How does parental contribution affect offspring performance in anadromous and resident brown trout, *Salmo trutta L.*?

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature Date

ABSTRACT

The brown trout, Salmo trutta L., displays one of the most variable and polymorphic life-history strategies of all the salmonids. In some populations, individuals spend their whole life-cycle in the river (freshwater-resident) whereas in others, a varying proportion migrates to sea for variable amounts of time to better feeding conditions before returning to spawn (anadromous). The 'decision' if an individual will migrate or not will be determined by the balance of the costs and benefits of following a particular life-history strategy. The balance of these, which do not affect males and females equally, will determine the future success (measured by fitness) of each strategy. This research addresses the influences of parental contribution, mainly maternal effect, of anadromous and freshwater-resident brown trout on offspring performance and subsequent life-history.

A partial migratory population of brown trout was studied in the Tadnoll Brook, one of the seven major tributaries on the River Frome. The tributary is classified as a circum-neutral chalk stream, 9.9 km long with a catchment approximately 50 km2. Carbon and nitrogen stable isotope analysis (SIA) was used to quantify maternal reproductive contribution of anadromous and freshwater-resident brown trout to offspring and determine the future success (measured by fitness) in terms of size and time of emergence. A panel of 12 microsatellite loci was used to assign parentage to 0+ parr. Using field data collected over 1.5 years on individual fish, this study tested parental influence on offspring performance in terms of size and growth rate and calculate the reproductive contribution of maternal/paternal anadromous and freshwater-residents. Adult life-history strategy was identified using a combination of results from SIA, PIT tag data and ecological data (body size, temperature). Parr life-history strategy (1+) was inferred using PIT tag detection data.

The results of the SIA indicated fry of anadromous females emerged earlier and at a larger size than fry of freshwater-resident females. Parentage assignment of parr was low (28 %), with 8 parr assigned to both parents and 43 assigned to only a single parent. There was no detectable effect of parental life-history on parr size and growth rate, however the raw data may suggest offspring of anadromous parents have an early size advantage but a slower growth compared to offspring of freshwater-resident parents during the first year of the parr stage. Twenty-four percent of the offspring were

identified as putative smolts at 2+ and both forms interbred and could produce offspring of each life-history. Estimates of reproductive contribution (SIA and growth) show a higher proportion of anadromous females and males (growth only) contributed to offspring production.

The results of this research indicate that the maternal anadromous contribution is higher in the Tadnoll Brook population, affording fitness benefits to their offspring during early ontogeny such as size advantages and emerging at a more profitable time to establish feeding territories. Adult life-history does not appear to influence juvenile (0+ parr) life-history but may have an effect on offspring performance. The presence of both forms in the population suggests the anadromous fitness benefits to offspring may only have an affect during ontogeny and early stages of growth. Then after juveniles reach a size threshold environmental factors influence offspring life-history, resulting in the largest parr with the fastest growth adopting an anadromous life-history.

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CHAPTER 1: INTRODUCTION

The factors affecting variations in early life-history traits in animals are important as they influence both population dynamics and evolution, and as such have received much interest from evolutionary ecologists (Rideout *et al.*, 2004; Uller, 2008; Badyaev and Uller, 2009). Of all the salmonids, brown trout (*Salmo trutta* L.) display one of the most variable life-histories; with strategies ranging from freshwater-resident to anadromous trout and populations can exist in both sympatric and allopatric forms. This thesis will address the effects of parental investment and maternal effect of freshwater-resident and anadromous brown trout on offspring performance and early life-history. Throughout the literature descriptions of parental forms of contribution and influence on offspring are sometimes confused; the following sections will define three main types (parental effect, investment and care) which ultimately all result in the same aim, i.e. to further reproductive success.

1.1 BROWN TROUT, *SALMO TRUTTA* L.

1.1.1 GEOGRAPHICAL DISTRIBUTION

Brown trout are indigenous to Europe, North Africa and Western Asia. Distribution ranges from Iceland, North Scandinavia and Russia in the north, the northern coastline of the Mediterranean Sea and the Atlas mountains of North Africa in the south and the European coastline in the west (Elliott, 1994) The eastern distribution is the Ural mountains and the AmuDar'ya River drainage of the Aral Sea in the east (MacCrimmon *et al.*, 1970) though this is the least defined.

Anadromous trout distribution is less extensive than their freshwater counterparts and populations have been found in latitudes north of 42° in Western Europe. Population distribution has been found in rivers flowing into the White Sea, Cheshkaya Gulf, North Sea, Baltic Sea, English Channel and the Irish Sea in the north and the Bay of Biscay in the Atlantic in the south (Frost and Brown, 1967; Elliott, 1994).

The ability to adapt to and colonise new habitats through their variability in ecological requirements mean brown trout have been introduced into at least twenty-four countries outside Europe successfully since 1850 and are now classed as a global species (Elliott, 1994). Varying populations of freshwater-residents and anadromous

trout living in allopatry and sympatry exist throughout the world. Internationally, wild (and hatchery reared) populations of brown trout are a valuable resource for both recreation and commercial fisheries.

1.1.2 LIFE-HISTORY

The brown trout is an iteroparous species that often reproduces two or more times (Berg *et al.*, 1998) during its life cycle. Maximum age has been found to be correlated with low water temperature (Jonsson *et al.*, 1991) and the average age between 5-8 years (Frost and Brown, 1967).

Mature adults return to their natal stream and spawn on gravel and stone beds (Crisp and Carling, 1989) usually in running water although lake spawning populations have been identified (Brabrand *et al.*, 2002; Heggenes *et al.*, 2009). Brown trout spawn in winter and in the UK typically between November and January. Females dig a depression in the gravel where she lays a batch of eggs, usually there are several larger males that compete but the most dominant will fertilise most if not all of the eggs (Largiader *et al.*, 2001). Multiple paternity within a redd is not uncommon in salmonids (Garcia-Vazquez *et al.*, 2001). Smaller male parr which sexually mature early in freshwater whilst still physically appear as juveniles, adopt sneaking behaviour to fertilise females While the female is being courted by the larger males, the smaller parr will rush in and fertilise a batch of eggs or can sneak in unnoticed while the males are competing against each other (Broberg *et al.*, 2000). After the eggs are fertilised the female immediately covers them with gravel and repeats the process a number of times moving slightly upstream, resulting in a series of pockets of eggs called a 'redd' (Ottaway *et al.*, 1981). The deeper the eggs are buried the more protected they are from being washed out, predators and other females digging (Steen and Quinn, 1999; Tonina *et al.*, 2008). Previous studies on brown trout redd structure have demonstrated that female body length is positively correlated with depth of egg burial, Elliott (1984b) showed that in a wild population of anadromous trout (fork length: 25-45 cm) egg burial was *c*. 17 cm compared with freshwater-residents (fork length: 17-29 cm) at only *c*. 4 cm. However, some studies suggest that in chalk streams there is no correlation between female body length and egg burial depth (Crisp and Carling, 1989).

Embryonic development is temperature dependant (Elliott and Hurley, 1998a) and degree day models can be used to predict date of emergence. Degree day models are based on calculating average daily temperatures from two time points. Alevins hatch in the spring and remain in the redd for five to six weeks while feeding endogenously on

their yolk sac until it is nearly used up then emerge as fry (~20 mm). This is the most critical period of the life-cycle as mortality is high (Elliott, 1994). Fry soon set up feeding territories, forming dominance hierarchies and compete for resources (Elliott, 1990); here size is a clear advantage and smaller individuals are often are forced to less productive habitats. Those that are successful at this stage will disperse throughout the stream to take advantage of the better feeding opportunities to attain increased growth. After a few weeks from emergence trout reach the parr stage with black stripes on the sides of their body and the characteristic orange/red adipose fin (Elliott, 1994).

Young of the year parr select shallow fast-flowing microhabitats to feed (Greenberg *et al.*, 1996). Trout are opportunistic feeders and parr often feed on a diet of insect larvae and adult insects, including chironomids. A larger gape-size through increased growth allows fish to forage on relatively larger terrestrial and aquatic prey including *Gammaus*, *Baetis* and *Simuliidae* (Langeland *et al.*, 1991; Steingrimsson and Gislason, 2002). In some populations fish may begin feeding piscivorously, Jonsson *et al.* (1999) showed that freshwater trout switched to fish feeding at 17.5 cm, with younger fast growers switching before smaller slow growers.

1.1.3 LIFE-HISTORY STRATEGY

Brown trout exhibit great variability in its life-history strategies (Jonsson, 1985) and four polymorphic life cycles have previously been described (Elliott, 1994). Figure 1.1 summarises the life-history of brown trout witnessed in populations in the South-West of England, natural populations in lakes here are rare (as opposed to stocked lakes for angling) therefore the life-history is not discussed in detail. Trout spend their first year of the life cycle in the natal stream to feed, this is usually a period of rapid growth, where the variation and size of prey increases with parr body size (Grey, 2001).

The simplest strategy; trout remain in the natal stream as freshwater-residents for their whole life cycle, this includes sexually mature precocious parr. The remaining strategies involve an ontogenetic habitat shift of varying degrees. In the second strategy, trout migrate to the parent river (or lake) usually when 1+ or 2+, then return to the natal stream to spawn. The third strategy involves partial migration as a smolt (see section 1.1.4) to the estuary, although this life cycle is not well documented in the study river. The fourth of the strategies involve parr migrating to sea as smolts for variable amounts of time before returning to spawn (anadromous).

Varying proportions of sympatric and allopatric populations of freshwater-resident and anadromous brown trout exist universally, exhibiting flexible life-history strategies and successful reproduction.

1.1.4 SMOLTIFICATION

Migration occurs during different developmental stages, the earliest being during emergence from the redd into the water column to feed and grow. In anadromous populations of brown trout parr can undergo parr-smolt transformation from 1+; usually this is determined in the previous autumn (Wright *et al.*, 1990; Thorpe *et al.*, 1998) and migration to the sea is in the spring.

While in freshwater, the parr-smolt transformation is driven by the endocrine system, increasing hypo-osmoregulatory capacity and influencing a series of morphological, physiological, biochemical and behavioural changes to pre-adapt to a migratory life in the marine environment (Arnesen *et al.*, 2003). The endocrine systems involved in the smoltification process include promotion of the growth hormone (GH), insulin-like growth factor I, cortisol and thyroid hormones and the inhibition of prolactin (Barron, 1986; McCormick, 2009). Increasing GH and cortisol induce salinity tolerance (Barron, 1986; Bjornsson, 1997), increased thyroid hormone plays part in morphological and behavioural changes (McCormick *et al.*, 2000; McCormick *et al.*, 2002; Ojima and Iwata, 2007) and prolactin is inhibitory to the development of salinity tolerance (McCormick, 2009). Morphological changes include a decrease in condition factor with a bigger increase in length compared to mass (Arnesen *et al.*, 2003) and a fusiform body shape adapted for migration (Jonsson, 1985). The deposition of metabolic by-products, guanine and hypoxanthine, in the skin and scales transform dark parr into silvering fish (Folmar and Dickhoff, 1980; McCormick *et al.*, 1998). This counter shading is common in many pelagic fish and is believed to be an adaptive predator avoidance tactic (McCormick *et al.*, 1998). The physiological changes include an increase in hypo-osmoregulatory capacity including gill Na^+, K^+ -ATPase activity (McCormick *et al.*, 1998; Arnesen *et al.*, 2003), lipid metabolism and protein synthesis (Folmar and Dickhoff, 1980; Dickhoff *et al.*, 1997) and an increase in buoyancy (Saunders, 1965). The behavioural changes incurred during parr-smolt transformation are an increase in negative rheotaxis (downstream migration) and reduced territorial behaviour (McCormick *et al.*, 1998).

The physiological and morphological changes associated with the process of smoltification are initiated by one or more environmental factors. Changes in

photoperiod in the spring months are thought to provide temporal cues to synchronise the parr-smolt transformation processes (Jonsson and Jonsson, 2002) and initiate downstream migration during darkness. The increasing water temperature in the spring may positively affect the developmental rate of pre-smolts (Jonsson and Jonsson, 2002) and to some extent high water flow (Jonsson, 1991).

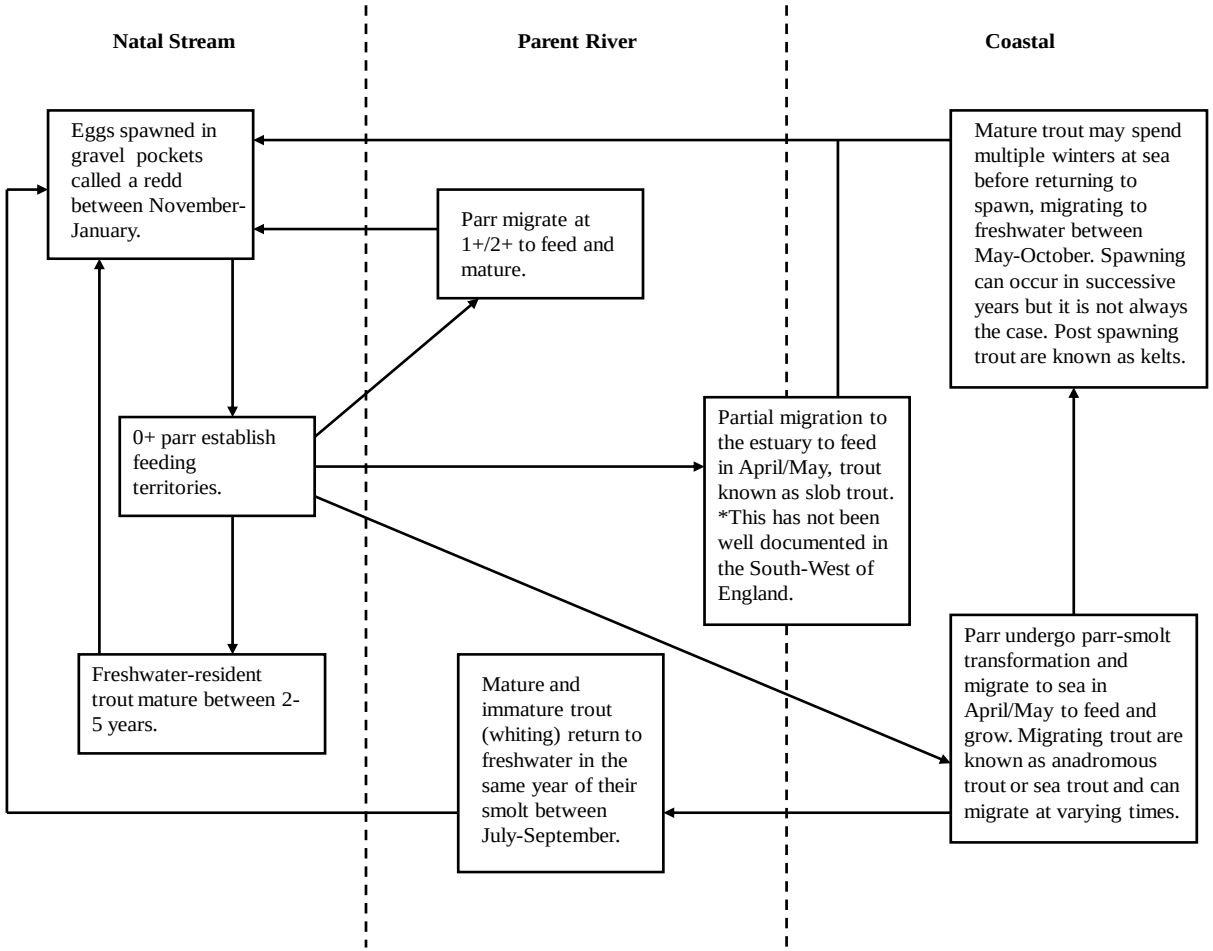

Figure 1. 1.1. Life-history strategies of brown trout

The theoretical identification of the determinants of smoltification in salmonids, particularly in Atlantic salmon *Salmo salar* are well studied (Morin and Døving, 1992; Økland *et al.*, 1993; McCormick *et al.*, 1998), yet in brown trout there is limited information. It is believed that genes influence smoltification (Jonsson, 1985) as only migratory morphs demonstrate morphological and developmental changes. Nichols *et al.* (2008) identified a region of the genome associated with smolt traits in Rainbow trout, *Oncorhynchus mykiss*. The onset of parr-smolt transformation is thought to be determined by size (McCormick and Naiman, 1984) irrespective if the environmental conditions have been reached. Smolt transformation may be triggered in the summer if fish exceed a genetically pre-determined threshold size in the following spring and if this size is not reached then parr remain in the river for another year (Metcalfe, 1998). Finstad and Ugedal (1998) showed that larger brown trout in hatchery reared experiments reached hypo-osmoregulatory capacity earlier before smaller parr under the same conditions and Bohlin *et al.*, (1996) found that larger, older brown trout parr smolted earlier than smaller, younger parr. However, growth rate appears to be an important factor in determining an ontogenetic niche shift, as faster growing parr tend to smolt when small and at an earlier age (Jonsson, 1985; Økland *et al.*, 1993) and often results in more than one year class of smolts in a population (L'Abeé-Lund *et al.*, 1989).

1.1.5 HOMING

It has been proposed that the homeward migration of brown trout to the natal stream is directed by population specific pheromones (the *pheromone* hypothesis) (Nordeng, 1977). Juvenile salmonids have shown positive rheotaxis to odorants of their own population in transplant experiments (Nordeng and Bratland, 2006).

1.1.6 MATURATION VS. SMOLTING AND MIGRATION

Maturation is an energetically expensive process (Thorpe, 1994), particularly in females who invest large amounts of energy into egg production compared to sperm production in males. Female Atlantic salmon can invest approximately six times more energy into offspring compared to males (Fleming, 1996). Brown trout freshwater-residents tend to mature between 2-5 years while anadromous trout gain maturity after a variable period of growth at sea. Once maturity is gained in freshwater, fish do not usually smolt. Fast growing parr may smolt at a smaller body size because they are energetically constrained in the freshwater environment and through migration can increase their metabolism and food intake (Økland *et al.*, 1993; Forseth *et al.*, 1999). Within anadromous populations there is bias to which sex remains resident and which adopt an

anadromous strategy. Usually males predominate in freshwater, maturing earlier and at a smaller size (precocious parr) than freshwater-resident females, whereas anadromous males mature at more variable ages (Jonsson, 1985). Female brown trout with a faster growth rate are generally more migratory (Forseth *et al.*, 1999) and can benefit from the increased reproductive advantages (fecundity and egg size) (Jonsson, 1985; Jonsson and Jonsson, 1999).

Sexual maturation appears to inhibit smolting in brown trout (Jonsson, 1985; Thorpe *et al.*, 1998) as there are two alternative strategies; mature and remain in freshwater or migrate and delay maturity. Defining what triggers fish to choose either of these strategies may be a response to increasing fitness by delaying maturation and migrating to a more profitable environment, a size-specific constraint or a genetic predisposition (see section 1.4.2).

1.1.7 MIGRATION VS RESIDENCY

Fish that migrate to saline environments, which tend to be richer in food resources, benefit from faster growth (Thorpe, 1987; Gross *et al.*, 1988) and a larger body size (Gross, 1987; Jonsson and Jonsson, 1993). Freshwater-resident fish tend to have lower growth and a smaller body size as they are constrained by feeding rates and food availability in the freshwater environment (Bachman, 1984). Jonsson (1985) showed that on average anadromous females and males were 17.5 and 18 cm larger compared to resident females and males respectively. In females, a large body size may result in anadromous females spawning in preferential sites and males obtaining higher competitive ability for females (Gross, 1985; Quinn and Foote, 1994). In comparison, male freshwater-residents may adapt to this size-dimorphism by adopting sneaking mating strategies to increase reproductive success (Garcia-Vazquez *et al.*, 2001). The increased energy acquired at sea and the larger final body size, promoted gonadal investment and fitness in anadromous fish (Jonsson and Jonsson, 1997; Klemetsen *et al.*, 2003). Another potential benefit of a large body size includes the ability to swim long distances against high current velocities (Jonsson *et al.*, 1990). Freshwater-resident fish are subject to adverse environmental conditions such as low water flow and icing up of streams (Nikolskii, 1963) whereas anadromous fish may avoid such conditions.

Migrating long distances is energetically costly and individuals face an osmoregulatory challenge (Gross, 1987). Freshwater-resident fish do not need to utilise energy on migration, however up to 70 % of energy can be invested for spawning (Jonsson *et al.*, 1997), therefore, fish may never replenish their energy in low productive

environments. Further costs of migration include: increased mortality; Nordeng *et al.* (1985) estimated annual survival rates for anadromous Arctic charr were 0.52 compared to 0.68 in freshwater-residents and an increase in predation both during smolt migration and at sea (Hvidsten and Lund, 1988; Dieperink *et al.*, 2001). Hvidsten and Lund (1988) estimated 20 % of Atlantic salmon smolts were being eaten by cod and saithe in the estuary of the River Orkla (Norway). Migration also exposes fish to a wider range of potential contaminants (Waring and Moore, 2004) and disease (Gross, 1987).

The 'decision' if an individual will migrate or not will be determined by the balance of the costs and benefits; growth and survivorship advantages of migration plus the costs of migration must outweigh the advantages to remain resident for migration to succeed (Gross, 1987). As more females adopt an anadromous life-history (Forseth *et al.*, 1999), it is likely that migration affords females better fitness benefits in terms of body size and fecundity (Jonsson and Jonsson, 1997).

1.2 FACTORS AFFECTING LIFE-HISTORY STRATEGIES

The variation within populations of life-history strategies, especially in the salmonids, has generated interest by applied researchers in asking the fundamental question- is life-history strategy controlled by environment or genetics? By testing specific hypotheses, this study will address this question in an attempt to understand the complexities of life-history. To investigate this, literature on parental investment and effect were reviewed in general, then in fish and then specifically in brown trout.

1.2.1 PARENTAL EFFECT

Parents can influence the phenotype and life-history traits of offspring through genetic and non-genetic contributions (Kirkpatrick and Lande, 1989; Shikano and Taniguchi, 2005; Räsänen and Kruuk, 2007; Uller, 2008). Parental effects are fundamental to offspring development and ultimately enable evolution by natural selection through the provision of resources (Badyaev and Uller, 2009). The majority of studies when assessing parental effect define the influences (and hence 'parental effect') as non-genetic effects (environment and/or parental phenotype) (Mousseau and Fox, 1998; Gilchrist and Huey, 2001; Rotem *et al.*, 2003). Genetic based studies have researched the effect of parental genotype on offspring phenotype (Branicky *et al.*, 2000; Benard *et al.*, 2001), though the effects appear more restricted by genetic variation of the parents. Maternal effects have been studied by many authors (Lindstrom, 1999; Einum and Fleming, 2000a; Plaistow *et al.*, 2006; Marshall and Uller, 2007; Benton *et al.*, 2008; Crean and Marshall, 2009) as differences in contribution result in variation in size of

eggs, offspring performance and affect in population structure. Paternal effects have been described as synonymous with genetic effects (Bang *et al.*, 2006) as the only significant contribution to offspring is sperm i.e DNA only. However, in some taxa paternal effects have been found to be influenced by environmental conditions (Watson and Hoffmann, 1995) and through providing a genetic contribution in the form of a nuptial gift, males provide nutrients to the offspring (Smedley and Eisner, 1996). When parents experience variable environmental conditions, they may select for non-genetic resources to be translated to offspring (Bonduriansky and Head, 2007) to maximise survival and fitness. Crean and Marshall (2009) suggested that when parents cannot predict the offspring environment, some maternal parents adopt a 'bet-hedging' strategy within clutches of varying offspring size to increase the chance of survival.

1.2.2 PARENTAL INVESTMENT AND CARE

Trivers (1972) views and discussion on parental investment has been one of the most valuable explanations to evolutionary ecologists on parental contribution. He developed the term *Parental Investment* and defined it as "any investment by the parent in an individual offspring that increases the offspring's chance of survival (and hence reproduction success) at the cost of the parent's ability to invest in other offspring". Trivers further proposed a hypothesis (Parental Investment Hypothesis) that females produced larger gametes compared to males (anisogamy) and invested larger energy resources into gamete production. Males would exhibit extra-pair mating strategies as they could replenish their supply of sperm more easily as it is relatively cheap compared to eggs (Kokko and Jennions, 2008). By mating more, males would provide less care to increase their reproductive success whereas females may be constrained due to the large investment but it would be more profitable to continue with care. The evolution of female-biased care was believed to originate from anisogamy and as investment and care differed between the sexes, sexual selection on males intensified.

Further to Trivers work, Clutton-Brock (1991) described parental care as any form of behaviour that will increase an offspring's fitness including production of eggs, care of offspring, feeding and protection. Parental investment includes only some of these behaviour or components of care that increase offspring survival and fitness at the cost of the parents residual reproductive value i.e. energy for egg production or nuptial gifts.

Parental care varies among and between species; in mammals ~90 % females provide care (Clutton-Brock, 1991), in birds 90% of species display biparental care,

with varying degrees of investment from each sex (Kokko and Jennions, 2008), reptiles usually display biparental or female care (Reynolds *et al.*, 2002), in invertebrates female care is the most common (Zeh and Smith, 1985) and female care is most common in insects (Tallamy and Brown, 1999). For parental care to evolve and be maintained the benefits must outweigh the costs as provision to young is costly in terms of time and energy and will potentially reduce further reproduction (Trivers, 1972).

The currency of parental investment is cost (Trivers, 1972) and for females large energy resources are required for egg production. Nilsson and Raberg (2001) measured the daily metabolic cost of egg formation in female great tits, *Parus major* at 27 % and Vezina and Williams (2002) estimated the egg production costs in female European starlings, *Sturnus vulgaris* at 22 %. By providing each egg with large energy resources females can potentially increase offspring fitness, however, they are constrained by body size and species can adapt a trade off between size and number of eggs to maximise success (Roff, 1992). Males have a more plastic approach to increasing their fitness; some employ sneaking strategies (Broberg *et al.*, 2000) or extra-pair mating (Parker and Tang-Martinez, 2005) and some develop secondary sexual characteristics (Von Hardenberg *et al.*, 2007). Attractiveness may increase male fitness through sexual display (Møller, 1994) or by mating with more attractive females (Møller and Thornhill, 1998). Sexual conflict occurs between the sexes as they have conflicting optimal fitness strategies, with all aspects of investment subject to conflict (Wedell *et al.*, 2006). Sexual selection acts differently on each sex, with morphological differences typically relating to body size (Fairbairn *et al.*, 2007) as many females are larger (sexual dimorphism) or differences in gamete size (gamete dimorphism). Sexual conflict may cause differences in sex ratio and allocation in offspring and in some species one sex may be more costly to invest in and rear, despite equal genetic contributions from each parent (Wedell *et al.*, 2006). In the fallow deer, *Dama dama*, male and female offspring both suckle for the same length of time but males drink faster and therefore are more energetically costly to the mother (Birgersson *et al.*, 1998). Therefore when there is a higher cost in investment and care in one sex, parents will select for the less costly sex and alter the sex ratio of offspring.

Trivers hypothesis paved the way for many studies in parental investment and care (Møller and Thornhill, 1998; Gross, 2005; Moore and Pizzari, 2005; Olson *et al.*, 2009) though some researchers highlighted flaws in his work (Kokko and Jennions, 2008) which lead to alternative hypotheses for sex-biased parental care (Queller, 1997).

Today, although there appears to be still some confusion with terminology, parental investment is defined as any behaviour or contribution pre-fertilisation in eggs and sperm and parental care is any behaviour post-fertilisation including nest guarding and feeding (Dziminski *et al.*, 2009).

1.2.2.1 *MATING STRATEGIES*

Through evolution there has been the development of different mating systems; monogamy, polygyny, polyandry and promiscuity (Clutton-Brock, 1991; Burley and Johnson, 2002). Different taxa display varying mating strategies, monogamy is typically common in species of birds while some reptile and fish species display promiscuity (Burley and Johnson, 2002; Kohda *et al.*, 2002; Theissinger *et al.*, 2009). It has been proposed that female care first evolved from promiscuous mating systems with no parental care (Burley and Johnson, 2002). As a consequence of anisogamy, females benefit from care and provision (nutrients/energy) for their offspring, which in some species care will involve nesting, protection and feeding. The development of (social) monogamy is believed to be a consequence of increasing male investment in reproductive contribution, which eventually confined species to adapting a monogamous mating strategy with some species developing male care (Trivers, 1972).

1.3 PARENTAL INFLUENCE IN FISH

1.3.1 PARENTAL CARE

Fish species display the most variable forms of parental care of all the taxa. Gross (2005) calculated that 20 % of families displayed care, of which ~40 % provide male only care, 30 % provide female care and 20 % provide bi-parental care. The remaining proportion of fish species provide no parental care (Reynolds *et al.*, 2002).

Despite male only care displaying in other taxa such as amphibians (Beck, 1998), male only parental care is of the highest proportion in fish. Gross (2005) suggested the evolution of male only parental care can be explained by the Williams Principle (cost/benefit analysis of current care and future care). Female reproductive success is shown to be positively related to body size (Jonsson, 1985) though not to the same degree in males. Current parental investment in offspring is likely to affect future investment in resources and may affect parental body size; this affect will be greater for females as size is important to fitness and success. Therefore, males are expected to obtain lower future costs compared to females for the same fitness benefits.

1.3.2 PARENTAL EFFECT AND INVESTMENT

Variation among and between eggs and offspring size is mainly due to maternal effect and provisioning of resources and the size of larvae has been found to be an important factor negatively related to starvation in some species (Miller *et al.*, 1988). The affect of maternal condition on offspring size and survival in fish has been studied by many authors; Gagliano and McCormick (2007) found egg quality (yolk sac and oil globule size) was significantly increased in food supplemented damselfish, *Pomacentrus amboinensis* females but egg size and offspring survival were not affected. Further, Donelson *et al.*, (2009) found offspring of supplemented damselfish, *Acanthochromis polycanthus* females were larger at hatching, had increased survival when reared on a low-quality diet but in offspring of both types of supplemented females (high and low) there was no change to survival. This suggests that maternal investment in eggs affects offspring performance and survival more under strong growth limitations in poor environments. Variation in egg size has been shown to increase with male mating size and attractiveness for example in the banggai cardinal fish, *Pterapogon kauderni*, females invested in larger, heavier eggs when paired with larger males (Kolm, 2001). Females may adjust individual investments in eggs in response to parental care; Taborsky *et al.*, (2007) found females of the cooperatively breeding cichlid, *Neolamprologus pulcher*, reduced egg size in response to increasing helper (alloparents) number as they protect offspring and potentially reduce mortality.

Compared to maternal investment, there have been relatively few studies on paternal investment in fish (Høie *et al.*, 1999a; b; Einum, 2003; Rideout *et al.*, 2004). This may be because some researchers believe paternal investment and influence on offspring is not as important as that of the mother due to the disproportionate energy resources between mother and father to the offspring. The effects of sperm competition and investment in gonadal tissues have been reported in many species (Stockley *et al.*, 1997; Pyron, 2000) and males are expected to invest more in gonadal tissues when sperm competition is high. Within fish species, competition is expected to be higher for external fertilising fish due to dilution in water (Petersen, 1991) and the competition with sneakers (Stockley *et al.*, 1997). The family Syngnathidae of pipefishes and seahorses, display a wide range of paternal care and mating strategies from externally fertilised eggs attached to the skin of the male (pipefish) to eggs being internally fertilised and protected in a brood pouch (seahorse) (Dawson, 85 reviewed in Kvarnemo and Simmons, 2004). However, Kvarnemo and Simmons (2004) found no difference in testes mass between species or brood type.

1.4 PARENTAL INFLUENCE IN BROWN TROUT

1.4.1 PARENTAL INVESTMENT AND MATERNAL EFFECTS

Brown trout display a polygynandrous-promiscious mating system, after fertilisation neither sex provides care to eggs or future offspring, only pre-fertilisation provisioning (investment). It is believed the importance of male provisioning is less compared to the female who invest large resources into egg production. Males may increase there reproductive success by displaying extra-pair mating strategies (Largiader *et al.*, 2001). There is evidence that employing 'sneaking' strategies to compete with larger aggressive males may increase reproductive success in mature parr but at a risk of injury and often anadromous males benefit by increasing their own success (Broberg *et al.*, 2000). Maternal traits are shaped by natural selection as a phenotypic response to environment heterogeneity (Mousseau and Fox, 1998). Maternal effects can influence egg size, offspring size, performance and survival (Bagenal, 1969; Elliott and Hurley, 1998b; Einum and Fleming, 1999). Reproductive success has been shown to increase exponentially with body size (Wootton, 1990). In fish, fecundity increases with body size as there is more energy available for production and a larger body cavity (Jonsson and Jonsson, 1997), egg size can also increase with body size (Thorpe *et al.*, 1984).

In separate allopatric populations of anadromous and freshwater-resident brown trout in Windermere (UK), anadromous fish produced more, heavier eggs (*c*. 510 eggs, 100 mg mean wet mass) compared to freshwater-resident trout (*c*. 220 eggs, 60 mg) (Elliott, 1984b). The advantage of producing large eggs may increase the offspring's fitness by providing larger energy resources (yolk) to withstand extreme environmental conditions (Einum and Fleming, 1999; Armstrong and Nislow, 2006), increased growth (and survival) and the ability to avoid predators more efficiently (Hinckley, 1990).

Females may maximise their fitness by increasing resources to each individual, therefore increasing offspring fitness. However, production of larger eggs is costly to the female (Trivers, 1972), therefore there cannot be a simultaneous maximisation of both size and number of eggs thus a trade-off must occur (Roff, 1992). Trade-offs in egg size and number in salmonids have been witnessed in anadromous and freshwater-resident populations (Fleming, 1996; Jonsson and Jonsson, 1999; Olofsson and Mosegaard, 1999). In partial migratory populations of brown trout, freshwater-residents produced relatively large but few eggs compared to similar sized anadromous trout (Jonsson and Jonsson, 1999). Klemetsen *et al*. (2003) suggested that smaller freshwater-residents produced large eggs (for their body size) as a competitive mechanism to

compensate for larger anadromous females providing offspring with larger resources. This adaption suggests sympatric freshwater-residents should produce larger eggs than those living in allopatry (Klemetsen *et al.*, 2003). This was shown in a brown trout population in Jörlandaån (Sweden), freshwater-resident trout had larger eggs (65.9-108.5 mg mean wet weight) than both sympatric anadromous (76.8-84.2 mg) and freshwater-residents living in allopatry (23.7-80.1 mg) (Olofsson and Mosegaard, 1999). It is not clear why egg size varies with female size, but females may have to trade-off egg size and number because of limited resources (Roff, 1992).

Studies have shown that egg size is positively related to offspring size (Einum and Fleming, 1999; Olsen and Vøllestad, 2001). One way parents may influence offspring survival and fitness is the time they spawn and hence the time offspring emerge. The benefits of offspring emerging earlier are they can establish territories first and begin feeding (Elliott, 1990), have increased competitive ability and survival compared to smaller offspring (Elliott, 1984b) however, parents which spawn first may be at a disadvantage as eggs may be overcut by later spawning adults (Fukushima *et al.*, 1998). Offspring which emerge later may be forced to migrate to less productive areas from competition as feeding territories are already set up (Héland, 1980a; b). In a hatchery reared experiment, maternal effect had an affect on offspring egg size, fry size, swimming stamina and survival. Egg size was positively related to fry size and body size was positively related to swimming stamina which influenced the survival in early life (Ojanguren *et al.*, 1996).

There has been surprisingly no studies comparing anadromous and freshwater-resident brown trout parental investment and/or maternal effects and the fitness benefits afforded to offspring.

1.4.2 IS LIFE-HISTORY STRATEGY CONTROLLED BY ENVIRONMENT OR GENETICS?

The variation of life-history strategies within populations has led researchers in asking the fundamental questions over the respective roles of genetics and environment in the manifestation of particular life-history strategies.

Previous studies have suggested that migration is stimulated by growth efficiency in the freshwater environment (Forseth *et al.*, 1999; Morinville and Rasmussen, 2003). Brook trout (*Salvelinus fontinalis*) pre-smolts, in a tributary of the Ste. Marguerite River (Canada) displayed higher metabolic costs and lower growth efficiencies compared to their freshwater counterparts. Therefore, despite consuming

more than 1.4 times as much as freshwater-residents, the energy acquired was growth limiting. Thus the onset of migration is believed to be a consequence of energetic limitation (Morinville and Rasmussen, 2003). Food availability is also an important factor influencing growth and hence migration, as resources tend to be lower in freshwater habitats, individuals may select to migrate to more favourable areas to attain increased growth and size (Gross, 1987; Thorpe, 1987). In a partially migratory Norwegian population of Arctic charr, *Salvelinus alpinus*, which freely interbred, Nordeng (1983) produced crosses and reared the progeny under manipulated food conditions. Under high food availability, the proportion of freshwater-residents was significantly higher compared to anadromous individuals. This was further supported by a transplant experiment with freshwater-resident brown trout, when transplanted to low productively habitats they undertook parr-smolt transformation and migrated (Zalewski *et al.*, 1985). Therefore through lab and field studies, the influences of environmental effects indicate that migration is a consequence of food and growth limitation.

Despite phenotypic polymorphism within populations, there has been no detectable genetic differentiation (differences in allelic frequencies) between anadromous and freshwater-resident individuals which spawn together (Hindar *et al.*, 1991; Pettersson *et al.*, 2001). If migration is genetically determined the trait must be favoured by natural selection in a partial migratory population to increase reproductive success. Studies have shown that populations separated by impassable barriers, i.e. waterfalls, individuals above the barrier do not show a migratory strategy and are selected for a freshwater-resident life-history (Fleming, 1983). Life-history strategy may be based on a threshold level of both environmental and genetic influences (Piche *et al.*, 2008) and anadromy would be initiated when both multiple genetic traits and environmental conditions exceed the threshold level (Harris and Milner, 2006). However, it remains unclear the contribution of environment and genetic influences on population dynamics.

The degree of phenotypic plasticity may be an adaptation to differing and annually variable environments, favouring the life-history strategy that results in the greatest fitness (Olsson *et al.*, 2006). However, the very existence of both morphs within a population suggests there is a genetic component influencing life-history with further studies needed. For example, identifying the genes expressed during smoltification (Seidelin *et al.*, 2001; Dann *et al.*, 2003), although the expression of genes does not necessarily indicate migration is under genetic control.

1.5 OFFSPRING PERFORMANCE

In salmonids there have been a number of studies focussing on how differences in life-history strategies affect offspring performance including variation in growth rate (Rikardsen and Elliott, 2000; Morinville and Rasmussen, 2003; Chernoff and Curry, 2007), survival (Hutchings, 1991; Jonsson and Svavarsson, 2000), feeding (Jonsson and Jonsson, 1993; Jonsson and Jonsson, 1998) and swimming performance (Ojanguren *et al.*, 1996).

Studying offspring performance, particularly during early life when factors like mortality are critical to offspring (Elliott, 1994), are important to the understanding of population dynamics (how population structure is influenced by genetics and the environment). Understanding the survival, growth and habitat of a partial migratory population has important uses for fisheries management, particularly for stocking of fry which are used to increase fish production and for population restoration (Armstrong and Nislow, 2006). Identifying the effects on offspring performance, hatcheries can recreate the conditions for eggs and fry and have control over when is suitable for release and often modify habitats (Armstrong and Nislow, 2006). Determining the effects of density-dependant factors on offspring performance, ultimately researchers can determine which factors influence life-history strategy.

Measuring offspring performance can be done in a laboratory (Hutchings, 1991; Chernoff and Curry, 2007), semi-natural (Dahl *et al.*, 2006) or in the wild (Elliott, 1984a; Rikardsen and Elliott, 2000). Problems arise, for example studying maternal effects on offspring as the effect is likely to be influenced by the current environment and the effect may not necessarily manifest in different environmental conditions (Einum and Fleming, 1999). Therefore, there should be caution when comparing results which are from different environment types.

1.6 TOOLS FOR ANALYSIS OF LIFE-HISTORY

When developing an experiment or study the methods or tools used are based on a cost benefit analysis; often factoring in productivity (potential result outcome), cost and time. Developments in chemical and molecular tools over the last few decades have facilitated substantial advancements in ecology and evolutionary ecology, and there are a range of widely used methods which are suitable to specific studies that can be used to test hypotheses.

1.6.1 STABLE ISOTOPE ANALYSIS (SIA)

The use of stable isotope analysis (SIA) in ecological studies, particularly in aquatic science, has increased over the past twenty years. Naturally occurring heavy isotopes of elements undergo certain physical and chemical processes at a different rate to their more frequent lighter counterparts, resulting in differing characteristic ratios in biological material dependent upon its history. Isotopic ratios of carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), oxygen ($^{18}O/^{16}O$), sulphur ($^{34}S/^{32}S$) and hydrogen ($^{2}H/^{1}H$) are typically determined and with continuing advances in mass spectrometry, samples can be analysed faster and cheaper. This technique has been used from the microscopic level assessing methanogenic pathways (Conrad, 2005) to the global level assessing global patterns of isotope ratios in precipitation for wildlife forensics (Bowen *et al.*, 2005). Isotopic ratios are typically described using the δ notation where:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \text{ x } 1000$$

where X is the isotope of interest (here either ^{13}C of ^{15}N) and R is the ratio of this isotope to the lighter isotope (either ^{12}C or ^{14}N). δX is expressed as the 'permille' (‰) deviation of that sample from the recognised standard (either Pee Dee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$).

The ratios of stable isotope signatures of certain elements vary in a predictable manner between consumer and diet, so the origin of organic matter can be identified (DeNiro and Epstein, 1978). Hence, naturally occurring stable isotopes can be used to identify the assimilated history of feeding (DeNiro and Epstein, 1978), unlike stomach content analysis which only provide a 'snapshot' of feeding habits at a particular point in time (Fry and Sherr, 1984). Further, they can be used to indicate the diet in an environment which is not easily accessible (Grey, 2006). The ratio of carbon isotopes ($^{13}C/^{12}C$) in organic matter is largely determined by the environmental conditions where the carbon is fixed. Freshwater environments are $\delta^{13}C$ deplete in comparison to marine environments (Rau, 1978) with the estuarine environment represented by a gradient of $\delta^{13}C$ between the two (Fry and Sherr, 1984). Freshwater environments typically are more $\delta^{15}N$ deplete than marine as a consequence of inputs from terrestrial biomass (Owens, 1987). However, the assimilation and fractionation of nitrogen isotopes result in the accumulation of the heavier isotope in animal tissue, causing an additional enrichment of *c.* 3-5 ‰ for $\delta^{15}N$ and a relatively minor *c.* 1 ‰ enrichment for $\delta^{13}C$ per trophic level above primary consumers (Peterson and Fry, 1987). Hence, it should be

possible to distinguish between individuals' feeding history, either marine or freshwater, by the $\delta^{13}C$ and $\delta^{15}N$ of their tissues.

Carbon and nitrogen are two of the most widely used isotopes in SIA. Applications for use include: analysing the trophic position of consumers and tracing the flux of biomass and energy between diet and consumer (Minagawa and Wada, 1984; Peterson and Fry, 1987; Vander Zanden and Rasmussen, 1999; Grey, 2001), detecting anthropogenic impacts on the ecosystem (Fry, 1999; Fisk *et al.*, 2002), identifying escaped farmed fish (Dempson and Power, 2004), determining physiological condition (Hobson *et al.*, 1993), resolving migration patterns (Cunjak *et al.*, 2005) and identifying anadromous life-history strategies (Doucett *et al.*, 1999b; McCarthy and Waldron, 2000; Jardine *et al.*, 2005).

Females invest large amounts of energy and organic matter into egg production and thus the stable isotopic signature of eggs and newly emerging fry reflect the feeding environment of the maternal parent (Doucett *et al.*, 1999a). When emerging fry begin exogenous feeding, the start of a period of rapid growth, the isotopic signal in their tissues begins to reflect the signal of the freshwater feeding environment as tissues are laid down, repaired or replaced. Hence, over time the marine isotopic signal of fry of anadromous mothers becomes dilute (Doucett *et al.*, 1999a).

Doucett *et al*. (1999a) demonstrated the first use of SIA to identify the maternal origin of anadromous and freshwater-resident 0+ brook trout, *S. fontinalis* in the Tabusintac River (Canada), while McCarthy and Waldron (2000) demonstrated that carbon and nitrogen stable isotopes could identify eggs as the progeny of anadromous or freshwater-resident brown trout in Northern Ireland and Scotland. Further studies have shown that stable isotope analysis of newly emerged fry in sympatric populations can be used to estimate the maternal reproductive contribution to juvenile production (Charles *et al.*, 2004; Curry, 2005) and determine the extent to which spawning grounds of anadromous and freshwater-residents overlap (Charles *et al.*, 2004). Despite this technique being widely used in studies to identify the life-history of salmonid fish, there has been very little research in using SIA to quantify the maternal investment in offspring. Jardine *et al*. (2008) used SIA to show that the progeny of anadromous brook trout in the Miramichi River (Canada) emerged on average later and at a larger size compared with their freshwater counterparts. They hypothesised that this difference was due to a potential adaptive advantage to fry of anadromous mothers, where increased resources were gained through migration and then passed to the offspring.

The benefits of using SIA to quantify parental investment include the potential to resolve the relative reproductive contribution of anadromous and freshwater-resident females to juvenile production. The relative contribution of anadromous to freshwater-resident to recruitment can have important management implications in rivers where over fishing is a problem (Curry, 2005). As females typically predominate in the marine environment and attain a larger final body size (Forseth *et al.*, 1999), they are often targeted by anglers; SIA can be used as an important tool to identify and conserve populations supported by migratory females. The future life-history strategy of offspring cannot be ascertained through SIA. However, parental investment can be quantified through conventional technologies, such as molecular analysis and measuring growth and survival.

The drawbacks of using stable isotopes as a tool to determine parental investment include: stable isotope analysis only identifies the maternal feeding environment when identifying progeny of anadromous or freshwater-resident adults. This is because the paternal investment of organic matter to the fertilised egg is relatively small in comparison with the female's contribution (Doucett *et al.*, 1999a), therefore a signal cannot be detected. To provide sufficient mass to determine the isotopic signature of the maternal parent in eggs or fry, the whole egg/body is needed, therefore terminal sampling is unavoidable. Finally, the maternal isotopic signal becomes dilute once the fry begin exogenous feeding (Doucett *et al.*, 1999a), this only gives a limited time to collect samples.

1.6.2 MICROSATELLITES

Microsatellites are short tandemly repeated sequences of DNA, with the unit of repetition between 1-6 base pairs (bp) long, repeated up to a few hundred times. The most common units of repetition are di-, tri- or tetranucleotide repeats (Park and Moran, 1994). Microsatellites are co-dominant and inherited in a Mendelian fashion (Jarne and Lagoda, 1996) and are considered to be selectively neutral. Each microsatellite locus has a unique flanking sequence, when this sequence is known, primers can bind to the complimentary sequence of the microsatellite locus and amplified using polymerase chain reaction (PCR). Microsatellite repeats are highly susceptible to mutation with changes in length. The primary mutation process that causes this change is called slip-strand mispairing (Wright, 1994), and occurs only along a single DNA strand often involving a change in a single repeat unit (the stepwise mutation model). The second mechanism that can evoke mutation involves recombination by unequal crossing-over,

which gives rise to a deletion in one chromosome and an insertion in the other (Smith, 1976). Mutation rates have been estimated between 10^{-2} to 10^{-6} mutations per locus per generation (Schlotterer, 2000). Microsatellites are highly polymorphic therefore they have wide applications in population biology (Presa and Guyomard, 1996) and within aquaculture and fisheries can be used to study inbred populations (i.e. when aquaculture is involved), pedigree analysis and low genetic differentiation within and between populations.

The advantages of using microsatellite loci are that they are typically short, approximately 80-400 bp long compared to allozymes loci, making amplification of loci relatively easier using PCR. Microsatellites have a higher variability compared to allozymes, therefore can be used in many applications including parentage assignment of individuals and populations. For allozymes studies, samples are required to be fresh or frozen however for microsatellite analysis, a broader range of preservation and storage techniques can be utilised (alcohol, dried) which is important when dealing with historical samples. The main problems of using microsatellites are null alleles, which occur when mutations take place at the primer binding region of the microsatellite locus. This results in the primer not annealing at the flanking region and not successfully being amplified which often results in the locus being read as a homozygote.

Biologists are increasingly interested in assigning unknown individuals to populations and/or their parents using a range of different methods (i.e. exclusion, maximum-likelihood) to answer important questions relating to evolutionary biology and conservation. Parentage assignment is used to determine mating patterns (Jones and Avise, 1997) and the degree of multiple mating (DeYoung *et al.*, 2002; Myers and Zamudio, 2004), kinship (Norris *et al.*, 2000), the effect of stocking (Baumsteiger *et al.*, 2008) and quantify reproductive success (Rico *et al.*, 1992; Jones *et al.*, 1998; Curry, 2005). Parentage analysis assigns parents to an individual based on their multi-locus genotype (Jones and Ardren, 2003). When all the parental genotypes are known (i.e. in laboratory experiments), an exclusion-based method is used (Danzmann, 1997; Taggart, 2007) until only one parental pair matches the genotype of the progeny. In some instances, particularly in sampling in the wild, not all the parental genotypes are known therefore fractional parental assignment are made based on the relative likelihood of different potential parents (Nielsen *et al.*, 2001; Duchesne *et al.*, 2005). Some methods allow for genotyping error (Kalinowski *et al.*, 2007) or can reconstruct parental genotypes when they are unknown (Jones and Ardren, 2003).

1.7 OBJECTIVES AND HYPOTHESES

The overall objective of this project is to determine the effects of parental investment and maternal effects of anadromous and freshwater-resident brown trout on offspring performance and early life-history.

The following hypotheses will be tested in the following chapters:

1.7.1 CHAPTER 3

In fish, fecundity and egg size increases with body size (Thorpe *et al.*, 1984; Jonsson and Jonsson, 1999), increasing reproductive success (Wootton, 1990). Maternal effects influence the size of eggs and offspring at hatching and can influence early life-history traits (Einum and Fleming, 1999). As females invest large amounts of energy and organic matter into egg production, the stable isotopic signature of eggs and endogenous feeding offspring will reflect the maternal feeding environment (Doucett *et al.*, 1999a). The following hypotheses will be tested regarding size and timing of emergence:

Size at emergence

H_0: The size of emerging fry from anadromous and freshwater-resident female adults will be the same, i.e. there will be no significant difference.

H_1: Fry of anadromous female adults will emerge at a larger size than fry of freshwater-resident female adults.

Time of emergence

H_0: Fry of anadromous and freshwater-resident female adults will emerge at the same time.

H_1: Fry of anadromous female adults will emerge earlier than fry of freshwater-resident female adults.

Maternal reproductive contribution

H_0: The relative maternal reproductive contribution will be equal between anadromous and freshwater-residents to offspring production.

H_1: Anadromous maternal reproductive contribution will be higher than freshwater-residents females to offspring production.

1.7.2 CHAPTER 4 & 5

Parental life-history affects offspring performance and life-history in partially migratory salmonid populations. Differences in parental life-history strategy significantly affect

offspring growth and survival (Hutchings, 1991; Morinville and Rasmussen, 2003; Chernoff and Curry, 2007). Using a panel of twelve microsatellite loci, parents were assigned to individual offspring based on their genotypes. The mean size and growth rates were compared between offspring from anadromous and freshwater-resident parents. The following hypotheses will be tested:

Relative maternal/paternal reproductive contribution

H_0: The relative maternal/paternal reproductive contribution will be equal between anadromous and freshwater-residents to offspring production.

H_1: Anadromous maternal/paternal reproductive contribution will be higher than freshwater-residents females/males to offspring production.

Size of 0+parr

H_0: The size of parr from anadromous and freshwater-resident females/males will be the same, i.e. there will be no significant difference.

H_1: Parr of anadromous females/males will be larger than parr of freshwater-resident females/males.

Growth rate of 0+parr

H_0: The growth rate of parr from anadromous and freshwater-resident females/males will be the same, i.e. there will be no significant difference.

H_1: The growth rate of parr of anadromous females/males will be higher than parr of freshwater-resident females/males.

Offspring life-history strategy

H_0: Parental life-history does not affect subsequent offspring life-history strategy (i.e. the decision to mature or smolt).

H_1: Anadromous parents produce offspring which adopt an anadromous life-history and freshwater-resident parents produce offspring which adopt a freshwater-resident life-history.

CHAPTER 2: METHODS

2.1 STUDY SITE

2.1.1 CATCHMENT HYDROLOGY AND GEOLOGY

The River Frome drains a largely chalk catchment in Dorset, southwest England. The River Frome rises near Evershot (ST573050) and flows southeast through Dorset before reaching Poole harbour at Wareham, the tidal limit. The confluence with the sea is at Sandbanks (SY941878). The river is 59 km in length (to the tidal limit) with a drainage area of 464 km^2. The Tadnoll Brook (Figure 2.1) is one of seven major tributaries on the River Frome; it is 9.9 km long, has a catchment of approximately 50 km^2 and joins the Frome 10 km above the tidal limit (SY815878). The catchment of the Tadnoll Brook is comprised of chalk bed-rock with overlaying tertiary clays and sands. As a consequence Tadnoll Brook is a circum-neutral stream (Crisp *et al.*, 1982), with alkaline springs rising through watercress beds at Empool (SY745846) and at other points along the stream supplemented with acidic surface drainage. Water is abstracted for use in a fish farm just below the cress beds. The upper to mid section of the Tadnoll has been characterised as having suitable sediment of pebble and gravel for salmonid spawning (Ibbotson *et al.*, 2006). The initial study site was at the Tadnoll Mill house (SY792869) however construction of a fish pass in the winter of 2005/6 affected the collection of spawning adults so an alternative site upstream was chosen. The Cider Museum site (SY774871) is a 300 m study site, moderately covered by canopy and known to have a partial migratory population of brown trout.

2.1.2 FISH POPULATION

The Frome catchment was historically known to have extensive spawning populations of freshwater-resident and anadromous brown trout and Atlantic salmon. However, in the 1980s a period of low winter flows meant spawning salmon were unable to pass a barrier at the Mill House on the Tadnoll Brook. The spawning population declined as adults were unable to reach suitable spawning habitat and by the 1990s the salmon population was extinct (Ibbotson *et al.*, 2006). A fish pass was constructed in 2006 and salmon parr (from artificial rearing experiment at CEH) were successfully reintroduced the same year as part of another study.

Despite the decline and eventual extinction of the salmon population in the Tadnoll Brook, the anadromous brown trout population remained stable (A.T. Ibbotson, pers. comms.). One reason may be the adaptive life-history of brown trout where

anadromous adults refrained from spawning and returned to sea for another year. The freshwater-resident population in the stream would have been able to find suitable spawning habitat below the barrier.

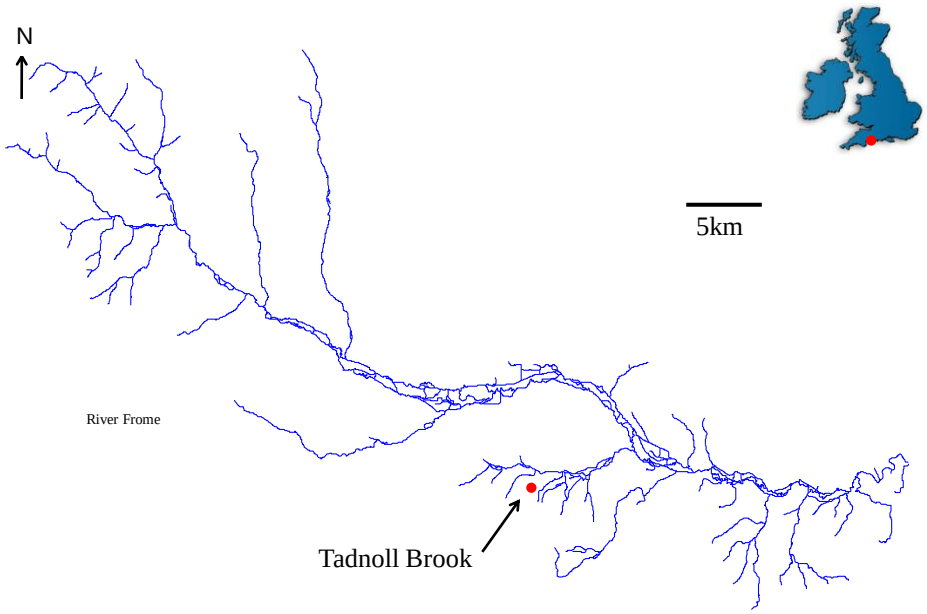

Figure 2. 1. Location of the Tadnoll Brook on the river Frome in South-West England.

2.1.3 LAND USE AND ANTHROPOGENIC ACTIVITY

The Frome catchment is predominantly rural and contains one of the highest concentrations of designated areas for nature conservation in England (EA, 2005). Arable farming and grassland are the highest proportion of land use throughout the catchment. Anthropogenic activity on the Frome is light and concentrated around the main towns of Dorchester and Wareham, with relatively little activity on the Tadnoll.

2.1.4 RECREACTION

The majority of the access to the Tadnoll Brook and along the Frome is owned privately or by organisations including the Environment Agency (EA) and the Freshwater Biological Association (FBA). Coarse angling is restricted to the lower sections of the catchment and throughout the catchment for salmon and anadromous trout.

2.2 SAMPLE COLLECTION

2.2.1 SPAWNING ADULTS

Regular visits to the river were made to record any spawning activity (courting behaviour, nest making) from the bank side, ensuring not to disturb any adults. The

location of established redds was recorded by placing markers on the bank side, measurements of width (m) and tail length (m) were taken for all redds.

A personal licence from the Home Office (PIL- 80/9925) was obtained to use anaesthesia, PIT tagging and the removal of a fin sample. This was through the Centre for Ecology and Hydrology (CEH) in Dorset.

2.2.2 EMERGING FRY

The initial design for collecting emerging fry included putting out ten drift nets (mesh-1 mm^2) twice a week at dusk and collecting them at dawn, and then electrofishing the stretch. However, after each collection the nets were full with debris and only one fry was caught after the first two weeks. The drift nets were deemed an inefficient method for fry collection, especially as samples needed to be collected whole for stable isotope analysis. This was particularly important as it was necessary to identify which fry had yolk sacs and which had begun exogenous feeding, this was not possible if the samples were damaged in the nets.

The emergence period was identified by the absence of fry on the first sampling dates, then the increasing number of exogenous fry over the weeks, concluding with a higher proportion of endogenous feeding fry.

2.3 PIT TAGGING

Untagged trout collected during this study were tagged with passive integrated transponder tags (PIT), except during the spawning season and after April 2008 onwards. The minimum body size for tagging trout was 6.6 cm. Fish were lightly anaesthetised in 2-Phenoxyethanol (0.333ml/l) and, after being weighed and measured, those large enough were prepared for tagging. A 0.3 cm incision was made along the mid ventral line anterior to the pelvic fin and the tag implanted into the peritoneal by gently pushing into the body cavity, demonstrated by Figure 2.2. PIT tags used were 1.2 cm long x 0.2 cm diameter (Full duplex (Fdx) Wyre Micro Design (WMD)) (Fig. 2.3). Tagged fish were held in recovery tanks and then released immediately or once electrofishing of that stretch had been completed. During the spawning season no mature fish were PIT tagged to avoid undue stress on the spawning adults. In subsequent sampling recapture, fish were identified by using a handheld PIT tag scanner (WMD LID500), the unique tag number along with other measurements (Length, mass, location, sex) were recorded. This data was used to identify individual movement and potentially migration within the river system.

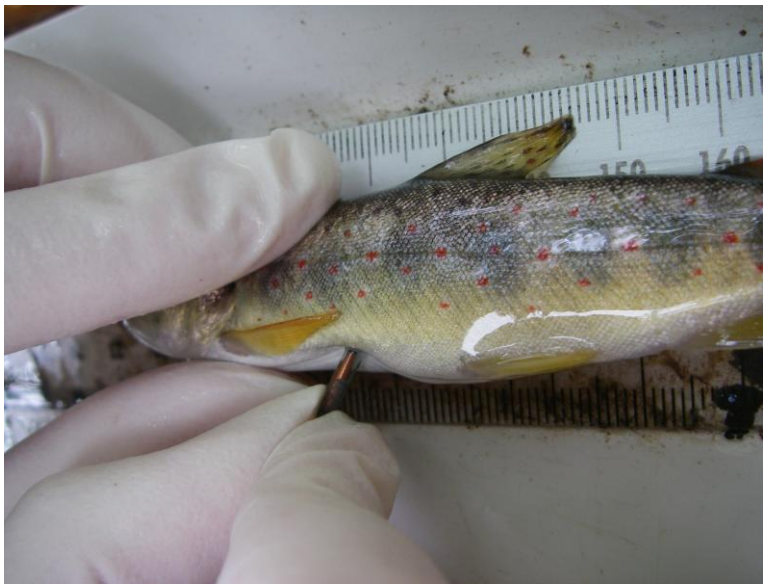

Figure 2. 2. PIT tagging in brown trout. The tag is inserted through a small incision into the body cavity.

Figure 2. 3. 12x2mm PIT tags used in this study (Fdx model, MWD)

2.4 PIT TELEMETRY

The recent development of PIT technology allows individual fish movement and behaviour to be monitored (Bruyndoncx *et al.*, 2002) from the detection of a unique ID from antennae in the river. This study was able to utilise five separate PIT detectors already installed in the river, currently being used as part of a separate study (Tadnoll Mill- SY792869, Fig.2.4). Each individual detector did not indicate direction of movement; however, a combination of 'downloads' in time from more than one geographical scanner did indicate upstream or downstream migration.

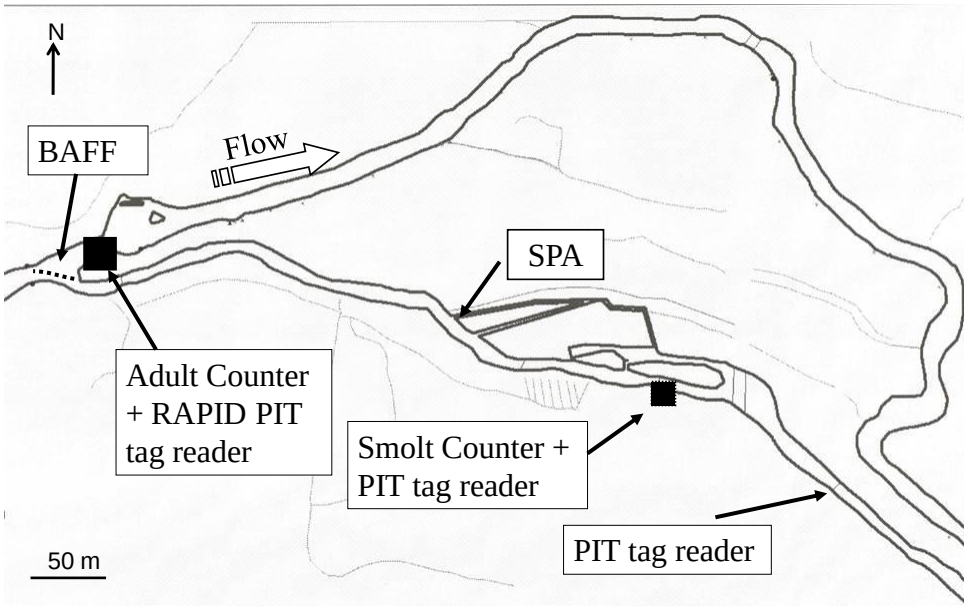

Figure 2. 4. Locations of PIT detectors along the River Frome

2.4.1 TADNOLL MILL

Tadnoll Mill (downstream of the study site) has previously been seen as the only barrier to migrating fish on the Tadnoll Brook; this problem was rectified in 2006 when the Environment Agency installed a fish pass. Subsequently, water was able to flow through the mill, a flood relief hatch, a secondary channel and the fish pass. The water levels are monitored during high flow; this is particularly important during the spawning season and at the time of upstream migration to ensure fish can navigate the pass. Three flat-bed antennae have been installed (1x0.3m, MWD), one on the flood relief hatch and two on the steps of the pass (Fig. 2.5).

2.4.2 RIVER FROME

The main river PIT detector is installed at the weir in East Stoke (Fig. 2.6) and consists of thirteen radio frequency identification (RFID) antennae, each housed within a fibreglass vane (3.5 x 48 x 200 cm), which are staggered across the weir to avoid obstructions (Ibbotson *et al.*, 2004). In this often fast flowing river, debris collecting along the vanes poses a problem and therefore each vane is connected to a pneumatic system encased by a galvanised steel support structure. When the sensor detects a build up of debris behind any of the vanes, this activates the pneumatic system to raise the vanes under high pressure horizontally above the water, allowing the obstruction to flow downstream. This process takes *c.* 20 seconds and an automatic timer ensures the vanes are raised every four hours (Ibbotson *et al.*, 2004). Each antenna has an area of 190 cm^2

for PIT detection. The PIT tags used in this study had a maximum of 2 cm detection, therefore, full coverage was achieved through the staggered formation of antennae 35 cm apart.

This system is used to detect movements both upstream and downstream in the main river. The antennae are set up to scan continuously for tagged fish. An above-water camera system is used in conjunction with the logged PIT data to determine direction of movement and acts as a back up if the antennae are not working, to determine fish numbers migrating.

Figure 2. 5.Tadnoll Mill fish pass on the Tadnoll Brook showing (a) the installation of the flat-bed PIT tag detectors on the steps (photo courtesy of W.R.C. Beaumont) and (b) the pass after completion.

Figure 2. 6. RFID detector at East Stoke on the River Frome showing (a) the thirteen antennae spaced equally apart and (b) being raised after the sensor detects pressure from accumulating debris.

2.4.3 FLUVARIUM

The fluvarium is located on the Millstream, branching from the River Frome at the weir for 1.2 km and then rejoining the main river. The fluvarium is set over the stream with two glass sided channels sunk into the water (Fig. 2.7), allowing visual observation of aquatic life in the stream. MWD rectangular tube PIT tag detectors are *in situ* during the spring smolt run of both salmon and trout. An above water video camera records fish movement and behaviour, this is also useful to calculate body size and determine species of migrating fish.

Figure 2. 7. The fluvarium on the Millstream at East Stoke showing the two channels.

2.4.4 MILLSTREAM WEIR

Downstream of the fluvarium *c.* 50 m there is an Environment Agency gauging weir where a 1 x 0.3 m flatbed PIT tag detector (MWD) is installed.

2.5 MANAGEMENT OF DATA

An Excel database was set up to store all of the sampling data collected. After each sampling the data was entered into a new file, this was then added to the master file. The database was used to identify recaptured fish, those appropriate for assignment (see Chapter 4 & 5 for further detail) and migrating fish.

The historical PIT tag records were made available to this study courtesy of the Fish Ecology Group, CEH. The Microsoft Access database was required to locate any fish logged that were recorded in this study. The main aims were to identify 1) the parent migrations, and 2) which offspring migrated after the first year.

CHAPTER 3: BROWN TROUT OFFSPRING FITNESS: A CONSEQUENCE OF MATERNAL LIFE-HISTORY STRATEGY.

Due to be submitted in the *Journal of Freshwater Biology* in July 2011, with the authors as follows:

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Collection and analysis of 0+ parr and macroinvertebrates was done by J. I. Jones, F. K. Edwards and R. B. Laurisden as part of a food web and salmon introduction project in the Tadnoll Brook.

3.1 INTRODUCTION

Brown trout *Salmo trutta L*. exhibit one of the most highly variable and polytypic life-history strategies of all the salmonids. Populations often exist where a proportion of individuals undergo smolting and migrate to the marine environment before returning to spawn in the natal stream (anadromous) whereas other individuals (freshwater-resident) complete their whole life-cycle in freshwater (Elliott, 1994). Both anadromous and freshwater-resident brown trout freely interbreed to produce fertile offspring (Frost and Brown, 1967).

Migration to more productive environments provides anadromous brown trout with access to increased food resources and enables them to achieve a larger final body size (Jonsson, 1985; Berg and Jonsson, 1990). However, migration is energetically costly (Dieperink *et al.*, 2001) and involves increased exposure to predators and disease (Bohlin *et al.*, 2001). The costs and benefits of migration versus residence do not affect males and females equally. Fitness is dependant on the number of offspring that an individual produces that, in turn, survive to reproductive age. In female brown trout individual fecundity increases with body size in both anadromous and freshwater-resident brown trout (Jonsson, 1985; Elliott, 1994; 1995). Females may maximise their fitness by increasing resources to each individual, therefore increasing offspring fitness. However, a number of studies have demonstrated that there can be no simultaneous maximisation of both size and number of eggs (Roff, 1992) and thus a trade-off must occur (Lobón-Cerviá *et al.*, 1997; Jonsson and Jonsson, 1999; Olofsson and Mosegaard, 1999; Acolas *et al.*, 2008). For males, reproductive success is determined by the number of eggs that an individual can fertilise. As sperm is cheap to produce relative to eggs, males often adopt a promiscuous: even small males can produce sufficient sperm to fertilise all the eggs of the largest of females. Large size has some advantage in attracting and defending females, such that large anadromous males are usually the principle spawners (Jones and Ball, 1954). However, smaller freshwater-resident males can adopt 'sneaking' strategies to achieve fertilisation success, thus reducing the advantage of being large in males and the pressure to migrate. Hence, male and female brown trout are likely to respond differently to any changes in the costs and benefits of migration versus freshwater residence, with the potential for profound changes for populations. The importance of understanding the influence of each parental life-history strategy and quantifying the relative reproductive contributions to future generations will provide vital information to fisheries managers.

An adaptive response to ensure offspring survival and thus maximise fitness for the maternal parent is to invest in maternal provisioning (Roff, 1992). In salmonids, the majority of studies that have investigated the influence of maternal traits have focussed on the effect of egg size on offspring size at hatching (Elliott, 1984b; Einum and Fleming, 1999; Einum and Fleming, 2000b; Berg *et al.*, 2001), size at emergence (Elliott, 1984b; Elliott and Hurley, 1998b), growth rate (Wallace and Aasjord, 1984; Ojanguren *et al.*, 1996) and survival (Hutchings, 1991; Jonsson and Svavarsson, 2000). Previous studies of maternal influence on offspring performance in brook trout *Salvelinus fontinalis* have shown conflicting results. Jardine *et al*. (2008) found progeny of anadromous brook trout emerged on average later and at a larger size, whereas Curry *et al*. (1995) found no evidence of female size influencing the size and time of emergence of offspring. Nevertheless, the fitness benefits in terms of size and time of emergence between anadromous and freshwater-resident brown trout remains unknown. Here we present a study of the influence of maternal life history on offspring performance in a wild population of brown trout.

Although anadromous fish undergo morphological changes that can usually be used to distinguish them from freshwater resident fish, independent verification of the life-history choices of individuals is often required. A number of laboratory techniques have been used to distinguish between anadromous and freshwater-resident life-histories in salmonids, including strontium concentration in scales (Eek and Bohlin, 1997), microchemistry of otoliths (Rieman *et al.*, 1994), carotenoid pigments (Youngson *et al.*, 1997) and parasite load (Black, 1981). Naturally occurring stable isotopes can be used to identify marine and freshwater feeding (DeNiro and Epstein, 1978) and are now becoming a superior alternative, providing a straightforward and relatively inexpensive technique (Doucett *et al.*, 1999a). The ratios of naturally occurring stable isotopes of certain elements vary in predictable ways that can be used to identify the origins of organic matter. The ratio of carbon isotopes (^{13}C:^{12}C) in organic matter is largely determined by the environmental conditions where the carbon was fixed. The combination of chemical fractionation between atmospheric CO_2 and bicarbonate in the sea and the uptake of dissolved inorganic carbon by phytoplankton results in a $\delta^{13}C$ value of ~ -19-24‰ (Fry, 2008). Freshwater environments are $\delta^{13}C$ deplete in comparison (Rau, 1978) and a gradient of $\delta^{13}C$ between the two reflects the estuarine environment (Fry and Sherr, 1984). Freshwater environments tend to be more $\delta^{15}N$ deplete compared with marine phytoplankton, a consequence of inputs from

terrestrial biomass (Owens, 1987). However, this marine $\delta^{15}N$ signal is confounded by predation: due to preferential excretion of the lighter isotope during protein transamination and deamination, the abundance of ^{15}N in consumer tissues tends to increase relative to their prey (Minagawa & Wada, 1984). Thus, the $\delta^{15}N$ of consumers is largely determined by their trophic position, which can vary among individuals.

Stable isotopes have a further advantage over other chemical techniques: as there is a large maternal investment of organic matter into egg production, the stable isotope composition of eggs and recently emerging fry reflect the feeding environment of the maternal parent (Doucett *et al.*, 1999a). Stable isotope analysis (SIA) has been used as a successful method for identifying eggs (McCarthy and Waldron, 2000) and recently emerging offspring (Doucett *et al.*, 1999a; Charles *et al.*, 2004; Curry, 2005; Jardine *et al.*, 2008) as the progeny of anadromous and freshwater-resident salmonids. However, once fry begin exogenous feeding over time the organic matter in their tissues begins to reflect the signal of the freshwater feeding environment, as a consequence of new growth, replacement or repair of tissues and, for fry of anadromous mothers, the marine isotopic signal becomes dilute (Doucett *et al.*, 1999a).

In this study of brown trout, the effects of maternal provisioning on offspring during early ontogeny were explored. The aims of this study were to (1) to identify recently emerging fry as the progeny of either anadromous or freshwater-resident brown trout by the use of carbon and nitrogen stable isotopes, (2) to assess differences in the fitness of offspring from anadromous or freshwater-resident maternal parents in terms time of emergence and size at hatching, (3) to calculate the relative contribution of the two maternal life history strategies to offspring production in the population.

3.2 MATERIALS AND METHODS

3.2.1 STUDY SITE

Please see section 2.1 for further details.

3.2.2 SAMPLE COLLECTION

Adult brown trout (n = 75) were caught by repeatedly electrofishing a 300 m stretch of the Tadnoll Brook throughout the 2006 spawning season (November to December). A Honda EU 1.0 generator connected to Electracatch WFC4 pulse box using 40 cm diameter anode with 300 cm braid cathode was used. The electrical waveform was 1/2 wave rectified at 50 Hz and circuit voltage ~220 volts and 1.25 Amps. The fish were visually identified in the field as either anadromous or freshwater-resident based on body size and colouration (Elliott, 1994). A sample of pelvic fin tissue was removed for isotopic analysis. Tissue samples were frozen and stored at -70 °C before sample preparation and analysis, except for tissues collected from the first sampling date (n = 27) which were stored in 96 % ethanol. To determine differential effects of preservation, 23 samples were split and were preserved by both techniques. Spawning activity was monitored from the bank side throughout the period, and the size and location of redds formed by this activity was marked.

Fry were collected from the same 300 m stretch of Tadnoll Brook over the period 27th February to 11th April 2007 by point sampling with an electrofisher. The timing of the fry collection period was based on the emergence date predicted from the degree day model of Elliott (1994) based on spawning date and temperature. Electrofishing was carried out once a week. Random points along the river were sampled using an Electracatch battery powered fishing gear with a 25 cm diameter anode and a 1 m copper braid cathode. The electrical supply was 1/2 wave rectified at 60 Hz and a circuit voltage ~200 volts, producing a fish capture radius ~0.7 m. A total of 119 fry were collected over the period, with no fry caught on the first two sampling occasions (27th February, 8th March). Fry were mainly caught in the shallows and slow-flowing areas, typical habitats for newly emerged fry (Greenberg *et al.*, 1996). Once captured, the fry were kept alive in a holding container until the whole stretch was fished. Fry were then over-anaesthetised by an overdose of 2-Phenoxyethanol (0.333 ml l^{-1}) and immediately frozen in the field. Upon return to the laboratory fry fork length was measured to the nearest 1 mm, the presence of yolk recorded and the digestive track removed and the remaining tissue washed with distilled water.

A further collection of juvenile trout and benthic macroinvertebrates was made in May 2006 in the same stretch of river, to determine the isotopic composition of parr that had been feeding in the river for some time and the prey items they were feeding on. Three species of benthic invertebrates were collected (*Baetis*, *Simuliidae*, and *Oligochaeta*) by kick sampling, sorted live in the laboratory and allowed to vacate their guts in distilled water before freezing: these three taxa comprise the majority of prey items of newly emerged fry in the Tadnoll Brook (as determined by direct observation of gut contents, F. Edwards pers. comm.) Each invertebrate sample used for isotopic analysis consisted of 16, 11 and 8 whole individuals for the *Baetis*, *Simuliidae*, and *Oligochaeta* respectively. The digestive tracks of the 0+ trout parr were removed and the sample was then washed in distilled water and frozen before further processing.

3.2.3 SAMPLE PROCESSING

All samples (fin clips, fry and benthic invertebrates) were dried at 85 ºC for 48 h and ground to a fine powder using a mortar and pestle. Approximately 0.56 mg, 0.58 mg and 1 mg for nitrogen and 0.50 mg, 0.60 mg and 1 mg for carbon of fry, fin and benthic invertebrates respectively were loaded into individual 4 x 6 mm tin capsules. The samples were combusted using a Eurovector elemental analyser and the resultant CO_2 and N_2 (subsequent reduction) was analysed for $\delta^{13}C$ and $\delta^{15}N$ using a Micromass Isoprime IRMS. The isotopic ratio is the relative difference between the isotope ratios of the sample and that of the standard. The standard (pure CO_2, NO_2) has a known value relative to the international standard (Peedee Belemnite for carbon, atmospheric air for nitrogen) given a value as zero (Dawson and Brooks, 2001). The results are expressed in parts per thousand (‰) and given the notation of delta (δ) calculated using the following equation:

$$X = \frac{\left[\left(R_{sample} - R_{standard}\right)\right]}{R_{standard}} \text{ x } 1000$$

Where X is $\delta^{13}C$ or $\delta^{15}N$, R_{sample} is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ and $R_{standard}$ for $\delta^{13}C$ is pure CO_2 and for $\delta^{15}N$ is pure N_2. Replicate samples of an internal standard (Daphnia) yielded results that were precise and accurate, $\delta^{13}C$ = -24.43 ± 0.10 ‰ (mean ± SD, N = 72), $\delta^{15}N$ = 16.96 ± 0.17 ‰ (mean ± SD, N = 71). Three adult fin samples and four fry samples were tested for sample reproducibility by taking repeated measurements (5 per sample). The coefficient of variation ranged between 0.001 and 0.017 for carbon and

0.005 and 0.028 for nitrogen, indicating reliable results. Single measurements were taken for all the remaining samples.

All stable isotope analysis (SIA) was undertaken at Lancaster Stable Isotope Facility (CEH).

3.2.4 SAMPLE PRESERVATION

It is important to eliminate contamination when collecting and storing samples for stable isotope analysis. In recent years, researchers have become increasingly interested in the effect of preservation on stable isotope signatures (Hobson *et al.*, 1997; Edwards *et al.*, 2002; Feuchtmayr and Grey, 2003; Kelly *et al.*, 2006), however, among these studies there are variations and differing results. Gloutney and Hobson (1998) reported no significant alteration of stable isotope values of quail eggs in both carbon and nitrogen after treatment with ethanol. However, other studies (Feuchtmayr and Grey, 2003; Kelly *et al.*, 2006; Syvaranta *et al.*, 2008) have shown preservation in ethanol to cause an enrichment of varying degrees in both isotopes, with Kaehler and Pakhomov (2001) reporting a range of 0.6-1.5 ‰ for carbon in marine species. Similar contradictory results have been found when testing the effects of freezing samples. Barrow *et al*. (2008) reported a significant depletion after 60 days in turtles in both carbon and nitrogen, whereas Feuchtmayr and Grey (2003) found the opposite effect with a depletion of 0.9 ‰ in carbon and an elevation of nitrogen in zooplankton. Other studies have shown no effects on isotopic integrity after freezing (Gloutney and Hobson, 1998; Kaehler and Pakhomov, 2001).

A correction factor was calculated to account for the effect of ethanol preservation in the 27 adult tissue samples. This was calculated from the 25 samples that were split and stored frozen and in ethanol. The difference in isotopic ratios between frozen and ethanol preserved samples was determined and the overall mean difference calculated (-0.12 for $\delta^{15}N$, 0.81 for $\delta^{13}C$), which compare well with previous findings of the effects of ethanol and freezing (Kaehler and Pakhomov, 2001; Feuchtmayr and Grey, 2003). The samples that were preserved were adjusted accordingly ($\Delta^{15}N$ +0.12, $\Delta^{13}C$ -0.81) to be compared with the frozen samples.

3.2.5 ISOTOPE MIXING MODEL

Trout embryos are carbon deplete relative to their respective maternal parents (Curry, 2005). This is a consequence of discriminative fractionation during the development of lipid rich egg tissues (DeNiro and Epstein, 1978). To account for this an independently

derived fractionation value (Grey, 2001) was applied to all fry samples before analysis ($\Delta^{15}N$ +0.8, $\Delta^{13}C$ +1.5).

To identify individual fry as having freshwater-resident or anadromous brown trout mothers, a single isotope, two-source mixing model was used (Phillips and Gregg, 2001-IsoError Version 1.04). This model returns the proportions of anadromous and river resident signal that if mixed would produce the isotopic ratio of the fry. To use these values to determine if fry had an anadromous or river resident mother it was necessary to set the range of isotopic ratios that could be reasonably assumed to correspond to freshwater-resident or anadromous mothers. To do this the mean and standard deviation of the isotopic ratios of tissues collected from adults identified from their morphology as freshwater-resident or anadromous were calculated. Assuming a normal distribution, the ratios that corresponded to 50 % and 95 % of potential observations (0.674 σ/1.96 σ) were calculated for both freshwater-resident or anadromous adults. Any fry that had isotopic signatures that fell within the 95 % limit were assumed to be recently emerged offspring of freshwater-resident or anadromous mothers. Ratios corresponding to 50 % of adult observations were included to provide more confidence in ascribing fry to anadromous or freshwater-resident mothers. For $\delta^{15}N$ the interval corresponding to 95 % of freshwater-resident observations overlapped with that calculated for anadromous trout making it impossible to discriminate between the offspring of anadromous and river resident mothers using $\delta^{15}N$. Since limits corresponding to 50 % of adult observations do not reflect the true range of fry isotopic signatures that could be derived from adult mothers, it was decided not to use $\delta^{15}N$ to determine the maternal life history of fry. Hence, all subsequent analysis on fry size and emergence was based on $\delta^{13}C$, where there was adequate separation between anadromous and river resident adults.

3.2.6 STATISTICS

T-tests were used to determine the significance of differences in adult $\delta^{13}C$ and $\delta^{15}N$, fry size, and time of emergence. Residual plots verified the assumptions of normality and homogeneity of variance. The level of critical significance in all statistical tests was set to 0.05. Statistical analyses were performed with Minitab 15.

3.3 RESULTS

3.3.1 REDD PRODUCTION

Despite not witnessing all the spawning events, the river was monitored every 3-7 days throughout the whole spawning period (plus one extra date in January) and data was collected on redd numbers and visual sightings of spawning activity. It is known that there is a positive correlation between size of redd and female fork length (Crisp and Carling, 1989), therefore it was possible to estimate the size of females, and thus their life-history, from the size of each redd. It was not possible to accurately identify the sex of all the adults caught. However, freshwater-resident females were smaller than anadromous females, fork length 18.9 to 24.8 cm *cf.* 46.0 to 74.8 cm (t-test: d.f. = 14, t = 9.23, $p < 0.001$). Out of the 12 redds recorded in the river, 9 were identified as having been cut by large females (redd tail length $\geq$ 1.6 m) and 3 by smaller females (redd tail length $\leq$ 1.5 m). The first redds to be produced were large; small redds were first observed 3 days later (Fig. 3.1). However, there was no distinction in the time of production of small and large redds, both were produced through the spawning period.

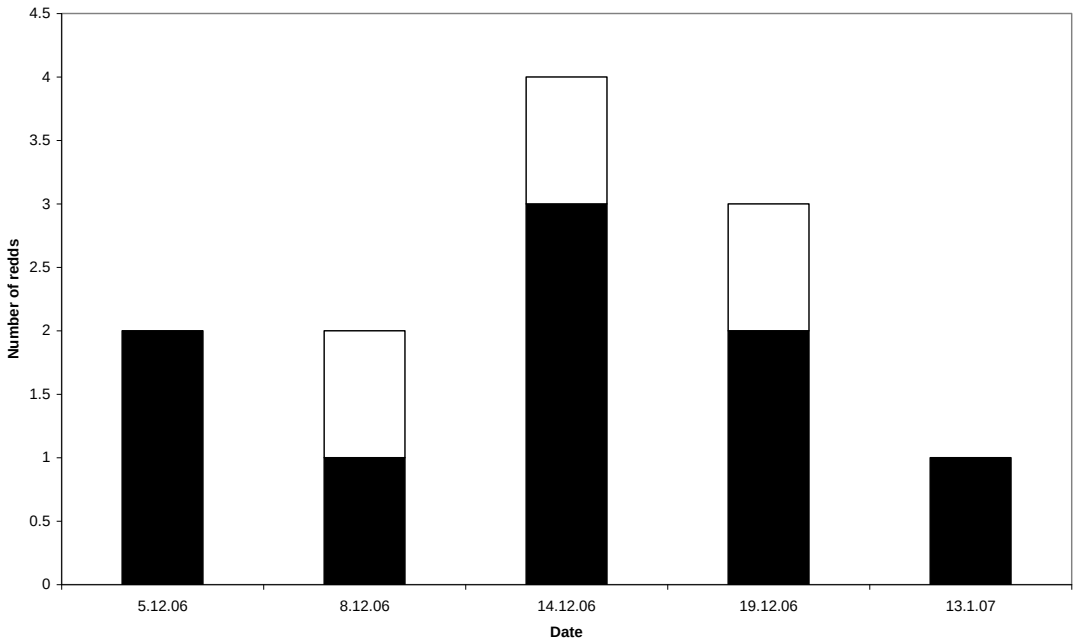

Figure 3. 1. Number of new anadromous (filled) and freshwater-resident (unfilled) brown trout redds created in the 300 m study section of Tadnoll Brook (UK) during the spawning period 2006-7.

3.3.2 IDENTIFICATION OF MATERNAL LIFE-HISTORY

Adult samples were split into two distinct groups by $\delta^{13}C$ and $\delta^{15}N$ (Fig. 3.2). Freshwater-resident trout were deplete in both isotopes compared with anadromous

trout (t-test, $\delta^{13}C$: d.f. = 73, t = 37.02, p = 0.0001; $\delta^{15}N$: d.f. = 73, t = 8.30, p <0.0001). Both groups of adult samples were enriched in stable isotopes compared with macroinvertebrates and juvenile trout that had been feeding in the river for some time (~ 4.5 ‰ and ~ 7.8 ‰ for $\delta^{15}N$ and by ~ 8.2 ‰ and ~ 17.6 ‰ for $\delta^{13}C$ for freshwater-resident and anadromous respectfully).

Unlike previous reports (Doucett *et al.*, 1999a; McCarthy and Waldron, 2000; Charles *et al.*, 2004), fry did not form two distinct groups based on their isotopic signatures but were scattered along a line between the anadromous and freshwater resident adults (Fig. 3.2). A single isotope, two-source mixing model (Phillips and Gregg, 2001) was used to estimate the proportion of marine (anadromous) and freshwater (resident) signal that if mixed would produce the $\delta^{13}C$ signature of individual fry, corrected for fractionation between mother and egg.

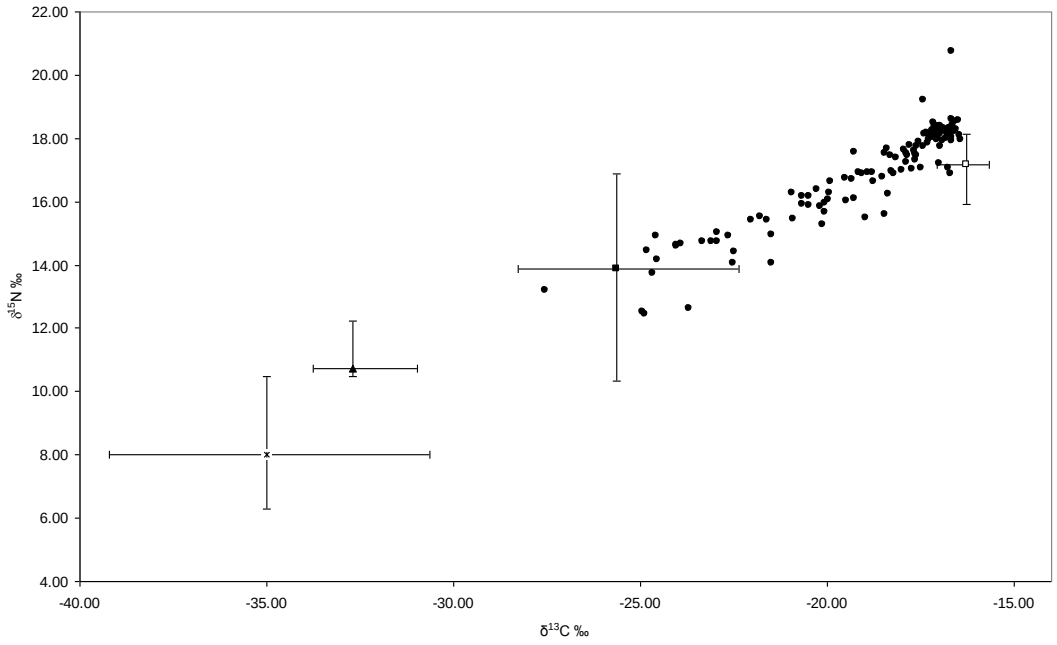

Figure 3. 2. Combined $\delta^{13}C$ and $\delta^{15}N$ of recently emerging brown trout (*Salmo trutta* L.) fry (●) collected in the Tadnoll Brook (UK) during the period of emergence in March – April 2007. Adult anadromous (□) and freshwater-resident (■) brown trout (collected in November-December 06), river feeding 0+ trout parr (▲) and macroinvertebrate prey (×) (collected in May 2006), all collected in the same stretch are also shown. Vertical and horizontal lines indicate the range of isotopic values (mean, minimum, maximum).

It was possible to identify recently emerged fry by the presence of visible yolk reserves (Fig. 3.3). Once yolk is exhausted fry can only sustain growth by exogenous feeding. Nevertheless, it is possible that fry start feeding exogenously before endogenous reserves are exhausted. Most fry with yolk had an isotopic signature that

was predominantly marine (Fig. 3.3). However, one individual was found with yolk that had a fresh water resident signal (Fig. 3.3). Fry of larger size and without obvious yolk tended to have progressively less of a marine signal with increasing size. Two smaller fry that did not have obvious yolk were identified as being within the freshwater-resident interval but not part of the cluster of other, larger fry (Fig. 3.3), indicating a putative freshwater-resident maternal origin. It seems unlikely that these two exogenous feeding fry are of anadromous origin due to the combination of small body size and freshwater signal. Fry within the limits set for anadromous origin that still had obvious yolk reserves are certainly of anadromous origin. However, 16 fry were identified as just outside or close to the limit defined for anadromous origin which when sampled still had yolk reserves (Fig. 3.3). These fry were of putative anadromous origin as their signal was not likely to have been derived from freshwater resident adults (Doucett *et al.*, 1999a) and they either had not started feeding exogenously or had not been doing so for long. A total of 12 fry within the limit set for anadromous origin when collected were identified in the laboratory as exogenous feeding.

With the exception of the three fry identified as being of freshwater-resident maternal origin, the relationship between fry length and % marine signal appears to follow a clear growth curve. Smaller fry feeding endogenously have a strong marine signal (associated with anadromous maternal origin) which becomes progressively dilute with increasing size presumably as larger fry lay down tissues using organic matter from freshwater prey. Thus, it is not unequivocal if the larger fry within the 95% freshwater-resident interval are of freshwater maternal origin or anadromous fry with a $\delta^{13}C$ signal diluted as a consequence of feeding (Fig. 3.3). Fry that could not be unequivocally identified as being of anadromous or freshwater-resident maternal origin were ascribed as of being undetermined source.

3.3.3 SIZE AT EMERGENCE

As maternal origin of some fry that had begun feeding could not be determined unequivocally (Fig. 3.3), only those fry of anadromous maternal origin with obvious yolk were used to determine size at emergence. Two groups of fry of anadromous maternal origin were used, those fry with yolk that were within the 95 % interval (within) and all fry with yolk within and outside the 95 % interval (pooled). Both groups of fry of anadromous maternal origin were larger (Within: FL = 2.80 cm ± 0.11 s.d., Pooled: FL = 2.77 cm ± 0.13 s.d.) than fry of freshwater-resident maternal origin

(FL = 2.55 cm ± 0.09 s.d., t-test: Within- d.f. = 18, t = 3.89, p = 0.001, Pooled- d.f. = 34, t = 2.80, p = 0.008).

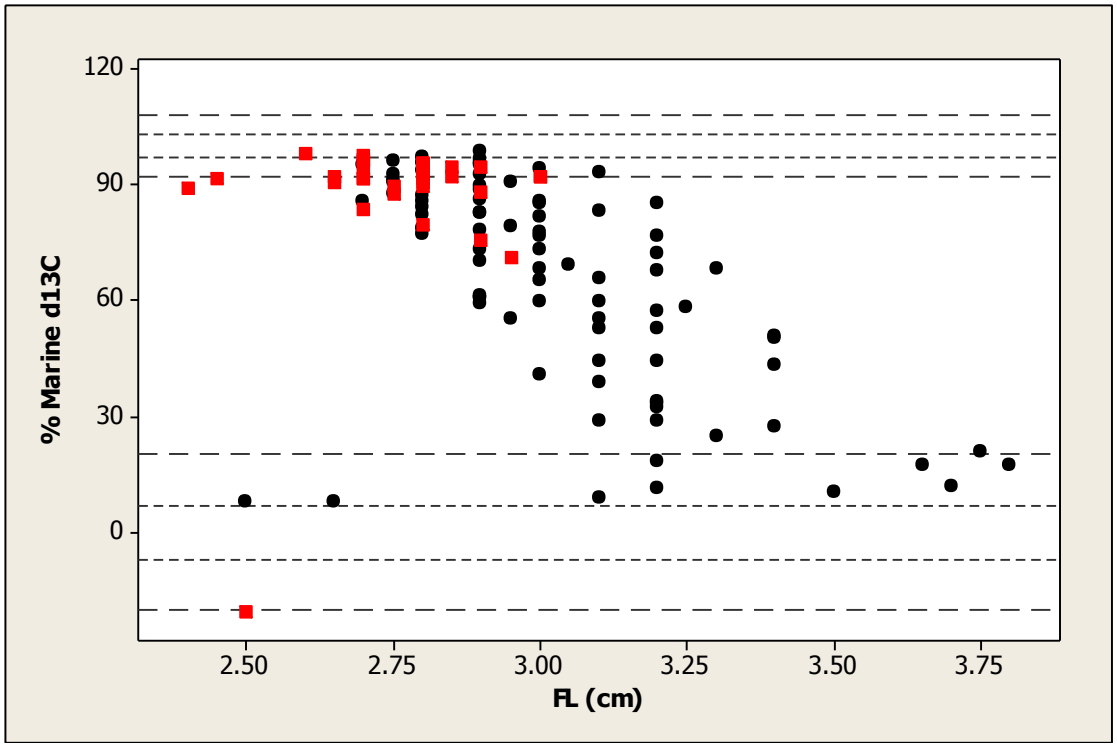

Figure 3. 3. Relationship between calculated proportion of marine $\delta^{13}C$ in each individual offspring and fork length in brown trout fry in the Tadnoll Brook, UK (0 % marine $\delta^{13}C$ corresponds to mean freshwater-resident adults, 100 % marine $\delta^{13}C$ corresponds to mean anadromous adults). The ranges corresponding to 95 % (large dashed line; 2 s.d.) and 50 % (small dashed line; 1 s.d.) of adult observations were used to define intervals that corresponded to the respective life history strategies. Any offspring that fell between the bounds were accepted as being identifiable as having mothers of that life history. Progeny with yolk (endogenous feeding - red squares) and without yolk (exogenous feeding - black circles) are identified. d13C refers to $\delta^{13}C$.

3.3.4 TIME OF EMERGENCE

Anadromous fry were first caught on the 13/3/07 and were collected throughout the whole period of emergence (Fig. 3.4). Fry feeding endogenously, indicating recent emergence, were caught on all but the final occasion (Fig. 3.5). Endogenous feeding fry of freshwater-resident maternal origin were first collected on 27/3/07 (Fig. 3.4 and 3.5) and the remaining fry of freshwater-resident maternal origin collected later. A small number of fry were collected on the first three occasions which had isotopic signatures between the anadromous and freshwater-resident signal, suggesting some degree of exogenous feeding and an earlier date of emergence.

3.3.5 MATERNAL REPRODUCTIVE CONTRIBUTION

Maternal life history was positively identified for a third of the fry caught (Fig. 3.3). It was found that 3 % of fry were attributed to freshwater-resident maternal origin whereas 14-28 % of the fry were attributed to anadromous maternal origin. The maternal origin of the remaining 69 % of fry was undetermined.

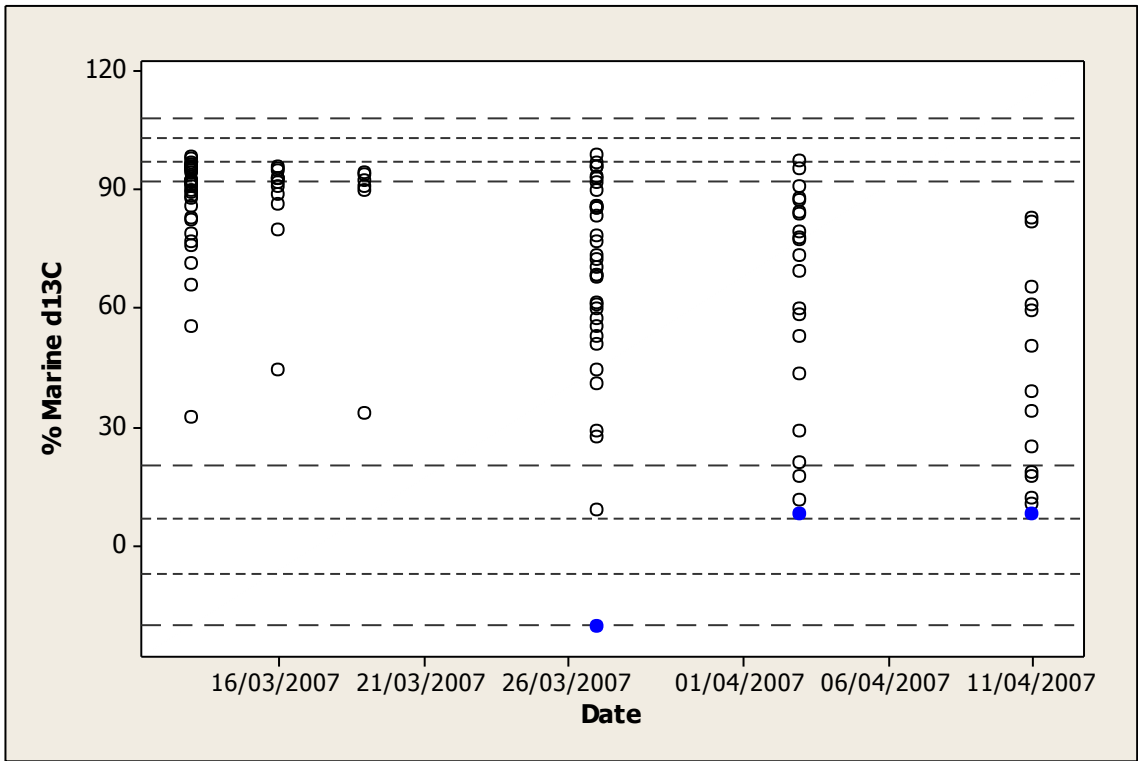

Figure 3. 4. Relationship between calculated proportion of marine $\delta^{13}C$ in each individual brown trout fry and date of capture in the Tadnoll Brook, UK (0 % marine $\delta^{13}C$ corresponds to mean freshwater-resident adults, 100 % marine $\delta^{13}C$ corresponds to mean anadromous adults). The ranges corresponding to 95 % (large dashed line; 2 s.d.) and 50 % (small dashed line; 1 s.d.) of adult observations were used to define intervals that corresponded to the respective life history strategies. Any offspring that fell between the bounds were accepted as being identifiable as having mothers of that life history. Fry identified as being of anadromous maternal origin (white circles) and freshwater-resident maternal origin (blue circles). d13C refers to $\delta^{13}C$.

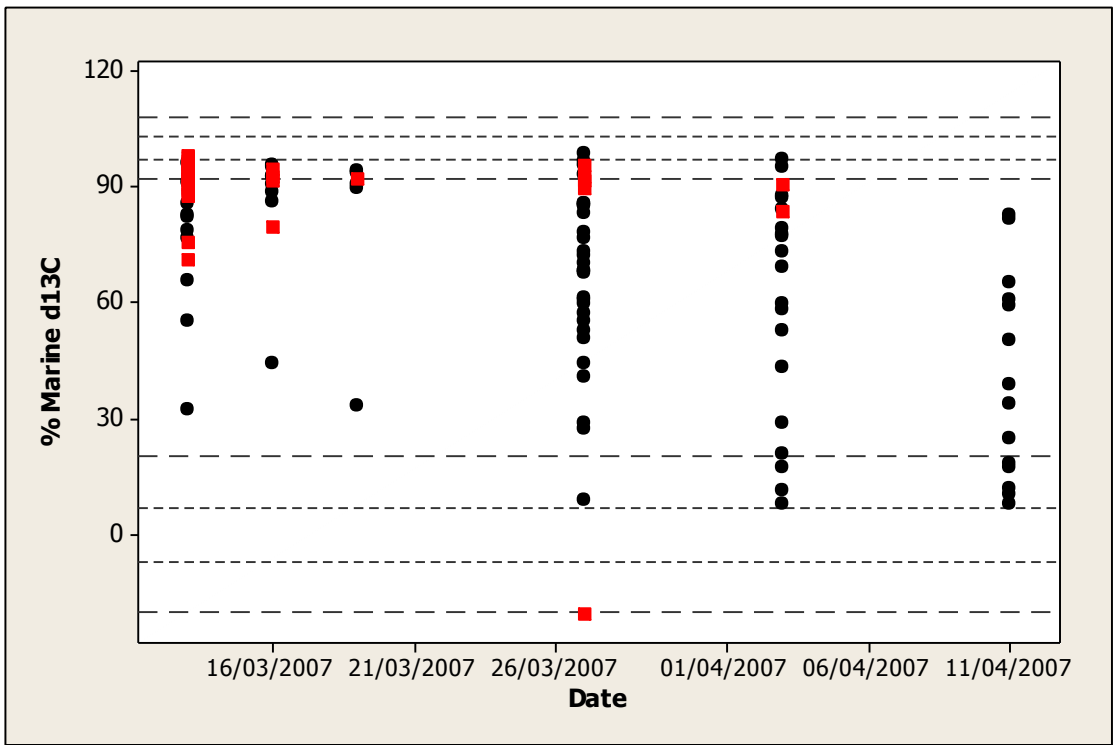

Figure 3. 5. Relationship between calculated proportion of marine $\delta^{13}C$ in each individual brown trout fry and date of capture in the Tadnoll Brook, UK (0 % marine $\delta^{13}C$ corresponds to mean freshwater-resident adults, 100 % marine $\delta^{13}C$ corresponds to mean anadromous adults). The ranges corresponding to 95 % (large dashed line; 2 s.d.) and 50 % (small dashed line; 1 s.d.) of adult observations were used to define intervals that corresponded to the respective life history strategies. Any offspring that fell between the bounds were accepted as being identifiable as having mothers of that life history. Fry have been distinguished between endogenous feeding (red squares) and exogenous feeding (black circles). d13C refers to $\delta^{13}C$.

3.4 DISCUSSION

3.4.1 IDENTIFICATION OF MATERNAL LIFE-HISTORY

The results of this study show how carbon and nitrogen stable isotopes are a valuable tool in analysing the effect of maternal provisioning on offspring fitness and early life-history. Using combined $\delta^{13}C$ and $\delta^{15}N$ it was possible to identify the feeding environment of the adult trout, thus verifying assumed differences in adult life history based on morphology. However, it was not possible to determine the maternal life history of fry using only $\delta^{15}N$: due to high variability in the $\delta^{15}N$ of freshwater resident adults, the range of values that could be returned from anadromous and freshwater-resident mothers overlapped. Freshwater resident brown trout feed on a wide variety of food including freshwater and terrestrial invertebrates with aquatic larvae (Steingrimsson and Gislason, 2002) and at a range of trophic levels including fish (L' Abèe-Lund *et al.,* 2002). In comparison anadromous trout in the marine environment feed on a diet of crustaceans, polychaetes and fish (Knutsen *et al.,* 2001; Rikardsen *et al.*, 2006). Since stable isotopes reflect trophic position and food sources of consumers, resulting in *c*. 3-5 ‰ enrichment for $\delta^{15}N$ and *c*. 1 ‰ enrichment for $\delta^{13}C$ (Peterson and Fry, 1987) per trophic level above primary consumers, the large variance of $\delta^{15}N$ and $\delta^{13}C$ of freshwater resident trout is likely to be a consequence of a more varied diet. Nevertheless, due to wider separation of adult $\delta^{13}C$ it was possible to separate fry of anadromous and freshwater maternal origin using $\delta^{13}C$.

3.4.2 IDENTIFICATION OF FRY LIFE-HISTORY

A mixing-model was used to determine if the $\delta^{13}C$ fry could be reasonably attributed to anadromous or freshwater resident mothers, i.e. if the isotopic signatures of fry were within the range covered by 95 % of observations for adults (Fig. 3.3). Confidence in attribution of maternal life history to fry was strengthened for individuals that still had yolk present. Previous studies have demonstrated that if progeny are feeding endogenously, the tissues will retain the isotopic signal of their maternal parent (McCarthy and Waldron, 2000; Charles *et al.*, 2004). Some individuals had yolk but fell outside the range of observations for adults, suggesting that either the range of accepted observations was too restrictive or that some fry with yolk had started feeding exogenously. The transition from endogenous to exogenous feeding is often identified by the absorption of the yolk-sac which occurs after swim up when the developing juveniles enter the water column to feed (De Leániz *et al.*, 2000). Despite being rare it is not uncommon for salmonid fry to begin feeding before the yolk is fully exhausted.

Hutchings (1991) reported a potential benefit of feeding before yolk absorption in larger brook trout to compensate against the increased developmental time in large eggs. Vignes and Heland (1995) demonstrated that in both Atlantic salmon *Salmo salar* and brown trout emerging fry in semi-natural conditions, first feeding began before the yolk-sac was fully used up. Studies by Doucett *et al*. (1999a) and Curry (2005) have shown that salmonid juveniles can still represent the stable isotope signature of their maternal parent after first feeding (~14 days).

Determining maternal parentage once offspring have begun feeding is possible (Charles *et al.*, 2004; Jardine *et al.*, 2008). However, there is a limited time before organic matter is taken up from the surrounding biome and used for growth and repair, thus gradually replacing the maternal signal with one characteristic of feeding in the freshwater environment (Doucett *et al.*, 1999a). Therefore, identification of fry of resident maternal origin was based on a combination of body size and isotopic signature. Charles *et al*. (2004) demonstrated that the size of emerging brown trout fry did not differ significantly between those which had just begun feeding and those still feeding endogenously. Since the size (Fig. 3.3) of fry feeding exogenously with an isotopic signature indicative of freshwater resident maternal origin (FL = 2.50 cm, 2.65 cm) was similar to that of the fry with a similar isotopic signature but feeding endogenously (FL = 2.50 cm), it was assumed little growth had occurred and that these exogenously feeding individuals had recently emerged.

Allocation of fry to marine and freshwater mothers using $\delta^{13}C$ sources was based on use of a mixing-model, however, such models are known to be sensitive to variation in the parameters used (Grey, 2006). The fry that were outside the interval covering 95 % of anadromous observations but had signs of endogenous feeding (Fig. 3.3), may have been erroneously placed outside the limits by using an incorrect value to correct for fractionation between mother and egg. The best available value from the literature for fractionation was from a study by (Grey, 2001). However, fractionation is dependent on the lipid content of eggs, and may vary among individuals dependent on the quality of eggs. It is also possible that processing errors, resulting in erroneous isotopic ratios, could have had a significant influence. Of the 12 fry identified as having anadromous mothers, a third had been identified as begun feeding with some yolk remaining and the remainder had been identified as collected just after first feeding (based on gut analysis), together with the fry having a high proportion of marine carbon these are likely to be progeny of anadromous mothers.

This study was unable to allocate fry that were collected later in the emergence period, after they had begun exogenous feeding. Fry which fell outside the predicted intervals for anadromous and freshwater-resident adults could not be confidently allocated to mothers of either life history. Even though some of these fry fell within the limits for freshwater-resident origin, their larger body size (FL > 3.10 cm) indicated that substantial growth had occurred as a consequence of feeding in the freshwater environment, thus the isotopic signal would reflect the environment and not the maternal parent. This was a cautious approach; fry with a high proportion of marine $\delta^{13}C$ were likely to be progeny of anadromous mothers. The negative relationship between $\delta^{13}C$ and body size (Fig. 3.4) is likely to reflect anadromous emerging fry starting to feed on benthic invertebrates, thus diluting their maternal signal, and increasingly adopting an isotopic signal representative of the river system as they grow.

3.4.3 SIZE AND TIME AT EMERGENCE

Offspring of anadromous brown trout emerged on average earlier and at a larger body size compared with offspring of freshwater-resident trout (Figs. 3.3, 3.4 and 3.5). These results do not correspond with all previous findings. Thériault and Dodson (2003) observed resident brook charr spawning *c*. 15 days before anadromous charr, with fry of resident mothers emerging earlier. Jardine *et al*. (2008) reported that the progeny of resident brook trout emerged earlier than progeny of anadromous individuals. However, both Chernoff and Curry (2007) and Jardine *et al*. (2008) observed a larger body size in emerging progeny of anadromous salmonids compared with progeny of freshwater-residents.

3.4.4 BENEFITS AND COSTS OF EARLY EMERGENCE

One way that parents can maximise offspring fitness is by spawning at a time that ensures their progeny emerge at an optimum time in terms of food availability and environmental conditions (Armstrong and Nislow, 2006). The advantages of emerging earlier are that it affords offspring access to better feeding territories (Cutts *et al.*, 1999) and increased growth (Hutchings, 1991). It has been hypothesised that when competition for space is high, subordinate fish are unable to establish territories and may adopt migration/dispersal strategies to less profitable habitats (Connell, 1983) and/or freshwater-residents were spawning mainly in a different area. This may explain why so few freshwater-resident trout were found during the period of emergence.

On the other hand, eggs that are spawned earlier in the season may be at a disadvantage as redds can be easily destroyed or disturbed by later spawning activity;

late spawners are known to superimpose redds on top of previously formed nests (Fukushima *et al.*, 1998). Early emergence is not without its disadvantages; post emergence is the period with the highest mortality for offspring (Elliott, 1986; Einum and Fleming, 2000b). Upon emergence and the onset of exogenous feeding, young must quickly establish feeding territories and be able to defend them (Cutts *et al.*, 1999). The influence of spawning time, and thus the influence on offspring fitness, may be offset by environmental conditions; offspring may experience a harsh climate if emerging early in winter/spring when the river system can be less than optimum (bad weather conditions). Early emerging fry may experience a higher number of predators, especially if fry density is relatively low in the early stages of emergence (Brannas, 1995).

3.4.5 BENEFITS AND COSTS OF A LARGER BODY SIZE

The influences of maternal traits are known to affect egg size in salmonids and, further, egg size has been found to be positively related to fry size (Bagenal, 1969; Ojanguren *et al.*, 1996; Lobón-Cerviá, 2000), and there are fitness benefits to juveniles of being a larger size. The volume of yolk resources increases with the size of egg (Elliott and Hurley, 1998b). Using hatchery reared brown trout Ojanguren *et al.* (1996) demonstrated that larger egg size afforded fry with greater energy reserves, which would allow for greater initial growth and increased swimming stamina. Larger fry have greater survival (Hutchings, 1991) and can feed earlier on larger items and a wider range of prey (Wootton, 1990). In a study by Keeley and Grant (1995), the body size of Atlantic salmon fry was found to correlated with territory size, however, food abundance did decrease with increasing territory size.

However, there are disadvantages of large egg size. During incubation in the gravel, eggs may be subjected to low levels of dissolved oxygen (Peterson and Quinn, 1996). As egg size increases the efficiency of oxygen transfer declines (Berg *et al.*, 2001). Therefore, larger eggs may have higher mortality in the redd. This may demonstrate potential adaptive advantages in freshwater-resident females producing smaller eggs that are at an optimum size for the spawning environment.

3.4.6 REPRODUCTIVE CONTRIBUTION

With such antagonistic pressures acting on egg size and timing of emergence, the relative advantages of a maternal anadromous or freshwater resident life history is likely to vary among sites dependent upon local conditions. Here a very small proportion of offspring (3 %) were attributed to freshwater resident mothers. Such a small proportion

may be a true reflection of the contribution of freshwater resident fish to the population or could be due to the sampling procedure. Before fry were collected fry emerging from redds created by freshwater-resident mothers may have migrated to more productive environments (Curry, 2005) or away from more aggressive individuals (Rhodes and Quinn, 1998). The small number of fry of freshwater-resident maternal origin caught may also be a consequence of smaller fry facing higher rates of mortality as they have smaller energy reserves compared with larger fry (Einum and Fleming, 1999).

3.4.7 CONCLUSIONS

The findings of this study suggest that in Tadnoll Brook anadromous females contribute a high proportion of the juvenile population, and that their progeny gain potential fitness benefits in early ontogeny as a result of higher energy resources. However, a large number of fry could not be confidently allocated to freshwater-resident or anadromous mothers as they fell outside the bounds identified for each adult morph: exogenous feeding had begun in some fry reducing the ability to confidently distinguish maternal origin. The impact of these fry of undetermined maternal origin on the estimate of the contribution to the population by freshwater-residents is not known. Future recommendations include recording the exact spawning time and location in the population of study to estimate any differences in hatching time between offspring of the two life-history strategies. Despite having used the Elliott (1994) degree day model to estimate the emergence date and the fact that no fry caught on the first two sampling dates, our results suggest that some of even the earliest fry had already begun exogenous feeding by the time they were caught. It is possible that fry may have emerged earlier and dispersed from another section of the river that was not sampled, however, this seems unlikely given the distances that fry migrate at this age. Heavy rainfall in the weeks up to the estimated emergence date may have washed away embryos, particularly of freshwater-resident mothers, that were not buried deep in the sediment (Crisp and Carling, 1989) or forced emerging fry to disperse downstream to more favourable habitats. To conclude, when using stable isotopes to determine maternal life history it is preferable to collect fry with yolk-sac before they begin exogenous feeding and dilute their maternal isotopic signal (Doucett *et al.*, 1999a), therefore fry can be quickly (visually) identified in the field based on their feeding state (Killeen *et al.*, 1999).

Differences in juvenile fitness in salmonids vary greatly within the same species (Elliott, 1984b; Acolas *et al.*, 2008). The occurrence of both anadromous and

freshwater-resident life histories in a single brown trout population would appear to suggest no overall advantage of either life-history strategy (Pettersson *et al.*, 2001). It is apparent that in Tadnoll Brook, the benefit of migrating to a more productive environment outweighs that of remaining in the river and these benefits are further passed onto their progeny. We hypothesise that in the population anadromous mothers invest more energy (in proportion to their size) into reproduction, and if offspring size is linearly related to maternal size (L' Abèe-Lund and Hinder, 1990), offspring of anadromous adults should be larger. There should be a trade off between size of eggs and fecundity (Elgar, 1990) which will be different for the two morphs as both characters are constrained by female body cavity and energy resources (Roff, 1992). Therefore, anadromous females should produce relatively fewer but large eggs compared with freshwater-resident females producing a higher number of smaller eggs. The larger eggs produced by anadromous females may be an adaptive advantage, as offspring have greater survival and this may compensate for the any reduction in fecundity (Svärdson, 1949). Varying degrees of genetic divergence have been reported between brown trout populations (Ferguson *et al.*, 1995; Fritzner *et al.*, 2001; Youngson *et al.*, 2003) and in rivers where life-history strategies overlap, no genetic differentiation has been detected (Hindar *et al.*, 1991; Pettersson *et al.*, 2001). However, if life-history is genetic and maternal provisioning does influence offspring fitness, at least in the early growth stages, molecular analysis should be employed to resolve this. Differences in phenotype have been linked to environmental conditions such as temperature (Ojanguren *et al.*, 1999) and dissolved oxygen (Roussel, 2007) which may influence offspring fitness. Whether life-history is predetermined by genotype or environment or a degree of both, the fitness benefits to offspring will have been shaped by the experiences of past generations.

CHAPTER 4: PARENTAGE AND RELATEDNESS OF A BROWN TROUT POPULATION.

4.1 INTRODUCTION

4.1.1 MOLECULAR MARKERS

The use of molecular markers has become popular for the identification of individuals and the assignment of offspring to parents. In natural habitats, where it is often not possible to make direct observations of parental success, natural genetic variation which can be 'visualised' with molecular markers has allowed the development of powerful tools for assigning related individuals within populations.

In fisheries, the application of molecular methods has opened up new avenues in ecology, evolution, conservation and management. The development of variable genetic markers (e.g. microsatellites) and associated analytical techniques has made it possible to study variation from the level of stocks and populations down to that of the individual. In assignment analysis, individuals of unknown origin can be assigned to the most likely population of origin, and applications include measuring the effects of stocking (Fritzner *et al.*, 2001) and understanding population structure (King *et al.*, 2001). Significantly, molecular markers can be used to calculate the probability of parentage, facilitating the testing of hypotheses on reproductive success (Taggart *et al.*, 2001; McLean *et al.*, 2004; Baumsteiger *et al.*, 2008) homing (Bentzen *et al.*, 2001) and kin-biased distribution (Carlsson *et al.*, 2004).

4.1.2 APPROACHES OF PARENTAGE ASSIGNMENT METHODS

The initial application of protein markers for parentage assignment had variable success as the markers had relatively low levels of polymorphism (Jones *et al.*, 2010), but with the development of microsatellites with much higher levels of polymorphism, successful assignment was achievable Microsatellites are selectively neutral and following the rules of Mendelian inheritance and the principles of Hardy-Weinberg Law (Jarne and Lagoda, 1996). This states in a population, allele frequencies will remain unchanged, generation after next, as long as rules are met (independent segregation of loci, random mating) and assuming no migration, mutation or selection (Beebee and Rowe, 2005) Parentage and relatedness analysis are hindered by incomplete sampling of candidate adults, errors (null alleles, scoring errors and mutation), relatedness between individuals when not considered and low marker discrimination (Jones and Ardren, 2003). Researchers therefore need to consider carefully which method of analysis will be the most informative and appropriate with the data they have collected. In this study, five methods of parentage analysis were assessed to determine which was the most

appropriate for this system. For each method there are a number of computer software packages available (not reviewed here).

4.1.2.1 *EXCLUSION*

The earliest form of pedigree analysis, exclusion, is based on Mendelian rules of inheritance for diploid organisms, i.e. that a parent and offspring will share at least one allele per locus. If there are incompatibilities with the genotype then the parent is rejected. The benefit of the method is that putative parents are excluded from the sample of candidate parents until the true parent is left (Jones *et al.*, 2010). However, there are some weaknesses to this approach, errors in the data can result in the false exclusion of true parents. To minimise the occurrence of false results, many strict exclusion programs allow for mismatches and often advise starting with more polymorphic loci with the prospect of removing any with null alleles.

4.1.2.2 *CATEGORICAL ALLOCATION*

This method is based on assigning the most likely parent or parent-pair based on likelihood scores to entire offspring. Categorical allocation has fast become the most widely used method in parentage assignment. Benefits of this method include the fact that candidate parents can be known *a priori* or can be designated not known for sampled offspring, which is important in wild populations, and scoring errors can be accommodated (Kalinowski *et al.*, 2007).

4.1.2.3 *FRACTIONAL ALLOCATION*

Fractional allocation uses similar likelihood calculations to categorical allocation, but offspring are split between all putative parents which are not a true representation of the population. A benefit of using this method is that it can be used for inferring reproductive success in a multiple mating population (Nielsen *et al.*, 2001).

4.1.2.4 *PARENTAL RECONSTRUCTION*

In some instances where one parent is known and the sample of offspring has known sibling groups (full-sibs and half-sibs), it is possible to reconstruct the unknown parental genotype by identifying alleles by descent (Jones and Avise, 1997), in which each offspring will have at least one allele per locus from each parent. When both parents are unknown it is also possible to reconstruct both parental genotypes, providing the offspring sample comprises full and half-siblings. This method can be used to assess mating strategies (Myers and Zamudio, 2004).

4.1.2.5 *SIBSHIP RECONSTRUCTION*

This method is appropriate when offspring are sampled, often without parental genotypes, and family relationships are not known *a priori*. Maximum-likelihood is used to infer groups of relatedness (full-sibs, half-sibs, unrelated) among a sample. When parental genetic data is known, this method can be used as a complimentary tool alongside parentage assignment.

4.1.3 CERVUS

The program CERVUS version 3.0.3, (Kalinowski *et al.*, 2007) was used for parentage assignment. This categorical allocation program, was chosen based on a number of criteria (see below), which would provide the most informative parentage assignment

Practically, one of the main concerns of this study was the sampling of the parent fish; despite regular sampling throughout the year and intensive sampling during the spawning season, it could not be assumed that all possible genetic parents had been collected. CERVUS allows incomplete sampling of adults, though there must be knowledge of the total number of breeding adults, which was estimated through visual observation during the spawning season and field observation during the sampling period. CERVUS accommodates scoring errors by allowing for mismatches, this results in assigning the most likely parent and/or the most likely parent with mismatches, which can then be cross checked against the genotype data to assess if the result represents a true relationship or if it is an incorrect assignment.

CERVUS calculates the natural logarithm of the likelihood ratio of all loci for each candidate parent. The overall likelihood ratio is calculated by multiplying the likelihood ratios of each locus and is expressed as the likelihood-odds ratio (LOD). A positive LOD score indicates that the individual is more likely to be a parent than a randomly selected individual, a negative LOD score indicates the individual is less likely to be a parent and a LOD score of zero indicates the individual is equally likely to be a parent of the offspring than a randomly selected individual. Simulations are calculated to determine the critical LOD score at which putative genetic parents are assigned as true relatives for a given confidence level. Parentage analysis on the real data is then carried out, using the same critical LOD score to assign putative parents for a given confidence level.

4.1.4 RELATENDESS AMONG SAMPLES

The ability to infer relatedness is important in many areas of genetics and evolutionary biology (Su *et al.*, 1996; Hansen *et al.*, 1997; Carlsson *et al.*, 2004). However, when

assigning parentage to offspring, one of the assumptions is that the sample of candidate parents is unrelated and if there is family structure within the candidate adults this will affect the outcome of assignment (Olsen *et al.*, 2001). Allele frequencies can be used to estimate relatedness when relationships between individuals are unknown. The program KINSHIP (Goodnight and Queller, 1999) was used to ascertain possible relationships between pairs (full-sibs, half-sibs and unrelated) and among candidate adults by calculating pairwise relatedness (r_{xy}).

4.1.5 AIMS

The aim was to assign the most likely parent-pairs and single parents to 0+ parr in the Tadnoll Brook, using a panel of 12 microsatellite loci. To estimate the extent of relatedness in the candidate parent population, the proportion of full-sibling, half-sibling and unrelated individuals will be determined by calculating pairwise relatedness. This will give an understanding of the genetic variation within the population and the importance of relatedness to fisheries managers.

4.2 METHODS

4.2.1 STUDY SITE AND SAMPLE COLLECTION

Please refer to sections 2.1 and the growth chapter for further information.

4.2.2 SAMPLE PRIORITISATION

With the large amount of tissue samples collected over the course of the sampling period it was not possible to genotype every sample. It was deemed necessary to identify the best adult and parr samples for assignment and the maximum number analysed would be 288 samples.

Adult samples were prioritised for parental assignment based on the following criteria:

- Body length > 20 cm
- Sex of fish is known
- Trout caught on a redd or spawning activity noted
- High recapture rate (from PIT records)

This was also the same criteria used for determining which adult samples would be analysed by SIA.

Molecular analysis of 0+ parr began in winter 2007, this meant that samples collected previously to this were prioritised for assignment. It was important to use parr that had a high recapture rate (to determine growth) as mortality is high in the first year (Elliott, 1994), however from first sampling to the start of analysis there were only four recapture dates. To determine the size range of parr and which samples were optimum for analysis, size frequency histograms were produced for the data collected (June – November 2007) (Table 4.1). Parr that were recaptured three or more times were genotyped first and then those recaptured less. Parr that had not been recaptured within a month from the last two sample collections were genotyped, as fish were not recaptured every month and therefore it was likely to collect them later in the sampling period.

In total, 96 adult trout and 179 0+ parr were prioritised for assignment and relatedness.

Table 4. 1. Prioritisation details of 2007 parr from the Cider Museum site for assignment.

Sampling date	Maximum Parr Length (cm)	Sample for assignment
5.7.07	9	Recaptured ≥ 3 times
9.8.07	9.8	Recaptured ≥ 3 times
4.9.07	10.8	All
16.10.07	10.3	All

4.2.3 DNA EXTRACTION

The Chelex DNA extraction is a simple, fast and inexpensive method however purity of DNA can be an issue (if there is contamination of the sample) and DNA degradation in storage (Estoup *et al.*, 1996). This method was regularly used in the Exeter laboratory on Brown trout and Atlantic salmon populations using microsatellite markers without problems. As the samples to be genotyped were fresh tissue (not historic samples) and cleaned thoroughly before extraction this method was deemed more appropriate than other lengthy protocols.

Chelex resins have an affinity under extreme conditions to metal ions. This method prevents DNA degradation by DNAases by removing magnesium.

Using this protocol, a 2 x 5mm section of tissue was placed in a 1.5 ml Eppendorf tube with 500 µl of 10 % Chelex and 7 µl Proteinase K. The sample was then heated in a water bath at 55 ºC for 75 minutes and vortexed every 15 minutes. The sample was then placed into a 100 ºC water bath for a further 15 minutes with a final vortex. Samples were stored short term in the fridge for immediate analysis or long term in the freezer at -20 ºC in the same tubes. For PCR reactions aliquots of DNA were then taken as needed from these stock solutions.

4.2.4 MULTIPLEX DESIGN

The first priority was to review the literature on individual assignment in salmonids, particularly brown trout and Atlantic salmon, using microsatellite markers (Hansen *et al.*, 2000; Norris *et al.*, 2000; Fritzner *et al.*, 2001). The maximum number of primers

would be fitted, based on size range, into two multiplexes. All potential primers to be tested were inputted into an excel database (including the published size range), this was used to help fit potential sets of primers together. After screening thirty four primer pairs, the final multiplex was designed.

4.2.5 PRIMER OPTIMISATION

A total of 12 microsatellite markers were optimised after each primer pair was tested under a range of PCR conditions and run out on agarose gel (Table 4.2). Unlabelled primer pairs (MWG Oligo) were run over a 12 ºC temperature gradient based around their melting point and over a range of magnesium concentrations every 0.5 mM between 1.5 - 2.5 Mm to determine the optimum annealing temperature and magnesium concentration. Finally once screened, the primer was run under optimum conditions in three reactions; forward primer only, reverse primer only and forward and reverse (control), the reaction which produced the least stutter on agarose was fluorescently labelled (Sigma Proligo).

4.2.6 POLYMERASE CHAIN REACTION (PCR)

The development of PCR technology allows simple amplification of the microsatelite by identifying the unique flanking region either side of the loci using short oligonucleotide primer sequences. The source DNA is incubated with *Taq* polymerase enzyme, oligonucleotide primers, four deoxynucleoside triphosphates (dNTPs), magnesium and reaction buffer.

The PCR reaction involves three steps: 1) Denaturing of the template DNA by heating, 2) Annealing of primers to complementary microsatellite sequence (at optimum annealing temperature) and 3) Elongation using polymerase enzyme for extension of the sequence. After the first PCR cycle the source DNA is amplified exponentially (Taylor, 1991).

All twelve microsatellite loci were amplified in a 10 µl reaction containing 1 unit of *Taq* polymerase (Bioline), 0.5 µM of each primer, 1x buffer, 1.5-2.0 $Mgcl_2$ (Table 4.2), and 0.2 M dNTPs. The PCR reaction consisted of 3 minutes at 94 ºC, 30 cycles for seconds at 94 ºC, 30 seconds at the optimum annealing temperature (Table 4.2), 30 seconds at 72 ºC and a final elongation cycle at 72 ºC for 10 minutes.

Table 4. 2. Details on the microsatellites used in the study.

Locus	Annealing Temp ºC	$MgCl_2$ mM	Size range	Multiplex	Volume µl	Dye	Source
Str85	53	1.5	154-182	1	0.8	Green	(Slettan *et al.*, 1995)
SsHaeIII 14.20	57	2.0	286-322	1	1.5	Blue	(Goodier unpub. Genbank U10050)
Str60	60	2.0	98-102	1	0.7	Green	(Estoup *et al*., 1993)
SS11	60	1.5	331-377	1	1.3	Green	(Martinez *et al.*, 1999)
Strutta58	55	1.5	102-166	1	0.7	Blue	(Poteaux. C 1995 thesis)
Str15	55	1.5	222-230	1	0.5	Blue	(Estoup *et al*., 1993)
Ssa197	60	1.5	130-166	1	0.8/1.5	Blue/Black	(O'Reilly *et al*., 1996)
SsoSL417	56	1.5	174-194	2	0.6	Blue	(Slettan *et al.*, 1995)
SsoSL25	59	1.5	114-134	2	2.0	Green	(Slettan *et al.*, 1995)
SsoSL85	55	2.0	163-177	2	2.0	Green	(Slettan *et al.*, 1995)
SsoSL438	57	1.5	100-110	2	0.7	Blue	(Slettan *et al*., 1996)
Str73	57	1.5	144-152	2	0.7	Blue	(Estoup *et al*., 1993)

4.2.7 AGAROSE GEL ELETROPHORESIS

PCR reactions were ran out on agarose gels during the screening process to determine what reaction conditions were optimum based on brightness of bands and sizing. Gel electrophoresis separates DNA fragments by size by applying an electric current where the smaller sized fragments move through the gel matrix faster. To visualise the different size fragments ethidium bromide was used and a size ladder (100 bp iteration) to estimate size at this stage.

In the laboratory 1.5 % agarose gel was made up using 0.5 TBE buffer Ph 8 to which 10 µl of ethidium bromide was added. Once the gel was set, between 5-10 µl of PCR product was loaded per well and 10 µl of size standard. The gel was run for 20-30 minutes at 100-130 volts then visualised under a UV camera.

4.2.8 GENOTYPING

The PCR product is run on an automated sequencing system to accurately size the alleles and detection of loci is through fluorescently labelling one of the primers. Alleles are eletrophoresed by size on a capillary gel and a fixed distance laser measured the amount of fluorescence in comparison with an internal size standard.

A Beckman Counter CEQ 8000 was used at Exeter University, this has a eight capillary system and uses a ninety six well format. This system allowed up to four dyes, one reserved for the size standard and a combination of the other three made up the two multiplexes of different sized microsatellites (Table 4.2).

All reagents were purchased through Beckman Counter and each sample was loaded together with 25 µl sample loading solution, 5 µl size standard (diluted between a row of 8) and a drop of pure mineral oil. The volume of each locus when multiplexed is shown in Table 4.2, if any samples were weak and needed rerunning singularly, their volumes were adjusted. Periodically samples were run to verify allele calling efficiency. The raw data was analysed using CEQ 8000 (*v*.7) software.

The black dye was the most problematic, showing degradation of sample brightness over time. Ssa197 was changed from black to green towards the end of the genotyping to prevent high numbers of reruns. However, this interfered with another locus of similar size range labelled green (Str85), therefore samples were run separately from the multiplex. A number of control samples were tested in Ssa197 in blue and green dye to check there was no difference to amplifying the microsatellite.

4.2.9 MATCHING SAMPLES

All samples prior to molecular analysis were ran through MICROSATELLITE TOOLKIT version 3.1.1 (Park, 2001) to detect any identical genotypes. Selecting for a tolerance of one non-matching allele, 14 pairs out of 109 adult samples matched 100 %. Each pair was cross checked against the sampling data and in all cases the second adult was classed as 'tag loss retagged'. This classification occurred when there could be no detection of a PIT tag but other signs of sampling were visible for example, a PIT tag scar or early re-growth of a fin clip. These samples were identified as the same fish and the matching samples were removed from the study (n = 13). For the purpose of the growth analysis (Chapter 5) I will use data from both samples.

4.2.10 STATISTICAL ANALYSIS

Adult and parr data was checked for null alleles, scoring errors and large allele drop-out using MICRO-CHECKER version 2.2.3 (Van Oosterhout *et al.*, 2004). The data was formatted using MICROSATELLITE ANALYSER (MSA) version 4.0 (Dieringer and Schlotterer, 2003). Allele numbers, observed (H_0) and expected (H_E) heterozygosity and F_{is} values were calculated using GENEPOP version 4.0.1 (Raymond and Rousset, 1995). The same program was used to test for conformity to Hardy-Weinberg equilibrium (HWE) within both samples across all loci using the method of Guo and Thompson (1992) and linkage disequilibrium across each pair of loci in both samples. Exact *P*-values were estimated using the Markov chain method through using 1000 dememorization steps, 100 batches and 1000 iterations. Significance levels for conformity to HWE and linkage disequilibrium were adjusted for multiple tests using sequential Bonferroni correction (Rice, 1989).

4.2.11 PARENTAGE ANALYSIS

The program CERVUS version 3.0.3; (Kalinowski *et al.*, 2007) was used to assign parentage of parr using the 'parent-pairs with unknown' sex test. This assigns the two most likely single parents and parent pair. Morphological sex assignment could only be accurately determined when fish reached maturation, typically during periods of spawning, therefore, not all adult fish were sexed. The program uses allele frequency data to simulate offspring genotypes and to calculate log-likelihood (LOD) ratios. The simulation provides a statistical means to determine critical values of LOD above which identified parent-offspring pairs can be considered true relatives for a given confidence level. The simulation was run with the following parameters: 10,000 offspring, 128 candidate parents, proportion parents sampled 0.75, proportion loci typed 0.97 and

proportion of loci error 0.01. Ninety-six putative parents were collected over the sampling period, as it was known that not all candidate parents were sampled it was estimated based on field observations that the total number of candidate parents was 128. Genotype error can occur through all stages of microsatellite amplification, a random 10% of samples were regenotyped and the error rate calculated as the number of incorrect alleles divided by the total number of alleles. For parentage analysis, CERVUS assigned the two most likely parents to each parr based on the threshold LOD score determine in the simulation analysis. Confidence levels were set to strict (95 %) and relaxed (80 %).

Mismatches between putative parents and offspring were checked against the genotypes, any scoring errors were corrected and the simulation and parentage was rerun to increase the confidence of assignment.

4.2.12 RELATEDNESS ANALYSIS

The KINSHIP 1.3.1 software (Goodnight and Queller, 1999) tested the likelihood of kin relationships between pairs of adults using allele frequencies. KINSHIP tests specific hypotheses to calculate the likelihood that individuals in a pair share alleles by the relationship specified. A simulation generated random pairs (1000) to specify a significance level for these likelihood ratios and the ratio that was needed to reject the null hypothesis. Statistical significance at $P < 0.01$ was used for likelihood ratios. Three hypotheses tests were performed, the relationship of full-sibling, half-sibling and unrelated on all adult individuals.

The same program was used to calculate pairwise relatedness values (r_{xy}) (Queller and Goodnight, 1989) on all adult pairs. This is an unbiased estimate of the true relationship between x and y. Theoretical expected values of r_{xy} are: full-sibs 0.5, half-sibs 0.25 and unrelated 0.

Misclassification rates were calculated as described in Blouin *et al* (1996). A cut-off r_{xy} between two relationships (i.e. full-sibs and half-sibs) for assigning a proportion of individuals was defined by the average of the mean values of each relationship.

4.3 RESULTS

4.3.1 STATISTICAL ANALYSIS

Genotypes were obtained for 100 % of adult samples at 9 microsatellite loci (n = 96), with the exception of SsHaeIII, Str73 and SsoSL85, for which 98-99 % of samples were genotyped. Genotypes were obtained for 100 % of parr samples at 10 microsatellite loci (n = 179), with the exception of 99 % of samples genotyped at SsHaeIII and Str73. The microsatellite loci used ranged from moderately (Str85, Str15, SsHaeIII, Ssa197, Str73, SsoSL438, SsoSL25, Str60) to highly (Strutta58, SS11, SsoSL85, SsoSL417) polymorphic, and allelic richness ranged from 2 to 17 alleles per locus for both adults and parr (Table 4.3). All 12 microsatellite loci conformed to HWE in the adult samples with comparable levels of heterozygosity. Six out of the 12 loci showed significant deviations from HWE (p <0.05) in the parr samples after sequential Bonferroni correction (Rice, 1989). Departure from HWE did not correlate with any significant heterozygote deficit or excess (Table 4.3). Positive adult F_{is} values did not reflect any deviation in HWE and 6 positive parr F_{is} values were observed, 3 of which indicated a deviation from HWE (Table 4.3). Significant genotypic phase disequilibrium was detected in two pairs (SS11/SsoSL85 and SsoSL85/ SsoSL25) out of 66 adult samples after correction for multiple tests (p <0.05) (Rice, 1989), which suggests physical linkage was negligible. Significant genotypic phase disequilibrium was detected in 41 pairs out of 66 (Appendix 1) after correction for multiple tests (Rice, 1989); this higher proportion expected by chance may be from sampling siblings within the same year class or the effect of small population size.

Table 4. 3. Genetic diversity of 12 microsatellite loci used for parentage and relatedness analysis.

Locus	Adults (*n*=96)					Parr (*n*=179)				
	a	Ho	He	HWE	F_{is}	a	Ho	He	HWE	F_{is}
Strutta58	17	0.854	0.834	0.3098	-0.0245	17	0.911	0.870	**0.0000**	-0.0465
Str85	7	0.729	0.701	0.9162	-0.0404	6	0.732	0.704	0.1918	-0.0396
Str60	2	0.438	0.460	0.6625	0.0489	2	0.290	0.354	0.0195	0.1802
Str15	5	0.583	0.574	0.1031	-0.0167	4	0.626	0.617	0.4328	-0.0148
SsHaeIII	11	0.851	0.835	0.6301	-0.0189	9	0.842	0.798	**0.0008**	-0.0556
Ssa197	8	0.781	0.754	0.6706	-0.0360	7	0.743	0.755	**0.0002**	0.0153
SS11	16	0.771	0.823	0.0082	0.0367	15	0.844	0.859	**0.0000**	0.0175
Str73	3	0.543	0.562	0.5844	0.0354	4	0.528	0.524	**0.0034**	-0.0088
SsoSL85	13	0.737	0.794	0.3101	0.0723	10	0.810	0.812	**0.0000**	0.0024
SsoSL438	4	0.541	0.621	0.3716	0.1281	4	0.721	0.710	0.3086	-0.0149
SsoSL417	11	0.729	0.800	0.0458	0.0894	11	0.788	0.790	0.3095	0.0034
SsoSL25	7	0.688	0.686	0.6834	-0.0026	6	0.687	0.743	0.0091	0.0751

a: number of alleles observed; Ho: Observed heterozygosity; He: Expected heterozygosity; HWE: Hardy-Weinberg Expectations (significant deviations from HWE at $p < 0.05$ after Bonferroni correction are indicated in bold); F_{is}: inbreeding coefficient.

4.3.2 PARENTAGE ANALYSIS

Initial analysis showed that one or two mismatches were identified between putative parent-offspring. The genotypes were reanalysed in which 2 out of 22 mismatches were identified as scoring error and the remaining as non-assignment (true genetic parents not sampled). The corrected genotype data was re-run through the simulation and the critical LOD score (Table 4.4) calculated and applied to the parentage analysis. Assignment was typed at 12 loci and putative parent-offspring was identified with zero mismatches. As sex was only known for a proportion of adult samples (76 %) the data could only be used post-assignment and the sex of adults was used to check that no same sex adults were assigned to an offspring. CERVUS simulated a proportion of parent-pair and single-parent assignments (Table 4.4); however, the observed values were considerably lower compared to the expected.

Putative parent-pair assignment was determined for 8 parr out 179 (Table 4.5) with 95% confidence. Of these, there were two sets of parent-pairs that were assigned to multiple offspring (51A/6A, 47A/52A) indicating successful pedigree inference. Further, female 51A was also assigned with another likely candidate male parent (53A) to another offspring suggesting multiple mating. Sex assignment was determined for all adults except one, however, the high LOD scores were deemed sufficient for confident assignment and thus 67A was identified as a female.

CERVUS assigned the most likely single parents from all possible parents. Assignment of putative single parents was achieved for 43 parr, 32 at 95 % confidence and 11 at 80 % confidence (Table 4.6). Five single putative parents (47A, 6A, 53A, 56A, 67A) which produced multiple offspring were also identified as a putative parent in the parent-pair analysis (Table 4.5). Of the remaining single parent assignments, 11 putative parents were assigned to multiple offspring. Caution has to be used when assigning single unknown parents (a known parent would be, for example, a maternal genotype) to offspring – assignment is more powerful with known parents and with known sex. Despite the inability to assign these offspring to both parents, the high LOD scores indicate successful assignment.

Table 4. 4. Parent-pair and single parent assignment results of 179 parr using the program CERVUS.

Single-Parent		
Assignment	80 %	95 %
Observed	156 (44 %)	63 (18 %)
Expected	313 (87 %)	210 (59 %)
Critical LOD	1.07	5.18
Parent-Pair		
Assignment	80 %	95 %
Observed	28 (16 %)	11 (6 %)
Expected	123 (69 %)	94 (52 %)
Critical LOD	6.75	11.97

Assignment rate and percentage for observed and expected values, relaxed (80 %) and strict (95 %) confidence shown. Critical LOD scores predicted by simulation of 10,000 offspring.

Table 4. 5. Parent-pair with unknown sex assignment results using the program CERVUS. LOD score is the log-likelihood ratio of a parent and offspring relationship.

Parent-Pair										
Offspring ID	First Candidate	Sex	Pair LOD	Pair Confidence	Second Candidate	Sex	Pair LOD	Pair Confidence	Trio LOD	Trio Confidence
38B	51A	♀*	4.70	80 %	6A	♂	10.11	95 %	19.31	95 %
12C	47A	♂*	11.33	95 %	52A	♀*	6.45	95 %	18.27	95 %
27C	51A	♀*	4.78	80 %	53A	♂	7.35	95 %	17.04	95 %
63C	56A	♂*	7.93	95 %	67A	?	5.76	95 %	16.67	95 %
24D	47A	♂*	9.51	95 %	52A	♀*	8.56	95 %	18.98	95 %
32D	47A	♂*	6.32	95 %	52A	♀*	10.02	95 %	17.62	95 %
34D	47A	♂*	9.50	95 %	52A	♀*	11.42	95 %	23.48	95 %
12E	51A	♀*	9.53	95 %	6A	♂	13.62	95 %	27.44	95 %

Offspring ID, first and second candidate (adult): putative offspring and parent-pairs; Pair LOD is between the 1st or the 2nd candidate parent and offspring; Trio LOD is between the most likely candidate parent-pair and offspring; Confidence for the most likely parent assignment was strict (95 %) or relaxed (80 %); * sex assigned only once, ? denotes unknown sex.

Table 4. 6. Single parent assignments using the program CERVUS. LOD score is the log-likelihood ratio of a parent and offspring relationship.

Single-Parent Offspring	Candidate Parent	Sex	Pair LOD	Confidence Level
12B	69A	?	9.82	95 %
19B	17A	♂	5.66	95 %
29B	27A	♀*	11.42	95 %
35B	12A	♀	5.86	95 %
4C	27A	♀*	7.89	95 %
5C	109A	?	5.40	95%
7C	53A	♂*	9.09	95 %
11C	67A	?	5.19	95 %
19C	47A	♂*	10.21	95 %
20C	47A	♂*	9.20	95 %
25C	53C	♂*	8.30	95 %
28C	69A	?	11.44	95 %
32C	63A	♂	6.35	95 %
37C	27A	♀*	5.39	95 %
44C	34A	♂*	5.77	95 %
45C	56A	♂*	8.60	95 %
46C	26A	?	5.81	95 %
53C	63A	♂	5.98	95 %
54C	53A	♂*	7.52	95 %
56C	46A	♂*	6.93	95 %
1D	99A	?	5.53	95 %
2D	73A	?	11.33	95 %
3D	53A	♂*	6.80	95 %
9D	63A	♂	5.55	95 %
14D	56A	♂*	7.16	95 %
23D	5A	♂	6.40	95 %
36D	67A	?	5.28	95 %
40D	87A	♀*	8.34	95 %
50D	102A	♀	5.51	95 %
7E	56A	♂*	10.57	95 %
8E	63A	♂	8.49	95 %
10E	6A	♂	13.48	95 %
2B	59A	♂*	4.72	80 %
22B	105A	♀	3.19	80 %
30B	72A	♂	4.74	80 %
6C	56A	♂*	4.97	80 %
16C	12A	♀	4.19	80 %
52C	25A	♂	3.85	80 %
10D	25A	♂	4.94	80 %
37D	109A	?	3.94	80 %
51D	63A	♂	3.65	80 %
52D	17A	♂	3.72	80 %
57D	12A	♀	3.42	80 %

Offspring ID, Candidate parent: putative parent-offspring assignment; Sex known for some adults but not used in analysis; Pair LOD: log-likelihood ratio for the most likely parent-offspring pair; Confidence of assignment given at strict (95 %) and relaxed (80 %); * sex assigned one once, ? denotes unknown sex.

4.3.3 RELATEDNESS ANALYSIS

The program KINSHIP tested the likelihood that pairs of individuals shared alleles for the relationships of full-sibling, half-sibling and unrelated. Statistical significance was set to $p < 0.01$. The pairwise relatedness statistic (r_{xy}) was calculated (Queller and

Goodnight, 1989) for all pairs of adult samples across all 12 loci. The distribution of r_{xy} values for full-siblings, half-siblings and unrelated adults is shown in Figure 4.1. The full-sibling mean is slightly lower than the theoretical expected value of 0.5, the half-sibling mean is higher than the expected value of 0.25 and the unrelated mean is lower compared to the theoretical expected value of 0.

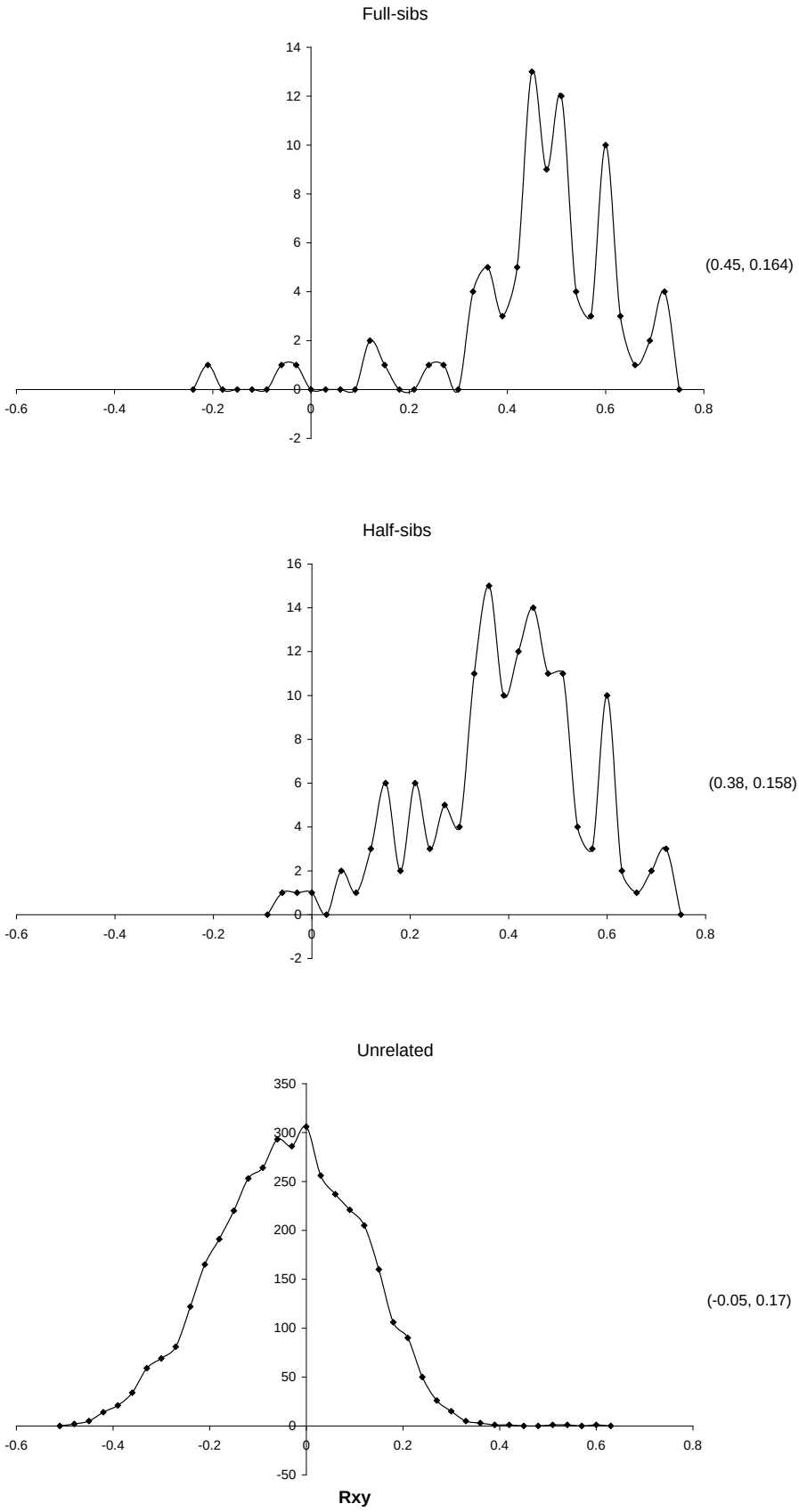

Figure 4. 1. Frequency distribution of r_{xy} for full-siblings, half-siblings and unrelated brown trout adults in the Tadnoll Brook. Mean and standard deviations shown in parentheses.

The distributions of relatedness values for full-siblings and half-siblings overlapped extensively as did the distributions of half-siblings and unrelated values. There was relatively small overlap between full-siblings and unrelated distributions (Figure 4.1). An error rate was calculated as described in Blouin *et al* (1996) to estimate the number of individuals misclassified as another relationship based on a cut-off point (i.e. the mid point between the mean of the two distributions) (Table 4.7). Misclassification rates were the highest between full-siblings and half-siblings (mean 40 %) and half-siblings and unrelated (mean 9 %). Estimated rates of misclassification between classifying full-siblings and unrelated were the lowest (mean 5 %).

Table 4. 7. Misclassification error rates based on r_{xy} values.

True relationship	Misclassified as	Misclassification rate
Full-sibling	Half-sibling	0.415
Half-sibling	Full-sibling	0.415
Full-sibling	Unrelated	0.2
Unrelated	Full-sibling	0.2
Half-sibling	Unrelated	0.165
Unrelated	Half-sibling	0.165

The analysis of the adult population indicates that 2.8 % of pairs have relatedness of 0.51 (full-siblings), 7.2 % of pairs have relatedness of 0.24 (half-siblings) and 78 % have relatedness of -0.07 (unrelated). These values are close to the theoretical expected values for relatedness.

4.4 DISCUSSION

Variable molecular markers such as microsatellites have made it possible to study parentage and relatedness in wild fish populations (Hansen *et al.*, 1997; Blanchfield *et al.*, 2003; McLean *et al.*, 2004; Baumsteiger *et al.*, 2008). This study used a categorical allocation method (CERVUS) to assign the most likely parent-pairs and single parents to parr. The results show that 72 % of the offspring could not be assigned to a parent-pair or single parent; reasons for low assignment are explained below. There is evidence of multiple mating strategies, despite this being a weak relationship, as only one female was assigned and identified as mating twice with different males. Multiple mating has been reported within the salmonids (Fleming, 1998; Garant *et al.*, 2005), with Taggart *et al,*. (2001) reporting up to 50 % of anadromous spawning adults contributing to one redd in Atlantic Salmon. It has been hypothesised that females increase their number of mates to increase genetic diversity, increase fitness in an often unstable spawning habitat (Garant *et al.*, 2001), avoid inbreeding depression and increase offspring heterozygosity (Foerster *et al.*, 2003). It is unknown if multiple matings were the result of synchronized contributions to the same redd or consecutive contributions to a number of redds or if any contributions were from 'sneaking' males (not witnessed in the river).

A higher proportion of parr were assigned to single parents (n = 43); it is probable that only one adult was assigned due to genotyping error, the inability to discriminate all loci and due to not having sampled all parental genotypes. Sources of error ranged from first collecting the sample, DNA extraction, through the PCR process and sizing alleles. Throughout this process steps were taken to minimise error, including multiplexing PCR to reduce handling of DNA, automated sequencers can analyse samples independently reducing gel error and repeat genotyping can be carried out on a subset of samples to calculate an error rate and estimate the sources of error. In this study scoring error was seen in two out of 22 mismatches, while the remaining were identified as incorrect assignments.

The low assignment values are not attributed to relatedness within adults as the candidate adult sample displayed high levels of unrelated estimates. One reason for low assignment may be the resolution power of the loci within the population. By increasing the number of loci or increasing the number of polymorphic loci one can increase allelic discrimination of pedigree relationships (Olsen and Vøllestad, 2001)

Another potential reason for low assignment is that not all parr in the river were sampled and may reflect parr movement within the freshwater environment. Incomplete

sampling of candidate adults may have caused low assignment, CERVUS requires good knowledge of the breeding population to be able to estimate the number of candidate parents. Despite the knowledge of the population through spawning and field observations, the estimate of the total number of candidate parents may have been incorrect; however, initial testing of this program (to see any differences) ran a larger number of candidate adults (results not shown) assuming only 50 % of the population was sampled, but the results did not differ significantly. It is assumed that the estimates of the number of candidate adults are correct but not all the candidate adults were sampled. Neff *et al.* (2000) calculated statistical confidence for parentage analysis when not all the offspring could be assigned with incomplete sampling of adults. Adults with low reproductive success (< 5 %) benefited from increasing the number of loci, whereas adults with high reproductive success (>80 %) benefited from increasing either the number of loci or number of offspring. Therefore, when parental reproductive success is estimated, researchers can determine the number of loci and number of offspring needed for parentage assignment with incomplete adult sampling.

Null alleles were not detected in any of the samples, but there may still have been null alleles that were undetected and therefore acted to cause heterozygote deficiencies and a deviation from Hardy-Weinberg equilibrium (HWE). MICRO-CHECKER detected the presence of possible null alleles in the parr sample, however, this was not detected in the adult sample and therefore Str60 was not identified as a null allele.

Despite no evidence of relatedness within the adult population, deviations from HWE may be an indication of non-random mating within a small population. If not all the adults have been collected, the population may be more related than expected.

Triploids can be found spontaneously in wild populations (Thorgaard and Gall, 1979) and are easily induced for commercial and recreational fisheries (Koenig *et al.*, 2011). Triploids have three sets of chromosomes instead of two in diploid organisms and they are generally sterile from irregular meiotic division of chromosomes (Tiwary *et al.*, 2004). Artificial triploidization can be induced via blocking the second meiotic division or preventing the extrusion of the second polar body of the eggs (Tiwary *et al.*, 2004) using temperature, pressure or chemical treatments (Thorgaard and Gall, 1979; Streisinger *et al.*, 1981; Johnstone *et al.*, 1989). The advantage to using sterile triploids is the reduced risk of interbreeding with the native wild stocks and reduced gonadal production results in the energy being used for growth (Teuscher *et al.*, 2003). There are

no obvious morphological differences in many triploid fish species (Leary *et al.*, 1985) but there have been reported differences in some, including lower jaw deformities in Atlantic Salmon (Sutterlin *et al.*, 1987). Despite being valued for helping management of fish stocks, growth rate and performance studies have shown mixed and often contradictory results between species and between individuals of the same species (Galbreath *et al.*, 1994; McGeachy *et al.*, 1995; Koenig *et al.*, 2011). The results of this parentage study did not show any evidence of triploid alleles therefore triploid individuals were not unknowingly sampled in the population.

In conclusion, this study has shown how assignment of unknown parents without known sex can assign a proportion of offspring to parent-pairs and single parents. The low assignment rate can be mainly linked to the effects of incomplete sampling of adults (and parr) and low allelic discrimination. Further study to improve assignment rate would look at more polymorphic loci to add to the 12 currently used and a more intensive sampling of adults during the spawning period to collect known spawning adults. While assignment without sex determination was possible using CERVUS, the results were not powerful enough to examine the complex mating systems within the population of brown trout in the Tadnoll Brook, however this study does begin to give an insight into the life-history.

CHAPTER 5: PARENTAL INFLUENCE ON OFFSPRING PERFORMANCE AND LIFE-HISTORY STRATEGY IN BROWN TROUT

PIT detection data was provided from the Salmon and Trout Research Centre, Game and Wildlife Conservation Trust (Dr A. T. Ibbotson, W. R. C. Beaumont and L. Scott).

Temperature data was recorded at the Cider Museum site by F. K. Edwards and R. B. Laurisden as part of a food web study.

5.1 INTRODUCTION

5.1.1 POLYMORPHISM IN BROWN TROUT

The success of brown trout populations inhabiting a wide range of habitats is due to their variability in life-history strategies, ranging from freshwater-resident trout to migratory anadromous trout and co-existence of both forms in a population is common (Pettersson *et al.*, 2001). Partially migratory populations of brown trout often exhibit polymorphism, with freshwater-resident trout being significantly smaller compared to their migratory counterparts (Jonsson, 1985). Within populations sexual size dimorphism is usually presented between the two forms, with small resident males predominating in the freshwater habitat (Jonsson, 1985) and large anadromous females predominating in the anadromous population (Forseth *et al.*, 1999). Both forms can spawn in the same areas (Charles *et al.*, 2005) and offspring are morphologically identical during early ontogeny until any smoltify (McCarthy and Waldron, 2000). Both forms can interbreed to produce fertile offspring (Frost and Brown, 1967) and both forms can produce offspring of each form (Rounsefell, 1958).

5.1.2 PARENTAL INVESTMENT

Maternal traits are shaped by natural selection as a phenotypic response to environmental heterogeneity (Mousseau and Fox, 1998). Females can increase their reproductive success through maternal provisioning therefore increasing the fitness benefits for their offspring (Roff, 1992). Reproductive success has been shown to increase linearly with body size (Wootton, 1990). Fecundity in salmonids increases with body size as there is more energy available to produce eggs and a larger body cavity to accommodate eggs (Jonsson and Jonsson, 1997), egg size also increases with body size (Thorpe *et al.*, 1984). Females develop mechanisms to increase their reproductive success by ensuring offspring can survive by providing more energy resources to each egg or by producing many eggs, for example to withstand harsh environmental conditions and competition (Einum and Fleming, 1999). Since there can be no simultaneous maximisation of egg size and number, trade-offs occur between the two and have been witnessed in sympatric brown trout populations (Jonsson and Jonsson, 1999; Olofsson and Mosegaard, 1999). Reproductive success in males is related to how many eggs they can fertilise and individuals may adopt extra-pair mating strategies (Largiader *et al.*, 2001). Smaller mature parr can employ ‘sneaking’ strategies to increase reproductive success and compete with the larger, aggressive males (Broberg *et al.*, 2000).

Time of spawning can influence time of emergence (Elliott, 1984b; Einum and Fleming, 1999) and affect offspring survival and fitness. Early emergence offers offspring a better chance of establishing feeding territories (Elliott, 1990) whereas late emerging offspring may be forced by competition to migrate to less productive feeding areas where there is less chance of survival (Héland, 1980a; b). On the other hand, eggs which are spawned earlier may be at a disadvantage from later spawning activity disturbing the redd and the survival of the eggs (Fukushima *et al.*, 1998) whereas later emerging fry may be at an advantage as they miss potential adverse conditions earlier in the season. Maternal traits influence offspring size at hatching as egg size is positively related to offspring size (Einum and Fleming, 1999; Olsen and Vøllestad, 2001). Jardine *et al.* (2008) studied the emergence and size of a partially migratory population of brook trout, *S. fontinalis* and found anadromous offspring emerged larger and on average at a later date than freshwater-residents.

Parental influence extends to juvenile size and growth rate; Chernoff and Curry (2007) demonstrated in laboratory experiments using brook charr over a three month period that maternal traits affect both size and growth rate. Offspring from anadromous parents were longer and heavier at emergence but showed a lower specific growth rate over the first two months. By the end of the third month anadromous offspring displayed a faster specific growth rate and remained larger compared to offspring of freshwater-resident parents. Rikardsen and Elliott (2000) studied a partially migratory population of Arctic charr in Norway to determine the differences in size and growth rate of parr that smolted and those that remained resident. They found parr which smolted were larger and the largest fish smolted first at a younger age. Growth rate of smolts was faster in the parr stage compared to freshwater-resident parr. Cucherousset *et al.* (2005) demonstrated in a partially migratory population of brown trout in France how juvenile growth is an important factor in determining whether to smolt or remain in the river as a resident. They found that fish with low metabolic rates remained in the freshwater environment as residents, where growth could be sustained by the habitat and faster growing parr smolted to a more productive environment.

5.1.3 THE ONSET OF SMOLTIFICATION

Why some fish adopt a migratory life-history and some adopt a freshwater life-history is not fully understood, particularly in brown trout. The onset of the parr-smolt transformation has been linked to both size and growth rate in salmonids. In the summer, if a pre-determined threshold size will be exceeded by the following spring,

migration will occur (Metcalfe, 1998). Growth rate in juvenile parr is an important factor to determining the decision to migrate (Rowe and Thorpe, 1990). Faster growing parr typically migrate at a smaller size as they have higher metabolic rates and are energy constrained in the freshwater environment (Jonsson and Jonsson, 1993) and migration to more productive habitats can increase their metabolic energy through the increased food resources (Økland *et al.*, 1993; Forseth *et al.*, 1999).

5.1.4 ENVIRONMENT OR GENETICS INFLUENCES THE DECISION TO MIGRATE

Growth is an important factor determining the decision to smolt in the freshwater environment, fast growing parr may be growth limited if they remain in an environment that cannot support their energetic needs (Morinville and Rasmussen, 2003). Transplant experiments of brown trout showed that in low productivity environments food availability is important with a higher proportion undergoing parr-smolt transformation (Zalewski *et al.*, 1985).

If life-history strategy is a genetic predisposition to select a feeding habitat it would be expected that there would be genetic differentiation between the two forms but as yet there has been no detectable differences (Pettersson *et al.*, 2001). The very existence of both forms within a partial migratory population suggests that there is some genetic aspect controlling part if not all of the decision to migrate.

The adoption of a particular life-history strategy is related to environmental and/or genetic influences but research indicates that this could be based on a threshold level of both influences (Piche *et al.*, 2008).

5.1.5 AIMS

Field data collected over one and half years during the juvenile parr stage was used to determine if parental life-history affected offspring performance and life-history using parr that were assigned to both parents and single parents in Chapter 4. Following individual parr the objectives were: 1) to determine if size and growth rate of parr of anadromous and fresh-water resident males/females differed; 2) to estimate the relative maternal/paternal reproductive contribution from anadromous and freshwater-resident trout to offspring production in the population; 3) to determine if parental life-history strategy affects the subsequent life-history of offspring in the first year of smolting.

5.2 METHODS

5.2.1 STUDY SITE

A description of the study site can be found in section 2.1.

5.2.2 SAMPLING AND TAGGING

Adult trout were sampled and monitored between November 2006 and August 2008 (~every month), where most of the fin samples used for analysis (see section 4.2) were collected during the two spawning seasons. In 2006 samples were collected on seven occasions between 21.11.06 and 19.12.06 and in 2007 samples were collected only on three occasions between 13.11.07 – 8.1.08. Trout were collected by electrofishing a 300 m section of the site which was split into four sections (90, 70, 60 and 80 m). Each section was fished twice to determine fishing efficiency (Seber and Lecren, 1967), except during he spawning season when they were fished once. Fish were processed after each section, then held until the completion of the second shock and then were released in the same section. Each fish was lightly anaesthetised in 2-Phenoxyethanol (0.333 ml/l) and the FL (nearest 1 mm), weight (W) (nearest 1 g), sex (if determined) was recorded. For each new fish (for this study) a sample of pelvic fin tissue was removed and stored in 95 % ethanol or frozen (see section 4.2) to be used for molecular and SIA analysis. Information on PIT tagging is found in section 2.3.

Between July 2007 and August 2009, the 0+ parr from the 2006/7 cohort were sampled and monitored on average every month a total of 14 times. A final fishing was carried out on the 11.3.09 to determine visually if any of the cohort was undergoing the parr-smolt transformation by identifying silvering fish (Fig. 5.1). Parr were collected at the same time as sampling adult trout, using the above method of electrofishing and processing.

Re-growth of fin tissue in all fish sampled was successful (Fig. 5.2), with no obvious effect on swimming performance or behaviour (through visual monitoring of released recaptures). Adult samples and data were also collected throughout the study for both new and recaptured trout (between November 2006 and March 2009) and all new fish were PIT tagged. (see section 2.3 for further details).

Figure 5. 1. Demonstration of a brown trout smolt (upper fish) identified by the ‘silver’ colouration, ready to migrate and a freshwater-resident brown trout (lower fish) in the Tadnoll Brook.

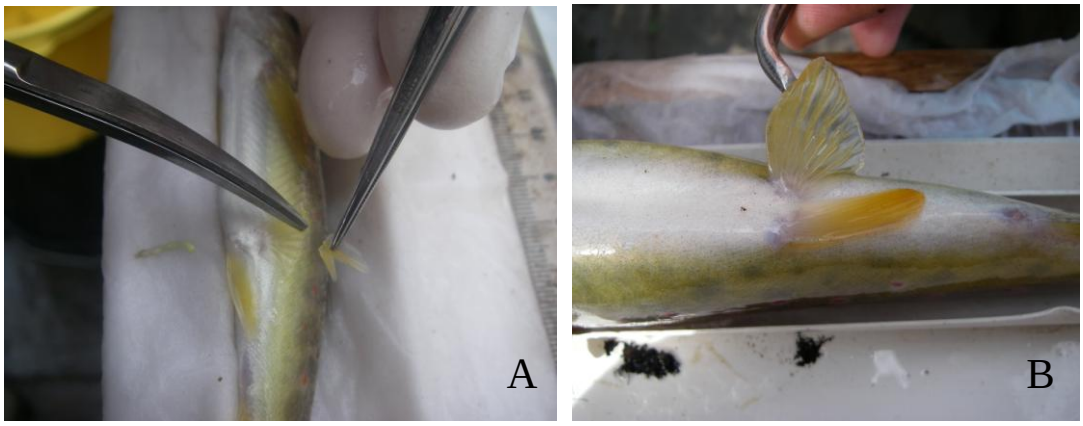

Figure 5. 2. Brown trout pelvic fin showing (a) clipping for molecular and stable isotope analysis and (b) re-growth after three months.

5.2.3 TEMPERATURE DATA

Water temperature in the Tadnoll Brook at Tadnoll Mill was recorded using a HOBO Pendant Temperature logger (Temcon) tethered to the stream bed by a metal rod. A red plastic disc was attached to the logger to mark the location. The data logger recorded temperature (°C) every 15 minutes. The logger was removed every 2-4 months, the data downloaded and returned the same day. Temperature was recorded between 19/9/06 – 10/12/07, problems with the logger resulted with incorrect data recordings for 6/7/07 – 2/9/07 and 1/4/08 – 5/8/08.

Mean daily temperature was recorded at the Cider museum as part of another project and for the periods temperature was recorded at Tadnoll Mill it was comparable to temperatures upstream at the Cider Museum. To display a continuous log of temperature I decided to use data from the Cider Museum for the period 1.7.07 – 31.7.08 collected by (Edwards *et al.*, 2009).

5.2.4 PIT TELEMETRY

Monitoring of fish movement was made possible at five locations along the Tadnoll Brook and River Frome using PIT tag detectors already installed (see section 2.4 for PIT Telemetry locations). The detectors do not indicate direction of movement, however it was possible to infer direction of migration when there was a combination of detections at different locations and times. The antennae do not always detect every fish, for example one detector may miss a fish migrating downstream in the Tadnoll Brook but it is then picked up in the main river (pers. comm. A. Ibbotson). When this occurs it is possible to use a combination of PIT detection data and the data collected in the field to determine putative life-history strategy.

5.2.5 DATA DOWNLOAD

The PIT tag data from all five detectors was downloaded by a member of the study group (Fish Ecology, CEH) into a MS Access database. This was carried out periodically as part of another study to collect data on the population of salmon and trout in the Frome. The detector on the main river was checked daily and data downloaded if there was a file stored. Data from both the Tadnoll Mill and the Millstream detectors were downloaded weekly. During the spring smolt run all readers except Tadnoll Mill were downloaded daily. I had access to this database over the period of sampling of study until the 5.6.09, including historical downloads of PIT tag records of any fish used in this study.

5.2.6 DATA ACQUISITION FOR LIFE-HISTORY STRATEGY

The data collected in the field in the two and a half years of monitoring the Cider Museum site was used to determine the life-history strategy of spawning adults. The resources used to identify an individual's life-history were: 1) SIA- ratios of carbon and nitrogen stable isotopes can identify marine and freshwater feeding (DeNiro and Epstein, 1978) at the time of sampling (see Chapter 3.), 2) PIT detections, 3) Age from scale samples (see section 2.9) and 4) Morphology (FL). During the spawning season it was possible to determine sex through morphological characteristics, however for some adults, sex was only determined once even if they were recaptured. I decided to assign

sex to all adults but use caution (marked with a *) for those only sexed once. For analysis of size, growth and reproductive contribution samples were split into offspring of anadromous and freshwater-resident females and males.

PIT detection was used to determine life-history strategy of the 2006/7 cohort. The final fishing in March 2009 when the parr would be 1+ was to visually identify any potential smolting parr. Parr analysed in this study that were detected at any one of the five PIT locations were classed as a putative smolt, thus indicating a migration. However, care needs to be used, as for some fish this may only indicate migration within the freshwater environment. As this study only focussed on the first two years of the brown trout's life-cycle it was not possible to fully conclude if the 1+ parr which had not been detected had adopted a freshwater-resident life-history, died or may smolt the following year. These parr were tentatively classed as putative freshwater-residents.

5.2.7 SCALIMETRY AND GROWTH

Fish scales are one of the hard structures that can be used to estimate the age and growth of an individual by analysing scale circuli spacing (Fisher and Pearcy, 1990). The life-history of an individual can be determined by counting the different growth rates inferred from circuli spacing that represent summer and winter growth, with the slowest growth in winter (Friedland *et al.*, 1997). An advantage of reading brown trout scales is the ability to determine the years spent feeding in freshwater and at sea and in some cases spawning.

5.2.7.1 COLLECTION AND MOUNTING

Scales were removed using forceps from all new untagged fish and placed in folded paper and stored in small envelopes with the recorded information about the individual fish. Scales were always taken from the area posterior to the dorsal fin and above the lateral line (Fig. 5.3) as scales here are the first to form and are often larger and more symmetric (Frost and Brown, 1967).

Three scales were sampled from each fish, firstly dry scales were checked for flaws under a light microscope then placed in distilled water to be cleaned. The scales were then mounted between two microscope slides and measured under a Projectina microscope at x 10 magnification.

Figure 5. 3. Location of area where scales were removed on brown trout.

5.2.7.2 READING OF SCALES

All three scales were read for each individual to check for any inconsistencies between scales. Standard terminology to describe scale formation and growth were used as followed:

- Focus: the centre of the concentric lines on the scale surface.
- Circuli: concentric lines which are laid down and often the posterior sections are not complete.
- Dark bands: circuli spaced close together formed during periods of slow growth, typically in winter. The outer edge is known as the annulus.
- Light bands: wide spacing between circuli formed during periods of faster growth, usually in summer.
- Annual zone: a measurement of one complete year of the life cycle determined by counting the annuli.
- River zone: period spent in freshwater.
- Sea zone: period spent at sea, this is often determined by the obvious faster growth (light bands) associated with better feeding.
- Plus growth: wider circuli on the scale edge represent an incomplete year in the life cycle.
- Spawning mark: reabsorption of part of the scale surface after spawning results in incomplete circuli.

When reading scales it is important to have a good understanding of the life cycle of the species and in brown trout Elliott's (1994) description of the life stages was

used. The first step in reading scales is to first count the annuli in the river zone to determine the number of years in freshwater. Next is to count the annuli in the sea zone, characterised by wide spaced circuli indicating rapid feeding in the marine environment. Finally any spawn marks are noted along with the plus growth, figures 5.4 & 5.5 show examples of both a freshwater-resident trout and an anadromous trout.

5.2.7.3 AGE NOTATION

The notation used when aging fish can often cause confusion; the 1st April is typically classed as the start of each year with a 0+ fish being less than 1 year old, a 1+ fish being between 1-2, and 2+ fish between 2-3 etc. A decimal point (·) separates the time spent in freshwater feeding and a sea and a '+' indicates plus growth. For aging the scales the terminology recommended by Alan and Ritter (1977), see (Elliott and Chambers, 1996) was used. For example:

- 2+ A fish that spent 2 years in freshwater.
- 3· 0+ 2SM+ A fish that smolted after three years, returned to spawn after less than one year at sea then spawned for a second time and would be entering the river to possibly spawn again.
- 2· 1+ 1SM+ A fish that smolted after two years, returned to freshwater but did not spawn, then spawned for the first time a year later and would be entering the river to possibly spawn again.

5.2.8 ANALYSIS

Analysis was carried out using 20 spawning adults and 42 0+ 2006/7 cohort parr. Parr were categorised as offspring of male or female/anadromous or freshwater-resident adults. There were 5 offspring from freshwater-resident females, 4 offspring from anadromous females, 13 offspring from freshwater-resident males, 12 offspring from anadromous males and 8 offspring with both known parents (7 both anadromous and 1 mixed parentage). Parr with both known parents were included in both male and female analysis. Recapture attempts were made on 14 occasions between 5.7.07 and 5.8.08 (with a final fishing on the 11.3.09 to only determine if any had the external appearance of smolting) and size and growth rates were calculated at and between sampling dates.

5.2.8.1 SIZE

All parr were analysed to calculate the differences in FL per sample date. Mean FL for offspring of male and female anadromous and freshwater-resident parents was compared using a two tailed t-test. Statistical analysis was performed using MINITAB

15. Residual plots verified the assumptions of normality and homogeneity of variance. The level of critical significance in all statistical tests was set to 0.05.

Parr recaptured ≥ 3 times (*n = 28*) were used to compare individual differences in FL over the sampling period. However, lower than expected recapture rates meant that most fish were not captured frequently enough to make direct comparisons, of size and growth rates, of the same fish at each sampling date. The resulting low sample sizes (Table 5.1) could not be analysed statistically and most of the results are restricted to observations of the data.

5.2.8.2 GROWTH

Analysis was carried out on parr which were recaptured over two consecutive months (*n = 35*). The specific growth rate (S*GR*) was calculated for each individual per month for body mass (W) using the formula $G = 100 \times \log (Y_2/Y_1)/(t_2-t_1)$, where Y_2 and Y_1 are body mass on days t_2 and t_1 respectively and expressed as % W day^{-1}. Average growth each month for parr from female/male anadromous/freshwater-resident parents was compared using a two tailed t-test.

5.2.8.3 REPRODUCTIVE CONTRIBUTION AND OFFSPRING LIFE-HISTORY

All parr (*n = 42*) that could be assigned to parents were used to estimate the relative reproductive contribution from male/female anadromous/freshwater-resident adults and subsequent offspring life-history.

Table 5. 1. Numbers of new fish recaptured on each subsequent month for all samples caught more than once (*n* = 33).

	New																			
	July					Aug					Sept					Oct				
	ANA M	RES M	ANA F	RES F	Total	ANA M	RES M	ANA F	RES F	Total	ANA M	RES M	ANA F	RES F	Total	ANA M	RES M	ANA F	RES F	Total
Recap																				
July	3	1	1	0	**5**	/	/	/	/		/	/	/	/		/	/	/	/	
Aug	2	1	1	0	**4**	4	4	4	2	**14**	/	/	/	/		/	/	/	/	
Sept	1	0	0	0	**1**	3	4	3	2	**12**	10	4	4	1	**19**	/	/	/	/	
Oct	2	0	1	0	**3**	2	2	2	2	**8**	5	4	2	0	**11**	1	2	0	1	**4**
Nov	2	0	1	0	**3**	2	2	2	1	**7**	4	1	2	1	**8**	1	1	0	1	**3**
Dec	1	0	0	0	**1**	1	1	1	0	**3**	2	1	1	0	**4**	1	1	0	0	**2**
Jan	1	1	0	0	**2**	0	2	0	1	**3**	1	2	2	0	**5**	1	0	0	0	**1**
Feb	0	0	0	0	**0**	0	0	1	0	**1**	2	1	2	0	**5**	0	0	0	0	**0**
Mar	0	1	0	0	**1**	0	0	0	1	**1**	2	1	1	0	**4**	0	0	0	0	**0**
Apr	1	1	0	0	**2**	2	0	2	1	**5**	2	1	1	0	**4**	1	0	0	0	**1**
May	0	1	0	0	**1**	1	0	1	0	**2**	0	1	0	0	**1**	0	0	0	0	**0**
Jun	1	0	0	0	**1**	0	1	0	0	**1**	1	1	1	0	**3**	0	0	0	0	**0**
Jul	1	0	0	0	**1**	1	0	1	0	**2**	3	1	2	0	**6**	0	0	0	0	**0**
Aug	0	0	0	0	**0**	1	0	1	0	**2**	1	1	2	0	**4**	1	0	0	0	**1**

New – New tagged fish; Recap – recaptured fish

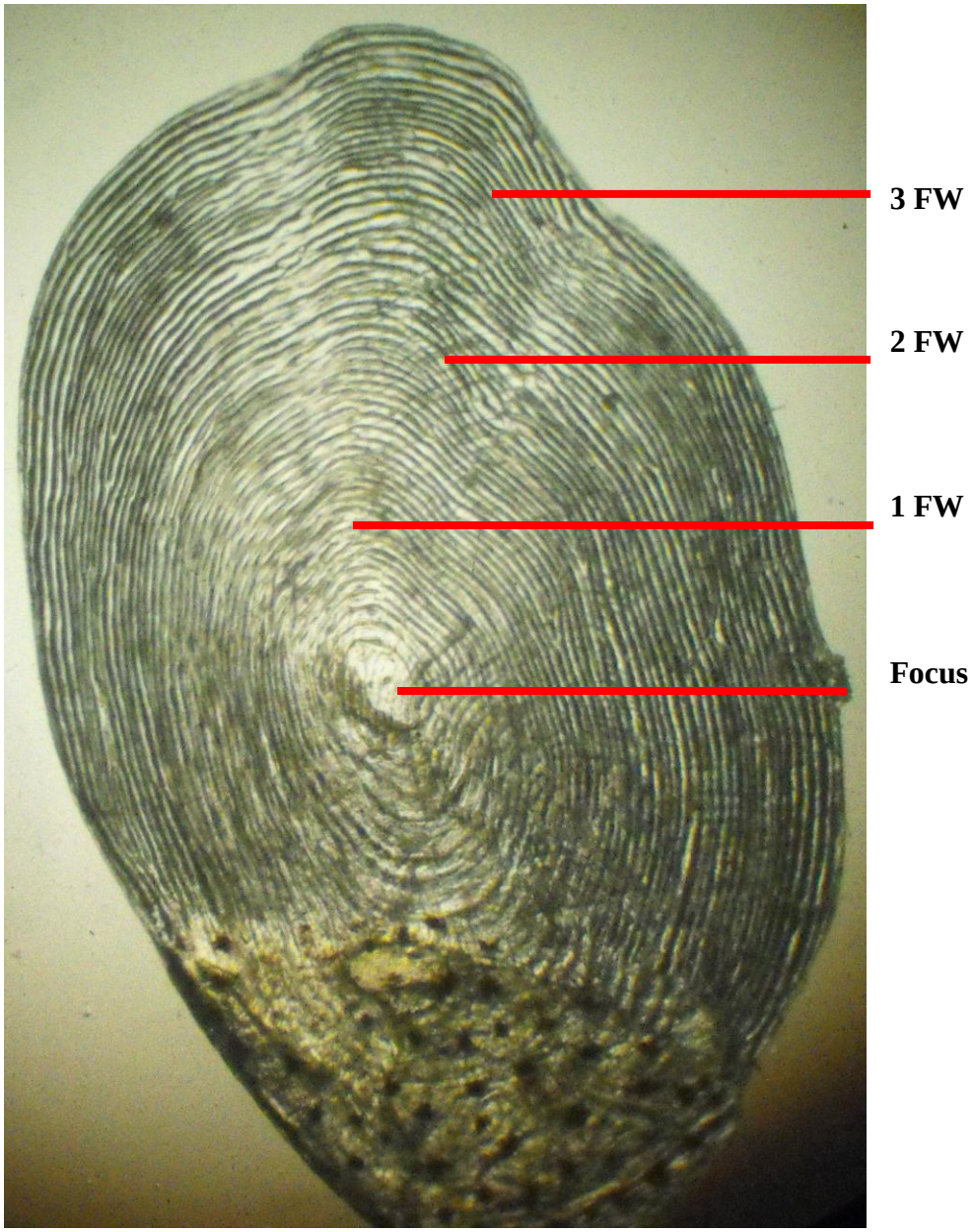

Figure 5. 4. Scale of a male brown trout caught on the 5.12.06 in the Tadnoll Brook measuring 27.5 cm aged 3+

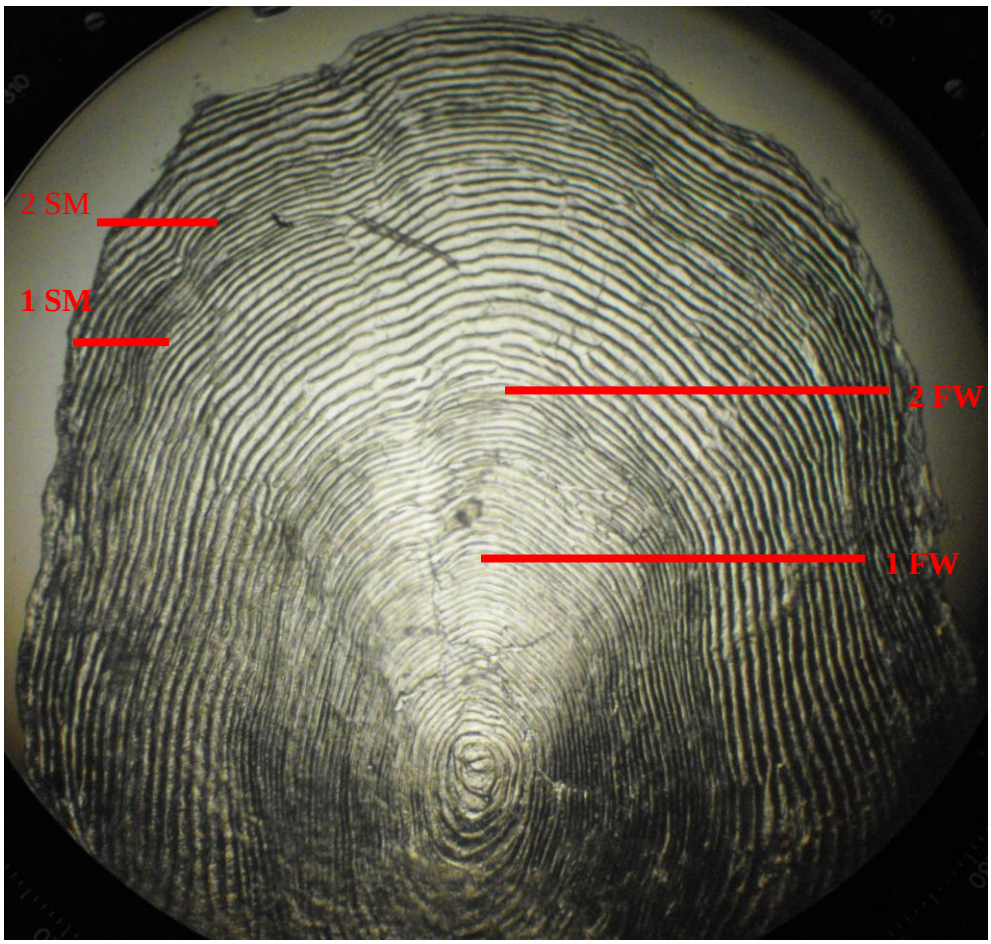

Figure 5. 5. Scale of a female sea trout sampled on the 12.1.206 in the Tadnoll Brook measuring 46 cm. The second annulus in the sea zone is much smaller after scale erosion and absorption after spawning. This fish was aged 2· 0+ 2SM /4+

5.3 RESULTS

5.3.1 ADULT LIFE-HISTORY IDENTIFICATION

The 20 adult samples were identified as either anadromous or freshwater-resident based on the collection of field data (Table 5.2). The majority of samples were mainly identified through SIA analysis, which identifies marine or freshwater feeding indicating the life-history strategy. Only one fish was detected by the PIT tag antennae at the Tadnoll Mill flat bed detectors. Based on the data for this fish it was identified as a freshwater-resident which is likely to have migrated further downstream to feed before returning to spawn. Morphological characteristics (FL, colouration, body form) were also used to aid identification. Size histograms were produced (not shown) on all adult data to identify the potential size range between anadromous and freshwater adults, with anadromous fish identified as ≥ 30 cm. In the instances where adults were not tagged (*n* = 3) or analysed by SIA, the data collected in the field was used to determine life-history. The majority of the adults could be aged (Table 5.3), ones which could not (*n* = 5) were due to the scale samples being unreadable. Age was determined and the presence of any spawn marks and/or marine growth on scales was noted to attempt to identify each individual's life-history.

5.3.2 ADULT SIZE

5.3.2.1 FEMALE ANADROMOUS AND FRESHWATER-RESDIENTS

The size of female anadromous and freshwater-resident adult trout (FL) were significantly different (t- test: df = 3, t = 6.07, *P* = 0.009) with anadromous females being larger (48. 0 – 74.8 cm) compared to resident trout (17.1 – 22.0 cm).

5.3.2.2 MALE ANADROMOUS AND FRESHWATER-RESIDENTS

The sizes of male anadromous and freshwater-resident adult trout (FL) were significantly different (t- test: df = 4, t = 5.20, *P* = 0.007) with anadromous males being larger (37.3 – 67.8 cm) compared to resident trout (16.1 – 26.5 cm).

Table 5. 2. Methods used to identify life-history of parent samples in the study.

Adult ID	Sex	FL (mm)	Date sampled	SIA	PIT detection	Morphology	Age	Life-history
102A	♀	206	19.12.06			Yes		Resident
105A	♀	183	21.11.06			Yes	Yes	Resident
12A	♀	220	12.12.06	Yes			Yes	Resident
17A	♂	228	21.11.06	Yes				Resident
25A	♂	265	12.12.06	Yes				Resident
27A	♀*	510	5.12.06	Yes		Yes	Yes	Anadromous
34A	♂*	227	12.12.06	Yes				Resident
46A	♂*	373	12.12.06	Yes			Yes	Anadromous
47A	♂*	661	12.12.06	Yes		Yes		Anadromous
51A	♀*	748	30.11.06	Yes		Yes		Anadromous
52A	♀*	480	30.11.06	Yes		Yes	Yes	Anadromous
53A	♂*	678	30.11.06	Yes		Yes		Anadromous
56A	♂*	456	5.12.06	Yes		Yes	Yes	Anadromous
59A	♂*	250	19.12.06	Yes				Resident
5A	♂	262	21.11.06	Yes				Resident
63A	♂	253	21.11.06	Yes	Yes			Resident
67A	♀**	171	30.11.06	Yes				Resident
6A	♂	597	19.12.06	Yes		Yes	Yes	Anadromous
72A	♂	173	15.12.06	Yes				Resident
87A	♀*	540	11.12.07			Yes	Yes	Anadromous

* Sexed only once. **Sex identification based on parentage-pair analysis

Table 5. 3. Age estimation of adults using scale samples.

Adult ID	Freshwater	Marine	Spawning	Age	Total Age	Date Sampled
102A	2	-	-	2+	2+	4.9.07
105A	2	-	1	1+1SM+	2+	21.11.06
12A	2	-	1	1+1SM+	2+	21.11.06
17A	2	-	-	2+	2+	21.11.06
25A	3	-	-	3+	3+	12.12.06
27A	3	1+	1	3·0+1SM+	4+	5.12.06
34A	2	-	-	2+	2+	12.12.06
46A	2	2	1	2·1+1SM+	4+	12.12.06
47A						12.12.06
51A						30.11.06
52A	2	2	1	2·1+1SM+	4+	30.11.06
53A						30.11.06
56A	?2	1+	1	?2·0+1SM+	?3+	5.12.06
59A	3	-	-	3+	3+	19.12.06
5A						21.11.06
63A	?2	-	-	?2+	?2+	21.11.06
67A	?1	-	-	?1+	?1+	30.11.06
6A	2	3+	3	2·0+3SM+	5+	19.12.06
72A						15.12.06
87A	?2	2+	2	?2·0+2SM+	?4+	11.12.07

? denotes unsure samples.

5.3.3 SAMPLE SIZE AND ANALYSIS

Sample sizes were too small to carry out sufficient statistical analysis on offspring size and growth rate (Table 5.1). The small sample size was attributed to low numbers of parr assigned to parents in Chapter 4 and the low recapture rates of offspring over the year (some only recaptured once). Mean FL was plotted for all fish caught each month, but different fish contributed to each month's data therefore analysis comparing mean values within each month constituted a different sample each time. Growth rates were plotted for individuals collected on two consecutive sampling dates which produced small sample sizes and only three months had sufficient data to carry out statistical comparisons between groups. The mean values for size and growth rate in Figures 5.6 & 5.9 are constructed from different fish at each sampling date.

5.3.4 PARR SIZE

5.3.4.1 OFFSPRING OF ANADROMOUS AND FRESHWATER-RESIDENT FEMALES

There were no significant differences within each month in mean FL between offspring of the two maternal forms, except during November (Fig. 5.6), where offspring of female anadromous parents were longer compared to freshwater-resident offspring (t-test: df = 4, t = 3.86, P = 0.018). Average FL for female anadromous offspring was longer in 6 (Fig. 5.6) out of the 7 months when both samples were present. On average individual offspring of anadromous females appear longer between August – November) (Fig. 5.7) compared to resident offspring.

5.3.4.2 OFFSPRING OF ANADROMOUS AND FRESHWATER-RESIDENT MALES

There were no significant differences in mean FL within each month between offspring of the two paternal forms, except during October and December (Fig. 5.6). Offspring of male anadromous parents were longer compared to freshwater-resident offspring in October (t- test: df = 17, t = 3.58, P = 0.002) and December (t- test: df = 7, t = 2.49, P = 0.042). Mean FL of male anadromous offspring was longer in 12 out of the 14 months sampled (Fig. 5.6). On average individual offspring of anadromous males appear longer between August and January (Fig. 5.8) compared to resident offspring.

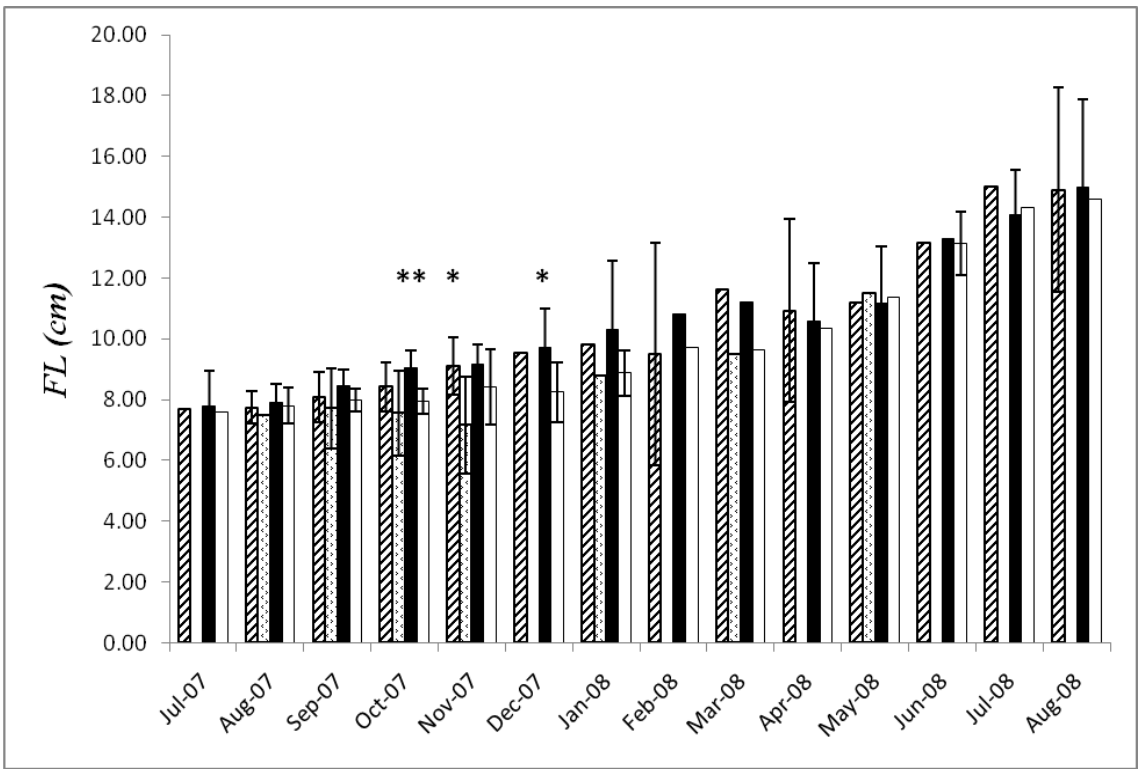

Figure 5. 6. Mean fork length of all offspring recaptured each month from female anadromous (diagonal lines) and freshwater-resident (dotted) and male anadromous (black) and freshwater-resident (white) parents, with 95 % CI for $n > 2$. Different offspring contributed to each months data, therefore each month represents a different sample each time. Significant differences in mean fork length between offspring of female anadromous and freshwater-resident (November) and male anadromous and freshwater-resident (October and December) are shown with significance of $P < 0.05$ - * and $P < 0.001$ - **.

5.3.5 0+ GROWTH RATE

5.3.5.1 *OFFSPRING OF ANADROMOUS AND FRESHWATER-RESIDENT FEMALES*

The mean SGR for body mass was calculated for offspring of anadromous and freshwater-resident females, recaptured over two consecutive months. When sample size was sufficient (> 2 samples), statistical analysis showed there was no significant difference in mean growth between the two groups each month (during August-September, September-October and October-November) (Fig. 5.9). Interestingly, the raw data suggests during August – November, freshwater-resident offspring display on average a faster growth compared to anadromous offspring (data not shown).

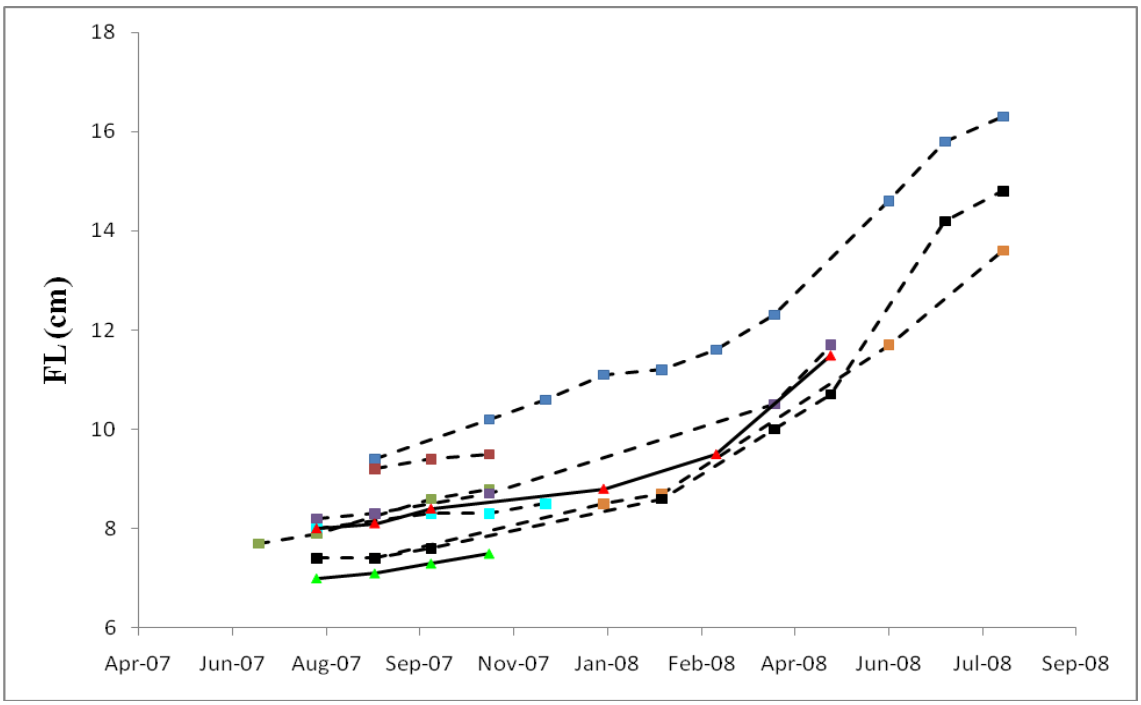

Figure 5. 7. Fork length of individual offspring from females that were either anadromous (dashed line, n = 7) or freshwater-resident (solid line, n = 2). Individual parr are identified by different coloured symbols (square: anadromous, triangle: freshwater-resident).

5.3.5.2 *OFFSPRING OF ANADROMOUS AND FRESHWATER-RESIDENT MALES*

The mean SGR for body mass was calculated for offspring of anadromous and freshwater-resident males, recaptured over two consecutive months. When sample size was sufficient (> 2 samples), statistical analysis showed there was no significant difference in mean growth between the two groups each month (during August-September, September-October, October-November and November-December) (Fig. 5.9). Interestingly, the raw data suggests during August – November, freshwater-resident offspring display on average faster growth rates compared to anadromous offspring. However, during January – April, anadromous offspring display on average faster growth rates (data not shown).

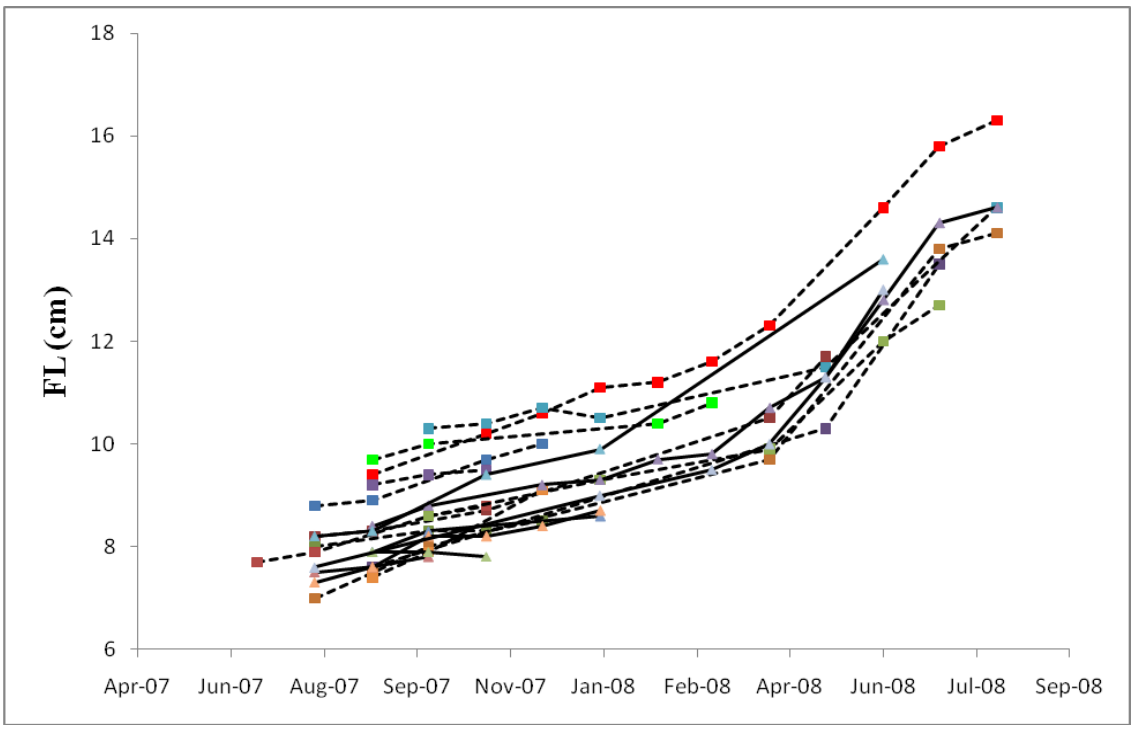

Figure 5. 8. Fork length of individual offspring from males that were either anadromous (dashed line, *n* = 12) or freshwater-resident (solid line, *n* = 7). Individual parr are identified by different coloured symbols (square: anadromous, triangle: freshwater-resident).

5.3.6 TEMPERATURE

5.3.6.1 *2007/8*

A seasonal pattern was recorded in water temperature at the Cider Museum site between July 2007 and July 2008 (Fig. 5.10). Water temperature ranged between 6.6 °C in November to 16.1 °C in July (08). There were no significant within month temperature variations, therefore all parr born or feeding at this site experienced the same temperature variations.

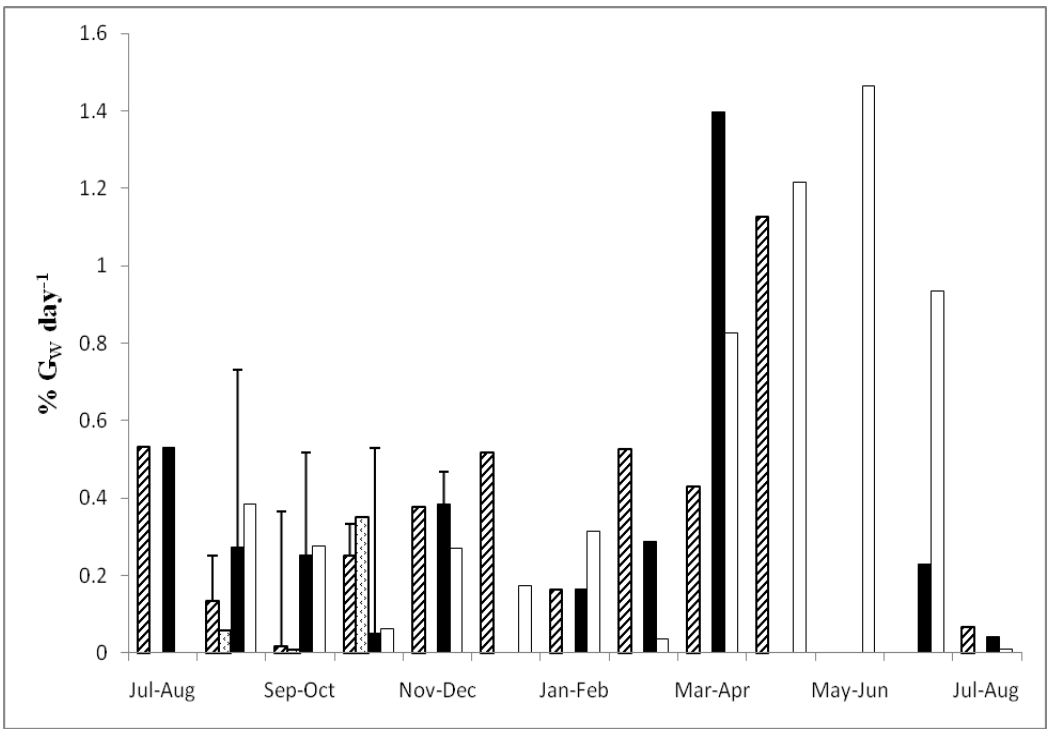

Figure 5. 9. Mean specific growth rate in body mass for offspring caught over two consecutive months for offspring of female anadromous (diagonal) and freshwater-resident (dotted) and male anadromous (black) and freshwater-resident (white) parents. with 95 % CI for $n > 2$. Different offspring contributed to each date interval, therefore each month represents a different sample each time.

5.3.7 REPRODUCTIVE CONTRIBUTION

5.3.7.1 *MATERNAL*

Of the parr assigned to female anadromous and freshwater-resident adults in Chapter 4 and used in this study, 22 % of parr were attributed to anadromous origin whereas 12 % of the parr were attributed to freshwater-resident origin.

5.3.7.2 *PATERNAL*

Of the parr assigned to male anadromous and freshwater-resident adults, 40 % were attributed to male anadromous origin whereas 26 % were attributed to male freshwater-resident origin.

Of the total number of parr analysed in this study ($n = 42$), 62 % were attributed to anadromous origin whereas 38 % were attributed to freshwater-resident origin.

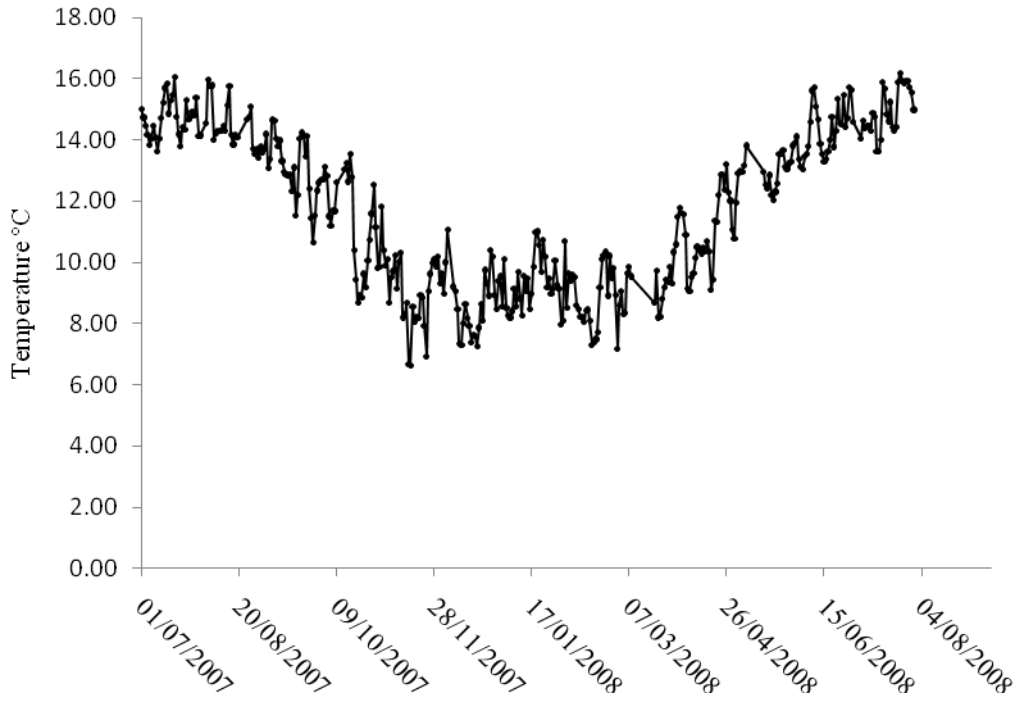

Figure 5. 10. Mean daily water temperature (°C) at the Cider Museum site on the Tadnoll Brook, between 1/7/07 to 31/07/08.

5.3.8 OFFSPRING LIFE-HISTORY STRATEGY

Of the parr that were assigned through parentage assignment in Chapter 4, 42 parr were used in this study.

Both parents were identified for eight offspring, with seven having anadromous parents and one offspring having an anadromous father and freshwater-resident mother (Table 5.4). PIT detection data collected in June 2009 indicated four parr were putative smolts. The anadromous parent pair 47A/52A produced multiple offspring, of which two were identified as putative smolts and two identified as putative freshwater-residents. Anadromous female 51A mated with at least two males and produced two putative smolts (with 53A) and one putative freshwater-resident (with 6A).

Single parents were identified for 34 parr (Table 5.5) while the other candidate parent genotype was not known (Chapter 4). Six parr were identified as putative smolts, three from anadromous males, one from a resident male and two from resident females. Twenty-eight parr were identified as putative freshwater-residents, with twelve from resident males, two from resident females, nine from anadromous males and four from anadromous females. Parr were assigned to parents from the parent-pair analysis (47A, 6A, 53A, 56A) and six single parents (17A, 27A, 12A, 63A, 56A, 25A) produced multiple offspring. The life-history of offspring was not related to parental life-history.

Of the 42 parr for which assignment analysis was successful, ten were identified as putative smolts (at 2+), seven were attributed to anadromous origin and three were attributed to freshwater-resident origin. Thirty-two parr were identified as putative freshwater-residents, sixteen from anadromous origin and sixteen from freshwater-resident origin.

Table 5. 4. Life-history identification of offspring assigned to parent-pairs.

Offspring ID	Life-history	First Candidate Parent	Sex	Life-history	Second Candidate Parent	Sex	Life-history
38B	Smolt	51A	♀*	ANA	6A	♂	ANA
12C	Smolt	47A	♂*	ANA	52A	♀*	ANA
27C		51A	♀*	ANA	53A	♂	ANA
63C		56A	♂*	ANA	67A	♀**	RES
24D		47A	♂*	ANA	52A	♀*	ANA
32D	Smolt	47A	♂*	ANA	52A	♀*	ANA
34D		47A	♂*	ANA	52A	♀*	ANA
12E	Smolt	51A	♀*	ANA	6A	♂	ANA

* sexed only once in the field; **sexed through parentage assignment ANA: anadromous; RES: Freshwater-residents.

Table 5. 5. Life-history identification of offspring assigned to single parents.

Offspring ID	Life-history	Candidate parent	Sex	Life-history
19B		17A	♂	RES
29B		27A	♀*	ANA
35B		12A	♀	RES
4C		27A	♀*	ANA
7C		53A	♂*	ANA
19C		47A	♂*	ANA
20C		47A	♂*	ANA
25C	Smolt	53A	♂*	ANA
32C		63A	♂	RES
37C		27A	♀*	ANA
44C		34A	♂*	RES
45C	Smolt	56A	♂*	ANA
53C	Smolt	63A	♂	RES
54C		53A	♂*	ANA
56C		46A	♂*	ANA
3D		53A	♂*	ANA
9D		63A	♂	RES
14D		56A	♂*	ANA
23D		5A	♂	RES
40D		87A	♀*	ANA
50D		102A	♀	RES
7E		56A	♂*	ANA
8E		63A	♂	RES
10E		6A	♂	ANA
2B		59A	♂*	RES
22B		105A	♀	RES
30B		72A	♂	RES
6C	Smolt	56A	♂*	ANA
16C	Smolt	12A	♀	RES
52C		25A	♂	RES
10D		25A	♂	RES
51D		63A	♂	RES
52D		17A	♂	RES
57D	Smolt	12A	♀	RES

* sexed only once; ANA: anadromous; RES: Freshwater-residents.

5.4 DISCUSSION

The main problem faced in this study was the low sample size and recapture rate of parr (Table 5.1). Parr samples were prioritised for molecular and growth analysis (see section 4.2.2) in September/October 2007. This was to have enough time to carry out laboratory work (primer optimisation, PCR and genotyping) and parentage assignment however, out of the 172 parr investigated, only 42 could be assigned to parents. Recapture rates of parr were low, with some fish only recaptured once. It is likely that some fish were not recaptured due to mortality or migration within the freshwater environment, after the samples were prioritised. The small sample size made it difficult to perform useful statistical analysis on

differences in size and growth rate. Mean values could only be used to test differences within each month and different fish were represented each month. Ideas about overcoming these problems are addressed in the further work section below (5.4.4).

5.4.1 OFFSPRING SIZE AND GROWTH RATE

There was no detectable effect of parental influence on offspring size during the first year of the parr stage. The original hypothesis, 'the size of parr from anadromous and freshwater-resident females/males will be the same, i.e. there will be no significant difference', cannot be rejected but the raw data suggests anadromous offspring are larger compared to their freshwater-resident counterparts, suggesting an early size advantage. Previous studies have shown that maternal traits affect offspring size (Einum and Fleming, 1999; Olsen and Vøllestad, 2001). The results of the present study agree with Chernoff and Curry (2007) who showed offspring of anadromous *S. fontinalis* were larger during three months after emergence compared to freshwater-resident offspring and support the findings in Chapter 3 that anadromous fry have a size advantage. Egg size (not studied here) is positively related to offspring size and anadromous females may maximise their fitness and survival of offspring by providing increased resources to their eggs compared to freshwater-resident females (Einum and Fleming, 1999; Olsen and Vøllestad, 2001). This size advantage may afford offspring better chances of survival (Elliott and Hurley, 1998b) and an increased competitive ability of establishing feeding territories (Elliott, 1984b). The male influence on offspring is only seen as a contribution of sperm therefore males can increase their reproductive success through extra-pair mating strategies. This size advantage may influence which individuals smolt and which remain resident, Rikardsen and Elliott (2000) demonstrated that in a population of partially migratory Arctic charr, those which smolted were the largest, though the parental life-history form was not studied.

There was no detectable effect of parental influence on offspring growth rate during the first year of the parr stage. The original hypothesis, 'growth rate of parr from anadromous and freshwater-resident females/males will be the same, i.e. there will be no significant difference', cannot be rejected, however interestingly the raw data suggests parental influence may have an effect. During August – November, parr from freshwater-resident parents show on average a faster growth rate compared to parr from anadromous parents and during January – April parr from anadromous parents (paternal parr only) display on average a faster growth rate. However, caution must be used as the data includes parr only assigned to a paternal parent and therefore that contribution will be different to that of a female parent

(Bang *et al.*, 2006). There have been no published studies of parental effect on growth rates of offspring of anadromous and freshwater-resident salmonids. Studies have focussed on the differing growth rates between individuals which smolt and those which remain resident. The findings of this present study contrast with those of Metcalfe and Thorpe (1992) who found in Atlantic Salmon, during winter larger fish could maintain their feeding whereas the smaller fish stopped feeding and suffered a decline in growth rate. The implications for offspring displaying varying degrees of growth during the early parr stage may be a mechanism for parr from freshwater-resident adults to compensate for their smaller size by having an increased growth rate to achieve a threshold size for smolting.

It is hypothesised based on the findings of this present study that offspring of anadromous parents are afforded a size advantage during the early parr stage and offspring of freshwater-resident parents have a faster growth during the autumn/winter months (August - November) to compensate for this size difference. As offspring from both parental life-history forms can smolt (see section 5.4.3), the decision to migrate may be triggered if a size threshold is exceeded by the following spring (Piche *et al.*, 2008). Therefore, the largest and/or fastest growing offspring from each form may smolt.

Water temperature recorded during the first year of the parr life-history was comparable with temperatures recorded in streams in South England with a range of 5-17 °C (Berrie, 1992). As temperature affects growth and survival (Elliott, 1994) the ranges detected within and between months in the Tadnoll Brook were not growth limiting.

5.4.2 REPRODUCTIVE CONTRIBUTION

Anadromous reproductive contribution to offspring in the Tadnoll Brook was greater than freshwater-resident contribution. These findings cannot reject the hypothesis, 'The relative maternal/paternal reproductive contribution will be equal between anadromous and freshwater-residents to offspring production' as the data was not strong enough because only a low proportion (23 %) of the parr could be assigned to parents. The higher anadromous reproductive contribution is supported by the findings in Chapter 3. As reproductive success increases with body size (Wootton, 1990) and anadromous females were significantly larger than freshwater-residents in the Tadnoll Brook, anadromous females may have afforded fitness benefits to their progeny through size differences at emergence (see Chapter 3). The assignment results also show a presence of multiple mating strategies of an anadromous female, a mechanism that has evolved to increase the reproductive success. Reproductive contribution between life-history forms to offspring production within rivers may vary from

one river to the next, as the advantages of maternal influence on offspring will be dependant on local conditions (Curry, 2005).

5.4.3 OFFSPRING LIFE-HISTORY STRATEGY

Offspring life-history strategy was not influenced by parental life-history, as adult pairings produced anadromous and freshwater-resident offspring, this is supported by previous studies (Rounsefell, 1958; Sckrochowska, 1969). However, the original hypothesis that parental life-history does not affect subsequent offspring life-history strategy (i.e. the decision to mature or smolt)', could not be rejected as the data was not strong enough. This is because only a low proportion of the parr could be assigned to parents, with 42 % assigned to a single parent. This study followed parr during the first two years of their life-history, therefore parr that were identified as putative freshwater-residents may either be freshwater-residents or pre-smolts which smolt the following year or later when a threshold body size or growth rate is attained. As the determination of a smolt was classed as all fish detected by the PIT antennae which are located along the tributary and main river, those detected, especially in the tributary, may be parr migrating to the main river to feed before returning to spawn in the Tadnoll (see Fig.1.1-brown trout life-history strategies). The low recapture rate over the sample period may indicate some individuals died and were then wrongly identified as parr that follow a freshwater-resident life-history strategy. The biological implications of parents producing offspring which follow varying life-histories may be a mechanism to increase reproductive success and offspring survival in adverse environmental conditions (Crean and Marshall, 2009). Anadromous fish are subject to higher energetic costs during migration (Gross, 1987) whereas freshwater-resident fish may never replenish their energy reserves after spawning in less productive environments (Nikolskii, 1963).

5.4.4 FUTURE WORK

Identification of adult life-history was achieved through a combination of methods with SIA being the most used method. SIA identifies the assimilated history of feeding in the marine and freshwater environment (DeNiro and Epstein, 1978), however, adults identified at the time of sampling as freshwater-residents may in fact be putative smolts (mature in freshwater but then smolt). One way to further clarify the identification of adult life-history would be to take scale samples at all recapture dates to calculate age over the period of sampling and detect spawn marks and marine growth.

Limitations on this study were the individuals prioritised for molecular analysis early in the sampling period, but by monitoring the population and recapture rates of offspring (until the first smolt year), individuals could have been prioritised based on a greater number of recapture data to test the individual hypotheses in this present study. Further work should entail utilising the PIT tag data to monitor movement within the freshwater environment, with the potential to sample further downstream in the main river to distinguish between putative smolts and the varied freshwater life-history migration strategies.

The small sample size made it difficult to make any formal conclusions therefore to develop this study further it would benefit from the following: 1) Parr may smolt as old as 3+, therefore to follow all parr until their subsequent life-history strategies are identified; 2) Quantify the food availability in the river (to calculate metabolic constraints) and collect long-term data on environmental variables (temperature, discharge); 3) Controlled mating experiments of different variations of adult forms to determine effect on offspring performance and life-history under controlled conditions. Either in a semi-natural/natural environment, where juveniles would be released once tagged and then followed periodically or in a laboratory environment where juveniles can grow under controlled conditions to determine if life-history is genetically determined.

5.4.5 CONCLUSION

This is a novel approach to determining the influence of parental investment and maternal effect on offspring performance and subsequent life-history in brown trout using assignment together with field data and SIA. These results indicate maternal anadromous contribution to the population is higher and both parental life-history forms can produce offspring of anadromous and freshwater-resident strategies. Parental life-history may influence offspring performance during the early parr stage, then once a size/growth threshold is reached (Hallerman, 2003; Piche *et al.*, 2008), environmental factors influence life-history strategy (Forseth *et al.*, 1999; Morinville and Rasmussen, 2003).

CHAPTER 6: GENERAL DISCUSSION

The overall objective of this research was to determine the effect of parental investment (mainly maternal effect) of anadromous and freshwater-resident brown trout on offspring performance (size and growth of 0+ parr) and subsequent life-history strategy. This is the first known study to test for differences between anadromous and freshwater-resident offspring in England, based on performance, utilising molecular and chemical tools. In other countries, studies of these individual research areas have been carried out on brown trout or in related salmonid species including: performance (Elliott, 1984a; Cucherousset *et al.*, 2005; Jardine *et al.*, 2008), parentage assignment (Norris *et al.*, 2000; Blanchfield *et al.*, 2003; McLean *et al.*, 2004; Baumsteiger *et al.*, 2008) and stable isotope analysis (SIA) (McCarthy and Waldron, 2000; Charles *et al.*, 2004; Curry, 2005; Acolas *et al.*, 2008). This research, with the combination of techniques used to test parental investment between anadromous and freshwater-resident offspring, will pave the way for further studies and have important implications for both management and conservation.

At each experimental stage, problems were faced testing the individual hypotheses. For the SIA, 69 % of fry could not be allocated to anadromous or freshwater-resident mothers confidently as they had already begun exogenous feeding, reducing the maternal signal and replacing it with one representing the freshwater feeding environment (Doucett *et al.*, 1999a). Heavy rainfall prior to sample collection could have had a major impact on the eggs and emerging fry, washing away shallow buried eggs (Crisp and Carling, 1989) and forcing emerging fry to migrate further downstream. Equally likely, fry could have emerged at an earlier date and already begun exogenous feeding when they were sampled. The implications for not identifying the maternal origin of the whole sample means estimates of the maternal contribution cannot be fully achieved. Parr prioritised for assignment and growth analysis were chosen early on in the sampling period to allow time for genetic analysis. Parentage assignment could only assign 28 % of the parr sample to putative parents, this was believed to be due to a number of factors including the resolution power of the loci and not sampling all of the adult population. The low assignment meant only a small sample of parr could be used in the growth study, with insufficient numbers to perform analysis. Problems were faced when a large proportion of parr were not recaptured (mortality and migration within freshwater) or had a low recapture rate making assumptions about offspring performance and

life-history difficult. The problem of a sufficient sample size in this study has been due to sampling a population in the wild and often many problems can be associated with this, included those above. These difficulties may have been avoided if fry were sampled earlier and only those with visible yolk reserves were collected to identify maternal origin and by prioritising samples for molecular and growth analysis after the population had been monitored for a minimum of one year to choose offspring with high recapture rates. Each hypothesis will be discussed below but the consequence of a small sample size means they can only be observations and at most only answer part of each hypothesis.

Maternal effect was found to have an influence on emerging fry (March 07) analysed through SIA. The results of the present study can begin to answer the original hypotheses that fry from anadromous females will emerge earlier and at a larger size compared to fry from freshwater-resident females. These results agree with previous studies on emerging fry size (Chernoff and Curry, 2007; Jardine *et al.*, 2008) but differ from studies on time of emergence, where fry from freshwater-resident females emerged earlier (Thériault and Dodson, 2003; Jardine *et al.*, 2008). Maternal effects are a fundamental factor in affecting the variation of offspring development and facilitate evolution by natural selection through environment heterogeneity (Mousseau and Fox, 1998). Early emergence of anadromous offspring may maximise offspring fitness by establishing optimum feeding territories first (Cutts *et al.*, 1999). As egg size is positively related to offspring size (Einum and Fleming, 1999; Olsen and Vøllestad, 2001), anadromous females could increase their fitness by providing increased resources to eggs compared to their freshwater-resident counterparts (Einum and Fleming, 1999). By potentially producing larger eggs for their body size (egg size not studied), increased yolk reserves offer offspring an advantage (Elliott and Hurley, 1998b) including increased growth (Hinckley, 1990) and higher survival in extreme environmental conditions (Einum and Fleming, 1999).

There was no detectable effect of parental investment on parr size and growth rate on offspring from the same cohort during their 0+/1+ stage (July 07 – August 08). The original hypotheses, 'size and growth rate of parr from anadromous and freshwater-resident males/females will be the same' cannot be rejected however the raw data suggests parental contribution may have an effect during 0+ life-history. Freshwater-resident offspring may exhibit a faster growth but smaller size during August – November compared to anadromous offspring which begin at a larger size but display on average a faster growth between November – April. The differences in offspring size agree with the findings of Chernoff and

Curry (2007) who showed offspring of anadromous *S. fontinalis* were larger compared to freshwater-resident parr, however, this only covered the first three months post-emergence. There has been no published research on parental influence on growth rate of offspring of anadromous and freshwater-resident salmonids. Research has focussed on the differing growth rates of individuals which smolt and those that remain resident within a population (Rowe and Thorpe, 1990; Jonsson and Jonsson, 1993; Cucherousset *et al.*, 2005). Rikardsen and Elliott (2000) demonstrated within populations of *S. alpinus*, over the first two years of the parr stage, those which smolted were larger and displayed a slower specific growth rate during winter – spring and a faster growth during the summer compared to those which remained resident. It is hypothesised, based on the findings of my study, that the parr with the largest size and fastest growth from anadromous and freshwater-resident parents will reach a size-threshold during early spring and will adopt an anadromous life-history strategy (Piche *et al.*, 2008).

Maternal reproductive contribution of anadromous and freshwater-resident adults to the production of fry was calculated and anadromous females contributed to more fry in the population. This was further supported by analysing the parr population through parentage assignment, which showed anadromous females produced significantly more offspring compared to freshwater-resident females. These findings cannot reject the original hypotheses, 'The relative maternal reproductive contribution will be equal between anadromous and freshwater-residents to offspring production' as the parental origin could not be identified for all fry. The impact of these offspring of undetermined parentage on the estimate of reproductive contribution to the population is unknown. These findings suggest anadromous females in the Tadnoll Brook contribute more offspring to the population and, in early development those offspring gain fitness benefits. However, as both forms exist in sympatry, it is apparent there is a lack of evidence that either form has achieved a reproductive advantage (Pettersson *et al.*, 2001; Curry, 2005).

Parental life-history did not influence offspring life-history strategy as anadromous and freshwater-pairings produced both anadromous and freshwater-resident offspring. These results cannot reject the original hypothesis, 'Parental life-history does not affect subsequent offspring life-history strategy (i.e. the decision to mature or smolt)' since during the study period it was only possible to identify putative smolts and residents without knowledge of offspring mortality and the potential for incorrectly identifying offspring as putative residents. However these observations agree with previous studies of parental influence on

offspring life-history (Rounsefell, 1958; Sckrochowska, 1969) in salmonids. This strategy of being able to produce offspring that follow variable life-histories may be a 'bet-hedging' strategy to increase survival (Crean and Marshall, 2009). Anadromous fish are subject to high energetic costs through migration to more productive feeding environments (Gross, 1987) and an increased chance of mortality by predation (Nordeng *et al.*, 1985), contamination (Waring and Moore, 2004) and disease (Gross, 1987). Despite freshwater-resident fish not utilising energy on migration, they may never replenish their energy resources after spawning in a relatively low productivity environment and could be subject to adverse environmental conditions such as low water flow (Nikolskii, 1963).

These results will ultimately advance the understanding of parental effects and influences on offspring and step closer to answering the fundamental question- does environment or genetics control life-history strategy?

The present study utilised three differing but complimentary techniques for analysing life-history strategy. SIA identifies marine and freshwater feeding and emerging fry can be identified as the progeny of anadromous and freshwater-resident females based on their isotopic symbol (Doucett *et al.*, 1999a). Parentage assignment using microsatellite markers could assign the most likely parent-pair and single parents to parr and PIT tagging and electro-fishing data (including ecologically relevant data i.e. growth) collected from two sites with PIT tag readers used to detect fish migration and visual inspection of fish recaptured during electro-fishing surveys. This is the first use of these techniques together in brown trout, contributing a further understanding to salmonid life-histories and the tools used to study them. The closest study to this has been by Charles *et al.* (2006) who tested genetic differentiation between offspring of anadromous and freshwater-resident brown trout using SIA in France. The benefit of using SIA in conjunction with parentage analysis is SIA can determine offspring maternal origin and reproduction contribution, and parentage analysis (together with ecological data) can determine offspring origin and parental reproductive contribution. Therefore, both forms of analysis can test a hypothesis and can each support the others findings, ultimately providing more confidence in the data. The present study has shown how it is possible to identify maternal origin in progeny that have used their yolk reserves before the isotopic signal becomes too dilute (Doucett *et al.*, 1999a), however, one drawback of this technique is whole fry samples are required for analysis. Parentage analysis was possible for a proportion of parr with adults of unknown sex, however the low

assignment rate may be from low allelic discrimination and further exploration into of the genetic analysis would be needed.

Trivers (1972) defined parental investment as 'any investment by the parent in an individual offspring that increases the offspring's chance of survival (and hence reproduction success) at the cost of the parent's ability to invest in other offspring'. Parents can influence offspring phenotype through parental effects (Mousseau and Fox, 1998) and increase offspring fitness through parental care (Clutton-Brock, 1991). Sexual conflict occurs between and among the sexes and between parental allocations to offspring (Wedell *et al.*, 2006) which results in varying forms of investment and care throughout the animal kingdom. Mammals invest heavily into gestation and lactation which has high energetic and nutrient constraints (Hayssen, 1993; Wedell *et al.*, 2006; Stockley and Bro-Jorgensen, 2011), therefore reproductive success in mammals is constrained by food availability (Stockley and Bro-Jorgensen, 2011) but for the benefits of investment and care to offspring survival to outweigh the costs, species adapt differing lactation lengths (Hayssen, 1993). In some species males are more costly for the female to carry and rear due to their larger size and energy requirements (Cappozzo *et al.*, 1991; Georges and Guinet, 2001), therefore mothers will bias for female offspring, whereas males who provide no care have the same costs for producing either sex (Wedell *et al.*, 2006). In birds, 90 % of species provide bi-parental care with varying degrees of investment from each sex (Kokko and Jennions, 2008). External incubation of eggs is thought to have evolved through adult endothermy and surface nests (Burley and Johnson, 2002). In some species, females gain fitness benefits through male care and investment in resources (nest site, territory defence and nuptial gifts) (Møller and Thornhill, 1998) and females may exhibit brood division, providing preferential care to some individuals to increase offspring survival (Draganoiu *et al.*, 2005). Maternal effect influences egg size and offspring size in fish (Bang *et al.*, 2006) and maternal condition has been shown to influence allocation of resources to eggs, where females in productive feeding environments provided eggs with better quality energy resources (Huang *et al.*, 1999; Donelson *et al.*, 2009). Variation in egg size has also shown to be influenced by male mating size (Kolm, 2001) and in cooperative breeding species (Taborsky *et al.*, 2007). Fish display the most variable forms of parental care (Reynolds *et al.*, 2002) with male only care being the highest out of all animals. In some species, males defend and maintain nests (fanning eggs to increase oxygenation) to attract females (Rios-Cardenas, 2005; Hale and St Mary, 2007), displaying their quality and nest caring capabilities.

This present study shows in relation to these theories of differing parental investments that in a non-caring strategy (males and female provide no care after fertilisation) egg size and number is important. Females may ensure offspring survival through the provision of resources (yolk quality and quantity) and/or number of individuals (Elliott and Hurley, 1998b; Einum and Fleming, 1999; Armstrong and Nislow, 2006). A non-caring strategy may have evolved in the case of salmonids as guarding of the nests would probably lead to heavy predation and a subsequent loss to future reproduction. By providing no care, parents can save energy expenditure for future reproduction. The existence of two differing life-history strategies within a population may have evolved through sexual-selection, with the costs and benefits of each strategy affecting the sexes differently. Migration in females is selected as they can achieve increased growth in the productive marine environment (Forseth *et al.*, 1999) and provide large energy resources to eggs whereas males can increase their reproductive success in the freshwater environment through extra-pair mating and sneaking strategies (Jonsson, 1985). Selection should favour females to produce offspring phenotypes which maximise maternal fitness (Mousseau and Fox, 1998; Uller, 2008) however this may not necessarily maximise offspring fitness (a size number conflict between mothers and offspring) (Trivers, 1974), therefore phenotypes should be influenced (in part) by the environment the offspring will experience (Crean and Marshall, 2009). When females cannot predict the conditions offspring will face based on their environmental experience, a bet-hedging strategy may be favoured (Crean and Marshall, 2009). As stated earlier, this study shows females can produce offspring which follow either life-history strategy. This variation in offspring phenotype may be a strategy to achieve reproductive success in unpredictable environments (freshwater vs. marine) and ensure at least one offspring phenotype will achieve fitness and survival (Crean and Marshall, 2009) and this has been witnessed in other species including soil mites (Plaistow *et al.*, 2006), coral reef fish (Gagliano and McCormick, 2007) and sea slugs (Marshall and Keough, 2003). Phenotypic plasticity may be important for species survival and understanding individual responses to the environment will help predict how populations respond and adapt to environmental change.

England and Wales supports many anadromous brown trout net and rod fisheries which are monitored by the EA (Evans and Greest, 2007). Stocking of hatchery salmonids has increased significantly over the last 15 years to accommodate the increased fishing pressure and salmonid population decline. It is important for ecologists and geneticists to understand the complex population dynamics within a river system and the influence of the

environment, as fisheries managers can address conservation issues and implement changes, particularly for re-stocking and recreating optimum hatchery conditions (Armstrong and Nislow, 2006). The results indicate that the Tadnoll Brook is a significant spawning tributary for anadromous brown trout. Understanding the reproductive contribution of a spawning population has important management and evolutionary implications (Curry, 2005; Armstrong and Nislow, 2006). As large anadromous females are typically targeted by anglers, techniques identifying populations that are supported by anadromous trout are useful to population protection and conservation. This knowledge will help influence fishing quotas and help fisheries managers understand temporal variation in each life-history strategy (Charles *et al.*, 2004). The information collected in this study was based on one population and may be more relevant to other populations from the same river type (chalk stream) but will have implications to other South England rivers and be useful in advancing knowledge on life-history to a wider audience.

This present study can begin to further the understanding of how environment controls migration strategy. The results indicate that growth and food availability can influence migration as the environment becomes growth limiting (Forseth *et al.*, 1999; Morinville and Rasmussen, 2003). This study did not try to identify specific genes but previous studies have identified genes expressed during smoltification (Richards *et al.*, 2003) although, this does not necessarily indicate that migration is controlled by genetics (Ferguson, 2006). Genes may also indirectly control energy efficiency and hence growth rate (Ferguson, 2006). Ferguson (2006) proposed migration is likely to be controlled by multiple genes together with the influence of environmental conditions and is initiated when a threshold level of these are exceeded (Hallerman, 2003; Piche *et al.*, 2008).

The combination of the tools and methods used in this study could be adapted to other conservation issues including: the forensic identification and foraging ecology of endangered species and identifying population origin of migratory species i.e. birds, and the importance of breeding sites and thus influence management and conservation.

Possible further research to explore the results found in the present study would include: 1) Determine sibling groupings (full-sibs) between the sample of fry and parr used for SIA and assignment to determine the maternal life-history identified through SIA. This may increase parentage assignment of parr that were only assigned to their fathers, therefore the maternal life-history can be inferred. If the fathers have not been assigned this technique cannot be used as SIA only reflects the mothers stable isotopic signature; 2) To gain a

broader understanding of the population structure of partially migratory brown trout, by determining the genetic variation of the study river with other catchments and analysing the results in light of the present study; 3) Radio track a number of parr to determine movements within the river and re-define the complex life-history and migration strategies that brown trout follow from the current concept of freshwater or marine feeding..

This PhD used a novel approach to determine the influence of parental investment on offspring performance and subsequent life-history using parentage assignment, SIA and detection of migration from the natal stream and into tidal reaches with PIT tags. The research indicates the Tadnoll Brook has a higher proportion of anadromous females contributing to the population. Anadromous maternal effects afford offspring fitness benefits, i.e. size and time of emergence, compared to freshwater-resident offspring. There is evidence that both forms interbreed and offspring of each life-history can be produced from both forms. Adult life-history does not appear to affect offspring life-history strategy but may influence juvenile performance. Anadromous offspring may have a size advantage during the winter of the first year of the parr stage but a slower growth compared to freshwater-resident offspring, however, further study on a larger sample would be needed to fully conclude this. However, the presence of both resident and anadromous forms in the population suggest that the anadromous maternal fitness benefits afforded to offspring are only apparent during early ontogeny and once offspring reach a size threshold, maternal effects are redundant. The results of this study imply that parental investment (maternal effect) affects offspring performance during ontogeny and environmental factors (for example food availably) determine offspring life-history. Determining parental contribution of anadromous and freshwater-residents and the affect on offspring performance has important management and conservation implications, especially in anadromous populations where large females may be targeted by anglers. The tools used in this research can be used as a way to identify adult and offspring of both life-history strategies to help conserve populations.

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APPENDIX ONE

The results testing for linkage disequilibrium across each pair of loci in the parr sample using the program GENEPOP (Raymond and Rousset, 1995). Significant genotypic phase disequilibrium was detected in 41 out of the 66 pairs after correction for multiple tests using sequential Bonferroni correction (Rice, 1989).

Locus 1	Locus 2	*P* value
Str58	Str85	**0**
Str58	Str60	**0**
Str58	SsHaeIII	**0**
Str60	SsHaeIII	**0**
Str58	Ssa197	**0**
Str85	Ssa197	**0**
SsHaeIII	Ssa197	**0**
Str58	SS11	**0**
Str85	SS11	**0**
Str60	SS11	**0**
Ssa197	SS11	**0**
Str58	Str73	**0**
Str85	SsoSL85	**0**
SsHaeIII	SsoSL85	**0**
Ssa197	SsoSL85	**0**
Str58	SsoSL438	**0**
Str85	SsoSL438	**0**
SS11	SsoSL438	**0**
Str58	SsoSL417	**0**
Str85	SsoSL417	**0**
Str60	SsoSL417	**0**
SsHaeIII	SsoSL417	**0**

Ssa197	SsoSL417	**0**
SsoSL85	SsoSL417	**0**
Str58	SsoSL25	**0**
Str85	SsoSL25	**0**
SsHaeIII	SsoSL25	**0**
Ssa197	SsoSL25	**0**
SS11	SsoSL25	**0**
SsoSL85	SsoSL25	**0**
Sso438	SsoSL25	**0**
SsoSL417	SsoSL25	**0**
Str60	SsoSL85	**0.00005**
SsoSL85	SsoSL438	**0.00018**
Str15	SS11	**0.00033**
SS11	Str73	**0.00061**
Ssa197	SsoSL438	**0.00076**
SsoSL438	SsoSL417	**0.0008**
Str60	SsoSL25	**0.00083**
SS11	SsoSL417	**0.0015**
SsHaeIII	SS11	**0.00189**
Str60	Ssa197	0.00267
Str60	SsoSL438	0.00275
SS11	SsoSL85	0.00292
SsHaeIII	Str73	0.0031
Str58	SsoSL85	0.009
Str73	SsoSL85	0.01027
Str60	Str73	0.01207
Str73	SsoSL25	0.01309
Str85	SsHaeIII	0.02626
SsHaeIII	SsoSL438	0.0307
Str60	Str15	0.0319

Str15	Ssa197	0.04511
Str85	Str73	0.04602
Str15	SsoSL85	0.062
Ssa197	Str73	0.08949
Str15	SsoSL417	0.09101
Str85	Str60	0.10092
Str15	SsoSL438	0.11573
Str73	SsoSL417	0.12008
Str15	SsHaeIII	0.14205
Str58	Str15	0.18259
Str85	Str15	0.22584
Str73	SsoSL438	0.36561
Str15	SsoSL25	0.44152
Str15	Str73	0.83423

Significant linkage disequilibrium at $p < 0.05$ after Bonferroni correction are indicated in bold.