1990), thus reducing predation by both ambush and active predators.

The susceptibility of prey to predation may also be affected by changes in prey morphology. Some contaminants act by increasing or decreasing the magnitude of a prey species' defence structures; for example, exposure of *Daphnia* spp. to pesticides can induce the growth of anti-predatory spines and neck teeth (Hanazato and Dodson, 1993; Hanazato, 2001). The induction of such defence structures can reduce susceptibility of prey to predation (Tollrian, 1995; Hammill *et al.*, 2008). Other contaminants act on non-defensive body structures, such as additional or missing limbs in amphibians (Kiesecker, 2002) and turtles (Bishop *et al.*, 1998), and spinal deformities in larval fish (Lemly, 2002). Such changes in the general body structures of prey may affect their behaviour, such as impaired swimming ability, and thus ability to escape predation (Lemly, 2002).

In cases where the susceptibility of sensitive prey species has been reduced, generalist predators may alter their prey choice. Such a response was observed in

Chapter 3, in which the ambush predator (*I. elegans*) fed on a greater proportion of *A. aquaticus* following the cadmium-induced reduction in activity of *C. dipterum*. Few studies have addressed this issue of shifting prey choices due to behavioural changes in prey (Clements *et al.*, 1989; Riddell *et al.*, 2005b), and none could be identified that investigated the consequences of morphological changes in prey on prey choice. However, these are important areas for future research to identify scenarios in which a prey species may not be sensitive to sublethal contaminant exposures but may experience reduced survival due to shifts in prey choice by generalist predators.

In addition to changes in susceptibility to predation, predators may experience reduced prey availability due to reductions in prey populations. Sublethal exposures to contaminants may act over longer periods to reduce the general fitness of prey, thereby reducing their reproductive output and hence their population sizes. For example, ability to detect female pheromones, and hence locate mates, was reduced in male amphipods (*Corophium volutator*) following exposure to naphthalene, an endocrine disruptor (Krang, 2007). Exposure of *Daphnia magna* to veterinary medicines resulted in fewer offspring being produced per female (Wollenberger *et al.*, 2000). Sensitive prey will therefore have reduced populations and thus may experience increased predation pressures from both specialist and generalist predators (Chapter 2). The ability of populations of sensitive prey species to resist local extinctions due to additional predation will depend on their ability to recolonise and the duration of contaminant exposure, as discussed previously with regard to lethally-reduced prey populations (Section 6.3.2.1).

6.3.3 Contaminants that are both bioaccumulative AND toxic

The effects of bioaccumulation within prey and shifts in their availability due to sublethal or lethal exposures were investigated separately within this thesis. However, contaminants exist that are both bioaccumulative and toxic, including anthracene, hexabromocyclododecane, benzo[*a*]pyrene and mercury (ECHA, 2009; EPA, 2009). Thus the effects studied in isolation within this thesis may occur concurrently in the environment. For example, sublethal effects on prey behaviour may reduce susceptibility of species X to predation, resulting in predators feeding

more on species Y. However, species Y accumulates more of the contaminant than species X, resulting in the predator exceeding its critical body residue more rapidly than it would have by feeding on species X. Such implications of prey switching for food chain transfer have not yet been investigated in the literature, though others have noted its potential (Riddell *et al.*, 2005b).

6.4 Conclusions

The main aim of this thesis was to address the hypothesis that changes in predator-prey interactions from contaminant exposure resulted in additional impacts, via toxic effects at either the prey or predator level. Three main scenarios were identified using the findings from Chapters 2 – 5 in which current practices in ecological risk assessment may underestimate the negative impacts of contaminants:

1. Bioaccumulative contaminants can be passed through food chains, with the extent of accumulation in predators depending on the exposure pathway to prey and the feeding behaviour of predators. Detection of subsequent toxic effects in predators requires consideration of not only the critical body residue of the predator, but also the mode of action of the contaminant and the traits of organisms that will be most sensitive to such effects.

2. Contaminants with low acute to chronic ratios for toxicity can act by reducing the survival of prey. Predators can respond to such reductions in availability by increasing their predation and thus further reducing prey survival. Alternatively they may switch to feeding on more abundant, alternative prey. The predator responses in this study could not be predicted *a priori* using a modelling approach. Scenarios for which such effects will be most detrimental are sensitive species that have a limited ability to recover, living in isolated habitats and exposed for long periods of time.

3. Contaminants with high acute to chronic ratios can have sublethal effects on prey, including behavioural, morphological and reproductive effects. These sublethal effects can result in changes in prey susceptibility to predation or reduced prey populations. Predators can respond to reductions in prey

availability by increasing their predation, thus causing reduced survival due to sublethal exposures of prey. The impact on sensitive prey species' populations will depend on the type of sublethal effect exhibited, the traits of the predator, and the ability of the prey species' population to recover.

This thesis demonstrates that changes in predator-prey interactions can result in additional negative impacts from contaminant exposure. Therefore the current risk assessment approach of focusing on direct exposures of single species to contaminants may be under protective. Instead, a scenario-based approach may be more appropriate, taking into account the interactions between contaminant properties, species traits, landscape profiles and exposure durations to identify situations where changes in predator-prey interactions may be particularly detrimental.

APPENDIX 4.1. HPLC method used by David Sanders, Unilever SEAC, Colworth, Bedford, UK

<u>HPLC analysis profiling the metabolites of [14C]Benzophenone after exposure to *Asellus, Chironomus* or *Cloëon*</u>

Method

Acetonitrile extracts (n=3/species) of macerated *Asellus, Cloëon* or *Chironomus* that had previously been exposed to sediment spiked with [14C]Benzophenone were supplied. In addition there were extracts of sediment that had been spiked with [14C]Benzophenone. Aliquots (10µl) of each extract were assayed by liquid scintillation counting to confirm that there was sufficient [14C] to perform radio-HPLC – all samples were deemed suitable for HPLC analysis.

Pooled samples of extracts from each species were prepared and analysed.

Standards of [14C]Benzophenone, benzophenone (0.14mg/ml), 4-hydroxybenzophenone (0.1mg/ml) and benzohydrol (diphenylmethanol @ 0.1mg/ml) were also analysed.

The radio-HPLC analysis method used for all samples can be summarised thus:

An Agilent 1100 quaternary pump, autosampler and variable wavelength UV absorbance detector.

A Beta-RAM radiodetector (LabLogic Systems, Sheffield) was fitted with a 500µl flow cell. Mixing of the column eluant was with Flowlogic scintillation cocktail (LabLogic Systems Ltd.) at a 1:1 by volume ratio and at a flow rate of 1ml/minute. LAURA 3.4.7.52 software (LabLogic Systems) was used to capture output from the detectors and for evaluation of the HPLC traces.

Prodigy C18 column (250 x 4.6mm) supplied by Phenomenex

UV detector set to 254nm

40µl injection volumes for the analysis samples (50µl for pooled extracts)

Column oven temperature set to 30ºC

1ml/min flow of in-line (vacuum) degassed mobile phase using the following gradient method:

0 - 5 mins 90% A/10% B

5-10 mins 90% A/10% B to 60% A/40% B

10-25 mins 60% A/40% B to 100% B

25-30 mins 100% B

30 - 31 mins 100% B to 90% A/10% B

31 – 35 mins 90% A/10% B

A = 0.01% (by vol) formic acid in Milli Q (ultrapure) water

B = 0.01% (by vol) formic acid in Acetonitrile

Formic Acid (AR grade) was from VWR International, Acetonitrile (HPLC grade) was from VWR International and water was from an in-house Millipore Q system.

The radio-trace was evaluated using the LAURA software and the amount of parent or metabolite component expressed as a percentage of the total [14C] evaluated. The UV traces of the standards were evaluated for retention time only.

Results

[14C]Benzophenone (14C trace) eluted after 21.3 minutes.

Benzophenone (UV trace) eluted after 21.4 minutes (0.1 minute offset error)

4-hydroxyphenone (UV trace) eluted after 17.3 minutes

Benzohydrol (UV trace) eluted after 18.7 minutes

The individual extracts from *Asellus*, *Cloëon*, *Chironomus* and sediment showed some variation within the species/sample type but different profiles between species. The results presented below are a summary of the profiles from the extracts for each species/sample type. Metabolite peaks contributing >10% of the evaluated [14C] were included.

Table A1.1. Mean percentages of metabolites found in extracts of sediment, *Asellus aquaticus*, *Chironomus riparius* and *Cloëon dipterum* after five days of exposure to [14C] benzophenone. Only metabolites contributing > 10% of the total activity have been included.

Compound	Retention time of compound (mm:ss)	% found in sample type			
		Sediment	*A. aquaticus*	*C. riparius*	*C. dipterum*
A	14:40		32.4 (3.0)		
B	15:33				27.9 (1.2)
C	16:05 – 16:25				11.1 (NA)*
D (benzohydrol)	18:36 – 18:41	12.3 (2.3)	35.2 (2.8)	15.5 (0.9)	25.0 (4.6)
E	19:53			50.8 (2.4)	47.6 (NA)*
F (parent)	21:04 – 21:05	84.9 (1.8)	19.6 (0.3)	26.7 (0.7)	13.5 (5.1)
Total % metabolized		12.3	80.4	73.3	86.5

NB. Numbers in parentheses denote standard errors associated with the means.
*Metabolite only detected in one of the extracts for *C. dipterum*

APPENDIX 2. Microautoradiography method used by Helen Minter, Unilever SEAC, Colworth, Bedford, UK

METHOD

The invertebrates species were examined on arrival and apart from a couple of dead individuals which were discarded, were in good condition and the live invertebrates were immediately frozen by mounting onto specimen discs (supplied by Leica Microsystems) using OCT compound (supplied by BDH) at -20°C and allowed to freeze. The animals were laid in a position with the objective to obtain longitudinal sections. Due to the small size of the *Chironomus reparius* larvae these were frozen in a clump of individuals and it was not possible to position or section individual larvae.

Micro-autoradiography procedures

Once frozen, the specimen disc plus OCT and sample were wrapped in parafilm and stored in a -20°C freezer until required. The samples destined for micro-autoradiography were processed in two batches due to the number of samples.

Slide preparation and emulsion application

Microscope slides were first pre cleaned in 1% hydrochloric acid in 95% ethanol/water (v/v) ("acid alcohol"), rinsed well in pure water and then "subbed" by dipping them into a gelatin/alum solution (gelatin 2.6g, chromium potassium sulphate 0.5g in 500ml Ultra pure water). The slides were drained and dried in an oven overnight.

The following day application of emulsion took place under dark room lighting (Kodak No 1 filter). Ilford K5 emulsion, Batch number 41AK51992 and 47CK51103 were obtained from Agar Scientific Limited. The K5 photographic emulsion was melted in a water bath at 43°C ± 2°C and decanted into a custom made dipping jar containing 30ml of 1% glycerol in Ultra pure water to give a 30/20ml

dilution (v/v) (glycerol solution/emulsion), total volume 50ml. The "subbed" slides were dipped individually into the emulsion, drained and the back of the slide wiped to remove excess emulsion. The "dipped" slides were placed onto an aluminium rack in a horizontal position and allowed to gel and dry, then placed in autoradiography boxes (20 slides per box), with the lids removed but sealed in a light proof box containing a sachet of silica gel overnight to ensure complete drying of the emulsion. The following morning the lids were replaced, under dark room safe lighting and the autoradiography boxes sealed using black electrician's tape. The boxes were placed in a -20°C freezer until required.

Sectioning

The samples were removed from the -20°C freezer and allowed to acclimatise in a Leica 3050 cryostat (supplied by Leica Microsystems Nussloch GmbH Heidelberg) for approximately 30 minutes. The samples were trimmed using a cryostat and sections picked up onto ordinary Superfrost™ microscope slides. The sections were examined using a microscope under bright field to determine section quality. Once representative areas were obtained cryosections at 8μm were cut and mounted onto the emulsion-coated slides under dark room safe lighting conditions. The slides were then placed in autoradiography boxes and stored in a -20°C freezer. The sections were exposed to the photographic emulsion for 120, 144 hours or 1 week.

Additional cryosections were obtained from each sample and mounted onto glass microscope slides and air dried. These sections were not subjected to the micro-autoradiography processing procedures and were further processed for histology.

Development and staining

The slides maintained in a -20°C freezer following exposure were split into two sets by selecting alternate slides. Following 'splitting' one set was air dried at room temperature, sealed in autoradiography boxes and the remaining set returned to the -20°C freezer sealed in autoradiography boxes to allow further exposure of the sections to the photographic emulsion.

The sections were developed either at 120 and 1 week or 144 hours and 1 week exposure using Ilford Phenisol developer diluted 1:4 (v/v) with Ultra pure water for 2 minutes. Development was stopped by immediate immersion for 1 minute in a "stop bath" of 1% acetic acid (v/v) in Ultra pure water and fixed in Ilford Hypam fixer diluted 1:4 (v/v) in Ultra pure water for 3 minutes. Following washing in slowly running tap water for approximately 10 minutes, the sections were fixed in Neutral Buffered Formalin 10% aq (v/v) for 10 minutes and rinsed in tap water. The sections were stained using a Jung (Leica) AutoStainer XL in haematoxylin (Gill's No. 3) for 3 minutes, rinsed in running tap water, differentiated in 1% hydrochloric acid in 70% alcohol, 'blued-up' for approximately 10 minutes and rinsed in running tap water. The sections were stained in eosin for approximately 1 minute, rinsed in running tap water and immersed in two changes of 95% and two changes of 100% ethanol, then immersed in two changes of xylene, each change for the duration of approximately 1 minute. Finally the sections were mounted in DPX under coverslips. The sections destined for histology were fixed in Neutral Buffered Formalin 10% aq (v/v) and stained using H&E as described above.

Photomicroscopy

Using a Leica DMRB microscope, the sections were examined for areas of silver grains under dark field illumination and the histology observed under bright field illumination. Photomicrographs were obtained using a digital 3CCD camera JVC KYF 75 attached to a Leica DMRB microscope and imported into OASIS 2000 via KY-LINK software. Images were formatted using Adobe PageMaker software and annotated with the slide and image file number.

Soluene™ digestion and liquid scintillation counting (LSC)

Spare animals (unweighed material) not used for sectioning were solubilised in 2ml Soluene™ over 24 – 48 hours at ambient temperature and counted using a LS6000TA counter to provide approximate estimation of levels of radioactivity in the body. 1ml of the 'pond water' used to transport the animals was counted and a blank control of tap water was used to estimate background levels of radioactivity. The duration of time exposed to 'pond water' was estimated to be less than 8 hours.

RESULTS

The three species of invertebrates were cryosectioned and exposed to photographic emulsion and the developed micrographs examined under a light microscope for areas of radioactivity. Images were captured to represent the areas of [14C] seen as white silver grains under dark field illumination (or black grains under bright field illumination) and compared to untreated control.

The aim was to section each species in the longitudinal plane and it should be noted that it was not possible to obtain the entire length in any one section due to the complexity of the structure and orientation of individuals. The *Chironomus riparius* larvae were frozen in a clump resulting in a mixture of longitudinal and transverse sections. Due to the size of the invertebrate species the Figures are a compilation of images to obtain as much of the entire section of the animal as possible. Note that the image compilations are labelled 'head' and 'tail' for orientation purposes and not necessarily the actual head or tail.

Liquid scintillation counting (LSC)

The results of the digestion of the invertebrates in Soluene™ and liquid scintillation counting can be seen in Table A2.1 and are for guidance only. The levels of radioactivity in the artificial pond water used to transport the animals was counted by LSC and showed varying levels of radioactivity. All water samples from the treated animals contained radioactivity. The water in which *Cloëon dipterum* was transported contained relatively high levels of radioactivity compared to the level in the body. The water used to transport *Chironomus riparius* compared to body levels was relatively high. The water used to transport *Asellus aquaticus* contained relatively low levels of [14C] compared to body levels. The body levels of [14C] were very low in *Cloëon dipterum* and relatively high in *Chironomus riparius* and very high in *Asellus aquaticus*.

Table A2.1. Radioactivity levels in artificial pond water and invertebrate species determined by LSC.

TEST SPECIES	Dose	[14C] dpm
Cloëon dipterum	Untreated control	682.68
Cloëon dipterum	[14C]benzophenone	1386.55
Artificial pond water	[14C]benzophenone	3118.77
Chironomus riparius	Untreated control	n/a
Chironomus riparius	[14C]benzophenone	15540.48
Artificial pond water	[14C]benzophenone	1779.96
Asellus aquaticus	Untreated control	116.47
Asellus aquaticus	[14C]benzophenone	46764.75
Artificial pond water	[14C]benzophenone	910.90

DISCUSSION

Carrying out micro-autoradiography on invertebrates was not an easy option compared to using mammalian tissues due to the structure and anatomy of invertebrates. The quality of the cryosections of unfixed tissue is inevitably compromised due to freezing artefacts occurring when ice crystals form in the tissues during the freezing process, causing tissue damage seen microscopically. Lack of preservation of anatomy is further compromised due to technical difficulties in cryosectioning the brittle exoskeleton contrasting with soft internal tissues. Add to this the sections then undergo the rigors of micro-autoradiography processing. The invertebrates were processed as frozen sections as it was believed that the test chemical benzophenone would be soluble and as a result removed from the tissues processed by the alternative wax embedded method. In spite of the rigors imposed the results were satisfactory.

The control and treated invertebrate species both showed bright white areas due to auto-fluorescence from the gut contents i.e. light reflecting from the gut contents and appears similar to silver grains under dark field. This should not be confused with localisation of radioactivity which is seen as individual silver grains under dark field and black grains under bright field illumination. The artificial sediment on which the species were fed comprised of sand, cellulose and kaolin and these were all thought to potentially reflect light under dark field illumination although was not tested in this study.

The micro-autoradiography demonstrated that the three species showed markedly different levels of radioactivity. There were very low levels of radioactivity which were only slightly above background in *Cloëon dipterum* and considerably higher levels in *Chironomus riparius* and *Asellus aquaticus*. *Chironomus riparius* appeared to have more uniform distribution and radioactivity was present in most tissues but with higher levels associated with the gut and contents. The *Asellus aquaticus* which contained overall the highest levels of radioactivity was mainly confined to the gut contents although some [14C] was seen in the body tissues.

There were very low levels of radioactivity in some areas adjacent to the exoskeleton and assumed to be vestigial contamination from the sediment. However none of the invertebrate species appeared to have significant localisation in the exoskeleton in spite of presumably being in contact with the radioactive labelled sediment. As the gut contained the highest levels of radioactivity it was assumed that this was due to ingestion of radiolabelled sediment (or potentially from consumption of each other).

It should be noted that interpretation was carried out over a number of slides and over a number of individuals. The images are a representation of the visual assessment and this is a non quantitative method. However the results are presumed to be representative but it should be noted that this is based on a single experiment in each case, little previous experimental evidence and relatively few samples processed.

The analysis of the radioactive levels in the transported water showed varying amounts of radioactivity. The level of radioactivity in the water used to transport *Cloëon dipterum* was higher than the level in the body of *Cloëon dipterum* and the micro-autoradiography showed background levels in this species. This result suggests that the *Cloëon dipterum* may have excreted the gut contents during the transportation period in the water. This may have happened in the other species to a lesser extent as all water levels of radioactivity were above background. It was thought that in preference the freezing of the animals should have been prior to transportation to reduce the chance of excretion during transit, but due to the logistics this was best option under the circumstances. The LSC was carried out on the water and spare animals to give an approximation only and acted as guidance for the micro-autoradiography. The LSC results were not intended for statistical analysis of radioactivity levels.

CONCLUSION

The micro-autoradiography of invertebrate species exposed to [14C]benzophenone radiolabelled sediment demonstrated that it was possible to use micro-autoradiography for determining localisation in these aquatic invertebrate species. The preservation of anatomy was compromised when compared to, for example,

mammalian tissues but overall it was considered to provide meaningful results. The localisation in both *Chironomus riparius* and *Asellus aquaticus* was mainly associated with the gut and contents indicating uptake either directly or indirectly from the sediment. There was very little localisation in the exoskeleton of all three species and some internal localisation seen in *Chironomus riparius* and *Asellus aquaticus*. There was no evidence of specific localisation in *Cloëon dipterum* as this species contained low levels of radioactivity. The results of liquid scintillation counting supported the micro-autoradiography results. The levels of [14C] determined by LSC in the body of each species was concomitant with levels seen in the micro-autoradiography. There was evidence of radioactivity in the water used to transport the invertebrates suggesting some excretion from the invertebrates during transit.

References

Abrams, P. & Matsuda, H. (1993) Effects of adaptive predatory and anti-predator behaviour in a two-prey-one-predator system. *Evolutionary Ecology*, **7**, 312-326.

Adcock, J. A. (1982) Energetics of a population of *Asellus aquaticus* (Crustacea, Isopoda): respiration and energy budgets. *Freshwater Biology*, **12**, 257-269.

Addicott, J. F. (1974) Predation and prey community structure: an experimental study of the effect of mosquito larvae on the protozoan communities of pitcher plants. *Ecology*, **55**, 475-492.

Ahrens, M. J., Hertz, J., Lamoureux, E. M., Lopez, G. R., McElroy, A. E. & Brownawell, B. J. (2001) The role of digestive surfactants in determining bioavailability of sediment-bound hydrophobic organic contaminants to 2 deposit-feeding polychaetes. *Marine Ecology Progress Series*, **212**, 145-157.

Akre, B. G. & Johnson, D. M. (1979) Switching and sigmoid functional response curves by damselfly naiads with alternative prey available. *Journal of Animal Ecology*, **48**, 703-720.

Andersen, N. H. & Sedell, J. R. (1979) Detritus processing by macroinvertebrates in stream ecosystems. *Annual Review of Entomology*, **24**, 351-377.

Ankley, G. T. & Collyard, S. A. (1995) Influence of piperonyl butoxide on the toxicity of organophosphate insecticides to three species of freshwater benthic invertebrates. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, **110**, 149-155.

Anon (2007) The world's worst polluted places. The top ten of the dirty thirty. (eds, pp. http://www.blacksmithinstitute.org/wwpp2007/finalReport2007.pdf. The Blacksmith Insitute, New York, USA.

Arnot, J. A. & Gobas, F. A. P. C. (2006) A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environmental Reviews,* **14,** 257-297.

Arukwe, A., Thibaut, R., Ingebrigtsen, K., Celius, T., Goksoyr, A. & Cravedi, J. P. (2000) In vivo and in vitro metabolism and organ distribution of nonylphenol in Atlantic salmon (*Salmo salar*). *Aquatic Toxicology,* **49,** 289-304.

Barron, M. G. (2002) Environmental contaminants altering behaviour. *Behavioural ecotoxicology* (eds G. Dell'Omo), pp. 167-186. John Wiley & Sons Ltd., Chichester, UK.

Béchard, K. M., Gillis, P. L. & Wood, C. M. (2008) Trophic transfer of Cd from larval chironomids (*Chironomus riparius*) exposed via sediment or waterborne routes, to zebrafish (*Danio rerio*): Tissue-specific and subcellular comparisons. *Aquatic Toxicology,* **90,** 310-321.

Begon, M., Harper, J. L. & Townsend, C. R. (1996) *Ecology: individuals, populations and communities. Third Edition.* Blackwell Science Ltd., Oxford, UK.

Beier, S., Bolley, M. & Traunspurger, W. (2004) Predator-prey interactions between *Dugesia gonocephala* and free-living nematodes. *Freshwater Biology,* **49,** 77-86.

Bejarano, A. C., Widenfalk, A., Decho, A. W. & Chandler, G. T. (2003) Bioavailability of the organophosphorus insecticide chlorpyrifos to the suspension-feeding bivalve, *Mercenaria mercenaria,* following exposure to dissolved and particulate matter. *Environmental Toxicology and Chemistry,* **22,** 2100-2105.

Beketov, M. A. & Liess, M. (2005) Acute contamination with esfenvalerate and food limitation: chronic effect on the mayfly *Cloeon dipterum. Environmental Toxicology and Chemistry,* **24,** 1281-1286.

Beketov, M. A., Schäfer, R. B., Marwitz, A., Paschke, A. & Liess, M. (2008) Long-term stream invertebrate community alterations induced by the insecticide

thiacloprid: Effect concentrations and recovery dynamics. *Science of the Total Environment,* **405,** 96-108.

Bergelson, J. M. (1985) A mechanistic interpretation of prey selection by *Anax junius* larvae (Odonata: Aeschnidae). *Ecology,* **66,** 1699-1705.

Berntssen, M. H. G., Lundebye, A.-K. & Maage, A. (1999) Effects of elevated dietary copper concentrations on growth, feed utilisation and nutritional status of Atlantic salmon *(Salmo salar* L.) fry. *Aquaculture,* **174,** 167-181.

Bervoets, L., De Bruyn, L., Van Ginneken, L. & Blust, R. (2003) Accumulation of ^{137}Cs by larvae of the midge *Chironomus riparius* from sediment: effect of potassium. *Environmental Toxicology and Chemistry,* **22,** 1589-1596.

Bishop, C. A., Ng, P., Pettit, K. E., Kennedy, S. W., Stegeman, J. J., Norstrom, R. J. & Brooks, R. J. (1998) Environmental contamination and developmental abnormalities in eggs and hatchlings of the common snapping turtle *(Chelydra serpentina serpentina)* from the Great Lakes-St Lawrence River basin (1989-1991). *Environmental Pollution,* **101,** 143-156.

Blackmore, G. & Morton, B. (2002) The influence of diet on comparative trace metal cadmium, copper and zinc accumulation in *Thais clavigera* (Gastropoda: Muricidae) preying on intertidal barnacles or mussels. *Marine Pollution Bulletin,* **44,** 870-876.

Blus, L., Cromartie, E., McNease, L. & Joanen, T. (1979) Brown pelican - population status, reproductive success, and organochlorine residues in Louisiana, 1971-1976. *Bulletin of Environmental Contamination and Toxicology,* **22,** 128-135.

Boening, D. W. (2000) Ecological effects, transport, and fate of mercury: a general review. *Chemosphere,* **40,** 1335-1351.

Bolker, B. (2006) Liklihood examples. *Ecological Models and Data in R* (eds, pp. Chapter 8. http://www.zoo.ufl.edu/bolker/emdbook/chap8A.pdf.

Borchert, J., Karbe, L. & Westendorf, J. (1997) Uptake and metabolism of benzo[a]pyrene absorbed to sediment by the freshwater invertebrate species *Chironomus riparius* and *Sphaerium corneum. Bulletin of Environmental Contaminantion and Toxicology,* **58,** 158-165.

Borgmann, U. & Ralph, K. M. (1986) Effects of cadmium, 2,4-dichlorophenol, and pentachlorophenol on feeding, growth, and particle-size-conversion efficiency of white sucker larvae and young common shiners. *Archives of Environmental Contamination and Toxicology,* **15,** 473-480.

Bott, T. L. & Standley, L. J. (2000) Transfer of benzo[*a*]pyrene and 2,2',5,5'-tetrachlorobiphenyl from bacteria and algae to sediment-associated freshwater invertebrates. *Environmental Science and Technology,* **34,** 4936-4942.

Boxall, A., Brown, C. & Barrett, K. (2001) Higher tier laboratory aquatic toxicity testing. Research report No. JF 4317E for DETR. (eds, pp. 70. Cranfield Centre for EcoChemistry, Cranfield, UK.

Boyd, W. A., Brewer, S. K. & Williams, P. L. (2002) Altered behaviour of invertebrates living in polluted environments. *Behavioural Ecotoxicology* (eds G. Dell'Omo), pp. 293-336. John Wiley & Sons, Ltd., Chichester, UK.

Boyle, T. P., Fairchild, J. F., Robinson-Wilson, E. F., Haverlands, P. S. & Lebo, J. A. (1996) Ecological restructuring in experimental aquatic mesocosms due to the application of diflubenzuron. *Environmental Toxicology and Chemistry,* **15,** 1806-1814.

Bronmark, C. (1992) Leech predation on juveline freshwater snails: effects of size, species and substrate. *Oecologia,* **91,** 526-529.

Bronmark, C. & Hansson, L. (2000) Chemical communication in aquatic systems: an introduction. *Oikos,* **88,** 103-109.

Brown, D. S. (1960) The ingestion and digestion of algae by *Chloeon dipterum* L. (Ephemeroptera). *Hydrobiologia,* **16,** 81-96.

Buet, A., Banas, D., Vollaire, Y., Coulet, E. & Roche, H. (2006) Biomarker responses in European eel (*Anguilla anguilla*) exposed to persistent organic pollutants. A field study in the Vaccarès lagoon (Camargue, France). *Chemosphere, 65*, 1846-1858.

Burkhardt, M. R., ReVello, R. C., Smith, S. G. & Zaugg, S. D. (2005) Pressurized liquid extraction using water/isopropanol coupled with solid-phase extraction cleanup for industrial and anthropogenic waste-indicator compounds in sediment. *Analytica Chimica Acta, 534*, 89-100.

Cairns, J. (1983) Are single species toxicity tests alone adequate for estimating environmental hazard? *Hydrobiologia, 100*, 47-57.

Cairns, J., Jr. (1986) The myth of the most sensitive species. *Bioscience, 36*, 670-672.

Campbell, N. A. (1993) *Biology. Third Edition.* The Benjamin/Cummings Publishing Company, Inc., Redwood City, California, USA.

Campbell, P. J., Arnold, D. J. S., Brock, T. C. M., Grandy, N. J., Heger, W., Heimbach, F., Maund, S. J. & Streloke, M. (1998) Guidance document on Higher-tier Aquatic Risk Assessment for Pesticides (HARAP). (eds. SETAC Europe, Brussels, Belgium.

Canty, M. N., Hagger, J. A., Moore, R. T. B., Cooper, L. & Galloway, T. S. (2007) Sublethal impact of short term exposure to the organophosphate pesticide azamethiphos in the marine mollusc *Mytilus edulis. Marine Pollution Bulletin, 54*, 396-402.

Carlson, R. W., Bradbury, S. P., Drummond, R. A. & Hammermeister, D. E. (1998) Neurological effects on startle response and escape from predation by medaka exposed to organic chemicals. *Aquatic Toxicology, 43*, 51-68.

Chan, S. M., Wang, W. X. & Ni, I. H. (2003) The uptake of Cd, Cr, and Zn by the macroalga Enteromorpha crinita and subsequent transfer to the marine herbivorous

rabbitfish, *Siganus canaliculatus*. *Archives of Environmental Contamination and Toxicology*, **44**, 298-306.

Chang, K. H., Sakamoto, M. & Hanazato, T. (2005) Impact of pesticide application on zooplankton communities with different densities of invertebrate predators: An experimental analysis using small-scale mesocosms. *Aquatic Toxicology*, **72**, 373-382.

Chesson, J. (1983) The estimation and analysis of preference and its relationship to foraging models. *Ecology*, **64**, 1297-1304.

Christensen, M., Andersen, O. & Banta, G. T. (2002) Metabolism of pyrene by the polychaetes *Nereis diversicolor* and *Arenicola marina*. *Aquatic Toxicology*, **58**, 15-25.

Cichna, M., Markl, P. & Huber, J. F. K. (1995) Determination of true octanol-water partition coefficients by means of solvent generated liquid-liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, **13**, 339-351.

Cid Montañés, J. F., Van Hattum, B. & Deneer, J. (1995) Bioconcentration of chlorpyrifos by the freshwater isopod *Asellus aquaticus* (L.) in outdoor experimental ditches. *Environmental Pollution*, **88**, 137-146.

Clements, W. H., Cherry, D. S. & Cairns, J. (1989) The influence of copper exposure on predator-prey interactions in aquatic insect communities. *Freshwater Biology*, **21**, 483-488.

Clements, W. H., Oris, J. T. & Wissing, T. E. (1994) Accumulation and food chain transfer of fluoranthene and benzo[a]pyrene in *Chironomus riparius* and *Lepomis macrochirus*. *Archives of Environmental Contamination and Toxicology*, **26**, 261-266.

Cock, M. J. W. (1978) The assessment of preference. *Journal of Animal Ecology*, **47**, 805-816.

Colton, T. F. (1987) Extending functional response models to include a second prey type: an experimental test. *Ecology*, **68**, 900-912.

Connell, J. H. (1961) The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology*, **42**, 710-723.

Connell, J. H. (1975) Some mechanisms producing structure in natural communities: a model and evidence from field experiments. *Ecology and evolution of communities* (eds M. L. Cody & J. M. Diamond), pp. 460-490. Belknap Press, Cambridge, MA, USA.

Craig, A., Hare, L., Charest, P.-M. & Tessier, A. (1998) Effect of exposure regime on the internal distribution of cadmium in *Chironomus staegeri* larvae (Insecta, Diptera). *Aquatic Toxicology*, **41**, 265-275.

Credland, P. F. (1973) A new method for establishing a permanent laboratory culture of *Chironomus riparius* Meigen (Diptera: Chironomidae). *Freshwater Biology*, **3**, 45-51.

Crossland, N. O. & Hillaby, J. M. (1985) Fate and effects of 3,4-dichloroaniline in the laboratory and in outdoor ponds: II. Chronic toxicity to *Daphnia* spp. and other invertebrates. *Environmental Toxicology and Chemistry*, **4**, 489-499.

Custer, C. M. & Custer, T. W. (2000) Organochlorine and trace element contamination in wintering and migrating diving ducks in the southern Great Lakes, USA, since the zebra mussel invasion. *Environmental Toxicology and Chemistry*, **19**, 2821-2829.

deNoyelles Jr., F., Dewey, S. L., Huggins, D. G. & Kettle, W. D. (1994) Aquatic mesocosms in ecological effects testing: detecting direct and indirect effects of pesticides. *Aquatic mesocosm studies in ecological risk assessment* (eds R. L. Graney, J. H. Kennedy & J. H. Rodgers Jr.), pp. 577-603. CRC Press, Inc., Boca Raton, Florida, USA.

Di Toro, D. M., Zarba, C. S., Hansen, D. J., Berry, W. J., Swartz, R. C., Cowan, C. E., Pavlou, S. P., Allen, H. E., Thomas, N. A. & Paquin, P. R. (1991) Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environmental Toxicology and Chemistry,* **10,** 1541-1583.

Dodson, S. I., Hanazato, T. & Gorski, P. R. (1995) Behavioural responses of *Daphnia pulex* exposed to carbaryl and *Chaoborus* kairomone. *Environmental Toxicology and Chemistry,* **14,** 43-50.

Drummond, R. A. & Russom, C. L. (1990) Behavioral toxicity syndromes: a promising tool for assessing toxicity mechanisms in juvenile fathead minnows. *Environmental Toxicology and Chemistry,* **9,** 37-46.

Dumas, J. & Hare, L. (2008) The Internal Distribution of Nickel and Thallium in Two Freshwater Invertebrates and its Relevance to Trophic Transfer. *Environmental Science and Technolology,* **42,** 5144-5149.

EC (2000) IUCLID Dataset: Benzophenone. (eds. European Commission - European Chemicals Bureau.

EC (2002) Working Document. Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC. Sanco/3268/2001 rev. 4 (final). (eds E. C. H. C. P. Directorate-General). Brussels.

EC (2003) Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment to New Notified Substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for existing Substances and Directive 98/8/EC of the European Parliament and the Council concerning the placing of biocidal products on the market. (eds, pp. 1009. Office for Official Publications of the European Communities, Luxembourg.

EC (2006) REACH in brief. (eds. Enterprise and Industry Directorate General and Environment Directorate General, European Commission, Brussels.

ECHA (2008) Guidance on information requirements and chemical safety assessment. Part C: PBT assessment. May 2008. *http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_part_c_en.pdf?vers=20_08_08*.

ECHA (2009) Candidate list of substances of very high concern for authorisation. *http://echa.europa.eu/chem_data/candidate_list_table_en.asp*.

Egeler, P., Meller, M., Roembke, J., Spoerlein, P., Streit, B. & Nagel, R. (2001) *Tubifex tubifex* as a link in food chain transfer of hexachlorobenzene from contaminated sediment to fish. *Hydrobiologia*, 463, 171-184.

Elliott, J. M. (2003) A comparative study of the functional response of four species of carnivorous stoneflies. *Freshwater Biology*, 48, 191-202.

Elliott, J. M. (2004) Prey switching in four species of carnivorous stoneflies. *Freshwater Biology*, 49, 709-720.

Elonen, G. E., Spehar, R. L., Holcombe, G. W., Johnson, R. D., Fernandez, J. D., Erickson, R. J., Tietge, J. E. & Cook, P. M. (1998) Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin to seven freshwater fish species during early life-stage development. *Environmental Toxicology and Chemistry*, 17, 472-483.

EPA (2009) Persistent Bioaccumulative and Toxic (PBT) Chemical Program: priority PBT profiles *http://www.epa.gov/pbt/pubs/cheminfo.htm*.

Escher, B. I. & Hermens, J. L. M. (2002) Modes of action in ecotoxicology: Their role in body burdens, species sensitivity, QSARs, and mixture effects. *Environmental Science and Technology*, 36, 4201-4217.

Falfushinska, H. I., Romanchuk, L. D. & Stolyar, O. B. (2008) Different responses of biochemical markers in frogs (*Rana ridibunda*) from urban and rural wetlands to the effect of carbamate fungicide. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 148, 223-229.

Feijtel, T., Kloepper-Sams, P., den Haan, K., van Egmond, R., Comber, M., Heusel, R., Wierich, P., Ten Berge, W., Gard, A., de Wolf, W. & Niessen, H. (1997) Integration of bioaccumulation in an environmental risk assessment. *Chemosphere*, **34**, 2337-2350.

Fisk, A. T., Hobson, K. A. & Norstrom, R. J. (2001) Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. *Environmental Science and Technology*, **35**, 732-738.

Fisk, A. T., Tomy, G. T. & Muir, D. C. G. (1999) Toxicity of C-10-, C-11-, C-12-, and C-14-polychlorinated alkanes to Japanese medaka (*Oryzias latipes*) embryos. *Environmental Toxicology and Chemistry*, **18**, 2894-2902.

Fisk, A. T., Yarechewski, A. L., Metner, D. A., Evans, R. E., Lockhart, W. L. & Muir, D. C. G. (1997) Accumulation, depuration and hepatic mixed-function oxidase enzyme induction in juvenile rainbow trout and lake whitefish exposed to dietary 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Aquatic Toxicology*, **37**, 201-220.

Fleeger, J. W., Carman, K. R. & Nisbet, R. M. (2003) Indirect effects of contaminants in aquatic ecosystems. *Science of the Total Environment*, **317**, 207-233.

Floyd, E. Y., Geist, J. P. & Werner, I. (2008) Acute, sublethal exposure to a pyrethroid insecticide alters behavior, growth, and predation risk in larvae of the fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Chemistry*, **27**, 1780-1787.

Forbes, V. E. & Calow, P. (2002) Extrapolation in ecological risk assessment: balancing pragmatism and precaution in chemical controls legislation. *Bioscience*, **52**, 249-257.

Forbes, V. E. & Forbes, T. L. (1994) *Ecotoxicology in theory and practice*. Chapman & Hall, London, UK.

Fox, L. R. & Murdoch, W. W. (1978) Effects of feeding history on short-term and long-term functional responses in *Notonecta hoffmanni*. *Journal of Animal Ecology*, **47**, 945-959.

Francioni, E., Wagener, A. d. L. R., Scofield, A. d. L., Depledge, M. H., Cavalier, B., Sette, C. B., Carvalhosa, L., Lozinsky, C. & Mariath, R. (2007) Polycyclic aromatic hydrocarbon in inter-tidal mussel *Perna perna*: Space-time observations, source investigation and genotoxicity. *Science of the Total Environment*, **372**, 515-531.

Friedmann, A. S., Watzin, M. C., Brinck-Johnsen, T. & Leiter, J. C. (1996) Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). *Aquatic Toxicology*, **35**, 265-278.

Fritz, R. S. & Morse, D. H. (1985) Reproductive success and foraging of the crab spider *Misumena vatia*. *Oecologia*, **65**, 194-200.

Gaskell, P. N., Brooks, A. C. & Maltby, L. (2007) Variation in the bioaccumulation of a sediment-sorbed hydrophobic compound by benthic macroinvertebrates: patterns and mechanisms. *Environmental Science and Technology*, **41**, 1783-1789.

Gerhardt, A. (1990) Effects of subacute doses of cadmium on pH-stressed *Leptophlebia marginata* (L.) and *Baetis rhodani* Pictet (Insecta: Ephemeroptera). *Environmental Pollution*, **67**, 29-42.

Gerhardt, A. (1995) Joint and single toxicity of Cd and Fe related to metal uptake in the mayfly Leptophlebia marginata (L.) (Insecta). *Hydrobiologia*, **306**, 229-240.

Giddings, J. M., Biever, R. C., Annunziato, M. F. & Hosmer, A. J. (1996) Effects of diazinon on large outdoor pond microcosms. *Environmental Toxicology and Chemistry*, **15**, 618-629.

Goodyear, K. L. & McNeill, S. (1999) Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: a review. *Science of the Total Environment*, **229**, 1-19.

Gossiaux, D. C., Landrum, P. F. & Fisher, S. W. (1998) The assimilation of contaminants from suspended sediment and algae by the zebra mussel, *Dreissena polymorpha. Chemosphere,* **36,** 3181-3197.

Graney, R. L., Kennedy, J. H. & Rodgers Jr., J. H. (1994) Aquatic mesocosm studies in ecological risk assessment. (eds C. H. Ward, B. T. Walton & T. W. La Point). CRC Press, Inc., Boca Raton, Florida, USA.

Green, D. W. J., Williams, K. A. & Pascoe, D. (1986) The acute and chronic toxicity of cadmium to different life history stages of the freshwater crustacean *Asellus aquaticus* (L). *Archives of Environmental Contamination and Toxicology,* **15,** 465-471.

Greene, C. H. (1986) Patterns of Prey Selection: Implications of Predator Foraging Tactics. *American Naturalist,* **128,** 824-839.

Grue, C. E., Gardner, S. C. & Gibert, P. L. (2002) On the significance of pollutant-induced alterations in the behaviour of fish and wildlife. *Behavioural Ecotoxicology* (eds G. Dell'Omo), pp. 1-90. John Wiley & Sons Ltd., Chichester, UK.

Ham, L., Quinn, R. & Pascoe, D. (1995) Effects of cadmium on the predator-prey interaction between the Turbellarian *Dendrocoelum lacteum* (Miiller, 1774) and the Isopod Crustacean *Asellus aquaticus* (L.). *Archives of Environmental Contamination and Toxicology,* **29,** 358-365.

Hamers, T. & Krogh, P. H. (1997) Predator-prey relationships in a two-species toxicity test system. *Ecotoxicology and Environmental Safety,* **37,** 203-212.

Hammerschmidt, C. R., Sandheinrich, M. B., Weiner, J. C. & Rada, R. G. (2002) Effects of dietary methylmercury on reproduction of fathead minnows. *Environmental Science and Technology,* **36,** 877-883.

Hammill, E., Rogers, A. & Beckerman, A. P. (2008) Costs, benefits and the evolution of inducible defences: a case study with *Daphnia pulex. Journal of Evolutionary Biology,* **21,** 705-715.

Hanazato, T. (1991) Effects of repeated application of carbaryl on zooplankton communities in experimental ponds with or without the predator *Chaoborus*. *Environmental Pollution,* **74,** 309-324.

Hanazato, T. (2001) Pesticide effects on freshwater zooplankton: an ecological perspective. *Environmental Pollution,* **112,** 1-10.

Hanazato, T. & Dodson, S. I. (1993) Morphological responses of four species of cyclomorphic *Daphnia* to a short-term exposure to the insecticide carbaryl. *Journal of Plankton Research,* **15,** 1087-1095.

Hanson, B. J., Cummins, K. W., Cargill, A. S. & Lowry, R. R. (1985) Lipid content, fatty acid composition, and the effect of diet on fats of aquatic insects. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology,* **80,** 257-276.

Hayashi, T., Okamoto, Y., Ueda, K. & Kojima, N. (2006) Formation of estrogenic products from benzophenone after exposure to sunlight. *Toxicology Letters,* **167,** 1-7.

Heads, P. A. (1985) The effect of invertebrate and vertebrate predators on the foraging movements of *Ischnura elegans* larvae (Odonata: Zygoptera). *Freshwater Biology,* **15,** 559-571.

Heaton, S. N., Bursian, S. J., Giesy, J. P., Tillitt, D. E., Render, J. A., Jones, P. D., Verbrugge, D. A., Kubiak, T. J. & Aulerich, R. J. (1995) Dietary exposure of mink to carp from Saginaw Bay, Michigan. 1. Effects on reproduction and survival, and the potential risks to wild mink populations. *Archives of Environmental Contamination and Toxicology,* **28,** 334-343.

Heiden, T. K., Hutz, R. J. & Carvan III, M. J. (2005) Accumulation, tissue distribution, and maternal transfer of dietary 2,3,7,8,-tetrachlorodibenzo-p-dioxin: impacts on reproductive success of zebrafish. *Toxicological Sciences,* **87,** 497-507.

Helgen, J. C., Larson, N. J. & Anderson, R. L. (1988) Responses of zooplankton and *Chaoborus* to temephos in a natural pond and in the laboratory. *Archives of Environmental Contamination and Toxicology,* **17,** 459-471.

Hickey, C. W., Roper, D. S., Holland, P. T. & Trower, T. M. (1995) Accumulation of organic contaminants in two sediment-dwelling shellfish with contrasting feeding modes: Deposit- (*Macomona liliana*) and filter-feeding (*Austrovenus stutchburyi*). *Archives of Environmental Contamination and Toxicology*, 29, 221-231.

Hirsch, M. P. (1998) Bioaccumulation of silver from laboratory-spiked sediments in the oligochaete (*Lumbriculus variegatus*). *Environmental Toxicology and Chemistry*, 17, 605-609.

Holling, C. S. (1959) Some characteristics of simple types of predation and parasitism. *Canadian Entomology*, 91, 385-398.

Hook, S. E. & Fisher, N. S. (2001a) Reproductive toxicity of metals in calanoid copepods. *Marine Biology*, 138, 1131-1140.

Hook, S. E. & Fisher, N. S. (2001b) Sublethal effects of silver in zooplankton: Importance of exposure pathways and implications for toxicity testing. *Environmental Toxicology and Chemistry*, 20, 568-574.

Hook, S. E. & Fisher, N. S. (2002) Relating the reproductive toxicity of five ingested metals in calanoid copepods with sulfur affinity. *Marine Environmental Research*, 53, 161-174.

HSE (1982) Health and Safety Executive Methods for the determination of ecotoxicity. Approved Code of Practice 8. (eds. Her Majesty's Stationary Office, London.

Huang, C. & Sih, A. (1990) Experimental studies on behaviourally mediated, indirect interactions through a shared predator. *Ecology*, 71, 1515-1522.

Inza, B., Rouleau, C., Tjälve, H., Ribeyre, F., Campbell, P. G. C., Pelletier, É. & Boudou, A. (2001) Fine-scale tissue distribution of cadmium, inorganic mercury, and methylmercury in nymphs of the burrowing mayfly *Hexagenia rigida* studied by whole-body autoradiography. *Environmental Research*, 85, 265-271.

Irving, E. C., Baird, D. J. & Culp, J. M. (2003) Ecotoxicological responses of the mayfly *Baetis tricaudatus* to dietary and waterborne cadmium: implications for toxicity testing. *Environmental Toxicology and Chemistry*, **22**, 1058-1064.

Johansson, F. (1995) Increased prey vulnerability as a result of prey-prey interactions. *Hydrobiologia*, **308**, 131-137.

Juliano, S. A. (2001) Nonlinear curve fitting. *Design and analysis of ecological experiments* (eds S. M. Scheiner & J. Gurevitch), pp. 178-196. Oxford University Press.

Kaag, N. H., Foekema, E. M., Scholten, M. C. & van Straalen, N. M. (1997) Comparison of contaminant accumulation in three species of marine invertebrates with different feeding habits. *Environmental Toxicology and Chemistry*, **16**, 837-842.

Kallander, D. B., Fisher, S. W. & Lydy, M. J. (1997) Recovery Following Pulsed Exposure to Organophosphorus and Carbamate Insecticides in the Midge, *Chironomus riparius*. *Archives of Environmental Contamination and Toxicology*, **33**, 29-33.

Kane Driscoll, S. B. & McElroy, A. E. (1997) Elimination of sediment-associated benzo[a]pyrene and its metabolites by polychaete worms exposed to 3-methylcholanthrene. *Aquatic Toxicology*, **39**, 77-91.

Kidd, K. A., Hesslein, R. H., Ross, B. J., Koczanski, K., Stephens, G. R. & Muir, D. C. G. (1998) Bioaccumulation of organochlorines through a remote freshwater food web in the Canadian Arctic. *Environmental Pollution*, **102**, 91-103.

Kiesecker, J. M. (2002) Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? . *Proceedings of the National Academy of Sciences*, **99**, 9900-9904.

Knezovich, J. P., Harrison, F. L. & Wilhelm, R. G. (1987) The bioavailability pf sediment-sorbed organic chemicals: a review. *Water, Air, and Soil Pollution*, **32**, 233-245.

Koivisto, S., Ketola, M. & Walls, M. (1992) Comparison of five cladoceran species in short- and long-term copper exposure. *Hydrobiologia,* **248,** 125-136.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B. & Buxton, H. B. (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. *Environmental Science and Technology,* **36,** 1202-1211.

Kowalik, R. A. & Ormerod, S. J. (2006) Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. *Freshwater Biology,* **51,** 180-191.

Krang, A. S. (2007) Naphthalene disrupts pheromone induced mate search in the amphipod *Corophium volutator* (Pallas). *Aquatic Toxicology,* **85,** 9-18.

Kukkonen, J. & Landrum, P. F. (1995) Measuring assimilation efficiencies for sediment-bound PAH and PCB congeners by benthic organisms. *Aquatic Toxicology,* **32,** 75-92.

Kukkonen, J. & Landrum, P. F. (1996) Distribution of organic carbon and organic xenobiotics among different particle-size fractions in sediments. *Chemosphere,* **32,** 1063-1076.

Lancaster, J. (1996) Scaling the effects of predation and disturbance in a patchy environment. *Oecologia,* **107,** 321-331.

Landrum, P. F. (1989) Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontopeoreia hoyl. Environmental Science and Technology,* **23,** 588-595.

Landrum, P. F., Lee, H. & Lydy, M. J. (1992) Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. *Environmental Toxicology and Chemistry,* **11,** 1709-1725.

Länge, R., Hutchinson, T. H., Scholz, N. & Solbe, J. (1998) Analysis of the ECETOC Aquatic Toxicity (EAT) Database II - comparison of acute to chronic ratios for various aquatic organisms and chemical substances. *Chemosphere*, **36**, 115-127.

Lawrence, A. L. & Mason, R. P. (2001) Factors controlling the bioaccumulation of mercury and methylmercury by the estuarine amphipod *Leptocheirus plumulosus*. *Environmental Pollution*, **111**, 217-231.

Lawton, J. H., Beddington, J. R. & Bonser, R. (1974) Switching in invertebrate predators. *Ecological Stability* (eds M. B. Usher & M. H. Williamson), pp. 141-158. Chapman and Hall, London.

Lawton, J. H., Thompson, B. & Thompson, D. J. (1980) The effects of prey density on survival and growth of damselfly larvae. *Ecological Entomology*, **5**, 39-51.

Lemly, A. D. (2002) Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. *Aquatic Toxicology*, **57**, 39-49.

LeRoy, K. D., Thomas, P. & Khan, I. A. (2006) Thyroid hormone status of Atlantic croaker exposed to Aroclor 1254 and selected PCB congeners. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **144**, 263-271.

Leversee, G. J., Giesy, J. P., Landrum, P. F., Gerould, S., Bowling, J. W., Fannin, T. E., Haddock, J. D. & Bartell, S. M. (1982) Kinetics and biotransformation of benzo(*a*)pyrene in *Chironomus riparius*. *Archives of Environmental Contamination and Toxicology*, **11**, 25-31.

Liess, M. & Schultz, R. (1996) Chronic effects of short-term contamination with pyrethroid insecticide fenvalerate on the caddisfly *Limnephilus lunatus*. *Hydrobiologia*, **324**, 99-106.

Liess, M. & Von der Ohe, P. C. (2005) Analyzing effects of pesticides on invertebrate communities in streams. *Environmental Toxicology and Chemistry*, **24**, 954-965.

Little, E. E. (1990) Behavioral toxicology: stimulating challenges for a growing discipline. *Environmental Toxicology and Chemistry*, **9**, 1-2.

Little, E. E. & Finger, S. E. (1990) Swimming behavior as an indicator of sublethal toxicity in fish. *Environmental Toxicology and Chemistry*, **9**, 13-19.

Liu, X.-J., Ni, I. H. & Wang, W.-X. (2002) Trophic transfer of heavy metals from freshwater zooplankton *Daphnia magna* to zebrafish *Danio reiro*. *Water Research*, **36**, 4563-4569.

Livingstone, D. R. (1998) The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. *Comparative Biochemistry and Physiology Part A*, **120**, 43-49.

Lotufo, G. R. (1998) Lethal and sublethal toxicity of sediment-associated fluoranthene to benthic copepods: application of the critical-body-residue approach. *Aquatic Toxicology*, **44**, 17-30.

Lozano, S. J., O'Halloran, S. L., Sargent, K. W. & Brazner, J. C. (1992) Effects of esfenvalerate on aquatic organisms in littoral enclosures. *Environmental Toxicology and Chemistry*, **11**, 35-47.

Lu, X., Reible, D. D. & Fleeger, J. W. (2004) Relative importance of ingested sediment versus pore water as uptake routes for PAHs to the deposit-feeding oligochaete *Ilyodrilus templetoni*. *Archives of Environmental Contamination and Toxicology*, **47**, 207-214.

Luoma, S. N. & Rainbow, P. S. (2005) Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environmental Science and Technology*, **39**, 1921-1931.

MacCausland, A. & McTammany, M. E. (2007) The impact of episodic coal mine drainage pollution on benthic macroinvertebrates in streams in the Anthracite region of Pennsylvania. *Environmental Pollution*, **149**, 216-226.

Mackay, D. (1982) Correlation of bioconcentration factors. *Environmental Science and Technology*, **16**, 274-278.

Maltby, L. (2006) Environmental risk assessment. *Chemicals in the environment: Assessing and Managing Risk* (eds R. E. Hester & R. M. Harrison). The Royal Society of Chemistry (Great Britain).

Marchini, S., Tosato, M. L., Norberg-King, T. J., Hammermeister, D. E. & Hoglund, M. D. (1992) Lethal and sublethal toxicity of benzene derivatives to the fathead minnow, using a short-term test. *Environmental Toxicology and Chemistry*, **11**, 187-195.

Marcus, J. H., Sutcliffe, D. W. & Willoughby, L. G. (1978) Feeding and growth of *Asellus aquaticus* (Isopoda) on food items from the littoral of Windermere, including green leaves of *Elodea canadensis. Freshwater Biology*, **8**, 505-519.

Maul, J. D., Belden, J. B., Schwab, B. A., Whiles, M. R., Spears, B., Farris, J. L. & Lydy, M. J. (2006) Bioaccumulation and trophic transfer of polychlorinated biphenyls by aquatic and terrestrial insects to tree swallows (*Tachycineta bicolor*). *Environmental Toxicology and Chemistry*, **25**, 1017-1025.

McCarty, L. S. (1986) The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. *Environmental Toxicology and Chemistry*, **5**, 1071-1080.

McCarty, L. S. & Mackay, D. (1993) Enhancing ecotoxicological modeling and assessment. Body residues and modes of action. *Environmental Science and Technology*, **27**, 1719-1728.

McElroy, A., Leitch, K. & Fay, A. (2000) A survey of in vivo benzo[a]pyrene metabolism in small benthic marine invertebrates. *Marine Environmental Research*, **50**, 33-38.

McKinnell, S. A. (1994) *The use of the non-biting midge species Chironomus riparius Meigen (Diptera: Chironomidae) as a toxicity test species with particular reference to surfactants*. Ph.D. Thesis.

Meador, J. P., Adams, N. G., Casillas, E. & Bolton, J. L. (1997) Comparative bioaccumulation of chlorinated hydrocarbons from sediment by two infaunal invertebrates. *Archives of Environmental Contamination and Toxicology*, **33**, 388-400.

Meador, J. P., Casillas, E., Sloan, C. A. & Varanasi, U. (1995) Comparative bioaccumulation of polycyclic aromatic hydrocarbons from sediment by two infaunal invertebrates. *Marine Ecology Progress Series*, **123**, 107-124.

Means, J. C. & McElroy, A. E. (1997) Bioaccumulation of tetrachlorobiphenyl and hexachlorobiphenyl congeners by *Yoldia limatula* and *Nephtys incise* from bedded sediments: effects of sediment- and animal-related parameters. *Environmental Toxicology and Chemistry*, **16**, 1277-1286.

Menge, B. A. (1976) Organization of the New England rocky intertidal community: role of predation, competition, and environmental heterogeneity. *Ecological Monographs*, **46**, 355-393.

Menge, B. A. (1995) Indirect effects in marine rocky intertidal interaction webs: patterns and importance. *Ecological Monographs*, **65**, 21-74.

Menge, B. A. & Sutherland, J. P. (1987) Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *American Naturalist*, **130**, 730-757.

Molles, M. C. & Pietruszka, R. D. (1983) Mechanisms of prey selection by predaceous stoneflies: roles of prey morphology, behavior and predator hunger. *Oecologia*, **57**, 25-31.

Mu, H., Ewald, G., Nilsson, E., Sundin, P. & Wesen, C. (2004) Fate of chlorinated fatty acids in migrating sockeye salmon and their transfer to Arctic grayling. *Environmental Science and Technology,* **38,** 5548-5554.

Munger, C. & Hare, L. (1997) Relative importance of water and food as cadmium sources to an aquatic insect (*Chaoborus punctipennis*): implications for predicting bioaccumulation in nature. *Environmental Science and Technology,* **31,** 891-895.

Munger, C. & Hare, L. (2000) Influence of ingestion rate and food types on cadmium accumulation by the aquatic insect *Chaoborus. Canadian Journal of Fisheries and Aquatic Sciences,* **57,** 327-332.

Munger, C., Hare, L., Craig, A. & Charest, P.-M. (1998) Influence of exposure time on the distribution of cadmium within the cladoceran *Ceriodaphnia dubia. Aquatic Toxicology,* **44,** 195-200.

Murdoch, W. W. (1969) Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecological Monographs,* **39,** 335-354.

Murdoch, W. W., Avery, S. & Smyth, M. E. B. (1975) Switching in predatory fish. *Ecology,* **56,** 1094-1105.

Naddy, R. B. & Klaine, S. J. (2001) Effect of pulse frequency and interval on the toxicity of chlorpyrifos to *Daphnia magna. Chemosphere,* **45,** 497-506.

Nakagawa, Y., Suzuki, T. & Tayama, S. (2000) Metabolism and toxicity of benzophenone in isolated rat hepatocytes and estrogenic activity of its metabolites in MCF-7 cells. *Toxicology,* **156,** 27-36.

O'Gara, B. A., Bohannon, V. K., Teague, M. W. & Smeaton, M. B. (2004) Copper-induced changes in locomotor behaviors and neuronal physiology of the freshwater oligochaete, *Lumbriculus variegatus. Aquatic Toxicology,* **69,** 51-66.

Oakley, B. & Palka, J. M. (1967) Prey capture by dragonfly larvae. *American Zoologist,* **7,** 727-728.

Oaten, A. & Murdoch, W. W. (1975) Switching, functional response, and stability in predator-prey systems. *American Naturalist,* **109,** 299-318.

OECD (1996) Proposal for updating guideline 305. Bioconcentration: flow-through fish test. Guideline No. 305. (eds. Organisation for Economic Co-operation and Development.

Oliver, D. R. (1971) Life history of the Chironomidae. *Annual Review of Entomology,* **16,** 211-230.

Ormerod, S. J. & Tyler, S. J. (1991) Exploitation of prey by a river bird, the dipper *Cinclus cinclus* (L.), along acidic and circumneutral streams in upland Wales. *Freshwater Biology,* **25,** 105-116.

Oros, D. R., Jarman, W. M., Lowe, T., David, N., Lowe, S. & Davis, J. A. (2003) Surveillance for previously unmonitored organic contaminants in the San Francisco Estuary. *Marine Pollution Bulletin,* **46,** 1102-1110.

Pääkkonen, J. P., Rantalainen, A. L., Karels, A., Nikkila, A. & Karjalainen, J. (2005) Bioaccumulation of PCBs in burbot (*Lota lota* L.) after delivery in natural food. *Archives of Environmental Contamination and Toxicology,* **49,** 223-231.

Paine, R. T. (1966) Food web complexity and species diversity. *American Naturalist,* **100,** 65-75.

Palmqvist, A., Selck, H., Rasmussen, L. J. & Forbes, V. E. (2003) Biotransformation and genotoxicity of fluoranthene in the deposit-feeding polychaete *Capitella* sp I. *Environmental Toxicology and Chemistry,* **22,** 2977-2985.

Peckarsky, B. L. (1982) Aquatic Insect Predator-Prey Relations. *Bioscience,* **32,** 261-266.

Peckarsky, B. L., Horn, S. C. & Statzner, B. (1990) Stonefly predation along a hydraulic gradient: a field test of the harsh-benign hypothesis. *Freshwater Biology,* **24,** 181-191.

Peckarsky, B. L. & Penton, M. A. (1989a) Early warning lowers risk of stonefly predation for a vulnerable mayfly. *Oikos,* **54,** 301-309.

Peckarsky, B. L. & Penton, M. A. (1989b) Mechanisms of prey selection by stream-dwelling stoneflies. *Ecology,* **70,** 1203-1218.

Peither, A., Juttner, I., Kettrup, A. & Lay, J. (1996) A pond mesocosm study to determine direct and indirect effects of lindane on a natural zooplankton community. *Environmental Pollution,* **93,** 49-56.

Peixoto, F. (2005) Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. *Chemosphere,* **61,** 1115-1122.

Penry, D. L. & Weston, D. P. (1998) Digestive determinants of benzo[a]pyrene and phenanthrene bioaccumulation by a deposit-feeding polychaete. *Environmental Toxicology and Chemistry,* **17,** 2254-2265.

Perez, M. H. & Wallace, W. G. (2004) Differences in prey capture in grass shrimp, *Palaemonetes pugio,* collected along an environmental impact gradient. *Archives of Environmental Contamination and Toxicology,* **46,** 81-89.

Pickford, K. A., Thomas-Jones, R. E., Wheals, B., Tyler, C. R. & Sumpter, J. P. (2003) Route of exposure affects the oestrogenic response of fish to 4-tert-nonylphenol. *Aquatic Toxicology,* **65,** 267-279.

Pojana, G., Bonfà, A., Busetti, F., Collarin, A. & Marcomini, A. (2004) Estrogenic potential of the Venice, Italy, lagoon waters. *Environmental Toxicology and Chemistry,* **23,** 1874–1880

Pojana, G., Gomiero, A., Jonkers, N. & Marcomini, A. (2007) Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. *Environment International,* **33,** 929-936.

Preston, B. L. (2002) Indirect effects in aquatic ecotoxicology: implications for ecological risk assessment. *Environmental Management,* **29,** 311-323.

Preston, B. L., Cecchine, G. & Snell, T. W. (1999a) Effects of pentachlorophenol on predator avoidance behaviour of the rotifer *Brachionus calyciflorus*. *Aquatic Toxicology*, **44**, 201-212.

Preston, B. L., Snell, T. W. & Dusenbery, D. B. (1999b) The effects of sublethal pentachlorophenol exposure on predation risk in freshwater rotifer species. *Aquatic Toxicology*, **47**, 93-105.

Primus, T., Wright, G. & Fisher, P. (2005) Accidental discharge of brodifacoum baits in a tidal marine environment: A case study. *Bulletin of Environmental Contamination and Toxicology*, **74**, 913-919.

Qiao, P., Gobas, F. A. P. C. & Farrell, A. P. (2000) Relative contributions of aqueous and dietary uptake of hydrophobic chemicals to the body burden in juvenile rainbow trout. *Archives of Environmental Contamination and Toxicology*, **39**, 369-377.

Qiu, X. & Davis, J. W. (2004) Environmental bioavailability of hydrophobic organochlorines in sediments - A review. *Remediation Journal*, **14**, 55-84.

R (2007) R: A language and environment for statistical computing. (eds., R Foundation for Statistical Computing, Vienna, Austria.

Rainbow, P. S. & Wang, W.-X. (2001) Comparative assimilation of Cd, Cr, Se, and Zn by the barnacle *Elminius modestus* from phytoplankton and zooplankton diets. *Marine Ecology Progress Series*, **218**, 239-248.

Rand, G. M. & Petrocelli, S. R. (1985) Fundamentals of aquatic toxicology. (eds. Taylor and Francis, Bristol, USA.

Relyea, R. A. & Hoverman, J. T. (2008) Interactive effects of predators and a pesticide on aquatic communities. *Oikos*, **117**, 1647-1658.

Reynaldi, S. & Liess, M. (2005) Influence of duration of exposure to the pyrethroid fenvalerate on sublethal responses and recovery of *Daphnia magna* straus. *Environmental Toxicology and Chemistry*, **24**, 1160–1164

Richter, S. & Nagel, R. (2007) Bioconcentration, biomagnification and metabolism of 14C-terbutryn and 14C-benzo[a]pyrene in *Gammarus fossarum* and *Asellus aquaticus. Chemosphere,* **66,** 603-610.

Riddell, D. J., Culp, J. M. & Baird, D. J. (2005a) Behavioural responses to sublethal cadmium exposure within an experimental aquatic food web. *Environmental Toxicology and Chemistry,* **24,** 431-441.

Riddell, D. J., Culp, J. M. & Baird, D. J. (2005b) Sublethal effects of cadmium on prey choice and capture efficiency in juvenile brook trout *(Salvelinus fontinalis). Environmental Toxicology and Chemistry,* **24,** 1751-1758.

Rivero, C. L. G., Barbosa, A. C., Ferreira, M. F. N., Dorea, J. G. & Grisolia, C. K. (2008) Evaluation of genotoxicity and effects on reproduction of nonylphenol in *Oreochromis niloticus* (Pisces: cichlidae). *Ecotoxicology,* **17,** 732-737.

Robards, K. & Worsfold, P. (1991) Cadmium: toxicology and analysis. *Analyst,* **116,** 549-568.

Rogers, D. (1972) Random search and insect population models. *Journal of Animal Ecology,* **41,** 369-383.

Rubinstein, N. I., Gilliam, W. T. & Gregory, N. R. (1984) Dietary accumulation of PCBs from a contaminanted sediment source by a demersal fish *(Leiostomus xanthurus). Aquatic Toxicology,* **5,** 331-342.

Russell, R. W., Gobas, F. A. P. C. & Haffner, G. D. (1999) Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. *Environmental Toxicology and Chemistry,* **18,** 1250-1257.

Russom, C. L., Bradbury, S. P., Broderius, S. J., Hammermeister, D. E. & Drummond, R. A. (1997) Predicting modes of toxic action from chemical structure: Acute toxicity in the fathead minnow *(Pimephales promelas). Environmental Toxicology and Chemistry,* **16,** 948-967.

Sanderson, R. A., Eyre, M. D. & Rushton, S. P. (2005) Distribution of selected macroinvertebrates in a mosaic of temporary and permanent freshwater ponds as explained by autologistic models. *Ecography,* **28,** 355-362.

Savage, A. A. (1989) *Adults of the British aquatic Hemiptera Heteroptera: a key with ecological notes.* Titus Wilson & Son Ltd., Kendal.

Schäfer, R. B., Caquet, T., Siimes, K., Mueller, R., Lagadic, L. & Liess, M. (2007) Effects of pesticides on community structure and ecosystem functions in agricultural headwater streams of three biogeographical regions in Europe. *Science of the Total Environment,* **384,** 272-285.

Schriever, C. A., Hansler Ball, M., Holmes, C., Maund, S. & Liess, M. (2007) Agricultural intensity and landscape structure: influences on the macroinvertebrate assemblages of small streams in northern Germany. *Environmental Toxicology and Chemistry,* **26,** 346-357.

Schuler, L. J., Wheeler, M., Bailer, A. J. & Lydy, M. J. (2003) Toxicokinetics of sediment-sorbed benzo[*a*]pyrene and hexachlorobiphenyl using the freshwater invertebrates *Hyalella azteca, Chironomus tentans* and *Lumbriculus variegatus. Environmental Toxicology and Chemistry,* **22,** 439-449.

Schultz, R. & Liess, M. (1995) Chronic effects of low insecticide concentrations on freshwater caddisfly larvae. *Hydrobiologia,* **299,** 103-113.

Schulz, R. & Dabrowski, J. M. (2001) Combined effects of predatory fish and sublethal pesticide contamination on the behaviour and mortality of mayfly nymphs. *Environmental Toxicology and Chemistry,* **20,** 2537-2543.

Selck, H., Palmqvist, A. & Forbes, V. E. (2003) Biotransformation of dissolved and sediment-bound fluoranthene in the polychaete, *Capitella* sp. I. *Environmental Toxicology and Chemistry,* **22,** 2364-2374.

Sherratt, T. N., Roberts, G., Williams, P., Whitfield, M., Biggs, J., Shillabeer, N. & Maund, S. J. (1999) A life-history approach to predicting the recovery of aquatic

invertebrate populations after exposure to xenobiotic chemicals. *Environmental Toxicology and Chemistry*, **18**, 2512-2518.

Simon, O. & Boudou, A. (2001) Direct and trophic contamination of the herbivorous carp *Ctenopharyngodon idella* by inorganic mercury and methylmercury. *Ecotoxicology and Environmental Safety*, **50**, 48-59.

Slooff, W. (1983) Benthic macroinvertebrates and water quality assessment: Some toxicological considerations. *Aquatic Toxicology*, **4**, 73-82.

Slooff, W., Canton, J. H. & Hermens, J. L. M. (1983) Comparison of the susceptibility of 22 freshwater species to 15 chemical compounds. I. (sub)acute toxcity tests. *Aquatic Toxicology*, **4**, 113-128.

Smith, G. M. & Weis, J. S. (1997) Predator-prey relationships in mummichogs (*Fundulus heteroclitus* (L.)): Effects of living in a polluted environment. *Journal of Experimental Marine Biology and Ecology*, **209**, 75-87.

Smith, P. N., Cobb, G. P., Godard-Codding, C., Hoff, D., McMurry, S. T., Rainwater, T. R. & Reynolds, K. D. (2007) Contaminant exposure in terrestrial vertebrates. *Environmental Pollution*, **150**, 41-64.

Smokorowski, K. E., Lasenby, D. C. & Evans, R. D. (1998) Quantifying the uptake and release of cadmium and copper by the opossum shrimp *Mysis relicta* preying upon the cladoceran *Daphnia magna* using stable isotope tracers. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 909-916.

Spitze, K. (1985) Functional response of an ambush predator: *Chaoborus americanus* predation on *Daphnia pulex*. *Ecology*, **66**, 938-949.

Standley, L. J., Sweeney, B. W. & Funk, D. H. (1994) Maternal transfer of chlordane and its metabolites to the eggs of a stream mayfly *Centroptilum triangulifer*. *Environmental Science and Technology*, **28**, 2105-2111.

Stark, J. D. (2005) How closely do acute lethal concentration estimates predict effects of toxicants on populations? *Integrated Environmental Assessment and Management,* **1,** 109-113.

Stark, J. D., Banks, J. E. & Vargas, R. (2004) How risky is risk assessment: the role that life history strategies play in susceptibility of species to stress. *Proceedings of the National Academy of Sciences,* **101,** 732-736.

Strauss, S. Y. (1991) Indirect effects in community ecology: their definition, study and importance. *Trends in Ecology and Evolution,* **6,** 206-210.

Sushchik, N. N., Gladyshev, M. I., Moskvichova, A. V., Makhutova, O. N. & Kalachova, G. S. (2003) Comparison of fatty acid composition in major lipid classes of the dominant benthic invertebrates of the Yenisei river. *Comparative biochemistry and physiology Part B,* **134,** 111-122.

Suter II, G. W. (1993) *Ecological risk assessment.* Lewis Publishers, Chelsea, Michigan, USA.

Tanabe, S., Iwata, H. & Tatsukawa, R. (1994) Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. *Science of the Total Environment,* **154,** 163-177.

Taylor, E. J., Morrison, J. E., Blockwell, S. J., Tarr, A. & Pascoe, D. (1995) Effects of lindane on the predator-prey interaction between *Hydra oligactis* Pallas and *Daphnia magna* Strauss. *Archives of Environmental Contamination and Toxicology,* **29,** 291-296.

Thompson, D. J. (1978) The natural prey of larvae of the damselfly, *Ischnura elegans* (Odonata: Zygoptera). *Freshwater Biology,* **8,** 377-384.

Thomson, J. R., Lake, P. S. & Downes, B. J. (2002) The effect of hydrological disturbance on the impact of a benthic invertebrate predator. *Ecology,* **83** 628-642.

Timmermans, K. R., Spijkerman, E., Tonkes, M. & Govers, H. (1992) Cadmium and zinc uptake by two species of aquatic invertebrate predators from dietary and aqueous sources. *Canadian Journal of Fisheries and Aquatic Sciences,* **49,** 655-662.

Tollrian, R. (1995) *Chaoborus crystallinus* predation on *Daphnia pulex*: can induced morphological changes balance effects of body size on vulnerability? *Oecologia,* **101,** 151-155.

Tosato, M. L., Vigano, L., Skagerberg, B. & Clementl, S. (1991) A new strategy for ranking chemical hazards. Framework and application. *Environmental Science and Technology,* **25,** 695-702.

Trexler, J. C., McCulloch, C. E. & Travis, J. (1988) How can the functional response best be determined? *Oecologia,* **76,** 206-214.

Trexler, J. C. & Travis, J. (1993) Nontraditional regression analyses. *Ecology,* **74,** 1629-1637.

Tschanz, B., Bersier, L. & Bacher, S. (2007) Functional responses: a question of alternative prey and predator density. *Ecology,* **88,** 1300-1308.

van den Brink, P. J., van Donk, E., Gylstra, R., Crum, S. J. H. & Brock, T. C. M. (1995) Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. *Chemosphere,* **31,** 3181-3200.

van den Brink, P. J., van Wijngaarden, R. P. A., Lucassen, W. G. H., Brock, T. C. M. & Leeuwangh, P. (1996) Effects of the insecticide Dursban® 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. *Environmental Toxicology and Chemistry,* **15,** 1143–1153

van Hattum, B. & Montanes, J. F. C. (1999) Toxicokinetics and bioconcentration of polycyclic aromatic hydrocarbons in freshwater isopods. *Environmental Science and Technology,* **33,** 2409-2417.

van Leeuwen, C. J. & Vermeire, T. G. (2007) Risk assessment of chemicals: an introduction. Second edition. (eds. Springer, Dordrecht, The Netherlands.

Veltman, K., Huijbregts, M. A. J., van Kolck, M., Wang, W.-X. & Hendricks, A. J. (2008) Metal bioaccumulation in aquatic species: quantification of uptake and elimination rate constants using physicochemical properties of metals and physiological characteristics of species. *Environmental Science and Technology*, **42**, 852-858.

Verrengia Guerrero, N. R., Taylor, M. G., Davies, N. A., Lawrence, M. A. M., Edwards, P. A., Simkiss, K. & Wider, E. A. (2002) Evidence of differences in the biotransformation of organic contaminants in three species of freshwater invertebrates. *Environmental Pollution*, **117**, 523-530.

Voparil, I. M. & Mayer, L. M. (2000) Dissolution of sedimentary polycyclic aromatic hydrocarbons into the lugworm's (*Arenicola marina*) digestive fluids. *Environmental Science and Technology*, **34**, 1221-1228.

Walde, S. J. (1986) Effect of an abiotic disturbance on a lotic predator-prey interaction. *Oecologia*, **69**, 243-247.

Walker, C. H., Hopkin, S. P., Sibley, R. M. & Peakall, D. B. (1996) *Principles of ecotoxicology*. Taylor & Francis Ltd, London.

Wallace, J. B. (1990) Recovery of lotic macroinvertebrate communities from disturbance. *Environmental Management*, **14**, 605-620.

Wallace, W. G., Brouwer, T. M. H., Brouwer, M. & Lopez, G. R. (2000) Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. *Environmental Toxicology and Chemistry*, **19**, 962-971.

Wallace, W. G. & Lopez, G. R. (1996) Relationship between subcellular distribution in prey and cadmium transfer to a predator. *Estuaries*, **19**, 923-930.

Wallace, W. G. & Luoma, S. N. (2003) Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). *Marine Ecology Progress Series,* **257,** 125-137.

Walters, D. M., Fritz, K. M. & Otter, R. R. (2008) The dark side of subsidies: adult stream insects export organic contaminants to riparian predators. *Ecological Applications,* **18,** 1835-1841.

Wang, W.-X. & Chow, A. T. S. (2002) Benzo[*a*]pyrene absorption and exposure pathways in the green mussel *Perna viridis. Environmental Toxicology and Chemistry,* **21,** 451-458.

Wang, W.-X. & Fisher, N. S. (1999) Assimilation efficiencies of chemical contaminants in aquatic invertebrates: a synthesis. *Environmental Toxicology and Chemistry,* **18,** 2034-2045.

Wang, W.-X. & Wong, R. S. K. (2003) Bioaccumulation kinetics and exposure pathways of inorganic mercury and methylmercury in a marine fish, the sweetlips *Plectorhinchus gibbosus. Marine Ecology Progress Series,* **261,** 257-268.

Wang, X. & Wang, W.-X. (2006) Bioaccumulation and transfer of benzo(a)pyrene in a simplified marine food chain. *Marine Ecology Progress Series,* **312,** 101-111.

Wang, X. H. & Wang, W. X. (2005) Uptake, absorption efficiency and elimination of DDT in marine phytoplankton, copepods and fish. *Environmental Pollution,* **136,** 453-464.

Ward, S., Arthington, A. H. & Pusey, B. J. (1995) The effects of a chronic application of chlorpyrifos on the macroinvertebrate fauna in an outdoor artificial stream system: species responses. *Ecotoxicology and Environmental Safety,* **30,** 2-23.

Warren, P. H. (1989) Spatial and temporal variation in the structure of a freshwater food web. *Oikos,* **55,** 299-311.

Watts, M. M. & Pascoe, D. (2000) A comparative study of *Chironomus riparius* Meigen and *Chironomus tentans* Fabricius (Diptera: Chironomidae) in aquatic toxicity tests. *Archives of Environmental Contamination and Toxicology*, **39**, 299-306.

Weis, J. S. & Weis, P. (1998) Effects of exposure to lead on behavior of mummichog (*Fundulus heteroclitus* L.) larvae. *Journal of Experimental Marine Biology and Ecology*, **222**, 1-10.

Wessel, N., Rousseau, S., Caisey, X., Quiniou, F. & Akcha, F. (2007) Investigating the relationship between embryotoxic and genotoxic effects of benzo[*a*]pyrene, 17[alpha]-ethinylestradiol and endosulfan on *Crassostrea gigas* embryos. *Aquatic Toxicology*, **85**, 133-142.

West, C. W., Ankley, G. T., Nichols, J. W., Elonen, G. E. & Nessa, D. E. (1997) Toxicity and bioaccumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin in long-term tests with the freshwater benthic invertebrates *Chironomus tentans* and *Lumbriculus variegatus*. *Environmental Toxicology and Chemistry*, **16**, 1287-1294.

Weston, D. P. & Mayer, L. M. (1998) Comparison of in vitro digestive fluid extraction and traditional in vivo approaches as measures of polycyclic aromatic hydrocarbon bioavailability from sediments. *Environmental Toxicology and Chemistry*, **17**, 830-840.

Whaley, M., Garcia, R. & Sy, J. (1989) Acute bioassays with benthic macroinvertebrates conducted in situ. *Bulletin of Environmental Contaminantion and Toxicology*, **43**, 570-575.

Wilding, J. (2004) *PhD Thesis. Laboratory single-species toxicity tests: predictive ability, sensitivity and ecological relevance.* The University of Sheffield, U.K.

Williamson, C. E., Stoeckel, M. E. & Schoeneck, L. J. (1989) Predation risk and the structure of freshwater zooplankton communities. *Oecologia*, **79**, 76-82.

Wogram, J. & Liess, M. (2001) Rank ordering of macroinvertebrate species sensitivityto toxic compounds by comparison with that of *Daphnia magna. Bulletin of Environmental Contamination and Toxicology,* **67,** 0360-0367.

Wollenberger, L., Halling-Sørensen, B. & Kusk, K. O. (2000) Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna. Chemosphere,* **40,** 723-730.

Wootton, J. T. (1994) The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecology and Systematics,* **25,** 443-466.

Wootton, J. T. (2002) Indirect effects in complex ecosystems: recent progress and future challenges. *Journal of Sea Research,* **48,** 157-172.

Xu, Y. & Wang, W. X. (2002) Exposure and potential food chain transfer factor of Cd, Se and Zn in marine fish *Lutjanus argentimaculatus. Marine Ecology Progress Series,* **238,** 173-186.

Young, J. O. (1981) A comparative study of the food niches of lake-dwelling triclads and leeches. *Hydrobiologia,* **84,** 91-102.

Zasada, K. A. & Smith, E. H. (1981) Freshwater invertebrates of the Sheffield district. *Sorby record Special Series No. 4* (eds. Sorby Natural History Society, Sheffield and Sheffield City Museums, Sheffield.

Zhou, T. & Weis, J. S. (1998) Swimming behaviour and predator avoidance in three populations of *Fundulus heteroclitus* larvae after embryonic and/or larval exposure to methylmercury. *Aquatic Toxicology,* **43,** 131-148.