Applied ecology and management of invasive topmouth gudgeon *Pseudorasbora parva* in England & Wales

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Abstract

Introduced species are a major threat to native biodiversity and, whilst there are socio-economic benefits often gained from introductions, there are also inherent risks to receiving ecosystems, particularly from those species that develop invasions. This is problematic, as once in the wild, their management is inherently difficult. The most effective method to avoid an invasion is preventing the initial introduction; if this is unsuccessful, then methods should aim to minimise the dispersal opportunities of the species into the wider environment.

This research used the Asian fish of the Cyprinidae family, topmouth gudgeon *Pseudorasbora parva*, as the model species, a highly invasive fish in mainland Europe. England and Wales was the model area, where 26 *P. parva* populations have been recorded but the species is not yet invasive. The research identified how aspects of *P. parva* ecology can underpin the application of methods to prevent their wider dispersal into the environment to prevent their invasion. It covered work on: (i) the expression of their life history traits; (ii) preventing their introduction into inland waters via fish movement audits; (iii) identifying the risk of their dispersal into river catchments from aquaculture sites; and (iv) assessing how their populations can be controlled to minimise dispersal opportunities.

Assessments of their somatic growth rates in relation to temperature and latitude revealed that whilst temperature was a strong regulator of growth in controlled conditions, in the wild, population density was important, with this having a stronger influence on growth rates than latitude (temperature). Individuals in recently founded populations and at low densities had very fast growth rates, with this advantageous in facilitating their establishment, with these growth rates slowing as population abundance increased. Assessments of their reproductive traits revealed that, contrary to European and Asian populations, they did not show an extended reproductive period involving fractional spawning, but instead had a discrete reproductive season that lasted no longer than three weeks in the early summer period.

Approximately 8000 fish movements (i.e. removals from fisheries, stocking into fisheries) are legally consented by the Environment Agency in England each year. On each of these stockings, there is a concomitant risk of also accidentally introducing a ‘hitch-hiking’ species that has not been detected in the batch of fish. To test whether a ‘fish movement audit’ scheme could reduce this risk, an experiment was completed on batches of native fish containing known proportions of *P. parva* and using auditors of varying non-native fish knowledge and pre-defined searching effort levels. Outputs revealed that although the scheme could be successful at preventing 95 % of accidental introductions, this required highly experienced auditors using high searching efforts.

The presence of *P. parva* on an aquaculture site in Southern England enabled completion of field studies on assessing their drifting rates from the farm and into an adjacent stream. Drift rates were negligible during normal summer operations. However, when aquaculture ponds on the site were drained down for husbandry purposes, the elevated flow of water displaced high numbers of mature *P. parva*, resulting in high dispersal rates. Whilst no *P. parva* were recorded in the three hours prior to ponds being drained down, over 200 were captured in drift nets in the 21 hours after draining commenced. Thus, whilst the overall risk of their dispersal was low, this was elevated considerably during specific on-site activities, and would require pro-active management to prevent accidental dispersal during these activities.

A long-term field experiment, allied with a field study, revealed that in small fishing and aquaculture ponds, *P. parva* population densities can be maintained at very low abundances over long time periods, but required a combination of biocontrol (via manipulating of numbers of native piscivorous fish) and targeted fish removals (by trapping in spring and autumn). Fish removals alone were unable to reduce population sizes due to the compensatory responses of the remaining fish. These responses were not, however, detected when native piscivorous fish were present, probably through their predation of nest-guarding male *P. parva* that then inhibited large numbers of in young-of-the-year being produced.

In summary, the research revealed utility of non-native fish management methods being applied to populations following their testing in more controlled conditions. It revealed that introductions into fisheries of fast growing, small-bodied, non-native fishes can be prevented by auditing, and where this is unsuccessful then their dispersal into the wider environment can be minimised, if not eliminated, by a variety of method that should reduce their invasion risk.

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Author’s declaration

I confirm that this thesis is all my own work, with the following exceptions:

Chapter 2 was published and was written in collaboration with Robert Britton as:

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Chapter 1

General introduction

This subject of this thesis is how aspects of the ecology of a model non-native fish (topmouth gudgeon, *Pseudorasbora parva*) can be applied to informing aspects of its management in a specific region (England and Wales) to assist prevention of their invasion. The research covers topics including non-native species generally and fishes specifically, the traits that can facilitate non-native fish establishment and invasion, and how these species can be managed when present in open systems.

1.1. Non-native species

Introduced non-native species are considered as a significant threat to native biodiversity and ecosystems, with consequences that include the extirpation of populations of native species and the loss of some ecosystem services (Chapin et al., 2000; IUCN, 2000; Millennium Ecosystem Assessment, 2005; Simberloff, 2005). To date, every continent has been subject to some non-native species introductions and their associated deleterious effects (IUCN, 2000). The introduction and proliferation of non-native species can often incur high costs to industries, including agriculture, forestry and fisheries, and can also impact some aspects of human health (Wittenberg and Cock, 2001). Indeed, Pimentel et al., (2000) estimated the total loss to the World economy as a result of non-native species was approximately 5 % of annual production. The estimated damage and costs associated with control of non-native species amount to $138 billion per annum (p.a.) in the U.S.A. alone and £1.7 billion p.a. in the UK (Williams et al., 2010).

Natural barriers to biotic exchange (e.g. hydrological, topographical and climatic) have resulted in speciation processes and biogeographical patterns that are reflected in distinct and heterogeneous biotic diversity between regions. Increasingly, however, factors including increased globalisation and free-trade has provided opportunities for species to disperse beyond their native ranges, with long-distance movements of species occurring between regions via both intentional transfers and their accidental transfer as contaminants (IUCN, 2000). In doing so, it has resulted in patterns of regional biodiversity becoming less distinct in a process often referred to as biotic homogenisation, whereby floral and faunal communities across the world are increasingly composed of similar species (Vitousek, 1997; Rahel, 2000; Olden and Poff, 2004). This process thus describes the manner by which species invasions and extinctions increase the genetic, taxonomic or functional similarity of two or more biotas over a specified time interval, with studies tending to focus more on taxonomic homogenization rather than genetic and functional homogenization (Olden, 2006). From a freshwater fish perspective, studies tend to suggest that taxonomic homogenization will continue to blur the current high level of taxonomic dissimilarity among freshwater fish faunas, primarily due to the issue of continued use of fishes within global trade and aquaculture (Villéger et al., 2015).

The issue of introduced non-native species is not new, however, with some species having been moved across Eurasia since Roman times, such as common carp *Cyprinus carpio* (Balon, 1995). Nevertheless, in the last 50 years, introduction processes have accelerated, aided by free trade movements, global transport processes and the increasing global use of some species, such as tilapias and Asian carp species, within intensive culturing systems (Gozlan et al., 2010a). Consequently, the rate of introductions of non-native species into Europe in the last 25 years is unprecedented (Hulme, 2009). Such changes in recent years have led to some scientists referring to the current period as the 'Homogocene' era, or the'Anthropocene' (Rosenzweig, 2001; Steffen et al., 2007, 2011).

This intentional long-distance transfer of non-native species into new regions can be highly advantageous, with introduced species often diversifying food production (Tesauro and Ehrenfeld, 2007; Pearson et al., 2000; Shapiro, 2002; Kats and Ferrer, 2003), providing new habitats for native species (Newman, 2002), and acting as beneficial ecosystem engineers (Salmon et al., 1995; Wonham et al., 2005), all of which can then provide high socio-economic benefits (Pierson et al., 2004; Reaser et al., 2007). However, when these introduced species become widely dispersed in an uncontrolled manner in the wider environment, either through lapses in biosecurity at aquaculture sites, or via their accidental release into open systems, then the potential for their development of sustainable populations can be high, resulting in the possibility of invasive populations to develop as the species undergoes a series of short-distance movements to colonise new areas (Gozlan et al., 2010b). Moreover, when a species has been accidentally introduced, such as one that has 'hitch-hiked' from its native range within a consignment of species being moved intentionally (Essl et al., 2013), it can pose considerable risks to the receiving environment as their introduction is likely to have occurred in the absence of any form of risk assessment or health-status checks (Copp et al., 2005).

The consequences of unregulated introductions of non-native species into the wider environment can then include ecological impacts including alterations in physical habitats, the detrimental consequences for populations of native species of increased predation rates and/ or inter-specific competition, hybridisation processes and pathogen transmission, with these usually considered as irreversible (Moyle, 1993; Vitule et al., 2009; Gozlan et al., 2010a). For example, the increased predation pressure by the introduced brown tree snake *Bioga irregularis* resulted in the local extinction of indigenous avian species in Guam (Savidge, 1987), and on the island of Uist, Scotland, wading bird egg depredation by the introduced European hedgehog *Erinaceus europaeus* has been attributed to the decline in wading bird numbers and nest success (Jackson and Green, 2000). Hybridisation and introgression is often overlooked as an impact associated with the introduction of non-native species (Rhymer, 2006), but the deterioration of genetic integrity of indigenous biota can lead to localised extinctions and ‘genetic swamping’ (Rhymer and Simberloff, 1996). Freed from the pressures associated with predators and other population controls in their native range through ‘enemy release’ (Heger and Jeschke, 2014), non-native species can outcompete native biota, suppressing or even extirpating indigenous species, such is the case of the non-native vase tunicate *Ciona intestinalis* in its introduced range and its impacts on indigenous sessile faunal species (Blum et al., 2009). The introduction of non-native species can also lead to the release of disease agents and the parasites associated with them (Sheath et al., 2015). For example, the importation of Asian eels *Anguilla japonica* to Germany has been associated with the release of the nematode parasite *Anguillicoloides crassus* which subsequently infected wild European eels *Anguilla anguilla* (Koops and Hartman, 1989; Kirk, 2003; Pegg et al., 2015). Additionally, habitat modification by non-native species can have far reaching consequences for native biota, for example, the invasive zebra mussel *Dreissena polymorpha* can attain vast populations in its introduced range and is capable of filtering large volumes of water daily (Hudson et al., 1996), filtering up to 80% of the planktonic biomass from the water column, leading to substantial food-web alterations (Strayer, 2009).

1.2. Non-native freshwater fish

Freshwater fishes are one of the most widely introduced aquatic animals at the global scale, with at least 625 freshwater fish species now having been introduced outside of their natural range (Gozlan, 2008; Gozlan et al., 2010a). This international transportation and release of non-native fishes does provide considerable socio-economic benefits through aquaculture and sport angling activities, as well as supporting the ornamental fish trade (Rixon et al., 2005; Gertzen et al., 2008; Gozlan, 2008; Gozlan et al., 2010a). Nevertheless, the increased presence of non-native fishes in new regions has also incurred ecological consequences, with a classic example being the release of the Nile perch *Lates niloticus* into East Africa's Lake Victoria, which has been implicated in the decline and, in some cases, extirpation of over 200 haplochromine cichlid species (Ogutu-Ohwayo, 1990; Barel et al., 1985: Kishe-Machumu et al., 2012). Other examples include the substantial declines (up to 95 %) in native fish diversity following the release of fishes of the *Cichla* genus for sport angling in reservoirs in Southern Brazil (Pelicice and Agostinho, 2009; Britton and Orsi, 2012), hybridisation of the already threatened native trout species (e.g. *Oncorhynchus apache* and *Oncorhynchus gilae*) with the introduced rainbow trout *Oncorhynchus mykiss* in North America (Vitule et al., 2009) and hybridisation of crucian carp *Carassius carassius* with introduced goldfish *Carassius auratus* and *C. carpio* in the UK (Wheeler, 2000; Hänfling et al., 2005). Additionally, *C. carpio* have also been shown to increase water turbidity and decrease aquatic macrophytes and zooplankton in invaded water bodies (Roberts et al., 1995; Lougheed, 1998).

Given this trade-off between gaining substantial socio-economic benefits from non-native fishes and the ecological risks they can pose should they reach open systems, then it is imperative the invasion processes of freshwater fishes are understood (Kolar and Lodge, 2002). This then enables identification of the key mechanisms and the implementation of policies and regulations to minimise the inherent ecological risks whilst still obtaining some of the benefits (Moyle and Light, 1996; Semmens et al., 2004; Bobeldyk et al., 2015). The invasion process (or 'invasion pathway') consists of four sequential stages (Figure 1): introduction, establishment, dispersal and impact, each with an independent probability of failure (Rosecchi et al., 2001). Success at each stage is then dependent on a number of environmental and biotic factors (Baltz and Moyle, 1993; Kolar and Lodge, 2002; Williamson and Fitter, 1996), with a species classed as ‘invasive’ should it successfully negotiate each stage of the invasion pathway, but critically, threaten or harm native biota or the receiving environment (Convention on Biological Diversity 2007).

The constraints on the ability of an introduced species to ultimately develop invasive populations include ‘biotic resistance’ (Simberloff and Von Holle, 1999), where species richness within the receiving environment can affect invasion outcomes (Lodge, 1993). The biotic resistance hypothesis suggests that species-rich systems offer the greatest resistance to non-native species invasions (Elton, 1958), as extant species already occupy the majority of the available niches, resulting in few opportunities for introduced species to exploit (Shea and Chesson, 2002; Stachowicz and Byrnes, 2006; Henriksson et al., 2014; Rius et al., 2014). Whilst examples of biotic resistance against introduced aquatic species are documented (Robinson and Wellborn, 1988; deRivera et al., 2005; Dzailowski et al., 2007), examples of biotic resistance against non-native fish tend to be rare (Britton, 2012). Habitat disturbances can also facilitate invasion success (Simberloff and Von Holle, 1999), since high disturbance can suppress native species interactions and create vacant niches for non-native species to exploit, and/ or can alter the turnover rate of resources, such as nutrient availability (Sher and Hyatt, 1999). Anthropogenic trophic subsidies (such as anglers bait) have also been shown to facilitate establishment of introduced fish, through increased food resource availability via introductions of high volumes of bait for angling (Jackson et al., 2013a).

A further mechanism by which the outcome of an introduction of a non-native fish species is influenced is through its ‘propagule pressure’ (Von Holle and Simberloff, 2005), in which the number of propagules (i.e. number of founders) and their flux (i.e. frequency) into a system can determine the probabilities of a successful invasion (Lockwood et al., 2005; Britton and Gozlan, 2013). Increased numbers of released individuals and/ or their increased frequency of release tends to increase the probability of their establishment as they are more likely to overcome demographic or environmental constraints on their survival and reproduction success (Lockwood et al., 2005).

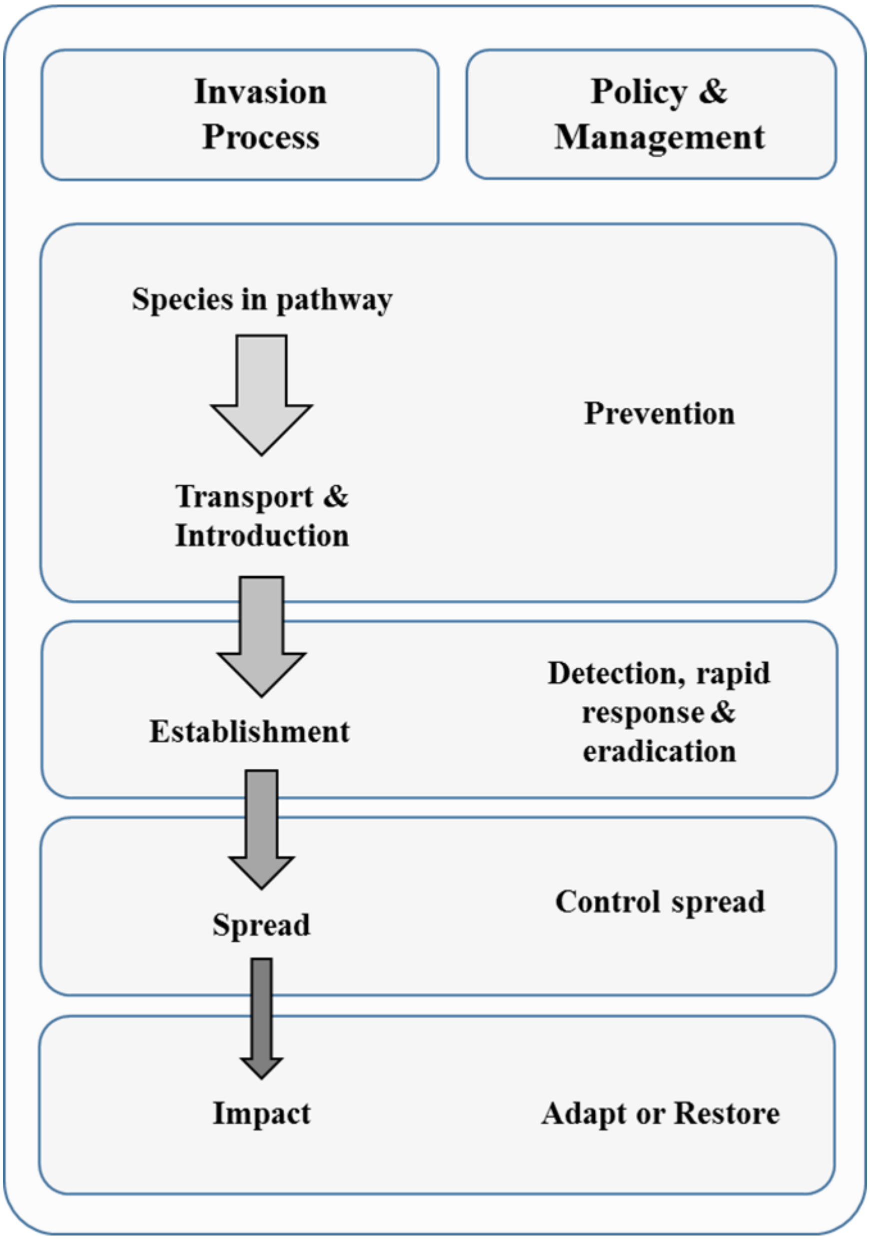

Figure 1. The stages of biological invasions and policy and management options at each stage. At each stage an organism has an independent probability of failing to achieve the next. Adapted from Lodge et al. (2009).

The net outcome of the invasion pathway (Figure 1) and the constraints imposed upon the released propagules by the recipient ecosystem and its communities at each stage, results in only a small proportion of introduced species going on to develop invasive populations, with even fewer then going on to cause detrimental effects within their new range (Williamson and Fitter, 1996; Gozlan, 2009; Leprieur et al., 2009). This process is termed the ‘tens rule’ (Williamson and Fitter, 1996), with the underlying principal that approximately 10% of non-native species transported outside of their indigenous range will be released or escape; from this 10%, a further 10% will be able to establish and about 10% of these will then go on to became invasive (Figure 1). Although this suggests that the overall level of invasion risk from introduced non-native fishes is low, this has enabled the development of a range of risk assessment processes that enable species-specific risk levels in a region to be quantified and species be prioritised accordingly (e.g. Copp et al., 2005, 2014a,b). This then enables resources to be prioritised towards preventing invasions of those species most at risk of causing ecological impacts in the environment.

It has been outlined that activities such as aquaculture and sport fishing remain the predominant drivers of introductions of non-native fishes (Copp et al., 2005; Gozlan et al., 2010a). Where freshwater fish are selected for use in aquaculture and sport angling, they tend to be selected for specific traits, such as their ease of culture and rearing in relatively high stock densities (e.g. *C. carpio* and many tilapia species). For sport angling, the traits of large body sizes and sporting qualities are often selected, as shown by the increased introduction of species such as European catfish *Silurus glanis* across Southern Europe (Copp et al., 2009). Freshwater fish have also been moved intentionally for their functional traits that make them useful as biological control agents, such as species of *Gambusia* genus for mosquito control (Fernandez-Delgado, 1989) and grass carp *Ctenopharyngodon idella* and silver carp *Hypophthalmichthys molitrix* for weed control (Starling, 1993; Silva et al., 2014). Some species were originally introduced for ornamental purposes, although they might now also be used for angling purposes, such as colour variant forms of orfe *Leuciscus idus* (Hickley and Chare, 2004) and *C. carpio* (Morgan et al., 2004). In addition, there are occasions where small-bodied fishes are intentionally released, such as *P. parva* being used as 'feed-fish' in Albanian aquaculture systems, supplementing the diet of cultured predatory fishes (Wildekamp et al., 1997).

From an environmental and ecological perspective, then the introduced non-native fishes that provide the greatest concerns are arguably those that are released without risk assessment. These include fishes that are introduced accidentally, with this usually occurring when a fish consignment has been purposely introduced for aquaculture and fish stock enhancement purposes but has been contaminated with a 'hitch-hiking' species, resulting in their unknowing introduction (Garcia-Bethou et al., 2005; Copp et al., 2007). Compared with other introduction pathways, this route has received relatively little attention (Copp et al., 2010), although Gozlan et al., (2010a) suggested that, overall, accidental introductions accounted for almost 8 % of all global fish introductions, potentially making the pathway a significant influence on the non-native fish fauna of a region. As the stocking of freshwater fish is a very common recreational fisheries management practice, driven by socio-economic forces (Gozlan and Newton, 2009), and with over 1.5 million fishes introduced per annum to England and Wales alone (Hickley and Chare, 2004), then this pathway could be responsible for a relatively high proportion of the accidental introductions that do occur.

1.3. Life history traits that promote invasion success

It was outlined in Section 1.2 that a series of abiotic and biotic constraints often prevent introduced non-native fishes from developing invasive populations. Conversely, the introduced fish species might have a suite of life-history traits that, when expressed in the new range, enables or even facilitates their establishment and subsequent invasion (Vila-Gispert et al., 2005; Olden et al., 2006). This facilitation is particularly enhanced when the introduced species' life-history traits are highly plastic (Davies and Britton, 2015a).

Kolar and Lodge (2002) investigated the life-history characteristics of a number of invasive fish species in the North American Great lakes and across different stages of the invasion process. They concluded that different traits were important at different stages of the invasion with, for example, fast somatic growth rates being important during the establishment phase, but with this then potentially limiting that species ability to then disperse (Kolar and Lodge, 2002). Generally, *r*-selected traits, such as short generation times, early maturation, high fecundity and rapid growth rates facilitate establishment and invasion success (Sakai et al., 2001; Fox et al., 2007). By contrast, *K* selected traits, such as late or delayed fecundity, and long generation times, can impede establishment and invasion processes (Olden et al., 2006).

The suite of life-history traits of introduced fishes that have successfully gone on to form invasive populations also vary by region, with Ribeiro et al., (2008) suggesting that successful traits tend to be those that are opposite of those expressed by the native species in that region. For example, in the Iberian Peninsula, invasive fishes tend to have large body sizes with low parental care, whereas native fishes tend to be small and have relatively high levels of parental care. By contrast, in Great Britain, invasive fish of the Cyprinidae family tend to be of small-body size, undergoing multiple spawning events and have nest guarding behaviours which are, again, a general contrast to the native cyprinid fishes (Britton et al., 2007; Ribeiro et al., 2008).

Thus, whilst the analysis of the life-history traits of an introduced freshwater fish can be a good predictor of its likelihood of subsequent invasion success, the advantageous traits for a defined spatial area analysis may vary. High plasticity in their expression tends to be important, with fast growth and early maturity especially important in the immediate post-introduction period, as this enables the newly introduced propagules to overcome the population demographics associated with low population sizes, and enables them to avoid going through population bottlenecks (Britton and Gozlan, 2013). Correspondingly, understanding the spatial and temporal variation in life history traits of specific species that have been introduced into a new region should assist their management by, for example, highlighting their ability to rapidly colonise new habitats and identifying the potential issues that could arise from aspects of their population control (Marchetti et al., 2004).

1.4. Management options in open systems

Non-native invasive species introductions are generally easier and cheaper to manage when their distribution are highly localised (IUCN, 2000; Manchester and Bullock, 2000; Genovesi, 2005; Cacho et al., 2006), increasing the likelihood of successful control and the limitation of impacts. The concept of ‘Rapid detection, rapid assessment, rapid response’ (Myers et al., 2000; Zavaleta et al., 2001; Zavaleta 2002; Anderson, 2005) highlights the importance of detecting a new introduction, especially when it followed by risk assessment and instigation of an appropriate management response in a swift and timely manner (Britton et al., 2011a,b). Rapid detection of an invasive fish population, prior to its initial introduction or whilst it is confined to a small spatial area, is particularly important for avoiding invasions developing from accidental introductions.

The ability to detect a newly introduced fish population is often compromised, however, due to factors including their low population abundances that inhibit their detection using traditional sampling techniques (Britton et al., 2011c). For some fishes, their discovery can be inadvertent, often occurring via reports from anglers or during routine fish population surveys (Pinder et al., 2005; Britton and Davies, 2007). Hayes et al. (2005) described the search effort required to detect an introduced species as being inversely proportional to the population size, with the difficulty of detection compounded by ‘false negative’ recordings, i.e. imperfect detection (Rout et al., 2009a,b; Britton et al., 2011b). Therefore, any surveillance or management strategies have to achieve a balanced between the amounts of effort, and therefore cost, required for detection and the associated costs of subsequent remediation protocols if there is a failure to detect a recent introduction (Hayes et al., 2005). The recent development of techniques based on genetic technologies that can detect the DNA of non-native species in the environment should, however, improve abilities to detect new introductions in future (Jerde et al., 2013; Keskin, 2014). Its application would nevertheless still require sufficient surveillance and intelligence gathering to enable the most appropriate water bodies to be sampled.

Ideally, contingency plans would already be in place to deal with a species that has been newly detected, with horizon scanning exercises appropriate for highlighting those species that are most likely to be introduced in the near future (Roy et al., 2014). Upon their detection, the most appropriate remediation responses can be implemented. The advantage of such rapid responses was apparent following the discovery of an invasive marine algae *Caulerpa taxifolia* in the coastal waters of California in 2000, where its containment and then eradication was initiated within 17 days of its discovery (Anderson, 2005). By contrast, its detection and subsequent spread in the Mediterranean was left unimpeded for over a decade (Boudouresque et al., 1995).

The inherent issue with managing non-native fishes in open systems is that management options are very limited, generally falling under control and/ or containment, as eradication of species and extirpations of local populations is difficult to achieve (Myers et al., 2000; Zavaleta et al., 2001; Cacho et al., 2006; Britton et al., 2011a). Indeed, eradication operations are usually controversial and their success is usually limited, particularly when the target species has established populations over large spatial areas. Additionally, eradication techniques are often described as ‘scorched earth’ methods, imposing collateral damage on native biota and incurring substantial costs (Myers et al., 1998; Simberloff, 2002; Britton et al., 2007). That said, where the impacts, or risk of impacts, resulting from the introduction of newly introduced species are assessed as high, then commensurate management actions should follow, which could include eradication, where feasible (Andersen et al., 2004; Britton et al., 2011c,d). To facilitate the success of control measures, knowledge of the introduced species, in particular its biology and life-history traits, will be important in the development of effective control and eradication procedures (Simberloff, 2003). The following sub-sections outline some of the more common approaches to avoid introductions and subsequent invasions of non-native fishes (Figure 1).

1.4.1. Legislation

Management of introduced fishes vary considerably across the world, with legislation in many developed nations used to regulate the release of certain species into the wild (Gozlan, 2010a). In England and Wales, three primary legislative controls regulate the keeping and introduction of non-native species (Table 1). The principal legislation is the Wildlife and Countryside Act (WCA) 1981, with the introduction of new species regulated by Section 14(1). Offences under the WCA Section 14 can incur considerable penalties, with an unlimited fine (commensurate to the offence committed) and/ or 2 years imprisonment. Specifically aimed at management of fish species, the Salmon and Freshwater Fisheries Act (SAFFA) 1975 Section 30 makes it an offence to introduce any fish into an inland water without the written consent of the water authority (i.e. Environment Agency in England and Natural Resources Wales in Wales). This legislation covers the translocation of native fish species as well as introductions of non-native fish species to England and Wales. Further protection is provided through the Import of Live Fish Act (ILFA) 1980, under which the import, keeping or release of live fish of listed species is restricted. The Prohibition of Keeping or Release of Live Fish (Specified Species) Order 1998, made under ILFA 1980 lists 47 non-native fish species which are prohibited from keeping or release without licence. A further Statutory Instrument, The Keeping and Introduction of Fish (England and River Esk Catchment Area) Regulations 2015 (KIF), came into force on 19th January 2015, which repeals ILFA 1980 in England. The Regulation requires any persons keeping or introducing any fish belonging to certain taxonomic orders to have a permit.

Penalties for offences committed under the Regulations are much greater than those imposed under ILFA 1980, with fines up to £50,000 on summary conviction.

However, such legislative protection is not always provided in other countries, such as Brazil, where legislation is weak and law enforcement is lax, and there are continued illegal introductions of non-native species (Vitule et al., 2009). Additionally, it is feared that proposed new laws in Brazil, designed to permit cultivation of non-native fish species in hydroelectric reservoirs, could lead to the widespread release of known invasive fish species (Vitule, 2012; Pelicice, 2014). Nevertheless, the Convention on Biological Diversity (1993) has the aim of developing strategies at national levels for the conservation and sustainable use of biological diversity, including the aim to “prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species” (Article 6a).

Table 1. Legislative controls on the keeping and introducing of non-native fish species in England and Wales.

Legislation	Extent	Offence	Penalty	Further provisions
WCA[1] 1981	England and Wales	To 'release or allow to escape into the wild any animal which is (a) not ordinarily resident in Great Britain and is not a regular visitor to Great Britain in a wild state, or (b) listed in Schedule 9 of the Act'.	Unlimited fine and/ or 2 years imprisonment (commensurate to offence).	Allow 'environmental authorities' to ensure that landowners tackle invasive non-native species or permit others onto their land to carry out their duties.
SAFFA[2] 1975	England and Wales	To 'offence to introduce any fish, or spawn of fish, into an inland water without the written consent of the water authority' (i.e. Environment Agency in England and Natural Resources Wales in Wales).		
ILFA[3] 1980	England and Wales	To import or attempt to import into, or keeps or releases, in any part of England and Wales any live fish, or the live eggs of fish, of a species specified in an order under section 1 without a valid licence.	Summary conviction to a fine not exceeding level 4 on the standard scale (£2500).	
KIF[4] 2015	England	Any persons keeping or introducing any fish belonging to a taxonomic order specified under Part 1 of the Schedule (and is not a species specified in Part II of the Schedule) without possession of a valid permit.	Up to £50,000 on summary conviction.	The 'Agency' (i.e. Environment Agency) can suspend or revoke any permit to keep (non-native fish species) in addition to serving notice to the responsible person, requiring the removal of the fish (i) in a manner specified in the notice, or (ii) at the expense of the holder of the permit.

[1] Wildlife and Countryside Act 1981

[2] Salmon And Freshwater Fisheries Act 1975

[3] Import of Live Fish Act 1980

[4] Keeping and Introduction of Fish Regulations 2015

1.4.2. Eradication

Eradication is the complete removal of every propagule of the target species within a defined spatial area so that the only possible means for its return into that area is via re-introduction (Britton et al., 2008a). Notwithstanding the drawbacks of eradication techniques outlined earlier, eradication programmes are often considered to be a commensurate response to biological invasions, such as when the perceived or measured negative impacts have been quantified in the native ecosystem and eradication is used to then eliminate these impacts (Britton et al., 2011a,b). Additionally, eradication may be considered more environmentally sensitive and ethical than long-term control methods whose cumulative cost can be considerable (Convention on Biological Diversity 2007). For example, the use of biocides, such as rotenone (Ling, 2003; Britton and Brazier, 2006), on a recently introduced fish population could be justified by the long-term ecological and socio-economic benefits that accrue should its application have prevented an invasion from developing (Britton et al., 2011a). Rotenone ($C_{23}H_{22}O_6$) is a natural toxin produced in a number of tropical plants, predominantly of the *Lonchocarpus* and *Derris* genera, and has been used as an insecticide and piscicide for centuries (Ling, 2003). The mechanism in which rotenone affects the target species is by blocking mitochondrial electron transport by inhibiting NADHuquinone reductase, thus cellular uptake of oxygen is blocked and death results from tissue anoxia with cardiac and neurological failure (Singer and Ramsay, 1994: Ling, 2003).

An example of a successful eradication operation is the extirpation of the invasive rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* from streams in Australian Capital Territory and Victoria using rotenone. The removal of these non-native salmonids permitted the successful re-colonisation of the nationally endangered galaxias *Galaxias olidus* and the establishment of a self-sustaining, breeding population within 3 years (Lintermans, 2000). Additionally, rotenone has successfully been used in the control of the non-native monogenean ectoparasite *Gyrodactylus salaris* in Norwegian rivers, where it is used to extirpate the fish communities, leading to concomitant eradication of the parasite due to the elimination of their host populations (Johnsen and Jensen, 1991). The biocide rotenone has been applied to 19 pond populations of *P. parva* in England and Wales since 2005 (Britton and Brazier, 2006; Britton et al., 2008a; Environment Agency unpublished data: Figure 2), with no evidence of re-introduction or re-establishment of population since the operations were carried out.

The application of non-specific piscicides, such as rotenone, to freshwaters can, however, incur adverse effects on non-target biota. Despite the high specificity of rotenone on fish (Ling, 2003), deleterious impacts on macro-invertebrates and zooplankton have been documented (Anderson, 1970; Kjærstad et al., 2015), with invertebrates from lotic environments showing high sensitivity to its effects (Magnum and Madrigal, 1999). The use of rotenone in the control of fish populations in England and Wales is restricted by SAFFA 1975 (Section 1.4.1), but its use may be permitted by approval of a specific Government Minister. The efficacy of rotenone-based eradications is also highly dependent on a number of biotic and abiotic factors, including water depth, chemistry and temperature. Rotenone is also unstable and undergoes rapid degradation when exposed to light or air, with its toxicity on the target species reduced at lower water temperatures (Ling, 2003). Moreover, its application to species' eradications is often subject to controversy, with public acceptance and knowledge of its use constituting a main concern for managers (McClay, 2000).

In addition, the application of rotenone to invaded freshwaters may not always be feasible, for example, in areas of high conservation status, such as 'Sites of Special Scientific Interest' that contain rare or protected species, such as the great crested newt *Triturus cristatus* (Ling, 2003). Loss of recreational opportunities or limitations on potable water supplies can also be incurred during chemical applications (Finlayson et al., 2000; McClay, 2000). Timing of the rotenone application must also be carefully considered, as rotenone does not affect fish eggs (Finlayson et al., 2000).

Furthermore, there are examples where eradications have had unexpected ecological consequences, such as the case of the eradication of largemouth bass *Micropterus salmoides* and bluegill *Lepomis macrochirus* from fish farm ponds in Japan. Their removal increased abundances of native fishes, shrimp and dragonfly species, but crayfish abundance also increased following the release of predation pressure from *M. salmoides*, leading to decreased macrophyte coverage (Maezono et al., 2005). Thus, whilst some fish eradications using chemical applications have had success, they are not always feasible for logistical, financial, or ethical reasons, and so alternative methodologies still require further development and consideration.

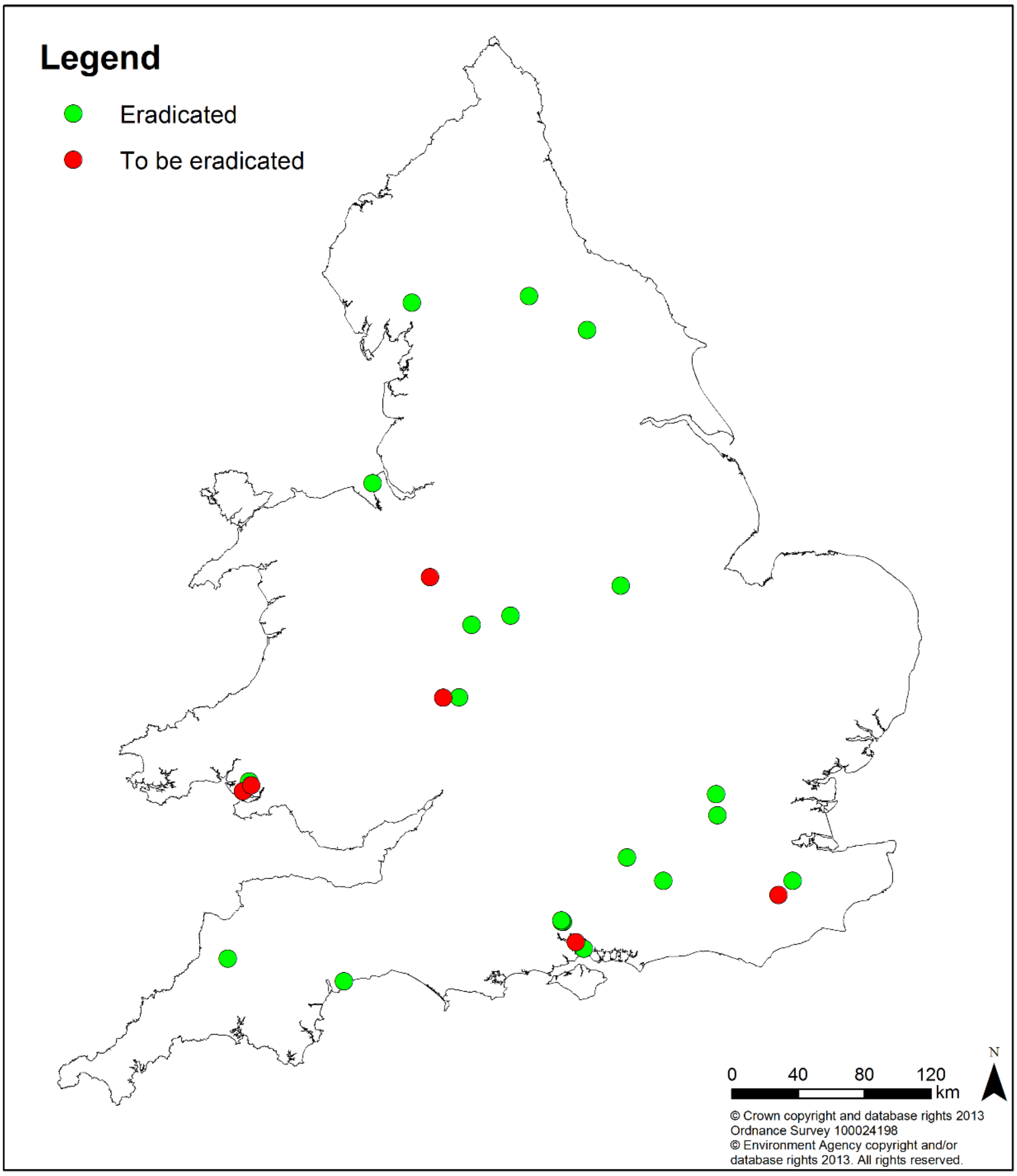

Figure 2. Topmouth gudgeon *Pseudorasbora parva* population locations in England and Wales in 2015.

1.4.3. Control and containment

Where eradication has been deemed un-feasible, control and containment methods may be employed to minimise the impact and limit the spread of the invasive fish (Figure 1), whilst recognising that population extirpation may never be achieved (Manchester and Bullock, 2000; Sorensen and Stacey, 2004). For example, the introduced zander *Sander lucioperca* or the European bitterling *Rhodeus amarus* are likely to be unable to be eradicated from the British Isles because of their wide geographical distribution in a number of major river catchments (Davies et al., 2004). However, prevention of these species moving into new river basins should represent worthwhile actions (Copp et al., 2003). Generally, control and containment management strategies aim to suppress invasive populations or contain their propagules in order to limit spatial distribution and their further dispersal into river catchments where they are not yet present (Britton and Brazier, 2006).

A number of management tools are available for the control and containment of an invasive fish population, with their implementation dependent upon the risks and/ or impacts posed by the target species. As per Gozlan et al. (2010a), invasive fish populations may be managed by (i) selective removal of propagules; (ii) eradication of satellite populations in lentic habitats with a high risk of further dispersal; (iii) prevention of movement of invasive species via anthropogenic means, through the implementation of existing legislation; (iv) prevention of water transfers from ‘infected’ catchments to ‘clean’ catchments; (v) increased bio-security, to limit dispersal opportunities; (vi) development of public awareness campaigns, to limit unintentional transfer of invasive species and (vii) bio-control programmes, that take advantage of negative interactions to suppress an introduced species’ abundance. Whilst each of these management options can be employed individually on a case-by-case basis, their integrated implementation in a holistic management programme could increase the chances of successful control.

1.4.4. Summary of management of non-native fish in the environment

There is much pessimism surrounding the control, and particularly the eradication, of invasive species (Dahlsten, 1986; Whitten and Mahon, 2005; Simberloff, 2009). Despite this, there are a number of examples of successful control and/ or eradication programmes of invasive fish species at small spatial scales, especially when the target species are in enclosed areas, such as ponds (Lintermans, 2000; Knapp and Matthews, 1998; Maezono et al., 2005; Britton and Brazier, 2006). However, there is also a plethora of examples of failed control attempts, with potentially an even greater number going un-reported (Simberloff, 2009). To date, there are no known cases of eradication of an invasive fish species that has been occupying a large spatial area, highlighting the requirement for rapid detection, rapid assessment and rapid response, to limit their distribution and therefore chance of successful eradication. Thus, there remains an on-going need for the development of holistic, risk-based suites of management tools and techniques for the control of potentially invasive fish species. These should aim to intervene at each stage of the invasion pathway and be tailored to the specific target species via the development of understandings of their ecology.

1.5. Research objectives

Given the issues identified in the ecology and management of introduced non-native fishes, and those factors that can constrain the application of certain eradication approaches in open systems and those of conservation importance, then the overall aim of this research is to identify aspects of the ecology of a model non-native fish in a defined spatial area, that can then be applied to inform a series of management approaches. Such approaches will seek to provide alternative management strategies to piscicide-based eradications, whilst still providing effectiveness against the model species developing invasive population. Using *P. parva* as the model species, and England and Wales as the model spatial area, the research objectives are to:

O1. Identify the environmental and biological factors influencing the plasticity of life history trait expression of *P. parva* in England and Wales, with assessment of whether these traits constrain or enhance their ability to develop invasive populations (Chapters 2 and 3);
O2. Quantify how accidental introductions of *P. parva* into inland waters could be prevented using fish introduction audit schemes (Chapter 4);
O3. Assess the risk posed by connected water bodies, especially infested aquaculture sites, of facilitating *P. parva* dispersal into the wider environment (Chapter 5); and

O4. Quantify the effectiveness of biocontrol and fish removal schemes as methods to control and contain the dispersal of small invasive fish species, and their potential utility as an alternative management method to population eradication operations. (Chapter 6).

With the research using an appropriate model species (Section 1.6) in a defined Northwest European and temperate context, it thus assists the development of management efforts at each stage of the invasion pathway, from introduction to impact (Figure. 1), and contributes to an overall management framework that should have wider applicability via informing he management of other, small-bodied, non-native fishes in other regions.

1.6. Topmouth gudgeon (*Pseudorasbora parva*): the model species

The topmouth gudgeon is a highly invasive cyprinid fish species in Europe that has achieved a pan-continental distribution (Gozlan et al., 2010b; Figure 3) following its introduction into Romania in 1960 (Pinder et al., 2005). Native to Japan, China, Korea and the River Amur basin, they are now present in a limited number of isolated lentic waterbodies in England and Wales after its initial discovery in the wild in 1996 (Domaniweski and Wheeler, 1996; Gozlan et al., 2010b). Whilst they are not classed as a lotic species, as they typically inhabit lakes, reservoirs and ponds, they use irrigation ditches, streams and even large rivers as conduits to navigate to more favourable environments (Asahina et al., 1990; Pollux and Korosi, 2006; Onikura and Nakajima, 2013). Individuals rarely exceed 100 mm in body length and have a number of highly plastic life history traits that facilitate their ability to invade new waters, including, early sexual maturity (1 year), batch spawning, nest guarding and wide environmental tolerances (Movchan and Smirnov, 1981; Ricciardi and Rasmussen, 1998; Section 1.3). Such traits facilitate *P. parva* to readily establish populations within their introduced range, where in some situations, they can develop high population densities, such as over 60 m^{-2}, where they are numerically dominant in the fish community (Britton et al., 2008a,b; Britton et al., 2010a). The ability of *P. parva* to rapidly colonise new water bodies, allied with their low commercial and angling value, has led them to be classed as an international pest species (Welcomme, 1992).

A development in the last decade is the discovery that *P. parva* is the 'healthy host' of the 'Rosette agent' *Sphaerothecum destruens*, an obligate intracellular eukaryotic pathogen, responsible for major salmonid mortalities in the United States (Arkush et al., 1998; Gozlan et al., 2005). Previous studies have shown that *S. destruens* is not species-specific, with susceptibility shown in salmonids and a number of cyprinid species (Arkush et al., 1998; Andreou et al., 2012). Gozlan et al., (2005) showed that sunbleak *Leucaspius delineatus* failed to reproduce and underwent a significant population decline when in cohabitation with *P. parva* with 67% of moribund *L. delineatus* found to be positive for *S. destruens* (Gozlan et al., 2005; Andreou et al., 2011). Other associated disease issues include the isolation of pike fry rhabdovirus (Ahne and Thomsen, 2006) and individual adults have been known to undertake facultative parasitism of larger fish species, causing open wounds which are then subject to penetration by infectious disease agents (Boltachev et al., 2006).

Ecologically, Britton et al., (2010a) demonstrated dietary overlap of *P. parva* with native fish communities, leading to stunted growth and poor juvenile recruitment in native species. More recent work, however, suggests their introductions tend to result in trophic niche divergence with native species, a process that avoids competition for food resources (Jackson et al., 2013b; Jackson and Britton, 2014; Tran et al., 2015).

The primary mechanism of *P. parva* introduction is accidental, generally where they are present as a contaminant species ('hitch-hikers') of recreational and aquaculture-related stocking activities. Indeed, this is how *P. parva* were introduced to Europe, including England, such as via contaminated batches of Asian carps (Simon et al., 2011). From a legislative perspective in England and Wales, they are specified in Part I of the Schedule under KIF 2015, in addition to Article 2 of The Prohibition of Keeping or Release of Live Fish (Specified Species) Order 1998 (ILFA). Under this Order, fish species are classified into one of 5 risk categories (1 to 5, from low to high risk), with *P. parva* listed as Category 5 ILFA species, where there is a general presumption against the release or keeping of this species, even in aquaria, due to their high risk rating.

Despite such controls, *P. parva* have been recorded in at least 26 lentic waterbodies in England and Wales since their initial detection (Figure 2), although unlike other European countries, they have yet to develop invasive populations and are yet widely present in the river network. The Environment Agency (EA), a non-departmental public body, tasked with the protection and enhancement of the environment in England (and Wales, up until 2013), initiated a control programme in 2005, using the piscicide rotenone (Ling, 2003; Britton and Brazier, 2006). To date, 20 water bodies, known to contain *P. parva* have been eradicated, predominantly using a piscicide-based approach (Environment Agency, unpublished data). However, all of the eradicated *P. parva* populations were pond based, albeit with some being hydrologically connected to adjacent rivers.

Aside from the prevention of introduction of *P. parva* by legislative controls, no other management or control techniques other than eradication have been explored in England and Wales. The development of management tools and techniques within this thesis will aim to thus provide managers with a suite of methods for preventing their introduction into new ponds and their natural dispersal into the river network of England and Wales, and identify the efficacy of control and containment methods. They will thus target each stage of the invasion process (Figure 1) and highlight the utility of management methods on waters where eradication might not be a feasible approach.

Figure 3. Topmouth gudgeon *Pseudorasbora parva* specimens (♀ top; ♂ bottom).

1.7. Thesis structure

The subsequent data chapters (Chapters 2 to 6) are each developed from objectives 1 to 4 (Section 1.5). They take the form of discrete pieces of work and so are presented in that format without an initial general chapter providing information on generic materials and methods. The final chapter (Chapter 7) will discuss the findings from Chapters 2 to 6, with recommendations for the management of invasive fish species and future research opportunities. A list of references and appendices will conclude the thesis.

1.8. Ethical considerations

The necessary ethical aspects and associated regulated scientific procedures carried out on the project were considered in an independent ethical review committee under Bournemouth University's Home Office (HO) Certificate of Designation. All procedures (anaesthesia, PIT tagging, biopsies of fin samples) were completed under appropriate project and personal licences. Permission to use *Pseudorasbora parva* in the manner described was granted by the Environment Agency (EA).

Chapter 2

Influences of population density, temperature and latitude on the growth of invasive topmouth gudgeon *Pseudorasbora parva*

A version of this chapter has been published as:
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2.1. Abstract

Plasticity in life history traits provides advantages for introduced fish in overcoming demographic bottlenecks that would otherwise inhibit establishment. Here, it was investigated how population density, temperature and latitude influenced the growth increments and growth rates of invasive populations of *P. parva*. Aquaria experiments tested the roles of fish number and water temperature on growth increments, pond experiments tested the role of density on growth increments, and a field study completed in England and Wales tested the influence of population density and latitude on growth rates. In the aquaria experiments, whilst growth increments were higher at 21 and 23 ^oC than at 19 and 25 ^oC, fish number had a greater influence on growth than temperature. Higher growth increments were produced at lower densities. In experimental ponds, growth increments were significantly higher in ponds with low densities of *P. parva* compared with those at elevated densities. In the field study comprising 10 wild populations across a latitudinal gradient of 4.0 ^oN and a difference in mean air temperatures of approximately 3 ^oC, and whose estimated population densities were 0.5 to 65.0 m^{-2}, population density was the only significant predictor of growth rates. Whilst populations at very low densities comprised of significantly faster growing individuals, there were no significant differences when densities were between 15 and 65 m^{-2}. Thus, invasive *P. parva* populations have considerable growth plasticity, especially at low densities, with this likely to be important in their ability to colonise new environments.

2.2. Introduction

Introduced non-native fishes raise considerable concern due to the detrimental ecological impacts arising from, for example, their predation of native fishes, increased inter-specific competition and disruptions to ecosystem functioning (Gozlan et al., 2010a; Cucherousset and Olden, 2011; Section 1.2). Understanding the processes that enable some introduced fishes to subsequently develop invasive populations and cause impacts is thus important for developing risk assessment and risk management processes (Britton et al., 2010a; Britton et al., 2011b). Analyses of life-history traits are especially useful for this as they can be strong predictors of establishment success (e.g. Fausch et al., 2001; Vila-Gispert et al., 2005; Olden et al., 2006; Section 1.3). Spatial trends in life-history traits are also useful for developing mechanistic-based understandings of patterns of fish invasions (Cucherousset et al., 2009). Consequently, studies have elucidated how life history traits are expressed between non-native and native fishes (Vila-Gispert et al., 2005), between introduced and native ranges (Britton et al., 2010b; Gozlan et al., 2010b), and within the introduced range of non-native fishes (Cucherousset et al., 2009).

Testing the drivers of variability in the expression of life-history traits across large spatial scales has primarily focused on identifying relationships across latitudinal gradients due to the significant relationship between latitude and temperature (Benejam et al., 2009; Cucherousset et al., 2009). Latitudinal gradients influencing the expression of somatic growth rates and reproductive traits are apparent in introduced fishes including pumpkinseed *Lepomis gibbosus* (Cucherousset et al., 2009), largemouth bass *Micropterus salmoides* (Britton et al., 2010b) and mosquitofish *Gambusia holbrooki* (Benejam et al., 2009). In these studies, traits such as growth rates and reproductive effort significantly decrease as latitude increases. For *L. gibbosus*, the associated thermal constraints on their trait expression have been correlated with inhibiting their ability to invade areas of increased latitude (Copp et al., 2004; Copp and Fox, 2007; Cucherousset et al., 2009).

The Asian topmouth gudgeon *Pseudorasbora parva* was introduced into Europe in the 1960s and has since proved highly invasive, dispersing to at least 32 countries to achieve a pan-continental invasion (Gozlan et al., 2010b). In comparing their native and invasive populations, Gozlan et al. (2010b) noted that a major determinant of their life history trait expression was latitude (as a surrogate of water temperature). However, whilst populations at lower latitudes generally achieved increased lengths and maturity at larger sizes, this was based on a small number of populations (7) over a very large spatial area. Moreover, at smaller spatial scales, considerable differences in the somatic growth and reproductive traits of populations have been observed that have been postulated, but not tested, as associated with population density rather than latitude (for example, Britton et al., 2008b; Záhorská and Kováč, 2009a). Consequently, the respective influences of population density, temperature and latitude were tested on the growth increments and growth rates (as key life history traits) of *P. parva* populations using England and Wales as the field study area. Through use of two experimental approaches and a field study, the research objectives were to: i) experimentally test the influence of fish number and water temperature on the growth increments of *P. parva* in controlled conditions in tank aquaria; ii) experimentally test the influence of population density on the growth increments of *P. parva* in replicated ponds; and iii) test the influence of latitude and population density on the growth rates of 10 wild *P. parva* populations across a latitudinal gradient of 4 ^{o}N.

2.3. Materials and Methods

2.3.1. Aquaria experimental design

The experiments were completed in 45-l tank aquaria across water temperatures of 19, 21, 23 and 25 ^{o}C. These temperature increases were achieved using water heaters and monitored for their accuracy using temperature loggers. Across these temperatures, the effect of fish abundance when under a fixed feeding regime was tested using three treatments where each was replicated three times. The first treatment used 9 *P. parva* of 40 to 50 mm. Only females were used to avoid reproduction and the fish were collected from the same population located in Southern England. Following their measurement (fork length, nearest mm) and acclimatization to the aquaria conditions, the fish were exposed to a daily ration of powdered fishmeal at mean 2 % body weight for 35 days. The rationale for this ration was that it was above a maintenance ration but also below ad libitum, so providing the fish with a food supply that was limited but not limiting. Also, by keeping the 2 % body weight ration constant across the temperatures, the effect of temperature on growth could also be tested under a limited food supply. At the conclusion of this period, the fish were removed and re-measured. The experiment was then repeated using 6 and 12 female *P. parva* of the same size range and at each temperature but keeping the same amount of food being fed daily as for 9 *P. parva*. For 6 fish, this provided a food ration at approximately 2.5 % body weight and for 12 fish, a food ration of approximately 1.5 % body weight.

The growth increment of *P. parva* in each aquarium was assessed using incremental fork length (IL) determined from $((Lt+1 - Lt) / t) / n$, where L*t* was the total starting fork length of fish in the aquarium, L*t*+1 was the total finishing fork length, *t* was the number of days between L*t* and L*t*+1 and n was the number of fish in the tank. Testing for differences in the mean length increments according to fish number and per density was completed using generalized linear models. The model testing for the effect of fish number used temperature as the covariate and the model testing the effect of temperature used fish number as the covariate. The mean starting length of fish per tank was also used initially as a co-variate in the models to identify if these influenced the growth outputs. Estimated marginal means provided the mean adjusted growth increments according to the dependent variable, with the effects of the covariate on this variable being controlled (‘mean adjusted incremental growth’). Differences between the treatments were compared using linearly independent pairwise comparisons with Bonferroni adjustment for multiple comparisons. The relative effects of fish number and water temperature on incremental length were then tested using multiple regression. For the independent variables, higher values of the standardised beta coefficient (β) indicated their increased explanation of variability in the incremental length data.

2.3.2. Pond experimental design

This experiment tested the effect of population density on the growth increments of *P. parva* in a series of small, adjacent, outdoor ponds that eliminated any effect of latitude on growth. Pond size was approximately 100 m^2 and maximum depth 1.5 m. They were set up at the beginning of June 2010, as this is nominally the beginning of the *P. parva* growth season, with minimal growth increments produced prior to June in UK populations, with the majority of growth produced in July and August as water temperatures are higher (Chapter 3). The ponds were located on a bio-secure aquaculture site near Southampton in Southern England where the species was present and licensed for keeping. *Pseudorasbora parva* of fork lengths 60 to 80 mm taken from a single pond on the site were introduced at densities of 0.5, 1.0, 5.0 and 10.0 m^{-2} at a sex ratio of 1:1 with each density replicated 3 times. These fish were recaptured in October 2010 (end of their growth season) using a baited fish trap. Throughout the sampling period, the water temperature in each mesocosm was recorded every 30 minutes to the nearest 0.1 ^{o}C using a temperature logger to test for any differences between the adjacent ponds.

Following the recapture of the fish in October, a random sub-sample of 40 fish per mesocosm was taken and scales removed. These were analysed on a projecting microscope (×30), aged (years) and the following measurements recorded: scale radius and distance between the last annulus and the scale edge. This enabled the length increment produced in the mesocosm to be determined by using these measurements in back-calculation (Francis, 1990). To test for differences in the mean length increments per density, a general linear model was used where the effect of the mesocosm (categorical, numbered 1 to 3 due to 3 replicates), temperature (daily mean per mesocosm), mean starting length (as recorded at the commencement of the experiment), and length, age and sex of the recaptured fish was controlled. Differences in adjusted means and their significance between densities were indicated by linearly independent pairwise comparisons with Bonferroni adjustments for multiple comparisons.

2.3.3. Field study

The field study sites were all ponds of < 4 ha with depths < 3 m and located in England and Wales. Their latitudes were rounded to 0.5 ^{o}N to enable them to be grouped into latitudinal categories, providing a latitudinal range between 50.5 and 54.5 ^{o}N. Of these waters, two were located at 50.5 ^{o}N, one at 51.5 ^{o}N, one at 53 ^{o}N, two at 54 ^{o}N and four at 54.5 ^{o}N. To identify whether there was a relationship of air temperature with latitude, i.e. was it valid to use latitude as a surrogate of temperature, daily air temperature data for 45 weather stations between 50.5 and 54.5 ^{o}N in England and Wales were collated for between 1990 and 1999 (National Climatic Data Center, 2009). For each weather station, supplementary data were provided on its altitude, longitude and precipitation. These data were grouped by latitude in increments of 1 °N and used in a general linear model to compare the temperature of each group between the months of June and October (to represent the fish growth season). The effects of altitude, longitude, and precipitation on temperature were controlled and provided an output of mean temperature per month (± SE) adjusted for the effects of the covariates. Linearly independent pairwise comparisons with Bonferroni adjustments for multiple comparisons were used for determining the mean temperature differences and their significance between each latitudinal group.

At each field study site, the fish were sampled through a combination of micro-mesh seine nets (25 m length, 2.5m depth) and point sample electric fishing. Population density estimates were determined using the samples collected by the seine net using a two or three catch depletion (number of catches being dependent on the depletion between catch one and two). The estimated number of *P. parva* $\left(\widehat{N}_{fs}\right)$ was estimated by: $\widehat{N}_{fs} = C_1^2 / (C_1 - C_2)$; as well as the probability of capture $\left(p_{fs}\right)$ by: $p_{fs} = (C_1 - C_2) / C_1$ where C_1 and C_2 are the number of fish caught in the first and second samples respectively (Seber and LeCren, 1967). The electric fishing provided additional samples for the analysis of life history traits. Due to existing non-native fish legislation (Hickley and Chare, 2004), all captured *P. parva* had to be removed; consequently, the density estimates were taken on the first sampling occasion so that the fish removal would not impact the density estimate. Samples were collected in March 2005, and March and April 2006.

During each sampling occasion, *P. parva* were collected and counted in the field, and then taken back to the laboratory where they were immediately euthanized using an overdose of MS-222. A random sub-sample of a maximum of 65 fish were then taken with all these fish measured (fork length, nearest mm), weighed (to 0.01 g) and a minimum of 3 scales removed for age and growth analysis. To analyse their growth, the initial step was to age the scales on a projecting microscope (×30) by counting the number of annuli, enabling back-calculation of their length at the last annulus to be determined using the scale proportional method (Francis, 1990). These were then used to determine the mean standardized growth residuals for each population (Benstead et al., 2007; Storm and Angilletta, 2007; Beardsley and Britton, 2012; Britton et al., 2012). The use of only the back-calculated length at the last growth check in the tests avoided statistical complications from using repeated measurements from individual fish in the same test (Beardsley and Britton, 2012). The predicted mean length at each age across all the populations was determined using the log-log quadratic function of Vilizzi and Walker, (1999) who identified this as the most precise and biologically meaningful growth model of five quadratic functions and the von Bertalanffy growth model. These values then enabled the standardized residual of the length at age of each fish to calculated (Beardsley and Britton, 2012). The mean growth residuals were then adjusted for the effect of sex in a general linear model (as *P. parva* are sexually dimorphic) before testing against latitude and population density using multiple regression, with the higher (irrespective of whether the value is above or below zero) standardised beta coefficients (β) of the independent variables indicating increased explanation of the growth variability.

2.4. Results

2.4.1. Aquaria experiment

In the initial generalized linear model, the effect of fish starting length was not significant ($P = 0.86$) and so it was removed from the final model. In the final model, a significant effect of fish number on incremental length was detected (Generalized linear model: Wald chi square = 268.14, $P < 0.01$), with significant increases in incremental length as fish number decreased ($P < 0.01$ in all cases; Figure 4). A significant effect of water temperature on incremental length was also found (Generalized linear model: Wald chi square = 16.06, $P = 0.01$), with the highest incremental lengths produced at 23 ^{o}C (Figure 4). Significantly higher increments were produced at 19 and 25 ^{o}C ($P < 0.01$) but not with 21 ^{o}C ($P > 0.05$) (Figure 4). Multiple regression revealed that fish number explained most of the variability in the incremental length data ($\beta = -0.89$, $P < 0.01$) with the effect of temperature found as not significant ($\beta = 0.04$, $P > 0.05$).

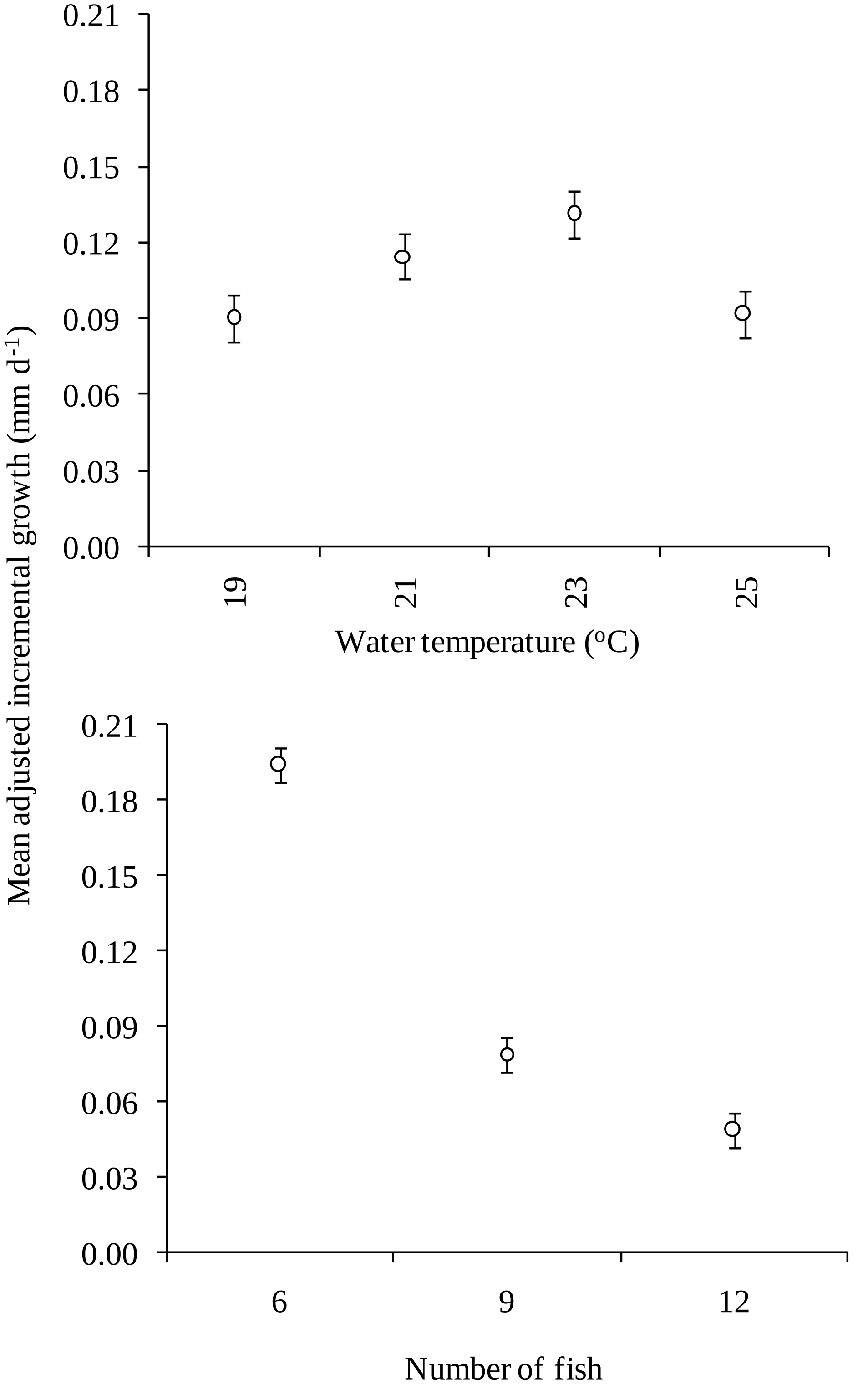

Figure 4. Relationship of mean incremental growth with water temperature (top) and fish number (bottom) where values are the estimated marginal means from the generalized linear model in which the effect of fish number and water temperature respectively have been controlled.

2.4.2. *Pond experiment*

The mean daily temperatures (± SD) in the ponds were 16.91 ± 2.55, 17.12 ± 2.92, 17.37 ± 3.19 and 17.14 ± 2.87 °C at densities 0.5, 1.0, 5.0 and 10.0 m^{-2} respectively. Between the densities, a significant difference was not detected for temperature ($F_{3,8} = 1.14$; $P > 0.05$). A significant influence of density on the mean growth increments was found (Table 2), with the interaction terms of recaptured length and age also significant. Length increments decreased as density increased (Figure 5). The effects of temperature, sex, mean starting length and mesocosm on the growth increments between the densities were found to be not significant. The difference in the adjusted mean length increment was not significant between densities 0.5 and 1.0 m^{-2} but was significant between all the other density combinations (Table 2; Figure 5).

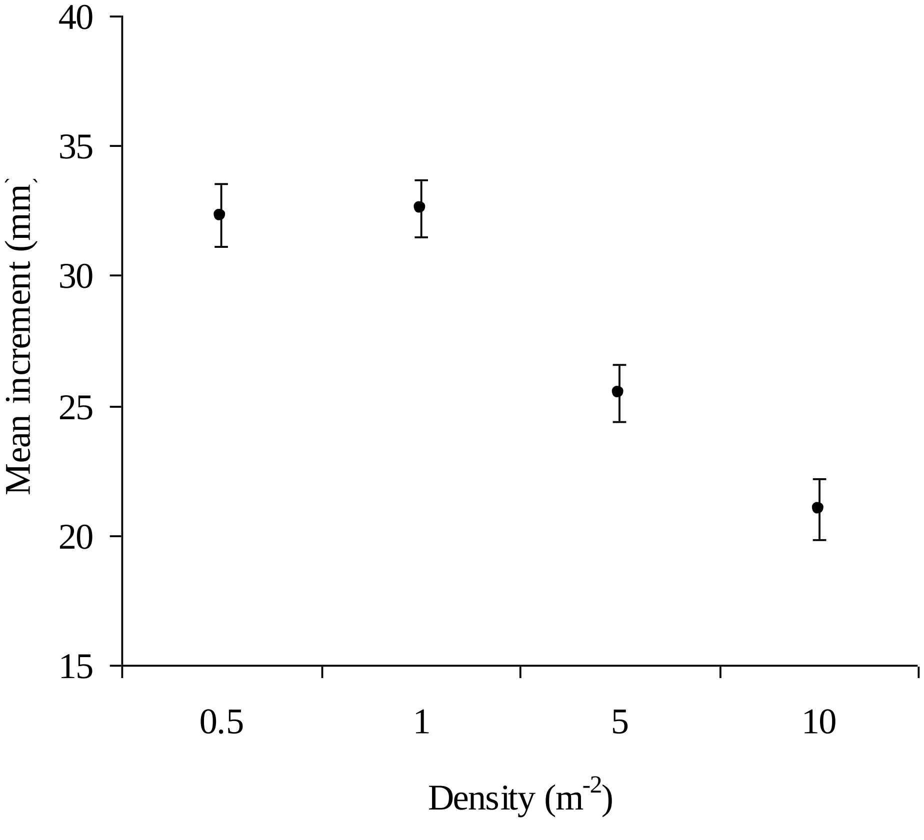

Figure 5. Mean length increment (± SE), determined in ANCOVA and adjusted for the effects of the covariates described in Table 2, between the population densities in the pond growth experiment.

Table 2. Effect of fish density, sex, age, tank position, mean starting length, recapture length, water temperature and pond on the length increments of *Pseudorasbora parva* in the pond experiment, determined in the general linear model. The corresponding differ between the densities, indicated by pairwise comparisons with Bonferroni adjustments for multiple comparisons, are displayed.

Effect	Length Increment (mm)
Density	$F_{3,714} = 18.01$, $P < 0.001$
Sex	$F_{1,716} = 0.03$, $P > 0.05$
Age	$F_{1,716} = 182.12$, $P < 0.001$
Length at recapture	$F_{1,716} = 75.81$, $P < 0.001$
Starting length	$F_{1,716} = 0.87$, $P > 0.05$
Temperature	$F_{1,716} = 1.13$, $P > 0.05$
Mesocosm	$F_{1,716} = 0.57$, $P > 0.05$

Difference between densities (mean ± S.E.)		
0.5	1.0	-0.24 ± 1.15
	5.0	$6.82 \pm 1.12^*$
	10.0	$11.33 \pm 1.18^*$
1.0	5.0	$7.10 \pm 1.63^*$
	10.0	$11.57 \pm 1.69^*$
5.0	10.0	$4.50 \pm 1.51^*$

* $P < 0.01$

2.4.3. Field study

Across the latitudinal range, the differences in the mean adjusted air temperatures were significant, with increased temperatures at lower latitudes (Figure 6). The pairwise comparisons with Bonferroni adjustments for multiple comparisons revealed the most significant differences in temperature were between latitudes of 52 and 54, and 52 and 55 ^{o}N. Between 52 and 55 ^{o}N, the mean difference ranged between 1.8 and 3.4 °C over the months, being significant at $P < 0.01$. Consequently, it was considered valid to use latitude as a surrogate of temperature in subsequent analyses.

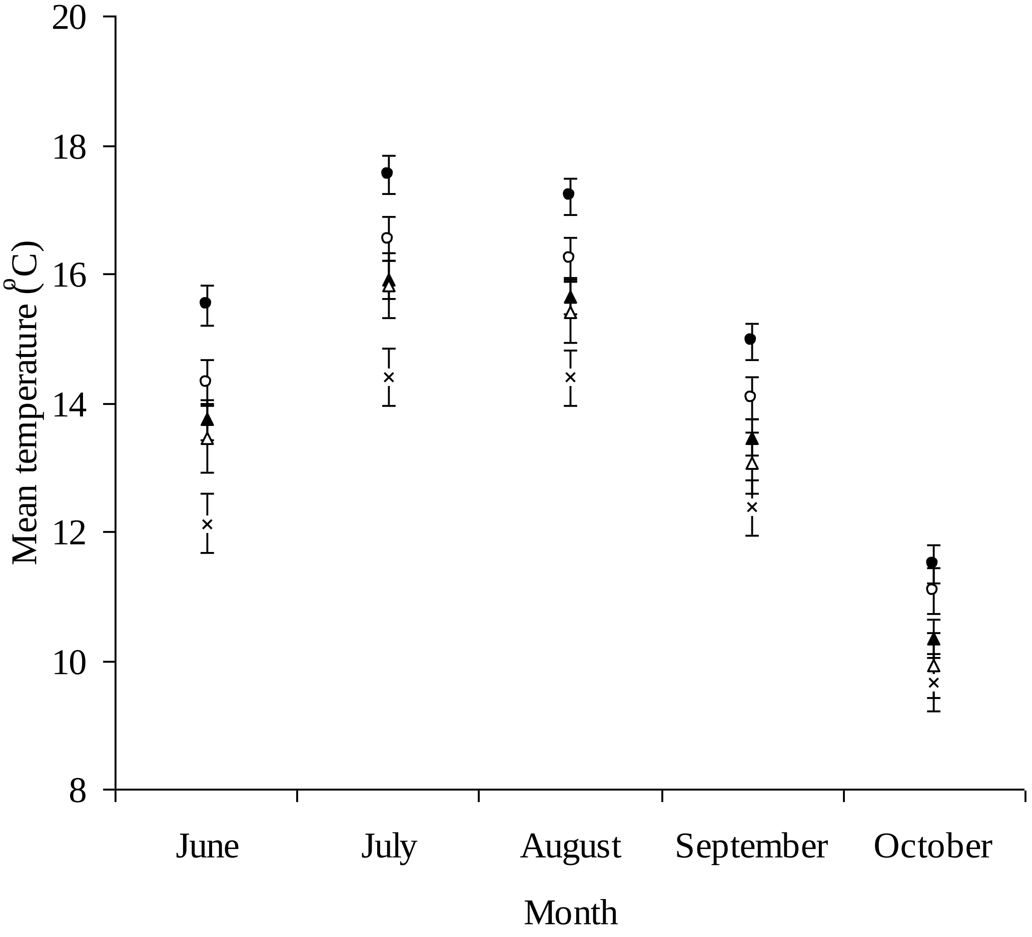

Figure 6. Mean monthly air temperature between June and October by latitude in England and Wales, determined in a General linear model where the covariates were latitude, longitude and precipitation; ○ 51 °N; ● 52 °N; ▲ 53 °N; Δ 54 °N; × 55 °N.

P. parva population density estimates ranged from very low (0.5 m^{-2}) to extremely high (65 m^{-2}), although the range of estimates was skewed to three populations with very low densities (< 1 m^{-2}) and the remaining being above 15 m^{-2} (Figure 7). The non-significant relationship between *P. parva* density and latitude ($R^2 = 0.10$; $F_{1,9} = 0.89$; $P > 0.05$) meant they were subsequently treated as independent variables. Prior to their use in the multiple regression model, the effect of sex on the standardized growth residuals was controlled and revealed that sex had a significant effect on growth with males significantly faster growing than females (General linear model: $F_{1,645} = 64.3$, $P < 0.01$). In the multiple regression model, the effects of fish density and latitude on the adjusted growth increments were significant ($R^2 = 0.67$, $F_{2,7} = 7.23$, $P = 0.02$). It revealed that fish density explained most of the variability in the mean growth residuals ($\beta = -0.81$, $P < 0.01$), with higher growth achieved at low densities (Figure 7). By contrast, a significant effect of latitude was not found, although this was marginal ($\beta = -0.52$, $P = 0.06$; Figure 7). However, when the populations of density below 1 m^{-2} were omitted from the model, the multiple regression model was not significant ($R^2 = 0.04$, $F_{2,4} = 0.44$, $P = 0.69$), with a significant effect of both variables not being detected ($P > 0.05$ in both cases).

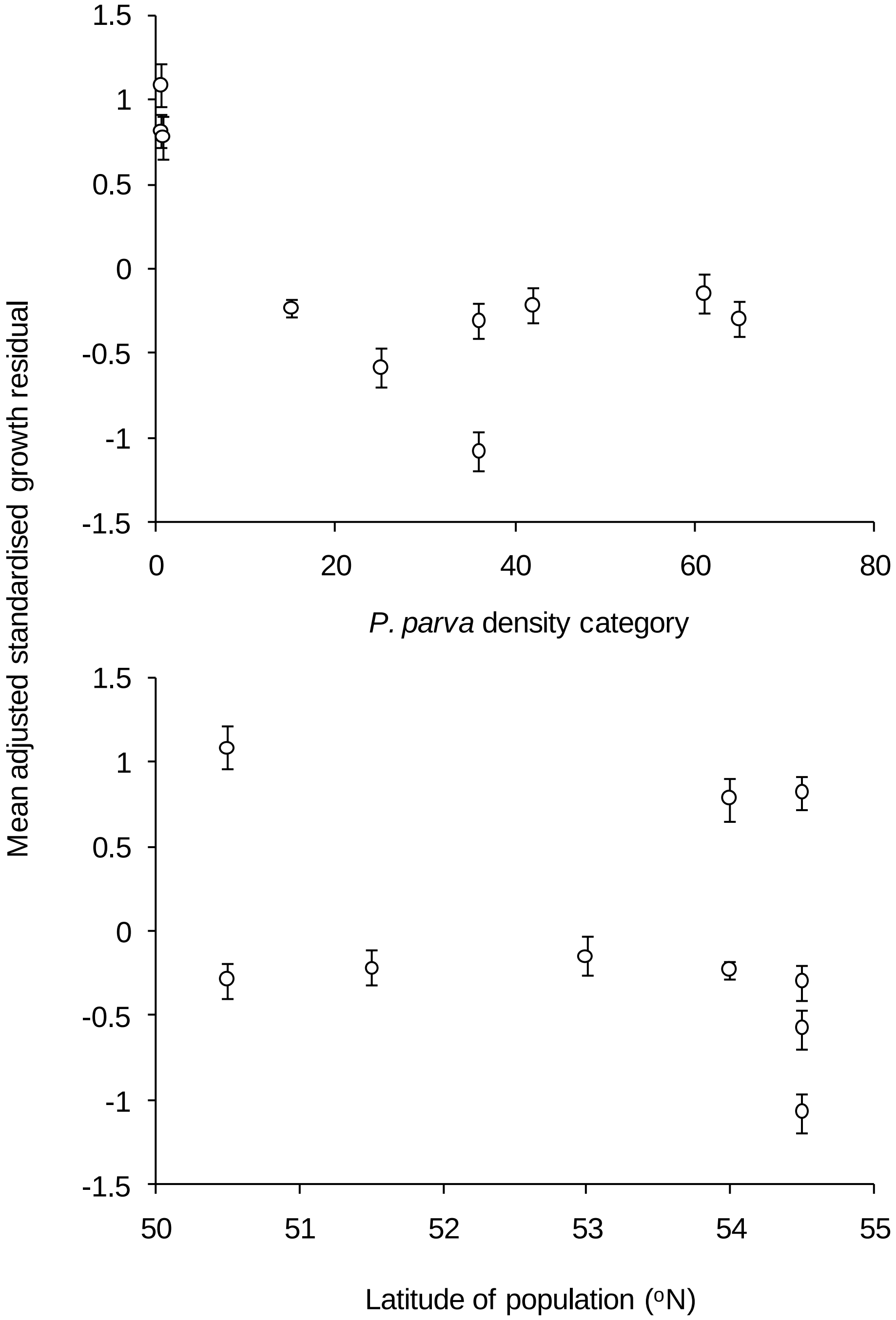

Figure 7. Mean adjusted growth residuals according to population density (*n* m^{-2}; top) and latitude (bottom).

2.5. Discussion

The two experiments and the field study consistently showed a significant effect of population density on the somatic growth of *P. parva*, with this more important in determining growth increments and growth rates than temperature and latitude. This was despite a relatively large temperature range being used, both in aquaria (range 19 to 25 ^{o}C) and in the field study (air temperature range of 3 ^{o}C). This output suggests that growth plasticity is an important trait that facilitates *P. parva* establishment as their fast growth at low density is likely to assist early life reproduction that enables newly introduced individuals to overcome population demographic bottlenecks relating to their low number (Britton and Gozlan, 2013). The aquaria experiment revealed that in higher densities, intra-specific competition will occur in *P. parva* when food resources are limiting and will result in depressed growth. Notwithstanding, some caution should also be applied to the outputs of the field studies, given that (i) the population densities of the field sites were either very low (< 1 m^{-2}) or relatively high (> 15 m^{-2}), preventing more fine-scale analysis of how low densities affect growth increments; and (ii) the effect of latitude on growth in the wild was still strong, albeit only just non-significant (P = 0.06), and so some effects might have been detected had a greater range of population data been available to the study. Thus, it could only be demonstrated that within the range of the data used that the effect of latitude was not significant on *P. parva* growth.

The finding that density was a stronger influence on growth than temperature and latitude is important given that, in general, the life history traits of invasive fishes tend to vary across latitudinal gradients, with faster growth, earlier reproduction and increased reproductive investment occurring at lower latitudes (e.g. Vondracek et al., 1988; Copp et al., 2004; Copp and Fox, 2007; Cucherousset et al., 2009). Indeed, latitude has been used to successfully explain patterns of invasions whereby populations are able to establish in some regions but not in others, such as the case of *L. gibbosus* across North West Europe (Cucherousset et al., 2009). In the case of *G. holbrooki* in France and Spain, significant shifts in their expression of life history traits were observed across a latitudinal gradient of only 5^{o}N where the maximum mean difference in temperature between populations was approximately 2.8 ^{o}C (Benejam et al., 2009). In this study, however, a significant effect of latitude on *P. parva* growth was not detected. Whilst this might relate to the latitudinal gradient covering only 4 ^{o}N, this was sufficient to have a significant influence on air temperature, with mean differences in some months and latitudes of over 3 ^{o}C, when adjusted for the effects of altitude, longitude and precipitation.

In the more controlled conditions of both experiments, this significant effect of density on the growth of *P. parva* remained. In the pond experiments, the effect of latitude was eliminated and there were no significant differences in water temperatures. Outputs revealed that individuals in low density populations (< 1 m^{-2}) produced significantly higher growth increments than at higher densities (> 5 m^{-2}). Similarly, in the tank experiments, fish number (and so density) had a much stronger influence on growth increments than the temperature range. Thus, both experiments provided some corroboration of the observations and tests completed in the field study. Nevertheless, some caution is needed in the interpretation of the field study in that only growth was studied rather than the full suite of life history traits, and there might have been trade-offs between growth and reproductive traits that were not ascertained. This is because in other studies on latitudinal effects on invasive fish life histories, there are often trends within the data that are difficult to explain ecologically due to the likelihood of complex trade-offs between somatic growth, reproduction and multiple environmental factors (e.g. Johnston and Leggett, 2002; Power et al., 2005; Benejam et al., 2009). Thus, there is a danger of over-simplifying life history meta-analyses to studying growth rates in relation to broad scale environmental parameters and so some caution in interpretation is recommended.

That introduced *P. parva* populations are able to develop such abundant population densities in England and Wales that generally comprise fish < 80 mm and numerically dominate fish communities may be associated with their traits that include fast growth in their early life, early maturity, and male nest building and guarding (Rosecchi et al., 2001; Britton et al., 2008b; Záhorská and Kováč, 2009a; Gozlan et al., 2010b). Sexual dimorphism was demonstrated in the wild populations of this study whereby males achieved greater lengths and grew faster, with female growth likely to be constrained by their tendency for batch spawning throughout the spawning season following maturity (Gozlan et al., 2010b). Given the apparent intra-specific density-dependent impacts on *P. parva* growth then this also raises concern over their potential to invoke the detrimental impacts of inter-specific competition in native fishes (Rahel and Olden, 2008). These effects tend to include reduced growth and fitness in coexisting fishes through asymmetric competition as the invasive fish may exploit common resources more effectively, be a superior competitor or be more numerous (Ruetz et al., 2003). In the case of *P. parva*, their tendency for developing high population density abundances suggest that irrespective of their competitiveness, their numerical dominance in invaded waters may play a key role in determining their ecological consequences for other species (Britton et al., 2012).

In summary, fish number and density exerted a strong density-dependent effect on the growth of *P. parva* in controlled conditions and on their growth in the wild, especially when population densities in the wild were very low. This suggests that following their introduction, when populations are likely to be at low density, somatic growth is likely to be relatively high, an ecological trait facilitating their population establishment and ability to adapt to new environments. This also has important management implications, as it suggests that where population control methods are being utilised then should the *P. parva* population be reduced to low abundance, the apparent plasticity in their growth rates, as detected here, could allow population ‘compensatory responses’. This is where the surviving fish respond to their new conditions by showing accelerated growth rates that enables, for example, their more rapid sexual development and thus reproduction earlier in life as they strive to re-establish their populations (Chapter 6).

Chapter 3

Consistency in length of the reproductive season and life-history trait expression in invasive *Pseudorasbora parva* population in Southern England

3.1. Abstract

The expression and plasticity of life-history of introduced fishes are important to understand as they can be strong predictors of establishment and invasion success. Here, the life-history traits of four *P. parva* populations in adjacent ponds in Southern England were measured throughout 2013 to determine the timing and length of their reproductive season and the extent of their trait plasticity. Across the four ponds, there was low variability in their catch per unit effort (a measure of relative abundance), and in traits relating to reproductive effort and somatic growth. All the populations were dominated by males, and both sexes matured at small body sizes (30 to 47 mm) and spawned from age 1+ years. Female fish invested heavily in reproduction, at least early in the spawning season. In all populations, the spawning season was relatively short, with peak female gonado-somatic index, oocyte diameter and fecundity occurring over a three week period in May and June in response to increasing water temperatures (range 13.2 to 14.6 °C in the reproductive period) and after approximately 210 degree-days > 12 ^{o}C. These reproductive metrics all significantly declined thereafter, with no evidence of any further reproductive activity in the year. These outputs reveal that these *P. parva* had a discrete, single annual spawning period, a contrast to other studies that suggest prolonged and fractional reproductive strategies. They also suggest whilst the expression of life-history traits of invasive fishes often vary with density, they were consistent between these populations at similar abundances.

3.2. Introduction

Non-native fishes have capacity to cause substantial ecological consequences in receiving ecosystems through the detrimental impacts of increased predation and inter-specific competition that can lead to food web alterations and shifts in ecosystem functioning (Gozlan et al., 2010a; Cucherousset and Olden, 2011). The risk-based management of non-native fishes is reliant on understanding the ability of that species to establish, impact and disperse in the spatial area in question, i.e. its potential to be invasive (Britton et al., 2010c; Britton et al., 2012; Chapter 2). Fundamental to this are analyses of the life-history traits of the species, as these tend to be strong predictors of establishment success (Vila-Gispert et al., 2005; Olden et al., 2006), especially when they are highly plastic (Davies and Britton 2015a; Chapter 2). Consequently, deriving spatial and temporal patterns in the life-history traits of introduced fishes assist understandings of why some species establish and become invasive, whilst others are unsuccessful (Cucherousset et al., 2009; Gozlan et al., 2010a). In this respect, studies have compared life-history traits of introduced and non-native fishes at a regional level, such as across Iberia (Garcia-Berthou, 2007), of species between their introduced and native ranges, such as in *Micropterus salmoides*, (Britton et al., 2010b) and *Cyprinus carpio* (Oyugi et al., 2010), and of non-native fishes across their introduced range, such as for *Gambusia holbrooki* (Benejam et al., 2009) (Chapter 2).

Whilst providing information suitable for understanding the ecology of invasive species at larger spatial scales, the studies cited above are less useful for indicating the variability in traits that might be apparent at different stages of invasion or different levels of population density, especially for populations in the same region (Britton and Gozlan, 2013; Davies and Britton, 2015a). Some life-history traits, such as fast growth and early maturation with a high reproductive effort, can increase the probability of an introduced fish establishing invasive populations (Fox et al., 2007). However, the expression of these traits in this manner might only be apparent in the initial stages of establishment, with slower growth, delayed maturity and reduced reproductive effort then apparent as the population establishes at higher population abundances (Chapter 2). This progression from rapid to slower development might involve trade-offs between individual investments in reproduction versus high population densities that could result in elevated levels of competition for food resources (MacArthur and Wilson, 1967: Bøhn et al., 2004; Fox et al., 2007).

The topmouth gudgeon has already been outlined in Chapter 1 as a highly invasive cyprinid fish species from Asia that has achieved pan-European distribution since its introduction in the 1960s (Gozlan et al., 2010b). Ecological consequences include modifications to food web structure (e.g. Britton et al., 2010a) and novel pathogen transmission (e.g. Andreou et al., 2012). Indeed, *P. parva* typically has a suite of life-history characteristics that contribute to its success as an invader, being of small body size with short generation time, capable of high reproductive effort, batch spawning and parental care (Adamek and Siddiqui, 1997; Rosecchi et al., 2001; Davies and Britton, 2015a). Recent studies have suggested high plasticity in life-history traits, such as somatic growth rates, is apparent following introductions, with initial fast growth when populations are at low density, followed by significantly slower growth post-establishment when populations are at higher abundance (Britton and Gozlan, 2013; Davies and Britton, 2015a; Chapter 2). Nevertheless, there remains some uncertainty as to how other life-history traits, such as reproductive investment, are expressed in relation to population densities, and there is also little information on how these change over the course of a spawning season, given the reported protracted and/ or fractional spawning behaviours of *P. parva* (Gozlan et al., 2010b).

Consequently, the aim here was to assess the timing and length of the *P. parva* reproductive season, and life-history traits, of four populations in adjacent ponds in Southern England through regular sampling over a 12 month period. The objectives were to: (i) for each population, analyse their duration of reproductive season, reproductive investment, length and age at maturity, fecundity and somatic growth rates; and (ii) test differences in these metrics between the populations and, where differences are detected, assess their causality. It was predicted that all populations would display an extended reproductive period, indicated by female reproductive effort and fecundity varying between individuals over a prolonged period during spring and summer, with variability in traits between populations according to their time since introduction and their abundance.

3.3. Materials and Methods

3.3.1. Site and pond characteristics

As with Chapter 2, the study site was located in Southern England, close to the city of Southampton, being a disused aquaculture site comprising of a number of adjacent small ponds (< 400 m^2) in which *P. parva* were present and licensed for temporary holding. Four rectangular ponds were selected for this study, all approximately 400 m^2 with depths up to 2 m. In Ponds 1 and 2, activities had resulted in *P. parva* only being present for 3 years prior to sample collection, whereas in Ponds 3 and 4, they had been present for at least 10 years. Ponds 1 and 2 were adjacent to each other, as were 3 and 4, separated by an elevated earth bank of 3 m. The distance between Ponds 2 and 3 was approximately 60 m. There were few native fishes present in the ponds and thus *P. parva* were dominant both numerically and by biomass. There were also no hydrological connections between the ponds and so fish were unable to move between them. The ponds also all had extensive beds of the submerged macrophyte *Elodea canadensis* present throughout the year.

3.3.2. Fish sampling

Sampling of populations commenced in January 2013, with samples then collected at least once per month throughout the year, with more regular collection during late spring and summer, i.e. the spawning period (Table 3). Due to the extensive macrophyte beds, sampling was restricted to the deployment 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 (Britton et al., 2011c). These traps provide representative samples of *P. parva* at lengths above 20 mm (Britton et al., 2011c). Each trap was baited with 5 fishmeal pellets of 21 mm diameter (Dynamite Baits, 2015) and were fished in triplicate in each pond and set in the morning (~ 09:00) and lifted one hour later. Following lifting of the traps, all fish were removed, counted, euthanized (anaesthetic overdose; MS-222) and frozen. Counts of the fish enabled calculation of catch per unit effort (CPUE) as a measure of their relative abundance (number of fish per trap per hour; n trap h^{-1}). In addition, the water temperatures of each pond were measured throughout the period of study using Tiny-Tag Aquatic 2 temperature loggers (Gemini Data Loggers Ltd., www.geminidataloggers.com) that recorded temperature every hour. These data were used to calculate the daily mean water temperature per month and the cumulative number of degree-days > 12 °C, with the latter calculated by totalling the number of degrees above 12 °C for each mean daily temperature recording.

3.3.3. *Data collection and initial analyses*

In the laboratory, samples were defrosted, measured (fork length, nearest mm) and weighed (to 0.01 g), before individuals were selected randomly and dissected to identify the sex (immature, male, female), with this concluding for a sample once 30 female fish had been identified. From these processed fish, between 3 and 5 scales were removed and used for subsequent age determination using a projecting microscope (×30 magnification). Ovaries were then removed and weighed for female fish, with egg counts then completed for estimates of fecundity. For an individual fish, total fecundity was determined gravimetrically, where fecundity was the product of gonad weight and oocyte density (as per Hunter et al., 1989). Subsamples were extracted from the ovaries and weighed to the nearest 0.001g.

These data were then analysed for the following metrics. Ages of fish were expressed as either 1+ or 2+ years (no fish were aged at 3+ years and above, and few were young-of-the-year). Reproductive effort (females only) was calculated using the gonado-somatic index (GSI: gonad weight/ (body weight−gonad weight)). Length at maturity was calculated from the percentage of mature fish in each 5 mm length class using a modification of the formula of DeMaster (1978) as the original formula is based on age classes, so the modification used 5 mm length classes instead (Trippel and Harvey, 1987):

$$Z_{Fl} = \frac{A_1 K_1 + A_2(K_2 - K_1) + A_3(K_3 - K_2) + A_n(K_n - K_{n-1})}{K_1 + (K_2 - K_1) + (K_3 - K_2) + (K_n - K_{n-1})}$$

Where; A_n = size class and K_n = percent of mature fish in the *n*th size class. A fish was classed as mature when developed gonads could be identified in the body cavity. The relationship between fecundity (F) and fork length (L_F) was then described by the logarithmic transformation of the power curve $\log(F) = \log(a) + b \log(L_F)$; where a and b are parameters (Bagenal and Braum, 1978).

3.3.4. Statistical analyses

The influence of water temperature was tested on catch per unit per pond using a generalized linear model, as the data were not normally distributed (Kolmogorov-Smirnov test, $P < 0.05$). The dependent variable was CPUE, the independent variable was pond and the covariate was water temperature at the time of sampling. Model outputs were the estimated marginal means of CPUE per pond, their standard error and the significance of differences in mean CPUE according to linearly independent pairwise comparisons with Bonferroni adjustment for multiple comparisons. Sex ratios between male and females were expressed as the ratio of females to males, and tested for the significance of their differences from 1M: 1F using chi-squared tests.

The effects of the date of sampling on reproductive effort (as female GSI) and total fecundity (F) were also tested using generalized linear models, as data were not normally distributed (Kolmogorov-Smirnov tests, $P < 0.05$, in both cases). In the models for each pond, GSI or F was the dependent variable, sample date was the independent variable and fish length was the covariate (due to its potential positive influence on both metrics). For GSI, linear distribution models were used; for fecundity models, poisson log-linear models were used, as the data represented count data of eggs. In addition, the fecundity models only used data up to the initial spawning episode of the fish, as fecundity was dramatically reduced thereafter (*cf.* Results). Model outputs were the estimated marginal means of GSI (i.e. mean GSI, adjusted for the effects of the covariates in the model) and fecundity per sample date and their standard error. The significance of differences in the estimated marginal means per sample date was indicated by linearly independent pairwise comparisons with Bonferroni adjustment for multiple comparisons. For both metrics, a final model was constructed where peak GSI or peak fecundity (i.e. their values in the period immediately before their initial spawning event) was the dependent variable, pond was the independent variable and fish length was the covariate. The estimated marginal means for each pond (i.e. mean adjusted values) and their pairwise comparisons indicated the significance of their differences and so also indicated the influence of fish density (as mean CPUE) on these metrics.

Differences in growth rates between the ponds were then assessed by testing differences in length at age 1+ and age 2+ between the ponds in generalised linear models, where the dependent variable was length at age, pond was the independent variable and sample date was the categorical covariate. Outputs were the estimated marginal means of length at age per pond (i.e. mean length at age, adjusted for the effects of the covariates in the model), adjusted for the date of capture, and their differences according to linearly independent pairwise comparisons, with Bonferroni adjustment for multiple comparisons. All statistics were completed in SPSS v. 21.0 for Windows (IBM Corp. Armonk, NY, USA). Where error is expressed around the mean, it is standard error unless otherwise stated.

3.4. Results

The generalized linear model testing differences in catch per unit effort across the ponds was not significant (Wald χ^2= 0.84, d.f. = 3, P = 0.84), where the effect of temperature as a covariate was also not significant (P = 0.31). Mean CPUE values (n trap $h^{-1)}$ per pond were 1: 116 ± 33; 2: 81 ± 23; 3: 97 ± 25; and 4: 95 ± 22. The generalized linear models (GLM) testing the effect of month on fish length was significant (Month: Wald χ^2= 262.87, d.f. = 10 $P < 0.01$; Pond: Wald χ^2= 1285.02, d.f. = 3, $P < 0.01$), with larger fish being captured in July (Table 3). The *P. parva* in pond 2 (44.40 ± 0.28 mm) were significantly larger than Ponds 1 (33.12 ± 0.16 mm), 3 (37.43 ± 0.23 mm) and 4 (35.49 ± 0.21 mm) ($P < 0.01$ in all cases).

Across all the analysed samples per pond, the mature fish were dominated by males, with sex ratios differing significantly from 1:1 (χ^2, $P < 0.05$ in all cases; Table 4). Lengths at 50% maturity for males in ponds 1, 2, 3 and 4 were 31.2, 47.0, 30.3 and 32.0 mm respectively; for females, they were 32.2, 47.0, 28.2 and 32.0 mm respectively. Scale ageing revealed all mature fish were at age 1+ years or above.

There was a significant effect of month on female gonado-somatic index in each pond (Generalized linear models: Pond 1: Wald χ^2= 866.4, d.f. = 11, $P < 0.01$; Pond 2: Wald χ^2= 456.8, d.f. = 8, $P < 0.01$; Pond 3: Wald χ^2= 641.3, d.f. = 10, $P < 0.01$; Pond 4: Wald χ^2= 638.3, d.f. = 10, $P < 0.01$). The effect of length as a covariate was also significant in Ponds 1, 2 and 4 ($P < 0.05$) but not pond 3 (P = 0.31). Female GSI increased from January, peaked in May and June, and reduced significantly thereafter (Figure 8; Table 5). Values then slowly increased later in the year in preparation for spawning the following year (Figure 8). Across the ponds, there were between 163 and 278 degree days > 12 ^{o}C prior to the post-spawning GSI decline (mean 212 ± 23.9) (Figure 9). The mean monthly temperatures with peak GSI values were between 13.2 and 14.6 ^{o}C, with higher water temperatures recorded later in the summer (Figure 8). Differences in female peak GSI between the ponds were not significant (Generalized linear model: Wald χ^2= 1.73, d.f. = 3, $P > 0.05$), with mean peak GSI being 18.6 ± 0.9, 20.1 ± 1.1, 19.2 ± 1.2 and 20.1 ± 1.1 in Ponds 1 to 4 respectively (Figure 8).

Table 3. Number, fork length range and mean length of *Pseudorasbora parva* captured in monthly samples from Ponds 1 to 4 between February 2013 and January 2014. All lengths are in mm and '-' indicates zero *P. parva* captured.

	Pond 1			Pond 2			Pond 3			Pond 4		
Month	*n*	Mean length	Length range	*n*	Mean length	Length range	*n*	Mean length	Length range	*n*	Mean length	Length range
February	313	34.3 ± 0.4	24 - 84	-	-	-	-	-	-	-	-	-
March	222	32.4 ± 0.5	15 – 69	28	46.5 ± 2.3	26 – 81	57	38.7 ± 0.8	21 – 57	135	35.8 ± 0.3	27 – 49
April	93	32.2 ± 0.8	22 – 58	47	50.0 ± 1.5	28 – 83	141	38.2 ± 0.9	23 – 78	318	35.3 ± 0.2	22 – 50
May	290	32.5 ± 0.4	20 – 63	68	48.2 ± 0.8	27 – 63	281	36.9 ± 0.5	6 – 84	142	35.4 ± 0.3	27 – 47
June	232	31.1 ± 0.4	21 – 52	72	45.2 ± 0.5	30 – 52	119	37.7 ± 0.6	26 – 69	100	36.5 ± 0.4	29 – 47
July	6	41.0 ± 4.2	34 – 61	163	47.8 ± 0.8	28 - 77	135	41.6 ± 0.6	29 - 62	84	36.7 ± 0.5	29 - 62
August	377	31.4 ± 0.4	21 – 66	117	40.2 ± 1.0	22 – 71	115	37.8 ± 0.7	22 – 77	279	36.4 ± 0.2	28 – 50
September	67	34.2 ± 1.3	24 – 64	62	45.8 ± 1.2	27 - 63	136	41 ± 0.7	23 – 79	23	37.2 ± 0.9	29 – 48
October	608	36.8 ± 0.3	22 – 77	30	46 ± 0.1	23 – 63	20	40.3 ± 2.8	24 – 69	27	39.6 ± 1.1	30 – 52
November	122	36.6 ± 0.7	25 – 67	-	-	-	4	40.8 ± 2.3	37 -47	14	44.8 ± 1.0	39 – 52
December	7	47.9 ± 2.7	34 – 63	1	30 ± 0	-	26	36.0 ± 1.7	25 – 58	36	39.8 ± 0.9	31 - 52
January	17	35.9 ± 1.7	25 - 54	-	-	-	31	41.4 ± 1.1	26 - 54	80	39.6 ± 0.5	31 - 53

Table 4. Sex ratio, fecundity, and oocyte diameters of *Pseudorasbora parva* in Ponds 1 to 4.

Pond	*n*	Sex Ratio (M:F)	Female total fecundity	Mean oocyte diameter (mm)	Oocyte diameter range (mm)
1	327	1:0.87	93.69 ± 10.30	0.75 ± 0.31	0.20 – 1.7
2	395	1:0.96	81.89 ± 11.08	0.89 ± 0.31	0.30 – 1.8
3	411	1:0.84	87.51 ± 9.81	1.01 ± 0.36	0.30 – 1.9
4	415	1:0.73	97.01 ± 10.41	0.90 ± 0.29	0.30 – 1.6

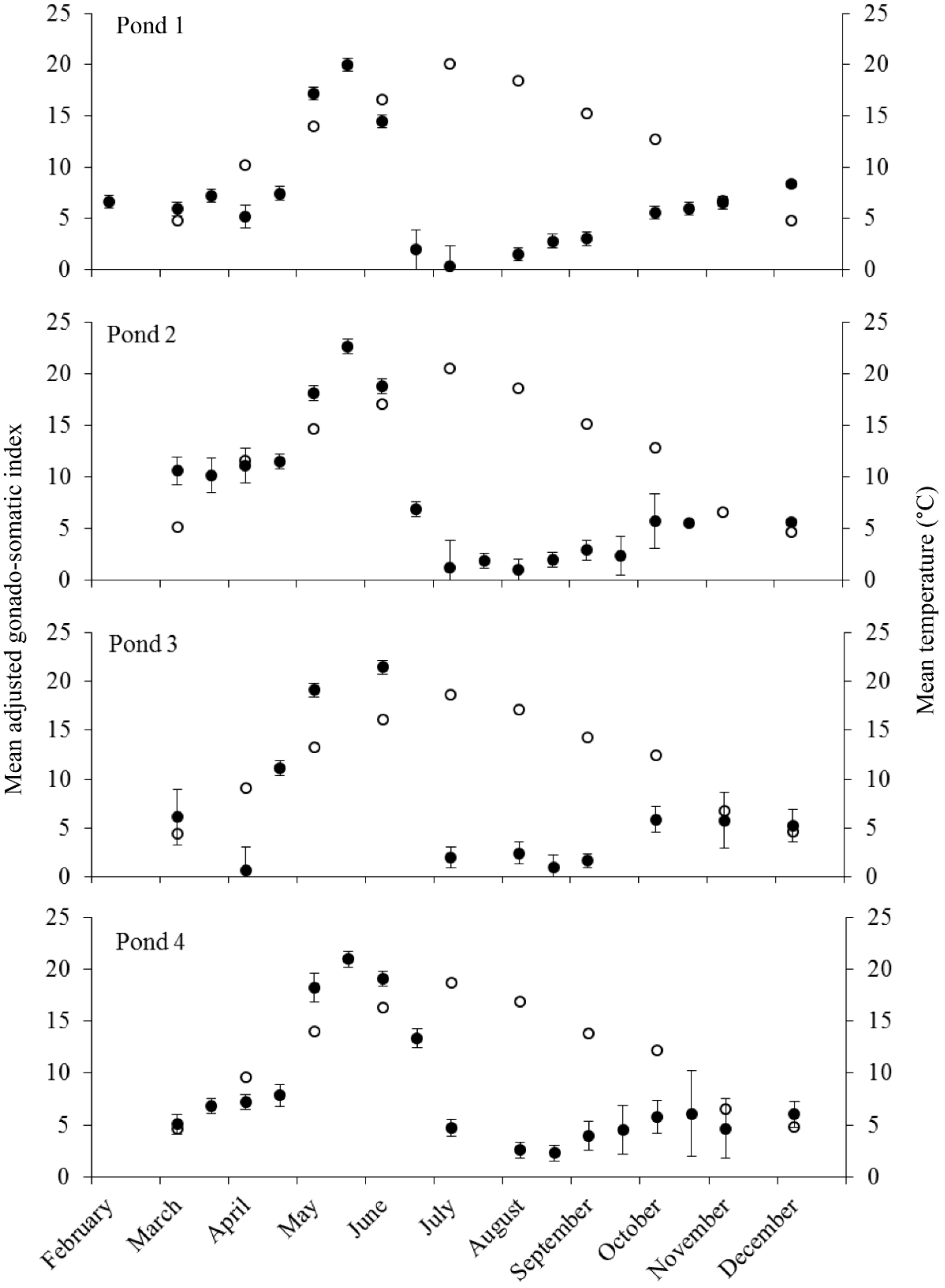

Figure 8. Mean gonado-somatic index (GSI; the means are adjusted via the control of the effects of the covariates on GSI within the model) per month and pond versus mean water temperature per month (°C) from February 2013 to December 2013. Error bars represent standard error; ● GSI; and ○ mean temperature per month.

Total fecundity of female fish also peaked in May and June, and decreased rapidly thereafter. The GLM testing fecundity between ponds was not significant (Wald χ^2= 0.883, d.f. = 3, $P > 0.05$), with differences in total female fecundity between ponds not being significantly different ($P > 0.05$ in all cases). Fecundity estimates per fish ranged between 81.9 ± 11.1 to 97.0 ± 10.4 eggs (Figure 10). The relationship of fish fork length and fecundity was significant in Ponds 2, 3 and 4, but not Pond 1 (Table 6).

Table 5. Pairwise comparisons of mean adjusted (for fish fork length) gonado-somatic index of female *Pseudorasbora parva* between May 2013 and all other months. Mean differences are displayed, with standard error. * difference significant at P < 0.05.

	Month	Pond 1	Pond 2	Pond 3	Pond 4
May	March	12.0 ± 0. 7*	10.0 ± 1.5*	13.1 ± 3.1*	14.2 ± 0.9*
	April	11.8 ± 0.8*	8.9 ± 1.0*	9.0 ± 1.1*	12.9 ± 0.9*
	June	5.2 ± 0.8*	7.6 ± 0.9*	-2.2 ± 1.1	3.7 ± 0.9*
	July	18.2 ± 2.1*	18.7 ± 1.1*	17.2 ± 1.3*	15.6 ± 1.1*
	August	16.5 ± 0.7*	18.9 ± 1.1*	17.4 ± 1.2*	17.8 ± 0.9*
	September	15.6 ± 0.8*	17.7 ± 1.3*	17.5 ± 1.1*	16.2 ± 1.4*
	October	12.8 ± 0.7*	14.9 ± 1.4*	13.3 ± 1.6*	14.5 ± 1.7*
	November	12.1 ± 0.8*	-	13.4 ± 3.1*	15.6 ± 3.1*
	December	10.2 ± 3.6	15.2 ± 4.9	14.0 ± 1.9*	14.3 ± 1.5*

The oocyte diameters revealed a bi-modal distribution in fish from all ponds in the pre-spawning period (May 2013), with diameters recorded between 0.20 and 1.90 mm (Table 4). No mature oocytes were observed in females in sampled subsequent to May. Mean oocyte diameters differed significantly between the ponds (Generalized linear model: Wald χ^2= 148.24, d.f. = 3, $P < 0.01$; Table 4); differences were between ponds 1 and 2, 3 and 4; 2 and 3; 3 and 4 and between pond 4 and 3 (pairwise comparisons, P < 0.05), but not between ponds 2 and 4 (pairwise comparisons, $P > 0.05$). Mean oocyte diameters with individual fish fecundity as a covariate were significant (Wald χ^2= 38.67.24, d.f. = 3, $P < 0.01$), with Bonferroni pairwise comparisons showing Pond 1 had significantly smaller mean oocyte diameters ($P < 0.05$) when compared to those from fish in Ponds 2 - 4 (Table 4).

Mean length at age 1+ was 31.9 ± 0.2; 45.6 ± 0.3; 38.7 ± 0.2 and 36.0 ± 0.2 mm for ponds 1 – 4 respectively, with mean length at age 2+ being 56.7 ± 1.2; 59.5 ± 0.9; 65.5 ± 1.44 and 48.6 ± 3.1 for ponds 1 to 4. Bonferroni pairwise comparisons showed significant differences in mean length at age 1+ between all ponds (Wald χ^2= 1335.25, d.f. = 3, $P < 0.01$.), with *P. parva* having a significantly greater mean length at age 2+ in Pond 3 ($P < 0.01$) than all others (Wald χ^2= 34.74, d.f. = 3, $P < 0.01$). Mean length at age 2+ for fish in Ponds 2 and 4 were not significantly different from those in Pond 1 ($P > 0.05$).

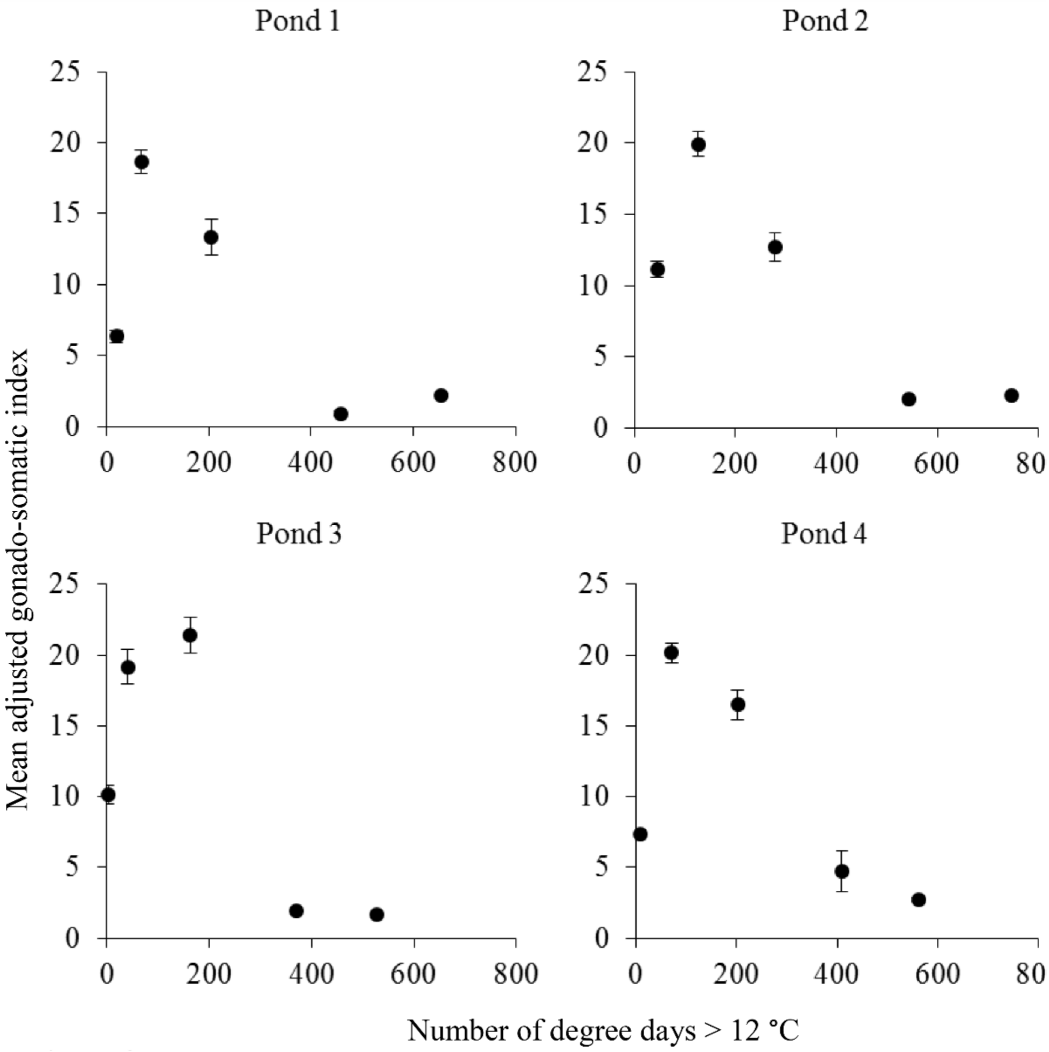

Figure 9. Number of degree days > 12 °C versus mean gonado-somatic index per pond (adjusted for the effect of covariates in the model). Error bars represent standard error.

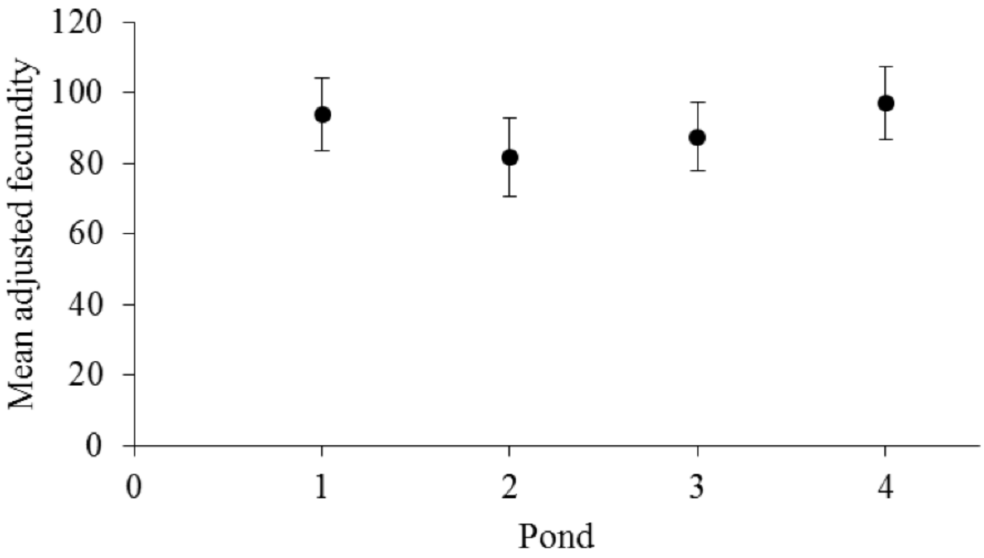

Figure 10. Mean fecundity (adjusted for the effect of fish fork length as a covariate in the model) per pond in May 2013.

Table 6. Fish length: fecundity relationships for female *Pseudorasbora parva* in Ponds 1 to 4, where log(F) = log(*a*) + *b* log(L_F). Values in parentheses are upper and lower 95% confidence levels. Error around mean is standard error.

Pond	*n*	Mean length (mm)	Length range (mm)	Log(a)	*b*	R^2	*F* statistic	*p*
1	15	37.3 ± 1.7	27 - 49	-2.53 (-9.50 to 4.43)	1.81 (-0.12 to 3.75)	0.20	$F_{(1,13)} = 4.10$	0.06
2	15	49.3 ± 1.8	37 - 61	-4.62 (-11.39 to 2.14)	2.40 (0.66 to 4.14)	0.40	$F_{(1,13)} = 8.92$	< 0.01
3	15	45.3 ± 2.2	35 - 60	-7.27 (-12.8 to -1.70)	3.07 (1.62 to 4.55)	0.61	$F_{(1,13)} = 20.66$	< 0.01
4	15	35.5 ± 1.0	30 - 45	-10.70 (-19.99 to -1.32)	4.11 (1.49 to 6.73)	0.50	$F_{(1,13)} = 11.49$	< 0.01

3.5. Discussion

Across the four ponds, there was consistency in the expression of the life-history traits of *P. parva*, with little evidence of significant differences in the reproductive or somatic growth metrics of the populations. The populations were all male dominated, comprising individuals that matured at small body sizes and spawned from age 1+ years, and with females that invested heavily in reproduction, at least early in the spawning season. These results are relatively consistent with other studies on invasive *P. parva* that demonstrate similar *r*-selected trait expression (Gozlan et al., 2010b), and with ages and lengths at maturity that were lower than reported from the native range (Záhorská et al., 2009), a common pattern in invasive fish more generally (Fox et al., 2007; Feiner et al., 2012). Other studies on *P. parva* in both their native and invasive ranges have suggested they are a fractional or batch spawning species, having a prolonged reproductive season with the production of young-of-the-year over a number of months (e.g. Gozlan et al., 2010b). Here, however, across all populations, the reproductive season appeared relatively short, with peak gonado-somatic index occurring in females sampled over a three week period in May and June in response to increasing water temperatures (range 13.2 to 14.6 °C in the spawning period) and after approximately 210 degree-days > 12 ^{o}C, and significantly declining thereafter with no evidence of any further spawning activity. Thus, the reproductive data, allied to oocyte diameter measurements, suggest these populations had a discrete spawning period early in the summer that lasted for approximately three weeks and whilst females might have batch-spawned within this period (this could not be measured here), this single reproductive period was contrary to the prediction.

Studies on the plasticity of *P. parva* somatic growth rates have revealed that population density is an important determinant of growth, often having greater effects than temperature (Britton and Gozlan, 2013; Davies and Britton, 2015a). This has been suggested as facilitating *P. parva* establishment as it assists the development and reproduction of newly released propagules early in life, enabling them to overcome population demographic bottlenecks relating to their low density (Britton and Gozlan, 2013; Davies and Britton, 2015a). In the four populations, however, there was little evidence of high inter-population plasticity in both reproductive and somatic growth metrics, with this consistency in trait expression being a key outcome of the study. During the study design, the ponds were selected on the basis of their age, with Ponds 1 and 2 being relatively new populations (approximately 3 years since introduction), whereas Ponds 3 and 4 were at least 10 years old. The rationale was that the populations in Ponds 1 and 2 would still be establishing, so potentially being at lower abundance when compared to Ponds 3 and 4, and thus were predicted to comprise of faster growing, earlier maturing fish that invested more heavily in reproduction. That this was not apparent relates to their relative abundances; despite the different ages of the populations, catch per unit effort suggested their densities of *P. parva* above 20 mm were not significantly different to each other, suggesting rapid population establishment in Ponds 1 and 2. Thus, their absolute densities were assumed to be relatively similar and so insufficient to result in significant plasticity in their life-history traits, with no evidence of any context-dependent differences.

These outputs are important in the context of the risk management of invasive *P. parva* populations. Highly invasive across Europe, impacts on native communities include the transmission of a novel pathogen (e.g. Andreou et al., 2012) and the detrimental effects of increased inter-specific competition (e.g. Britton et al., 2010a). In the case of the latter, this might only be apparent at very high population abundances that only occur in disturbed lentic environments, such as fishing ponds, where high trophic subsidies from anglers facilitate the production of unnaturally high *P. parva* densities (Jackson et al., 2013a; Britton et al., 2015; Tran et al., 2015). The outputs of this study, in conjunction with those of Chapter 2 and Britton and Gozlan (2013), suggest that following an introduction of *P. parva* into a new environment, it is likely that the expression of their growth and reproductive traits will enable rapid establishment of a sustainable population (< 2 years) that, where the environment is relatively undisturbed, will then have life-history traits that maintain a relatively stable population size, involving maturity at small sizes and single spawning events. Should the population be in a habitat that receives substantial trophic subsidies (such as bait introduced by anglers), their population abundances might then attain very high densities and where these interfere with recreational angling or risk dispersing high numbers of juvenile *P. parva* into the wider environment, then management interventions can either extirpate their population (e.g. Britton and Brazier, 2006; Britton et al., 2011a) or reduce their abundance (e.g. Davies and Britton, 2015b; Chapter 6). If the latter is used, however, then mechanisms in the population, arising from their plasticity in life-history traits, could enable populations to compensate for losses through production of high numbers of young-of-the-year (Davies and Britton, 2015b).

In summary, analysis of the life-history traits of these four *P. parva* populations over a 12 month period revealed consistent patterns in the length of their reproductive season and in the expression of important life-history traits. Their consistency was interpreted as relating to the low variability in relative abundances across the populations, suggesting that at specific densities, aspects of their life-history traits expression are predictable and lack stochasticity. These outputs contribute to knowledge on *P. parva* specifically, and small invasive fishes generally, by suggesting that the continuum of life-history trait expression, whilst variable according to their stage of invasion and thus population density, can act as an important predictor of the stability of invading populations and their likely response to management interventions, with the latter important to the on-going management of *P. parva* in England and Wales (Chapter 6).

Chapter 4

Can accidental introductions of non-native species be prevented by fish stocking audits?

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4.1. Abstract

Accidental introductions of non-native species into aquatic environments often result in invasive populations that cause substantial conservation concerns. They account for 8% of all fish introductions and often occur when fish consignments are intentionally released into the wild (‘stocked’) but are unknowingly contaminated with a ‘hitch-hiking’ species that is also released. Here, the efficacy of a fish stocking audit procedure designed to prevent fish introductions of non-native fish was tested using of a model hitch-hiking fish (*P. parva*) within a batch of model native fish (*Rutilus rutilus*). It was tested in relation to different *P. parva* contamination levels (1, 5, 10, 20 %), auditor expertise (Expert, Intermediate, Novice) and search effort. There was considerable variability in the detection thresholds between contamination levels, auditor experience and search effort; false-negative recordings reduced as all these parameters increased. Probability of *P. parva* detection (POD, 0 to 1) revealed that at the lowest search effort, POD was > 0.80 for Expert auditors only when contamination levels exceeded 10 %. At the highest search effort, POD was > 0.80 at the 1 % contamination level for Experts, but was at 3 and 8% contamination for Intermediates and Novices. Thus, whilst small non-native fishes are at risk of being accidentally introduced due to their difficulty of detection in stocking consignments, an effective audit procedure using experienced auditors and high searching effort reduces this risk. Implementation should help prevent subsequent invasions, protecting native species from their adverse ecological consequences.

4.2. Introduction

Introduced non-native species are a major threat to native biodiversity (Chapin et al., 2000) that raise global concern over biotic homogenisation (McKinney and Lockwood, 1999). Freshwater ecosystems are particularly susceptible to the adverse ecological consequences of introduced species (Marchetti et al., 2004; Clavero and Garcia-Berthou, 2006), with river catchments effectively representing biogeographical islands that provide a closed area for invasions (Leprieur et al., 2009; Saunders et al., 2010). Fish are among the most introduced group of aquatic animals in the world (Gozlan, 2008), with the number of fishes introduced worldwide more than doubling in the last 30 years (Williamson and Fitter, 1996; Gozlan, 2008). The growth in global trade and human mobility has been principally responsible for this (Gozlan et al., 2010a), with aquaculture, the ornamental fish trade and sport fishing all being major introduction pathways (Gozlan et al., 2010a; Section 1.2). If these introductions subsequently result in an invasion then negative ecological, conservation and socio-economic impacts are likely to develop (Pimentel et al., 2000). Thus, the regulation of fish introduction pathways using robust legislation and policies are valuable in limiting introduction opportunities and minimising subsequent detrimental impacts (Copp et al., 2005, 2009).

Non-native species can be introduced accidentally, with this usually occurring when contaminated fish consignments have been moved in the aquaculture and fish stocking trade (Garcia-Bethou et al., 2005; Copp et al., 2007). Thus, these ‘hitchhiking’ non-native fish are unknowingly introduced when the fish consignment is intentionally released (‘stocked’). Compared with other introduction pathways, this has received relatively little attention (Copp et al., 2010), although Gozlan et al., (2008) suggested that, overall, accidental introductions accounted for almost 8 % of all global fish introductions, potentially making the pathway a significant influence on the non-native fish diversity of a region. An example of such an introduction is *P. parva*, that has already been described as being accidentally introduced into Europe from China in the 1950s through the transfer of contaminated batches of large Asian carp being used in aquaculture (Section 1.2); *P. parva* has since achieved a pan-European distribution through both further accidental introductions and natural dispersal (Gozlan et al., 2010b). Such introductions are of concern because they are unregulated and largely undetectable, particularly during the initial stages of invasion when the species is at a low level of abundance (Britton et al., 2011a,c), with Hayes et al., (2005) describing the search effort required to detect an introduced species being inversely proportional to its population size. Indeed, the imperfect detection of cryptic species or small populations of newly introduced non-native populations may give rise to 'false-negative' detection data during surveillance surveys ('imperfect detection'; Rout et al., 2009a,b). Knowledge on imperfect detection has enabled probability models to be developed to enhance searching strategies (Harvey et al., 2009; Rout et al., 2009a,b; Christy et al., 2010). These models remain problematic, however, as uncertainty is difficult to eliminate (Britton et al., 2011c). Thus, preventing the initial release of the propagules into the wild is arguably the most effective management tool at preventing invasions. It minimises the need for subsequent surveillance surveys and, where detected, prevents the subsequent costly and often controversial eradication and control measures of the species (Simberloff, 2009; Williams et al., 2010; Britton et al., 2011a).

The deliberate movement of fish into inland waters for the enhancement of fisheries remains a commonplace management activity around the world (Britton and Orsi, 2012) and even where the activity is regulated, stocking events may be frequent. For example, in England and Wales, there are over 4500 legal fish movement events per year, involving over 1.5 million fish, despite relatively stringent fish movement legislation that requires the granting of written permission (consent) by a regulatory authority before a fish stocking event can occur (Hickley and Chare, 2004; Britton et al., 2010d). Each fish stocking event has an inherent risk of also introducing a hitch-hiking species, with this risk dependent on the source water of the stocked fish regarding its invertebrate, plant and non-native fish diversity. Where a fish stocking event has been consented, then it enables an 'audit' to be completed, especially if it is considered 'high risk' according to the source water of the fish, i.e. non-native species are either present or suspected to be present in that source (Cowx, 1994; Hickley and Chare, 2004). This audit involves the fish to be stocked being checked to ensure no hitch-hiking species are also about to be released. Despite the apparent value of such audit procedures in relation to preventing accidental introductions and subsequent invasions, there is a paucity of information on the efficacy of such schemes and how they should be completed in order to be most effective.

Consequently, the aim of this study was to identify the efficacy of a fish stocking audit procedure in preventing the release of a model hitch-hiking, non-native fish within a batch of model native fish about to be stocked into the wild. This was completed in relation to (i) different levels of contamination of the hitch-hiker within the batch of the fish; (ii) the experience of the auditor; and (iii) the search effort of the audit. The fish audit procedure used was that of the regulatory authority for fish stocking events in England (Environment Agency) (Hickley and Chare, 2004). This procedure is completed primarily to ensure compliance with the stocking consent and does not explicitly require the auditor to check for the presence of a hitch-hiking species. It also does not stipulate whether the auditor should be capable of identifying a range of non-native species. This procedure was tested using *P. parva* as the model hitch-hiker, as it has been well established that its accidental introduction in many European countries was via this pathway (Gozlan et al., 2010b; Copp et al., 2010; Chapter 1). The outputs of this Chapter will thus have management implications for preventing accidental introductions of this and similar non-native fish around the world.

4.3. Materials and Methods

4.3.1. Fish stocking audit trials

The audit scheme was tested using a fish stocking scenario typically encountered in England and Wales (Hickley and Chare, 2004). This required 4 fish transport tanks (110 cm x 60 cm x 60 cm; Figure 11) to be filled with water before the release of 100 roach *Rutilus rutilus* (fork length 70 to 120 mm) into each (the fish ‘consignment’). The roach is native to England and Wales, and is regularly stocked into inland fishes (Beardsley and Britton, 2012). The *P. parva* contaminants (fork length 50 to 80 mm) were introduced randomly into each tank at 1, 5, 10 and 20 % contamination levels. The fish were allowed to acclimatise to the transport tanks for 24 hours, with each tank having oxygen being pumped through the water to maintain high dissolved oxygen levels. Regular measurements of water temperature revealed it never exceeded 12 ^{o}C in any consignment.

The auditors comprised of 25 individuals, all employed by the Environment Agency but with varying degrees of fish identification ability and fish stocking audit experience. Each completed a questionnaire prior to completing their audits (Appendix I) that enabled their categorisation into Experienced (Group 1; *n* = 8), Intermediate (2; *n* = 9) and Novice (3; *n* = 8) auditors. Prior to the audit, each auditor was briefed that each consignment of 100 *R. rutilus* was intended to be stocked into the wild and their role was to identify, through the audit procedure, whether the stocking could proceed on the basis of the species actually present in the consignment. For each audit (i.e. the inspection of a single consignment by an auditor), the searching effort was limited to a maximum of 5 minutes using a visual inspection. Within this time, a maximum of 5 hand-nets (frame size 270 × 202.5 mm) of fish were allowed for removal to enable further visual inspection. The rationale for this was it provided a timeframe that is used realistically in actual audits and prevented excessive handling of the fish. Each audit was considered as complete when either the hitch-hiking species was detected or detection had not been achieved at the conclusion of the searching effort.

4.3.2. Data analysis

The primary outcome of each audit was recorded as 1 = detection of *P. parva* and 0 = no *P. parva* detected. The supplementary data recorded was the level of *P. parva* contamination, the auditor experience and the search effort required to detect the contamination (as number of nets, 1 to 5).

The initial step in testing the audit data was to determine the *P. parva* detection thresholds of each auditor experience category, expressed as the minimum contamination at which *P. parva* was detected by that category. To then test the efficacy of the audit trials in detecting *P. parva* in the consignments, the probability of detection (POD) was used (Delaney and Leung, 2010; Britton et al., 2011c). This was completed through logistic regression that, for each level of search effort (1 to 5 net removals), tested the relationship between the POD of *P. parva*, measured as the binary yes (detection) or no (non-detection), against the contamination level (n) and expertise of the auditor (S), where a, b and c were the regression coefficients, and where the compliment of POD is the probability of a false-negative recording: POD = $e^{(a+bn+cS)}$ / 1+ $e^{(a+bn+cS)}$ (Equation 1; Delaney and Leung 2010). From this model, the probability that an auditor would detect *P. parva* in a consignment according to their searching effort, experience and the level of contamination was determined and displayed using contour plots.

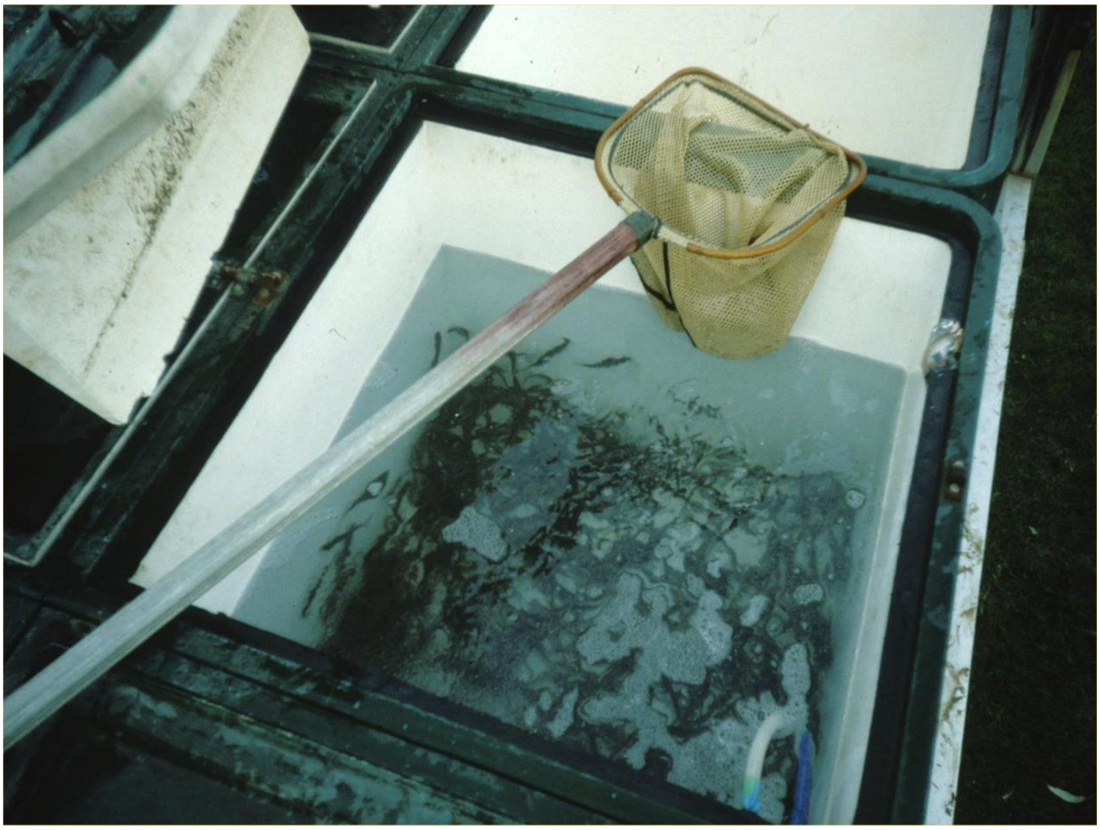

Figure 11. Typical fish transport tank set up.

4.4. Results

Overall, the detection threshold of *P. parva* in the audit trials was 1 %; i.e. even at a contamination level of 1 fish in the consignment, a low number of auditors successfully detected the presence of *P. parva* (Figure 12). Nevertheless, variability was evident in detection thresholds between experience categories, contamination levels and search effort (Figure 12). For example, at a search effort of 4 nets, the Expert category successfully detected *P. parva* in all consignments at all levels of contamination, whilst this level of detection was achieved at a search effort of 5 nets in the Intermediate category (Figure 12). For Novice users, however, detection was below 20 % at 1 % contamination and at search effort 5. Thus, whilst false-negative recordings of *P. parva* were evident throughout the trials, these reduced as search effort and auditor experience increased.

The multiple logistic regressions revealed that both auditor experience and *P. parva* contamination (%) had significant effects on POD across the different search efforts (Table 7). Use of the regression coefficients in Equation 1 enabled POD to be expressed as a function of auditor experience and *P. parva* contamination, and according to search effort (Figure 13). For Expert auditors at search effort 1, POD only exceeded 0.80 when contamination exceeded 10 %; this increased to 16 % for Intermediate users and was never exceeded for Novice users (Figure 13). At search effort 5, POD exceeded 0.80 at 1 % contamination level in Expert auditors, increased to 3 % contamination for Intermediate auditors and was > 8 % contamination in Novice users. At search effort 5, for *P. parva* POD to exceed 0.95, the required contamination level was 2 % for Expert auditors, 8 % for Intermediates and 13 % for Novices (Table 8). At search level 1, POD only exceeded 0.95 at a contamination level of 19 % (Expert auditors; Table 8).

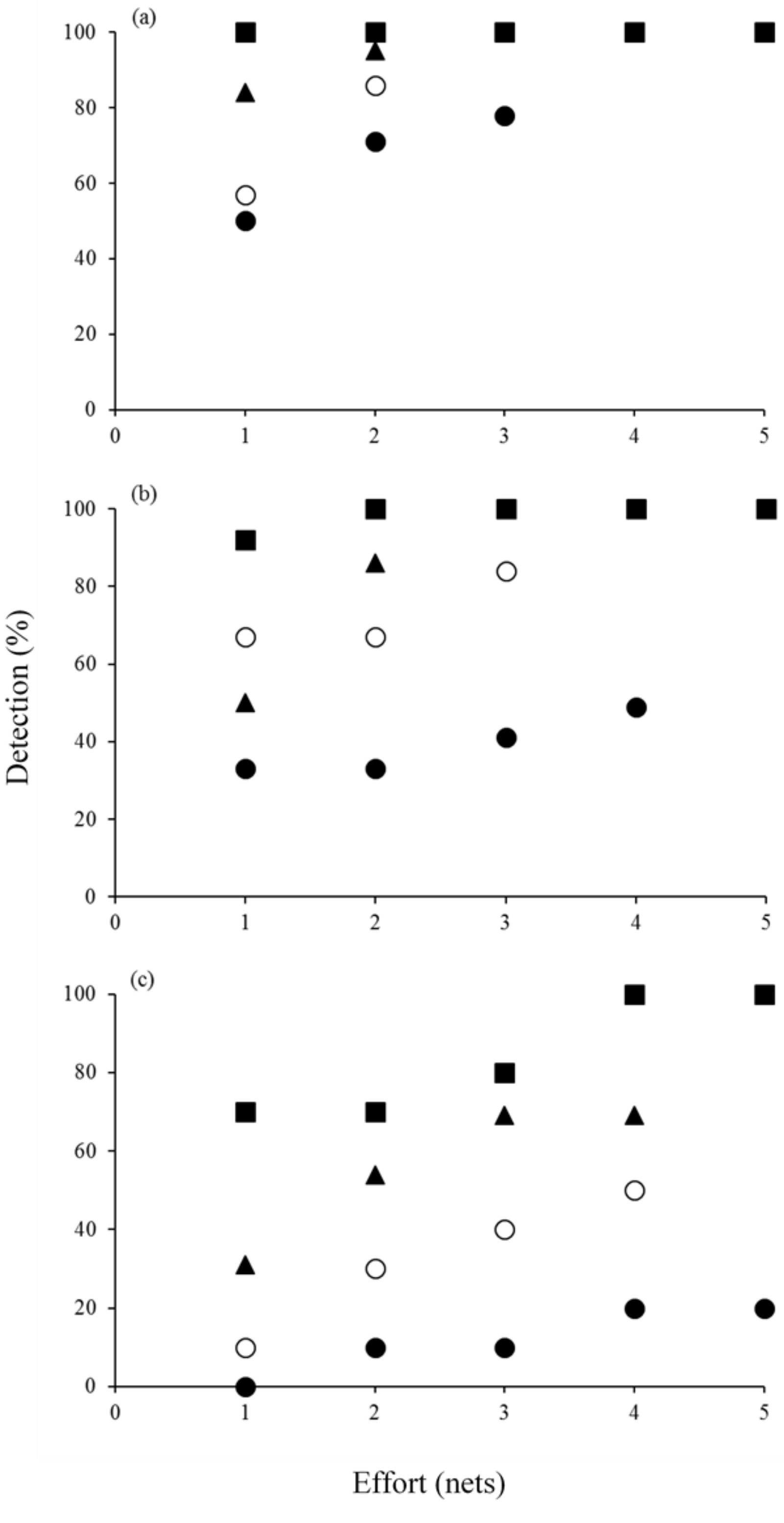

Figure 12. Proportion of audits (%) resulting in detection of *Pseudorasbora parva* according to levels of expertise, where (a) Expert auditors; (b) Intermediate auditors; and (c) Novice auditors, and *P*. *parva* contamination level where ●1%; ○ 5%; ▲10% and ■20%.

Table 7. Multiple logistic regression coefficients for determining the probability of detecting (POD) *Pseudorasbora parva* in audit trials according to search effort (1 to 5 net searches), *P. parva* contamination and auditor experience. * P < 0.01.

Effort	Parameter	Symbol in Equation 1	Coefficient	SE
1	Constant	*a*	0.78^*	0.25
	Contamination	*b*	0.18	0.03
	Experience	*c*	-1.10^*	0.49
2	Constant	*a*	1.77^*	0.04
	Contamination	*b*	0.21^*	0.27
	Experience	*c*	-1.28^*	0.55
3	Constant	*a*	4.34^*	0.05
	Contamination	*b*	0.36^*	0.29
	Experience	*c*	-2.02^*	0.63
4	Constant	*a*	3.84^*	0.08
	Contamination	*b*	0.34^*	0.40
	Experience	*c*	-1.90^*	0.92
5	Constant	*a*	4.34^*	0.09
	Contamination	*b*	0.36^*	0.45
	Experience	*c*	-2.02^*	1.06

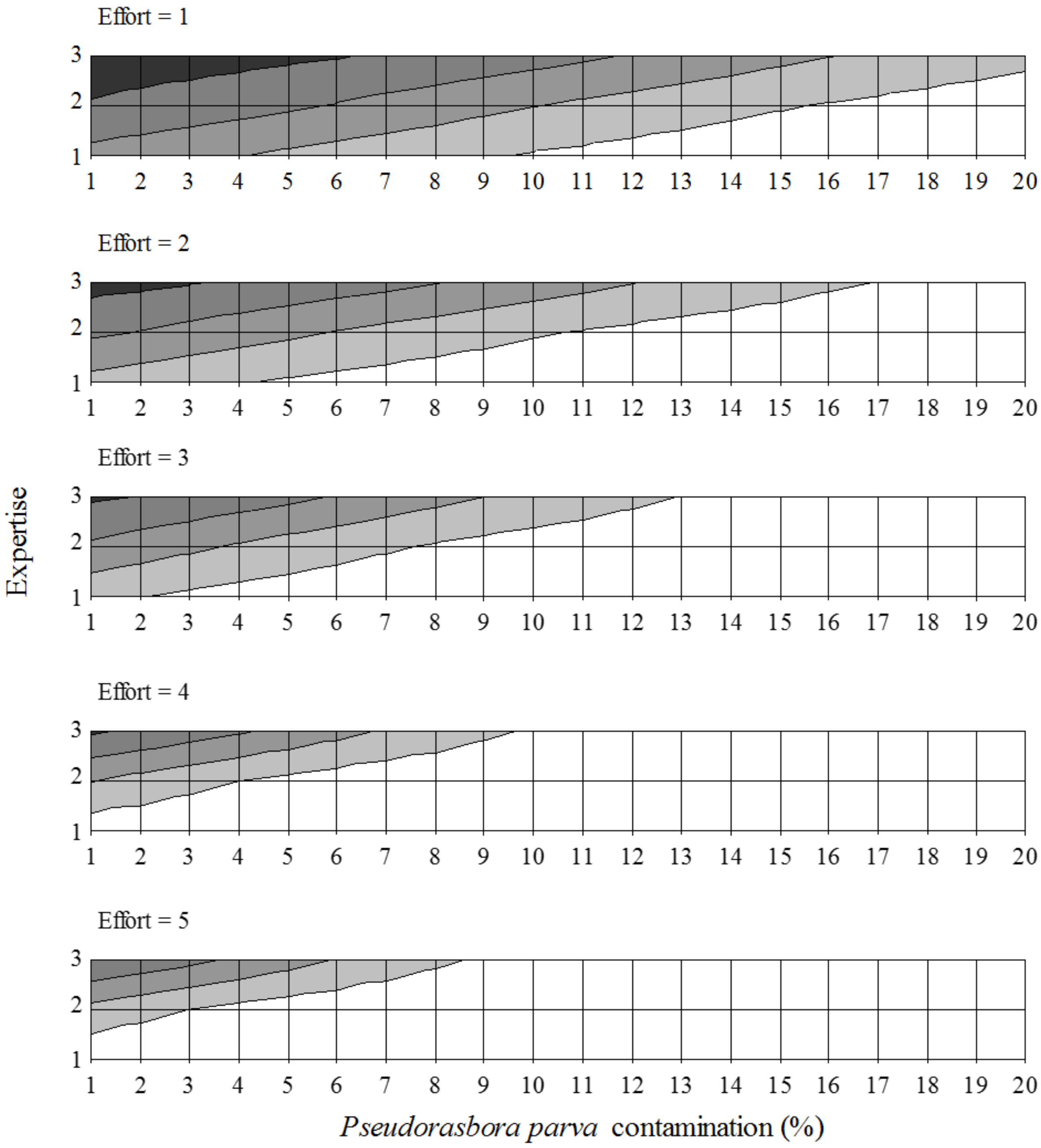

Figure 13. Contour plot of predicted probability of detection (POD) of *Pseudorasbora parva* in audits according to expertise level, where 1 = Expert, 2 = Intermediate and 3 = Novice, and *P. parva* contamination level. Key: Black shading: 0 to 0.2 POD, dark grey: > 0.2 to 0.4; grey > 0.4 to 0.6; light grey > 0.6 to 0.8; and white > 0.8 to 1.0.

Table 8. Levels of contamination (%) required to detect *Pseudorasbora parva* in audits at a probability of detection of ≥ 0.95 according to auditor experience (Expert, Intermediate, Novice) and search effort (1 to 5 net searches).

	Search effort				
	1	2	3	4	5
Expert	19	12	9	3	2
Intermediate	25	19	14	9	8
Novice	31	25	20	15	13

4.5. Discussion

Efforts to prevent introductions and limit incursions by newly introduced species are preferable to managing the subsequent ecological and conservation impacts of their invasions over large spatial areas due to the reduced expense and increased opportunity for success (Jarrad et al., 2010; Britton et al., 2011c). From the perspective of introduced non-native fish, introduction audit schemes can be incorporated into conservation management toolkits that also include surveillance surveys nested within rapid detection and reaction strategies (Britton et al., 2011a,c). Consequently, the audit trials completed here are important in the context of demonstrating that audits can detect hitch-hiking species within consignments of fish that are about to be legally released into the wild. They revealed that although false-negative data were a feature of the audits and may be difficult to eliminate completely, especially at low contamination levels, they reduced as both auditor experience and search effort increased. Thus, assuming that subsequent audit procedures utilise a relatively high search effort and are conducted by experienced auditors then introductions of hitch-hiking species into the wild could be reduced. Given that 8 % of all non-native fish introduced into the wild have been released accidentally (Gozlan, 2008) then a successful audit scheme could thus have a substantial benefit in protecting and conserving native flora and fauna from future fish invasions and associated ecological impacts.

A successful outcome from this study was the quantification of the efficacy of fish introduction audits using probabilities of detection. Thus, when managers design a fish stocking audit that aims to prevent the accidental release of small, non-native fishes, this can be done in relation to a quantified probability of detection. In effect, these POD data represent a form of assessed risk, i.e. they quantify the risk that the auditor will be unable to detect the hitch-hiker and it will be introduced. Correspondingly, it is this assessed risk that requires to be managed within the audit procedure. Thus, a risk management decision is required on deciding which probability of detection level is being managed by the audit. If the manager is relatively risk-averse (Stokes et al., 2006; Finnoff et al., 2007) then an audit process, which has a 95 % probability of detecting a contaminant, may be necessary, although it would require use of experienced auditors and relatively high search efforts. Conversely, if it is considered that a detection probability of 80 % is satisfactory (e.g. Jarrad et al., 2010), then the use of less experienced auditors may be considered (and perhaps be less expensive). The trade-off would be, however, that there is an increased chance of an accidental introduction occurring.

A consideration in the setting of acceptable levels of POD within an audit procedure is allied to the levels of contamination. Given that at the level of individual introduced populations, the primary determinant of establishment success is propagule pressure, i.e. the number of individuals introduced (Lockwood et al., 2005, 2009), then it is arguable whether the release of a low number of undetectable hitch-hiking propagules would actually result in subsequent population establishment and invasion. This is because propagule pressure relates to the concept of the minimum viable population size more prevalent in conservation biology (Terborgh and Winter, 1980; Traill et al., 2007; Lockwood et al., 2009). Thus, it may be argued that in an audit scheme design to minimise invasions, it may be more important to be able to always detect relatively high contaminations of hitch-hiking species than be able to occasionally detect low ones. This is because the release of a higher number of individuals is more likely to ensure a number of individuals survive factors including environmental and demographic stochasticity, overcome Allee effects and/ or have sufficient genetic variation to adapt to local conditions (Lockwood et al., 2005, 2009; Blackburn et al., 2009; Simberloff, 2009).

Allied to the design of an audit process is the species that specifically require to be searched for and, thus, what training requirements may be necessary for new auditors, given that inexperienced auditors are prone to misidentifying species (Fitzpatrick et al., 2009). This latter point is important in the context of being able to identify non-native species in batches of native species that not only belong to same family, as in this study, but also the same genus. In these cases, the species may look very similar. An example in England is the similarity between the native crucian carp *Carassius carassius*, non-native goldfish *Carassius auratus* and their hybrids, as these often require molecular techniques to distinguish properly (Hänfling et al., 2005). The prioritisation of species within training may be developed from existing risk analysis schemes that predict the potential invasiveness of non-native species (Copp et al., 2005, 2009; Britton et al., 2010d). For example, the Fish Invasiveness Scoring Kit (FISK) assesses the potential invasiveness of existing and potential future non-native freshwater fishes (Copp et al., 2005). It is based on a scoring system that assesses species on the basis of their biogeography and history, and biology and ecology (Copp et al., 2005). Higher scores indicate an increased risk of the species being invasive following an introduction (Copp et al., 2005), and calibration has revealed fishes with scores above 19 pose the greatest risk (Copp et al., 2009). Thus, within an audit scheme in England and Wales, all auditors could be trained to identify all the lifestages (larval, juvenile, adult) of the likely hitch-hiking fish that have FISK scores above 19. These currently include *P. parva*, fathead minnow *Pimephales promelas* and sunbleak *L. delineatus* (Britton et al., 2010d). Moreover, consideration could be given to identifying lifestages of other non-native organisms are now present in England and Wales, including macro-invertebrates such as non-native crayfishes of the *Pacifastacus* and *Procambarus* genera (Manchester and Bullock, 2000) and the ‘killer shrimp’ *Dikerogammarus villosus* (MacNeil et al., 2010).

In summary, the revelation that fish stocking audits can prevent the accidental introduction of non-native fishes provides a preventative tool that can be used in the risk management of invasive species and thus can be applied to the preventative approaches that aim to avoid further releases of *P. parva* into the wild in England and Wales. As the outputs suggest increased success with auditor experience and searching effort, its implementation may have resource implications, with this potentially important in an era of financial austerity in England and Wales.

Nevertheless, these are in a trade-off with the conservation benefits that will accrue from preventing an introduction that would otherwise have resulted in an invasion.

Chapter 5

Assessment of non-native fish dispersal from a freshwater aquaculture site

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5.1. Abstract

Freshwater fish aquaculture is highly dependent on the culture of non-native species, which are often transported across biogeographic regions. Routine aquaculture management activities, such as de-watering, can increase opportunities for release of fishes into the environment. The dispersal rates of the invasive topmouth gudgeon *P. parva* were investigated from an aquaculture site to assess escapement under normal operating conditions (low velocity) and increased discharge (high velocity) associated with pond drain-down. No dispersal of *P. parva* was detected during normal (0.07 and 0.08 ± 0.01 $m{\cdot}s^{-1}$) discharge conditions. In contrast, during elevated flow conditions (0.47 ± 0.13 $m{\cdot}s^{-1}$) a total of 241 *P. parva* were detected. *P. parva* length was not correlated to discharge velocity or time since commencement of pond drain-down. Number of *P. parva* dispersed per hour, mean velocity in that hour and time since commencement of drain down were also not significant. However, cumulative number of fish dispersed was significantly correlated to cumulative volume of water released and cumulative time since drain-down commenced. Thus, under flow regimes of normal aquaculture activities, propagule escapement was zero, but increased water flow significantly increased the escapement of this pest fish, highlighting the need for increased biosecurity during key management and operational activities.

5.2. Introduction

Global aquaculture activities tend to be highly dependent on culturing alien species, with their voluntary and/ or accidental introduction into the wider environment an increasing ecological concern (De Silva et al., 2009). The movement of fishes between countries and biogeographic regions for aquaculture can also result in the accidental transfer of 'hitch-hiking species' (Savini et al., 2010; Blakeslee et al., 2010), enabling the long-distance dispersal of non-commercial alien species that would otherwise never be introduced into new regions (Blakeslee et al., 2010). Following their introduction into aquaculture in a new region then, depending on the 'openness' of the aquaculture facility, opportunities for their dispersal into the wild potentially exist, particularly where the biosecurity of rearing facilities is low, providing minimal barriers to natural dispersal (Marchini et al., 2008).

Freshwater aquaculture of cyprinid fishes is routinely completed in pond systems that usually have some connection to adjacent watercourses, at least for short-time periods, such as during pond drain-down for fish harvesting (Horvath et al., 2002). This potentially provides opportunities for the unintentional release of fishes of non-commercial interest that might have been accidentally introduced to the site during fish transfers. Indeed, this has been implicated in the release of alien fishes into the wild from aquaculture sites in Europe, particularly *P. parva* (Gozlan et al., 2010b). Their local dispersal from aquaculture sites has facilitated their colonisation of fresh waters in at least 32 countries and whose initial long-distance dispersal from their native range in China was through being accidental contaminants of batches of Asian carps in aquaculture (Gozlan et al., 2010b; Section 1.6).

The risk management of alien fishes is strongly reliant on preventing the initial introduction (Davies et al., 2013; Chapter 4) and their wider dispersal (Britton et al., 2008a, 2010). Although there is extant knowledge on how to prevent *P. parva* being released accidentally into ponds via application of auditing (Davies et al., 2013), there is little known about their dispersal rates from sites following their release and how this is affected by on-site activities. Consequently, the aim here was to assess the dispersal of *P. parva* from an aquaculture site during normal operation and compare this to their dispersal during periods of pond drain-down.

5.3. Materials and Methods

As with Chapters 2 and 3, the study was completed on a former aquaculture facility in Southern England where *P. parva* was licensed for holding and where previous work had revealed limited natural dispersal (Pinder et al., 2005; Figure 14). As part of an on-going eradication programme for *P. parva* from the UK (Britton et al., 2010c), all of the ponds in this site were being de-watered prior to chemical treatment to extirpate the species. Each pond used was at least 300 m^{-2} in area and of 2 m maximum depth, and discharged into a rectangular overflow channel of 400 m length via an underground connecting pipe (12 cm diameter) that was plugged during normal operation, i.e. there was no water connection (Figure 14). This overflow channel then discharged into a settlement pond of approximately 200 m^{-2} via an unscreened outflow (50 cm width). Water then discharged from the settlement pond into an adjacent stream via an un-screened pipe (30.5 cm diameter).

Assessment of the dispersal rates of *P. parva* was completed in two phases. Phase one was completed in July 2012 and 2013, periods of the *P. parva* reproductive season when larvae and juveniles were most likely to be present and passively drifting from the site (Pinder et al., 2005), and was used to assess drift under normal conditions when no ponds were being drained. A drift net with a rectangular opening of 24 cm x 40 cm that lead to a 250 μm mesh net with a removable end-cap (EFE and GB Nets, Bodmin, UK) was installed in front of the discharge pipe of the settlement pond, so all water leaving the site passed through the net. This was set for one hour starting at 09.00. As this failed to capture any fish, it was re-set and fished continually over a 24 hour period, with removal every 6 hours when the net contents were emptied into a sorting tray. Captured fish were euthanized (anaesthetic overdose; MS-222) and subsequently counted and measured in laboratory conditions (fork length, nearest mm), enabling the number of dispersing fish per hour to be calculated. Concomitantly, the flow of water passing through the net was measured using a Stream Flowmeter (NHBS Ltd. Devon, UK).

The second phase was completed in July 2014 when two aquaculture ponds were being drained down that were both known to contain *P. parva*. A drift net was again placed in front of the discharge pipe, commencing three hours before the two ponds were drained, with checking hourly. The plugs on the two ponds were then released, with the drift net then checked hourly for the next 24 hours, using the same protocol as outlined above. For both phases of the study, the volume of water passing through the drift net was determined from $Q = A\bar{u}$ (Buchanan and Somers, 1969), where Q is the discharge ($m^{-3}\ s^{-1}$), A is the cross section of the drift net occupied by water (m^2) and $\bar{u}$ is the water velocity ($m{\cdot}s^{-1}$).

5.4. Results

During Phase 1, no fish were captured in the drift net in either 24 hour period and when mean flow rate of water passing through the drift net was 0.08 ± 0.01 $m{\cdot}s^{-1}$ in 2012 and 0.07 ± 0.01 $m{\cdot}s^{-1}$ in 2013. This was despite *P. parva* being observed in the aquaculture ponds on site, including larvae. In Phase 2, no fish were captured in the drift net in the initial three hours of fishing when the two ponds were still full and the mean flow was 0.07 ± 0.01 $m{\cdot}s^{-1}$. The water from the two ponds was then released, discharging their contents initially into the overflow pond, then the settlement pond, and then through the drift net prior to being discharged into the stream. Over the next 22 hours, the mean flow of water through the net was 0.47 ± 0.13 $m{\cdot}s^{-1}$, with *P. parva*, plus other fishes, captured in the drift net during each subsequent hour of monitoring. A total of 241 *P. parva* (42.1 ± 0.72 mm), 42 three-spined stickleback *Gasterosteus aculeatus* (L.) (34.8 ± 1.3 mm), 6 bullhead *Cottus gobio* (L.) (48.1 ± 2.2 mm) and 2 tench *Tinca tinca* (L.) (114.5 ± 6.5 mm) were captured. In the hours when more than 5 *P. parva* were captured, whilst there were some significant differences in *P. parva* lengths (ANOVA $F_{14,209} = 2.87$; $P < 0.01$), these were not significantly related to mean flow per hour or time since the drain-down started ($R^2 = 0.04$, $F_{1,13} = 0.55$, $P = 0.47$; $R^2 = 0.05$, $F_{1,13} = 0.53$, $P = 0.49$ respectively). The relationship between the number of dispersed fish per hour, the mean flow rate in that hour and time since drain-down commenced was also not significant ($R^2 = 0.04$, $F_{1,13} = 0.55$, $P = 0.47$; $R^2 = 0.05$, $F_{1,13} = 0.53$, $P = 0.49$, respectively; Figure 15a,b). During the period of pond discharge, 2.38 ML of water were calculated as passing through the drift net, with the cumulative number of fish dispersing from the site since the commencement of the drain-down being significantly related to the cumulative volume of water released ($R^2 = 0.94$, $F_{1,17} = 313.74$, $P < 0.01$; Figure 15c) and cumulative time since the drain down commenced ($R^2 = 0.83$, $F_{1,17} = 51.4$, $P < 0.01$; Figure 15d).

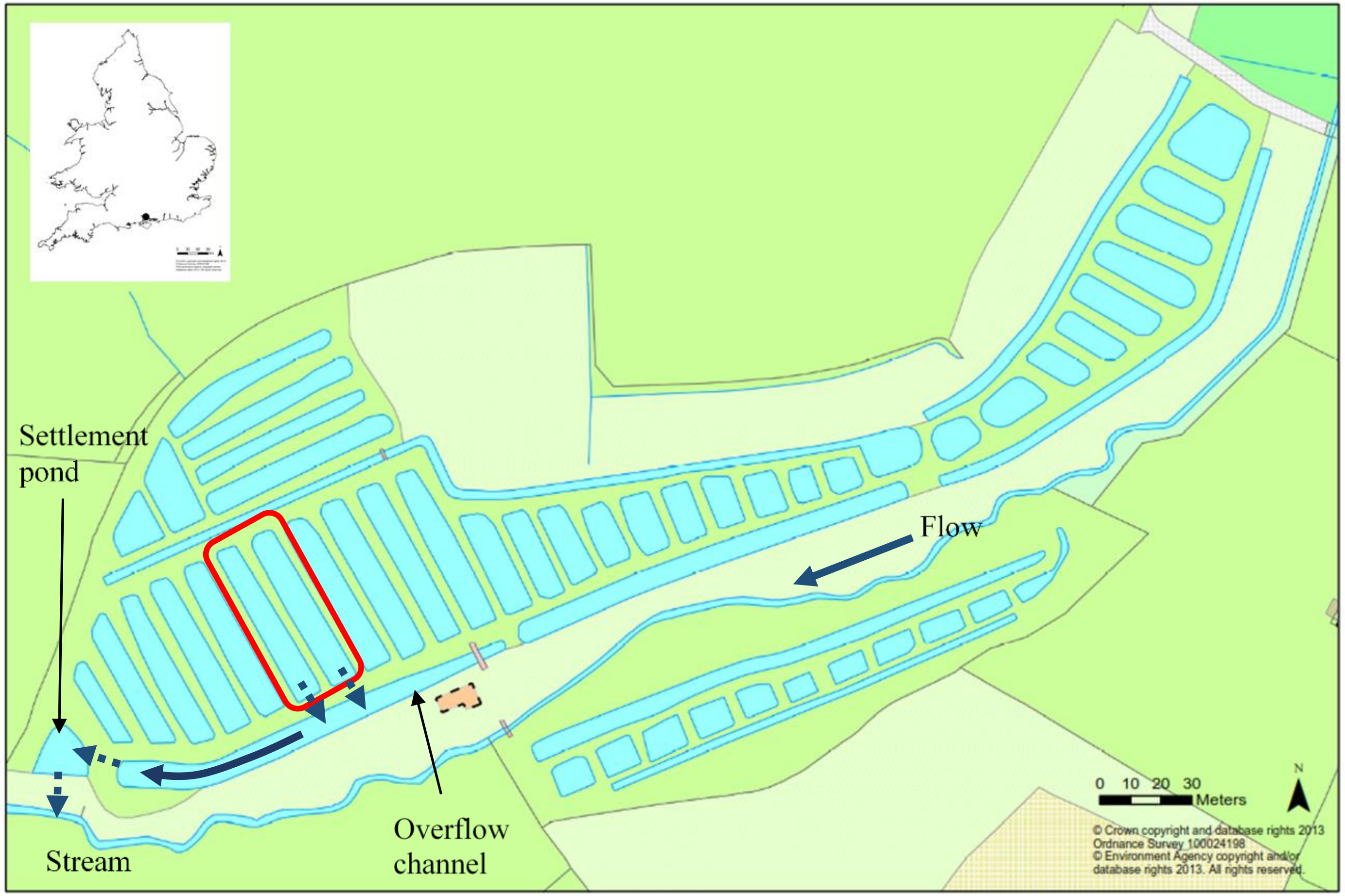

Figure 14. Aerial view of aquaculture facility in Southern England, UK. Blue arrows show water flow during site drain-down and red rectangle shows ponds drained.

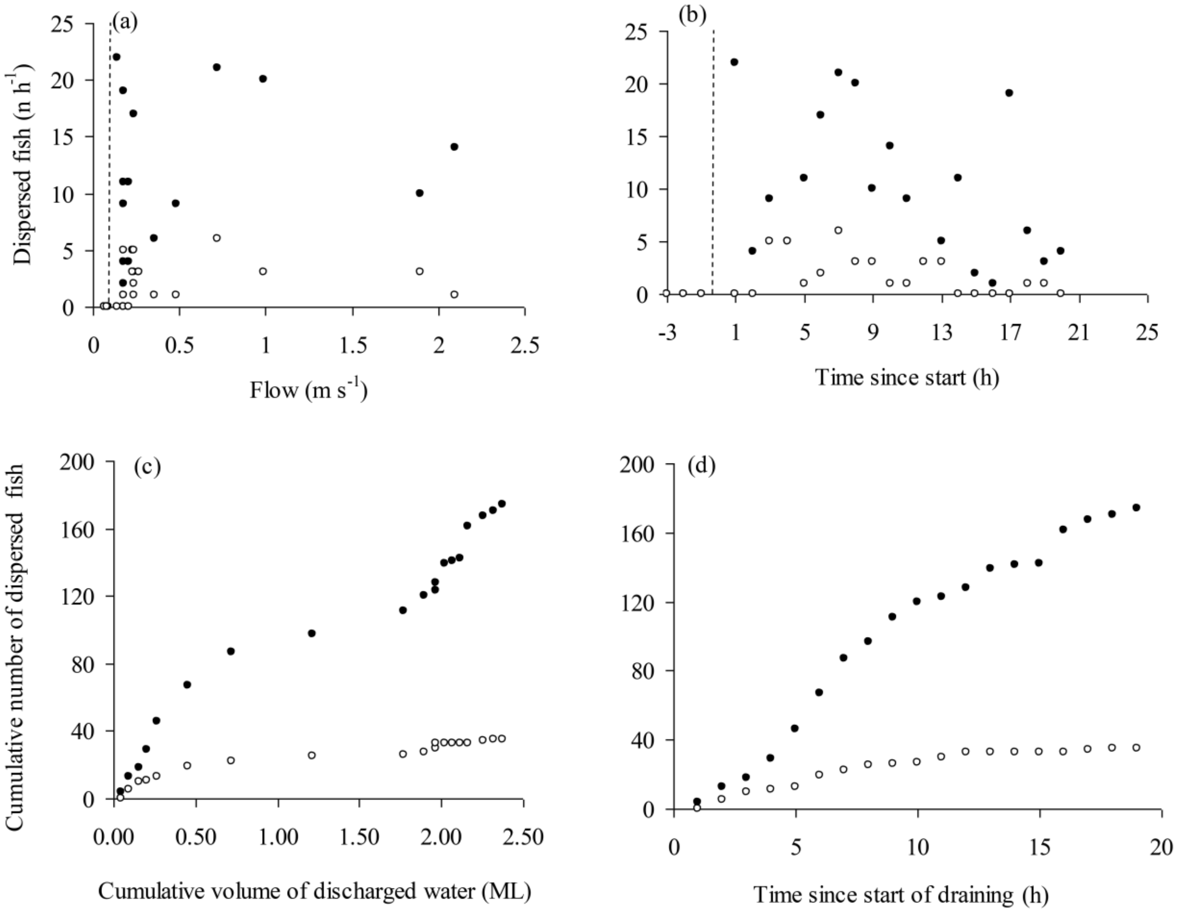

Figure 15. Relationship of the number of fishes dispersed from aquaculture site with (a) discharge velocity and (b) time and just before and during drain down exercise; and cumulative number of fish dispersed from the aquaculture site versus cumulative volume of water released (c) and cumulative time (d). ● *Pseudorasbora parva*; ○ *Gasterosteus aculeatus*; vertical dashed lines denote the flow rates pre- (left) and post-pond draining (right).

5.5. Discussion

Under the normal activities of the aquaculture site, no *P. parva* were detected drifting out of the site, but this changed during the drain-down operation when relatively high numbers of *P. parva* were captured passively dispersing from the site following their displacement by elevated flows. Moreover, their lengths indicated they were primarily mature fish, not larvae or juveniles, and thus their potential release into the wider environment during their reproductive season could have potentially resulted in the rapid establishment of populations (Chapters 2 and 3), particularly given this apparently high 'propagule pressure' (Britton and Gozlan, 2013).

Despite *P. parva* being predominantly found in lentic environments in England and Wales (Section 1.6), they have been shown to inhabit rivers within their invaded range, with the assumption that they use rivers as 'dispersal corridors' (Pollux and Korosi, 2006). Indeed, in the European invasion of *P. parva*, their long-distance dispersal was achieved through their 'hitch-hiking' within contaminated batches of fish in aquaculture (Gozlan et al., 2010b), but their subsequent short-distance dispersal was most likely to be achieved in a manner similar to that outlined here. Biological invasion theory predicts that establishment success is more favourable under higher propagule pressure and given the plasticity in life-history traits displayed by *P. parva* according to different population abundances (Chapter 2), high propagule pressure could potentially lead to established lotic populations where sufficient habitat is available for their spawning activities and nursery areas. Such populations could subsequently use this hydrological connection to find suitable online lentic waterbodies and establish further populations. Additionally, this 'drip-feeding' of *P. parva* propagules into the lotic environment could sustain *P. parva* populations, even when conditions are not conducive for their reproduction. Indeed, where *L. gibbosus* is present in rivers in England, extant populations persist without in-stream reproduction, with populations maintained by escapees from lakes with hydrological connections to the rivers (Klaar et al., 2004). Also, given the disease implications of *P. parva* via their hosting of *S. destruens* (Gozlan et al., 2005; Section 1.6), then their dispersal into river catchments with recreationally and economically important fish populations and communities would be an additional concern.

Given the weak microhabitat electivity of *P. parva* and strong positive interactions with native riverine species (Beyer et al., 2007), there is potential for adverse impacts should *P. parva* successfully establish riverine populations in England and Wales. Additionally, considering the difficulties associated with the control of non-native fishes in an open system (Section 1.4), the escapement of *P. parva* during drain-down operations or water management on aquaculture facilities poses a significant risk to the receiving environment. It suggests that on pond aquaculture and fishery sites where non-native fish are present and could disperse more widely (e.g. Fobert et al., 2013), consideration is needed to increase biosecurity during key management activities to negate dispersal opportunities, especially when the fishes are small bodied like *P. parva* (Gozlan et al., 2010). Actions taken would thus help prevent the small-scale dispersal of non-native fishes into the wild that often occurs following a long-distance dispersal event, and thus assist invasive populations from developing, lessening the need for subsequent management activities (Section 1.4; Chapter 6).

Chapter 6

Assessing the efficacy and ecology of biocontrol and biomanipulation for managing invasive pest fish

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6.1. Abstract

Management of non-native species aims to prevent biological invasions using actions including control and containment of the potential invader. Biocontrol and biomanipulation strategies are used frequently to reduce population sizes of non-native species, and reduce their ecological impacts and dispersal rates. Assessments of the efficacy of biocontrol and biomanipulation actions for managing non-native pest fish, and the ecological mechanisms involved, were studied here using lentic populations of *P. parva*. Biocontrol was through release of the indigenous piscivorous fish *Perca fluviatilis* and biomanipulation through intensive fish removals. A combined biocontrol and removal programme was completed in an invaded pond over 2 reproductive seasons. Almost 10000 *P. parva* were removed, with cumulative removal numbers significantly related to their decreased abundance (> 60 to < 0.1 m^{-2}). Ten adult *P. fluviatilis* were also released initially and reproduced each season. Analyses revealed *P. parva* contribution to their diet was high initially, but decreased as *P. parva* abundance reduced. Individual contributions of the management actions to declined *P. parva* abundance were difficult to isolate. The individual effects of biocontrol and removals on *P. parva* populations were then tested using a field trial in replicated pond mesocosms over 3 reproductive seasons. Replicates started with 1500 *P. parva*. The control (no interventions) revealed no significant temporal changes in *P. parva* abundances. In the removal treatment, where over 17000 *P. parva* were removed per replicate over the trial, abundance declined initially, but increased significantly after each reproductive season as remaining fish compensated through increased reproductive output. In the biocontrol, abundance declined and remained low; analyses revealed *P. parva* were an important dietary component of larger *P. fluviatilis*, with predation suppressing compensatory responses. Thus, biocontrol and removals can significantly reduce abundances of lentic populations of small invasive fishes. Removals provide short-term population suppression, but high effort is needed to overcome compensatory responses. Biocontrol can provide longer-term suppression but could invoke unintended ecological consequences via ‘stocking-up’ food webs. Application of these results to decision-making frameworks should enable managers to make more objective decisions on risk-commensurate methodologies for controlling small invasive fishes.

6.2. Introduction

The effective prevention of biological invasions requires activities such as horizon scanning (Roy et al., 2014), import controls and screening (Lodge et al., 2006), auditing of regulated animal movements (Chapter 4) and the rapid detection of new introductions (Britton et al., 2011a). If these activities fail to prevent a non-native species from being introduced, the species can colonise and disperse, initiating an invasion. Whilst eradication of new populations of non-native species might be the preferred option to prevent these invasions developing, eradication can be difficult and controversial (Myers et al., 1998; Simberloff, 2002). Many methods are non-specific in their target species, such as chemical biocides that also result in mortalities of non-target species (Simberloff, 2009; Chapter 1). Biocide applications are also often inappropriate when the area of invasion has high conservation value, such as habitats containing protected species (Britton et al., 2011a).

Alternative approaches to managing populations of invasive species include control and containment programmes that aim to reduce population abundance and dispersal probabilities, and decrease ecological impacts on native biota (Britton et al., 2011b). Although unlikely to achieve eradication (Manchester and Bullock, 2000), these provide less controversial approaches that can limit the invasion’s spatial extent (Allendorf and Lundquist, 2003). This is important as river basins generally represent discrete biogeographic islands (Gozlan et al., 2010a), so minimising dispersal rates of non-native fish from ponds into river catchments can inhibit their invasion (Britton et al., 2011b). Preventing these invasions either requires population extirpation by biocide, eliminating dispersal (Britton and Brazier, 2006), or actions that reduce population abundance, minimising dispersal, which also reduces impacts on native species (Jackson et al., 2014). Although control and containment strategies are often used in attempts to control non-native fish populations, there is limited knowledge on the efficacy of their long-term applications and the ecological mechanisms involved, constraining the ability of managers to make objective decisions on their application (Britton et al., 2011a).

Control techniques for managing invasive fish populations typically include their physical removal (biomanipulation) and enhancing populations of piscivorous fish to increase predation pressure (biocontrol) (Kolar and Lodge, 2001; Lee, 2001). The removal of individuals from non-native fish populations can be effective when applied to spatially limited, isolated populations (e.g. Knapp and Matthews, 1998). Classical biocontrol programmes introduce a predator or pathogen from the native range of the invasive species to limit its population growth and has been used effectively for managing non-native plants (e.g. Gassmann et al., 2006). However, the introduced predator may expand their prey range to non-target native species, leading to irreversible effects (Simberloff, 2009). Consequently, for non-native fish, classical biocontrol is rarely feasible, with options limited to enhancing their predator populations using indigenous fish from the introduced range (Gozlan et al., 2010a).

As the ecological consequences of *P. parva* include modifications to food web structure (e.g. Britton et al., 2010a) and novel pathogen transmission (Andreou et al., 2012) then in aspects of their invasive range, there is a desire to prevent their further spread and reduce their impacts (Britton et al., 2011c). Whilst this has been achieved in the UK through rotenone application to pond populations (Britton and Brazier, 2006), this is a non-species specific biocide whose application potentially incurs relatively high costs initially (Britton et al., 2011c; Chapter 1). In areas of the *P. parva* invasive range in Europe, its application is prohibited and so alternative management approaches are required. Consequently, *P. parva* is used here as the in wild and semi-controlled conditions to assess the efficacy and ecological mechanisms of biomanipulation (by removals) and biocontrol (population enhancement of a facultative piscivorous fish) on their established populations.

Objectives were to: (1) measure the effect on *P. parva* population abundance of a combined biomanipulation and biocontrol programme on a field site; (2) determine the individual effects of biomanipulation and biocontrol measures on *P. parva* population abundance in a field trial using pond mesocosms; and (3) assess the ecological mechanisms involved in the consequent reductions of the *P. parva* populations and their subsequent population responses. Results are assessed in relation to the mechanisms and efficacy of the two methodologies, and their practical application to managing their presence in, and preventing their invasion of England and Wales.

6.3. Materials and Methods

6.3.1. Field site

The field site was a 0.3 ha, shallow (< 1.5 m) pond in North West England (N53°22’33’’, W3° 08’19’’) where *P. parva* was detected in an initial survey in November 2005 (Figure 16; Pond 1). Sampling commenced in April 2006 using a series of 25 m micro-mesh seine nets; population density estimates were derived from depletion estimates from successive deployments of the net in specific locations of the ponds (Cowx 1983). The presence of a very high *P. parva* density (Table 9) meant a biomanipulation programme (hereafter referred to as ‘removal’) was initiated to reduce their abundance by cropping (i.e. mass removal) at approximately 6 month intervals for two years, covering two *P. parva* reproductive seasons, using the same sets of micromesh seine nets. The rationale for these time periods was the mature fish would be removed in the spring prior to their spawning and the young-of-the-year (YoY) produced by the remaining mature fish in the spawning season would be cropped in autumn. On each sampling occasion, depletion sampling was completed in advance to obtain the *P. parva* population estimate before the removal exercise was completed. The removals netted the pond until all major habitat areas had been netted at least once.

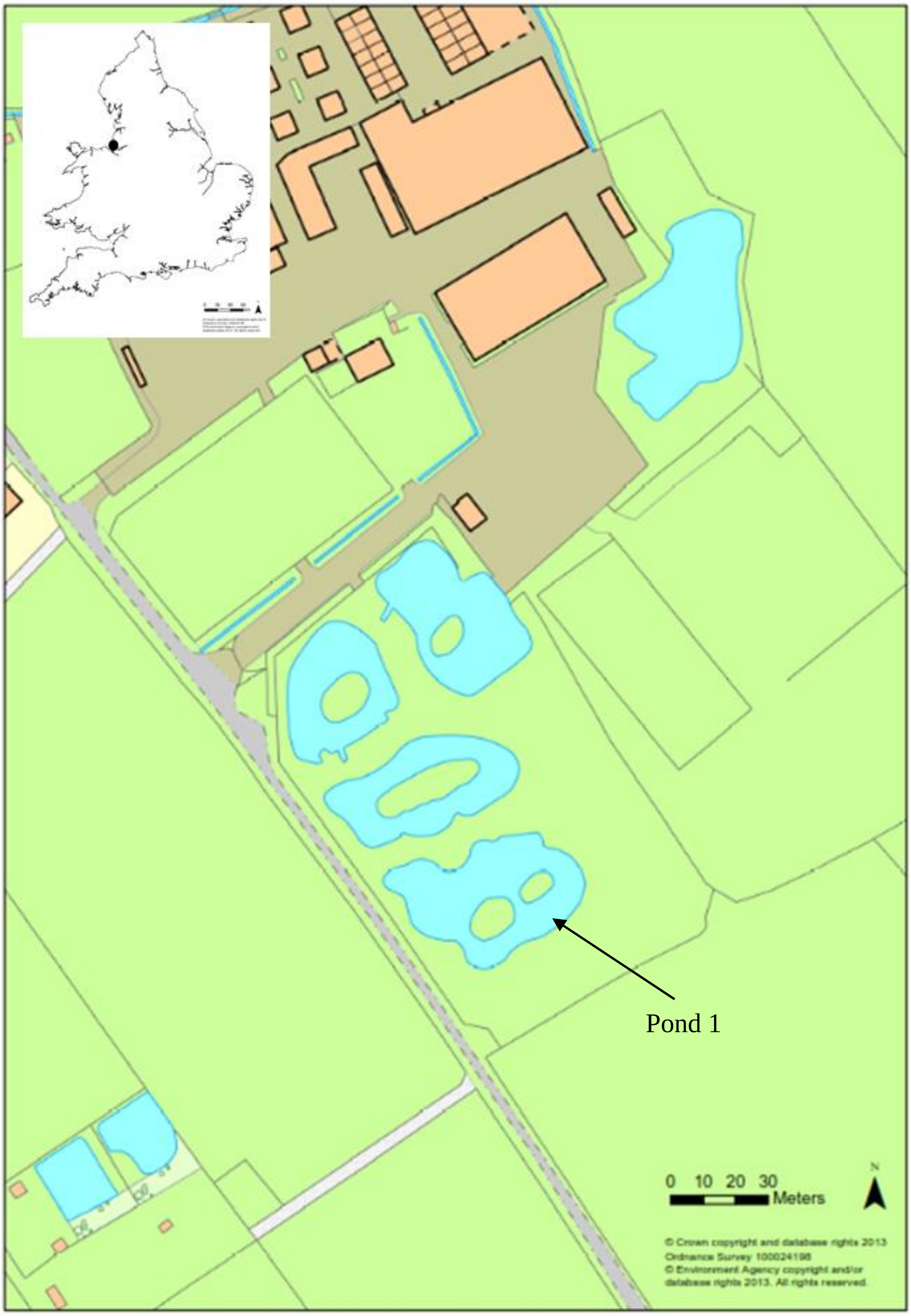

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Figure 16. Aerial view of field site in Northwest England, UK. Pond used in the study was Pond 1.

The effects of these removals on the *P. parva* population densities were reported in Britton et al. (2010c). However, this management programme also incorporated the stocking of the native facultative piscivorous fish perch *Perca fluviatilis*, with the species also indigenous to the watershed. A total of 10 fish (210 - 325 mm) were released in April 2006. No obligate piscivorous fish were present in the pond and the other species were all of the family Cyprinidae. Initially, the efficacy of this aspect was not assessed, as it was not perceived to have contributed to the effectiveness of the removal programme. However, opportunities to test the contribution of *P. parva* to the diet of *P. fluviatilis* were available subsequently via scales for stable isotope analysis. The stable isotope data derived from fish scales significantly relate to those of dorsal muscle, which is used more generally, enabling their application in this manner (e.g. Grey, 2006). Thus, this assessed whether the *P. fluviatilis* were assisting the removals by consuming *P. parva* (as biocontrol). Stable isotope analyses reveal trophic linkages through the naturally occurring ratios of ^{15}N:^{14}N and ^{13}C:^{12}C (Grey, 2006); carbon ratios reflect the consumer diet with typical enrichment of 0 to 1 ‰ and nitrogen ratios show greater enrichment of 2 to 4‰ from resource to consumer, indicating trophic position (Post, 2002; McCutchan et al., 2003).

On each sampling occasion, between 3 and 5 scales were removed from a sub-sample of *P. parva* and from all sampled *P. fluviatilis*. During sampling of April 2007 and September 2007, macro-invertebrate samples had also been collected (n = 3 to 10 per resource). In the laboratory, the scales were prepared for analysis by taking material from only the very outer portions of scales, i.e. material produced through the most recent growth (Hutchinson and Trueman, 2006). All stable isotope samples were oven dried to a constant weight at 60ºC for 48 hours, before being processed at Cornell Isotope Laboratory, New York, USA. Here, they were ground into a homogenous powder, approximately 0.5 mg weighed out into a tin cup, with the actual weight recorded using a Satorius MC5 microbalance. The nitrogen and carbon isotopes were then analysed, using a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer. Data outputs were reported relative to conventional standards in the δ notation, where $\delta^{13}C$ or $\delta^{15}N$ = [Rsample/Rstandard-1] x 1000, and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (‰). Reference standards 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 standard deviation for both $\delta^{13}C$ and $\delta^{15}N$ to determine reliability of analyses. Overall standard deviation of the mink standard was no greater than 0.23 ‰ for δ^{15}N and 0.14 ‰ for δ^{13}C.

The stable isotope data were then analysed in two ways. Firstly, data from each sampling occasion were tested for differences in δ^{13}C and δ^{15}N between *P. parva* and *P. fluviatilis* using a generalized linear model (GLM). The dependent variable was either δ^{13}C or δ^{15}N and the independent variable was the interaction of species and sampling date. Given the large size range of *P. fluviatilis* (approximately 40 to > 300 mm), their data were split into different size classes (‘small’, < 100 mm; ‘large’ > 101 mm), as ontogenetic changes in gape size influences the body size of their prey fish (Dörner et al., 2003). Differences in δ^{13}C or δ^{15}N of the fishes were determined using estimated marginal means and multiple pairwise comparisons with Bonferroni adjustment for multiple comparisons. Secondly, for data from April and October 2007 when the macro-invertebrate data were available as putative food resources, *P. fluviatilis* diet composition by size classes was estimated using Bayesian mixing models in the SIAR package in the R computing programme (Parnell et al., 2010; R Core Development Team, 2013). Data for putative resources with similar isotope signatures were combined *a priori* to optimise model performance (Phillips et al., 2005). Thus, they were pooled into: macro-invertebrates (*Gammarus pulex* and Chironomid larvae), ‘small’ *P. fluviatilis* (< 50 mm, to allow for cannibalism) and *P. parva*. To correct for isotopic fractionation between resources and consumers, 2.9 ‰ (±0.32 ‰) was used for δ^{15}N and 1.3 ‰ (±0.3 ‰) for δ^{13}C (McCutchan, 2003). Outputs were the predicted contribution to diet of each resource.

6.3.2. Field trial

The field trial ran between February 2011 and October 2013, covering three *P. parva* reproductive seasons and, as per Chapter 2, 3 and 5, was completed in the disused aquaculture site in Southern England (Figure 14). It comprised the following treatments, each replicated four times in identical pond mesocosms of approximately 200 m^{-2} where depths were to 2 m: control (no interventions), removal (involving cropping at 6 month intervals) and biocontrol (using released and indigenous *P. fluviatilis*). Prior to use, each pond was drained and dried in spring 2010 to ensure complete fish absence, followed by natural refilling. Measures to deter avian predators were then deployed, including anti-predator netting, before 1500 mature *P. parva* (fork lengths 40 to 70 mm and of approximately equal sex ratios) were introduced to each pond in June 2010 that were sampled randomly from 10 other ponds on the site.

These fish were left until the trial commenced in February 2011 when an initial sampling of all mesocosms was undertaken. This used rectangular fish traps comprising of a circle alloy frame of length 107 cm, width and height 27.5 cm, mesh diameter 2 mm and with funnel shaped holes (6.5 cm diameter) at either end to allow fish entry and capture. They were baited using fishmeal pellets (21 mm diameter) as these baited traps provide reliable *P. parva* catch per unit effort estimates (n fish h^{-1}; CPUE) (Britton et al., 2011c). Once the initial CPUE of each mesocosm had been determined, 20 *P. fluviatilis* of 100 to 140 mm were released into each biocontrol replicate, with each individual already tagged with passive integrated transponder (PIT) tags. The first *P. parva* removal event was also completed on all removal ponds, when traps were set in triplicate for 2 hours before lifting and removing all fish. The removal concluded when the CPUE of the trapping reduced to levels <10 fish per trap per hour. Following these removals, all ponds were re-sampled in March 2011 to estimate CPUE once more.

Thereafter, until October 2013, the control and biocontrol ponds were left, other than sampling for CPUE each spring and autumn when a random sub-sample of 30 fish was removed per pond for subsequent analysis. For the removal ponds, sampling also occurred each spring and autumn until October 2013, but after each sampling event, a removal event was also completed, as described above. In October 2013, the trial concluded by sampling and then draining each pond; for the biocontrol, all of the surviving *P. fluviatilis* and their progeny were collected, along with samples of *P. parva* and macro-invertebrates, including signal crayfish *Pacifastacus leniusculus*.

For the *P. parva* sub-samples, individuals were measured (fork length, mm) and scales removed that were viewed on a projecting microscope (×30) and their ages estimated. For the samples of *P. fluviatilis* and *P. parva* collected from the biocontrol treatment mesocosms in October 2013, each fish was measured and samples of dorsal muscle removed and dried for stable isotope analysis (Perga and Gerdeaux, 2009). The macro-invertebrate samples were treated as per those from the field site.

6.3.3. Field trial data analysis

CPUE per treatment over the trial was analysed using a GLM using the interaction of CPUE and sampling date as the dependent variable and treatment as the independent variable; outputs were the estimated marginal means of CPUE per treatment over time and the significance of their differences (pairwise comparisons with Bonferroni adjustment for multiple comparisons). The *P. parva* age data were used to estimate the contribution (%) of young-of-the-year (YoY) fish to their population, with fish sampled in spring that were produced the previous summer still classed as YoY. These data were tested in a GLM as per CPUE. Significant differences in the *P. parva* YoY age and length data between treatments and over time were tested in a linear mixed model, with pond used as a random effect on the intercept to avoid inflating the residual degrees of freedom by using individual fish as true replicates. Differences in YoY age and lengths were determined using estimated marginal means and multiple comparison post-hoc analyses (general linear hypothesis test).

The stable isotope data for the biocontrol from October 2013 contained data for *P. fluviatilis* between 47 and 295 mm and could be split into three size ranges: small (< 100 mm; $n = 8$); medium (101-200 mm; $n = 13$) and large (> 201 mm, $n = 5$). Initially, these data were used to determine the significance of differences between *P. parva* and the *P. fluviatilis* size classes in $\delta^{13}C$ and $\delta^{15}N$, with data were combined across replicates, as differences between the stable isotope data of the macro-invertebrates in each mesocosm were not significant (Mann Whitney U-test, $Z = 0.02$, $P > 0.05$ for *Asellus aquaticus* and Chironomid larvae). These data were used in a linear mixed model, with pond used as the random factor to avoid inflating residual degrees of freedom. Differences in $\delta^{13}C$ and $\delta^{15}N$ between the species and size classes were detected using multiple comparison post-hoc analyses (general linear hypothesis test). The diet composition of the perch size classes were then estimated from their putative food resources (*P. parva*, macro-invertebrates, *P. leniusculus* and smaller *P. fluviatilis*) using Bayesian mixing models, as per the Field site. All of the stable isotope data for *P. parva* and small *P. fluviatilis* were included in medium and large *P. fluviatilis* mixing models. For small *P. fluviatilis*, the only fish prey entered were < 50 mm.

6.4. Results

6.4.1. Field site

In the field site, *P. parva* population density estimates reduced from 63.1 to < 0.1 m^{-2} over the study period (see Table 9). The relationship between the cumulative number of *P. parva* removed and their subsequent population estimate was significant; abundance decreased as removal number increased (linear regression: $R^2 = 0.95$; $F_{1,3} = 53.17$, $P < 0.01$; Figure. 17a). Following the release of *P. fluviatilis* into the pond in spring 2006, they reproduced, with their progeny present in samples from April 2007 (Tables 10 and 11).

The stable isotope data of the *P. fluviatilis* size classes and *P. parva* varied between April 2006 and April 2008 (Table 10). The GLMs testing differences in $\delta^{13}C$ and $\delta^{15}N$ between *P. fluviatilis* and *P. parva* on each sampling occasion were significant ($\delta^{13}C$: Wald $\chi^2 = 275.48$, d.f. = 12, $P < 0.01$; $\delta^{15}N$: Wald $\chi^2 = 198.74$, d.f. = 12, $P < 0.01$). Excluding data from February 2006 (values for *P. fluviatilis* were from their original pond and not the field site), these data revealed significant higher values of $\delta^{15}N$ (to 4.24 ‰) in both size classes of *P. fluviatilis* than *P. parva* in samples to April 2007, but not thereafter (Table 11). For $\delta^{13}C$, there was a significant difference between the large *P. fluviatilis* size class and *P. parva* in April 2007 (mean difference 1.99 ‰) but not in any other sample (Table 11).

Stable isotope mixing models using data from April 2007 predicted the large *P. fluviatilis* were highly piscivorous, with mean *P. parva* contribution to their diet being 49 % (Table 12). In October 2007, whilst the models predicted that these large perch were still mainly piscivorous, *P. parva* contribution reduced to a mean of 21 %, with an increase in diet of small *P. fluviatilis* and macro-invertebrates (Table 12). The mixing models for small perch revealed some piscivory of *P. parva* < 60 mm in April 2007 that declined to a very low level by October 2007 (Table 12).

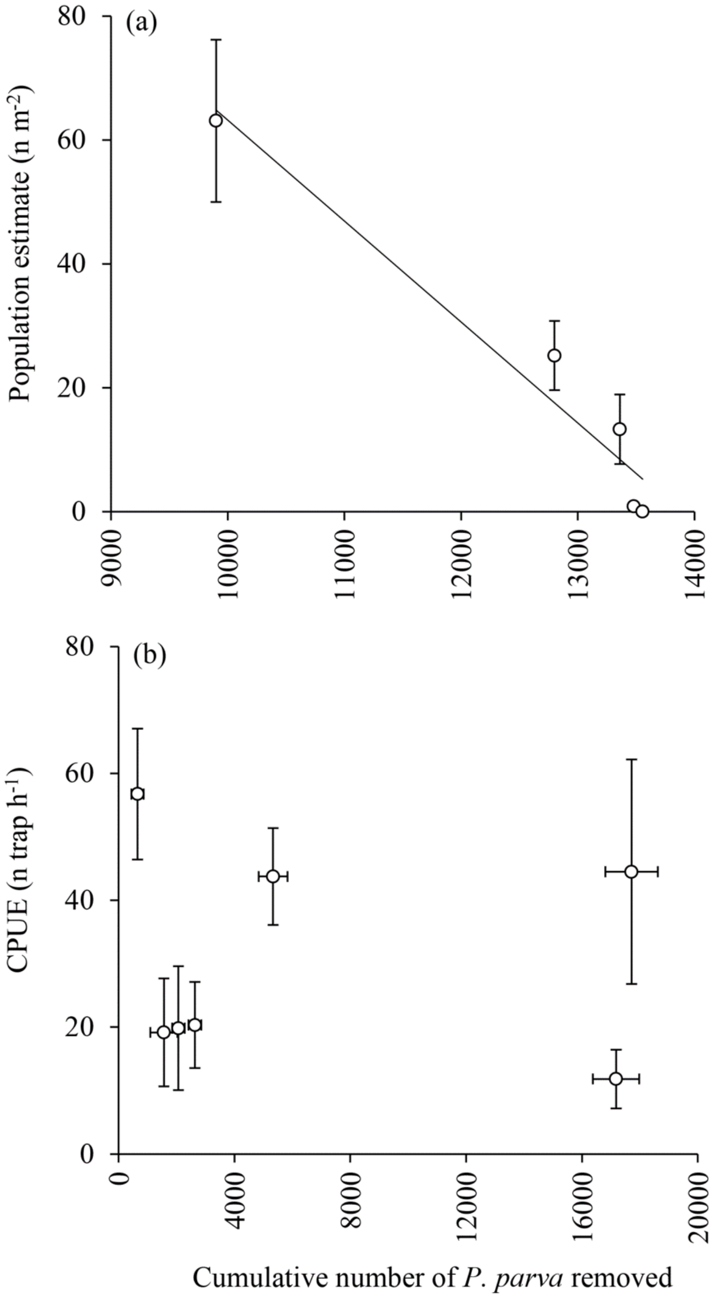

Figure 17. Relationship of CPUE and cumulative number of *Pseudorasbora parva* removed from (a) the field site; and (b) from the Removal treatment in the field trial. The solid line denotes significant relationships between variables (linear regression) and error bars represent standard error.

Table 9. Population estimates of *Pseudorasbora parva* at the field site and the number and weight of *P. parva* removed (Britton et al., 2010c).

	P. parva population estimate (n m^{-2})	Approximate number of *P. parva* removed	Approximate weight of *P. parva* removed (g)
April 2006	63.1 ± 13.1	9900	9090
Sept 2006	25.2 ± 5.6	2900	4970
April 2007	13.3 ± 5.5	560	1360
Sept2007	0.9 ± 0.3	120	255
April 2008	0.1 ± 0.0	75	129

Table 10. Numbers of analysed fish, mean lengths and length range (mm) of *Perca fluviatilis* and *Pseudorasbora parva* from the field site. 'Large' *P. fluviatilis* were > 101 mm, 'small' were ≤ 100mm.

Date	Species	n	Mean length (mm)	Length range (mm)
Apr 2006	Large *P. fluviatilis*	6	276 ± 35	235 - 323
	P. parva	6	40 ± 7	33 - 54
Sept 2006	Large *P. fluviatilis*	10	147 ± 26	112 - 214
	P. parva	6	55 ± 22	41 - 98
Apr 2007	Large *P. fluviatilis*	5	196 ± 94	132 - 359
	Small *P. fluviatilis*	6	55 ± 8	49 - 70
	P. parva	16	56 ± 15	38 - 95
Sept 2007	Large *P. fluviatilis*	6	266 ± 60	206 - 352
	Small *P. fluviatilis*	9	80 ± 8	68 - 90
	P. parva	15	60 ± 23	23 - 93
Apr 2008	Large *P. fluviatilis*	2	239 ± 171	118 - 360
	Small *P. fluviatilis*	8	90 ± 8	76 - 99
	P. parva	10	55 ± 16	25 - 77

Table 11. Mean adjusted $\delta^{13}C$ and $\delta^{15}N$ for *Perca fluviatilis* in (a) 'small' and (b) large size classes and *Pseudorasbora parva*, and their mean difference and significance according to pairwise comparisons (with Bonferroni adjustment for multiple comparisons) by sampling date at the field site. *Difference significant at $P < 0.05$; ** $P < 0.01$.

(a)	'Small' *P. fluviatilis*		*P. parva*		Mean difference	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
April 2007	-30.34 ± 0.57	16.83 ± 0.45	-26.46 ± 0.38	13.71 ± 0.30	3.88**	3.11**
Sept 2007	-28.83 ± 0.45	14.57 ± 0.36	-29.35 ± 0.38	15.41 ± 0.30	0.52	0.84
Apr 2008	-27.54 ± 0.47	16.16 ± 0.37	-27.55 ± 0.46	15.02 ± 0.36	0.01	1.14

(b)	'Large' *P. fluviatilis*		*P. parva*		Mean difference	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Feb 2006	-23.29 ± 0.87	12.05 ± 0.56	-28.12 ± 0.59	15.03 ± 0.47	4.83**	2.98
Sept 2006	-26.46 ± 0.45	16.87 ± 0.36	-25.88 ± 0.57	13.89 ± 0.45	0.57	2.97**
April 2007	-28.45 ± 0.70	17.95 ± 0.34	-26.46 ± 0.38	13.71 ± 0.30	1.99*	4.24**
Sept 2007	-29.62 ± 0.84	17.42 ± 0.66	-29.35 ± 0.38	15.41 ± 0.30	0.27	2.00
Apr 2008	-27.41 ± 1.10	16.06 ± 0.85	-27.55 ± 0.46	15.02 ± 0.36	0.14	2.39

Table 12. Predicted mean proportions (%) and 95 % confidence limits from Bayesian mixing models of putative food resources to the diet of (a) ‘small’ and (b) ‘large’ *Perca fluviatilis* in the field site.

(a)	*Pseudorasbora parva*	*Perca fluviatilis* (< 50 mm)	Macro-invertebrates
April 2007	36 (1 - 64)	n/a	64 (36 - 99)
Sept 2007	13 (0 - 44)	n/a	87 (56 - 100)

(b)	*Pseudorasbora parva*	*Perca fluviatilis* (< 50mm)	Macro-invertebrates
April 2007	49 (24 - 73)	22 (1 - 41)	29 (3 - 53)
Sept 2007	21 (0 - 50)	45 (4 - 86)	35 (0 - 67)

6.4.2. *Field trial*

The GLM testing CPUE from the Control, Removal and Biocontrol treatments revealed the effect of the interaction of treatment and date was significant ($P < 0.01$), with estimated marginal means and pairwise comparisons revealing no significant differences in CPUE in the control over the trial, but with significant differences in the removal and biocontrol treatments (Figure 18). Comparison of CPUE in the removal versus the control on each sampling occasion revealed significantly reduced *P. parva* CPUE from October 2011 to March 2012, and in March 2013, but not in October 2012 and October 2013 when CPUE increased (Table 13; Figure 18). Whilst the highest cumulative number of *P. parva* removed from a replicate in the Removal treatment was over 18500 fish, the relationship between the cumulative number of *P. parva* removed and CPUE was not significant ($R^2 = 0.08$; $F_{1,5} = 0.04$, $P = 0.84$; Figure 17b). By contrast, there was a significant reduction in CPUE in the biocontrol compared to the control from October 2011 that remained through to October 2013 (Table 13; Figure 18).

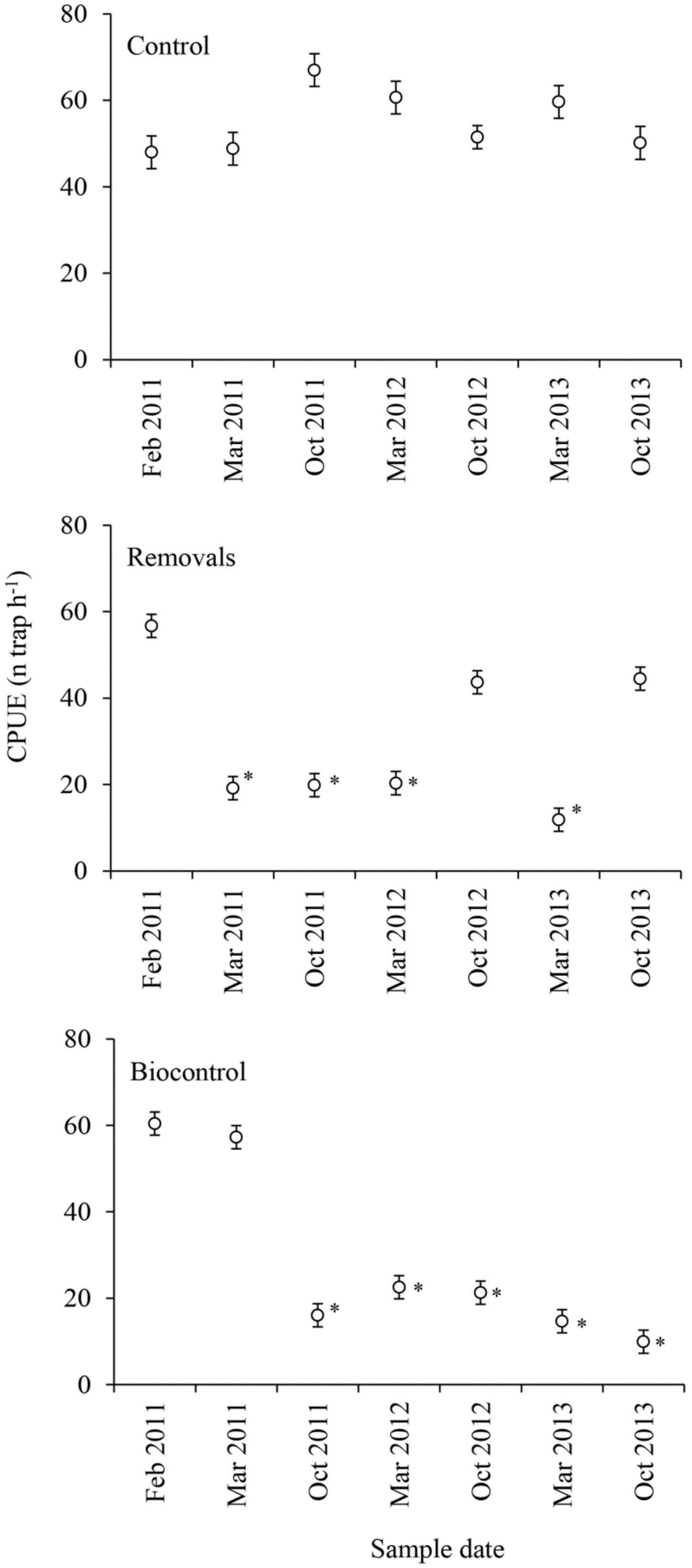

Figure 18. Mean relative abundance estimates between February 2011 and October 2013 in the field trial for the control, removal and biocontrol. Error bars represent standard error. *$P < 0.01$ for CPUE on that date and initial CPUE (February 2011).

Table 13. Mean differences in the CPUE of *Pseudorasbora parva* in the control and treatments by sampling date in the field trial. * $P < 0.01$.

	Control - Removal	Control - Biocontrol	Removal -. Biocontrol
Feb 2011	-8.8	-12.4	3.7
Mar 2011	29.7*	8.4	38.1*
Oct 2011	47.2*	51.0*	3.8
Mar 2012	40.3*	38.2*	2.2
Oct 2012	7.8	30.3*	-22.4*
Mar 2013	47.8*	45.0*	-2.83
Oct 2013	5.7	40.3*	-34.6*

The linear mixed effects model testing the proportion of YoY *P. parva* on each sampling date in the control and treatments revealed the interaction of treatment and date was significant ($P < 0.01$). Significant increases in the proportion of YoY were apparent in both the Control and Removal treatment, but not in the Biocontrol treatment ($P < 0.01$; Figure 19). The linear mixed effects model testing the mean length of YoY on each sampling date from the control and treatments revealed the effect of the interaction of treatment and date was also significant ($P < 0.01$). Whilst there were no significant changes in mean lengths in the control and biocontrol, significantly reduced YoY mean length was recorded in October 2012 and October 2013 in the Removal treatment (Figure 19).

Following their release, *P. fluviatilis* reproduced in the biocontrol and so by the conclusion of the trial, there were three age classes present, age 0+ to 2+ years, plus a low number of tagged original fish (Table 10). The linear mixed effects model using stable isotope data from the biocontrol treatment from samples taken in October 2013 revealed that the effect of species/ size-class was significant for both $\delta^{13}C$ and $\delta^{15}N$, with significant differences apparent in $\delta^{15}N$ between *P. parva* and medium and large *P. fluviatilis*, and between all *P. fluviatilis* size classes (Table 14). Stable isotope mixing models indicated all *P. fluviatilis* size classes predated upon *P. parva*, with the contribution to diet increasing as mean body size increased (Table 14c).

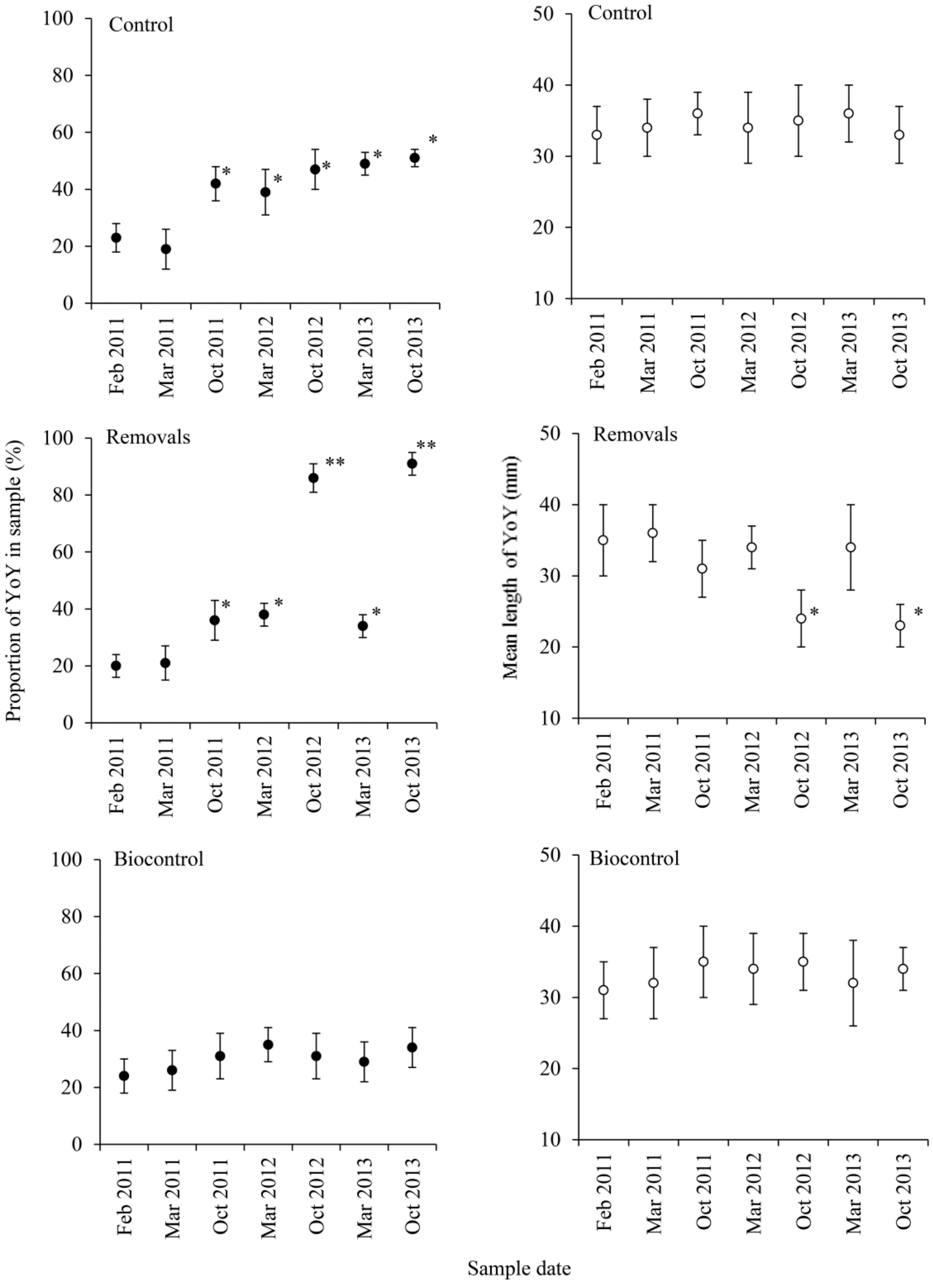

Figure 19. Mean proportion of *Pseudorasbora parva* young-of-the-year (YoY; filled circle) in October of each year and March the following year (i.e. at age 1), and their mean length of (open circle) in the field trial for the control, removal and biocontrol. $*P < 0.05$, $**P < 0.01$ for proportion between that date and the initial estimate in February 2011.

Table 14. a) Information on the fish analysed from the biocontrol treatment sampled at the conclusion of the trial; (b) Pairwise comparisons of δ^{13}C and δ^{15}N of *Pseudorasbora parva* and the three size classes of *P. fluviatilis*; **P* < 0.01; (c) predicted mean proportions (%) and 95 % confidence limits of putative food resources to the diet of *Perca fluviatilis* from the field trial.

(a)

Species	n	Mean length (mm)	Length range (mm)	Mean δ^{13}C (‰)	Mean δ^{15}N (‰)
P. parva	10	50 ± 11	33 – 72	-29.53 ± 0.39	5.92 ± 0.15
Small *P. fluviatilis*	8	64 ± 11	47 – 90	-26.45 ± 0.44	5.92 ± 0.17
Medium *P. fluviatilis*	13	147 ± 24	105 – 181	-28.55 ± 0.34	7.68 ± 0.13
Large *P. fluviatilis*	5	282 ± 14	261 – 295	-27.79 ± 0.55	9.60 ± 0.21

(b)

Comparison	δ^{13}C	δ^{15}N
P. parva vs. Small *P. fluviatilis*	3.08 ± 0.59*	0.01 ± 0.23
P. parva vs. Medium *P. fluviatilis*	0.97 ± 0.52	1.76 ± 0.20*
P. parva vs. Large *P. fluviatilis*	1.73 ± 0.68	3.67 ± 0.26*
Small *P. fluviatilis* vs. Medium *P. fluviatilis*	2.10 ± 0.56*	1.77 ± 0.22*
Small *P. fluviatilis* vs. Large *P. fluviatilis*	1.34 ± 0.71	3.68 ± 0.28*
Medium *P. fluviatilis* vs. Large *P. fluviatilis*	0.76 ± 0.65	1.92 ± 0.25*

(c)

	Perca fluviatilis size class		
	Small	Medium	Large
Pseudorasbora parva	20 (0 - 48)	27 (0- 46)	34 (7 - 60)
Perca fluviatilis (< 110mm)	-	5 (0 - 15)	15 (0 - 33)
Pacifastacus leniusculus	36 (0 - 71)	22 (0 - 44)	29 (1 - 54)
Macro-invertebrates	44 (2 - 86)	47 (0 - 60)	21 (0 - 42)

6.5. Discussion

The results of the field site and trial indicated that invasive *P. parva* pond population abundances can be significantly reduced using removals and biocontrol. Given the considerable presence of other small, invasive pest fishes in lentic environments around the world, such as *Gambusia* species (e.g. Pyke, 2008), Trinidadian guppy *Poecilia reticulata* Peters (Deacon et al., 2011) and minnow *Phoxinus phoxinus* (Museth et al., 2007), these outputs have high application to the management of non-native fishes generally. It should be noted, however, that population extirpations were not achieved by these methods. If the management aim is extirpation then chemical biocide application remains the most effective method to achieve this (Britton et al., 2011a).

Here, the use of removals to suppress *P. parva* populations was effective initially, with rapid and significant reductions in population sizes. There was limited success thereafter as populations compensated for losses by increasing their reproductive output. Other studies using removals to manage invasive fish populations have also shown some effectiveness in suppressing populations of target species. For example, removals of invasive brook trout *Salvelinus fontinalis* by gill netting in California, USA, were effective in reducing abundances in small lakes (Knapp and Matthews, 1998). Although trout below 110 mm were less susceptible to capture, the method provided some population control when biocide application was not possible for conservation reasons (Knapp and Matthews, 1998; Knapp et al., 2007). Other operations have been less successful due to compensatory responses in the target species. The population suppression of invasive *P. fluviatilis* in New Zealand resulted in increased juvenile abundances as the cannibalistic adults were removed only after they had spawned (Ludgate and Closs, 2003). The application of trapping and electric fishing to controlling black bullhead *Ameiurus melas* was relatively effective in a French lake as no compensatory responses were recorded (Cucherousset et al., 2006). In contrast, compensatory responses were detected in *A. melas* populations elsewhere following mass removals (Hanson et al., 1983). Thus, where the management aim is suppression of invasive fish populations then removals can provide an effective short-term measure. Its long-term effectiveness is, however, reduced substantially if the remaining fish exhibit compensatory responses, such as increased survival, growth and fecundity (Wydoski and Wiley, 1999).

Correspondingly, long-term population suppression using removals is likely to require sustained management efforts, potentially accruing high resource costs (Britton et al., 2011b).

The use of fish as biocontrol agents has generally been applied to managing insects such as mosquito *Aedes aegypti* (Martínez-Ibarra et al., 2002), particularly using *Gambusia* species (Pyke, 2008). Wild fish populations, particularly of European eel *Anguilla anguilla*, are also recognised as strong resistors of invasions of non-native crayfishes (e.g. Musseau et al., 2015). However, there are no reported large-scale programmes of bio-control that have successfully utilised piscivorous fish to suppress the invasion of a non-native fish (Britton et al., 2011b). The outcome of this study suggest it has considerable potential for suppressing populations of small, invasive fishes, such as *P. parva* and *Gambusia* spp., particularly in lentic environments. Despite its action being less immediate than for removals it has the potential benefit of negligible long-term management costs.

Managers pursuing the implementation of this form of biocontrol face practical and ethical challenges. Primarily, they must consider the predatory species used, as although the release of piscivorous fish into invaded ponds can suppress invasive populations, it might also result in the undesirable consequences of ‘stocking-up’ food webs (Eby et al., 2006). This is where the stocked fish either increase the species richness of top predators or replace other ones. This can result in additional predation pressure on native fish communities, increasing top-down effects (Eby et al., 2006). Releasing a native piscivorous fish is arguably more ethical than introducing a non-native one, given the reported impacts on native fish communities by non-native piscivorous fish released for sport angling, such as *Cichla* species (Britton and Orsi, 2012). A recent study found native pike *Esox lucius*, an obligate piscivore, was effective at suppressing *P. parva* populations in Belgium (Lemmens et al., 2014). However, the potential of *E. lucius* to grow to relatively large sizes (>10 kg), allied to their relatively large gape size (Nilsson and Brönmark, 2000), means their potential prey species cover a substantially wider size range than *P. fluviatilis* (Dörner and Wagner, 2003). This increases their risk of invoking undesirable cascading consequences in native prey fish populations. Correspondingly, in practical and ethical decisions over whether native predator enhancement is appropriate for suppressing invasive fish populations, managers must firstly consider the potential risk of altering food-web structure and causing ecosystem-level effects. This risk should then be balanced against the ecological risk of the target species and their invasion probability if their populations are left uncontrolled.

The field study used the biocontrol and removals in combination, whereas the field trial used them individually. This meant that the field trial identified the mechanisms involved in the actions of each method in isolation, but it could not assess their efficacy in combination. A final treatment involving the two methods was not completed due to logistical constraints. Considering the outputs of the field study and field trial together suggests that their effects were either additive or synergistic. Removals of mature *P. parva* prior to their spawning season reduced their reproductive effort, biocontrol minimised their compensatory responses through increased predation pressure, and removals at the end of the reproductive season reduced their recruitment. Where managers are only able to use one of these methods then consideration is between using removals that achieve short-term population suppression with the likelihood of long-term effort to maintain this, versus the longer-term suppression achieved by biocontrol but that potentially incurs negative cascading effects in the ecosystem.

In conclusion, the study revealed biocontrol and removals provide effective methods for suppressing populations of lentic *P. parva* populations. As *P. parva* represent a strong model of small, invasive fish more generally (Gozlan et al., 2010b), the results should be highly applicable to the management of small, invasive fishes in other systems and regions. In particular, these results can be applied to informing decision-making processes for invasive fishes, including ponds in England and Wales where populations have established. For example, where the management objective is extirpation of the target population then these methods are unlikely to be effective. If the objective is reducing their population abundance and controlling their dispersal (Chapter 5), then both methods could be effective when applied individually, with the method applied dependent on the timeframe of the objective, the resources available and the risk of incurring ecological consequences via stocking-up food webs. If the methods are used in combination, there is high potential that the population of the target species will be reduced to very low levels of abundance, but are nevertheless unlikely to lead to extirpation.

Chapter 7. General discussion

7.1. Introduction

Invasion ecology is developing into a significant ecological discipline with an associated rise in the number of publications on the subject (Lockwood et al., 2013). The exponential increase in such articles since the 1990s has led to significant advances in understandings of the invasion process, the associated impacts of invasive species and their population control (Kolar and Lodge, 2001; Blackburn et al., 2009; Lockwood et al., 2013). Nevertheless, rates of introductions of non-native species have been unprecedented in the last 25 years (Hulme, 2009), predominantly due to anthropogenic globalisation and free-trade, which has provided a plethora of opportunities for the long-distance dispersal of numerous species (IUCN, 2000) and has the potential to cause rapid and radical changes to the structure of biological communities globally (Clout and Veitch, 2002). Such a rise in the number of non-native species has led to the demand for robust research on introduced biota, given that research will assist policy makers and environment managers in the optimisation and best use of resources as well as the ability to prioritise non-native biota which pose the greatest threat. It is notable that not every introduced species will become invasive and problematic (Williamson and Fitter, 1996), a consequence of the abiotic and biotic factors present at each stage of the invasion pathway (Figure 1). Knowledge of the target species and its interaction with the native biota and ecosystems is thus critical in the development of preventative, control and mitigation strategies.

Here, the research aim was to describe relevant aspects of the ecology of a model non-native fish (*P. parva*) within a defined spatial area (England and Wales) in order to demonstrate how this knowledge could assist the development of management approaches that assist prevention of their invasion and provide alternative methods to piscicide-based extirpations. These outputs would then have wider applicability for the prevention and management of other small-bodied non-native fishes elsewhere, particularly those currently restricted to lentic sites. Knowledge on the processes that facilitate the invasion successes of invasive species is critical in their population management (Britton et al., 2010a), and life-history traits and their plasticity are linked to establishment success (Kolar and Lodge, 2001). As the life-history traits of a species can vary temporally and spatially, it is thus important that spatial patterns are considered when investigating invasion processes (Hamilton et al., 2005). Consequently, the influence of *P. parva* population density, temperature and latitude on their somatic growth increments and growth rates in England was determined (Chapter 2). Experiments in tank aquaria revealed that whilst temperature does play a role in determining growth rates, density had a greater influence, with higher increments produced at lower densities. This was corroborated though the use of experimental ponds and field trials, where the fastest growth was again at the lower densities, revealing that *P. parva* have considerable growth plasticity, facilitating their invasion success as small numbers of introduced founders can grow rapidly to attain sexually maturity, avoiding a demographic bottle-neck (Britton and Gozlan, 2013). Across four populations of similar abundance, the expression of their life-history traits was highly consistent, with similar reproductive effort and somatic growth increments produced over a 12 month period. These fish also revealed a relatively short spawning season, contrary to some populations investigated elsewhere in Europe (Chapter 3).

The efficacy of a fish stocking audit procedure as a prevention measure against the accidental introduction of hitch-hiking species revealed that the use of experienced auditors and a high searching effort can reduce the risk of accidental introductions (Chapter 4). If this is unsuccessful then to prevent individuals from dispersing into the wider environment is critical to preventing their invasion of neighbouring watercourses. It was demonstrated here that their dispersal rates from an aquaculture site were minimal during their reproductive period under normal summer husbandry operations, but when ponds were being manipulated, such as drain-down operations, then the elevated flow conditions resulted in substantial numbers of mature fish being displaced and potentially being dispersed into the wider environment, highlighting the need for enhanced biosecurity during critical operational periods (Chapter 5). Population management strategies and their efficacy were then examined (Chapter 6) and suggested that biological control, via manipulation of populations of a native piscivorous fish, and physical removal of *P. parva* individuals, can significantly reduce the abundance of lentic *P. parva* populations. However, some differences were noted in the efficacy of the two methods. Whilst physical removal was shown to lead to short-term population suppression, high effort was required to overcome the compensatory responses of the remaining fish (*cf*. Chapter 2). Biocontrol offered a longer-term suppression and thus potentially better ecological outcomes, although the manipulation of numbers of piscivorous fish could result in some unexpected consequences. Nonetheless, this revealed that these methods can provide effective methods for reducing population abundances in small ponds, thus further reducing dispersal opportunities, although neither was capable of eradicating pond populations in the manner than application of biocides can (Britton and Brazier, 2006).

For the following sub-sections, the outputs of each investigation are assessed in more detail (Chapters 2 to 6) and their applicability to managing *P. parva* in England and Wales, and their potential application for other non-native fish species, are discussed. Conclusions and future research opportunities are then summarised.

7.2. Latitudinal and density effects on *Pseudorasbora parva* growth

One of the most important questions in invasion ecology is why some organisms are able to establish and disperse following their introduction into a new environment, and thus develop invasive populations, while others fail. Indeed, why does a known invasive species, fail to establish in some areas of within its extended invaded range, yet can thrive in others (Copp et al., 2007)? Viewing the invasion process as a series of sequential stages (Figure 1), with biotic and abiotic constraints present at each stage, has assisted analysis of the traits that enable a species to progress through this process (Kolar and Lodge, 2001; Cucherousset et al., 2009). In general, an introduced species with a ‘fast’ or ‘*r*’ type life-history strategy, with traits including high reproductive effort, early maturity, rapid growth, are considered advantageous for the establishment of a non-native species (Lodge, 2003), with these also favouring their dispersal and facilitating their attainment of high population densities (Rehage and Sih, 2004). Thus, the analysis of life-history traits of a specific introduced species and how these are expressed over spatial scales within the invaded area can be used to inform and develop understandings of not only the invasion process (Cucherousset et al., 2009), but how these can be utilised to inform their management. Testing the factors influencing the expression of life-history traits of non-native species across large spatial scales has primarily focused on identifying relationships across latitudinal gradients (Benejam et al., 2009; Cucherousset et al., 2009), with these strongly influencing the somatic growth rates of *L. gibbosus*, *M.*

salmoides and *G. holbrooki* (Benejam et al., 2009; Cucherousset et al., 2009; Britton et al., 2010b). Previous studies on the expression of life-history traits in *P. parva* showed latitude was an important determinant across their entire range (Gozlan et al., 2010b), although it has also been postulated that population density can also significantly influence growth rates (Britton et al., 2008b; Britton and Gozlan, 2013). Here, aquaria experiments using fixed food supplies revealed that fish number (i.e. *P. parva* density) had a greater effect on *P. parva* growth rates than temperature, despite a range of 6 ^{o}C being used (19 to 25 ^{o}C). This contrasts to *L. gibbosus*, which suggests that it is thermal constraints imposed by the climate of England that inhibit their ability to invade and establish new populations (Copp et al., 2004; Copp and Fox, 2007). Indeed, the experimental pond study and field data then revealed that *P. parva* density had the greatest effect on their growth, despite use of latitudinal (and thus temperature) gradient. This suggests that plasticity in growth of *P. parva* is important in facilitating their establishment, with their fast growth at low densities assisting their avoidance of demographic bottlenecks (Britton and Gozlan, 2013). It also indicates that where operations to control or eradicate populations only reduce their numbers to low levels, rather than zero, the remaining fish might be able to re-establish their populations relatively rapidly due to elevated growth rates that potentially leads to their earlier development and thus reproduction.

Given that somatic growth rates, rather than the full suite of life history traits, were studied, some caution must be applied to extrapolating these data too far. This is because there are often complex trade-offs between somatic growth rates and reproductive effort, with other abiotic and biotic pressures also providing constraints (Johnston and Leggett, 2002; Power et al., 2005). It could thus be important to consider trait flexibility over larger spatial scales and thus, thermal variability, in addition to population densities, and even time since the initial introduction, given that all of these parameters can influence life-history traits of non-native species.

7.3. Consistency in life-history trait expression and spawning season length of *Pseudorasbora parva*

In addition to determining the influences of spatial effects on non-native fish populations, researchers have also identified the differences in the expression of life-history traits of non-native species between their native and introduced ranges (Britton et al., 2010b; Oyugi et al., 2010). Whilst such studies contribute to the knowledge of non-native species, they do not determine the variability of traits such as somatic growth, at differing stages of the invasion, which has already been demonstrated to be plastic in the case of *P. parva* (Britton and Gozlan, 2013; Davies and Britton, 2015a). Reproductive effort of *P. parva*, specifically in its relation to invasion stage and how it changes over time and population density has received relatively little attention. The outcomes of this investigation thus contribute to current understandings of the mechanisms involved in the establishment of new *P. parva* populations by demonstrating that reproductive effort and somatic growth were consistent across four populations in adjacent ponds with similar densities and, as per other studies on *P. parva*, they generally revealed *r*-selected traits (Gozlan et al., 2010b). However, previous studies on the reproduction of *P. parva* in both native and introduced range mainly suggested fractional or batch spawning occurs, where batches of eggs are developed over the reproductive season and laid in a series of events over extended time periods (e.g. Gozlan et al., 2010b). In these four populations, however, a relatively short reproductive season was noted, with peak gonado-somatic index occurring over a three week period in May and June at temperatures between 13.2 to 14.6 °C, with a subsequent decline with no further peaks that year. These findings, along with oocyte diameters, indicated that the study populations underwent a single, discrete spawning event, at least in 2013.

The experimental design chose four populations of differing age, or time since *P. parva* introduction, with the expected outcome being a greater investment in reproductive effort and somatic growth in the younger populations. This was not so, with consistency shown across all four populations. The lack of variability was attributed to be a result of *P. parva* attaining the same consistency in their densities in their ponds relatively quickly, indicating their potential for rapid colonisation of new ecosystems within their introduced range (< 2 years). These findings are not only important in the understanding of *P. parva* specifically and small pest-fishes generally, but also their management and their likely response to management actions. Current management of lentic *P. parva* populations in England and Wales relies on piscicide-based extirpations (Britton et al., 2008b), the use of rotenone in such extirpations means they can only be conducted when there are no viable eggs of the target species present (Ling, 2003). Based on the literature available at the time (e.g. Gozlan et al., 2010b), which suggested a protracted reproductive season and batch spawning events, the current window for these operations was limited to the period of late autumn through to early spring periods, when temperatures tend to be low that constrains the efficacy of the toxin (Britton and Brazier, 2006). Consequently, should populations in England and Wales generally show a discrete spawning pattern in the same manner then these outcomes should assist the design of management programmes that enable operations to be implemented throughout much of the late summer and autumn period.

7.4. Preventing introductions of *Pseudorasbora parva*

The prevention of accidental introductions of non-native species is the most effective management option for preventing subsequent invasions (Jarrad et al., 2010; Britton et al., 2011c). Indeed, the ability to quantify the efficacy of such a scheme through probability of detection allows environment managers to best target their resources. Where risk is deemed to be high or the manager is risk-averse, increased effort and resources can be attributed to this (Stokes et al., 2006; Finnoff et al., 2007). Thus, fish movements from waters known, or suspected to have non-native fish species present, can be specifically targeted, limiting resource expenditure. Despite this, there are few schemes designed to prevent such introductions and, furthermore, their efficacy is unknown.

Here, the testing of an audit scheme that was in use at the time across England and Wales revealed that hitch-hiking species can be detected in batches of other fish prior to their release into the wider environment, providing skilled practitioners and sufficient search effort is utilised. False-negative results during these assessments did reveal an elevated risk of introductions occurring when these were not used. Consequently, unless regulatory bodies invest in their staff to ensure their training and issues awareness are sufficiently high then there is a high likelihood of accidental introductions occurring, irrespective of the structure of the prevention scheme in place.

Where there is a desire to prevent or decrease such false-negative results, the use of environmental DNA could be used. For example, Egan et al., (2015) demonstrated the efficacy of environmental DNA in the detection of non-native bivalve species quagga mussel *Dreissena bugensis* and zebra *D. polymorpha* in the ballast water of commercial ships, with up to 100% detection rates. However, such technology may not be readily available yet and its costs may prove prohibitive for wider application at the present stage, given budgetary constraints.

The current audit procedure is conducted by the Environment Agency, which regulates the introduction of freshwater fish into the wild, as part of its statutory duties. However, with at least 4500 regulated stockings of fish taking place each year (Hickley and Chare, 2004), environment managers have to select the most appropriate fish stocking to audit. Given that aquaculture is the primary driver behind non-native fish movement (Gozlan et al., 2010a), then those events utilising fish from aquaculture sites should presumably be deemed as being high risk and thus be the target of audits.

7.5. Reducing risks of *Pseudorasbora parva* dispersal into watercourses

Dispersal of non-native species can be a result of anthropogenic interference, or natural mechanisms, such as drift (e.g. Copp et al., 2002; Gozlan et al., 2010a). Indeed, it was the result of an import of fish destined for an aquaculture facility to be reared for recreational fisheries that led to the inadvertent introduction of *P. parva* to England (Gozlan et al., 2005). Given that such facilities rely on a constant flux of water though the rearing ponds in order to maintain good water quality (Baluyut, 1989), or at least have some connection to adjacent watercourses in periods of pond drain-down (Hovarth et al., 2008), opportunities for fish escapement can occur. Whilst the assessment of non-native fish dispersal from an aquaculture facility (Chapter 5) showed high numbers of *P. parva* passively dispersing from the site, this only occurred under elevated flows. With the success of a non-native establishing a population determined, at least partly, by propagule pressure (Britton and Gozlan, 2013), it is clear that aquaculture sites with non-natives present require increased biosecurity during these key management activities, to lower or prevent such propagule pressure of non-native species into the wider environment.

The establishment of a population by some of the most successful non-native species has been partly attributed to their dispersal abilities, with this as a key aspect of the invasion success of species such as the common carp *C. carpio* and *D. polymorpha* (Moyle, 1986; Lodge, 1993). Thus, species that have similar dispersal attributes may be more likely to establish new populations following an initial introduction. Limiting, or preventing the drift of propagules from an infested site is one of the most desirable of management strategies, given that preventative measures are often far outweighed by the costs of controlling or eradicating a species once it is established and spread (Vander Zanden et al., 2010). With populations of *P. parva* known to still exist in the wild in England and Wales, some with direct connections to the lotic environment, it is critical this 'secondary spread' is curtailed (Vander Zanden and Olden, 2008), if their establishment of sustainable populations in the wider environment is to be avoided.

Also of note was that the dispersing *P. parva* from the aquaculture facility were adults, rather than larvae or juveniles, as the likelihood of establishment success is greater. Not only are adult *P. parva* capable of passive drift, but active migration too (Pollux and Korosi, 2006). Additionally, if pond drain-downs occurred during the reproductive season (Chapter 3), establishment of new populations could be rapid. However, subsequent to a non-native species' secondary dispersal, successful establishment within the recipient environment is dependent on multiple factors (Lonsdale, 1999). Biotic resistance, whether that be from the extant biotic community processes such as competition (Case, 1990) or predation (Baltz and Moyle, 1993), can prevent a non-native species establishing. Native riverine fish species in England and Wales include a number of piscivores, such as the obligate piscivore *E. lucius* and facultative piscivores including *P. fluviatilis* brown trout *S. trutta* and chub *Squalius cephalus*. Such species could thus resist invasive populations from developing in the wider environment (Chapter 6), particularly if propagule pressure was low and dispersal events sporadic.

7.6. Control and containment of *Pseudorasbora parva*

The suite of measures available for the effective prevention of biological invasions include horizon scanning (Roy et al., 2014), legislative controls on import (Lodge et al., 2006) and auditing of regulated consignments (Davies et al., 2013; Chapter 4), with these all seeking to prevent the initial introduction of a non-native species and/or their secondary spread (Vander Zanden and Olden, 2008). Should a non-native species still manage to be introduced, establish and risk dispersing then the decision has to be made of how to deal with a species now at risk of invasion. Whilst over 700 eradication operations have been used to successfully eradicate non-native species on islands, there are very few examples of continental-wide eradications (Howald et al., 2007), with management of non-native fish populations over large spatial areas rare or perhaps even non-existent (Britton et al., 2011a). Additionally, eradications can be expensive with success not always guaranteed (Britton et al., 2010c; 2011a). Britton et al., (2011b) determined that the cost of a single rotenone-based eradication of *P. parva* in a relatively confined waterbody was exceeded after approximately 9 years of cumulative costs of long-term control and containment, suggesting that despite the high initial cost, eradication was deemed to be the appropriate response. Thus, eradications should be considered when long-term costs of damage or control exceed the short-term cost of eradication (Myers et al., 2000).

The quandary whether to eradicate or not is further compounded upon the discovery of a new non-native species. Should managers initiate a costly eradication programme, without knowing the total distribution of the new species (i.e. the likely success of management), or instead implement immediate control and containment measures to prevent the species from spreading to provide time to obtain further information on the species and its potential impacts and distribution? Here, the use of a native predator (*P. fluviatilis*) and low-technology removals of *P. parva* (Chapter 5) demonstrated high efficacy of reducing lentic *P. parva* populations for a nominal cost, particularly when compared to the elevated cost of piscicide-based extirpations (Britton et al., 2008a). Extirpation of *P. parva* through the use of rotenone has been successful in 19 lentic waterbodies since 2005 (Environment Agency, unpublished data), however, very few operations are carried out each year due to limited resources and the fact that rotenone does not affect fish eggs, limiting the time periods when it can be applied (Ling, 2003). Therefore, known *P. parva* populations will remain for potentially long time periods, irrespective of the desire of regulatory authorities to extirpate them. These populations can act as reservoirs for *P. parva* further dispersal (Chapter 4 and 5) and, as such, should be managed accordingly.

Should *P. parva* populations be detected in an area where rotenone-based eradications are not feasible, in areas of high conservation value for example, or where the cost of rotenone would be prohibitive, then the methods described here could provide a viable alternative control. Additionally, human health concerns remain over the use of rotenone (e.g. Sherer et al., 2003), which may impact on its future application. That said, artificially increasing the predation pressure on a native fish community can increase top-down effects and induce food-web alterations (Eby et al., 2006), so caution still has to be applied when considering biocontrol.

Given the low cost and demonstrated efficacy of the introduction of *P. fluviatilis*, it is recommended that the remaining, un-managed *P. parva* populations should be intentionally stocked with *P. fluviatilis* where they are not present (they are often removed or absent in managed pond and lake fisheries due to a belief that their predation will have a deleterious effect on the fish community). Not only will this suppress the *P. parva* populations, reducing their impacts on the native ecosystem, but it will also lower the propagule pressure, reducing the likelihood of further dispersal (Chapter 5). This can be complemented by targeted removals at pre- and post-spawning times.

7.7. Contribution to management of *Pseudorasbora parva*

Despite the concerns and clearly demonstrated impacts of *P. parva* on native species and ecosystems (Welcomme, 1992; Arkush et al., 1998; Gozlan et al., 2005; Britton et al., 2008; Britton et al., 2010a; Gozlan et al., 2010b), the only management technique currently employed on their populations in England and Wales has been extirpation via rotenone application. Indeed, the outcomes presented in this thesis are the only known studies of alternative control techniques for such small pest fishes in England and Wales to date. Each study targeted a specific stage of the invasion process (Figure 1) and demonstrated the variety of management methods where the use of piscicides may not be feasible.

Piscicide-based eradications can be costly (Britton et al., 2008a), with non-target species also affected (Ling, 2003). There are still *P. parva* populations in England

and Wales despite an on-going eradication programme, and the recent discovery of 4 more lentic populations in just 2 years (Environment Agency, unpublished data) suggests that there may be a number of 'new' sites to be identified. This presents a problem for environment managers (e.g. Defra and Environment Agency), who have invested substantial amounts of money into the extirpation of *P. parva* from England and Wales since 2005. Heavy budgetary cuts of over 30% (Howard, 2015) have left Defra, and the Environment Agency, with even fewer resources, imposing greater pressures on environment mangers to make best use of the finances available. The need for the development of holistic, risk-based studies of management tools and techniques for the control of *P. parva* in England and Wales is clear. The design of a robust audit procedure (Chapter 4) can minimise the further dispersal of *P. parva* via anthropogenic mechanisms, from waters known, or suspected to contain *P. parva*, preventing their secondary spread and colonisation of new waterbodies. The assessment of *P. parva* drift from an aquaculture site (Chapter 5) highlighted the importance of increased biosecurity during key management operations on not just aquaculture sites but any water body containing invasive pest fishes and a direct hydrological connection to the wider environment. These findings can be used to ensure the correct preventative measures are in place prior to such actions, reducing resource allocation, yet still offering sufficient protection of the environment.

The impacts of *P. parva* on native biota in England and Wales has been well documented (e.g. Britton et al., 2007; 2009; 2010a; Gozlan et al., 2009; Andreou et al., 2012), yet little resource has been invested in aspects of their ecology and how this compares to their native range and other invaded areas. Such information may offer insights into specific stages of their invasion, or life cycle, at which control measures are best targeted. Chapter 2 identified that intraspecific density had the greatest influence on *P. parva* somatic growth and plasticity of growth in *P. parva* was a fundamental aspect of their ability to rapidly establish populations. Upon detection of a *P. parva* population, knowledge of the age structure and their growth can assist in determining in whether the population is still undergoing expansion, indicating time since introduction. Additionally, the development of novel management techniques (Chapter 6) has shown to provide an effective means of population suppression, overcoming compensatory effects in *P. parva* reproduction.

Subject to the desired management objective, if population extirpation is desired, then the use of rotenone may be the best option, resource and practicability permitting, given that both the biocontrol and removals did not lead to extirpation. However, the limited number of piscicide-based operations per annum means some *P. parva* populations go un-managed. The introduction of *P. fluviatilis* into such waters, or a structured removal programme (via trapping) could help suppress these populations, minimising impacts within the waterbody and reducing the wider ecological risk. Moreover, if both methods are used in combination, the reduction of a population to its minimum viable population size could yet its extirpation through the effects of environmental stochasticity or Allee effects (Allee et al., 1949; Lande, 1993). If this did occur, then these control methods could be very useful, relatively cheap and non-controversial control methodology for the management of extant *P. parva* populations.

Thus, the increased knowledge of our understandings of the ecology of this highly invasive, pest-fish species and the development of tools and techniques described in this thesis will provide environment managers with a suite of control methods for the prevention of the introduction of *P. parva*, their further dispersal and population suppression.

7.8. Contribution to management of introduced fishes

The use of *P. parva* as the model species is justified given the suite of life-history traits they exhibit that are advantageous in colonisation of new environments (Movchan and Smirnov, 1981; Ricardi and Rasmussen, 1998), allied to their broad habitat requirements (Asahina, 1990; Domaniweski and Wheeler, 1996; Pollux and Korosi, 2006; Gozlan et al., 2010b; Onikura and Nakajima, 2013), potential to harbour highly detrimental diseases (Arkush et al., 1998; Gozlan et al., 2005; Ahne and Thomsen, 2006) and their classification as a pest species (Welcomme, 1992). Such traits are also exhibited in many other small-bodied, non-native fishes in Europe, for example pumpkinseed *Lepomis gibbosus* (Fox et al., 2007); fathead minnow *Pimephales promelas* (Marchetti et al., 2004); sunbleak *Leucaspius delineatus* (Beyer et al., 2005) and black bullhead *Ameiurus melas* (Ruiz-Navarro et al., 2015). Thus, the management techniques outlined in this thesis are equally applicable to the control and management of other small-bodies non-native fishes. Additionally, despite the focus of the work described in previous chapters being undertaken in a defined Northwest European and temperate context, the suite of control measures described can still be utilised in other climatic regions and indeed, has global applications, particularly given the invaded range of the model species, *P. parva*.

The need for adequate management of non-native species has led to the development of numerous techniques, such as ‘Trojan genes’ for the control of introduced Nile tilapia *Oreochromis niloticus* (Gutierrez and Teem, 2006) and pheromone laced traps for sea lamprey *Petromyzon marinus* (Johnson et al., 2008) with varying degrees of success, some more than others (Gozlan et al., 2010a). Here, the use of a native predator (Chapter 6) was contrary to ‘classical biocontrol’, where one non-native species is introduced to control another (Howarth, 1983). However, the use of non-native species in such a way can have undesirable outcomes, particularly if the ‘control’ species develops a preference for native species. Such adverse interactions are often apparent where generalist predators are used, for example, the introduction of foxes *Vulpes vulpes* into Australia to control rabbits *Oryctolagus cuniculus* resulted in the decimation of several marsupial species, while the rabbits did not suffer heavy predation (Sih et al., 2010). Nonetheless, supplementary introductions of native generalist predators are capable of population suppression in non-native populations. These findings provide a framework, on which environment managers can construct effective control strategies for other non-native fish species, relying on predators naturally occurring within the target area but that are otherwise often controlled or removed for fishery management purposes. Such control mechanisms are more likely to be accepted by the public and environment managers, more so than piscicide-based eradications, for example, the attempted extirpation of *E. Lucius* from Lake Davis, California (Lee, 2001). Additionally, the considerable presence of other small, non-native pest fishes in lentic environments globally, such as *Gambusia* species (e.g. Pyke, 2008), Trinidadian guppy *Poecilia reticulata* Peters (Deacon et al., 2011) and especially minnow *Phoxinus phoxinus* in countries such as Norway (Museth et al., 2007), suggests that the findings here have high application to the management of small, pest fishes generally.

Given that preventing the initial introduction of a non-native species is the most desirable management action (Jarrad et al., 2010), a commensurate resource should be allocated. Should managers fail in preventing the introduction, it is desirable to know the ecology of the introduced species, to adequately target management action.

Literature on the life-history and ecology of *P. parva* within its native and introduced range was readily available, and indeed, used to influence the timing of piscicide extirpations. However, the findings in Chapter 3 are contrary to that described (e.g. Gozlan et al., 2010b), which indicates a much larger operational window than originally anticipated. This demonstrates the necessity of understanding how an introduced species performs and interacts with the indigenous biota and ecosystem and its likely response to management activities.

7.9. Conclusions and future directions

Analysis of the life-history traits, specifically reproduction, of *P. parva* in Chapter 3 revealed a single, short reproductive season coinciding with temperatures between 13.2 to 14.6 °C, contrary to findings on their populations in other countries (e.g. Gozlan et al., 2010b). It is unclear whether this was a particular characteristic of the *P. parva* populations in the field trail, or representative of other populations in England and Wales. It is suggested that further studies, based on the experimental design and procedure carried out here, are conducted on extant *P. parva* populations to determine whether this trait was site specific. Further to this, a lack of plasticity in reproductive traits was also noted, again, contrary to current literature (Adamek and Siddiqui, 1997; Rosecchi et al., 2001; Davies and Britton, 2015a), although this was measured across four similar population densities. The lack of plasticity in reproductive effort was assumed to be due to *P. parva* already attaining their maximum densities (i.e. their population abundances were no longer growing), despite a difference in population age. Thus, it would be beneficial to observe how such traits differ in recently introduced populations (< 2 years old), when *P. parva* are still in their exponential phase of colonisation. It is hypothesised that they may undergo 2 or 3 distinct spawning events over a protracted reproductive season (Záhorská and Kováč, 2009b), to rapidly generate progeny and overcome resistance offered by the native environment and biota within.

The ability of a fish stocking audit to prevent the accidental introduction of non-native fishes ensures it will be a valuable tool in the management of non-native species. Expansion of this procedure, to include other likely hitch-hiking species, could provide a measure of probability of detection for a number of non-native species. In place of *P. parva*, model species could also include macro-invertebrates such as non-native crayfishes (Manchester and Bullock, 2000) and Ponto-Caspian species such as the 'killer' *Dikerogammarus villosus* and 'demon' shrimps *D. haemobaphes* (MacNeil et al., 2010), plus species highlighted as likely to arrive in England and Wales in the future via horizon-scanning exercises (Roy et al., 2014). This will provide environment mangers with information on likelihood of detection of a hitch-hiker, given a specific resource allocation. Allied to preventing the introduction of a non-native is determining likely sources. To date, no known surveillance of waterbodies for *P. parva* populations exists, other than the possible capture of individuals through routine fisheries monitoring surveys or via angler reports. That said, the sampling gears typically used in such routine surveys have been shown to yield poor detection of *P. parva* even when present within a waterbody in relatively high densities (Britton et al., 2011c). Indeed, the discovery of the *D. haemobaphes* in the River Severn in 2012 (GB NNSS, 2012) highlighted the importance of a surveillance programme for non-native species, where subsequent to this discovery, it was found in many other river catchments over a large spatial area. This suggests that the initial introduction went un-noticed, allowing the further spread of this species. The use of environmental and biological parameters of waters known to contain *P. parva* should be collated and modelled to predict other waterbodies with similar parameters. These sites can then be specifically targeted for audit, using the appropriate gears, or for application of environmental-DNA technologies to identify presence/ absence.

The findings from Chapter 5 highlight the risk of increased propagule drift rates from a site containing non-native species during periods of elevated flows. This was only quantification of the risk and the work undertaken as part of this thesis did not seek to address how to prevent such drift. It is impossible to prevent water egress from an on-line site, particularly during pond-drain down. However, a procedure or mechanism should be tested, which reduces or even eliminates non-native propagule drift. Mechanical screens to intercept individuals and prevent them from escaping via a hydrological connection are already stipulated as a condition for keeping certain species. However, they are not designed to prevent the escapement of small-bodied fish, nor juveniles or eggs. Given that they cannot impede water flow from a site, lest it increases the flooding risk and greater escapement of propagules, a design has to still permit water flow, but retain any drifting life stages. This could be achieved through the use of 'gravel traps' where water cascades onto a large surface area of gravel and percolates away. Such a scheme would need to be tested to determine its efficacy on preventing different species and different life stages from drifting.

Biocontrol of *P. parva* populations using *P. fluviatilis* as a predator was shown to significantly reduce their abundance. Despite *P. fluviatilis* being native, there is a perceived concern over their introduction to lentic waterbodies by fishery managers, as it is believed that they rapidly reproduce and can have significant impacts on the densities of other species. The ‘stocking-up’ of food webs can also incur detrimental top-down effects (Eby et al., 2006). Thus, it is necessary to trail alternative native predatory species, such as *E. lucius*, *S. cephalus* and *S. trutta*. The added benefit of using *S. trutta* as a biocontrol is that they can be reproduced as triploid (Arai and Wilkins, 1987), significantly reducing any natural reproduction and population growth. This would limit any ‘stocking-up’ of food webs and allay fishery managers concerns.

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9. Appendix I

Questionnaire used to determine auditor level of experience

Invasive non-native fish movement audit trails

Auditor questionnaire:

Name	

Please put an ‘x’ in the most appropriate box for questions 1 – 7.

Background:			**x**		**x**		**x**
1	Number of years in relevant post	< 2 years		2-5 Years		> 5 Years	
2	Level of experience of fish netting exercises	Novice		Intermediate		Experienced	
3	Level of experience of non-native fish	Novice		Intermediate		Experienced	
4	**Native** fish ID skills	Low		Average		Excellent	
5	**Non-native** fish ID skills	Low		Average		Excellent	
Specifics:							
6	Knowledge of current non-native issues (impacts, benefits, etc)	Low		Average		Excellent	
7	Experience of fisheries audit scenarios	None		Few		Many	

~~~~~ Please leave this row blank ~~~~
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Fish ID:

Please mark the following fish as '**native**' or '**non-native**' and add **common name** where known.

Native/ non-native? **Common name:**

Native/ non-native? **Common name:**

Native/ non-native? **Common name:**

Fish ID (cont.):

Native/ non-native?

Common name:

Native/ non-native?

Common name:

Native/ non-native?

Common name: