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The impact of introduced European catfish (*Silurus glanis* L.) in UK waters: a three pond study

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Abstract

Invasive fish species pose a threat to native fish species diversity across the globe with the extinction of many native and endemic fish species. There are concerns about the increase in non-native *Silurus glanis* introductions for trophy angling into the UK and possible harmful impacts to native fish communities. This study aimed to determine a) how different *S. glanis* size groups may influence establishment success into new environments by using parameters such as growth and trophic alterations and b) the potential risks posed by holding non-native *S. glanis*.

Three ponds and a lake situated in the flood zone to the surrounding river catchment in southern England were used as study sites. A first application in Europe of the European Non-native Species in Aquaculture Risk Analysis (ENSARS) and stable isotope analysis contributed to address the study aims.

From this study of the three *S. glanis* size groups there was a suggestion of differences in growth and trophic impact were correlated with fish size suggesting that life history traits can influence establishment into invaded ponds. Large sized *S. glanis* exhibited high trophic position in food web which was indicative of an apex predator in pond communities. Across all *S. glanis* size groups, individuals had distinct differences in isotopic $\delta^{13}C$ range of diet. This suggests that *S. glanis* have plasticity in trophic strategy and phenotypic response within new environments which may influence establishment success.

The ENSARS analysis distinguished between the potential risks of harmful ecological and socio-economic impacts posed by the likelihood of *S. glanis* establishment and spread of disease transmission to native fish communities. The analysis suggested that shallow drainage channels and floodplains were likely to be favourable spawning and breeding habitats. A novel ancyrocephalid monogenean parasite *Thaparocleidus vistulensis* was detected upon *S. glanis* which was a new finding for the UK.

Climate type and habitat niche also influence establishment of non-native fish with predicted thermal changes in aquatic habitats driven by climate change likely to facilitate *S. glanis* invasiveness into aquatic habitats throughout the UK. Consequently the present risk status of non-native *S. glanis* invasive potential is liable to change in future years which should be taken into account by updating appropriate risk and regulatory policies in non-native fisheries management.

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1.2 Current Research

1.2.1 Life history traits

Non-native species are organisms that have been introduced into new environments intentionally or accidentally via anthropogenic activities. These may involve global trade exchanges with transport between countries enabling non-native species to cross geographical barriers, resulting in their dispersal beyond natural ranges. Non-native species may invade into introduced ranges if climate or habitats provide similar conditions to those of their natural range (Hubbs, 1977; Gozlan et al. 2010, Cucherousset et al. 2018). For example, in the UK, an invasive plant species such as Oxford ragwort (*Senecio squalidus*) has spread from its original site at Oxford botanical gardens into the wild by seed dispersal from trains travelling along railway tracks adjacent to these gardens. Studies have indicated that their dispersal throughout the UK is related to development in railway networks from Oxford to other geographical regions in the UK (Lenda et al. 2014).

In recent decades, there has been a growing concern about the frequency of accidental and deliberate introductions of non-native fish species and their risk of invasion into freshwater habitats around the world (Garcia-Berthou et al. 2005; Britton et al. 2010; Copp et al. 2016b). Invasive species are considered a major threat to biodiversity. Exchanges of non-native fish species from formerly isolated freshwater habitats is likely to facilitate species homogenisation resulting in loss of native species because they lack the evolutionary experience to compete or avoid predation from invaders (Vila-Gispert & Moreno-Amich, 2003; Bohn et al. 2004; Britton et al. 2010; Cucherousset et al. 2018).

Consequently gaining an understanding of the processes related to colonisation of non-native fish species into new environments is instrumental in minimising their impact. Colonisation involves interrelated phases such as introduction, establishment, dispersal and impact with invasion success of introduced species influenced by ecological drivers such as species life history traits (Kolar & Lodge, 2002; Vila-Gispert & Moreno-Amich, 2003; Copp et al. 2007a; Guillerault et al. 2015) (See Figure 1.1).

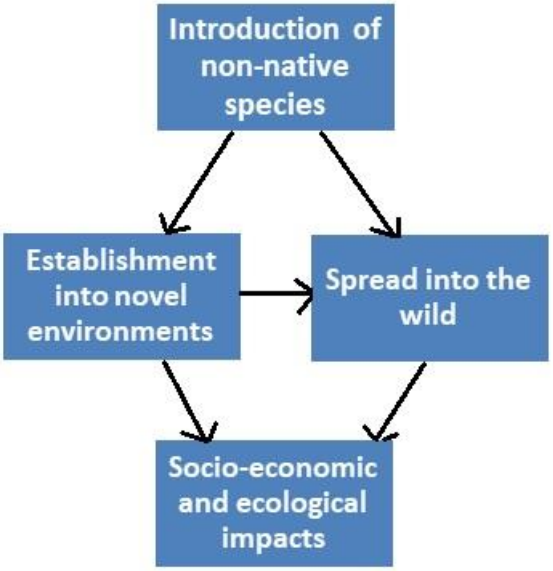

Figure 1.1. Phases of non-native fish species colonisation into new environments adopted from (Kolar & Lodge, 2002; Vila-Gispert & Moreno-Amich, 2003).

Predicting the invasiveness of non-native fish species into new environments is important in fisheries management with non-native fish species global databases providing information about r and K life history traits as an indicator of potential invasiveness. Information is provided about fish species growth, fecundity, lifespan, reproductive, trophic strategies, and other factors such as climate or habitat suitability so as to categorise invasiveness risks (Bohn et al. 2004; Copp et al. 2005b; Copp et al. 2007a; Piria et al. 2016).

The impact of life history traits in invasion success is well documented, for example the invasion of non-native large mouth bass (*Micropterus salmoides*, pumpkinseed (*Lepomis gibbosus*) and roach (*Rutilus rutilus*) into Lake Banyoles in the Iberian Peninsula, southern Europe. These introduced species dominate fish communities throughout the lake, displacing native species such as Mediterranean barbel (*Barbus meridionalis*) and chub (*Leuciscus cephalus*) owing to their higher fecundity or larger size (Mooney & Cleland, 2001; Vila-Gispert & Moreno-Amich, 2003; Boavida et al. 2015). Reproductive strategies such as parental care of eggs and guarding of young may facilitate invasiveness, with higher survival of young owing to parental care by guarders and bearers (Marchetti et al. 2004; Copp et al. 2009a). Similar results were found

elsewhere in England and Wales, with over 50% of established fish invaders demonstrated parental care of young, contributing to their invasion success (Gozlan et al 2003; Britton et al. 2010; Gozlan et al. 2010; Cucherousset et al. 2018).

In addition to this, life history traits such as plasticity in trophic strategy may enable invaders to exploit seasonal and local food resources. For example, the invasion success of non-native racer goby (*Neogobius gymnotrachelus*) populations into major river catchments of Poland and Laurentian Great lakes in North America were attributed to their trophic plasticity in feeding upon abundant molluscs and crustaceans avoided by native fish species (Kostrzewa & Grabowski, 2003; Kakareko et al. 2013).

1.2.2 Environment & non-native fish species impacts

Colonisation of non-native fish species in the wild may be influenced by environmental characteristics of recipient aquatic habitats, particularly species tolerance to environmental extremes. Fish invaders resilience to low ($\leq 13^0$C) water temperatures during winter time may be critical to their survival, particularly in larvae and fry stages (Kostrzewa & Grabowski, 2003; David, 2006; Ricciardi, 2007; Copp et al. 2009a; Cucherousset et al. 2018).Variation in disturbance levels, ecosystem trophic functioning and resilience of native species may influence colonisation. Depauperate aquatic habitats with few predators are more likely to be colonised than those with high diversity of species (Strauss et al. 2006; Gozlan et al. 2010; Capra et al. 2014).

High propagule pressure of non-native species released into aquatic habitats may trigger invasion meltdown processes which will result in biodiversity loss. For example, multiple introductions of non-native large mouth bass (*Micropterus salmoides*) and cyprinids released into Lake Naivasha in South Africa for recreational angling accelerated ecological degradation with loss of endemic fish species such as black lampeye (*Aplocheilichthys antinorii*). It is likely that these naïve fish lacked the evolutionary experience to avoid predation or successfully compete with invaders resulting in evolutionary change (Ricciardi & Rasmussen 1998; Garcia-Berthou et al. 2005; Hickley et al. 2008; Sheaves et al. 2015).

Other detrimental impacts to native fish species caused by the introduction of non-native fish species include disease transmission, changes in trophic structure, increased competition, predation of native species and hybridisation (Cambray, 2003; Martino et al. 2011; Cucherousset et al. 2018). The risks of disease emergence from novel pathogens and parasites caused by non-native fish species exchanges to native fish species is of wide concern. For example, the

transmission of novel parasite (*Anguillicolloides crassus*) from cultured European eels (*Anguilla anguilla*) to wild populations has been linked to inadequate health and quarantine measures at aquaculture units involving species transfers from mainland Europe to Africa and America. The infection to wild *A. anguilla* populations that followed was linked to their decline with infected specimens unable to migrate to spawning grounds for reproduction (Kirk, 2003; Gozlan et al. 2010; Cresci et al. 2017).

Similar disease related mortalities have been reported with the accidental release of non-native topmouth gudgeon (*Pseudorasbora parva*) into the wild via lapses in biosecurity at fisheries. This highly invasive species acts as a healthy host carrier for a novel parasite known as the rosette agent (*Sphaerothecum destruens*) which can switch host causing the mortality of susceptible fish species such as sunbleak (*Leucaspius delineatus*), Chinook salmon (*Oncorhynchus tshawytscha*) and Atlantic salmon (*Salmo salar*) (Gozlan et al. 2005; Laverty et al. 2017). These cases highlight the threat to biodiversity due to introduced pathogen spread by non-native fish species movements and prove the need for better quarantine regulations to prevent transmission of disease epidemics to wild fish populations (Gozlan et al. 2010; Cucherousset et al. 2018).

The dispersal of non-native fish species into the wild may adversely impact trophic functioning in habitats with increased interspecific competition for food resources with native fish species. For example, the introduction of non-native brook trout (*Salvelinus fontinalis*) into mountain streams in south west France resulted in the restricted foraging of native brown trout (*Salmo trutta*) due to the aggressive dominance of these larger invaders (Cucherousset et al. 2007; Cucherousset & Olden, 2011; Závorka et al. 2017).

It seems that intensity in predation impacts by invaders upon native fish species may vary in aquatic habitats due to the differences in water temperature related to geographical latitude. The addition of non-native zander (*Sander lucioperca*) specimens released into some lake fisheries in Turkey, southern Europe contributed to the collapse of Cyprinidae prey stocks because of their active predation in warm waters. In contrast, thermal constraints in temperate lake fisheries of northern Europe, Canada and the UK, restrained their predation impacts with introduced *S. lucioperca* specimens living in balance with large densities of prey fish (Lewin et al. 2006; Gozlan et al. 2010; Slynko & Kiyashko, 2012).

Hybridization of indigenous fish populations with subsequent loss in genetic integrity of wild fish stocks are some of the detrimental impacts related to non-native fish species introductions.

For example, wild crucian carp (*Carassius carassius*) populations are under threat in some ponds in south east England because of hybridization by non-native goldfish (*Carassius auratus*) which were released into the wild (Wheeler, 2000; Gozlan et al. 2010; Brennan et al. 2015).

1.2.3 Introductory pathways

The increase in non-native fish species invasion into freshwater habitats around the world is related to the expansion in freshwater aquaculture industries. The demand for cultured non-native fish species is endorsed by economic drivers and supportive government subsidies as fish consumption cannot be met from wild capture fisheries alone. Globally, aquaculture supplies over 40% of fish consumption and plays an important role in supporting local and regional economies particularly in developing countries (Pauly & Froese 2012). Aquaculture is an important pathway for non-native fish species exchanges. Better regulatory enforcement is required given the threats posed by non-native fish species escaping into the wild causing ecological and economic harm (Vila-Gispert & Moreno-Amich, 2003; Gozlan et al. 2010; Piria et al. 2016).

Recreational fishing can also facilitate the spread of invasive fish species into the wild. In the last decade, angling has increased by 10% globally and is a popular and highly lucrative sport with over 25 million anglers recorded in Europe. The associated angling annual expenditure in England and Wales was valued at over £1.18 billion in 2017 from fisheries and estimated to increase (Peirson et al. 2001; Cooke & Cowx, 2004; Arlinghaus et al. 2015; Rees et al. 2017). This economic driver accelerates the importation of non-native fish species into lake fisheries. For example non-native round goby (*Neogobius melanostomus*) is frequently stocked into fisheries in Europe with some specimens escaping into major river catchments in Germany where they have established invasive populations, with over 50% species abundance documented in fish catches (Brandner et al. 2013).

Most freshwater anglers fish for salmonids or cyprinids with these species constantly introduced into fisheries around the world, yet with little awareness of their adverse ecological impact upon native species. For some factions of the angling community, the release of non-native fish species into the wild was promoted as an improvement to freshwater habitats (Cowx, 1998; McDowall, 2004; Browman et al. 2019). In many National parks, for example, in South Africa and Italy, introduced fish species outnumber native fish species resulting in ecological homogenisation and loss of endemic fish species from lakes. Similarly, in New Zealand, several native *galaxiidae* species are absent from major river catchments owing to displacement by

invasive salmonid species released into the wild for angling (Cambray, 2003; Cooke & Cowx, 2004; McDowall, 2004; Arlinghaus et al. 2015).

In contrast to aquaculture and recreational fisheries, other activities such as research play a minor role in invasive fish species dispersal into the wild (Gozlan et al. 2010). Biological control is another introductory pathway, with ecological harm from some deliberate non-native fish species introductions caused by shortcomings in regulatory control or gaps in knowledge about their adverse impact. For example, non-native American western mosquito fish (*Gambusia affinis*) and eastern mosquito fish (*Gambusia holbrooki*) were intentionally introduced into Australia in the 1920`s to control mosquitoes. These fish have since become a highly invasive pest in major rivers throughout Australia, with an associated decline in native fish species (Simberloff & Stiling, 1996; Britton et al. 2010; O`dea et al. 2014).

Finally, ballast water from ships is another introductory pathway for invasive fish species. This is likely to be underestimated due to scarcity in information about fish species transported by ballast water. Nevertheless, it seems that ballast water from ships may be an important driver in the spread of several non-native species into the wild (Gozlan et al. 2010; Balaji et al. 2014). For example, non-native Eurasian Ruffe (*Gymnocephalus cernuus*) specimens were accidentally released into the Laurentian Great lakes of North America via ship ballast water. These fish have since become invasive throughout the lakes, displacing native fish species such as yellow perch (*Perca flavescens*) an important species for fisheries and local economies (Ricciardi & Rasmussen, 1998; Padilla & Williams, 2004; Sieracki et al. 2014).

1. 2.4 Trade offs

There is concern about the irreversible ecological harm and economic costs caused by non-native species fish introductions via aquaculture with associated annual costs valued as over US $ 120 billion in North America, US $ 6.9 billion in China and over £20 billion in Europe in 2010. These costs are set to increase with expansion of aquaculture industries globally (Naylor & Burke, 2005; Gozlan et al. 2010; Cao et al. 2015; Rees et al. 2017). For example, it is estimated that over 2 million farmed Atlantic salmon (*Salmo salar*) escape into the Atlantic Ocean every year from open net pens in North America with deleterious impacts to native wild fish populations such as increased competition, interbreeding and disease transmission (Naylor & Burke, 2005; Buschmann et al. 2009). Recent studies found that over 40% of wild *S. salar* specimens captured in the Atlantic Ocean were of farmed origin. This is a clear sign of the decline in genetic diversity of wild fish stocks following hybridisation with farmed fish (Pimental et al. 2000; Naylor & Burke, 2005; Cao et al. 2015).

There is a strong need to prevent these harmful impacts from invasive fish species and as such the challenge for policy makers is to implement effective safeguards. International legislation and risk protocols should be in line with the precautionary approach advocated by the Convention on Biological Diversity in maintaining global biodiversity (Pimental et al. 2000; Wonham et al, 2000; Padilla & Williams, 2004; Copp et al. 2005a; Ricciardi, 2007; Piria et al. 2016).

The development of global databases for non-native fish species is a toolkit used to support communication between countries around the world in managing the risk of invasive fish species. There are several global databases in existence such as the Invasive Marine Pests Database in Australia and Sea Grant Non-Indigenous Species Site used in America. Other databases elsewhere in Europe and UK are the United Nations Database on Introductions of Aquatic Species, FishBase and Database on Introductions of Aquatic Species (DIAS) (Copp et al. 2005b; Britton et al. 2010; Roy et al. 2018). These databases provide information about fish species life history traits and possible adverse socio-economic and ecological impacts as a guideline in non-native fisheries management (Wonham et al, 2000; Padilla & Williams, 2004; Copp et al. 2005b; Bostock et al. 2010; Piria et al. 2016).

Nevertheless, for such schemes to be successful in managing non-native fish species invasions, a review about the variation in data quality for fish species recorded in these databases should be addressed. In general, there seems to be gaps in knowledge about many non-native fish species, with poor record keeping of non-native fish species in developing countries. This in turn is likely to restrict forecasting efficacy in assessing risks of potential non-native fish invasions in fisheries management (Copp et al, 2005c; Copp et al. 2009a; Britton et al. 2010; Britton et al. 2011a; Gozlan et al. 2013; Roy et al. 2018).

The challenge for policy makers has been to implement protective measures with international legislation and non-native fish species risk protocols to protect biodiversity (Ricciardi, 2007; Gallardo et al. 2016a). Overall, it seems that the current legislation and regulatory control protocols for non-native fish species introductions needs to be reassessed with more stringent enforcement required in developing countries (Copp et al. 2005b; Gozlan et al. 2010; Piria et al. 2016; Rees et al. 2017).

In the last decade, there have been several developments in environmental risk protocols for non-native fish species management in developed countries (Copp et al. 2005b; Roy et al. 2018). Some counties such as the UK, France, Belgium and the Netherlands have focused on using environmental risk strategies with horizon scanning in order to improve identification of invasive fish species and prevent their introduction into new environments. These measures involve the rapid detection of the presence of new species in management control so as to prevent their escape into the wild, yet these measures need to be further developed and adopted by other countries (Wonham et al. 2000; Padilla & Williams, 2004; Copp et al. 2005c; Roy et al. 2014a; Piria et al. 2016).

Risk assessment toolkits such as Fish Invasiveness Scoring Kit (FISK) and European Non-native Species in Aquaculture Risk Assessment Scheme (ENSARS) are widely used in fisheries management in the UK with similar adaptations in Europe. The adoption of these risk assessment protocols, coupled with a better understanding of invasive threats has led to the identification of several invasive species such as topmouth gudgeon (*Pseudorasbora parva*) killer shrimp (*Dikerogammarus villosus*) zebra mussel (*Dreissena polymorpha*) and signal crayfish (*Pacifastacus leniusculus*). These species have now been given high risk priority status and require a rapid response by regulatory bodies in the UK to prevent their release into the wild (Copp et al. 2005b; Britton et al. 2010; Gozlan et al. 2010; Copp et al. 2016a).

Unfortunately risk assessment with regulatory enforcement for all routes of non-native fish species introductions has been addressed only in a few countries around the world, in Slovenia and Croatia and other developing countries lack legislative enforcement to control new species introductions (Copp et al. 2005b; Gozlan et al. 2010; Piria et al. 2016). Consequently, the challenge for policy makers is for global enforcement of standardised risk assessment protocols for all non-native fish species introductions. These changes are necessary for more responsible fisheries management practises. For example, in Spain, *S. glanis* are listed in the National invasive non-native species inventory with fish stocking and transfer subject to strict regulatory controls. On the other hand, in Belgium and France *S. glanis* angling is permitted all year round with fewer restrictions (Padilla & Williams, 2004; Ricciardi, 2007; Copp et al. 2009b; Britton et al. 2010; Britton et al. 2011a; Piria et al. 2016; Cucherousset et al. 2018).

Following important pieces of legislation to protect species biodiversity such as Invasive Alien Species (Enforcement and Permitting) Order 2019, EU Water Framework Directive (2000/60/EC) (G05), the Prohibition of Keeping or Release of Live Fish (specified species) Amendment (England) Order 2003, Import of Live Fish Act 1980 (ILFA) and Wildlife and Countryside Act 1981 section 9, the introduction prevention approach is practised in the UK for the control of non-native fish species. Consequently, under these regulations *S. glanis* are classified as a non-native fish species with importation subject to strict regulatory protocols in the UK (Copp et al. 2009a; Britton et al. 2010; Rees et al. 2014; Tarkan et al. 2014; Copp et al. 2016a; Gallardo et al. 2016a; Piria et al. 2016) (See Table 1.1).

Table 1.1 Legislation relevant in protecting native fish species diversity in the UK (Copp et al. 2016a).

Legislation	Policy context	Implications
Import of Live Fish Act England & Wales 1980 (ILFA)	This act made it an offence to import, hold or release certain non-native fish species without a licence such as *S. glanis*	Controls the introduction of non-native fish species into England & Wales to protect native fish species diversity
EC Habitats Directive 1992 (92/43/EEC)	Designated Special Areas of Conservation (SACs) for species and habitats for European Union countries	Protection of habitat diversity by designation of Special Areas of Conservation (SACs)
UK Biodiversity Action Plan 1994	Creation of a national Biodiversity Action Plan for species and habitats under threat	Protection of species diversity in the UK with designated species such as salmonids
Natura 2000	Designated Special Areas of Conservation and Special Protection Areas under the EC Habitats Directive and Birds Directive	Largest network of protected conservation areas for European Union countries in the world
EU Water Framework Directive (2000/60/EC)	Maintain good ecological and chemical status in surface and ground waters by 2015	Obligation to enhance aquatic habitats to protect biodiversity.
EC Freshwater Fisheries Directive (2006/44/EC)	Protection and enhancement of freshwaters to support native fish species	Protection of salmonid and cyprinid fisheries.
The EU Regulation (1143/2014) on invasive alien (non-native) species 2015	Preventative action to fulfil Aichi Target 9 of the Strategic Plan and EU 2020 Biodiversity Strategy	Restrictions on the spread of 37 invasive species listed of concern for European Union countries.
Invasive Alien Species (Enforcement and Permitting) Order 2019	Further enforcement of non-native species introductions into England & Wales	Restrictions upon the release of a list of non-native species harmful to species diversity.

S. glanis introduced into lake fisheries go through coordinated regulatory assessments by governmental bodies such as the Environment Agency and Centre for Environment Fisheries and Aquaculture Science (CEFAS) and Department of Environment, Food and Rural Affairs (DEFRA). These bodies are responsible for fish health inspections, environmental risks, recreational angling and license permits for the introduction of live fish into England and Wales (Copp et al. 2005b; Copp et al. 2009a; Gozlan et al. 2010; Copp et al. 2016a). Further enforcement control is carried out by Natural England and the Home Office which holds supreme legislative enforcement of non-native fish species introductions into the UK (Copp et al. 2005b; Gozlan et al. 2010; Piria et al. 2017). The stocking of *S. glanis* into fisheries requires authorised consent with an ILFA licence permit obtained from (DEFRA) that needs to comply with specific protocols to ensure good biosecurity. Lakes should be enclosed and lie outside of the flood zone to mitigate threats of flooding and fish dispersal into the wild (Copp et al. 2009a; Britton et al. 2010; Rees et al. 2017).

Such concerns underline the need for better cooperation between regulatory bodies and angling organisations in raising awareness about the threats to biodiversity posed by *S. glanis* dispersal into the wild. There is a need for stricter protocols in the early detection and prevention of *S. glanis* dispersal into the wild so as to minimise potential risks to native fish species as often species introductions into lake fisheries are done without regulatory compliance with insufficient monitoring in control management (Copp et al. 2007b; Copp et al. 2009a; Guillerault et al. 2015; Sagouis et al. 2015; Rees et al. 2017). To this end, to facilitate better control and understanding of *S. glanis* harmful impacts, some gaps in knowledge about their potential trophic and predation impacts to native species in aquatic habitats in the UK has been addressed in the study (Rees et al. 2017; Cucherousset et al. 2018).

1.2.5 *Siluris glanis* in native Eastern Europe

S. glanis, as depicted in Plate 1.1 (approximate fish total length 55cm) is a freshwater fish species of the family Siluridae originally from European, Asian and African descent and is a native species in Europe. This is the largest bodied fish species of the order of Siluriformes and characterised by a wide jaw gape with six feelers around its mouth with olfactory chemoreceptors to assist in detection of prey in benthic freshwater habitats (Copp et al. 2009a; Cucherousset et al. 2018). Their long scale less body is tapered with a long anal fin of 75 rays which extends over half of its body length whereas its dorsal fin is small with only 4 rays. *S. glanis* are capable of fast growth with some specimens able to attain body lengths of over three metres (TL) in aquatic habitats in south west Europe with warm water temperatures during summer time providing ideal conditions for optimal growth (Alp et al. 2004; Britton et al. 2007; Copp et al. 2009a; Boulêtreau & Santoul, 2016; Slavik et al. 2016).

Plate 1.1. Photo of *Siluris glanis* specimen (Fishbase, 2013).

S. glanis is a predatory fish species, native to eastern Europe and western Asian countries such as Germany, Poland, southern Sweden, southern Turkey, Greece, northern Iran, Baltic states, Russia, the Aral Sea of Kazakhstan and Uzbekistan (Bora & Gul, 2004) (See Figure 1.2). Some remnant populations in lakes in western Greece are at risk of becoming endangered owing to displacement from introduced species such as Aristotle catfish (*Silurus aristotelis*) released into these habitats. These isolated populations are at risk of becoming genetic bottlenecked with low genetic variability due to small *S. glanis* populations (Triantafyllidis et al. 1999; Economidis et al. 2000; Copp et al. 2009a).

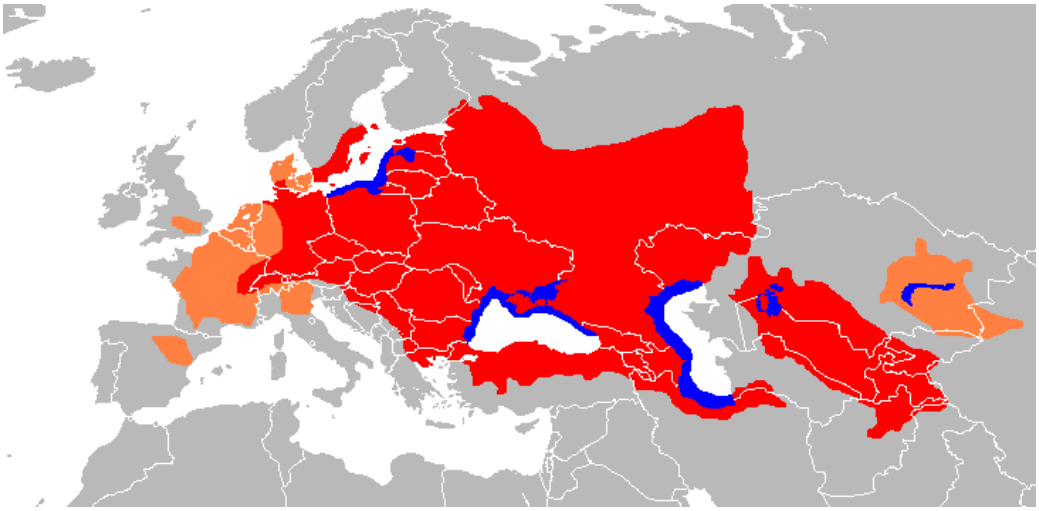

Figure 1.2. Distribution of *S. glanis* populations around the world. (Source: modified from IUCN, 2018).

- Native freshwater populations highlighted in red
- Coastal populations highlighted in blue
- Non-native populations highlighted in orange

Native *S. glanis* populations are well established throughout major rivers in Eastern Europe such as the River Volga, Don and Ural in Russia and Kazakhstan and River Dnieper that flows from Russia to the Ukraine. Other native populations are found in the River Vistula in Poland, River Vag in Slovakia, River Tisza in Hungary and River Danube in Germany (Harka, 1984; Copp et al. 2009a; Horoszewicz & Backiel, 2012). They are also established in several reservoirs throughout Eastern Europe such as Orlice in Czech Republic, Kakhovsk in Ukraine and Zegrzyński in Poland (Horoszewicz & Backiel, 2012).

1.2.6 *Silurus glanis* aquaculture status

In European freshwater aquaculture, the main fish species cultivated are rainbow trout (*Oncorhynchus mykiss)*, brown trout (*Salmo trutta*) and common carp (*Cyprinus carpio*) rather than *S. glanis*. The predominant aquaculture industries cultivate *Salmonidae* in Western Europe where as Cyprinidae aquaculture is more widespread in Eastern Europe. Cyprinid pond farming in the vast waterways of native Eastern and central Europe is practised in Romania, Bulgaria, Poland, Hungary, Poland, Austria, Czech Republic, former Yugoslavia and Russia (Linhart et al. 2002; Dokuchaeva, 2005; Bekcan et al. 2006; Omeragié, 2009; Copp et al. 2009a; Talpeş et al. 2009; Hadjinikolova et al. 2010; Dokuchaeva, 2011; Yakhchalil et al. 2012).

Traditional Cyprinidae pond farming produces low annual yields, and as such contributes to only 5% of the total freshwater aquaculture production in Europe (Verdi et al. 2001; Bostock et al. 2010; FAO, 2012). *S. glanis* cultivation plays a subsidiary role in Cyprinidae pond farming with low annual yields of 700 tonnes whereas, in contrast *O. mykiss* production is over 700,000 tonnes annually (Varadi et al. 2001b; Linhart et al. 2002; FAO, 2012). Cyprinidae and *S. glanis* low aquaculture production in Eastern Europe may be related to socio- economic drivers with low fish consumption due to market forces as fish is more expensive than meat to purchase, thus, most freshwater fish production is exported to other countries such as Germany (Varadi et al. 2001a; Linhart et al. 2002; Omeragié, 2009; Muscalu et al. 2010; FAO, 2012).

Traditionally, *S. glanis* were reared to control wild forage fish (small pelagic fish) during seasonal flooding in ponds so as to reduce competition for cultivated cyprinids. *S. glanis* supplementary yields contribute to 10% of annual production in cyprinid farming (Bokor et al. 2012). However, in the longer term with advancements in intensive aquaculture farming *S. glanis* production may change. Developments in intensive recirculation and flow-through systems allow higher yields and turnover of fish compared to traditional pond farming. This has led to a renewed interest in cultivating *S. glanis* in some regions of Germany, France and Italy

(Ghittino, 1979; Flajšhans et al. 1999; Pruszyński & Pistelok, 1999; Mazurkiewicz et al. 2008; Muscalu et al. 2010; Bokor et al. 2012; Slavik et al. 2016). There has also been some interest in the nutritional benefits of *S. glanis* because, like herring and mackerel, these are high in protein and essential omega-3,-6 fatty acids. Changes in marketing may be required to increase demand and public awareness. At present, *S. glanis* fillets are mainly smoked and consumed in their native countries rather than marketed around the world. These fish are marketed as a smoked delicacy rather than part of a staple diet, and are more expensive compared to other fish such as rainbow trout or common carp (Jäger, 1992; Fűllner & Wirth, 1996; Bogut et al. 2002; Linhart et al. 2002; Copp et al. 2009a; Cucherousset et al. 2018).

1.2.7 Non-native *Silurus glanis* in mainland Europe

In recent decades, *S. glanis* have been introduced into recreational fisheries and for aquaculture in western Europe and northern hemisphere countries such as the UK, Spain, Italy, Portugal, France, Belgium, Denmark, The Netherlands and Albania, Tunisia, China and Brazil (Shumka et al. 2008; Copp et al. 2009a; Syväranta et al. 2009; Cunico & Vitule, 2014; Gkenas et al. 2015). *S. glanis* are increasingly imported for recreational angling owing to demand for large bodied specimens by trophy anglers as their addition to lake fisheries is likely to boost annual revenue. Anglers are prepared to travel and spend on bait and angling equipment in these specialist fisheries with repeat visits determined by travel costs and catch challenge (Ricciardi, 2007; Copp et al. 2009a; Rees et al. 2017).

There has been some concern about the risks of large bodied *S. glanis* specimens dispersing into the wild in introduced ranges causing ecological harm to native fish species and environmental degradation in habitats (Carol et al. 2009; Copp et al. 2009a; Cucherousset & Olden, 2011; Cucherousset et al. 2012). Some of these detrimental ecological impacts include increased risks in disease transmission, predation and competition to native fish species (Copp et al. 2009a; Britton et al. 2010; Cucherousset et al. 2012; Guillerault et al. 2015).

The spread in disease emergence is of increasing interest as non-native *S. glanis* specimens may act as host carriers of generalist pathogens and parasites that can switch host to native fish species resulting in their mortality and subsequent biodiversity loss. Wild *S. glanis* specimens are carriers of several parasites including Protists, Monogenea, Trematoda, Cestodea, Nematoda and Acanthocephala. These may be harmful to native fish species (Danilkiewicz, 1981; Dezfuli, 1992; Yakhchali et al. 2012; Copp et al. 2016a).

Such risks of disease outbreaks to native species are likely to increase in warmer water temperatures over 20ºC so in turn native fish species in invaded habitats at lower geographical latitudes may be more vulnerable (Hamáčková et al. 1992; Gurevitch & Padilla, 2004; Sweetman et al. 2006; Has-Schön et al. 2015). Moreover, the spread in disease emergence to native species via invaders is likely to be exacerbated by increasing water temperatures owing to climate change. Indeed, these thermal changes are likely to accelerate *S. glanis* growth and predation impacts, facilitating their colonisation into environments formerly unsuitable for invasion (Rahen & Olden, 2008; Daufresne and Boêt, 2007; Copp et al. 2009a; Cucherousset et al. 2018) (See Table 1.2).

Table 1.2. Variation of the thermal requirements and physiological behaviour strategies of *S. glanis* (Carol et al. 2007; Carol et al. 2009; Copp et al. 2009a; Cucherousset et al. 2018).

Water Temperature (ºC)	***S. glanis* behaviour**
8-10	Upstream migration to spawn
>12	Onset of foraging
<13-14	Larvae mortality
25- 28	Optimum food assimilation
15-23	Reduction in food assimilation

Recent studies have reported several non-native *S. glanis* populations established within major river catchments in Iberian Peninsula, Italy and south west France in mainland Europe. Large bodied *S. glanis* specimens can attain sizes > 1500mm TL in these warm water environments (Syväranta et al. 2010; Boulêtreau et al. 2011; Boulêtreau & Santoul, 2016).

This latter point is important as large bodied *S. glanis* specimens may threaten diversity of native species and long-term ecosystem functioning with predation of anadromous fish species, wildfowl and small mammals (Benejam et al. 2007; Carol et al. 2009; Syväranta et al. 2010; Cucherousset et al. 2012; Boulêtreau et al. 2018; Guillerault et al. 2017). There is a growing body of evidence to suggest that predation impacts by large bodied *S. glanis* specimens >1200mm (TL) might carry harmful consequences to biodiversity (Carol et al. 2007; Copp et al. 2009a; Guillerault et al. 2017; Cucherousset et al. 2018).

The extent of non-native *S. glanis* predation impact upon endangered eel (*Anguilla anguilla*) seems at present equivocal. Studies in the Camargue in France revealed strong predation by *S. glanis* and consequently reduced species abundance in their presence (Bevacqua et al. 2011). However this contrasted with other results which suggested little predation impact upon *A. anguilla* by *S. glanis* instead they displayed a greater consumption of crayfish and cyprinid species (Syväranta et al. 2009; Martino et al. 2011).

Study results in Iberian Peninsula revealed that introduced *S. glanis* may affect different trophic levels in aquatic habitats owing to their plasticity in foraging (Copp et al. 2009a; Cucherousset et al. 2018). They are able to switch to feeding upon the most abundant prey available and profit

from being able to feed from different food resources (Orlova & Popova, 1987; Czarnecki et al. 2003; Bora & Gul, 2004; Syväranta et al. 2009; Horoszewicz & Backiel, 2012; Guillerault et al. 2017; Boulêtreau et al. 2018).

1.2.8 Non-native *Silurus glanis* in the UK

In the UK, there is concern about the increase in *S. glanis* introductions into highly stocked carp fisheries with these conditions likely to accelerate their growth and adverse ecological impacts. In recent years, there has been regular import of large bodied *S. glanis* specimens into the UK due to demand from trophy anglers for large specimens weighing over 27kg. There is some apprehension about the risks of large bodied *S. glanis* specimens dispersing into the wild from lake fisheries causing harm to native fish species, in particular with unregulated introductions into lakes in floodplains at higher risk of flooding. These escapees dispersing into rivers catchments may threaten native species diversity via disease emergence, predation impacts and changes in competition and trophic functioning (Peirson et al. 2001; Copp et al. 2007b; Copp et al. 2009a; Britton et al. 2010; Rees, 2010; Rees et al. 2017; Cucherousset et al. 2018).

There is evidence to suggest that thermal changes in rivers coupled with higher incidences of flooding spates in England and Wales, due to climate change impacts, may in turn facilitate *S. glanis* colonisation into the wild and exacerbate their ecological impacts. For example, there is a greater risk of disease virulence to native fish species (Britton et al. 2010; Rees, 2017; Cucherousset et al. 2018). In recent years, there was some heavy flooding resulting in *S. glanis* specimens escaping from lake fisheries into major river catchments. Consequently, changes in water temperature and warming up of shallow channels in floodplains of river catchments during summer time may be ideal conditions for *S. glanis* establishment, particularly in regions of lower geographical latitudes such as southern England which would be more vulnerable to invasion. Climate change impacts will likely eliminate cold water temperatures in these aquatic habitats during winter, and as such facilitate *S. glanis* invasion (Brown et al. 2007; Daufresne & Boet, 2007; Rahel & Olden, 2008; Cucherousset et al. 2009; Rees et al. 2010; Keller & Seehausen, 2012; Rees et al. 2017).

At present, there is some ambiguity about the significance in harmful ecological impacts to native fish species diversity caused by non-native *S. glanis* specimens escaping from lake fisheries into the wild. Assessing their invasiveness has proved difficult given the limited knowledge about their ecological impacts threatening the long-term integrity of native species in

riverine ecosystems in England and Wales. The majority of studies about non-native *S. glanis* ecological impacts in rivers are polarised to Mediterranean regions in western Europe rather than in the UK and as such these gaps in knowledge need to be addressed (Britton et al. 2007; Copp et al. 2005a; Copp et al. 2007a; Britton et al. 2010; Cucherousset et al. 2018).

1.3 Aims and objectives

The aim of this research is to investigate the ecological impacts and variation in growth, trophic impact and risk status of *S. glanis* released into ponds in England. The specific objectives of the study are:

To assess the variability in growth of three different size groups of *S. glanis* in adjacent ponds, and to establish fish size relationships within introduced habitats;
To investigate any changes in Fulton`s condition and trophic position of these *S. glanis* size groups and to estimate fish size relationships with diet and thus their potential trophic impacts within introduced habitats;
To identify the likelihood of ecological, socio-economic risks and potential disease transmission of introduced *S. glanis* dispersing into a major river catchment in England using European Non-native Species in Aquaculture Risk Analysis (ENSARS) and climate change modelling CLIMATCH.

2. Study sites

2.1 Introduction

This chapter outlines the key features of the study sites for *S. glanis* field studies that were carried out during the study. The accessibility of suitable study sites were the main determinants in site selection such as the ease of access in draining ponds down for fish recapture. Suitable study sites for *S. glanis* were selected following site checks and approval by three experts (Copp, G.H., Davies, G.D. &Wright, R.M.) in fisheries from the Environment Agency and Centre for Environment Fisheries and Aquaculture Science (CEFAS) to ensure that the sites were fit for purpose for the study objectives. Suggestions and co-operation with the field studies were provided by *S. glanis* fishery experts from Catfish Conservation Group, bailiffs and fishery owners. As a result of accessibility and limited time frame, the sites described in this chapter were selected. The timing of fish sampling and duration of the field studies were influenced by practical logistics and technical limitations.

The field studies took place in the south of England at two locations (See Table 2.1). One site was at a lake fishery in Mayland, near Maldon in Essex (See Figure 2.1) and the other was at a commercial angling venue Northfield Main Pit Lake near Ringwood in Dorset (See Figure 2.2).

Table 2.1. Grid references for the two study sites which was the three ponds at Mayland near Maldon in Essex and Northfield Main Pit Lake near Ringwood in Dorset, England.

Geographic variables	**3 ponds at Mayland**	**Northfield Main Pit Lake**
National grid reference	TL91200240	SU160075
Latitude	51.687792N	50.866786N
Longitude	0.764687E	1.774008E
Substrate	Mud / Silt	Mud/Silt
Depth (m)	1	0.5 - 4.26

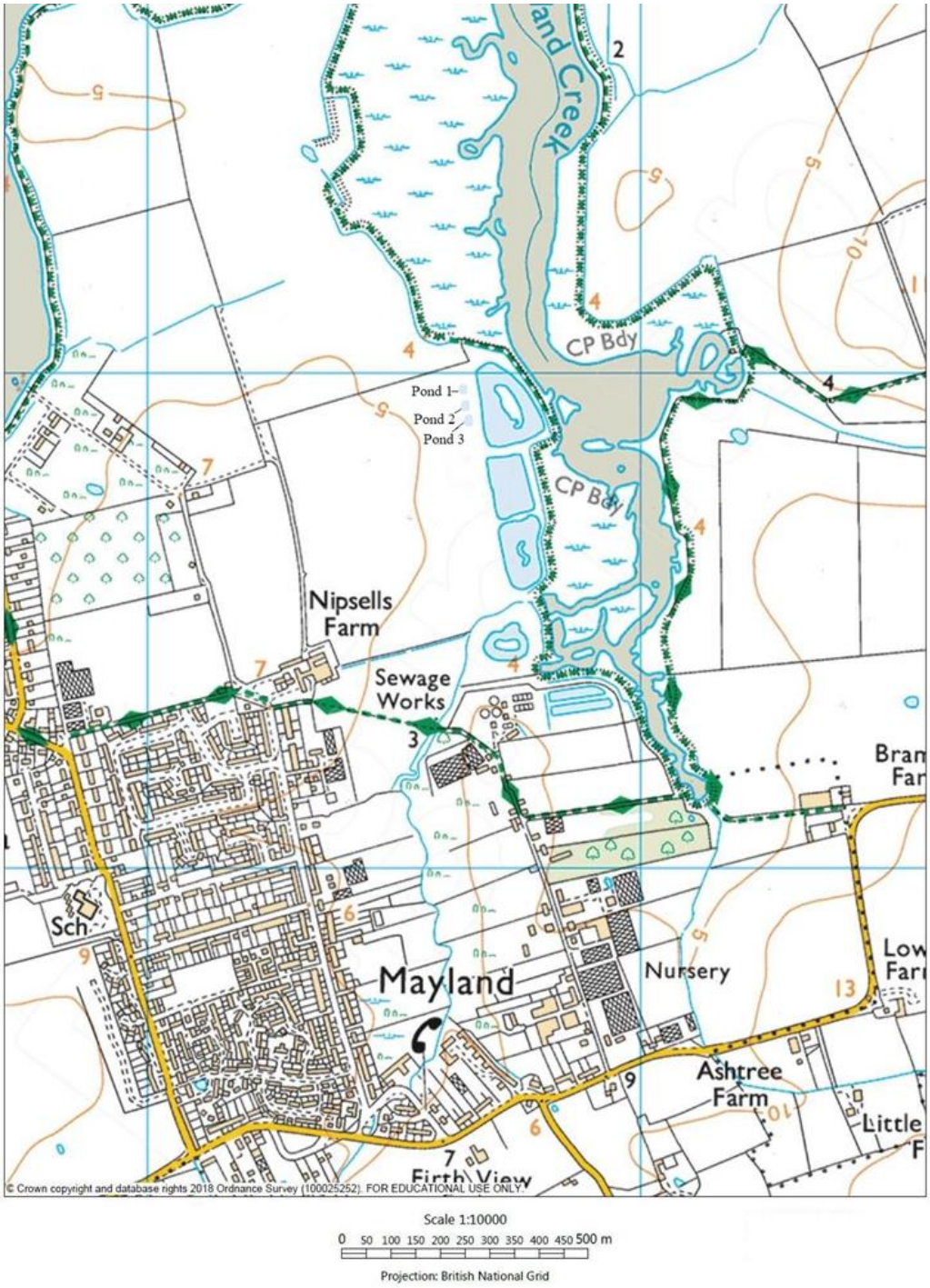

Figure 2.1 Location of field study ponds at Mayland lake fishery, near Maldon, Essex (Digimap O.S. copyright 2018).

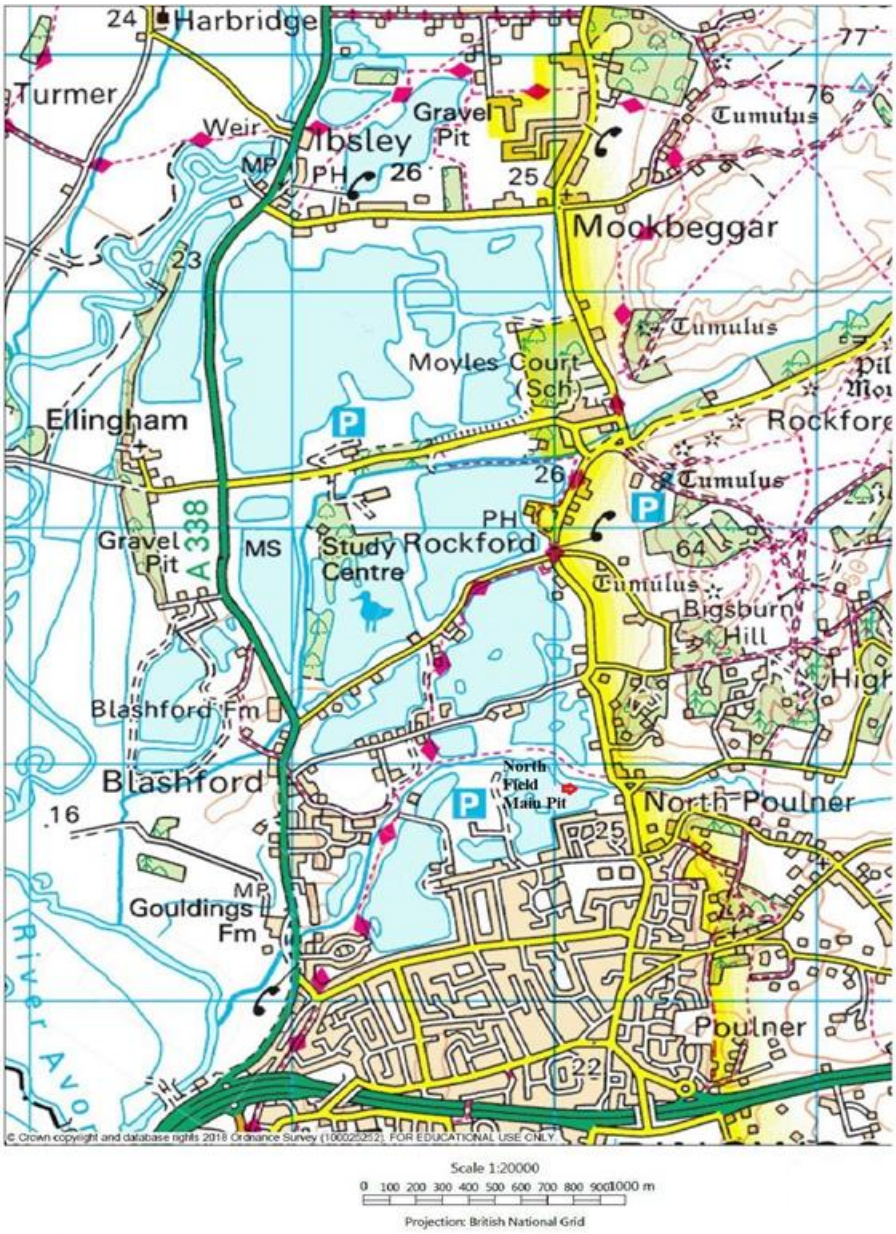

Figure 2.2. Location of Northfield Main Pit Lake near Ringwood, Dorset, which is highlighted by a red arrow on the map (Digimap O.S. copyright, 2018).

2.2 Global and UK distribution of non-native *Silurus glanis*

2.2.1 *Silurus glanis* global distribution

S. glanis are native to western Asia and eastern European countries such as Germany, Poland, Russia and the Baltic states. They have spread into several counties west and south of their native range owing to economic drivers such as recreational angling accelerating introductions. Some of these are often unregulated owing to lapses in biosecurity and poor regulatory control. *S. glanis* expansion in range is likely to be facilitated by thermal changes in aquatic habitats as a consequence of climate change resulting in changes in species distribution globally. *S. glanis* are introduced but not established in the UK, Cyprus, Belgium, Algeria, Tunisia, Brazil, Bosnia-Herzegovina, Italy, Portugal, Denmark, Tunisia, Syria, Portugal, Croatia, Turkey, the Netherlands and China (Bora & Gul, 2004; Copp et al. 2009a; Cunico & Vitule 2014; Cucherousset et al. 2018) (See Figure 2.3).

S. glanis are introduced and established into major river basins in some parts of France, Belgium and Spain. More research is needed as there are gaps in knowledge about the potential risks of adverse ecological impacts of invasive *S. glanis* populations upon native fish communities in these areas (Copp et al. 2009a; Cucherousset et al. 2018).

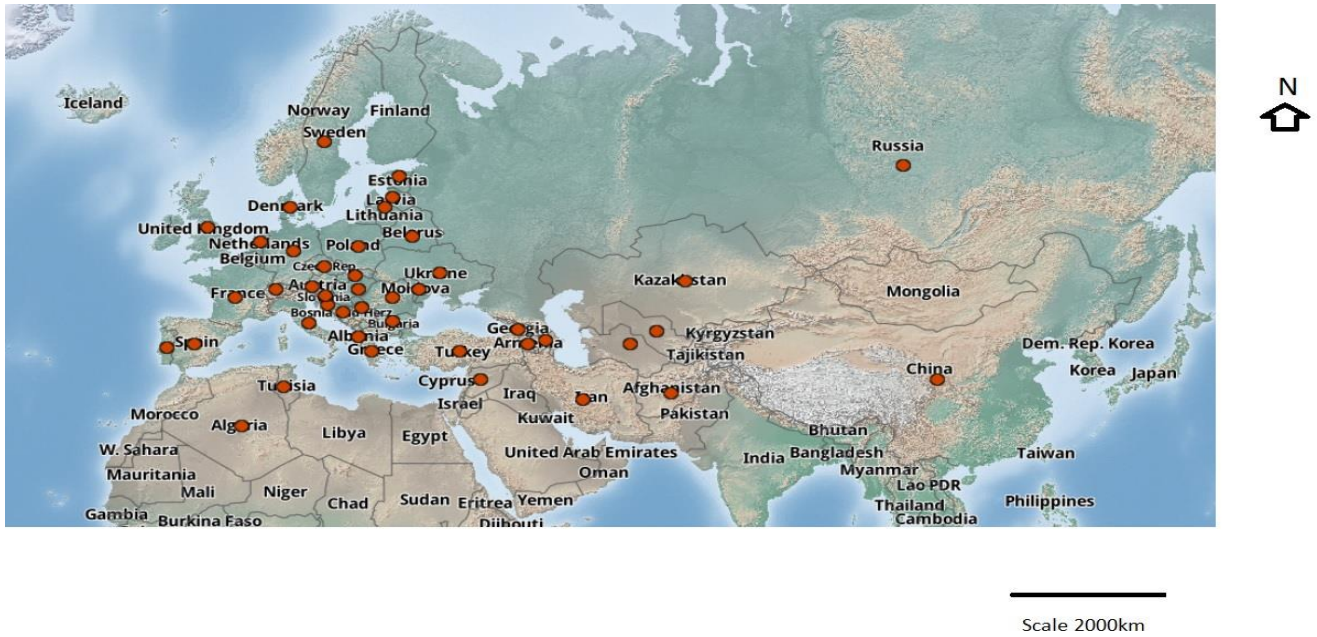

Figure 2.3 Global distribution of native and introduced records of *S. glanis* shown as red circles on the map from CABI, 2018 Invasive Species Compendium. Wallingford UK CABInternational www.cabi.org/isc.

2.2.2 *Silurus glanis* distribution in the UK

S. glanis were first imported from Germany into the UK during the 1880s into some privately owned lakes at Woburn Abbey Estate in Bedfordshire and from this stock the species were established into other lakes nearby (Copp et al. 2009a). In recent decades, *S. glanis* introductions into lake fisheries for angling have increased and they are known to be present in over 500 lake fisheries mainly in south east England. They are not established in aquatic habitats further north as their expansion seems restrained by thermal barriers with low ($\leq 13^0$C) water temperatures likely to inhibit spawning and fry over wintering survival (David, 2006; Copp et al. 2009a; Rees et al. 2017) (See Figure 2.4).

S. glanis are rarely recaptured from riverine catchments in England and Wales. However, there is some evidence that they are present in several rivers such as the Thames, Colne, Chelmer, Kennet and Ouse in southern England (Rees et al. 2017). In future decades, species distribution is predicted to change with thermal shifts of their preferred temperature range owing to climate change likely to facilitate their invasion throughout the UK (Copp et al. 2009a; Rees et al. 2017).

Figure 2.4. Distribution of *S. glanis* populations recorded in the UK. (Source: modified from National Biodiversity Network NBN Atlas for *S. glanis*, 2018).

2.3 Study sites holding *Silurus glanis*

2.3.1 The field study at Mayland lake fishery, near Malden in Essex

The lake fishery at Mayland was located 9 km south east of Maldon, Essex (grid reference, TL91200240) which is privately owned and run by angler membership only. The venue operates as a catch and release fishery and holds four lakes. The lakes are restocked every few years with cyprinids such as common carp (*Cyprinus carpio*), gudgeon (*Gobio gobio*), silver bream (*Blicca bjoerkna*), roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*), common bleak (*Alburnus alburnus*), tench (*Tinca tinca*) and other coarse species such as European perch (*Perca fluviatilis*). The fishery covers a total area of about 150 hectares surrounded by agricultural land in a rural catchment near the Black water estuary. The estuary flows into the North Sea at Mersea island near Maldon in Essex.

The field studies for the investigation of growth and trophic interactions of *S. glanis* were undertaken in three outdoor ponds located at the fishery. The ponds were constructed specifically for research of *S. glanis* ecological impacts (including the pilot study). Situated on the grounds of the fishery, all ponds were of similar bathymetry, shallow (~ 0.5 - 1 m) and rectangular in shape so as to facilitate draining down of ponds for fish recapture. The macrophyte cover in the ponds were managed and cut by the owner annually so as to prevent too dense shading around the pond edges. The system of adjacent ponds covered a total area of about 0.04 ha in a marshy field of 4.05 ha which was separated from angling lakes by a hedgerow and some overhanging trees (See Figure 2.5, Plate 2.1). The ponds were enclosed by high embankments ~ 0.5 m above water level and were in permanent hydro period. The embankment interface between each pond was ~ 6.78m.

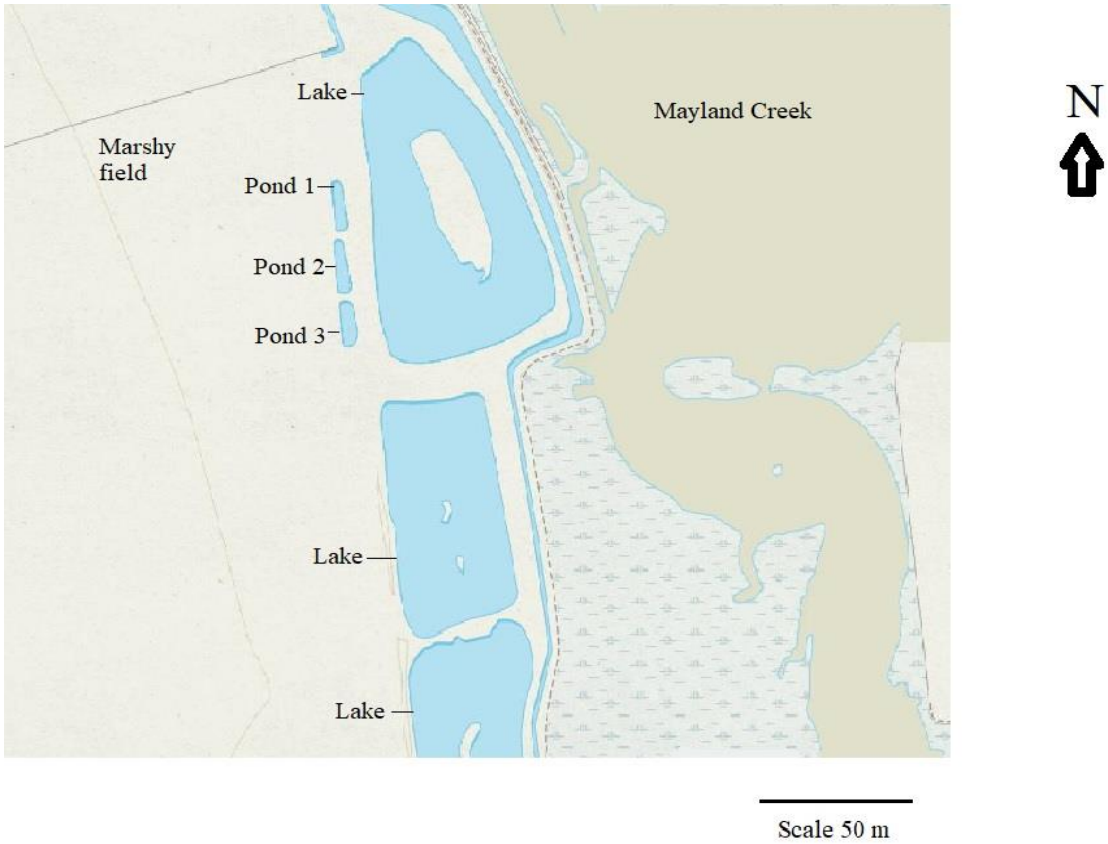

Figure 2.5. Location of the three ponds at Mayland, near Maldon, Essex (Google earth, 2018).

Plate 2.1. Aerial photo of the three ponds at Mayland, near Maldon, Essex (Google earth, 2018).

2.3.2 Pond one, at Mayland, near Maldon, Essex

The uppermost, largest and most northern of the enclosed ponds was Pond 1 which was ~1 m deep with ~ 0.017 ha surface area. Some surface areas of the pond were shaded by in pond vegetation mainly; yellow water lilies (*Nuphar lutea*) and arrowhead (*Sagittaria sagittifolia*). Some emergent macrophytes such as reedmace (*Typha latifolia*), burreed (*Sparganium erectum*), common reed (*Phragmites australis*) and reed grass (*Glyceria maxima*) were present yet these were mainly around pond margins (0 - 0.5 m from the bank).

Bankside vegetation were mainly overhanging bushes and trees such as crack willow (*Salix fragilis*), alder (*Alnus glutinosa*), hawthorn (*Crataegus Monogyna*), sycamore (*Acer pseudoplatanus*) ash (*Fraxinus excelsior*) and bramble (*Rubus fruticosus*) which provided some shade. Overhanging trees helped stabilise pond banks and are likely to have provided some habitats and refuge cover for fish by their underwater roots and wooden debris in the muddy substrate (Garner, 1995; Langler & Smith, 2001; Copp et al. 2009a) (See Plate 2.2, Table 2.2).

Plate 2.2. Photo of pond one at Mayland, near Maldon, Essex (Rees, 2014).

Table 2.2 Habitat diversity in ponds at Mayland, near Maldon, Essex.

Habitat classification		
In pond vegetation	**Woody debris and tree roots**	**Emergent vegetation**
Arrow head (*Sagittaria sagittifolia)*	Woody debris	Reedmace (*Typha latifolia)*
Yellow water lilies (*Nuphar lutea*)	Willow roots (*Salix fragilis*)	Burreed (*Sparganium erectum*)
	Overhanging trees	Common reed *(Phragmites australis*)
		Reed grass (*Glyceria maxima*)

2.3.3 Pond two at Mayland, near Maldon, Essex

The second-most of the ponds was ~1 m deep and ~ 0.015 ha in surface area which was situated between pond 1 and pond 3. Pond 2 had similar substrate and macrophyte cover to pond 1 with *N. lutea*, *H. vulgaris*, *S. emersum* and *S. sagittifolia* around the pond margins. In all three ponds, habitat complexity was evident with submerged, emergent macrophytes and overhanging trees providing spawning and refuge areas for fish. Dense macrophyte cover was provided by *T. latifolia*, *P. australis*, *G. maxima* which were ~ 0.5 m from the pond embankment (See Plate 2.3).

Plate 2.3. Photo of pond two at Mayland, near Maldon, Essex (Rees, 2014).

2.3.4 Pond three at Mayland, near Maldon, Essex

The most southern and smallest pond was pond 3 which was ~1 m deep and ~ 0.015 ha in surface area. The pond had some overhanging vegetation from marginal macrophytes and *S. fragilis* and *A. glutinosa* trees which provided some shade. Parts of the pond surface were covered by *N. lutea* and *H. vulgaris*. Around the pond margins were emergent macrophytes such as *T. latifolia*, *P. australis*, *G. maxima* which were ~ 0.5 m from the pond embankment (See Plate 2.4).

Plate 2.4. Photo of pond three in Mayland, near Maldon, Essex (Rees, 2014).

2.3.5 Biotic sampling at Mayland

The experimental design involved a pilot study about the efficacy of tagging *S. glanis* size groups and after studies which investigated *S. glanis* size groups growth and trophic interactions in ponds. At the Mayland site *S. glanis* were divided into three size groups; small (36- 43 cm TL), medium (41- 44 cm TL) and large (49- 51 cm TL) (See Appendix A, Table 6A1.10, Table 6A1.11, Table 6A1.12). The selection of size groups were in line with other studies of *S. glanis* and recapture proficiency from small ponds (Zaikov et al. 2008; Carol et al. 2009; Cucherousset et al. 2018). These fish were then individually tagged with fish lengths and weights recorded before being released into ponds. The large size *S. glanis* group was released into pond 1, medium size *S. glanis* group into pond 2 and small size *S. glanis* group into pond 3 and recaptured annually (See Appendix A Table 6A1.16, Table 6A1.17, Table 6A1.18). All *S. glanis* size groups in the study were sourced from aquaculture and were originally from Croatia.

Prior to the field study, no fish were stocked in the ponds. The field study involved the simulation of a model native fish community which comprised of three cyprinid prey species: *R. rutilus, S. erythrophthalmus* and *B. bjoerkna* likely to be present in invaded ponds. All prey fish were sourced from large angling lakes used for angling on the site with fish size selection related to the approximate jaw gape of *S. glanis* size groups (Refer to Appendix A, Table 6A1.16, Table 6A1.17, Table 6A1.18). The experimental ponds were then left for two weeks to allow the forage fish communities to establish before *S. glanis* were released into the ponds. All ponds were open with no anti bird netting fixed at posts so that fish communities were potentially at risk from terrestrial and avian predation. There were some anectdotal reports from the site owner of possible fish loss by avian predation which may have occurred from some angling lakes at the venue although no avian or terrestrial predation were observed from the three study ponds during the field studies (M. White 2012, personal communication, 14 November). The fish communities were also subject to no angling interference or supplementary food sources such as bait so as to simulate natural conditions in invaded habitats.

2.3.6 Water temperature in ponds & Degree-days approach

Abiotic factors such as water temperature in ponds were recorded continuously (24 hr) with Tinytaggers throughout the study. A TinyTag temperature logger (Gemini Data Loggers Ltd, U.K.) was placed in the same location for each pond (~1 m deep and ~ 3 m from pond margin) for recording of water temperature. Tinytag loggers were set at 15 minute readings for all ponds. However, for pond 1 there was an error in Tinytag recording in 2014, so water temperature data was obtained which was the predicted mean temperature for 2014. A comparative analysis was undertaken to assess the appropriateness of the two alternative data series (pond 2 and pond 3). Available data from pond 1 in 2014 was compared to that of pond 2 and pond 3 with the aim of identifying the most suitable match. The average of water temperature in pond 3 in 2014 was used in place of the missing data for pond 1 in 2014.

S. glanis are ectotherms (unable to regulate own thermal homeostasis) so their metabolism and growth were mainly determined by ambient water temperature in ponds when food resources were not a limiting factor. Fish growth was quantified using Degree-days (DD) approach to determine the thermal opportunity for growth by the aggregation of water temperatures as there is strong linear relationship between fish growth and Degree-days (Honsey et al. 2018; Honsey et al. 2019).

The Degree-days metric was used to determine *S. glanis* size groups growth in ponds. A lower temperature threshold (T_0) was incorporated to define the onset of growth which varies with different fish species (Honsey et al. 2018; Honsey et al. 2019). From peer reviewed literature and using similar studies about *S. glanis* growth and development in invaded habitats, the (T_0) for the onset of growth was determined as $\geq 17^0$C with optimum growth as $\geq$ 25 - 28^0C in the study. Although these baselines may vary with *S. glanis* populations in different geographic environments (Linhart et al. 2002; Britton et al. 2007; Carol et al. 2007; Gullu et al. 2008; Zaikov et al. 2008; Carol et al. 2009; Copp et al. 2009a; Muscalu et al. 2010; Cirkovic, 2012; Cucherousset et al. 2018). Similarly, previous studies about cultivated *S. glanis* growth were reviewed to elicit understanding about the application of (T_0) metric for *S. glanis* size groups in the study (Harka, 1984; Hilge, 1985; Hilge, 1989; Mareš et al. 1996; Bogut et al. 2002; Ulikowski et al. 2003; Jamróz et al. 2008; Dediu et al. 2010; Alp et al. 2011; Kumar et al. 2017).

Equation 1 The Degree- days model

The Degree- days metric (DD ^{0}C·days) was calculated using:

$DD = [T_{Max} + T_{Min}]/2\text{-}T_0$

where T_{Max} is the maximum daily water temperature, T_{Min} is the minimum daily water temperature in ponds and T_0 is the threshold water temperature for *S. glanis* growth (Honsey et al. 2018; Honsey et al. 2019).

In the study, the frequencies of mean daily water temperatures $\geq 17^0$C in ponds were determined. This indicated the average cumulative Degree-days as an index of thermal energy spent for *S. glanis* growth over that period (See Table 2.3). The Degree- day model used the standard assumption that fish growth was linear and only a function of ambient temperature (Chezik et al. 2014; Honsey et al. 2018; Honsey et al. 2019). The statistical analysis was performed using IBM SPSS statistics version 21.

Table 2.3 Frequencies of the mean daily water temperature ≥17^{0}C Degree- days (DD) in ponds during the study (2013 & 2014). Standard Deviation (SD), Standard Error (SE) and 95% Confidence Interval (95%CI) given.

Month	Pond 1 2013	Pond 1 2014	Pond 2 2013	Pond 2 2014	Pond 3 2013	Pond 3 2014
April	1	0	0	0	1	0
May	29	6	30	4	30	6
June	16	28	16	21	16	28
July	31	29	31	29	31	29
August	31	21	30	18	30	21
September	7	13	7	8	6	13
Total	115	97	114	80	114	97
SD	13	12	13	11	13	12
SE	5	5	5	5	5	5
95% CI	From 5 to 33	From 4 to 29	From 5 to 33	From 2 to 25	From 5 to 33	From 4 to 29

2.3.7 Water quality in ponds

In the study, all the ponds were quarterly monitored for water quality parameters such as; pH, dissolved oxygen, phosphate and nitrate concentrations in 2013 (25th September 2012, 26th January, 25th May, 21st September) and in 2014 (13th November 2013, 15th March, 12th July, 13th November). These water quality variables were measured by Hach HI 9146 meter and Hach biotector B3500eTOC analyser respectively.

In the study, the mean dissolved oxygen was over 80% saturation in ponds in 2013 and 2014 which were well within good water quality status recommended by the General Quality Assessment (GQA) guidelines for fish species and EC Freshwater Fisheries Directive (2006/44/EC) (Rees, 2010). The mean pH across all ponds was slightly alkaline (7.2) and within the range of 6.0 - 9.0 recommended for fish health (See Table 2.4) (Environment Agency, 2007).

Table 2.4. Variation in mean pH, nitrate, phosphate concentrations in ponds (2013 & 2014).

Ponds	0_2 % of saturation		pH		Nitrate (mg/L)		Phosphate(mg/L)	
	2013	2014	2013	2014	2013	2014	2013	2014
1	85.64	86.99	7.2	7.2	0.03	0.04	0.06	0.15
2	84.71	84.96	7.2	7.2	0.03	0.04	0.05	0.12
3	86.65	85.36	7.2	7.2	0.02	0.03	0.05	0.06

In 2014, compared to the increased phosphate concentrations recorded for ponds 1 and 2, there was an inconsistent result for the phosphate reading in pond 3 (See Table 2.4). There is no definite explanation, although phosphate levels in ponds may increase following hydrological seasonal changes. In addition, it may be possible that following heavy rainfall and runoff, ponds 1 and 2 were previously more exposed to drying up than pond 3 which might result in slight variation in phosphate levels (Serrano et al. 1999; Zhang & Sun, 2017).

2.3.8. Study site, Northfield main pit Lake, near Ringwood, Dorset

The Northfield Main Pit Lake fishery near Ringwood in Dorset was used in the ENSARS study to investigate any harmful risks of potential invasiveness of *S. glanis* which were released into the lake without regulatory consent (Refer to Appendix E6). The lake fishery was located in the flood zone near the Hampshire Avon river catchment and other Avon Valley Lakes which are used for angling. There were concerns about the potential risks of *S. glanis* species transfer to other water bodies because the Hampshire Avon is within 1.8 km from the lake, with a brook and footpath that fringed around the lake margins allowing public access into the surrounding area (Environment Agency, 2007) (See Figure 2.6, Plate 2.5).

Figure 2.6. Location of North Main Pit Lake near Ringwood, Dorset. The red arrows on the lake indicate the sites at which there is a risk of fish transfer to other water bodies (Environment Agency O.S. copyright, 2018).

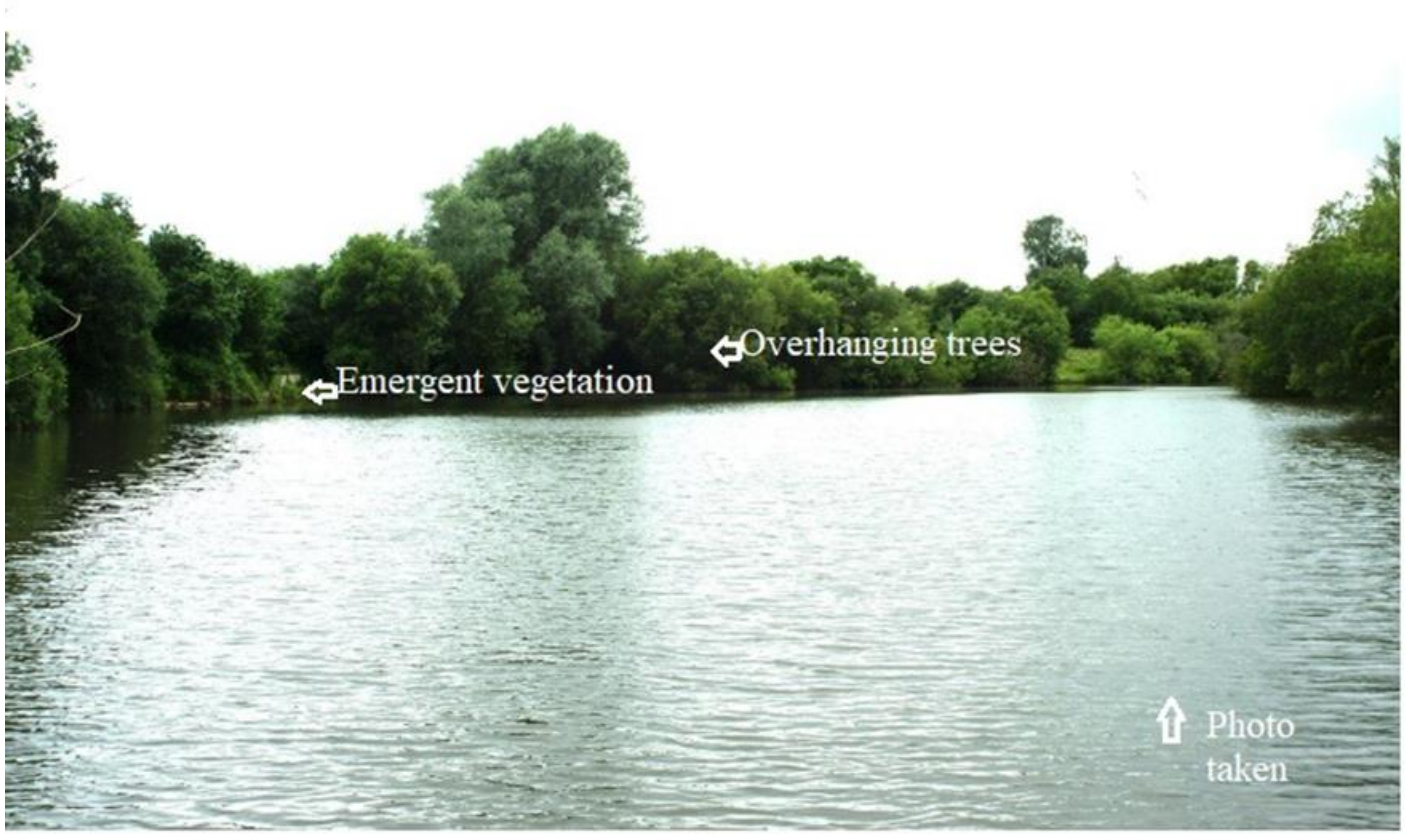

Plate 2.5 Photo of Northfield Main Pit Lake, near Ringwood, Dorset, view taken from west bankside of lake (Ringwood angling, 2018).

The Avon Valley Lakes are located near the lower reach of the river catchment between Ringwood and Bicton area in Dorset. These lakes are flooded quarry pits fed by groundwater springs. They are situated in the flood zone and prone to flooding into the river catchment. The mosaic of Avon Valley Lakes includes: Hightown Lake, Wash Pit, Kingfisher Lake, Linbrook Lake (Half Pit), Roach Lake, Somerley Lake, Blashford Lake, Ellingham Lake, Rockford Pit, Ivy Lake, Mockbeggar Lake, Ibsley Water, Hucklesbrook Lake and New Forest Water Park (Environment Agency, 2007).

The Northfield Main Pit Lake fishery is situated 3km north of Ringwood, in Dorset (grid reference, SU160075). This fishery is privately owned and run by angler membership only. The venue operates as a catch and release fishery and has one lake for specialist angling. The lake is restocked every two years with cyprinids such *C. carpio*, *B. bjoerkna*, *R. rutilus*, *S. erythrophthalmus* and *T. tinca*. The fishery covers a total area of about 15 hectares.

2.3.9. Biotic sampling of Northfield Main Pit Lake

The Northfield Main Pit Lake is fed by groundwater springs and the lake has a surface area of ~ 10 ha and mean depth ~ 0.5 - 4.26 m. The lake had some vegetation from overhanging trees such as *C. Monogyna*, *S. fragilis, A. glutinosa, A. pseudoplatanus* and *F.excelsior* which provided some shade. Some areas of the lake (3 m) were covered by large patches of *N. lutea* at the lake margins (0 - 3 m from the bank). The lake substrate was muddy with some woody debris and tree roots. The northern section of the lake was heavily shaded with overhanging trees and woodland. Parts of the lake supported diverse habitats of submerged and emergent macrophytes such as *P. austalix* and *S.erectum* which provided potential spawning and refuge areas for fish (Environment Agency, 2007).

S. glanis were known to be introduced without regulatory consent into the Northfield Main Pit Lake during the 1990s and has since become established into the lake (Rees, 2010). Established *S. glanis* populations were confirmed by Environment Agency annual fish surveys carried out since 2010 as part of a removal action plan by regulatory bodies to reduce *S. glanis* abundance. Recaptured *S. glanis* specimens were killed with anaesthesia (5 ml L^{-1} of 2-phenoxyethanol) under Home Office licence due to their non-native status and in accordance with the UK Animals (Scientific Procedures) Act 1986. Evaluation of the potential ecological and socio-economic risks posed by *S. glanis* escapees into the surrounding area involved an appraisal of the river catchment and its conservation status.

2.3.10. The Hampshire Avon catchment

The River Hampshire Avon flows approximately 71 km from its source which covers the north of Vale of Pewsey to its tidal limit at Christchurch, through the borders of Wiltshire, Hampshire and Dorset counties. Most of the catchment lies in Wiltshire, whilst the floodplains and estuary are in Dorset. The main tributaries are the Bourne, Wylye, Nadder and Ebble. The river lies mainly in a rural catchment surrounded by agricultural land. Some of the catchment includes the Avon Valley Lakes which supports a range of diverse habitats that hold European and International conservation status. These designated habitats include several Sites of Special Scientific Interest (SSSIs) and Special Area of Conservation (SACs) with protected species such as bullhead (*Cottus gobio*), brook lamprey (*Lampetra planeri*), sea lamprey (*Petromyzon marinus*) and Atlantic salmon (*Salmo salar*). Most of the Avon valley lake floodplain supports diverse wetland habitats for native wild fowl species with designated areas holding Special Protection Areas (SPA) and Ramsar conservation status (Environment Agency, 2005).

Key to understanding the potential ecological risks of *S. glanis* dispersal and establishment into the river catchment, the study investigated the variation in seasonal water temperature of several sites of the river catchment. The upper reach sites of the river which were monitored were Wylye, Ebble, Bourne and Nadder and the lower reach sites included Ibsley, East Mills, Ringwood, Avon Causeway, Knapp Mill and Christchurch (See Figure 2.7, Table 2.5). The water temperature of the riverine sites was measured by using Tinytag loggers (Gemini Data Loggers Ltd, U.K.) which were placed at the same depth for each site (~ 0.5 m deep and ~ 1 m from the river bank). The Tinytag loggers were set at 15 minute readings for all riverine sites and recorded continuously (24 hr) throughout the year from 2007- 2009.

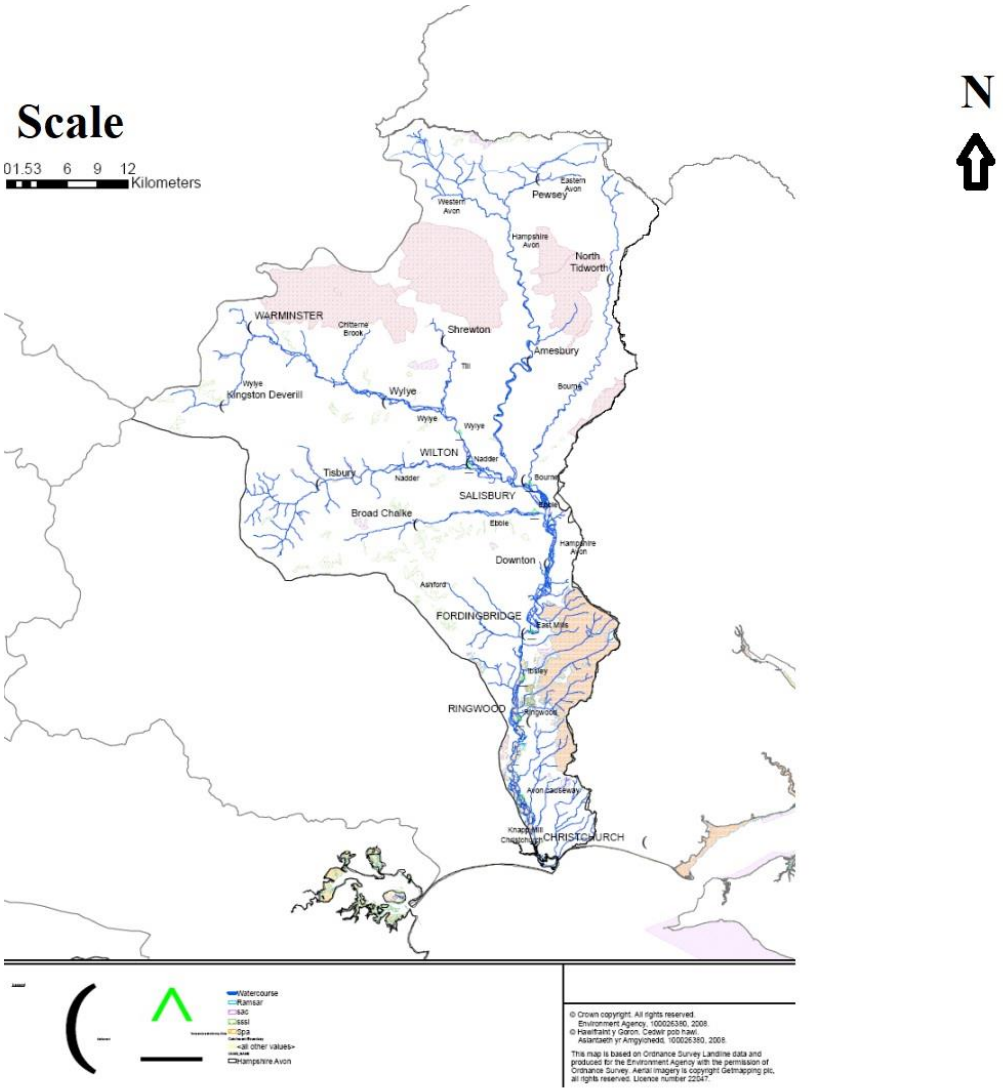

Figure 2.7 Map of River Hampshire Avon catchment (Environment Agency, 0.S. copyright, 2018).

Table 2.5. Selection of sites of the River Hampshire Avon used for water temperature monitoring in the study.

Sites	**Reach**	**NGR**
Wylye	Upper	SU0862634244
Ebble	Upper	SU1607626360
Bourne	Upper	SU1562829131
Nadder	Upper	SU0985230824
Ibsley	Lower	SU1495909670
East Mills	Lower	SU1585514340
Ringwood	Lower	SU1457205550
Avon Causeway	Lower	SZ1494297867
Knapp Mill	Lower	SZ1547093794
Christchurch	Lower	SZ1606893237

3. Growth and trophic interactions study

*Data from parts of the study were published in peer reviewed papers with presentations given at the Freshwater Biological Association, London Freshwater group and UH School of Life & Medical Sciences Research Conference.

Rees EMA, Britton JR, Godard MJ, Crooks N, Miller JI, Wesley KJ, & Copp GH (2014) Efficacy of tagging European catfish *Silurus glanis* (L., 1758) released into ponds. Journal of Applied Ichthyology 1: 127-129.

Rees EMA, Edmonds-Brown VR, Alam MF, Wright RM, Britton JR, Davies GD & Cowx IG (2017) Socio-economic drivers of specialist anglers targeting the non-native European catfish (*Silurus glanis*) in the UK. PloS one 12: e0178805.

3.1 Introduction

In the UK, in recent decades, in response to demand from trophy anglers large bodied *S. glanis* weighing 27kg and above have been imported from mainland Europe into the UK. However, how *S. glanis* impacts on native fish species diversity in freshwater habitats in England and Wales is not known. Similarly to the stocking into cyprinid lake fisheries, the addition of *S. glanis* has substantial financial benefits for fishery owners and to the local economy. Specialist *S. glanis* angling can attract non-residential anglers to the region and thus increase revenue in angling related tourism, employment and land estate. As a consequence of these socio-economic drivers it seems likely that *S. glanis* introductions may increase in future years (Britton et al. 2010; Rees et al. 2014; Arlinghaus et al. 2016; Cooke et al. 2016; Rees et al. 2017; Cucherousset et al. 2018).

The number of lake fisheries stocking non-native *S. glanis* species has increased to over 500 in recent years into England and Wales, with high fish stocking rates ~1000kg ha $^{-1}$ in syndicate fisheries promoting rapid fish growth and size. However, there is evidence that sometimes *S. glanis* introductions into lake fisheries are completed without risk appraisal or authorised consent from regulatory bodies (Rees et al. 2017). *S. glanis* can be introduced into lakes located within floodplains and this poses higher risk of flooding and fish dispersal into the wild (Copp et al. 2009a; Britton et al. 2011a; Arlinghaus et al. 2016; Venturelli et al. 2017; Rees et al. 2017).

In addition, extrinsic factors such as climate and habitat niche may facilitate invasion of non-native *S. glanis*. Thermal changes in freshwater habitats driven by climate change are likely to contribute to their expansion further northwards in the UK. It is predicted that in the UK the water temperature will increase by 2-3ºC by 2050 yet research about their potential adverse ecological impacts to native fish communities in the UK remains limited (Britton et al. 2010; Gozlan et al. 2010; Datry et al. 2016). Some studies in southern France have reported strong predation impacts upon native waterfowl (Cucherousset et al. 2012) and European freshwater native crayfish (*Austropotamobius pallipes*) (Martino et al. 2011; Cucherousset et al. 2018).

It seems that life history traits such as rapid growth and large fish size may confer a competitive edge to the invasiveness of *S. glanis* which might influence fish invasions. In some cases, the introduction of *S. glanis* into new environments may affect the composition of fish taxa in a recipient water body (Slavik et al. 2014; Boulêtreau & Santoul, 2016; Vejřík et al. 2017). Those fish whose growth rates permit rapid growth to a size that puts them beyond the gape of *S. glanis* are likely to be favoured over slow growers (Czarnecki et al. 2003; Britton et al. 2007; Copp et al. 2009a; Alp et al. 2011; Thao et al. 2016). *S. glanis* is already invasive in major river

catchments of the Iberian Peninsula and southern France at the expense of native fish species (Copp et al. 2009a; Cucherousset et al. 2018).

Understanding the novel trophic interactions of *S. glanis* released into aquatic habitats is essential given the risks of ecological harm such as predation and disturbance to native species communities (Grey, 2006; Copp et al. 2009a; Cucherousset et al. 2011; Guillerault et al. 2017). There remains some uncertainty about *S. glanis* being categorized as a top predator akin to Zander (*Sander lucioperca*) or pike (*Esox lucius*) and whether it occupies similar high trophic positions within introduced freshwater habitats. This may shift their non-native risk status to a higher ranking which would call for stricter regulatory controls about their movements in the UK (Kopp et al. 2009; Syväranta et al. 2010; Boulêtreau et al. 2011; Slavek et al. 2014; Vejřík et al. 2017).

The latter point is important given some equivocal study findings about their predation impacts upon native fish species communities in recent years. For example, comprehensive reviews of *S. glanis* fish behaviour in invaded habitats implied that because of their solitary scavenging behaviour, their predation impacts were likely to be opportunistic and not harmful to native fish species (Copp et al. 2009a; Martino et al. 2011; Capra et al. 2018). However, other studies have indicated intense predation of native eel (*Anguilla anguilla)* populations by large aggregations of *S. glanis* in some river catchments in France (Bevacqua et al. 2011; Cucherousset et al. 2012; Cucherousset et al. 2018). These invaders exhibited trophic plasticity and preyed upon a broad diet range of freshwater and anadromous fish species including cyprinid, Atlantic salmon (*Salmo salar*) and allis shad (*Alosa alosa*) (Carol et al. 2007; Carol et al. 2009; Syväranta et al. 2009; Syväranta et al. 2010; Boulêtreau et al. 2011; Boulêtreau et al 2018; Cucherousset et al. 2018).

Over the last decade, understanding the impacts of non-native fish in freshwater habitats has been assisted by developments in stable isotope analysis (SIA) about the trophic interactions of invasive species and disruption of food web particularly to prey species. Stable isotope analysis approach gives the possibility of tracing key elements such as carbon or nitrogen using distinctive isotope ratios so that essential ecological processes in the food web can be identified at a fine scale (Grey, 2006; Slavik et al. 2014; Cucherousset et al. 2015; Busst & Britton, 2017). In the present study, the stable carbon isotope values ($\delta^{13}C$) of consumers such as *S. glanis* were used to determine the dietary sources of carbon consumed in ponds. Enriched mean $\delta^{13}C$ values of *S. glanis* indicated higher proportion of cyprinid prey rather than macro-invertebrates in their diet due to variation in isotopic signatures of putative prey organisms (Syväranta et al. 2010; Green et al. 2012).

Similarly, mean ratios of nitrogen isotopes ($\delta^{15}N$) values were used to estimate the trophic position of *S. glanis* relative to a baseline indicator species such as Chironomidae which are primary consumers and were abundant in ponds (Vander Zanden & Rasmussen, 1996; Grey, 2006; Cucherousset et al. 2018). Variation in enrichment of $\delta^{15}N$ values of *S. glanis* size groups may relate to differences in trophic position and possible piscivory (Kopp et al. 2009; Nelson et al. 2017; Cucherousset et al. 2018).

Stable isotope analysis using mean ratios of carbon isotopes and nitrogen isotopes are widespread in food webs and may provide insights into the relationship between consumers and diet, allowing differentiation in trophic interactions between species (Grey, 2006; Tran et al. 2015). The application of stable isotope analysis provides reliable and robust data comparable to conventional gut content analysis of fish yet non-destructively (Kopp et al. 2009; Nelson et al. 2017). It is based on a predictable trophic relationship between consumer and diet with isotopic signatures increasing across trophic levels over a given time period. In contrast, the gut analysis approach with fish is based upon an examination of a specimen`s gut contents following fish kill or nonlethal methods (stomach flushing) to determine dietary contents, this method, however, is also hampered by a high frequency of empty fish guts (72%) within predatory fish (Syväranta et al. 2010; Vejřík et al. 2017).

Developments in stable isotope analysis have aimed to refine isotopic dietary analysis of organisms as the presence of lipids in a sample may cause bias in $\delta^{13}C$ values with misinterpretations in diet. Consequently, lipids are extracted from samples or corrected by use of a lipid normalisation model before stable isotope analysis (Grey, 2006; Green et al. 2012). In other cases, variability in isotopic values of organisms may occur due to seasonality with changes in water temperature or diet quality. As such, a certain level of ecological experience and competence is necessary in interpreting isotopic values with reference to trophic interactions of non-native fish species (Grey & Jones, 1999; Grey, 2001; Marchetti et al. 2004; Grey, 2006; Kopp et al. 2009; Nelson et al. 2017).

Determining non-native fish species invasiveness may benefit from stable isotope analysis approach as it provides a tool in understanding the drivers of trophic interactions of invasive fish species and disruption of food web functioning (Jones et al. 1998; Chirwa, 2008; Gherardi et al. 2009; Kopp et al. 2009; Syväranta et al. 2010; Jackson et al. 2012). Some studies have investigated the trophic position of non-native predators such as *S. lucioperca* so as to determine the scale of their trophic impacts upon prey species. The study outcomes implied that these fish

hold a high trophic position in food web and behave as apex predators in some river catchments in southern France (Kopp et al. 2009). Other studies using stable isotope analysis determined that prey species adapted their foraging activities in order to avoid predatory invaders. For example, significant shifts in diet of native brown trout (*Salmo trutta*) and displacement from foraging niche were revealed owing to aggression from introduced brook trout (*Salvelinus fontinalis*) in mountain streams in south west France (Cucherousset et al. 2007; Cucherousset et al. 2012).

Given the risks of dispersal of non-native *S. glanis* escapees from lake fisheries into rivers over the years, there is a need to understand their growth and trophic impacts to native fish species in the UK (Feuchtmayr et al. 2004; Copp et al. 2009a; Chezik et al. 2014; Rees et al. 2017; Honsey et al. 2018). The aim of this chapter was to investigate the variation in growth, condition, survival, estimated trophic position and trophic interactions of different size *S. glanis* groups (small, medium and large) in freshwater ponds in southern England.

The specific objectives were to:

1) investigate growth (length and weight), Fulton`s condition factor and survivorship of different size groups of *S. glanis* (small, medium and large) stocked at different predator: prey ratios in ponds.

2) determine the influence of abiotic factors such as water temperature upon growth, condition and survival of different size *S. glanis* groups (small, medium and large) in ponds.

3) estimate trophic position of different size class *S. glanis* groups (small, medium and large) stocked with cyprinid species in ponds;

4) determine any variation of mean $\delta^{15}N$ isotope signatures with different sized *S. glanis* groups (small, medium and large) in ponds;

5) assess any variation of mean $\delta^{13}C$ isotope signatures with different sized *S. glanis* groups (small, medium and large) in ponds.

3.2 Materials and methods

A pilot study followed by growth and trophic interactions of *S. glanis* studies in 2013 and 2014 which were used to address the aim and the objectives of this thesis (Copp et al. 2017; Cucherousset et al. 2018).

3.2.1 Sampling and fish survey

The three ponds at Mayland, near Maldon, Essex were used for the pilot study and following studies investigating variation in growth and trophic interactions of *S. glanis* size groups.

3.2.2 Pilot study for efficacy of tagging of *Silurus glanis*

Prior to the *S. glanis* growth and trophic interactions studies, a pilot study was carried out at the same site (three ponds, Mayland, Maldon, Essex) from (23rd May 2011- 4th May 2012) to investigate the efficacy of tagging of *S. glanis* with passive integrated transponder (PIT) tags in the field (See Plate 3.1). The aims were to assess tag retention and potential sub-lethal impacts in using (PIT) tags with non-native *S. glanis* as there are risks of high tag loss (within 7 days) and adverse impacts on fish growth and swimming performance in avoiding predation (Rees et al. 2014).

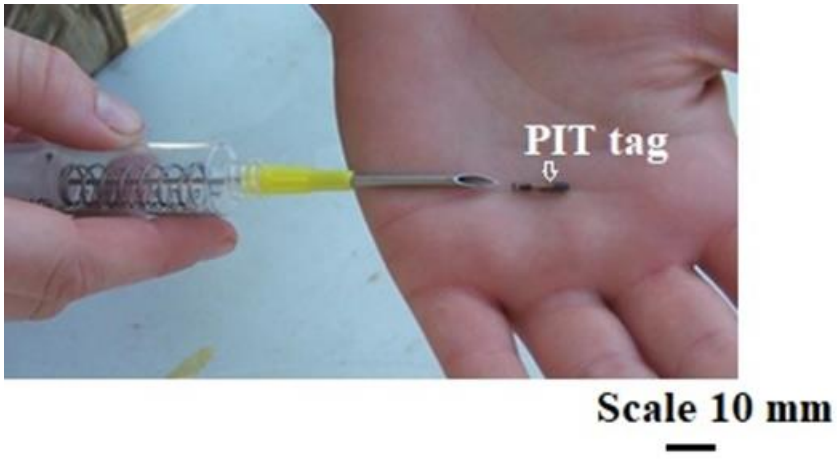

Plate 3.1. Photo of PIT tags used for tagging *S. glanis* in the study (www.bobluskoutdoors.com)

Sixty *S. glanis* originally captured from the wild in Croatia were obtained from an aquaculture source in the UK. Fish length ranged between 39 to 51 cm total length (TL) with *S. glanis* placed into equal numbers (n=20) and selected into 3 size class groups: a) small (36- 40 cm TL), b) medium (41- 44 cm TL) and c) large (49- 51 cm TL) (See Appendix A, Table 6A1.1, Table 6A1.3, Table 6A1.4 and Table 6A1.5). Determination of the size range of *S. glanis* assemblages in the study were consistent with other studies of *S. glanis* and recapture efficiency from small study ponds (Zaikov et al. 2008; Carol et al. 2009; Cucherousset et al. 2018). Selection of the size range of *S. glanis* assemblages were used to determine any potential effects of using passive integrated transponder (PIT) tagging with fish size. It is possible that PIT tag retention might vary with fish size and tag loss was a potential concern with little information available about tagging efficiency with *S. glanis* (Rees et al. 2014). Each fish was measured for total length TL (cm), weight (g) and jaw gape (cm) and tagged under anaesthesia before released separately into ponds. The stocking density of *S. glanis* ranged from 907.47 Kg ha^{-1} biomass in the large *S. glanis* group, 507.44 Kg ha^{-1} in the medium group and 396.26 Kg ha^{-1} biomass in the small size *S. glanis* group (See Appendix A, Table 6A1.1).

Prior to release into ponds at Mayland, *S. glanis* were individually tagged by insertion of a Passive Integrated Transponder (PIT) tag (Wyre Micro Design Ltd: 2.2 x 12 mm, 0.1g) into the ventral area of the peritoneal body cavity between the pelvic fins and anus. Before surgical insertion of PIT tags, fish were anaesthetised by immersion in 2-phenoxy ethanol at the same surgical level (1 ml per dm^3 in anaesthetic bath). Insertion of a PIT tag into *S. glanis* was undertaken manually, using a PIT tag needle to place the tag with incision suture closed using Orahesive. Tagging time (anaesthesia and surgical procedure) was kept to a minimum (~3-4 min for each fish). All tagging was carried out under Home Office licence and in accordance with the UK Animals (Scientific Procedures) Act 1986. All fish were placed in a recovery bath until normal swimming behaviour was observed.

The pilot study revealed high PIT tag retention (>91%) across all *S. glanis* size assemblages over a twelve month interval which suggested that PIT tagging was suitable for long-term field studies for *S. glanis* less than 70cm TL (See Appendix A, Table 6A1.1). However, there were some limitations in the study with sampling difficulties in fyke net efficiency for fish recapture. There was some weight loss among the *S. glanis* assemblages recaptured but it is unlikely that this caused any bias in the tag retention rates observed (See Appendix A, Table 6A1.2). Subsequently, in the following *S. glanis* field studies at Mayland, all ponds were drained down and seine nets were used for better fish recapture efficiency instead of using fyke nets. There

were also adjustments in stocking of forage fish prey in ponds to increase growth in body weight and length across all *S. glanis* assemblages with predator: prey ratios (fish number) of 1:2 and 1:3 in subsequent field studies investigating growth and trophic behaviour of *S. glanis*. In the pilot study, forage prey fish were stocked at approximately 1:1 predator-prey ratios (in fish numbers) with stocking densities of 171.79 Kg ha^{-1} biomass in the large *S. glanis* size group, 58.94 Kg ha^{-1} in the medium *S. glanis* size group, and 47.89 Kg ha^{-1} for the smallest size *S. glanis* group (See Appendix A, Table 6A1.6). Forage prey fish were selected in reference to the approximate jaw gape of *S. glanis* assemblages. The number of prey fish were identified to cyprinid species and each fish was measured for total length (cm) and weight (g) prior to release into all three ponds (Refer to Appendix A, Table6A1.7, Table 6A1.8, Table 6A1.9).

3.2.3 Variation in *Siluris glanis* two year growth study (2012- 2014)

The first year study (25th September 2012- 4th October 2013), took place at the three ponds at Mayland, near Maldon, Essex (grid reference TL 912021) in south east England. The surface area of all ponds were similar e.g. pond 1 (large size *S. glanis* group) was ~ 0.017 ha, pond 2 (medium size *S. glanis* group) was ~ 0.015 ha and pond 3 (small *S. glanis* group) was ~ 0.015 ha. Pond 2 in Mayland is pictured in Plate 3.2. The first year growth study of *S. glanis* ended on 4th October 2013 with follow up recapture of 42 *S. glanis* were caught. Similarly the second year growth study of *S. glanis* commenced with restocking of forage fish prey at predator: prey ratio 1:3 on the 13th November 2013 with follow up recapture on 13th December 2014 with 31 *S. glanis* recaptured.

Plate 3.2 Photo of pond two at Mayland, Essex in 2013 (Rees 2013).

Forty eight PIT tagged *S. glanis* were recaptured from ponds (from the pilot study) by draining down ponds and recapturing all fish (*S. glanis* and forage fish separately for each pond). All *S. glanis* in the three assemblages ranged between 39 to 51 cm total length (TL) (See Plate 3.3). *S. glanis* were placed into relatively equal numbers into 3 size class groups to simulate similar biomass likely to be present in invaded ponds as well as minimising fish aggression and cannabilism. Post PIT tag identification of individual *S. glanis* were carried out using PIT tag reader (in all ponds only PIT tagged *S. glanis* were present). Fish were measured for TL (cm), weight (g) and then categorised into 3 groups by size (large, medium and small size groups) and released separately into 3 adjacent ponds of ~ 1m mean depth which were empty of forage fish. Annual recapture of *S. glanis* was carried out from the ponds (drained down) from September 2012- October 2013.

Plate 3.3 Photo of *S. glanis* in the growth study, Mayland, Essex (Rees 2013).

For each pond, the number of prey fish were identified to species and each fish was measured for total length (cm) and weight (g) (Refer to Appendix A, Table 6A1.21, Table 6A1.22, Table 6A1.23). Overall, forage prey were stocked at 1:2 predator-prey ratios (in fish numbers) for *S. glanis* size groups in the first year (2013) and restocked at 1:3 in the following year (2014) (See Table 3.1).

Table 3.1. Variation in mean length TL and weight of forage fish prey species stocked at different forage prey biomass in *S. glanis* size groups in ponds in 2013 & 2014. Standard Error (SE), Standard Deviation (SD) and 95% Confidence Interval (95%CI) are given.

Group	**n forage prey species 2013**	**Biomass Kg ha^{-1} Forage prey fish**	**Mean TL 2013 (cm)** (± SE)	**Mean W 2013 (g)** (± SE)	**n forage prey species 2014**	**Biomass Kg ha^{-1} Forage prey fish**	**Mean TL 2014 (cm)** (± SE)	**Mean W 2014(g)** (± SE)
Large	45	361.67	19.33 ± 0.20	123.77 ± 4.22	60	821.42	23.52 ± 4.22	210.83 ± 9.12
SD			1.35	28.33			2.77	70.66
95%CI			From 18.93 to 19.74	From 115.26 to 132.28			From 22.80 to 24.23	From 192.58 to 229.09
Medium	36	110.98	14.88 ± 0.11	51.79 ± 1.90	45	350.06	20.62 ± 0.17	130.69 ± 4.62
SD			0.66	11.43			1.17	31.01
95%CI			From 14.66 to 15.11	From 47.92 to 55.66			From 20.27 to 20.97	From 121.38 to 140.02
Small	30	74.71	13.03 ± 0.25	36.61 ± 3.31	45	289.68	18.40 ± 0.25	94.63 ± 4.31
SD			1.39	18.15			1.69	28.94
95%CI			From 12.52 to 13.55	From 29.83 to 43.39			From 17.90 to 18.91	From 85.94 to 103.33

Forage fish prey size was selected in relation to the approximate jaw gape of *S. glanis* size groups. For the smallest size *S. glanis* group, forage fish prey was selected between ~7.62- 12.70 cm TL in length. Larger forage fish prey of body lengths ~ 10.16- 15.24 cm TL were released into the medium size *S. glanis* group. The largest forage fish prey of body lengths ~ 12.70-20.32cm TL were released into the largest size *S. glanis* group. Individual forage fish prey weights (g) ranged from ~ 20 - 190g released into ponds.

Temperature in all ponds was monitored by Tiny Tag loggers set at 15 minute readings which recorded continuously (24 hr) throughout the 2 year study trial. All ponds were quarterly monitored for chemical water parameters such as; pH, dissolved oxygen, phosphate and nitrate concentrations which were measured by Hach HI 9146 meter and Hach biotector B3500eTOC analyser respectively.

Long-term survivorship was measured as the proportion of recaptured fish relative to the total number of tagged fish released. After draining the ponds the *S. glanis* were removed, counted and re-measured for length (TL cm) and weight (g). Age determination in the estimation of *S. glanis* growth (by examination of otoliths following fish kill) was not included in this study and all *S. glanis* were rehomed at the end of the study. The forage fish were sourced from the large angling lakes at the site and all cyprinid species such as *C. carpio, R. rutilus, B. bjoerkna* and *S. erythrophthalmus* were recorded individually with their lengths and weights measured before being released into the ponds according to size.

As demonstrated in the pilot study, sampling difficulties associated with *S. glanis* habitat use of crevices and concealment under tree roots in ponds resulted in poor recapture rates of *S. glanis* with fyke nets. Consequently, monthly fyke netting for individual fish growth rates was not practical in the field studies. The practical logistics involved in draining down 3 ponds within a narrow time frame (8 hrs) and associated financial costs affected the study conduct. The *S. glanis* assemblages were measured annually, gut content analysis, eDNA metabarcoding of fish faeces and prey fish biomass recapture were not included in the study. However, despite the limitations in experimental design and the lack of replicates of *S. glanis* size groups in ponds in the study, data at each follow up time revealed good recapture rates and survival of *S. glanis* across all size groups.

Survival, Fulton`s condition factor *K*, median growth and specific growth rate of *S. glanis* were calculated at the end of each study.

Equation 2 specific growth rate (SGR) of *S. glanis* size groups

This parameter was calculated with the formula:

$SGR\ (\%\ day^{-1}) = 100 \times (\ln W_t - \ln W_0) \times t^{-1}$

Where ln is the natural logarithm; W_0 and W_t are the initial and final fish mass (g); t is the number of days in the trial (Acolas et al. 2007; Jepsen et al. 2008).

To investigate median growth of *S. glanis* size groups (in length and weight) between release (time point t1) and follow up (time point t2) at end of trial, a Mann-Whitney U test was used for each group. For incremental growth (in length and weight) of *S. glanis* size groups between release (t1) and follow up (t2) a Wilcoxon signed-rank test was used for the non-parametric data.

Equation 3 Fulton's condition factor (*K*) of *S. glanis* size groups

This parameter was calculated with the formula:

$K = W \times 10^5 \times TL^{-3}$

Where W is mass (g); TL is the fish total length (cm) (Copp & Mann 1993).

Fulton's condition factor (*K*) was calculated for *S. glanis* size groups at follow up (time point t2). Comparisons in *K* of *S. glanis* size groups from their release (time point t1) into the ponds and follow up (t2) were investigated by use of a Wilcoxon signed- rank test. The process involved attempting to normalise the data, by using a natural logarithmic transformation (ln) which succeeded in normalising data of Fulton`s condition factor *K*. A t-test was used to ascertain any differences in the mean log Fulton`s condition factor *K* between *S. glanis* size groups.

Fulton's condition factor *K* is based on an idealized isometric growth pattern for individual fish with an assumption that a fish is in better 'condition' or 'well being' if it has a higher weight-to-length ratio at a given length. Fulton`s condition factor *K* of a fish may vary with species, food source availability, age, sex and habitat type (Copp & Mann 1993; Skov et al. 2005).

Fish growth may be quantified using Degree-days (DD) approach of the thermal opportunity for *S. glanis* growth by the aggregation of water temperatures in ponds (Honsey et al. 2018; Honsey et al. 2019). Many studies have adopted this approach and, when studying the *S. glanis* growth in invaded habitats, the (T_0) baseline temperature for the onset of growth was determined as $\geq 17^0$C with optimum growth as $\geq$ 25 - 28^0C (Linhart et al. 2002; Ulikowski et al. 2003; Britton et al. 2007; Carol et al. 2007; Gullu et al. 2008; Zaikov et al. 2008; Carol et al. 2009; Copp et al.

2009a; Muscalu et al. 2010; Cirkovic, 2012; Cucherousset et al. 2018). Similarly, available evidence was used to determining the (T_0) metric of *S. glanis* size groups in the study (Bogut et al. 2002; Jamróz et al. 2008; Dediu et al. 2010; Alp et al. 2011; Kumar et al. 2017).

3.2.4 Trophic interactions of *Silurus glanis* study (2013- 2014)

3.2.4.1 Putative prey cyprinids & S*ilurus glanis* sampling

The trophic interaction study of *S. glanis* was undertaken concomitantly with the second year growth study at the three ponds in Mayland, Essex. 31 PIT tagged *S. glanis* were recaptured with lengths ranging from 36-59 cm in total length (TL) from ponds.

Foraging activity of *S. glanis* was quantified using Degree-days (DD) approach (Honsey et al. 2018; Honsey et al. 2019). The (T_0) baseline temperature for the onset of foraging was determined as $\geq 12^0$C (David, 2006; Britton et al. 2007; Copp et al. 2009a; Cucherousset et al. 2018).

Fish (prey fish and *S. glanis*) were individually measured for total length (TL) and weight before release into ponds. In addition, in order to allow for stable isotope analysis (See Table 3.2) samples of prey fish (approximately 10 individuals from each cyprinid species from each pond) were fin clipped whilst fish were under general anaesthesia (5 ml-1 Lof 2-phenoxyethanol) before release into ponds again. Similarly, cyprinid fin samples included *R. rutilus, B. bjoerkna* and *S. erythrophthalmus*) from each pond whilst all tagged *S. glanis* size groups were sampled for fin clipping before release into ponds and upon annual recapture.

Table 3.2 Variation in mean length (TL ± SE) of forage fish prey species stocked in ponds (different sized *S. glanis* groups) in the trophic interactions study from 2013-2014 .

		Pond1 **Large *S. glanis* gp**		**Pond 2** **Medium *S. glanis* gp**		**Pond 3** **Small *S. glanis* gp**
Species	**n**	**Mean TL (cm)**	**n**	**Mean TL (cm)**	**n**	**Mean TL (cm)**
Silver bream	20	23.44 ± 0.62	15	20.56 ± 0.20	15	18.26 ± 0.44
Roach	20	23.26 ± 0.69	15	20.55 ± 0.32	15	18.51 ± 0.41
Rudd	20	23.86 ± 0.56	15	20.75 ± 0.31	15	18.44 ± 0.48

Fin clipping of *S. glanis* and cyprinids correlates to the muscle tissue of the fish specimens, this allows for optimal stable isotope analysis without lipid extraction procedure. The same methodology was used before (Kopp et al. 2009; Cucherousset et al. 2012). This method allowed for non-lethal sampling of fish as their fin clips correlated closely to muscle tissue for quantifying fish diet with stable isotope analysis (Syväranta et al. 2009; Cucherousset et al. 2012).

Standard fin clipping procedure of fish specimens were implemented using surgically sharp scissors, sterilized with alcohol, a small fin clip of $1cm^2$ was taken from each specimen. The fin sample was washed with distilled water using a pipette, placed in an Eppendorf tube which was labelled with date and species recorded. Eppendorf samples from each pond were chilled in ice boxes and later frozen in freezers before laboratory stable isotope analysis. Survival, variation in estimated trophic position and mean $\delta^{13}C$ and $\delta^{15}N$ isotopes values of different *S. glanis* size groups were calculated at the end of the study. In addition, estimated trophic position of cyprinid forage fish species were calculated for each ponds. The mean $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of cyprinid prey fish and other invertebrate trophic groups were also determined.

Mean δ^{15} N and δ^{13}C signatures

The ratio of heavy to light nitrogen ^{15}N:^{14}N and carbon ^{13}C:^{12}C stable isotopes of *S. glanis* were used to determine the scale of trophic interactions upon putative prey species in ponds. The heavy isotopes of N and C react differently from the light isotopes in chemical reactions of trophic processes which results in isotopic fractionation and can be used as ecological tracers. Consequently, the mean δ^{15} N and $\delta^{13}C$ signatures of a specimen are isotopically distinct and carry an isotopic imprint known as a signature which can be used to determine nitrogen and carbon sources in a food web (Werner et al. 2012).

Estimated trophic position

Estimated trophic position is the trophic level that an organism occupies in the food chain which is hierarchical in food webs. Stable isotope ratios of $\delta^{15}N$ and $\delta^{13}C$ of organism can reveal the flow of carbon and nitrogen in the food web. The stable isotopes $\delta^{15}N$ of fish may be used to estimate their trophic positions in food webs by the use of an appropriate isotopic baseline species which reflects the $\delta^{15}N$ isotopic signature at the base of the food web. Baseline indicator species are primary consumers such as Chironomidae in the food web that capture $\delta^{15}N$ isotopic signature variation at the base of food webs (referred to as baseline isotopic signatures). As a

general rule, baseline indicator species allow comparison between $\delta^{15}N$ values of secondary consumers (fish) which reflect the nitrogen source upon which they are reliant. Chironomidae were abundant in the ponds and are important dietary source of *S. glanis* (Grey, 2006; Kopp et al. 2009; Syväranta et al. 2010; Copp et al. 2017).

Estimated mean trophic positions for fish were calculated using the formula from (Vander Zanden & Rasmussen, 1996; Vander Zanden et al. 1997; Grey, 2006).

Equation 4 Estimated mean trophic position

$$\text{Estimated mean trophic position} = [(\delta^{15}N_{\text{Secondary consumer}} - \delta^{15}N_{\text{prey}}) / 3.4] + 2$$

Where ($\delta^{15}N_{\text{Secondary consumer}}$) was the mean *S. glanis* size class $\delta^{15}N$ value; ($\delta^{15}N_{\text{prey}}$) was the mean $\delta^{15}N$ value of Chironomidae from pond; 3.4 represented the fixed trophic fractionation factor; 2 was the baseline trophic level of Chironomidae as a primary consumer in the food web.

Mean estimated trophic position of fish (*S. glanis* and cyprinids) were calculated as the $\delta^{15}N$ values alone cannot be used as an absolute measure of trophic position owing to variation in $\delta^{15}N$ isotopic signatures at the base of food webs in ponds (Vander Zanden et al. 1997; Grey, 2006).

The mean estimated trophic position of predatory *S. glanis* size groups and cyprinid prey fish was calculated using their mean $\delta^{15}N$ isotopic signatures. The formulae assumed that nitrogen in an organism`s tissues increased with each trophic level with trophic fractionation of 3.4‰ for nitrogen (Kopp et al. 2009; Syväranta et al. 2010).

To test for differences between *S. glanis* median length from between release and recapture a non-parametric Wilcoxon signed- rank test was employed for each *S. glanis* size group. Following examination of normality and homoscedasticity, and to determine any differences in estimated mean trophic postion relationships with *S. glanis* size groups, a one-way ANOVA was employed with post-hoc tests (Tukey`s multiple comparisons).

In addition, a pooled ordinary least square regression method was used to determine the relationship between dependent variable ($\delta^{13}C$ isotopic pooled values of *S. glanis*) and independent variable (increasing length of *S. glanis*) calculated by using the formula:

Equation 5 Pooled ordinary least square regression method

$$y_i = \beta_0 + \beta_1 X_i + e_i$$

where y_i was the dependent variable of $\delta^{13}C$ isotopic pooled values of *S. glanis* size groups; β_0 was the constant; β_1 was the parameter estimate for the ith independent length variable; X_i was the vector of independent length variable and e_i was the error term. The effects of independent length variable of *S. glanis* size groups were determined with dependent *S. glanis* size groups $\delta^{13}C$ isotopic values which indicated either a positive or negative correlation between these variables which may or not be significant (Wooldrige, 2003).

3.2.5 Macro-invertebrates sampling

The sampling for SIA of macro-invertebrate samples were undertaken as they are considered important putative prey resources for the *S. glanis* size groups at the total lengths (TL) they were released into the ponds (Carol et al. 2007; Carol et al. 2009; Cucherousset et al. 2018).

The macro- invertebrate sampling was undertaken in May and September 2014 (over one day period). Semi-quantitative samples for macro-invertebrates were collected for each pond at these times to cover for possible seasonal variation in fish diet. Individual omnivorous food items were collected and assigned into trophic groups such as: plankton, algae, detritus, detritivores and zooplankton. Different macro-invertebrate taxa were assigned into functional feeding groups of: grazers, shredders and predators for SIA (Vander Zanden & Rasmussen, 1999; Grey, 2006; Britton & Busst, 2018) (See Table 3.3).

Table 3.3 Frequency of various primary producer and consumer groups analysed for stable isotope analysis in ponds

Trophic group	**Prey category**	**n**	**includes**
Primary producer	Algae	8	
Primary producer	Plankton	5	
Primary producer	Detritus	11	Detritus, plants, mud
Primary consumer	Detritivores	9	Chironomidae, Asellidae
Primary consumer	Zooplankton	15	Planktonic copepods, *Cyclops bicuspidatus*, planktonic crustaceans *Daphnia*, zooplankton micro-crustacea
Primary consumer	Grazer	1	Ephemeroptera
Primary consumer	Shredder	5	Amphipod crustacean *Gammarus pulex*, lesser water boatman *Corixa punctata*
Primary consumer	Predator	13	Damselfly *Coenagrion puella*, great diving beetle *Dytiscus marginalis*

All three ponds were sampled using the National Pond Survey (NPS) protocol (Biggs et al. 1994; Biggs et al. 2005) for plant and macro-invertebrates groups. All samples were duplicated for each trophic group to ensure sampling efficacy (Grey, 2001; Grey, 2006). Samples of algae and plankton from ponds were collected by two horizontal drags across the pond surface using a 110 µm mesh plankton net. Then, plankton samples were washed with distilled water using a pipette, placed in Eppendorf tubes, labelled and then frozen in fridge freezer before laboratory analysis for SIA.

Macro-invertebrate samples were collected using an invertebrate kick net of 0.5 mm mesh size, with the net swept through vegetation for ~ three minutes in each pond. There was also some net kicking of the pond substratum to disturb macro-invertebrates following NPS protocols in each pond.

Macro-invertebrates were collected in a tray were identified and counted directly by hand using forceps. The specimens were classified by family and separated into functional feeding trophic groups with sufficient number of individuals (n=9 per pond) collected for SIA. Specimens were washed with distilled water and placed into Eppendorf tubes, labelled and then frozen at -180^{0}C in fridge freezer. Dominant trophic groups such as Chironomidae were collected from each pond in preparation for SIA and for the estimation of trophic positions of *S. glanis* and cyprinids (Vander Zanden & Rasmussen, 1996; Grey & Jones, 1999; Feuchtmayr et al. 2004; Grey, 2006; Britton & Busst, 2018).

3.2.6 Laboratory procedures for stable isotope analysis

The fish tissue samples were oven dried for 24 hour at 60°C and then ground in a drying cabinet with a built in grinder whilst the invertebrate tissue samples were freeze dried overnight and then ground in situ. The carbon and nitrogen isotope analysis of fish and invertebrate tissue samples were performed by Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS) (Kopp et al. 2009).

All samples and references were weighed in tin capsules, sealed, and loaded into an auto-sampler on a Europa Scientific elemental analyser, and then dropped in sequence into a furnace of 1000°C and combusted in the presence of oxygen, for C and N isotope analysis. The resultant gases were swept away in a continuous flow to an Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS). All fish and invertebrate samples and references were converted to N_2 and CO_2 and analysed using this method (Vander Zanden et al.1999; Kopp et al. 2009).

The analysis proceeded in a batch process by which a reference of soy protein, L-alanine, tuna protein, oxalic acid and ammonia sulphate standards were analysed followed by a number of samples and then another reference as a quality control check of samples analysis. These standard references were calibrated against the inter-laboratory comparison standards distributed by the International Atomic Energy Agency (IAEA) standards. The isotopic analyses of samples were performed at ISO Analytical laboratory, Crewe, UK.

Isotope ratios of sample results were expressed in delta (δ) notation, measured as the parts per thousand (‰) deflection from the standard material,

for example for $\delta^{13}C$ or $\delta^{15}N = ([R_{SAMPLE} / R_{STANDARD}] - 1) X 1000$

Where R is either $^{13}C/ ^{12}C$ or $^{15}N/ ^{14}N$.

The standard material was Pee Dee belemite limestone for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The more positive isotopic value was reflective of isotopic enrichment with higher proportion of the heavier isotopes for example of ^{13}C or ^{15}N in the sample. Twenty percent of the fish and invertebrate samples were analysed in duplicate, of which the mean value of the duplicated and original sample of ^{13}C or ^{15}N were used in data analysis (Vander Zanden & Rasmussen, 1996; Cucherousset et al. 2007).

3.3 Results

3.3.1 Variation in growth of *Silurus glanis*

In the first year study (2013), with stocking at predator-prey density of 1:2 for *S. glanis* size groups and at prey fish stocking densities of 361.67 Kg ha^{-1} the mean growth for the largest *S. glanis* group, of mean length 53.08 ± 0.81 cm TL, had a mean growth increment of 2.14 ± 0.39 cm TL, (4%). At prey fish stocking densities of 110.98 Kg ha^{-1} in pond, the medium size *S. glanis* group mean length was 44.78 ± 0.74 cm TL with mean increment of 1.89 ± 0.49 cm TL (4%). The smallest *S. glanis* size group had a mean length of 41.63 ± 0.78 cm TL with highest mean growth increment of 2.27 ± 0.33 cm TL (6%) with prey fish stocking density of 74.71 Kg ha^{-1} (See Table 3.4 & 3.5).

The mean growth increment in weight was highest for the smallest size *S. glanis* group with 134.41 ± 14.72g. Mean weight was 400.69± 31.00 g, (34%). The medium size *S. glanis* group had lower mean growth increment in weight of 56.85 ± 18.45 g and mean weight of 427.69 ± 21.00 g, (17%). The largest *S. glanis* group mean growth increment in weight was 83.52 ± 14.00 with mean weight of 739.64 ± 39.17 g (11%) (See Table 3.4 & 3.5).

In 2014, with forage fish restocked at predator: prey ratio of 1:3, there was a higher mean growth in length and weight for *S. glanis* assemblages than in the previous year (2013). At prey fish stocking densities of 821.42 Kg ha^{-1} in the pond the highest mean growth was for largest *S. glanis* group with mean length of 56.49 ± 1.65 cm TL and mean increment of 4.39 ± 0.87 cm TL in length (8%). Their mean weight was 967.38 ± 100.47 g with a mean increment of 266.64 ± 75.39g (28%). For the medium size *S. glanis* group, with prey fish stocking densities of 350.06 Kg ha^{-1} in pond, *S. glanis* mean length was 45.68 ± 1.17 cm TL with mean increment of 1.29 ± 0.41 cm TL in length (2%). Their mean weight was 516.69 ± 43.12 g with increment of 98.56 ± 19.58 g (13%). Regarding the smallest *S. glanis* size group with prey fish stocking densities of 289.68 Kg ha^{-1} in pond, their mean length was 45.81 ± 1.53 cm TL with increment of 3.86 ± 1.25 cm TL (8%). Their mean weight was 627.00 ± 72.08 g with increment of 223.60 ± 47.84 g in body weight (36%) (See Table 3.4 & 3.5).

Table 3.4. Variation in mean growth (W & TL± SE) & biomass of *S. glanis* size groups in the first year (2013) and second year growth study (2014). Standard deviation (SD) and 95% Confidence Interval (95%CI) are given.

	Mean length (TL) (cm) (± SE)						**Mean weight (W) (g) (**± SE)		
Group	**Baseline TL**	**2013 TL**	**2014 TL**	**Baseline Biomass Kg ha^{-1}**	**Baseline W**	**2013 Biomass Kg ha^{-1}**	**2013 W**	**2014 Biomass Kg ha^{-1}**	**2014 W**
Large	50.55± 0.72	53.08± 0.81	56.49± 1.65	742.66	635.39 ±33.49	768.46	739.64 ±39.17	816.62	967.38 ± 100.47
SD	3.05	3.24	5.95		142.08		156.70		362.25
95%CI	From 49.03 to 52.07	From 51.35 to 54.81	From 52.89 to 60.09		From 564.73 to 706.04		From 656.14 to 823.14		From 748.48 to 1186.29
Medium	42.47± 0.53	44.78± 0.74	45.68± 1.17	334.85	330.91 ±23.83	330.95	427.69 ±21.00	246.04	516.69 ± 43.12
SD	2.21	2.68	3.30		98.27		75.71		122.10
95%CI	From 41.33 to 43.61	From 43.16 to 46.41	From 42.93 to 48.45		From 280.38 to 381.43		From 381.94 to 473.44		From 414.60 to 618.77
Small	39.15± 0.55	41.63± 0.78	45.81± 1.53	232.41	262.80 ±19.23	354.35	400.69 ±31.00	426.53	627.00 ± 72.08
SD	1.98	2.83	4.83		69.34		111.76		227.94
95%CI	From 37.95 to 40.34	From 39.92 to 43.34	From 42.35 to 49.27		From 220.90 to 304.70		From 333.15 to 468.23		From 463.95 to 790.05

Table 3.5. Variation in mean incremental growth (weight and length) of different *S. glanis* size groups from 2012- 2013 and 2013 -2014. Standard deviation (SD), 95% Confidence Interval (95%CI) and P values (*P*) given.

Group	**Length (TL) (cm) (**±SE) **2013**	**Weight (g) (** ±SE) **2013**	**Length (TL) (cm) (**±SE) **2014**	**Weight (g) (**±SE) **2014**
Large group				
Mean difference	2.14± 0.39	83.52 ± 14.00	4.39± 0.87	266.64 ± 75.39
SD	1.54	55.99	2.89	75.39
P value	0.000	0.000	0.001	0.005
95%CI	From 1.32 to 2.97	From 53.69 to 113.35	From 2.45 to 6.33	From 98.65 to 434.62
Medium group				
Mean difference	1.89± 0.49	56.85 ± 18.45	1.29 ± 0.41	98.56 ± 19.58
SD	1.78	61.19	1.16	55.38
P value	0.002	0.007	0.017	0.002
95%CI	From 0.82 to 2.96	From 15.74 to 97.97	From 0.31 to 2.26	From 52.26 to 144.86
Small group				
Mean difference	2.27± 0.33	134.41 ±14.72	3.86± 1.25	223.60 ± 47.84
SD	1.28	56.99	3.95	151.28
P value	0.000	0.000	0.013	0.001
95%CI	From 1.56 to 2.98	From 102.85 to 165.97	From 1.03 to 6.69	From 115.38 to 331.82

A non-parametric Wilcoxon signed-rank test was performed for each *S. glanis* size group, with the Null hypothesis that there was no difference between median length or weight for each group between the two time points (release and follow up recapture). The results indicated that in 2013 and 2014 for each *S. glanis* size group, differences in median weight and length were highly significant between release and recapture so the null hypothesis was rejected.

In 2013, differences in median length for large size *S. glanis* group were significant z = -3.52, $P < 0.001$, median weight z = -3.52, $P < 0.05$. For the medium *S. glanis* group, median length z = -3.18, $P < 0.05$, median weight z = -3.18, $P < 0.05$ and with the small *S. glanis* size group, median length z = -3.18, $P < 0.05$ and median weight z = -3.18, $P < 0.05$. Similarly in 2014, results indicated that small size *S. glanis* group, median length z =-2.67, $P=0.008$ with the median weight z = -2.80, $P =0.005$. Median length for large *S. glanis* size group was z =-2.93, $P=0.003$, median weight z = -2.93, $P<0.05$. Regarding the medium *S. glanis* size group, median length z = -2.52, $P=0.012$ and median weight z = -2.52, $P < 0.05$ (See Table 3.6).

Table 3.6. Significant statistical comparisons (Wilcoxon signed- rank tests) of growth and differences in median length (TL) and weight (W) of *S. glanis* size groups from release and follow up in the study.

Group	**Median $_{TL}$ z- value 2013**	***p*- value**	**Median $_{W}$ z- value 2013**	***p*- value**	**Median $_{TL}$ z- value 2014**	***p*- value**	**Median $_{W}$ z- value 2014**	***p*- value**
Large	-3.52	<0.001	-3.52	<0.05	-2.93	0.003	-2.93	0.003
Medium	-3.18	<0.05	-3.18	<0.05	-2.52	0.012	-2.52	<0.05
Small	-3.18	<0.05	-3.18	<0.05	-2.67	0.008	-2.80	0.005

3.3.2 Variation in mean specific growth rate of *Silurus glanis* groups

For all *S. glanis* size groups, the mean specific growth rate in length $(SGR)_{TL}$ and in weight $(SGR)_W$ were positive in both years (2013 and 2014).

In 2013, the mean $(SGR)_{TL}$ for the large *S. glanis* size group was +0.01 cm/day in length and +0.04g/ day in weight for mean $(SGR)_W$. For the medium sized *S. glanis* group the mean $(SGR)_{TL}$ was +0.01cm/day with $(SGR)_W$ + 0.06g/day in body weight. For small sized *S. glanis* group, their mean $(SGR)_{TL}$ was + 0.02 cm/day and + 0.12g/day for mean $(SGR)_{W,}$ and was the highest out of all other *S. glanis* size groups in the study.

In 2014, the smallest *S. glanis* group mean $(SGR)_{TL}$ was + 0.02 cm/day and mean $(SGR)_W$ + 0.12g/day. The medium size *S. glanis* group mean $(SGR)_{TL}$ was +0.01cm/day and $(SGR)_W$ was + 0.04g/day whereas with the largest *S. glanis* mean $(SGR)_{TL}$ was +0.02 cm/day and mean $(SGR)_W$ of +0.07g/ day (See Table 3.7).

Table 3.7. Variation in mean specific growth rate (% day^{-1} in weight and length ±SE) of different *S. glanis* groups in 2013 and 2014 in the study. Standard deviation (SD).

Group	**SGR_W mean (% day^{-1}) 2013** (±SE)	**SGR_L mean (% day^{-1}) 2013** (±SE)	**SGR_W mean (% day^{-1}) 2014** (±SE)	**SGR_L mean (% day^{-1}) 2014** (±SE)
	Weight (g)	**Length (cm)**	**Weight (g)**	**Length (cm)**
Large	+0.04 (±0.01)	+0.01 (±0.00)	+0.07 (±0.02)	+0.02 (±0.00)
SD	0.20	0.01	0.05	0.01
Medium	+0.06 (±0.02)	+0.01 (±0.00)	+0.04 (±0.02)	+0.01 (±0.00)
SD	0.01	0.01	0.05	0.01
Small	+0.12 (±0.01)	+0.02 (±0.00)	+0.12 (±0.02)	+0.02 (±0.01)
SD	0.04	0.01	0.06	0.02

3.3.3 Variation in Fulton`s condition factor *K* for *Silurus glanis* groups.

The results of Fulton`s condition factor (*K*) and the growth (length and weight) of *S.glanis* indicated that the smallest *S. glanis* had the highest mean log Fulton`s condition factor *K* of 6.28 ±0.07 compared to other *S. glanis* size groups in 2013 which was significant different ($P < 0.05$).The mean log Fulton`s *K* of the medium *S. glanis* size group was 6.17 ± 0.06 and for the large *S. glanis* size group was 6.21 ± 0.01. There were significant differences in mean log Fulton`s *K* between large and small size *S. glanis* groups t = - 3.32, ($P < 0.05$) and between medium and small size *S. glanis* groups, t = - 2.69, ($P < 0.05$). However, there was no significant difference in the mean log value of condition factor *K* between large and medium sized *S. glanis* groups at follow up, t = 0.34, ($P = 0.74$) (See Table 3.8).

In 2014, the results revealed that all *S. glanis* size groups had a higher mean log Fulton`s condition factor *K* than in the previous year, yet no significant differences between *S. glanis* size groups. In 2014, the small *S. glanis* size group had the highest mean log Fulton`s condition factor *K* of 6.43 ± 0.07 out of all groups. The medium *S. glanis* size group had mean log Fulton`s condition factor K of 6.26 ±0.71 and the largest *S. glanis* size group was 6.25 ± 0.04 respectively (See Table 3.8, Figure 3.1 & 3.2).

Table 3.8. Variation in mean Log Fulton`s condition factor *(K*) for all *S. glanis* size groups in 2013 and 2014 in the study. Standard deviation (SD) and 95% Confidence Interval (95% CI).

Group	**Mean log Fulton`s Condition *K* 2013** ± SE	**Mean log Fulton`s Condition *K* 2014** ± SE
Large	6.21 ± 0.01	6.25 ± 0.04
SD	0.05	0.13
95% CI	From 6.18 to 6.24	From 6.16 to 6.33
Medium	6.17 ± 0.06	6.26 ± 0.71
SD	0.16	0.20
95% CI	From 6.04 to 6.31	From 6.09 to 6.43
Small	6.28 ± 0.07	6.43 ± 0.07
SD	0.21	0.22
95% CI	From 6.12 to 6.43	From 6.28 to 6.59

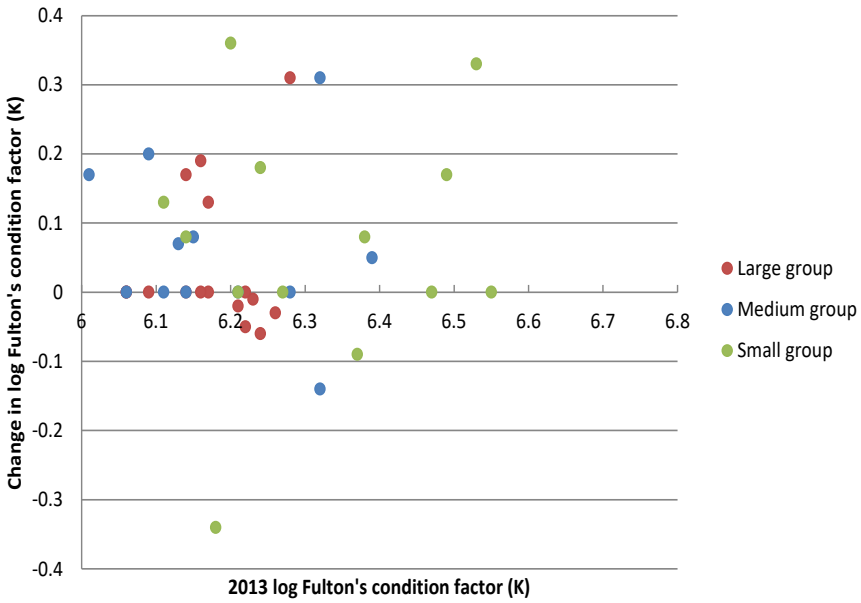

Figure 3.1 Changes in mean log Fulton`s condition factor (*K*) between 2013 to 2014 for all *S. glanis* size groups. Logarithmic transformation of (*K*) has compressed strong trends between *S. glanis* size groups 2013-2014.

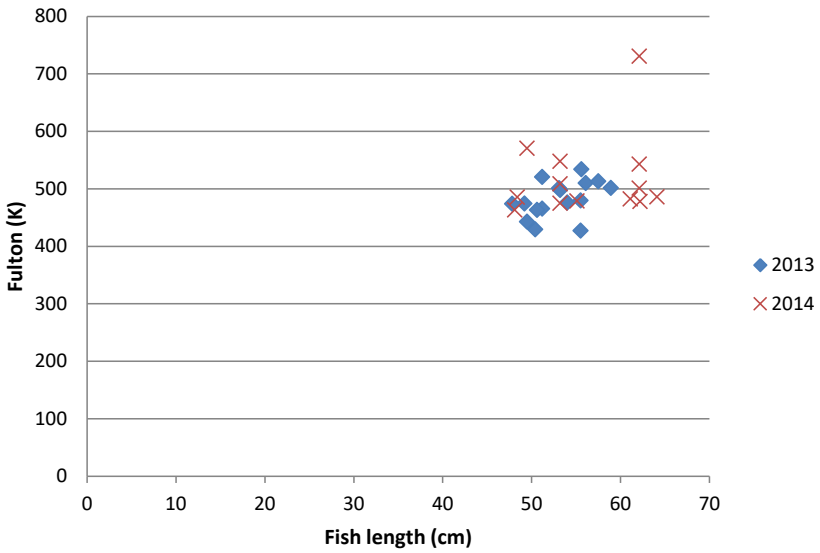

Figure 3.2 Variation in mean Fulton`s condition factor (*K*) in 2013 to 2014 for all *S. glanis* size groups. Each point represents a *S. glanis* specimen with variation between fish length and Fulton`s condition factor *K*. Larger specimens had higher values (*K*) in 2014 than the previous year.

3.3.5 Variation in water temperature in ponds

Seasonal variation in water temperature was recorded in all ponds in 2013 and 2014 during the study. S. *glanis* require water temperatures over 17 ºC to initiate growth in aquatic habitats (Linhart et al. 2002; Ulikowski et al. 2003; Britton et al. 2007; Carol et al. 2007; Gullu et al. 2008; Zaikov et al. 2008; Carol et al. 2009; Copp et al. 2009a; Muscalu et al. 2010; Cirkovic, 2012; Cucherousset et al. 2018). Mean daily water temperatures in the ponds were suitable for the onset of *S. glanis* growth which ranged from over 17 to 23ºC from April to September.

Overall, the mean daily water temperature compatible for *S. glanis* growth was higher in 2013 than in 2014 for all ponds. In 2013 there was a higher frequency in mean daily Degree- days >17ºC with 115 degree days in pond 1 (large *S. glanis* size group), 114 Degree- days for the other *S. glanis* groups where as in 2014, there was a lower number of Degree- days for example 97 Degree- days in pond 1 for large size *S. glanis* group (Refer to Ch 2, Table 2.3).

The water quality in ponds was good with mean dissolved oxygen levels within range supportive for *S. glanis* fish health and survival. In 2013 there was 89% survival for large size *S. glanis* group, 76% for medium size *S. glanis* group and 100% for small size *S. glanis* group in the study. In 2014 there was 81% survival for large size *S. glanis*, 62% for medium size *S. glanis* group and 77% for small *S. glanis* group.

In terms of *S. glanis* foraging activity there was some seasonal variation of mean daily water temperatures that ranged from 12- 23ºC in ponds from April to October (See Figure 3.3). The baseline to initiate foraging activity of *S. glanis* is ≥ 12ºC (Carol et al. 2009; Copp et al. 2009a; Cucherousset et al. 2018).

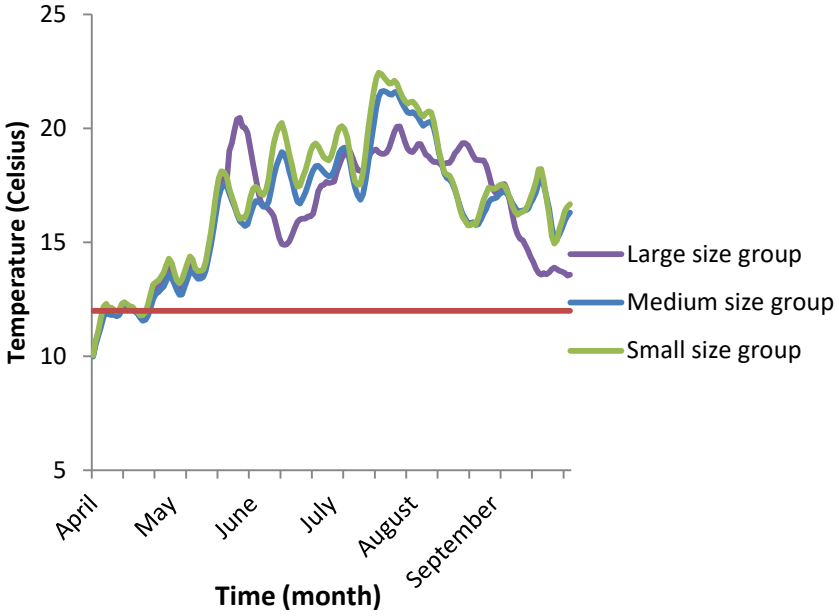

Figure 3.3 Variation in mean daily water temperatures and foraging activity of *S. glanis* in ponds in 2014. The red line represents the baseline water temperature for onset of foraging ($\geq 12^0$C) (Carol et al. 2009; Copp et al. 2009a; Cucherousset et al. 2018).

3.3.5 Variation in trophic position with *Silurus glanis* groups.

A total of 31 *S. glanis* were recaptured and sampled for stable isotope analysis from ponds in 2014. Thirteen specimens were recaptured from the large size group of median total length 56.49 cm ± 1.65, 8 specimens were recaptured from medium size group of median total length 45.69 cm ± 1.17. 10 specimens were recaptured from the small size group of median total length 45.81 cm ± 1.53 (See Table 3.9). A non-parametric Wilcoxon signed- rank test was performed for each *S. glanis* size group, with the Null hypothesis that there was no difference between median length for each group between release and follow up recapture. The results indicated for each *S. glanis* size group, differences in median length were highly significant between release and recapture so the null hypothesis was rejected. For the small size group, median length z = -2.67, $P = 0.008$ where as for the medium size group was z = -1.82, $P = 0.068$ and large size *S. glanis* z = -2.77, $P = 0.006$.

The largest size *S. glanis* group had the highest estimated mean trophic position of 4.37 ± 0.04, with slightly lower values for smaller groups which possibly suggested a trend with fish length and trophic position in food webs. The medium size *S. glanis* group estimated mean trophic position was 4.35 ± 0.02 whilst the smallest *S. glanis* group was 4.29 ± 0.06. Specifically, differences in trophic position between *S. glanis* size groups were not significantly different between the large, medium and small size *S. glanis* groups (ANOVA, $F_{(2, 28)} = 0.794$, $P = 0.462$) (See Table 3.9, Figure 3.4).

Table 3.9 Variation of median total lengths of *S. glanis* size groups at recapture (2014), with minimum, maximum total lengths and estimated mean trophic position. Standard error **(**±SE), Standard Deviation (SD) and 95% Confidence Intervals (95% CI) are given.

***S. glanis* group**	***n***	**Median TL (cm) (**± SE)	**Min TL (cm)**	**Max TL (cm)**	**Mean TP (**±SE)
Large	13	56.49 ± 1.65	48.10	64.10	4.37 ± 0.04
SD		5.95			0.14
95% CI		From 52.89 to 60.09			From 4.28 to 4.45
Medium	8	45.69 ± 1.17	42.20	53.10	4.35 ± 0.02
SD		3.30			0.07
95% CI		From 42.93 to 48.45			From 4.29 to 4.41
Small	10	45.81 ± 1.53	41.70	58.10	4.29 ± 0.06
SD		4.83			0.20
95% CI		From 42.35 to 49.27			From 4.15 to 4.43

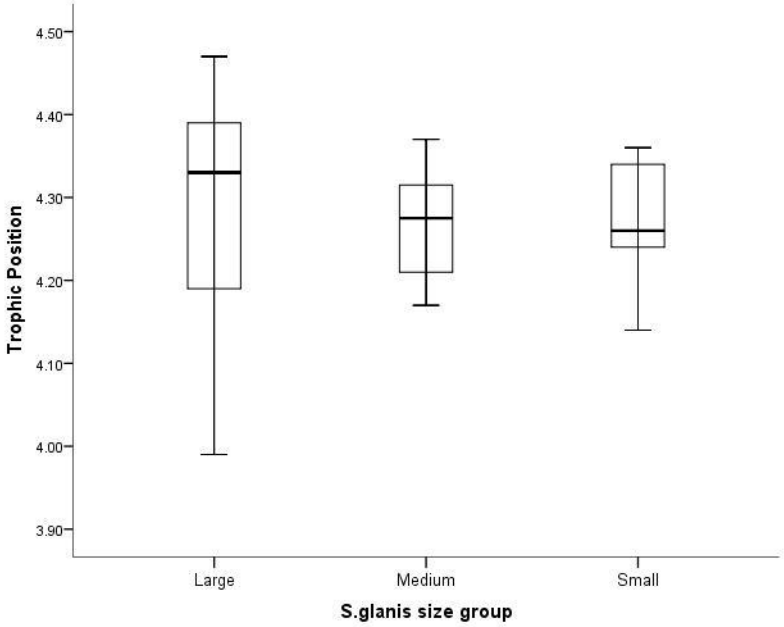

Figure 3.4 Trophic position of different *S. glanis* size groups in ponds. The boxes show the interquartile range, the horizontal line is the mean value whilst the whiskers represent the highest and lowest values of trophic position.

3.3.6 Variation in $\delta^{15}N$ isotopic values of *Silurus glanis* groups

There was enrichment in mean $\delta^{15}N$ isotopic values with increasing length of *S. glanis* specimens. Larger bodied *S. glanis* exhibited higher $\delta^{15}N$ isotopic values than smaller fish in the study. The large sized *S. glanis* group had the highest mean $\delta^{15}N$ value with 15.06 ± 0.13 ‰ and a range of 1.62‰ for individuals in the group. In contrast, there were lower mean $\delta^{15}N$ values for medium and small sized *S. glanis* groups (See Table 3.10).

For the medium size *S. glanis* group, their mean $\delta^{15}N$ value was 14.99 ± 0.82 ‰ with a range of 0.70 ‰ within the group whilst the smallest size *S. glanis* group was 14.78 ± 0.21 ‰ with a range of 2.23 ‰. Similar trends were evident in relationship of size of *S. glanis* with trophic position in the study and larger bodied *S. glanis* exhibited a higher trophic position and mean $\delta^{15}N$ isotopic signature value than their smaller counterparts in the ponds. However, there were no significant differences between mean $\delta^{15}N$ isotopic values and *S. glanis* size groups (ANOVA, $F_{2,28} = 0.94$, $P = 0.40$).

All *S. glanis* size groups had mean $\delta^{15}N$ isotopic values higher than those of their putative prey fish which were significantly different across all ponds. The largest *S. glanis* specimens had the highest increment of 3.62‰ compared to their putative prey (ANOVA, $F_{3,31} = 409.32$, $P < 0.05$). Similar patterns were evident for the medium size *S. glanis* groups with 3.18‰ increment to cyprinid prey (ANOVA, $F_{3,25} = 278.14$, $P < 0.05$). The smallest size *S. glanis* group had increment of 3.47‰ to prey fish (ANOVA, $F_{3,32} = 241.48$, $P < 0.05$) (See Table 3.10, 3.11& Figure 3.5).

Table 3.10 Variation in mean total length of *S. glanis* size groups with mean isotopic $\delta^{15}N$, $\delta^{13}C$ signatures and trophic position upon recapture in 2014. Standard error **(**±SE), Standard Deviation (SD) and 95% Confidence Intervals (95% CI) of variables included.

***S. glanis* group**	**Mean TL (cm) (**± SE)	**Mean $\delta^{15}N$ (‰) (**± SE)	**Mean $\delta^{13}C$ (‰) (**± SE)	**TP (**± SE)
Large	56.49 ± 1.65	15.06 ± 0.13	-25.85 ± 0.19	4.37 ± 0.04
SD	5.95	0.45	0.68	0.14
95% CI	From 52.89 to 60.09	From 14.78 to 15.34	From -26.26 to -25.44	From 4.28 to 4.45
Medium	45.69 ± 1.17	14.99 ± 0.82	-26.43 ± 0.18	4.35 ± 0.02
SD	3.30	0.23	0.51	0.07
95% CI	From 42.93 to 48.45	From 14.80 to 15.19	From -26.85 to -25.99	From 4.29 to 4.41
Small	45.81 ± 1.53	14.78 ± 0.21	-26.41 ± 0.35	4.29 ± 0.06
SD	4.83	0.65	1.11	0.20
95% CI	From 42.35 to 49.27	From 14.32 to 15.25	From -27.19 to -25.61	From 4.15 to 4.43

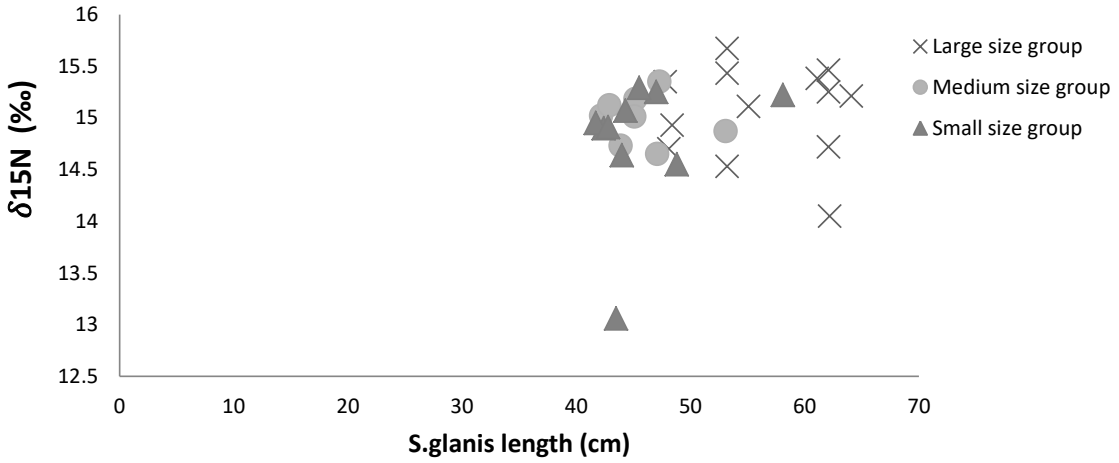

Figure 3.5 Variation in mean $\delta^{15}N$ isotope values of *S. glanis* size groups with total length in cm, in 2014. The large *S. glanis* size group had higher $\delta^{15}N$ than smaller groups.

Table 3.11 Variation in minimum and maximum $\delta^{15}N$ and $\delta^{13}C$ isotopic values of *S. glanis* size groups upon recapture in 2014.

***S. glanis* group**	**n**	**Total length (cm)**		**$\delta^{15}N$ (‰)**		**$\delta^{13}C$ (‰)**	
		Min	**Max**	**Min**	**Max**	**Min**	**Max**
Large	13	48.10	64.10	14.05	15.67	-26.75	-24.70
Medium	8	42.20	53.10	14.65	15.35	-27.16	-25.49
Small	10	41.70	58.10	13.06	15.29	-28.13	-24.36

3.3.7 Variation of $\delta^{13}C$ isotopic signatures of *Silurus glanis* groups.

A pooled linear regression showed an insignificant positive correlation between increasing fish length of *S. glanis* with mean $\delta^{13}C$ isotopic values. Larger bodied *S. glanis* exhibited higher $\delta^{13}C$ isotopic values compared to smaller fish in the study although differences in $\delta^{13}C$ mean values between size groups were not significant (ANOVA, $F_{2, 28} = 1.83$, $P = 0.18$) (See Table 3.10, 3.11, Figure 3.6).

The largest size *S. glanis* group had the highest mean $\delta^{13}C$ values with -25.85 ± 0.19 ‰ and 2.05‰ range between individuals in the same group. Lower mean $\delta^{13}C$ values were observed for other groups with -26.43 ± 0.18 ‰ and 1.67‰ range for medium size *S. glanis* group and -26.41 ± 0.35 ‰ with 3.77‰ range for smallest group.

The $\delta^{13}C$ isotopic signatures of large bodied *S. glanis* were enriched by over 5‰ increment compared to macro-invertebrate prey with similar patterns evident for other size *S. glanis* groups. The results suggested a shift in diet range with increasing size of *S. glanis* with 0.7‰ mean difference between large and small size *S. glanis* specimens in $\delta^{13}C$ isotopic values.

There was a wider range of $\delta^{13}C$ isotopic signatures among individuals of the smallest size *S. glanis* group with 4‰ range difference may imply consumption of a highly diverse diet compared to larger *S. glanis* specimens. This may suggest that smaller bodied *S. glanis* individuals consumed high proportion of diverse omnivorous prey of lower $\delta^{13}C$ isotopic values such as macro-invertebrates, detritus and plankton in their diet.

Overall the results revealed that *S. glanis* size groups displayed $\delta^{13}C$ isotopic range of 2- 4‰ among individuals in each group which may suggest individual differences in diet diversity for *S. glanis* specimens. All *S. glanis* size groups had similar mean $\delta^{13}C$ isotopic values in concurrence to those of putative cyprinid prey fish which indicated consumption.

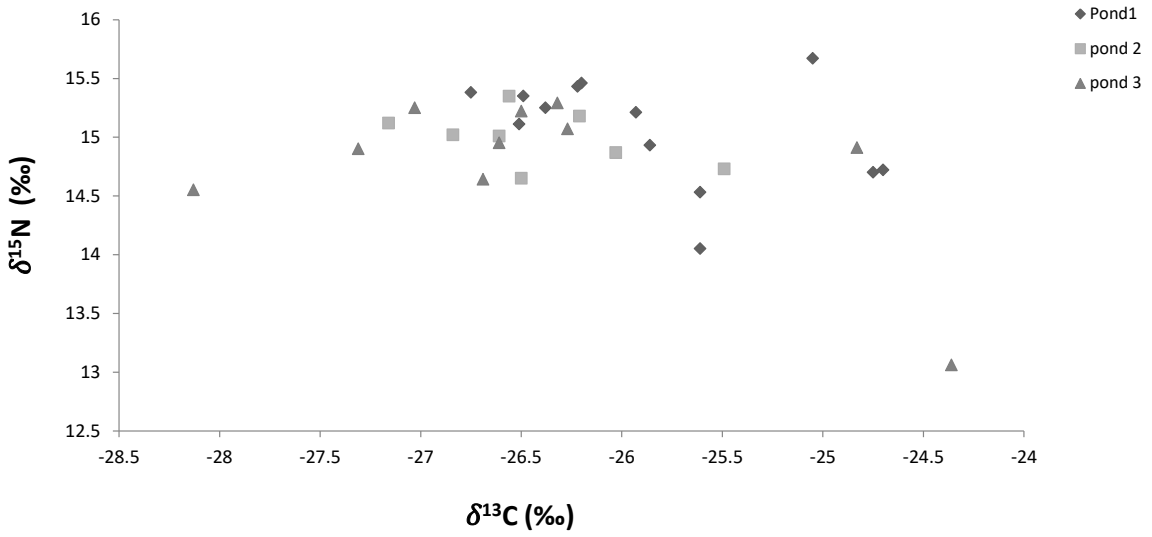

Figure 3.6 Variation in mean $\delta^{15}N$ and $\delta^{13}C$ isotopic values of different *S. glanis* size groups in the study showing large size *S. glanis* group have higher $\delta^{15}N$ and $\delta^{13}C$ isotopic values than other groups.

3.3.8 Trophic interactions of Cyprinidae species and other trophic groups in ponds.

The trophic position of secondary consumers such as Cyprinidae species was lower than the top predator *S. glanis* in ponds. This reflected cyprinids omnivorous trophic strategy, feeding on a diverse range of plants, detritus, zooplankton and macro- invertebrates with lower isotopic $\delta^{15}N$ signatures and trophic levels.

The Cyprinidae species exhibited an estimated mean trophic position ranging from 3.19 to 3.56 whereas *S. glanis* size groups had higher values in ponds. Among the Cyprinidae species, *B. bjoerkna* exhibited the highest estimated mean trophic position of 3.56± 0.08, with similar estimates of 3.27± 0.10 for *S. erythrophthalmus* and 3.44 ± 0.07 for *R. rutilus* respectively (See Table 3.12).

Similarly the mean $\delta^{15}N$ isotopic values for Cyprinidae species ranged from 11.03 to 11.90‰ and as such were lower than those of *S. glanis*, indicative of their lower trophic level as secondary consumers in food web. Among different Cyprinidae species there were no significant differences in mean $\delta^{15}N$ isotopic values between ponds (ANOVA, $F_{(2,81)}$=2.58, P = 0.08) (See Table 3.13).

In food web dynamics, the mean $\delta^{13}C$ values of Cyprinidae species varied from – 26.06 to – 24.07‰ and were not significantly different between ponds (ANOVA, $F_{(2,81)}$=0.53, P = 0.59). However there were significant differences in mean $\delta^{13}C$ signatures between *B. bjoerkna* and *R. rutilus* in large size *S. glanis* pond (ANOVA, $F_{(2,19)}$= 4.49, P = 0.03) and in the small size *S. glanis* pond (ANOVA, $F_{(2,33)}$= 3.65, P = 0.04). Specifically, *R. rutilus* exhibited significantly higher mean $\delta^{13}C$ isotopic values compared to than *B. bjoerkna* in large size *S. glanis* pond (Tukey`s test, $P$ = 0.03) and small *S. glanis* pond (Tukey`s test, P = 0.04).

Table 3.12 Variation in mean trophic positions of cyprinid species in ponds. Standard error **(**±SE), Standard Deviation (SD) and 95% Confidence Intervals (95% CI) of variables are included.

		Pond1		**Pond 2**		**Pond 3**
		Large *S. glanis* gp		**Medium *S. glanis* gp**		**Small *S. glanis* gp**
Species	**n**	**Mean TP (**± SE)	**n**	**Mean TP (**± SE)	**n**	**Mean TP (**± SE)
Silver bream	10	3.33 ± 0.07	10	3.56 ± 0.08	17	3.39 ± 0.07
SD		0.24		0.25		0.23
95% CI		From 3.16 to 3.51		From 3.38 to 3.74		From 3.24 to 3.54
Roach	9	3.23± 0.06	9	3.44 ± 0.07	13	3.19 ± 0.04
SD		0.19		0.20		0.15
95% CI		From 3.09 to 3.38		From 3.29 to 3.59		From 3.09 to 3.28
Rudd	3	3.36 ± 0.10	5	3.27 ± 0.10	6	3.23 ± 0.14
SD		0.18		0.23		0.34
95% CI		From 2.90 to 3.80		From 2.99 to 3.55		From 2.87 to 3.59

Table 3.13 Variation in mean $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures (‰) of cyprinid species in ponds. Standard error **(**±SE), Standard Deviation (SD) and 95% Confidence Intervals (95% CI) of variables included.

	Pond1		**Pond 2**		**Pond 3**	
	Large *S. glanis* gp		**Medium *S. glanis* gp**		**Small *S. glanis* gp**	
Species	**Mean $\delta^{15}N$**	**Mean $\delta^{13}C$**	**Mean $\delta^{15}N$**	**Mean $\delta^{13}C$**	**Mean $\delta^{15}N$**	**Mean $\delta^{13}C$**
	(± SE)	**(**± SE)	**(**± SE)	**(**± SE)	**(**± SE)	**(**± SE)
Silver bream	11.53 ± 0.26	-26.06 ± 0.53	12.21± 0.26	-24.84 ± 0.21	11.71 ± 0.24	-25.03 ± 0.19
SD	0.81	1.67	0.86	0.69	0.99	0.78
95%CI	From 10.95 to 12.11	From -27.25 to -24.87	From 11.63 to 12.78	From -25.30 to -24.38	From 11.20 to 12.22	From -25.43 to -24.63
Roach	11.19 ± 0.21	-24.11 ± 0.47	11.90 ± 0.23	-25.15 ± 0.40	11.03 ± 0.15	-24.14 ± 0.14
SD	0.64	1.40	0.68	1.19	0.53	0.50
95%CI	From 10.69 to 11.68	From -25.19 to -23.03	From 11.38 to 12.43	From -26.07 to -24.24	From 10.71 to 11.35	From -24.44 to -23.84
Rudd	11.60 ± 0.35	-24.07 ± 0.74	11.31 ± 0.35	-24.07 ± 0.80	11.19 ± 0.47	-24.27 ± 0.75
SD	0.61	1.28	0.77	1.79	1.16	1.83
95%CI	From 10.09 to 13.12	From -27.25 to -20.89	From 10.35 to 12.27	From -26.29 to -21.84	From 9.97 to 12.41	From -26.19 to -22.36

There was some variation in isotopic signatures of primary consumers such as zooplankton and macro- invertebrate groups in ponds. The study results indicated that predatory macro-invertebrates such as damselfly *Nehalenmia gracilis* and great diving beetle *Dytiscus marginalis* exhibited higher mean δ15N isotopic values (9.01- 10.97‰) than grazers, shredders or zooplankton. Mean nitrogen isotopic signatures of shredders such as amphipod crustacean *Gammarus pulex* and lesser water boatman *Corixa punctata* ranged from 9.45 to 10.36‰ whilst grazers including Ephemeroptera specimens were 8.65‰. In contrast, zooplankton with specimens of planktonic copepods e.g. *Cyclops bicuspidatus*, planktonic crustaceans *Daphnia* and zooplankton micro-crustacea had lower isotopic values (6.96- 8.89‰) (See Table 3.13 & 3.14).

In this study the mean $\delta^{13}C$ values of macro-invertebrates groups exhibited isotopic range from -36.19 to -22.28‰. Grazers had the lowest $\delta^{13}C$ signatures compared to other macro-invertebrates, yet were more $\delta^{13}C$ enriched than zooplankton.

Primary producers for example; algae, plankton and detritus including leaf litter exhibited trends of lower $\delta^{15}N$ isotopic values than higher consumer trophic groups in ponds. Algae`s mean $\delta^{15}N$ signature varied from 6.56 -7.16‰ with similar values for plankton and detritus. The wide range in mean $\delta^{13}C$ isotopic signatures of these primary producers (algae, plankton and detritus) indicated diverse basal food sources of carbon in the food web of ponds (See Table 3.14, Figure 3.7).

Table 3.14 Variation in mean $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures of primary producer and consumer groups in ponds. Standard Deviation (SD) and 95% Confidence Intervals (95% CI) of variables included.

	Pond1 large *S. glanis* gp		**Pond 2 medium *S. glanis* gp**		**Pond 3 small *S. glanis* gp**	
Trophic gp	**$\delta^{15}N$ (‰) (± SE)**	**$\delta^{13}C$ (‰) (± SE)**	**$\delta^{15}N$ (‰) (± SE)**	**$\delta^{13}C$ (‰) (± SE)**	**$\delta^{15}N$ (‰) (± SE)**	**$\delta^{13}C$ (‰) (± SE)**
Algae	7.16 ± 0.12	-33.13 ± 0.03	6.56 ± 0.18	-29.25 ± 1.07	6.20 ± 0.50	-35.96 ± 0.59
SD	0.21	0.05	0.31	1.85	0.71	0.83
95%CI	From 6.65 to 7.67	From -33.26 to -32.99	From 5.77 to 7.34	From -33.84 to -24.66	From -0.15 to 12.55	From -43.39 to -28.53
Plankton	7.71 ± 0.01	-30.59 ± 0.01	-	-	7.83 ± 1.45	-31.53 ± 1.36
SD	0.01	0.01	-	-	2.05	1.92
95%CI	From 7.64 to 7.76	From -30.64 to -30.52	-	-	From -10.59 to 26.25	From -48.81 to -14.24
Detritus	7.25± 0.14	-28.38± 0.23	8.32 ± 0.52	-29.25 ± 0.69	5.02 ± 0.46	-30.05 ± 0.41
SD	0.28	0.45	1.05	1.39	0.79	0.72
95%CI	From 6.80 to 7.69	From -29.10 to -27.65	From 6.65 to 9.98	From -31.45 to -27.04	From 3.06 to 6.97	From -31.83 to -28.26
Detritivores	7.42 ± 0.13	-29.85 ± 0.55	5.00 ± 1.79	-22.28 ± 7.47	6.86 ± 0.03	-28.76 ± 1.49
SD	0.23	0.96	1.64	2.02	0.04	2.11
95%CI	From 6.84 to 7.99	From -32.24 to -27.46	From 2.59 to 10.72	From -34.72 to -24.69	From 6.47 to 7.24	From -47.69 to -9.82
Zooplankton	6.96± 0.24	-32.64± 0.57	8.76 ± 0.41	-35.36 ± 0.60	8.89 ± 0.18	-34.72 ± 0.63
SD	0.42	0.10	1.01	1.47	0.43	1.53
95%CI	From 5.91 to 8.01	From -32.88 to -32.39	From 7.70 to 9.82	From -36.89 to -33.81	From 8.43 to 9.34	From -36.33 to -33.11
Grazer	8.65	-36.19	-	-	-	-
SD	-	-	-	-	-	-
95%CI	-	-	-	-	-	-
Shredder	10.21± 0.38	-31.21± 0.76	9.45	-31.41	10.36	-30.34
SD	0.66	1.31	1.01	1.47	-	-
95%CI	From 8.57 to 11.84	From -34.46 to -27.95	-	-	-	-
Predator	10.97 ± 0.28	-34.40 ± 0.23	9.14 ± 0.54	-33.81 ± 0.34	9.01 ± 0.24	-31.75 ± 0.38
SD	0.40	0.33	1.21	0.77	0.58	0.94
95%CI	From 7.41 to 14.52	From -37.32 to -31.47	From 7.63 to 10.64	From -34.76 to -32.85	From 8.40 to 9.62	From -32.73 to -30.75

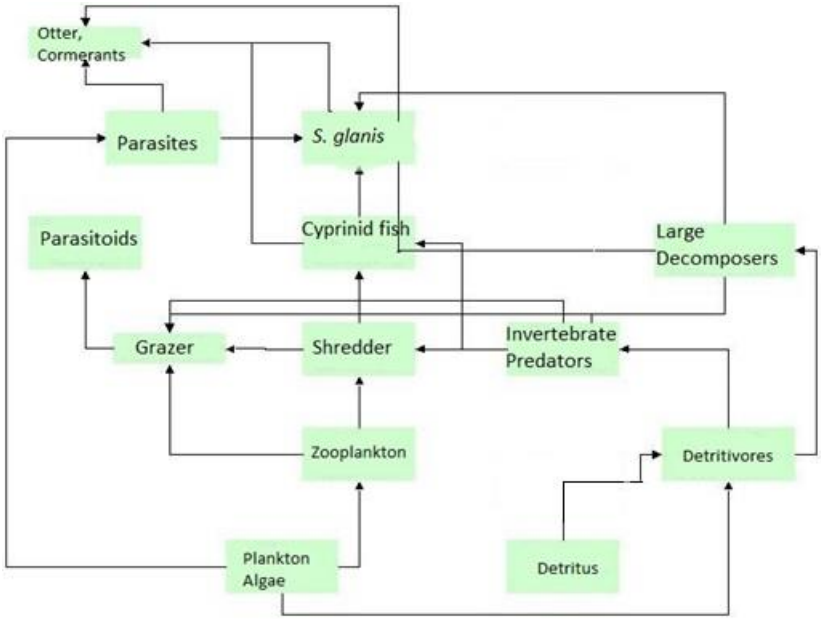

Figure 3.7 Food web and trophic network with each functional group (highlighted in green) may contain many species with variation in isotopic enrichment at trophic levels in ponds (Edmonds- Brown, 2018).

3.4 Discussion

3.4.1 Background

There is concern about the risks of adverse ecological impacts to native fish species with disruption in trophic functioning in freshwater habitats by *S. glanis* escapees released into major river catchments in the UK (Copp et al. 2009a; Britton et al. 2011a; Sagouis et al. 2015; Rees et al. 2017). From this it follows a need to increase understanding of non-native *S. glanis* species trophic interactions with prey species. In some instances, some introduced species dominate fish communities displacing native fish species because of their larger fish size leading to increased predation impact and competition for food resources (Vander Zanden et al. 1997; Grey, 2001; Sagouis et al. 2015; Nolan & Britton, 2018; Nyqvist et al. 2018).

Predicting the invasiveness of non-native *S. glanis* species into new environments is important in non-native fisheries management with r and K life history traits used as a general indicator of fish species invasiveness. Consequently rapid growth, fish condition and large body size may be advantageous to invaders as their predation risk is minimised by quickly exceeding the jaw gape of native predators. These traits confer a competitive edge to *S. glanis* over native resident fish which can result in changes in the trophic functioning of the recipient waterbody and loss in biodiversity (Hendry et al. 2000; Copp et al. 2009a; Squadrone et al. 2015; Tran et al. 2015).

Limited fat reserves and lower resilience over winter might affect larval and juvenile fish mortality, this, in turn, can influence fish invasions (Gozlan et al. 2003; Copp et al. 2009a; Britton & Busst, 2018). Study results indicated high mortality rates of *S. glanis* fry when exposed to water temperatures less than 13ºC during the winter and this may restrain *S. glanis* establishment success into northern hemispheres (David, 2006; Copp et al. 2009a; Muscalu et al. 2010; Cucherousset & Villéger, 2015).

Although there is some research about the growth and condition of cultured *S. glanis* from aquaculture facilities in parts of Eastern Europe there are very few studies about their non-native impacts in invaded habitats in England and Wales owing to lack of available funding (Kim et al. 2005; Jamróz et al. 2008; Dediu et al. 2010; Muscalu et al. 2010; Alp et al. 2011; Rees et al. 2017). Given the high propagule pressure of *S. glanis* introductions into lake fisheries in the UK and risks of dispersal into the wild, there is a need to understand their invasiveness in the UK (Copp et al. 2009a; Nyqvist et al. 2018).

The present study investigated variation in growth and trophic impacts of *S. glanis* size groups upon cyprinid prey species in ponds. Variation in trophic impacts by *S. glanis* size groups were

indicated by differences in mean trophic position or mean $\delta^{13}C$ and δ^{15} N isotopic signatures reflective of predation and diet among fish (Vander Zanden et al. 1999; Kopp et al. 2009; Cucherousset et al. 2018).

Large fish size is considered an important life history trait with predation impacts to native fish species likely to be exacerbated with larger fish size. Moreover with rising water temperatures predicted for aquatic habitats in the UK due to climate change, this may intensify *S. glanis* harmful impacts (Copp et al. 2009a; Britton et al. 2010; Busst & Britton, 2017).

There are gaps in knowledge about *S. glanis* trophic interactions with native fish prey species in freshwater habitats in the UK and the risks of these adverse ecological impacts may be underestimated (Britton et al. 2010; Rees et al. 2017). Over the years, research in non-native fish predatory impacts using conventional sampling methods with gut analysis has been compromised by a high frequency of empty guts from predators. Other limitations are that the gut contents from fish may yield diet snapshots of a highly temporal nature which provide little information about the trophic interactions of fish invaders (Vander Zaden et al. 1997; Grey, 2006; Kopp et al. 2009; Syväranta et al. 2010; Britton & Busst, 2018). Consequently, it has been difficult to distinguish the drivers in trophic interactions with invasive species such as *S. glanis*. Advances in stable isotope analysis may start to provide some useful insights about their trophic impacts (Vander Zanden et al. 2000; Syväranta et al. 2009; Kopp et al. 2009; Cucherousset et al. 2015; Winter et al 2019).

3.4.2 Variation in growth

In this study, the relative growth of different *S.glanis* size groups was observed. The median growth for all *S. glanis* size groups when stocked at predator: prey ratio of 1:2 was limited in comparison to mean growth (~ 900-1800 g *W*) of specimens (over 27 Kg) held in highly stocked cyprinid fisheries in the UK (Britton et al.2007; Copp et al. 2009a; Rees et al. 2017). Variation in fish growth may be related to several factors such as age, sex, habitat conditions, pollution, food resources and ambient water temperature (Zaikov et al. 2008; Carol et al. 2009; Alp et al. 2011; Cucherousset et al. 2018).

S. glanis are a warm water fish species which grow well in water temperatures over 20^0C with optimum growth reported at 25-28^0C in aquatic habitats. The study results indicated that the thermal opportunity for growth with ambient water temperatures (115 degree days $\geq 17^0$C) and food availability (361.67Kg ha^{-1} forage prey biomass) in ponds were suboptimal for rapid growth. Other studies elsewhere have revealed that *S. glanis* foraging and growth was constrained at water temperatures 15-23^0C with growth only taken in spurts during the warmer months. Restrained growth of *S. glanis* was reported in some lakes in the northern midlands where water temperatures during the summer were predominantly below 20^0C (Britton et al. 2007; Copp et al. 2009a).

Variation in fish growth may also relate to invasion stage of *S. glanis* colonisation. Recently introduced populations had significantly higher growth than those at a more advanced stage of invasion. These differences may be related to fish diet and food resources with highest growth reported for juveniles (48 cm TL per year) which eased after two years of age (40 cm TL per year) (Carol et al. 2009; Copp et al. 2009a; Cucherousset et al. 2018).

In this study, significant differences in median length and weight were observed for *S. glanis* size groups, between release and recapture $P < 0.05$ in all cases. The smallest size *S. glanis* group had the highest increment in length than larger fish with 2.27 ±0.33 cm TL. In terms of median weight gain, the smallest *S. glanis* group also had the highest increment with 134.41 ± 14.72 g *W* than other groups These differences related possibly to fish age, plasticity in diet and ambient water temperature in ponds (See Table 3.4 & 3.6).

The smallest and youngest *S. glanis* specimens exhibited the highest mean specific growth rate in weight and mean log Fulton`s condition factor *K* compared to other larger size groups in 2013 and 2014. Their mean specific growth rate in weight was the highest with 0.12 % $day^{-1}$ mean weight with significantly higher mean log Fulton`s condition *K* than larger size *S. glanis* groups

in 2013 (See Table 3.7). Smaller fish grew faster than larger bodied fish, this coupled with their wider $\delta^{13}C$ isotopic range could reflect a diverse diet spectrum of cyprinids, macro-invertebrates, detritus and plankton. Common to most organisms, younger fish may grow relatively faster than their older counterparts as they adapt better to novel habitats and to trophic plasticity in their diet. Similar results were indicated of small sized *S. glanis* specimens of body length (30 cm TL) recaptured from River Ebro and Catalan reservoirs in Spain and River Tarn in southwest France. These fish had significantly higher condition than larger fish which may be related to trophic plasticity in diet compared to larger fish confined to less catholic diets and piscivory (Carol et al. 2009; Cucherousset et al. 2018).

There was a suggestion from the stable isotope results that the addition of forage fish prey by increasing the predator - prey ratio to 1:3 in ponds during the following year (2014) could have contributed to the higher median growth for *S. glanis* size groups, this, however requires more robust data. In addition to this, all three ponds were open and juvenile waterfowl, amphibians and small mammals may have contributed as prey. These were not included in the stable isotope analysis which was a limitation in the study. Nevertheless, the study results were consistent with growth rates observed in short- term studies (30 days) of one year old *S. glanis* (n= 28) which consumed cyprinids in ponds at water temperatures 19.5 - 21.5ºC in Bulgaria, Eastern Europe. Mean growth in weight was 25.43 g and 0.5 cm TL in length with variation in growth likely related to food resources, ambient water temperature and social hierarchy in ponds (Alp et al. 2004; Zaikov et al. 2008; Grecu et al. 2019).

In this study, in 2014 compared to the other groups the large *S. glanis* group experienced the highest incremental growth in weight (266.64 ± 75.39g) and total length (4.39 ± 0.87cm TL)). This might be related to increased piscivory because large bodied *S. glanis* specimens had a wider jaw gape and were able to consume prey fish of varying body size thus, having greater access to food resources. However, this requires further quantification as it might be possible that small mammals and waterfowl may have fallen prey to these opportunistic predators in ponds. Other studies found in southern Europe in some waterbodies in the Catalan region in Spain evidence of large bodied *S. glanis* (>60 cm TL) utilisation of prey food sources which included waterfowl and small mammals as well as cyprinids (Syväranta et al. 2010; Cucherousset et al. 2018).

3.4.3 Water temperature and fish growth

A quantitative understanding of the seasonal variation of the water temperature in the ponds was useful as it revealed the limited thermal opportunity for fish growth (short period and suboptimal water temperature). The investigation of water temperature variation in ponds involved the estimation of the mean daily water temperatures above 17ºC using cumulative Degree- days. This approach assessed the thermal opportunity for *S. glanis* growth in ponds as described in chapter 2. The study outcomes revealed that the frequency in mean daily temperatures above 17ºC during the growing season were from April - September was 115 days for large size *S. glanis* group and 114 days for the other groups in 2013 (Refer Ch 2, Table 2.3). In contrast, a lower number of cumulative Degree- days were recorded in the following year (2014) for all *S. glanis* assemblages.

For all ponds, the mean daily ambient temperatures varied from 17°C to 23ºC during the growth season, well below the threshold of 25ºC needed for *S. glanis* optimal growth. Consequently the ambient water temperatures in ponds did not support rapid growth or optimum foraging activity (Copp et al. 2009a). However, other studies have indicated that shallow channels in floodplains in southern England which warm up rapidly (≥20 ºC) may provide suitable conditions for elevated *S. glanis* growth and establishment success (Britton et al. 2010; Rees, 2010; Rees et al. 2017).

It has been reported that growth rates of introduced *S. glanis* populations are higher in southern Europe compared to native populations in eastern Europe. Rapid growth of non-native *S. glanis* specimens had been documented in major river catchments of Hungary, France, Turkey, Spain and Italy. Variation in *S. glanis* growth may be related to differences in geographical latitude, demography and ambient water temperature. For example rapid growth rates were reported with some *S. glanis* specimens recaptured from the Menzelet reservoir in Turkey where water temperatures were above 20ºC for eight months of the year. These environmental drivers may facilitate fast fish growth (~ 5- 10 cm TL per year) and high Fulton`s condition factor *K* (~ 0.48-1.11per year) for invaders (Alp et al. 2004; Copp et al. 2009a; Cirkovic, 2012; Horoszewicz & Backiel, 2012).

The risks of climate driven thermal changes in aquatic habitats are likely to facilitate invasion of *S. glanis* non-native range particularly in southern Europe and the northern hemisphere. Increased water temperatures (>20ºC) are likely to ease growth restraints and accelerate reproduction success, predation, trophic impacts and disease transmission to native fish species.

Spawning and fertilisation processes of *S. glanis* are temperature related occurring only above 21- 23ºC. Similarly larvae and fry development stages require water temperatures above 22ºC for survival with warming conditions likely to favour larval development times and fry survival (Gullu et al. 2008; Combe & Gozlan, 2018). Thermal changes in aquatic habitats are likely to increase the spread of infectious disease outbreaks related to non-native fish species exchanges with native fish species which may accelerate loss in biodiversity (Ulikowski et al. 2003; Mazurkiewicz et al. 2008; Copp et al. 2009a; Britton et al. 2010; Combe & Gozlan, 2018).

3.4.5 Water quality and fish survival

There were risks to *S. glanis* from avian and terrestrial predation such as from cormorants (*Phalacrocorax carbo*) and otters (*Lutra lutra*) possibly from the estuarine floodplains near the study ponds which may have affected fish survival during the study. Foraging activity may elicit some trade- offs with higher risk of mortality with individuals that took greater risks while foraging may grow faster but have lower survival than those that are less active (Copp et al. 2009a; Cucherousset et al. 2018). *S. glanis* mortality may also have arisen from fish aggression in ponds as cannibalism (40%) was observed among large bodied individuals which reduced with fish size (18%) in other studies (Dediu et al. 2010). Cannibalism is an important concern with stocking *S. glanis* in ponds so to minimise these risks, size selection of *S. glanis* specimens was attempted by placing fish into separate groups before release into ponds during the study.

Overall, *S. glanis* exhibited species resilience and good survival over winter in ponds with water temperatures below 2ºC. In the first year, survival varied across *S. glanis* size groups, with 100% recapture for the smallest size *S. glanis,* followed by 89% for the largest *S. glanis* group and 76% for medium size group. In the following year, recapture was 81% for the largest *S. glanis* group, 77% for the smallest group and 62% for the medium *S. glanis* size class. However, there may be some bias in the survival results recorded for *S. glanis* in this study as some specimens could have avoided recapture by seine net avoidance when ponds were drained down, seine netted and fish removed upon annual recapture. Similarly, other studies have demonstrated good survival (90%) of two year old *S. glanis* recaptured from ponds in Poland (Cirkovic et al. 2012).

In this study, the physio-chemical water quality in the ponds was within range of good ecological and chemical status supportive of fish life recommended by General Quality Assessment (GQA) guidelines for fish species and the EC Freshwater Fisheries Directive (2006/44/EC) (Rees, 2010) and likely have contributed to fish survival in the study. The mean variation of dissolved oxygen was above 80% saturation in ponds and supportive of fish health. The mean pH was slightly alkaline yet within the accepted range for fish health. Variation in mean nitrate and phosphate concentrations during the sampling periods were also found to be within the acceptable range for fish health (Refer to Ch 2, Table 2.4). Good water quality in ponds was essential for *S. glanis* during the study. *S. glanis* survival has shown to be significantly related to water quality in ponds with fish over stocking can result in low dissolved oxygen concentrations causing fish stress and mortality (Zaikov et al. 2008; Muscalu et al. 2010; Plăcintă et al. 2012).

3.4.6 Variation in trophic impact & risks of climate change

In this study, estimated trophic positions varied slightly for all *S. glanis* size groups with largest *S. glanis* specimens presenting the highest mean trophic position compared to smaller size groups. This may be indicative of a fish size predation relationship with *S. glanis*. Other results have found that *S. glanis* of similar size had trophic positions positively correlated to fish length. Trophic positions ranged from 4.3 to 4.7 of large bodied *S. glanis* specimens over 60 cm TL in some river catchments in southern France (Syväranta et al. 2010; Cucherousset et al. 2018).
In the present study, large sized *S. glanis* group mean length was 56.49 (± 1.65) cm TL and ranged from 48.10 to 64.10 cm TL with mean trophic position of 4.4 (± 0.04) in the food web. In turn, some large bodied *S. glanis* specimens can exhibit higher trophic position of predators such as *E. lucius* (4.5 ± 0.1) but lower than *S. lucioperca* (5.1 ± 0.0) in some riverine habitats in France (Kopp et al. 2009; Syväranta et al. 2009). This study results indicated that some large bodied *S. glanis* may occupy high trophic position similar to that of an apex predator in the food web with piscivorous predation likely to be a component of their diet. However, due to practical logistics and costs there were some limitations in the study. These may have caused some bias in the SIA study results. Firstly, the full diet spectrum of potential prey sources (amphibians and small mammals) likely to be available for *S. glanis* consumption in the ponds were not monitored for SIA. In addition, the lack of gut content analyses and eDNA metabarcoding of *S. glanis* during the study may constrain some of the SIA inferences of trophic interactions findings in the study.

Other study results revealed similar trophic positions to those in the present study yet these fish were at a more advanced stage of invasion. Non-native *S. glanis* (n=18) of similar mean lengths (40.1± 2.7 cm TL) recaptured from the River Tarn in the Garonne basin, southwest France had mean trophic position of (4.3±0.1). Their diet was mainly piscivorous with consumption of cyprinids, crayfish and anadromous fish such as *A. alosa*. Elevated marine δ^{13} C signatures in tissues of recaptured *S. glanis* indicated that anadromous prey were an important dietary component yet several of these prey species (*A. anguilla*) are classified as endangered in the EC Habitats Directive. Moreover the diets of larger bodied *S. glanis* (152.2 ± 28.2 cm TL) revealed some consumption of terrestrial prey including waterfowl and coypu (*Myocastor coypus*). The results indicated that the addition of *S. glanis* contributed to increased level of predation and competition with native species in invaded habitats (Vadeboncoeur et al. 2005; Syväranta et al. 2009; Jackson et al. 2012; Sagouis et al. 2015). However, given the intrinsic differences in trophic functioning between lentic and lotic freshwater habitats and variation in prey abundance,

it is highly probable that *S. glanis* trophic interactions with prey species in recipient habitats may vary. For example, some *S. glanis* specimens recaptured from River Lot in the Garonne basin in southwest France had lower trophic positions (3.8 ± 0.1) than those in the study possibly reflective of seasonal food resources (Czarnecki et al. 2003; Kopp et al. 2009; Cucherousset et al. 2018).

In the present study there was slight variation in the mean trophic positions of large, medium and small *S. glanis* size groups yet with no significant differences between *S. glanis* size groups, $P > 0.05$ in all cases. There was some size class overlap between classes revealed upon recapture which may have influenced the results and caused some bias.

Other studies suggested some trends in fish size predation relationships with *S. glanis* recaptured from River Ebro and Susqueda reservoir in the Iberian Peninsula. Small sized *S. glanis* specimens (<30 cm TL) were mainly omnivorous, whereas larger fish (>30 cm TL) profited from piscivorous predation with diet switches from cyprinids to crayfish related to prey abundance. Ontogenetic changes in diet of *S. glanis* were significantly related to fish size, with small sized *S. glanis* consumed mainly macro-invertebrates such as trichopteran (*Hydropsyche exocellata*), freshwater shrimps (*Ataephyra desmaresti*), ephemeropterans and detritus. This in turn illustrated the relevance of rapid fish growth and size in contributing to the invasion success of non-native *S. glanis* into new environments (Benejam et al. 2007; Carol et al. 2009; Copp et al. 2009a; Boulêtreau & Santoul, 2016).

The present study results indicated some trophic interactions between *S. glanis* and cyprinids with differences in trophic position in ponds. The application of SIA assisted in determining some trophic relationships of the four trophic levels in the ponds which were producer (plankton) followed by primary consumer (macro-invertebrates) then secondary consumers (cyprinids) and finally tertiary consumer (*S. glanis*) (refer to Figure 3.7, Table 3.14). The trophic position of cyprinid species was lower than *S. glanis* size groups which reflected their lower trophic level in ponds. Cyprinid mean trophic position ranged from 3.27 (± 0.10) for *S. erythrophthalmus* to 3.56 (± 0.08) for *B. bjoerkna* in ponds (See Table 3.12). The results suggested that cyprinids behaved as secondary consumers in the food web feeding upon diverse herbivorous and omnivorous food resources from primary consumers and producers which had lower nitrogen and carbon isotopic signatures (Jones et al. 1998; Grey et al. 2009; Dalu et al. 2016).

Overall, variation in trophic position of *S. glanis* in food webs may indicate changes in *S. glanis* trophic strategy with increased jaw gape and body size allowing consumption of larger putative prey from higher trophic levels. Large sized specimens (56 cm TL ± 1.65) exhibited higher

trophic position than smaller individuals probably due to these size related differences and diet range.

In addition, the risks of climate change are likely to exacerbate trophic impacts caused by fish invaders with warming conditions forecasted for England and Wales likely to accelerate *S. glanis* rapid fish growth and size. Large bodied *S. glanis* are able to hold high trophic positions during the early phase of invasion and their addition is likely to lengthen the food chain and narrow the isotopic niche of native species. The study results suggest wider implications of *S. glanis* trophic impacts beyond England and Wales as they may potentially impact native and anadromous fish populations in invaded habitats elsewhere (Britton et al. 2010; Britton & Busst, 2018; Cucherousset et al. 2018).

3.4.7 Variation in $\delta^{15}N$ isotopic values

In the study, the results of mean $\delta^{15}N$ isotopic values of different sized *S. glanis* groups indicated positive correlation with increasing fish length yet were not significantly different between groups.

Large bodied *S. glanis* specimens exhibited enriched mean $\delta^{15}N$ isotopic signature of 15.06 ± 0.13 whereas other smaller size groups had lower isotopic values. The smallest *S. glanis* specimens had the lowest mean $\delta^{15}N$ isotopic signature with 0.3‰ mean difference with large size *S. glanis* group. These trends suggested that mean $\delta^{15}N$ isotopic values of *S. glanis* were likely to increase with size, ontogeny and trophic position in ponds.

The results suggested that larger *S. glanis* specimens were likely to have consumed some cyprinid prey in their diet which reflected their heavier $\delta^{15}N$ isotopic signatures than other groups. Smaller size groups had lower $\delta^{15}N$ isotopic signatures yet wider isotopic range (2.3‰) which suggested a wide breadth in diet spectrum than larger bodied counterparts. Similar results have been found elsewhere with positive correlation of $\delta^{15}N$ isotopic values in relation to increasing fish size which indicated a progressive change in diet with fish ontogeny. The mean difference in $\delta^{15}N$ isotopic signatures between small (40.1± 2.7cm TL) and large bodied *S. glanis* size groups was 1.2‰ which were recaptured from the River Tarn in southwest France (Carol et al. 2007; Carol et al. 2009; Copp et al. 2009a; Syväranta et al. 2010; Cucherousset et al. 2018).

Results of the present study indicated the $\delta^{15}N$ isotopic values of large bodied *S. glanis* individuals were enriched by 3 to 4‰ isotopic increment compared to those of analysed prey with similar patterns evident for other groups. This was consistent with other studies with large bodied specimens which consumed small sized cyprinids (4.8± 6 cm TL) such as *S. erythrophthalmus* with avoidance of larger prey fish beyond jaw gape. *S. glanis* indicated preference for small sized prey and all *S. erythrophthalmus* were consumed during the trial (Adamek et al. 1999; Copp et al. 2009a; Kopp et al. 2009; Squadrone et al. 2015).

3.4.8 Variation in $\delta^{13}C$ isotopic values

In this study, there were no significant differences in mean $\delta^{13}C$ isotopic values between different sized *S. glanis* groups. The results indicated a positive correlation with increasing fish length and the largest size specimens exhibited higher $\delta^{13}C$ isotopic signatures in contrast to smaller size *S. glanis* groups. Large bodied *S. glanis* displayed the highest mean $\delta^{13}C$ signature of -25.85 ± 0.19 where as other groups had lower isotopic values. It is possible that the results reflect a progressive shift in dietary constituents which resulted in a 0.7‰ mean difference between large and small size *S. glanis* specimens in $\delta^{13}C$ isotopic values.

The $\delta^{13}C$ isotopic signatures of large bodied *S. glanis* were higher by over 5‰ increment compared to the mean $\delta^{13}C$ isotopic signatures of macro-invertebrate prey with similar patterns evident for other groups. Their putative prey is likely to have ranged from cyprinids to secondary producers such as macro-invertebrates and primary producers such as algae. The large bodied *S. glanis* specimens might have also consumed some small mammals and waterfowl prey which also contributed to a higher $\delta^{13}C$ isotopic value compared to those feeding exclusively upon cyprinids.

The results indicated a wider $\delta^{13}C$ isotopic range of 4‰ for the smallest size *S. glanis* group compared to larger *S. glanis* groups. This suggested that small bodied *S. glanis* specimens consumed as part of their diet a broad spectrum of plants, detritus, zooplankton and macro-invertebrates of lower $\delta^{13}C$ isotopic signatures and trophic levels in the food web. The pond margins supported a rich diversity of aquatic invertebrates from emergent macrophytes such as *S. emersum, P. australis* and *G. maxima*.

Similar results have been found elsewhere with the diet of non-native *S. glanis* in the Catalan reservoirs in Spain which were related to fish size with distinct ontogenetic dietary changes evident among *S. glanis* size classes. Large bodied *S. glanis* (60-130 cm TL) had significantly enriched $\delta^{13}C$ isotopic signatures reflective of some consumption of waterfowl and small mammals with 4‰ increment in $\delta^{13}C$ values than putative cyprinid prey. The $\delta^{13}C$ isotopic range of the small and medium *S. glanis* size groups reflected the $\delta^{13}C$ isotopic values of their predominant putative prey which was confirmed by stomach contents analysis (Syväranta et al. 2010; Cucherousset et al. 2018).

Gut analysis and SIA of *S. glanis* over a two month period revealed a predominance of molluscs (n=40), crustaceans such as red swamp crayfish (*Procambarus clarkia*) (n=20) and cyprinids (n=60) among smaller *S. glanis* (<60 cm TL). Large *S. glanis* (60-130 cm TL) consumed some waterfowl (n=10) while individuals (>130 cm TL) predated upon small mammals such as *M. coypus* from the River Tarn, southwest France (n=10). The range of $\delta^{13}C$ signatures of *S. glanis* ranged over 5‰ which indicated a wide consumption of available prey sources and flexibility in foraging. The range of $\delta^{13}C$ values for *S. glanis* appears broader than other piscivorous predators such *E. lucius* or *S. lucioperca* possibly indicative of scavenging (Copp et al. 2009a; Kopp et al. 2009; Syväranta et al. 2010; Cucherousset et al. 2018).

Overall, the study results may suggest that *S. glanis* size groups were opportunistic foragers, able to consume a highly diverse range of putative prey organisms from different trophic levels to satisfy their omnivory and piscivory energy demands in ponds. All *S. glanis* size groups displayed isotopic $\delta^{13}C$ range of 2 to 4‰ among individuals in each pond which might be indicative of distinctive differences in diet composition for each *S. glanis* specimen. Such a response might suggest some trophic plasticity in *S. glanis* foraging which may favour survival into new environments.

3.5. Conclusions

This study provides some evidence that variation in growth and survival of non-native *S. glanis* released into ponds in southern England might be related to differences in food abundance, habitat niche diversity and ambient water temperature. In open ponds stocked with cyprinids and at ambient water temperatures *S. glanis* assemblages growth were restrained in comparison to those stocked in syndicate fisheries in England and Wales. However during the study, ambient water temperatures in all ponds were below the threshold required for optimum growth for *S. glanis* (Copp et al. 2009a; Britton et al. 2010; Rees et al. 2017).

In year one, mean growth variablity and Fulton`s condition factor *K* of *S. glanis* size groups could be related to food availability in ponds. Survival varied across all group sizes with the small *S. glanis* group showing highest survival rate. Small sized *S. glanis* group exhibited a broader $\delta^{13}C$ isotopic range, this might suggest increased consumption of omnivory in their diet compared to their larger counterparts. Differences in $\delta^{13}C$ isotopic range between *S. glanis* specimens within groups could suggest distinct individual diets and opportunistic foraging. This in turn may facilitate invasiveness as trophic plasticity is likely to contribute to higher survival into new environments and might give them a competitive advantage over larger predatory fish in conditions of limited food sources.

In year two, it is possible that the higher prey stocking densities (821.42 Kg ha^{-1}) enabled the largest size *S. glanis* group which had the highest incremental growth and survival in comparison to other groups. Larger bodied specimens had enriched $\delta^{13}C$ isotopic signatures and higher trophic position than smaller *S. glanis* specimens which might be related to predation fish size relationship and increased piscivory in ponds (Copp et al. 2009a; Kopp et al. 2009; Cucherousset et al. 2018).

The data provides some baseline evidence that large bodied *S. glanis* specimens might occupy high trophic positions in food webs with potential to behave as apex predators in new environments. The risks associated with climate change will exacerbate non-native fish trophic impacts because of increased predation and competition by *S. glanis* on the isotopic niche of native species in ponds across England and Wales.

4. Synthesis of study findings regarding *Silurus glanis*

4.1 Synthesis of findings

4.1.1 Aims and objectives

The aim of thesis was to investigate *S. glanis* ecological impacts following their release into ponds in England. The specific objectives were to:

- Determine variability in growth and Fulton`s condition factor *K* of three different sized *S. glanis* groups in adjacent ponds at the same site, so as to assess life history traits of *S. glanis* and their survival into introduced habitats (Chapter 3).
- Assess the trophic interactions of three different sized *S. glanis* groups in adjacent ponds at the same site, so as to quantify fish size relationships with reference to predation and trophic impacts into introduced habitats (Chapter 3).
- Identify the level of ecological and socio-economic risks of introduced *S. glanis* dispersing into a major river catchment in England using European Non-native Species in Aquaculture Risk Analysis (ENSARS) modules (Appendix E6).

4.1.2 Synthesis of the findings: *Silurus glanis* variation in growth

Introductions of *S. glanis* for angling are frequent in the UK and may pose a threat to native fish species with increased predation, competition, trophic impacts and disease transmission in freshwater fish communities. However, there are only a few studies in England and Wales of *S. glanis* ecological impacts on native fish species. This study is the first attempt to examine variation of life history traits such as growth and Fulton`s condition factor *K* of different *S. glanis* size groups survival and establishment into invaded ponds. The results could suggest that differences in food abundance, habitat niche diversity and ambient water temperature in ponds may influence *S. glanis* growth, Fulton`s condition factor *K* and survival.

S. glanis growth was greatest when stocked with higher prey stocking densities in ponds with the largest size *S. glanis* group having the highest incremental growth in weight and survival (89%) compared to the smaller *S. glanis* groups. Vice versa, at lower prey fish stocking densities, the smallest size *S. glanis* group had the highest survival, mean incremental growth in weight and significantly higher condition than larger *S. glanis* size groups. This may suggest that small sized *S. glanis* plasticity in phenotypic response to limited food sources in invaded ponds may facilitate establishment. Differences in growth and condition of *S. glanis* may be attributed to the ambient water temperatures in the study ponds with growth probably constrained by low water

temperatures as observed with other studies elsewhere in Bulgaria (Alp et al. 2004; Zaikov et al. 2008).

S. glanis invasions in major river ecosystems have been documented in southern Europe yet their ecological impacts related to the decline of native and anadromous fish populations remains unclear (Cucherousset et al. 2018). In order to quantify better the impact of thermal changes upon *S. glanis* spread, future research may benefit from the development of predictive models using National and regional climate databases such as the Meteorological Office (Met Office) (Britton et al. 2010; Vilizzi & Copp, 2017).

4.1.3 Synthesis of the findings: *Silurus glanis* trophic interactions in ponds

The release of non-native *S. glanis* into recipient aquatic habitats may cause ecological damage by disruption of trophic functioning in native fish communities with increased predation and competition by invaders. This study is the first occasion to examine *S. glanis* trophic interactions in ponds holding cyprinids in southern England which were assessed using stable isotope analysis (SIA). Differences in trophic position of *S. glanis* were positively correlated with fish length. The results suggested a trend of mean $\delta^{15}N$ isotopic values of *S. glanis* increasing with fish size and ontogeny which corresponded with mean trophic position in ponds. The largest *S. glanis* had the highest trophic position indicative of an apex predator in invaded ponds. Similar trophic position results were observed with large bodied *S. glanis* recaptured from several river catchments in France with predation impacts likely to be exacerbated with rapid growth and large body fish size (Syväranta et al. 2010; Cucherousset et al. 2012). In the study, *S. glanis* isotopic $\delta^{13}C$ range among individuals in each pond might be indicative of distinctive differences in diet. Such a response might suggest trophic plasticity in *S. glanis* foraging which may favour survival into new environments.

There remains some uncertainty about the evidence of *S. glanis* predation impacts upon native fish species as some researchers report this fish to be a scavenger and not an effective bio manipulator in the wild. Nevertheless, impacts on water fowl and *A. anguilla* populations have been attributed to *S. glanis* in several aquatic habitats in Iberia and France (Copp et al. 2009a; Almeida et al. 2013; Cucherousset et al. 2018) and more research is needed. *S. glanis* trophic interactions are likely to benefit from forecasted climate change impacts in riverine catchments, this also has received little attention in the UK and needs to be investigated.

4.1.4 Synthesis of the findings: ENSARS

There is some concern about the risks of harmful ecological impacts from non-native *S. glanis* introductions into lake fisheries in the UK which are often completed without authorised consent by regulatory bodies and thus, at higher risk of dispersal into the wild. Previous risk assessment studies have used FISK application which identified *S. glanis* as a highly invasive species although their establishment is at a lag phase owing to thermal constraints in aquatic habitats in England and Wales (Britton et al. 2010; Roy et al. 2014b; Copp et al. 2016a; Copp et al. 2016b; Gallardo et al. 2016a; Rees et al. 2017). However, *S. glanis* risk status is liable to change with better understanding of their invasive potential and developments in risk analysis protocols. This study is the first ENSARS risk assessment for *S. glanis* which investigated their impacts on native fish species from a lake fishery near Ringwood, Dorset in southern England.

Differences in potential risks and impacts of ecological and socio-economic harm of *S. glanis* escaping from the lake into the river catchment were identified using ENSARS modules (Organism, Facility, Pathway, Socio-economic and Infectious agent) in the study. The highest risk outcomes and confidence ranking were for the Organism, Facility and Pathway modules results. These outcomes identified natural dispersal of *S. glanis* into the surrounding waters via flooding events or by angling involvement as important risks from an unlicensed fishery which was poorly managed.

The results could suggest that the lower reach of the river catchment and flood plain waters which had water temperatures over 18ºC during summertime were likely to favour establishment of a self-sustaining population (Copp et al. 2009a; Rees, 2010; Rees et al. 2017). Similarly the Climatch model outcomes predicted that the river catchment was favourable for *S. glanis* colonisation with forecasted thermal changes related to climate change impacts likely to facilitate invasion. However, from the Infectious agent results it remains unclear whether introduced *S. glanis* may spread disease to native fish communities as there were gaps in knowledge about the harmful impacts to native fish species of the parasites detected upon *S. glanis* in the study. It is likely that the risks of ecological harm were underestimated and were given low confidence scores in the study. To address these gaps in knowledge on disease transmission by *S. glanis* in non-native habitats in the UK and southern Europe, further research is needed as the infectious agents associated with *S. glanis* are poorly studied (Gozlan et al. 2014; Copp et al. 2016a).

The Socio-economic module results suggested low risk of economic impacts to the local area or lake fishery as *S. glanis* were known to be present in other lakes with little evidence of adverse impacts on biodiversity. However this may change if *S. glanis* populations become invasive in the river catchment.

4.2. Management &Recommendations

4.2.1 Legislative and risk protocols

In recent years, attention has intensified about the potential crisis with biodiversity loss caused by the influx of non-native fish species introductions globally. These harmful impacts are exacerbated by expansion in freshwater aquaculture and other activities such as recreational fishing (Naylor & Burke, 2005; Gozlan et al. 2010; Copp et al. 2016b). There is an urgent need to update management policies with non-native fish species introductions between countries so as to prevent invasions. The task for decision makers has been to execute effective safeguards with international legislation and non-native fish species risk protocols in line with the precautionary approach advocated by the Convention on Biological Diversity 1992 in protecting biodiversity (Copp et al. 2005b; Ricciardi, 2007; Gozlan et al. 2010; Gallardo et al. 2016a).

There are several important pieces of legislation relevant to the protection of fish species diversity and conservation of habitats in the UK such as the Wildlife and Countryside Act 1981. Legislation such as the Import of Live Fish Act 1980 (ILFA) and Prohibition of Keeping and Release of Live Fish (Specified Species) (England) Order 2014 amendment are designed to control non-native fish species into England and Wales. These regulations control the importation and holding of listed non-native fish species such as *S. glanis* into lake fisheries (Copp et al. 2005a: Gozlan et al. 2010; Roy et al. 2014a; Copp et al. 2016a; Gallardo et al. 2016a; Piria et al. 2016).

Other legislation designed to protect threatened native habitats and species diversity in Europe includes Natura 2000 and EU Water Framework Directive 2000. The key emphasis is on rehabilitation schemes so as to enhance aquatic ecosystems in order to achieve good ecological status. This in turn has driven improvements in fish habitats with non-native species management schemes implemented in the UK and across Europe (Copp et al. 2005a; Copp et al. 2010; Almeida et al. 2013; Copp et al. 2016a; Piria et al. 2017).

Nevertheless for such approaches to be successful in managing fish invasions there needs to be better communication between countries with mandatory regulatory enforcement across the globe. This latter point is important as several European countries such as Slovenia and Croatia and developing countries lack the legislation and regulatory infrastructure to control non-native fish species introductions. In particular, there is concern about the lack of risk assessment protocols and quarantine for non-native fish introductions in developing Asian countries. These

countries are leaders in aquaculture production and as such it is inevitable that economic drivers will determine the scale of non-native fish invasions and biodiversity loss around the world (Copp et al. 2005b; Gozlan et al. 2010; Britton et al. 2011a; Gallardo et al. 2016a).

One approach has been the development of global databases for non-native fish species to provide better communication about the threats of invasive fish species and biodiversity loss. In recent years, there have been developments in environmental risk protocols with horizon scanning and risk assessment toolkits such as FISK and ENSARS in the UK and similar adaptations across Europe (Wonham et al. 2000; Copp et al. 2005b). However, for such measures to be successful in preventing or minimising the risk of non-native fish species invasions, international cooperation between countries is needed. For example, there needs to be standardisation of environmental risk protocols for all pathways of non-native fish species introductions in forecasting invasive impacts globally which at present is absent (Naylor & Burke, 2005; Lin et al. 2007; Copp et al. 2009a; Gozlan et al. 2010; Gallardo et al. 2016a).

Another related issue is the poor record keeping of transfer of non-native species movements in developing countries (Padilla & Williams, 2004; Copp et al. 2005b; Ricciardi, 2007; Britton et al. 2010; Gozlan et al. 2010). As a general rule there is insufficient monitoring for early detection of invasive fish species in freshwater habitats. These problems are evident across the globe as the management of invasive fish species requires more funding, research and educational networks. Such issues need to be given priority so as to raise awareness about the threats posed by invasive fish species with more responsible fisheries management (Copp et al. 2005a; Lin et al. 2007; Britton et al. 2010; Gozlan et al. 2010; Piria et al. 2017).

4.2.2 Recommendations for the management of *Silurus glanis* in the UK

4.2.2.1 Preventative measures

In the control of non-native fish species, the introduction prevention approach is endorsed by important pieces of legislation such as Wildlife and Countryside Act 1981 section 9, the Import of Live Fish England and Wales Act 1980 and the Prohibition of Keeping or Release of Live Fish Specified Species Amendment Order 2003. Under these regulations *S. glanis* species are classified as a non-native fish species and their introductions are subject to control by regulatory bodies in the UK (Copp et al. 2009a; Britton et al. 2010; Rees et al. 2014; Copp et al. 2016a).

This in turn signifies that *S. glanis* stocked into lake fisheries are subject to coordinated regulatory control by governmental bodies such as the Environment Agency, Centre for Environment Fisheries and Aquaculture Science (CEFAS) and Department of Environment, Food and Rural Affairs (DEFRA). These agencies share responsibility for environmental protection, fish health inspectorates, recreational angling and license permits for importation of live fish into England and Wales (Copp et al. 2005b; Copp et al. 2009a; Gozlan et al. 2010; Copp et al. 2016a). The regulatory infrastructure is hierarchical in enforcement control. Natural England and the Home Office hold ultimate legislative enforcement in non-native fish species introductions with border control allocated to HM Revenue and Customs agency in the UK (Copp et al. 2005b; Gozlan et al. 2010; Piria et al. 2017).

The introduction and holding of *S. glanis* in lake fisheries requires authorised consent with an ILFA licence permit issued from (DEFRA) that needs to meets specific criteria and good biosecurity status for the licensed site. For example, lakes should be enclosed waters with no inlets or outlets and have good site security so as to ensure there are no risks of fish escaping via flooding events. However, problems still persist with unregulated introductions of *S. glanis* into lake fisheries situated in the flood zone with higher risks of dispersal into the wild (Copp et al. 2009a; Britton et al. 2010, Rees et al. 2017).

Such issues underline the need for closer cooperation between regulatory bodies and angling organisations in raising awareness about the threats posed to native fish species by *S. glanis* escapees into the wild. In a study about socio-economic factors of *S. glanis* angling in the UK one in five anglers (21%) appeared unaware of the threats posed to biodiversity by the introduction of non-native fish. Anglers demonstrated little awareness of the risks posed by *S. glanis* such as disease transmission, predation, trophic impact and hybridisation into native fish communities. Such a response may be related to inadequate training and educational workshops

available to anglers by angling organisations which needs reassessment (See Appendix F6, Table F61.1) (Rees et al. 2017).

This body of literature underlined the need for more training and educational networks to be available from angling organisations and regulatory bodies for anglers to raise public awareness about non-native species. It is recommended a need to:

Develop more educational networks with regular newsletters and social media channels updates, study visits to lake fisheries and workshops about non-native fish species. This will increase sharing of knowledge and news about non-native fish species including *S. glanis* and help raise public awareness in England and Wales.

1) Update the supply and distribution of ID for *S. glanis* species and their adverse ecological impacts via collaboration with angling organisations, regulatory bodies and media. This will increase ecological awareness with fishery managers and anglers about their invasive risks.

2) Develop and update educational networks with International collaboration in sharing information about non-native fish species on an institutional and global scale via open data networks with published information freely available (Copp et al. 2009a; Bean et al. 2017).

4.2.3 *Silurus glanis* control & risks of climate change

Effective control of non-native fish species incorporates environmental risk strategies with regulatory enforcement. The focus is upon introduction prevention of invasive fish species so as to minimise any harmful impacts by preventing their introduction into new environments. However, introduction prevention approach represents only one aspect in the management of non-native fish species. In practice, accidental and unregulated introductions of *S. glanis* often occur in lake fisheries with fish released without any risk assessment or awareness of the potential risks of ecological damage to freshwater habitats (Wonham et al. 2000; Copp et al. 2005b; Gozlan et al. 2010).

In tackling unregulated introductions of *S. glanis*, remediation and mitigation protocols are practised by regulatory bodies so as to prevent species establishment into water bodies. Such an approach is reliant upon early detection and quick response to the introduced species (Britton et

al. 2010; Gozlan et al. 2010; Copp et al. 2016a). However, the problem is that in most cases there is inadequate monitoring and regulatory enforcement for early detection and removal of *S. glanis* from unauthorised waters owing to lack of funding available (Copp et al. 2009a; Britton et al. 2010; Rees et al. 2017).

In theory, effective management of *S. glanis* introductions are reliant on early detection, yet in practice they have already become established in water bodies before detection or warranting a risk appraisal by regulatory authorities. In most cases unless an introduced population has becomes a severe pest issue then control methods rather than eradication are practised (Copp et al. 2009a; Britton et al. 2011a; Copp et al. 2016a). Eradication programmes of non-native species from water bodies may be determined if the scale of ecological and socio-economic benefits outweigh the magnitude in costs in eliminating the population completely (Britton & Brazier, 2006; Copp et al. 2016a).

It is apparent that in many cases the "Do nothing" approach seems integral to non-native fisheries management which is influenced by risk appraisal outcomes and available funding by regulatory bodies. This line of approach is undertaken when the associated costs in the removal of *S. glanis* from unregulated water bodies exceeds the risks of adverse ecological and socio-economic impacts to local areas. This may be reversed if they become an invasive pest (Britton et al. 2010; Gozlan et al. 2010).

S. glanis has been identified as a highly invasive species in England and Wales in a FISK study, yet owing to thermal constraints in aquatic habitats they are present but not established in river catchments (Copp et al. 2009a; Britton et al. 2010). *S. glanis* does not easily spawn in England as generally the climate is cooler than in it`s native range. However, lower reach river catchments and floodplain water bodies which warm up in late summer are likely to provide adequate high temperatures favourable for spawning (≥18ºC) and reproduction.

Climatch modelling approach predicted water temperatures supportive for *S. glanis* establishment in a river catchment (Hampshire Avon) in southern England (See Appendix E, Table E6.5). *S. glanis* spawning, larval and fry stages require water temperatures over 22^0C for development with survival susceptible at low (≤14^0C) water temperatures during these life stages. These factors are likely to contribute to *S. glanis* invasion into a range of riverine habitats in southern Europe where climates are generally warmer than their native range or in the UK (Carol et al. 2007; Gullu et al. 2008; Copp et al. 2009a; Cucherousset et al. 2018).

The investment in parental care strategies where males guard and protect eggs in nests against predation may facilitate reproduction success of *S. glanis* into new environments. In addition, thermal changes (2-5^{0}C) via climate change impacts may facilitate species colonisation throughout the UK. Increased flooding events as a consequence of climate change are likely to accelerate *S. glanis* dispersal into the neighbouring riverine catchments from lake fisheries. Migratory strategies may facilitate the spread of invaders as *S. glanis* are known to undertake some migrations into neighbouring river catchments in predation of anadromous fish (Carol et al. 2007; Copp et al. 2009a; Cucherousset et al. 2018). In assessing the potential spread and invasion risks of *S. glanis* in the UK it must be noted that *S. glanis* are a long lived species and known to be tolerant to changes in water quality and pollution impacts which may explain their persistence in invaded habitats in England and Wales (Copp et al. 2009a; Rees et al. 2017). Given these outcomes from the study it is recommended that:

1) Review tools used in freshwater monitoring programmes in assessment of non-native *S. glanis* distribution with application of new techniques. These include using fish pheromones to increase efficiency in net trapping of specimens and environmental DNA molecular analysis in forecasting species distribution and presence of infectious agents (Gozlan et al. 2010; Davison et al. 2017).

2) Adoption of integrated modelling approach with data collection and monitoring of non-native *S. glanis* species with application of multi-species, total ecosystem approach and Climatch models. These applications will identify areas at high risk from *S. glanis* introductions in England and Wales with predicted climate change impacts (Bean et al. 2017).

3) Review for stricter regulatory measures for large bodied *S. glanis* specimens introductions into the UK so as to minimise invasive risks as currently consents are given with no size restrictions (Copp et al. 2009a; Britton et al. 2010; Rees et al. 2017).

4) Investigate genetic selection in sex determination of *S. glanis* specimens approach as a possible tool in controlling species invasiveness into the UK (Linhart et al. 2002).

5) Update existing environmental risk assessment toolkits for *S. glanis* so as to provide holistic assessments of aquatic habitats and integration of models. This will involve accounting for uncertainty in data and climate change impacts so as to improve the

decision making process in determining invasive risks (Copp et al. 2016a; Bean et al. 2017).

4.2.4 Control of infectious agents

Increasing risks of disease emergence and harmful impacts from novel pathogens and parasites caused by *S. glanis* exchanges to native fish species is becoming more evident. This may be related to variability in quarantine standards and inadequate fish health inspections for *S. glanis* exported from Eastern Europe into the UK for recreational angling. Non-native *S. glanis* species are host carriers of generalist pathogens and parasites which may switch host to native fish species and cause fish mortality. Disease emergence related to *S. glanis* introductions is likely to increase with popularity in trophy angling and poor fishery practises (Carol et al. 2009; Copp et al. 2009a; Cucherousset & Olden, 2011; Cucherousset et al. 2012).

In assessing the impacts of disease emergence with the transfer of exotic parasites and pathogens from *S. glanis* to native fish communities it must be noted that in general there are gaps in knowledge about the potentially harmful impacts to native fish species. For example, a novel ancyrocephalid monogenean parasite *T. vistulensis* was identified upon recaptured *S. glanis* from a lake fishery, near Ringwood, Dorset in this study (Appendix E6). This was a new finding for this novel siluriform parasite in freshwater habitats in the UK yet it remains unclear whether it is potentially harmful to native fish species and capable of host switching (Reading et al. 2012; Copp et al. 2016a).

There appears to be a need for further research with infectious agents associated with *S. glanis* and stricter quarantine measures for non-native fish transfers around the world. It is highly probable that in future decades, thermal changes related to climate change may increase disease emergence from fish invaders to native fish species communities in the UK. Higher water temperatures can accelerate reproductive life cycles of parasites to the detriment of fish survival. The survival of novel fish parasites are likely to be accelerated in warmer waters with disease spreading from Europe into the UK (Sweetman et al. 2006; Gozlan et al. 2010; Cucherousset & Olden, 2011; Reading et al. 2012; Cucherousset et al. 2012; Copp et al. 2016a). It is recommended to:

1) Develop and update fish health criteria in the classification of Category 2 non-native parasite and pathogen check lists for non-native *S. glanis* species by regulatory bodies in the UK (Reading et al. 2012; Copp et al. 2016a).

2) Carry out more detailed research for infectious agents such as *T. vistulensis* and *E. sieboldi* identified in the study so as to improve decision making process in determining *S. glanis* invasive risks (Reading et al. 2012; Copp et al. 2016a).

3) Investigate the spread of non-native fish species pathogens in relation to climate change effects in aquatic habitats using parasitological and modelling approaches so as to identify areas at higher risk from *S. glanis* introductions in the UK (Gozlan et al. 2005; Copp et al. 2009a; Busst & Britton, 2017).

4) Lay out a mandatory enforcement of stricter biosecurity controls for all routes of introduction of non-native *S. glanis* between countries with better quarantine standards, fish health inspectorates and risk assessments before fish transfer (Gozlan et al. 2005; Copp et al. 2009a; Copp et al. 2016a).

4.3 Further research

4.3.1 Spread and trophic interactions

1) Likelihood of establishment and spread of *Silurus glanis* into the UK

The aim of the study was to investigate using existing literature data and databases on fish invasions and life history traits, what determines the potential invasion of *S. glanis* in the UK. The review provides an assessment of the likelihood spread of *S. glanis* into the UK, potential impacts upon recipient habitats (lakes, reservoirs, water courses, rivers), likelihood of reproduction success, diet assimilation efficiency and potential impacts of disease emergence (novel parasites and pathogens) upon native fish communities.

Investigating predictors of establishment success of *S. glanis* involves estimation of *S. glanis* growth. This is measured by the following parameters: length-at-age (L_t) relationships, mass-length (W- L_t) relationships, von Bertalanffy growth function parameters (VBGF), specific growth rate (SGR), Fulton`s condition factor *K* and Feed conversion ratio (FCR). By the use of databases (FishBase, Database on Introductions of Aquatic species), and applications from population dynamics models and Climatch modelling will determine predicted growth, reproduction success, diet assimilation, and risks of disease emergence by climate matching. This will provide a state of knowledge of *S. glanis* ecological impacts at a global scale and may shed some light on whether establishment success of *S. glanis* may vary with different recipient habitats (lentic and lotic) or between habitats and climates in reference to the UK (Copp et al. 2017; Cucherousset et al. 2018).

2) Prey selectivity and application of DNA metabarcoding on faeces to identify *Silurus glanis* diet

Understanding aspects of *S. glanis* predation and prey selectivity may provide useful insights in determining their invasive impacts in invaded habitats. Some of these objectives may be investigated using DNA metabarcoding on faeces of *S. glanis* as well as conventional stomach content gut analysis in identifying their diet in ponds. All *S. glanis* specimens would be tagged to allow for individual identification for stomach content and faeces collection. *S. glanis* would be classed into size groups and released in ponds stocked with cyprinids. The total prey fish biomass should be recorded and prey size selected with jaw gape of *S. glanis* size groups. The experimental set up should simulate natural conditions as closely as possible including a wide range of cyprinid prey species available and mitigate possible experimental constraints such as

annual fish recapture (quarterly recapture of *S. glanis* recommended with prey fish recapture biomass data recorded) which may influence study findings (Carol et al. 2009; Copp et al. 2009a; Guillerault et al. 2017).

4.3.2 Management and control

There is some variability in the success of targeted removal of *S. glanis* using conventional netting and electro-fishing practises in aquatic habitats. Some of the difficulties are that *S. glanis* are a benthic fish species and known to lie hidden in deep crevices and muddy substrates in river catchments and lakes. Consequently, new applications in recapturing invasive *S. glanis* populations may be beneficial with the integration of environmental DNA analysis and fish pheromone applications for species control (Copp et al. 2009a; Davison et al. 2017).

1) Application of environmental DNA analysis for detection of *Silurus glanis*

Fisheries control management of invasive species may benefit from the new field of environmental DNA analysis approach. Fishery surveys for the detection of environmental DNA have been successfully used in the control and eradication programmes for invasive *P. parva* populations in England and Wales. Environmental DNA surveys may be used to protect native fish species communities as DNA shed by non-native species can be detected accurately and are less environmentally damaging than convention sampling techniques. In the case of invasive *S. glanis* this approach may detect and provide information of their spatial distribution in England and Wales with reduction of costs in fisheries management (Copp et al. 2009a; Davison et al. 2017)

2) Fish pheromone applications

The application of fish pheromones as an integrated pest control programme has been successful with introduced species such sea lamprey (*Petromyzon marinus*) in the North American Great Lakes via disruption of spawning and fish movements and subsequent suppression of invasive populations (Sorensen & Stacy, 2004; Gozlan et al. 2010). It is recommended that research in the development of fish pheromones in the control of *S. glanis* species in England and Wales be considered, with initial approaches such as:

a) Pheromone trapping

The application of synthesized fish pheromones as an attractant for non-native *S. glanis* to be used as bait in fish traps and fish nets so as to enhance trapping efficiency and fish removal in aquatic habitats (Copp et al. 2009a; Gozlan et al. 2010).

b) Disruption of reproduction success

The application of synthesized sex fish pheromones adapted to disrupt spawning for non-native *S. glanis* as a control strategy so as to reduce reproduction success and fish abundance (Copp et al. 2009a; Gozlan et al. 2010)

4.3.3 Thermal adaptation of parasites

Fish species are vulnerable to several infectious agents (viruses, bacteria, pathogens and parasites) with risks of infectious outbreaks from non-native fish exchanges to native fish communities (Peeler et al. 2011; Gozlan et al. 2014).

Climate driven thermal changes in aquatic habitats may increase the spread in disease emergence to native fish species communities. However, there are some gaps in knowledge about the parasitology of invasive fish species with little information available about novel infectious agents carried by *S. glanis* into introduced habitats. Potentially these risks could be investigated using experimental studies and modelling application (Ecopath) to determine thermal adaptations of novel parasites occurring on *S. glanis* from different populations (northern Europe vs. southern Europe). These two populations are chosen because of species adaptations to different climates and water temperature variation (Gozlan et al. 2014; Bean et al. 2017).

4.3.4 Phylogeography and adaption of invasive *Silurus glanis*

Understanding non-native fish species ecological impacts may benefit from new approaches such as conservation genetics in predicting the risks of species invasive potential. This may be useful in understanding species origin, genetic diversity and migration patterns. In the case *S. glanis*, understanding their spatial distribution may provide information about their adaptive mechanisms and invasive characteristics. A genetic approach is a valuable tool in tackling these questions by following *S. glanis* populations at a fine-scale with identification of potential impacts to native fish species and risks of dispersal (Gozlan et al. 2010).

1) Conservation genetics of non-native *Silurus glanis* populations in the UK

It is recommended that research in the development of conservation genetics of invasive fish species is intensified by using novel approaches such as the conservation genetics of non-native *S. glanis* populations in the UK. *S. glanis* is a popular trophy species for angling in the UK and has originated from a single refugium around the Ponto-Caspian region which has spread into central and southern Europe. Although the genetic structure and phylogeography of *S. glanis* has been studied in its native range, no information is known about its genetic characteristics in introduced ranges. The origin and genetic structure of *S. glanis* populations in England and Wales may be investigated by application of genetic markers (DNA sequence) such as mitochondrial and autosomal markers. This approach would provide information about the genetic variability of *S. glanis* populations, the risks of inbreeding and species dispersal into the UK (Triantafyllidis et al. 2002; Copp et al. 2009a; Vittas et al. 2011).

4.4 Concluding remarks

S. glanis is a popular trophy species for recreational angling in the UK and across Europe yet little is known about their invasiveness potential into introduced ranges and risks of ecological harm to native fish species. Ultimately, species protection requires regulatory enforcement and environmental risk protocols to be undertaken yet with large bodied *S. glanis* introductions into the UK, present quarantine measures and regulatory control of fish movements between countries needs to be reassessed (Copp et al. 2005b; Gozlan et al. 2010; Rees et al. 2017).

In order to predict the risks of *S. glanis* invasiveness potential in water bodies in the UK, it is important to understand their life history traits coupled with updating of risk assessment protocols to improve the decision making process and appropriate management response. The present study investigated several aspects of invasion ecology which may influence *S. glanis* invasion into new environments and provide new information of potentially harmful impacts to native fish species. These findings brought attention to the risks of disease emergence to native fish species as well as possible predation and trophic impacts from large bodied *S. glanis* in ponds. Ultimately, these new findings may help identify and increase understanding about the potential scale of *S. glanis* ecological impacts in invaded habitats which are likely to be exacerbated with climate change impacts in future decades.

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6. Appendices

This includes data from the study in Appendices A, B, C, D and E.

Appendix A

Appendix A contains growth data from Chapter 3 of the Pilot study

Table 6A1.1 *S. glanis* size groups stocking densities (kg /ha^{-1}) , mean total length and weight and PIT tag recapture rates (using fyke nets) in ponds during the pilot study at Mayland, Essex, 2011- 2012. Standard deviation (SD) and 95% Confidence Interval (95%CI) are given.

Group	**n *S. glanis* in 2011**	**Biomass Kg ha^{-1} of *S. glanis* in 2011**	**Mean TL 2011 (cm)** (± SE)	**Mean W 2011 (g)** (± SE)	**N_R (Total no of *S. glanis* recaptured) in 2012**	**N_{PT} (Total of *S. glanis* recaptured with a tag) in 2012**	**PIT tag retention rate % of *S. glanis* in 2012**
Large	20	907.47	50.25 ± 0.55	698.75 ± 25.49	19	19	(100%)
SD			2.48	113.98			
95%CI			From 49.09 to 51.41	From 645.41to 752.09			
Medium	20	507.44	42.78 ± 0.42	426.25 ± 13.39	15	15	(100%)
SD			1.87	59.87			
95%CI			From 41.90 to 43.66	From 398.23 to 454.27			
Small	20	396.26	38.25 ± 0.62	291.25 ± 13.61	11	10	(91%)
SD			2.78	60.85			
95%CI			From 36.95 to 39.55	From 262.77 to 319.73			

Table 6A1.2. Variation in mean growth (Length TL & Weight ± SE) of *S. glanis* size groups in the pilot study (2011). Standard deviation (SD) and 95% Confidence level Interval (95%CI) are given.

Group	**Length (TL) (cm) (**±SE)	**Weight (g) (** ±SE)
	2011	**2011**
Large group Mean difference	0.18± 0.20	-62.94 ± 14.48
SD	0.87	61.43
P value	0.369	0.000
95%CI	From -0.24 to 0.62	From -93.49 to -32.39
Medium group Mean difference	-0.21± 0.13	-97.04 ± 18.75
SD	0.52	72.30
P value	0.104	0.000
95%CI	From -0.48 to 0.04	From -136.78 to -57.29
Small group Mean difference	0.12± 0.06	-37.20 ±19.78
SD	0.22	71.31
P value	0.077	0.084
95%CI	From -0.01 to 0.25	From -80.29 to 5.89

Table 6A1.3 Raw data for jaw gape, length TL and weight of large size *S. glanis* group in the pilot study 2011-2012.

Large *S. glanis* group PIT tag No	Jaw gape cm	Length (TL) cm 2011	Weight g 2011	Length (TL) cm 2012	Weight g 2012
DC003FEC9B	7.3	52.5	775.0	53.2	758.5
DC003FEE14	6.9	49.4	625.0	50.2	550.0
DC003FF1EA	7.3	53.2	775.0	53.2	730.0
DC003FF376	7.6	54.3	900.0	54.4	812.1
DC003FF7A7	6.2	47.4	600.0	46.6	462.3
DC003FFAFA	7.5	53.4	800.0	53.9	746.5
DC003FFB1C	7.2	50.6	700.0	51.1	626.8
DC003FFECA	6.4	48.1	675.0	47.9	539.8
DC004000B1	7.5	54.4	950.0	54.9	843.1
DC004001FE	6.6	49.6	675.0	47.9	592.5
DC0040053C	6.8	49.2	600.0	48.9	540.6
DC0040082A	6.5	47.5	525.0	47.3	510.9
DC004009C4	6.7	48.0	575.0	48	498.3
DC00400E52	6.2	47.5	625.0	47.1	474.6
DC0040103F	6.8	50.5	700.0	50.6	661.2
DC0040167A	7.6	53.5	850.0	54.9	873.5
DC00401846	7.2	50.5	700.0	52.9	775.6
DC00401B68	6.4	46.9	575.0	46.9	440.7
DC00400AA5	6.6	49.0	625.0	-	-
DC003FF21B	6.8	49.5	725.0	-	-

Table 6A1.4 Raw data for jaw gape, length TL and weight of medium size *S. glanis* group in the pilot study 2011-2012.

PIT tag No	Jaw gape (cm)	Length (TL) cm 2011	Weight g 2011	Length (TL) cm 2012	Weight g 2012
DC00401116	4.8	38.4	350.0	38.1	216.3
DC00400DEA	5.4	43.2	425.0	43.3	218.8
DC0040066B	4.9	39.4	350.0	38.6	190.2
DC003FF402	5.8	46.4	550.0	46.1	543.3
DC003FFEFA	6.0	46.0	575.0	46.8	483.2
DC003FEEDC	5.3	43.1	400.0	42.5	289.1
DC00401892	5.3	44.0	475.0	43.8	270.8
DC00400631	5.3	44.5	475.0	-	-
DC00400A65	5.3	43.4	400.0	-	-
DC003AOE19	5.4	43.00	400.0	42.9	387.5
DC00400703	5.2	42.0	425.0	41.7	280.5
DC00400964	5.1	41.4	350.0	41.4	350.0
DC003FFE49	5.2	42.0	450.0	41.5	296.6
DC003FFF66	5.2	42.0	375.0	-	-
DC0040090A	5.3	44.1	425.0	44.9	473.6
DC00401B42	5.2	42.7	425.0	42.4	305.6
DC0040090E	5.2	42.5	425.0	41.8	262.8
DC00400C20	5.2	42.5	450.0	42.5	329.4
DC003FF714	4.9	41.6	375.0	41.6	375.0
DC003FF88B	5.3	43.4	425.0	42.1	352.7

Table 6A1.5 Raw data for jaw gape, length TL and weight of small size *S. glanis* group in the pilot study 2011-2012.

PIT tag No	Jaw gape (cm)	Length (TL) cm 2011	Weight g 2011	Length (TL) cm 2012	Weight g 2012
DC00400531	4.6	39.0	300.0	39.0	244.6
DC003FFE00	4.5	37.8	300.0	37.6	197.3
DC003FF946	4.5	38.4	300.0	-	-
DC00400AD8	4.8	38.2	300.0	38.6	225.0
DC00401AF1	4.7	38.9	325.0	-	-
DC004011AC	4.5	38.2	300.0	-	-
DC00401A42	4.7	38.5	150.0	38.8	291.2
DC00400E82	5.0	39.9	350.0	40.1	299.3
DC00401068	4.6	39.5	325.0	39.5	325.0
DC00401250	4.9	40.0	325.0	39.9	286.1
DC003FFFE1	5.1	40.6	350.0	40.9	363.4
DC00401565	4.7	36.1	275.0	36.3	203.5
DC00401388	4.8	38.6	300.0	38.6	180.7
DC004000F7	4.4	35.8	250.0	35.7	181.7
DC00400879	4.5	37.7	250.0	-	-
DC00401735	5.0	40.6	325.0	40.6	226.2
DC003FFD76	5.1	42.8	350.0	43.3	392.4
DC003FFEE2	4.5	37.0	275.0	-	-
DC00400E73	4.8	38.9	350.0	-	-
DC003FF6E8	4.0	28.6	125.0	-	-

Table 6A1.6 Prey fish stocking densities (kg /ha^{-1}**),** mean total length (TL) and weight (W) in ponds for *S. glanis* size groups during the pilot study at Mayland, Essex, 2011-2012.

Group	**n forage prey species 2013**	**Biomass Kg ha^{-1} Forage prey fish**	**Mean TL 2011 (cm)** (± SE)	**Mean W 2011 (g)** (± SE)
Large	24	171.79	17.79 ± 0.24	110.23 ± 5.55
SD			1.16	27.17
95%CI			From 17.30 to 18.28	From 98.76 to 121.71
Medium	25	58.94	14.60 ± 0.20	41.26 ± 1.02
SD			0.98	5.08
95%CI			From 14.20 to 15.00	From 39.16 to 43.36
Small	24	47.89	12.61 ± 0.13	29.33 ± 1.61
SD			0.62	7.88
95%CI			From 12.34 to 12.87	From 26.01 to 32.66

Table 6A1.7 Raw data for the prey fish species length and weight stocked in large *S. glanis* size group in the pilot study at Mayland, Essex, 2011-2012.

Prey species	Length (TL) cm	Weight g
C. carpio	16.3	116.2
C. carpio	16.6	114.2
C. carpio	19.2	133.8
C. carpio	19.0	131.5
C. carpio	19.2	134.7
C. carpio	19.0	133.4
C. carpio	18.2	115.3
C. carpio	18.8	118.9
C. carpio	16.2	64.3
C. carpio	17.3	61.2
C. carpio	18.2	118.9
S. erythrophthalmus	17.2	121.8
S. erythrophthalmus	16.6	62.3
S. erythrophthalmus	17.5	63.4
S. erythrophthalmus	19.0	135.2
S. erythrophthalmus	16.1	115.6
S. erythrophthalmus	18.7	116.6
S. erythrophthalmus	17.3	51.8
S. erythrophthalmus	17.5	119.3
S. erythrophthalmus	16.2	117.9
S. erythrophthalmus	19.3	135.1
S. erythrophthalmus	18.1	114.2
S. erythrophthalmus	16.3	116.3
S. erythrophthalmus	19.1	133.7

Table 6A1.8 Raw data for the prey fish species length and weight stocked in medium *S. glanis* size group in the pilot study at Mayland, Essex, 2011-2012.

Prey species	Length (TL) cm	Weight g
C. carpio	**15.6**	**45.6**
C. carpio	15.8	45.6
C. carpio	15.6	41.5
C. carpio	15.3	44.8
C. carpio	15.8	43.9
C. carpio	15.2	41.9
C. carpio	15.7	42.8
C. carpio	15.4	44.6
C. carpio	15.5	45.8
C. carpio	15.3	49.8
C. carpio	15.8	49.4
C. carpio	14.3	46.7
S. erythrophthalmus	13.3	33.3
S. erythrophthalmus	13.2	39.2
S. erythrophthalmus	13.5	38.3
S. erythrophthalmus	14.3	33.2
S. erythrophthalmus	13.1	36.3
S. erythrophthalmus	14.3	45.9
S. erythrophthalmus	13.4	36.2
S. erythrophthalmus	14.8	33.2
S. erythrophthalmus	14.3	38.7
S. erythrophthalmus	14.7	39.1
S. erythrophthalmus	13.4	35.3
S. erythrophthalmus	14.2	44.2
S. erythrophthalmus	13.2	36.2

Table 6A1.9 Raw data for the prey fish species length and weight stocked in small *S. glanis* size group in the pilot study at Mayland, Essex, 2011-2012.

Prey species	Length (TL) cm	Weight g
C. carpio	13.2	41.3
C. carpio	12.3	35.8
C. carpio	13.2	43.8
C. carpio	12.4	32.3
C. carpio	12.2	37.4
C. carpio	12.1	38.4
C. carpio	12.3	39.9
C. carpio	14.2	31.3
C. carpio	13.8	32.9
C. carpio	13.7	41.7
C. carpio	13.1	32.7
S. erythrophthalmus	12.3	24.3
S. erythrophthalmus	12.5	22.8
S. erythrophthalmus	12.2	23.9
S. erythrophthalmus	12.4	23.8
S. erythrophthalmus	12.6	23.7
S. erythrophthalmus	12.8	20.1
S. erythrophthalmus	11.6	18.8
S. erythrophthalmus	12.2	22.7
S. erythrophthalmus	12.3	22.9
S. erythrophthalmus	12.1	23.8
S. erythrophthalmus	12.2	23.1
S. erythrophthalmus	12.8	23.7
S. erythrophthalmus	12.1	22.9

Appendix A growth data from Chapter 3, Growth & trophic study

Table 6A1.10 Variation in mean length TL and weight, Fulton`s condition factor (K) ± SE of large size *S. glanis* group stocked at 1:2 predator-prey ratio in the first year growth study, Mayland Essex, 2013.

***S. glanis* characteristics**	**N**	**Mean (SE)**	**Minimum**	**Maximum**
Baseline Fish length (cm)	18	50.55 ±0.72	46.60	54.90
Baseline Fish weight (g)	18	635.39 ±39.49	440.70	873.50
Follow up Fish length (cm)	16	53.08 ±0.81	49.20	58.90
Follow up Fish weight (g)	16	739.64±39.17	537.00	1025.00
Fulton`s condition factor (*K*)	16	499.75 ±7.10	427.02	534.09

Table 6A1.11 Variation in mean length TL and weight, Fulton`s condition factor (K) ±SE of medium size *S. glanis* group stocked at 1:2 predator-prey ratio in the first year growth study, Mayland Essex, 2013.

***S. glanis* characteristics**	**N**	**Mean (SE)**	**Minimum**	**Maximum**
Baseline Fish length (cm)	17	42.47 ±0.53	38.10	46.80
Baseline Fish weight (g)	17	330.91 ±23.83	190.20	543.30
Follow up Fish length (cm)	13	44.78 ±0.74	41.60	49.50
Follow up Fish weight (g)	13	427.69 ±21.00	300.00	560.00
Fulton`s condition factor (*K*)	13	485.58 ±26.96	364.46	593.28

Table 6A1.12 Variation in mean length TL and weight, Fulton`s condition factor (K) ±SE of small size *S. glanis* group stocked at 1:2 predator-prey ratio in the first year growth study, Mayland Essex, 2013.

***S. glanis* characteristics**	**N**	**Mean (SE)**	**Minimum**	**Maximum**
Baseline Fish length (cm)	13	39.15 ±0.55	35.70	43.30
Baseline Fish weight (g)	13	262.80±19.23	180.70	392.40
Follow up Fish length (cm)	13	41.63 ±0.78	36.20	48.10
Follow up Fish weight (g)	13	400.69 ±31.00	250.00	650.00
Fulton`s condition factor (*K*)	13	541.81 ±34.21	449.56	699.56

Table 6A1.13 Variation in mean length TL and weight, Fulton`s condition factor (K) ±SE of large size *S. glanis* group stocked at 1:3 predator-prey ratio in the second year growth study, Mayland Essex, 2014.

***S. glanis* characteristics**	**N**	**Mean (SE)**	**Minimum**	**Maximum**
Baseline Fish length (cm)	16	53.08 ±0.81	49.20	58.90
Baseline Fish weight (g)	16	739.64 ±39.17	537.00	1025.00
Follow up Fish length (cm)	13	56.49 ±1.65	47.80	64.10
Follow up Fish weight (g)	13	967.38 ±100.47	515.70	1750.00
Fulton`s condition factor (*K*)	13	522.26 ±22.21	463.41	730.74

Table 6A1.14 Variation in mean length TL and weight, Fulton`s condition factor (K) ±SE of medium size *S. glanis* group stocked at 1:3 predator-prey ratio in the second year growth study, Mayland Essex, 2014.

***S. glanis* characteristics**	**N**	**Mean (SE)**	**Minimum**	**Maximum**
Baseline Fish length (cm)	13	44.78 ± 0.74	41.60	49.50
Baseline Fish weight (g)	13	427.69 ± 21.00	300.00	560.00
Follow up Fish length (cm)	8	45.68 ± 1.17	42.20	53.10
Follow up Fish weight (g)	8	516.69± 43.12	342.00	735.00
Fulton`s condition factor (*K*)	8	531.86 ±40.09	404.23	759.13

Table 6A1.15 Variation in mean length TL and weight, Fulton`s condition factor (K) ±SE of small size *S. glanis* group stocked at 1:3 predator-prey ratio in the second year growth study, Mayland Essex, 2014.

***S. glanis* characteristics**	**N**	**Mean (SE)**	**Minimum**	**Maximum**
Baseline Fish length (cm)	13	41.63 ±0.78	36.20	48.10
Baseline Fish weight (g)	13	400.69 ± 31.00	250.00	650.00
Follow up Fish length (cm)	13	45.81 ±1.53	41.70	58.10
Follow up Fish weight (g)	10	627.00 ± 72.08	249.00	1050.00
Fulton`s condition factor (*K*)	10	637.42 ±46.52	343.39	955.45

Table 6A1.16 Large size *S. glanis* group growth study 2013 & 2014. Raw data for jaw gape, length, weight, Fulton`s condition factor K, specific growth rate in length and weight (SGRL, SGR*W*).

Large *S. glanis* group PIT tag No	**Jaw gape cm**	**Length (TL) cm 2012**	**Weight g 2012**	**Length (TL) cm 2013**	**Weight g 2013**	**Length (TL) cm 2014**	**Weight g 2014**	**Fulton *K* 2013**	**Fulton *K* 2014**	**SGR (L) 2013**	**SGR (*W*) 2013**	**SGR (L) 2014**	**SGR (*W*) 2014**
DC003FEC9B	7.3	53.2	758.5	55.5	820.0	62.1	1300.0	479.66	542.84	0.01	0.02	0.03	0.12
DC003FEE14	6.9	50.2	550.0	50.4	560.3			437.65		0	0.01		
DC003FF1EA	7.3	53.2	730.0	55.5	785.0			459.19		0.01	0.02		
DC003FF376	7.6	54.4	812.1	56.1	900.0	62.1	1200.0	509.75	501.08	0.01	0.03	0.03	0.07
DC003FF7A7	6.2	46.6	462.3	49.5	537.0			442.75		0.02	0.04		
DC003FFAFA	7.5	53.9	746.5	54	750.0	55.1	801.0	476.3	478.83	0	0	0.01	0.02
DC003FFB1C	7.2	51.1	626.8	53.1	750.0			500.93		0.01	0.05		
DC003FFECA	6.4	47.9	539.8	51.2	625.0			465.66		0.02	0.04		
DC004000B1	7.5	54.9	843.1	55.6	918.0	62.1	1750.0	534.09	730.74	0	0.02	0.03	0.17
DC004001FE	6.6	47.9	592.5	51.2	699.0	53.2	766.0	520.8	508.74	0.02	0.05	0.01	0.02
DC0040053C	6.8	48.9	540.6	49.2	565.0	53.2	715.0	474.41	474.87	0	0.01	0.02	0.06
DC0040082A	6.5	47.3	510.9	50.6	600.0	53.2	825.0	463.13	547.92	0.02	0.04	0.01	0.08
DC004009C4	6.7	48	498.3			48.4	550.3		485.36				
DC00400E52	6.2	47.1	474.6	47.8	575.0	49.5	623.0	526.48	513.66	0.01	0.05	0.01	0.02
DC0040103F	6.8	50.6	661.2	53.2	750.0	64.1	1280.0	498.11	486	0.01	0.03	0.05	0.13
DC0040167A	7.6	54.9	873.5	57.5	975.0	61.1	1100.0	512.86	482.25	0.01	0.03	0.02	0.03
DC00401846	7.2	52.9	775.6	58.9	1025.0	62.2	1150.0	501.62	477.89	0.03	0.08	0.01	0.03
DC00401B68	6.4	46.9	440.7			48.1	515.7		463.41				

Table 6A1.17 Medium size *S. glanis* group growth study 2013 & 2014. Raw data for jaw gape, length, weight, Fulton`s condition factor K, specific growth rate in length and weight (SGRL) and (SGRW).

Medium *S. glanis* group PIT tag No	**Jaw gape cm**	**Length (TL) cm 2012**	**Weight g 2012**	**Length (TL) cm 2013**	**Weight g 2013**	**Length (TL) cm 2014**	**Weight g 2014**	**Fulton *K* 2013**	**Fulto n *K* 2014**	**SGR (L) 2013**	**SGR (*W*) 2013**	**SGR (L) 2014**	**SGR (*W*) 2014**
DC003AOE19	5.4	42.9	387.5	44.3	390.0			448.59				0.01	0
DC003FEEDC	5.3	42.5	289.1										
DC003FF402	5.8	46.1	543.3	49.5	560.0	53.1	735.0	461.71	490.91	0.02	0.07	0.02	0.01
DC003FF714	4.9	41.6	375	42.9	380.0	44.8	500.0	556.08	481.3	-0.01	-0.07	0.02	0.08
DC003FF88B	5.3	42.1	352.7	42.4	400.0			524.76				0	0.03
DC003FFE49	5.2	41.5	296.6	41.6	400.0	42.2	570.5	555.62	759.13	0	0.09	0	0.08
DC003FFEFA	6.0	46.8	483.2	49.1	550.0			464.64				0.01	0.03
DC0040066B	4.9	38.6	190.2										
DC00400703	5.2	41.7	280.5	43.1	475.0	45.1	577.0	593.28	628.99	0.01	0.05	0.01	0.14
DC0040090A	5.5	44.9	473.6	46.8	480.0	47.1	533.0	468.28	510.11	0	0.03	0.01	0
DC0040090E	5.2	41.8	262.8	43.5	300.0	43.9	342.0	364.46	404.23	0	0.03	0.01	0.03
DC00400964	5.1	41.4	350	48.1	475.0			426.83				0.04	0.08
DC00400C20	5.2	42.5	329.4	42.9	350.0	44.1	380.0	443.3	443.07	0.01	0.02	0	0.02
DC00400DEA	5.4	43.3	218.8										
DC00401116	4.8	38.1	216.3										
DC00401892	5.3	43.8	270.8	44.9	400.0	45.2	496.0	441.9	537.11	0	0.06	0.01	0.1
DC00401B42	5.2	42.4	305.6	43.1	400.0			499.61				0	0.07

Table 6A1.18 Small size *S. glanis* group growth study 2013 & 2014. Raw data for jaw gape, length, weight, Fulton`s condition factor K, specific growth rate in length and weight (SGRL) and (SGRW).

Small *S. glanis* group PIT tag No	**Jaw gape cm**	**Length (TL) cm 2012**	**Weight g 2012**	**Length (TL) cm 2013**	**Weight g 2013**	**Length (TL) cm 2014**	**Weight g 2014**	**Fulton *K* 2013**	**Fulton *K* 2014**	**SGR (L) 2013**	**SGR (*W*) 2013**	**SGR (L) 2014**	**SGR (*W*) 2014**
DC003FFD76	5.1	43.3	392.4	48.1	650.0	58.1	1050	584.09	535.38	0.05	0.12	0.03	0.14
DC003FFE00	4.5	37.6	197.3	40.4	325.0	47	730	492.88	703.12	0.04	0.21	0.02	0.13
DC003FFFE1	5.1	40.9	363.4	42.5	525.0	45.5	900	683.9	955.45	0.02	0.14	0.01	0.1
DC004000F7	4.4	35.7	181.7	36.2	250.0			527				0	0.09
DC00400531	4.6	39.0	244.6	40.9	450.0	42.4	595	657.72	780.58	0.01	0.07	0.01	0.16
DC00400AD8	4.8	38.6	225.0	40.8	400.0	44.3	556	588.95	639.53	0.02	0.08	0.02	0.15
DC00400E82	5.0	40.1	299.3	41.5	500.0			699.56				0.01	0.14
DC00401068	4.6	39.5	325.0	44.0	425.0			498.92				0.03	0.07
DC00401250	4.9	39.9	286.1	42.7	350.0	44	435	449.56	510.66	0.01	0.06	0.02	0.05
DC00401388	4.8	38.6	180.7	41.7	249.0	41.7	350	343.39	482.68	0	0.09	0.02	0.09
DC00401565	4.7	36.3	203.5	37.9	350.0	48.8	752	642.91	647.08	0.07	0.19	0.01	0.14
DC00401735	5.0	40.6	226.2	42.3	350.0	42.8	395	462.43	503.81	0.01	0.03	0.01	0.12
DC00401A42	4.7	38.8	291.2	42.2	385.0	43.5	507	512.3	615.94	0.01	0.07	0.02	0.08

Table 6A1.19 *S. glanis* size groups stocking densities (kg /ha^{-1}**)** in ponds at Mayland, Essex during the two year growth study at Mayland, Essex, 2013 & 2014.

Study year	**Small size *S. glanis* stocking densities biomass kg /ha^{-1}**	**Medium size *S. glanis* stocking densities biomass kg /ha^{-1}**	**Large size *S. glanis* stocking densities biomass kg /ha^{-1}**
2012 (Baseline)	232.41	334.85	742.66
2013	354.35	330.95	768.46
2014	426.53	246.04	816.62

Table 6A1.20 Prey fish stocking densities (kg /ha^{-1}**)** in ponds for *S. glanis* size groups at Mayland, Essex during the two year growth study at Mayland, Essex, 2013 & 2014.

Study year	**Prey fish stocking densities biomass kg /ha^{-1} (small *S. glanis* group)**	**Prey fish stocking densities biomass kg /ha^{-1} (Medium *S. glanis* group)**	**Prey fish stocking densities biomass kg /ha^{-1} (Large *S. glanis* group)**
2013	74.71	110.98	361.67
2014	289.68	350.06	821.42

Table 6A1.21 Large size *S. glanis* group growth study at Mayland, Essex, 2013 prey fish species length and weight data stocked in ponds.

Cyprinid species	Length (TL) cm	Weight g	Cyprinid species	Length (TL) cm	Weight g
B. bjoerkna	20.3	170.6	*R. rutilus*	20.1	112.6
B. bjoerkna	20.6	167.9	*R. rutilus*	19.6	101.7
B. bjoerkna	19.0	120.8	*R. rutilus*	18.6	106.7
B. bjoerkna	20.7	169.0	*R. rutilus*	17.7	81.6
B. bjoerkna	20.2	169.9	*R. rutilus*	18.8	95.7
B. bjoerkna	20.7	165.8	*R. rutilus*	19.9	105.6
B. bjoerkna	19.7	143.7	*R. rutilus*	16.8	78.7
B. bjoerkna	18.5	115.7	*R. rutilus*	19.6	103.4
B. bjoerkna	16.2	76.3	*S. erythrophthalmus*	20.2	124.7
B. bjoerkna	18.3	110.7	*S. erythrophthalmus*	20.6	125.3
B. bjoerkna	19.3	190.7	*S. erythrophthalmus*	18.1	122.4
B. bjoerkna	20.7	168.9	*S. erythrophthalmus*	20.7	124.7
B. bjoerkna	21.7	170.8	*S. erythrophthalmus*	20.1	125.7
B. bjoerkna	17.7	180.8	*S. erythrophthalmus*	20.7	124.7
B. bjoerkna	18.1	121.7	*S. erythrophthalmus*	19.0	120.7
R. rutilus	20.1	113.6	*S. erythrophthalmus*	18.5	118.3
R. rutilus	19.7	105.8	*S. erythrophthalmus*	15.3	83.7
R. rutilus	19.9	106.7	*S. erythrophthalmus*	18.3	124.7
R. rutilus	20.5	112.7	*S. erythrophthalmus*	19.1	123.7
R. rutilus	18.7	99.6	*S. erythrophthalmus*	20.3	125.5
R. rutilus	19.6	102.5	*S. erythrophthalmus*	21.7	126.1
R. rutilus	20.2	113.8	*S. erythrophthalmus*	18.3	106.9
			S. erythrophthalmus	18.1	109.5

Table 6A1.22 Medium size *S. glanis* group growth study at Mayland, Essex, 2013 prey fish species length and weight data stocked in ponds.

Cyprinid species	Length (TL) cm	Weight g	Cyprinid species	Length (TL) cm	Weight g
B. bjoerkna	15.9	69.6	*R. rutilus*	15.0	43.7
B. bjoerkna	15.6	70.5	*R. rutilus*	15.2	45.5
B. bjoerkna	14.1	63.7	*R. rutilus*	15.1	46.7
B. bjoerkna	15.2	68.9	*R. rutilus*	15.1	45.3
B. bjoerkna	15.0	65.7	*R. rutilus*	15.0	41.6
B. bjoerkna	15.6	67.5	*R. rutilus*	13.7	39.6
B. bjoerkna	14.3	64.3	*S. erythrophthalmus*	14.0	44.3
B. bjoerkna	13.5	59.7	*S. erythrophthalmus*	15.1	47.2
B. bjoerkna	15.2	69.8	*S. erythrophthalmus*	15.2	47.3
B. bjoerkna	15.2	69.3	*S. erythrophthalmus*	15.0	46.2
B. bjoerkna	15.2	62.4	*S. erythrophthalmus*	15.2	47.3
B. bjoerkna	15.1	68.1	*S. erythrophthalmus*	15.1	46.9
R. rutilus	15.1	45.2	*S. erythrophthalmus*	15.0	47.2
R. rutilus	15.0	44.9	*S. erythrophthalmus*	14.8	44.2
R. rutilus	15.0	42.5	*S. erythrophthalmus*	15.1	47.7
R. rutilus	15.2	45.9	*S. erythrophthalmus*	15.0	47.1
R. rutilus	14.4	41.6	*S. erythrophthalmus*	12.4	25.3
R. rutilus	15.1	44.7	*S. erythrophthalmus*	15.1	47.2

Table 6A1.23 Small size *S. glanis* group growth study at Mayland, Essex, 2013 prey fish species length and weight data stocked in ponds.

Cyprinids	Length (TL) cm	Weight g	Cyprinids	Length (TL) cm	Weight g
B. bjoerkna	15.1	60.2	*R. rutilus*	12.1	45.5
B. bjoerkna	13.2	45.7	*R. rutilus*	11.7	46.7
B. bjoerkna	14.1	62.7	*R. rutilus*	12.5	45.3
B. bjoerkna	15.3	69.2	*R. rutilus*	12.7	41.6
B. bjoerkna	15.4	64.3	*R. rutilus*	12.7	39.6
B. bjoerkna	14.2	65.3	*S. erythrophthalmus*	12.5	26.3
B. bjoerkna	15.8	65.8	*S. erythrophthalmus*	12.3	26.1
B. bjoerkna	15.0	66.2	*S. erythrophthalmus*	11.4	24.3
B. bjoerkna	13.7	49.8	*S. erythrophthalmus*	12.1	22.7
B. bjoerkna	15.9	61.6	*S. erythrophthalmus*	12.1	26.0
R. rutilus	12.2	22.1	*S. erythrophthalmus*	11.9	22.1
R. rutilus	12.3	25.4	*S. erythrophthalmus*	11.0	26.0
R. rutilus	12.0	21.0	*S. erythrophthalmus*	12.3	25.1
R. rutilus	12.6	24.9	*S. erythrophthalmus*	12.4	26.3
R. rutilus	12.7	25.8	*S. erythrophthalmus*	12.0	25.1

Table 6A1.24 Large size *S. glanis* group growth study at Mayland, Essex, 2014 prey fish species length and weight data stocked in pond.

Cyprinid species	Length (TL) cm	Weight g	Cyprinid species	Length (TL) cm	Weight g
B. bjoerkna	25.7	308.2	*R. rutilus*	25.1	220.1
B. bjoerkna	24.8	289.5	*R. rutilus*	25.4	224.7
B. bjoerkna	25.8	300.1	*R. rutilus*	25.1	227.4
B. bjoerkna	22.2	202.1	*R. rutilus*	24.0	218.5
B. bjoerkna	24.8	270.4	*R. rutilus*	17.3	65.7
B. bjoerkna	20.7	150.3	*R. rutilus*	16.4	54.2
B. bjoerkna	19.7	125.6	*R. rutilus*	24.6	212.6
B. bjoerkna	25.9	303.1	*R. rutilus*	21.8	129.4
B. bjoerkna	24.7	260.6	*R. rutilus*	20.3	111.6
B. bjoerkna	25.6	306.2	*R. rutilus*	25.1	215.6
B. bjoerkna	24.4	300.1	*S. erythrophthalmus*	20.4	120.1
B. bjoerkna	21.7	200.4	*S. erythrophthalmus*	25.5	243.6
B. bjoerkna	26.3	300.1	*S. erythrophthalmus*	25.2	246.7
B. bjoerkna	17.7	90.3	*S. erythrophthalmus*	26.3	281.3
B. bjoerkna	20.4	148.7	*S. erythrophthalmus*	25.4	245.2
B. bjoerkna	18.1	106.5	*S. erythrophthalmus*	23.6	222.1
B. bjoerkna	25.4	298.6	*S. erythrophthalmus*	25.1	243.6
B. bjoerkna	24.3	258.2	*S. erythrophthalmus*	25.4	241.7
B. bjoerkna	25.5	299.1	*S. erythrophthalmus*	25.1	240.4
B. bjoerkna	25.1	288.6	*S. erythrophthalmus*	23.0	189.4
R. rutilus	26.1	257.43	*S. erythrophthalmus*	25.6	243.6
R. rutilus	25.3	220.5	*S. erythrophthalmus*	18.7	85.3
R. rutilus	22.3	160.4	*S. erythrophthalmus*	17.9	70.3
R. rutilus	24.5	190.4	*S. erythrophthalmus*	24.6	216.5
R. rutilus	17.2	63.7	*S. erythrophthalmus*	25.1	247.3
R. rutilus	25.5	219.5	*S. erythrophthalmus*	26.3	280.2
R. rutilus	25.2	215.7	*S. erythrophthalmus*	24.4	214.7
R. rutilus	26.1	253.5	*S. erythrophthalmus*	20.1	121.5
R. rutilus	25.4	218.4	*S. erythrophthalmus*	24.3	217.4
R. rutilus	22.4	150.5	*S. erythrophthalmus*	25.1	242.5

Table 6A1.25 Medium size *S. glanis* group growth study at Mayland, Essex, 2014 prey fish species length and weight data stocked in pond.

Cyprinid species	Length (TL) cm	Weight g	Cyprinid species	Length (TL) cm	Weight g
B. bjoerkna	22.1	201.6	*R. rutilus*	19.2	94.1
B. bjoerkna	20.3	151.3	*R. rutilus*	20.6	109.3
B. bjoerkna	18.6	106.7	*R. rutilus*	22.1	148.5
B. bjoerkna	19	128.3	*R. rutilus*	20.3	111.4
B. bjoerkna	19.8	127.4	*R. rutilus*	21.6	109.5
B. bjoerkna	20.3	151.7	*R. rutilus*	22.1	150.2
B. bjoerkna	21.5	176.5	*R. rutilus*	20.7	108.3
B. bjoerkna	19.9	127.6	*S. erythrophthalmus*	22.0	163.4
B. bjoerkna	20.8	148.7	*S. erythrophthalmus*	20.5	120.3
B. bjoerkna	22.1	200.5	*S. erythrophthalmus*	21.7	140.3
B. bjoerkna	19.9	126.4	*S. erythrophthalmus*	20.6	119.4
B. bjoerkna	20.7	142.3	*S. erythrophthalmus*	21.9	140.5
B. bjoerkna	21.6	175.6	*S. erythrophthalmus*	20.6	120.3
B. bjoerkna	22	203.6	*S. erythrophthalmus*	19.9	101.4
B. bjoerkna	19.8	126.7	*S. erythrophthalmus*	17.7	70.3
R. rutilus	22	150.2	*S. erythrophthalmus*	19.2	101.6
R. rutilus	19.3	92.3	*S. erythrophthalmus*	20.6	118.9
R. rutilus	18.3	84.1	*S. erythrophthalmus*	22.1	162.6
R. rutilus	20.7	111.2	*S. erythrophthalmus*	20.3	120.6
R. rutilus	22.1	151.3	*S. erythrophthalmus*	21.6	138.3
R. rutilus	20.6	108.6	*S. erythrophthalmus*	20.8	118.7
R. rutilus	19.9	93.2	*S. erythrophthalmus*	21.7	141.3
R. rutilus	18.8	86.4			

Table 6A1.26 Small size *S. glanis* group growth study at Mayland, Essex, 2014 prey fish species length and weight data stocked in pond.

Cyprinid species	Length (TL) cm	Weight g	Cyprinid species	Length (TL) cm	Weight g
B. bjoerkna	20.1	151.4	*R. rutilus*	18.8	80.4
B. bjoerkna	19.3	128.4	*R. rutilus*	19.2	93.1
B. bjoerkna	19.4	125.4	*R. rutilus*	16.4	63.8
B. bjoerkna	20	126.7	*R. rutilus*	15.3	41.3
B. bjoerkna	18.3	105.3	*R. rutilus*	20.3	109.7
B. bjoerkna	16.2	73.5	*R. rutilus*	19.6	93.6
B. bjoerkna	14.3	41.3	*R. rutilus*	18.3	81.2
B. bjoerkna	17.3	90.3	*R. rutilus*	17.2	65.3
B. bjoerkna	19.1	126.3	*S. erythrophthalmus*	20	120.6
B. bjoerkna	20	155.2	*S. erythrophthalmus*	19.2	101.3
B. bjoerkna	18.2	108.3	*S. erythrophthalmus*	18.3	85.4
B. bjoerkna	20	151.3	*S. erythrophthalmus*	19.2	102.5
B. bjoerkna	18.2	103.5	*S. erythrophthalmus*	20.1	119.4
B. bjoerkna	17.3	89.4	*S. erythrophthalmus*	20.1	120.2
B. bjoerkna	16.2	72.3	*S. erythrophthalmus*	19.9	100.2
R. rutilus	20	111.5	*S. erythrophthalmus*	16.3	58.3
R. rutilus	19.3	93.5	*S. erythrophthalmus*	15.3	46.4
R. rutilus	18.3	81.2	*S. erythrophthalmus*	20.6	118.2
R. rutilus	18.2	80.3	*S. erythrophthalmus*	19.4	103.2
R. rutilus	16.3	54.2	*S. erythrophthalmus*	19.3	98.8
R. rutilus	20.6	106.6	*S. erythrophthalmus*	16.4	58.2
R. rutilus	19.9	103.7	*S. erythrophthalmus*	15.3	47.2
			S. erythrophthalmus	17.2	70.5

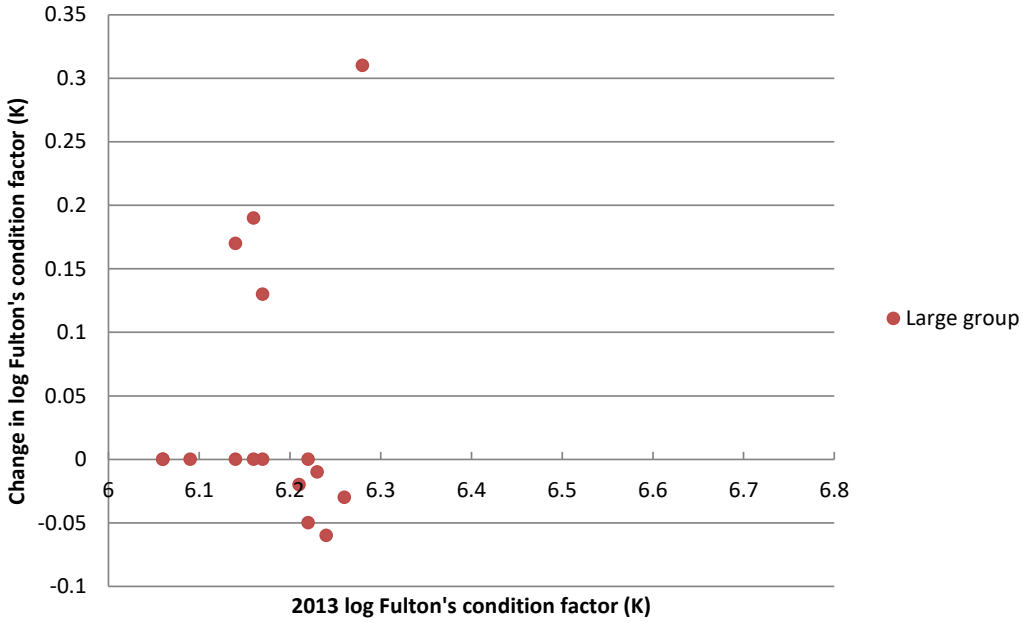

Figure 6A1.1 Change in log Fulton`s condition factor (*K*) between 2013 to 2014 for large *S. glanis* group in ponds in the growth study, (Chapter3).

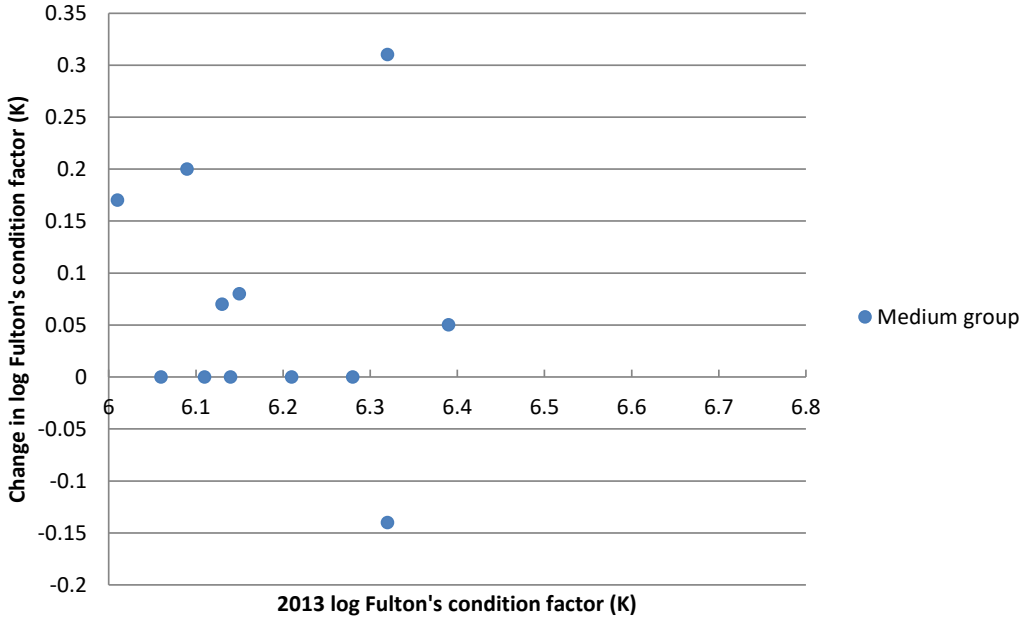

Figure 6A1.2 Change in log Fulton`s condition factor (*K*) between 2013 to 2014 for medium *S. glanis* group in ponds in the growth study, (Chapter3).

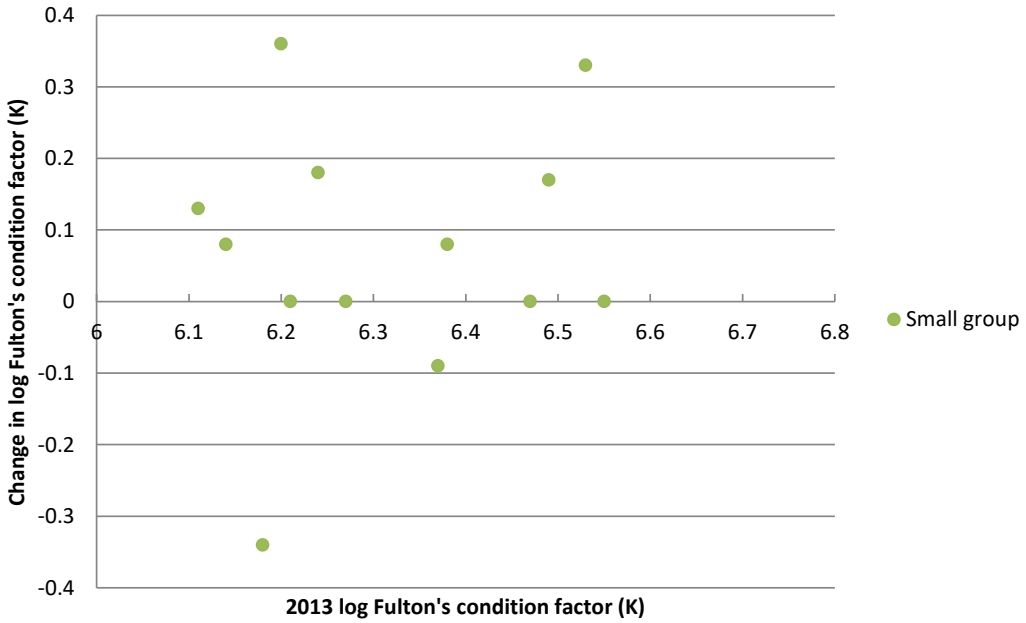

Fig 6A1.3 Change in mean log Fulton`s condition factor (*K*) between 2013 to 2014 for small *S. glanis* group in ponds in the growth study, (Chapter3).

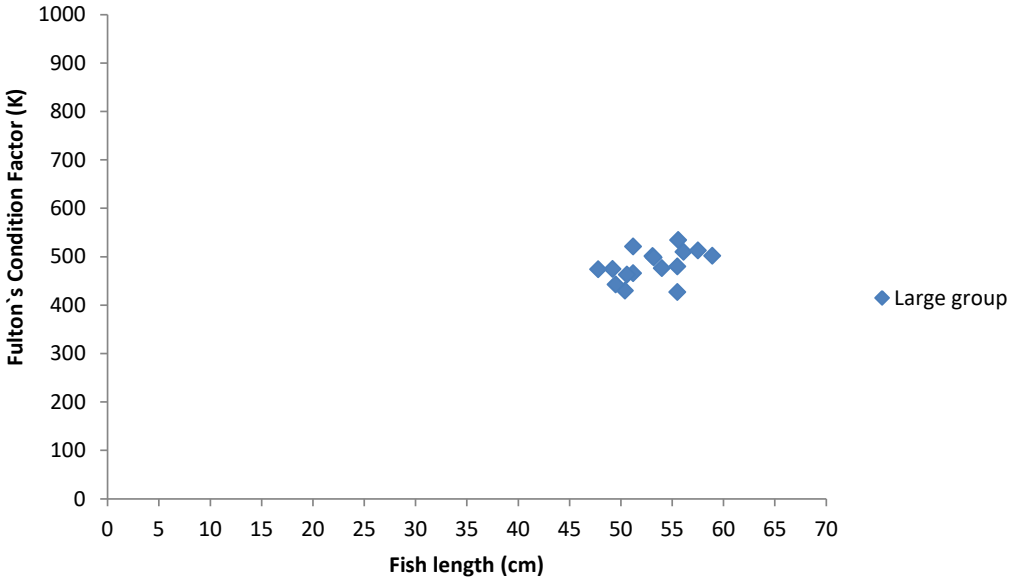

Figure 6A1.4 Variation in mean Fulton`s condition factor (*K*) for large *S. glanis* group in ponds stocked at predator prey ratio 1:2. in the first year growth study in 2013 (Chapter 3).

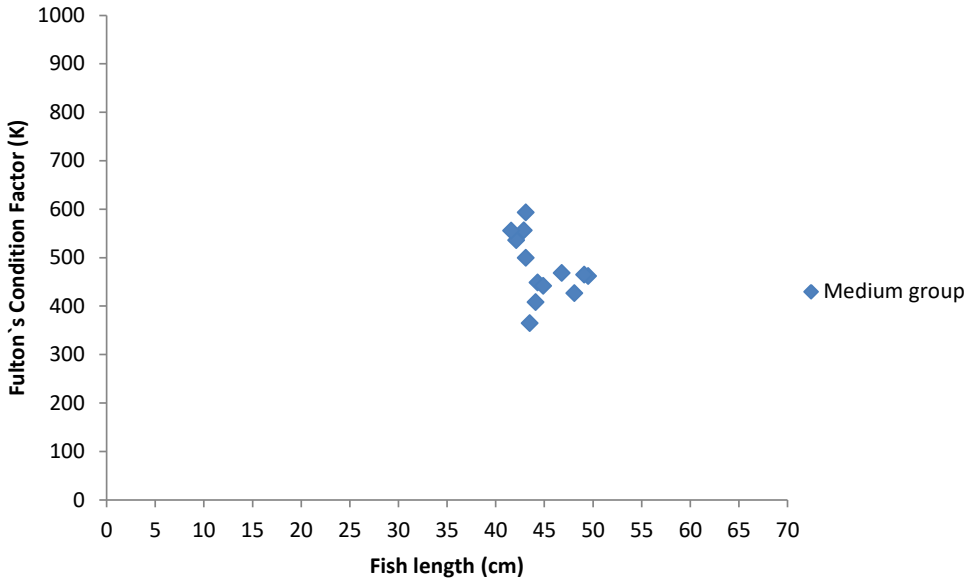

Figure 6A1.5 Variation in mean Fulton`s condition factor (*K*) for medium *S. glanis* group in ponds stocked at predator prey ratio 1:2. in the first year growth study in 2013 (Chapter 3).

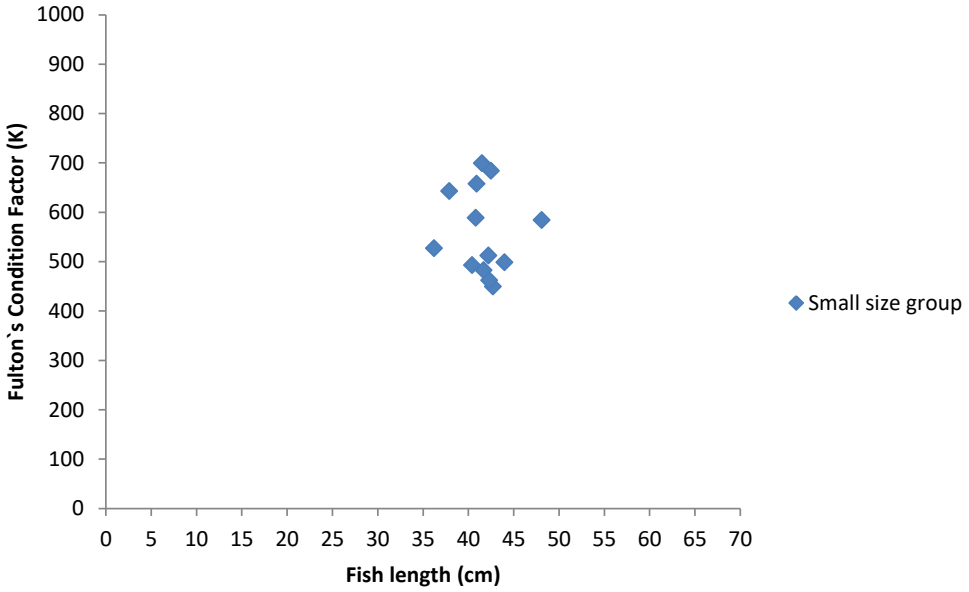

Figure 6A1.6 Variation in mean Fulton`s condition factor (*K*) for small *S. glanis* group in ponds stocked at predator prey ratio 1:2. in the first year growth study in 2013 (Chapter 3).

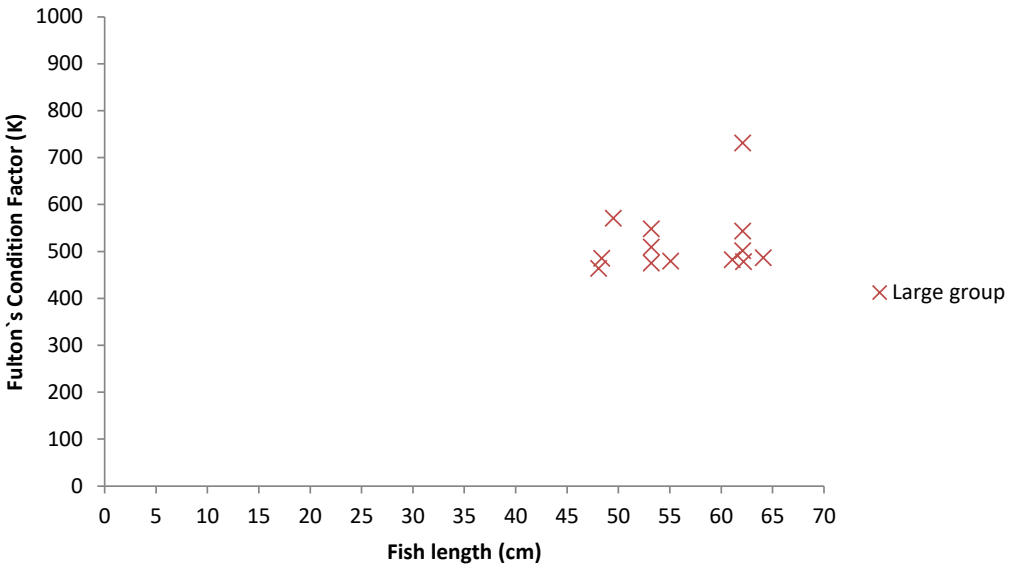

Figure 6A1.7 Variation in mean Fulton`s condition factor (*K*) for large *S. glanis* group in ponds stocked at predator prey ratio 1:3. in the second year growth study in 2014 (Chapter 3).

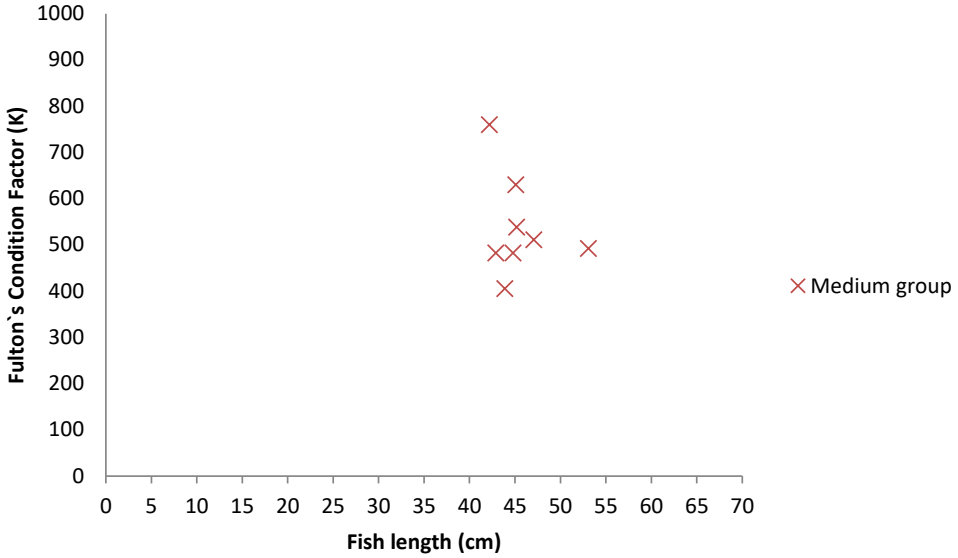

Figure 6A1.8 Variation in mean Fulton`s condition factor (*K*) for medium *S. glanis* group in ponds stocked at predator prey ratio 1:3. in the second year growth study in 2014 (Chapter 3).

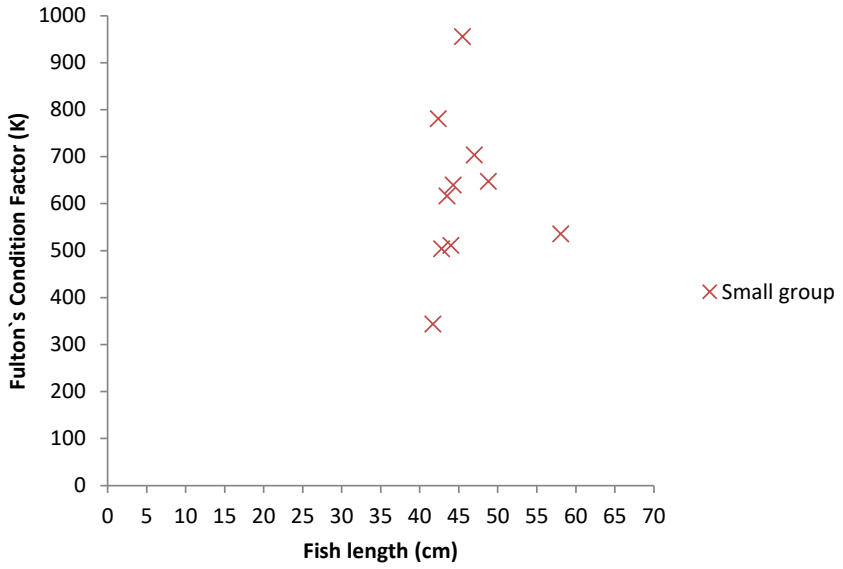

Figure 6A1.9 Variation in mean Fulton`s condition factor (*K*) for small *S. glanis* group in ponds stocked at predator prey ratio 1:3. in the second year growth study in 2014 (Chapter 3).

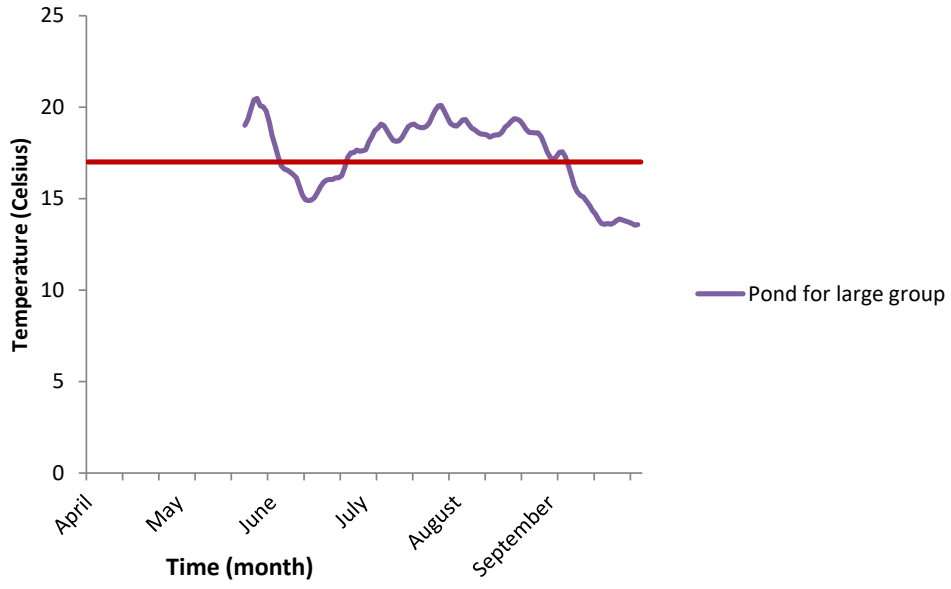

Figure 6A1.10 Variation in mean daily water temperatures in ponds for large *S. glanis* size group during the growing season in 2014, Mayland Essex (Chapter 3).

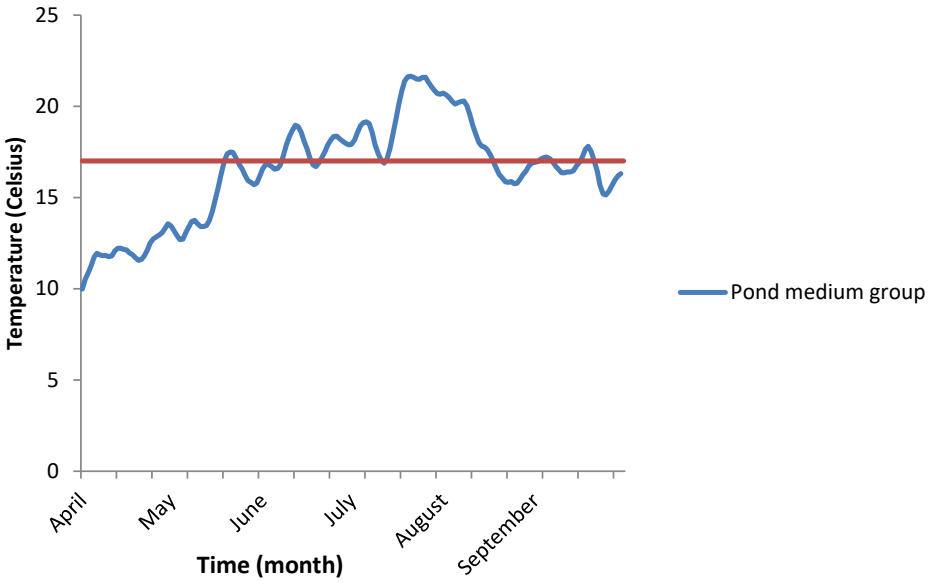

Figure 6A1.11 Variation in mean daily water temperatures in ponds for medium *S. glanis* size group during the growing season in 2014, Mayland, Essex (Chapter 3).

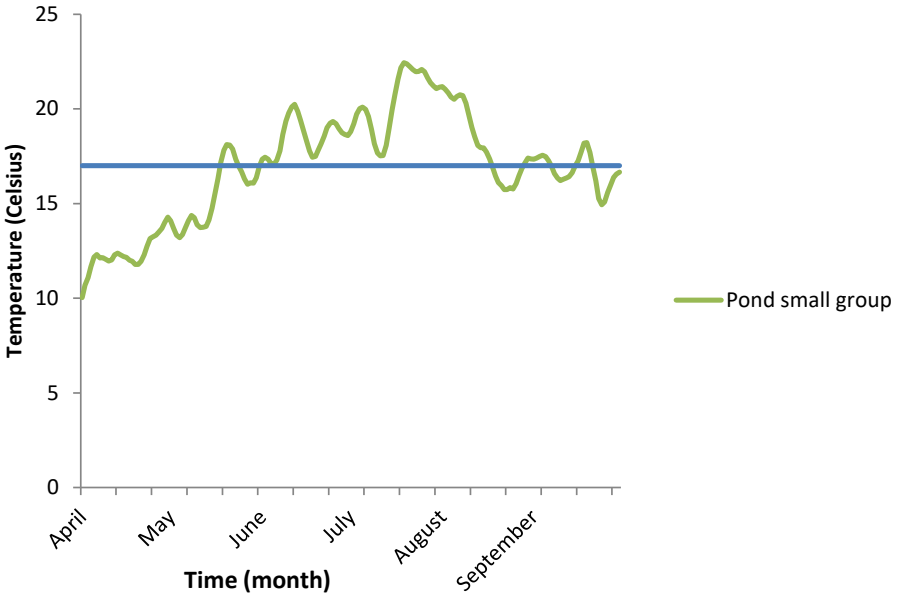

Figure 6A1.12 Variation in mean daily water temperatures in ponds for small *S. glanis* size group during the growing season in 2014, Mayland, Essex (Chapter 3).

Appendix B

Appendix B contains trophic interactions data from Chapter 3, Growth & trophic study

Appendix B

Table 6B1.1 Variation in mean length TL and weight, $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures of large size *S. glanis* group, Mayland Essex, 2014

***S. glanis* characteristics**	**N**	**Mean**	**Minimum**	**Maximum**
Length (cm)	13	56.49 ±1.65	47.80	64.10
Nitrogen isotope value ($\delta^{15}N$)	13	15.06 ±0.13	14.05	15.67
Carbon isotope value ($\delta^{13}C$)	13	-25.83 ± 0.19	-26.75	-24.70
Estimated trophic postion	13	4.37 ± 0.04	4.05	4.55

Table 6B1.2 Variation in mean length TL and weight, $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures of medium size *S. glanis* group, Mayland Essex, 2014

***S. glanis* characteristics**	**N**	**Mean**	**Minimum**	**Maximum**
Length (cm)	8	45.45± 1.22	42.20	53.10
Nitrogen isotope value ($\delta^{15}N$)	8	14.99 ± 0.82	14.65	15.35
Carbon isotope value ($\delta^{13}C$)	8	-26.43 ± 0.18	-27.16	-25.49
Estimated trophic postion	8	4.35 ± 0.02	4.25	4.46

Table 6B1.3 Variation in mean length TL and weight, $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures of small size *S. glanis* group, Mayland Essex, 2014

***S. glanis* characteristics**	**N**	**Mean**	**Minimum**	**Maximum**
Length (cm)	10	45.81± 1.53	41.70	58.10
Nitrogen isotope value ($\delta^{15}N$)	10	14.78 ±0.21	13.06	15.29
Carbon isotope value ($\delta^{13}C$)	10	-26.41±0.35	-28.13	-24.36
Estimated trophic postion	10	4.29 ± 0.06	3.78	4.44

Table 6B1.4 Variability in mean estimated trophic position, nitrogen isotope ($\delta^{15}N$), carbon isotope signatures ($\delta^{13}C$) of cyprinids released into (*S. glanis* size groups) ponds.

Pond	Cyprinidae Species	N	Mean trophic position	Isotopic signature	Isotopic values Mean	Minimum	Maximum
Large group	*B. bjoerkna*	10	3.33± 0.07	$\delta^{15}N$	11.53±0.26	10.34	12.94
				$\delta^{13}C$	-26.06± 0.53	-28.46	-23.85
	R. rutilus	9	3.23± 0.06	$\delta^{15}N$	11.19± 0.21	10.39	12.23
				$\delta^{13}C$	-24.11± 0.47	-27.62	-22.60
	S. erythrophthalmus	3	3.36± 0.10	$\delta^{15}N$	11.60±0.35	11.00	12.22
				$\delta^{13}C$	-24.07± 0.74	-25.53	-23.14
Medium group	*B. bjoerkna*	10	3.56± 0.08	$\delta^{15}N$	12.21± 0.26	10.84	13.28
				$\delta^{13}C$	-24.84 ± 0.21	-26.05	-23.53
	R. rutilus	9	3.44± 0.07	$\delta^{15}N$	11.03±0.15	10.68	12.65
				$\delta^{13}C$	-25.15± 0.40	-26.61	-23.13
	S. erythrophthalmus	5	3.27± 0.10	$\delta^{15}N$	11.31±0.35	10.47	12.10
				$\delta^{13}C$	-24.07±0.80	-26.85	-22.44
Small group	*B. bjoerkna*	17	3.39 ±0.07	$\delta^{15}N$	11.71±0.24	10.20	13.61
				$\delta^{13}C$	-25.03± 0.19	-26.47	-23.67
	R. rutilus	13	3.19 ±0.04	$\delta^{15}N$	11.03±0.17	10.13	11.97
				$\delta^{13}C$	-24.14± 0.14	-25.00	-23.51
	S. erythrophthalmus	6	3.23± 0.14	$\delta^{15}N$	11.19± 0.47	9.52	12.71
				$\delta^{13}C$	-24.27± 0.75	-26.25	-21.89

Table 6B1.5 Variability in mean, minimum and maximum nitrogen isotope signatures ($\delta^{15}N$), carbon isotope signatures ($\delta^{13}C$) of invertebrate trophic groups in (large *S. glanis* size group) pond.

Trophic groups	N	Isotopic signature	Isotopic values Mean	Minimum	Maximum
Algae	3	$\delta^{15}N$	7.16±0.12	6.92	7.29
		$\delta^{13}C$	-33.13±0.03	-33.17	-33.07
Detritivores	3	$\delta^{15}N$	7.42±0.13	7.16	7.61
		$\delta^{13}C$	-29.85±0.55	-30.72	-28.82
Detritus	4	$\delta^{15}N$	7.25±0.14	6.90	7.58
		$\delta^{13}C$	-28.38± 0.23	-29.06	-28.12
Grazers	1	$\delta^{15}N$	8.65	8.65	8.65
		$\delta^{13}C$	-36.19	-36.19	-36.19
Plankton	2	$\delta^{15}N$	7.71±0.01	7.70	7.71
		$\delta^{13}C$	-30.59±0.01	-30.59	-30.58
Predators	2	$\delta^{15}N$	10.97±0.28	10.69	11.25
		$\delta^{13}C$	-34.40±0.23	-34.63	-34.17
Shredder	3	$\delta^{15}N$	10.21±0.38	9.45	10.64
		$\delta^{13}C$	-31.21±0.76	-32.14	-29.71
Zooplankton	3	$\delta^{15}N$	6.96±0.24	6.53	7.37
		$\delta^{13}C$	-32.64± 0.57	-32.75	-32.57

Table 6B1.6 Variability in mean, minimum and maximum nitrogen isotope signatures ($\delta^{15}N$), carbon isotope signatures ($\delta^{13}C$) ± SE of invertebrate trophic groups in (medium *S.glanis* size group) pond.

Trophic groups	N	Isotopic signature	Isotopic values		
			Mean	Minimum	Maximum
Algae	3	$\delta^{15}N$	6.56±0.18	6.22	6.84
		$\delta^{13}C$	-29.25± 1.07	-31.05	-27.36
Detritivores	4	$\delta^{15}N$	5.00± 1.79	-	8.35
		$\delta^{13}C$	-22.28±7.47	-32.00	-
Detritus	4	$\delta^{15}N$	8.32±0.52	7.26	9.53
		$\delta^{13}C$	-29.25±0.69	-30.72	-27.21
Predators	2	$\delta^{15}N$	9.14±0.54	7.77	10.27
		$\delta^{13}C$	-33.81±0.34	-34.56	-32.59
Shredder	1	$\delta^{15}N$	9.45	9.45	9.45
		$\delta^{13}C$	-31.41	31.41	31.41
Zooplankton	6	$\delta^{15}N$	8.76±0.41	7.49	9.64
		$\delta^{13}C$	-35.36±0.60	-36.97	-33.77

Table 6B1.7 Variability in mean, minimum and maximum nitrogen isotope signatures ($\delta^{15}N$), carbon isotope signatures ($\delta^{13}C$) ± SE of invertebrate trophic groups in (small *S. glanis* size group) pond.

Trophic groups	N	Isotopic signature	Isotopic values Mean	Minimum	Maximum
Algae	2	$\delta^{15}N$	6.20±0.50	5.70	6.70
		$\delta^{13}C$	-35.96± 0.59	-36.55	-35.38
Detritivores	2	$\delta^{15}N$	6.86± 0.03	6.83	6.89
		$\delta^{13}C$	-28.76±1.49	-30.25	-27.27
Detritus	3	$\delta^{15}N$	5.02±0.46	4.11	5.50
		$\delta^{13}C$	-30.05± 0.41	-30.72	-29.29
Plankton	2	$\delta^{15}N$	7.83±1.45	6.83	9.28
		$\delta^{13}C$	-31.53±1.36	-32.89	-30.17
Predators	6	$\delta^{15}N$	9.01±0.24	8.40	9.65
		$\delta^{13}C$	-31.75±0.38	-32.59	-29.90
Shredder	1	$\delta^{15}N$	10.36	10.36	10.36
		$\delta^{13}C$	-30.34	-30.34	-30.34
Zooplankton	6	$\delta^{15}N$	8.89±0.18	8.20	9.44
		$\delta^{13}C$	-34.72±0.63	-36.82	-33.15

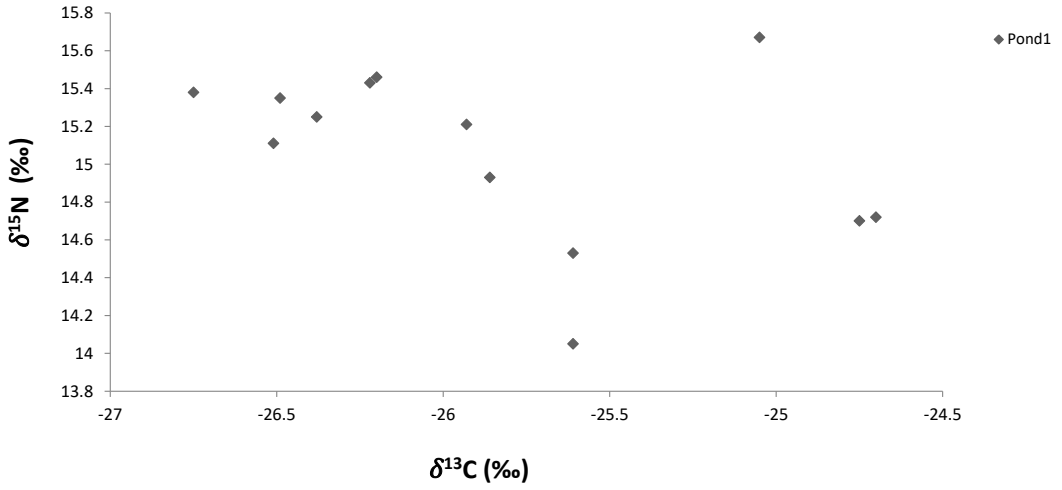

Figure 6B1.1 Variation in mean nitrogen isotope signatures ($\delta^{15}N$) and mean carbon isotope values of large *S. glanis* size group in 2014

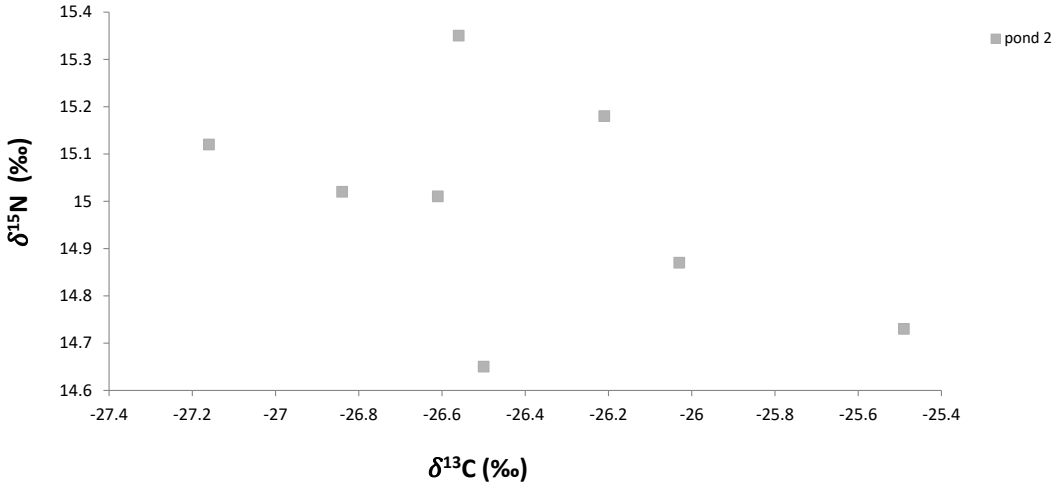

Figure 6B1.2 Variation in mean nitrogen isotope signatures ($\delta^{15}N$) and mean carbon isotope values of medium *S. glanis* size group in 2014

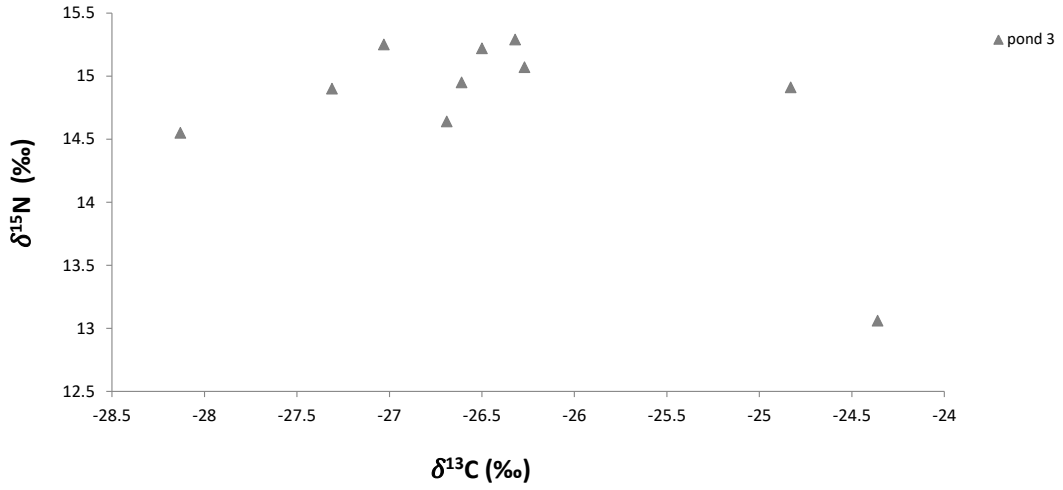

Figure6B1.3 Variation in mean nitrogen isotope signatures ($\delta^{15}N$) and mean carbon isotope values of small *S. glanis* size group in 2014

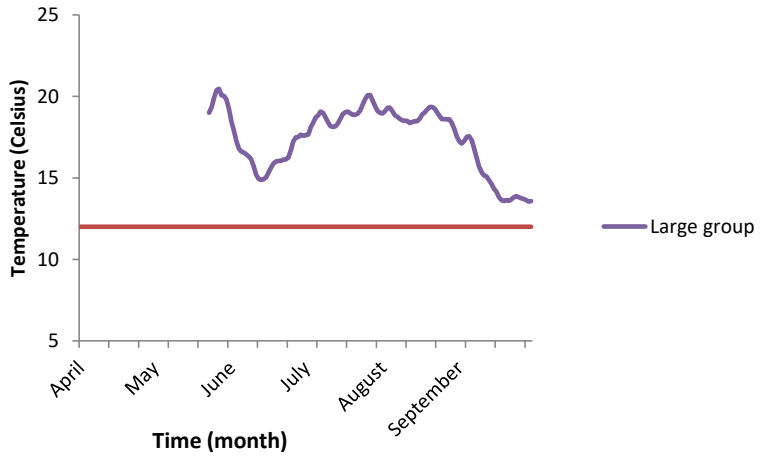

Figure6B1.4 Variation in mean daily water temperatures in pond for large *S. glanis* size group, with 12ºC required for onset of foraging activity in 2014.

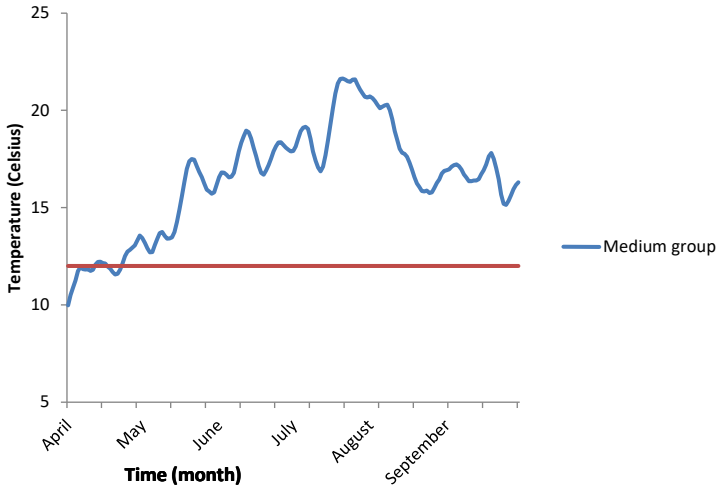

Figure6B1.5 Variation in mean daily water temperatures in pond for medium *S. glanis* size group, with 12ºC required for onset of foraging activity in 2014.

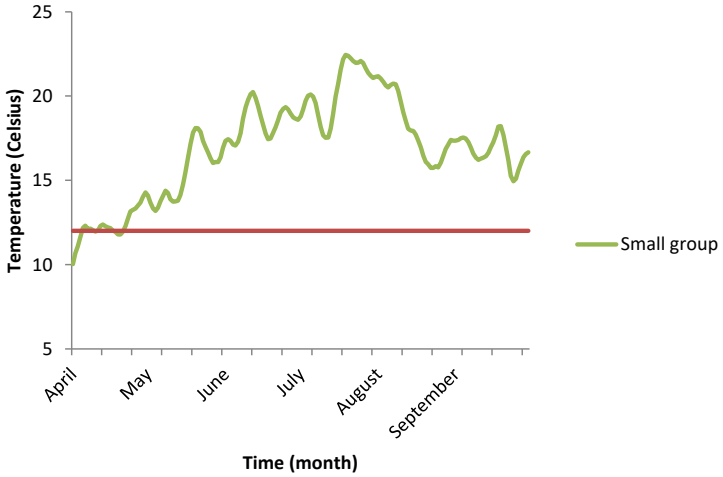

Figure6B1.6 Variation in mean daily water temperatures in pond for small *S. glanis* size group, with 12ºC required for onset of foraging activity in 2014.

Appendix C

Appendix C contains ENSARS data from ENSARS study (Appendix E)

Appendix C

Table 6C1.1 ENSARS Organism risk assessment questions, part A and part B.

Part A: Invasive screening assessment of Organism module
1.1) Is the organism likely to be accompanied by more non-target organisms that are not present but that could persist in the risk assessed area? 1.2)Is the climate of the organism's native range similar to RA area to facilitate establishment? 1.3)Is there a habitat or host suitable for the survival of the organism occur in the RA area? 1.4) Does at least one essential habitat or host (necessary for the organism to persist and to complete its life cycle) occur in the RA area? 1.5) Is the organism an infectious agent? 1.6) Is the organism a fish, invertebrate or Amphibian? 1.7) Did the invasiveness pre-screening tool indicate the organism is potentially of medium or high risk of being invasive or harmful? **PARTB: Detailed assessment** 1.8) From outcome of Pathways module: what is the overall risk of escape of the organism into the wild during import procedures? 1.9) Using the outcome of the Pathways Module: what is the overall risk of escape of the organism into the wild during farming procedures? 1.10) Using the outcome of the Pathways Module: what is the overall risk of escape of the organism due to destination/uses of farmed non-native organisms? 1.11) Using the outcome of the Facility Module: indicate the likelihood of target organisms escaping from any of the facilities involved in its production. 1.12) Using the outcome of the Facility Module: indicate the likelihood of non-target organisms (other than infectious agents) escaping from any of the facilities involved in its production. 1.13) Using the outcome of the Facility Module: indicate the likelihood of non-target infectious agents escaping from any of the facilities involved in its production.

Table 6C1.2 ENSARS Organism: risks of establishment questions in assessment

Risks of establishment assessment in Organism module
1.1) How similar are climatic conditions affecting establishment in the RA area and in the area of current distribution? 1.2) How similar are other abiotic factors that would affect establishment in the RA area and in the area of present distribution ? 1.3) What proportion of the habitats, hosts, or partners (for symbiotic taxa) vital for the survival, development and reproduction of the organism are present in the RA area? 1.4) How widespread are the habitats, hosts, or partners (for symbiotic taxa) vital for the survival, development and reproduction of the organism in the RA area ? 1.5) If the organism requires a host or symbiotic partner, then how likely is the organism to become associated with such species in the RA area? 1.6) How likely is competition (with existing species in the RA area) to prevent the organism's establishment in the RA area? 1.7) How likely is predation/foraging (by existing organisms in the RA area) to prevent the organism's establishment in the RA area? 1.8) How likely is existing environmental management in the RA area to aid establishment? 1.9) How likely is it that existing control or husbandry measures (e.g. use of triploids) will fail to prevent establishment of the organism? 1.10) How widely distributed is the intended use of the organism in the RA area in either closed or open systems? 1.11) How likely is establishment to be facilitated by the organism's reproductive strategy or life-cycle duration? 1.11) How likely is establishment facilitated by the organism's natural capacity disperse 1.12) How adaptable is the organism? 1.13) How likely is low genetic diversity of the founder population to be a constraining factor in the organism's establishment of a self-sustaining, persistent population? (1.14) How often has the organism established self-sustaining populations outside its original range as a result of man's activities? 1.15) How likely is the organism to resist existing infectious agents in the RA area? 1.16) Even if establishment of the organism is unlikely, how likely is it that transient populations (casuals) will persist in the RA area?

Table 6C1.3 ENSARS Organism: risks of dispersal questions in assessment

Risks of dispersal assessment in Organism module
1.1) How rapidly is the organism likely to disperse in the RA area by natural means? 1.2) How rapidly is the organism likely to disperse in the RA area with human assistance? 1.3) How difficult would it be to contain/control the organism within the RA area? 1.4) Based on the answers to questions on the potential for establishment and spread, how wide/important is the area threatened by the organism within the RA area?

Table 6C1.4 ENSARS Organism: risks of impact questions in assessment.

Risks of impact assessment
1.1) Using the outcome of the Socio-economic Impact Assessment Module: indicate the likely level of economic costs to eradicate an infestation by the organism from the RA area. 1.2) Using the outcome of the Socio-economic Impact Assessment Module: indicate the likely level of economic losses incurred to local economies should the organism escape captivity and become a pest in the RA area. 1.3) Using the outcome of the Socio-economic Impact Assessment Module: Please indicate the likely level of economic losses incurred to wider /national/EU economies should the organism escape captivity and become a pest in the RA area. 1.4) How likely are consignments of organism contain non-target (non-infectious) organisms? 1.5) What is the magnitude of threat posed by non-target (non-infectious) organism(s)? 1.6) Using the outcome of the Infectious Agent Risk Assessment Module: Please indicate how likely is the target organism to be a susceptible species for infectious agents or act as a vector of infectious agents? 1.7) Indicate the likelihood of the non-target infectious agent establishing in the RA area. 1.8) If infectious agents have been identified, then indicate the likelihood of the non-target infectious agent dispersing in the RA area. 1.9) If infectious agents have been identified, indicate the likely magnitude of harm posed by the non-target, infectious agents? 1.10) Indicate the level of harm in the species diversity by the organism in areas where it has already escaped captivity. 1.11) Indicate the level of harm in ecosystem function by the organism in areas where it has already escaped captivity? 1.12) Indicate the likely level of harm in the species diversity if the organism were to escape captivity (or be released into) the RA area. 1.13) Indicate the likely level of harm to ecosystem function if the organism escaped captivity (or released into RA area? 1.14) How likely is the organism would adversely impact ecosystem services in the RA area? 1.15) How likely is organism have an adverse impact on the gene pool of native species? 1.16) How likely is it that management measures (to control the organism) will have adverse impacts on non-target organisms in the recipient ecosystems? 1.17) Indicate how widely the ecosystems at risk in the RA are to be impacted .

Table 6C1.5 ENSARS Organism risk summary of introduction, establishment, dispersal and impact risks in module.

Overall summary of 4 sections of Organism module
Summarise introduction (entry) risks Summarise establishment risks Summarise dispersal risks Summarise risks of impacts

Table 6C1.6 ENSARS Facility risk assessment part A for facility, target species and fishery management.

Part A Facility, target species & management
1.1) What type of facility is being assessed? 1.2) What non-native taxon/taxa (target species) will be reared at the facility? 1.3) How many taxa (target species) will be reared simultaneously? 1.4) What life stages(s) will be reared at the facility? 1.5) How precise is the written procedure for running the facility? 1.6) How accurate and precise are the records of activities at the facility? 1.7) How accurate and precise are the records of goods and services at the facility? 1.8) Is there a maintenance plan for all equipment? 1.9) If there is a treatment system, then what is the level of training of personnel authorised to use the treatment system? (1.10) Is there a fail-safe back-up system for treatment of effluent, solid waste and dead animals? (1.11) What is the efficacy of the contingency plan in case of accidental effluent discharge without treatment? (1.12) What is the magnitude (i.e. volume) of effluent will be produced by the facility? (1.13) Overall, how effective is the quality management system? (1.14) What is the magnitude (i.e. volume) of effluent will be produced by the facility?

Table 6C1.7 ENSARS Facility risk assessment questions for Part B risk of unintentional release of target organism from facility.

Part B Risk of unintentional release of target organisms from facility
(1.1) What is the effectiveness of mechanisms (e.g. gates, screens, meshes) aimed at preventing the unintentional release of target organisms? (1.2) How frequently will live target organisms be transported to and from the facility? (1.3) What is the likelihood of live target organisms (or their propagules) escaping the facility in the effluent? (1.4) What is the likelihood of live target organisms (or their propagules) escaping the facility in the solid waste (i.e. waste products, excess food, dead organisms, etc.)? (1.5) How vulnerable is the facility to environmental, climatic and/or geological perturbations (e.g. storms, floods, sea-level rise, earthquakes)? (1.6) Overall, what is the likelihood of unintentional release of target organisms from the facility?

Table 6C1.8. ENSARS Facility risk assessment questions for part C risks of unintentional release of non-target organisms from the facility.

Part C Risk of unintentional release of non-target organisms from the facility
(1.2) How frequently are the mechanisms checked and maintained? (1.3) How frequently will live or dead target organisms be transported to and from the facility? (1.4) How frequently is the facility inspected for non-target organisms? (1.5) How frequently is the facility cleaned/disinfected/drained/emptied. (1.6) How effective is the quarantine procedure/structure present at the facility. (1.7) What is the likelihood of live non-target organisms (or their propagules) escaping the facility in the effluent. (1.8) What is the likelihood of live non-target organisms (or their propagules) escaping the facility in the solid waste (i.e. waste products, excess food, dead organisms, etc.). (1.9) How vulnerable is the facility to environmental, climatic and/or geological perturbations (e.g. storms, floods, sea-level rise, earthquakes). (1.10) How likely are non-target organisms to reproduce in the facility? (1.11)Summarise the overall likelihood of non-target (non-infectious) organisms escaping the facility. (1.12) Summarise the overall likelihood of a non-target infectious agents escaping the facility.

Table 6C1.9 ENSARS Pathway risk assessment module with part A import procedures, part B farming procedures and part C destination use questions in assessment.

Part A Import procedures
(1.1) From how many geographical sources could the organism be introduced? (1.2) What is the frequency of introduction of the organism? (1.3) What is the magnitude (i.e. tonnes/year or individual/year) of the total transfer of the organism along all its pathways of introduction? (1.4) How long is the transit time of the organism during import procedures? (1.5)What risk of release of the target and non-target organisms in the transfer procedures? (1.6) What likelihood of the organism reaching the RA area by natural range expansion or secondary introduction? (1.7)What likelihood that the organism will be imported during its reproductive season? (1.8) What is the risk level with potential escape with any existing procedures or mitigation actions that may prevent an accidental introduction of the target and its associated non-target organisms into the wild during import? (1.9) What overall risk of spreading of the organism into the wild during import procedures? **Part B Farming procedures** (1.10) How complex is the farming process of the organism? (1.11) What overall risk of spread of the organism into the wild during farming procedures? **Part C Destination final use** (1.12) How many final destinations/uses (e.g. food market; ornamental, stocking; biocontrol; research; social) does the organism have in the RA area? (1.13) How likely the major destination/use of the organism to be an effective pathway of introduction into the wild? (1.14) What is the level of national enforcement of regulations concerning deliberate release of non-native organisms into the wild? (1.15) What is the level of public awareness in the country of introduction regarding non-native organisms? (1.16) How likely is a release of the organism into the wild due to human activities? (1.17) What the overall risk of dispersal of the organism due to destination/uses of farmed non-native organisms? (1.18) What is the overall risk of dispersal of the organism due to destination/uses of farmed non-native organisms?

Table 6C1.10 Pathway summary module and overall summary for import, farming and destination use sections in assessment.

Pathway summary
Summarise overall risk of escape of the organism into the wild during import procedures? Summarise overall risk of escape of the organism into the wild during farming procedures? Summarise overall risk of escape of the organism due to destination or uses of farmed non-native organisms? Likelihood of escape by the organism after the farming phase has been completed

Table 6C1.11 ENSARS Infectious agent risk assessment questions for introduction of infectious agent into risk assessed area.

Introduction of infectious agent into risk assessed area
(1.1) How often has the infectious agent entered and established in new areas outside its original range as a result of man's activities? (1.2) How widespread is the infectious agent in the exporting country? (1.3) How likely is the infectious agent to be present at the location where the target organism is sourced? (1.4) How likely is the infectious agent to be present in the exported animals? (1.5) How likely is the infectious agent to exist in a sub-clinical or latent state in the target organism? (1.6) Is the infectious agent 'notifiable' in the exporting country? (1.7) How likely is vaccination against the infectious agent to be practised at the exporting site? (1.8) How reliable are the diagnostic tests?

Table 6C1.12 Infectious agent risk assessment questions for risks of establishment of infectious agent into risk assessed area.

Risks of establishment of infectious agent into risk assessed area
(1.1) Does at least one host species for the infectious agent exist in the RA area? (1.2) How many known host species exist in the RA area (in the wild and/ or in farms?) (1.3) Does the infectious agent need an intermediate host to complete its lifecycle? (1.4) How abundant are the intermediate host(s) in the RA area? (1.5) If the infectious agent has an intermediate host, how likely is it to become associated with such organisms at the site of introduction? (1.6) How likely is it that the water temperatures in the RA area will be conducive to establishment of the infectious agent? (1.7) How likely is it that the target organism (or non-target organisms) will excrete the pathogen/shed the parasite at the site of introduction? (1.8) How likely is it that excretion of the infectious agent will result in its establishment in the RA area (i.e. on average more than one new infection per infected animal?)

Table 6C1.13 Risks of spread of infectious agent into the risk assessed area questions in assessment.

Risks of spread of infectious agent into risk assessed area
(1.1) How widespread is the host organism in the RA area? (1.2) How abundant is the host organism in areas where it is present? (1.3) How widespread are the intermediate host organisms in the RA area? (1.4) How likely is the infectious agent to be rapidly detected? (1.5) How frequent are human movements of host or intermediate host species between river catchments in the RA area? (1.6) How long can the infectious agent survive off the host in the aquatic environment? (1.7) How long can the infectious agent survive desiccation? (1.8) How important is the mechanical spread of free-living infectious agent between drainage basins in its natural range? (1.10) How rapidly on average has the infectious agent spread when introduced into new areas?

Table 6C1.14 Risks of impact for infectious agent into the risk assessed area questions in assessment.

Risks of impacts of infectious agent into risk assessed area
(1.1) How likely is it that the infectious agent is a potential threat to human health? (1.2) How important is environmental harm caused by the infectious agent (through impact on wild aquatic animal populations) within its existing geographic range? (1.3) How easily can the infectious agent be controlled? (1.4) How likely is it that management measures (to control the infectious agent) will have adverse impacts on non-target organisms in the recipient ecosystems? (1.5) Using Socio-economic impact outcome: indicate the likely magnitude of economic losses to local economies should infectious agent escape captivity and become a pest in the RA area. (1.6) Using Socio-economic impact outcome: indicate likely magnitude of economic costs to eradicate an infestation by the infectious agent from the RA area. (1.7) Using Socio-economic impact outcome: indicate likely magnitude of economic losses to wider national economies should infectious agent escape and become a pest in the RA area. (1.8) How widespread in the RA area the economic and environmental impacts may occur?

Table 6C1.15 Infectious agent module for overall summary for introduction, establishment, spread and impact of infectious agent into risk assessed area.

Overall summary of 4 sections of Infectious agent risk assessment module
Summarise likelihood of target organism as a vector of infectious agent dispersal into RA area Summarise likelihood of the non-target infectious agent establishing in the RA area Summarise likelihood of the non-target infectious agent dispersing in the RA area Summarise magnitude of harm posed by non-target, infectious agent into RA area

Table 6C1.16 ENSARS Socio-economic impact part A assessment and market impacts of the risk assessed area questions in the module.

Part A Market socio-economic impacts
(1.1) What is the magnitude of economic loss from direct market/commercial impacts caused by the organism within its introduced geographic range? (1.2) How significant are such losses? (1.3) What is the likely magnitude of potential economic loss from direct market/commercial impacts caused by the organism within the RA Area? (1.4) How significant are such losses likely to be? (1.5) What is the magnitude of economic loss from indirect market/commercial impacts caused by the organism within its introduced geographic range? (1.6) How significant are such losses? (1.7) What is the likely magnitude of potential economic loss from indirect market/commercial impacts caused by the organism within the RA Area? (1.8) How significant are such losses likely to be?

Table 6C1.17 Risks of Socio-economic impact part B assessment questions related to eradication costs to the risk assessed area.

Part B Eradication costs
(1.1) Estimate the magnitude of costs for surveys or surveillance for eradication attempt. (1.2) Estimate the magnitude of the cost for containment during an eradication attempt (1.3) Estimate the magnitude of the cost of treatment for eradication. (1.4) Estimate the magnitude of the cost to verify eradication. (1.5) What is the likely magnitude of eradication costs on producer's profits? (1.6) How significant are eradication costs likely to be?

Table 6C1.18 Risks of Socio-economic impact part C assessment questions, related to local or national scale economic impacts to the risk assessed area.

Part C Impacts at a wider local or national scale
If eradication is not feasible with costs are unacceptable or otherwise, what is the likely magnitude of costs to "manage" the introduced species on a non-statutory basis, i.e. deal with it as a domestic "pest"? How significant are such costs likely to be? How great a change in commodity prices is the organism likely to cause in the RA area? How likely is the presence of the organism in the RA area to cause market losses? What is the magnitude/value of such export markets? What is the magnitude of social harm caused by the organism within its introduced geographic range? What is the magnitude of social harm likely to be in the RA Area? What is the magnitude of other economic costs resulting from introduction likely to be in the RA Area? How significant are such costs likely to be?

Table 6C1.19 Socio-economic risk assessment overall summary of market impacts, eradication costs, local and national impacts to risk assessed area in the module.

Overall summary of 3 sections of Socio-economic risk assessment module
Summarise likelihood of market socio-economic impacts to the RA area Summarise likelihood of eradication costs to the RA area Summarise likelihood of impact at local and national scale to the RA area

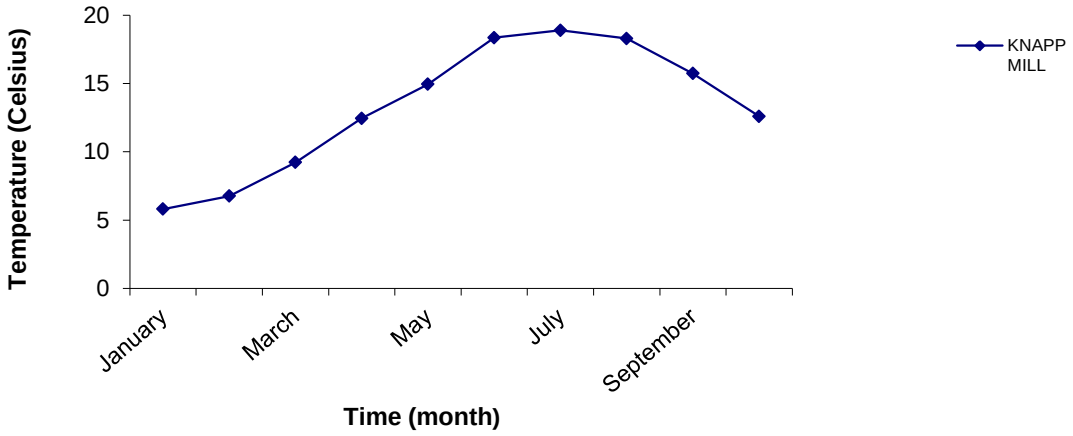

Figure C61.1 Variation in water temperature at Knapp Mill site of the River Hampshire Avon in 2009 in the ENSARS study.

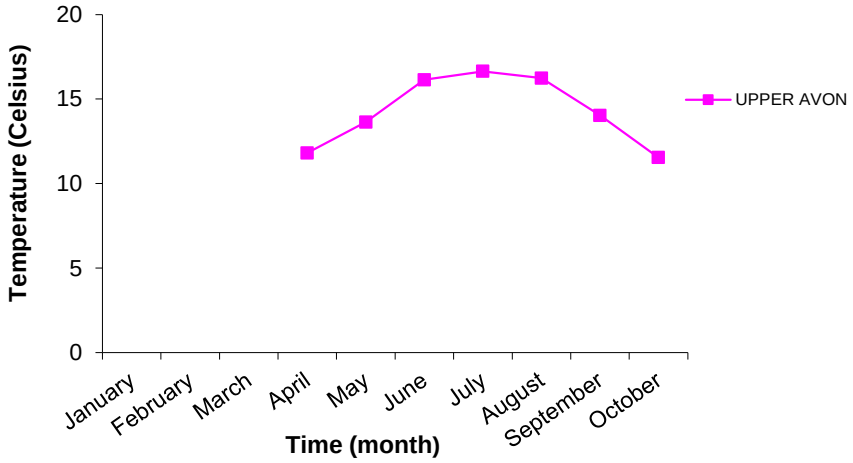

Figure C61.2 Variation in water temperature at Upper Avon site of the River Hampshire Avon in 2009 in the ENSARS study.

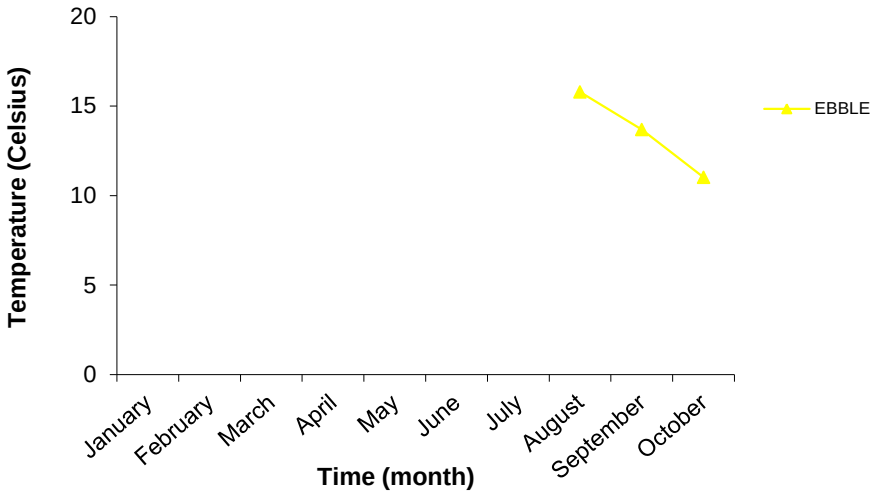

Figure C61.3 Variation in water temperature at Ebble site of the River Hampshire Avon in 2009 in the ENSARS study.

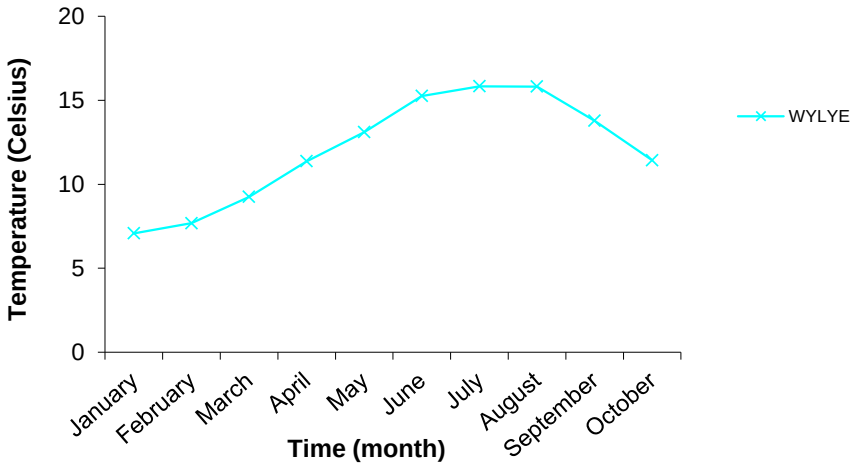

Figure C61.4 Variation in water temperature at Wylye site of the River Hampshire Avon in 2009 in the ENSARS study.

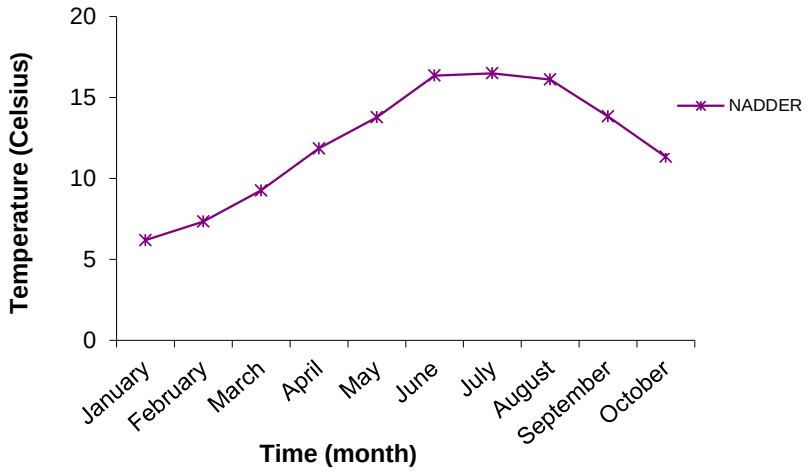

Figure C61.5 Variation in water temperature at Nadder of the River Hampshire Avon in 2009 in the ENSARS study.

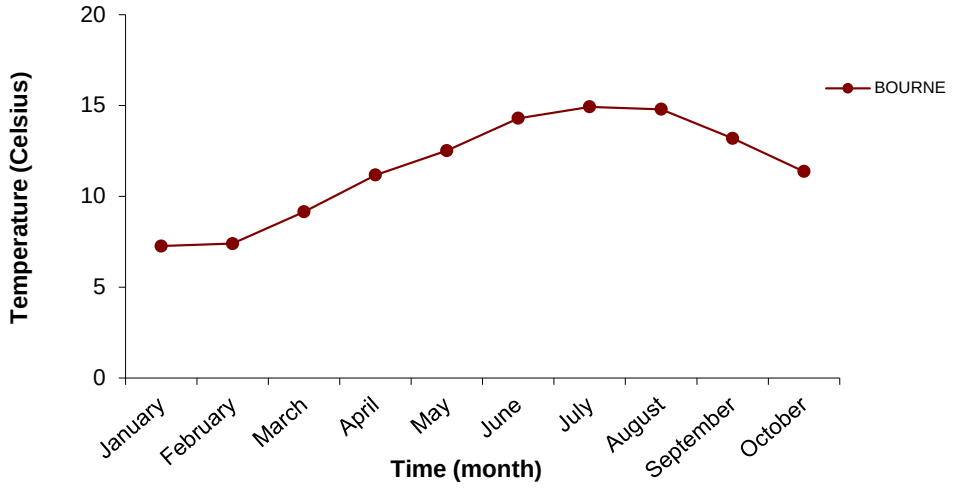

Figure C61.6 Variation in water temperature at Bourne site of the River Hampshire Avon in 2009 in the ENSARS study.

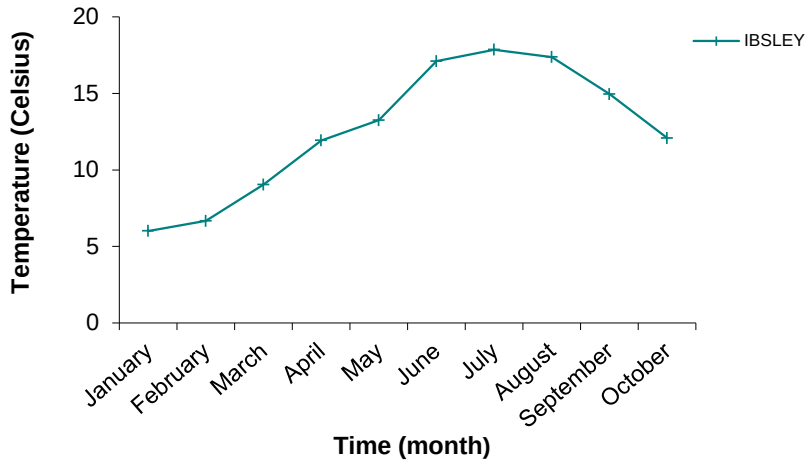

Figure C61.7 Variation in water temperature at Ibsley site of the River Hampshire Avon in 2009 in the ENSARS study.

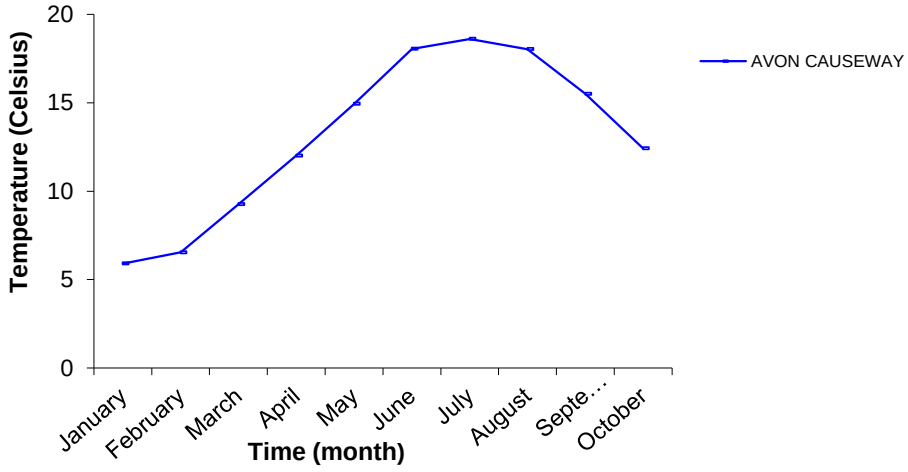

Figure C61.8 Variation in water temperature at Avon Causeway site of the River Hampshire Avon in 2009 in the ENSARS study.

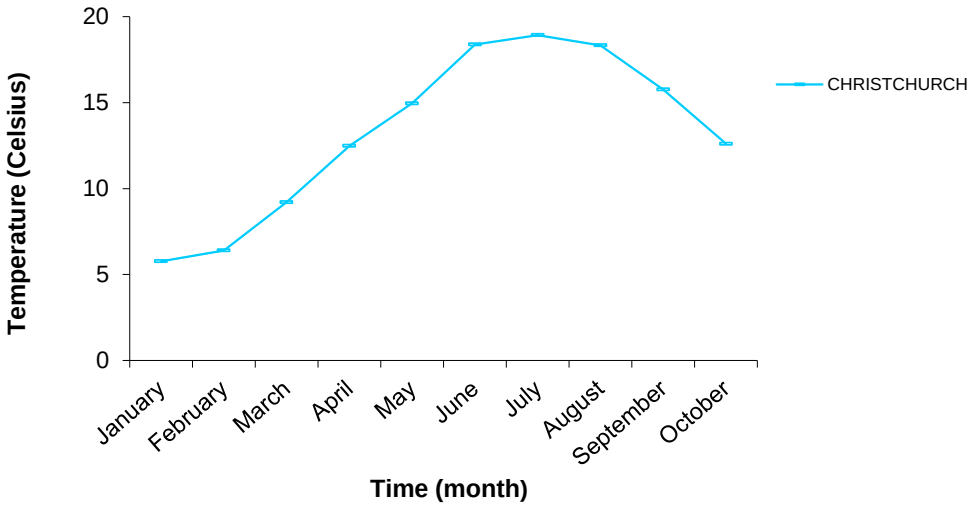

Figure C61.9 Variation in water temperature at Christchurch site of the River Hampshire Avon in 2009 in the ENSARS study.

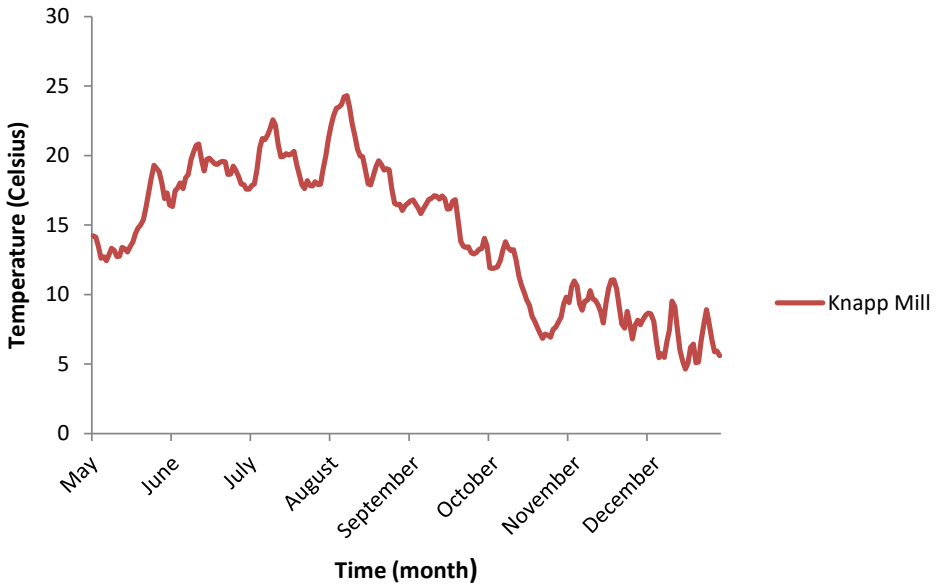

Figure C61.10 Variation in water temperature at Knapp Mill of the River Hampshire Avon in 2007 in the ENSARS study.

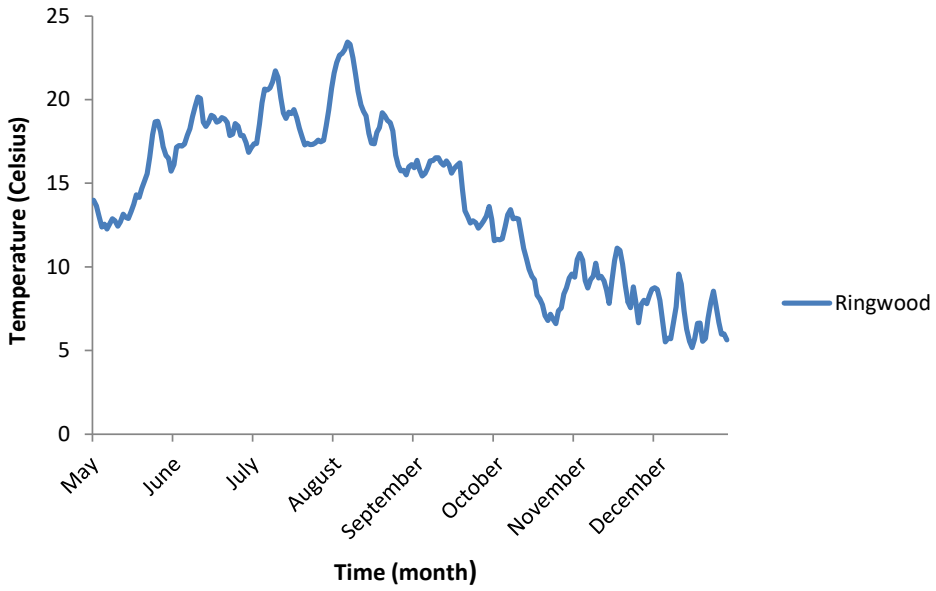

Figure C61.11 Variation in water temperature at Ringwood of the River Hampshire Avon in 2007 in the ENSARS study.

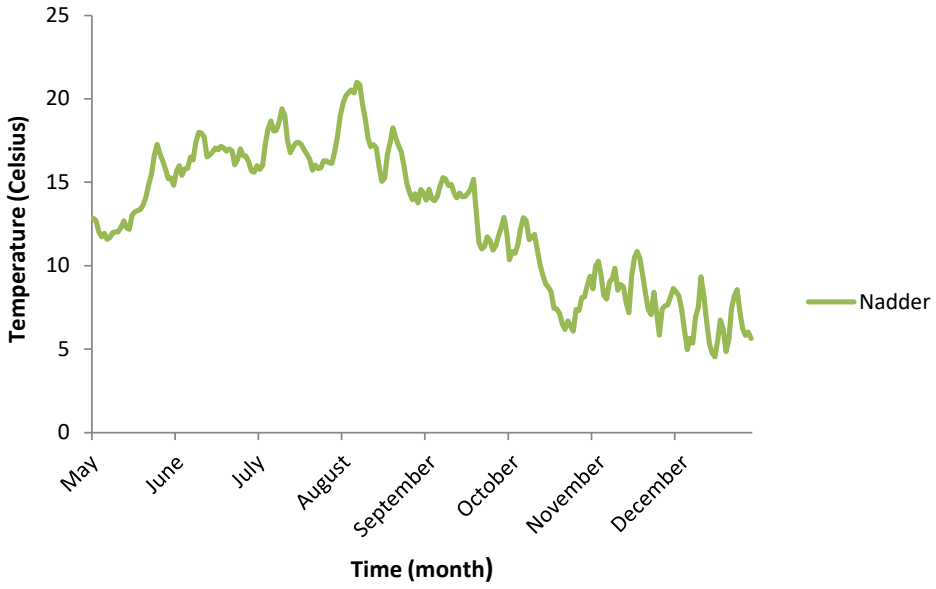

Figure C61.12 Variation in water temperature at Nadder site of the River Hampshire Avon in 2007 in the ENSARS study.

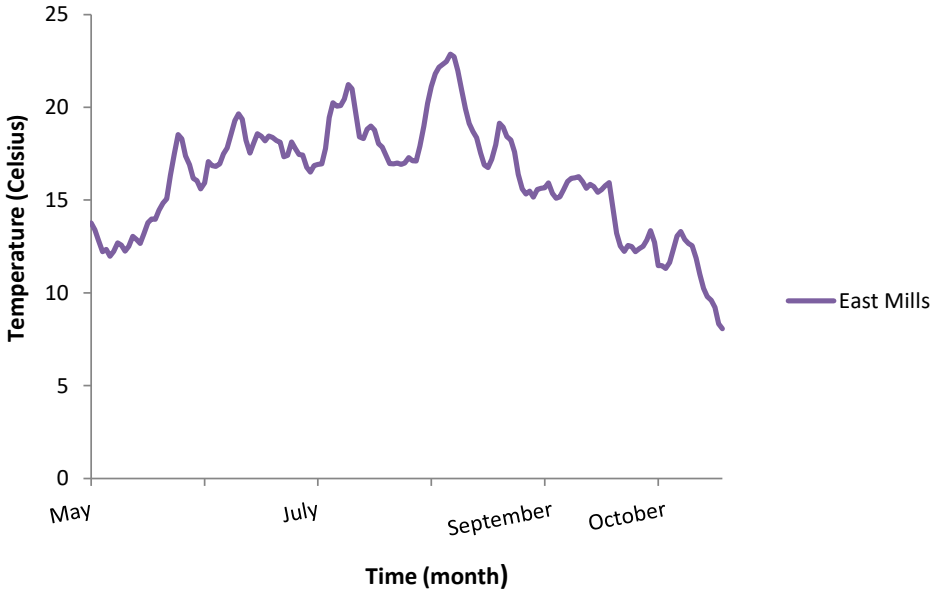

Figure C61.13 Variation in water temperature at East Mills of the River Hampshire Avon in 2007 in the ENSARS study.

Appendix D

Appendix D contains information about glossary of terms used in study and angling questionnaire about *S. glanis* specialist angling study in the UK, parts of which were used in this thesis (Rees et al. 2017).

Appendix D

Table D61.1 Glossary of terms used in study

Terminology	Meaning
Non-native (alien, exotic, introduced)	Transfer of a species outside of it's native range by human activities
Invasive species	A non-native species that has become established and poses ecological and socio-economic impacts potentially a risk to native species
Establishment	A process where a non-native species is able to reproduce independent sustaining populations into a new environment
Dispersal	A natural process involving movement of a species to new environment without human assistance
Lag phase	A time period of suspended invasion for a non-native species already introduced into new environment
Stable isotope analysis	The identification of natural occurring chemical element isotopes such as hydrogen, oxygen, nitrogen, carbon and sulphur using isotopic signatures to trace ecological processes in food web and ecosystems
Trophic interactions	To trace foraging impacts between predator and prey organisms in food webs using elemental isotopes signatures and feedback processes such as carbon and nitrogen cycle in food web
Omnivorous putative prey	Prey from a variety of plant and animal sources in the foodweb
Carbon isotope ($\delta^{13}C$) and Nitrogen isotope ($\delta^{15}N$) in stable isotope analysis with non-native species	The stable isotope analysis of the light elements (carbon-13, nitrogen-15) using isotope ratio mass spectrometry of animal tissue samples in trophic ecology to determine interactions between introduced predator and prey species in food web

Terminology	Meaning
Mean $\delta^{15}N$ and $\delta^{13}C$ signatures	The ratio of heavy and light isotopes of nitrogen and carbon react differently in chemical reactions of trophic processes which via isotopic fractionation can be used to determine ecological tracers ($\delta^{15}N$ and $\delta^{13}C$ signatures) of an organism in food web
Baseline isotopic signatures	The isotopic signatures such as ($\delta^{15}N$ and $\delta^{13}C$ signatures) of an organism that are primary consumers in the food web that capture isotopic variation at the base of food webs
Baseline indicator species	These are species which are primary consumers at the base of food web that allow comparison of isotopic signatures with organisms higher up in the food chain such as secondary consumers
Estimated trophic position	The estimation of the position an organism occupies in the food web. The estimated trophic position of an organism can be calculated by its mean $\delta^{15}N$ signature value with that of a baseline indicator species using mathematical formulae from Vander Zanden & Rasmussen; 1996, Vander Zanden et al.1997; Grey, 2006).
Estimated mean trophic position	Estimated mean trophic position of an organism are calculated from mathematical formulae from Vander Zanden & Rasmussen; 1996, Vander Zanden et al.1997; Grey, 2006). Mean $\delta^{15}N$ signature values of an organism are calculated because of variation in $\delta^{15}N$ isotopic signatures
Chironomidae	Chironomidae are primary consumers widespread in food web. They are generally used as a baseline indicator species in estimating mean trophic position of secondary consumers (fish)

Table D61.2 Questionnaire (a) used in *Silurus glanis* specialist angling study (Rees et al. 2017).

Questions for respondants in the study
Your gender?
Your age? (yr)
Your marital status?
How many members in your household?
What region do you live?
What is your level of education?
What is your employment status?
What is your monthly income (£)?
What are your annual expenses spent on equipment & bait for specialist European catfish angling (£)?
What are your annual expenses spent on travel to specialist European catfish angling lakes (£)?
What are your annual expenses spent on fishing licence, membership fee or day tickets at specialist European catfish lakes (£)?
How many specialist European catfish angling trips do you spend per year?
How long have you been a general angler (in years)?
How long have you been a specialist European catfish angler (in years)?
How far is the average distance from your home to a specialist European catfish angling lake (in miles)?
How long do you spend on average in specialist European catfish angling from your arrival to departure time at the fishery?
How many days do you spend on specialist European catfish angling in a year?
How many hours do you actively spend in specialist European catfish angling at a specimen lake?
Do you think there are any adverse risks in high fish stocking density in this European catfish specimen lake?
What is your view of bait allowed in recreational fishing?
What is your preferable catch size of European catfish when specialist European catfish angling?
How well are you informed about ecological concerns and risks of European catfish by media, angling and environmental organisations e.g. CEFAS, Environment Agency, wildlife trusts, angling in events, training, newsletters & meetings?

Table D61.3 Questionnaire (b) used in *S.glanis* specialist angling study (Rees et al. 2017).

Questions for respondants in the study
Please rank in importance the different motivations you have in specialist European catfish angling:
To be relaxed, enjoy nature & tranquillity?
To escape from every day life & be alone?
To socialise with family?
To enjoy angling as a social gathering with friends?
To experience adventure & excitement?
To experience a sense of accomplishment and as a sport?
To chase or catch a personal trophy fish & experience a fighting fish?
To improve angling skills & catch fish?
To learn new angling techniques?
To enjoy angling with a partner?
Are there any factors that influence specialist European catfish angling such as challenge & catchability of these fish?
Is the size of European catfish in angling important? e.g. large size more than 60lbs?
Do you have any knowledge of non-native fish legislation concerning European catfish?
Are you aware of any ecological risks about European catfish e.g. disease transmission, trophic impact predation, hybridisation, establishment and dispersal concerns?
What is your opinion about the costs involved in specialist angling of European catfish?
How many fishing licenses do you hold (per angler)?
What is your opinion about the price of a fishing license?
What is your view of the closed season period?
What is your view of the fish stocking density of this European catfish specimen lake?

Appendix E

Appendix E contains the ENSARS study

E6. European Non-native Species in Aquaculture Risk Scheme (ENSARS)

*Data from parts of the study were published in peer reviewed papers:

Copp, GH, Godard, MJ, Russell, IC, Peeler, EJ, Gherardi, F, Tricarico, E, Miossec, L, Goulletquer, P, Almeida, D, Britton, JR, Mumford, J, Williams, C, Reading, A, Rees, EMA, Merino-Aquire, R & Vilizzi, L (2016a) A preliminary evaluation of the European Non-native Species in Aquaculture Risk Assessment Scheme applied to species listed on Annex IV of the EU Alien Species Regulation. Fisheries Management and Ecology 23: 12- 20.

E6.1 Introduction

Invasive fish species pose a major threat to biodiversity and are linked to the extinction of many native and endemic fish species with non-native fish species introductions accelerated by anthropogenic activities around the world (Ricciardi & Rasmussen, 1998; García-Berthouet al. 2005; Wonham et al. 2000; Gallardo et al. 2016b) as described in Chapter 1. This potential crisis has led to the introduction of several International legislative pieces and non-native fisheries policies with the focus being on developing environmental risk strategies so as to identify highly invasive species with a rapid response. However, there is a need for standardisation of risk protocols across countries so as to mitigate the management costs of invasive fish species. Costs currently estimated to range from £12.5- 20 billion annually in Europe and are expected to escalate across the globe (Savini et al. 2010; Britton et al. 2011a; Gallardo et al. 2016a).

The need to update management policies and risk protocols with non-native fish species introductions between countries is essential so as to prevent invasions and harmful impacts. However, despite the Convention on Biological Diversity, only a few countries set appropriate policies so as to maintain biodiversity. As result, it has proved difficult to control invasive species on a global scale. In an attempt to manage non-native fish invasions, the focus has been on developing environmental risk strategies with horizon scanning to identify highly invasive species as part of the introduction prevention approach in non-native fisheries management in the UK and across Europe (Copp et al. 2005b; Ricciardi, 2007; Gozlan et al. 2010; Gallardo et al. 2016a).

In the UK, the research focus has been on developing reliable predictive risk analysis toolkits so as to assess invasive potential of non-native fish species, particularly as the time lag between introductions of new fish species has accelerated from 30yrs to 5yrs into the UK owing to expansion of fish farming practises (Gozlan, 2008; Gozlan et al. 2010; Gallardo & Aldridge, 2013). The development in environmental risk protocols in the UK has been influenced by guidelines set by important pieces of legislation such as the EU Water Framework Directive (2000/60/EC) (G05), Import of Live Fish Act 1980 (ILFA), the Prohibition of Keeping or Release of Live Fish (specified species) Amendment (England) Order 2003 and Wildlife and Countryside Act 1980 (See Table E6.1). All designed to protect species diversity (Copp et al. 2005b; Roy et al. 2014a; Copp et al. 2016c).

Table E6.1 Key relevant legislation related to ENSARS risk assessment in the study are listed below (Copp et al. 2005b; Copp et al. 2016a).

Legislation	**Implications for risk assessment in study**
The Ramsar Convention 1975	Protection of wetland habitats of International importance. Some of the river catchment is a designated Ramsar site
EC Birds Directive (79/409/EEC)	Protection of waterfowl with Special Protection Areas (SPAs) habitats for migratory wildfowl. Part of the river floodplain in the study is (SPA).
Import of Live Fish Act England & Wales 1980 (ILFA)	Controls the introduction of non-native fish species into England & Wales. A licence is needed to import, hold or release *S. glanis* from regulatory authorities
Wildlife and Countryside Act 1981	Conservation and protection of habitat and species diversity with list of designated Sites of Special Scientific Interest (SSSIs). Some of the floodplains are SSSIs, in the study
EC Habitats Directive (92/43/EEC)	Protecting habitat diversity with designation of Special Areas of Conservation (SACs) for species and habitats. Some of the river catchments is (SAC) in the study
UK Biodiversity Action Plan 1994	Protection of species diversity in the UK with designated species such as salmonids which are present in the river catchment
Eel Management Plan (2000/60/EC	Protected status and long term viability of eels (*Anguilla anguilla*). These species are present in river catchment
EU Water Framework Directive (2000/60/EC)	Requirement for good ecological and chemical status in surface and ground waters by 2015 and prevent environmental degradation
EC Freshwater Fisheries Directive (2006/44/EC)	Protection of fresh waters to support fish life with water quality standards for salmonid and cyprinid fisheries. These species are present in river catchment
Invasive Alien Species (Enforcement & Permitting) Order 2019	Further restrictions upon release of invasive non-native species that threaten native species diversity

Subsequent risk assessment toolkits such as the Fish Invasiveness Screening Kit (FISK) and European Non-native Species in Aquaculture Risk Analysis Scheme (ENSARS) were adapted from the Weed Risk Assessment (WRA) Pheloung, 1999 as part of the introduction prevention approach in non-native species management (Copp et al. 2009b; Almeida et al. 2013; Copp, 2013; Simonovic et al. 2013; Tarkan et al. 2014; Copp et al. 2016b). The first steps involve rapid detection of the presence of new species with effective control actions so as to prevent their dispersal and spread. However, these protocols need to be further developed and adopted by other countries so as to mitigate serious threats to loss in biodiversity (Copp et al. 2005b; Copp et al. 2005c; Britton et al. 2011b; Britton & Gozlan, 2013; Gallardo et al. 2016b)

FISK was specifically designed as a risk assessment toolkit for freshwater fish species in temperate climates which was widely used in the UK and across Europe and was a valuable tool for distinguishing between invasive and non-invasive fish species (Copp et al. 2005a; Tricarico et al. 2010; Tarkan et al. 2014; Perdikaris et al. 2016). ENSARS has a taxon generic approach with a broader scope than FISK. ENSARS can be used in all climatic zones such as tropical, marine, freshwater and brackish ecosystems around the world. This has increased comparability in risk protocols in assessing non-native species impacts across countries (Copp et al. 2016a; Copp et al. 2016b; Gallardo et al. 2016b).

Central to ENSARS, is the inclusion of the uncertainty ranking by multidisciplinary assessment team assessors for the non-native species under review. Each assessment is combined with a confidence score. This aims to improve transparency about the risk estimates given as often information about species fish behaviour and invasiveness may be fragmentary and unreliable. Additional factors used in the risk assessment are the inclusion of socio-economic costs and the inclusion of a set of modular sections using predicted climate change models so as to assess species invasive potential with environment suitability (Copp et al. 2016a; Copp et al. 2016b).

Overall, ENSARS has provided a replicable, science based risk assessment toolkit, allowing assessors to distinguish important threats with recently introduced species and risks of introduction, establishment, dispersal and adverse impacts (Copp et al. 2016a; Copp et al. 2016b).

The present study examined the risks of dispersal and adverse ecological impacts with non-native *S. glanis* from a lake fishery. The aim of the study was to assess the risk status of *S. glanis* held in a lake fishery without regulatory consent in southern England with ENSARS using: Organism, Facility, Infectious Agent, Pathway and Socio-economic impact risk assessment modules.

The specific objectives were to:

1) evaluate the risks of introduction, establishment, dispersal and impact of *S. glanis* from the lake fishery using the Organism risk assessment module with water temperature data of the river catchment and Climatch modelling;

2) examine the risks of unintentional release of *S. glanis* and non-target organisms from the facility into the surrounding area with the Facility risk assessment module;

3) determine the risks of escape of *S. glanis* specimens from the lake fishery into the surrounding area with the Pathway risk assessment module;

4) assess the likelihood of *S. glanis* as a vector of infectious agent and the risks of infectious agent impacts using the Infectious agent risk assessment module, and

5) determine the magnitude of socio- economic loss caused by *S. glanis* escaping into the surrounding area with the Socio-economic impact risk assessment module.

E6.2 Materials and methods

E6.2.1 Sampling and fish survey

Annual fish surveys involving *S. glanis* removal from the lake fishery (Northfield Main Pit Lake) near Ringwood, Dorset (grid reference SU160075), were carried out from August 2009- October 2010 with 5 *S. glanis* specimens recaptured overall. The enclosed lake has a surface area of ~10 ha with mean depth of ~0.5- 4.26 m as described in Chapter 2 (See Figure E6.1). The venue operated as a catch and release fishery but holding of *S. glanis* in the lake was without regulatory consent.

The fisheries survey for *S. glanis* removal from the lake used a series of 5 fyke nets, with 6 baited ends set up in a section of the lake cordoned off from anglers. The section of lake netted was approximately a fifth of the total lake area. The fyke nets were left overnight and retrieved the following morning. In addition to fyke netting, a 100 m length of seine netting (5m depth) was also used. Recaptured *S. glanis* specimens were removed from lake and killed by overdose of anaesthesia (benzocaine solution 5% w/v) as per Home Office guidelines and then preserved in 4% formalin for further examination in the laboratory. All work was carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

Figure E6.1 Map of lake fishery (Northfield Main Pit Lake) near Ringwood, red arrows highlighted bio security risks for fish transfer to other waters (Google earth, 2018).

E6.2.2 Water temperature data

E6.2.2.1 Selection of sites

Data was obtained from Environment Agency database of water monitoring of ten sites of the upper and lower reach of the River Hampshire Avon from 2003–2009. The data consisted of 15 minute water temperature readings by TinyTag loggers which recorded continuously throughout the year. From the water temperature data, mean maximum daily temperatures were obtained to fit the study purpose in determining water temperatures which were compatible for *S. glanis* establishment.

There were some limitations in the database with gaps in water temperature monitoring for a few sites, which may have caused some bias in the study. There was also no data available in the years preceding 2003, so records were from 2003-2009, which was a fairly narrow time frame to assess water temperature variation of the river catchment (See Table E6.2).

Table E6.2 Sites of the upper and lower reach of the River Hampshire Avon

Site	Reach	National grid reference	Flow rate Km u/s TL	Monitoring period
Wylye, South Newton	Upper	SU0862634244	66.1	2003, 2007-09
Ebble, Nunton bridge GS	Upper	SU1607626360	-	2007-09
Bourne, Salisbury B&Q	Upper	SU1562829131	-	2003, 2007-09
Nadder, at Wilton	Upper	SU0985230824		2003, 2007-09
Ibsley	Lower	SU1495909670	-	2007-09
East Mills GS	Lower	SU1585514340	35.5	2003
Ringwood GS	Lower	SU1457205550	22.4	2003, 2006-07
Avon Causeway	Lower	SZ1494297867	-	2007-09
Knapp Mill	Lower	SZ1547093794	0.9	2003, 2007-09
Christchurch Bypass bridge	Lower	SZ1606893237	0.9	2004-05, 2007-09

Water temperature analysis of the river catchment was used to determine the likelihood of *S. glanis* becoming established into the river catchment as they require warm waters of over 18ºC to initiate spawning in aquatic habitats (See Table E6.3) (Copp et al. 2009a; Cucherousset et al. 2018).

Table E6.3 Thermal requirements for *S. glanis* reproduction (Gullu et al. 2008; Copp et al. 2009a; Cucherousset et al. 2018).

Water Temperature requirement (ºC)	**Behaviour strategy**
13-14	Death of larvae
15	50% reduction of food assimilation and growth
18-22	Initiation of spawning
22-23	Spawning
23-25	Embryos hatching
22	Larvae & fry development
25-28	Optimum growth

E6.2.3 Climatch application of the River Hampshire Avon catchment, Dorset

The Climatch Euclidean algorithm model was used to predict the risk of *S. glanis* distribution into the River Hampshire Avon catchment using climatic variables. These included the annual mean temperature, annual temperature range, temperature of the coldest and warmest month obtained from world meteorological stations (9460) for climatic matching between target and source regions.

The Euclidean algorithm (Closest Euclidean Match) was used to calculate the climate distance between input sites and target site (Hampshire Avon catchment) for climatic matching with *S. glanis* native ranges (eastern Europe and western Asian countries) and predict climate match scores. The Climatch match scores for the target area may range from 0-10 with high scores indicative of very similar climate between the target and source regions. In addition to this the predicted species distribution counts were also calculated to estimate species establishment (Crombie et al. 2008).

E6.2.4 ENSARS data collation

Data was obtained from responses to ENSARS modules with 49 questions broken down into sections (See Appendix C, Table 6C1.1). There were 5 ENSARS modules used in the study; Organism risk assessment module; 2) Facility risk assessment; 3) Infectious agent risk assessment; 4) Pathway risk assessment and 5) Socio-economic impact risk assessment (See Figure E6.2). The Entry and Pre-screening modules of ENSARS were not used in the study as the species was already listed on the Annex IV of the EU Alien Species Regulation and thus preselected (Copp et al. 2016a).

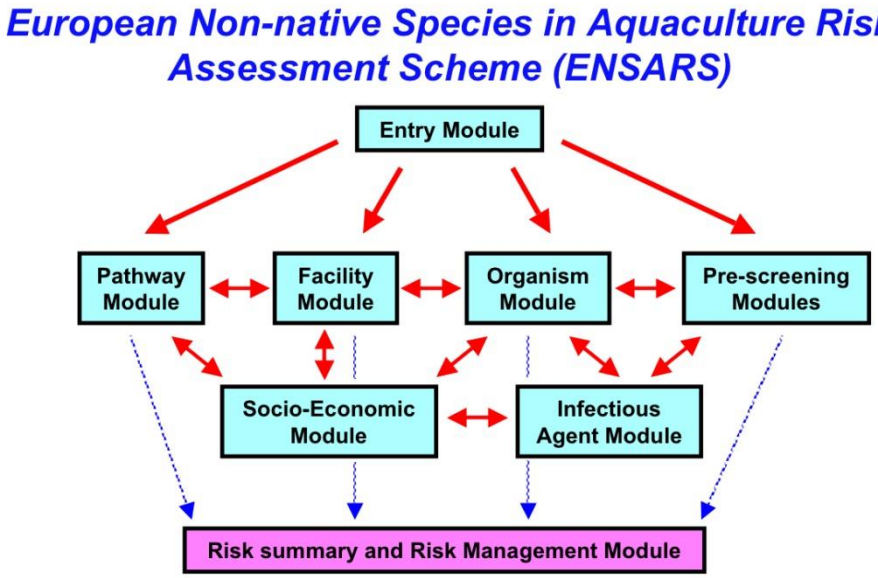

Figure E6.2. (ENSARS) modules used in the study (Copp et al. 2016a).

Responses for the ENSARS modules were collated with liaison from the fishery bailiff (Clements, M.) using a chain sampling method (snow ball sampling or referral) with the co-operation and written consent of the bailiff who agreed to participate in the study conducted under environmental organisation auspices and as such did not require ethics approval. The study relied on good communication from the bailiff and authors including (Rees, E.M.A) and three fishery experts (Copp, G.H., Lightfoot, G. & Peeler, E.P.) from Environment Agency and CEFAS who have specific research expertise in invasive fish risk analysis and disease emergence. All assessors in the study confirmed that they had no competing interests to declare. The ENSARS risk assessments were adapted from peer reviewed risk assessment guidelines from the GB Non-native Species Risk Assessment Scheme, the European and Mediterranean Plant Protection Organisation (EPPO) and the International Plant Protection Convention (IPPC) based on plant and animal non-native species introduction risk assessments (Baker et al. 2008; Roy et al. 2014b; Copp et al. 2016a; Copp et al. 2016b).

For reasons of ethics, cost-effectiveness, statistical reliability and in order to minimise bias, responses to the ENSARS modular questions were transparent with the inclusion of confidence level scores using the confidence rankings approved by the Intergovernmental Panel on Climate Change (IPPC). Moreover, to clarify the level of certainty in response by assessors with the ENSARS modules questionnaires, questions were answered with a certainty response. Assessors were asked to answer from a list of certainty rankings to each question selected from 4 groups of confidence level response scores (Copp et al. 2016a; Copp et al. 2016b).

E6.2.5 The ENSARS modules

The overall design of each ENSARS modules consisted of a series of 49 questions divided into two sections. The first section examined the ecological and life history traits of species whereas the other section assessed the environmental and socio-economic impacts (Baker et al. 2008; Copp et al. 2016a; Copp et al. 2016b).

The second section included a summary of the risks assessed with an overall mean risk summary scores for each section and the confidence levels scores given. Each ENSARS module response scores ranged from 0- 4, from which the overall mean risk summary score for the module was derived. Risk categories of the overall mean score were ranked into 5 groups from score intervals:

0) [0-0.8] - low risk;

1) [0.8-1.6] - moderately low risk;

2) [1.6-2.4] - medium risk;

3) [2.4-3.2] - moderately high risk; and

4) [3.2-4.0] - for high risk (Copp et al. 2016b).

The response scoring system and sequence of questions in ENSARS followed the same format of (FISK v2) and (WRA) with confidence level response scores ranked into 4 groups from: 1) 0, low confidence (2 out of 10 chance of the score being correct); 2) 1, moderate confidence (5 out of 10 chance of the score being correct); 3) 2, high confidence (8 out of 10 chance of the score being correct) and 4) 3, very high confidence (9 out of 10 chance of the score being correct). From these confidence level response scores, an overall mean confidence score was obtained for each module (Copp et al. 2016a).

E6.2.5.1 The Organism risk assessment module

The ENSARS Organism risk assessment module was used to assess the risks of introduction, establishment, dispersal and impacts of *S. glanis* specimens should they escape from the lake fishery into the surrounding area (See Figure E6.3). The module was divided into 2 sections (A and B). Section A included a list of questions about the recipient area and details of the type of non-native organism being assessed whether it was a fish, invertebrate or an infectious agent. Questions about climate matching and environment suitability of the recipient habitat were also addressed (See Appendix C, Table 6C1.1) (Roy et al. 2014b; Copp et al. 2016a; Copp et al. 2016b).

The second section of the Organism module (Part B, the full risk assessment of 45 questions) was in 4 parts with questions about: 1) the likelihood of introduction, 2) likelihood of establishment, 3) likelihood of dispersal, and 4) likelihood of environmental and socio-economic adverse impacts (See Appendix C, Table 6C1.2, Table 6C1.3, Table 6C1.4, Table 6C1.5).

Regarding the likelihood of *S. glanis* introduction and establishment into the surrounding, questions about life history traits, climate suitability and possible competition or predation on native species were included. For dispersal risks, estimates on how quickly *S. glanis* would spread into the risk assessed area was assessed and effectiveness in control and eradication options. The socio-economic impact risks section assessed the likelihood of harm of *S. glanis* escaping into the surrounding area and possible damage (Copp et al. 2016a)

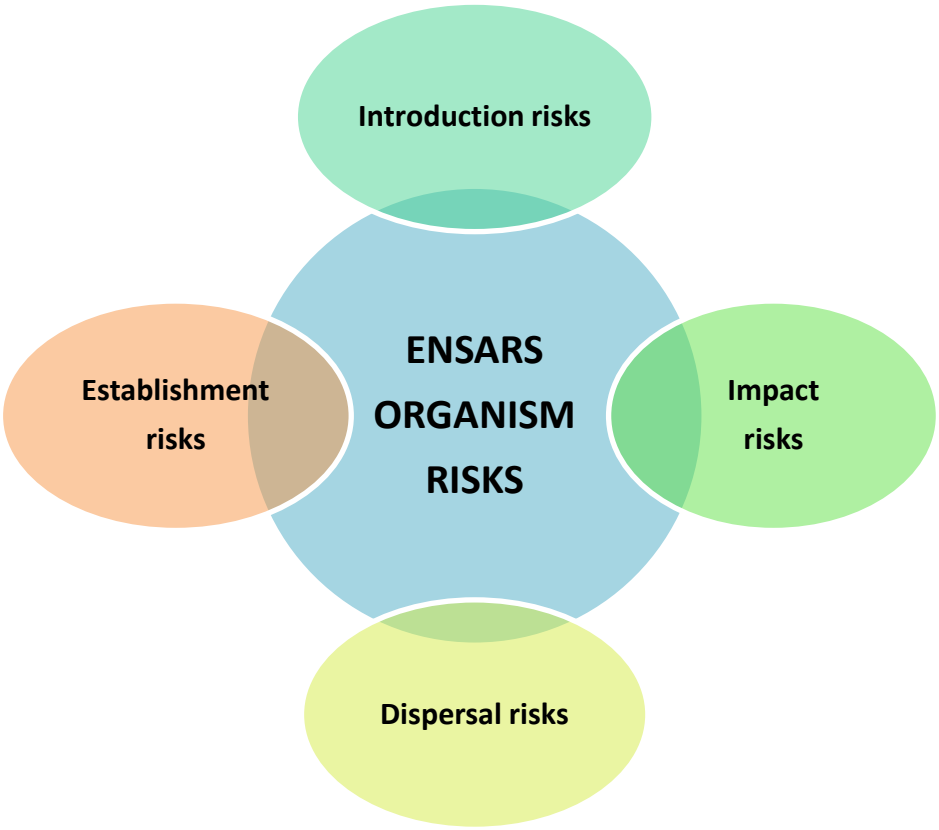

Figure E6.3. ENSARS Organism risk assessment module with the summary of introduction, establishment, dispersal & impact risks (adapted from Copp et al. 2016a).

E6.2.5.2 Pathway risk assessment module

The Pathway risk assessment module included a series of questions to assess the overall risk of escape of *S. glanis* specimens into the surrounding area by 3 different pathways: 1) from importation procedures; 2) from farming procedures; and 3) from final destination and use. Reference to importation procedures, a list of questions addressed the frequency and magnitude of *S. glanis* introductions into the fishery and likelihood of fish escaping into the wild. Farming procedures included questions about biosecurity of farming process of *S. glanis* and overall risk of escapees into the wild. The final section assessed the dispersal risks of *S. glanis* specimens from the lake fishery into the surrounding area and level of regulatory enforcement with deliberate fish release from the fishery (See Appendix C, Table 6C1.9, Table 6C1.10) (Copp et al. 2016a).

E6.2.5.3 Facility Risk Assessment module

Responses for the Facility Risk Assessment Module data was obtained from 31 questions broken down into 3 components: 1) assessment of management operations efficacy practised at the fishery; 2) assessment of the risks of unintentional release of *S. glanis* specimens from lake fishery by escape via screens, or waste effluent into the surrounding area; 3) assessment of the risks of unintentional release of any non- target organisms or non-target infectious agents from the fishery via screens or waste effluent into the wild (Copp et al. 2016a).

The Facility risk assessment module was designed to elicit understanding of possible risks of non-native species escaping via operation procedures at the fishery into the wider environment. The first section determined the stocking density of *S. glanis* in the lake and competence in fishery management, with questions about whether there was a written procedure for fishery management, records of daily activities and maintenance plans for equipment. Other questions determined if there was a treatment system for effluent discharge or contingency plan so as to give an overall background about the fishery management practised at the lake fishery (Copp et al. 2016a).

To clarify the likelihood of *S. glanis* and associated non-native organisms escaping from the fishery into the surrounding area, the module assessed the efficacy of preventative mechanisms (gates, screens, meshes) and the effluent discharge treatment systems at the fishery were in

operation. Frequency of inspections and quarantine procedures at the fishery were also included (See Appendix C, Table 6C1.6, Table 6C1.7, Table 6C1.8) (Copp et al. 2016a).

E6.2.5.4 Socio-economic impact risk assessment module

The Socio-economic impact risk assessment module estimated the magnitude of economic loss caused by *S. glanis* escaping into the surrounding area with the associated costs of eradication, removal or containment of *S. glanis* from the lake fishery.

The final section of the module addressed the likelihood of socio-economic and ecological harm to the local area following *S. glanis* escapees from the lake fishery into the surrounding area. Estimates of the likelihood of adverse socio-economic impacts were categorised using a 5-point rating scale with the options of: 1) minimal; 2) minor; 3) moderate; 4) major; and 5) massive (See Appendix C, Table 6C1.16, Table 6C1.17, Table 6C1.18, Table 6C1.19) (Copp et al. 2016a).

E6.2.5.5 Infectious Agent Risk module

The Infectious Agent risk module section for infectious agents detected upon *S. glanis* specimens, followed The Office International des Epizooties (OIE) protocols on import risk analysis with questions broken down into four components: 1) risks of introduction of the infectious agent into the risk assessed area, 2) risks of establishment into the risk assessed area, 3) risks of spread and 4) risks of impact into the risk assessed area. The risks of introduction section assessed how widespread the infectious agent was outside its native range, the likelihood of subclinical infection and reliability of diagnosis and vaccination against such infectious agents.

The second part investigated the risks of establishment of infectious agents into the recipient area and whether they needed an intermediate host to complete their life cycle. Disease virulence in relation to the water temperature profile of the surrounding area was also assessed (Copp et al. 2016a).

Risks of spread by infectious agents related to the stocking density of *S. glanis* released into the lake with rapid detection of disease on fish. The frequency of angler transference in releasing *S. glanis* specimens from the lake into the surrounding area was also estimated. The final section addressed the risks of adverse impacts by infectious agents to native species human health and disease control (See Appendix C, Table 6C1.11, Table 6C1.12, Table 6C1.13, Table 6C1.14, Table 6C1.15) (Rees, 2010; Copp et al. 2016a).

E6.3 Results

E6.3.1 Variation in water temperature

There was seasonal variation in water temperature in the upper and lower reach of the River Hampshire Avon. Water temperatures were fairly low (~10^0C) in spring, with summer water temperatures peaked in June- August (~19^0C). The results implied that over a 6yr interval from 2003–2009, there were some annual differences in water temperature variation where some years (e.g.2003) were warmer than others (e.g.2009), due to natural fluctuations in climate.

Overall, the results indicated differences in variation of water temperatures between the upper and lower reach of the Hampshire Avon due to geographical latitude. In general, the mean summer water temperatures were higher for sites of the lower reach of the river than those of the upper reach. All lower reach sites such as Avon Causeway, Christchurch, Knapp mill, East Mill and Ringwood attained mean maximum daily temperatures over 18ºC during the summer which were suitable temperatures for *S. glanis* spawning and growth (Gullu et al. 2008; Copp et al. 2009a; Cucherousset et al. 2018) (See Table E6.4, Fig E6.4-E6.5).

Table E6.4 Variation of the mean maximum daily water temperatures in the summer for lower reach sites;Knap Mill, Ringwood, East Mills, Christchurch and Avon Causeway of the R. Hampshire Avon in 2003 and 2009. Mean maximum daily water temperatures (ºC) by month. Standard Deviation (SD) given in brackets.

	2003			2009		
Month	**Knap Mill**	**Ringwood**	**East Mills**	**Knap Mill**	**Christchurch**	**Avon Causeway**
May	13.60 (1.88)	13.40 (1.83)	13.30 (1.78)	14.35 (1.73)	14.41 (1.72)	14.28 (1.85)
June	19.00 (1.26)	18.30 (1.27)	17.80 (1.18)	18.60 (1.68)	18.67 (1.66)	18.23 (1.74)
July	19.20 (1.60)	18.30 (1.53)	17.80 (1.48)	18.48 (1.05)	18.52 (1.60)	18.12 (1.64)
August	19.60 (2.33)	19.20 (2.28)	18.70 (2.26)	18.28 (1.05)	18.35 (1.04)	17.96 (1.04)
September	16.40 (1.46)	15.70 (1.50)	15.30 (1.46)	15.71 (0.83)	15.73 (0.81)	15.50 (0.86)

The mean maximum daily water temperatures of lower reach sites such as Knap Mill, Ringwood and Christchurch attained water temperatures over 18ºC favourable for *S. glanis* spawning during the summer. Although larval and fry stages require higher water temperatures for development (22-25ºC) (Gullu et al. 2008; Copp et al. 2009a; Cucherousset et al. 2018).

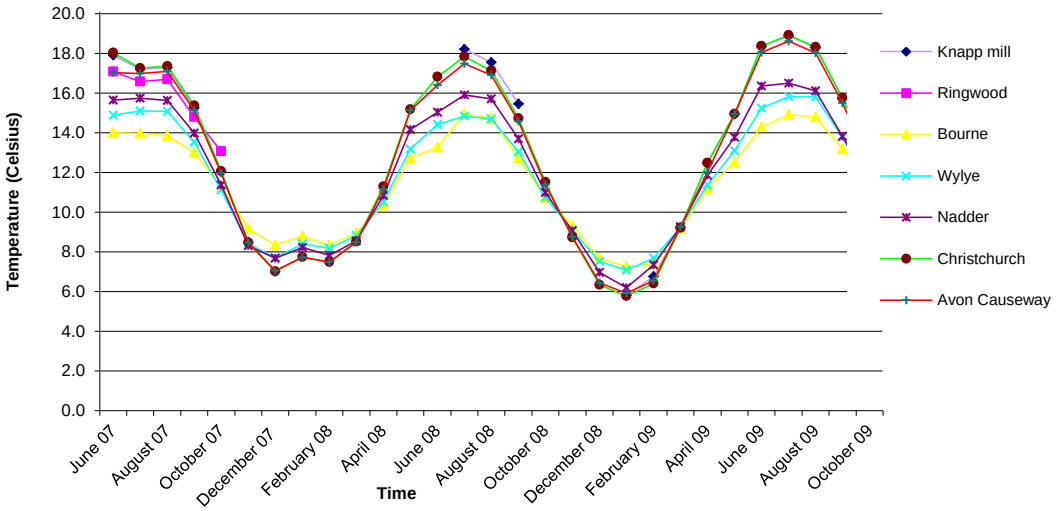

Figure E6.4. Differences in temperature gradient in monthly mean water temperatures between the upper and lower reach of the R. Hampshire Avon during 2007-2009.

Sites of the lower reach such as Avon Causeway, Knapp Mill, Christchurch exhibited mean water temperatures over 18ºC favourable for *S. glanis* spawning in the summer.

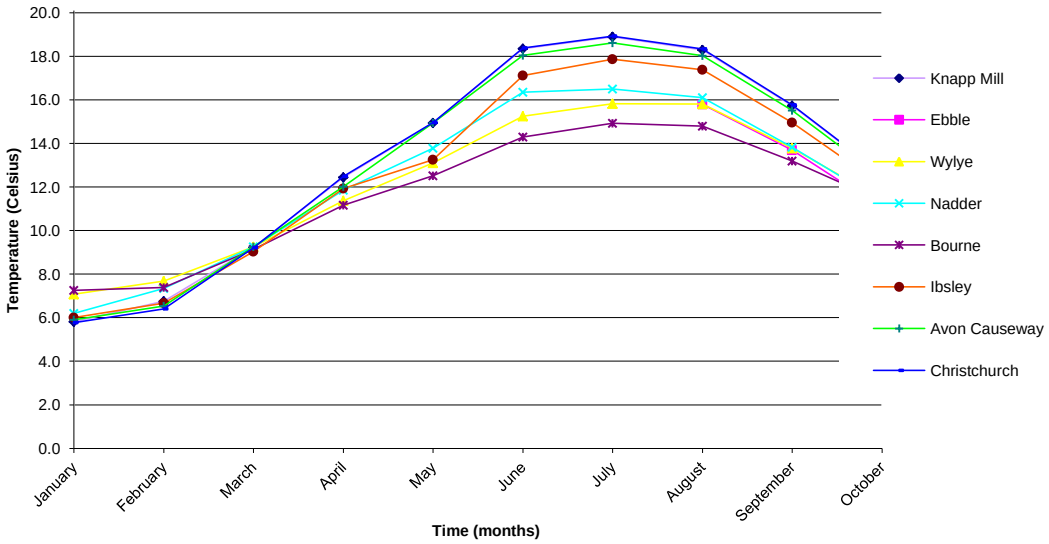

Figure E6.5. Variation of mean monthly water temperatures of the upper and lower reach of the R. Hampshire Avon in 2009.

E6.3.2 Climatch results of the River Hampshire Avon catchment, Dorset

The Climatch Euclidean algorithm model predicted the risks of *S. glanis* establishment and distribution into the Hampshire Avon catchment by matching climatic variables with those of native ranges. The Climatch match predicted scores were high (7) for *S. glanis* distribution (species count of 67) and establishment into the river catchment (See Table E6.5, Figure E6.6).

Table E6.5 Climatch scores predicted for *S. glanis* distribution and establishment into the R. Hampshire Avon catchment, Dorset.

Climatch Score	Species Count
1	0
2	0
3	0
4	0
5	10
6	33
7	67
8	45
9	4
10	0

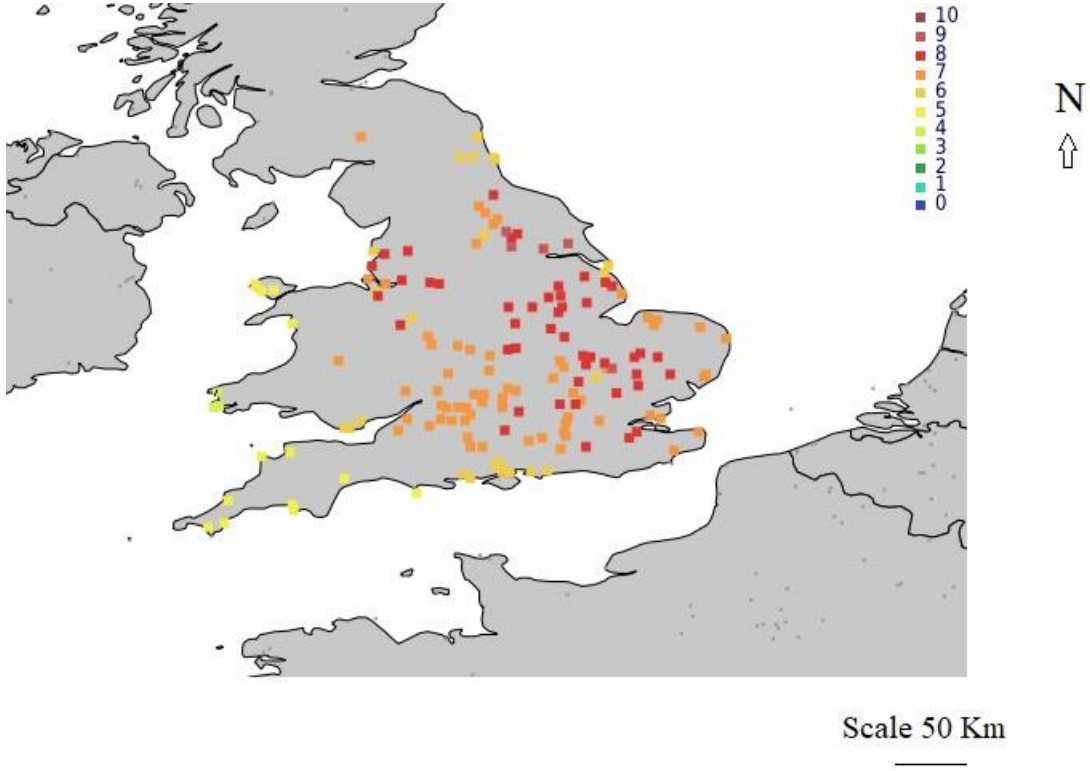

Figure E6.6 Climatch predicted match scores for the R. Hampshire Avon catchment, Dorset, England for *S. glanis* distribution (Climatch, 2019).

The predicted Climatch scores (0-10 are colour coded) and indicate variation in climate favourable for *S. glanis* establishment in England & Wales.

E6.3.3 The Organism ENSARS Module results

The Organism Module with its overview of the risks of *S. glanis* introduction, establishment success, dispersal and ecological impacts into the surrounding risk area was given an overall mean score of 1.9 (Confidence scoring of 2.0) and categorised as medium risk with a high confidence score. The highest risk mean scores and confidence levels were for the risks of introduction and dispersal of *S. glanis* into the surrounding area. The summary of introduction risks was given 2.4 (Confidence scoring of 3.0) with dispersal risks 2.3 (Confidence scoring of 2.0) and were determined as medium risk with high confidence levels.

Lower risk scores were given for the overall risks of adverse impacts caused by *S. glanis* specimens following escape and establishment into the surrounding area with 0.9 (Confidence scoring of 1.0) and 1.9 (Confidence scoring of 2.0) scores respectively. The scores were ranked as moderately low risk for adverse impacts and low medium risk for *S. glanis* establishment into the surrounding area. However, low confidence scoring was given with these risks owing to gaps in knowledge about their invasive potential in aquatic habitats in the UK (See Table E6.6).

Table E6.6 ENSARS Organism risk summary for *S. glanis* in the study. For the Organism Risk Summary module, the introduction risks were ranked with overall mean of moderately high risk (2.4) and very high confidence scoring of (3).

Organism Risk Summary	No of responses	Risk categories				Overall mean	Overall confidence score
		0	1	2	3		
Summary introduction risks	5		1	1	3	2.4	3
Summary establishment risks	17	1	4	7	5	1.9	2
Summary dispersal risks	4		1	1	2	2.3	2
Summary impact risks	17	7	5	5		0.9	1

Table E6.7 ENSARS Facility risk summary for *S. glanis* in the study.

Facility Risk Summary	No of responses	Risk categories					Overall mean	Overall confidence score
		0	1	2	3	4		
Summary of *S. glanis* escaping facility	6			5	1		2.2	2
Summary of non-target organisms escaping facility	12		1	7	2	2	2.5	2

For the Facility module, the risks of *S. glanis* escaping from the lake fishery into the surrounding area were ranked with an overall mean of medium risk (2.2) and high confidence scoring of (2).

Table E6.8 ENSARS module summary risks of *S. glanis* in the study.

ENSARS Module Summary Risks	Mean scores	Mean confidence	Overall mean	Overall confidence	Overall risk category
Organism Module			1.9	2.0	Medium
Introduction	2.4	3.0			
Establishment	1.9	2.0			
Dispersal	2.3	2.0			
Impact	0.9	1.0			
Facility Module			2.4	2.0	Moderate high
S. glanis escape facility	2.2	2.0			
Non-target organisms escape facility	2.5	2.0			
Pathway Module			2.5	2.3	Moderate high
Import procedures	2.3	2.0			
Farming procedures	2.3	1.7			
Destination use	2.8	3.0			
Infectious agent Module			1.5	1.8	Moderate low
Introduction	1.8	1.9			
Establishment	2.3	2.5			
Dispersal	1.4	1.8			
Impact	0.6	1.9			
Socio-economic Module			1.5	2.1	Moderate low
Market impacts	0.9	2.0			
Eradication costs	1.8	2.1			

Key risks were distinguished using ENSARS modules of the likelihood of *S.glanis* dispersing into the surrounding area via the Pathway module. The risk of *S. glanis* dispersal owing to facility operations at fishery were determined by Facility module and risks of *S. glanis* introduction, establishment and dispersal into the surrounding area with the Organism module. The risks of socio-economic impacts, likelihood of spread and harmful impacts of infectious agents from *S. glanis* into the river catchment were determined by the Socio-economic and Infectious agent module respectively.

E6.3.4 The Pathway ENSARS Module

The overall mean scores for the Pathway module in the study ranged from medium to moderately high risk. The Pathway module distinguished the risks of *S. glanis* dispersal into the wild via three pathways; importation procedures, farming procedures and final destination use. The Destination use pathway in the study scored the highest risk with a mean score of 2.8 and very high confidence score of 3.0. The overall risk of escape of *S. glanis* into the surrounding area by importation or farming procedures were ranked as of medium risk for importation 2.3 (confidence score of 2.0) and for farming 2.3 (confidence score of 1.7) in the assessment (See Table E6.8).

E6.3.5 The Facility ENSARS Module

For the Facility module, the overall mean score was medium risk 2.2 and high confidence scores of 2.0 regarding the risks of unintentional release of *S. glanis* specimens escaping into the surrounding area. The risks of non-target organisms escaping from the lake fishery into the surrounding area was given mean score of 2.5 and high confidence score of 2.0 in the assessment by EMAR following liaison with fishery bailiff during visits. Security mechanisms such as gates, screens and meshes to prevent fish escapees from the lake into the surrounding area was assessed, including waste effluent systems. Other bio security risks such as flooding and frequency of fish inspections and quarantine measures practised at the lake fishery were also included in the assessment (See Table E6.7, E6.8).

E6.3.6 The Socio-economic ENSARS Module

The results for the socio-economic module indicated that the likelihood of significant economic loss caused by *S. glanis* specimens escaping from the fishery into the surrounding area was determined as overall moderately low risk with high confidence levels for all assessed sections. The potential socio-economic loss was assessed as low with mean score of 0.9 and high confidence score of 2.0. Estimates in fishery surveys removal, surveillance or containment of *S. glanis* in the lake were ranked as medium risk 1.8 and confidence score of 2.1 and were unlikely to adversely impact fishery profits in the assessment. The estimates did not include eradication costs as eradication was considered unfeasible owing to the risks of rotenone contamination to other lakes as the lake was sourced from ground water springs (See Table E6.8).

E6.3.7 The Infectious Agent ENSARS Module

The outcomes from the Infectious Agent module revealed that the overall mean risk score of disease transmission by *S. glanis* in establishing and spreading infectious agents into the surrounding area were moderately low (1.5) and given a moderate confidence scoring of 1.8. Fish health inspection of recaptured *S. glanis* specimens from the lake detected a novel ancyrocephalid monogenean parasite *Thaparocleidus vistulensis* (Sivak, 1932) occurring upon the gills (intensity range 1-35 per gill arch) as a moderate infection. This parasite was identified as a specialist parasite of siluriform fishes and is a new recording for aquatic habitats in the UK (See Plate E6.1). There are some records of *T. vistulensis* occurring upon *S. glanis* specimens in the wild in Italy and parts of Eastern Europe e.g. Czech Republic, the Slovak Republic and Poland. However there are gaps in knowledge about it's adverse impacts and spread into non-native ranges (Reading et al. 2012; Copp et al. 2016a).

Another infectious agent detected upon recaptured *S. glanis* specimens was the non-native generalist parasite *Ergasilus sieboldi* (Nordmann, 1832) as an infection on the gill laminae of fish (intensity range 1-10 per gill arch). *E. sieboldi* is a parasitic gill infection that may affect freshwater fish species in the UK and heavy infestations may cause fish mortality (Reading et al. 2012).

The overall mean risk scores in the spread and adverse impacts of infectious agents identified on *S. glanis* specimens were considered fairly low risk and of little impact as *T. vistulensis* is a specialist parasite and unlikely to switch host to other native fish species in the surrounding area. Moreover, *E. sieboldi* is a generalist parasite already widespread on most native fish species in the wild in the UK. The risks of the infectious agents identified on *S. glanis* specimens were ranked as of medium risk for introduction 1.8 (confidence score of 1.9) and establishment 2.3 (confidence score of 2.5) with low risks for infectious agents impacts 0.6 (confidence score of 1.9) and dispersal into the surrounding area 1.4 (confidence score of 1.8) (See Table E6.8).

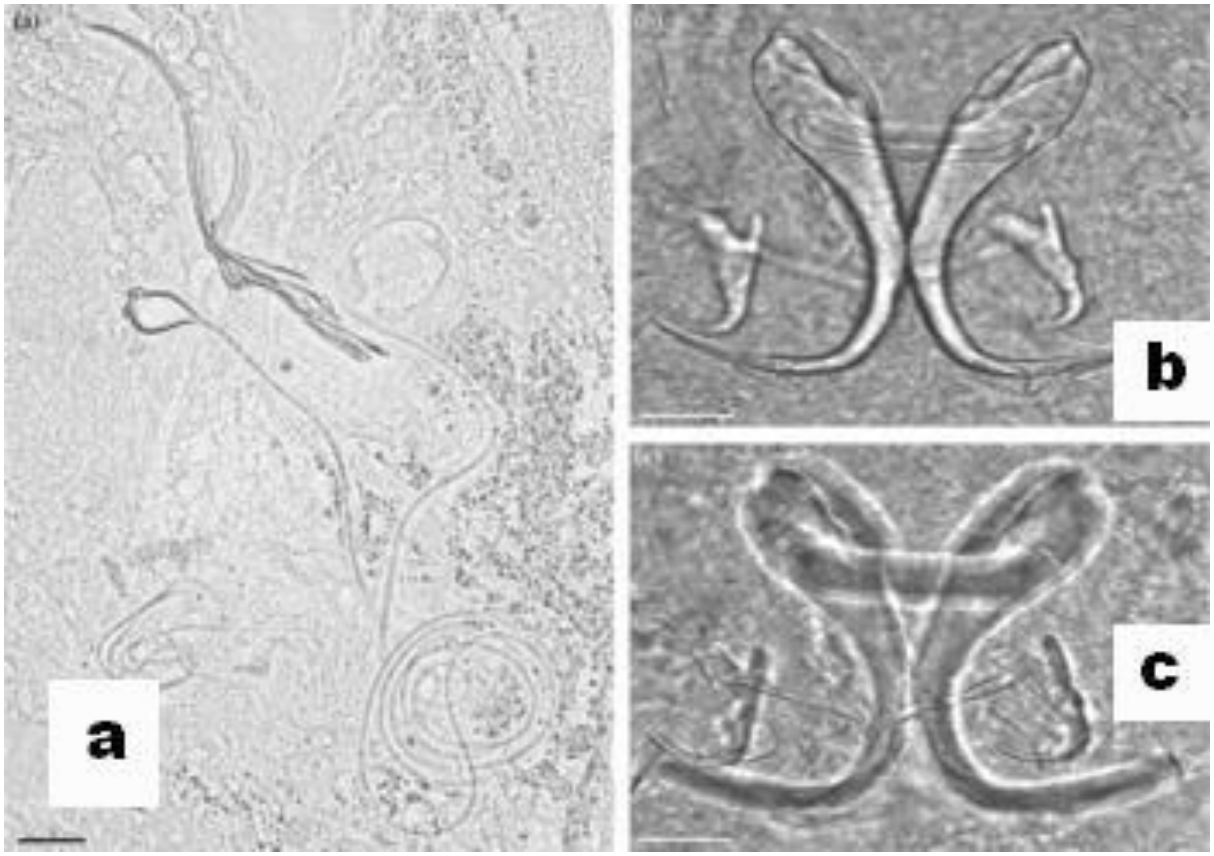

Plate E6.1. Parasitological examinations of *T. vistulensis* (Siwak, 1932) showing (a) their copulatory complex, (b, c) and their haptoral sclerites. Scale bar = 20µm (Reading et al. 2012).

The novel ancyrocephalid monogenean parasite *Thaparocleidus vistulensis* was identified on the gills of recaptured *S. glanis* specimens from the lake (Northfield Main Pit) by parasitological examination in the study (Reading et al. 2012; Copp et al. 2016a).

E6.4 Discussion
E6.4.1 Background

There is a need to investigate the risks posed by non-native *S. glanis* species introductions into lake fisheries without regulatory consent as the ecological damage posed to other waters is likely to be underestimated. Over the years, increased propagule pressure due to popularity of *S. glanis* trophy angling has resulted in introductions into lakes (~500) in England and Wales which are often completed without adequate environmental risk assessment and are likely to be harmful (Copp et al. 2007b; Britton et al. 2010; Zięba et al. 2010; Rees et al. 2017).

It has proved difficult to predict the potential invasive abilities of *S. glanis* species owing to gaps in knowledge about their adverse ecological impacts to native fish species and recipient habitats (Britton et al. 2007; Syväranta et al. 2009; Gozlan et al. 2010; Cucherousset et al. 2018). In general, there is insufficient monitoring for early detection of invasive fish species in aquatic habitats and there needs to be better cooperation between fishery managers and regulatory bodies with stricter regulatory enforcement in the control of *S. glanis* in the UK (Copp et al. 2007a; Britton et al. 2011a; Rees et al. 2017).

In an attempt to manage non-native fish species, research has focused in developing risk assessments toolkits such as FISK so as to predict species invasiveness and provide information for policy makers so that appropriate management policies can be implemented. The FISK approach provided a predictive profile of the invasive potential of non-native fish species. This ranged from invasive pest to harmless and was based on species life history traits and habitat suitability so that potentially invasive and non-invasive species could be distinguished (Copp et al. 2005b; Tricarico et al. 2010; Copp, 2013; Lawson et al. 2013; Tarkan et al. 2014).

Other study results used the FISK approach with some non-native *S. glanis* introductions into England and Wales and identified *S. glanis* as a highly invasive species. However owing to thermal barriers ($\leq 20^0$C) they are at a lag phase and not established in riverine habitats in the UK (Copp et al. 2009a; Britton et al. 2010). It is anticipated that this may change with climate driven thermal changes (2-5^0C) in aquatic habitats are likely to facilitate *S. glanis* invasion (Carol et al. 2007; Rahel & Olden, 2008; Gozlan, 2010; Syväranta et al. 2010; Rees et al. 2017).

Moreover, non-native *S. glanis* species present invasive FISK risk status is liable to change with advances in risk assessment protocols and greater understanding of their invasive characteristics. For example the ENSARS approach involves the standardization of risk protocols for all climate zones which can assist decision makers to update management policies for control of invasive fish species globally. The latter point is important given that non-native fish species account for over 25% in freshwater communities in European climates with increasing loss of native fish species and biodiversity (Copp et al. 2009a; Britton et al. 2010; Gozlan et al. 2010; Britton et al. 2011b; Copp, 2013).

In this study, ENSARS modules were used to determine any risks of potential ecological and socio-economic impacts caused by *S. glanis* escaping from the lake fishery into the surrounding area.

E6.2 ENSARS modules
E6.2.1 Organism risk assessment module

The Organism module results brought attention to the risks of *S. glanis* introductions into the lake fishery (2.4) and dispersal (2.3) into the surrounding area (river catchment). The study results indicated that some sites of the lower reach of the river catchment during summertime may provide conditions compatible for *S. glanis* establishment. For example, several downstream riverine sites such as Avon Causeway, Knapp Mill, East Mills, Ringwood and Christchurch had mean daily water temperatures over 18ºC from June-August which may be favourable for *S. glanis* establishment. In addition, the Climatch model predicted strong climate matching of the river catchment with *S. glanis* native compatible for establishment success (mean climate match score of 7). Other studies have predicted similar climate match scores for *S. glanis* establishment (mean climate match score >7) into several river catchments in England and Wales (Britton et al. 2010).

In the present study, some of the riverine habitat included shallow drainage channels and floodplain water bodies which warm up rapidly ($\geq 20^0$C) during the summertime which may be favourable spawning and breeding habitats (Copp et al. 2009a; Rees, 2010; Rees et al. 2017). Nevertheless, the UK climate is generally cooler and less extreme than *S. glanis* native range and the species does not easily spawn in England at present. Spawning, embryo, larval and fry development are temperature dependent and require water temperatures over 22^0C and it is likely that cooler water temperature regimes in river catchments may constrain species establishment. *S. glanis* are long lived species (males 22 years, females 16 years) and appear to persist without becoming invasive in temperate fish communities (Britton et al. 2007; Gullu et al. 2008; Rees et al. 2017).

Other studies using FISK ranked *S. glanis* as a highly risk invasive species (21.5 FISK score) by a multidisciplinary assessment team, expert advisors to policy makers in non-native fisheries management issues. *S. glanis* has become established into major river catchments of six of the seven countries where they were introduced including Belgium, Spain, Iberian Peninsula and France. *S. glanis* invaders are known to be resilient to environmental changes and are tolerant to changes in water quality (oxygen levels) and elevated water temperature in new environments (Britton et al. 2010; Gallardo et al. 2016a; Cucherousset et al. 2018).

The overall outcomes from the Organism module indicated that the risks about *S. glanis* at the lake fishery were of concern particularly as they were known to be present in other lakes (Avon Valley Lakes) in the same area. Moreover, there were some limitations in the study that may bias

S. glanis risk outcomes as only one lake fishery (North Field Main Pit Lake) from the Avon Valley Lakes complex was used in the ENSARS study. Determining the likelihood of *S. glanis* ecological impacts upon native fish communities in the river catchment was hampered by the gaps in knowledge of *S. glanis* potential invasiveness in England and Wales (Copp et al. 2009a; Rees et al. 2017).

E6.2.2 Pathway risk assessment module

In the study, the outcomes from the Pathway module indicated that the destination use and importation procedures posed a high risk of *S. glanis* riverine dispersal from the lake fishery. Destination use scored the highest risk with a mean score of 2.8 and a high confidence score of 3.0. Importation and farming pathways were ranked lower. The study involved co-operation of the bailiff and fishery owner who agreed to participate during visits to assess whether the lake fishery facilities were fit for purpose for holding *S. glanis* by EMAR and several fishery experts from the Environment Agency. The risk assessment was carried out under the regulatory guidelines from EU Water Framework Directive (2000/60/EC) (G05), Import of Live Fish Act 1980 (ILFA) and Prohibition of Keeping and Release of Live Fish (Specified Species) (England) Order 2014. These restrictions come into force into holding of non-native *S. glanis* into lake fisheries (Copp et al. 2016a; Gallardo et al. 2016a; Piria et al. 2017). The presence of *S. glanis* indicated that illegal (unregulated) releases of fish into the lake had taken place with the purpose to establish the species for long-term angling. As a result, it was likely that all life stages were present in the lake.

The enclosed lake was situated in the flood zone two which was classed as vulnerable to flooding risks (1 in 100 chance) with natural dispersal of *S. glanis* during flood events was considered likely. The lake had no flood defence structures present and was situated in close proximity to other floodplain water bodies with risk of water exchanges from the lake into the river catchment.

There were concerns about the bio security risks of the lake fishery as the lake was unmanned with no boundary fencing or gate around the northern edge. Anglers were likely to transfer fish between waters due to low site security. Other studies elsewhere have reported high risks of dispersal of non-native fish by human transfer into water bodies which were close to public access footpaths and unlikely to be noticed (Lintermans, 2004; Radinger &Wolter, 2014).

Moreover, some factions of the angling community have advocated the release of *S. glanis* into the wild in the UK as the addition of *S. glanis* is perceived as filling up empty niches in riverine catchments, yet these practises are likely to inflict significant damage upon native fish communities (Rees, 2010; Gozlan et al. 2013; Cucherousset et al. 2018). Consequently given the risk outcomes identified in this study there is a need to improve better fisheries management practises and public awareness around the detrimental impacts related to *S. glanis* dispersal into the wild (Arlinghaus et al. 2014; Rees et al. 2017).

E6.2.3 Facility risk assessment module

The study outcomes for the risks of *S. glanis* or non-target organisms escaping from the lake fishery unintentionally into the surrounding waters were moderately high risk (2.2) and given with high confidence scores (2.0). There were high risks of flooding from the lake into the river catchment as the lake fishery was situated in flood zone two and prone to flooding. There were no flood defence structures present at the lake fishery which suggested low efficacy in preventing water exchanges between the lake and river catchment. In addition, there was low site security at the lake (unmanned) so anglers were able to transfer *S. glanis* between waters. Unregulated releases of *S. glanis* into other waters were likely to take place occasionally. There were also gaps in information about the record keeping and maintenance of the facility with no quarantine procedures in place which indicated low efficacy in fisheries management.

There were possible risks of natural dispersal of *S. glanis* and cyprinids held in the lake being released into the river catchment during flood events. These dispersal pathways appear to play a lesser role in the spread of invasive fish because they are more likely to go unnoticed, and the ecological impacts underestimated as a result. *S. glanis* are present in several river catchments in southern England such as the River Colne, River Thames and River Chelmer with some of these introductions related to natural dispersal of *S. glanis* during flooding spates from lake fisheries in recent years (Copp et al. 2009a; Rees et al. 2017*).*

S. glanis are broadly tolerant to changes in water quality and pollutants and these factors are likely to favour adaptation into new environments in response to flooding and dispersal activity (Copp et al. 2009a; Cucherousset et al. 2018). Other studies assessing dispersal impacts from some lake fisheries in New Zealand reported that fish invaders such as *S. trutta* were able to adapt to environmental fluctuations. These fish were able to response rapidly to dispersal in

floodplain annexes and main channels and were more likely to become established into new environments (McDowall, 2004; Cucherousset et al. 2018).

Further research is needed in understanding the ecological impacts from flooding events as these multiple dispersal routes for non-native species introductions are likely to be exacerbated with climate change impacts. Damage caused by non-native fish is increasing with over 21% of total species in fish communities are exotic species in freshwater habitats in the UK (Almeida et al. 2013; Britton & Gozlan, 2013).

E6.2.4 Socio-economic risk assessment module

The results of Socio-economic module indicated moderately low risk with high confidence ranking for any potential economic losses to the local rural area or impacts to the lake fishery profits as a result of *S. glanis* specimens escaping from the lake into the surrounding area. The outcomes indicated that the magnitude in costs of *S. glanis* eradication from the lake were low (<£100K), although a mitigation action was recommended via regulatory enforcement and a targeted removal action plan for *S. glanis* in the lake.

This would involve regular fishery surveys carried out at the lake for *S. glanis* removal using seine netting, elecro-fishing and targeted angling so as to reduce fish abundance in the lake. Estimates in the costs for *S. glanis* removal from the lake were relatively low (£ 5-10K per annum), this excluded eradication costs. Results elsewhere have shown that periodic targeted angling (over 18 days) effectively reduced *S. glanis* abundance by 10% as population control in some lakes in the Czech Republic (Vejřík et al. 2019). In the study, the eradication option for *S. glanis* in the lake was relatively inexpensive (~ £80 K) but was considered unfeasible given the risks of rotenone contamination to other waters as the lake was sourced from groundwater springs. Rotenone (pesticide) treatments are widely used to control and eliminate invasive fish species from water bodies, yet growing awareness of the damage it causes to native biodiversity, particularly aquatic invertebrates, has led to alternative methods being used in the control of invasive species (Koupal et al. 2013).

Some telemetry studies have indicated that *S. glanis* are a sedentary species but are known to undertake migrations in watercourses including movements between main channels and floodplain annexes, so natural dispersal in the river catchment is likely to be slow. However, *S. glanis* are long lived species and undertake parental care of the young and these factors are likely

to favour establishment of self- sustaining populations into the catchment (Carol et al. 2007; Copp et al. 2009a; Vejřík et al. 2019).

In assessing the likelihood of reproduction and trophic impacts of *S. glanis*, climate change impacts and wide niche breadths may facilitate their invasion into the river catchment. Predicted warmer temperatures (2-5^{0}C) and increased frequency in flooding spates in the river catchment are likely to facilitate *S. glanis* downstream dispersal and establishment into the surrounding catchment. For example, several lower reach sites such as Avon Causeway, Knapp Mill, East Mills, Ringwood and Christchurch had water temperatures over 18ºC during summertime which may facilitate *S. glanis* colonisation. Shallow drainage channels and floodplain water bodies which warm up rapidly may be favourable spawning and breeding habitats (Copp et al. 2009a; Rees, 2010; Rees et al. 2017). In addition, the Climatch model results predicted climate matching of the river catchment with *S. glanis* native range which was likely to be favourable for establishment (mean climate match score of 7). Moreover, other studies have predicted similar climate match scores for *S. glanis* colonisation in major river catchments into the UK (Copp et al. 2009a; Britton et al. 2010; Copp et al. 2016a).

Nocturnal foraging and opportunistic trophic strategies may give *S. glanis* a competitive edge over native fish as by feeding at night they are able to exploit empty feeding niches in the river catchment where few native fish are nocturnal foragers (Copp et al. 2009a; Cucherousset et al. 2018). The addition of large bodied *S. glanis* (>30 cm TL) are likely to increase predation and competition for food resources with some impacts on the rest of the food chain and ecosystem functioning. Another aspect that may contribute to *S. glanis* potential impacts is that native species in the river catchment are likely to be more vulnerable to predation as they have not evolved phenotypic adaptations to coexist with invaders due to lack of evolutionary experience. Large bodied *S. glanis* predation, competition impacts may potentially affect endangered anadromous species such as *A. anguilla*, salmonid and resident water fowl populations in unique riverine habitats designated European and International conservation status. Other studies have indicated reduced abundance of these species by introduced *S. glanis* populations which have become invasive in the River Ebro in Iberia and other major river catchments in southwest France (Carol et al. 2009; Boulêtreau et al. 2018; Cucherousset et al. 2018).

There were some concerns that anglers were likely to transfer *S. glanis* into adjacent water bodies especially once the catchment had become known to have the species. Repetitive transfers by anglers of fish in small numbers due to their size or as juveniles were likely to have taken place as all life stages of *S. glanis* were present in the lake. These introductions are likely to go

unnoticed yet the repetitive nature of fish transfers by anglers may inflict significant damage to native fish communities. For example, the involvement of anglers was related to the invasion of *C. carpio* into isolated freshwater habitats in large countries such as Australia which are particularly vulnerable to invaders because of the potential habitat niches available (Cambray, 2003; Copeland et al. 2017).

In most cases, mitigation or eradication measures of non-native fish species are difficult to implement, once they have become introduced into riverine catchments. The associated expenses with fish removal are likely to escalate with few successful cases documented (Copp et al.2010; Britton et al. 2011a; Gozlan et al. 2010; Copp et al. 2016a). In this study the magnitude of potential market loss or local impacts caused by *S. glanis* escapees to the surrounding area was ranked as low risk at1.5. *S. glanis* were already present in other lakes and valued as a sport fish, the economic impact would likely be positive with little evidence of adverse impacts on biodiversity and ecosystem functioning. Nevertheless, there were possible risks of disease transmission with *S. glanis* as some of the parasites found on specimens in the study may cause exposure to diseases and parasites to native fish. *S. glanis* are known to host pathogens of concern such as epizootic haematopoietic necrosis virus (EHNV) and European sheatfish virus (ESV). These may be harmful to native fish species and amphibians (Peeler et al. 2011).

E6.2.5 Infectious agent risk assessment module

The results of the Infectious agent module indicated that the likelihood of disease emergence from infectious agents detected upon recaptured *S. glanis* specimens in the lake fishery were moderately low risk but were given with low confidence scoring. The risk outcomes and confidence score ranking were given by the multidisciplinary team of expert assessors from the Environment Agency and EMAR. The variation in responses and patterns in the confidence scoring were transparent in determining the possible risks of disease emergence from *S. glanis* to fish communities.

In the study, a novel specialist parasite, ancyrocephalid monogenean parasite *T. vistulensis* (Sival, 1932) was detected on recaptured *S. glanis* specimens, as a gill infection. It was a new recording in the UK with little known about its potential adverse impacts to native fish species or spread of disease emergence in *S. glanis* introduced ranges. Gaps in knowledge are a recurrent issue with many exotic fish parasites with little research known about their biology or likelihood of host switching to other fish which is a key concern. For example, *S. glanis* are carriers of exotic specialist parasite *Pseudotracheliastes stellifer* and non-native pathogen European Sheatfish Virus (ESV) which are thought to be pathogenic to other fish species (Sweetman et al. 2006; Copp et al. 2009a; Peeler et al. 2011; Reading et al. 2012).

The other infectious agent in the present study was a non-native generalist parasite *E. sieboldi* (Nordmann, 1832) which was detected as a gill infection on recaptured *S. glanis*. This is a widespread parasitic gill infection found on most freshwater fish species in the UK yet heavy infestations may cause fish mortality (Reading et al. 2012; Copp et al. 2016a). Both infectious agents identified on *S. glanis* specimens were classed as low risk because *T. vistulensis* is a specialist parasite and considered unlikely to switch host to other native fish species whereas *E. sieboldi* is a generalist parasite already widespread in England and Wales. However, the study findings have brought attention to the gaps in knowledge about the risks of infectious agents detected upon *S. glanis* which can be harmful to native fish species. Changes in risk status of non-native fish species has been illustrated with *P. parva* with growing awareness and identification that the species is a carrier of the rosette agent parasite (*Sphaerothecum destruens*) which can switch host to other native fish species and cause mortalities (Copp et al. 2005a; Gozlan et al. 2005; Gozlan et al. 2006; Britton, 2013).

The risks of disease transmission with non-native fish species introductions are likely to be exacerbated in future with increasing water temperatures (>20^0C) driven by climate change in aquatic habitats. Increasing water temperatures can accelerate reproductive life cycles of parasites and disrupt parasite-host relationship in favour of parasites to the detriment of fish survival. It is thought that seasonal changes contributed to several disease outbreaks of ESV in *S. glanis* farming which decimated fish stocks in Germany and Hungary (Whittington et al. 2009). Consequently, the survival and persistence of exotic fish parasites are likely to be enhanced with warmer waters with higher risks of disease spreading from Europe into the UK (Rahel, 2007; Reading et al. 2012; Britton, 2013; Fobert et al. 2013; Gozlan et al. 2014).

The risk outcomes underlined some weakness in the risk assessment such as the gaps in knowledge of exotic infectious agents carried by *S. glanis*, and as a result, the risks of disease transmission to native fish communities maybe underestimated. Determining the likelihood of exotic parasites carried by *S. glanis* likely to be harmful was difficult and may have some bias and representative of the subjective responses given by the expert assessors included in the study. Nonetheless, there is a clear need for more research around raising awareness about the potential damage inflicted on native biodiversity by *S. glanis* and risks of disease transmission. From this more responsible management practises can be implemented (Gozlan et al. 2006; Peeler et al. 2011; Andreou et al. 2012; Sheath et al. 2015).

E6.4. Conclusions

In the study, the ENSARS modules results distinguished between differences in the risks and potential impacts of ecological and socio-economic harm with *S. glanis* escaping from the lake into the river catchment. The highest risk outcomes and confidence ranking were for the Organism, Facility and Pathway modules which indicated that natural dispersal via flooding events or by angling involvement were likely to result in *S. glanis* being released into the river catchment from an unlicensed fishery that was poorly managed. However, following compliance with targeted improvements of an action plan for non-native fisheries management provided by regulatory bodies (Environment agency and CEFAS) the fishery has remained open.

The risks of predicted warmer temperatures (2-5^{0}C) and increased frequency in flooding events related to climate change were likely to facilitate *S. glanis* establishment into the river catchment. Some lower reach sites and flood plain waters had water temperatures over 18ºC during summertime which may be potential spawning and breeding habitats (Copp et al. 2009a; Rees, 2010; Rees et al. 2017). The Climatch model results predicted climate matching of the river catchment with *S. glanis* native range which was likely favourable for colonisation.

The Infectious agent module risk outcomes brought attention to the gaps in knowledge about the risks of disease transmission and harmful impacts to native fish species from the parasites detected on the *S. glanis*. Subsequently, the risks of adverse ecological impacts were likely to be underestimated and were given low confidence ranking in the study (Gozlan et al. 2014; Copp et al. 2016a).

Finally, the Socio-economic module results indicated low risks of economic depression to the local area or lake fishery profits as *S. glanis* were already present in other lakes with little evidence of adverse impacts on biodiversity. Although, this may be liable to change in future years with *S. glanis* populations becoming invasive in the river catchment facilitated by thermal changes related to climate change.