Characterizing the trophic niche of non-native *Pseudorasbora parva* and the consequences for native fish communities

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Abstract

Introductions of non–native fish can be a key driver of environmental change that has major implications for biodiversity and ecosystem functioning, including the adverse consequences of increased inter-specific competition for native fishes. Here, the consequences of an introduction of a model non-native fish on the trophic position and trophic niche size of native fishes were investigated, along with assessment of the mechanisms of resource partitioning or sharing between the co-existing species. The model non-native fish was topmouth gudgeon *Pseudorasbora parva*, a highly invasive fish in Europe that originates from Southeast Asia. The study was completed over three spatial scales: experimental mesocosms over 100 days, small and established aquaculture ponds where *P. parva* had co-existed with native species for approximately 8 years, and wild ponds colonized by *P. parva*. Given difficulties in using stomach contents analysis for small cyprinid fishes, stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) was used to determine the effects of *P. parva* on the trophic ecology of co-existing fishes.

The experimental mesocosms used *P. parva* and three native fishes in allopatric and sympatric contexts. At the end of the 100 day period, in all cases it

was revealed that there was strong trophic niche divergence between *P. parva* and the sympatric native fishes, with no evidence of food resource sharing, and with *P. parva* always feeding at a significantly lower trophic level. For all species, trophic niche sizes were reduced in sympatry when compared with allopatric contexts. This pattern was also observed in the small aquaculture ponds, with strong divergence between *P. parva* and all co-existing species, with no sharing of food resources between species, and with *P. parva* again always feeding at lower trophic levels than the native fishes. In four wild fish communities, the situation was more complex, as *P. parva* was present in multi-species communities that also contained other non-native fishes. In these communities, there was some evidence of trophic niche overlap between *P. parva* and the other fishes, although the extent of this was always low. Moreover, *P. parva* tended to have a limited trophic niche breadth compared with the other fishes, with little evidence suggesting *P. parva* was strongly influencing food web structure and the feeding relationships of the other species.

In entirety, these outputs suggest that introductions of *P. parva* rarely compete directly with native fishes for food resources, with trophic niche divergence more evident. This suggests that following *P. parva* introduction, their consequent resource partitioning with native fishes avoids the adverse consequences of inter-specific competition, promoting their co-existence in the community. Given that current risk assessments for *P. parva* tend to indicate high risks to native fishes due to impacts including the adverse consequences of inter-specific competition, then these outputs might have important implications for their risk management.

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Author’s declaration

I confirm that the work presented in this thesis is my own work.

1. INTRODUCTION

The subject of this thesis is the trophic relationships that develop within native fish communities when a non-native fish is introduced and establishes an invasive population. To do this, the research uses the Asian fish topmouth gudgeon *Pseudorasbora parva* as its model species. In this first chapter, the research area is introduced and discussed in order to develop the project aim and objectives. The issues relating to introduced non-native species and fishes are outlined, the general ecological theory is discussed, the model species is introduced and the project aims and objectives are outlined.

1.1 Introductions of non-native species

The rate of introductions of non-native species has more than doubled at the global scale compared with estimates of nearly three decades ago (Gozlan et al. 2010a). These introductions of non-native species have principally been the result of human activity, usually associated with enhancing ecosystem services such as agriculture and aquaculture, and have been both deliberate and accidental (Vitousek et al. 1997; Koo and Mattson 2004; Gozlan et al. 2010 a,b). Despite this large volume of introductions, the majority of introduced species fail to establish sustainable populations (Meffe 1991; Marchetti et al. 2004). This is aligned to the 'tens rule' of Williamson (1996) in which only 10 % of introduced species establish and 10 % of established species develop invasive populations, a finding that is also consistent with more recent studies (e.g. Gozlan 2008).

When a non-native species establishes a sustainable population then the receiving ecosystem is at risk of ecological changes as the new population integrates into the receiving ecosystem (Gozlan and Newton 2009). Whilst many of these species might only result in minor ecological consequences (Gozlan 2008), there are numerous examples where more severe ecological consequences have developed in the receiving ecosystem, such as detrimental interactions with native species or the alteration of ecosystem functioning (Gozlan et al. 2010a). For example, where the introduced species is taxonomically similar to the native species then reproduction can result in hybridization and the loss of genetic integrity (Hänfling et al. 2005). Introduced species can also introduce novel parasites into the receiving ecosystem that spill-over into the native populations; the native species might then be vulnerable to infections through a lack of immunity due to their lack of co-evolution with the parasite that results in poor anti-parasite behaviours and low immune responses (Gozlan et al. 2010a). The establishment of a new population also means a new species is present in the food web and this might then have direct trophic consequences for other populations in the community through increased grazing and/or predation pressure, and through the increased sharing of food resources leading to competitive processes that can result in detrimental ecological consequences (e.g. reduced somatic growth and reproductive investment; Gozlan et al. 2010a). The integration of this new species into the food web can also potentially cause shifts in food web structure and aspects of ecosystem functioning, such as decomposition rates (Cucherousset and Olden 2011).

There are some good examples of where an established non-native species has caused dramatic negative consequences in the receiving ecosystem. The population of the crayfish native to the UK, *Austropotamobius pallipes*, has been adversely impacted through the spread of the fungal pathogen *Aphanomyces astaci* that was introduced with the North American signal crayfish *Pacifastacus leniusculus* (Reynolds 1988). The signal crayfish was introduced as a new species in aquaculture (Richards 1983; Lowery and Holdich 1988) and it acts as a healthy host for *A. astaci;* when transmitted to native crayfish, however, it causes crayfish plague (Unestam 1976) that causes high mortality rates in native crayfish and threatens population sustainability and even species extinction (Alderman et al. 1990; Taugbol and Skurdal 1999). The Zebra mussel, *Dreissena polymorpha*, is native to the Black and Caspian Sea region and has been introduced to many lakes and rivers of Central and Western Europe, and North America, via ship ballast water (Mellina and Rasmussen 1994; Karatayev et at. 1997). Their impacts include reduced population abundances of native unionid mussels and substantial changes in both water quality (including increased water clarity) and ecosystem functioning (Hebert et al. 1989; Schloesser and Nalepa 1994; Nalepa et al. 1996; Ricciardi et al. 1996, 1998; Martel et al. 2001). The improvement of water clarity in rivers and lakes in Europe caused by *D. polymorpha* leads to deeper light penetration and enhances benthic photosynthesis (Vanderploeg et al. 2002). This then affects the distribution and community composition of submerged macrophytes (Wetzel 1983; Chambers and Kalff 1985).

A further example of where a non-native species has caused substantial consequences for native species is the case of the Grey squirrel, *Sciurus carolinensis*. Native to North America, it has been introduced into Europe, South Africa and Australia (Davis 1950; Corbet 1978; Seebeck 1984; Gurnell 1987), causing some substantial economic and ecological impacts (Gurnell 1996). In Britain, their introduction has damaged timber through their bark-stripping and has caused the displacement of European red squirrel *Sciurus vulgaris* through both competitive processes and the introduction of a novel pathogen (Gurnell 1994). Thus, whilst the 'tens rule' suggests only a relatively small proportion of introduced species will develop invasive populations, some of these invasive species have the capacity to cause substantial ecological and economic impacts.

1.2 Introductions of non-native freshwater fish

1.2.1 Introduction pathways

As with non-native species generally, the rate of introductions of non-native fish have increased dramatically in recent decades (Vitousek et al. 1997; Koo and Mattson 2004). This rate has been estimated as having doubled in the last 30 years as a result of increased global trade (Gozlan et al. 2010a). There are a number of introduction pathways for non-native fish, with Gozlan et al. (2008) suggesting that these were aquaculture (providing 51 % of introduced fishes), the ornamental fish trade (21 %), sport fishing (12 %) and fisheries (7 %). Figures from the FAO suggest slightly different proportions (Fig. 1.1). In addition, the motives for introducing fish vary from country to country with, for example, the brown bullhead *Ameiurus nebulosus* introduced for aquaculture in Poland and

Germany, but was introduced for improving wild fish stocks in Finland (Holčík 1991).

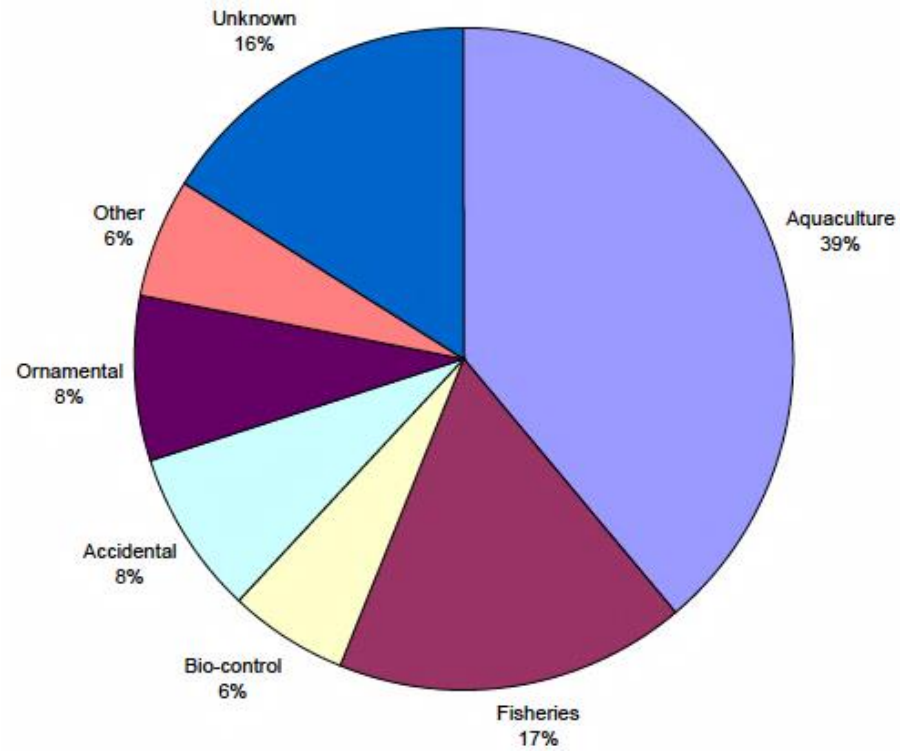

Figure 1.1 Reasons for introductions of aquatic species, as a percentage of DIAS records. Source: FAO Fisheries Department – Database on introductions of aquatic species (DIAS) (FAO, 1990).

The aquaculture introduction pathway has been responsible for the introduction of a number of invasive fishes, including non-native salmonid fishes (such as rainbow trout *Oncorhynchus mykiss*), species of large cyprinid fishes including common carp *Cyprinus carpio* and grass carp *Ctenopharyngodon idella*, and species of the Cichlidae family, such as the Nile tilapia *Oreochromis niloticus* (Lever 1996). Some of these species can have substantial consequences for receiving environments, with introductions of *C. carpio* associated with

reduced water quality and degraded aquatic habitats (McCrimmon 1968; Roberts et al. 1995; King et al. 1997; Koehn et al. 2000, Jones and Stuart 2006).

Fishes from the ornamental introduction pathway, such as the goldfish *Carassius auratus*, have been widely distributed for breeding in ornamental fish ponds. In England, goldfish are also present in the wild through disposal of unwanted pet fish and through the enhancement stocking of fishing ponds (Wheeler 2000; Copp et al. 2005). Regarding the sport fishing introduction pathway, some of the most common introduced species at the global scale include the largemouth bass *Micropterus salmoides*, a North American species that has been introduced across much of Africa and Europe (Britton et al. 2010a). Their impacts on native fish can include decreased abundance of small native fishes, as *M. salmoides* is a piscivorous species (Gratwicke and Marshall 2001).

1.2.2 Ecological consequences of non-native fish

In Section 1.2.1, the impacts of some important non-native fishes introduced through the primary introduction pathways were mentioned briefly. Indeed, introduced fishes can have substantial consequences for native species and ecosystems through a variety of mechanisms and processes. These are related to aspects including increased predation pressure (Arthington 1991), habitat alteration (Manchester and Bullock 2000), lost of genetic integrity (Cambray 2003) in native fish species, introduced pathogens (Cambray 2003; Gozlan et al. 2010a) and increased inter-specific competition (Harwood et al. 2002) in the native fish community.

Increased predation pressure

Introduced fish species can reduce the population of resident species through increasing predation, with this including predation on other fish species and on invertebrate communities (Arthington 1991). The introduction of Nile perch (*Lates niloticus*, Latidae) in Lake Victoria (Ogutu-Ohwayo 1990; Ogutu-Ohwayo and Hecky 1991; Pitcher and Bundy 1994; Pitcher 1995) is a strong example of how increased predation pressure can impact indigenous fish communities. Following their introduction in 1963 in an attempt to increase the economic value and use of the lake's fisheries, the population of Nile perch boomed in the 1980s where it contributed to very high fishery catches (Cucherousset and Olden 2011). This was, however, also coincident with an apparent large decline in the number of haplochromine fishes in the lake (Ogutu-Ohwayo 1990; Witte et al. 1992; Hauser et al. 1998). Whilst this decline was also related to more general environmental changes arising from the boom in Nile perch catches that lead to large increases in human populations and their associated disturbances around the lake, the increased predation pressure on the haplochromine fishes is still believed to have been a major factor in their decline (Achieng 1990).

Habitat alteration

When introduced into a new ecosystem, ecosystem engineering species (Cucherousset and Olden 2011) can cause substantial alterations in the receiving environment. These have the potential to alter biogeochemical, hydrological and geomorphological processes of the ecosystem. Examples of freshwater fish that act in this manner include *C. carpio* (Koehn 2004; Pipalova 2006). They disrupt the submerged macrophyte communities through their benthic foraging

behaviours, increasing nutrient availability for algae and increasing water turbidity; ultimately, they have the capacity to shift lakes from oligotrophic to eutrophic status (Koehn 2004). Another example is the Chinook salmon *Oncorhynchus tshawytscha* which can decrease the abundance of mosses, algae, and macrophytes in river channels that results in substantial geomorphic modification of pool-riffle sequences (Field-Dodgson 1987).

Loss of genetic integrity

An ecological consequence arising from introduced fishes is the loss of genetic integrity in native species that can occur when a closely related species is introduced and is able to interbreed with the native fish (Hänfling et al. 2005). Hybridization related to introduced fishes accounts for 17% of known fish hybridization (Scribner et al. 2001). Consequently, whilst its effects might not be widespread, its consequences could be substantial at more local spatial scales (Allendorf 1991; Allendorf et al. 2004; D'Amato et al. 2007). An example is the hybridization that occurs in the UK between the native cyprinid fish crucian carp *Carassius carassius* and its invasive congener *C. auratus*, native to East Asia (Hänfling et al. 2005). The consequence of goldfish introduction for crucian carp is rapid population declines as a result of their populations becoming composed of fertile hybrids that are then able to reproduce with other hybrids, as well as the original two species, impacting the integrity of the crucian carp gene pool (Hänfling et al. 2005; Tóth et al. 2005). Nevertheless, other factors have also been related to the decline of crucian carp in the UK, including habitat loss and introduced parasites (Gozlan 2008).

Introduced pathogens

The transfer of diseases and parasite from non-native fishes to native fishes can represent one of the most severe threats for native fishes from an introduced species (Boxshall and Frear 1990; Kennedy et al. 1991; Clifford et al. 1998; Kirk 2003; Beyer et al. 2005; Gozlan et al. 2005, 2006). When free-living species are introduced, they also potentially introduce their parasites and whilst the number of parasites that are introduced tends to be low overall through a mechanism known as 'enemy release' (Torchin et al. 2003), those that are introduced can have substantial consequences for native hosts (Kirk 2003). Novel fish pathogens have been introduced into native fish communities in Europe (Holčík 1991), Asia, the Americas (Fernando 1991; Krueger and May 1991) and Australia (Arthington 1991). Native fishes are at risk through their lack of co-evolution with the pathogen, resulting in poor anti-infection behaviours and low auto-immune responses (Gozlan et al. 2010a). Should the introduced pathogen 'host-switch' to native fishes then negative consequences include both lethal (i.e. high mortality rates) and sub-lethal consequences, including modified behaviours, shifts in life history traits and energetics, and reduced fitness (Gozlan et al. 2010a). An example of an introduced pathogen in Europe that has been able to host-switch is the nematode parasite *Anguillicolloides crassus.* Its European introduction was via the aquaculture trade in Japanese eel *Anguilla japonica* in the early 1980s and it arrived in the UK via Billingsgate market (Kirk 2000). The parasite is native to *A. japonica* but in European freshwaters has been able to infect European eel *Anguilla anguilla* where both parasite abundance and parasite prevalence can be high in infected populations (Kirk 2003). The parasite infects the swim bladder, destroying its functionality, with this hypothesized as a

factor in the European decline of eels as it potentially impedes their ability to return to their spawning grounds in the Southern Atlantic Ocean (Starkie 2003).

Increased competition in native fish community

Following an introduction, non-native fish must access adequate food resources if they are to survive, reproduce and establish, i.e. develop invasive populations (Jackson and Britton 2013). In accessing these food resources, there is a likelihood these resources will be shared with native fishes, potentially resulting in interspecific competition (Gozlan et al. 2010a). For competitive effects to be detected, there is the requirement for resource sharing to be measured between the invader and at least one native species, demonstration that these resources are limiting and so actually produce a competitive effect, and quantification of a fitness-related consequence in at least one of the competitors (Crowder 1990). Competitive mechanisms between non-native and native fish have been outlined in a number of reviews (e.g. Gozlan et al. 2010a, Cucherousset and Olden 2011). For the purposes of this research project, these feeding interactions are crucial in the development of how introduced fish affect food web structure. Consequently, the next section will focus on the ecological theory relating to the development of trophic niches, as this is important underpinning information.

1.3 Trophic niche theory

In Section 1.2.2, it was outlined that increased competition for food resources can be a potential consequence arising from the establishment of a non-native fish population. However, this is a rather simplistic perspective given that niche

theory predicts that rather than resource sharing, species-specific specialization in resource use is a primary mechanism that allows the stable coexistence among competing species within a local community (Chesson 2000, Kylafis and Loreau 2011). It thus suggests it is specialization - rather than generalization - in the exploitation of food resources that is important as it is this that enhances the coexistence of species as it reduces interspecific competition (Gabler and Amundsen 2010; Kleynhans et al. 2011). Thus, rather than an introduced fish increasing inter-specific competition for food resources that ultimately leads to the decline of native species, trophic niche theory suggests that instead, the competing species will segregate their resource use, reduce the extent of competition and thus the introduced and native species will be able to co-exist in the system by exploiting different food resources.

Thus, resource partitioning relates to how sympatric species differ in their resource use (Toft 1985) and has been used to study how species with similar functional traits and diet composition coexist by avoiding the negative consequences of interspecific competition (MacArthur 1965; Schoener 1974; Roughgarden 1976). Resource partitioning can be a challenging subject to study in fishes, for their growth is indeterminate, resulting in a complex size structure in many populations and communities (Nilsson 1955; Werner 1977; Werner and Gilliam 1984). This means that differences in diet composition between species that appear to be resource partitioning might instead relate to ontogenetic dietary differences that stem from differences in, for example, gape size and the ability of individual fish to capture and handle food items of different sizes (Werner and Gilliam 1984). Nevertheless, when non-native and native species are in sympatry

then resource partitioning can develop in aspects including food, habitat utilization and/or time segregation (e.g. Pianka 1973; Schoener 1974). Within fish communities, trophic segregation tends to be more important than habitat partitioning, primarily because the latter can be difficult to determine due to factors including sampling bias and the difficulty in determining the importance of separation along spatial and trophic dimensions equally (i.e. trophic partitioning and habitat partitioning might in effect be measured as the same process even if the reason for the spatial and/or temporal segregation is driven by reducing inter-specific competition (Ross 1986)).

There are numerous examples of trophic partitioning in fishes generally (e.g. Sibbing and Nagelkerke 2000; Layman et al. 2007; Jackson et al. 2013; Sepulveda et al. 2012). In the native fishes of the Great Lakes of North America, partitioning among the species was more related to segregation in diet than in habitat (Crowder et al. 1981). By contrast, the non-native species differed more by their habitat utilization, suggesting that their diet composition was less flexible than for native species (Crowder et al. 1981). The importance of partitioning in terms of habitat and food resources can also be related to the characteristics of the ecosystem, with habitat often playing important roles in large freshwater systems (Mendelson 1975; Baker and Ross 1981). Moreover, the prevailing conditions that the species are being exposed to can affect partitioning, with Zaret and Rand (1971) and Greenfield et al. (1973) revealing that when food resources were limiting during the dry season in Central American rivers, the overlap in the trophic niche of stream fishes was much reduced. Nilsson (1955) also recorded reduced trophic overlap between trout

Salmo trutta and charr *Salvelinus alpinus* when food abundance was limiting. This is a contrast to salt marsh and estuarine fishes that have demonstrated increased overlap in diet during periods of reduced food abundance (Harrington and Harrington 1961; Thorman 1982).

When an introduced predator establishes in a new ecosystem, they have the potential to cause a dramatic decline in the density of the native prey populations (Elton 1958; Preisser et al. 2005; Salo et al. 2007). This effect can shift the trophic niche (TN) of native species and has the potential to then reduce the growth and survival rates of the native species, and result in their population decline. For example, Correa and Hendry (2012) explored how the density of invasive salmonid fishes altered the TN of the native populations of *Galaxias platei* in lakes of Chilean Patagonia. The invasive brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* were both acting as apex predators (Cambray 2003; Casal 2006) whose predation pressure was having negative impacts on the galaxiid populations (McDowall 2006; Young et al. 2010; Correa and Hendry 2012). From a food web perspective, the result was a shift in the trophic height of the galaxiid population, as the predation by the invasive salmonids prevented their normal ontogenetic shift to feeding on items higher in the food web that would normally have facilitated their faster growth and larger body sizes (Correa and Hendry 2012). Overall, the outputs revealed the trophic level of the invasive salmonids was higher in the presence of *G. platei*, whilst the trophic position of *G. platei* was reduced in the presence of the salmonids (Correa and Hendry 2012).

In summary, ecological theory on trophic niches suggest that following an introduction of a non-native fish into a new ecosystem, where that species initially exploits food resources that are shared with native species, then partitioning in terms of diet composition and habitat utilization might develop. Whilst this would assist their coexistence by minimizing the extent of the inter-specific competition, this would also result in shifts in food web structure and thus the trophic position of the species might be different between their allopatric and sympatric contexts.

1.4 Stable isotope ecology

1.4.1 Analysis of the diet composition of fishes

The analysis of competitive relationships - or resource partitioning - in fishes can be inherently difficult to assess in wild situations as resource abundance and fitness metrics are often challenging to measure in many field situations (Galster et al. 2012). Many fish studies infer competitive interactions from the sharing of food resources alone, with this often identified through the completion of gut contents analysis (GCA; e.g., Rosecchi et al. 1993). The use of GCA can, however, be problematic in fishes unless long-term studies are completed, with issues arising over, for example, the occurrence of empty stomachs in fish samples, the requirement for large sample sizes in a destructive technique, sampling periodicity affecting diet composition due to diurnal differences in feeding behaviour and prey availability, and difficulties of identifying macerated items (e.g. macro-invertebrate and zoo-plankton species) to an acceptable taxonomic level in fishes with pharyngeal teeth and agastric stomachs (Britton et

al. 2010a). The recent development of analyses of trophic relationships using metrics of stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ now enables the extent of resource sharing between sympatric species (such as an introduced fish and native fishes) to be quantified more easily (Cucherousset et al. 2012; Jackson et al. 2012). Moreover, compared with GCA, stable isotope analysis provides a longer-term perspective on resource acquisition and trophic relationships, making it more advantageous to use in the context of determining metrics such as trophic niche width and the extent to which these overlap between species (Grey et al. 2009; Jackson et al. 2012). Consequently, stable isotopes have been used widely in ecological studies in the last 10 years to answer a series of questions relating to aspects such as food web structure, energy flux through ecosystems and how introduced species integrate into native food webs (e.g. Grey 2006; Fry 2006; Cucherousset et al. 2012).

The initial applications of stable isotope analysis (SIA) in food-web analyses provided important advances in the determination of the trophic relationships of consumers and their resources (Haines and Montague 1979; Peterson et at. 1985; Zieman et at. 1984). For ecological research, there are three main elements that used in SIA: carbon, nitrogen and sulphur. The carbon-12 isotope is the main form of carbon (98.9 %) and a small fraction (1.11 %) is carbon-13; nitrogen is present mainly as the nitrogen-14 isotope (99.64 %), with nitrogen-15 making up the remainder (0.36 %). Sulphur exists in four forms. The most common is sulphur-32 (95.02 %), the other contributions are also made from sulphur-34 (4.21 %), sulphur-33 (0.75 %) and sulphur-36 (0.02 %) (Jardine et al. 2003). The most common stable isotope used in freshwater ecology are carbon ($\delta^{13}C$) and

nitrogen ($\delta^{15}N$) because their relative isotopic similarities between diet and consumer of $\delta^{13}C$ (~1 ‰; DeNiro and Epstein 1978; Post 2002b) allows identification of the diet source of consumers, and the predictable incremental increase of $\delta^{15}N$ (~3.4 ‰) indicates an increase in trophic level (DeNiro and Epstein 1981; Minagawa and Wada 1984; Post 2002b) (Fig. 1.2). The traditional approach in analyzing these data is through stable isotope biplots, where $\delta^{13}C$ is plotted on the X-axis and $\delta^{15}N$ on the Y-axis. The predictable increases in both stable isotopes then allow the trophic level of each species to be determined and the trophic relationships between sympatric species to be inferred (Fig. 1.2). Indeed, these bi-plots have been used to calculate trophic position of consumers (Vander Zanden et al. 1997; Post et al. 2000; Post 2002a, Layman et al. 2005), the relative contribution of prey items to consumers (Vander Zanden and Vadeboncoeur 2002), niche shifts (Post 2003), and intraspecific diet variability (Bolnick et al. 2003; Bearhop et al. 2004; Matthews and Mazumder 2004).

In more recent years, the analysis of stable isotope data in food web analysis has progressed from relatively simple relationships presented on bi-plots (e.g. Fig. 1.2) to more quantitative analyses that provide stable isotope metrics based on community relationships (e.g. Layman et al. 2007; Jackson et al. 2011; Jackson et al. 2012). In doing so, the development of these metrics has provided increased insights into the trophic relationships of sympatric species, allowing better quantification of the extent of a species' trophic niche (e.g. trophic niche breadth through standard ellipse area, SEA) and how this might overlap with a sympatric species (i.e. indicating the sharing of resources; Jackson et al. 2012). In addition, mixing models have been developed that allow the estimation of the

diet composition of consumers from their isotope data and those of their putative prey items. The stable isotope metrics that will be used in this research are defined and explained fully in Materials and Methods (Chapter 2).

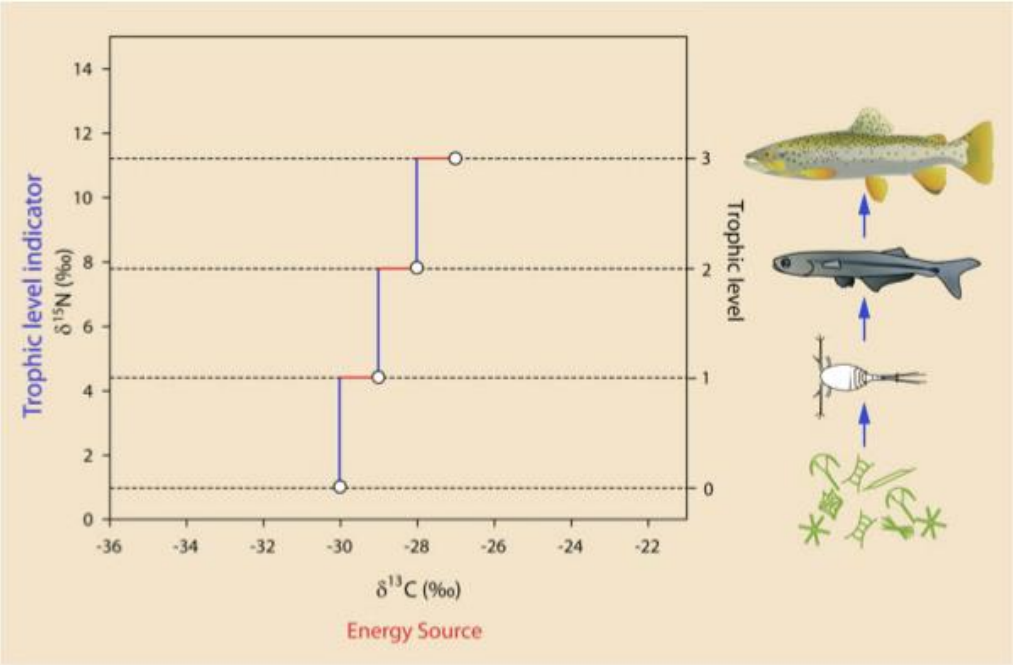

Figure 1.2. A stable isotope bi-plot showing mean values of $\delta^{15}N$ and $\delta^{13}C$ of freshwater producers and consumers, where the blue and red lines indicate the predictive increases in trophic levels according to the stable isotope values (Source: C. Harrod).

1.4.2. Stable isotope analysis for studying invasive fishes

Stable isotope analyses have provided a highly useful tool for studying how non-native fishes integrate into native food webs and, for example, might share resources with native fishes. For instance, introduced fishes of the Salmonidae family, including *Oncorhynchus* spp., *Salmo* spp. and *Salvelinus* spp., have been shown to alter food web structure firstly by occupying high trophic positions and

secondly through then impacting prey fish abundances that has cascading effects on the phyto- and zoo-plankton communities (e.g., Vander Zanden et al. 1999). Initial applications of SIA to invasive fish ecology revealed that the predation by two introduced fishes, the small-mouth bass, *Micropterus dolomieu* and rock bass *Ambloplites rupestrisi,* in North America were responsible for both decreased diversity and abundance of littoral prey fish (Vander Zanden et al. 1999). The use of the stable isotopes indicated lower trophic positions of the prey fishes in lakes with the introduced fishes compared with lakes without them.

The utility of using stable isotope analysis in assessing the trophic relationships of other non-native fishes has been demonstrated in a number of studies. Syväranta et al. (2009) revealed that the contribution of native anadromous fish species, such as Allis shad, *Alosa alosa*, to the diet of the non-native European catfish *Silurus glanis* in the Garonne River (South-western France) was high when the shad returned to the river for spawning, albeit their contribution in diet was highly variable between individuals, although this was not correlated with the sizes of the catfish. For *C. carpio*, stable isotopes have been used to determine their diet composition. For example, Britton et al. (2007) revealed their diet in Lake Naivasha, Kenya, was varied but included predation of the invasive crayfish *Procambarus clarkii*. Matsuzaki et al. (2010) combined SIA and molecular tools to quantify the functional consequences of hybridization between native *C. carpio* and introduced domesticated *C. carpio* in Lake Kasumigaura, Japan. This revealed a significant correlation between values of $\delta^{13}C$ in individual fish and their degree of hybridization. By contrast, there was no similar relationship for $\delta^{15}N$. This suggested that *C. carpio* with higher levels

of hybridization used littoral habitats more frequently than other individuals (Matsuzaki et al. 2010).

Consequently, stable isotope analyses using data on $\delta^{13}C$ and $\delta^{15}N$ is now a well-established ecological tool used for the study of food webs, including the analysis of trophic relationships between species in the communities. In invasive fish ecology, it has been applied to a wide number of species and case studies, providing insights into the diet of piscivorous non-native fish and the diets and feeding relationships of more generalist species. It provides a different perspective on diet than GCA and is also able to overcome many of the inherent problems associated with that method.

1.5 Topmouth gudgeon *Pseudorasbora parva*

1.5.1 Pseudorasbora parva as the model species

The basis of this research project is investigating the food web and trophic consequences of invasive fishes (Section 1.2), in relation to trophic niche theory (Section 1.3), with the methodology to be used being stable isotope analysis (Section 1.4). Rather than investigating a number of invasive fishes, the research will focus on the topmouth gudgeon *Pseudorasbora parva*, a fish species that is native to South East Asia and is highly invasive across Europe, and is now also present in the Middle East and North Africa (Gozlan et al. 2010b). The purpose of this section is to provide some background information on the species and the current state of knowledge on their invasion so that the rationale for their use as the sole model invasive fish in the research will become apparent.

1.5.2 European invasion of Pseudorasbora parva

Pseudorasbora parva is native to Japan, China, Korea and the River Amur basin and, because of its small size (< 10 cm; Fig. 1.3) is now considered a pest fish across much of Europe (Pinder et al. 2005). The first European recording of *P. parva* was in Romania in 1960 (Banarescu 1964), with this being an accidental introduction through their contamination of batches of Asian carp species being moved from China into Eastern Europe for aquaculture. Following that initial introduction, *P. parva* was subsequently detected in many regions of Romania in the 1960s, including the Danube delta. This enabled it to disperse along the river, with the species recorded in Hungary in 1963. The Danube provided a strong dispersal pathway for the introduction of *P. parva* into many other European countries (Bianco 1988; Gozlan et al. 2002; Pollux and Korosi 2006). In addition to this natural dispersal mechanism, accidental introductions via the movement of other fish in aquaculture have enabled their introduction into countries such as Spain (Elvira and Almodóva 2001). They have now achieved pan-European distribution (Gozlan et al. 2010b).

In the UK, *P. parva* was first recorded in Southern England at an aquaculture site in 1986 and then in the wild in 1996 (Domaniewski and Wheeler 1996; Gozlan et al. 2002). Records have since increased, with 32 waters having recordings of their introduction, although these have been reduced through management operations that have extirpated some populations through chemical treatment (Britton et al. 2010b). The majority of these records are in lakes that are used for angling, with *P. parva* having been introduced accidentally into these during the enhancement stocking of fish such as *C. carpio* (Britton et al.

2007). The mechanism tends to be the batch of fish for stocking has been accidentally contaminated by *P. parva* and, due to their small size, they remain undetected and are released into the lake with the other fishes (Davies et al. 2013). They can then form very large populations comprising of fish that are mainly below 50 mm in body size through their life history and reproductive traits that include rapid growth to sexual maturation, maturation at ages < 1 year old and multiple spawning events through the reproductive season (Pinder et al. 2005). This raises ecological concerns relating to their potential for sharing food resources with native fishes (Section 1.5.3) and their status as a healthy host of an obligate inter-cellular eukaryote pathogen *Sphaerothecum destruens* whose transmission to a range of other cyprinid and salmonid fishes can cause high mortality rates (Gozlan et al. 2005). Given the focus of this research on food web issues, then *S. destruens* will not be discussed further in the thesis.

Figure 1.3 Top: Sexually mature *Pseudorasbora parva*, where the fish at the top of the photo is a male and the one below is a female. Bottom: A 25 m micro-mesh seine net containing 63 kg of *P. parva*, sampled from a fishing pond in the West Midlands of England. Source: R. Britton.

1.5.3 Trophic ecology of Pseudorasbora parva

Studies on the trophic ecology of *P. parva* have been rather limited, with the majority focusing on the use of GCA. These have revealed that invasive *P. parva* do exploit common food resources that are also exploited by native fishes, such as Chironomid larvae (e.g. Rosecchi et al. 1993; Declerck et al. 2002). Whilst these studies have inferred that the species must thus be competing, no evidence is presented that suggests the sharing of the food resources is resulting in limiting food availability or that adverse effects are occurring in the species concerned,

such as reduced growth rates. Moreover, many of these studies suffer from the issues already highlighted with GCA as a methodology, particularly in relation to the collection of samples over time. Consequently, stable isotope approaches arguably provide more robust outputs on how invasive *P. parva* and native fishes interact trophically.

The initial study on this was completed by Britton et al. (2010c) and this revealed that in an invaded fishing lake, a highly abundant *P. parva* population (Fig. 1.3) was sharing food resources with *C. carpio* and roach *Rutilus rutilus*, but not with other native fishes, including rudd *Scardinius erythrophthalmus* (Fig. 1.4). This sharing of food resources between the abundant *P. parva* population and *R. rutilus* had a strong negative consequence for the growth rates of *R. rutilus*, indicating an adverse effect of inter-specific competition. Thus, for *P. parva*, *R. rutilus* and *C. carpio*, resource partitioning was not evident (Britton et al. 2010c).

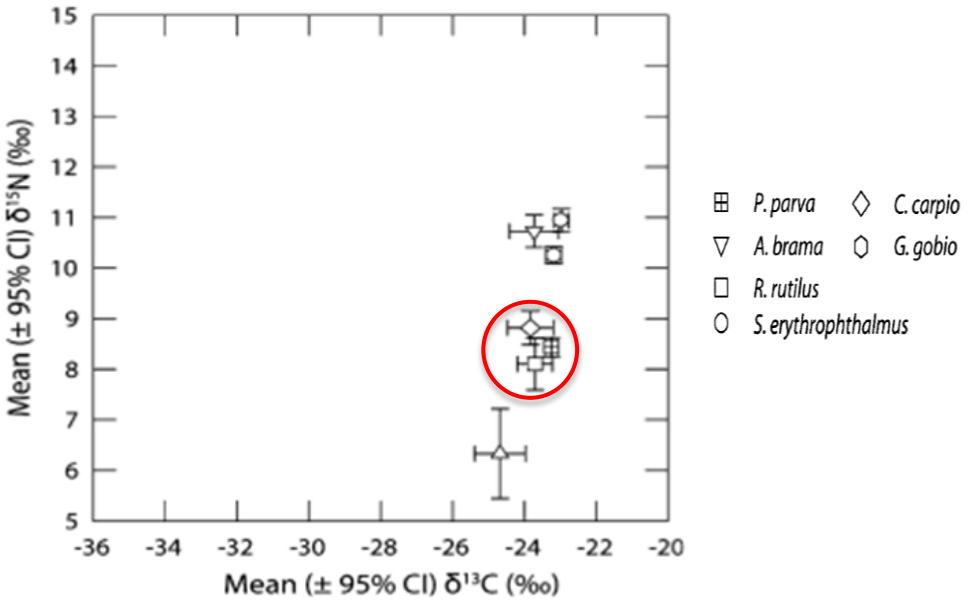

Figure 1.4 Stable isotope bi-plot showing overlaps in the diet of *Pseudorasbora parva*, *Cyprinus carpio* and *Rutilus rutilus* (and highlighted by the red circle) and the lack of overlap between *P. parva*, *Scardinius erythrophthalmus*, gudgeon *Gobio gobio* and common bream *Abramis brama* (Britton et al. 2010c).

One issue with the study outlined above and shown in Figure 1.4 was that it was based on a single fish community and so may not be representative of trophic consequences elsewhere. Consequently, a further study was completed on UK populations of *P. parva* where five invaded fish communities were studied (Jackson and Britton 2013). As shown in Table 1.1, the study revealed variable trophic consequences associated with *P. parva*. For example, the extent of the trophic niche overlap between *P. parva* and *S. erythropthalmus* was high when in sympatry (86 % and 92 %), whereas it did not overlap between *P. parva* and *A. brama*. For *P. parva* and *R. rutilus*, their sharing of trophic space was relatively high at 73 % and 48 %. Thus, rather than revealing a common pattern of *P. parva*

always sharing food resources with native fishes (as would be indicated by the overlap of their trophic niches) and so potentially competing, these outputs suggest some context dependency, with little evidence that common patterns can easily be identified (Table 1.1).

Table 1.1. Species, sample size, fork length range and mean fork length (mm, ± SD), and estimated density (n m^{-2}) of the fish communities used in the study and their mean stable isotope metrics where CR_b= $\delta^{13}C$ range; NR_b= $\delta^{15}N$ range; SEA_c=standard ellipse area (trophic niche size); % overlap= percentage of niche (SEA_c) shared with *P. parva*. Numbers in parentheses show the 2.50-97.50 % quantile range. *S. erythr.*= *Scardinius erythropthalmus*. The column in bold font indicates the extent to the overlap in trophic niche size (from Jackson and Britton 2013).

Site	Species	N	Length range	Mean length	Density	CR_b	NR_b	SEA_c	**% overlap**
1	*P. parva*	16	37-72	52±12	6.1	3.46 (1.93-4.00)	2.79 (1.18-3.97)	3.58	
	R. rutilus	11	49-86	64±19	1.2	2.43 (1.79-2.67)	2.14 (1.64-2.40)	2.57	**73±2**
2	*P. parva*	13	38-78	60±14	0.3	3.83 (1.71-4.40)	2.27 (0.95-2.95)	2.05	
	A. brama	11	78-104	88±6	0.2	1.04 (0.25-1.45)	0.51 (0.28-0.61)	0.23	**0**
3	*P. parva*	13	22-72	47±16	2.4	5.86 (3.78-6.75)	3.54 (2.33-4.03)	7.03	
	R. rutilus	11	33-68	57±15	1.5	3.88 (2.06-4.35)	1.85 (0.87-2.32)	1.81	**48±1**
	S. *erythr.*	10	52-63	58±4	1.1	2.50 (0.57-2.80)	1.53 (0.40-1.71)	0.78	**86±1**
4	*P. parva*	14	37-84	52±14	3.5	3.60 (1.35-4.28)	1.21 (0.41-1.75)	2.66	
	C. carpio	10	34-102	73±35	1.4	1.38 (0.61-1.60)	0.83 (0.31-1.11)	0.66	**49±0**
5	*P. parva*	14	38-95	57±16	0.5	5.25 (2.64-7.08)	4.74 (2.37-6.61)	6.69	
	S. *erythr.*	6	115-146	78±22	1.5	1.99 (0.77-2.30)	0.63 (0.30-0.71)	1.12	**92±1**
	A. brama	6	106-119	92±4	0.1	1.63 (0.40-2.09)	0.78 (0.42-0.94)	1.01	**10±1**

Moreover, a recent study on the trophic relationships of *P. parva*, *C. carpio* and signal crayfish *Pacifastacus leniusculus* revealed little overlap in the trophic niches of these species from across 6 communities, but with strong patterns of resource partitioning in 5 of the 6 communities (Fig. 1.5; Jackson and Britton 2014).

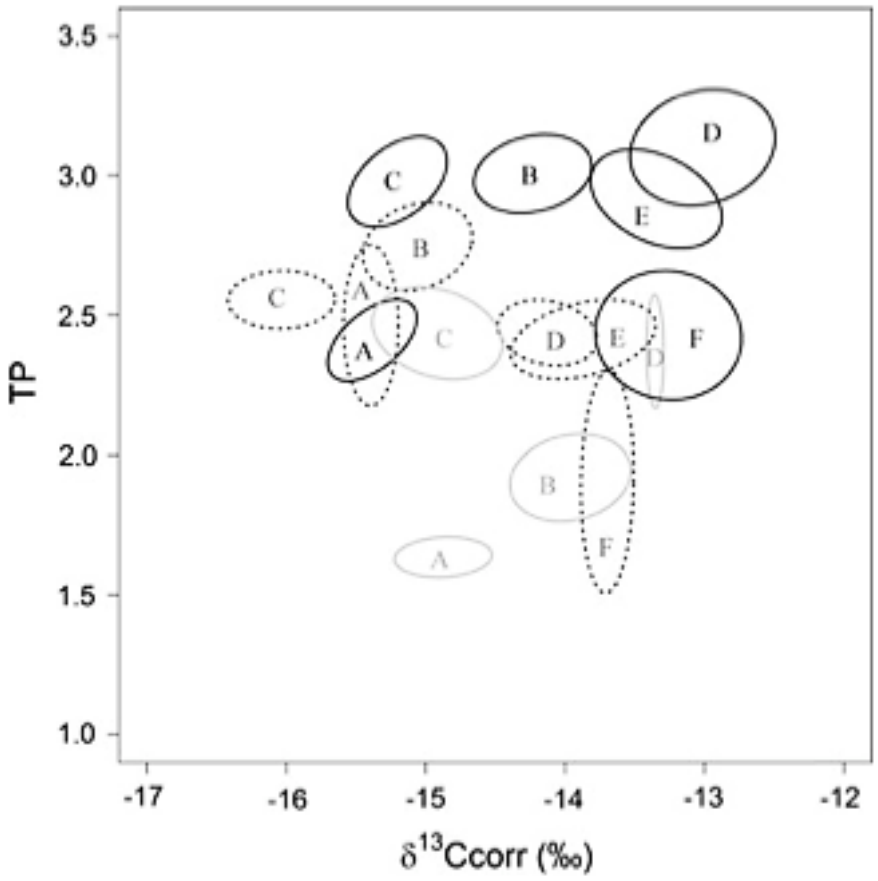

Figure 1.5 Stable isotope biplot of trophic position (TP) and corrected values of $\delta^{13}C$ across 6 ponds (A to F). Each ellipse encloses the core trophic niche width (SEA_c) of *Cyprinus carpio* (black), *Pacifastacus leniusculus* (grey) and *Pseudorasbora parva* (dashed) (Jackson and Britton 2014).

Consequently, current knowledge on the trophic ecology and trophic relationships of invasive *P. parva* is rather limited and aspects of it are contradictory, with both inter-specific competitive relationships and resource partitioning evident. This suggests that these relationships could be context dependent, varying according to factors such as the native species concerned and *P. parva* population density. Nevertheless, the lack of replicated and controlled studies completed on the species means that attempts to decipher general patterns and formulate ecological rules are difficult, despite there being a strong management driver for this information (Britton et al. 2010c).

1.6 Research aims and objectives

The overall aim of the research is to investigate the trophic consequences of introductions of invasive fish. Using *P. parva* as the model species, the research will identify how the introduced fish modify the trophic niche size of native fishes, assess the mechanisms of resource partitioning and resource sharing between *P. parva* and native fishes, and where feasible, assess the ecological consequences of these.

Given the issues already outlined over the lack of replication and control in previous studies on *P. parva* trophic ecology then this research will be completed using approaches over three spatial scales. The first approach provides data from relatively controlled and replicated conditions through the use of experimental mesocosms in which known numbers and sizes of *P. parva* and native fishes will be used over discrete and pre-determined periods. The experimental design will

use allopatric and sympatric contexts to assess how *P. parva* modifies trophic niche size and the trophic position of native fishes. The second approach is the use of small ponds that have previously been used for aquaculture. In these ponds, *P. parva* and a range of native fishes have been present for approximately 8 years. The ponds are relatively small and have simple fish communities comprising of a low number of species, enabling patterns in the trophic relationships to be identified with relative ease and compared with the outputs from the experimental mesocosms. The third and final approach is the use of field sites. Four invaded ponds are used; three of these are located in Belgium and have relatively complex fish communities that include a number of invasive fishes. The rationale for their use is that the Belgian temperate climate is broadly similar to the UK and so this will enable the generated data to be comparable to UK data. The fourth pond is located in South Wales, UK, and has a fish community broadly similar to other UK sites that have been investigated (Britton et al. 2010c; Jackson and Britton 2013). Consequently, through employing the use of stable isotope analysis, the research objectives (O) are to:

O1. Quantify the influence of *P. parva* on the trophic niche size and trophic position of native fishes in experimental mesocosms through completion of treatments in which the fishes are used in allopatric and sympatric contexts;

O2. Identify the trophic relationships and basic food web structure of small aquaculture ponds containing low numbers of native fishes and invasive *P. parva*, and assess whether general patterns that are apparent in the outputs have synergies with those of O1;

O3. Assess the trophic relationships, basic food web structure and the ecological consequences of *P. parva* invasion in four wild ponds and assess whether patterns apparent in the data outputs have synergies with those from data generated in more controlled environments in O1 and O2; and

O4. Using the outputs of O1 to O3, draw conclusions on the trophic relationships of invasive *P. parva* in the context of trophic niche theory, and identify any management implications.

2. Materials and methods

The purpose of this chapter is to provide an overview and explanation of the experimental designs and analytical methods used in the study. To meet the demands of Research objectives 1 to 3 (Section 1.6), research was completed in experimental mesocosms, small aquaculture ponds and wild fish communities. To explain the approach used at each of these, the chapter is broken up into three large sub-sections, each detailing the approach used (Sections 2.1, 2.2 and 2.3). Note that in all cases, the licences and legal permissions required to work on the species concerned and in the locations outlined had been granted from the appropriate authorities, including ethical approval. As such, these licences will not be mentioned again. Also, note that although *C. carpio* is not a native fish to the UK, it is considered naturalized and legislation and policy treats it as a native fish in England and Wales. Thus, it is referred to as a native fish within the study.

2.1 Experimental mesocosms

2.1.1 Experimental design

The aim of the experimental mesocosms was to complete Objective 1: 'Quantify the influence of *P. parva* on the trophic niche size and trophic position of native fishes in experimental mesocosms through completion of treatments in which the fishes are used in allopatric and sympatric contexts'. Consequently, the experimental design used *P. parva, C. carpio,* tench *Tinca tinca* and three-spined stickleback *Gasterosteus aculeatus* in allopatric and sympatric contexts, where each context and species combination was replicated three times (Table 2.1).

These species were used due to both their frequent co-existence with *P. parva* in fisheries in the UK and their potential for similar diets, as they are all omnivorous and are bentho-pelagic, except *T. tinca* which is benthic (www.fishbase.org). Consequently, there is high potential for dietary interactions between them and thus sharing of food resources. These replicated contexts were completed in fibre-glass mesocosms that were constructed within larger aquaculture ponds that were separated, using pond liner, to create four compartments of approximately 1000 l volume and 1 m depth (Fig. 2.1). These were situated in the open-air, on grass and close to tree-cover (within 15 m). This meant that inputs of terrestrial material into each mesocosm would be similar, and all ponds would receive similar amounts of shade and direct sunlight on a daily basis. They were located at a disused aquaculture site located close to the city of Winchester in Southern England (National Grid Reference SU38712242).

Table 2.1 Overview of the experimental design used in the experimental mesocosms.

Context	Species	Number of fish used per replicate
Allopatric	*P. parva*	8 *P. parva*
Allopatric	*C. carpio*	8 *C. carpio*
Allopatric	*T. tinca*	8 *T. tinca*
Allopatric	*G. aculeatus*	8 *G. aculeatus*
Sympatric	*P. parva/ C. carpio*	4 *P. parva* / 4 *C. carpio*
Sympatric	*P. parva/ T. tinca*	4 *P. parva*/ 4 *T. tinca*
Sympatric	*P. parva/ G. aculeatus*	4 *P. parva*/ 4 *G. aculeatus*

The experiments were completed in 2012 (allopatric *P. parva* and sympatric *P. parva* and *C. carpio*) and in 2013 (a repeat of allopatric *P. parva*, plus sympatric *P. parva* and *T. tinca* and sympatric *P. parva* and *G. aculeatus*). Each experiment ran for 100 days between late July and October when water temperatures were recorded between 7.6 and 19.2 °C (mean ± SE: 13.6 ± 0.9 °C; measured hourly using a data logger (TinyTag). Each mesocosm was also covered with 20 mm nylon mesh to prevent access for predators (Fig. 2.1). The fish used in the mesocosms were female *P. parva* and female *G. aculeatus* that were available from a small pond on the disused aquaculture site, and immature *T. tinca* and *C. carpio* that were sourced from aquaculture. The use of female fish prevented any reproduction and the immature fish were used as these were the smallest fish available for that species and they needed to be relatively similar in size to the *P. parva* and *G. aculeatus* to prevent confounding issues in the experiment (Chapter 3). This was because dietary differences arising from ontogenetic differences could have otherwise occurred.

Figure 2.1 Four of the experimental mesocosms following their initial filling with water. The mesh covering the mesocosms is to prevent ingress of fish-eating fauna.

One month before the start of the experiments, the mesocosms were set-up by filling them with water from a nearby fishless pond using a 3.5 inch petrol powered water pump. They were then each provided with a gravel (approximately 6 mm diameter) substrata (1.5 cm depth), provided with fish refuge structures (two open-ended circular plastic tubes of 15 cm length and 6 cm diameter) and a native pond lily (*Nymphoides peltata*; uniform wet mass were 10 ± 1 g). They were then seeded with Chironomidae, *Asellus aquaticus* and *Gammarus pulex* (20 of each) to enable establishment of a macro-invertebrate community. At the end of the month, the fish were released and the mesocosms were then left for 100 days. The rationale for 100 days period is explained in Section 2.1.3.

2.1.2 Sample collection

On day 100, each mesocosm was partially emptied of its water using buckets and the fish recaptured using hand nets. At the same time, samples of algae, macrophyte, zooplankton and the macro-invertebrates (Chironomidae, *Asellus aquaticus* and *Gammarus pulex*) were collected. The macro-invertebrates were collected using a hand net of 0.25 mm mesh that was swept through the water column and across the benthos. Captured macro-invertebrates were then removed from the net, taken back to the laboratory, identified to species level and sorted into eppendorf tubes of 1.5 ml for subsequent drying. Zooplankton samples were collected using by passing a standard 20 L of water through a zooplankton net of mesh size 250 μm. Macrophye samples were collected by hand and algal samples from scaping the side of ponds with a cover slip, with the scraping then transferred to an eppendorf tube (1.5 ml), with three tubes collected per pond. The recaptured fish were euthanized using an overdose of anaesthetic (MS-222) and taken back to the laboratory where a proportion of muscle tissue was removed for stable isotope analyses. The fish were also measured (fork length, nearest mm). These data were used to test for differences in the starting lengths between the fishes (ANOVA, as data normally distributed), and the relationship of recaptured length with the stable isotope data (linear regression). Where significant differences in lengths were detected between species or between length and the stable isotope data, then they were treated as a covariate in subsequent tests (Section 2.1.4).

2.1.3 Stable isotope analysis

Fish diet composition has traditionally been completed through gut content analysis (GCA), but the method is disadvantageous through it being incapable of elucidating the extent to which the fish are assimilating their energy from their putative food resources (Paradis et al. 2008; Section 1.4). More recently, increased understandings of the trophic relationships between animals and their putative food sources have been gained by the use of stable isotope analyses (SIA) (Vander Zanden et al. 1999; Grey 2006; Section 1.4). The ratios of the stable isotopes ($\delta^{13}C/\delta^{12}C$; $\delta^{15}N/\delta^{14}N$) reveal the trophic structure and pathways of energy flow in the studied food web as they vary predictably from resource to consumer (Fry 2006). Consumer $\delta^{13}C$ is an indicator of energy source (DeNiro and Epstein 1978; Fry and Sherr 1989; Section 1.4). The stable nitrogen isotope ($\delta^{15}N$) typically becomes enriched by 3 to 4 ‰ between prey and predator tissue and so is an indicator of consumer trophic position (DeNiro and Epstein 1981; Minagawa and Wada 1984; Section 1.4). The use of stable isotope analysis to determine the trophic relationships between the fishes when in sympatry was preferred to GCA as the latter would have been restricted to only understanding dietary differences on day 100, rather than reflecting the 100 days period, as per SIA. Stomach flushing of the fishes during the experimental period was not feasible as it was likely to rupture the intestine of the agastric fishes.

In order for the isotopic signature of the fish to reflect their diet under the experimental conditions, sufficient time was needed for isotopic turnover in the muscle tissue; 100 days during the summer and autumn period is sufficient for this in *P. parva*, based on published data on turnover rates in fish and is also

consistent with Jackson et al. (2013) who also worked experimentally on *P. parva* at similar water temperatures (7.5 to 18.8 °C; Jackson et al. 2013).

In addition to the analysis of the fish tissues, samples of three putative fish food resources (algae, chironomidae and zooplankton) that were collected from each mesocosm on day 100 were also analyzed for their stable isotopes to enable their relative importance to the diet of the fishes to be assessed. All of the samples were dried at 60ºC for 48 hours before being processed at the Cornell Stable Isotope Laboratory, Ithaca, USA. At this laboratory, each sample was prepared by grinding and then weighing approximately 0.5 mg into a tin cup, with the actual weight recorded accurately using a Sartorius MC5 microbalance. The samples were then analysed for their carbon and nitrogen isotopes using a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer. The outputs from the spectrometer included data on the carbon and nitrogen stable isotope ratios that could be then be expressed relative to conventional standards as $\delta^{13}C$ and $\delta^{15}N$, respectively (Section 1.4), where $\delta^{13}C$ or $\delta^{15}N$ = [Rsample/Rstandard-1] x 1000, and R is $\delta^{13}C/\delta^{12}C$ or $\delta^{15}N/\delta^{14}N$. Standards references were Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. A standard of animal (mink) was run every 10 samples to calculate an overall standard deviation for both $\delta^{15}N$ and $\delta^{13}C$ to ascertain the reliability of the analyses. The overall standard deviation of the animal standard was not more than 0.23 ‰ for $\delta^{15}N$ and 0.14 ‰ for $\delta^{13}C$.

2.1.4 Stable isotope data testing and metrics

The initial stable isotope data analyses were straight forward, with plotting of uncorrected stable isotope data in isotopic biplots (Fig. 1.2). This enabled an initial assessment to be made of the distribution of the stable isotope data between the sympatric fishes and their relationship to their putative food resources. Differences between species in the sympatric contexts and between the same species between their allopatric and sympatric contexts were tested for significance using Mann Whitney U tests when length was not used as a covariate and in generalized linear models where it was used as covariate (Section 2.1.2).

The next step was to identify whether the data from each replicate in each context and species combination could be combined in order to increase the statistical power in subsequent tests by increasing sample size. This was completed by testing the $\delta^{13}C$ and $\delta^{15}N$ data of the putative fish food resources (i.e. the macro-invertebrates, hereafter referred to as the isotopic 'baseline') for each replicate in each context. Where these indicated significant differences between replicates in their isotopic values, the $\delta^{13}C$ and $\delta^{15}N$ were corrected. For $\delta^{15}N$, correction was by calculating trophic position (TP) using:

$$TP_i = \frac{\delta^{15}N_i - \delta^{15}N_{base}}{3.4} + 2$$

Where TP_i is the trophic position of the individual fish, $\delta^{15}N_i$ is the isotopic ratio of that fish, $\delta^{15}N_{base}$ is the isotopic ratio of the primary consumers (i.e. the 'baseline' invertebrates), 3.4 is the fractionation between trophic levels (i.e. 3.4 ‰; Section 1.4) and 2 is the trophic position of the baseline organism (Post

2002b). For $\delta^{13}C$, correction was according to the equation below following Olsson et al. (2009):

$$\delta^{13}C_{corr} = \frac{\delta^{13}C_i - \delta^{13}C_{meaninv}}{CR_{inv}}$$

Where $\delta^{13}C_{corr}$ is the corrected carbon isotope ratio of the individual fish, $\delta^{13}C_i$ is the uncorrected isotope ratio of that fish, $\delta^{13}C_{meaninv}$ is the mean invertebrate isotope ratio (the 'baseline' invertebrates) and CR_{inv} is the invertebrate carbon range (δ^{13}Cmax - δ^{13}Cmin). Once the stable isotope data had been corrected (where necessary), the data for each replicate per context were combined.

The corrected data were tested for the significance of their differences between the $\delta^{13}C$ of each species in sympatry, and then for each species between their allopatric and sympatric contexts (Table 2.1). This was completed using the non-parametric Mann Whitney U test, as the stable isotope data were not normally distributed. The testing of $\delta^{15}N$ between each species in sympatry, and between the allopatric and sympatric contexts for each species, was also completed using the same test (Table 2.2).

Table 2.2 Overview of the combination of species by context used in testing for differences between $\delta^{13}C$ and $\delta^{15}N$ using Mann-Whitney U tests.

Contexts	Species tested
Sympatric	*P. parva* vs. *C. carpio*
Sympatric	*P. parva* vs. *T. tinca*
Sympatric	*P. parva* vs. *G. aculeatus*
Allopatric vs. Sympatric	*P. parva* vs. *P. parva*
Allopatric vs. Sympatric	*C. carpio* vs. *C. carpio*
Allopatric vs. Sympatric	*T. tinca* vs. *T. tinca*
Allopatric vs. Sympatric	*G. aculeatus* vs. *G. aculeatus*

The next step was to then use the stable isotope data within population metrics that provide information on the trophic structure and the isotopic niche of the fishes. Using the combined and corrected isotope data as appropriate (i.e. TP and $\delta^{13}C_{corr}$), the first metric that was calculated was the standard ellipse area (SEA_c) for each species in each context (Table 2.1). These were calculated using the SIAR package (Jackson et al. 2011) in the R computing program (R Core Team 2012). The subscript 'c' in SEA_c indicates that a small sample size correction was used (due to the limited number of fish used in the experiments; Table 2.1). SEA_c is a bivariate measure of the distribution of individuals in trophic space; each ellipse encloses ~ 40 % of the data and, therefore, represents the core dietary niche, indicating typical resource use within a species or population (Jackson et al. 2011; Jackson et al. 2012). Thus, standard ellipse areas basically represent a measure of the trophic niche of each species, where a higher value represents a larger trophic niche (i.e. a more broad diet comprising of a wider

variety of food items). Should there be a situation where the SEA_c's overlapped between the sympatric fishes within a context then the area and percentage of overlap was also calculated. This overlap would indicate the sharing of resources by the species and is the initial assessment used to quantify the extent of inter-specific competition between them. These outputs were also supplemented by the metrics carbon range (CR) and nitrogen range (NR) that indicate the extent of the isotopes' ranges in the species (Jackson et al. 2012).

In entirety, this testing of the stable isotope data and its application within the metrics enabled quantification of the influence of *P. parva* on the trophic niche size and trophic position of the three native fishes in experimental mesocosms through testing of data between the allopatric and sympatric contexts.

2.2 Small aquaculture ponds

2.2.1 Fish communities of the small ponds

The aim of the use of the small aquaculture ponds was to complete Objective 2: 'Identify the trophic relationships and basic food web structure of small aquaculture ponds containing a relatively low diversity of native fishes and invasive *P. parva*, and assess whether general patterns that are apparent in the outputs have synergies with those of O1'. To meet the objective, eight ponds were sampled on the disused aquaculture site on which the experimental mesocosms were located. The ponds were generally 30 to 40 m in length, 8 to 10 m in width and had a maximum depth of 2 m (Fig. 2.2). Extensive beds of the submerged macrophyte *Elodea canadensis* were present in all of these ponds. Although previously used for fish culture, none of the ponds had been used for this purpose since the mid-2000s and they had not been sampled or manipulated since then. Thus, whilst the experimental mesocosms assessed the trophic relationships of *P. parva* with other species over a 100 days period, these small ponds were representing trophic relationships that had developed over a much longer time period.

Figure 2.2 A typical disused aquaculture pond sampled during Objective 2 (*cf*. Table 2.3).

In the experimental mesocosms, it had been possible to manipulate the fish species present and their numbers present in each pond. This was not possible within these small ponds. Consequently, whereas in the experimental mesocosms where each context was able to be replicated three times, there was a greater degree of randomness in the fish community structure of each sampled pond, making replication more difficult (Table 2.3). In addition, the signal crayfish *Pacifastacus leniusculus* was present in some of the ponds (Table 2.3). Nevertheless, some replication was possible and even where communities were not identical in terms of species composition, there were some broad similarities (Table 2.3). As important, at least one of the contexts matched one of the contexts from the experimental mesocosms (*P. parva* and *G. aculeatus*; Table

2.1, 2.3). The maximum number of fish species present in the ponds was four, emphasizing their relatively simple fish community structure and low fish species diversity.

Table 2.3 Species composition of the fish communities present in the 'Small ponds' that were used in Research objective 2.

Fishes present in the community	*Pacifastacus leniusculus* present?	Number of ponds
P. parva, G. aculeatus	×	4
P. parva, T. tinca	✓	2
P. parva, T. tinca, G. aculeatus,	✓	1
P. parva, T. tinca, G. aculeatus, C. carpio	×	1

2.2.2 Sampling the small ponds

The small ponds were all sampled in July 2013 using a series of rectangular fish traps that comprised of a circular alloy frame of length 107 cm, width and height 27.5 cm, mesh diameter 2 mm and with funnel shaped holes of 6.5 cm diameter at either end to allow fish entry and hence their capture (Fig. 2.3). Each trap was baited with 5 fishmeal pellets of 21 mm diameter (Dynamite Baits 2010). Trapping was required as the heavy growth of *E. canadensis* prevented effective use of seine nets and electric fishing. The traps were fished in triplicate in each pond and set in the morning (~ 9 am) and lifted approximately two hours later, where the two hours provided the opportunity for a high catch of *P. parva* (J.R. Britton personal communication). Fishing the traps overnight was found to decrease sampling efficiency due to the increased activity of *P. leniusculus* that

resulted in their increased capture in traps and as they were in the traps for extended periods, they tended to start consuming the trapped fish (J.R. Britton personal communication). Where *P. leniusculus* was present in the ponds (Table 2.3), their samples for stable isotope analysis were collected using these traps.

Figure 2.3 Example of the fish trap used in the study. The fishmeal pellets used as bait can be observed in the center of the trap.

Following lifting of the fish traps, all of the fish were removed and identified to species level. For the ponds with only *P. parva* and *G. aculeatus* present, a random sub-sample of a minimum of 8 fish per species was selected. All fish to be used in stable isotope analysis were euthanized using an overdose of anaesthetic (MS-222), before being put on ice and transported back to the laboratory where they were measured (*G. aculeatus*: total length, all other species: fork length; nearest mm) and a sample of dorsal muscle taken. At the same time as the fish sampling, sweep nets were used to capture macro-invertebrates and samples of juvenile *P. leniusculus*. These were collected as triplicate samples for the purposes of stable isotope analysis. As such, the

samples of fish dorsal muscle, signal crayfish (where present; Table 2.3) and macro-invertebrates (as putative food resources for the fish) were dried at 60 ºC for 48 hours in preparation for stable isotope analysis.

2.2.3 Stable isotope analysis and data analysis.

The stable isotope analysis and the testing of these data were as per Section 2.1.3 and 2.1.4. There were no differences in the manner in which the data were tested between Research objectives 1 and 2.

2.3 Wild sites

2.3.1 Fish community complexity of the sites

The aim of the use of the wild sites was to complete Research objective 3: 'Assess the trophic relationships, basic food web structure and the ecological consequences of *P. parva* invasion in four wild ponds and assess whether patterns apparent in the data outputs have synergies with those from data generated in more controlled environments in O1 and O2'. The characteristics of the ponds are provided in Table 2.4. The issue with the four wild fish communities that were used in the study was their complexity; they mainly consisted of fish communities comprising of multiple species (native and non-native) in wild freshwater ponds in which climatic and environmental parameters were uncontrolled (Table 2.5). Each pond was also sufficiently different to each other that their only similarity tended to be invasion by *P. parva* (Table 2.5).

Table 2.4 Characteristics of the four wild ponds used to complete Research objective 3.

Pond	Country	Location	Size (m^2)
1	Belgium	51°2'7.73"N 4°10'40.64"E	600
2	Belgium	51°2'17.64"N 4°10'54.86"E	1900
3	Belgium	50°2'59'3.35"N 5°20'10.52"E	1300
4	Wales	51°41'10.0"N 4°12'06.00"W	1200

Table 2.5 Fish species sampled from the four wild fish communities used in the study to complete Research objective 3. *indicates that fish species is non-native to that country.

Pond	Fish species present
1	*P. parva*, G. aculeatus, Rhodeus amarus** & *Carassius gibelio**
2	*P. parva*, G. aculeatus, Leucaspius delineatus**; *R. amarus*, Blicca bjoerkna, C. gibelio*, Scardinius erythropthalmus, C. carpio** & *R. rutilus*
3	*P. parva*, G. aculeatus, R. amarus*, C. gibelio*, Scardinius erythropthalmus** & *Pungitius pungitius*
4	*P. parva*, S. erythropthalmus, T. tinca, Carassius auratus**

2.3.2 Sample collection

The four ponds were sampled in March 2013 using a variety of fishing techniques, including fish traps, electric fishing, seine nets and fyke nets. The invertebrate samples were collected using sweep netting in the littoral zone. The sampling of the Belgian ponds (i.e. Ponds 1 to 3) was completed on behalf of this study by the Research Institute for Nature and Forest (INBO), a scientific institute of the Flemish Government in Belgium. Pond 4 was sampled on behalf of this study by the Environment Agency. All fish samples from the ponds were euthanized (over-anaesthetized, MS-222) and transferred to the laboratory where they were frozen. The macro-invertebrate and plankton samples were also frozen on their return to the laboratory. These samples were then all transferred to the laboratories at Bournemouth University where they were processed after defrosting. The fish were identified to species level, measured (fork length or

total length as appropriate for the species, nearest mm). Dorsal muscle samples were taken for stable isotope analysis along with triplicate samples of macro-invertebrates for the purposes of stable isotope analysis. As such, the samples of fish dorsal muscle and macro-invertebrates (as putative food resources for the fish) were dried at 60 ºC for 48 hours in preparation for stable isotope analysis.

2.3.3 Stable isotope analysis and data analysis.

The stable isotope analysis and the testing of these data were as already outlined in Section 2.1.3 and 2.1.4. There were no differences in the manner in which the data were tested between Research objectives 1, 2 and 3.

2.3.4 Fish scale age and growth analysis

Where the stable isotope data analysis suggested some substantial resource partitioning between species in the wild fish communities, then the ecological consequence of this was determined by analysing the somatic growth rates of the fishes concerned. This was completed through the collection of scales from the species concerned, with 3 to 5 scales taken from between the base of the dorsal fin and the lateral line. The scales were then viewed on a microfiche reader at ×48 magnification, where their scale patterns enabled the age of the fish to be determined through counting their annual growth checks (‘annuli’; Fig. 2.4). Following age determination, the distance from the scale focus (the centre of the scale, representing when the scale was formed early in the life of the fish) to the scale radius (edge of the scale, representing the length of the fish when it was captured) was measured and recorded. The distance from the scale focus to each annulus was then measured along the same axis. These distances enabled the

length at each age of the fish to be calculated using a process known as back-calculation (Francis 1990). This is based on the premise that the scales grow in proportion with the length of the fish. The back-calculation equation used to determine the length at age at each annulus was the Dahl-Lea body proportional equation:

$$L_t = (S_c / S_R) \times L_c$$

Where L_t = back-calculated length at time t; S_c = distance from scale focus to annulus representing time t; S_R = total scale radius and L_c = length at capture. Although this equation does not correct for the length of the fish when the scales are formed (generally 10 to 15 mm; Francis 1990), it has the advantage that the estimated length at capture is the same as the actual length at capture and is not affected by the use of constants from the regression relationship between scale radius and fish length.

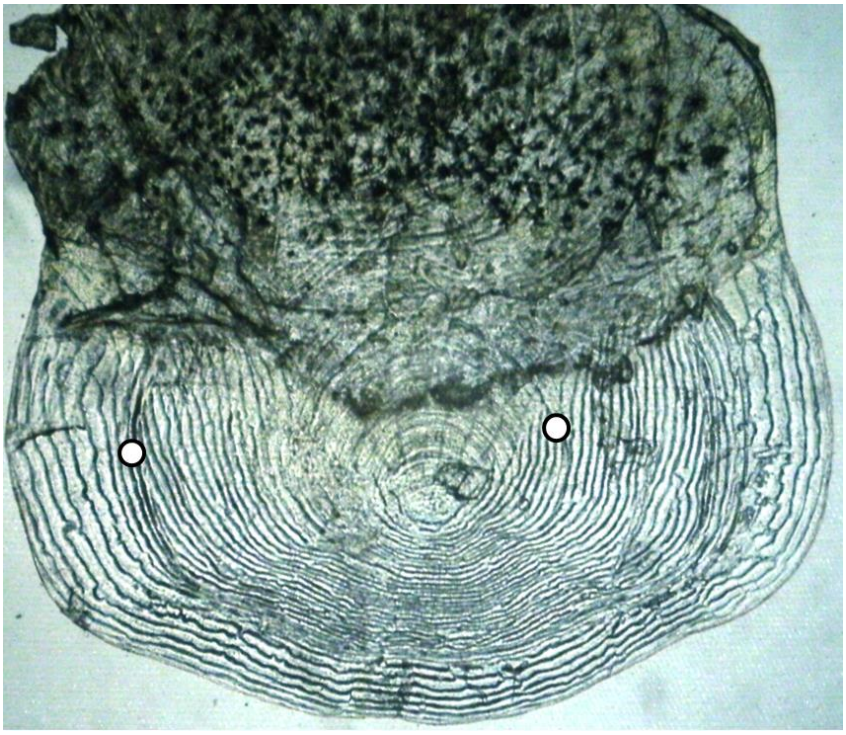

Figure 2.4 A scale taken from a *Pseudorasbora parva* of 83 mm. The white circles mark two annual marks on the scale, identified by the circuli 'cutting-over' as the growth of the scale slowed over the winter period and then accelerated in the following spring.

The growth rates of the fishes being analyzed were completed through initially calculating the last full annual growth increment for that fish population. The growth rate analysis was then completed by determining the mean standardized growth residuals for each age class of that fish population (Jones 2000; Benstead et al. 2007; Storm and Angilletta 2007). The use of only one growth increment per fish in the analyses avoided statistical complications from using repeated measurements from individual fish in the same test (i.e. pseudo-replication; Britton et al. 2010d; Beardsley and Britton 2012). The mean increment per age was calculated and the extent of the difference of the

increments for each individual fish from the mean was calculated as the standardized residual (Britton et al. 2010c; Beardsley and Britton 2012). These were then compared between the age groups of the fish (and, therefore, different years of growth) using ANOVA with Tukeys post-hoc tests. Differences in growth rates over time were then compared in relation to the outputs of the stable isotope analysis.

2.4 Research objective 4

Research objective 4 is 'Using the outputs of O1 to O3, draw conclusions on the trophic relationships of invasive *P. parva* in the context of trophic niche theory, and identify any management implications'. Thus, it is completed in Chapter 4, Discussion, which brings together the outputs from Research objectives 1 to 3 and discusses them in their wider ecological context and in relation to the conservation management of *P. parva* specifically, and non-native fishes more generally, in the UK and beyond.

3. Results

3.1 Experimental mesocosms

3.1.1 *Gasterosteus aculeatus* and *Pseudorasbora parva* in allopatric and sympatric contexts

Starting lengths of fish and recovery rates from the mesocosms

The lengths of the fish used were not significantly different between the three different contexts (Table 3.1, 3.2). The recovery rates of the introduced fish from the mesocosms was high, with 85 % of all fish recovered, including 75 % from the allopatric *G. aculeatus* and 100 % from the allopatric *P. parva* (Table 3.3).

Table 3.1 Outputs of analysis of variance (ANOVA) testing for differences in the starting lengths of *Pseudorasbora parva* and *Gasterosteus aculeatus* in their allopatric and sympatric contexts.

Treatment	*d.f.*	F	P
Allopatric *G. aculeatus* – sympatric *G. aculeatus*	1,34	2.65	0.12
Allopatric *P. parva* – sympatric *P. parva*	1,34	0.73	0.40
Allopatric *G. aculeatus* - allopatric *P. parva*	1,46	0.06	0.80
Sympatric *P. parva* – allopatric *G. aculeatus*	1,34	0.02	0.90

Stable isotope: fish length relationships

The relationship between $\delta^{13}C$ and $\delta^{15}N$ was tested against fish length using linear regression to determine if there were any ontogenetic shifts in diet that

would have to be accounted for in subsequent tests. The test outputs revealed no significant relationship between fish length and $\delta^{13}C$ and $\delta^{15}N$ in any context (Table 3.2).

Table 3.2 Outputs of linear regression testing the effect of fish length (mm) on $\delta^{13}C$ and $\delta^{15}N$.

Context	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
Allopatric *P. parva*	0.06	1,23	1.44	0.24	0.01	1,23	0.08	0.78
Allopatric *G. aculeatus*	0.19	1,16	3.90	0.07	0.15	1,16	13.18	0.09
Sympatric *P. parva*	0.07	1,90	0.63	0.44	0.02	1,90	0.20	0.67
Sympatric *G. aculeatus*	0.14	1,60	0.96	0.36	0.44	1,60	4.78	0.07

Stable isotope data between the contexts

There were significant differences in $\delta^{13}C$ and $\delta^{15}N$ between the two species when in sympatry (Table 3.3, 3.4); $\delta^{15}N$ indicated *G. aculeatus* were occupying a higher trophic position than *P. parva* and $\delta^{13}C$ indicated they were exploiting different food resources (Fig. 3.1).

Comparison of the stable isotope data for the allopatric and sympatric *G. aculeatus* revealed no significant differences in $\delta^{13}C$ and $\delta^{15}N$ between the two contexts (Table 3.1, 3.4; Fig. 3.1, 3.2). By comparison, both $\delta^{13}C$ and $\delta^{15}N$ were significantly different for *P. parva* in their allopatric and sympatric context (Table 3.1, 3.4), with $\delta^{15}N$ indicating the allopatric *P. parva* were feeding at higher trophic positions (Table 3.4; Fig. 3.1, 3.3).

Table 3.3 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for *Gasterosteus aculeatus* and *Pseudorasbora parva* across the three contexts. Variation around the mean represents standard error.

Context	n	Mean length (mm)	Length Range (mm)	Mean $\delta^{13}C$ (‰)	$\delta^{13}C$ range (‰)	Mean $\delta^{15}N$ (‰)	$\delta^{15}N$ range (‰)
Sympatric *G. aculeatus*	8	35.38 ± 1.16	30 to 40	-28.56 ± 0.45	-30.4 to 26.48	8.99 ± 0.15	8.38 to 9.51
Sympatric *P. parva*	11	34.91 ± 2.76	23 to 58	-26.76 ± 0.29	-28.49 to -25.24	6.36 ± 0.11	5.78 to 6.93
Allopatric *G. aculeatus*	18	31.78 ± 1.37	23 to 42	-28.71 ± 0.39	-31.3 to -25.31	8.56 ± 0.28	6.41 to 10.15
Allopatric *P. parva*	24	32.32 ± 1.62	18 to 40	-27.96 ± 0.20	-30.84 to -25.89	6.89 ± 0.11	6.09 to 8.51

Table 3.4 Outputs of Mann Whitney U tests to determine the difference in the stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) of *Gasterosteus aculeatus* and *Pseudorasbora parva* between the different contexts.

	$\delta^{13}C$		$\delta^{15}N$	
Context	Z	P	Z	P
Sympatric *G. aculeatus* vs. sympatric *P. parva*	-2.73	< 0.01	-3.63	< 0.01
Sympatric *G. aculeatus* vs. allopatric *G. aculeatus*	0.85	> 0.05	0.66	> 0.05
Sympatric *P. parva* vs. allopatric *P. parva*	-2.95	0.02	-2.84	< 0.01

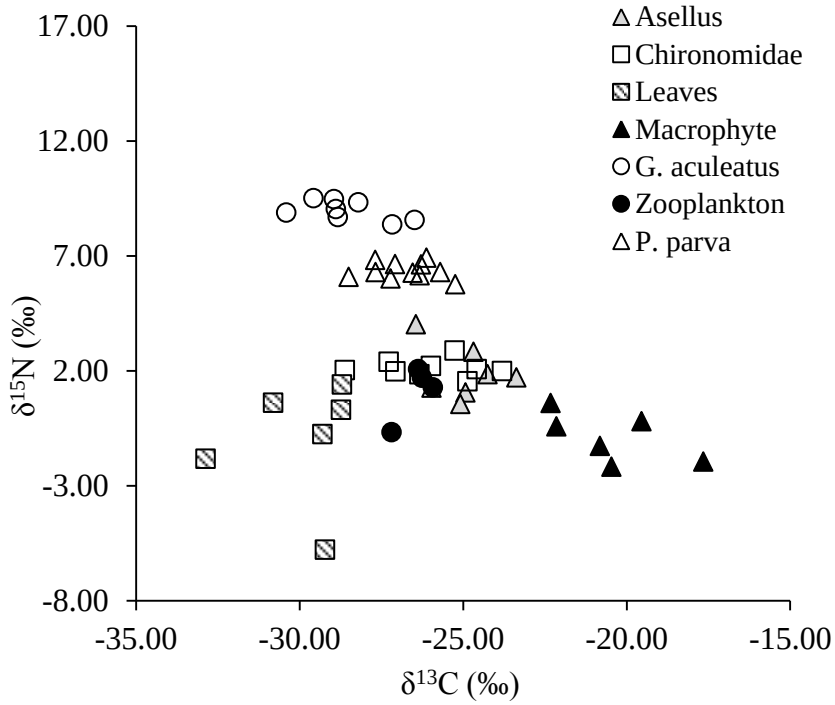

Figure 3.1 Stable isotope bi-plot showing data from the sympatric *Gasterosteus aculeatus* and *Pseudorasbora parva* context.

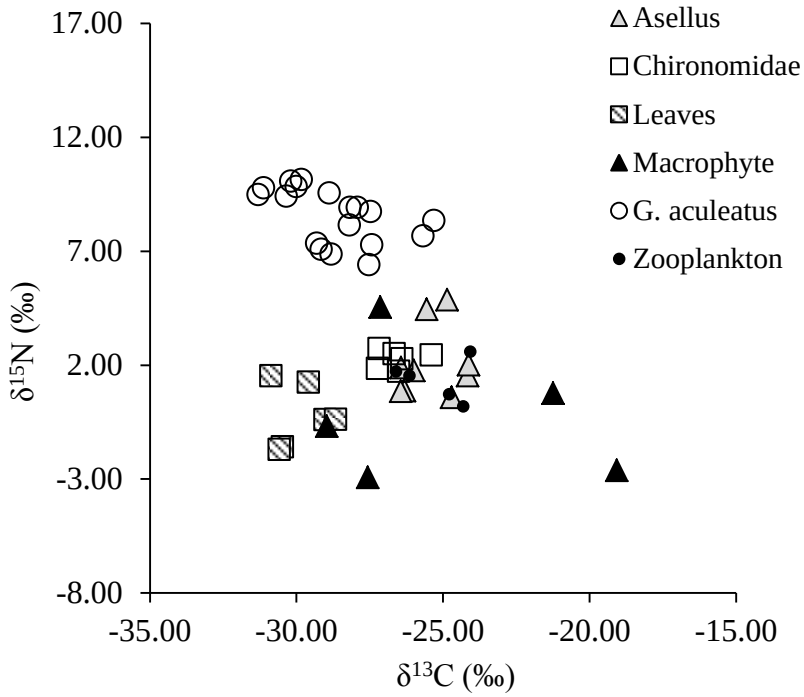

Figure 3.2 Stable isotope bi-plot showing data from the allopatric *Gasterosteus aculeatus* context.

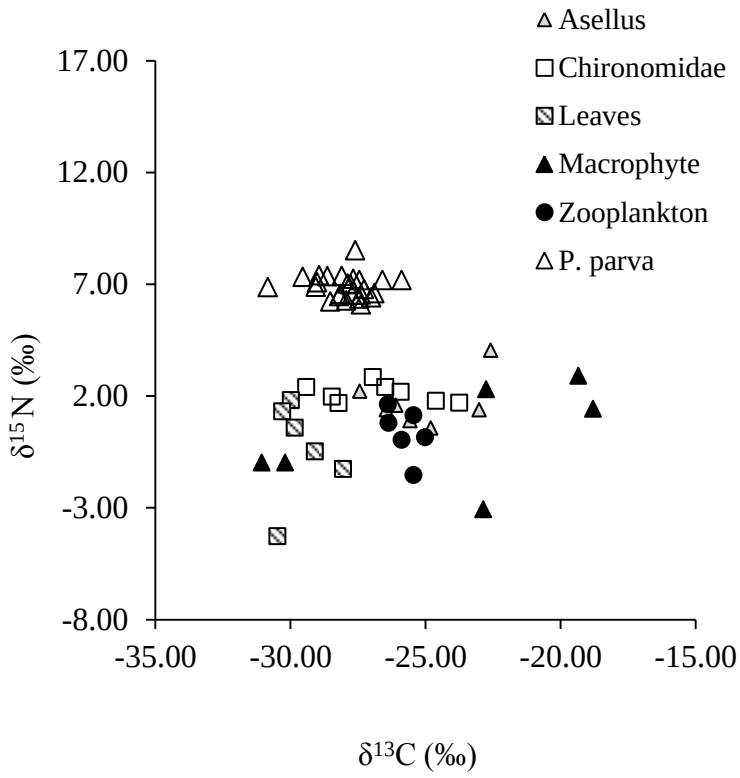

Figure 3.3 Stable isotope bi-plot showing data from the allopatric *Pseudorasbora parva* context.

Stable isotope metrics

There were no significant differences in the stable isotope data of the baseline invertebrates between three contexts (*Asellus aquaticus*: $\delta^{13}C$ $F_{2,20} = 0.25$, $P > 0.05$; $\delta^{15}N$ $F_{2,20} = 0.15$, $P > 0.05$; Chironomidae: $\delta^{13}C$ $F_{2,20} = 0.56$, $P > 0.05$; $\delta^{15}N$ $F_{2,20} = 0.31$, $P > 0.05$; zooplankton sp. $\delta^{13}C$ $F_{2,20} = 0.10$, $P > 0.05$; $\delta^{15}N$ $F_{2,20} = 0.12$, $P > 0.05$; Table 3.5). However, there were some significant differences in the baseline data between the experiments involving the different species (i.e. between the *G. aculeatus*, *T. tinca* and *C. carpio* treatments; Section 3.1.1 to 3.1.3) (*Asellus aquaticus*: $\delta^{13}C$ $F_{5,39} = 4.19$, $P < 0.05$; Chironomidae: $\delta^{15}N$ $F_{5,43} = 8.18$, $P < 0.01$). Thus, to enable comparison of data between the different experiments, rather than just between contexts in each experiment, the stable isotope data were corrected for use in the metric calculations (Section 2.1.4). Thus, the data for *G. aculeatus* and *P. parva* here were converted to trophic position (TP) (from $\delta^{15}N$) and $\delta^{13}C_{corr}$ (from $\delta^{13}C$) (Section 2.1.4; Table 3.6). The trophic position data indicated that *G. aculeatus* were occupying significant higher trophic positions than *P. parva*, irrespective of context ($F_{1,17} = 183.30$, $P < 0.05$) (Table 3.6). These converted data were then used to calculate the stable isotope metrics.

Comparison of the metrics standard ellipse area (SEA_c), nitrogen range (NR) and carbon range (CR) revealed that in sympatry, *G. aculeatus* had the larger trophic niche of the two species (Table 3.7; Fig. 3.4) with their trophic niches showing no overlap (Fig. 3.4). Comparison of SEA_c for *G. aculeatus* between their allopatric and sympatric contexts revealed that their trophic niche size was

approximately twice as large in allopatry, despite the number of fish present in each context being the same, i.e. the presence of *P. parva* appeared to constrict the SEA_c of *G. aculeatus* (Table 3.7; Fig. 3.5). However, for *P. parva*, SEA_c was approximately three times larger in allopatry than sympatry, and so the presence of *G. aculeatus* also constricted their SEA_c (Table 3.7; Fig. 3.6).

Table 3.5 Mean $\delta^{13}C$ and $\delta^{15}N$ values for basal resources in each treatment.

	Allopatric *G. aculeatus*		Allopatric *P. parva*		Sympatric *G. aculeatus*: sympatric *P. parva*	
Taxa	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Asellus aquaticus	-25.39 ± 0.32	2.09 ± 0.51	-25.14 ± 0.68	1.72 ± 0.43	-24.96 ± 0.39	1.91 ± 0.45
Chironomidae	-26.57 ± 0.27	2.26 ± 0.16	-26.73 ± 0.69	2.12 ± 0.15	-25.97 ± 0.50	2.11 ± 0.12
Leaves	-29.87 ± 0.37	-0.21 ± 0.56	-29.63 ± 0.37	-0.39 ± 0.91	-29.94 ± 0.67	-1.00 ± 1.06
Macrophyte	-24.79 ± 1.95	-0.19 ± 1.36	-24.17 ± 2.16	0.26 ± 2.16	-20.49 ± 0.71	-0.89 ± 0.44
Zooplankton	-25.17 ±0.50	1.34 ± 0.42	-25.76 ± 0.23	0.37 ± 0.45	-26.43 ± 0.27	1.10 ± 0.61
Phytoplankton	-	-	-26.44 ± 0.37	2.47 ± 0.21	-	-

Table 3.6 $\delta^{13}C_{corr}$ and trophic position values of *Gasterosteus aculeatus* and *Pseudorasbora parva*.

	$\delta^{13}C_{corr}$	Range	TP	Range
Allopatric *P. parva*	-0.94 ± 0.18	-2.39 to 0.54	3.46 ± 0.03	3.22 to 3.89
Allopatric *G. aculeatus*	-1.10 ± 0.17	-2.15 to 0.39	3.85 ± 0.11	2.96 to 4.50
Sympatric *P. parva*	-0.36 ± 0.12	-0.98 to 0.53	3.28 ± 0.03	3.14 to 3.48
Sympatric *G. aculeatus*	-1.18 ± 0.34	-3.06 to -0.31	4.04 ± 0.05	3.81 to 4.24

Table 3.7 Stable isotope metrics for *Gasterosteus aculeatus* and *Pseudorasbora parva* in allopatric and sympatric contexts and where SEA_c = Standard ellipse area, NR= $\delta^{15}N$ range and CR= $\delta^{13}C$ range.

Species	SEA_c	NR	CR
Sympatric *G. aculeatus*	0.44	0.43	2.75
Sympatric *P. parva*	0.13	0.34	1.51
Allopatric *P. parva*	0.45	0.67	2.93
Allopatric *G. aculeatus*	1.06	1.54	2.54

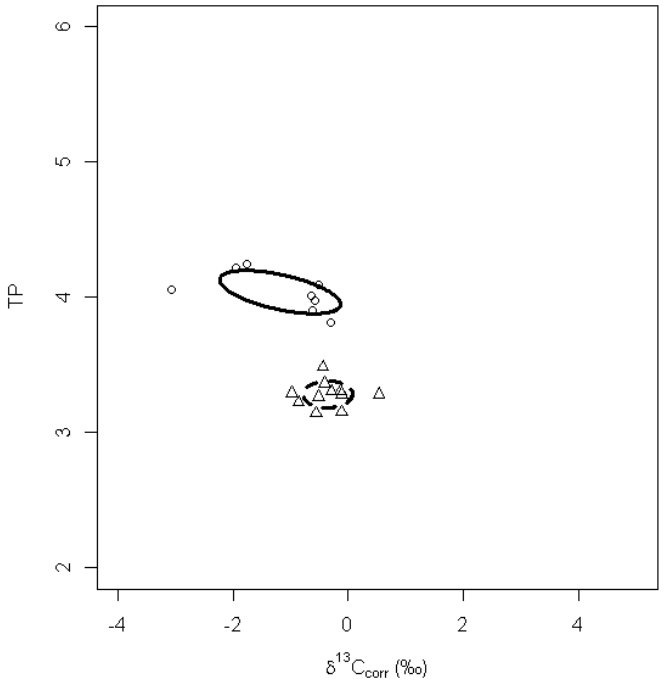

Figure 3.4. Standard ellipse areas of sympatric *Gasterosteus aculeatus* and *Pseudorasbora parva*. Open triangles represent individual *P. parva* and open circles represent individual *G. aculeatus*. The lines enclose the standard ellipse area (SEA_c) for *G. aculeatus* (solid) and *P. parva* (dashed).

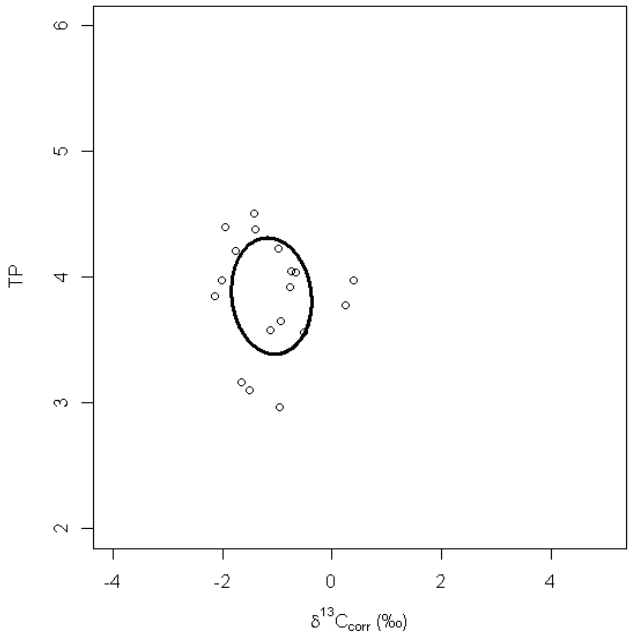

Figure 3.5 Standard ellipse areas of allopatric *Gasterosteus aculeatus*. Open circles represent individual *G. aculeatus* and the line encloses the standard ellipse area (SEA_c).

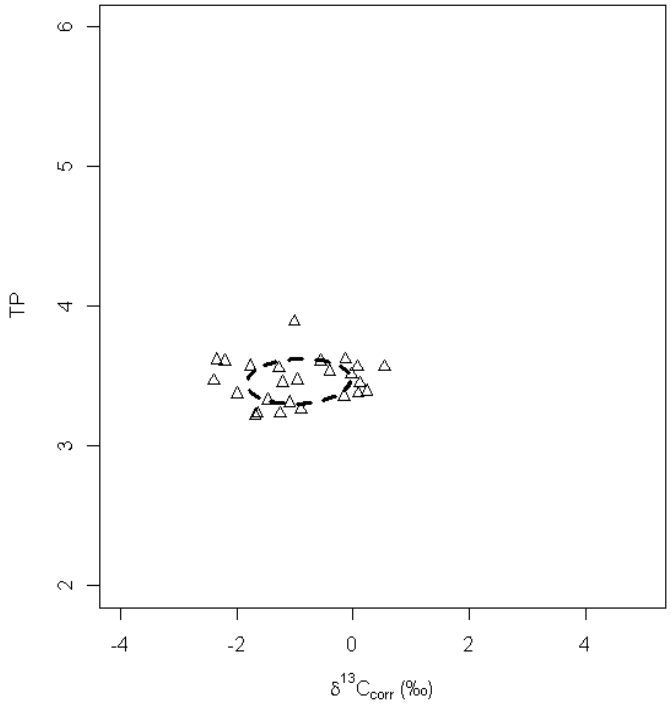

Figure 3.6 Standard ellipse area of allopatric *Pseudorasbora parva*. Open triangles represent individual *P. parva* and the lines encloses the standard ellipse area (SEA_c).

3.1.2 *Tinca tinca* and *Pseudorasbora parva* in allopatric and sympatric contexts

Starting lengths of fish and recovery rates from the mesocosms

The availability of *T. tinca* of only above 50 mm fork length from aquaculture meant that there were significant differences in the fish lengths between some of the contexts, with only the lengths of the allopatric and sympatric *T. tinca* not being significantly different to each other (Table 3.8). Across the three contexts, the recovery rates of the fish from each replicate were high, with all fish recaptured (100 %).

Table 3.8 Outputs of ANOVA testing for differences in the fork lengths of *Pseudorasbora parva* and *Tinca tinca* in their allopatric and sympatric contexts.

Treatment	*d.f.*	F	P
Allopatric *T. tinca* – sympatric *T. tinca*	1,46	0.22	0.63
Allopatric *P. parva* – sympatric *P. parva*	1,46	6.13	< 0.02
Allopatric *T. tinca* - allopatric *P. parva*	1,46	358.30	< 0.01
Sympatric *T. tinca* – sympatric *P. parva*	1,46	143.95	< 0.01

Stable isotope: fish length relationships

Linear regression revealed there was a significant relationship between fish length and $\delta^{13}C$ and $\delta^{15}N$ for the sympatric *P. parva* but not for the allopatric *P. parva* (Table 3.9). For allopatric *T. tinca*, there was a significant relationship in their length and $\delta^{15}N$ but not length and $\delta^{13}C$, with the relationships for sympatric *T. tinca* non-significant for length and both stable isotopes (Table 3.9).

Table 3.9 Outputs of linear regression testing the effect of fish length (mm) on $\delta^{13}C$ and $\delta^{15}N$ for each species and context.

Context	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
Allopatric *T. tinca*	0.13	1,22	3.29	0.08	0.18	1,22	4.91	0.04
Allopatric *P. parva*	0.06	1,23	1.44	0.24	0.01	1,23	0.08	0.78
Sympatric *T. tinca*	0.04	1,10	0.38	0.55	0.29	1,10	4.10	0.07
Sympatric *P. parva*	0.46	1,10	8.37	0.02	0.36	1,10	5.67	0.04

Stable isotope data between the contexts

Given that some of the relationships between fish length and the stable isotope data were significant (Table 3.9), allied with the significant differences in fish length between the species (Table 3.8), then testing for differences in the stable isotope data between species and contexts needed to control for fish length in generalized linear models. The model output indicated that between the species, the only significant difference in $\delta^{13}C$ and $\delta^{15}N$ was between sympatric *T. tinca* and *P. parva*, but not for either species in their allopatric and sympatric contexts (Table 3.10, 3.11; Fig. 3.7 to 3.9). Thus, when in sympatry, *T. tinca* had significantly higher trophic positions than *P. parva* and were exploiting different food resources (Fig. 3.7), but for each species in their allopatric and sympatric contexts, trophic positions and trophic niche sizes were similar.

Table 3.10 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for *Tinca tinca* and *Pseudorasbora parva* across the three contexts. Variation around the mean represents standard error.

Context	n	Mean length (mm)	Range (mm)	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
Sympatric *T. tinca*	12	70.42 ± 1.43	63 to 80	-23.82 ± 0.25	-25.55 to -22.01	12.20 ± 0.11	11.69 to 12.79
Sympatric *P. parva*	12	48.42 ± 1.14	42 to 53	-27.93 ± 0.28	-29.16 to -26.52	6.74 ± 0.12	6.11 to 7.32
Allopatric *T. tinca*	24	69.54 ± 1.09	60 to 80	-24.04 ± 0.14	-25.29 to -22.79	12.38 ± 0.08	11.73 to 13.14
Allopatric *P. parva*	24	42.32 ± 1.62	38 to 45	-27.96 ± 0.20	-30.84 to -25.89	6.89 ± 0.10	6.09 to 8.51

Table 3.11 Outputs of generalized linear model testing for differences in $\delta^{13}C$ and $\delta^{15}N$ *Pseudorasbora parva* and *Tinca tinca* where length is the effect of fork length as a covariate in the model (as Wald χ^2), group describes the two species used in each context and group difference is the output of pairwise comparisons with Bonferroni adjustment for multiple comparisons. * $P < 0.05$; ** $P < 0.01$.

	$\delta^{13}C$			$\delta^{15}N$		
	Length	Group	Group difference	Length	Group	Group difference
Sympatric *T. tinca vs.* sympatric *P. parva*	1.81	27.61**	5.10**	0.01	172.30**	5.46**
Sympatric *T. tinca vs.* allopatric *T. tinca*	3.50	0.59	0.19	9.65**	1.79	0.12
Sympatric *P. parva vs.* allopatric *P. parva*	3.14	2.29	0.74	0.06	0.61	0.19

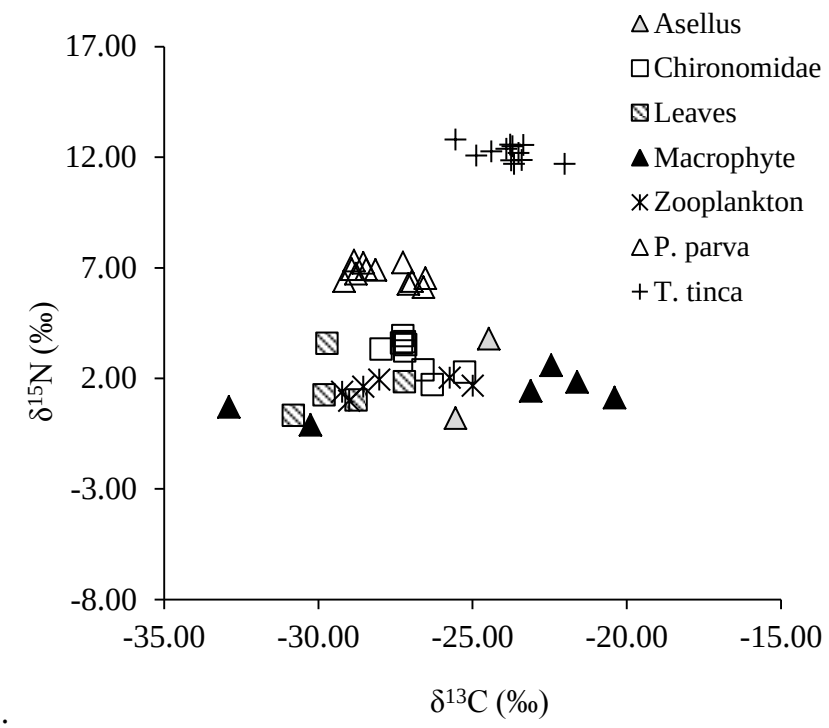

Figure 3.7 Stable isotope bi-plot showing data from the sympatric *Tinca tinca* and *Pseudorasbora parva* context.

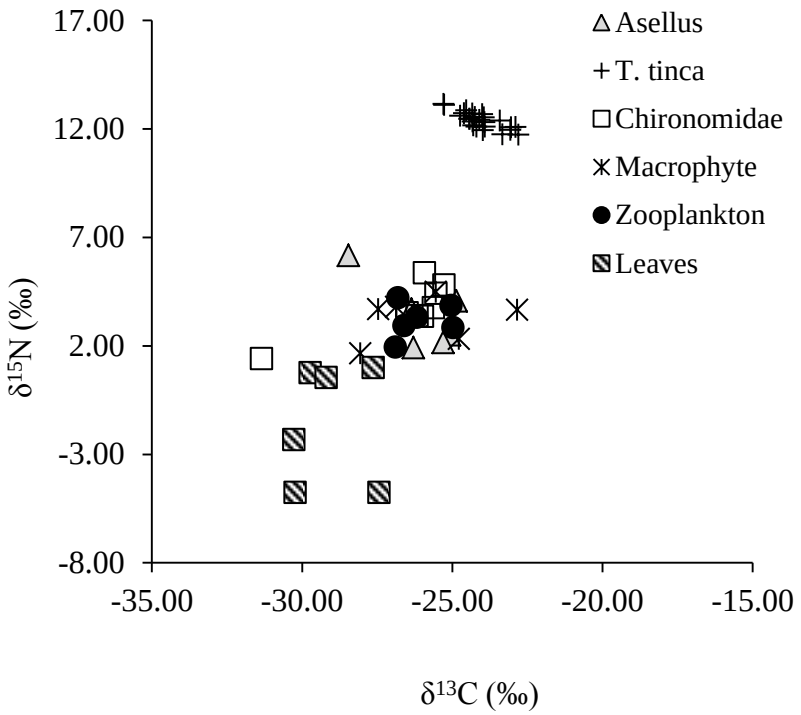

Figure 3.8 Stable isotope bi-plot showing data from the allopatric *Tinca tinca* context.

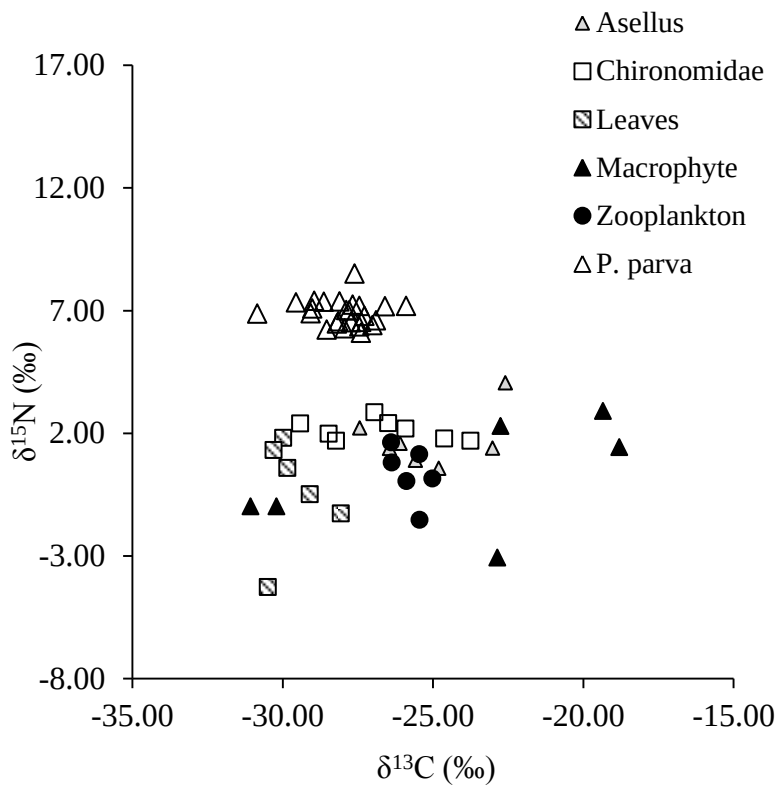

Figure 3.9 Stable isotope bi-plot showing data from the allopatric *Pseudorasbora parva* context.

Stable isotope metrics

There were no significant differences in the stable isotope data of the baseline invertebrates between the contexts (*Asellus aquaticus*: $\delta^{13}C$ $F_{2,12}$ = 0.68, P > 0.05; $\delta^{15}N$ $F_{2,12}$ = 2.14, P > 0.05; Chironomidae: $\delta^{13}C$ $F_{2,22}$ = 0.11, P > 0.05; $\delta^{15}N$ $F_{2,22}$ = 7.52, P > 0.05; Table 3.12). However, given the significant differences between the experiments (Section 3.1.1) then the data were corrected (Section 2.1.4, Table 3.13). The trophic position data indicated that *T. tinca* occupied higher trophic positions than *P. parva*, irrespective of context ($F_{3,69}$ = 191.33, P < 0.05) (Table 3.13). These converted data were then used to calculate the stable isotope metrics.

Table 3.12 Mean $\delta^{13}C$ and $\delta^{15}N$ values for basal resources in each treatment.

	Allopatric *T. tinca*		Allopatic *P. parva*		Sympatric *T. tinca :* Sympatric *P. parva*	
Taxa	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Asellus aquaticus	-26.06 ± 0.54	3.42 ± 0.65	-25.14 ± 0.68	1.72 ± 0.43	-25.0 ± 0.54	2.00 ± 1.80
Chironomidae	-26.55 ± 0.70	3.76 ± 0.42	-26.73 ± 0.69	2.12 ± 0.15	-26.92 ± 0.26	3.08 ± 0.25
Leaves	-29.09 ± 0.52	-1.59 ± 1.12	-29.63 ± 0.37	-0.39 ± 0.91	-29.27 ± 0.61	1.61 ± 0.55
Macrophyte	-25.93 ± 0.79	3.27 ± 0.43	-24.17 ± 2.16	0.26 ± 2.16	-25.12 ± 2.10	1.26 ± 0.38
Zooplankton	-26.09 ± 0.36	3.19 ± 0.33	-25.76 ± 0.23	0.37 ± 0.45	-27.58 ± 0.73	1.60 ± 0.16
Phytoplankton	-	-	-26.44 ± 0.37	2.47 ± 0.21	-	-

Table 3.13 $\delta^{13}C_{corr}$ and trophic position values of *Tinca tinca* and *Pseudorasbora parva*.

Context	$\delta^{13}C_{corr}$	Range	TP	Range
Sympatric *T. tinca*	1.62 ± 0.28	0.36 to 3.56	4.77 ± 0.06	4.49 to 5.06
Sympatric *P. parva*	-0.79 ± 0.12	-1.63 to -0.24	3.17 ± 0.03	2.94 to 3.31
Allopatric *T. tinca*	0.86 ± 0.10	0.21 to 2.21	4.60 ± 0.07	3.99 to 5.11
Allopatric *P. parva*	-0.94 ± 0.18	-2.39 to 0.54	3.46 ± 0.03	3.22 to 3.89

Comparison of the metrics standard ellipse area (SEA_c), nitrogen range (NR) and carbon range (CR) revealed that in sympatry, *T. tinca* had the larger trophic niche of the two species (Table 3.14; Fig. 3.14) with their trophic niches showing no overlap (Fig. 3.10). Comparison of SEA_c for *T. tinca* between their allopatric and sympatric contexts revealed that their trophic niche size was larger in allopatry, despite the number of fish present in each context being the same, i.e. the presence of *P. parva* appeared to constrict the SEA_c of *T. tinca* (Table 3.14; Fig. 3.11). For *P. parva*, SEA_c was approximately five times larger in allopatry than sympatry, suggesting that the presence of *T. tinca* had influence on their SEA_c (Table 3.14; Fig. 3.12).

Table 3.14 Stable isotope metrics for *Tinca tinca* and *Pseudorasbora parva* in allopatric and sympatric contexts and where SEA_c= Standard ellipse area, NR= $\delta^{15}N$ range and CR= $\delta^{13}C$ range.

Species	SEA_c	NR	CR
Sympatric *T. tinca*	0.37	0.57	3.20
Sympatric *P. parva*	0.10	1.39	0.37
Allopatric *T. tinca*	0.54	1.12	2.00
Allopatric *P. parva*	0.45	0.67	2.93

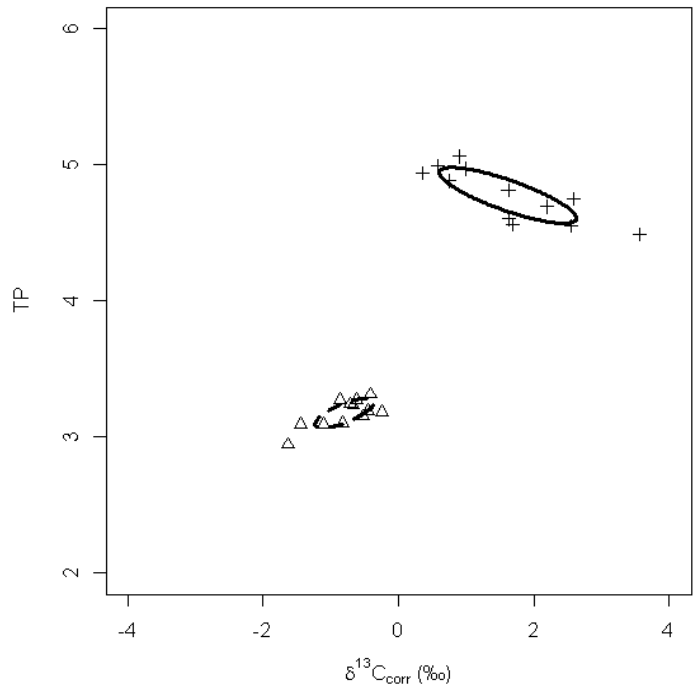

Figure 3.10. Standard ellipse areas of sympatric *Tinca tinca* and *Pseudorasbora parva*. (+) symbols represent individual *T. tinca* and open triangles represent individual *P. parva*. The lines enclose standard ellipse areas (SEA_c) for *T. tinca* (solid) and *P. parva* (dashed).

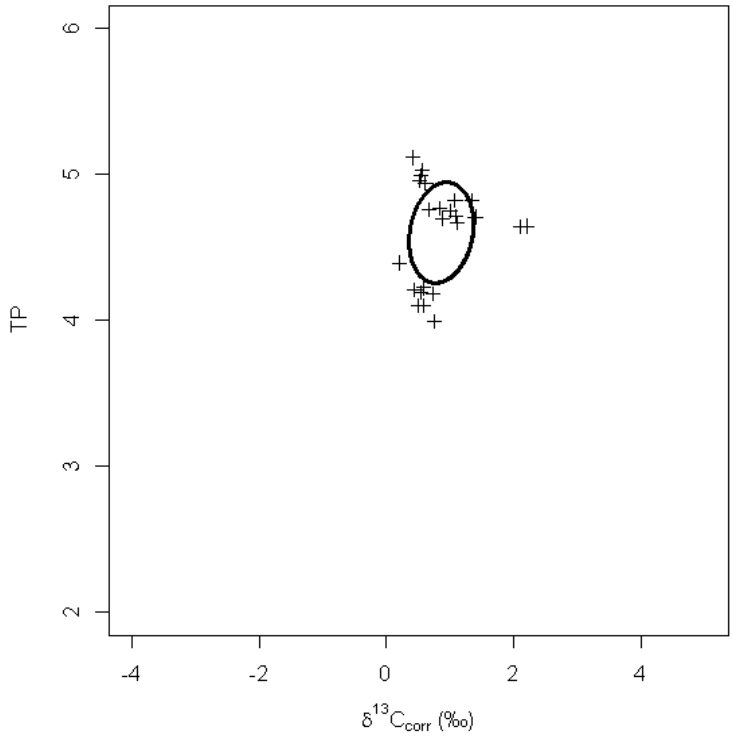

Figure 3.11 Standard ellipse areas of allopatric *Tinca tinca*. (+) symbols represent individual *T. tinca* and the line encloses the standard ellipse area (SEA_c).

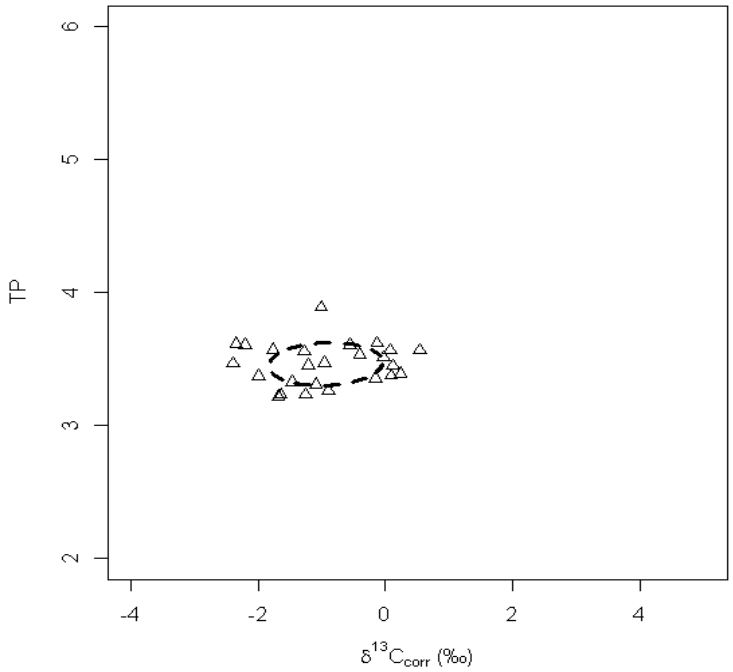

Figure 3.12 Standard ellipse area of allopatric *Pseudorasbora parva*. Open triangles represent individual *P. parva* and the lines encloses the standard ellipse area (SEA_c).

3.1.3 *Cyprinus carpio* and *Pseudorasbora parva* in allopatric and sympatric contexts

Starting lengths of fish and recovery rates from the mesocosms

The availability of *C. carpio* of only above 50 mm fork length from aquaculture meant that there were significant differences in the fish lengths between some of the contexts, with only the lengths of the allopatric and sympatric *C. carpio* and *P. parva* not being significantly different to each other (Table 3.15, 3.16). Across the three contexts, the recovery rates of the fish from each replicate were high, with all fish recaptured (100 %).

Table 3.15 Outputs of ANOVA testing for differences in the fork lengths of *Pseudorasbora parva* and *Cyprinus carpio* in their allopatric and sympatric contexts.

Treatment	*d.f.*	F	P
Allopatric *C. carpio* - sympatric *C. carpio*	1,46	0.69	> 0.05
Allopatric *P. parva* - sympatric *P. parva*	1,46	1.14	> 0.05
Allopatric *C. carpio* - allopatric *P. parva*	1,46	45.54	< 0.01
Sympatric *C. carpio* - sympatric *P. parva*	1,46	56.78	< 0.01

Table 3.16 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for *Cyprinus carpio* and *Pseudorasbora parva* across the three contexts. Variation around the mean represents standard error.

Context	n	Mean length (mm)	Range (mm)	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
Sympatric *C. carpio*	12	56.12 ± 1.41	51 to 61	-21.32 ± 0.31	-23.28 to -19.64	10.81 ± 0.14	9.68 to 11.56
Sympatric *P. parva*	12	49.31 ± 1.22	43 to 53	-27.01 ± 0.26	-29.24 to -25.52	7.10 ± 0.20	5.67 to 8.51
Allopatric *C. carpio*	24	54.23 ± 1.13	50 to 61	-20.40 ± 0.12	-21.93 to -19.50	11.66 ± 0.08	10.77 to 12.37
Allopatric *P. parva*	24	48.31 ± 1.34	44 to 54	-26.85 ± 0.15	-28.05 to -24.96	6.90 ± 0.25	5.36 to 10.42

Stable isotope: fish length relationships

Linear regression revealed there were no significant relationships between fish length and $\delta^{13}C$ and $\delta^{15}N$ for either species in any context (Table 3.17).

Table 3.17 Outputs of linear regression testing the effect of fish length (mm) on $\delta^{13}C$ and $\delta^{15}N$ for each species and context.

Context	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
Allopatric *C. carpio*	0.09	1,22	1.34	0.44	0.11	1,22	1.88	0.21
Allopatric *P. parva*	0.08	1,22	1.28	0.46	0.03	1,22	0.77	0.62
Sympatric *C. carpio*	0.11	1,10	2.12	0.29	0.12	1,10	2.87	0.11
Sympatric *P. parva*	0.03	1,10	0.88	0.51	0.11	1,10	2.79	0.11

Stable isotope data between the contexts

Mann Whitney U tests were used to test data between the contexts, as length was not significantly influencing the stable isotope data (Table 3.17). In the sympatric context, there were significant differences in $\delta^{13}C$ and $\delta^{15}N$ between *C. carpio* and *P. parva* (Table 3.18); the $\delta^{15}N$ data indicated that *C. carpio* were occupying a higher trophic position than *P. parva* and $\delta^{13}C$ indicated they were exploiting different food resources (Fig. 3.13). Between the allopatric and sympatric *C. carpio*, there were also significant differences in their $\delta^{13}C$ and $\delta^{15}N$ data (Table 3.18) and revealed *C. carpio* feeding at a higher trophic position (Fig. 3.13, 3.14). By contrast, there were no significant differences in $\delta^{13}C$ and $\delta^{15}N$ between the allopatric and sympatric *P. parva* (Table 3.18; Fig. 3.13, 3.15).

Table 3.18 Outputs of Mann Whitney U determining the significance of differences in the stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) of *Cyprinus carpio* and *Pseudorasbora parva* between the different contexts.

	$\delta^{13}C$		$\delta^{15}N$	
Context	Z	P	Z	P
Sympatric *C. carpio* vs. sympatric *P. parva*	-4.67	<0.01	-4.67	<0.01
Sympatric *C. carpio* vs. allopatric *C. carpio*	-2.42	0.02	-4.33	<0.01
Sympatric *P. parva* vs. allopatric *P. parva*	-0.59	>0.05	-0.85	>0.05

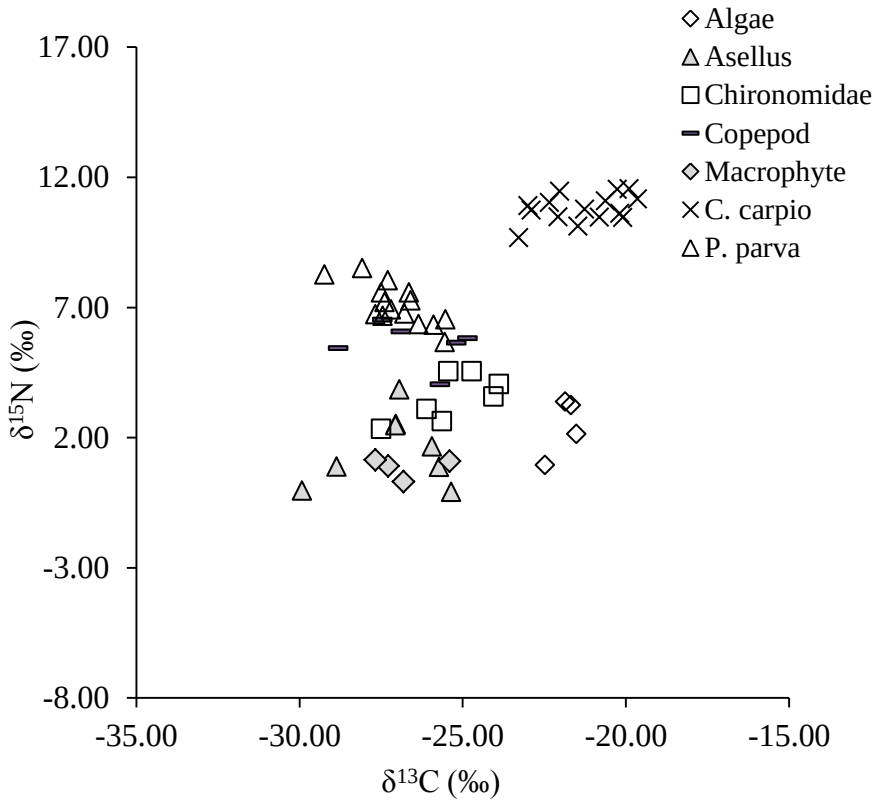

Figure 3.13 Stable isotope bi-plot showing data from the sympatric *Cyprinus carpio* and *Pseudorasbora parva* context.

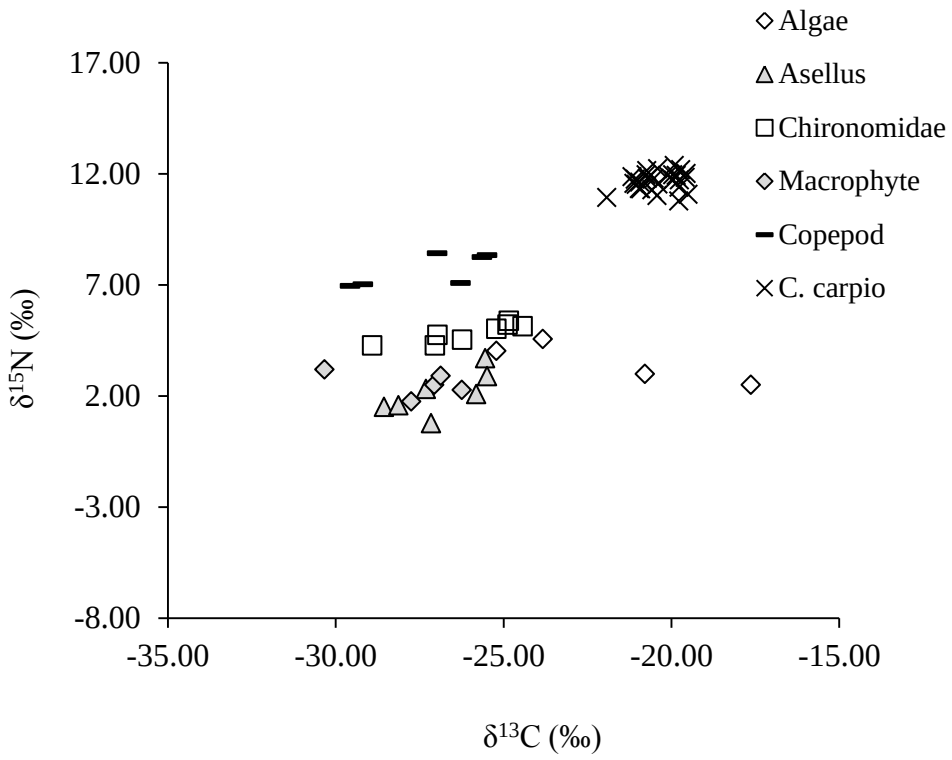

Figure 3.14 Stable isotope bi-plot showing data from the allopatric *Cyprinus carpio* context.

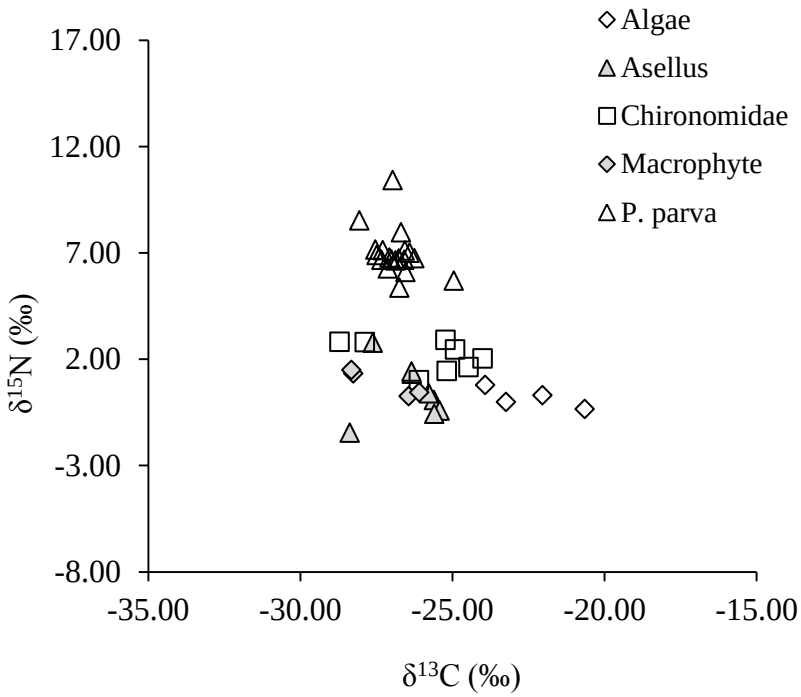

Figure 3.15 Stable isotope bi-plot showing data from the allopatric *Pseudorasbora parva* context.

Stable isotope metrics

There were no significant differences in $\delta^{13}C$ of the baseline invertebrates between the contexts (Algae: $\delta^{13}C$ $F_{2,9} = 0.10$, $P > 0.05$; *Asellus aquaticus*: $\delta^{13}C$ $F_{2,20} = 0.63$, $P > 0.05$; Chironomidae: $\delta^{13}C$ $F_{2,20} = 0.45$, $P > 0.05$; Copepod: $\delta^{13}C$ $F_{1,10} = 0.54$, $P > 0.05$; Macrophyte: $\delta^{13}C$ $F_{2,10} = 0.47$, $P > 0.05$; Table 3.19). However, given the significant differences between the experiments that have already been determined (Section 3.1.1) then the data were corrected (Section 2.1.4, Table 3.13). These corrected data revealed that the trophic position of *C. carpio* was always significantly higher than *P. parva*, irrespective of context ($F_{2,74} = 16.4$, $P < 0.05$) (Table 3.20). These corrected data were then used to calculate the stable isotope metrics.

Comparison of the metrics standard ellipse area (SEA_c), nitrogen range (NR) and carbon range (CR) revealed that when in sympatry, *P. parva* had a slightly larger trophic niche than *C. carpio* (Table 3.21) but with no overlap in their trophic niches (Fig. 3.16). The trophic niche size (SEA_c) of *C. carpio* was lower in sympatry than in allopatry, with a marked reduction in nitrogen and carbon range (Table 3.21; Fig. 3.16, 3.17). For *P. parva*, SEA_c was considerably larger in allopatry than in sympatry (Table 3.21; Fig. 3.16, 3.18).

Table 3.19 Mean $\delta^{13}C$ and $\delta^{15}N$ values for basal resources in each treatment.

	Allopatric *C. carpio*		Allopatric *P. parva*		Sympatric *C. carpio*: sympatric *P. parva*	
Taxa	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Algae	-21.87 ± 1.69	3.52 ± 0.47	-22.46 ± 0.72	0.17 ± 0.24	-21.89 ± 0.21	2.43 ± 0.57
Asellus aquaticus	-26.86 ± 0.48	2.12 ± 0.36	-26.36 ± 0.38	0.44 ± 0.48	-27.11 ± 0.56	1.52 ± 0.48
Chironomidae	-26.06 ± 0.54	4.82 ± 0.15	-25.81 ± 0.59	2.13 ± 0.25	-25.34 ± 0.47	3.55 ± 0.34
Copepod	-27.19 ± 0.73	7.68 ± 0.30	-	-	-26.49 ± 0.62	5.59 ± 0.34
Macrophyte	-27.65 ± 0.71	2.50 ± 0.25	-27.28 ± 0.59	0.88 ± 0.31	-26.80 ± 0.50	0.86 ± 0.19

Table 3.20 $\delta^{13}C_{corr}$ and trophic position values of *Cyprinus carpio* and *Pseudorasbora parva*

Context	$\delta^{13}C_{corr}$	Range	TP	Range
Sympatric *C. carpio*	1.89 ± 0.15	1.23 to 2.78	4.50 ± 0.06	3.88 to 4.77
Sympatric *P. parva*	-0.38 ± 0.16	-1.59 to 0.30	3.38 ± 0.07	2.94 to 3.74
Allopatric *C. carpio*	2.96 ± 0.22	1.16 to 4.51	4.39 ± 0.02	4.14 to 4.55
Allopatric *P. parva*	-0.29 ± 0.20	-1.12 to 1.43	3.70 ± 0.08	3.15 to 4.54

Table 3.21 Stable isotope metrics for *Cyprinus carpio* and *Pseudorasbora parva* in allopatric and sympatric contexts and where SEA_c= Standard ellipse area, NR= $\delta^{15}N$ range and CR= $\delta^{13}C$ range.

Species	SEA_c	NR	CR
Sympatric *C. carpio*	0.43	0.89	1.55
Sympatric *P. parva*	0.50	0.80	1.89
Allopatric *C. carpio*	0.34	0.41	3.35
Allopatric *P. parva*	0.85	1.39	2.55

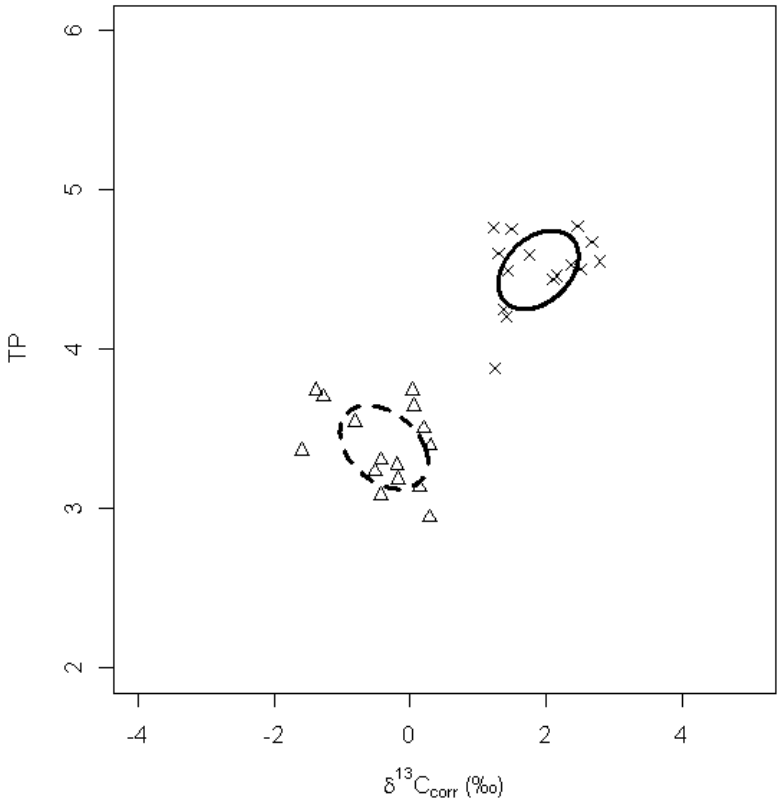

Figure 3.16 Standard ellipse areas of sympatric *Cyprinus carpio* and *Pseudorasbora parva*. (×) symbols represent individual *C. carpio* and open triangles represent individual *P. parva*. The lines enclose the standard ellipse area (SEA_c) for *C. carpio* (solid) and *P. parva* (dashed).

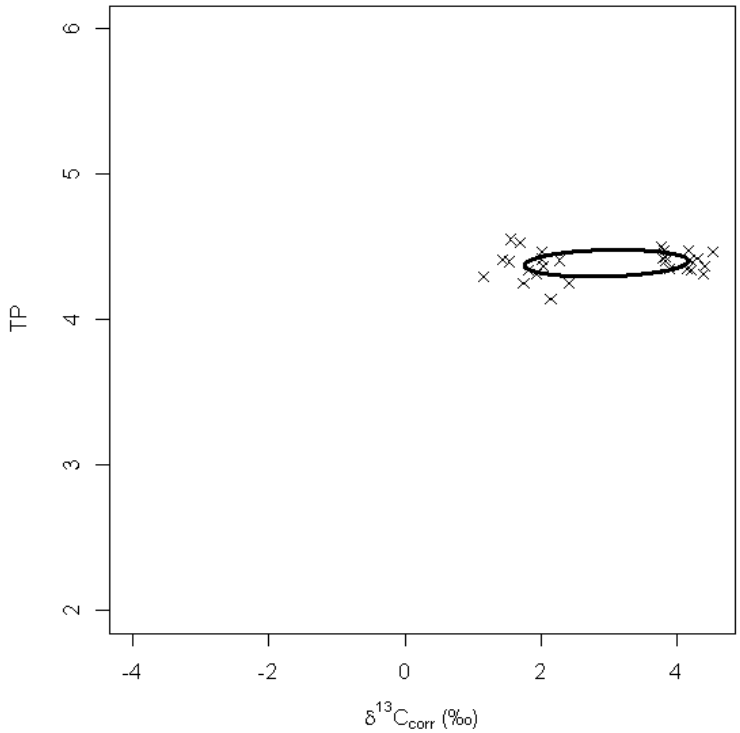

Figure 3.17 Standard ellipse areas of allopatric *Cyprinus carpio*. (×) represent individual *C. carpio* and the line encloses the standard ellipse area (SEA_c).

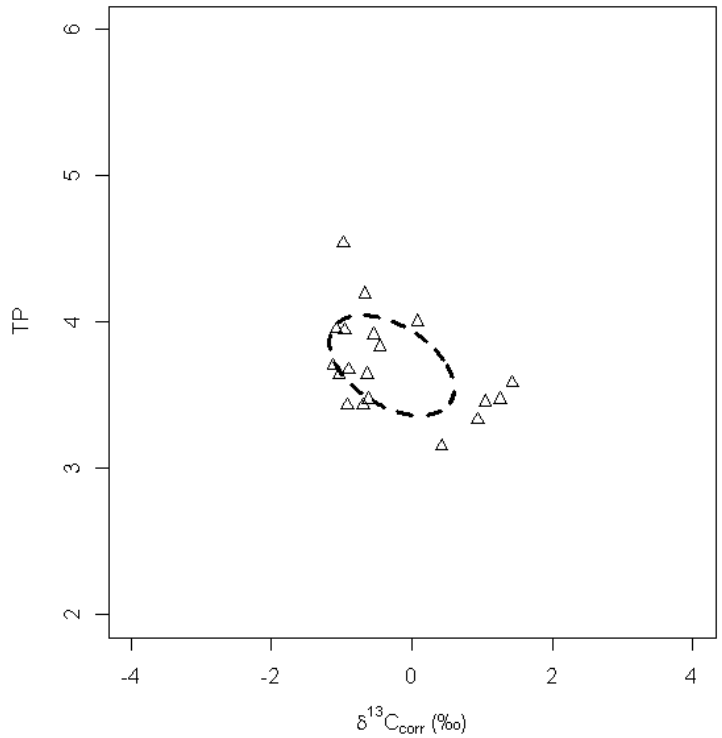

Figure 3.18 Standard ellipse area of allopatric *Pseudorasbora parva*. Open triangles represent individual *P. parva* and the lines encloses the standard ellipse area (SEA_c).

3.1.4 Summary of outputs from the mesocosm experiments

In summary, across the three experiments, the trophic position of sympatric *P. parva* was always lower than the co-habiting species. Comparison of their stable isotope metrics revealed that when in sympatry, *G. aculeatus* and *T. tinca* had larger trophic niche than *P. parva*, but was similar with *C. carpio*. Importantly, when in sympatry, there were no overlaps in the trophic niche space of *P. parva* with the other species, indicating a divergence in their trophic niches. Moreover, comparison of SEA_c for *G. aculeatus* and *T. tinca* between their allopatric and sympatric contexts revealed that their trophic niche sizes were larger in allopatry than in sympatry with *P. parva*, although as this was also apparent in *P. parva* then it was not clear whether this was caused by the divergence of both species or whether it was *P. parva* mediated (i.e. it was caused only by *P. parva* presence).

3.2 Small aquaculture ponds

In the small aquaculture ponds, *P. parva* was present in sympatry with a range of different fish species, ranging in number from one to four species, of which some of these scenarios were replicated (Table 2.3). In this section, the results are presented starting with the least complex scenario, *P. parva* and *G. aculeatus* in sympatry replicated four times, and finishing with the most complex scenario, *P. parva* with three other species with no replication. In these subsequent sections, the data are combined across each set of ponds wherever appropriate and are reported in the same manner. Where there were no statistical differences in the baseline stable isotope data for ponds with the same composition of their fish community, their data were combined to increase the statisitical power to detect significant differences.

3.2.1 Sympatric *Pseudorasbora parva and Gasterosteus aculeatus*

In this sub-section, the trophic relationships of *P. parva* and *G. aculeatus* in four small ponds are discussed where they were the only fish species present.

Fish lengths

The mean length of the analysed *P. parva* was 48.5 ± 2.0 mm and *G. aculeatus* was 42.2 ± 1.4 mm, with these significantly different from each other (ANOVA; $F_{1,78} = 5.44$, $P = 0.02$). The differences related to *P. parva* being a larger species than *G. aculeatus*. These differences also arose through these ponds were being

under uncontrolled conditions and so, unlike the mesocosms, were not able to be manipulated.

Stable isotope: fish length relationships

There was a significant relationship between length of *G. aculeatus* and $\delta^{15}N$, but not with $\delta^{13}C$ (Table 3.22). In contrast, for *P. parva*, the relationship between length and $\delta^{15}N$ was not significant, but was significant between length and $\delta^{13}C$ (Table 3.22).

Table 3.22 Outputs of linear regression testing the effects of fish length (mm) on $\delta^{13}C$ and $\delta^{15}N$.

Species	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
G. aculeatus	0.01	1,44	0.01	0.95	0.20	1,44	10.73	< 0.01
P. parva	0.19	1,32	7.44	0.01	0.01	1,32	0.01	0.94

Stable isotope data between the species

Due to significant relationships on fish length and the stable isotope data (Table 3.22), then generalized linear models were used to test for differences in stable isotope data using fish length as the covariate. Model outputs revealed the differences in both $\delta^{13}C$ and $\delta^{15}N$ were significant between the species (Table 3.23, 3.24), with *G. aculeatus* occupying a significantly higher trophic position than *P. parva* in these ponds (Fig. 3.19).

Table 3.23 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for *Gasterosteus aculeatus* and *Pseudorasbora parva* across the four small ponds where they were present in sympatry. Variation around the mean represents standard error.

Species	n	Mean length (mm)	Length range (mm)	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
G. aculeatus	46	42.15 ± 1.43	22 to 64	-31.29 ± 0.64	-36.93 to -23.63	7.91 ± 0.13	6.21 to 9.52
P. parva	34	48.50 ± 1.99	34 to 76	-28.60 ± 0.59	-35.46 to -24.56	6.57 ± 0.12	5.34 to 8.06

Table 3.24 Outputs of generalized linear model testing for differences in $\delta^{13}C$ and $\delta^{15}N$ for *Gasterosteus aculeatus* and *Pseudorasbora parva* in the small ponds where length is the effect of fork length as a covariate in the model (as Wald χ^2), group describes the two species used in context and group difference is the output of pairwise comparisons with Bonferroni adjustment for multiple comparisons. *P<0.05; **P<0.01.

	$\delta^{13}C$			$\delta^{15}N$		
	Length	Group	Group difference	Length	Group	Group difference
G. aculeatus vs. *P. parva*	1.75	10.93**	-3.00**	7.32	75.11**	1.52**

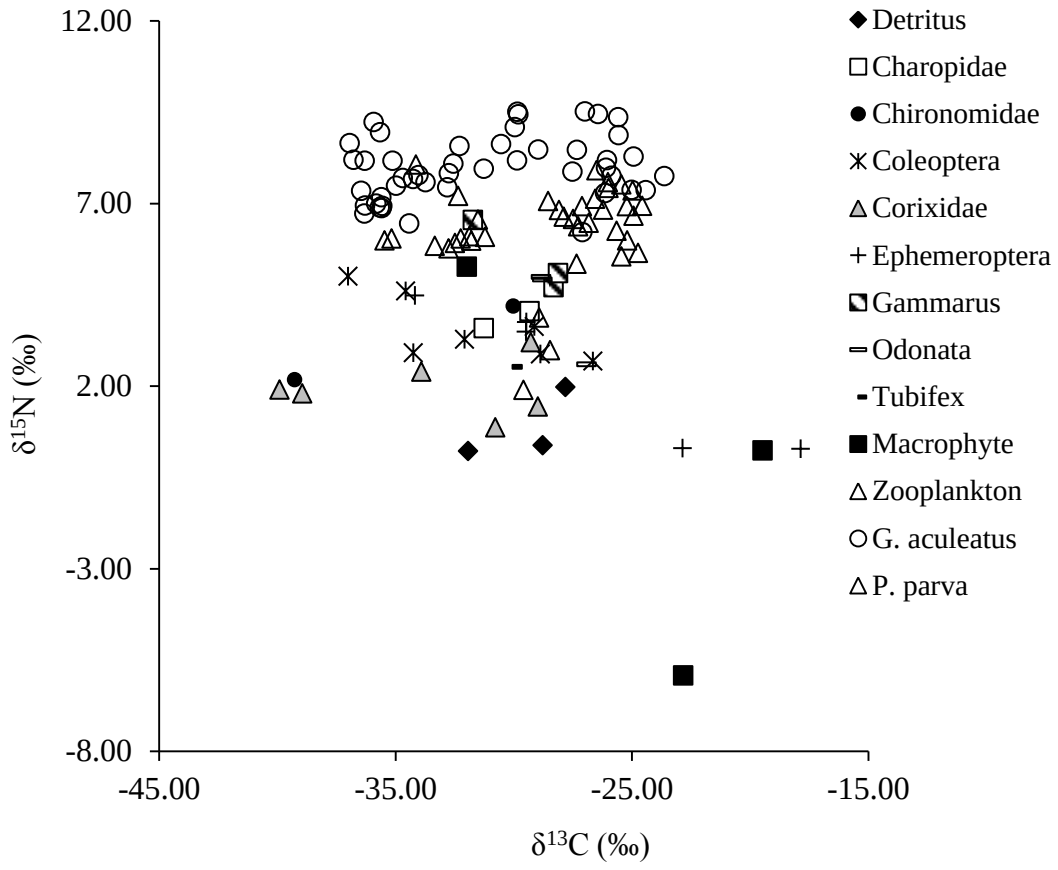

Figure 3.19 Stable isotope bi-plot showing the combined data from across the four small ponds in which the only species present were *Gasterosteus aculeatus* and *Pseudorasbora parva*.

Stable isotope metrics

There were no significant differences in the stable isotope data of the majority of the baseline invertebrates across the four aquaculture ponds (Coleoptera: $\delta^{15}N$ $F_{1,4} = 0.95$, $P > 0.05$; Corixidae: $\delta^{13}C$ $F_{1,3} = 1.45$, $P > 0.05$; $\delta^{15}N$ $F_{1,3} = 0.5$, $P > 0.05$; Table 3.25). However, there was a significant difference in $\delta^{13}C$ between the ponds in Coleoptera ($F_{1,4} = 15.15$, $P < 0.05$). As Coleoptera is a putative fish food resource then to enable comparison of data between the four ponds, the stable isotope data were corrected to TP and $\delta^{13}C_{corr}$ for use in the metric

calculations (Section 2.1.4; Table 3.26). The TP data indicated that *G. aculeatus* occupied a significantly higher trophic position than *P. parva* ($F_{1,17}$ = 186.29, P < 0.05) (Table 3.26). These converted data were then used to calculate the stable isotope metrics.

Table 3.25 Mean (± SE) stable isotope values of $\delta^{13}C$ and $\delta^{15}N$ for basal resources across the four ponds where only *Gasterosteus aculeatus* and *Pseudorasbora parva* were present.

Taxa	n	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
Detritus	3	-29.51 ± 1.24	-31.93 to -27.82	0.86 ± 0.56	0.22 to 1.98
Charopidae	2	-30.30 ± 0.97	-31.27 to -29.33	3.82 ± 0.24	3.58 to 4.05
Chironomidae	2	-34.65 ± 4.63	-39.27 to -30.02	3.18 ± 1.01	2.17 to 4.19
Coleoptera	7	-31.79 ± 1.41	-37.00 to -26.65	3.57 ± 0.34	2.68 to 5.01
Corixidae	6	-33.63 ± 1.97	-39.90 to -28.98	1.93 ± 0.33	0.87 to 3.20
Ephemeroptera	5	-26.77 ± 2.86	-34.17 to -17.86	2.46 ± 0.90	0.28 to 4.48
Gammarus	3	-29.39 ± 1.17	-31.72 to -28.12	5.45 ± 0.56	4.71 to 6.55
Odonata	3	-28.18 ± 0.63	-28.84 to -26.92	4.17 ± 0.79	2.60 to 4.99
Macrophyte	3	-24.76 ± 3.74	-31.98 to -19.47	-0.14 ± 3.24	-5.92 to 5.27
Zooplankton	3	-28.99 ± 0.33	-29.59 to -28.46	2.92 ± 0.57	1.90 to 3.87

Table 3.26 $\delta^{13}C_{corr}$ and trophic position (TP) values of *Gasterosteus aculeatus* and *Pseudorasbora parva* in the four small ponds

	$\delta^{13}C_{corr}$	Range	TP	Range
G. aculeatus	0.14 ± 0.06	-0.9 to 1.02	3.39 ± 0.05	2.90 to 4.04
P. parva	0.42 ± 0.05	-0.06 to 1.09	2.99 ± 0.05	2.65 to 3.61

The metrics of standard ellipse area (SEA_c), nitrogen range (NR) and carbon range (CR), revealed that *G. aculeatus* had a larger trophic niche than *P. parva* (Table 3.27), with no overlap in their trophic niches (Fig. 3.20).

Table 3.27 Stable isotope metrics for *Gasterosteus aculeatus* and *Pseudorasbora parva* in sympatric context and where SEA_c= Standard ellipse area, NR= $\delta^{15}N$ range and CR= $\delta^{13}C$ range.

Species	SEA_c	NR	CR
G. aculeatus	11.50	3.31	13.31
P. parva	7.46	2.72	10.90

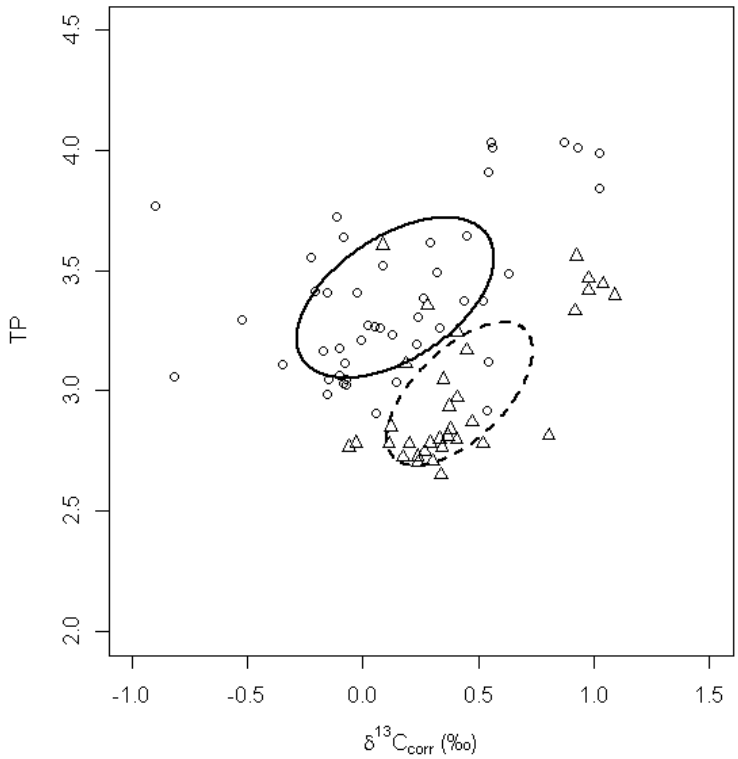

Figure 3.20 Standard ellipse areas of *Gasterosteus aculeatus* and *Pseudorasbora parva*. Open circles represent individual *G. aculeatus* and open triangles represent individual *P. parva*. The lines enclose the standard ellipse area (SEA_c) for *G. aculeatus* (solid) and *P. parva* (dashed).

3.2.2. Sympatric *Pseudorasbora parva, Tinca tinca and Pacifastacus leniusculus* in small ponds

In this sub-section, the trophic relationships of *P. parva* and *T. tinca*, and *Pacifastacus leniusculus* in two small ponds are discussed where they were the only fish and crayfish species present.

Fish and crayfish lengths

The mean length of *T. tinca* from the two ponds was 99.88 ± 2.87 mm and *P. parva* was 53.69 ± 2.86 mm, with the length difference between the species being significant ($F_{1,30}$ = 129.91; P < 0.01). The mean carapace length of *P. leniusculus* was 51.81 ± 1.86 mm; given the morphological differences between them and the fish species, their lengths are not compared.

Stable isotope: fish length relationships

The relationship between $\delta^{13}C$ and $\delta^{15}N$ was tested against body and carapace length for the fish and crayfish respectively using linear regression to determine if there were any ontogenetic shifts in diet that would have to be accounted for in subsequent tests. The results revealed there was no significant relationship between length and $\delta^{13}C$ and $\delta^{15}N$ (Table 3.28).

Stable isotope data between the species

There were some significant differences in $\delta^{13}C$ and $\delta^{15}N$ between *T. tinca*, *P. parva* and *P. leniusculus* (Table 3.29, 3.30), with $\delta^{15}N$ suggesting *T. tinca* occupied a higher trophic position than both *P. parva* and *P. leniusculus*, and *P. parva* occupied a higher trophic position than *P. leniusculus* (Table 3.29; Fig. 3.21).

Table 3.28 Outputs of linear regression testing the effect of body and carapace length (mm) on $\delta^{13}C$ and $\delta^{15}N$.

Species	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
P. leniusculus	0.09	1,14	1.38	0.26	0.04	1,14	0.64	0.44
T. tinca	0.03	1,14	0.48	0.50	0.09	1,14	1.44	0.25
P. parva	0.11	1,14	1.72	0.21	0.04	1,14	0.55	0.47

Table 3.29 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for *Pseudorasbora parva, Tinca tinca and Pacifastacus leniusculus* across the two small ponds where they were present in sympatry. Variation around the mean represents standard error.

Species	n	Mean length (mm)	Length range (mm)	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
P. leniusculus	16	51.81 ± 1.86	40 to 65	-28.90 ± 0.22	-30.50 to -27.39	8.68 ± 0.17	7.76 to 9.76
T. tinca	16	99.88 ± 2.87	77 to 121	-32.93 ± 0.29	-34.28 to -30.42	12.76 ± 0.29	11.30 to 14.35
P. parva	16	53.69 ± 2.86	33 to 71	-32.76 ± 0.39	-34.89 to -29.14	10.80 ± 0.57	7.30 to 13.37

Table 3.30 Outputs of Mann Whitney U tests to determine the difference in the stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) of *Pseudorasbora parva, Tinca tinca and Pacifastacus leniusculus* across the two small ponds where they were present in sympatry.

Test	$\delta^{13}C$		$\delta^{15}N$	
	Z	P	Z	P
P. leniusculus vs. *P. parva*	-4.79	<0.01	-4.82	<0.01
P. leniusculus vs. *T. tinca*	-4.56	<0.01	-2.26	<0.05
T. tinca vs. *P. parva*	-0.26	>0.05	-2.04	<0.05

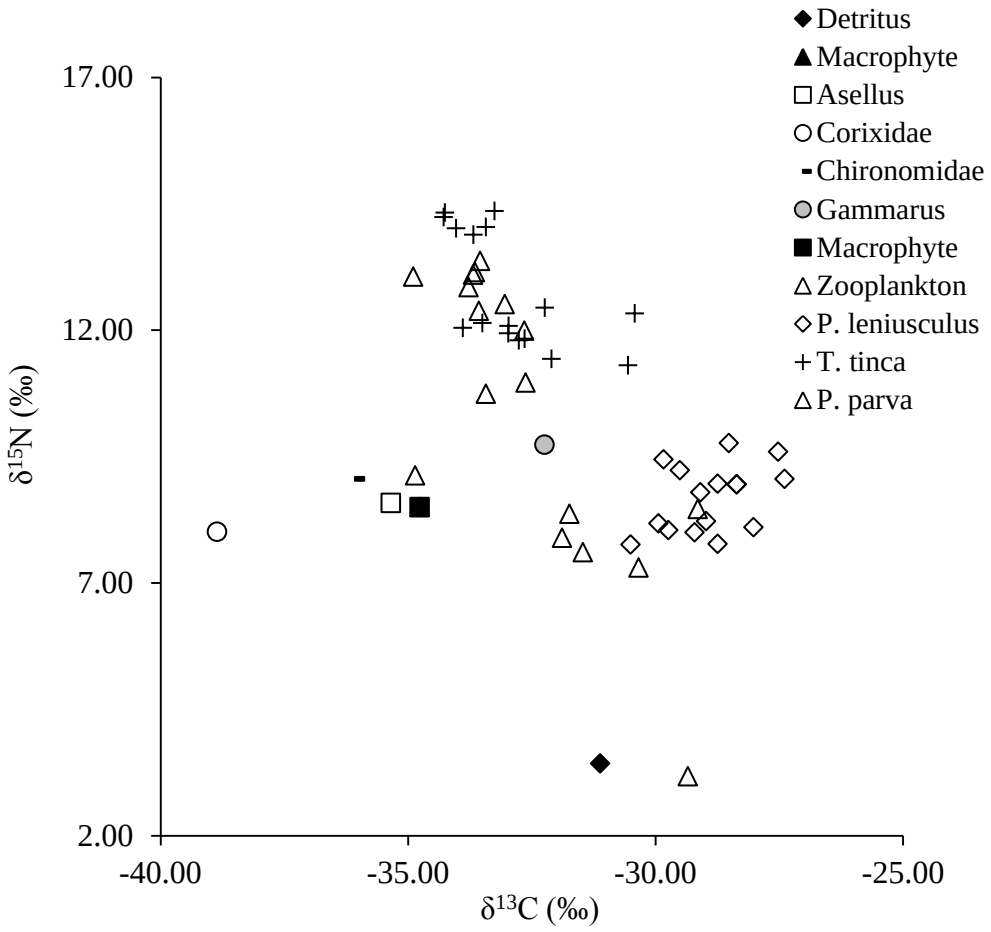

Figure 3.21 Stable isotope bi-plot for *Pseudorasbora parva, Tinca tinca and Pacifastacus leniusculus* in sympatric context from the two ponds (combined data)

Stable isotope metrics

In these two ponds, replicated samples of the baseline data were unavailable, with only single samples available for analysis. Although these cannot be tested statistically, there were only slight differences between each of the samples taken (Table 3.31). Consequently, it was decided that there was no requirement to correct the data prior to combining the stable isotope data to enable their metrics

to be calculated. These were the data used within the subsequent calculations of the stable isotope metrics (Table 3.32).

Table 3.31 Stable isotope values of the single samples of the baseline items for the two ponds with *Pseudorasbora parva*, *Tinca tinca* and *Pacifastacus leniusculus* present, where 1 and 2 indicate the different ponds.

	$\delta^{13}C$		$\delta^{15}N$	
Item	1	2	1	2
Gammarid	-32.15	-32.24	9.73	9.68
Corixid	-38.98	-38.86	8.01	8.15
Chironomid	-36.24	-36.07	9.05	9.09
Asellus	-35.56	-35.34	8.58	8.64
Detritus	-32.76	-31.12	3.43	3.59
Macrophyte	-34.67	-34.76	8.50	8.78
Zooplankton	-30.14	-29.94	3.17	3.54

Comparison of the metrics standard ellipse area (SEA_c), nitrogen range (NR) and carbon range (CR) revealed that *P. parva* had a larger trophic niche than *P. leniusculus* and *T. tinca* (Table 3.33), with a small trophic overlap between *T. tinca* and *P. parva* of 16.80 % (Fig. 3.22). Trophic position indicated *T. tinca* occupied significant higher trophic position than *P. parva* and *P. leniusculus* (*T. tinca* vs. *P. leniusculus* $F_{1,30} = 52.56$, $P < 0.05$; *T. tinca* vs. *P. parva* $F_{1,30} = 9.37$, $P < 0.05$) (Table 3.32).

Table 3.32 Stable isotope metrics for *Pseudorasbora parva, Tinca tinca and Pacifastacus leniusculus* in sympatric context and where SEA_c= Standard ellipse area, NR= $\delta^{15}N$ range and CR= $\delta^{13}C$ range, TP= trophic position.

Species	SEA_c	NR	CR	TP
P. leniusculus	1.78	2.00	3.11	2.43
T. tinca	3.45	3.05	3.86	3.63
P. parva	8.48	6.07	5.75	3.06

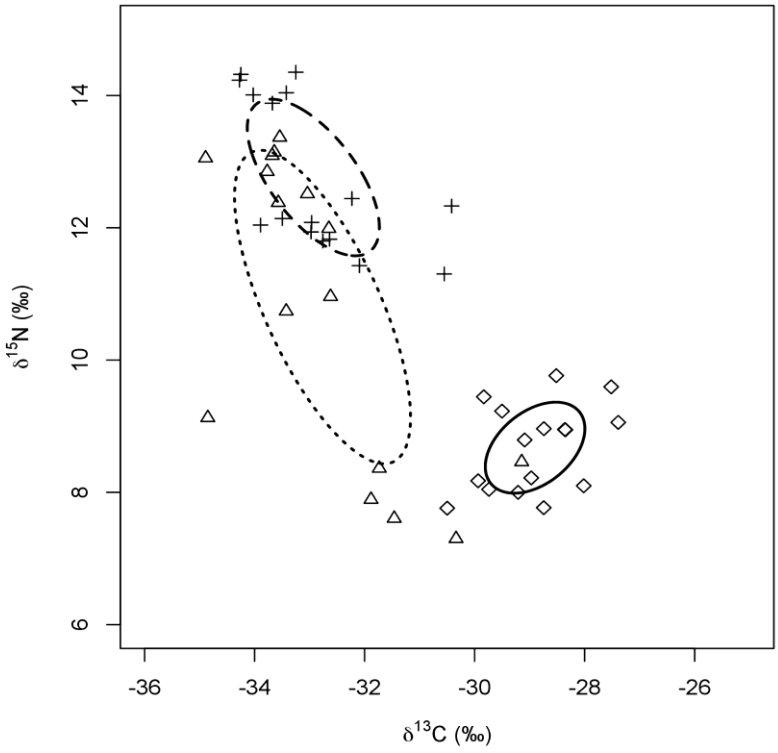

Figure 3.22 Standard ellipse areas of *Pseudorasbora parva, Tinca tinca and Pacifastacus leniusculus.* Open diamonds represent individual *P. leniusculus,* (+) symbols represent individual *T. tinca* and open triangles represent individual *P. parva*. The lines enclose the standard ellipse area (SEA_c) for *P. leniusculus* (solid), *T. tinca* (dashed) and *P. parva* (dotted).

3.2.3 Sympatric *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Pacifastacus leniusculus*

In this sub-section, the trophic relationships of *P. parva*, *G. aculeatus*, *T. tinca* and *P. leniusculus* in a single small pond are discussed, in which they were the only fish and crayfish species present.

Fish length

In the stable isotope analyses, the mean fork length of *P. parva* was 48.00 ± 2.09 mm, *T. tinca* was 108.88 ± 4.76 mm, and *G. aculeatus* was 45.62 ± 1.54 mm; *T. tinca* were significantly higher in length than the other two fish species, whilst the lengths of *P. parva* and *G. aculeatus* were not significantly different (Table 3.33). Mean carapace length of *P. leniusculus* was 25.63 ± 2.58 mm.

Table 3.33 Outputs of ANOVA testing for differences in the fork lengths of *Pseudorasbora parva, Tinca tinca* and *Gasterosteus aculeatus and Pacifastacus leniusculus.*

Species	*d.f.*	F	P
T. tinca - P. parva	1,14	137.71	<0.01
T. tinca - G. aculeatus	1,19	229.61	<0.01
P. parva - G. aculeatus	1,19	0.87	0.36

Stable isotope: fish length relationships

Linear regression revealed there were no significant relationship between fish and carapace length and $\delta^{13}C$ and $\delta^{15}N$ in any of the fish species (Table 3.34).

Table 3.34 Outputs of linear regression testing the effect of fish and carapace length (mm) on $\delta^{13}C$ and $\delta^{15}N$.

Species	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
P. leniusculus	0.21	1,60	1.62	0.25	0.36	1,60	3.42	0.11
T. tinca	0.33	1,60	2.94	0.14	0.28	1,60	2.28	0.18
P. parva	0.01	1,60	0.03	0.86	0.01	1,60	0.01	0.96
G. aculeatus	0.03	1,11	0.34	0.57	0.10	1,11	1.22	0.29

Stable isotope data between species

As there were no length effects on the stable isotope values then Mann Whitney U tests were used to test for differences in stable isotope values between the species. These revealed some significant inter-specific differences in $\delta^{13}C$ and $\delta^{15}N$ (Table 3.35, 3.36); for *P. parva*, their $\delta^{15}N$ was significantly different to *T. tinca* and *G. aculeatus*, but not *P. leniusculus* (Table 3.36).

Table 3.35 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Pacifastacus leniusculus* in their sympatric context. Variation around the mean represents standard error.

Species	n	Mean length (mm)	Length range (mm)	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
P. leniusculus	8	25.63 ± 2.58	15 to 35	-31.20 ± 0.26	-32.43 to -30.24	9.76 ± 0.37	8.59 to 11.20
T. tinca	8	108.88 ± 4.76	97 to 138	-32.32 ± 0.45	-34.25 to -30.40	12.70 ± 0.25	11.42 to 13.42
P. parva	8	48.00 ± 2.09	40 to 59	-32.02 ± 1.08	-35.04 to -27.09	9.93 ± 0.85	6.42 to 12.79
G. aculeatus	13	45.62 ± 1.54	31 to 53	-34.12 ± 0.50	-35.70 to -30.02	13.96 ± 0.68	7.63 to 16.61

Table 3.36 Outputs of Mann Whitney U tests testing the difference in $\delta^{13}C$ and $\delta^{15}N$ between sympatric *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Pacifastacus leniusculus*.

Species	$\delta^{13}C$		$\delta^{15}N$	
	Z	P	Z	P
P. leniusculus vs. T. tinca	-1.79	>0.05	-3.36	<0.01
P. leniusculus vs. P. parva	-0.95	>0.05	-0.63	>0.05
P. leniusculus vs. G. aculeatus	-3.04	<0.01	-3.19	<0.01
T. tinca vs. P. parva	-0.32	>0.05	-2.79	<0.05
T. tinca vs. G. aculeatus	-2.32	<0.05	-2.10	<0.05
P. parva vs. G. aculeatus	-1.81	>0.05	-2.93	<0.05

Stable isotope metrics

As there was only one pond then there was no requirement for stable isotope data correction; the baseline stable isotope data for the pond are shown in Figure 3.23. The $\delta^{15}N$ data suggested *G. aculeatus* occupied higher trophic positions than both *P. parva* and *T. tinca*, and *P. parva* occupied the lowest trophic position (Fig. 3.23, Table 3.37). Comparison of the metrics standard ellipse area (SEA_c), nitrogen range (NR) and carbon range (CR) revealed that *P. parva* had the larger trophic niche among the four species (Table 3.37; Fig. 3.24), with their trophic niche having an overlap with *P. leniusculus* of 29.32 % (Fig. 3.24). Also, the trophic niches of *T. tinca* and *G. aculeatus* had minor overlap of 8.34 %, with no overlap between the other species (Fig. 3.24). Calculation of TP revealed *P. parva* occupied a significant lower trophic position than *G. aculeatus* and *T. tinca* (*P. parva* vs. *G. aculeatus* $F_{1,19} = 13.45$, $P < 0.05$; *P. parva* vs. *T. tinca* $F_{1,14}$

= 9.81, P < 0.05), but not *P. leniusculus* (*P. parva* vs. *P. leniusculus* $F_{1,14}$ = 0.03, P > 0.05) (Table 3.37).

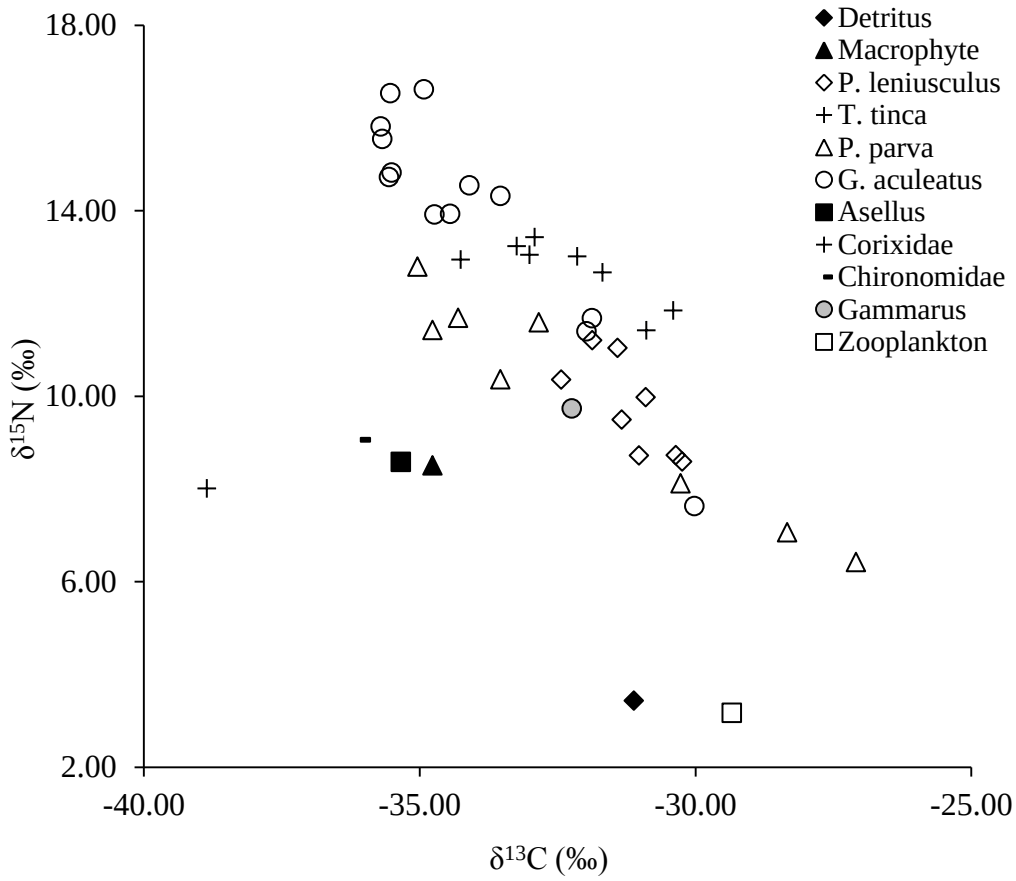

Figure 3.23 Stable isotope bi-plot showing data from the sympatric *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Pacifastacus leniusculus* in the single pond.

Table 3.37 Stable isotope metrics for *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Pacifastacus leniusculus* in sympatric context and where SEA_c= Standard ellipse area, NR= $\delta^{15}N$ range and CR= $\delta^{13}C$ range, TP= trophic position.

Species	SEA_c	NR	CR	TP
P. leniusculus	1.87	2.61	2.19	2.75
T. tinca	1.99	2.01	3.85	3.61
P. parva	6.80	6.37	7.95	2.80
G. aculeatus	5.46	8.98	5.69	3.98

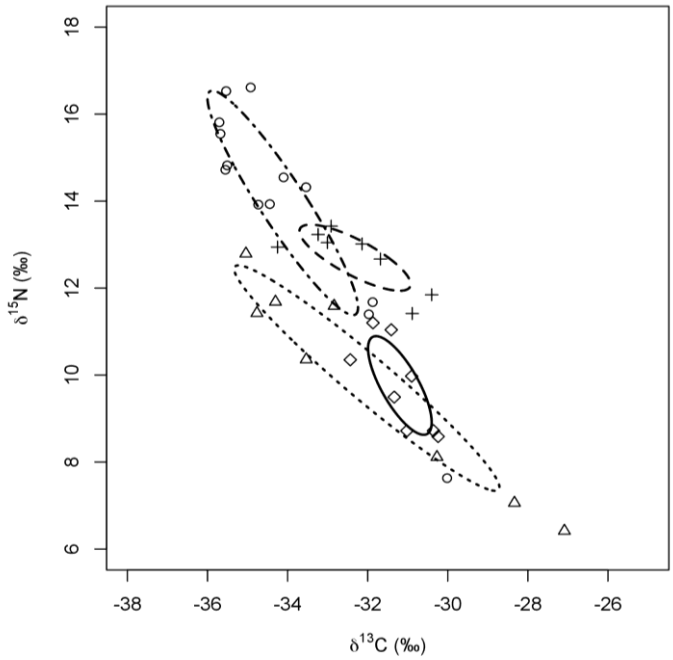

Figure 3.24 Standard ellipse areas of *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Pacifastacus leniusculus.* Open diamonds represent individual *P. leniusculus*, (+) symbols represent individual *T. tinca*, open circles represent individual *G. aculeatus* and open triangles represent individual *P. parva*. The lines enclose the standard ellipse area (SEA_c) for *P. leniusculus* (solid), *T. tinca* (dashed), *G. aculeatus* (dotdash) and *P. parva* (dotted).

3.2.4 Sympatric *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Cyprinus carpio*

In this sub-section, the trophic relationships of *P. parva*, *G. aculeatus*, *T. tinca* and *C. carpio* in a single small pond are discussed, in which they were the only fish species present.

Fish lengths

In the stable isotope analyses, the mean fork length of *P. parva* was 37.38 ± 2.55 mm, *T. tinca* was 104.50 ± 11.68 mm, *G. aculeatus* was 36.50 ± 0.53 mm and *C. carpio* was 135.33 ± 28.17 mm. The length differences between *C. carpio* and *T. tinca* were not significantly different, but both of these species were significantly greater in length than *P. parva* and *G. aculeatus* (Table 3.38).

Stable isotope: fish length relationships

Linear regression revealed there were no significant relationships between fish length and $\delta^{13}C$ and $\delta^{15}N$ for any of the species (Table 3.39). Thus, subsequent stable isotope analyses did not require length to be controlled as a covariate.

Table 3.38 Outputs of ANOVA testing for differences in the fork lengths of *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Cyprinus carpio*.

Species	*d.f.*	F	P
C. carpio - T. tinca	1,70	1.50	0.26
C. carpio - P. parva	1,90	36.77	<0.01
C. carpio - G. aculeatus	1,90	40.16	<0.01
T. tinca - P. parva	1,12	41.63	<0.01
T. tinca - G. aculeatus	1,12	46.34	<0.01
P. parva - G. aculeatus	1,14	0.11	0.74

Table 3.39 Outputs of linear regression testing the effect of fish length (mm) on $\delta^{13}C$ and $\delta^{15}N$.

Species	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
C. carpio	0.91	1,10	10.4	0.19	0.96	1,10	21.3	0.14
T. tinca	0.43	1,40	3.07	0.15	0.16	1,40	0.79	0.43
P. parva	0.16	1,60	1.11	0.33	0.06	1,60	0.40	0.55
G. aculeatus	0.02	1,60	0.15	0.71	0.07	1,60	0.46	0.52

Stable isotope data between species

Mann Whitney U tests revealed significant differences in $\delta^{13}C$ and $\delta^{15}N$ between *C. carpio, T. tinca* and *G. aculeatus*, but not in $\delta^{15}N$ between *P. parva, C. carpio* and *T. tinca* (Table 3.40, 3.41; Fig. 3.25). The *G. aculeatus* also occupied a significantly higher trophic position than other species (Table 3.41).

Table 3.40 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for sympatric *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Cyprinus carpio*. Variation around the mean represents standard error.

Species	n	Mean length (mm)	Length range (mm)	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
C. carpio	3	135.33 ± 28.17	100 to 191	-26.80 ± 0.43	-27.52 to -26.02	8.23 ± 0.41	7.78 to 9.04
T. tinca	6	104.50 ± 11.68	74 to 149	-30.10 ± 0.36	-31.33 to -29.11	9.64 ± 0.20	9.07 to 10.46
P. parva	8	37.38 ± 2.55	24 to 45	-31.90 ± 0.27	-32.63 to -30.53	7.39 ± 0.17	6.46 to 7.90
G. aculeatus	8	36.50 ± 0.53	35 to 39	-31.25 ± 0.23	-32.24 to -30.45	9.88 ± 0.17	9.12 to 10.86

Table 3.41 Outputs of Mann Whitney U tests to determine the difference in the stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) of *Pseudorasbora parva*, *Tinca tinca*, *Gasterosteus aculeatus and Cyprinus carpio*.

Species	$\delta^{13}C$		$\delta^{15}N$	
	Z	P	Z	P
C. carpio vs. *T. tinca*	-2.32	<0.05	-2.32	<0.05
C. carpio vs. *P. parva*	-2.45	<0.05	-1.74	>0.05
C. carpio vs. *G. aculeatus*	-2.46	<0.05	-2.45	<0.05
T. tinca vs. *G. aculeatus*	-2.71	<0.05	-3.10	<0.05
T. tinca vs. *P. parva*	-2.20	<0.05	-1.23	>0.05
P. parva vs. *G. aculeatus*	-1.68	>0.05	-3.36	<0.05

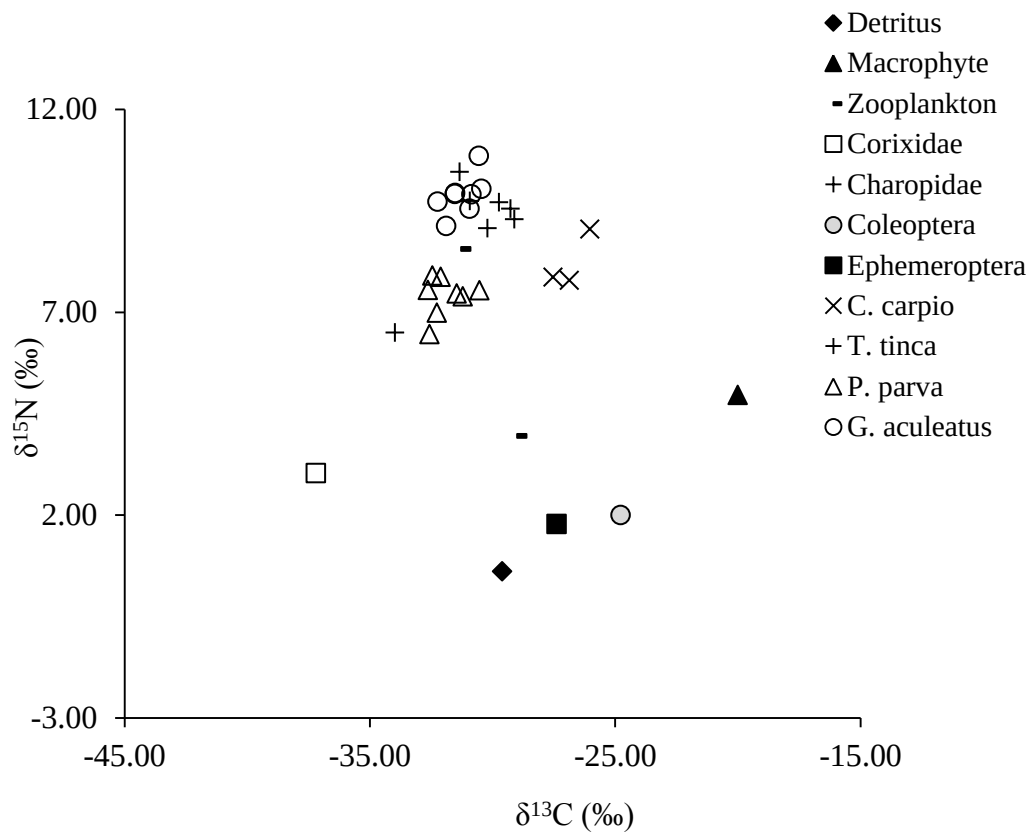

Figure 3.25 Stable isotope bi-plot showing data from the small pond where *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Cyprinus carpio* were present in sympatry.

Stable isotope metrics

With data on these species only available from a single pond then there was no requirement for data correction and the data for the stable isotope baseline are displayed in Figure 3.25. Comparison of the metrics standard ellipse area (SEA_c), nitrogen range (NR) and carbon range (CR) revealed that *C. carpio* had the highest SEA_c among the species, albeit this was measured from only 3 fish and all of the species had relatively small trophic niche sizes (Table 3.42; Fig. 3.26). Moreover, there was minimal overlap in the trophic niches of any of the species

(Fig. 3.26). Calculation of TP indicated that *P. parva* occupied significantly lower trophic positions than *G. aculeatus, C. carpio* and *T. tinca (P. parva* vs. *G. aculeatus* $F_{1,14} = 106.18$, $P < 0.05$; *P. parva* vs. *C. carpio* $F_{1,9} = 5.35$, $P < 0.05$; *P. parva* vs. *T. tinca* $F_{1,12} = 76.35$, $P < 0.05$).

Table 3.42 Stable isotope metrics for *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Cyprinus carpio* in sympatric context and where SEA_c= Standard ellipse area, NR= $\delta^{15}N$ range and CR= $\delta^{13}C$ range, TP= trophic position.

Species	SEA_c	NR	CR	TP
C. carpio	1.64	1.26	1.50	3.13
T. tinca	1.24	1.38	2.22	3.54
P. parva	1.29	1.45	2.10	2.88
G. aculeatus	0.93	1.73	1.79	3.61

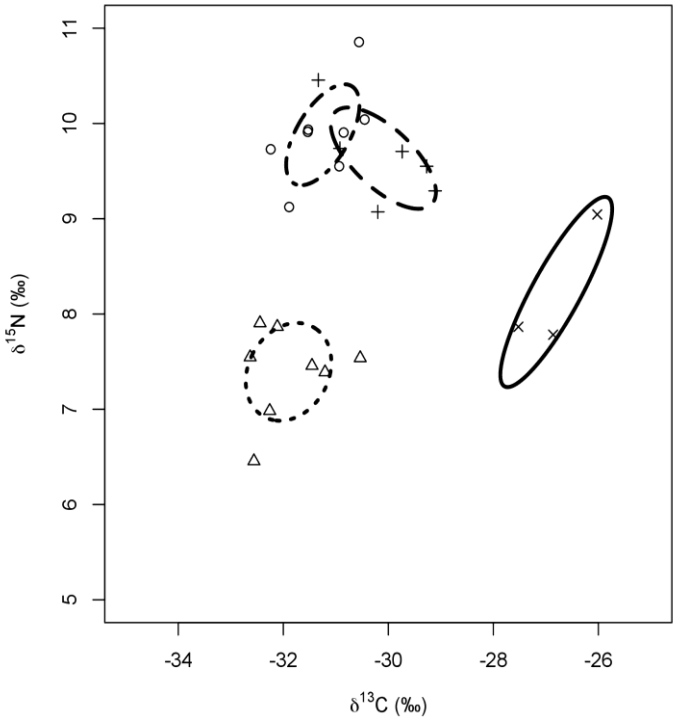

Figure 3.26 Standard ellipse areas of sympatric *Pseudorasbora parva, Tinca tinca, Gasterosteus aculeatus and Cyprinus carpio.* (×) symbols represent individual *C. carpio,* (+) symbols represent individual *T. tinca,* open circles represent individual *G. aculeatus* and open triangles represent individual *P. parva*. The lines enclose the standard ellipse area (SEA_c) for *C. carpio* (solid), *T. tinca* (dashed), *G. aculeatus* (dotdash) and *P. parva* (dotted).

3.2.5 Summary of outputs from the small ponds

In these small ponds, the stable isotope metrics revealed that *P. parva* tended to occupy low trophic positions compared to the other fish species, including *G. aculeatus*. The extent to which the trophic niches of *P. parva* and the other fishes overlapped was generally low and thus, similar to the mesocosm experiments,

there was a tendency for trophic niche divergence between these species, rather than convergence. This suggests the food resources exploited by the sympatric species differed and thus competition was being avoided.

3.3 Wild pond sites

There were four ponds sampled in the wild, Belgium Ponds 1, 2 and 3, and the Millennium Coastal Park pond. The results are presented sequentially in the following sub-sections.

3.3.1 Belgium pond 1

Fish species and lengths

The fish species sampled from Belgium Pond 1, and their length range and mean lengths, are provided in Table 3.43. There were some significant differences in the lengths of these fish between the species, most notably for *Carassius gibelio* and all other species (Table 3.43, 3.44).

Table 3.43 Number, mean length and length range of fish sampled from Belgium Pond 1, where N (sampled) represents the number of fish captured, n (analysed) is the number of fish used in stable isotope analysis and the lengths represent the analysed fish only.

Species	N (sampled)	n (analysed)	Mean length (mm)	Length range (mm)
P. parva	505	12	60.00 ± 3.74	42 to 82
C. gibelio	19	15	121.6 ± 13.77	44 to 209
R. amarus	4	4	67.00 ± 1.22	65 to 70
P. pungitius	20	10	52.60 ± 1.05	48 to 60

Table 3.44 Outputs of Mann Whitney U tests that tested the significance of differences in the lengths of the fish species in Belgium Pond 1.

Species	Fork length	
	Z	P
P. parva vs. *C. gibelio*	-3.59	<0.01
P. parva vs. *R. amarus*	-0.91	>0.05
P. parva vs. *P. pungitius*	-1.22	>0.05
C. gibelio vs. *R. amarus*	-2.55	<0.01
C. gibelio vs. *P. pungitius*	-3.61	<0.01
R. amarus vs. *P. pungitius*	-2.85	<0.01

Stable isotope: fish length relationships

For the majority of the fish species, linear regression revealed no significant relationships between fish length and $\delta^{13}C$ and $\delta^{15}N$ (Table 3.45), the exception being $\delta^{15}N$ in *P. pungitius* (Table 3.45).

Table 3.45 Outputs of linear regression testing the effect of fish length (mm) on $\delta^{13}C$ and $\delta^{15}N$.

Species	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
P. parva	0.01	1,10	0.03	0.87	0.02	1,10	0.18	0.68
C. gibelio	0.21	1,13	3.43	0.09	0.05	1,13	0.75	0.40
R. amarus	0.01	1,20	0.01	0.97	0.08	1,20	0.17	0.72
P. pungitius	0.19	1,80	1.85	0.21	0.47	1,80	7.10	0.03

Stable isotope data of fish species in Pond 1

Given that the relationship of $\delta^{15}N$ and length of *P. pungitius* was significant (Table 3.45) then testing for differences in the stable isotope data between

species needed to control for fish length as a covariate within a generalized linear model. This revealed that significant differences in $\delta^{15}N$ were apparent between all the species, and in $\delta^{13}C$, for all but *P. pungitius* and *P. parva* (Table 3.46, 3.47; Fig. 3.27).

Table 3.46 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for species in Belgium Pond 1. Variation around the mean represents standard error.

Species	n	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
P. parva	12	-37.38 ± 0.17	-38.7 to -36.44	15.75 ± 0.13	15.24 to 16.93
C. gibelio	15	-36.41 ± 0.12	-37.35 to -35.75	14.93 ± 0.12	13.69 to 15.80
R. amarus	4	-35.71 ± 0.12	-35.99 to -35.42	14.22 ± 0.15	13.92 to 14.52
P. pungitius	10	-38.31 ± 0.61	-40.16 to -34.63	13.33 ± 0.66	10.13 to 16.47

Table 3.47 Outputs of generalized linear model testing for differences in $\delta^{13}C$ and $\delta^{15}N$ for species in Belgium Pond 1 where length is the effect of fork length as a covariate in the model (as Wald χ^2), group describes the two species used in each aspect of the test, and group difference is the pairwise comparisons of the species with Bonferroni adjustment for multiple comparisons where * $P < 0.05$; ** $P < 0.01$.

	$\delta^{13}C$			$\delta^{15}N$		
	Length	Group	Group difference	Length	Group	Group difference
P. parva vs. *C. gibelio*	2.26	25.43**	-1.20**	0.71	19.07**	0.93**
P. parva vs. *R. amarus*	0.01	29.30**	-1.65**	0.21	42.90**	1.51**
P. parva vs. *P. pungitius*	0.26	1.84	0.80	0.33	13.53**	2.29**
C. gibelio vs. *R. amarus*	4.63*	4.72**	-0.48**	1.04	5.32**	0.26**
C. gibelio vs. *P. pungitius*	0.28	10.85**	2.12**	0.24	4.01*	0.69**
R. amarus vs. *P. pungitius*	2.60	0.06	-0.49	9.55*	5.54*	-4.42**

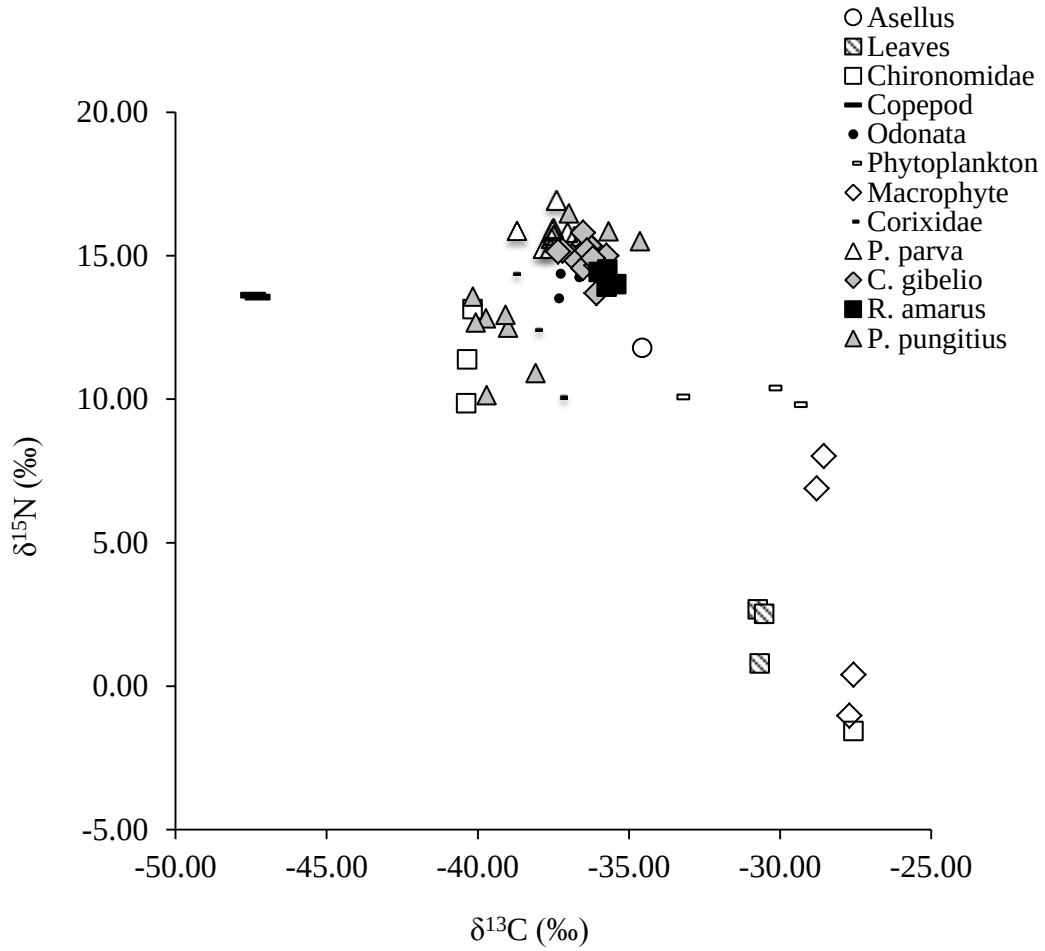

Figure 3.27 Stable isotope bi-plot showing data from the sampled fish and basal resources data from Belgium Pond 1.

Stable isotope metrics

There was no requirement to correct the data for subsequent analyses, as only one pond was being analysed. The stable isotope data for the baseline data are displayed in Figure 3.27. Calculation of TP revealed *P. parva* occupied significantly higher trophic positions than the others species sampled from the pond (*P. parva* vs. *C. gibelio*: $F_{1,25} = 21.45$, $P < 0.05$; *P. parva* vs. *R. amarus:* $F_{1,14} = 41.21$, $P < 0.05$; *P. parva* vs. *G. aculeatus:* $F_{1,20} = 15.61$, $P < 0.05$) (Table 3.48). Comparison of the metrics standard ellipse area (SEA_c), nitrogen range

(NR) and carbon range (CR) revealed that *G. aculeatus* had a comparatively large trophic niche compared with the other species (Table 3.48) and had minimal overlap with both *P. parva* (0.98 %) and *C. gibelio* (4.25 %) (Fig. 3.28). There were no other overlapping trophic niches between the species (Fig. 3.28).

Table 3.48 Stable isotope metrics for the fish species from Belgium Pond 1, where SEA_c= Standard ellipse area, NR= $\delta^{15}N$ range, CR= $\delta^{13}C$ range and TP = trophic position.

Species	SEA_c	NR	CR	TP
P. parva	0.91	1.70	2.26	3.22 ± 0.04
C. gibelio	0.67	2.11	1.60	2.98 ± 0.04
R. amarus	0.28	0.60	0.58	2.77 ± 0.04
G. aculeatus	10.25	6.34	5.53	2.51 ± 0.19

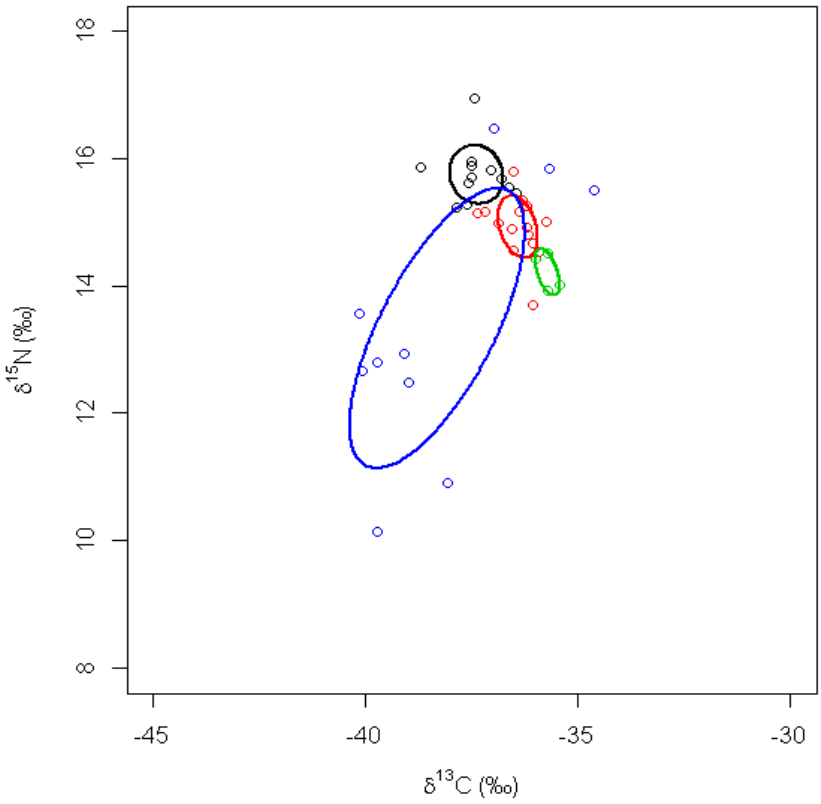

Figure 3.28 Standard ellipse areas of *Pseudorasbora parva*, *Carassius gibelio*, *Rhodeus amarus* and *Pungitius pungitius*. Open circles represent individual species and different colour represent different species. The line encloses the standard ellipse area (SEA_c) for *P. parva* (black), *C. gibelio* (red), *R. amarus* (green) and *P. pungitius* (blue).

3.3.2 Belgium pond 2

Fish species and lengths

There were 9 fish species sampled from Belgium Pond 2, of which five were non-native (Table 3.49). Their sample size, length range and mean lengths are shown in Table 3.49; between the fish species, there were significant differences in the lengths of these used in subsequent analyses (Table 3.50)

Table 3.49 Number, mean length and length range of fish sampled from Belgium Pond 2, where N (sampled) represents the number of fish captured, n (analysed) is the number of fish used in stable isotope analysis and the lengths represent the analysed fish only, and where * indicates the species is non-native to Belgium.

Species	N (sampled)	n (analysed)	Mean length (mm)	Length range (mm)
*P. parva**	1460	10	72.10 ± 2.30	62 to 85
*C. gibelio**	27	10	85.60 ± 4.80	53 to 103
*R. amarus**	30	10	51.80 ± 4.40	35 to 70
G. aculeatus	> 20	10	48.40 ± 1.00	43 to 53
Scardinius erythropthalmus	> 20	14	92.80 ± 11.00	44 to 183
Blicca bjoerkna	7	6	55.50 ± 2.00	51 to 65
*Leucaspius delineatus**	24	10	58.50 ± 3.50	38 to 73
Rutilus rutilus	> 20	12	97.70 ± 12.80	43 to 150
*C. carpio**	27	10	70.30 ± 2.70	58 to 85

Table 3.50 Outputs of Mann Whitney U tests testing the significance of differences in the lengths of the species from Belgium Pond 2.

Species	Length	
	Z	P
P. parva vs. *C. gibelio*	-2.42	<0.05
P. parva vs. *R. amarus*	-2.91	<0.01
P. parva vs. *G. aculeatus*	-3.79	<0.01
P. parva vs. *S. erythropthalmus*	-1.11	>0.05
P. parva vs. *B. bjoerkna*	-3.10	<0.01
P. parva vs. *L. delineatus*	-2.65	<0.05
P. parva vs. *R. rutilus*	-0.73	>0.05
P. parva vs. *C. carpio*	-0.46	>0.05
C. gibelio vs. *R. amarus*	-3.48	<0.01
C. gibelio vs. *G. aculeatus*	-3.75	<0.01
C. gibelio vs. *S. erythropthalmus*	-0.15	>0.05
C. gibelio vs. *B. bjoerkna*	-2.83	<0.05
C. gibelio vs. *L. delineatus*	-3.21	<0.01
C. gibelio vs. *R. rutilus*	-0.66	>0.05
C. gibelio vs. *C. carpio*	-2.46	<0.05
R. amarus vs. *G. aculeatus*	-0.27	>0.05
R. amarus vs. *S. erythropthalmus*	-2.99	<0.01
R. amarus vs. *B. bjoerkna*	-0.76	>0.05
R. amarus vs. *L. delineatus*	-1.21	>0.05
R. amarus vs. *R. rutilus*	-2.24	<0.05
R. amarus vs. *C. carpio*	-2.72	<0.05
G. aculeatus vs. *S. erythropthalmus*	-3.08	<0.01
G. aculeatus vs. *B. bjoerkna*	-3.01	<0.01
G. aculeatus vs. *L. delineatus*	-2.28	<0.05
G. aculeatus vs. *R. rutilus*	-2.32	<0.05
G. aculeatus vs. *C. carpio*	-3.79	<0.01
S. erythropthalmus vs. *B. bjoerkna*	-1.90	>0.05
S. erythropthalmus vs. *L. delineatus*	-2.26	<0.05

Table 3.50 (cont.)

S. erythropthalmus vs. *R. rutilus*	-0.23	>0.05
S. erythropthalmus vs. *C. carpio*	-1.20	>0.05
B. bjoerkna vs. *L. delineatus*	-0.71	>0.05
B. bjoerkna vs. *R. rutilus*	-1.22	>0.05
B. bjoerkna vs. *C. carpio*	-2.99	<0.01
L. delineatus vs. *R. rutilus*	-1.48	>0.05
L. delineatus vs. *C. carpio*	-2.23	<0.05
R. rutilus vs. *C. carpio*	-0.79	>0.05

Stable isotope: fish length relationships

Linear regression revealed that there were significant relationships between fish length and $\delta^{13}C$ and $\delta^{15}N$ for *L. delineatus* and between $\delta^{15}N$ and length for *C. gibelio*, *B.bjoerkna* and *R. rutilus* (Table 3.51). None of the other relationships between fish length and the stable isotope data were significant.

Table 3.51 Outputs of linear regression testing the effect of fish length (mm) on $\delta^{13}C$ and $\delta^{15}N$ for each species.

Species	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
P. parva	0.01	1,80	0.04	0.84	0.01	1,80	0.06	0.81
C. gibelio	0.01	1,80	0.01	0.98	0.47	1,80	7.10	0.03
R. amarus	0.19	1,80	1.88	0.21	0.14	1,80	1.31	0.29
G. aculeatus	0.18	1,80	1.72	0.23	0.19	1,80	1.88	0.21
S. erythropthalmus	0.01	1,12	0.01	0.98	0.16	1,12	2.24	0.16
B. bjoerkna	0.54	1,40	4.75	0.09	0.71	1,40	9.68	0.04
L. delineatus	0.50	1,80	8.04	0.02	0.70	1,80	18.97	< 0.01
R. rutilus	0.09	1,10	0.96	0.35	0.51	1,10	10.53	0.01
C. carpio	0.10	1,80	0.92	0.36	0.01	1,80	0.09	0.78

Stable isotope data of species in Belgium Pond 2

Given that some of the relationship between fish length and the stable isotope data were significant (Table 3.51) then testing for differences in the stable isotope data used a generalized linear model in which fish length was the covariate (Table 5.52). This revealed that there were significant differences in the stable isotope values of the majority of species (Table 5.53).

Table 3.52 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for species in Belgium Pond 2. Variation around the mean represents standard error.

Context	n	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
P. parva	10	-35.84 ± 0.41	-36.35 to -34.88	13.50 ± 0.10	12.92 to 13.89
C. gibelio	10	-36.64 ± 0.11	-37.26 to -36.22	13.06 ± 0.11	12.63 to 14.00
R. amarus	10	-35.70 ± 0.25	-36.99 to -34.46	12.86 ± 0.08	12.46 to 13.26
G. aculeatus	10	-38.84 ± 0.31	-39.84 to -36.64	10.78 ± 0.41	9.73 to 14.27
S. erythropthalmus	14	-33.27 ± 0.35	-36.98 to -31.83	12.99 ± 0.16	12.00 to 13.85
B. bjoerkna	6	-35.49 ± 0.17	-35.93 to -34.84	13.79 ± 0.24	12.63 to 14.32
L. delineatus	10	-37.46 ± 0.17	-37.99 to -36.60	14.92 ± 0.14	14.10 to 15.42
R. rutilus	12	-33.62 ± 0.58	-36.11 to -30.37	13.43 ± 0.22	12.19 to 15.11
C. carpio	10	-34.99 ± 0.35	-36.90 to -33.14	12.84 ± 0.35	10.41 to 13.88

Table 3.53 Outputs of generalized linear model testing for differences in $\delta^{13}C$ and $\delta^{15}N$ of species in Pond 2 where length is the effect of fork length as a covariate in the model (as Wald χ^2), group describes the two species used in each context (also as Wald χ^2) and group difference is the output of pairwise comparisons with Bonferroni adjustment for multiple comparisons. * $P < 0.05$; ** $P < 0.01$.

	$\delta^{13}C$			$\delta^{15}N$		
	Length	Group	Group difference	Length	Group	Group difference
P. parva vs. *C. gibelio*	0.02	15.41**	0.79**	4.79**	3.44**	0.27**
P. parva vs. *R. amarus*	2.81	0.60	0.28	0.72	19.68**	-0.75**
P. parva vs. *G. aculeatus*	0.67	20.50**	3.60**	0.46	11.73**	3.33**
P. parva vs. *S. erythropthalmus*	0.01	34.03**	-2.56**	3.44	4.31**	0.40*
P. parva vs. *B. bjoerkna*	0.30	2.03	-0.50	1.27	0.01	0.02
P. parva vs. *L. delineatus*	5.74**	71.01**	1.97**	11.77**	112.28**	-1.76**
P. parva vs. *R. rutilus*	1.95	8.69*	-1.88**	17.03**	1.34	-0.23
P. parva vs. *C. carpio*	1.28	5.33*	-0.81**	0.16	3.51	0.65
L. delineatus vs. *R. rutilus*	1.40	23.22**	-3.4**	8.52**	17.10**	1.09**
L. delineatus vs. *C. carpio*	4.25*	54.72**	2.91**	2.12	34.79**	2.40
R. rutilus vs. *C. carpio*	1.31	2.11	1.04	4.57*	5.74*	0.91*
R. amarus vs. *G. aculeatus*	2.57	80.55**	3.25**	0.58	29.17**	2.13**

Table 3.53 (cont.)

	$\delta^{13}C$			$\delta^{15}N$		
	Length	Group	Group difference	Length	Group	Group difference
R. amarus vs. *S. erythropthalmus*	0.03	21.82**	-2.48**	4.38*	3.11	-0.37
R. amarus vs. *B. bjoerkna*	1.86	0.78	-0.28	2.61	27.43**	-0.98**
R. amarus vs. *L. delineatus*	7.75**	39.64**	1.58**	2.03	161.44	-2.00**
R. amarus vs. *R. rutilus*	1.23	4.31*	-1.59*	19.66**	26.68**	-1.13**
R. amarus vs. *C. carpio*	2.85	6.36*	-1.23*	0.01	0.01	-0.01
G. aculeatus vs. *S. erythropthalmus*	0.00	91.79**	-5.57**	1.16	30.93**	-2.49
G. aculeatus vs. *B. bjoerkna*	0.13	40.36**	-3.48**	4.64*	38.36**	-3.96**
G. aculeatus vs. *L. delineatus*	4.33*	24.28**	-1.79**	0.52	66.24**	-3.95**
G. aculeatus vs. *R. rutilus*	1.59	31.81**	-4.58**	4.66*	45.66**	-3.29**
G. aculeatus vs. *C. carpio*	2.29	33.57**	-4.99**	0.05	4.66*	-2.26*
S. erythropthalmus vs. *B. bjoerkna*	0.02	13.69**	2.18**	3.15	3.96*	-0.57*
S. erythropthalmus vs. *L. delineatus*	0.02	77.76**	4.22**	1.30	57.58**	-1.80**
S. erythropthalmus vs. *R. rutilus*	0.85	0.39	0.38	12.31**	5.16*	-0.48*
S. erythropthalmus vs. *C. carpio*	0.01	11.13**	1.73**	0.94	0.60	0.27
B. bjoerkna vs. *L. delineatus*	4.28*	80.16**	1.90**	2.80	21.50**	-1.07**
B. bjoerkna vs. *R. rutilus*	14.10**	0.40	-0.18	1.79	2.21	-1.29
B. bjoerkna vs. *C. carpio*	0.70	2.00	-0.88	0.02	1.78	0.88

Stable isotope metrics

There was no requirement to correct the data for subsequent analyses, as only one pond was being analysed. The stable isotope data for the baseline data are displayed in Figure 3.29, along with the data for the fishes.

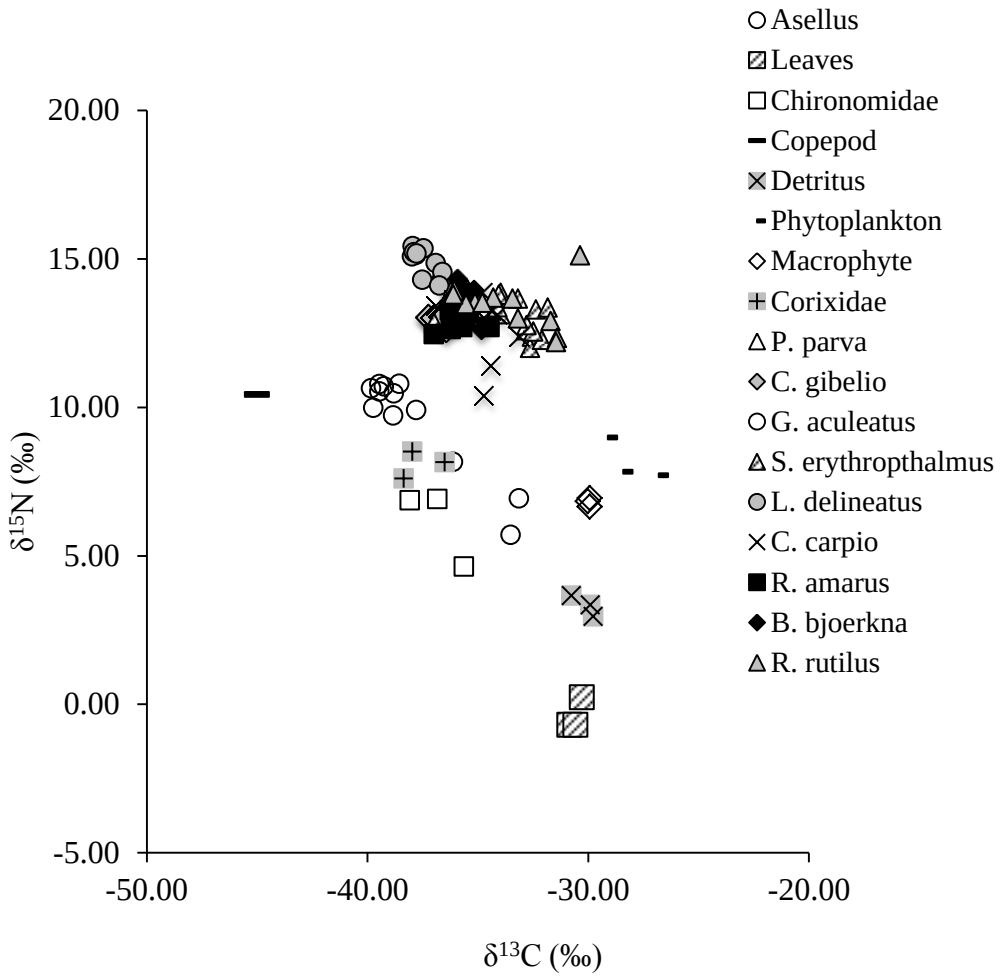

Figure 3.29 Stable isotope bi-plot showing the stable isotope data of fish and basal resources data from Belgium Pond 2.

Comparison of the metrics standard ellipse area (SEA_c), nitrogen range (NR) and carbon range (CR) revealed that *R. rutilus* had the larger trophic niche of the nine species (Table 3.55; Fig. 3.30), with their trophic niche overlapping with *P.*

parva (2.45 %), *R. amarus* (0.52 %), *C. gibelio* (2.21 %) and *C. carpio* (13.58 %) (Fig. 3.30). For *P. parva*, there was also an overlap in trophic niche with *B. bjoerkna* (8.05 %) and *C. carpio* (10.25 %). The trophic position data of *P. parva* indicated they occupied a significantly higher position than some of the species (*P. parva* vs. *C. gibelio*: $F_{1,18} = 8.76$, $P < 0.05$; *P. parva* vs. *R. amarus:* $F_{1,18} = 24.8$, $P < 0.05$; *P. parva* vs. *G. aculeatus:* $F_{1,18} = 43.33$, $P < 0.05$; *P. parva* vs. *S. erythropthalmus:* $F_{1,22} = 6.31$, $P < 0.05$; Table 3.54). For some species, there was no signficant difference in TP with *P. parva* (*P. parva* vs. *B. bjoerkna:* $F_{1,14} = 1.63$, $P > 0.05$; *P. parva* vs. *C. carpio:* $F_{1,18} = 3.34$, $P > 0.05$; *P. parva* vs. *R. rutilus:* $F_{1,20} = 0.81$, $P > 0.05$) (Table 3.55). However, *P. parva* trophic position was significantly lower than *L. delineatus* ($F_{1,18} = 66.0$, $P < 0.05$; Table 3.54).

Table 3.54 Stable isotope metrics for the fish species from Belgium Pond 2, where SEA_c= Standard ellipse area, NR= $\delta^{15}N$ range, CR= $\delta^{13}C$ range.

Species	SEA_c	NR	CR	TP
P. parva	0.50	0.97	1.48	4.08 ± 0.03
C. gibelio	0.40	1.37	1.04	3.95 ± 0.03
R. amarus	0.74	0.80	2.53	3.89 ± 0.02
G. aculeatus	3.27	4.55	3.19	3.27 ± 0.12
S. erythropthalmus	2.52	1.85	5.14	3.92 ± 0.05
B. bjoerkna	0.49	1.68	1.09	4.16 ± 0.07
L. delineatus	0.60	1.32	1.38	4.49 ± 0.40
R. rutilus	5.23	2.92	5.74	4.05 ± 0.07
C. carpio	3.99	3.47	3.76	3.88 ± 0.10

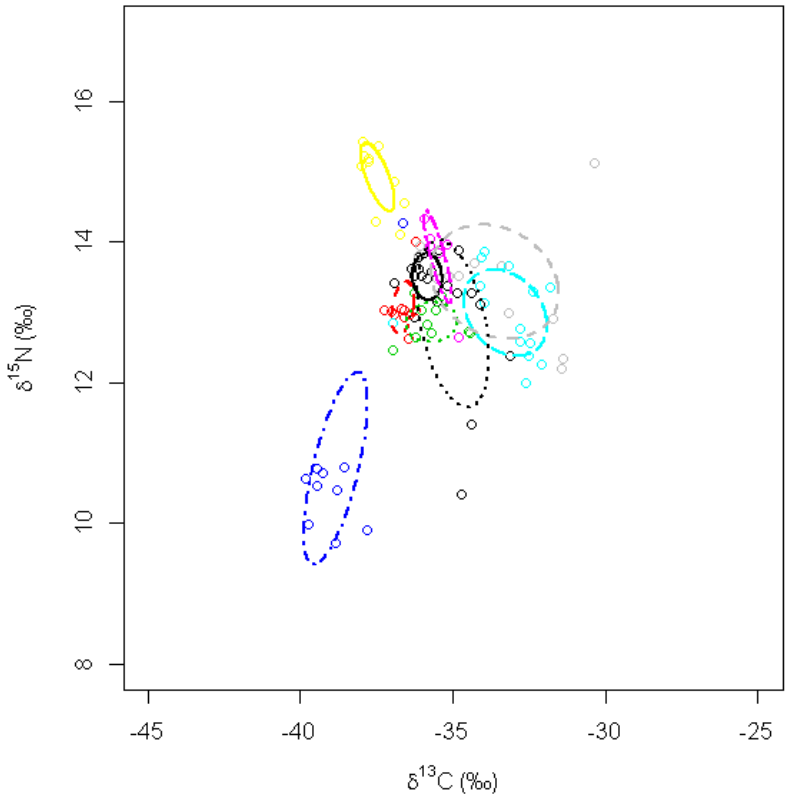

Figure 3.30 Standard ellipse areas of species in Belgium Pond 2. Open circles represent individual species and different colour represents different species. The line encloses the standard ellipse area (SEA_c) for *P. parva* (black solid), *C. gibelio* (red dashed), *R. amarus* (green) and *G. aculeatus* (blue dash-dotted), *S. erythropthalmus* (bright blue long dashed), *B. bjoerkna* (pink long dashed), *L. delineatus* (yellow solid), *R. rutilus* (grey dashed), *C. carpio* (black dotted).

3.3.3 Belgium pond 3

Fish species and lengths

The six fish species sampled from Belgium Pond 3, and their length range and mean lengths, are provided in Table 3.55. Three of the six species were non-native to Belgium. There were some significant differences in the lengths of

these fish between the species, most notably for *P. parva* and all other species (Table 3.56). Of the fishes sampled, *P. parva*, *C. gibelio* and *Rhodeus amarus* are all non-native to Belgium.

Table 3.55 Number, mean length and length range of fish sampled from Belgium Pond 3, where N (sampled) represents the number of fish captured, n (analysed) is the number of fish used in stable isotope analysis and the lengths represent the analysed fish only, and where * indicates the species is non-native to Belgium.

Species	N (sampled)	n (analysed)	Mean length (mm)	Length range (mm)
*P. parva**	125	10	73.60 ± 2.20	62 to 82
*C. gibelio**	6	6	236.67 ± 23.83	168 to 321
*R. amarus**	20	10	63.80 ± 2.20	57 to 75
G. aculeatus	25	9	42.90 ± 1.50	38 to 50
S. erythropthalmus	7	7	183.86 ± 5.95	163 to 205
P. pungitius	20	8	43.80 ± 1.50	38 to 50

Table 3.56 Outputs of Mann Whitney U tests testing the significance of differences in fish lengths between species in Belgium Pond 3.

Context	Length	
	Z	P
P. parva vs. *C. gibelio*	-3.26	<0.05
P. parva vs. *R. amarus*	-2.69	<0.05
P. parva vs. *G. aculeatus*	-3.68	<0.05
P. parva vs. *S. erythropthalmus*	-3.42	<0.05
P. parva vs. *P. pungitius*	-3.57	<0.05
C. gibelio vs. *R. amarus*	-3.27	<0.05
C. gibelio vs. *G. aculeatus*	-3.19	>0.05
C. gibelio vs. *S. erythropthalmus*	-1.16	>0.05

Table 3.56 (cont.)

C. gibelio vs. *P. pungitius*	-1.72	<0.05
R. amarus vs. *G. aculeatus*	-3.69	<0.05
R. amarus vs. *S. erythropthalmus*	-3.43	<0.05
R. amarus vs. *P. pungitius*	-3.57	<0.05
G. aculeatus vs. *S. erythropthalmus*	-3.34	<0.05
G. aculeatus vs. *P. pungitius*	-0.83	>0.05
S. erythropthalmus vs. *P. pungitius*	-3.26	<0.05

Stable isotope: fish length relationships

Linear regression revealed that there were no significant relationships between fish length and $\delta^{13}C$ and $\delta^{15}N$ for any of the fish species in Belgium Pond 3 (Table 3.57).

Table 3.57 Outputs of linear regression testing the effect of fish length (mm) on $\delta^{13}C$ and $\delta^{15}N$ for each species in Belgium Pond 3.

Context	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
P. parva	0.02	1,80	0.16	0.70	0.01	1,80	0.01	0.96
C. gibelio	0.35	1,40	0.81	0.21	0.14	1,40	0.01	0.46
R. amarus	0.25	1,80	2.60	0.15	0.02	1,80	0.13	0.73
G. aculeatus	0.02	1,70	0.12	0.74	0.10	1,70	0.79	0.41
S. erythropthalmus	0.01	1,50	0.09	0.81	0.63	1,50	1.36	0.06
P. pungitius	0.34	1,60	3.16	0.13	0.01	1,60	0.01	0.93

Stable isotope data between species

As there was no effect of fish length on the stable isotope data (Table 3.57), Mann Whitney U tests were used to test for differences in the stable isotope data of the fish species. There were significant differences in $\delta^{13}C$ and $\delta^{15}N$ between

G. aculeatus, *S. erythropthalmus* and *P. pungitius* (Table 3.58, 3.59; Fig. 3.31). By contrast, there were no significant differences in $\delta^{13}C$ and $\delta^{15}N$ between *P. parva* and *C. gibelio*, *C. gibelio* and *R. amarus* and between *R. amarus* and *P. pungitius* (Table 3.58, 3.59; Fig. 3.31).

Stable isotope metrics

There was no requirement to correct the data for subsequent analyses, as only one pond was being analysed. The stable isotope data for the baseline data are displayed in Figure 3.31. The stable isotope data for the fishes are also shown in Fig. 3.31 with further details in Table 3.58. The $\delta^{15}N$ data indicated that when compared to the other species, *C. gibelio* occupied the highest trophic position and *S. erythropthalmus* occupied the lowest trophic position.

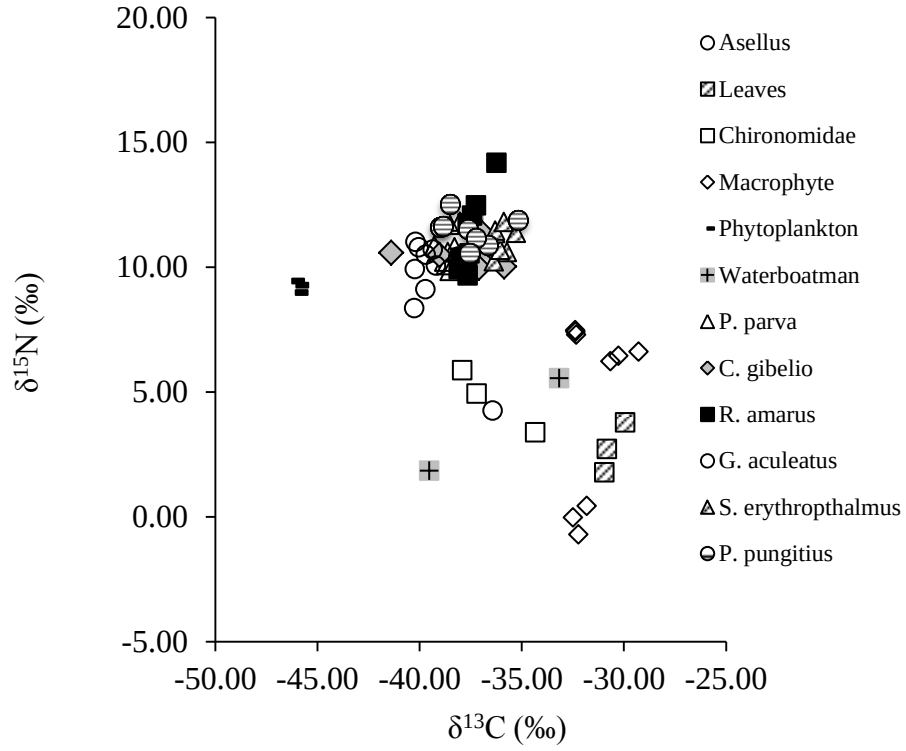

Figure 3.31 Stable isotope bi-plot showing the stable isotope data for fish and basal resources from Belgium Pond 3.

Table 3.58 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for the fish species in Belgium Pond 3. Variation around the mean represents standard error.

Species	n	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
P. parva	10	-38.58 ± 0.13	-39.28 to -38.02	10.70 ± 0.20	9.84 to 11.82
C. gibelio	6	-37.73 ± 0.87	-41.40 to -35.85	10.48 ± 0.20	9.97 to 11.36
R. amarus	10	-37.53 ± 0.16	-38.08 to -36.24	11.10 ± 0.46	9.66 to 14.18
G. aculeatus	9	-39.64 ± 0.23	-40.26 to -38.10	10.10 ± 0.29	8.35 to 11.02
S. erythropthalmus	7	-35.98 ± 0.15	-36.36 to -35.29	11.07 ± 0.22	10.23 to 11.81
P. pungitius	8	-37.59 ± 0.45	-39.01 to -35.19	11.47 ± 0.22	10.56 to 12.53

Table 3.59 Outputs of Mann Whitney U tests testing the significance of differences in the $\delta^{13}C$ and $\delta^{15}N$ data of fish species in Belgium Pond 3.

Species	$\delta^{13}C$		$\delta^{15}N$	
	Z	P	Z	P
P. parva vs. *C. gibelio*	-1.30	>0.05	-1.03	>0.05
P. parva vs. *R. amarus*	-3.63	<0.01	0.01	>0.05
P. parva vs. *G. aculeatus*	-2.94	<0.01	-1.35	>0.05
P. parva vs. *S. erythropthalmus*	-3.42	<0.01	-1.22	>0.05
P. parva vs. *P. pungitius*	-1.73	>0.05	-2.13	<0.05
C. gibelio vs. *R. amarus*	0.87	>0.05	-0.22	>0.05
C. gibelio vs. *G. aculeatus*	-2.00	0.05	-0.47	>0.05
C. gibelio vs. *S. erythropthalmus*	-1.86	>0.05	-2.07	<0.05
C. gibelio vs. *P. pungitius*	-0.26	>0.05	-2.58	<0.05
R. amarus vs. *G. aculeatus*	-3.67	<0.01	-1.02	>0.05
R. amarus vs. *S. erythropthalmus*	-3.13	<0.01	-0.59	>0.05
R. amarus vs. *P. pungitius*	-0.09	>0.05	-1.07	>0.05
G. aculeatus vs. *S. erythropthalmus*	-3.34	<0.01	-2.17	<0.05
G. aculeatus vs. *P. pungitius*	-3.18	<0.01	-3.08	<0.01
S. erythropthalmus vs. *P. pungitius*	-2.43	<0.05	-1.27	>0.05

Comparison of the metrics standard ellipse area (SEA_c), nitrogen range (NR) and carbon range (CR) revealed that *C. gibelio* had the largest trophic niche of the six species (Table 3.60; Fig. 3.32), with their trophic niche overlapping with *P. parva* (16.44 %), *R. amarus* (10.37 %), *G. aculeatus* (13.65 %), *S. erythropthalmus* (3.79 %) and *P. pungitius* (3.57 %) (Fig. 3.32). For *P. parva*, there was also an overlap in their trophic niche with *G. aculeatus* (1.52 %) and *P. pungitius* (4.08 %) (Table 3.60; Fig. 3.32). Trophic position data indicated that other than with *P. pungitius*, there were no significant differences in the TP of *P. parva* with the other species (*P. parva* vs. *C. gibelio*: $F_{1,14} = 0.52$, $P > 0.05$; *P.*

parva vs. *R. amarus:* $F_{1,18} = 0.63$, $P > 0.05$; *P. parva* vs. *G. aculeatus:* $F_{1,17} = 2.97$, $P > 0.05$; *P. parva* vs. *S. erythropthalmus:* $F_{1,15} = 1.54$, $P > 0.05$; *P. parva* vs. *P. pungitius:* $F_{1,16} = 6.76$, $P < 0.05$) (Table 3.60).

Table 3.60 Stable isotope metrics for the fish species from Belgium Pond 3, where SEA_c= Standard ellipse area, NR= $\delta^{15}N$ range, CR= $\delta^{13}C$ range.

Species	SEA_c	NR	CR	TP
P. parva	0.89	1.98	1.26	3.56 ± 0.06
C. gibelio	4.11	1.39	5.55	3.50 ± 0.06
R. amarus	1.29	4.52	1.85	3.68 ± 0.14
G. aculeatus	2.10	2.66	2.16	3.39 ± 0.08
S. erythropthalmus	0.81	1.58	1.07	3.67 ± 0.06
P. pungitius	2.79	1.97	3.82	3.79 ± 0.06

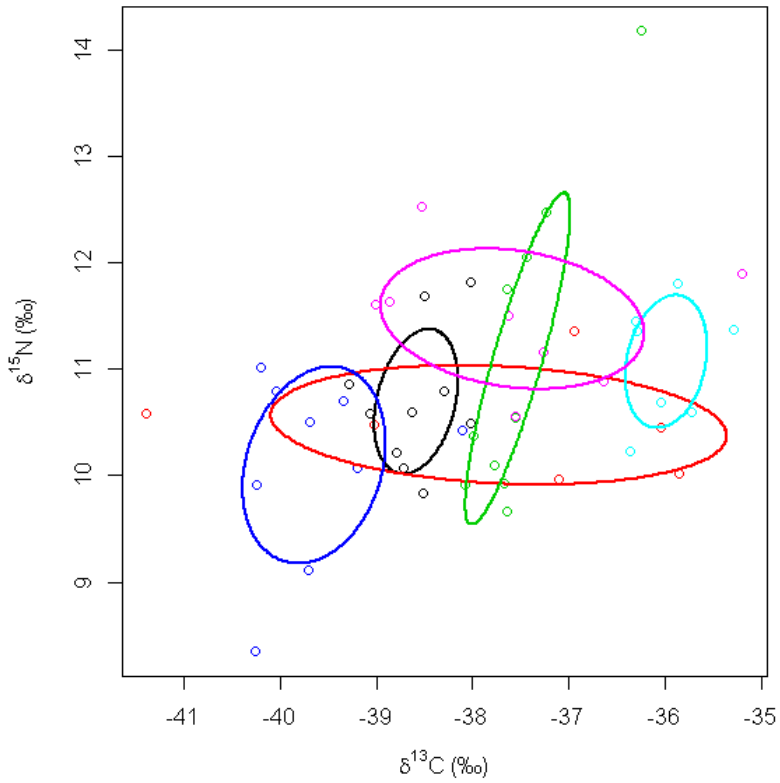

Figure. 3.32 Standard ellipse areas of the fish species analysed from Belgium Pond 3. Open circles represent individual species and different colour represents different species. The line encloses the standard ellipse area (SEA_c) for *P. parva* (black), *C. gibelio* (red), *R. amarus* (green), *G. aculeatus* (blue), *S. erythropthalmus* (bright blue) and *P. pungitius* (pink).

3.3.4 Millennium Coastal Park, Wales

Fish species and lengths

The four fish species sampled from the site included *P. parva* and the non-native wild goldfish *Carassius auratus* (Table 3.61). Of the *S. erythropthalmus* present in the samples, there were two apparent size classes present, < 100 mm ('Small') and > 100 mm ('Large'), with their lengths being significantly different (Table

3.62). Regarding *P. parva*, their lengths were only significantly different to the 'Large' *S. erythropthalmus* and *T. tinca* (Table 3.62).

Table 3.61 Number, mean length and length range of fish sampled from in the Millennium Coastal Park, Wales, where n (analysed) is the number of fish used in stable isotope analysis.

Species	n	Mean length (mm)	Length range (mm)
P. parva	20	63.00 ± 3.90	39 to 110
C. auratus	12	89.00 ± 12.70	53 to 160
T. tinca	12	95.70 ± 10.10	45 to 146
'Small' *S. erythropthalmus*	10	61.50 ± 3.50	35 to 86
'Large' *S. erythropthalmus*	17	174.30 ± 10.10	108 to 205

Stable isotope: fish length relationships

Linear regression revealed that there were significant relationships between fish length and $\delta^{13}C$ for *C. auratus* and the 'Large' *S. erythropthalmus*, and between $\delta^{15}N$ and length of *P. parva* (Table 3.63).

Table 3.62 Outputs of Mann Whitney U tests testing the significance of differences in length between the analysed fish in the Millennium Coastal Park, Wales.

Species	Length	
	Z	P
P. parva vs. *C. auratus*	-1.42	>0.05
P. parva vs. *T. tinca*	-2.55	<0.05
P. parva vs. 'Small' *S. erythropthalmus*	-0.15	>0.05
P. parva vs. 'Large' *S. erythropthalmus*	-4.36	<0.01
C. auratus vs. *T. tinca*	-0.46	>0.05
C. auratus vs. 'Small' *S. erythropthalmus*	-0.84	>0.05
C. auratus vs. 'Large' *S. erythropthalmus*	-3.46	<0.01
T. tinca vs. 'Small' *S. erythropthalmus*	-2.48	>0.05
T. tinca vs. 'Large' *S. erythropthalmus*	-3.43	<0.01
'Small' *S. erythropthalmus* vs. 'Large' *S. erythropthalmus*	-4.27	<0.01

Table 3.63 Outputs of linear regression testing the effect of fish length (mm) on $\delta^{13}C$ and $\delta^{15}N$ for each species in the Millennium Coastal Park, Wales.

Context	$\delta^{13}C$				$\delta^{15}N$			
	R^2	*d.f.*	F	P	R^2	*d.f.*	F	P
P. parva	0.13	1,18	2.68	0.12	0.48	1,18	16.64	<0.01
C. auratus	0.61	1,10	15.35	<0.01	0.05	1,10	0.49	0.50
T. tinca	0.07	1,10	0.78	0.40	0.19	1,10	2.30	0.16
'Small' *S. erythropthalmus*	0.01	1,15	0.02	0.90	0.15	1,15	2.57	0.13
'Large' *S. erythropthalmus*	0.78	1,80	28.21	<0.01	0.12	1,80	1.13	0.32

Stable isotope data between species

As some of the relationships between fish length and the stable isotope data were significant (Table 3.63) then testing for differences in the stable isotope data between species needed to control for fish length using generalized linear models, where fish length was the covariate. This revealed that in the majority of species (including *P. parva*), at least one of the stable isotopes (i.e. $\delta^{13}C$ and $\delta^{15}N$) was significantly different between the species and size classes of *S. erythropthalmus* (Table 3.64, 3.65; Fig 3.33). The exception was between *T. tinca* and 'Small' *S. erythropthalmus* (Table 3.65; Fig. 3.33). It should be noted that the difference in $\delta^{13}C$ and $\delta^{15}N$ between the size classes of *S. erythropthalmus* was also significant (Table 3.65; Fig. 3.33).

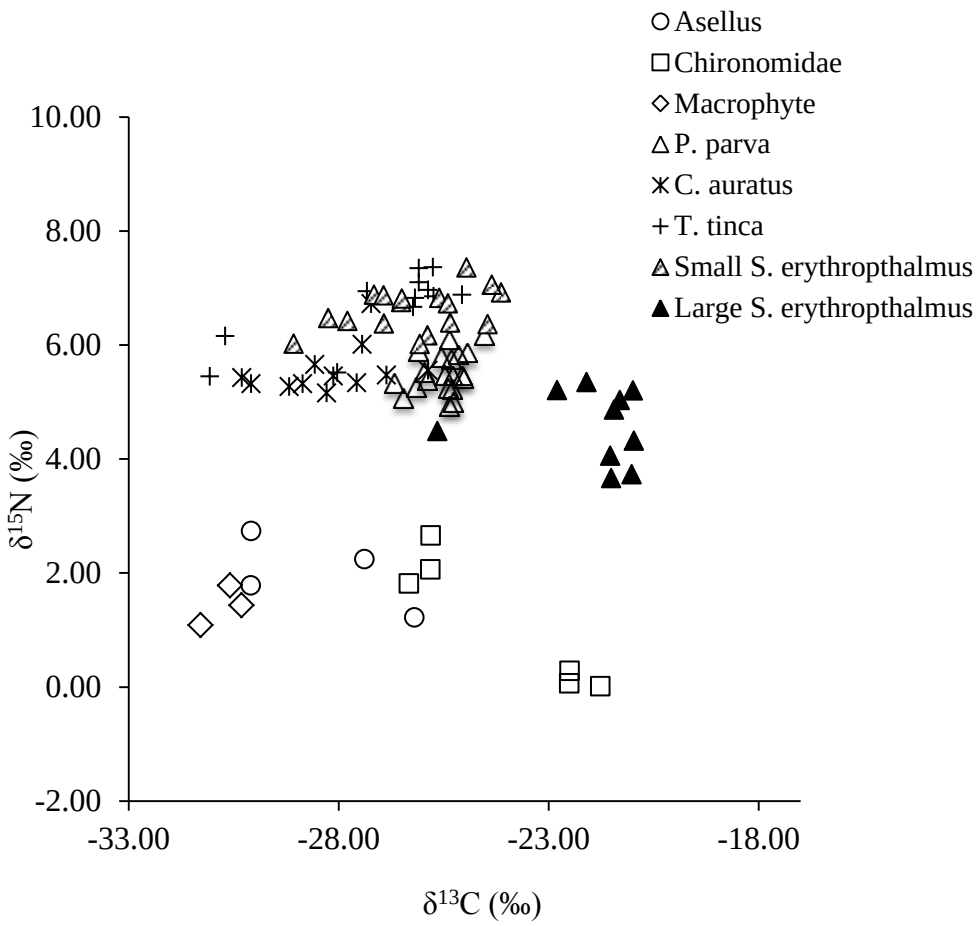

Figure 3.33 Stable isotope bi-plot showing the stable isotope data of fish and basal resources data from the Millennium Coastal Park, Wales.

Table 3.64 Overview of the $\delta^{13}C$ and $\delta^{15}N$ data for species in the Millennium Coastal Park, Wales. Variation around the mean represents standard error.

	n	Mean $\delta^{13}C$ (‰)	Range (‰)	Mean $\delta^{15}N$ (‰)	Range (‰)
P. parva	20	-25.51 ± 0.12	-26.68 to -24.54	5.51 ± 0.08	4.93 to 6.17
C. auratus	12	-28.20 ± 0.38	-30.31 to -25.87	5.56 ± 0.12	5.16 to 6.72
T. tinca	12	-27.01 ± 0.57	-31.07 to -25.06	6.67 ± 0.18	5.45 to 7.36
'Small' *S. erythropthalmus*	10	-26.19 ± 0.34	-29.07 to -24.13	6.61 ± 0.09	6.01 to 7.35
'Large' *S. erythropthalmus*	17	-21.93 ± 0.45	-25.65 to -20.96	4.59 ± 0.20	3.66 to 5.35

Table 3.65 Outputs of generalized linear model testing for differences in $\delta^{13}C$ and $\delta^{15}N$ of species in the Millennium Coastal Park, Wales, where length is the effect of fork length as a covariate in the model (as Wald χ^2), group describes the two species used in each context and group difference is the output of pairwise comparisons with Bonferroni adjustment for multiple comparisons. * $P < 0.05$; ** $P < 0.01$.

	$\delta^{13}C$			$\delta^{15}N$		
	Length	Group	Group difference	Length	Group	Group difference
P. parva vs. *C. auratus*	12.52**	60.29**	2.28**	0.34	0.01	-0.02
P. parva vs. *T. tinca*	0.69	5.73*	1.26*	0.16	36.18**	-1.21**
P. parva vs. 'Small' *S. erythropthalmus*	0.31	4.25*	0.68*	2.03	96.31**	-1.11**
P. parva vs. 'Large' *S. erythropthalmus*	31.24**	0.42	-0.40	0.10	5.61*	1.04*
C. auratus vs. *T. tinca*	7.05*	5.27*	-1.32**	2.68	31.94**	-1.14**
C. auratus vs. 'Small' *S. erythropthalmus*	7.48*	8.83**	-1.44**	1.80	39.38**	-0.97**
C. auratus vs. 'Large' *S. erythropthalmus*	0.35	63.31**	-6.64**	1.62	4.45*	0.67*
T. tinca vs. 'Small' *S. erythropthalmus*	1.11	0.28	-0.38	6.05*	2.91	0.34
T. tinca vs. 'Large' *S. erythropthalmus*	0.40	16.53**	-4.53**	4.14*	15.71**	1.49**
'Small' *S. erythropthalmus* vs. 'Large' *S. erythropthalmus*	6.57*	13.67	3.60**	4.02*	5.97*	1.15*

Stable isotope metrics

There was no requirement to correct the data for subsequent analyses, as only one pond was being analysed. The stable isotope data for the baseline data are displayed in Figure 3.33, with $\delta^{15}N$ suggesting *T. tinca* had the highest trophic position and *P. parva* occupied a significant higher trophic position than 'Large' *S. erythropthalmus*.

Calculations of TP were completed for the fishes and revealed *P. parva* had a significantly different position to the other species (*P. parva* vs. *T. tinca:* $F_{1,30}$ = 44.46, $P < 0.05$; *P. parva* vs. 'Small' *S. erythropthalmus:* $F_{1,35} = 85.33$, $P < 0.05$; *P. parva* vs. 'Large' *S. erythropthalmus:* $F_{1,28} = 26.51$, $P < 0.05$), with goldfish the only exception (*P. parva* vs. *C. auratus*: $F_{1,30} = 0.13$, $P > 0.05$) (Table 3.66). Comparison of the metrics standard ellipse area (SEA_c), nitrogen range (NR) and carbon range (CR) then revealed *P. parva* has a relatively narrow trophic niche compared with all the other species, and it showed no overlap with either 'Small' *S. erythropthalmus* or 'Large' *S. erythropthalmus* (Table 3.66; Fig. 3.34). Indeed, there was no overlap in the trophic niches of the two size classes of *S. erythropthalmus* (Fig. 3.34), with the stable isotope outputs suggesting the 'Large' *S. erythropthalmus* were mainly feeding in a different chain in the food web to the other fishes, with this chain based on Chironomid larvae, whereas for other fishes, their chain appeared to be more related to macro-invertebrates such as *Asellus aquaticus* (Fig. 3.33, 3.34). The consequence of this difference for the growth rates of *S. erythropthalmus* is explored in the next sub-section.

Table 3.66 Stable isotope metrics for the fish species in the Millennium Coastal Park, Wales, where SEA_c= Standard ellipse area, NR= $\delta^{15}N$ range, CR= $\delta^{13}C$ range.

Species	SEA_c	NR	CR	TP
P. parva	0.59	1.24	2.14	3.18 ± 0.02
C. auratus	1.77	1.56	4.44	3.20 ± 0.04
T. tinca	2.64	1.91	6.01	3.52 ± 0.05
'Small' *S. erythropthalmus*	1.57	1.34	4.94	3.51 ± 0.03
'Large' *S. erythropthalmus*	3.14	1.69	4.68	2.91 ± 0.06

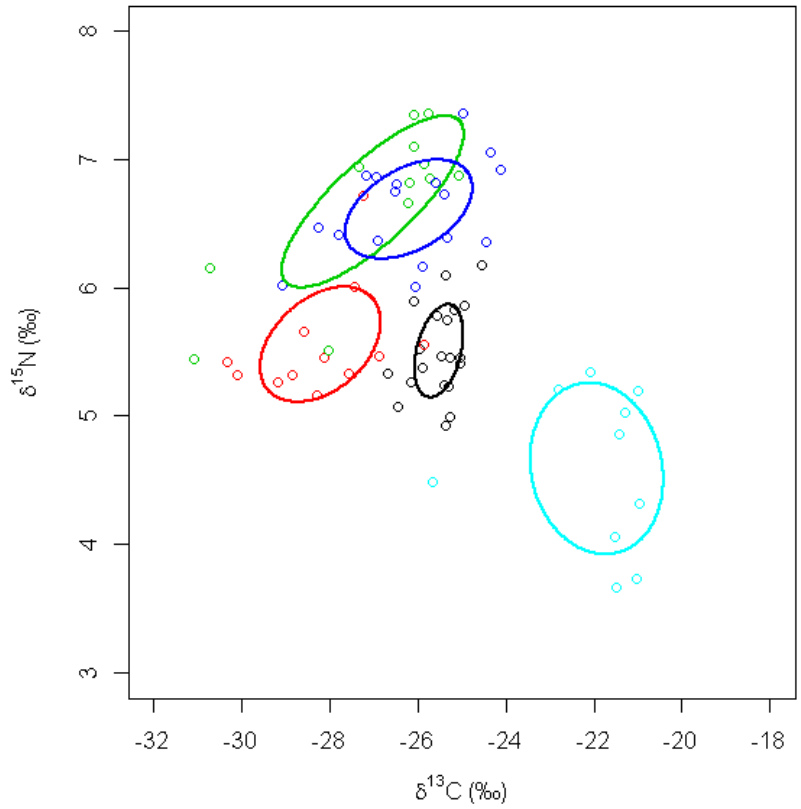

Figure 3.34 Standard ellipse areas of fish species in the Millennium Coastal Park, Wales. Open circles represent individual species and different colour represent different species. The line encloses the standard ellipse area (SEA_c) for *P. parva* (black), *C. auratus* (red), *T. tinca* (green), 'Small' *S. erythropthalmus* (blue) and 'Large' *S. erythropthalmus* (bright blue).

Age and somatic growth rates of the S. erythropthalmus size classes

The 'Large' *S. erythropthalmus* were aged between 3 and 7 years old, whilst the 'Small' *S. erythropthalmus* were aged between 2 and 4 years old, i.e. the two length classes both contained fish at age 3 and 4 years old (Fig. 3.35). For back-calculated length at age 3, mean length was significantly larger for the 'Large' size class (105.1 ± 2.9 mm) than the 'Small' size class (62.7 ± 0.7) (ANOVA: $F_{1,21} = 41.27$, $P < 0.01$). This could not be done for age 4 due to too few fish at that age in the Small length class. Calculation of the length increment between age 1 and 2 years old of all the fish (Fig. 3.35) enabled their standardized residuals to be calculated and then compared between the two size classes using a generalized linear model where fish size at capture was the covariate. The model was significant (Wald $\chi^2 = 8.60$, $P < 0.01$), with the effect of fish length as the covariate being significant ($P = 0.05$). The adjusted mean standardized residual for the 'Small' *S. erythropthalmus* was 0.28 ± 0.12 and for the 'Large' *S. erythropthalmus* was 0.87 ± 0.31, with the difference between these significant according to pairwise comparisons with Bonferroni adjustment for multiple comparisons ($P < 0.01$). Thus, the 'Large' *S. erythropthalmus* length class comprised of fish that were significantly faster growing, both between age 1 and 2 years, and as shown by their length at their third annulus.

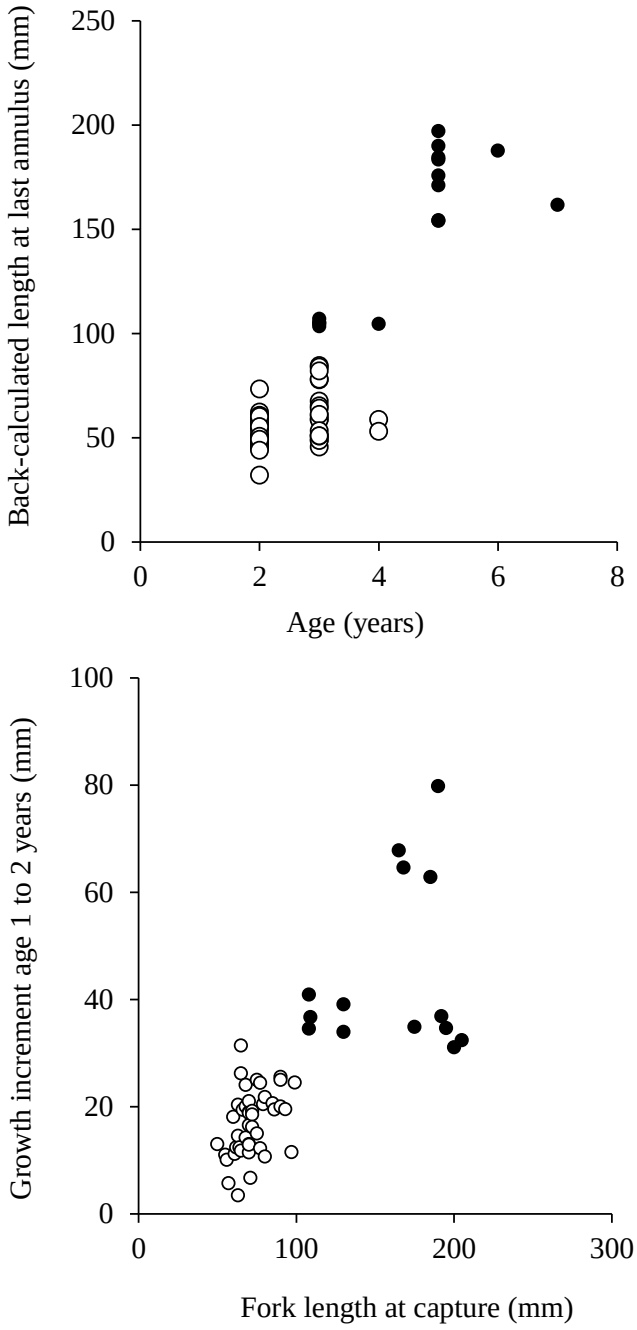

Figure 3.35 (top) Back-calculated lengths at the last annulus of 'Large' (●) and 'Small' *S. erythropthalmus*; (bottom) Growth increment between age 1 and 2 of 'Large' (●) and 'Small' *S. erythropthalmus*.

3.3.5 Summary of outputs from the wild sites

The stable isotope data from the wild sites revealed a more complex pattern than observed in both the mesocosms and small ponds. Whereas in the mesocosms and small ponds *P. parva* tended to have the lowest trophic position of the fish species studied, in the wild ponds this was not the case and often they had relatively high trophic positions compared with some species. In contrast to the mesocosm and small ponds, *P. parva* revealed a greater extent of trophic niche overlap with other fish species in the wild. However, the extent tended to be low and allied with *P. parva* often having a relatively small trophic niche size, it would be difficult to suggest that their establishment in these ponds was a key ecological driver that impacted the trophic ecology and feeding relationships of the other species. Indeed, in all of the wild sites, *P. parva* were not the only invasive species and thus this greater complexity in community composition makes drawing firm conclusions on patterns in the data inherently difficult. This thus adds credence to the use of the initial experimental approaches. Finally, the stable isotope outputs of the two size classes of *S. erythropthalmus* in the Millennium Coastal Park indicated they were exploiting different chains in the food web, resulting in no overlap between them and enabling the 'Large' length group to be significantly faster growing. However, there was no supporting evidence to suggest this was related to the presence of *P. parva* in the site.

Finally, whilst the data were not used in the thesis, age determination from scales revealed the *P. parva* of each population in the wild pond were present between 0+ (i.e. young-of-the-year) and 3+ years. Thus, from a population

perspective, the fish were thus typical of *P. parva* populations more generally (Gozlan et al. 2010b). These data were not used in this chapter due to the lack of evidence supporting resource sharing between *P. parva* and the other fishes, suggesting consequences for their somatic growth rates (such as those arising from increased inter-specific competition) would be minimal and thus non-significant.

4. Discussion

4.1 Overview of the research

The increasingly rapid spread of biological invaders comprises a key driver of global environmental change with major implications for biodiversity and ecosystem functioning. For non-native fish, their rate of introductions has increased dramatically in recent decades (Vitousek et al. 1997; Koo and Mattson 2004) and their pathways of introduction are varied and include aquaculture, ornamental fish trade, sport fishing and fisheries (Gozlan et al. 2008). Whilst non-native fishes can have substantial consequences for native species through processes including increased predation pressure, habitat alteration, loss of genetic integrity and introduced pathogens, this research focused on the issue of whether a model non-native fish, topmouth gudgeon, impacted native fish communities through competitive processes. It was also explored as to whether alternative processes were apparent in invaded communities, particularly trophic niche divergence. This is ecologically important for European fish communities as *P. parva*, native to South East Asia, is now highly invasive across Europe. Moreover, it is now dispersing more widely, with populations now present in the Middle East and North Africa (Gozlan et al. 2010b). Given the issues outlined earlier with analyzing trophic interaction using gut contents analysis (Chapter 1, 2), the research focused on using stable isotope analysis to investigate the feeding relationships of *P. parva* with native fishes across three spatial scales: experimental mesocosms over 100 days, small and established aquaculture ponds, and wild ponds. This enabled testing of ecological theory on trophic niche

divergence (or convergence) under a range of experimental and uncontrolled conditions.

The research outputs provided important insights into how *P. parva* affects native fish food webs by revealing how trophic niche size, via standard ellipse areas calculated from stable isotope data, and trophic position, calculated using a standard equation that quantifies the position of each species in the food web using the $\delta^{15}N$ data, of native fishes might be modified by the presence of *P. parva*. Completion of this within the mesocosms enabled control and replication in the work, and so this provided some relatively precise outputs on how the trophic niche and trophic position of three native fishes were modified in *P. parva* presence. Work on the small aquaculture ponds provided some additional information from populations with greater complexity and less control, but still within relatively simple communities, whilst the wild ponds - being completely non-replicated and uncontrolled - provided a snap-shot of feeding relationships of multiple fish species in four invaded food webs. The outputs of these works are discussed next and then in relation to the implications for the risk assessment and risk management of *P. parva*, particularly in the UK.

4.2 Trophic interactions of *Pseudorasbora parva* with native fishes over varying spatial scales

4.2.1 Experimental mesocosms

The experimental outputs were consistent in indicating that after 100 days in sympatry with *C. carpio*, *T. tinca* or *G. aculeatus*, the trophic position of *P.*

parva was always significantly lower than with the co-habiting species, indicating their feeding on different food resources. There was no evidence of the species' trophic niches overlapping when in sympatry, with their trophic niche sizes always being larger in allopatry than in sympatry.

Consequently, the completion of Objective 1 ('Quantify the influence of *P. parva* on the trophic niche size and trophic position of native fishes in experimental mesocosms through completion of treatments in which the fishes are used in allopatric and sympatric contexts') revealed that in the sympatric treatments, there was relatively rapid niche divergence between the three sympatric species, with no evidence of inter-specific competition. Although the presence of *P. parva* appeared to constrict the trophic niche size and trophic position of the three sympatric native fishes, this constriction was also apparent in the *P. parva* trophic niche when in sympatry. Thus, as both species' trophic niche were constricted in sympatry, it was likely to be a result of the co-habitation, rather than *P. parva* invasion. This suggests that there could have been some initial resource sharing between the sympatric species in mesocosm prior to their divergence when these resources presumably became exhausted.

4.2.2 Small aquaculture ponds

In the more complex environment provided by the small aquaculture ponds, *P. parva* was present with a wider range of native species and also signal crayfish. Replication was evident in some contexts but not in others. Nevertheless, similar to the experimental mesocosms, there was some consistency in the outputs of the

analyses. As per the mesocosms, the trophic position of *P. parva* was always lower than the sympatric fishes, but tended to be higher than signal crayfish. In the simplest context with just two sympatric species present, there was no overlap in their trophic niches and the trophic niche size of *G. aculeatus* was larger than *P. parva*. In the other contexts, whilst there was variation in trophic niche size between *P. parva* and the other fishes, there was little evidence suggesting sharing of trophic niche space; instead, there was strong evidence of trophic niche divergence between all of the species in the majority of the ponds.

Consequently, the output of Objective 2 ('Identify the trophic relationships and basic food web structure of small aquaculture ponds containing a relatively low diversity of native fishes and invasive *P. parva*, and assess whether general patterns that are apparent in the outputs have synergies with those of O1') revealed that in these ponds - undisturbed for several years - the outputs were very similar to those from the mesocosms, with minimal evidence for resource sharing between *P. parva* and sympatric fishes, and relatively low trophic positions of *P. parva*.

4.2.3 Wild ponds

The four wild ponds provided greater complexity in their communities, with higher numbers of fishes present and with little replication between the ponds and their fish community composition. In contrast to the mesocosms and small ponds where *P. parva* had relatively low trophic positions to the other fishes, in these wild communities, *P. parva* had relatively high trophic positions compared

with some species. For instance, the $\delta^{15}N$ data indicated *P. parva* tended to have higher trophic positions than *G. aculeatus* in the wild ponds, a complete reversal of patterns observed previously.

In the wild ponds, the trophic niche size of *P. parva* was, however, relatively restricted compared to other species and often showed some overlap with sympatric fishes, albeit not necessarily to a great extent. However, given their relatively small trophic niche size, it would be difficult to suggest that even where *P. parva* had an overlapping trophic niche with other species, they represented a food web perturbation that was having impacts or cascading influences on other species. Conversely, given the presence of other invasive fishes in most of the ponds, *P. parva* influence on the trophic niche and position of the other fishes appeared minimal. Indeed, this complexity and the lack of opportunity in the project for long-term study and manipulation, that inhibited the ability to draw more firm conclusions on the patterns and processes evident, supports the earlier use of the experimental approaches, even if some patterns were inconsistent between all three objectives.

It was interesting to note that in the Millennium Coastal Park, the stable isotope outputs of the two size classes of *S. erythropthalmus* indicated that they were exploiting different food chains within the food web, with the suggestion that the large *S. erythropthalmus* were mainly feeding on Chironomid larvae, whereas for other fishes, their food chain related to macro invertebrate such as *Asellus aquaticus*. There was no apparent trophic niche overlap between the two class size of *S. erythropthalmus*, with the 'Large' length group being

significantly faster growing, suggesting their exploitation of the different food resources provided them with a distinct ecological advantage arising from their trophic niche divergence. There was no evidence to suggest this divergence was related in any way to the presence of invasive *P. parva*.

Consequently, the outputs of Objective 3 ('Assess the trophic relationships, basic food web structure and the ecological consequences of *P. parva* invasion in four wild ponds and assess whether patterns apparent in the data outputs have synergies with those from data generated in more controlled environments in O1 and O2') revealed a more complex situation than observed in Objectives 1 and 2. Nevertheless, there was some consistency in outputs with Objectives 1 and 2, as there was minimal evidence that *P. parva* were a key ecological driver impacting the food web and trophic niche and position of the sympatric fishes. Instead, their relatively small trophic niche size indicated that they might have actually been having only very minor consequences for the feeding relationships of other fishes in the community, with these fishes potentially being more ecologically significant.

4.2.4 Summary of trophic interactions of invasive topmouth gudgeon

In Chapter 1, it was discussed that in earlier work on the trophic ecology of *P. parva*, an initial study had indicated their high sharing of trophic space with *R. rutilus* and *C. carpio*, with negative consequences for the growth rate of *R. rutilus* (Britton et al. 2010c). It was also discussed that subsequent work had suggested that this pattern was not repeated in other invaded fisheries, with often

minimal or no sharing of trophic space between *P. parva* and sympatric species (Jackson and Britton 2013, 2014). It was also noted that in the study where there was high sharing of trophic space, the *P. parva* population was highly abundant (> 60 n m^{-2}; Britton et al. 2010c), whereas in the other studied waters, their abundances were lower but variable. Thus, this suggests some context dependency in the trophic relationships of *P. parva*, with only high trophic overlaps with sympatric species when present in extreme population sizes that might develop only under certain circumstances, such as elevated productivity arising through angling and the introduction of high levels of angling bait (Jackson et al. 2013) with subsequent exhaustion of natural food supplies resulting in niche convergence.

The outputs of Objectives 1 to 3 in this study supported the theory that *P. parva* do not compete directly with the native species used studied in this research, with trophic niche divergence the more common mechanism. This suggests the fish were actually avoiding inter-specific competition by divergence that promotes their co-existence. However, it should be noted that in this research, no populations were used that would represent extreme *P. parva* population abundances. This was because these were not present in the wild ponds or small aquaculture ponds, and it was not considered ethical to use extreme fish abundances in experimental conditions due to the potential for starvation of the fishes.

4.3 Research outputs in the context of trophic niche theory

Trophic niche theory suggests that when an invasive fish establishes in a native environment, instead of increasing inter-specific competition for food resources which lead to the decline of native fishes, they will segregate their resource use and reduce the extent of competition thus the invasive and native species will be able to co-exist in the system by exploiting different food resources (Chesson 2000, Kylafis and Loreau 2011). Consequently, it is food resource specialization, not generalization, that is the key mechanism in ensuring species' co-existence, as it should reduce interspecific competition (Gabler and Amundsen 2010; Kleynhans et al. 2011). Thus, the outputs of this study (Section 4.2) are highly consistent with trophic niche theory as the *P. parva* did not generally compete directly for food resources when in sympatry with the native fish species used in the study, with divergence the predominant mechanism observed between the fishes.

According to Toft (1985), resource partitioning has been used to study how sympatric species differ in their resource use and how they coexist with species with similar functional traits and diet composition by avoiding negative consequences of food resource competition (MacArthur 1965; Schoener 1974; Roughgarden 1976). When compared between allopatric and sympatric contexts in the mesocosms, data outputs revealed sympatric *P. parva* had smaller trophic niche breadths than allopatric *P. parva*. Whilst it is difficult to allocate this specifically to a shift in specific food resource use, given the use of stable isotope analysis rather than GCA, this constriction may have been related to *P. parva*

diverging their habitat use in the mesocosms under sympatric contexts, resulting in subtle shifts in their resource use. However, this is not supported by Beyer et al. (2007) who revealed *P. parva* had habitat associations with co-existing bull head *Cottus gobio*, European chub *Leuciscus cephalus* and brown trout *Salmo trutta* in the Tadburn Lake stream that is close to the aquaculture site that was used in England. Nevertheless, in larger systems, habitat partitioning can play an important ecological role in determining trophic niche sizes and divergences between coexisting species (Mendelson 1975; Baker and Ross 1981). It should be noted that it was beyond the scope of this study to study habitat use in the sympatric and allopatric fishes but this is something that is recommended for further work.

4.4 Management implications of the study outputs

Pseudorasbora parva is considered a pest fish across much of Europe (Pinder et al. 2005) and in non-native fish risk assessments it tends to score highly and thus be assessed as of high ecological risk (Britton et al. 2010e, 2011). This assessment results from two issues: (1) their propensity for producing extreme population abundances, with these comprising of individual fish that compete strongly with native fishes (e.g. Britton et al. 2010c); and (2) their status as a health host of *S. destruens* that could cause high mortality rates in native fish populations (Gozlan et al. 2005; Andreou et al. 2012).

Risk assessments of non-native species are important as they form the basis of species' risk management, particularly for species that are already introduced

(Britton et al. 2011). Non-native fish risk management identifies, evaluates and implements actions to reduce their risk to the native biodiversity and ecosystem functioning - and in the case of fish, the fishery interests (Britton et al. 2010d). Consequently, in the UK, a risk-based programme of sustained control of *P. parva* was instigated by the Environment Agency of England and Wales in the 2000s on the basis of their high ecological risk combined with their restricted distribution (< 30 invaded water; Britton et al 2010b). This was developed within a basic evaluation framework for managing populations in high risk, lentic environments (Britton et al. 2010b, 2011). Thus, the commensurate actions in waters providing a high risk for *P. parva* dispersal, such as ponds connected to river catchment (i.e. 'open' waters) was eradication, usually with application of the piscicide rotenone (Meadows 1973; Allen et al. 2006; Britton and Brazier 2006). Eradication by rotenone is a highly effective management intervention, and although it was recommended by Britton et al. (2010b) to be only used in high risk waters (i.e. that provide natural dispersal opportunities), it has now been used more widely by the Environment Agency through its application to *P. parva* invaded lakes which are also fully enclosed (Gozlan et al. 2013). This is because the Environment Agency now perceives the risk to native fishes from *P. parva* to be sufficiently high to warrant their complete eradication, with this possible due to their restricted distribution and presence in only lentic systems.

The results of this research suggest, however, that the risk from *P. parva* for native fishes in the UK is not necessarily associated with competitive processes, with these only apparent in managed fisheries with high fish stocks that facilitate *P. parva* proliferation through input of angler bait (Jackson et al. 2013). Indeed,

the outputs here suggest that whilst there may be some food web perturbations resulting from *P. parva* introduction, there will be trophic niche divergence with co-existing species that facilitates their co-existence, rather than competition that results in negative consequences. This means that rather than managing *P. parva* on the basis of their ecological impact, their risk management ought to focus more on the management of their dispersal of *S. destruens* (Gozlan et al. 2005). Given the potential of this generalist pathogen to invoke considerable mortality rates on native UK fish, then it is likely the 'high risk' of the species will remain in assessments (Andreou et al. 2012). However, it does mean that some shift in emphasis might be required in the reporting and justification of eradication operations, as these tend to focus on aspects of the adverse effects of inter-specific competition *from P. parva* (e.g. Environment Agency 2013). Consequently, the outputs of this research have an important management and applied context, as well as its contribution to theoretical perspectives on trophic niche divergence in invasion ecology.

4.5 Study limitations and recommendations for future work

The study was inherently constrained by its ability to work on fish communities known to be invaded by *P. parva* and where permissions and sampling teams could be set up to collect samples. This meant that alternative non-native fishes were not studied, despite their potential for also showing similar patterns in their trophic ecology in relation to native fishes. For example, sunbleak *Leucaspius delineates* is an invasive fish in the UK that could have similar trophic ecology to *P. parva* and might have made an equally strong model species, but logistics

meant they were unable to be studied. Similarly, a restricted number of native fishes were selected for study in the experiments, using just three. Species that had been used in previous field-based studies, such as *R. rutilus* and *A. brama*, were not used (e.g. Britton et al. 2010b). The reasons for this were two-fold. Firstly, use of multiple species in the experiments was not feasible due to a limited number of ponds being available. Secondly, some cyprinid species, such as *R. rutilus* and *A. brama*, can be difficult to use experimentally and a proportion may die prior to the end of the experiment, inhibiting the ability of that work to then draw any conclusions (JR Britton, personal communication). However, this did mean that in the experiments, the conclusions drawn might not be consistent across all native fishes, and are only applicable at this stage to those fishes used in the study, even if these were relatively consistent across the three spatial scales.

It was discussed that stomach contents analysis were not used in the study due to issues of the difficulty of identifying small, semi-digested items in fish intestines and in obtaining sufficient samples across time and space to draw strong conclusions. Instead, stable isotope analysis was used that is now considered as a powerful tool to investigate the ecological effects of non-native fish species by assess their diet (Cucherousset *et al*. 2012). However, it should be noted that the method was not used to quantify the actual food items being taken by each species and did not quantify any short-term dietary changes that might have occurred and resulted in aspects of the trophic niche divergence that was measured at the end of the experiment. Whilst samples could have been taken during experiments to try and quantify short-term diet changes, such as

sampling the fish and taking samples of mucus whose stable isotopes can reflected very short time periods (e.g. 3 days; Church *et al*. 2008), this would have disturbed the mesocosms, impacted the food resources and potentially disrupted the development of the long-term stable isotope patterns. Moreover, the outputs of studies that use both stable isotope analysis and stomach contents analysis often show contrasting outcomes form each method, suggesting each indicates important dietary patterns but ones that can be difficult to interpret when put together (e.g. Locke *et al.* 2013).

This difficulty in interpretation of stable isotope data also extends to the data available on the fish. For example, fish diet is strongly affected by ontogeny and thus fish size (and so their gape size) is a key consideration when comparing the diet of two species (Cucherousset *et al*. 2012). Where possible, only fish covering the same length range were used, although circumstances distated this was not always possible. In addition, samples of muscle collected from fish sampled early in the year, prior to the commencement of their growth season, will also be problematic in that the stable isotope data will reflect the diet of the fish the previous summer rather than than the winter, and this should be factored into any interpretation (Perga and Gerdeaux 2005).

Consequently, in terms of future work, then the following recommendations are made:

1. The completion of more mesocosm experiments involving *P. parva* in sympatry with other native cyprinid species such as *R. rutilus* and *A.*

brama, and also within more complex communities of native fishes (e.g. two species and above) involving species of different feeding guilds.

2. The completion of mesocosm experiments using a different model non-native fish, such as *L. delineatus*, to identify whether the patterns observed in this study are applicable to small, non-native cyprinid fish more generally.
3. The application of alternative methods of dietary analyses in studies to determine how the limitations of stable istope analysis using dorsal muscle (as outlined above) might be overcome by using either alternative tissues or material in the analysis (such as mucus) or alternative methods such as stomach contents analysis or DNA barcoding of stomach contents (Jo *et al*. 2014). If done experimentally then for barcoding and stomach contents analysis, this would require regular removal and replacement of fish as the emthods are destructive. The advantages of all these methods is their indication of diet over a much shorter timeframe than the stable isotope analysis of dorsal muscle, but their use would mean a change in experimental design.
4. More integrative studies could be used that amalgamate trophic and feeding studies with use of different macro- and micro-habitats by the different fish species. By using more advanced approaches in passive intgegrated transponder tags (PIT tags), shifts in the habitat of the non-native and native fishes could be measured between their allopatric and sympatric contexts, and related to dieary shifts (e.g. Peterson et al. 2004). This would help indicate whether the process of diet partitioning was related to aspects of habitat partitioning.

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