MATERNAL EFFECTS OF MIGRATION ON SYMPATRIC OFFSPRING OF RESIDENT AND ANADROMOUS ATLANTIC SALMON *(Salmo salar)*

By

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Abstract

Many of the coastal rivers in Newfoundland contain Atlantic salmon populations which include both anadromous (i.e. migrate to sea) and non-anadromous (i.e. freshwater resident) phenotypes. However, little is known about the relationship between the two types and particularly, how early offspring performance (i.e. growth, dominance and survival) may differ as a result of maternal effects (e.g., marine versus freshwater derived nutrients in the eggs). Six pairs of paternal half sib families were created by crossing unique anadromous and resident mothers with a single male (anadromous or resident). Samples were collected at each of four stages (i.e. unfertilized eggs, eyed eggs, yolk-sac larvae and newly emerged fish), weighed and used for lipid analyses. All samples were processed and lipid profiles were determined by Iatroscan instrumentation and further characterized and quantified by gas chromatography. There were no significant differences in triacylglycerols (TAG) or phospholipids (PL) between the two offspring types, however the anadromous offspring had higher amounts of eicosapentaenoic acid (EPA, 20:5ω3), docosahexaenoic acid (DHA, 22:6ω3) and ω3:ω6 fatty acids. The resident offspring had higher amounts of arachidonic acid (AA, 20:4ω6). Results also showed that resident mothers had larger eggs and their offspring were larger at emergence. Pair-wise dominance trials between newly emerged anadromous and resident offspring revealed no significant difference in positioning relative to a defensible food source. Similarly, growth and survival of the newly emerged offspring, tested over a four

week period in stream channels and across three treatments (12 resident offspring, 12 anadromous offspring, and 6 offspring of each type; five replicates of each), differed little. Thus, while there were apparent differences in maternal contributions to the offspring, there were no indications under the experimental conditions examined that these strongly influenced offspring performance after emergence.

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List of Abbreviations and Symbols

AA (20:4ω-6)	Arachidonic acid
AMPL	Acetone mobile polar lipids
Ddpf	Degree days post fertilization
DHA (22:6ω3)	Docosahexaenoic acid
EPA (20:5ω3)	Eicosapentaenoic acid
mg	milligrams (measurement unit)
mm	millimetres (measurement unit)
MUFA	Monounsaturated Fatty Acid
Other PUFA	Other polyunsaturated fatty acids
PC	Principal component
PCA	Principal component analysis (statistical analysis)
PL	Phospholipids
PUFA	Polyunsaturated fatty acids
SD	Standard deviation
TAG	Triacylglycerols
µg	micrograms (measurement unit)

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Chapter 1: General Introduction

1.1 Conditional Strategy

Environments are constantly changing and to be successful organisms must be able to respond appropriately. There are two major ways for organisms to respond to their surroundings, longer term options (across generations) stemming from natural selection, and phenotypic plasticity which are shorter term solutions (within generations) such as behavioural or physiological changes based on environmental conditions (Hutchings 2011). Behavioural changes include migration where a population may seek a better-suited environment for specific activities, i.e. mating, spawning, nurseries or feeding. Partial migration, in which a single population contains both migratory and resident individuals, is a fundamental behaviour in avian ecology and is the basis for the evolution of migration in general (Kerr *et al*. 2009). In fish ecology, migration has its own distinct terminology with the general migration of fish from salt water to fresh water and vice versa known as diadromy. This is further delineated as anadromy which refers to migration from salt water to fresh water for breeding (e.g., Atlantic salmon), catadromy which refers to migration from fresh water to salt water for spawning (e.g., American eel) and amphidromy which refers to fish that migrate either way but not for breeding purposes (e.g., Bull shark), (McDowall 1992). Fish that migrate entirely within the inland waters of a river system are referred to as potadromous (Northcote 1999). Many salmonids have both anadromous and non-anadromous (resident/potadromous) life history forms although it is unclear which form came first or if the common ancestor pre-dating the family was already diadromous (McDowall 2002). Partial migration is also well documented among salmonids (Power *et al*. 1987; Verspoor and Cole 1989). Two

main mechanisms may explain this phenomenon: (1) they are genetically distinct populations that overlap or (2) they are alternative life history phenotypes making up a single population with individuals capable of potentially adopting any of the alternative phenotypes. Predictably, there is evidence to support both theories (Robitaille *et al.* 1986; Tessier and Bernatchez 1999; Schluter and McPhail 1993; Adams 2007; Chapman *et al*. 2011; Dodson *et al*. 2013).

Alternative life history phenotypes occur when an individual assumes a tactic according to its environmental or physiological state (i.e. status, body size), which is referred to as a conditional strategy (Dawkins 1980; Gross 1996; Taborsky *et al*. 2008). According to the environmental threshold model, there is genetic variation among organisms in the response to the switch point, which is the value of the environmental cue necessary to express one alternative phenotype over the other (Hazel *et al*. 1990; Roff 1998; Tomkins and Hazel 2007). Game theory states the average fitnesses of the tactics are not equal, but the fitnesses of the alternatives at the switchpoint are equal. This helps to explain the differences in migratory patterns among individuals of a single population; however, the exact mechanisms responsible remain unclear. It is largely accepted that a threshold body size at a particular age is a determining factor for migration among salmonids (Thorpe *et al*. 1998; Paez *et al*. 2011), however others have argued that size alone cannot account for life history selection (Økland *et al*. 1993; Bohlin *et al*. 1996) and other factors such as maturity, smolt age (Letcher and Gries 2003) and genetic variation all play a part (Paez *et al*. 2011; Dodson *et al*. 2013). Frequently involved in developmental plasticity are parental effects, which allow for transmission of various

adaptive phenotypic strategies that span generations. This can have important ecological and evolutionary consequences (e.g. Mousseau and Dingle 1991; Reznick 1991; Riska 1991; Fox 1994; Einum and Fleming 2000a,b), as this potential to generate rapid phenotypic change can either speed up or otherwise interfere with evolutionary responses to natural selection (Kirkpatrick and Lande 1989; Rasanen and Kruuk 2007).

For most organisms, the phenotypes of the offspring tend to be influenced more by the mother than the father (Green 2008). For example, mothers control the location, timing and dispersal of offspring/eggs as well as offspring size and protection, parental care and provisioning to developing young, as well as characteristics of the father if mate choice is present (Mousseau and Fox 1998). This influence is referred to as a maternal effect, and can be broadly defined as the causal influence of the maternal genotype or phenotype on the offspring phenotype (Lacy 1998; Roff 1998; Wolf and Wade 2009). This phenotypic plasticity ensures that there are a greater quantity of phenotypes available than there are genotypes and allows these traits to be subject to natural selection and genetic modification hence affecting the evolution of a species. The degree to which maternal environment and behaviour influence progeny phenotype and fitness will determine the likelihood that such maternal effects themselves will be selected upon (Wade 1998; Fox *et al*. 1999; Rasanen and Kruuk 2007).

Maternal effects have garnered much interest since the 1980s and the plethora of articles on the subject illustrates the excitement this topic incites among those in the evolutionary and ecological communities. Fish research abounds claiming links between maternal size, condition and age (Kjesbu *et al*. 1996) and egg/offspring size, condition

and viability (Chambers and Waiwood 1996; Trippel and Neil 2004; Rideout *et al*. 2005; Higashitani *et al*. 2007). Size of the hatching larvae may also be affected by the chemical composition of the eggs (Morley *et al*. 1999) a product of maternal provisioning. Hormones provided by the mother are present in the egg and act as developmental and physiological signals modifying behaviour for the developing offspring as well as the adult. Hormones also regulate transitions between life history stages. Variation in behavioural phenotype due to maternal hormone signalling to embryo/fetus is widespread in many taxa (Groothuis and Schwabl 2008) thus producing multiple behavioural phenotypes within families, in populations and among populations. Individuals within partially migrating populations would not only differ potentially in the quantity of hormones and genetic material provided to offspring but mothers would offer different nutritional resources because of their own different nutritional environments (marine versus freshwater), creating further possible phenotypic variation and providing a unique opportunity to study effects of maternal provisioning.

1.2 Atlantic salmon

Some organisms provide a better opportunity for investigating the effects of maternal environments than others. One of the more commonly studied species is *Salmo salar*, the Atlantic salmon, which is found in the temperate and sub-Arctic regions of the North Atlantic Ocean. It is a popular sport fish and was a commercial target species until a decline in abundance and range over the last century that now has it listed as endangered in regions such as the Inner Bay of Fundy, and recommended for listing in Eastern Cape Breton Island, Anticosti Island, the Nova Scotia Southern Uplands and the Outer Bay of

Fundy (COSEWIC 2010). In general, it is an iteroparous, anadromous species demonstrating immense within-population variability in size and age at maturity (Fleming 1998; Hutchings and Jones 1998; Jonsson and Jonsson 2011). Atlantic salmon (*Salmo salar*) populations commonly include two major life history pathways; 1) maturation following an anadromous migration to the ocean and 2) precocial maturation as parr in freshwater prior to any anadromous migration. Anadromous salmon undergo physiological transformations (smolting) and migrate seaward for 1-3 years before returning to their natal river systems to mate and spawn (Jonsson and Jonsson 2011). Mature parr remain in the rivers, mature at an earlier age and smaller size than anadromous males and may smolt and migrate to sea before breeding again (Berglund *et al*. 1992, Fleming and Reynolds 2004), however most will spend their entire lives in freshwater (Aubin-Horth *et al*. 2005). This latter life history is limited to mature males in most instances. In most of the ponds/lakes of Newfoundland a third option frequently appears, freshwater residency (non-anadromy) (Klemetsen *et al*. 2003; Fleming and Einum 2011) leading to populations made up exclusively of freshwater residents, as well as mixed populations of sympatric anadromous and resident individuals (Power *et al.* 1987; Verspoor and Cole 1989; Adams 2007). Freshwater residents are generally smaller than anadromous salmon and live out their entire life cycles in fresh water moving between the rivers and connecting lakes or ponds (Hutchings 2002). Considered non-migratory because they never leave the freshwater systems, some residents do however move from the bigger reservoirs into the rivers to spawn (Verspoor and Cole 1989).

Atlantic salmon females do not provide any type of active parental care for offspring after the fertilization of eggs (Fleming 1996), therefore the key aspect of maternal provisioning is the size, biochemical composition and energy content of the eggs. These maternal effects can impact offspring fitness, behaviour and survival as well as other characteristics (Heath and Blouw 1998; Mousseau and Fox 1998; Lindstrom 1999; Einum and Fleming 1999, 2000a). Larger salmon usually lay larger eggs and size, as well as energy content of the eggs, is a critical feature of maternal provisioning. Larger eggs are usually associated with larger juvenile body size and higher growth and survival rates (Hutchings 1991; Einum and Fleming 1999, 2000a). Nonetheless, offspring can increase their fitness by efficiently converting energy stores provided by their mothers into somatic tissue, preparing themselves for exogenous feeding (Berg *et al*. 2001). Furthermore, according to Berg *et al*. (2001), egg size and energy stores vary much more among families than within them and although resident salmon are generally smaller than anadromous salmon, they often have only marginally smaller eggs (Wood and Foote 1996; Fleming 1998). The success of newly emerged offspring establishing territories depends on time of emergence, metabolic rate and body size (Cutts *et al*. 1999a, b), which is also directly influenced by resources provided by the mother in the eggs. Salmon eggs, which are large compared to many other species of fish, have a long incubation period and once hatched the offspring continue to rely on endogenous food sources for some time before active feeding begins. These traits in particular give researchers time and opportunity to investigate maternal effects and due to the partially migratory populations, Atlantic salmon also provide a unique opportunity to consider the

differing kinds of maternal provisioning and their effects within an eco-evolutionary context.

Numerous *Salmo salar* populations in the rivers of Newfoundland are composed of anadromous and resident, as well as mature parr phenotypes. In general, resident salmon have evolved as landlocked salmon either through naturally occurring events such as glaciation or anthropogenic events such as stocking (Hendry and Stearns 2004). These landlocked salmon are physically isolated from the saltwater and have therefore adapted to an entirely freshwater existence. However, resident salmon in Newfoundland do not appear to have evolved this way. Although some populations are physically isolated, residents are abundant in many watersheds where the sea is accessible. Research indicates that these two strategies are most likely exhibiting phenotypic plasticity under environmental conditions prevalent in the area and the two groups are not genetically distinct (Adams 2007). Research on brown trout (Jonsson and Jonsson 2011) and Arctic char (Nordeng *et al*. 1989) show that resident fish can be produced from diadromous parents and vice versa and it is likely the case with Atlantic salmon as well. Burton and Idler (1984) discovered that the Newfoundland resident population does include individuals that have the capacity to adapt to seawater. The environmental cues that trigger the life history choice remain unclear and present an interesting avenue of exploration. The existing knowledge of this species makes it particularly practical and interesting for examining fundamental questions about life history and migratory strategies in fish.

1.3 Offspring origin

Eggs and fry of both anadromous and resident parentage are physically very similar and it is impossible to accurately classify young offspring using morphometrics and/or meristics (Riley *et al*. 1989). In a population where both phenotypes are present, it may be difficult to determine the contribution of each to the overall population. As the expression of the migratory phenotype in many of these populations is plastic, genetic separation of the contribution would not be feasible. Studies on brown trout and brook trout have used stable isotope analysis to identify offspring of anadromous and freshwater-resident parents (Charles *et al*. 2004; Curry 2005). Other methods used to distinguish freshwater residents from anadromous individuals in various salmonid populations include analysis of carotenoid pigment profiles in muscle tissue extracts (Youngson *et al*. 1997), strontium content of the scales (Eek and Bohlin 1997), and analysis of otolith microchemistry (Howland *et al*. 2001; Zimmerman and Reeves 2002).

Lipid analysis can help determine where a fish has been feeding based on prey type (Parrish 1999). This can be useful in determining feeding behaviour, foodweb relationships, ecological niches and the division of food sources among various species (Ackman 1994). Specifically, marine and freshwater prey items contain specific fatty acids which accumulate in the fishes lipid stores. Many marine fish larvae must have essential fatty acids, such as eicosapentaenoic acid (EPA, 20:5ω3), docosahexaenoic acid (DHA, 22:6ω3) and arachidonic acid (AA, 20:4ω6), in their diet due to an inability to synthesize these components (Tocher *et al*., 1989). Measurements of these essential fatty acids and their ratios can be used as indicators of egg quality (Penney *et al*., 2006).

Inadequate quantities of DHA can result in impaired larval behaviour, while insufficient EPA and AA can have an impact on structural phospholipids (Wiegand *et al*., 2004). Lipids from aquatic and terrestrial plants have distinctive distinguishable fatty acid signatures and can be used to classify the predominant energy source of an aquatic system. Various studies have shown that the concentrations of fatty acids such as, 18:2ω6, 18:3ω3 and 20:4ω6 in the lipids of freshwater animals are higher than their marine counterparts (Linko *et al*. 1992; Kakela and Hyvarinen 1998; Gonzalez-Baro and Pollero 1988). These distinct lipid profiles can help to identify whether or not a fish has been to sea. Since salmonids are entirely dependent on the lipid reserves provided by the mother until they begin feeding, newly emerged offspring can be sampled and should be able to be classified as being either 1) fry from an anadromous mother, or 2) fry from a resident mother, via lipid analysis. Using this method, population structure in natural stream environments can be determined, advancing studies in partially migratory populations.

Research indicates select fatty acids have an effect upon the performance of various fishes. McKenzie *et al*. (1998) found that there was a positive relationship between maximum swimming speed and total ω6/ω3 fatty acid ratio of muscle lipids in Atlantic salmon. Other studies have shown that higher dietary concentrations of ω3 fatty acids improve egg viability in European sea bass (Carrillo *et al*. 1995) and lower mortality rates and the incidence of heart lesions among Atlantic salmon during live transport (Bell *et al*. 1994).

If resources provided by the mothers differ in quality, it may be that offspring with comparatively better resources have a greater capacity to achieve higher social status thus effecting their survival. Typically, dominant individuals acquire more advantages than do their subordinate counterparts when it comes to survival, feeding occurrences, growth and mating (Fausch 1984; Metcalfe *et al*. 1989; Nielsen 1992). Dominant individuals also appear to experience lower stress (Abbott and Dill 1989) and inhabit more energetically profitable territories (Fausch 1984, Metcalfe 1986). Faster growth is generally associated with dominance in the laboratory and in natural pools in rivers (Metcalfe *et al*. 1989; Nakano 1995; Martin-Smith *et al*. 2004). Investigations into early emergence behaviour and performance as well as growth rates should provide insight into social rank and survival.

1.4 The Thesis

The first aim here was to test quantitatively for differences in lipid composition of egg, embryos and larval offspring of sympatric resident and migratory Atlantic salmon. By doing so, existence of differences in lipid profiles could be established to determine if lipid profiles are unique to each alternate strategy, which would make it possible to determine the contribution of each phenotype to a sympatric river population. The second aim of this study was to experimentally test if differences in maternal provisioning influence offspring performance (competitive ability, growth and survival) of first feeding juveniles (i.e. at the start of exogenous feeding) of the two alternate migratory strategies of Atlantic salmon. By looking at competitive ability, growth rate and

mortality, this study will help answer fundamental questions about life history and migratory strategies, adding to our understanding about the evolution, coexistence and maintenance of the two alternative life history phenotypes. Specifically, the thesis provides a framework for studying the relationships and interactions between the anadromous and resident Atlantic salmon from a single population, addressing how maternal effects might influence the occurrence of partially migratory populations.

Chapter 2: Maternal effects and early life performance and interactions among offspring of resident and migratory Atlantic salmon *(Salmo salar)*.

2.1 Introduction

Salmonid life histories show considerable variability, including in age and size at first reproduction, degree of iteroparity, the phenology of reproduction, degree of reproductive investment, sexual dimorphism, parental care and the presence and degree of diadromy (Hutchings and Jones 1998, Fleming 1998, Fleming and Reynolds 2004, Jonsson and Jonsson 2011). Much of the research regarding salmonid phenotypic plasticity has focused on the alternative male reproductive tactics and comparatively little on the existence of alternative migratory tactics among Atlantic salmon. This has led to a slightly skewed view on alternate life history phenotypes in the Atlantic salmon and the partially migratory populations, especially the alternative forms of females (Adams 2007) tend to be somewhat neglected. While many Atlantic salmon populations do tend to be a blend of anadromous and resident spawners (mature male parr), there are also populations which exhibit partial migration where individuals of both sexes within a cohort either migrate or remain resident all year round. Studies of partially migratory populations of Atlantic salmon have tended to focus on the mechanisms (i.e. genetic and environmental determinants) behind the expression of the alternative migratory phenotypes, with little attention paid to maternal effects on the offspring. These blended populations are thought to be either exhibiting polyphenism (Robitaille *et al*. 1986; Adams 2007) or are genetically distinct reproductively isolated populations (Verspoor and Cole 1989). In populations exhibiting polyphenism, the expression of the alternative life history phenotypes is thought to be a conditional strategy, whereby the tactic adopted

is dependent on the individual's social and/or physiological state (i.e. status, body size; sensu Dawkins 1980, Gross 1996, Oliveira *et al*. 2008).

Since the 1990s, there has been a surge in recognition of the role maternal effects as a driving mechanism of phenotypic variability (Bernardo 1996; Mousseau and Fox 1998). Traditionally, differences in phenotypes were considered a direct product of genetic and environmental variation. However the phenomenon of maternal (transgenerational) effects has quickly gained recognition and is now widely accepted as an important mechanism that plays a unique role in various evolutionary and ecological processes including phenotypic plasticity (Wolfe and Wade 2009).

Recent research on maternal effects in Atlantic salmon have stressed the idea that fitness outcomes of egg size variation are more pronounced during early juvenile stages rather than the egg or larval stage (Rollinson and Hutchings 2011; Louhi *et al*. 2014). If maternal provisioning can account for differences in behaviour, competitive ability and size and growth, by extension maternal effects are likely to affect offspring survival, and ultimately the fitness of mothers adopting the alternative tactics (i.e. the evolutionary switch point between the tactics). Furthermore, such maternal effects may influence an offspring's subsequent phenotype (e.g. status or state) when the expression of the alternative tactics is cued, thus playing a non-genetic role in the expression of partial migration. Offspring of a given phenotype at that time may be better off remaining resident than migrating or vice versa.

In addition to the amount of resources a mother allocates to each of her eggs, the form or quality of those resources is likely to have maternal effects on offspring

performance. Since anadromous female Atlantic salmon derive much of their resources from feeding in a marine environment while the resident females do so from feeding in a freshwater environment, the resources they provide to their corresponding eggs and offspring may differ significantly. This may lead to differences in offspring growth, competitive ability and survival that may be attributed to the differences in the form of maternal provisioning (e.g. fatty acids). Fish feeding in a marine environment typically have different fatty acid compositions than their freshwater counterparts due in most part to their differing diets. In general, freshwater fish have higher levels of ω6 fatty acids (FA) while marine fish have higher levels of ω3 long chain FA. If maternal provisioning influences growth rate, better resources may encourage a higher growth rate and provide a fitness advantage which may cause a shift in the evolutionary equilibrium in the expression of the alternative tactics.

The rivers and watersheds in Newfoundland provide a unique opportunity to study partially migratory populations and provide insight into the mechanisms involved in the expression of the alternative migratory tactics (migration versus residency). Both tactics coexist in many of the same spawning sites despite each potentially affording different maternal resources to their offspring that may affect fitness. By investigating the maternal allocation of resources to eggs and offspring, and the ensuing social interactions between offspring of the two phenotypes, behaviour that may impact fitness can be compared and quantified. Such differences in behaviour may shed light on the degree to which maternal effects influence the co-existence of the alternative life history tactics.

The objectives of this study were to test: (1) for differences in maternal allocation to eggs by females of the two life-history phenotypes, particularly in terms of egg size and lipid content and composition; (2) whether any differences persist through offspring emergence to the onset of exogenous feeding; and if so, whether these differences affect (3) the competitive ability of newly emerged juveniles and (4) their growth and mortality in a near natural stream environment. This research provides a framework for studying the relationship between the anadromous and resident Atlantic salmon from a single population. It investigates differences in performances of both types of offspring due to maternal effects which may help to answer fundamental questions about life history and migratory strategies, adding our understanding about the evolution, coexistence and maintenance of the two alternative life history phenotypes.

2.2 Methods

2.2.1 Experimental Crosses

Five anadromous and 12 resident female, and 6 anadromous and 4 resident male Atlantic salmon were captured by electro fishing from Indian Bay Brook, Newfoundland (49^{o}03' N, 54^{o}03' W). Anadromous fish were distinguished as being over 550 mm in length while the resident fish were under 450 mm (Adams 2007). Gametes were collected by stripping over a two day period and kept cool while being transported to the Ocean Sciences Centre of Memorial University (St. John's). Within the same time frame, eggs were also stripped from an additional three anadromous females captured at the Grand Falls Fishway on the Exploits River, Newfoundland (49^{o}04' N, 55^{o}20' W). On 5

November 2005, 300-400 eggs from each female were crossed with sperm from one of the males to produce 20 different families. Each sire was crossed with at least one anadromous and one resident dam to create paternal half sib families and thus control for paternal effects (Table 2.1). The offspring from each sub-family could then be tested for growth rate, competitive ability and mortality. When creating the crosses, ten eggs from each female were weighed individually for wet and dry mass. Dry masses were obtained by placing the eggs on small, pre-weighed aluminum foil pans and then in a desiccating oven (70^{o}C) until mass stabilized (~ 48 h).

Embryos from each family were raised in separate containers within a vertical incubation system (i.e. a "Heath" tray incubator) until emergence at approximately 760 ddpf (degrees days post fertilization). As there was no gravel in the incubators, emergence was estimated to have occurred when fish were free swimming, no longer laying on the bottom, and had absorbed most of their yolk sac (i.e. "buttoned-up"). All offspring for lipid analyses were taken directly from the incubator prior to exogenous feeding. At emergence, the remainder of each family was transferred to glass aquaria, one per family, provided with air stones and fed *ad libitum* with live *Artemia* nauplii.

Table 2.1: Experimental crosses using resident and anadromous females and males from Indian Bay (IB) and anadromous females from the Exploits River (ER) to create families, * Identifies families that were used for experiments following the start of exogenous feeding.

	Female			Male		
Cross (Family)	ID	Origin	Length (mm)	ID	Origin	Length (mm)
1*	R3	Resident (IB)	321	A3	Anadromous (IB)	571
2*	A2	Anadromous (IB)	565	A3	Anadromous (IB)	571
3*	R6	Resident (IB)	396	A3	Anadromous (IB)	571
4*	A7	Anadromous (ER)	557	A3	Anadromous (IB)	571
5*	R2	Resident (IB)	312	R8	Resident (IB)	315
6*	A1	Anadromous (IB)	530	R8	Resident (IB)	315
7	R8	Resident (IB)	323	R8	Resident (IB)	315
8	A5	Anadromous (ER)	612	R8	Resident (IB)	315
9*	R7	Resident (IB)	320	R11	Resident (IB)	300
10*	A5	Anadromous (IB)	650	R11	Resident (IB)	300
11	R11	Resident (IB)	330	R11	Resident (IB)	300
12*	R4	Resident (IB)	406	R12	Resident (IB)	285
13*	A4	Anadromous (IB)	650	R12	Resident (IB)	285
14	R9	Resident (IB)	392	R12	Resident (IB)	285
15	R12	Resident (IB)	332	A2	Anadromous (IB)	550
16	A3	Anadromous (IB)	537	A2	Anadromous (IB)	550
17	R5	Resident (IB)	331	A2	Anadromous (IB)	550
18*	R10	Resident (IB)	331	R7	Resident (IB)	401
19*	A6	Anadromous (ER)	550	R7	Resident (IB)	401
20	R1	Resident (IB)	365	R7	Resident (IB)	401

2.2.2 Lipid analyses

Eggs and offspring from each family were sampled at four stages to track lipid profiles: (1) unfertilized eggs, (2) eyed eggs (~225-230 ddpf), (3) hatched alevins (newly hatched larvae feeding endogenously from the yolk sac; ~510 ddpf) and (4) newly emerged fry (~760 ddpf). At the unfertilized and eyed egg stages, three eggs from each female were taken for lipid analyses and dry mass (N = 60). Due to mortality during hatching and the start of exogenous feeding (i.e. emergence), only 12 families created from the gametes of 6 resident females, 6 anadromous females and the appropriate corresponding 5 males were found suitable (i.e. adequate number of individuals) for analysis of the alevins and emergent stages (Table 2.1). To keep analysis consistent, only these twelve families were used in this study.

Because of the limited number of individuals only one sample from each family and at each stage was used to determine dry mass. However egg mass typically varies little [< 3%] within as compared to among females (Einum and Fleming 2004) and this was also found to be the case with the ten eggs taken for dry mass from each female previously. Another two samples from each family were used for lipid analysis. In preparation for lipid analysis, whole samples were individually weighed, placed in lipid cleaned tubes containing 2 ml of chloroform, flushed with nitrogen and sealed with Teflon lined caps and Teflon tape. Specimens were stored at -20^{o}C until extraction.

Lipids were extracted using a variation of the Folch procedure (Folch *et al*. 1957) as described by Parrish (1999). Lipid classes of these extracts were determined by Chromarod thin-layer chromatography with flame ionization detection (TLC/FID) using

a MARK V Iatroscan (Iatron Laboratories). The extracts were spotted on silica gel coated Chromarods-SIII and a three-stage development system was used to separate lipid classes according to Parrish (1999). After each separation, the rods were scanned and the three resulting chromatograms were combined using T Data Scan 3.0 (RSS Inc. Bennis. Tenn., USA). The signal (detected in millivolts) was quantified using lipid standards (nanodecane, cholesteryl stearate, 3-hexdecanone, tripalmitin, palmic acid, cetyl alcohol, cholesterol, monopalmitoyl, phosphatidylcoline dipalmitoyl) from Sigma Chemicals (Sigma Chemicals, St. Louis, MO, USA). Standards used were composed of saturated fatty acids so they would be stable, however sample compounds would contain significant proportions of polyunsaturated fatty acids. Parrish *et al.* (1992) found that polyunsaturated standards gave lower responses but the difference in response was small by comparison with the error of repeat analyses at any one level or the error in regression equations obtained from calibration data.

Fatty acid methyl esters (FAME) were prepared by transesterfication, with 14% BF3/MeOH at 85°C for 1.5 hrs. The derivatives were analysed with a HP 6890 Gas Chromatograph (GC) Flame Ionization Detector (FID) equipped with a 7683 autosampler and a ZB wax+ GC column (Phenomenex, U.S.A.) using hydrogen as the carrier gas. The column length was 30m with an internal diameter of 0.32mm. The column temperature began at 65ºC and held this temperature for 0.5 minutes. The temperature ramped to 195 ºC at a rate of 40 ºC/min, held for 15 minutes then ramped to a final temperature of 220 ºC at a rate of 2 ºC /min. This final temperature was held for 0.75 minutes. The carrier gas was hydrogen and flowed at a rate of 2 ml/minute. The injector temperature

started at 150 ºC and ramped to a final temperature of 250 ºC at a rate of 120 ºC /minute. The detector temperature stayed constant at 260 ºC. Chromatograms were integrated and analyzed using Galaxie Chromatography Data System, version 1.9.3.2 (Varian Inc.) and individual fatty acid peaks were identified using retention times from standards purchased from Sigma Chemicals (37 component FAME mix (product number 47885-U), bacterial acid methyl ester mix (product number 47080-U), PUFA 1 (product number 47033) and PUFA 3 (product number 47085-U).

2.2.3 Offspring measurements at the start of exogenous feeding

At emergence and just prior to the start of exogenous feeding, 20 of the surviving emergent fry from each of the 12 families were sampled simultaneously, weighed and photographed using the Pixera Viewfinder 2.6 software application (Pixera Corp.,Los Gatos, USA). Total body area and the area of remaining yolk was measured using Matrox Inspector 3.0 digital image software (Matrox Electronic Systems Ltd., Dorval, Canada) to provide yolk sac to body size ratio which accounts for any body size differences. The goal was to compare the two phenotypes and discover how much yolk sac remained at this stage. Generally, alevins from small eggs (less yolk reserves) are expected to survive less time without food than alevins from large eggs.

2.2.4 Competition experiment

Two identical fiberglass troughs (50 cm wide × 18 cm high × 2.61 m long), each divided longitudinally with a solid divider to make two separate stream channels. A current was

generated within each stream channel using an inflow spray bar positioned behind window screening at the upstream end of each channel. Each trough consisted of one channel with a spray bar attached directly to a facility freshwater supply and the other attached to a pump, creating a partially recirculating, unidirectional flow. Five test arenas (18 cm long × 8 cm wide × 8-10 cm depth), each composed of Coruplas plastic sheeting along the bottom with gradually sloping sides and screening along the front and back, were placed at equal intervals within each channel to provide a total of 20 separate testing arenas (Figure 2.1). Within the arenas, water flowed at a depth of 8-10 cm at 3-5 cm sec^{-1} over a layer of 1 - 3 cm diameter gravel and an ambient light cycle was followed. Taking the depth of the water into account, the fish had a relatively square area within which to swim and interact.

For Atlantic salmon, the ability to compete at low densities with a highly localised food source indicates similar performance at high densities with a more dispersed food supply (Adams and Huntingford 1996). To this end, ninety pair-wise (anadromous *vs* resident) dominance trials were performed with recently emerged fish (~ 900 ddpf) competing for food. These fish had all been provided with food and were presumed to be feeding in the holding aquaria for approximately five days before the start of this experiment. All offspring were paired with a paternal half sibling. The fry were anaesthetized with MS-222 (Western Chemical Inc., Ferndale, WA, USA), weighed and tagged by injecting a small aliquot of elastomer (NorthWest Technologies, WA, USA) into the musculature directly in front of the dorsal fins with a 29G needle. Anadromous and resident fish were randomly selected and marked with different colours to control for

effects due to the marking procedure. Elastomer tagging has been found to have better retention rates and be less intrusive compared to previously used external tags, and to have little to no impact on mortality in fish (Willis and Babcock 1998).

Pairs of fish (half-siblings) were transferred into the testing arenas, where they were allowed 24 hrs to acclimate and learn the location from which food was dispensed. They were fed brine shrimp (*Artemia*) nauplii delivered via a tygon tube located at the centre of the upstream end of each arena. During the acclimation, the fish were fed at approximately every 3 hours during daylight. During the experiments, observations were made through slits in tarps surrounding each trough to minimize disturbance. Within each channel, testing was conducted from the downstream- to the upstream-most arena to prevent uneaten food from drifting down to untested fish.

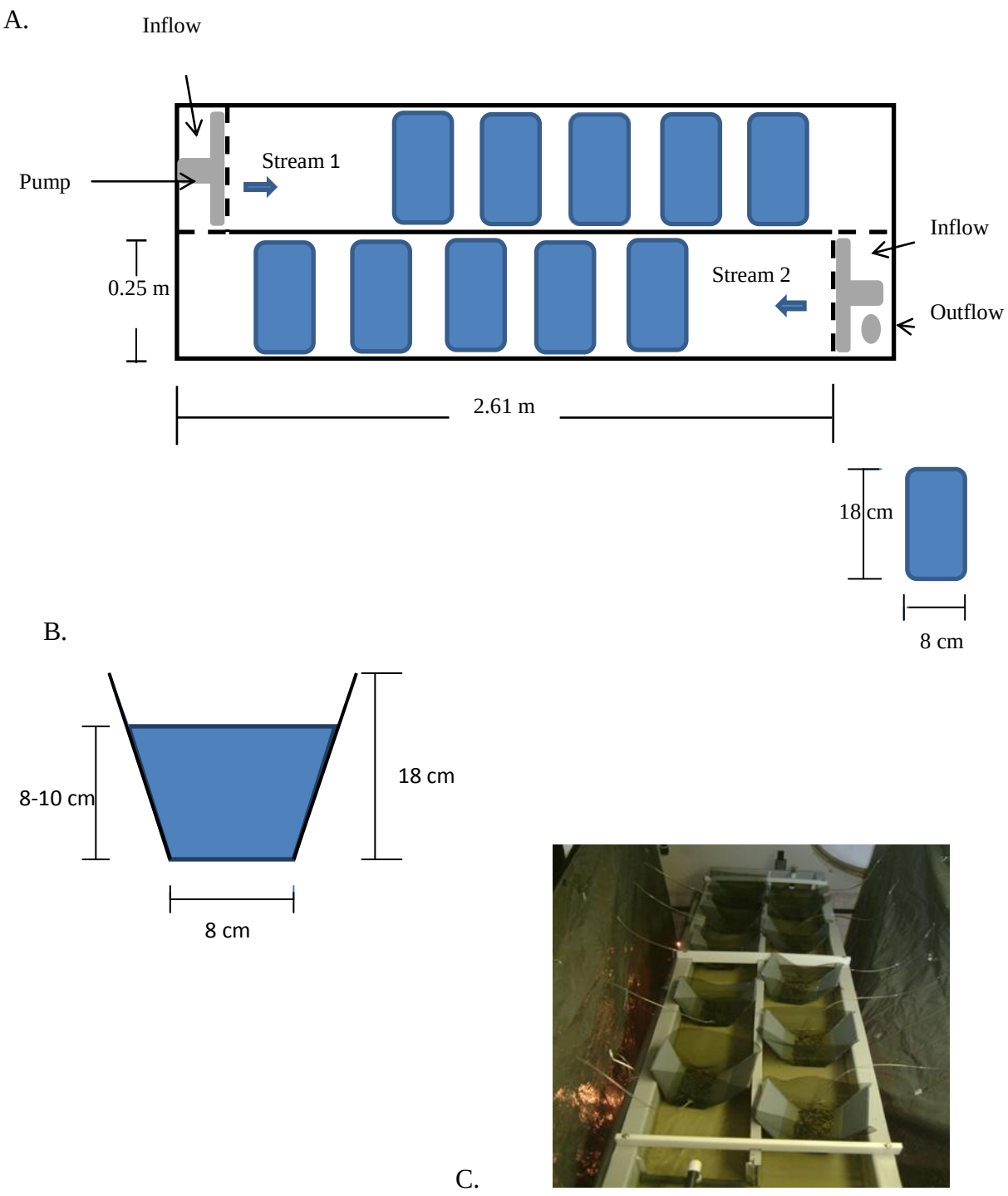


Figure 2.1: Schematic (A) and photograph (C) of stream channels with 10 separate testing areas for behavioural experiments; (B) sideview of arena showing water depth and area available to fish.

During each trial, similar quantities of live *Artemia* spp. (~ 10) were delivered through a feeding tube centered at the upstream end of the contest arena and feeding attempts recorded for each individual.

Dominance was assessed with a point system accounting for feeding attempts and spatial position (Metcalfe *et al*., 1992, 1995; Cutts *et al*., 1999b; Moreau *et al*. 2011). Fish received a score of 1 for each feeding attempt or 2 if the attempt was contested (distinct biting or lunging motion) by another fish (Metcalfe *et al.* 1989). Position of individuals in relation to a defensible food source and food consumption have been used successfully to measure dominance and aggression in stream-living salmonids (Metcalfe *et al*.. 1989, 1995; Huntingford *et al*. 1990; Riley *et al*. 2005; Moreau *et al*. 2011). Dominant juvenile salmonids usually occupy central-rear positions within a feeding territory, often keeping position just off the substrate with subordinates remaining on the side lines (Metcalfe *et al*. 1989; Johnsson *et al*. 1996). Therefore fish were also scored according to an index that combined both spatial position and ability to obtain a contested food item (Metcalfe *et al*. 2003; Moreau *et al*. 2011). The position values were based on optimal feeding locations with the square occupied by the fish's head recorded both before and during presentation of the food item. Each pair was observed 5 times with an hour between each trial and obtained a feeding attempt score (0-2) as well as a position score (Metcalfe *et al*. 2003). Each individual observation lasted about one minute. Overall scores included both scores for each of the five trials. Only in contests where there was a minimum difference of 3 points between overall scores was an individual declared dominant (Metcalfe *et al*. 2003).

2.2.5 G*rowth and survival experiment*

Five troughs, identical to those described in the competition experiment, were used. Each of the two parallel channels within a trough was partitioned into two equally sized sections separated by a screen (Figure 2.2). To simulate the abiotic conditions of a natural stream, a 3 cm thick layer of 1 - 3 cm diameter gravel was added to each section, and water depth was kept at 8-10 cm with water flow at 3-5 cm sec^{-1} and an ambient light cycle was followed. The density of fish in rivers and streams is highly variable, however according to Grant and Kramer (1990), territory size of recently emerged salmonids ranges from 0.010-0.037 m^2. Based on this information, each stream section was stocked with one of 3 treatments (5 replicates per treatment), with each replicate located in a different quadrant in a subsequent trough each time and leaving one stream section empty in each trough to control for position effects. The quadrants in the fifth trough were assigned treatments randomly, again with one quadrant left empty. Each treatment consisted of 12 fish (~ $37m^{-2}$), with treatment one having 6 anadromous and 6 resident offspring (Allopatric - matched according to families), treatment two had 12 resident offspring (Sympatric Resident Group – all resident families represented) and treatment three consisted of 12 anadromous offspring (Sympatric Anadromous Group – all anadromous families represented) (Table 2.2). All fish were anaesthetized, measured for length and weighed and marked using the same procedure as above, however, each fish within a stream section received a unique colour so individual growth rates could be tracked. Fish in each section were fed similar volumes (4% of fish biomass) of *Artemia* 2-3 times daily. This food level and its pulsated delivery was designed to create a

competitive environment. Estimates of invertebrate drift in natural streams suggest that this food level would be representative of a food-limited environment (Wilzbach *et al*. 1986; Keeley and Grant 1995).

At the termination of the experiment, 30 days later, the fish were remeasured. Specific growth rate was calculated using the formula:

$G = (Ln(w_t) - Ln(w_i))/t$

where $Ln\ (w_t)$ is the natural logarithm of the mass at time t (number of days) and $Ln\ (w_i)$ is the natural logarithm of the initial mass. Instantaneous growth rate (G) is particularly useful for reporting the growth of small fish (Ricker 1979).

All animals were treated in accordance with the guidelines of the Canadian Council on Animal Care during holding and experimentation, and approval was granted by Memorial University's Institutional Animal Care Committee (AUP 06-04-IF).

A.

B.

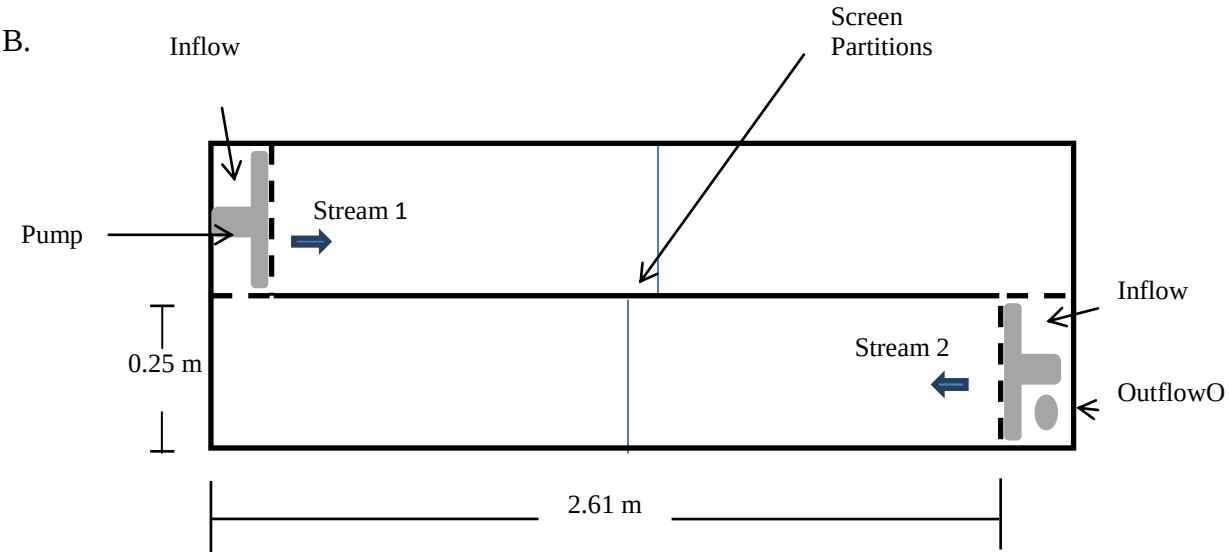


Figure 2.2: Photograph (A) and schematic (B) of stream channels with 4 separate areas for the growth and survival study.

Table 2.2: Mean ± SD mass of the anadromous and resident offspring in each treatment of the growth and survival experiment.

Treatment	Resident mass (g)	Anadromous mass (g)
Sympatric	0.185 ± 0.026 (n=30)	0.177 ± 0.021 (n=30)
Allopatric	0.187 ± 0.029 (n=60)	0.170 ± 0.020 (n=60)

2.2.6 Statistical analysis

General linear models, ANOVAs (SPSS©21, SPSS Inc.), were used to test for differences in fatty acids by maternal origin (anadromous vs resident) and developmental stage (unfertilized egg, eyed egg, alevin and first feeding fry). There were no significant origin-×-stage interactions ($P > 0.19$) and thus the models were rerun excluding the interaction term. Significance levels were adjusted for multiple comparisons using sequential Bonferroni correction. A selection of lipid classes and fatty acids were analysed in terms of mg/g dry weight but results did not differ from results obtained from using percentages. The analyses resulting from using the percentages were tested for normality and equal variance and passed both tests.

Principal component analysis (PCA) was used to condense the amount of information contained in a larger number of original variables into a smaller set of variables with minimum loss of information (McCure & Grace, 2002). In this way previously unsuspected relationships may become more obvious. PCA was performed using the correlation matrix (SPSS©16, SPSS Inc.) to help identify the patterns associated with female phenotype, life stage (unfertilized egg, eyed egg, alevin, emerged fry), lipid classes (phospholipids, TAG) other lipid classes [hydrocarbons, steryl esters/wax esters, methyl ketones, glyceryl ethers, triacylglycerols, free fatty acids, alcohols, sterols, diacylglycerols and acetone mobile polar lipids] and selected fatty acids (AA, EPA, DHA, saturated fatty acids, MUFA, signature terrestrial fatty acids [18:2ω6 and 18:3ω3], and other PUFAs). Results of the PCA are based on the rotated component matrix (Varimax rotation with Kaiser Normalization).

To compare the relationship between yolk sac area and body area (excluding yolk area) at emergence of offspring of anadromous and resident females (fixed effect), a nested ANOVA (SPSS©21, SPSS Inc.) was performed on natural logarithm transformed data with female nested within origin (random effect).

For trials of the relative competitive abilities of half siblings, a logistic regression with a binomial distribution was used. The formula was:

wins/total = type + error

where wins = # of non-losses; total = total # of trials and type = resident or anadromous. The generalized linear model procedure of SAS (1988) was used to evaluate logistic regressions. Differences in initial and final mass and specific growth rate of fish in the growth and survival experiment were tested using ANOVA with fish origin and treatment as fixed effects and replicate as a random effect. The level of significance for all tests was $\alpha = 0.05$.

2.3 Results

Despite their smaller body sizes (Table 2.1), resident females had significantly larger eggs than anadromous females, both in terms of wet (nested ANOVA $F_{1,18} = 15.81$, $P = 0.001$) and dry mass ($F_{1,18} = 10.47$, $P = 0.005$; Table 2.3). There was also a significant difference in egg size between andromous females from Indian Bay Brook and the Exploits River (wet mass $F_{1,6} = 33.46$, $P = 0.001$; dry mass $F_{1,6} = 43.85$, $P = 0.001$; Table 3). When comparing only the females from Indian Bay Brook, resident females continued

to show significantly heavier egg wet mass ($F_{1,15} = 6.27$, $P = 0.024$), but the difference was marginally nonsignificant in terms of egg dry mass ($F_{1,14.9} = 3.69$, $P = 0.074$).

Table 2.3: Mean ± SD wet and dry mass of unfertilized eggs of the resident and anadromous females.

	Resident	Anadromous	
	Indian Bay Brook (n = 12 females)	Indian Bay Brook (n = 5 females)	Exploits River (n = 3 females)
Egg Wet Mass (mg)	144 ± 20	124 ± 11	102 ± 6
Egg Dry Mass (mg)	54.7 ± 9.0	47.4 ± 1.9	38.6 ± 3.2

2.3.1 Total lipids, lipid classes and fatty acids

There were no significant differences in lipid profiles between the offspring of andromous females from Indian Bay Brook and those from the Exploits River ($P > 0.20$), and thus the two groups were combined in subsequent analyses. Total lipids of whole individuals (mg/g) did not differ by maternal origin (i.e. anadromous vs. resident mother) across the developmental stages examined ($F_{1,43} = 0.16$, $P = 0.694$; Figure 2.3), nor did phospholipids ($F_{1,43} = 4.02$, $P = 0.052$) and triacylglycerols as a percent of total lipid mass (TAG; $F_{1,43} = 0.16$, $P = 0.694$; Figure 2.4) (see Appendix 1 for complete list of lipid classes per family across developmental stages). There was also no significant difference between offspring of resident and anadromous females in total saturated fatty acids (SAT; Table 2.4, Figure 2.5). There were, however, a number of significant differences in the fatty acid profiles of eggs and offspring of resident versus anadromous females (Table 2.4) (see Appendix 2 for complete list of fatty acids per family across developmental stages), particularly in relation to monounsaturated (MUFA; Figure 2.5) and polyunsaturated fatty acids (PUFA), including arachidonic acid (AA; Figure 2.6), eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA). Moreover, the ratios of AA:EPA (Figure 2.7), DHA:EPA (Figure 2.8) and ω3:ω6 (Figure 2.9) showed significant differences between offspring origin at each life stage.

There was generally little effect of developmental stage on lipid and fatty acid profiles (Table 2.4). Neither total lipids ($F_{1,43} = 0.85$, $P = 0.476$; Figure 2.3) nor phospholipids as a percent of total lipid mass varied significantly with developmental stage ($F_{1,43} = 1.12$, $P = 0.354$; Figure 2.4). However, triacylglycerols (TAG) as a percent

of total lipid mass did vary significantly with developmental stage ($F_{1,43}$ = 6.96, P = 0.001; Figure 2.4). It declined particularly during the latter stages of development, from representing ~ 46% of total lipids at unfertilized egg, eyed egg and alevin stages to 37% at the emergent fry stage. There was also a similar decline in stearic acid (18:0), with a pronounced change from the alevin to emergent fry stage, though it was not significant following sequential Bonferroni correction (Table 2.4). The only other changes with development stage were in regard to palmitic acid (16:0) and gondoic acid (20:1ω9), and both were relatively weak and not significant following sequential Bonferroni correction (Table 2.4).

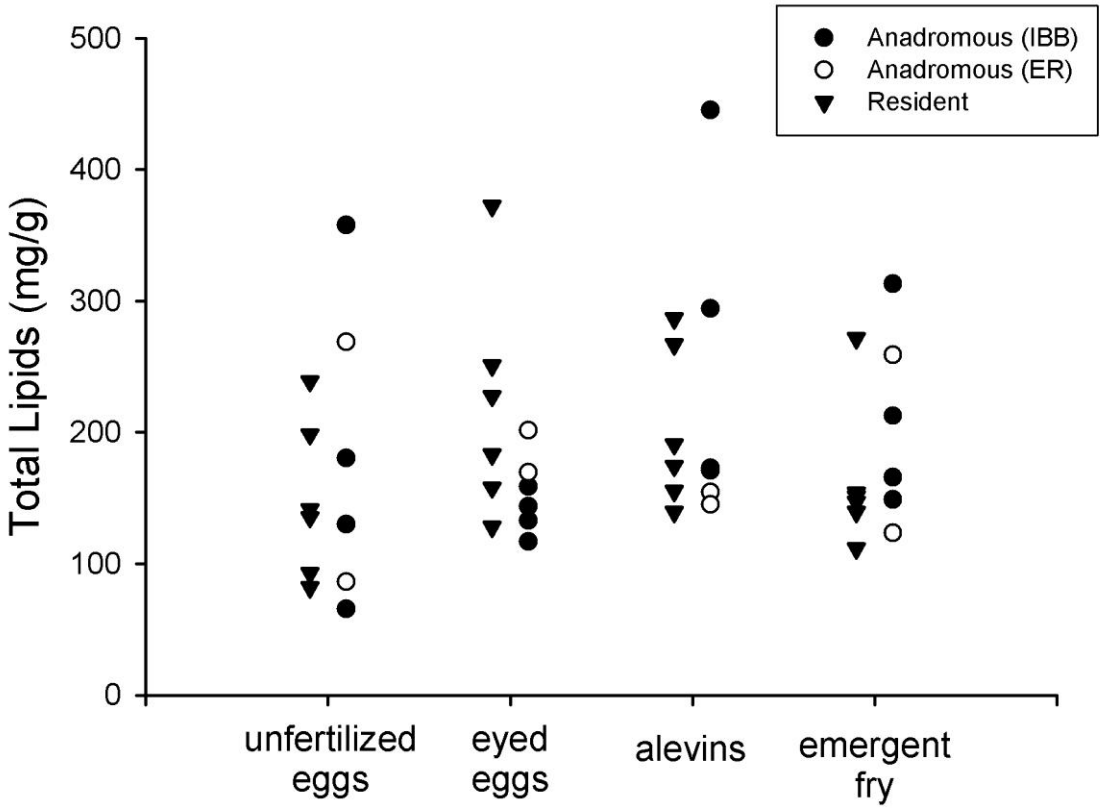


Figure 2.3: Total lipids (mg/g) for offspring of anadromous and resident mothers at the four developmental stages studied. ($F_{1,43} = 0.85$, $P = 0.476$)

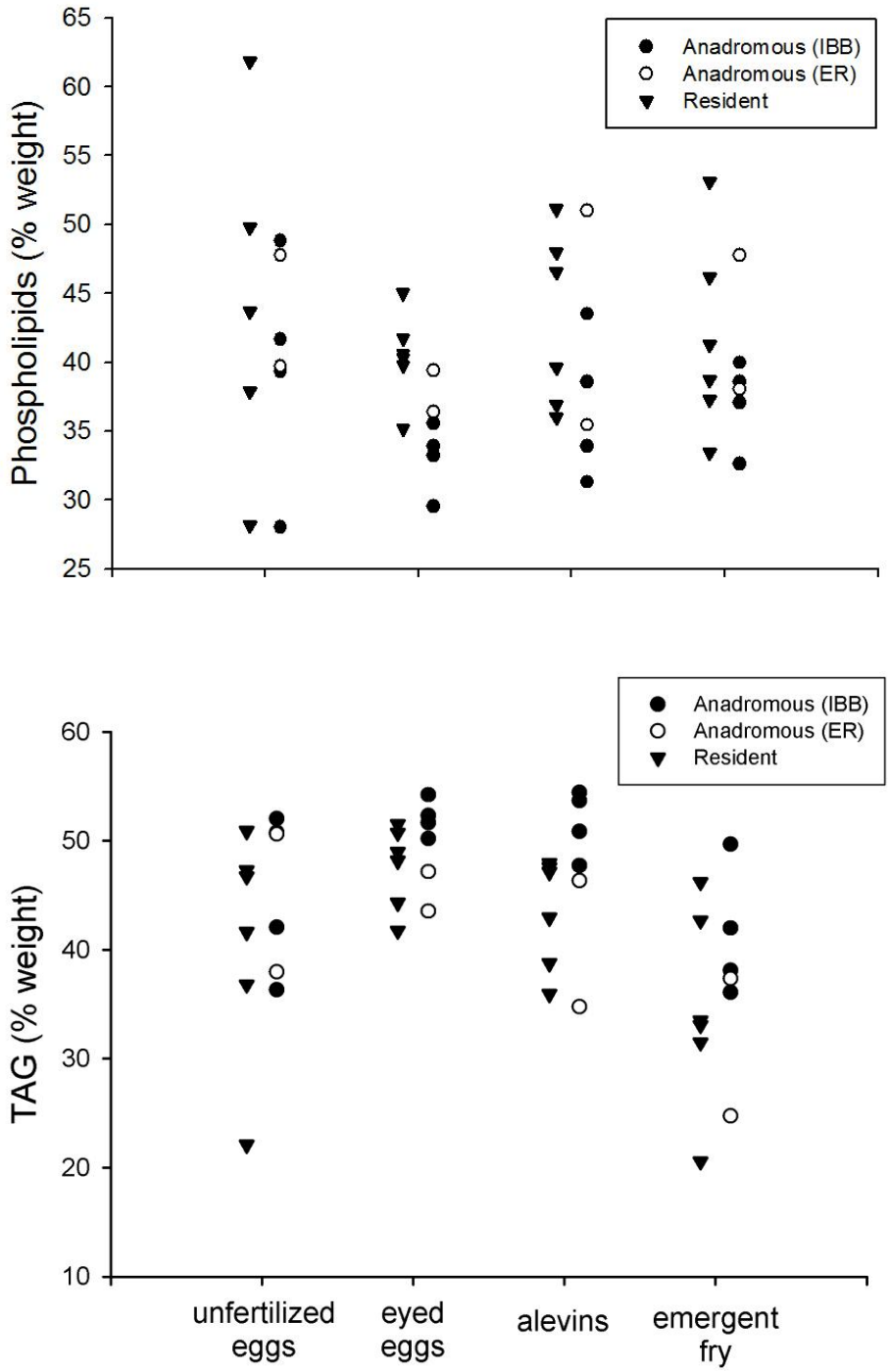


Figure 2.4: Phospholipids (PL, $F_{1,43} = 4.02$, $P = 0.052$) and triacylglycerols (TAG, $F_{1,43} = 0.16$, $P = 0.694$) as a percent of total lipid mass across the four developmental stages for offspring of anadromous and resident mothers.

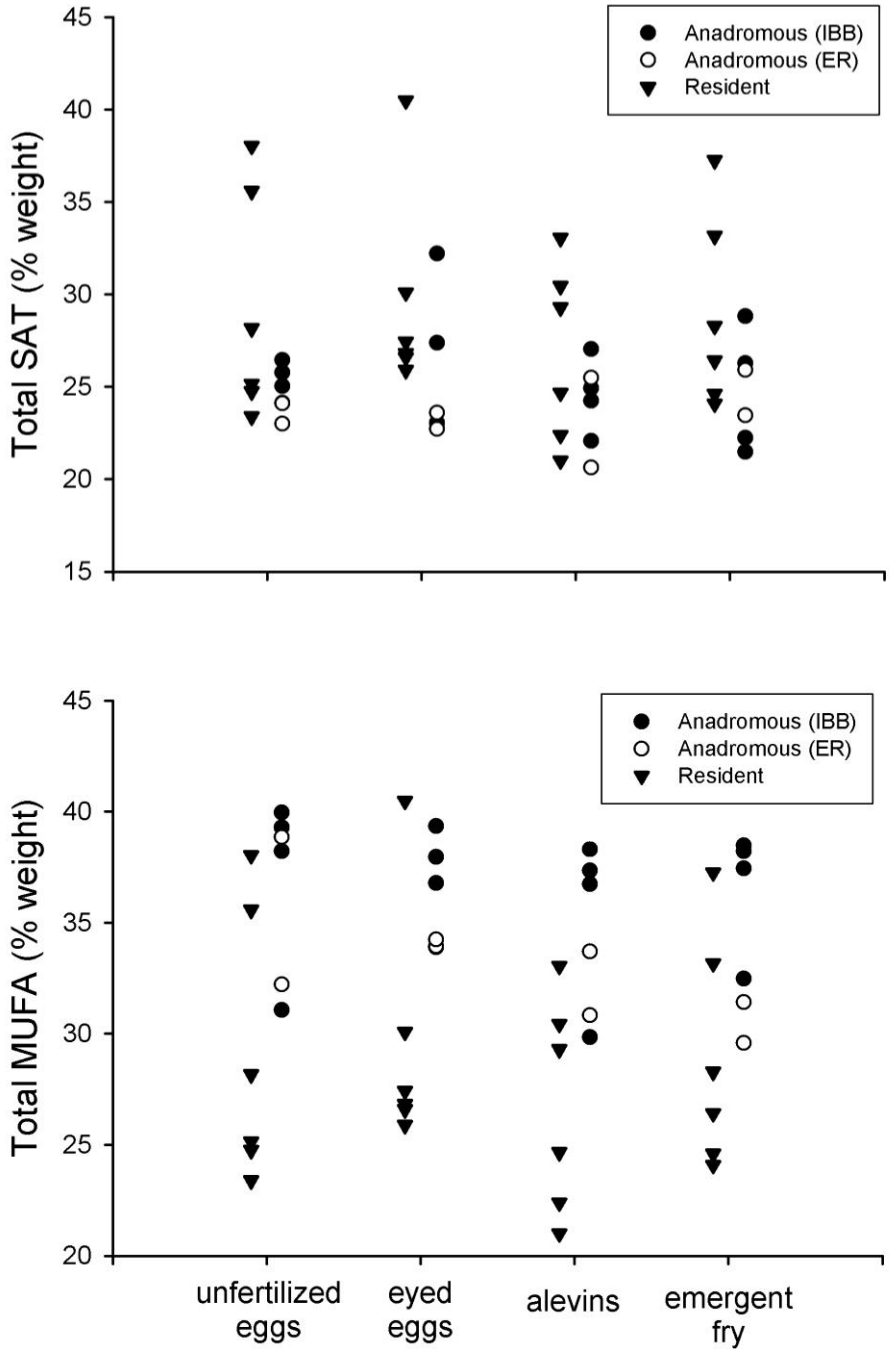


Figure 2.5: Total saturated fatty acids (SAT) and monounsaturated fatty acids (MUFA) as a percent of total fatty acid mass across the four developmental stages for offspring of anadromous and resident females. (See Table 2.4)

Table 2.4: Profiles of major fatty acids (i.e. > 1% of total fatty acid composition) of offspring of resident and anadromous females at four different development stages (unfertilized egg, eyed egg, alevin and emergent fry). Data are means ± SD and p-values from ANOVA results are shown.
The following fatty acids were below detection limits in all samples: 18:2a, 18:2b, 22:2NMIDa?, 22:2NMIDb?, trimethyltridecanoic acid (TMTD), pristanic acid?, 16:1ω11?, *ai*17:0, 16:4ω3?, 18:1ω11?, 19:0, 20:0, 18:5ω3, 21:0, 22:0.
The following fatty acids and fatty acid markers were present at proportions < 1 % and were not included in the above Table: 14:1, *i*15:0, ai15:0, 15:1, 16:1ω5, *i*17:0, 17:1b, 16:4ω1, 18:3ω6, 20:1ω11?, 20:2ω6, 20:3ω6, 22:1ω7, 22:1ω11(13), 23:0, 22:4ω6?, 22:4ω3?.

	Unfertilized Eggs		Eyed Eggs		Alevins		Fry		ANOVA (p values)	
Fatty Acid	Resident	Anad.	Resident	Anad.	Resident	Anad.	Resident	Anad.	Origin	Stage
14:0	1.2 ± 0.5	1.6 ± 0.5	1.3 ± 0.6	1.6 ± 0.4)	1.2 ± 0.5	1.5 ± 0.5	1.2 ± 0.4	1.3 ± 0.4	**0.027**	0.785
16:0	14.2 ± 1.5	14.3 ± 0.7	14.1 ± 1.2	14.3 ± 0.7	14.1 ± 0.6	13.6 ± 2.2	14.6 ± 1.0	16.2 ± 2.0	0.372	**0.044**
16:1ω7	5.3 ± 2.1	6.3 ± 2.5	5.3 ± 1.9	6.4 ± 2.2	4.3 ± 1.4	5.5 ± 1.2	4.7 ± 2.1	5.4 ± 3.8	0.178	0.706
18:0	9.7 ± 1.7	7.7 ± 1.7	9.9 ± 2.9	8.3 ± 1.6	9.3 ± 1.5	8.6 ± 1.5	6.7 ± 2.0	6.6 ± 1.0	**0.042**	**0.005**
18:1ω9	16.0 ± 4.0	20.6 ± 1.4	14.5 ± 4.5	20.8 ± 1.7	14.7 ± 3.4	20.4 ± 2.9	12.0 ± 3.7	19.6 ± 1.6	**<.001***	0.205
18:1ω7	5.3 ± 0.9	4.1 ± 0.6	6.8 ± 2.6	4.3 ± 0.9	5.1 ± 0.8	4.1 ± 0.8	6.4 ± 4.3	4.2 ± 0.4	**0.002***	0.494
18:2ω6	2.6 ± 1.0	0.9 ± 0.1	2.7 ± 0.5	0.9 ± 0.2	2.4 ± 1.0	0.9 ± 0.9	2.3 ± 0.7	0.9 ± 0.1	**<.001***	0.851
18:3ω3	1.1 ± 0.4	0.3 ± 0.1	1.2 ± 0.5	0.3 ± 0.1	1.1 ± 0.4	0.3 ± 0.3	1.1 ± 0.3	0.3 ± 0.1	**<.001***	0.988
20:1ω9	0.5 ± 0.3	1.7 ± 0.4	0.5 ± 0.2	1.8 ± 0.3	0.6 ± 0.2	1.9 ± 0.2	0.4 ± 0.1	1.4 ± 0.2	**<.001***	**0.044**
20:3ω6	1.2 ± 0.6	0.1 ± 0.1	1.1 ± 0.5	0.2 ± 0.0	1.0 ± 0.5	0.2 ± 0.5	0.6± 0.4	0.4 ± 0.4	**<.001***	0.208

Table 4 (continued)

	Unfertilized Eggs		Eyed Eggs		Alevins		Fry		ANOVA (p values)	
Fatty Acid	Resident	Anad.	Resident	Anad.	Resident	Anad.	Resident	Anad.	Origin	Stage
22:5ω3	4.5 ± 1.0	6.4 ± 0.6	4.3 ± 1.0	6.3 ± 0.3	4.0 ± 1.2	6.3 ± 1.1	4.1 ± 0.6	5.3 ± 0.7	**<.001***	0.127
20:4ω6 (AA)	9.3 ± 1.0	0.8 ± 0.3	9.3 ± 1.1	0.9 ± 0.3	9.7 ± 0.9	1.0 ± 0.8	10.0 ± 0.8	1.4 ± 0.5	**<.001***	0.135
20:5ω3 (EPA)	5.0 ± 1.5	8.4 ± 0.7	5.1 ± 1.6	8.5 ± 0.6	5.8 ± 1.6	8.9 ± 1.4	5.7 ± 1.4	8.9 ± 0.6	**<.001***	0.340
22:5ω6	1.3 ± 0.4	0.0 ± 0.0	1.3 ± 0.4	0.1 ± 0.0	1.2 ± 0.5	0.0 ± 0.3	1.2 ± 0.5	0.0 ± 0.3	**<.001***	0.953
22:6ω3 (DHA)	16.2 ± 4.3	19.7 ± 2.7	14.5 ± 3.9	17.9 ± 5.0	17.2 ± 4.3	21.0 ± 3.7	17.7 ± 3.9	21.1 ± 4.2	**0.003***	0.175
24:1	0.7 ± 1.0	1.7 ± 0.8	0.6 ± 0.2	1.1 ± 0.5	0.9 ± 0.8	1.2 ± 0.8	3.2 ± 5.9	1.8 ± 1.1	0.873	0.252
ΣSAT	26.1 ± 3.0	24.7 ± 1.6	26.2 ± 3.9	26.1 ± 5.7	24.2 ± 3.1	23.7± 4.9	23.7 ± 2.2	24.7 ± 3.1	0.483	0.367
ΣMUFA	29.2 ± 6.2	36.6 ± 4.8	29.6 ± 5.7	36.0 ± 2.9	26.8 ± 4.9	34.5 ± 3.7	29.0 ± 7.6	34.6 ± 4.4	**<.001***	0.573
ΣPUFA	44.0 ± 6.6	38.3 ± 4.4	43.5 ± 6.0	37.6 ± 6.5	43.0 ± 6.3	37.4 ± 5.2	46.7 ± 7.7	40.4 ± 4.7	**<.001***	0.245
AA/EPA	2.0 ± 0.6	0.1 ± 0.0	2.1 ± 0.7	0.1 ± 0.0	1.9 ± 0.7	0.1 ± 0.1	1.9 ± 0.6	0.2 ± 0.1	**<.001***	0.472
DHA/EPA	3.1 ± 0.6	2.4 ± 0.4	3.0 ± 0.4	2.1 ± 0.7	2.9 ± 0.4	2.2 ± 0.3	3.2 ± 1.1	2.4 ± 0.6	**<.001***	0.954
ω3:ω6	4.6 ± 2.2	28.3 ± 6.5	4.3 ± 1.7	26.1 ± 5.6	4.8 ± 2.3	27.1 ± 5.6	5.5 ± 2.2	29.5 ± 8.5	**<.001***	0.668
Σω3	27.1 ± 7.5	35.4 ± 3.4	26.7 ± 7.5	34.4 ± 5.1	30.1 ± 7.8	37.9 ± 2.8	30.1 ± 5.9	36.7 ± 3.3	**<.001***	0.751

Table 4 (continued)

Fatty Acid	Unfertilized Eggs		Eyed Eggs		Alevins		Fry		ANOVA (p values)	
	Resident	Anad.	Resident	Anad.	Resident	Anad.	Resident	Anad.	Origin	Stage
$\Sigma\omega 6$	6.5 ± 1.7	1.3 ± 0.3	6.5 ± 1.2	1.4 ± 0.3	6.0 ± 1.6	1.4 ± 0.3	5.7 ± 0.8	1.3 ± 0.2	**<.001***	0.639

* Significant following sequential Bonferroni adjustment

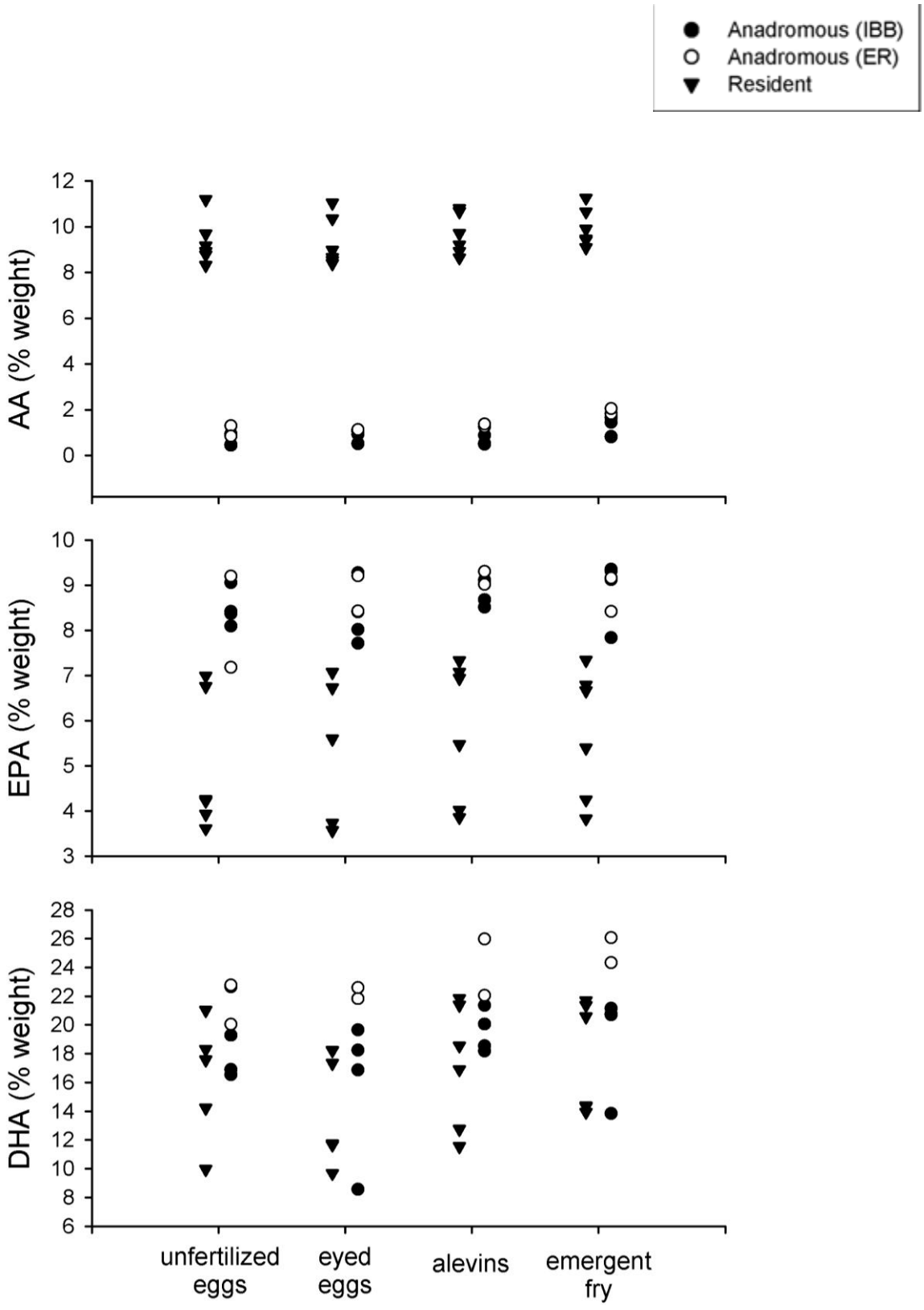

Anadromous (IBB)
Anadromous (ER)
Resident
AA (% weight)
EPA (% weight)
DHA (% weight)
unfertilized eggs
eyed eggs
alevins
emergent fry

Figure 2.6: Total arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosapentaenoiec acid (DHA) as a percent of total fatty acid mass across the four developmental stages for offspring of anadromous and resident females. (See Table 2.4)

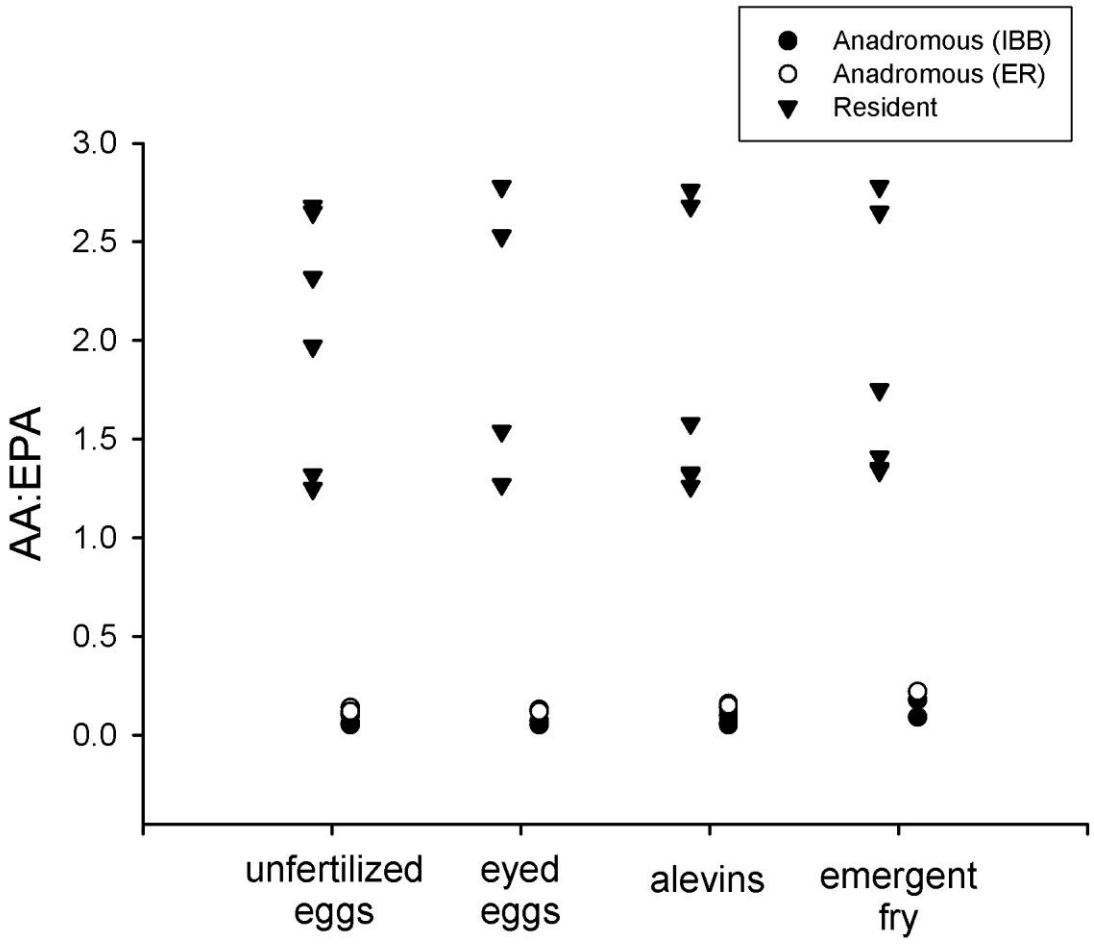


Figure 2.7: Ratio of arachidonic acid (AA) to eicosapentaenoic acid (EPA) across the four developmental stages for offspring of anadromous and resident females. (See Table 2.4)

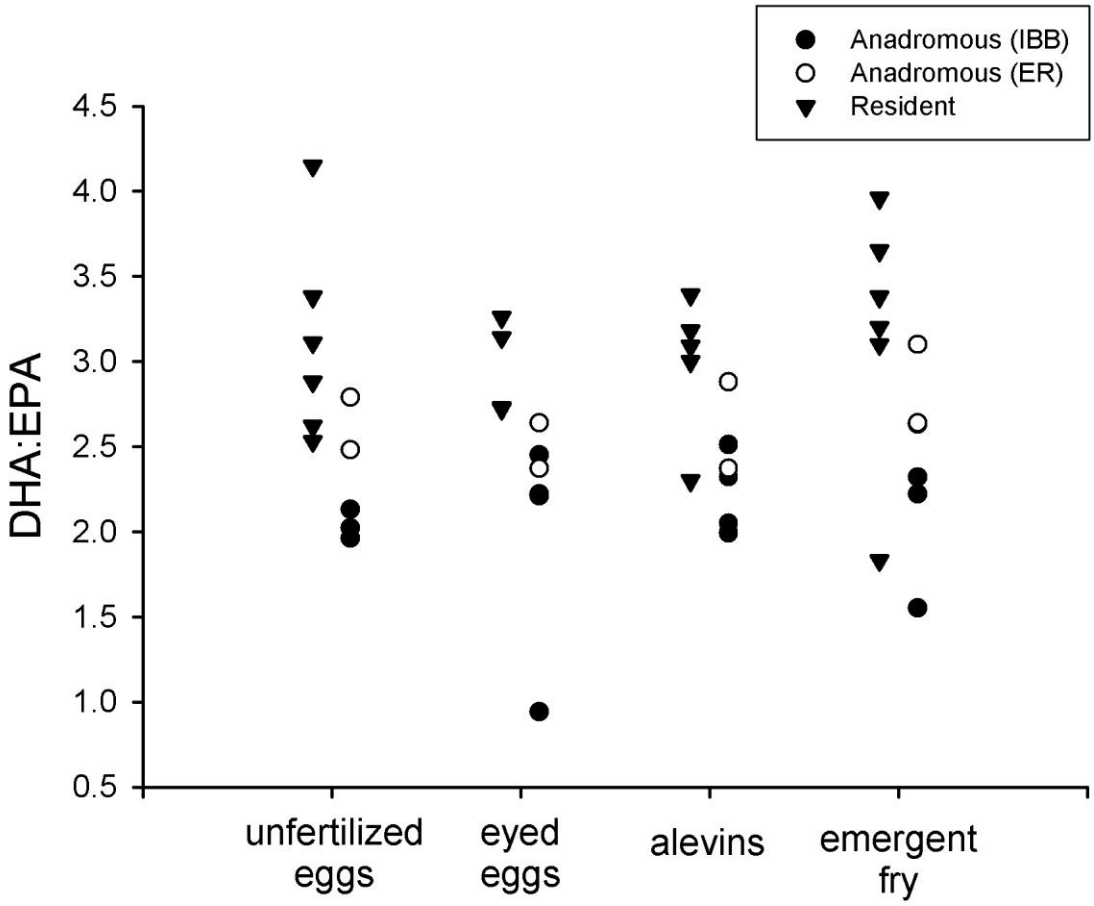


Figure 2.8: Ratio of docosapentaenoic acid (DHA) to eicosapentaenoic acid (EPA) across the four developmental stages for offspring of anadromous and resident females. (See Table 2.4)

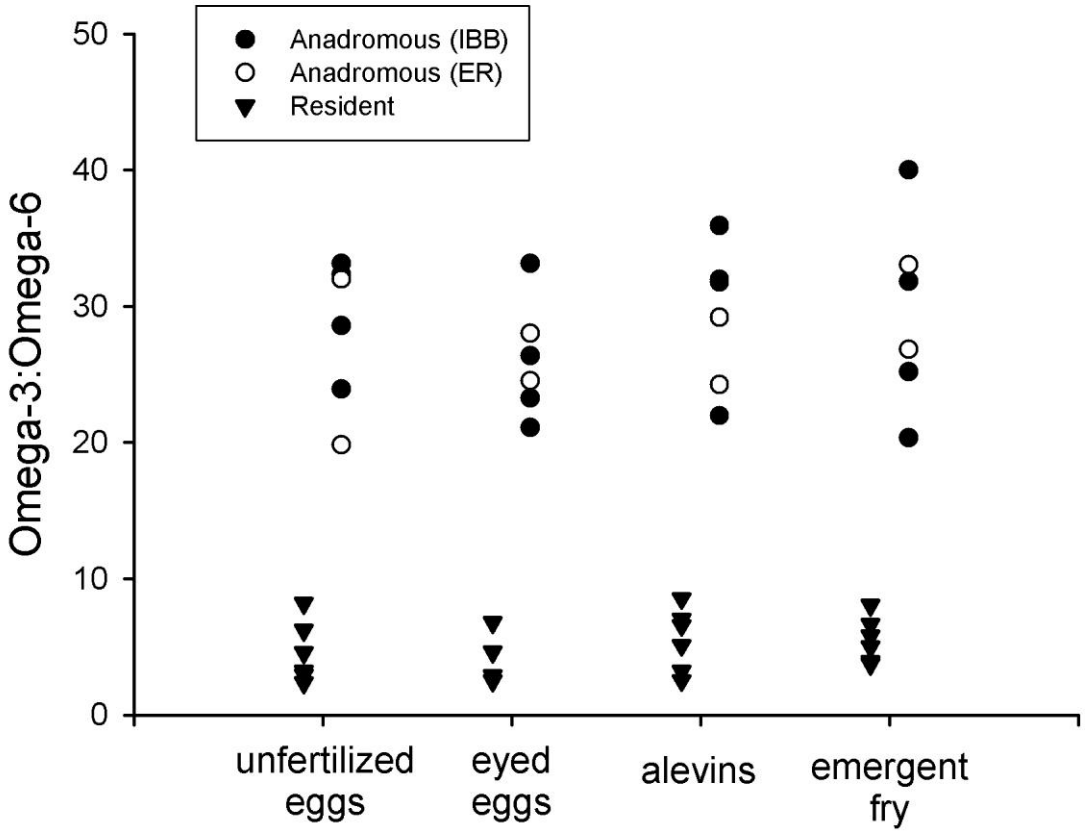


Figure 2.9: Ratio of ω3 to ω6 fatty acids across the four developmental stages for offspring of anadromous and resident females. (See Table 2.4)

2.3.2 Principal Component Analysis

Approximately 87% of variance observed between the offspring of the different origins can be explained by the first three components of the rotated data matrix (Table 2.5). Principal component 1 (PC 1) explains 51% of the variance with strong positive loadings for phospholipids (PL), triacylglycerols (TAG), saturated fatty acids (SAT), monounsaturated fatty acids (MUFA), eicosapentaeonic acid (EPA), docosahexaenoic acid (DHA) and other polyunsaturated fatty acids (other PUFA), and a weaker negative loading for offspring origin (Table 2.6, Figure 2.10). The highest loadings for principal component 2 (PC 2) were offspring origin, arachidonic acid (AA) and terrestrial fatty acids (terrestrial FA). For principal component 3 (PC 3) the highest loadings were for developmental stage and other lipids.

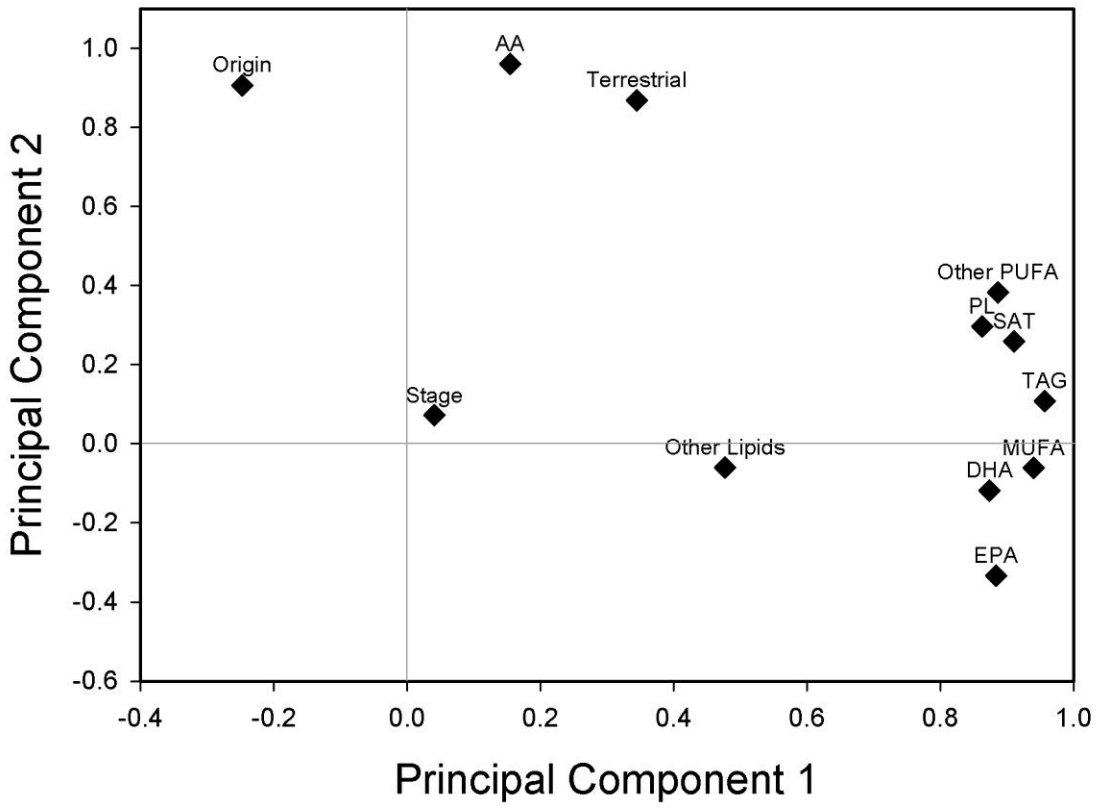
Table 2.5: Sums of squared loadings, percent of variance between offspring explained and cumulative percent for the first three principal components (Eigenvalues > 1) derived using Varimax rotation with Kaiser normalization to examine patterns in offspring origin, developmental stage and absolute amounts of selected lipid classes and fatty acids.

Principal Component	Rotation Sums of Squared Loadings		
	Eigenvalue	% of Variance	Cumulative %
1	6.141	51.177	51.177
2	2.952	24.604	75.781
3	1.443	12.022	87.803

Table 2.6: Rotated component matrix (Varimax with Kaiser normalization) for the first three components of the PCA to examine patterns in offspring origin, developmental stage and absolute amounts of selected lipid classes and fatty acids.

	Principal Component Scores		
	PC1	**PC2**	**PC3**
Origin	-0.247	**0.907**	0.005
Stage	0.041	0.072	**0.917**
Other Lipids	0.477	-0.060	**0.618**
PL	**0.863**	0.297	0.169
TAG	**0.957**	0.108	0.062
SAT	**0.911**	0.259	0.074
MUFA	**0.940**	-0.061	0.043
AA	0.155	**0.961**	0.043
EPA	**0.884**	-0.333	0.251
DHA	**0.874**	-0.118	0.326
Terrestrial FA	0.345	**0.869**	-0.017
Other PUFA	**0.887**	0.383	0.091

A.


Origin
AA
Terrestrial
Other PUFA
PL
SAT
TAG
Stage
Other Lipids
MUFA
DHA
EPA
Principal Component 2
Principal Component 1
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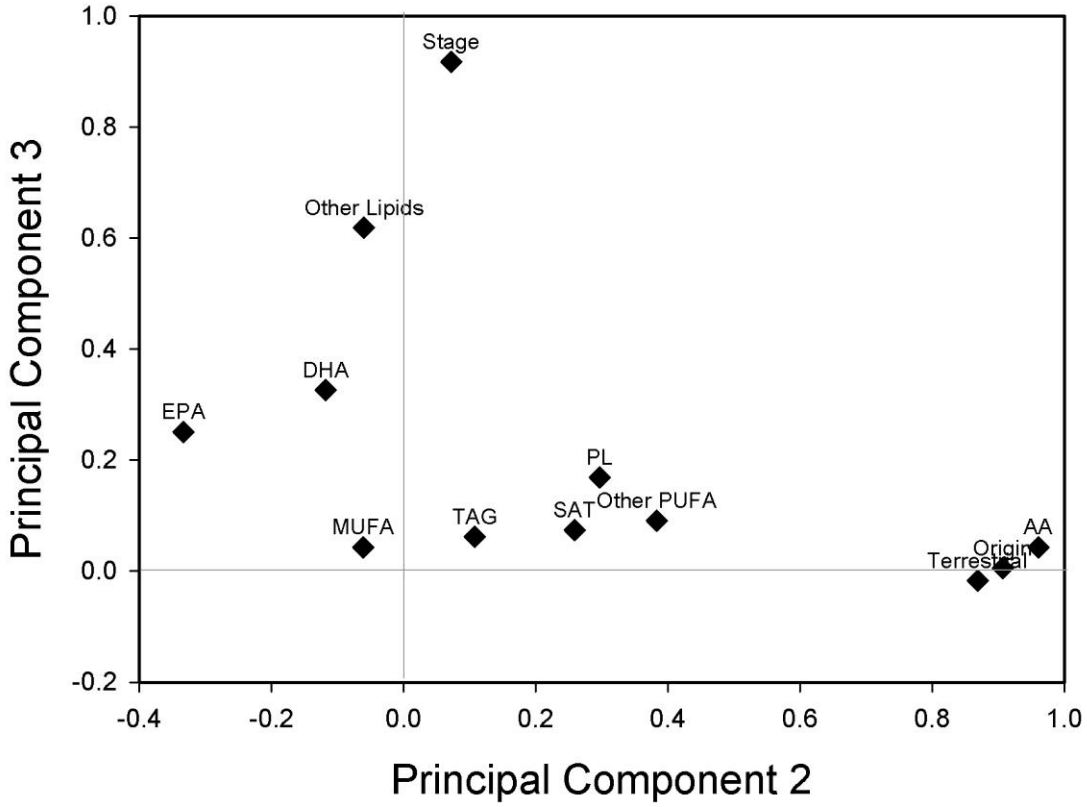
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0

B.


Principal Component 3
Principal Component 2
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0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
Stage
Other Lipids
DHA
EPA
PL
Other PUFA
SAT
TAG
MUFA
AA
Origin
Terrestrial

C.

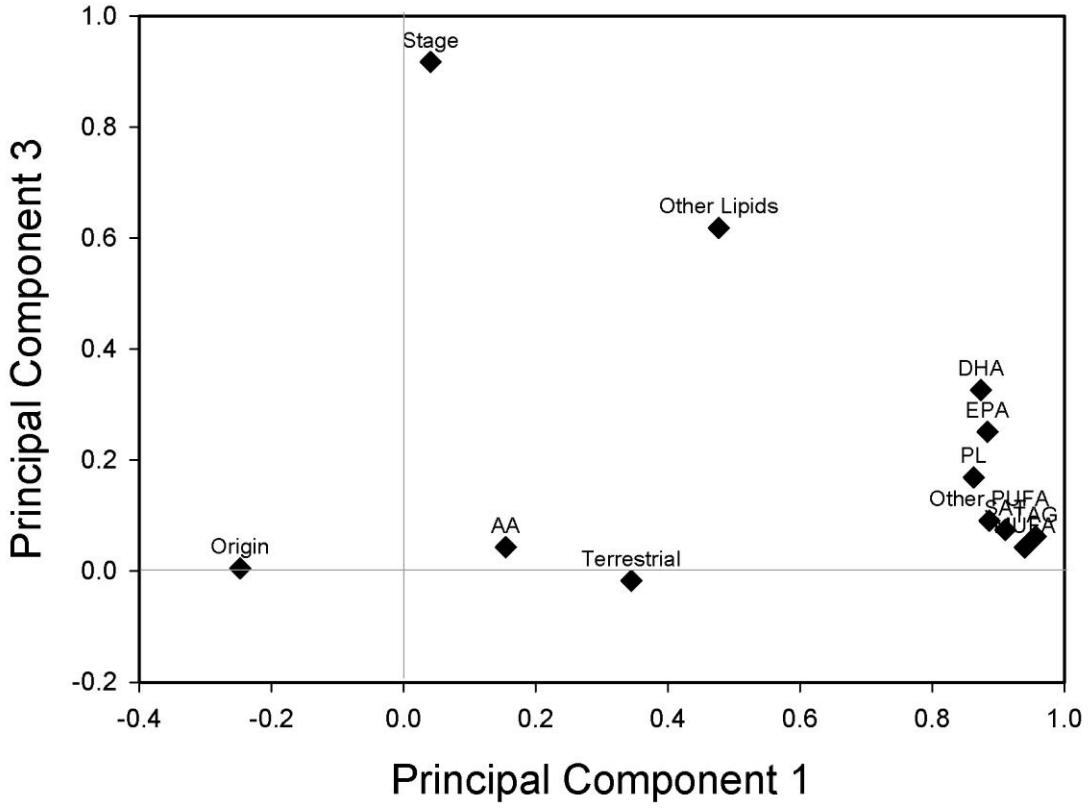

Figure 2.10: Two-factor plots of the rotated principal component data matrix of the absolute amount of selected lipid classes and fatty acids showing the loadings for: (A) the first two principal components, (B) principal components two and three and (C) the principal components one and three. Origin = female phenotype; Stage = one of four developmental stages (unfertilized egg, eyed egg, alevin, emerged fry); PL = phospholipids; Other Lipids = hydrocarbons, steryl esters/wax esters, methyl ketones, glyceryl ethers, triacylglycerols, free fatty acids, alcohols, sterols, diacylglycerols and acetone mobile polar lipids ; TAG = triacylglycerols; SAT = saturated fatty acids ; AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; MUFA = mono unsaturated fatty acids ; Terrestrial = signature terrestrial fatty acids ; Other PUFA = polyunsaturated fatty acids minus AA, DHA, EPA and Terrestrial.

2.3.3 Emergent measurements

The emergent fry of resident mothers were larger, having greater total surface areas (body plus yolk sac surface area; mean ± SD = 94.5 ± 8.8 mm^2) than those of anadromous mothers (84.3 ± 6.5 mm^2; nested ANOVA: $F_{1,10} = 5.95$, $P = 0.035$). They were also heavier (resident 206 ± 28 mg, anadromous 169 ± 18 mg; $F_{1,10} = 8.04$, $P = 0.018$) and tended to be longer (resident 27.1 ± 1.3 mm; anadromous 26.1 ± 0.7 mm^2), though not significantly so ($F_{1,10} = 4.14$, $P = 0.069$). There was a strong relationship between unfertilized egg mass and emergent fry mass (adjusted $r^2 = .941$, $P < 0.001$; fry mass = 0.748 (egg mass) – 9.11). In terms of the yolk sac area remaining at this developmental stage, there was a nonsignificant tendency for resident offspring to have both absolutely (resident 12.1 ± 2.7 mm^2; anadromous 9.5 ± 2.9 mm^2; nested ANOVA: $F_{1,10} = 3.11$, $P = 0.109$) and relatively larger yolk sacs than that of anadromous offspring, though not significantly so ($F_{1,10.1} = 3.87$, $P = 0.077$, covariate body area $F_{1,235.2} = 7.43$, $P = 0.007$; Figure 2.11). There was a significant relationship between the body area and yolk sac area for the anadromous offspring ($F_{1,118} = 24.90$, $P = 0.00$) but not for the resident offspring ($F_{1,118} = 0.380$, $P = 0.54$).

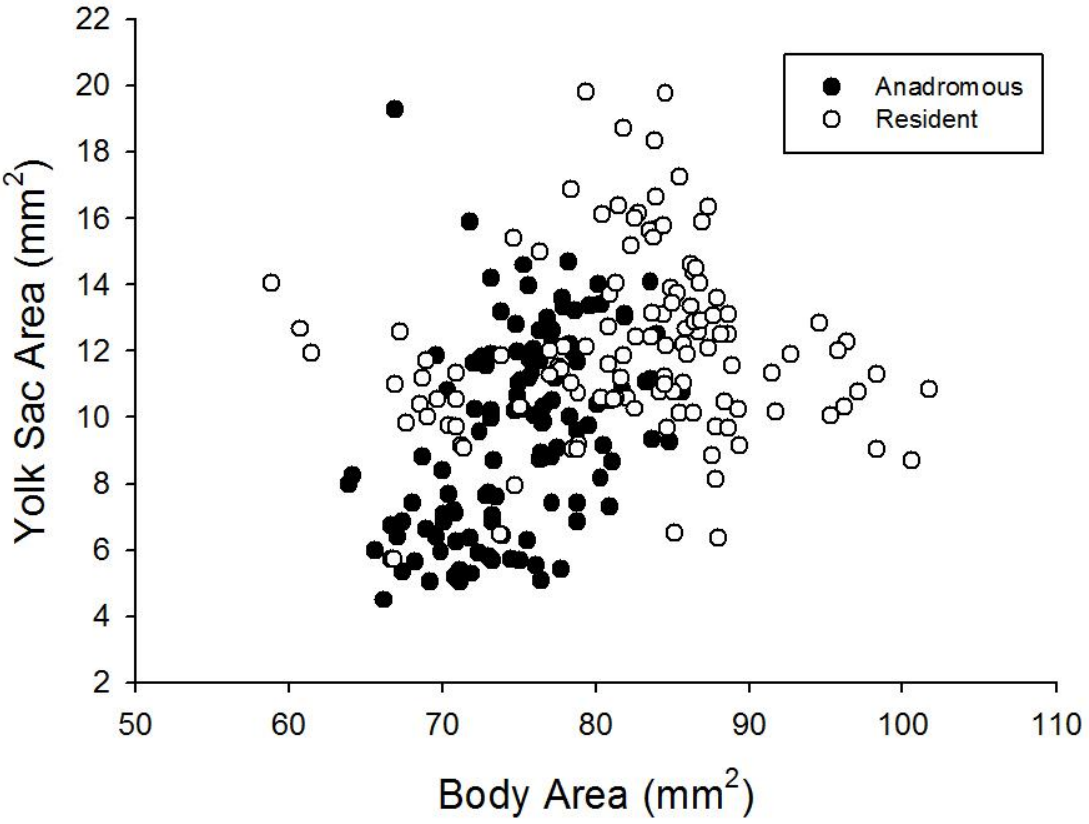

Figure 2.11: Relation between yolk sac and total body area at the emergent fry stage for offspring of anadromous and resident females.

2.3.4 Competition trials

The majority of the pairwise competition trials ended as a draw with neither individual declared as dominant (Figure 2.12). Statistical analysis confirmed this result among all pairs and showed no significant differences between the two strains of salmon ($p = 0.31$). There were no obvious signs of aggression noted (biting or chasing). Most of the results were based on position as the fry were not taking food regularly during observations even though fish had all been provided with food and were presumed to be feeding in the holding aquaria for approximately five days before the start of this experiment.

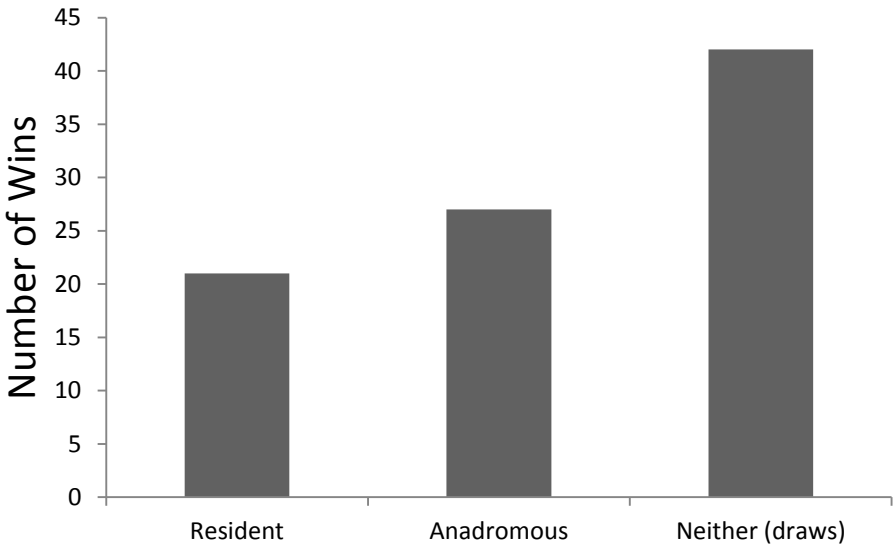

Figure 2.12: Number of wins for offspring of resident and anadromous females in pairwise competition trials, as well as the number of draws.

2.3.5 Growth and Survival

Results from the growth and survival experiment indicate that fish of both phenotypes lost mass over the course of the experiment; however, there was no significant difference in the growth rate between offspring origin, treatments or replicates (all interaction terms $P > 0.30$; reduced model: origin $F_{1,171} = 0.86$, $P = 0.354$, treatment $F_{1,171} = 0.57$, $P = 0.452$, replicate $F_{4,171} = 0.25$, $P = 0.911$). At the onset of the experiment, the offspring from resident mothers were significantly heavier than those of the anadromous mothers (all interaction terms $P > 0.20$; reduced model: origin $F_{1,173} = 13.71$, $P < 0.001$, treatment and replicate $P > 0.20$), but by the end of the experiment, 30 days later, there was no significant difference between the masses of the two strains (all interaction terms $P > 0.20$; reduced model: origin $F_{1,171} = 0.00$, $P = 0.966$, treatment and replicate $P > 0.40$; Table 2.7, Figure 2.13). There were only two mortalities during this experiment, one from each phenotype; an anadromous offspring from a pure treatment and a resident offspring from a mixed treatment.

Table 2.7: Mean ± SD initial and final offspring mass and growth rate from the growth and survival experiment broken down by treatment.

	Resident			Anadromous		
Treatment	Initial mass (g)	Final mass (g)	Growth Rate	Initial mass (g)	Final mass (g)	Growth Rate
Sympatric	0.185 ± 0.026 (n=30)	0.143 ± 0.023 (n=29)	- 0.008 ± 0.003	0.177 ± 0.021 (n=30)	0.138 ± 0.020 (n=30)	- 0.009 ± 0.003
Allopatric	0.187 ± 0.029 (n=60)	0.145 ± 0.023 (n=60)	- 0.009 ± 0.002	0.170 ± 0.020 (n=60)	0.147 ± 0.112 (n=59)	- 0.008 ± 0.002

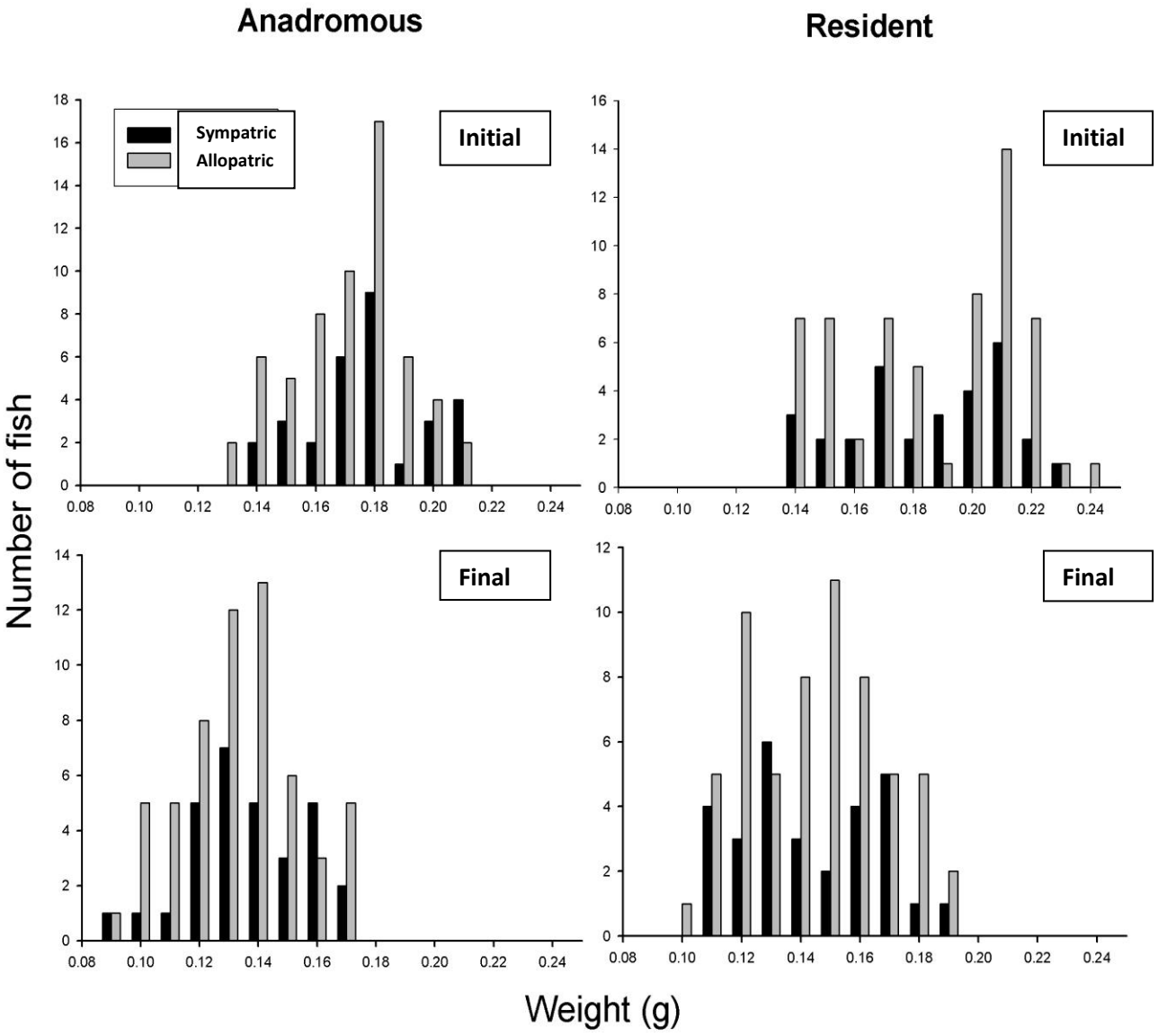

Figure 2.13: Initial and final mass (g) of offspring from anadromous and resident females by treatment (sympatric or allopatric) in the growth and survival experiment.

2.4 Discussion

Many of the coastal rivers in Newfoundland contain Atlantic salmon populations made up of both anadromous and resident individuals. It was the aim of this study to discover if maternal effects (specifically the lipid composition of the resources provided) differed between the two phenotypes and how it may affect offspring performance. Our findings indicate that there are clear differences in maternal allocation of resources to eggs by sympatric anadromous and resident female Atlantic salmon. This goes beyond any differences in egg size and includes differences in fatty acid profiles. Differences in arachidonic acid (AA), terrestrial fatty acids (linoleic and α-linolenic acid), eicosapentaenoic acid (EPA), and the ratios of AA to EPA, DHA to EPA and ω3 to ω6 fatty acids, in particular, distinguish fully the eggs and offspring (at least to the start of exogenous feeding) of the two phenotypes of females. Moreover, there was strong relation between initial egg size and the size of emergent fry (760 degree days post fertilization; $r^2 = 0.941$), such that the newly emerged offspring of resident females weighed significantly more than did those of anadromous females. The anadromous offspring, however, showed a nonsignificant trend to have proportionately (relative to body area) more yolk sac remaining at this developmental stage. Despite the differences in egg size and lipid profiles, they did not translate into differences in competitive behaviour, growth rates or mortality of the emergent offspring following the start of exogenous feeding.

As expected, there were many differences between phenotypes at every stage when it came to fatty acid composition, especially with regards to the ω3 and ω6 fatty acids. As expected the resident offspring had higher percentages of arachidonic acid (AA)(20:4ω6), linoleic acid (LA)(18:2ω6) and α-linolenic acid (LNA) (18:3ω3) as the natural prey of many freshwater fish (e.g. freshwater algae, crustacea, and aquatic larvae of insects) are generally, rich in these particular fatty acids although important season-dependent differences do occur (Wood 1974; Takahashi and Yamada 1976; Hanson *et al*. 1985; Wiegand 1996). Many freshwater fish are also capable of producing DHA (22:6ω3) and EPA (20:5ω3) from LNA, and AA from LA. When the long chain ω3 FA are in short supply, and LNA is either unavailable or inefficiently converted to DHA to supply tissue needs the body synthesizes a substitute from ω6 precursors. The replacement FA is docosopentaenoic acid (22:5ω6) (Mohrhauer and Holman 1963), which appears essential in the early life stages of fishes such as cod (*Gadus morhua*) (Parrish *et al*. 2007). This was one of the fatty acids which was consistently found to be in greater supply among the resident offspring. Some studies have suggested that some freshwater species (e.g. pike *Esox lucius* L.), may not be able to synthesize AA and EPA and that Atlantic salmon in particular are not able to produce AA from LA and must obtain it from their diet (Henderson et al. 1995; Ackman and Takeuchi 1986). Marine species generally are characterized by low levels of LA and LNA, as well as higher levels of long chain ω3 PUFAs predominantly EPA and DHA (22:6ω3) (Anderson *et al*. 1990; Wiegand 1996), which was the case for the anadromous offspring in this study as well. EPA and DHA are copious in the marine environment coming from diatoms and

flagellates respectively, where they are passed on from zooplankton to fish (Klenk and Eberhagen 1962; Hilditch and Williams 1964; Yamada and Hayashi 1975; Sargent *et al*. 1995). Clearly the essential fatty acids ingested by the mothers are being passed on to the offspring through maternal provisioning.

DHA, EPA, AA (and their associated ratios) are significantly correlated with egg quality, fertilization success, hatching success and larval development in many fish species such as cod, spotted wolfish and common snook (Pickova *et al*., 1997; Tveiten *et al*., 2004; Yanes-Roca *et al*., 2009). In particular, the rate and extent of production of these biologically active eicosanoids, which are involved in various stress reactions from blood clotting to inflammatory reactions, are determined by the ratio of AA:EPA which in turn is influenced by the dietary, or in this case the yolk sac ratio of ω6 :ω3 PUFA (Sargent *et al*. 1995). Ackman and Takeuchi (1986) suggested that low levels of AA in hatchery-reared Atlantic salmon smolts compared to wild fish may be the cause of various skin pathologies. The current study found the resident offspring to have significantly higher ratios of AA:EPA, which implies that these fish may be better able to withstand stress related issues than anadromous offspring.

For marine fish a DHA:EPA ratio of 2:1is generally accepted as adequate for normal growth and development with higher ratios being associated with high quality eggs and normal development(Sargent 1995, Sargent *et al*. 1999). For the anadromous offspring, the mean DHA:EPA ratios ranged from 2.1 ± 0.7 up to 2.4 ± 0.6 implying that these fish were developing normally while the mean ratios for the resident offspring

ranged from 2.9 ± 0.4 to 3.2 ± 1.1 were significantly higher and may imply a high quality of eggs. Marine fish naturally experience a much higher input of ω3 than ω6 PUFA, while freshwater fish tend to have lower levels of ω3 PUFA but higher levels of ω6 PUFAs, especially linoleic acid and AA (Steffens 1997; Sargent *et al*. 1995), which is the preferred substrate and produces eicosanoids of higher biological activity (Bell *et al*. 1994; Sargent *et al*. 1995). The ratio of ω3 to ω6 fatty acids was significantly higher in the fish originating from an anadromous mother, which was expected and is consistent with previous research. Interestingly, even though Atlantic salmon are not exclusively marine fish, due to maternal provisioning the eggs and newly emerged anadromous offspring clearly follow this pattern. This is useful data in attempting to distinguish phenotypes, a goal of this study. Given the total lack of overlap in the ratios of ω3:ω6 FA between offspring of the two phenotypes it would be relatively easy to determine from which phenotype of mother an individual derived.

There was generally little effect of developmental stage on lipid and fatty acid profiles which is quite different from what is generally seen in many other species of fish (Wiegand 1996). However, preferential retention of PUFA especially DHA and AA is very common throughout development as was evidenced in this study. These fatty acids are generally essential to membrane fluidity and possibly the nervous system as well which may explain why demand for these particular fatty acids increase during development (Cowey *et al*. 1985). Since most of the lipids in fertilized fish eggs are either converted into structural components, such as cell membranes, or are channeled into energy production, total lipid content of fertilized eggs tends to remain relatively

consistent until hatch (Heming and Buddington 1988). This is consistent with the findings of this study as well, as total lipids were not significantly different between stages for both phenotypes or between phenotypes.

Fish eggs must contain levels of nutrient that meet the energy and growth demand of the embryo and eventual fry through to, and during the onset of exogenous feeding. These nutrients must be provided by the mother. Triacylglycerols (TAG) are a key neutral lipid class in the diet of marine fish and are generally the predominant lipid class in the diet of freshwater fish. These lipids are a major long-term source of energy. Phospholipids (PL) are required for optimal growth and survival and play a central role in the structure of cell membrane bilayers in fishes. They are crucial for the prevention of skeletal deformities and possibly stress resistance in larval and early juvenile fish of both marine and freshwater species (Tocher *et al*. 2008). TAG was the predominant lipid in the unfertilized and eyed stages, with PL becoming the predominant lipid by the emergent fry stage (start of exogenous feeding). While there was no significant difference in TAG or PL between the two offspring origins, there was a significant difference in TAG among the developmental stages with amounts decreasing throughout the latter stages, which coincided with the depletion of the yolk sac/maternal resources. This is common, as it is generally accepted that neutral lipid- rich eggs, like Atlantic salmon eggs, utilize primarily TAG and also steryl and wax esters where present for growth and development (Tocher *et al*. 2008).

2.4.1 Egg and Offspring Size at Emergence

Contrary to other findings (Wood and Foote 1996, Fleming 1998), the resident eggs here were larger than the anadromous eggs and therefore the resident offspring were significantly larger from the outset. This is an interesting finding as the resident mothers were smaller than the anadromous mothers and most research indicates that larger fish within a species have larger eggs (Roff 1992; Einum and Fleming 2002). It is possible that since their offspring must compete directly with offspring of anadromous mothers the resident mothers are trading egg quantity for egg quality and supplying their eggs with more lipids and laying fewer eggs. Research indicates that resident mothers do in fact lay fewer eggs than their anadromous counterparts in a population (Klemetsen *et al*. 2003), however this is not surprising as resident salmon are generally smaller than anadromous salmon.

Size at emergence, as well as size of energy reserves have important fitness consequences for salmonids (Einum and Fleming 2000; Rollinson and Hutchings 2013). Upon emergence, offspring rely on the remainder of their yolk sac while they become accustomed to exogenous feeding. Size of yolk sac remaining may affect swim ability and allow individuals with larger sacs more time to adapt, establish territories and make the switch to active feeding (Dill 1977). Both the emergent anadromous offspring and the resident offspring were sampled at the same age and both types were free swimming, however the fry of the resident females were significantly larger and also showed a non-significant tendency to have larger yolk sacs. This fits in well with previous research as generally, larger larvae have larger yolk reserves, which provide them with prolonged

food reserves before the need for exogenous feeding (Rideout *et al*. 2005). All offspring were compared to their paternal half sibs before exogenous feeding began so any differences were attributed to maternal effects.

Most mortality during the first year of life takes place between the time of emergence from the gravel and the founding of territories, when swimming abilities have improved (Ottaway and Clarke 1981; Einum and Fleming 2000a). Upon emergence, fry are highly vulnerable to predation and therefore stand a better chance of survival by emerging synchronously. Nonetheless those that emerge first have preferential access to feeding territories. However, there is also a risk that by emerging too early they may be ahead of the food availability. Here, the resident offspring are at a definite advantage by being able to rely on their endogenous resources longer while learning how to feed from outside sources. The anadromous offspring must switch to exogenous feeding sooner or risk starvation. Rottiers (1993) found that landlocked Atlantic salmon juveniles had higher lipid content than those of anadromous strains when fed an identical diet, which indicates that the landlocked salmon may have a higher capacity to use dietary lipids reflecting an adaptation to freshwater where the food chain is typically poorer in lipids than in a marine environment (Pickova *et al*. 1999). Obviously both phenotypes have adaptations to freshwater as juveniles but they may be using the lipids differently as a result of maternal provisioning. The resident offspring may be using their resources more efficiently and therefore have more yolk sac remaining upon emergence. Again this may be a case of quantity over quality, more resources supplied by the mothers so that the resident offspring are able to compete with anadromous conspecifics. Resident offspring

may also be genetically predisposed to using freshwater lipids more efficiently as their mothers have spent their entire lives in a freshwater environment. Alternatively, it is possible since the emergent fish did not actually emerge from gravel on their own but rather were estimated to have emerged based on observations (i.e. they were free swimming and had 'buttoned up'), that the resident offspring would not have actually left the gravel yet (e.g. resident offspring have later emergent times) and would have had later access to territories. This may explain why their yolk sacs were larger upon sampling.

2.4.2 Competition Trials

This particular experiment suggests that there is no discernible difference between phenotypes at this stage when it comes to dominance status. The idea that larger size and dominance go hand in hand has been well documented among salmonids (Newman 1956; Jenkins 1969; Wankowski and Thorpe 1979; Abbot *et al*. 1985), but Huntingford *et al*. (1990) found that in Atlantic salmon size was likely a consequence of social status and not a cause. Metcalfe *et al*. (1995) also found that the standard metabolic rate was a better indicator of social status than relative size or mass of Atlantic salmon. When they corrected for metabolic rate, size had no effect on the outcome of the competitive encounters. In this study, even though the resident offspring were significantly larger there was no difference in the competitive abilities of the two phenotypes under these experimental conditions.

There were few if any outwardly aggressive acts and hardly any competition over food acquisition in these trials. Since resident offspring still had endogenous resources they may not have been a threat to the anadromous fry and hence no direct competition

was observed. The absence of marked aggression may also be due to kin discrimination or low genetic diversity as pairs tested were paternal half siblings. Brown and Brown (1992) demonstrated that Atlantic salmon can discriminate kin from non-kin and subsequently, reported that kin discrimination abilities allow individuals to reduce the levels of aggression associated with territorial defence towards related conspecifics and to defend smaller territories near kin versus non-kin (Brown and Brown 1996).

This study also agrees with previous research which suggests that bigger does not always mean more dominant (Huntingford *et al*. 1990; Metcalfe *et al*. 1995). The resident offspring were larger and had slightly larger remaining yolk sacs, but were clearly not more dominant. It appears that neither resident nor anadromous mothers have more dominant offspring upon emergence under the conditions tested.

2.4.3 Growth and Survival

Competitive interactions for food are an important source of growth rate variation since they result in dominant individuals consuming a disproportionate quantity of food and growing disproportionately fast compared to less aggressive fish. Even though the resident offspring were statistically larger at the onset of the experiment there was no significant difference in growth rates or end mass between the two phenotypes. All individuals lost mass during this experiment which was not unexpected. The decrease in specific growth rate observed may have been influenced by an initial period of acclimation to both unfamiliar food items (Wang and White, 1994) and environment.

These newly emerged offspring were living off the remains of their yolk sacs while learning how to feed in an environment which was purposely designed to be food limited and generate competition.

Rottiers (1993) reported a higher growth rate in landlocked Atlantic salmon juveniles than in anadromous salmon of same age. However that study was done on fish older than those studied here and therefore may have already established their particular life history phenotypes. Those populations studied were also from two distinct populations unlike those of this current study which were likely from one population exhibiting alternative phenotypes (Adams, 2007). Peng *et al*. (2003) found that landlocked offspring had a noticeably higher body mass than the anadromous fry as well as a higher growth rate, although the two strains tested were also from different populations. The current investigation did not find a perceptible difference in the mean growth rates of the offspring of the two phenotypes, however according to Elliot and Hurley (1997) salmon obtained from populations from a narrow geographical area had no intraspecific differences when it came to growth rates. It is possible however, that any differences in behaviour were not apparent under the conditions tested and different conditions may produce different results.

According to this study, offspring of the two alternate origins differ in lipid profiles, egg size and size at emergence with the resident offspring seemingly at a short term advantage upon emergence due to their overall larger size and quantity of endogenous resources, however there are many variables that affect growth, fitness and

survival and anadromous offspring may have an advantage early on by having access to better quality resources through maternal provisioning. Only a couple of specific behaviours that may have been influenced by maternal effects were investigated in this short term study. Further study is needed to shed light on the interactions of the two phenotypes after endogenous resources have been completely depleted and territories have been established.

Chapter 3: Summary and Conclusions

3.1 Summary and Conclusions

To the best of my knowledge, this study is the first in-depth look at the differences in the lipid content and composition of various early life history stages for wild Atlantic salmon and offers a practical solution to the task of discovering the contribution made to the population by both phenotypes. The results indicate that there are sufficient differences between specific fatty acids of the offspring of anadromous mothers and resident mothers to create distinct fatty acid profiles and is therefore a realistic way to identify offspring enabling researchers to determine the contributions resident and anadromous females make to a population. This information would improve fisheries management and policies by allowing a more thorough understanding of the population structure of the Atlantic salmon in a given region since anadromous salmon are a popular game fish and governed by much tighter fishing regulations than the resident fish. Fishing pressure on anadromous phenotypes may cause decreased competition for the resident phenotype thereby possibly allowing them to become the predominant presence in the river systems. Also since the anadromous fish are more likely to be harvested it is possible that the evolutionary threshold for adopting one tactic over another could shift promoting rapid life history evolution, changing the population dynamics of a river over time and possibly threaten sustainability of fisheries yields. As adoption of a life history tactic is likely to be a conditional strategy with unequal fitness for both tactics, there may be ramifications of a shift in population structure as the anadromous phenotype declines (Gross 1996; Brockman 2001).

Even though resources provided by the two types of mothers were different with regards to lipid composition and content this did not appear to have an impact on the behaviour of the offspring, at least at the developmental stages, and under the conditions investigated. While the anadromous offspring had more ω3 FA than the resident offspring, the resident offspring make up for this deficiency by converting other FA into the required ω3 FA. This seems to be a successful strategy for the resident offspring as they did not appear to be any less fit than the anadromous offspring and were able to hold their own in the dominance competitions.

An anadromous parent is likely capable of producing a resident offspring and vice versa. Therefore the purpose of this study was not to predict life history choice based upon maternal provisioning but rather to investigate if there were differences in maternal provisioning for the two types and if those dissimilarities would produce differences in early behaviour that may ultimately affect the fitness associated with the two tactics. While there was a distinct difference in the fatty acid profiles of the resources provided to offspring there also seemed to be a difference in the method of allocation. According to this study the anadromous mothers may opt for the highest quality of egg they can achieve in their environments while the resident mothers seem to provide more resources and hence have larger eggs. However results suggest that behavioural differences in early life performance and interactions between the offspring of the anadromous mothers and the offspring of the resident mothers are minimal at best under these experimental conditions and may not directly affect the coexistence of the two strains. This makes sense as the two phenotypes have evolved to spawn within the same environment and

even though quality and quantity of resources differed for the offspring their competitive abilities seem to be evenly matched. While there does appear to be a link between egg/offspring size and increased fitness (Einum and Fleming 1999, 2000), studies have stressed the idea that fitness consequences of egg size differences are more pronounced during early juvenile stages rather than the egg or larval stage (Rollinson and Hutchings 2011; Fleming and Einum 2011; Louhi *et al*. 2014). So even though the resident eggs and offspring were larger, behavioural differences may not be apparent until offspring are older however, as both types of offspring have been coexisting in the same population it is likely that their competitive abilities would be similar. This may be due in part to the resources provided by their mothers. The anadromous offspring had more long chain fatty acids (DHA, EPA) while the resident offspring were supplied with less of the long chain fatty acids but more of ω6 fatty acids. This seemingly allows both types of offspring to coexist and perform similarly within a population.

The success of newly emerged offspring establishing territories depends on time of emergence, metabolic rate and body size (Cutts *et al*. 1999a, b). Higher metabolic rates seemingly cause a greater quantity of the yolk reserves to be used in respiration over a set amount of time leading to an earlier requirement for exogenous food (Metcalfe *et al*. 1995). In this study anadromous offspring appeared to have less yolk sac reserves than the resident fry upon emergence and it may be that they have a higher metabolic rate. A higher metabolic rate has been hypothesized to coincide with a larger otolith at first feeding, faster digestion, a higher dominance status, increased growth rates and an increased likelihood of early seaward migration (Metcalfe *et al*. 1992; McCarthy 2000;

Millidine *et al*. 2009; Reid *et al*. 2011). Again these characteristics may not be apparent until offspring were older.

This study opens up several avenues for future research including: 1) presumably both types of offspring would be ingesting the same diet as they occupy the same habitat and it would be interesting to see how long the offspring of the two phenotypes retain their different lipid profiles. 2) If the anadromous offspring use up their resources quicker than resident offspring this interestingly may suggest a higher metabolic rate that may lead to a higher growth rate in the future. Metabolic rates of both types should be measured and compared. 3) Overall lipid profiles were unique to each type and it would be interesting to discover if in fact any of the resident offspring became anadromous upon maturity and how their lipid profiles would change. 4) In aquaculture, marine fish must be fed an enriched diet to increase their DHA intake and thereby provide a higher DHA:EPA ratio necessary for normal growth and development and for pigmentation. If resident (or fish from a partial migratory population) rather than strictly anadromous Atlantic salmon were used for aquaculture, diet supplementation with DHA might not be necessary as freshwater fish may have the ability to make their own DHA and utilize dietary lipids more efficiently. 5) Offspring of anadromous and resident females could be reared until smoltification occurs in some and the maternity of both phenotypes could then be determined.

References

Abbott, J. C. and Dill, M. 1989. The relative growth of dominant and subordinate juvenile steelhead trout (*Salmo gairdneri*) fed equal rations. *Behaviour*. 108:104-1 13.

Ackman, R. G. 1994. Animal and marine lipids. In B. S. Kamel and Y. Kakuda (eds.), Technological advances in improved and alternate sources of lipids. Blackie Academic and Professional, Chapman and Hall. 292-328.

Ackman, R. G., and Takeuchi, T. 1986. Comparison of fatty acids and lipids of smolting hatchery-fed and wild Atlantic salmon *Salmo salar*. *Lipids*. 21(2):117-120.

Adams, Blair K. 2007. Migratory strategies of Atlantic salmon (*Salmo salar*) of Newfoundland and Labrador. *PhD Thesis*. Dalhousie University. Halifax, Canada.

Adams, Colin E. and Huntingford, F.A. 1996. What is a successful fish? Determinants of competitive success in Arctic char (*Salvelinus alpinus*) in different social contexts. *Canadian Journal of Fisheries and Aquatic Sciences*. 53:2446-2450.

Anderson, A. J., Arthington, A. H. and Anderson, S. 1990. Lipid classes and fatty acid composition of the eggs of some Australian fish. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry (United Kingdom*). 96:267-270.

Aubin-Horth, N., Letcher, B. H., & Hofmann, H. A. 2005. Interaction of rearing environment and reproductive tactic on gene expression profiles in Atlantic salmon. *Journal of Heredity*. 96:261-278.

Bell, J. G., Ghioni, C. and Sargent, J. R. 1994. Fatty acid compositions of ten freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*); a comparison with commercial diets. *Aquaculture*. 128:301-313.

Berg, O. K., Hendry, A. P., Svendsen, B., Bech, C., Arnekleiv, J. V. and Lohrmann, A. 2001. Maternal provisioning of offspring and the use of those resources during ontogeny: variation within and between Atlantic salmon families. *Functional Ecology*. 15:13-23.

Berglund, I., Schmitz, M. and Lundqvist, H. 1992. Seawater adaptability in Baltic salmon (*Salmo salar*) : a bimodal smoltification pattern in previously mature males. *Canadian Journal of Fisheries and Aquatic Sciences*. 49:1097-1106.

Bernardo, J. 1996. Maternal effects in animal ecology. *American. Zoology*. 36:83-105.

Bohlin, T., Dellefors, C. and Faremo, U. 1996. Date of smolt migration depends upon body size but not age in wild sea-run brown trout. *Journal of Fish Biology*. 49:157-164.

Brown, G. E., Brown, J. A. 1992. Do rainbow trout and Atlantic salmon discriminate kin? *Canadian Journal of Zoology*. 70:1636-1640.

Brown, G. E. and J. A. Brown. 1996. Kin discrimination in salmonids. *Reviews in Fish Biology and Fisheries*. 6:201-219.

Burton, M. P. and Idler, D. R. 1984. Can Newfoundland land-locked salmon Salmo salar L., adapt to sea water? *Journal of Fish Biology*. 24:59-64.

Carrillo, M., Zanuy, S., Prat, F., Cerdá, J.L., Ramos, J., Mañanós E. and Bromage, N. 1995. Sea bass (Dicentrarchus labrax). In: N.R. Bromage and R.J. Roberts, Editors, *Broodstock Management and Egg and Larval Quality*. Blackwell Science, Oxford. 138-168.

Chambers, R.C. and Waiwood, K.G. 1996. Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua. Canadian Journal of Fisheries and Aquatic Sciences.* 53:1986–2003.

Chapman, Ben B., Bronmark, C., Nilsson, Jan-Ake and Hansson, Lars-Anders. 2011. The ecology and evolution of partial migration. *Oikos*. 120:1764-1775.

Charles, K., Roussel, J. M. and Cunjak, R. A. 2004. Estimating the contribution of sympatric anadromous and freshwater resident brown trout to juvenile production. *Marine and Freshwater Research*. 55:185-191.

Committee on the Status of Endangered Wildlife in Canada [COSEWIC]. 2010. COSEWIC assessment and status report on the Atlantic salmon, *Salmo salar* (anadromous form) in Canada. Committee in the Status of Endangered Wildlife in Canada, Ottawa, Canada.

Cowey, C. B., Bell, J. G., Knox, D., Fraser, A., and Youngson, A. 1985. Lipids and lipid antioxidant systems in developing eggs of salmon (*Salmo salar*). *Lipids*. 20(9):567-572.

Curry, R. A. 2005. Assessing the reproductive contributions of sympatric anadromous and freshwater-resident brook trout. *Journal of Fish Biology*. 66:741-757.

Cutts, C. J., Brembs, B., Metcalfe, N. B. and Taylor, A. C. 1999a. Prior residence, territory quality and life history strategies in juvenile Atlantic salmon (*Salmo salar*). *Journal of Fish Biology*. 55:784-794.

Cutts, C. J., Metcalfe, N. B. and Taylor, A. C. 1999b. Competitive asymmetries in territorial juvenile Atlantic salmon, *Salmo salar*. *Oikos*. 86:479-486.

Dawkins, R. 1980. Good strategy or evolutionarily stable strategy? *Sociobiology: Beyond Nature/Nurture* (Barlow, G.W. and Silverberg, J., eds), Westview. 331–367.

Dill, P. A. 1977. Development of behaviour in alevins of Atlantic salmon, *Salmo salar*, and rainbow trout, *S. gairdneri*. *Animal Behaviour*. 25:116-121.

Dodson, J. J., Aubin-Horth, N., Thériault, V. and Páez, D. J. 2013.. . The evolutionary ecology of alternative migratory tactics in salmonid fishes. *Biological Reviews*. 88:602-625

Eek, D. and Bohlin, T. 1997. Strontium in scales verifies that sympatric sea-run and stream-resident brown trout can be distinguished by coloration. *Journal of Fish Biology*. 51:659-661.

Einum, S. and Fleming, I. A. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proceedings of the Royal Society B: Biological Sciences*. 266:2095-2100.

Einum, S. and Fleming, I. A. 2000*a*. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution*. 54:628-639.

Einum, S. and Fleming, I.A. 2000*b*. Highly fecund mothers sacrifice offspring survival to maximize fitness. *Nature*. 405: 565-567.

Einum, S. and Fleming, I.A. 2002. Does within-population variation in fish egg size reflect maternal influences on optimal values? *American Naturalist* 160:756-765.

Einum, S. and Fleming, I.A. 2004. Environmental unpredictability: conservative vs. diversified bet-hedging. *Evolutionary Ecology Research*. 6:443-455.

Fausch, K. D. 1984. Profitable stream positions for salmonids: Relating specific growth rate to net energy gain. *Canadian Journal of Zoology*. 62:441-451.

Fleming, I. A. 1996. Reproductive strategies of Atlantic salmon: ecology and evolution. *Reviews in Fish Biology and Fisheries*. 6:397-416.

Fleming, I. A. 1998. Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Canadian Journal of Fisheries and Aquatic Sciences*. 55:59-76.

Fleming, I. A. and Reynolds, J. D. 2004. Salmonid breeding systems. In Evolution illuminated: salmon and their relatives. A. P. Hendry and S. C. Stearns (Eds.), pp. 264–294. Oxford University Press.

Fleming, I.A. and Einum, S. 2011. Reproductive Ecology: a tale of two sexes, pp 33-65. In: Ø. Aas, S. Einum, A. Klemetsen and J. Skurdal (eds.) Atlantic salmon ecology. Wiley-Blackwell, Oxford, UK.

Folch J., Lees, M. and Sloane-Stanley G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*. 226: 497-509.

Fox, C. W. 1994. Maternal and genetic influences on egg size and larval performance in a seed beetle (*Callosobruchus maculates*): multigenerational transmission of a maternal effect? *Heredity*. 73:509-517.

Fox, C. W., Czesak, M. E., Mousseau, T. A. and Rott, D. A. 1999. The evolutionary genetics of an adaptive maternal effect: egg size plasticity in a seed beetle. *Evolution*. 53:552-560.

Gonzalez-Baro, M.,and Pollero, R. J. 1988. Lipid characterization and distribution among tissues of the freshwater crustacean *Macrobrachium borelli* during an annual cycle. *Comparative Biochemistry and Physiology and Physiology B-Biochemestry and Molecular Biology*. 91:711-715.

Green, B.S. 2008. Chapter 1 Maternal effects in fish populations. *Advances in Marine Biology*. 54:1-105.

Grant, J. W. A. and Kramer, D. L. 1990. Territory size as a predictor of the upper limit to population density of juvenile salmonids in streams. *Canadian Journal of Fisheries and Aquatic Sciences*. 47:1724-1737.

Groothuis, T. G. G., and H. Schwabl. 2008. Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them? *Philosophical Transactions of the Royal Society B: Biological Sciences*. 363:1647-1661.

Gross, M. R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology and Evolution*. 11:92-98.

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

Hazel, J. R. and Williams, E. E. 1990. The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Progress in Lipid Research*. 29:167-227.

Heath, D. D. and Blouw, D. M. 1998. Maternal effects in fish: are they adaptive or merely physiological side-effects? In: *Adaptive maternal effects*. T. A. Mousseau and C. W. Fox (Eds.) Pp. 178-201. Oxford University Press, Oxford.

Heming, T. A. and Buddington, R .K. 1988. Yolk absorption in embryonic and larval fishes. In *Fish Physiology, Vol. XI, The Physiology of Developing Fish, Part A, Eggs and Larvae.* W. S. Hoar and D. J. Randall (Eds.), pp. 407-446. San Diego: Academic Press.

Henderson, R.J., Park, M.T. and Sargent, J.R. 1995. The desaturation and elongation of ^{14}C-labelled polyunsaturated fatty acids by pike (*Esox lucius L.) in vivo. Fish Physiology and Biochemistry*. 14:223-235.

Hendry, A. P. and Stearns, S. C. 2004. Evolution illuminated: salmon and their relatives. A. P. Hendry and S. C. Stearns (Eds.), pp. 264–294. Oxford University Press, Oxford.

Higashitani, T., Takatsu, T., Nakaya, M., Joh, M., and Takahashi, T. 2007. Maternal effects and larval survival of marbled sole (*Pseudopleuronectes yokohamae*). *Journal of Sea Research*. 58(1):78-89.

Hilditch, T. P. and Williams, P. N. 1964. Chemical Constitution of Natural Fats, 4th ed., p. 207. London: Chapman and Hall Ltd.

Howland, K. L., Tonn, W. M., Babaluk, J. A. and Tallman, R. F. 2001. Identification of freshwater and anadromous inconnu in the Mackenzie River system by analysis of otolith strontium. *Transactions of the American Fisheries Society*. 130:725-741.

Huntingford, F.A., Metcalfe, N. B., Thorpe, J. E., Graham, W. D. and Adams, C. E. 1990. Social dominance and body size in Atlantic salmon parr, *Salmo salar L*. *Journal of Fish Biology*. 36:877–881.

Hutchings, J.A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout, *Salvelinus fontinalis*. *Evolution*. 45:1162-1168.

Hutchings, J.A. 2002. Sustaining Atlantic salmon in the Northwest Atlantic: considerations from a life history perspective. *In:* K.D. Lynch, M.L. Jones, and W.W. Taylor [eds], *Sustaining North American Salmon: Perspectives Across Regions and Disciplines*, American Fisheries Society, Bethesda, MD, Pp. 33-60

Hutchings, J.A. and Jones, M.E.B. 1998. Life history variation and growth rate thresholds for maturity in Atlantic salmon, *Salmo salar*. Canadian Journal of Fisheries and Aquatic Sciences. 55: 22-47.

Hutchings, J.A. 2011. Old wine in new bottles: reaction norms in salmonid fishes. *Heredity* 106: 421-437

Jenkins, T. M. Jr. 1969. Social structure, position choice and micro-distribution of two trout species (*Salmo trutta* and *Salmo gairdneri*) resident in mountain streams. *Animal Behaviour Monographs*. 2:56-123.

Jonsson, B. and N. Jonsson. 2011. Ecology of Atlantic salmon and brown trout. Springer, Dordrecht. *The Netherland*. 708.

Johnsson, J. I., Petersson, E., Jönsson, E., Björnsson, B. T. and Järvi, T. 1996. Domestication and growth hormone alter antipredator behaviour and growth patterns in juvenile brown trout, *Salmo trutta*. *Canadian Journal of Fisheries and Aquatic Sciences*. 53:1546-1554.

Kakela R. and Hyvarinen, H. 1998. Composition of polyunsaturated fatty acids in the liver of freshwater and marine ringed seals (*Phoca hispida* ssp.) differs largely due to the

diet of the seals. *Comparative Biochemistry and Physiology and Physiology B-Biochemestry and Molecular Biology*. 120:231-237.

Keeley, E. R. & Grant, J. W. A. 1995. Allometric and environmental correlates of territory size in juvenile Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*. 52:186-196.

Kerr, Lisa A., Secor, David H. and Piccoli, Philip M. 2009. Partial migration of fishes as exemplified by the estuarine-dependent white perch. *American Fisheries Society*. 34:114-123.

Kirkpatrick, M. and Lande, R. 1989. The evolution of maternal characters. *Evolution*. 43:485-503.

Kjesbu, O.S., Solemdal, P., Bratland, P. and Fonn, M. 1996. Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences.* 53:610–620

Klemetsen, A., Amundsen, P.A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F. and Mortensen, E. 2003. Atlantic salmon, *Salmo salar*(L.), brown trout, *Salmo trutta* (L.) and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish*. 12:1-59.

Klenk, E., and Eberhagen, D. 1962. Über die ungesättigten C16 Fettsäuren des Meeresplanktions und das Vorkommen der Delta 6,9,12,15-Hexadecateraensäure. *Hoppe-Seylers Zeitschrift für physiologische Chemie* . 328:189-197.

Lacy E.P. What is an adaptive environmentally induced parental effect? In Maternal effects as adaptations, Mousseau, T. and Fox, C. 1998. pp. 54-66. Eds. Oxford, UK:Oxford University Press.

Letcher, B. H. and Gries, G. 2003. Effects of life history variation on size and growth in stream-dwelling Atlantic salmon. *Journal of Fish Biology*. 62: 97-114.

Lindstrom, J. 1999. Early development and fitness in birds and mammals. *Trends in Ecology and Evolution*. 14:343-348.

Linko, R.R., Rajasilta, M. and Hiltunen, R. 1992. Comparison of lipid and fatty acid composition in vendace (*Coregonus albula* L.) and available plankton feed. *Comparative Biochemistry and Physiology*. 103A: 205–212.

Louhi, P., Robertson, G., Fleming, I.A. and Einum, S. 2014. Can timing of spawning explain the increase in egg size with female size in salmonid fish? *Ecology of Freshwater Fish* (doi: 10.1111/eff.121210)

Martin-Smith, K. M., Armstrong, J. D., Johnsson, J. I. and Bjornsson, B. Th. 2004. Growth hormone increases growth and dominance of wild juvenile Atlantic salmon without affecting space use. *Journal of Fish Biology*. 65:156-172.

McCarthy ID. 2000. Temporal repeatability of relative standard metabolic rate in juvenile Atlantic salmon and its relation to life history variation. *Journal of Fish Biology*. 57:224 - 238.

McCure, B. and Grace, J.B. 2002. Chapter 14: Principle Component Analysis. In: *Analysis of Ecological Communities*. MJM Press pp: 114-121

McDowall, R. M. 1992. Particular problems for the conservation of diadromous fish. *Aquatic Conservation: Marine and Freshwater Ecosystems*. 2:351-355.

McDowall, R.M. 2002. Accumulating evidence for a dispersal biogeography of southern cool temperate freshwater fishes. *Journal of Biogeography*. 29:207-220.

McKenzie, D. J., Higgs, D. A., Dosanjh, B. S., Deacon, G. and Randall, D. J. 1998. Dietary fatty acid composition influences swimming performance in Atlantic salmon (*Salmo salar*) in seawater. *Fish Physiology and Biochemistry*. 19:111-122

Metcalfe, N. B. 1986. Intraspecific variation in competitive ability and food intake in salmonids: Consequences for energy budgets and growth rates. *Journal of Fish Biology*. 28:525-531.

Metcalfe, N.B., Huntingford, F. A., Graham, W. D., and Thorpe, J. E. 1989. Early social status and the development of life-history strategies in Atlantic salmon. *Proceedings of the Royal Society of London Services B: Biological Sciences*. 236: 7–19.

Metcalfe, N. B., Wright, P. J. and Thorpe, J. E. 1992. Relationships between social status, otolith size at first feeding and subsequent growth in Atlantic salmon (*Salmo salar*). *Journal of Animal Behaviour*. 61:585-589.

Metcalfe, N. B., Taylor, A. C. and Thorpe, J. E. 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. *Animal Behaviour*. 49:431-436.

Metcalfe, N. B., Valdimarsson, S. K. and Morgan, I. J. 2003. The relative roles of domestication, rearing environment, prior residence and body size in deciding territorial contests between hatchery and wild juvenile salmon. *Journal of Applied Ecology*. 40:535-544.

Millidine KJ, Armstrong JD and Metcalfe NB. 2009. Juvenile salmon with high standard metabolic rates have higher energy costs but can process meals faster. *Proceedings of the Royal Society of London Services B: Biological Sciences*. 276:2103 - 2108.

Mohrhauer, M. and Holman, R.T. 1963. The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. *Journal of Lipid Research*. 4:151-159.

Moreau, D.T.R., Fleming, I.A., Fletcher, G.L. and Brown, J.A. 2011. Growth hormone transgenesis does not influence territorial dominance or growth and survival of first-feeding Atlantic salmon *Salmo salar* in food-limited stream microcosms. *Journal of Fish Biology* 78:726-740.

Morley, S. A., Batty, R. S., Geffen, A. J., and Tytler, P. 1999. Egg size manipulation: a technique for investigating maternal effects on the hatching characteristics of herring. *Journal of Fish Biology*. 55(sA):233-238.

Mousseau, T. A. and Dingle, H. 1991. Maternal effects in insect life histories. *Annual Review of Entomology*. 36:511-534.

Mousseau, T. A. and Fox, C. W. 1998. The adaptive significance of maternal effects. *Trends in Ecology and Evolution*. 13:403-407.

Nakano, S. 1995. Individual differences in resource use, growth and emigration under the influence of a dominance hierarchy in fluvial red-spotted masu salmon in a natural habitat. *Journal of Animal Ecology*. 64:75–84.

Newman, M. A. 1956. Social behavior and interspecific competition in two trout species. *Physiological Zoology*. 29:64–81.

Nielsen, J. 1992. Microhabitat-specific foraging behavior, diet, and growth of juvenile coho salmon. *Transactions of the American Fisheries Society*. 121:617–634.

Nordeng, H., Bratland, P.and Skurdal, J. 1989. Pattern of smolt transformation in the resident fraction of anadromous Arctic charr *Salvelinus alpinus* genetic and environmental influence. *Physiology and Ecology Japan*. 1 (spl.):483–488.

Northcote T.G. 1999. Migratory behaviour of fish and its significance to movement through riverine fish passage facilities. In: M.Jungwirth, S.Schmutz & S.Weiss (eds) *Fish Migration and Fish Bypasses*. Oxford: Fishing News Books, Blackwell Science, pp. 3-18.

ØKland, F., Jonsson, B., Jensen, A. J. and Hansen, L. P. 1993. Is there a threshold size regulating seaward migration of brown trout and Atlantic salmon? *Journal of Fish Biology*. 42: 541–550.

Ottaway, E. M. and Clarke, A. 1981. A preliminary investigation into the vulnerability of young trout (*Salmo trutta*, L.) and Atlantic salmon (*S salar*, L.) to downstream displacement by high water velocities. *Journal of Fish Biology*. 19:135-145.

Paez, D.J, Brisson-Bonenfant, C., Rossignol, O., Guderley, H.E., Bernatchez, L. and Dodson, J.J. 2011. Alternative developmental pathways and the propensity to migrate: a case study in the Atlantic salmon. *Journal of Evolutionary Biology*. 24:245-255.

Parrish, C. C. 1992. Separation of polyunsaturated and saturated lipids from marine phytoplankton on silica gel-coated Chromarods. *Journal of Chromatography*. 607:97-104.

Parrish, C. C. 1999. Determination of total lipid, lipid classes and fatty acids in aquatic samples. In *Lipids in Freshwater Ecosystems* Arts, M. T. and Wainman, B. C. (Eds.). Pp.4-20. New York, N.Y., USA: Springer Verlag.

Peng, J., Larondellea, Y., Phama, D., Ackmanb, R. G. and Rollinc, X. 2003. Polyunsaturated fatty acid profiles of whole body phospholipids and triacylglycerols in anadromous and landlocked Atlantic salmon (*Salmo salar* L.) fry. *Comparative Biochemistry and Physiology Part B*. 134:335–348.

Pickova, J., Dutta, P.C., Larsson, P-O. and Kiessling, A., 1997. Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: Comparison between two cod (*Gadus morhua*) stocks. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 2410-2416

Pickova, J., Kiessling, A., Petterson, A. and Dutta, P. C. 1999. Fatty acid and carotenoid composition of eggs from two non-anadromous Atlantic salmon stocks of cultured and wild origin. *Fish Physiology and Biochemistry*. 21:147–156.

Power, G., Power, M. V, Dumas, R. and Gordon, A. 1987. Marine migrations of Atlantic salmon from rivers in Ungava Bay, Quebec. *American Fisheries Society Symposium*. 1:364-76.

Rasanen, K. and Kruuk, L. E. B. 2007. Maternal effects and evolution at ecological time-scales. *Functional Ecology*. 21:408–421

Reid D, Armstrong JD, and Metcalfe NB. 2011. Estimated standard metabolic rate interacts with territory quality and density to determine the growth rates of juvenile Atlantic salmon. *Functional Ecology*. 25:1360-1367.

Reznick, D.N. 1991. Maternal effects in fish life histories, in *The Unity of Evolutionary Biology*. Dudley, E.C., (Ed.), pp. 780–793, Dioscorides Press, Portland, OR.

Ricker, W. E. 1979. Growth rates and models. *Fish Physiology*. 8:677-743.

Rideout, R. M., Rose, G. A. and Burton, M. P. M. 2005. Skipped spawning in female iteroparous fishes. *Fish and Fisheries*. 6:50–72.

Riley, S. C., Power, G. and Ihssen, P. E. 1989. Transactions of the American Fisheries Society. 118:515–522

Riley, S. C., Tatara, C. P. and Scheurer, J. A. 2005. Aggression and feeding of hatchery-reared and naturally reared steelhead (*Oncorhynchus mykiss*) fry in a laboratory flume and a comparison with observations in natural streams. *Canadian Journal of Fisheries and Aquatic Sciences*. 62:1400-1409.

Riska, B. 1991. Introduction to the symposium, in *The Unity of Evolutionary Biology Dudley*, E.C., (Ed.), pp. 719–724, Dioscorides Press, Portland, OR.

Robitaille, J. A., Côté, Y., Shooner, G. and Hayeur, G. 1986. Growth and maturation patterns of Atlantic salmon, *Salmo salar*, in the Koksoak River, Ungava, Quebec. In *Salmonid age at maturity*.

Roff, D. A. 1992. The evolution of life histories: theory and analysis. Chapman & Hall, New York

Roff, D.A. 1998. The detection and measurement of maternal effects. In *Maternal effects as adaptations*. Mousseau, T. and Fox, C. pp. 11–83. Eds. Oxford, UK:Oxford University Press.

Rollinson, N., and Hutchings, J. A. 2011. Body size-specific maternal effects on the offspring environment shape juvenile phenotypes in Atlantic salmon. *Oecologia*. 166: 889-898.

Rollinson, N. and Hutchings, JA. 2013. Environmental quality predicts optimal egg size in the wild. *American Naturalist*. 182:76-90.

Rottiers, D.V. 1993. Elemental composition of a migratory and a landlocked strain of Atlantic salmon, *Salmo salar*. *Comparative Biochemistry and Physiology. Part A* 104: 93–100.

Sargent, J.R. 1995. (n-3) polyunsaturated fatty acids and farmed fish. In: *Fish Oil: Technology, Nutrition and Marketing*. Hamilton, R.J., Rice, R.D. (Eds.), pp. 67–94. Barnes and Associates, Bucks.

Sargent, J. R., Bell, J. G., Bell, M. V., Henderson, R. J. and Tocher, D. R. 1995. Requirement criteria for essential fatty acids. Symposium of European Inland Fisheries Advisory Commission. *Journal of Applied Ichthyology*. 11:183–198.

Sargent, J. R., Bell, G., McEvoy, L., Tocher, D. R. and Estevez, A. 1999. Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*. 177:191-199.

SAS, 1988. SAS/STAT release 6.03. SAS Institute Inc., Cary, North Carolina.

Schluter, D, and McPhail, J. D. 1993. Character displacement and replicate adaptive radiation. *Trends in Ecology and Evolution*. 8:197-200.

Steffens, W. 1997. Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish. *Aquaculture*. 151:97-119.

Taborsky, M., Brockmann, H.J. and Oliveria, R.F. 2008. The evolution of alternative reproductive tactics: concepts and questions. In R.F. Oliveria, M. Taborsky and H.J. Brockmann (eds.), *Alternative reproductive tactics: an intergrative approach*. Cambridge University Press, Cambridge, UK, pp. 1-22.

Takahashi, H. and Yamada, M. 1976. Lipid composition of seven species of crustacean plankton. *Bulletin of the Japanese Society of Scientific Fisheries*.

Tessier, N. and Bernatchez, l. 1999. Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar* L.) *Molecular Ecology*. 8:168-179.

Thorpe, J. E., Mangel, M., Metcalfe, N.B. and Huntingford, F.A. 1998. Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, *Salmo salar* L. *Evolutionary Ecology*. 12:581-599.

Tocher, D., Carr, J. and Sargent, J.R. 1989. Polyunsaturated fatty acid metabolism in fish cells: differential metabolism of (n−3) and (n−6) series acids by cultured cells originating from fresh water teleost fish and from marine teleost fish. *Comparative Biochemistry and Physiology,* B. 94:367-374.

Tocher, D. R., Bendiksen, E. A., Campbell, P. J. and Bell, J. G. 2008. The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture*. 280:21–34.

Tomkins, J.L. and Hazel, W. 2007. The status of the conditional evolutionarily stable strategy. *Trends in Ecology and Evolution*. 22:522-528.

Trippel, E.A. and Neil, S.R.E. 2004. Maternal and seasonal differences in egg sizes and spawning activity of northwest Atlantic haddock (*Melanogrammus aeglefinus*) in relation to body size and condition. *Canadian Journal of Fisheries and Aquatic Sciences*. 61: 2097-2110.

Tveiten, H., Jobling, M. and Andreassen, I. 2004. Influence of egg lipids and fatty acids on egg viability, and their utilization during embryonic development of spotted wolf-fish, *Anarhichas minor* Olafsen. *Aquaculture Research.* 35:152-161

Verspoor, E. and Cole, L. J. 1989. Genetically distinct sympatric populations of resident and anadromous Atlantic salmon, *Salmo salar*. *Canadian Journal of Zoology*. 67:1453-1461.

Wade, M. J. 1998. The evolutionary genetics of maternal effects. In TA Mousseau, CW Fox, eds. *Maternal effects as adaptations*. Pp. 5-21. Oxford University Press, New York.

Wang, L. and White, R. J. 1994. Competition between wild brown trout and hatchery greenback cutthroat trout of largely wild parentage. North American Journal of Fisheries Management. 14:475–487.

Wankowski, J. W. S. and Thorpe, J. E. 1979. Spatial distribution and feeding in Atlantic salmon *Salmo salar* L., juveniles. *Journal of Fish Biology*. 14:239-247.

Wiegand, M. D. 1996. Composition, accumulation and utilization of yolk lipids in teleost fish Reviews in Fish Biology and Fisheries. 6:259-286.

Wiegand M.D., Johnston T.A., Martin J. and Leggett W.C. 2004. Variation in neutral and polar lipid compositions in ova in ten reproductively isolated populations of walleye (*Sandrus vitreus*). *Canadian Journal of Fisheries and Aquatic Sciences.* 61:110–121

Willis, T.J., and Babcock, R.C. 1998. Retention and *in situ* detectability of visible implant fluorescent elastomer (VIFE) tags in *Pagrus auratus* (Sparidae). *New Zealand Journal of Marine and Freshwater Research*. 32:247-254.

Wilzbach, M. A., Cummins, K. W. & Hall, J. D. 1986. Influence of habitat manipulations on interactions between cutthroat trout and invertebrate drift. *Ecology.* 67: 898-911.

Wolf, J.B., and Wade, M.J. 2009. What are maternal effects (and what are they not)? *Phil. Trans. R. Soc. B.* 364:1107-1115.

Wood, B. J. 1974. Fatty acids and saponifiable lipids. In: Algal Physiology and Biochemistry, Stewart, W. D. P. (Ed.), pp. 236–65, Blackwell, London.

Wood, C. and Foote, C. J. 1996. Evidence for sympatric genetic divergence of anadromous and non-anadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution*. 50:1265-1279.

Yamada, M. and Hayashi, K. 1975. The lipids of marine animals from various habitat depths. I. Fatty acid composition of lipids from 22 species of fish and molluscs. *Bulletin of the Japanese Society of Scientific Fisheries [Nihon Suisan Gakkai-shi]* 41:1143-1152.

Yanes-Roca, C., Rhody, N., Nystrom, M. and Main, K.L. 2009. Effects of fatty acid composition and spawning season patterns on egg quality and larval survival in common snook (*Centropomus undecimalis*). *Aquaculture*. 287: 335-340

Youngson, A. F., Mitchell, A. I., Noack, P.TTT. and Laird, L. M. 1997. Carotenoid pigment profiles distinguish anadromous and nonanadromous brown trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences*. 54:1064-1066.

Zimmerman, C. E. and Reeves, G. H. 2002. Identification of steelhead and resident Rainbow trout progeny in the Deschutes River Oregon, revealed with otolith microchemistry. *Transacations of the American Fisheries Society*. 131:986-993.

Appendices

Appendix 1: Lipid class means expressed as a % of total lipids (means <1 % excluded) of individual families of anadromous females from Exploits River (E) and Indian Bay Brook (S) and resident females from Indian Bay Brook (O) at four different developmental stages. Shaded areas indicate means < 1%.

Family	Total Lipids (mg/g)	% Phospholipids	% TAG	% Sterols	% AMPL	% FFA	% Hydrocarbons	% Steryl Esters/ Wax Esters	% Methyl Ketones
	Unfertilized								
E6	268.78	39.72	50.64	9.64	0.00				
E7	135.91	47.77	37.98	10.50	3.34				
S1	65.47	48.81	36.33	12.35	0.00				
S2	129.96	41.64	42.07	14.07	1.87				
S4	180.37	39.34	50.77	9.23	0.00				
S5	357.72	28.00	52.04	6.28	13.65				
O10	92.73	61.84	22.09	15.17	0.00				
O2	141.30	43.66	41.63	13.58	0.00				
O3	135.27	49.78	36.80	11.99	0.89				
O4	238.86	43.67	47.27	7.94	1.07				
O6	81.88	28.16	50.90	18.16	1.40				
O7	198.39	37.86	46.73	10.68	1.71				
	Eyed								
E6	169.41	36.39	47.16	9.70	3.73	2.02			
E7	201.47	39.40	43.54	9.48	3.86	2.34			
S1	116.84	29.51	51.66	5.29	8.08	5.24			
S2	132.67	33.22	52.31	9.14	3.25	1.72			
S4	143.44	35.53	50.20	9.76	1.42	2.18			
S5	158.81	33.87	54.22	7.61	2.34	1.39			
O10	127.96	41.75	44.32	11.57	1.83	0.53			
O2	251.01	40.29	48.18	8.45	1.54	0.53			
O3	183.07	45.01	41.75	7.77	3.16	1.77			
O4	227.59	39.76	48.96	8.87	2.02	0.00			
O6	157.79	35.19	50.73	7.55	1.86	2.67			
O7	372.33	40.61	51.51	5.85	0.79	1.24			

Appendix 1: (Continued)

Family	Total Lipids (mg/g)	% Phospholipids	% TAG	% Sterols	% AMPL	% FFA	% Hydrocarbons	% Steryl Esters/ Wax Esters	% Methyl Ketones
	Alevin								
E6	154.31	35.44	46.33	12.98	3.02				
E7	145.06	50.99	34.75	11.72	0.87				
S1	445.21	38.57	50.88	5.87	1.90				
S2	170.77	31.29	53.69	9.63	2.65				
S4	172.80	33.89	54.45	9.40	1.40				
S5	294.30	43.50	47.73	7.22	0.68				
O10	175.04	46.54	42.93	9.40	1.14				
O2	155.30	36.92	47.54	11.61	1.66				
O3	174.36	51.11	38.77	8.08	1.61				
O4	190.93	35.99	47.94	9.18	3.49				
O6	266.93	39.62	47.14	8.28	1.62				
O7	286.54	47.96	35.93	9.22	1.79				
	Emergent								
E6	123.41	47.74	24.73	8.05	10.85		2.56	0.00	3.08
E7	258.72	38.06	37.35	13.21	4.77		2.48	0.38	3.75
S1	148.96	39.97	36.07	15.19	2.12		1.36	1.81	2.35
S2	165.92	38.57	38.12	14.19	4.65		2.45	0.00	2.03
S4	212.61	37.03	41.99	11.95	4.63		2.01	0.88	1.51
S5	313.11	32.60	49.68	10.31	3.58		0.80	0.74	2.30
O10	139.15	46.15	33.09	14.52	2.80		1.58	0.00	1.86
O2	271.62	37.30	46.22	12.21	0.86		0.38	1.14	0.48
O3	146.82	41.26	33.50	13.82	6.06		1.42	0.76	2.42
O4	151.15	33.40	42.69	13.47	4.48		1.44	1.51	3.01
O6	111.50	38.69	31.49	15.39	4.89		2.23	3.73	3.56
O7	154.15	53.13	20.56	15.49	5.70		0.79	0.39	3.88

Appendix 2: Fatty acid means expressed as a % of total fatty acids (means <1 % excluded) of individual families of anadromous females from Exploits River (E) and Indian Bay Brook (S) and resident females from Indian Bay Brook (O) at four different developmental stages.

Family	14:0	16:0	16:1w7	18:0	18:1w9	18:1w7	18:2w6	20:1w9	20:4w6	20:5w3	22:5w3	22:6w3	24:1
	Unfertilized												
E6	1.15	14.22	4.67	7.27	23.20	4.01	0.91	2.40	0.85	7.18	5.47	20.05	2.91
E7	1.34	14.05	4.42	8.16	19.46	3.56	0.87	1.73	1.29	9.20	6.62	22.77	2.03
S1	1.17	15.32	3.97	9.37	19.35	4.06	0.88	1.88	0.91	8.09	6.65	22.64	0.58
S2	1.77	13.26	5.94	4.64	20.20	3.53	1.04	2.15	0.93	9.06	7.22	19.28	1.68
S4	2.10	14.21	9.25	8.47	20.39	5.23	0.73	1.71	0.44	8.42	6.44	16.54	1.98
S5	2.13	14.68	9.72	8.55	21.12	4.16	0.78	1.68	0.47	8.37	6.06	16.90	1.14
O10	0.85	13.48	5.13	9.76	15.78	5.64	3.54	0.35	11.18	4.22	4.15	14.23	0
O2	0.85	14.63	8.37	9.82	21.51	5.97	2.43	0.28	9.68	3.61	3.37	10.38	0.71
O3	0.95	16.82	2.16	12.75	12.89	5.59	1.71	0.99	8.31	4.26	3.91	17.59	2.33
O4	2.05	12.53	5.38	7.56	13.26	3.97	2.09	0.75	8.77	6.99	5.86	18.31	0.21
O6	1.60	13.98	4.19	9.26	12.25	4.47	1.73	0.58	8.92	6.76	5.20	21.02	1.57
O7	1.12	13.64	6.53	9.29	20.30	6.24	4.21	0.30	9.16	3.93	3.47	9.97	0.28
	Eyed												
E6	1.32	14.85	4.25	6.86	20.78	4.44	0.78	2.20	1.02	8.47	5.91	22.35	1.00
E7	1.44	13.70	5.55	7.04	22.57	2.71	1.09	1.72	1.12	9.21	6.31	21.83	0.54
S1	1.29	13.90	5.27	7.41	23.06	4.40	1.17	1.77	0.92	8.02	6.23	19.66	0.74
S2	1.68	14.76	5.00	10.28	18.50	4.99	0.93	2.08	1.03	8.41	6.76	18.25	1.28
S4	1.83	14.70	7.97	10.48	19.79	4.15	0.73	1.75	0.52	7.71	6.18	16.86	2.05
S5	2.29	13.36	10.09	7.72	20.20	5.14	0.83	1.52	0.51	9.27	6.38	8.56	0.78
O10	0.82	14.03	4.32	11.59	14.28	8.53	3.58	0.43	10.35	3.73	3.71	11.66	0.79
O2	0.92	14.15	8.70	8.93	22.79	6.46	2.81	0.32	8.97	3.57	3.13	9.69	0.49
O3	1.25	14.48	4.96	8.37	18.98	10.54	2.85	0.82	8.65	5.60	4.14	18.23	0.28
O4	2.14	11.98	5.94	6.78	15.44	3.22	2.24	0.69	8.39	7.07	5.96	17.35	0.48
O6	1.75	14.01	4.93	8.59	13.08	5.15	2.29	0.48	8.52	6.73	5.07	18.25	0.88
O7	0.76	15.77	3.08	14.97	12.14	7.02	2.23	0.44	11.04	3.73	3.89	11.73	0.73

Appendix 2: (Continued)

Family	14:0	16:0	16:1w7	18:0	18:1w9	18:1w7	18:2w6	20:1w9	20:4w6	20:5w3	22:5w3	22:6w3	24:1
	Alevin												
E6	0.56	9.29	1.57	10.38	22.10	5.14	0.80	2.81	1.26	9.02	5.15	25.97	0.78
E7	1.40	15.37	2.31	8.10	17.59	3.57	1.06	1.70	1.36	9.30	6.36	22.04	1.63
S1	1.37	13.41	5.51	6.73	21.97	3.81	1.19	1.56	0.88	8.68	6.19	20.06	2.62
S2	1.36	14.84	4.28	10.12	16.60	4.07	0.74	1.94	1.36	8.51	7.31	21.36	1.58
S4	1.98	14.38	9.42	8.26	20.37	4.02	0.74	1.58	0.53	9.02	6.46	18.55	0.40
S5	2.07	14.08	9.80	7.83	21.01	3.96	0.84	1.62	0.48	9.11	6.48	18.17	0.41
O10	0.88	13.53	5.76	9.03	17.05	5.38	4.16	0.31	10.79	4.02	3.65	12.76	0.30
O2	0.69	15.20	6.07	12.09	18.27	6.59	2.14	0.30	10.66	3.86	3.66	11.55	0.48
O3	1.16	13.77	4.22	7.76	18.08	4.36	2.65	0.80	8.64	5.47	4.10	18.55	0.65
O4	2.03	13.93	2.78	8.40	12.38	4.98	2.12	0.73	9.70	7.33	4.04	16.91	2.44
O6	1.50	14.26	4.43	9.31	11.43	4.52	1.62	0.60	9.20	6.94	5.34	21.39	0.38
O7	1.05	13.86	2.66	9.28	11.26	4.53	1.48	0.62	8.92	7.07	5.05	21.85	0.93
	Emergent												
E6	0.89	16.00	3.29	6.11	18.17	3.97	0.89	1.51	1.82	8.42	4.63	26.07	3.02
E7	1.10	17.24	2.87	7.06	17.82	3.58	0.82	1.15	2.05	9.16	5.06	24.34	1.92
S1	1.02	19.24	3.56	8.03	18.67	4.58	0.89	1.34	1.45	7.84	4.64	20.76	3.16
S2	1.71	16.69	6.34	7.27	21.71	4.42	1.09	1.74	1.65	9.31	6.37	13.84	1.53
S4	1.88	14.41	9.91	5.70	20.12	4.59	0.89	1.15	0.82	9.35	5.27	20.71	0.70
S5	1.78	13.74	9.59	5.71	20.89	4.28	0.69	1.33	0.81	9.12	5.87	21.15	0.74
O10	0.86	13.57	5.16	9.83	15.88	5.68	3.56	0.35	9.27	4.25	4.18	14.33	0
O2	0.84	16.42	8.45	3.70	21.63	14.97	2.65	0.30	10.65	3.83	3.24	13.94	1.06
O3	0.99	14.63	3.32	6.84	15.52	5.03	2.43	0.55	9.46	5.40	3.53	21.38	0.78
O4	1.72	13.89	3.80	6.63	12.02	4.64	1.92	0.52	9.41	6.66	5.13	20.57	2.16
O6	1.60	14.25	5.14	7.15	13.26	4.23	2.07	0.44	9.04	6.79	4.61	21.68	0.19
O7	1.03	14.58	2.28	6.27	10.30	3.82	1.45	0.36	9.89	7.34	3.92	28.74	3.38

Appendix 3: Total masses (g) of individual families of anadromous females from Exploits River (E) and Indian Bay Brook (S) and resident females from Indian Bay Brook (O) at four different developmental stages.

Family	Total mass (g)			
	Unfertilized	Eyed	Alevins	Emergent
E6	0.096 ± 0.003	0.124 ± 0.006	0.099 ± 0.001	0.147 ± 0.009
E7	0.101 ± 0.004	0.127 ± 0.007	0.106 ± 0.002	0.146 ± 0.009
S1	0.131 ± 0.017	0.146 ± 0.000	0.123 ± 0.005	0.189 ± 0.007
S2	0.127 ± 0.015	0.138 ± 0.005	0.125 ± 0.002	0.177 ± 0.008
S4	0.121 ± 0.005	0.142 ± 0.007	0.115 ± 0.002	0.174 ± 0.007
S5	0.119 ± 0.003	0.149 ± 0.002	0.198 ± 0.109	0.181 ± 0.010
O10	0.157 ± 0.023	0.147 ± 0.072	0.193 ± 0.026	0.232 ± 0.030
O2	0.110 ± 0.004	0.128 ± 0.003	0.114 ± 0.004	0.156 ± 0.008
O3	0.154 ± 0.003	0.179 ± 0.003	0.162 ± 0.003	0.221 ± 0.009
O4	0.149 ± 0.005	0.185 ± 0.001	0.154 ± 0.002	0.202 ± 0.011
O6	0.156 ± 0.005	0.174 ± 0.017	0.155 ± 0.001	0.217 ± 0.008
O7	0.154 ± 0.006	0.185 ± 0.001	0.128 ± 0.010	0.209 ± 0.015