PARENTAL CONTRIBUTIONS TO THE EARLY LIFE HISTORY TRAITS OF JUVENILE SOCKEYE SALMON (*ONCORHYNCHUS NERKA*): THE ROLES OF SPAWNER IDENTITY AND MIGRATORY EXPERIENCE

by

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ABSTRACT

Pacific salmon (*Oncorhynchus* spp.) undergo arduous upstream migrations in order to spawn. To date, much scientific attention has focused on why certain migrants succeed in reaching their destination while others die trying. Less is known about how 'successful' spawners differ in the quality of the progeny they produce. Using sockeye salmon *O. nerka* (Walbaum) as a model, two artificial fertilization experiments were conducted to investigate the relationships between individual salmon and their offspring. In the first experiment, I evaluated survival, size, and burst swimming ability in fry of known parentage (spawners from the Weaver Creek population). After four months of exogenous feeding, fry size remained under significant maternal influence. Paternal identity did not affect size but significantly influenced both egg and fry survival. Burst swimming ability was not affected by parentage and only weakly associated with offspring size. In the second experiment, I evaluated an 'energetic trade-off' hypothesis which proposes that because adults migrate with a fixed energy budget while completing sexual maturation, investments to reproductive development may be impaired by an increase in the costs of swimming to reach spawning grounds. This hypothesis was evaluated by subjecting migrants to two different 'migration difficulties' (i.e. current speeds). Fish in the 'fast' treatment expended more energy than those in the 'slow' and also showed signs of greater physiological stress. However, these differences did not appear to influence allocations to reproductive development in terms of sex trait morphology, ovulation timing, and reproductive hormone levels. Likewise, the survival, incubation time, and size of progeny were not related to the treatments experienced by their parents. These traits were nonetheless influenced by parental identity, with

significant contributions from both male and female parents. Regression models showed that offspring size and survival were linked to certain aspects of maternal condition at the time of fertilization, including size, stress, and energy levels.

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DEDICATION

À ma famille. Finis les 'petits coups'.

CO-AUTHORSHIP STATEMENT

Patrick Nadeau held primary responsibility for the experimental design, fieldwork, data analysis, and writing of manuscripts inherent to the present thesis. Use of the first person plural in Chapters 2 and 3 acknowledges the following co-authors, who will be named in the manuscript versions of these chapters (currently in preparation for submission to peer-reviewed publications): in Chapter 2, Scott Hinch and David Patterson, who provided important technical expertise and manuscript reviews; in Chapter 3, both of the above in addition to Kim Hruska, who assisted with experimental design and manuscript reviews. Experimental manipulations occurring prior to January 2005 (described in Chapter 2) were conducted under the leadership of David Patterson and colleagues. Patrick Nadeau retained principal responsibility for all other aspects of the study.

CHAPTER 1

INTRODUCTION

Background

Pacific salmon are key constituents of the marine and freshwater ecosystems they occupy, in addition to playing a vital role in the economy and culture of North Pacific communities. The seven species comprising Pacific salmon (*Oncorhynchus* spp.) span an incredibly diverse range of life history strategies (Quinn 2005), such that it is difficult to make sweeping generalizations to describe them. Most populations are anadromous (freshwater spawning and oceanic rearing) and semelparous (a single spawning season per lifetime), both ecologically important characteristics which have garnered much scientific attention (Groot and Margolis 1991; Quinn 2005).

Salmonid spawning migrations are among the most remarkable feats of the animal kingdom. Central to the success of the adults undertaking these migrations is a strong genetic control over the timing of both river entry and subsequent spawning. It has been suggested that migration and spawning timing result from adaptations to the long-term average river conditions which optimize migratory efficiency and offspring viability (Quinn 2005). From year to year, however, the physical environment encountered by migrating salmon is not always representative of historical conditions. Particularly challenging migrations may occur when temperatures or flows are higher than usual, both of which can impede migratory progress (Macdonald 2000; Standen et al. 2002). Often, segments of spawning runs die before reaching spawning grounds, presumably as a consequence of encountering sub-optimal migratory conditions (Macdonald 2000; Hinch and Bratty 2000).

An important feature of spawning migrations lies in the fact that sexual maturation occurs concurrently with upstream movement (Hendry and Berg 1999; Kinnison et al. 2001). If the environment experienced by maturing fish affects the maturation process (Rossiter 1996; Mousseau and Fox 1998), then particularly difficult migratory conditions may interfere with salmon reproduction. Although this idea is supported indirectly by observations that inter-annual differences in population-level reproductive success are related to migration difficulty (Patterson 2004), little is known about the impacts of migratory conditions on individual-level reproduction. Only recently have studies begun to consider this question directly, via the experimental manipulation of migratory difficulty (Kinnison et al. 2001, 2003; Patterson et al. 2004). However, important knowledge gaps remain, notably concerning the extent to which increasing migratory difficulty can impact gamete quality and consequently fertilization success and offspring traits.

The overarching purpose of this thesis was to explore the relationships between individual salmon and their progeny. To this effect, I followed a two-pronged approach. Using sockeye salmon *O. nerka* (Walbaum) as a model, my first step was to evaluate the importance of parental identity in shaping juvenile traits, namely survival, size, and swimming performance (Chapter 2). In a separate study, I experimentally increased the difficulty of migrations faced by maturing sockeye. I then used artificial fertilization to monitor the consequences of difficult migrations on the resulting generation of juveniles (Chapter 3).

Migration and reproduction

In order to consider the effects of migratory conditions on reproductive success, it is important to understand the physiological processes inherent to migration and their relationship with the reproductive system (Hinch et al. 2006). Below I review these processes in terms of 'energetics' (i.e. energy reserves and allocation) and 'physiology' (i.e. endocrinology, ion balance, and stress). Although certainly not independent of each other, treating energetics and physiology separately highlights their respective roles in reproductive development and possible pathways for effects on gamete and offspring quality.

Energetics

Salmon stop feeding prior to the freshwater phase of their homing migrations, thus migration must be entirely fueled by the energy stores acquired during ocean residency. In sockeye, energy levels at the onset of migration typically range from 7–10 MJ kg^{-1}, and vary depending on prevailing oceanic conditions (Crossin et al. 2004a) and population-level adaptations (Crossin et al. 2004b). During migration, the majority of energy mobilization occurs via lipid catabolism (Patterson et al. 2004a; Magnoni et al. 2006), with a transition to protein catabolism as senescence progresses and lipid reserves fall (Hendry and Berg 1999). In this manner, sockeye generally deplete 50–80% of initial energy reserves between river entry and death (Brett 1995; Hendry and Berg 1999; Crossin et al. 2003), the latter occurring at an energy density of approximately 4 MJ kg^{-1}, an apparent lower threshold for sustaining life (Crossin et al. 2004b).

Due to energetic constraints, reproductive success ultimately requires that energy be adequately partitioned into the various demands of migration, including routine metabolic costs, upstream locomotion (Rand and Hinch 1998; Hinch and Rand 2000), development of gonads and secondary sexual traits (Hendry and Berg 1999; Kinnison et al. 2001; Kinnison et al. 2003), and spawning ground behaviours such as site acquisition, displays, and the building and defending of redds (Healey et al. 2003). Of these, swimming and reproductive development are the most costly (Crossin et al. 2004b), but the relative energy allocated to each can vary. Locomotory costs are generally believed to surpass costs related to reproductive development (Idler and Clemens 1959; Rand and Hinch 1998; Magnoni et al. 2006), although others have inferred roughly equal investment in either function, particularly in females (Crossin et al. 2003; Crossin et al. 2004b). Both reproductive and swimming allocations are briefly discussed below.

In migrating sockeye, reproductive development entails the maturation of gonads and the appearance of secondary sexual characteristics such as dorsal humps and kypes (mandibular protrusions). In females, the mobilization of energy into ovarian development consists primarily of vitellogenesis, a process by which the hepatically-derived lipoprotein vitellogenin (Vtg) is taken up by maturing oocytes. The subsequent proteolytic cleavage of Vtg into yolk derivatives results in the accumulation of yolk in the developing egg (Brooks et al. 1997; Coward et al. 2002). In this manner, eggs continue to gain in mass until just prior to spawning, vitellogenesis potentially accounting for more than 90% of final egg volume (Tyler 1991). In males, gonadal mass does not undergo a substantial increase during migration (Hendry and Berg 1999; Crossin et al. 2004b), although secondary sexual characteristics require significant energetic inputs (Hendry

and Berg 1999; Kinnison et al. 2003). Protein mobilization is the key pathway of these allocations, given the cartilaginous composition of humps and kypes (Hendry and Berg 1999).

Throughout this thesis, I consider an increase in 'migration difficulty' to reflect an increase in the energy spent swimming. Thus, a primary driver of migration difficulty is the work (in its physical sense, the product of distance and elevation) required to reach spawning grounds (Crossin et al. 2004b). For a given individual, the total energy spent swimming depends on energetic efficiency, or the cost of transportation (COT, the energy required per unit of distance traveled). Many factors can increase the COT, including poor health (Jain et al. 1998) or extreme temperatures (Lee et al. 2003). Whether a fish is swimming at an energetically optimal speed (*sensu* Brett 1995) can also be influenced by river discharge. For example, assessments of energy use in sockeye (*O. nerka*) and pink salmon (*O. gorbuscha*) via electromyogram (EMG) telemetry revealed that migrants faced with higher velocity currents and more complex hydraulic patterns adopted behaviours resulting in increased energetic depletion (Hinch et al. 2002; Standen et al. 2002). Difficult migratory conditions (e.g. fast currents) could increase energetic depletion by decreasing the energetic efficiency of locomotion and/or adding to the time required to reach spawning grounds.

Energetic trade-offs could occur in migrating salmon if increasing resources spent on locomotion came at the expense of reproductive development (Kinnison et al. 2001; Kinnison et al. 2003) via a reduction in offspring size or number. Smith and Fretwell (1974) set the theoretical basis for such trade-offs, stipulating that there exists an optimal proportion of energy that should be allocated to reproduction to maximize parental

fitness, and that from this allotment there is an optimal combination of offspring size versus number.

Physiology

Migration entails a suite of dramatic physiological changes, beginning in the ocean and ending with post-spawning mortality. The endocrine system, for example, is marked by an increase in key reproductive hormones such as testosterone and 17β-estradiol, which stimulate upstream migration (Munakata et al. 2001) and control final sexual maturation (Coward et al. 2002). Moreover, late maturation and spawning are marked by the loss of control over the interrenal secretion and excretion of cortisol, a hormone involved in both reproductive development and stress responses, resulting in a net increase in plasma concentrations (Schreck et al. 2001).

The transition from saltwater to freshwater involves a putatively irreversible loss of hypoosmoregulatory capability (Shrimpton et al. 2005). Underlying this process are changes in the distribution of two types of gill chloride cells, osmoregulatory centres which host gill Na^+, K^+ -ATPase, a key enzyme in ion transport. Specifically, filament gill chloride cells undergo apoptosis while the number of lamellar gill chloride cells increase: the result being hyperosmoregulatory preparedness and a decrease in ATPase activity (Uchida et al. 1997). This decrease, accompanied by a gradual decline in plasma ion concentrations, has been observed to continue throughout migration and eventually compromises homeostasis (Shrimpton et al. 2005).

Given the scope of the physiological transformations operating during migration, particularly difficult migratory conditions are likely to exacerbate the already 'stressed' state of migrants. For example, high river discharge may lead to more frequent bouts of exhaustive exercise, thereby increasing circulating levels of cortisol, lactate, and glucose (Barton 2002; Portz et al. 2006). Prolonged or exhaustive swimming may also lead to osmotic imbalances (Postlethwaite and McDonald 1995; McDonald and Milligan 1997), changes in plasma erythrocyte concentrations, or decreases in plasma pH (McDonald and Milligan 1997).

The exact mechanisms by which stress might affect reproductive success and offspring qualities are varied and not fully understood (Pankhurst and Van Der Kraak 1997; Portz et al. 2006). Both direct and indirect pathways may be involved. Under a direct scenario, the developing gonads themselves would be exposed to parental stressors, such as through the permeability of eggs to circulating hormones (Brooks et al. 1997). Eggs, for example, take up cortisol in amounts related to maternal levels (McCormick 1998; Eriksen et al. 2006), a process which has been associated with reduced offspring size and survival (McCormick 2006; Mingist et al. 2007). Parental physiology could also affect progeny in an indirect manner. Chronic stress, for instance, tends to suppress reproductive hormone levels (Pankhurst and Van Der Kraak 1997; Portz et al. 2006). Stress in migrants could thus affect the rate of sexual maturation (*sensu* Patterson et al. 2004a), which in turn might lead to a suboptimal spawning date in terms of offspring survival and development potential. Likewise, stress may interfere in the partitioning of energy to reproductive development, given the role of reproductive hormones in the

control of nutrient allocation to the egg via vitellogenesis (Brooks et al. 1997; Pankhurst and Van Der Kraak 1997).

Thesis rationale

Despite the strong connections between migration and reproduction, sockeye spawning migrations have rarely been considered with individual-level fertilization success or offspring traits as endpoints (Patterson 2004). Currently, "successful" migrants are often defined as those which manage to reach spawning grounds, irrespective of subsequent fate or offspring viability (e.g. Cooke et al. 2006a, b). A better understanding of spawner influences, including the physiological factors that may drive them, is essential in developing a more holistic view of population-level spawning success. From a management perspective, such knowledge is crucial because the early life survival of juveniles sets the cap for future spawner returns. Moreover, the ongoing changes in climate will likely affect migratory conditions in years to come (Rand et al. 2006), increasing the potential for reproductive and intergenerational impacts.

Approach

This thesis consists of two separate but complementary studies summarized in Figure 1.1 and described below. Chapter 2 is an experiment on juvenile attributes and their underlying parental influences. Chapter 3 builds on this knowledge and methodology to evaluate the effects of the parental migratory experience. Below I describe the approaches of both chapters with regard to the related background literature.

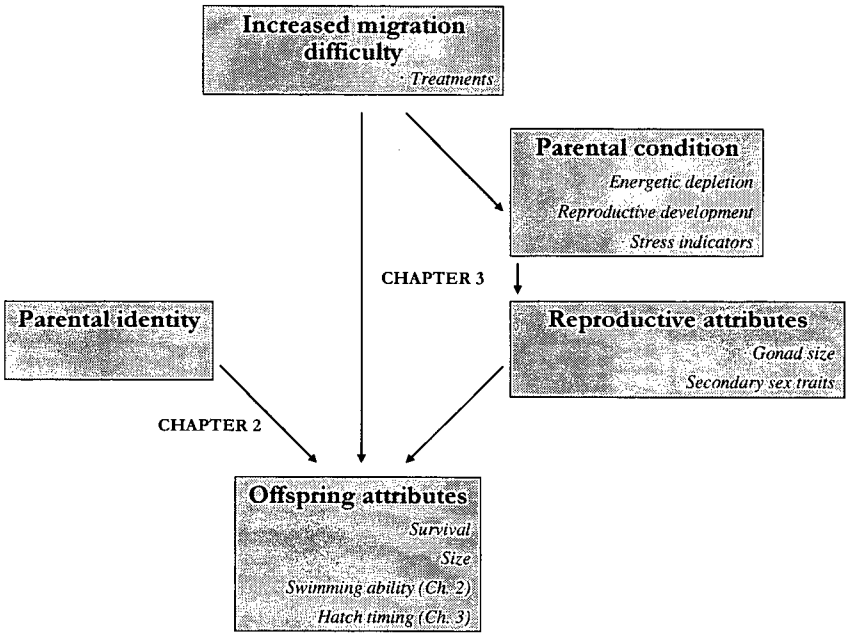

Figure 1.1. Overview of the experimental approach.

Chapter 2

The overall approach taken in this chapter (a full factorial mating design and subsequent offspring measurements) is akin to that of many studies which aim to describe juvenile traits as a function of 'maternal' or 'paternal' effects. The use of these terms in the genetics literature usually refers to the epigenetic contributions of parents to offspring phenotypes, such as 'inherited environmental effects' (Rossiter 1996; Mousseau and Fox 1998), through which the parental environment has an intergenerational impact on offspring traits. In other studies (e.g. Yamamoto and Reinhardt 2003; Rideout et al. 2004; Green and McCormick 2005; the present thesis), however, maternal and paternal effects refer more generally to the effects of 'parental identity' without formally discerning genetic from environmental contributions. Even without this distinction (such as an estimate of heritability, h^2), these studies can provide valuable insight into the relative

importance of maternal vs. paternal contributions, can identify juvenile traits which might be more susceptible to impacts from the parental environment, and can establish the magnitude, persistence, and biological relevance of parental influences.

Swimming ability was included as an offspring trait of interest partly due to recent findings in juvenile sockeye. Patterson et al. (2004b) demonstrated an important maternal influence on juvenile sockeye enzyme activity, including lactate dehydrogenase (LDH), an indicator of anaerobic glycolytic capacity. This finding was suggestive of a possible maternal influence on burst swimming ability, although it was not tested at the time. Moreover, assessing swimming ability was facilitated by the recent development of a burst swimming protocol for juvenile sockeye (Pon et al. 2007).

Chapter 3

In this chapter, I address the central question of how difficult migratory conditions might affect reproductive success. This was done using an experimental approach, by creating two treatments whereby maturing sockeye were subjected to different flow regimes. To gain the best possible understanding of parental influences, offspring attributes were evaluated from two perspectives: i) directly in terms of the parental migratory experience (treatments), and ii) indirectly via the links between parental attributes and progeny traits (Figure 1.1). Few studies thus far have explicitly examined the relationship between migratory experience and reproduction: those that have, briefly described below, were at the origins of my approach for this chapter.

In a large-scale reciprocal transplant experiment on chinook salmon, Kinnison et al. (2001, 2003) tagged juveniles from two populations and released them at either a near-

coastal site (17km from the ocean) or further inland (100km). After oceanic rearing, the adults homing to the site of their release were recaptured and examined. It was found that the females returning to the farther site had significantly lower ovarian mass, egg size, and muscle solids than those from the same original population (or even family) returning to the closer site (Kinnison et al. 2001). Likewise, males recaptured after migrating the longer distance had smaller humps and snouts, in addition to lower tissue energy reserves (Kinnison et al. 2003). Collectively, these results strongly supported the role of migratory difficulty in affecting reproductive attributes, thus setting the stage for my experiment. In these studies, however, the authors limited their examination of adults to morphological measurements (as opposed to the physiological assessments in Chapter 3) and did not evaluate fertilization success or offspring traits.

A subsequent Fraser River study by Patterson et al. (2004a) showed that it was possible to intercept migrating sockeye, bring them to maturity in a captive setting, and collect their gametes for artificial fertilization. In this experiment, long-distance migrants from the Early Stuart population were 'released' from their demanding migrations and made to swim either at moderate speeds or in the absence of current. Results suggested that experimental manipulation could indeed affect the rate of energetic depletion in migrants, but that sockeye could not reallocate energy into reproductive development when encountering relatively benign conditions. Given that energy saved on swimming did not go to reproductive development, I decided to investigate the opposite (whether energy used on swimming diverts from reproductive development): similar to the premise of Kinnison et al. (2001, 2003), but with a treatment and fertilization protocol adapted from Patterson et al. (2004a).

Objectives and research questions

The overarching objective of the present thesis was to evaluate the role of individual spawners in shaping the early life history traits of their offspring. In line with this objective, Chapter 2 aimed to determine: i) to what extent the variability in juvenile sockeye survival, size, and swimming ability can be explained by maternal identity, and ii) whether juveniles continue to display maternal influences beyond the onset of exogenous feeding. Likewise, Chapter 3 was intended to establish: i) whether experimental manipulation can be used to differentially deplete energy stores in short-distance migrants, ii) how the migratory experience of adults affects sex traits and the timing of reproductive development, and iii) to what extent offspring attributes (e.g. survival, size) can be affected by parental migratory experience and/or specific aspects of parental physiology.

In general, I predicted that females would have important impacts on offspring attributes, given their role in the provisioning of nutrients and other compounds to the eggs (Brooks et al. 1997; Berg et al. 2001), and that the more challenging experimental treatment would result in adults with lower energy levels and reduced gonadal investment, with correspondingly smaller and less viable progeny.

REFERENCES

Barton, B. A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42: 517-525.

Berg, O. K., A. P. Hendry, B. Svendsen, C. Bech, J. V. Arnekleiv, and A. Lohrmann. 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.

Brett, R. J. 1995. Energetics. Pp. 1-68 *In* C. Groot, L. Margolis, and W. C. Clarke, eds. Physiological ecology of Pacific salmon. UBC Press, Vancouver.

Brooks, S., C. R. Tyler, and J. P. Sumpter. 1997. Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7: 387-416.

Cooke, S. J., S. G. Hinch, G. T. Crossin, D. A. Patterson, K. K. English, M. C. Healey, J. M. Shrimpton, G. Van Der Kraak, and A. P. Farrell. 2006a. Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. Ecology 87: 1575-1586.

Cooke, S. J., S. G. Hinch, G. T. Crossin, D. A. Patterson, K. K. English, M. J. Shrimpton, G. Van Der Kraak, and A. P. Farrell. 2006b. Physiology of individual late-run Fraser River sockeye salmon (*Oncorhynchus nerka*) sampled in the ocean correlates with fate during spawning migration. Canadian Journal of Fisheries and Aquatic Sciences 63: 1469-1480.

Coward, K., N. R. Bromage, O. Hibbitt, and J. Parrington. 2002. Gamete physiology, fertilization and egg activation in teleost fish. Reviews in Fish Biology and Fisheries 12: 33-58.

Crossin, G. T., S. G. Hinch, A. P. Farrell, D. A. Higgs, and M. C. Healey. 2004a. Somatic energy of sockeye salmon *Oncorhynchus nerka* at the onset of upriver migration: a comparison among ocean climate regimes. Fisheries Oceanography 13: 345-349.

Crossin, G. T., S. G. Hinch, A. P. Farrell, D. A. Higgs, A. G. Lotto, J. D. Oakes, and M. C. Healey. 2004b. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. Journal of Fish Biology 65: 788-810.

Crossin, G. T., S. G. Hinch, A. P. Farrell, M. P. Whelly, and M. C. Healey. 2003. Pink salmon (*Oncorhynchus gorbuscha*) migratory energetics: response to migratory difficulty and comparisons with sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Zoology 81: 1986-1995.

Eriksen, M. S., M. Bakken, Å. Espmark, B. O. Braastad, and R. Salte. 2006. Prespawning stress in farmed Atlantic salmon *Salmo salar*: maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. Journal of Fish Biology 69: 114-129.

Green, B. S., and M. I. McCormick. 2005. Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. Marine Ecology Progress Series 289: 263-272.

Groot, C., and L. Margolis. 1991. Pacific salmon life histories. UBC Press, Vancouver.

Healey, M. C., R. Lake, and S. G. Hinch. 2003. Energy expenditures during reproduction by sockeye salmon (*Oncorhynchus nerka*). Behaviour 140: 161-182.

Hendry, A. P., and O. K. Berg. 1999. Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. Canadian Journal of Zoology 77: 1663-1675.

Hinch, S. G., and J. Bratty. 2000. Effects of swim speed and activity pattern on success of adult sockeye salmon migration through an area of difficult passage. Transactions of the American Fisheries Society 129: 598-606.

Hinch, S. G., S. J. Cooke, M. C. Healey, and A. P. Farrell. 2006. Behavioural physiology of fish migrations: salmon as a model approach. Pp. 239-295 *In* K. Sloman, R. W. Wilson, and S. Balshine, eds. Fish physiology, Volume 24. Academic Press, London, U.K.

Hinch, S. G., and P. S. Rand. 2000. Optimal swimming speeds and forward-assisted propulsion: energy-conserving behaviours of upriver-migrating adult salmon. Canadian Journal of Fisheries and Aquatic Sciences 57: 2470-2478.

Hinch, S. G., E. M. Standen, M. C. Healey, and A. P. Farrell. 2002. Swimming patterns and behaviour of upriver-migrating adult pink (*Oncorhynchus gorbuscha*) and sockeye (*O. nerka*) salmon as assessed by EMG telemetry in the Fraser River, British Columbia, Canada. Hydrobiologia 483: 147-160.

Idler, D. R., and W. A. Clemens. 1959. The energy expenditures of Fraser River sockeye salmon during the spawning migration to Chilko and Stuart Lakes. International Pacific Salmon Fisheries Commission Progress Report 6: 1-80.

Jain, K. E., I. K. Birtwell, and A. P. Farrell. 1998. Repeat swimming performance of mature sockeye salmon following a brief recovery period: A proposed measure of fish health and water quality. Canadian Journal of Zoology 76: 1488-1496.

Kinnison, M. T., M. J. Unwin, and T. P. Quinn. 2003. Migratory costs and contemporary evolution of reproductive allocation in male chinook salmon. Journal of Evolutionary Biology 16: 1257-1269.

Kinnison, M. T., M. J. Unwin, A. P. Hendry, and T. P. Quinn. 2001. Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution 55: 1656-1667.

Lee, C. G., A. P. Farrell, A. Lotto, M. J. MacNutt, S. G. Hinch, and M. C. Healey. 2003. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. Journal of Experimental Biology 206: 3239-3251.

Macdonald, J. S. 2000. Mortality during the migration of Fraser River sockeye salmon (*Oncorhynchus nerka*): a study of the effect of ocean and river environmental conditions in 1997. Canadian Technical Report of Fisheries and Aquatic Sciences: 2315.

Magnoni, L. J., D. A. Patterson, A. P. Farrell, and J.-M. Weber. 2006. Effects of long-distance migration on circulating lipids of sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 63: 1822-1829.

McCormick, M. I. 1998. Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. Ecology 79: 1873-1883.

McCormick, M. I. 2006. Mothers matter: crowding leads to stressed mothers and smaller offspring in marine fish. Ecology 87: 1104-1109.

McDonald G., and L. Milligan. 1997. Ionic, osmotic and acid-base regulation in stress. Pp. 119-144 *In* G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. B. Schreck, eds. Fish stress and health in aquaculture. Cambridge University Press, Cambridge, U.K.

Mingist, M., T. Kitani, N. Koide, and H. Ueda. 2007. Relationship between eyed-egg percentage and levels of cortisol and thyroid hormone in masu salmon *Oncorhynchus masou*. Journal of Fish Biology 70: 1045-1056.

Mousseau, T. A., and C. W. Fox. 1998. Maternal effects as adaptations. Oxford University Press, New York.

Munakata, A., M. Amano, K. Ikuta, S. Kitamura, and K. Aida. 2001. The involvement of sex steroid hormones in downstream and upstream migratory behavior of masu salmon. Comparative Biochemistry and Physiology Part B 129: 661-669.

Pankhurst, N. W., and G. Van Der Kraak. 1997. Effects of stress on reproduction and growth in fish. Pp. 73-94 *In* G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. B. Schreck, eds. Fish stress and health in aquaculture. Cambridge University Press, Cambridge, U.K.

Patterson, D. A. 2004. Relating the sockeye salmon (*Oncorhynchus nerka*) spawning migration experience with offspring fitness: a study of intergenerational effects. Masters thesis, Simon Fraser University. Burnaby, Canada.

Patterson, D. A., J. S. MacDonald, S. G. Hinch, M. C. Healey, and A. P. Farrell. 2004a. The effect of exercise and captivity on energy partitioning, reproductive maturation and fertilization success in adult sockeye salmon. Journal of Fish Biology 64: 1039-1059.

Patterson, D. A., H. Guderley, P. Bouchard, J. S. Macdonald, and A. P. Farrell. 2004b. Maternal influence and population differences in activities of mitochondrial and glycolytic enzymes in emergent sockeye salmon (*Oncorhynchus nerka*) fry. Canadian Journal of Fisheries and Aquatic Sciences 61: 1225-1234.

Pon, L. B., S. G. Hinch, G. N. Wagner, A. G. Lotto, and S. J. Cooke. 2007. Swimming performance and morphology of juvenile sockeye salmon, *Oncorhynchus nerka*: comparison of inlet and outlet fry populations. Environmental Biology of Fishes 78: 257-269.

Portz, D. E., C. M. Woodley, and J. J. J. Cech. 2006. Stress-associated impacts of short-term holding on fishes. Reviews in Fish Biology and Fisheries 16: 125-170.

Postlethwaite, E., and D. McDonald. 1995. Mechanisms of Na^+ and Cl^- regulation in freshwater-adapted rainbow trout (*Oncorhynchus mykiss*) during exercise and stress. Journal of Experimental Biology 198: 295-304.

Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. UBC Press, Vancouver.

Rand, P. S., and S. G. Hinch. 1998. Swim speeds and energy use of upriver-migrating sockeye salmon (*Oncorhynchus nerka*): Simulating metabolic power and assessing risk of energy depletion. Canadian Journal of Fisheries and Aquatic Sciences 55: 1832-1841.

Rand, P. S., S. G. Hinch, J. Morrison, M. G. G. Foreman, M. J. MacNutt, J. S. MacDonald, M. C. Healey, A. P. Farrell, and D. A. Higgs. 2006. Effects of river discharge, temperature, and future climates on energetics and mortality of adult migrating Fraser River sockeye salmon. Transactions of the American Fisheries Society 135: 655-667.

Rideout, R. M., E. A. Trippel, and M. K. Litvak. 2004. Paternal effects on haddock early life history traits. Journal of Fish Biology 64: 695-701.

Rossiter, M. 1996. Incidence and consequences of inherited environmental effects. Annual Review of Ecology and Systematics 27: 451-476.

Schreck, C. B., W. Contreras-Sanchez, and M. S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197: 3-24.

Shrimpton, J. M., D. A. Patterson, J. G. Richards, S. J. Cooke, P. M. Schulte, S. G. Hinch, and A. P. Farrell. 2005. Ionoregulatory changes in different populations of maturing sockeye salmon *Oncorhynchus nerka* during ocean and river migration. Journal of Experimental Biology 208: 4069-4078.

Smith, C. C., and S. D. Fretwell. 1974. The optimal balance between size and number of offspring. American Naturalist 108: 499-506.

Standen, E. M., S. G. Hinch, M. C. Healey, and A. P. Farrell. 2002. Energetic costs of migration through the Fraser River Canyon, British Columbia, in adult pink (*Oncorhynchus gorbuscha*) and sockeye (*Oncorhynchus nerka*) salmon as assessed by EMG telemetry. Canadian Journal of Fisheries and Aquatic Sciences 59: 1809-1818.

Tyler, C. R. 1991. Vitellogenesis in salmonids. Pages 295-299 *In* A. P. Scott, J. P. Sumpter, D. E. Kime, and J. Rolfe, eds. Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish. Sheffield University Press, Sheffield, U.K.

Uchida, K., T. Kaneko, A. Yamaguchi, T. Ogasawara, and T. Hirano. 1997. Reduced hypoosmoregulatory ability and alteration in gill chloride cell distribution in mature chum salmon (*Oncorhynchus keta*) migrating upstream for spawning. Marine Biology 129: 247-253.

Yamamoto, T., and U. G. Reinhardt. 2003. Paternal effect on otolith size at emergence in masu salmon. Journal of Fish Biology 62: 1226-1231.

CHAPTER 2

PERSISTENT PARENTAL EFFECTS ON THE SURVIVAL AND SIZE, BUT NOT BURST SWIMMING PERFORMANCE, OF JUVENILE SOCKEYE SALMON (*ONCORHYNCHUS NERKA*)[1]

INTRODUCTION

Pacific salmon (*Oncorhynchus* spp.) face a wide range of selective pressures over the course of their early development – to the extent that the average survival of eggs and yolk-sac alevin in the wild has been estimated at around 8% (Bradford 1995). After hatching and emerging from their gravel redds, fry must compete for food while avoiding predation, and in some cases must migrate within their watershed to reach freshwater rearing grounds (Groot and Margolis 1991; Hinch et al. 2006). Fish must then attempt to increase their size as much as possible in order to improve their odds of survival (Quinn 2005).

Early life history traits can be influenced by both extrinsic (e.g. water quality, food availability) and intrinsic (e.g. yolk supply) factors. Among the latter, a key consideration is the extent to which juvenile traits are a function of their parentage. Instances of 'parental influences' have been described in many fish species. Maternal effects on progeny traits (typically size) are often documented (Berg et al. 2001; Trippel et al. 2005; McCormick 2006), given the importance of the female-mediated provisioning of nutrients and other compounds (e.g. hormones) to the eggs (Brooks et al. 1997; Berg et al. 2001). Nonetheless, the relative impact of maternal identity in affecting offspring fitness can vary by species, populations, or by a range of environmental conditions. In

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addition, the strength of effects can vary in time, according to the developmental stage (Kamler 2005; Trippel et al. 2005). In an experimental setting, the detection of maternal influences may depend on which progeny responses (e.g. size, survival, etc.) are under consideration (Keckeis et al. 2000).

The majority of studies examining maternal influences in fish have been limited to eggs or hatchlings, much less frequently including fry beyond the onset of exogenous feeding. This is presumably because of logistic limitations in long-term rearing, and the fact that parental influences on offspring traits are known to decrease over time (Mousseau and Fox 1998; Heath et al. 1999). Even so, effects of considerable magnitude and temporal persistence have been documented among the relatively few studies which did monitor juveniles beyond exogenous feeding (e.g. Garenc et al. 1998; Heath et al. 1999).

The existence of persistent maternal effects in salmon (e.g. lasting effects of the initial yolk supply) could allow for parentage to shape population dynamics through effects on alevin and fry viability. Juvenile sockeye salmon *O. nerka* (Walbaum), for example, generally spawn in streams and migrate to lakes where they rear for at least one year (Burgner 1991). Given that these migrations typically occur a few months after hatching and can be associated with heightened water flows and predation risk, in particular when rearing lakes are situated upstream of redds (Brannon 1972), persistent maternal effects on swimming ability could be an important factor underlying migration success.

Experimental assessments of salmonid swimming ability have been undertaken for decades, largely pioneered by the work of Brett (e.g. Brett 1964, 1967). In studies

specific to juveniles, comparisons have been made between species (Hawkins and Quinn 1996; McDonald et al. 1998) and between populations of conspecifics differing in life history and/or morphological traits (Taylor and McPhail 1985; Taylor and Foote 1991; Pon et al. 2007). To our knowledge, the swimming ability of juvenile salmonids has never been investigated as a function of parental identity, and has only rarely been considered in non-salmonid species (e.g. Garenc et al. 1998; Green and McCormick 2005).

The potential for maternal influences to affect swimming performance in salmon is supported by previous work on juvenile enzymatic activity. In comparing maternal broodlines of sockeye salmon fry from Weaver and Gates creeks, Patterson et al. (2004a) found significant differences in the whole-body activity of several enzymes, including lactate dehydrogenase (LDH), an indicator of anaerobic glycolytic capacity. Given the primarily anaerobic pathways invoked by burst swimming, maternal influences on enzyme activity could also translate into important effects on this type of swimming performance.

Our objective in the present study was to investigate the role of female sockeye in shaping juvenile traits (namely survival, size, and burst swimming ability), as well as the magnitude and persistence of these effects. To this end, we created full-sib families from wild Weaver Creek spawners and reared them separately for several months beyond hatching. Following this period, we evaluated fry survival, size, and burst swimming ability in order to test the hypothesis that maternal identity accounted for a significant proportion of the variability in these traits.

METHODS

Study population and fertilizations

Our study population consisted of sockeye salmon from Weaver Creek, one of the major sockeye stocks in the Fraser River basin of southern British Columbia, Canada. From mid-September to late October, adults return to their native spawning grounds (which include both Weaver Creek itself and the adjacent artificial spawning channel), located approximately 117 km from the mouth of the Fraser River and 10 m above sea level (Figure 2.1). Fry begin to emerge in March and undertake an approximately 8 km migration to Harrison Lake, the last 5 km of which requires upstream swimming in the Harrison River (D. Patterson, Fisheries and Oceans Canada, pers. comm.).

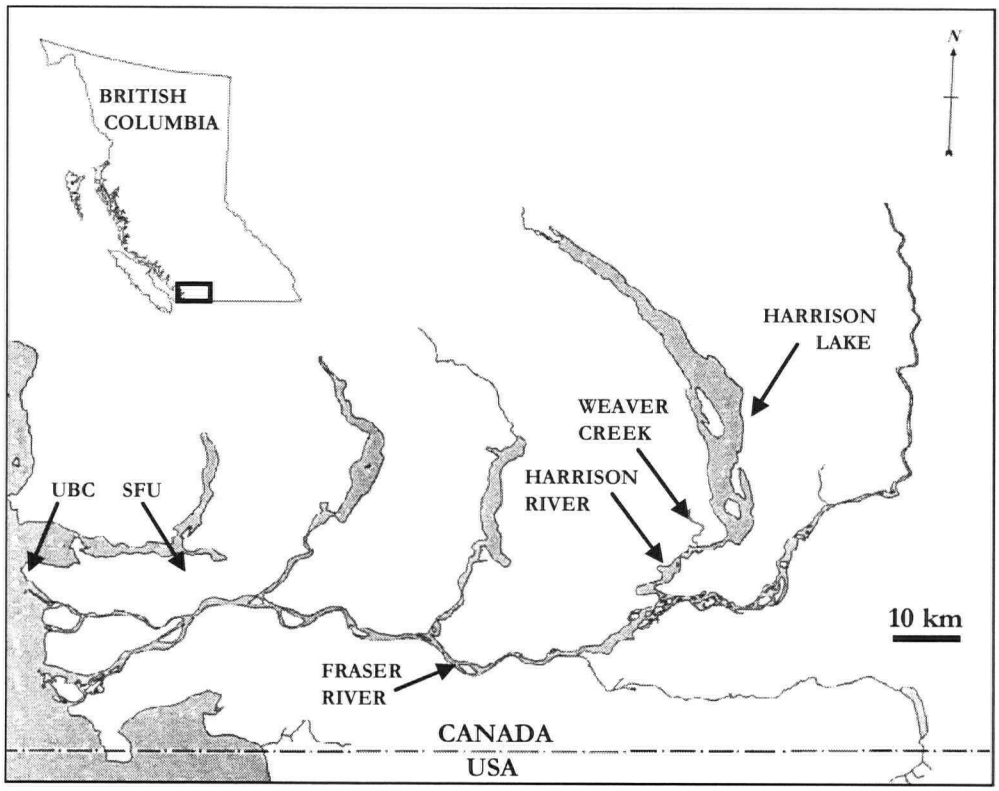

Figure 2.1. Features of the study area and relative location within British Columbia (inset). Laboratory locations are abbreviated: University of British Columbia (UBC); Simon Fraser University (SFU). Scale is approximate.

All procedures described below were conducted in accordance with guidelines by the Canadian Council on Animal Care and approved by the University of British Columbia Animal Care Committee (protocol number A05-0424).

On 26 October 2004, we dip-netted eight female and four male sockeye from the Weaver Creek spawning channel. Ripeness was confirmed by the extrusion of eggs or milt after gentle abdominal pressure, after which fish were immediately sacrificed by cerebral concussion. Eggs and milt were extracted by abdominal pressure into individual plastic containers, taking care to avoid water and blood contamination. Three 10-egg subsamples were weighed (± 0.1 mg) and averaged to calculate individual egg mass. Gametes were stored at 4 °C and transported to Simon Fraser University (Figure 2.1, <2 h transport time) for fertilization. A full factorial complement of crosses (8 females × 4 replicate males) was created following the fertilization protocol of Patterson et al. (2004b).

The resulting 32 families were transferred to separate netted capsules for incubation in Heath trays. However, not all families could be reared in a common tray. To account for putative positional effects, a randomized complete block design (RCBD) was implemented whereby progeny from each female were distributed across four discrete blocks (each consisting of one half of a Heath tray). Each block was established with offspring from a single male, such that any paternal variability would also be accounted for. Thus, all eight females were represented once per block. Incubating eggs were checked routinely until yolk-sac absorption, and dead eggs removed. During this period, lab water temperature followed seasonal decline from 16 °C to 7 °C.

Rearing

Upon yolk sac absorption (early March), families were transported to the University of British Columbia (Figure 2.1, <1 h transport time), where they were transferred to 10 L netted rearing enclosures placed inside 400 L flow-through tanks. Maternal broodlines were assigned to placement within tanks following the RCBD described above, with each block (tank) retaining its original eight families. Every effort was made to maintain homogenous rearing conditions for all fry. Enclosures were routinely rearranged within a given block to avoid subjecting maternal broodlines to positional effects. Lighting conditions were similar throughout the laboratory and adjusted bi-weekly to reflect natural photoperiod. Fish were fed powdered fishmeal (EWOS Canada Ltd.) in two daily instalments of 1% total body mass.

Comparable enclosure densities (initially approx. 1 fish $\cdot$ dL^{-1}) were maintained and adjusted by removing fish from families with the most individuals when necessary. We were unable to correct densities in five enclosures with markedly lower densities than any of the others due to high mortality or low original number of fertilized eggs. (We did not wish to potentially compromise sample sizes by excessive removals in all enclosures to match the lowest densities). Since the lower densities were maintained for several months prior to swim testing, thereby increasing the likelihood of confounding size and swim performance comparisons, we excluded the five families from these analyses. This was confirmed to be a conservative approach, since their inclusion resulted in lower p-values than those reported hereafter. Thus, 27 families were included in size and swimming assessments, among which all but one of the eight females were represented in a minimum of three blocks (one female accounted for two of the five excluded families).

Swim tests and measurements

We assessed burst swimming ability as a measure of swim performance and a component of overall fitness. Burst swimming performance was defined as an effort that could be sustained for a short period (generally 20 s or less, *sensu* Beamish 1978; Pon et al. 2007), but of longer duration than a startle response spanning mere fractions of a second, for which the term "burst swimming" has also been used (e.g. Guderley et al. 2001; Franklin et al. 2003). A logistical advantage of using burst swimming tests rather than prolonged swimming trials (i.e. U_{crit}) is that the former require less time per trial, allowing for a greater number of individuals to be tested. In addition, burst swimming is biologically relevant to the life history stage we observed – both in terms of predator avoidance (Taylor and McPhail 1985) and for enabling fry whose nursery lakes are upstream from their redds (e.g. Weaver sockeye, Figure 2.1) to reach these lakes during spring migrations (Pon et al. 2007).

We conducted burst swimming tests on 16 fry per maternal broodline, consisting of four randomly selected full sibs from each experimental block (4 offspring × 4 blocks × 8 females). A maximum of 16 fry could be tested per day. Consequently, all offspring were tested in eight days from 30 June to 12 July 2005, with no testing taking place between 5–9 July. To minimize effects of testing date on swim performance, maternal half sib families were staggered over the full duration of the experiment, such that offspring from each female were tested on four separate days.

Preliminary experiments showed that fish would not burst without prior acclimation to directional current. Consequently, fry were first removed from their rearing enclosures 48 hours before testing and placed in open-top acclimation raceways

(30 cm length × 10 cm width × 5 cm depth), where they were subjected to a constant 'current acclimation velocity' of 4.5 cm s^{-1}. Food was withheld during this period.

Burst trials were conducted in an open-top rectangular flume (230 cm length × 17 cm width × 4.75 cm depth, described in detail by Pon et al. 2007) with an available 'swimming area' of 50 cm length × 5 cm width in the forward section. Mesh gates were located upstream and downstream to prevent escapes. To initiate all trials with fish in a standard starting position, an opaque screen was placed above the foremost 10 cm of the swimming area. This screen cast a shadow which effectively enticed fish to remain within 10 cm of the front mesh gate. At the downstream end of the flume, well beyond the swimming area, a removable acrylic gate was installed to control current velocity. In its 'lowered' configuration, the gate allowed for partial spill-over of flume water, producing an initial 'flume acclimation velocity' of 10 cm s^{-1} in the swimming area. On test day, a single fish at a time was transferred from its acclimation raceway into the swimming area of the flume, where it was subjected to the acclimation velocity for four minutes. After this period the rear gate was rapidly removed, thereby allowing water to flow out of the flume unhindered and increasing current speed in the swimming area to 22 cm s^{-1} (the burst test velocity) while reducing water depth to 2.2 cm. The transition from acclimation velocity to test velocity required approximately two seconds following gate removal. Therefore, the trial was deemed to begin at $T_0 = T_{\text{gate lifted}} + 2$ s, and ended (T_F) when the fish had drifted back 20 cm (approximately five body lengths) from its position at T_0, measured at the tip of the snout. Two video cameras (Panasonic WV-BP312) simultaneously recorded the trial from overhead (Camera 1) and through the transparent lateral wall of the swimming area (Camera 2). A VHS time-lapse recorder (Panasonic

AG-6124) captured the images from both cameras onto the same video frames for split-screen viewing, which enabled the determination of T_0 and T_F to the nearest 0.1 s (full details on recording equipment and cameras available in Hinch and Rand 2000). Water temperature throughout the trials was maintained at 10.5 °C (± 0.2). Immediately following a trial, fish were removed from the flume and sacrificed by overexposure to tricaine methanesulfonate (MS-222), after which they were blotted dry, measured (± 0.1 mm), and weighed (± 0.1 mg).

While most fish oriented into the current when first introduced to the flume and then moved to the front of the swimming area, some did not immediately swim but rather became pinned against the rear mesh. In such cases, a blunt probe was used to stimulate swimming. If the fish remained unresponsive after three seconds of probing, two further attempts were made at 30 second intervals. The required number of these interventions was recorded and considered to be indicative of an individual's 'willingness to swim'. In this manner, fish were classified as having 'high', 'medium', or 'low' willingness to swim (0, 1, or ≥2 interventions needed, respectively).

Data analysis

Maternal influences on progeny survival were analyzed by random-effects ANOVAs. Offspring size and swimming performance were analyzed similarly, with family means as response variables (full sibs were not considered independent) and including test date as a covariate. When the latter was not significant, models were reduced to two-way ANOVAs. F-tests (type III SS) were used to determine statistical significance, and variance components were estimated by restricted maximum likelihood

(REML). Size (length and mass), swimming performance, and survivorship variables were transformed (log, square-root, and arcsine-square-root, respectively) in order to meet normality and homoskedasticity assumptions. Given the significant maternal influences on egg survival to hatch and fry mass (see Results), the role of initial egg mass on these variables was investigated by linear regression, using overall female averages as response variables (n = 8). When no maternal effects on burst swimming were detected (see Results), the swimming performance of individuals was further analyzed by linear regression using offspring length, mass, and testing order as independent variables. Pre-trial willingness to swim was analyzed as a function of maternal identity (by contingency analysis) and of individual size and/or testing order (by multinomial regression). Statistical procedures were performed with SAS 9.1 (SAS Institute, USA) with the significance level set at $\alpha = 0.05$ for all analyses.

RESULTS

All crosses yielded viable offspring. Survivorship of incubating eggs was generally very high, with an average of 95% of eggs in a family surviving to hatching (n = 32, Table 2.1). Both maternal identity and blocks significantly affected survival to hatch (P = 0.049 and P = 0.041, respectively, Table 2.1). Egg size alone did not significantly predict mean egg survival for a given female (n = 8, P = 0.21). Between the onset of exogenous feeding and swim tests, two unexplained instances of elevated mortality occurred throughout the lab: one in mid-March and another in mid-April. Overall fry survival during this period was not attributable to maternal influence (P = 0.28) and was not related to pre-hatch survival (P = 0.38), but a significant block effect

(P = 0.001) was noted (Table 2.1). To visualize the magnitude of inter-female variability on progeny survival, as well as the unexpected significant influence of blocks, survivorship data were plotted for each family (Figure 2.2).

Mean family length on test day was 38.1 mm, while mean mass was 553.8 mg (Table 2.1). Mass differences were relatively large between fry from different maternal families (approximately 200 mg between the smallest and largest maternal averages, Figure 2.2). Mass was significantly predicted by female identity (P = 0.027), but not by original egg mass, although a trend in this direction was apparent (n = 8, r^2 = 0.45, P = 0.068). Maternal effects on length were not significant (P = 0.07) despite the high correlation between mass and length (r = 0.97).

Table 2.1. Full-sib family means and ranges of offspring attributes (n = 32 for survival; n = 27 for size and swimming performance variables) and associated analysis of variance outputs. Significant effects are in bold. Variance components are REML estimates.

	Mean and range			**Analysis of variance**				
Offspring attribute	Mean (S.D.)	Min.	Max.	Effect	D.F.	F	*P*	% Var.
Survival to hatch (%)	95.0 (6.0)	78.0	100.0	Female	7	2.51	**0.049**	22.7
				Block	3	3.29	**0.041**	17.2
Fry survival (%)	86.0 (10.0)	61.0	100.0	Female	7	1.35	0.277	4.5
				Block	3	7.66	**0.001**	43.4
Fry mass (mg)	553.8 (86.0)	420.6	771.5	Female	7	3.23	**0.027**	32.2
				Block	3	1.72	0.205	0.1
				Test day	1	5.27	**0.037**	
Fry length (mm)	38.1 (1.6)	35.3	41.9	Female	7	2.37	0.073	7.9
				Block	3	2.02	0.152	0.0
Burst swimming performance (s)	14.5 (4.2)	4.9	24.2	Female	7	1.61	0.203	13.8
				Block	3	0.71	0.558	0.0

There was considerable behavioural variation in the willingness of fish to swim at the flume acclimation speed when first introduced into the swimming area, although all fish eventually showed the ability to swim at this speed. The majority of individuals (58 fish, 56.9%) began swimming without intervention ('high' willingness to swim). A further 25 fish (24.5%) showed 'medium' motivation, while 19 (18.6%) were considered to have 'low' willingness to swim. Willingness to swim was unaffected by maternal identity (chi-square $P = 0.20$), and did not depend on offspring size and/or testing order (all models $P > 0.40$). Ultimately, groups of fish showing differences in initial motivational status did not differ in burst swimming abilities ($P = 0.34$).

Given the range of juvenile lengths on test day (Table 2.1), fish encountered the absolute burst test speed (22 cm s^{-1}) at a relative velocity (in body lengths per second, BL s^{-1}) of 5.3 to 6.2 BL s^{-1}. Mean burst swimming performance was 14.5 s, after excluding an outlier in one family which swam for 132.4 s (video analysis showed this fish exploiting a region of irregular current within the flume). Maternal identity did not significantly affect burst swimming performance ($P = 0.20$, Table 2.1), nor did offspring mass ($P > 0.40$) when included as a covariate. The swimming performances of individual fish (irrespective of maternal identity) were not significantly affected by testing order ($P = 0.39$), length ($P = 0.10$), or mass ($P = 0.08$, Figure 2.3), underscoring very large inter-individual differences in burst swimming ability.

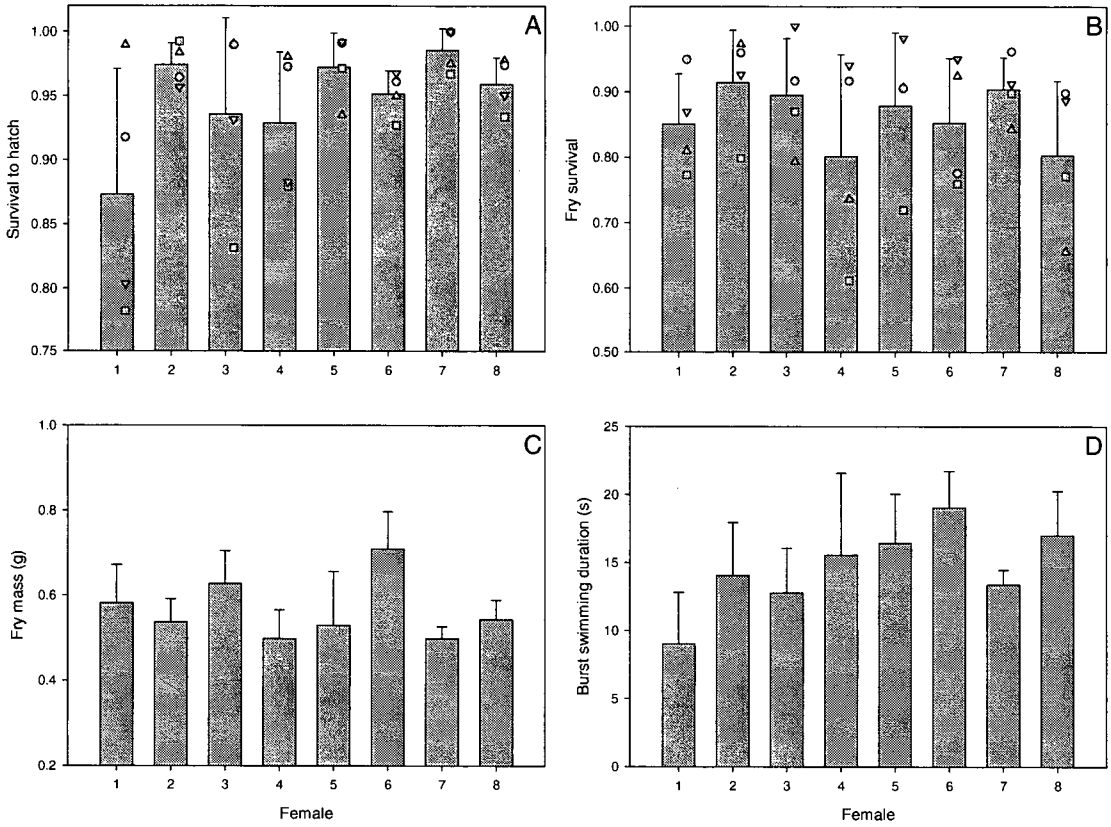

Figure 2.2. Maternal family means (+ S.D.) for selected offspring traits. To illustrate the significant effect of blocks on egg survival to hatch (A) and on fry survival between emergence and swim trials (B), each block is identified by a unique symbol. Raw data are used for presentation; date-adjusted masses (not shown) yielded very similar maternal means to those in (C).

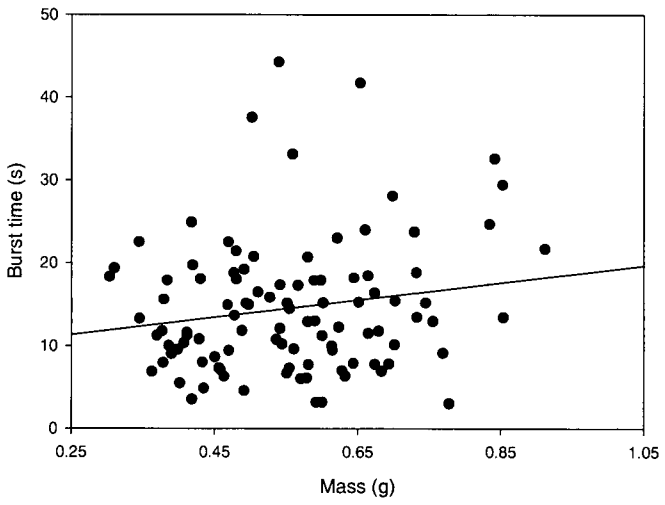

Figure 2.3. Linear regression of burst swimming times for individual fry as a function of mass ($r^2 = 0.03$, $P = 0.08$).

DISCUSSION

We studied the role of female sockeye salmon in shaping survival, size, and burst swimming ability in their exogenously feeding progeny. Maternal identity was a significant factor in determining egg survival, but this effect did not extend to fry. Our results were suggestive of a male effect (discussed below) on both egg and fry survival, even though we did not originally seek to evaluate paternal influences. Progeny mass, but not length, was under significant maternal influence. Burst swimming performances were highly variable and not influenced by maternal identity or individual fry size.

Survival

Females significantly affected egg survival, in line with the findings of previous studies (e.g. Nagler et al. 2000, Patterson et al. 2004b, Johnston et al. 2007). Factors other than egg quality likely increase in their influence on survival as juveniles age, potentially explaining why females did not significantly affect fry survival. Original egg mass was not a significant predictor of survival to hatching, akin to findings reported elsewhere (e.g. Keckeis et al. 2000; Patterson 2004; Chapter 3 of this thesis), suggesting that the maternal effect on egg viability was being influenced by other egg quality parameters not measured in the present study.

We did not anticipate the experimental block influence observed on both egg and fry survival. Providing a definitive mechanism to explain this finding was complicated by two factors. First, the proximate causes of mortality in eggs and fry were not generally known, making it difficult to identify the underlying sources of block variability. Second, due to the nature of the blocks (i.e. eight maternal broodlines sharing a common sire and

reared together), it was not possible to conclusively isolate male effects from enclosure effects. Since all reasonable efforts were made to maintain uniform rearing conditions throughout the lab, we propose that paternal influences were an important driver of the significant block effects on progeny survival. It is conceivable that the genetic contributions of different males yielded offspring with differential resistance to pathogens, confinement stress, or other unknown selective pressures. There was empirical evidence to support this suggestion. For example, the block with the overall lowest egg survival (identified by squares in Figure 2.2a) also had the lowest survival as fry (Figure 2.2b). If enclosure effects had been a driving factor, it is unlikely that this block would have been particularly affected both at Simon Fraser University (Heath trays) and at the University of British Columbia (tanks). Even so, enclosure effects could not be ruled out. These effects were more likely to have occurred in tanks (where we found a strong block influence) because of the flow-through setup: any undetected pathogens or water quality problems could have caused mortality in a particular tank without affecting the others. Conversely, Heath trays were less susceptible to environmental effects since blocks were in close proximity and did not have a self-contained water supply. Previous work did not find egg survival to be affected by location within Heath trays identical to those used in the present study (Patterson 2004).

Potentially confounding effects could be avoided in the future by making every attempt to assess male contributions in their own right. Several recent studies have considered male effects explicitly by abandoning the traditionally common practice of pooling milt from multiple males to fertilize females (Rideout et al. 2004). Evidence of paternal influences has been found in various progeny traits (e.g. Garant et al. 2002;

Yamamoto and Reinhardt 2003; Green and McCormick 2005), including cases where effects exceeded those attributable to maternal identity (Rideout et al. 2004).

Size

The detection of significant maternal effects on offspring mass was concurrent with findings from other studies: in fact, progeny size variability is the most frequently reported manifestation of maternal influences (Heath and Blouw 1998). We also noted a trend whereby greater initial egg mass yielded heavier fry on test day ($P = 0.068$). These findings were particularly interesting, suggesting that differences in initial egg provisioning can have lasting consequences on fry, even after four months of exogenous feeding. A similar experiment on a Skeena River sockeye population found that the size of juveniles reflected considerable maternal influences after three months but not after nine months of captive rearing (Bilton 1971). Persistent maternal influences such as these could have important ecological consequences which would be overlooked by researchers if progeny were not monitored beyond exogenous feeding, as is typically the case in this type of study. Additional research is needed to determine the magnitude and persistence of maternal influences at various developmental stages (e.g. during smolt migrations) and under different environmental conditions.

Large fry are commonly considered to hold a fitness advantage over smaller conspecifics due to improved predator avoidance, resistance to starvation, and competitive ability (Einum and Fleming 1999, 2000; Kamler 2005). The degree to which larger fish are also better swimmers may depend on several factors. While this relationship has been confirmed by several authors (Brett 1967; Taylor and McPhail

1985), with size accounting for more than 60% of the variability in juvenile trout sprinting performance (McDonald et al. 1998), others have not found swimming performance to be correlated with individual morphology (Gregory and Wood 1998; Pon et al. 2007; the present study). It is possible that the range of sizes we observed was sufficiently narrow to preclude any major impacts on swimming – individuals at the upper end of the size range did tend to perform better (Figure 2.3). Larger fish may also have been affected by altered flows associated with closer proximity to surfaces (*sensu* Webb 1988) because of the relatively low water level during burst trials. Even so, differences in hydrodynamics due to water depth probably had little overall effect, since all fish were completely submerged during trials and the range of individual body depths, although not directly measured, spanned a few millimetres at most. In any case, the fact that we found individual size to be a very poor predictor of burst swimming ability was suggestive of inherent inter-individual differences, as discussed further below.

Swimming ability

Our prediction that maternal identity would influence burst swimming performance was in part derived from previous work on sockeye juveniles by Patterson et al. (2004a), who found significant maternal differences in the whole-body activity of several enzymes including lactate dehydrogenase (LDH), an indicator of anaerobic glycolytic capacity. Despite the primarily anaerobic pathways invoked by burst swimming, we did not find maternal identity to explain a significant proportion of the variability in swimming performance. Traits under considerable selective pressure such as swimming ability are generally found to be less heritable than their tissue-level

determinants (Falconer and Mackay 1996). For example, Garenc et al. (1998) found that juvenile threespine sticklebacks displayed greater heritability for the biochemical correlates of burst swimming (i.e. enzyme activity) than for burst swimming itself. Our findings highlight the need for caution when attempting to attribute direct ecological relevance to sub-organismal parental effects.

Maternal influences on burst swimming might have been detected by the present study if tests had occurred sooner after fry emergence. We note that the maternal broodline differences in enzyme activities reported by Patterson et al. (2004a) were in unfed emergent fry, while the fish in the current study were much older, having been feeding exogenously for four months. Garenc et al. (1998) found burst swimming to be significantly heritable in two-month-old sticklebacks, but not in those aged 3.6 months, supporting the notion that parental influences eventually decrease with age (Mousseau and Fox 1998; Heath et al. 1999).

Burst trials revealed unexpectedly large individual variability in swimming capacity. Performances were highly variable even within the offspring of a given female, including among full-sibs of similar size tested on the same day (Figures 2.2 and 2.3). These differences were not likely to be caused by artefacts of pre-trial handling, since initial 'willingness to swim', a potential indicator of prior stress or exhaustion, was not a function of testing order and did not predict subsequent burst swimming performance. Given that the present study did not find parentage and size to be good predictors of burst swimming, the underlying causes of individual variability in swimming capacity warrant future investigation. Indeed, individual variation in swimming performance is increasingly being viewed as "something of real importance worthy of study" (Gregory

and Wood 1998) rather than an annoyance to be dismissed as statistical noise. Findings from the present study are among the few to emphasize that this variation can be important even within juveniles of common parentage.

Collectively, our results highlight the need for scientists and managers to consider both maternal and paternal influences as an integral component of population dynamics in salmon, including in the months following emergence. An understanding of the magnitude and persistence of parental effects, of the spawner attributes that may generate them (e.g. the environmental conditions encountered by migrating adults), and of the factors underlying inter-individual variability will be essential in developing a more holistic view of population-level spawning success and fry survival.

REFERENCES

Beamish, F. W. H. 1978. Swimming capacity. Pp. 101-187 *In* W. S. Hoar and D. J. Randall, eds. Fish physiology, Volume 7. Academic Press, New York.

Berg, O. K., A. P. Hendry, B. Svendsen, C. Bech, J. V. Arnekleiv, and A. Lohrmann. 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.

Bilton, H. T. 1971. A hypothesis of alternation of age of return in successive generations of Skeena River sockeye salmon (*Oncorhynchus nerka*). Journal of the Fisheries Research Board of Canada 28: 513-516.

Bradford, M. J. 1995. Comparative review of Pacific salmon survival rates. Canadian Journal of Fisheries and Aquatic Sciences 52: 1327-1338.

Brannon, E. L. 1972. Mechanisms controlling migration of sockeye salmon fry. International Pacific Salmon Fisheries Commission Bulletin 21: 1-86.

Brett, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. Journal of the Fisheries Research Board of Canada 21: 1183-1226.

Brett, J. R. 1967. Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. Journal of the Fisheries Research Board of Canada 24: 1731-1741.

Brooks, S., C. R. Tyler, and J. P. Sumpter. 1997. Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7: 387-416.

Burgner, R. L. 1991. Life history of sockeye salmon (*Oncorhynchus nerka*). Pp. 1-118 *In* C. Groot and L. Margolis, eds. Pacific salmon life histories. UBC Press, Vancouver.

Einum, S. 2003. Atlantic salmon growth in strongly food-limited environments: effects of egg size and paternal phenotype? Environmental Biology of Fishes 67: 263-268.

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

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

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Longman, Essex, U.K.

Franklin, C. E., R. S. Wilson, and W. Davison. 2003. Locomotion at -1.0°C: burst swimming performance of five species of Antarctic fish. Journal of Thermal Biology 28: 59-65.

Garant, D., P.-M. Fontaine, S. P. Good, J. J. Dodson, and L. Bernatchez. 2002. The influence of male parental identity on growth and survival of offspring in Atlantic salmon (*Salmo salar*). Evolutionary Ecology Research 4: 537-549.

Garenc, C., F. G. Silversides, and H. Guderley. 1998. Burst swimming and its enzymatic correlates in the threespine stickleback (*Gasterosteus aculeatus*): full-sib heritabilities. Canadian Journal of Zoology 76: 680-688.

Green, B. S., and M. I. McCormick. 2005. Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. Marine Ecology Progress Series 289: 263-272.

Gregory, T. R., and C. M. Wood. 1998. Individual variation and interrelationships between swimming performance, growth rate, and feeding in juvenile rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 55: 1583-1590.

Groot, C., and L. Margolis. 1991. Pacific salmon life histories. UBC Press, Vancouver.

Guderley, H., P. H. Leroy, and A. Gagné. 2001. Thermal acclimation, growth, and burst swimming of threespine stickleback: enzymatic correlates and influence of photoperiod. Physiological and Biochemical Zoology 74: 66-74.

Hawkins, D. K., and T. P. Quinn. 1996. Critical swimming velocity and associated morphology of juvenile coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead trout (*Oncorhynchus mykiss*), and their hybrids. Canadian Journal of Fisheries and Aquatic Sciences 53: 1487-1496.

Heath, D. D., and D. M. Blouw. 1998. Are maternal effects in fish adaptive or merely physiological side effects? Pp. 178-201 *In* T. A. Mousseau and C. W. Fox, eds. Maternal effects as adaptations. Oxford University Press, New York.

Heath, D. D., C. W. Fox, and J. W. Heath. 1999. Maternal effects on offspring size: variation through early development of chinook salmon. Evolution 53: 1605-1611.

Hinch, S. G., S. J. Cooke, M. C. Healey, and A. P. Farrell. 2006. Behavioural physiology of fish migrations: salmon as a model approach. Pp. 239-295 *In* K. A. Sloman, R. W. Wilson, and S. Balshine, eds. Fish physiology, Volume 24. Academic Press, London.

Hinch, S. G., and P. S. Rand. 2000. Optimal swimming speeds and forward-assisted propulsion: energy-conserving behaviours of upriver-migrating adult salmon. Canadian Journal of Fisheries and Aquatic Sciences 57: 2470-2478.

Johnston, T. A., M. D. Wiegand, W. C. Leggett, R. J. Pronyk, S. D. Dyal, K. E. Watchorn, S. Kollar, and J. M. Casselman. 2007. Hatching success of walleye embryos in relation to maternal and ova characteristics. Ecology of Freshwater Fish 16: 295-306.

Kamler, E. 2005. Parent-egg-progeny relationships in teleost fishes: an energetics perspective. Reviews in Fish Biology and Fisheries 15: 399-421.

Keckeis, H., E. Bauer-Nemeschkal, V. V. Menshutkin, H. L. Nemeschkal, and E. Kamler. 2000. Effects of female attributes and egg properties on offspring viability in a rheophilic cyprinid, *Chondrostoma nasus*. Canadian Journal of Fisheries and Aquatic Sciences 57: 789-796.

McCormick, M. I. 2006. Mothers matter: crowding leads to stressed mothers and smaller offspring in marine fish. Ecology 87: 1104-1109.

McDonald, D. G., W. J. McFarlane, and C. L. Milligan. 1998. Anaerobic capacity and swim performance of juvenile salmonids. Canadian Journal of Fisheries and Aquatic Sciences 55: 1198-1207.

Mousseau, T. A., and C. W. Fox. 1998. Maternal effects as adaptations. Oxford University Press, New York.

Nagler, J. J., J. E. Parsons, and J. G. Cloud. 2000. Single pair mating indicates maternal effects on embryo survival in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 184: 177-183.

Patterson, D. A. 2004. Relating the sockeye salmon (*Oncorhynchus nerka*) spawning migration experience with offspring fitness: a study of intergenerational effects. Masters thesis, Simon Fraser University. Burnaby, Canada.

Patterson, D. A., H. Guderley, P. Bouchard, J. S. Macdonald, and A. P. Farrell. 2004a. Maternal influence and population differences in activities of mitochondrial and glycolytic enzymes in emergent sockeye salmon (*Oncorhynchus nerka*) fry. Canadian Journal of Fisheries and Aquatic Sciences 61: 1225-1234.

Patterson, D. A., J. S. MacDonald, S. G. Hinch, M. C. Healey, and A. P. Farrell. 2004b. The effect of exercise and captivity on energy partitioning, reproductive maturation and fertilization success in adult sockeye salmon. Journal of Fish Biology 64: 1039-1059.

Pon, L. B., S. G. Hinch, G. N. Wagner, A. G. Lotto, and S. J. Cooke. 2007. Swimming performance and morphology of juvenile sockeye salmon, *Oncorhynchus nerka*: comparison of inlet and outlet fry populations. Environmental Biology of Fishes 78: 257-269.

Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. UBC Press, Vancouver.

Rideout, R. M., E. A. Trippel, and M. K. Litvak. 2004. Paternal effects on haddock early life history traits. Journal of Fish Biology 64: 695-701.

Rossiter, M. 1996. Incidence and consequences of inherited environmental effects. Annual Review of Ecology and Systematics 27: 451-476.

Taylor, E. B., and C. J. Foote. 1991. Critical swimming velocities of juvenile sockeye salmon and kokanee, the anadromous and non-anadromous forms of *Oncorhynchus nerka* (Walbaum). Journal of Fish Biology 38: 407-419.

Taylor, E. B., and J. D. McPhail. 1985. Burst swimming and size-related predation of newly emerged coho salmon *Oncorhynchus kisutch*. Transactions of the American Fisheries Society 114: 546-551.

Trippel, E. A., G. Kraus, and F. W. Köster. 2005. Maternal and paternal influences on early life history traits and processes of Baltic cod *Gadus morhua*. Marine Ecology Progress Series 303: 259-267.

Webb, P. W. 1988. Simple physical principles and vertebrate aquatic locomotion. American Zoologist 28: 709-725.

Yamamoto, T., U. G. Reinhardt. 2003. Paternal effect on otolith size at emergence in masu salmon. Journal of Fish Biology 62: 1226-1231.

CHAPTER 3

ENERGY ALLOCATIONS, REPRODUCTIVE DEVELOPMENT, AND PARENTAL EFFECTS IN SOCKEYE SALMON (*ONCORHYNCHUS NERKA*): INFLUENCES OF A SIMULATED MIGRATORY CHALLENGE[2]

INTRODUCTION

Pacific salmon (*Oncorhynchus* spp.) are well known for spawning migrations which involve remarkable feats of navigation and endurance. Beginning in the ocean where adults cease feeding, migrations end when fish reach their native freshwater spawning grounds, sometimes in excess of 1000 km upstream. Spawning occurs over a matter of days or weeks and death inevitably follows (Groot and Margolis 1991).

Many aspects of migration are under strong genetic control, a result of adaptations to the river conditions which optimize migratory efficiency and offspring fitness (Quinn 2005). From year to year, however, river conditions may depart from the historical baseline to which migrants have adapted. High water temperatures or increased flows may create particularly challenging conditions with potential impacts on migration success. Difficult migratory conditions have been shown to impede upstream progress (Standen et al. 2002), to amplify stress responses (Macdonald 2000), and to contribute to in-river mortality (Rand and Hinch 1998; Macdonald 2000).

Because upstream migration occurs concurrently with the most important stages of sexual maturation (Hendry and Berg 1999), it might be expected that reproductive functions could also be influenced by migratory difficulty. Yet relatively little is known

[2] A version of this chapter (describing the 2006 experiment only) will be submitted for publication in a peer-reviewed journal. Nadeau, P. S., S. G. Hinch, K. A. Hruska, and D. A. Patterson. Energy allocations, reproductive development, and parental effects in sockeye salmon (*Oncorhynchus nerka*): influences of a simulated migratory challenge.

about how difficult conditions might affect reproductive development, and lesser still about the potential for such effects to have an intergenerational impact on the early life history traits of progeny.

One possibility is that difficult migrations could deleteriously affect reproductive traits via energetic trade-offs. Since migrations are entirely fueled by the energy stores acquired during ocean residency (fish do not feed during migration), a fixed energy budget must be adequately partitioned into various necessities, including upstream locomotion (Rand and Hinch 1998; Hinch and Rand 2000), development of gonads and secondary sexual traits, (Hendry and Berg 1999; Kinnison et al. 2001, 2003), and spawning ground behaviours such as site acquisition, displays, and the building and defending of redds (Healey et al. 2003). In this manner, difficult migrations could lead to an increase in the resources spent on locomotion, at the expense of reproductive development (e.g. egg size).

Previous studies have shown that adverse environmental conditions can indeed increase locomotory costs in migrating salmon. For example, assessments of energy use in sockeye (*O. nerka*) and pink salmon (*O. gorbuscha*) via electromyogram (EMG) telemetry revealed that migrants faced with higher velocity currents and more complex hydraulic patterns adopted behaviours that resulted in increased energetic depletion (Hinch et al. 2002; Standen et al. 2002). A study of Early Stuart sockeye suggested that adults migrating during a year of unusually high Fraser River flows tended to invoke catabolism of muscle lipids and proteins at a relatively early stage in their migration (Macdonald 2000). Other studies corroborated the link between migration difficulty and increased energy use while also providing evidence for energetic trade-offs. Upper Fraser

River sockeye populations have been shown to use more energy while migrating and to invest less in reproductive development (e.g. lower fecundity and egg mass) than lower river populations (Idler and Clemens 1959; Crossin et al. 2004), reflecting the greater trade-offs between swimming and reproductive allocations imposed by more difficult migrations. Perhaps the strongest support for the existence of energetic trade-offs came from the work of Kinnison et al. (2001, 2003) on New Zealand chinook salmon (*O. tshawytscha*). In a large-scale reciprocal transplant experiment, the authors tagged juveniles from two populations and retrieved them several years later as adults returning to spawn. Females returning to a distant inland site (100 km up-river) had smaller ovarian mass, egg size, and muscle solids than those from the same original population (or even family) returning to the closer site (17 km; Kinnison et al. 2001). Likewise, males migrating the longer distance had smaller humps and snouts, in addition to lower tissue energy reserves (Kinnison et al. 2003).

Difficult migrations could also impact reproductive success by increasing physiological stress in migrants. Prolonged and/or exhaustive exercise is known to induce stress responses such as osmotic imbalances (Postlethwaite and McDonald 1995; McDonald and Milligan 1997) and increases in plasma glucose and lactate (Wedemeyer et al. 1990). Stressed fish may become immunosuppressed (Pickering and Pottinger 1989; Wendelaar Bonga 1997), thus more vulnerable to opportunistic infections which in turn further increase stress levels. Physiological stress could impact reproductive success by suppressing reproductive hormone concentrations (Pankhurst and Van Der Kraak 1997; Kubokawa et al. 1999), which could delay maturation and lead to a suboptimal spawning date. Stress could also have a direct impact on progeny viability by reducing the quality

of gametes themselves (Campbell et al. 1994; McCormick 2006). Nonetheless, the mechanisms by which reproductive traits might be affected by stress are complex and not fully understood (Pankhurst and Van Der Kraak 1997).

Few studies have experimentally assessed the effects of migratory conditions on reproductive parameters. A notable exception was the previously described work of Kinnison et al. (2001, 2003). Another was a study of Fraser River sockeye by Patterson et al. (2004), who intercepted Early Stuart adults at the onset of migration and 'released' them from their normally difficult migrations (>1000 km), allowing them instead to reach sexual maturity in one of two captive treatments – exposure to a moderate current (0.55 m s^{-1}) or holding in the absence of directional flow. After several weeks, gametes were collected from mature fish and artificial fertilizations were conducted. The authors found that although fish held in the absence of current maintained higher energy levels, this 'surplus' energy was not reallocated into sexual traits (e.g. gonad size, fecundity). This implied that energetic trade-offs in migrating salmon might be unidirectional: contrary to the potential inhibitory effects of energetically demanding migrations on reproductive traits (e.g. Kinnison et al. 2001, 2003), favourable conditions did not lead to enhanced reproductive development.

Despite the above findings, considerable knowledge gaps remain. For example, the endpoint assessment of migrants by Kinnison et al. (2001, 2003) did not allow for quantifying individual-level physiological status (e.g. energy stores, stress responses) throughout migration – an approach that might have yielded information on the underlying causes of the observed trade-offs. Moreover, gamete viability and offspring fitness were not examined, such that the potential intergenerational impacts of these

trade-offs was not explicitly determined. Conversely, Patterson et al. (2004) assessed the energetic and physiological status of the fish in their captive treatments and conducted artificial fertilizations to evaluate progeny. However, these treatments involved a reduction in migratory difficulty relative to the natural migration faced by these fish. Thus, fish were not challenged to a point where energetic constraints might have hindered reproductive development.

Our overall approach in the present study was to assign sexually maturing sockeye salmon to treatments representing two different 'migration difficulties', both intended to be more challenging than natural migrations. Both before and after these treatments, we measured a suite of variables (i.e. morphology, energy levels, osmoregulatory status, sex traits, and stress) to determine how migratory difficulty affected fish condition, especially in terms of reproductive development (*sensu* Kinnison et al. 2001, 2003) and subsequent progeny fitness. We predicted that migrants subjected to the more difficult migration (i.e. faster current speeds) would undergo greater energetic depletion and be more stressed, and thus display reduced investment in sexual traits (e.g. smaller eggs), resulting in poorer progeny fitness. We also anticipated that treatment effects would be more pronounced in females, given their greater energetic investment into gonad development (Hendry and Berg 1999; Crossin et al. 2004).

METHODS

Study population

Our study focused on sockeye salmon from the Weaver Creek population, one of the major sockeye stocks in the Fraser River basin of southern British Columbia, Canada (Figure 3.1). Weaver sockeye are classified as a 'late-run' stock, with river migrations normally starting in mid-September and spawning taking place from early to late October (R. Stitt, Fisheries and Oceans Canada, pers. comm.). Most migrants spend one to three weeks holding in nearby Harrison Lake or Harrison River (Figure 3.1) prior to returning to spawn in Weaver Creek or the adjacent artificial spawning channel (Cooke and Hinch 2005). Weaver adults experience a relatively short upriver spawning migration (approximately 117 km) and unlike the majority of Fraser sockeye populations, are not required to pass through the Fraser River canyon and Hell's Gate, an area of high flows notorious for impeding migratory progress (Hinch and Bratty 2000). Moreover, Weaver sockeye are known to be among the least energetically efficient migrants of the Fraser populations (Crossin et al. 2004) and those which experience the most dramatic ionoregulatory changes per unit of migratory distance (Shrimpton et al. 2005). These were considered desirable characteristics for the purposes of the present study, since it was expected that such fish would respond more readily to the different migratory conditions imposed by the experiment.

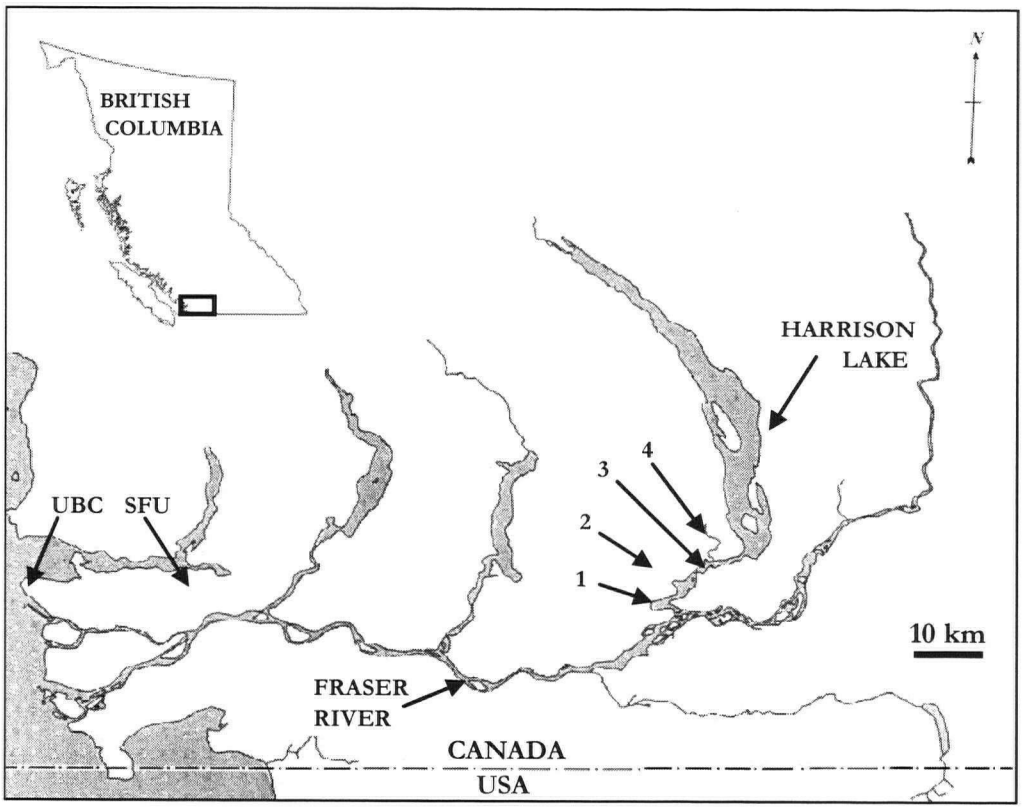

Figure 3.1. Features of the study area: Harrison River (1), Chehalis hatchery (2), capture site (3), Weaver Creek (4), and relative location within British Columbia (inset). Laboratory locations are abbreviated: University of British Columbia (UBC), Simon Fraser University (SFU). Scale is approximate.

Fish capture and initial sampling

All procedures were conducted in accordance with guidelines by the Canadian Council on Animal Care and approved by the University of British Columbia Animal Care Committee (protocol number A05-0424).

Our initial protocol involved three steps: i) intercepting migrating Weaver adults, ii) measuring a suite of variables to determine their overall condition, and iii) transferring them to one of two experimental treatments representing different 'migration difficulties'. The study was originally conducted in 2005 and repeated in 2006: methods were identical in both years unless otherwise noted.

Migrating adults were captured on 27 and 28 September 2005 (26 to 29 September in 2006) using beach seines deployed from the bank of the Harrison River, approximately 2 km downstream of the Harrison-Weaver confluence and 5.5 km from terminal spawning grounds (Figure 3.1). Following capture, fish were held in a shallow water netpen while awaiting biosampling and tagging. Those which were confined longer than approximately one hour were released and excluded from the study.

Biosampling consisted of a suite of procedures aimed at determining overall fish condition, and was performed by a team of researchers while fish were immobilized in a flow-through trough supplied with river water, the process generally lasting less than 150 seconds. Samples were taken according to a routine protocol that has no known detrimental effects on sockeye migration or survival, described at greater length in Cooke et al. (2005). Briefly, a small gill tissue sample was removed from the first gill arch and immediately stored on dry ice, pending transfer to storage at -80 °C and subsequent analysis of gill Na^+, K^+-ATPase activity (an indicator of osmoregulatory status). Two to three scales were collected from a standard landmark above the lateral line for stock identification (Gable and Cox-Rogers 1993). An adipose fin clipping was removed for backup stock identification via tissue DNA (Beacham et al. 1995; Beacham et al. 2004) in the event of inconclusive scale analysis. A blood sample was taken to analyze osmoregulatory status (plasma osmolality and concentrations of chloride, sodium, and potassium), metabolite loading (plasma lactate and glucose) and packed cell volume (hematocrit) – to be collectively interpreted as a measure of physiological stress; in addition to reproductive hormone concentrations (testosterone and 17β-estradiol) - to assess reproductive status. Blood samples were obtained by caudal puncture, collected in

3 mL heparinized vacutainers, and immediately stored in an ice water slurry until centrifugation. Two separate centrifugation protocols were followed. First, a capillary tube was filled and centrifuged for 4.5 minutes at 5900 *g* in a Readacrit centrifuge (Clay Adams, USA). Hematocrit was measured as the ratio of packed cells in the capillary tube relative to the entire sample. The remainder of the blood was centrifuged for six minutes at 1163 *g* in a Compact II centrifuge (Clay Adams, USA). Plasma was stored on dry ice and later transferred to -80 °C storage pending assays for ion, metabolite, and hormone concentrations. Following these procedures, fish were marked with unique cinch tags and transferred to an aerated holding tank for transportation (<15 minutes) to the experimental site at Chehalis hatchery (Figure 3.1), where they were randomly assigned to one of the two experimental treatments.

In 2005, 100 migrants were captured and biosampled in the above manner. An additional 27 fish (10 males and 17 females) were captured on 28 September and immediately sacrificed by cerebral concussion, in order to obtain representative baseline values for traits which could not be assessed in the live experimental fish (i.e. gross somatic energy and gonad morphology). To determine gross somatic energy, a 200–300 g portion of dorsal musculature was removed from the left side of the fish anterior to the insertion of the dorsal fin, and stored at -20 °C until proximate constituent analysis. Fish were weighed to the nearest 1 g (including muscle sample), measured (i.e. fork length, post-orbital-hypural length, and snout length – a secondary sex trait; all ± 0.5 cm) and dissected to obtain gonad mass (± 1 g). A similar procedure was followed in 2006, with the following additional protocols. A first set of eight females was captured on 26 September for the assessment of gonad morphology. Eggs were weighed (± 1 g) to

determine total gonad mass (M_G). Three 10-egg subsamples were weighed (± 0.1 mg) and averaged to calculate individual egg mass (M_E). Fecundity (total number of eggs) was approximated using $M_G M_E^{-1}$. A second set of eight fish (four males and four females) were captured on 29 September for the determination of gross somatic energy as described above.

Experimental treatments

Two raceways were built at the Chehalis hatchery by constricting an existing concrete channel into 1 m-wide enclosures along either wall, using plywood as inner partitions. Raceways each measured 14.7 m in length and 0.4 m in water depth, with submerged pumps (Model LB3-750, Tsurumi Manufacturing Co., Ltd., Japan) positioned upstream, at mid-point, and downstream to maintain current speeds of approximately 38 cm s^{-1} (the 'fast' treatment) and 10 cm s^{-1} (the 'slow' treatment). In 2006, pumps were activated 48 h after the first experimental fish were introduced into the raceways, before which time all fish faced a current of approximately 10 cm s^{-1}. Mean current speeds were calculated from daily measurements taken at set positions within the raceways (5 lengthwise × 3 widthwise × 2 depths) using a Model 2100 current meter (Swoffer Instruments Inc., USA). Speeds were relatively constant and uniform throughout the raceways (Table 3.1) although a decreasing gradient (1-3 cm s^{-1}) was noted between upstream and downstream ends and between the surface and the bottom.

Treatment speeds fell within the low to moderate range of currents that adult Fraser sockeye salmon have been observed to encounter during natural migrations (Hinch and Rand 2000). Indeed, even though mean river speeds often exceed those used in our

treatments, sockeye migrating through reaches with relatively slow currents (approx. <0.6 m s^{-1}) minimize energetic depletion by seeking out areas where currents are slower than the average speeds recorded in these reaches (Standen et al. 2004). Treatment currents were substantially slower than the critical swimming speed (U_{crit}) reported for Weaver Creek sockeye (approximately 1 m s^{-1}; Lee et al. 2003). Thus, while our two treatments represented considerably different challenges (the fast treatment invoking three- to four-fold greater swimming speeds than the slow), both were within a range of naturally occurring current speeds, with the fast treatment not so rapid as to result in excessive energetic exhaustion.

Fish were monitored throughout the experiment to assess behaviour and condition, and to confirm that continuous swimming was necessary for maintaining position within the raceways. This was done through daily visual assessments and twice-weekly remote observations, the latter using underwater and suspended video cameras (Panasonic WV-BP312) connected to a VHS time-lapse recorder (Panasonic AG-6124) to obtain five-minute recordings of a fixed area in each raceway (see Hinch and Rand 2000 for full details on video equipment). In both years, visual and video observations confirmed that fish were swimming continuously in order to maintain position. For each treatment, total distance 'traveled' while in captivity was therefore approximated as the product of mean raceway velocity and experimental duration: first calculated for each individual depending on capture date, then averaged across all fish surviving to the end of the experiment (Table 3.1).

Table 3.1. Mean (+ S.D.) treatment attributes for 2005 and 2006. Sample sizes include fish from the Weaver Creek stock only.

	2005		2006	
Treatment attribute	Slow	Fast	Slow	Fast
Mean raceway speed ($m\ s^{-1}$)	0.10 (0.01)	0.38 (0.01)	0.12 (0.01)	0.39 (0.01)
Total distance traveled in captivity (km)[a]	143	540	153	476
Females				
Initial number *n*	8	18	26	23
Total mortality *n* (%)	4 (50.0)	7 (38.9)	13 (50.0)	10 (43.5)
Males				
Initial number *n*	18	15	20	18
Total mortality *n* (%)	1 (5.6)	3 (20.0)	5 (25.0)	2 (11.1)
Mean water temperature (°C)	11.52 (0.57)		12.10 (1.58)	

a. Distances are approximations based on mean raceway current speeds and the time spent in treatments by individual fish (see text), thus standard deviations are not presented.

The maximum amount of time spent in raceways (as experienced by fish caught on the first sampling day) was 17 days (18 days in 2006). This duration was selected to approximate the normal time lapse between freshwater entry and spawning ground arrival (i.e. 2-3 weeks) in Weaver sockeye and many other Fraser River sockeye populations (Gilhousen 1990; Crossin et al. 2004). It was therefore expected that most fish would be fully mature by the end of the experiment.

Termination of treatments and fertilizations

Termination of treatments involved three steps: i) capturing fish and conducting biosampling, ii) gamete collection, and iii) transferring gametes to the lab for fertilization and incubation.

On the last day of the experiment (14 October in both years), surviving fish were dip-netted from the raceways and sacrificed by cerebral concussion. Biosampling was immediately performed as described previously, after which gametes were extracted by abdominal pressure into individual plastic containers, taking care to avoid water and blood contamination. In 2006, all females were first checked for ripeness (by the extrusion of eggs with gentle abdominal pressure) on 11 October. Ripe females (six fast and four slow) were removed from treatments on this date, while the remainder were allowed a few additional days to ovulate prior to being recaptured on 14 October (three fast and four slow).

Concurrent with the end of experiments, a number of wild migrants were captured upon arrival at the Weaver Creek spawning grounds. These 'native arrivals' served three functions. First, they provided a common source of gametes for crosses with the treated fish used in fertilizations (i.e. males in 2005 and females in 2006, Table 3.2) – the reciprocal crosses (i.e. treated females × native arrival males in 2005, vice-versa in 2006) were not performed (see Results). Second, in order to compare the progeny of captive and wild fish, five native arrival females were themselves fertilized along with the slow and fast females (in 2006), all by the same native arrival males (Table 3.2). Finally, all native arrivals were biosampled (as above) as a benchmark to evaluate the overall impact of handling and captivity on experimental fish. In 2005, biosampling data were obtained from 10 males and 14 females sampled on 12 October for a separate experiment not described here (instead of the native arrival females, Table 3.2) due to logistic difficulties in preserving samples from the latter.

Table 3.2. Number of ripe adults used in fertilizations, per sex and treatment. 'Arrival' refers to wild migrants captured at the spawning grounds.

		2005			2006		
Fertilized adults		Slow	Fast	Arrival	Slow	Fast	Arrival
Males (*n*)		5[a]	5	-	-	-	6[c]
Females (*n*)	*11 October*	-	-	-	4	6	5
	14 October	-	-	5[b]	4	3	-

a. One male proved to be infertile.
b. Females crossed with all slow and fast males.
c. Males crossed with all slow, fast, and native arrival females (same milt used both 11 and 14 October).

Following gamete extractions, fish were weighed and measured as detailed previously. In males, an aliquot of milt was kept to determine spermatocrit (a proxy of sperm cell concentration; Hoysak and Liley 2001) using the same method as for hematocrit. Testes were removed by dissection and weighed (± 1 g). In females, total egg mass, individual egg mass, and fecundity were measured as detailed above. In addition, eggs were photographed with a digital camera (Finepix S4000, Fujifilm, Japan) to estimate egg area using image analysis software (Scion Image, Scion Corporation, USA). Ovarian fluid pH (a putative biomarker of egg viability; Lahnsteiner et al. 1999) was determined by probing an aliquot of fluid with a pH meter (Checker model, Hanna Instruments, USA). Gamete containers were supplemented with medical-grade oxygen and stored at 4 °C for transportation to Simon Fraser University (in 2005, transport time <3 h) or the University of British Columbia (in 2006, transport time <4 h, Figure 3.1), where 'dry' fertilizations were performed according to the method of Patterson et al. (2004) within 24 hours of gamete collection. Full factorial complements of crosses were created (Table 3.2).

In 2006, milt from native arrival males was preserved at 4 °C under oxygenated conditions in order to fertilize both the first and second set of females (11 and 14 October), with native arrival females fertilized concurrently with the October 11 group.

Incubation and rearing

Fertilized eggs were transferred to netted egg capsules and randomly assigned incubation space in Heath stacks, where flows were maintained at a rate of 0.25 L s^{-1}. Each full-sib family was represented once only because limited space precluded replication. Previous studies did not find egg survival to be affected by location within Heath stacks identical to those used in the present study (Patterson 2004; P. Nadeau, unpublished data). Daily assessments of water temperature and dissolved oxygen confirmed that incubation environments were homogenous throughout. Between fertilization and yolk-sac absorption, water temperature followed natural seasonal decrease from 13 °C to 8 °C (13 °C to 5 °C in 2006). Capsules were initially checked weekly to remove dead eggs. At the eyed stage, survival was calculated as the proportion of live embryos relative to the original number of eggs. Beyond this stage, capsules were checked every second day. During this period in 2006, a mechanical malfunction resulted in high or complete mortality in several capsules. Progeny survival was therefore not evaluated beyond the eyed stage. Following complete hatching, the proportion of alevins with severe morphological malformations was recorded in each family (2006 only).

Date of emergence was used as a reference point for beginning to feed fry and for subsequent offspring measurements. In 2005, this date was approximated based on the thermal history of incubating eggs and hatchlings (Jensen et al. 1992). In 2006, progeny

development beyond hatching was more staggered. An emergence date specific to each family was therefore approximated by visual assessments (depletion of yolk reserves) – in this manner, measurements such as mass at emergence were not taken concurrently for all families.

In both years, individual families having reached emergence were transferred to 10 L netted rearing enclosures at the University of British Columbia (transported from Simon Fraser University in 2005, <1 h), where they were randomly assigned to placement within 400 L flow-through tanks. The order of rearing enclosures within a tank was rearranged on a weekly basis to avoid potential positional effects. Fry were fed powdered fishmeal (EWOS Canada Ltd.) in two daily instalments of 1% total body weight. Comparable enclosure densities were maintained and adjusted if necessary. Progeny length (± 0.1 mm) and mass (± 0.1 mg) were taken at the estimated date of emergence and again three weeks thereafter. In these instances, 10 fish per family (or all survivors if fewer than 10 remained) were euthanized by overexposure to tricaine methane sulfonate (MS-222) and their measurements averaged to produce one value per full-sib family. In 2005, fry survival was monitored for a period of three months. Fry survival was not monitored in 2006 due to the above-mentioned mechanical malfunction.

Laboratory Assays

Gross somatic energy (GSE) in adults was estimated by proximate constituent analysis (as per Crossin et al. 2004), using the homogenized dorsal musculature samples – valid proxies for entire carcasses under our circumstances (David Patterson, Fisheries and Oceans Canada, pers. comm.). Determination of plasma osmolality, lactate, glucose,

chloride, potassium (2005 samples only), and sodium followed Farrell et al. (2001). Gill Na^+, K^+-ATPase (hereafter gill ATPase) activity was determined by microassay (2005 samples only) as per Shrimpton et al. (2005). Plasma testosterone and 17β-estradiol levels were measured (2006 samples only) by ELISA (M. Shrimpton, University of Northern British Columbia, pers. comm.).

Data analysis

Energetics and mortality

Gross somatic energy (GSE) of fish at the start of the experiment (i.e. Harrison River baseline samples), after treatments, and in native arrivals was analyzed by one-way ANOVA followed by pairwise comparisons (Tukey HSD). Survival during treatments was assessed by comparing product-limit (Kaplan-Meier) survivor functions (Wilcoxon tests). This approach allowed for the inclusion of right-censored observations, such as fish that were removed from ongoing treatments for a separate study.

Reproductive development

Morphological indicators of reproductive development (e.g. testes mass, fecundity) at the start of the experiment (i.e. Harrison River baseline samples), after treatments, and in native arrivals were analyzed by ANCOVA – reduced to ANOVAs if covariates were not significant. Length (post-orbital to hypural, L_{POH}) was used as the preferred size covariate, given its reduced susceptibility to fluctuating in mature fish (approx. 2% shrinkage during spawning; Hendry and Berg 1999) compared to body mass (approx. 10% reduction in females; Hendry et al. 2000).

Indicators of osmoregulation, stress, and reproductive hormone levels

Three sets of tests were performed, taking advantage of the information gained from sampling the same fish both before and after treatments. First, two-tailed paired t-tests were conducted for each physiological variable to determine whether fish in a given treatment underwent a significant change over time (i.e. a net increase or decrease). Second, t-tests were used to evaluate whether the magnitude of these changes differed between fast and slow fish. Finally, t-tests were performed to compare captive fish (final values from slow and fast treatments combined) and native arrivals. Wilcoxon rank sum tests were used when group variances differed significantly (Levene's test). In 2005, when data for both males and females were available (see Results), sexes were pooled unless significant gender differences were detected *a priori.*

Offspring traits

Treatment effects on offspring traits were tested with split-plot ANOVAs. The 2005 models evaluated offspring responses to treatments as experienced by their fathers (nested within treatments), with native arrival females included as the split-plot factor (sexes were reversed in 2006). Males and females were considered random effects, with variance components estimated by restricted maximum likelihood (REML). Progeny responses were log-transformed when necessary to meet linear model assumptions, except survival ratios which were arcsine-square root transformed. Responses were not adjusted for maternal length because of heterogeneous slopes between treatments. In 2006, treatment and parental effects on the incidence of offspring malformations were

evaluated by contingency analysis, with each family coded for the presence or absence of malformed hatchlings.

Multiple regression models were constructed by backward elimination to investigate the overall effects of maternal traits (irrespective of treatment) on eyed-stage survival, mass at emergence, and length at emergence. Adjusted R^2 values were retained for interpretation. Only females (2006 data) were used since they explained substantially more variability in progeny traits than males (see Results). Thus, offspring responses in the regressions were grand means for each female. Factor analysis (using the correlation matrix and equamax axis rotation) was performed *a priori* to reduce the number of dependent variables and to avoid multicollinearity in the regression. Four factors were retained ($\lambda > 1$) and labelled based on variables with loadings of $r \geq |0.70|$. To minimize bias between batches of fish handled and fertilized at different times (native arrivals, 11 October, and 14 October), an additional variable was created to categorize females by 'fertilization group'. All analyses were performed with SAS 9.1 (SAS Institute, USA), with the threshold for significance set at $\alpha = 0.05$ and not corrected for the total number of tests performed.

Stock identity and sample size constraints

The timing and location of sampling on the Harrison River was intended to maximize the number of fish captured from the target stock (Weaver Creek) relative to other co-migrating populations. However, stock identification (scale patterns and/or DNA) in the days following sampling revealed that a substantial number of individuals were from the co-migrating Harrison population (in both years). These fish were

excluded from our analyses and not used in fertilizations, due to marked differences in life history (i.e. timing and location of spawning) between the two populations. Since stock identity was not known at the time of treatment assignment, Weaver fish were not evenly distributed across treatments or by gender (Table 3.1). Among these, only a subset was available for fertilizations at the end of the experiment (Table 3.2) because sample sizes were reduced by mortality, the non-ripeness of some individuals, and by a subsequent study which required energy-depleted adults (not described here). In 2005, it was deemed that the number of ripe females (one slow and four fast) was too low to perform fertilizations. Data from available treated females (three slow and six fast, including the above ripe individuals) were nonetheless included in analyses of GSE, survival, and physiological parameters to compare responses by gender and to bolster sample sizes.

The low number of females in 2005 was the main reason for repeating the experiment in 2006 with a focus on females from the outset. Males were included in 2006 treatments for the purposes of another study (not described here), although data on GSE and survival were readily available and thus retained for analyses. Inter-annual comparisons were not an initial objective of this study, therefore results from the 2005 and 2006 experiments are hereafter described in tandem – notable inter-annual differences are nonetheless highlighted.

RESULTS

Energetics and mortality

In 2005, experimental fish underwent significant decreases in gross somatic energy (GSE). Based on the GSE of destructively-sampled Harrison River migrants ('baseline', 5.29 MJ kg^{-1}), fast fish had 'depleted' an average of 0.91 MJ kg^{-1} (17.2%), significantly greater than slow fish (0.43 MJ kg^{-1}, 8.1%, Figure 3.2). By comparison, non-experimental native arrivals had consumed an intermediate amount (0.63 MJ kg^{-1}). No gender differences in GSE were apparent at any stage.

Fifteen fish (25.4%) died during the 2005 treatments (Table 3.1). Females had significantly higher mortality than males ($P = 0.005$) and generally began dying earlier (Figure 3.3). Fast fish from both sexes began dying earlier than the slow, but overall treatment differences in mortality curves were not significant (Wilcoxon test, $P > 0.40$, gender-corrected).

In 2006, GSE decreased relative to baseline levels (5.61 MJ kg^{-1}) by an average of 0.72 MJ kg^{-1} (12.8%) in fast fish and by 0.37 MJ kg^{-1} (6.6%) in slow fish, a non-significant difference under multiple pairwise comparisons (Figure 3.2). Native arrivals had lower mean GSE than both the fast and slow fish (by 4.1% and 10.5%, respectively), significantly so for the latter. Relative to 2005, energy levels in 2006 were greater for baseline, slow and fast fish (by approx. 0.3-0.5 MJ kg^{-1}, 6-12% among corresponding groups) but nearly identical for native arrivals (Figure 3.2).

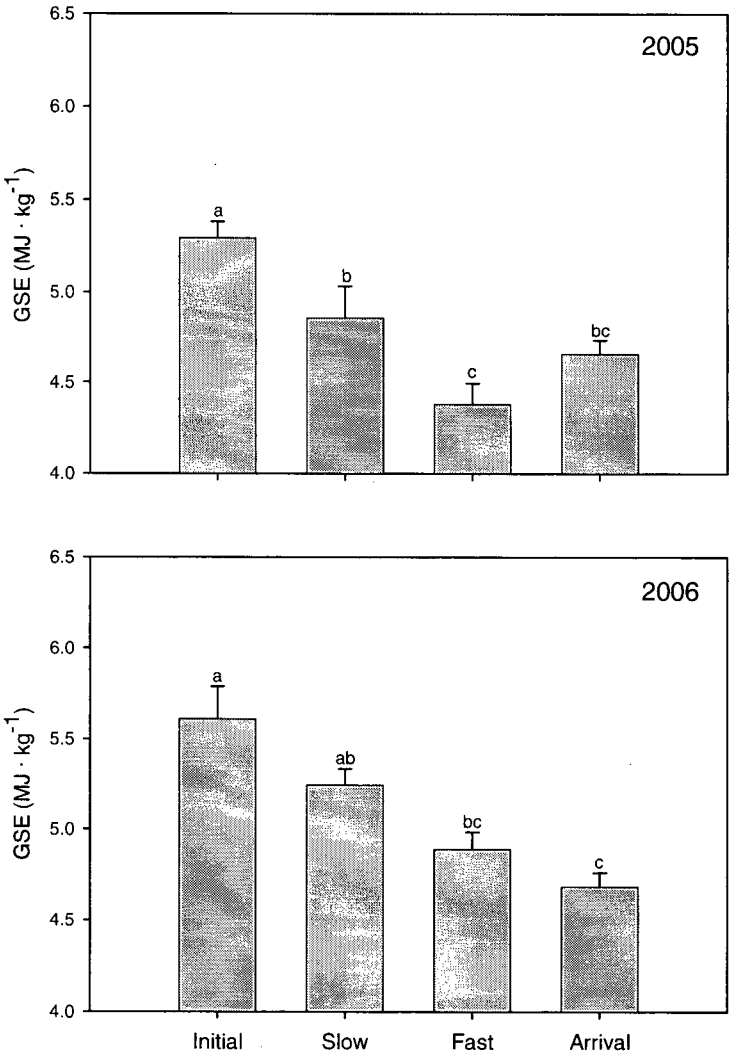

Figure 3.2. Mean (+ S.E.) gross somatic energy (GSE) of terminally sampled fish in the Harrison River (Initial), after the slow and fast treatments, and in untreated fish arriving at spawning grounds (Arrival). Sample sizes for the respective categories were: (2005) 27, 9, 10, 24; (2006) 8, 8, 9, 11. Significantly different means (Tukey HSD, $P < 0.05$) are indicated by different letters.

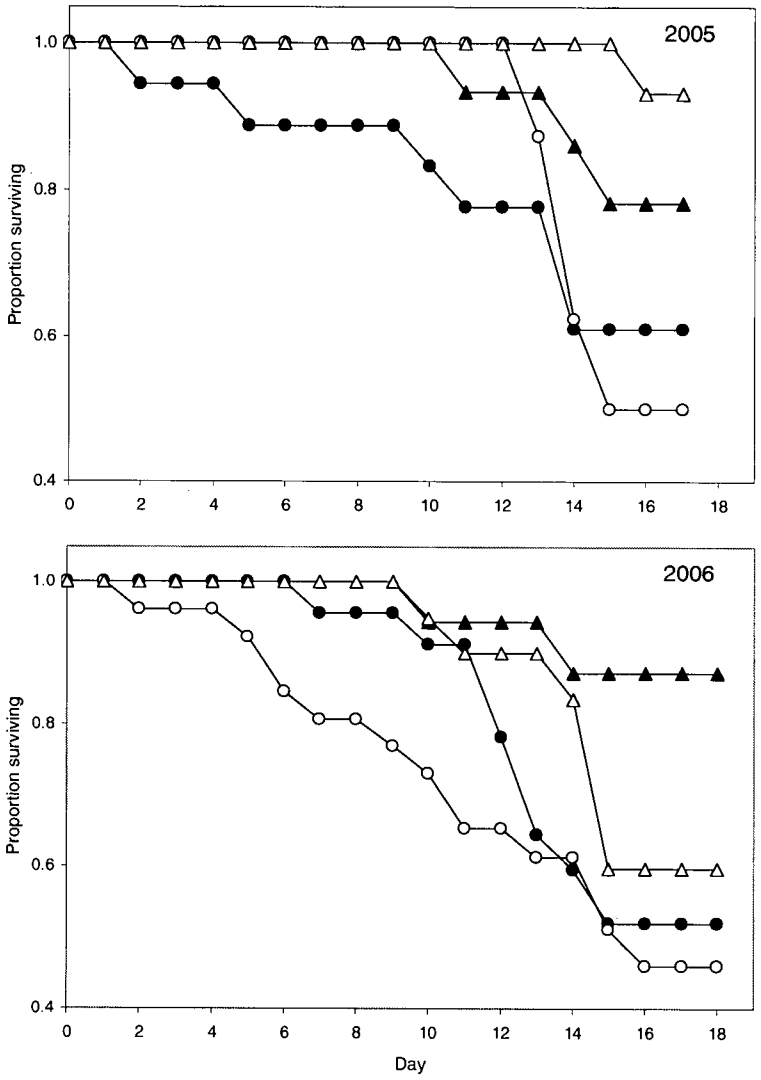

Figure 3.3. Product-limit estimates for the proportion of fish surviving treatments over time. Symbols as follow: fast females (solid circles), slow females (open circles), fast males (solid triangles), slow males (open triangles). Initial sample sizes for each group are given in Table 3.1.

Thirty of 87 fish (34.5%) died prior to the end of the 2006 experiment (Table 3.1). Similar to 2005, females experienced significantly higher mortality than males (P = 0.006, Figure 3.3) but no significant treatment differences were apparent (P = 0.21, gender-corrected).

Physiological condition

The physiological condition of treated fish was summarized by testing: i) whether variables changed over time (i.e. increases or decreases) in each treatment, ii) whether these changes were different in slow vs. fast fish, and iii) how treated fish (final values) compared with native arrivals.

In 2005 (see Figure 3.4), fish underwent significant decreases in plasma osmolality (two-tailed paired t-tests, d. f. = 6 and 8 for slow and fast treatments respectively, both $P < 0.01$), chloride ($P < 0.01$), and lactate (slow fish only, $P = 0.015$), and significant increases in plasma potassium and glucose (all $P < 0.01$). Changes in gill ATPase activity differed by gender in the slow treatment: activity in males decreased ($P = 0.013$) while that of females did not change ($P = 0.08$).

Fast and slow fish had some different physiological responses to treatments. In fast fish, the average decrease in plasma chloride was twice that of the slow (27.6 vs. 13.6 mmol L^{-1}, d. f. = 14, $P = 0.037$, Figure 3.4a). The same trend was apparent for plasma osmolality ($P = 0.058$). Visual assessments showed fast fish to be in worse external physical condition, particularly in the incidence of skin and gill infections. Changes in plasma lactate, glucose, potassium, hematocrit, or gill ATPase activity did not differ significantly between fast and slow fish (all $P > 0.05$, Figure 3.4).

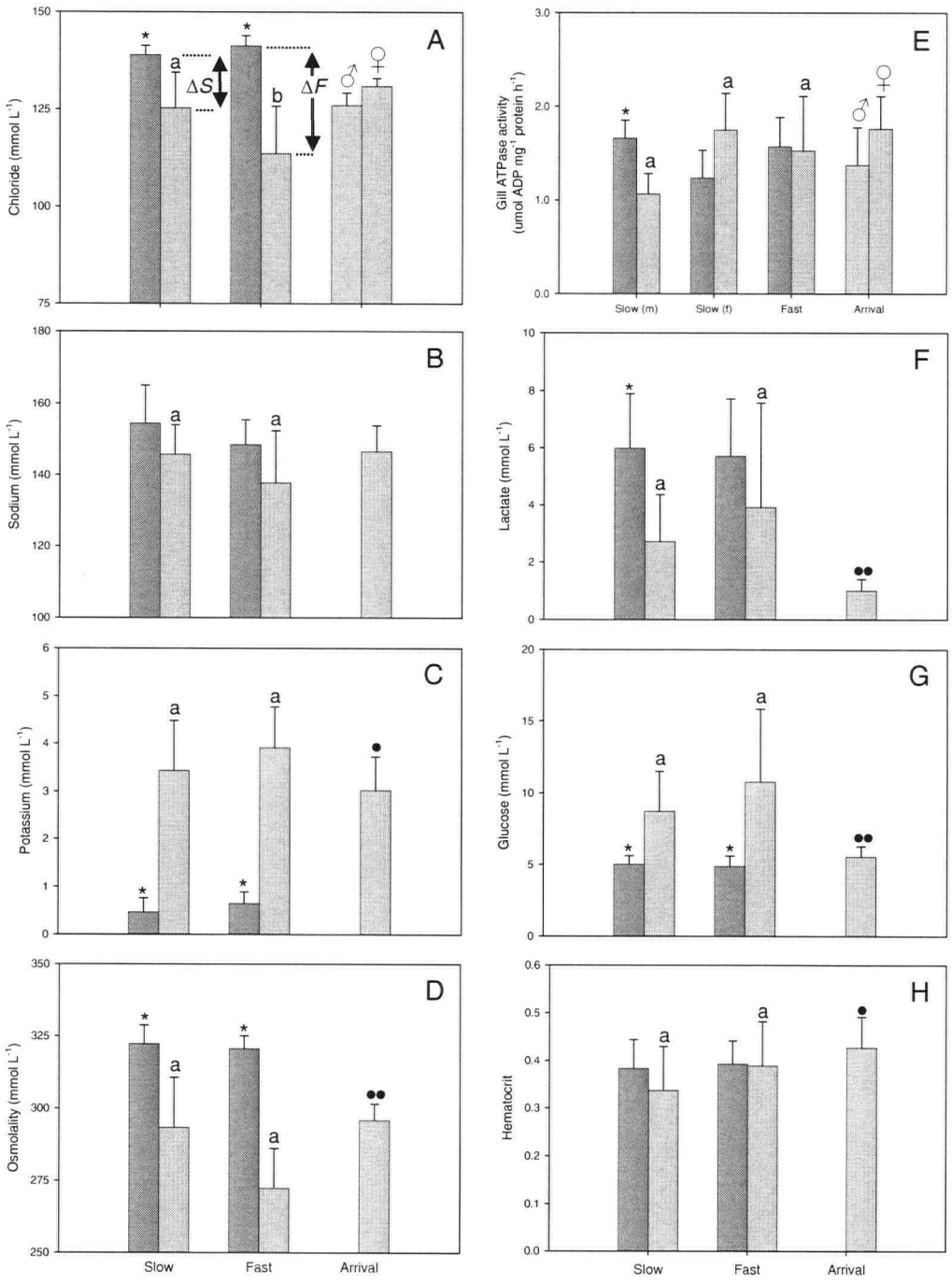

Figure 3.4. Means (+ S.D.) for physiological parameters sampled in the same individuals before (dark grey) and after (light grey) the slow and fast treatments in 2005. Symbols represent the following: *within* a treatment, an asterisk indicates a significant before-and-after difference (paired t-test); *between* treatments, different letters indicate a significant difference in the magnitude of changes (t-test, see illustration panel A); *between* captive fish (slow and fast values combined) and native arrivals, significant differences at the $P < 0.05$ and $P < 0.005$ level are indicated by • and ••, respectively. Sample sizes as follow: slow (n = 4 males, 3 females); fast (n = 4 males, 5 females); native arrivals (n = 10 males, 14 females); except in (E), n = 2 slow females. Significant gender differences were detected in native arrivals for chloride and gill ATPase activity (panels A and E), and in slow fish for gill ATPase activity (panel E): in these cases, the above analyses were performed on each sex separately.

Collectively, captive fish differed from native arrivals. The former had approximately twice the glucose and three times the lactate concentrations (d. f. = 38, both $P < 0.01$, Figure 3.4). Osmolality and hematocrit were also significantly lower in captive fish ($P = 0.005$ and $P = 0.029$, respectively), while potassium was significantly higher ($P = 0.012$). Native arrivals were in better external condition, with little evidence of skin or gill infections.

In 2006 (see Figure 3.5), fish underwent significant decreases in plasma chloride (two-tailed paired t-tests, d.f. = 7 and 8 for slow and fast treatments respectively, both $P < 0.001$), osmolality ($P < 0.001$), sodium ($P < 0.001$), hematocrit (fast fish only, $P = 0.012$), and lactate (slow fish only, $P = 0.039$) – and significant increases in glucose ($P < 0.001$).

There were notable physiological differences between the fast and slow treatments. Decreases in chloride concentrations were significantly more pronounced in fast fish (37.7 vs. 20.9 mmol L^{-1}, d. f. = 15, $P = 0.020$). Changes in hematocrit were significantly different between treatments ($P = 0.015$): while every fast fish showed a decrease (mean = -0.08, $P = 0.012$), slow fish did not change significantly (mean = +0.02, $P > 0.40$). Likewise, changes in lactate differed significantly ($P = 0.022$), with a significant decrease in the slow (mean = -4.03 mmol L^{-1}, $P = 0.039$) versus no change in the fast (mean = +2.39 mmol L^{-1}, $P = 0.24$). As in 2005, skin and gill infections were more widespread in fast fish. There were no significant differences between fast and slow fish for changes in changes plasma osmolality, sodium, or glucose (all $P > 0.20$, Figure 3.5).

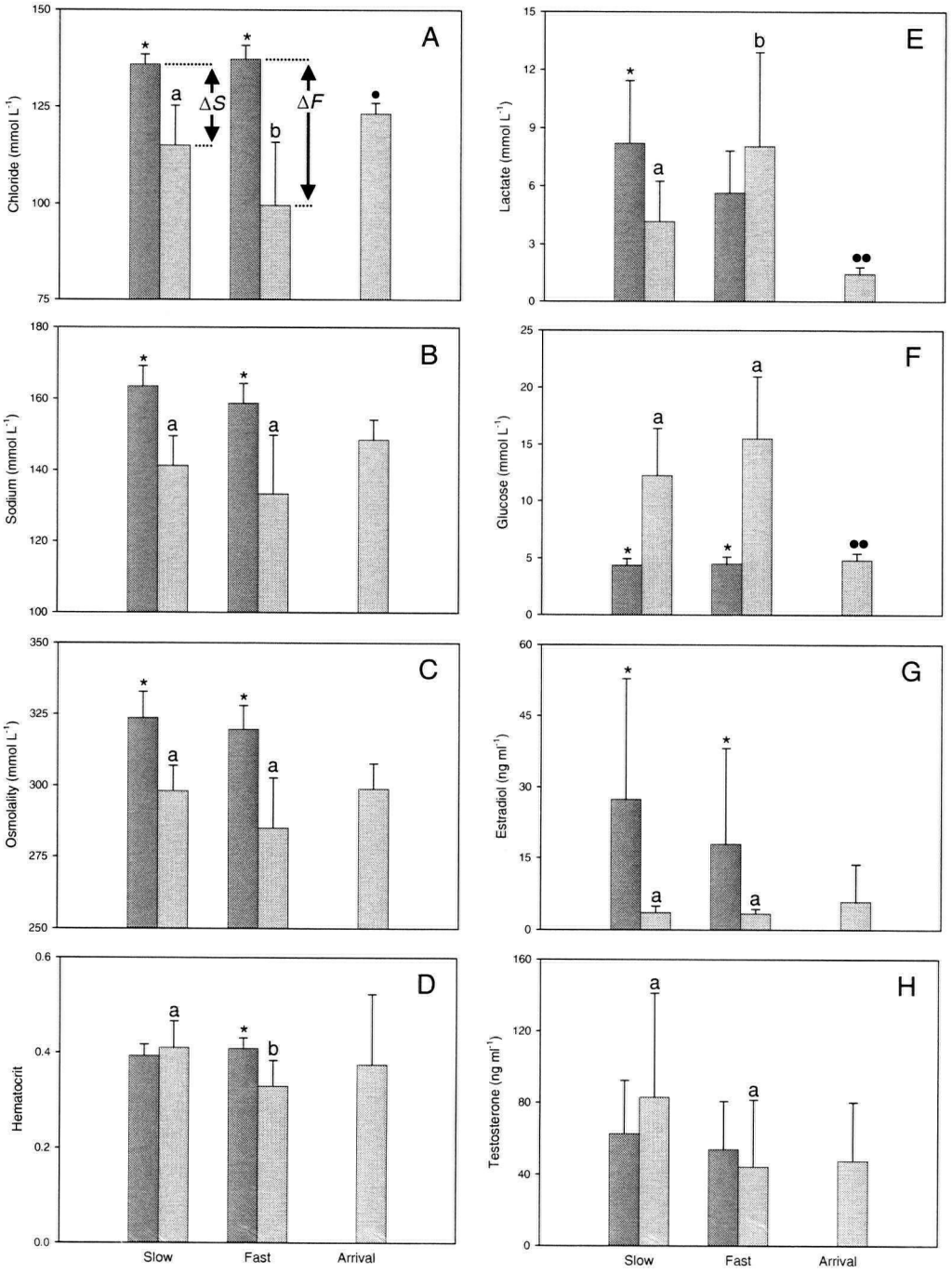

Figure 3.5. Means (+ S.D.) for plasma parameters sampled in the same females before (dark grey) and after (light grey) the slow and fast treatments in 2006 (n = 8 and 9, respectively). Symbols represent the following: *within* a treatment, an asterisk indicates a significant before-and-after difference (paired t-test); *between* treatments, different letters indicate a significant difference in the magnitude of changes (t-test, see illustration panel A); *between* captive fish (slow and fast values combined) and native arrivals (n = 5), significant differences at the $P < 0.05$ and $P < 0.005$ level are indicated by • and ••, respectively.

Captive fish (fast and slow combined) were markedly different from native arrivals, the former having nearly three times the glucose and more than four times the lactate (both $P < 0.005$). Ion concentrations were generally lower in treated fish, significantly so for chloride ($P = 0.046$).

Reproductive development

In 2005, male sex traits did not vary significantly with length (L_{POH}), thus uncorrected data were analyzed. Fast and slow males did not differ significantly in testes mass, snout length, or spermatocrit (Figure 3.6). Among untreated fish, baseline males sampled on the Harrison River had significantly greater testes masses than native arrivals ($P = 0.013$, Figure 3.6a) – treated and untreated fish were not compared directly for this trait because the former were weighed after milt was extracted for fertilizations. Native arrivals had larger snouts than both baseline and treated fish (Figure 3.6b).

In 2006, there were no significant differences in gonad mass, individual egg mass, and fecundity between Harrison River baseline, fast, slow, and native arrival females (all $P > 0.20$, Figure 3.7). ANCOVAs explained a substantial amount of the variability in these traits (R^2 = 0.65, 0.60, and 0.42, respectively), but this was driven almost exclusively by the highly significant effect of length (L_{POH}) covariates (all $P < 0.001$; fecundity was analyzed with post-orbital-to-fork length (L_{POF}) due to a violation of the slope homogeneity assumption with L_{POH}). Egg area and ovarian fluid pH did not require size correction and did not differ between slow and fast fish. However, ovarian fluid pH was significantly higher in native arrivals than in slow and fast fish (both $P < 0.01$, Figure 3.7). During the experiment, plasma 17β-estradiol concentrations significantly

decreased in slow and fast females (paired t-tests, d. f. = 7 and 8 respectively, both $P < 0.05$, Figure 3.5), but the magnitude of this change was not significantly different between treatments ($P > 0.40$). Testosterone levels did not change significantly over time or between treatments (all $P > 0.20$, Figure 3.5).

It was inferred that most females ovulated relatively late in the experiment. Indeed, of the 23 females that died during the treatments (Table 3.1), 78% (18) had not yet ovulated. Moreover, 40% of survivors were known to have ovulated within the final three days of the study since they were ripe on 14 October but not when first assessed on 11 October. There was no significant difference between fast and slow females in terms of propensity to have ovulated during the study, with a nearly equal proportion of fish from each treatment having ovulated by 14 October (11 of 23 fast vs. 12 of 26 slow, $P > 0.40$).

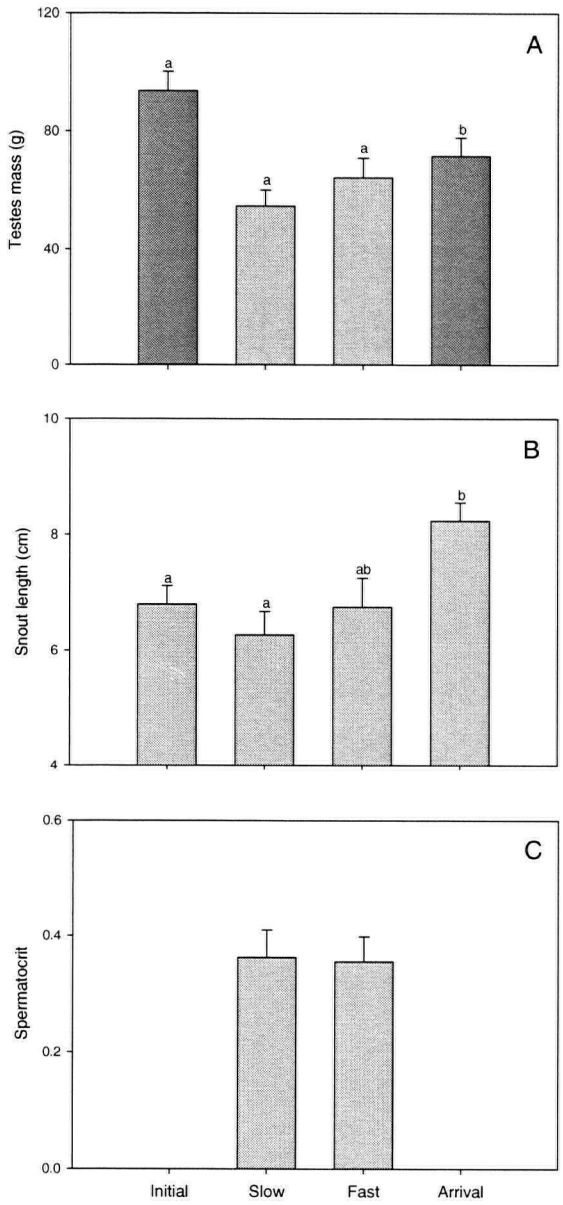

Figure 3.6. Means (+ S.E.) for the sex traits of males captured in 2005 on the Harrison River (Initial), after the slow and fast treatments, and in native arrivals. Significantly different means (Tukey HSD, $P < 0.05$) are indicated by different letters. Respective sample sizes for Initial, Slow, Fast, and Arrival fish were: (A) 9, 6, 4, 10; (B) 10, 6, 4, 10; and (C) 5, 4 (spermatocrit was measured only in treated fish). Groups represented by different colours in panel A were not compared with each other in multiple comparisons (see text).

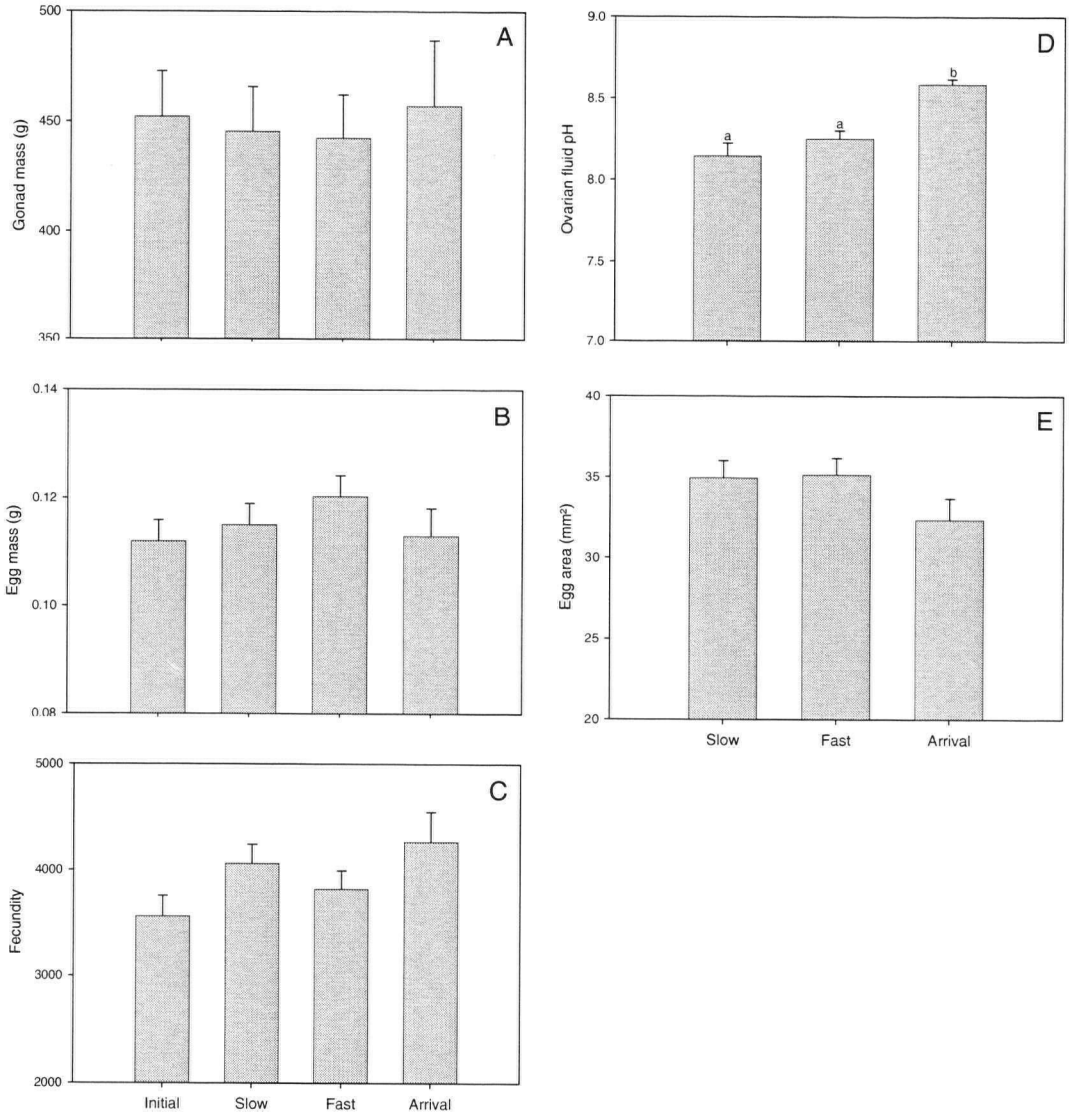

Figure 3.7. Means (+ S.E.) for the sex traits of females captured in 2006 on the Harrison River (Initial), after the slow and fast treatments, and in native arrivals. Significantly different means (Tukey HSD, $P < 0.05$) are indicated by different letters. Respective sample sizes for Initial, Slow, Fast, and Arrival fish were: (A, C) 8, 8, 9, 4; (B) 8, 8, 9, 5; (D-E) 8, 9, 5 (not measured in Harrison River fish). Size covariates were significant for A-C, adjusted means are presented.

Effects on offspring

In 2005, treatment effects on offspring were analyzed for males only (Table 3.2). One slow male proved to be infertile (dissection had revealed torsion of both testes). The remaining 45 crosses all produced viable eggs. Survival to the eyed stage was generally very high (median = 90.3%) and did not differ significantly between the progeny of fast and slow males (mean = 74.5% and 84.6%, respectively, $P = 0.12$, Table 3.3). Very few eggs died beyond the eyed stage, such that survival to hatching and emergence were virtually unchanged ($r > 0.99$) and are not presented herein. Eggs from all families hatched more or less synchronously (within a one-week span) with 50% hatch occurring 65 and 66 days post-fertilization in families from slow and fast males, respectively (Table 3.3). Consequently, there were no treatment effects on hatch timing ($P = 0.40$), although male identity (irrespective of treatments) had a significant influence ($P = 0.005$, Table 3.3).

Fry size at emergence varied substantially across families – individuals from the smallest family measured 12% (3.2 mm) and weighed 29% (54.3 mg) less than the largest. The progeny of slow and fast males did not differ significantly in mean length (26.71 vs. 26.86 mm, respectively) or mass (163.8 vs. 167.2 mg) although male identity significantly affected length at emergence ($P < 0.001$, Table 3.3). Between the measurements taken at emergence and those taken three weeks later, overall progeny length increased by approximately 4% (1.0 mm) while mass increased by 2% (3.9 mg). Family size hierarchies remained relatively constant during this period, as measurements at emergence were highly correlated to those taken three weeks later ($r = 0.92$ and $r = 0.74$ for length and mass, respectively). There were no significant treatment effects on

size at three weeks post-emergence, but male influences on length remained significant (P = 0.003, Table 3.3). The influence of the non-treated (native arrival) females outweighed all male effects and was highly significant for all progeny responses (all $P <$ 0.001) with the exception of survival to three months post-emergence, for which no factor in our model was significant. Female-by-treatment interactions were detected for all size measurements except weight at emergence (Table 3.3).

Table 3.3. Family means (+ S.D.) for offspring responses to 2005 treatments and associated analysis of variance outputs (see Table 3.2 for fertilization design). Significant effects are in bold. Variance components are REML estimates.

	Means (S.D.)		**Analysis of variance**					
Offspring response	Slow	Fast	Effect	D.F.	MS	F	*P*	% Var.
Survival to eyed stage (%)	74.5 (26.6)	84.6 (21.7)	Treatment	1	661.67	3.20	0.117	
			Male (Treatment)	7	206.88	1.32	0.277	3.1
			Female	4	1685.60	10.77	**<0.001**	51.0
			Female*Treatment	4	145.40	0.93	0.461	0.0
Days to 50% hatch	65 (2)	66 (2)	Treatment	1	1.96	0.81	0.397	
			Male (Treatment)	7	2.41	3.81	**0.005**	10.3
			Female	4	22.71	36.00	**<0.001**	72.2
			Female*Treatment	4	0.44	0.70	0.599	0.0
Mass at emergence (mg)	163.8 (18.0)	167.2 (14.3)	Treatment	1	0.14	2.72	0.143	
			Male (Treatment)	7	0.05	1.42	0.235	1.0
			Female	4	2.41	68.33	**<0.001**	87.3
			Female*Treatment	4	0.04	0.99	0.428	0.0
Length at emergence (mm)	26.71 (0.88)	26.86 (0.70)	Treatment	1	0.01	0.51	0.497	
			Male (Treatment)	7	0.02	5.18	**<0.001**	8.2
			Female	4	0.22	73.78	**<0.001**	78.1
			Female*Treatment	4	0.01	2.76	**0.047**	3.9
Mass 3 wks post-emerg. (mg)	166.7 (12.1)	171.8 (23.8)	Treatment	1	0.23	4.76	0.066	
			Male (Treatment)	7	0.05	0.42	0.884	0.0
			Female	4	2.18	18.45	**<0.001**	45.3
			Female*Treatment	4	0.62	5.21	**0.003**	29.1
Length 3 wks post-emerg. (mm)	27.67 (0.71)	27.93 (0.90)	Treatment	1	0.60	2.30	0.173	
			Male (Treatment)	7	0.26	4.11	**0.003**	5.3
			Female	4	5.55	86.97	**<0.001**	80.3
			Female*Treatment	4	0.27	4.24	**0.009**	6.1

In 2006, treatment effects on offspring were analyzed for females only, including native arrivals for comparison (Table 3.2). Analyses were restricted to those sampled on 11 October (not 14 October – the second set of ripe females) because: i) the temporal lag between the two rounds of fertilizations complicated direct comparisons, and ii) despite efforts to preserve milt from the same males for both sets, fertilization success was much lower in families from the second group – with 25 of 42 families producing viable offspring (vs. 86 of 90, $P < 0.001$) and 41.5% surviving to the eyed stage (vs. 63.7%, $P = 0.005$).

Eyed-stage survival in 2006 was lower than in 2005 (Table 3.3 vs. 3.4) and was significantly influenced by males and females (both $P < 0.001$), but not by the treatments experienced by the latter (Table 3.4). Beyond the eyed stage, 14 families suffered complete mortality due to a mechanical malfunction. The remaining families reached 50% hatch date within one week of each other. The average incubation period of 60 days (identical for all treatments) was shorter than in 2005, although nearly equal when expressed in terms of accumulated thermal units (A.T.U., the sum of mean daily temperatures): 648 (± 14) and 647 (± 16) for 2005 and 2006, respectively. Hatch timing was significantly affected by male identity ($P = 0.036$) but not by females ($P = 0.21$) – after removing 'treatment' as an effect in the model, since no effects were significant in its presence (Table 3.4). Relative to hatching, emergence dates (estimated from yolk-sac depletion) spanned a long period (22 days between the earliest and latest family). Emergence date for a given family was weakly correlated to its hatch date ($r = 0.39$, $P = 0.002$) and was not significantly affected by treatments (Table 3.4), but remained under significant paternal influence ($P = 0.022$).

Analyses of progeny morphology did not include seven families with fewer than three surviving members – deemed inadequate for calculating representative family means. Of the remaining 65 families, mass and length at emergence were significantly affected by treatments (P = 0.044 and P = 0.008, respectively). Post-hoc pairwise comparisons (Tukey HSD) revealed that this effect was driven by a difference between captive fish and native arrivals (rather than a difference between slow and fast fish), whereby progeny from native arrivals were smaller than captives, significantly so in comparisons with fast fish (P = 0.007 and P = 0.035 for length and mass, respectively, Table 3.4). Correlations between the measurements taken at emergence and three weeks later were high for both length and mass (r = 0.89 and r = 0.86, respectively), but no treatment effects were detected on size at three weeks post-emergence. As in 2005, both males and females influenced length at emergence (P = 0.009 and P < 0.001, respectively) while only females influenced mass at emergence and three weeks thereafter (both P < 0.001). Fifteen of the 65 families (23%) had individuals with severe morphological malformations. These included abnormalities of the yolk sac (i.e. oedema), the spine (i.e. scoliosis, lordosis, kyphosis), and various forms of conjoined twins, which generally represented a small proportion of individuals in the affected families (median = 2.5%). The presence or absence of these malformations within a family was not significantly influenced by treatment, female, or male effects (all P > 0.40).

Table 3.4. Family means (+ S.D.) for offspring responses to 2006 treatments (with native arrivals included for comparison) and associated analysis of variance outputs (see Table 3.2 for fertilization design). Significant effects are in bold. Variance components are REML estimates.

	Means (S.D.)			**Analysis of variance**					
Offspring response	Slow	Fast	Arrival	Effect	D.F.	MS	F	*P*	% Var.
Survival to eyed stage (%)	65.8 (39.3)	55.2 (35.4)	72.5 (25.2)	Treatment	2	1185.96	0.36	0.704	
				Female (Trt)	12	3281.22	25.09	**< 0.001**	71.4
				Male	5	762.28	5.83	**< 0.001**	6.8
				Male*Treatment	10	89.36	0.68	0.736	0.0
Days to 50% hatch[a]	60 (2)	60 (2)	60 (2)	Female	14	6.04	1.34	0.212	4.9
				Male	5	11.54	2.56	**0.036**	11.1
Days to emergence	122 (6)	124 (6)	126 (5)	Treatment	2	82.95	2.60	0.116	
				Female (Trt)	12	31.94	1.26	0.275	8.0
				Male	5	74.44	2.95	**0.022**	13.5
				Male*Treatment	10	22.35	0.88	0.555	4.3
Mass at emergence (mg)	159.9 (5.1)	173.9 (19.0)	144.4 (18.6)	Treatment	2	4.95	4.11	**0.044**	
				Female (Trt)	12	1.20	57.53	**< 0.001**	92.8
				Male	5	0.03	1.31	0.281	0.8
				Male*Treatment	10	0.01	0.70	0.715	0.0
Length at emergence (mm)	26.85 (0.28)	27.77 (0.78)	26.14 (0.90)	Treatment	2	14.83	7.30	**0.008**	
				Female (Trt)	12	2.03	35.79	**< 0.001**	86.2
				Male	5	0.21	3.68	**0.009**	2.4
				Male*Treatment	10	0.10	1.68	0.125	2.5
Mass 3 wks post-emerg.[b] (mg)	175.9 (14.8)	173.7 (23.6)	150.8 (29.2)	Treatment	2	2.43	1.74	0.224	
				Female (Trt)	10	1.39	15.99	**< 0.001**	85.3
				Male	4	0.04	0.50	0.733	0.0
				Male*Treatment	8	0.05	0.57	0.788	0.0
Length 3 wks post-emerg.[b] (mm)	27.71 (0.44)	28.19 (0.92)	26.79 (1.13)	Treatment	2	2.07	3.25	0.082	
				Female (Trt)	10	0.64	12.46	**< 0.001**	81.7
				Male	4	0.10	1.88	0.160	3.0
				Male*Treatment	8	0.14	0.28	0.965	0.0

a. Treatment was excluded as a factor when the original model was not significant.
b. One male was excluded because none of its offspring remained in the slow treatment, thus preventing estimation of an interaction term. One slow and one fast female had no offspring remaining.

To construct an overall model linking maternal condition to progeny traits, data from all females fertilized in 2006 (n = 22, Table 3.2) were submitted to factor analysis as a precursor to multiple regression. The four retained factors collectively explained 82% of the dataset variance, with corresponding variable loadings forming a straightforward structure (Table 3.5). Multiple regression revealed that eyed-stage survival was significantly affected by 'sampling event' (F3) – essentially an experimental artefact, and negatively affected by female 'energy level' (F4), both of which collectively accounted

for 40% of the adjusted variance (Table 3.5). This proportion increased to 48% by including the marginally significant ($P = 0.062$) negative effect of 'stress' (F2). Female 'size' (F1) largely determined progeny mass and length, explaining 65% and 50% of the variance in these traits, respectively. Interestingly, 'stress' (F2) also had a significantly positive effect on length ($P = 0.026$, Table 3.5).

Table 3.5. *Upper panel*: Factor analysis of female traits in 2006 ($n = 22$). Following an equamax axis rotation, factor loadings of $r \geq |0.70|$ were retained. *Lower panel*: Multiple regressions for selected progeny responses as a function of the four factors summarizing maternal traits. Significant factors and their cumulative contribution to the adjusted R^2 are bolded, the sign of the t value indicating the direction of the relationship. For comparison, all other factors are presented in order of importance. P and t values are those obtained when including all previously listed factors.

Factor (% Var[a])	**Attributed maternal trait**	**Variables**		r
F1 (36.4)	Size	Fork length		0.91
		Total mass		0.88
		Gonad mass		0.87
		Single egg mass		0.83
F2 (32.8)	Stress	Plasma [Cl^-]		-0.94
		Osmolality		-0.92
		Plasma [Na^+]		-0.77
		Plasma glucose		0.76
		Plasma lactate		0.70
F3 (16.7)	Sampling event[b]	Fertilization group		0.96
		Ovarian fluid pH		-0.79
F4 (14.1)	Energy level	GSE		0.91
Offspring response	Factor	T	P	Cum. $R^2_{adj.}$
Survival to eyed stage	**F3**	-3.25	**0.005**	**0.22**
	F4	-2.86	**0.010**	**0.40**
	F2	-1.99	0.062	0.48
	F1	0.12	0.902	0.45
Mass at emergence	**F1**	6.64	**< 0.001**	**0.65**
	F2	1.88	0.076	0.68
	F3	1.08	0.293	0.69
	F4	-0.21	0.836	0.67
Length at emergence	**F1**	5.19	**< 0.001**	**0.50**
	F2	2.42	**0.026**	**0.59**
	F3	1.63	0.120	0.63
	F4	-0.42	0.677	0.61

a. Percentages of variance explained refer to factor communality (82%), thus sum to 100.
b. The variables strongly associated with F3 were both connected to the sampling event: 'fertilization group' was a variable created to account for bias between groups fertilized at different times, while 'ovarian fluid pH' was significantly higher in native arrival females (see Figure 3.7) for uncertain reasons, but possibly due to differences in pH meter calibration between sampling events.

DISCUSSION

Subjecting sockeye salmon to two different current velocities during the final stages of sexual maturation resulted in notable differences between the two groups. Fish from the fast treatment expended more energy than the slow and showed some signs of greater stress. However, these differences did not appear to influence allocations to reproductive development in terms of sex trait morphology, ovulation timing and reproductive hormone levels. Progeny traits were significantly affected by either one or both parents, but this was largely a function of variability among parents rather than an effect of the treatments they experienced. Regression models showed that offspring size and survival were linked to certain aspects of maternal condition at the time of fertilization, including size, stress, and energy levels.

Energetics

As expected, fish in the fast treatment depleted more energy than those in the slow, thus experimentally confirming the role of current velocity in affecting the rate of energy consumption (the fact that the difference was more pronounced in 2005 was perhaps reflective of the greater differentiation in travel distances that year). In a study of long-distance migrating sockeye populations, Hinch and Rand (2000) found that migrants encountering currents slower than approximately 0.25 m s^{-1} swam at energetically optimal speeds, whereas those encountering higher speeds were less efficient. Although encounter and swimming speeds were synonymous in the present study (fish were not making forward progress), the threshold of 0.25 m s^{-1} (approximately midway between

our treatment speeds) would be concurrent with our observation that fast fish depleted more energy overall.

Given that fast fish had lower energy and were closer to the minimum energetic threshold for sustaining life of 4 MJ kg^{-1} (Crossin et al. 2004), it might have followed that these fish would have experienced higher mortality. On the contrary, survivor functions were not significantly different between treatments. Relative mortality was in fact higher in the slow treatment for all gender-by-year combinations except 2005 males (Table 3.1, Figure 3.3), but this may have been an artefact of small and unequal sample sizes. For example, 7 of the 11 deaths of 2005 females were in the fast treatment, yet the slow treatment had higher relative mortality (Table 3.1).

We noted that females in both years experienced higher mortality than males and generally began dying earlier. Crossin et al. (in review) recently observed a similar trend in captive Weaver Creek sockeye (also intercepted during migration) and suggested that the greater energetic investments required by females for reproductive development reduced the energy available for 'buffering' against adverse conditions. In the present study, factors other than energetics were probably also involved, since some females (but no males) died in the early days of the experiment in both years (Figure 3.3) – a timeframe likely too short for different energetic requirements alone to have had this effect.

Despite energetic differences between fast and slow fish, there were no significant treatment effects on sex traits or progeny attributes. This was contrary to our expectation that energetic trade-offs would impair reproductive development in fast fish. However, our ability to detect these trade-offs was limited by our capacity to experimentally

generate them, namely because treatments were applied late in maturation. Indeed, effects might have been observed if fish had been captured earlier in their migration. For example, differences in egg mass would be expected if vitellogenesis (the process by which the lipoprotein vitellogenin (Vtg) is taken up by maturing oocytes to form a yolk supply) was relatively impaired in fast females. By contrast, our findings suggested that vitellogenesis was already well underway when fish were initially captured, as evidenced by the lack of significant differences between pre- and post-treatment sex trait measurements (e.g. egg mass), and by the significant decrease of 17β-estradiol levels during the experiment, as this hormone is a stimulator of Vtg synthesis (Brooks et al. 1997). Most females probably underwent treatments with at least some potential for vitellogenesis to continue, given that eggs continue to sequester Vtg until just prior to ovulation (Tyler and Sumpter 1996) and that most fish ovulated near the end of the experiment. Even so, we acknowledge that our ability to test hypotheses regarding trade-offs in energetic allocations to sex traits was limited, since much of these allocations had already occurred prior to the experiment. Our findings did suggest that other reproductive mechanisms are unaffected by differences in energetic status during late maturation. For example, ovulation timing did not differ between treatments, even though fast fish might have gained an advantage by ovulating earlier to compensate for the lower energy available for spawning ground interactions. In addition, there was no evidence to suggest that fish are capable of mobilizing energy by reabsorbing nutrients previously allocated to eggs or secondary sex traits – a process which can occur (in eggs) during earlier stages of sexual maturation (i.e. atresia; Schreck et al. 2001).

Interestingly, native arrivals had energy levels comparable to those of treated fish and significantly lower than the Harrison River baseline. This initially appeared counterintuitive because sampling locations for baseline fish and native arrivals (Harrison River and Weaver Creek, respectively) were less than 10 km apart, versus the approximately 150 km and 500 km traveled by slow and fast fish during the experiment. Previous telemetry studies have shown that Weaver sockeye can spend 1–3 weeks 'milling' about in the Harrison River or Harrison Lake (Figure 3.1) prior to traveling into spawning areas (Cooke and Hinch 2005). This milling behaviour has an energetic cost, but likely occurs so that fish can complete sexual maturation, seek thermal refuge in the lake's hypolimnion to recover from physiological stress (Hinch et al. 2006), and because low water levels in Weaver Creek often prevent fish from moving upstream until the end of the first week of October (R. Stitt, Fisheries and Oceans Canada, pers. comm.). The last factor was concurrent with our finding that native arrivals in 2006 actually had lower energy stores than captive fish, since water levels in that year were particularly low – potentially increasing holding times and energetic depletion. Thus, it appears that contrary to our initial expectations, our two treatments (while differing in their relative difficulty) fell within a natural range of migratory expenditures for this population – even if they required swimming the equivalent of two to four times the distance between the Fraser River estuary and Weaver Creek. Previous work has shown that coastal stocks such as Weaver Creek have larger gonads and greater egg numbers than upper river populations, allocations made available because of the reduced energetic requirements of powering upstream locomotion (Crossin et al. 2004). Nevertheless, the adaptive value of

this apparent 'energetic surplus', as well as the factors that may be limiting further investment into reproductive traits, remain unclear and warrant future investigation.

Physiological condition

An underlying assumption in our interpretation of physiological variables was that values reflected fish condition immediately prior to sampling. While we acknowledge that the sampling event itself can be stressful, in our opinion such a bias was not a major cause for concern in the present study. Even if stress indicators were inflated, this would not have affected our comparison of fast and slow fish since they were subjected to the same handling. By contrast, our interpretation of temporal changes in physiology could have been affected, because the pre-treatment capture method (beach seining, with greater struggling and sampling delays) was likely more stressful than the post-treatment method (dip-netting and immediate sampling). Since most variables suggested that stress increased over time, a handling bias would in fact have caused us to underestimate the magnitude of these increases.

Compared to native arrivals, captive fish (fast and slow combined) had significantly higher plasma lactate and glucose and significantly lower ion concentrations, which we interpreted as evidence that the latter group was more stressed. Indeed, high lactate and glucose levels are secondary responses to acute and/or chronic stress (Wedemeyer et al. 1990), while electrolyte losses (such as decreases in chloride) can occur as a secondary stress response (Postlethwaite and McDonald 1995; McDonald and Milligan 1997; Wendelaar Bonga 1997) and also reflect increasingly compromised homeostasis in migrating salmon (Shrimpton et al. 2005). The fact that low ions and high

metabolites grouped together in factor analysis (Table 3.5) supported our interpretation of physiological stress with respect to these variables.

It was not altogether surprising that captive fish were more stressed than wild migrants, since the conditions inherent to captivity (e.g. higher densities, abrasive enclosure surfaces) were probably added stressors (Portz et al. 2006). Nonetheless, a previous study involving maturing Weaver Creek sockeye held in captivity for longer periods did not find physiological differences between captive and wild individuals (Crossin et al. in review), indicating that some aspects of our treatments may have been inherently stressful. To illustrate the relative magnitude of these stress levels, we briefly compare our results to others found in the literature.

Lactate concentrations in captive fish (approx. 5–8 mmol L^{-1}) were generally below levels associated with critical stress (>12 mmol L^{-1}; Jain and Farrell 2003), yet well above those of native arrivals (approx. 1 mmol L^{-1}) and comparable values reported by Hruska et al. (2007) for Weaver native arrivals (approx. 1.8 mmol L^{-1}) and by Jain et al. (1998) for resting sockeye (approx. 1 mmol L^{-1}) – suggesting a substantial but not incapacitating level of stress. Glucose in captive fish was two to three times higher than in native arrivals. By comparison, however, concentrations in captive fish were similar to those seen in other sockeye populations near spawning (approx. 11–12 mmol L^{-1}; Kubokawa et al. 1999; Patterson et al. 2004). Plasma chloride, osmolality, and to a lesser extent sodium levels were lower in captive fish than in native arrivals, albeit still the within normal ranges reported for this life history stage (e.g. chloride concentrations of 100–120 mmol L^{-1} in Weaver Creek native arrivals; Shrimpton et al. 2005).

Within captive fish, there was evidence that fast fish were more stressed than the slow. In both years, the former showed a significantly greater decline in chloride levels. The magnitude of this difference was important, as low plasma chloride (hypochloremia) and can become life-threatening at concentrations below 90 mmol L^{-1} (Wedemeyer et al. 1990). By comparison, mean plasma chloride in fast fish (in 2006) was 100 mmol L^{-1}, including all three individuals in the experiment with concentrations below 90 mmol L^{-1}. Furthermore, fast fish in both years were in worse external condition, including more frequent and severe cases of skin and gill infections. This greater morbidity was presumably a consequence of stress-induced immunosuppression (*sensu* Pickering and Pottinger 1989; Wendelaar Bonga 1997), with the infections themselves further exacerbating stress levels. Visual and video observations did not reveal obvious behavioural differences (e.g. number of antagonistic interactions) that would have led to more dermal abrasions in fast fish, which could have otherwise contributed to their susceptibility to infections (Portz et al. 2006). In 2006, fast fish underwent additional changes that were significantly different in magnitude from the slow: a significant decrease in hematocrit (vs. a non-significant increase in the slow), and a non-significant increase in lactate (vs. a significant decrease in the slow). Given that low hematocrit (hemodilution) can be a sign of impaired osmoregulation (Wedemeyer et al. 1990) and that higher lactate indicates a greater stress response (see above), both differences could be cautiously interpreted as further evidence of the greater stress in fast fish. Conclusively interpreting the changes in hematocrit was difficult because their magnitude was relatively small, and values were well within the 'normal' range for sockeye previously reported by Jain et al. (1998), Magnoni et al. (2006), and Crossin et al. (in

review; 0.35, 0.45-0.55, and ~ 0.35, respectively). Interpreting lactate changes was complicated by a relatively large, although non-significant (P = 0.07) difference in pre-treatment levels (Figure 3.5e). Thus, we further investigated the treatment effect by comparing final values directly (instead of change from initial levels), and found that the difference between fast and slow fish remained marginally significant (P = 0.055). Furthermore, we noted that all but one fish in each treatment underwent changes in the same direction (decreases in the slow and increases in the fast), suggesting that lactate differences were indeed reflective of higher stress in fast fish.

Despite marked differences in stress levels between native arrivals and captive fish, and further differences between fast and slow fish, no groups differed in terms of sex traits or progeny attributes (as previously discussed in terms of energetic trade-offs). This was contrary to our expectation that stress might impair reproductive traits. Indeed, others have found that reproductive timing can be altered by stress (Contreras-Sánchez et al. 1998; Patterson et al. 2004), that fertilization and hatching success are often higher in wild versus captive fish (reviewed in Brooks et al. 1997; Portz et al. 2006), and that stressed females may yield smaller progeny (Campbell et al. 1994; McCormick 2006). By contrast, findings from the present study suggested that during late maturation, salmon may have the capacity to buffer reproductive systems from the effects of stressful conditions (*sensu* Schreck et al. 2001).

Parental identity and offspring traits

The lack of treatment effects on reproductive and progeny traits underscored the very high inter-individual variability in these traits. For example, eyed-stage survival

ranged from nearly 0–100% among families in a given year, including those originating from the same treatment (Tables 3.3 and 3.4). Such variability, coupled with relatively low sample sizes, highlighted the limitations of treatment-based approaches for testing the effects of migratory conditions on reproduction. Nonetheless, important results (discussed below) were obtained by investigating progeny traits (i.e. incubation time, survival, and size) directly in terms of parental influences (irrespective of treatments).

Incubation time

We found a significant male effect on incubation time in both years, whereas female effects were only significant in 2005. Paternal influences are increasingly gaining attention for their contributions to progeny traits such as size and/or survival (Garant et al. 2002; Yamamoto and Reinhardt 2003; Green and McCormick 2005; Chapter 2 of this thesis), including cases where these effects exceed those attributable to maternal identity (Rideout et al. 2004). Less attention has been given to the putative influence of males on incubation time, despite mounting evidence in this regard (e.g. Gilbey et al. 2005; D. Patterson, Fisheries and Oceans Canada, pers. comm.; the present study). Future studies should attempt to quantify paternal effects on incubation time under various environmental conditions, since such influences could have a significant impact on juvenile survival rates (*sensu* Einum and Fleming 2000).

Progeny survival

Survival was strongly influenced by maternal identity in both years, concurrent with findings from other studies (e.g. Patterson et al. 2004; Chapter 2 of this thesis). Using 2006 data, we investigated the role of female condition on eyed-stage survival using multiple regression. 'Sampling event' was found to be the primary factor driving the variability in this trait, essentially an artefact of our unsuccessful preservation of milt for fertilizing successive sets of females. We focus instead on the significant effect of the second factor in importance, energetic status. Recall that at the treatment level, differences in energy levels were not reflected in progeny traits, contrary to our prediction that more difficult migrations would reduce allocations to gametes (thus potentially affecting progeny survival). By contrast, multiple regression suggested that survival was in fact highest in the progeny of females with the lowest energy levels. This finding was difficult to interpret, implying that females with the lowest energy somehow positively contributed to gamete quality, perhaps via investments in egg constituents that we did not measure (reviewed in Brooks et al. 1997). Another possibility is that females with the lowest energy were also those that ovulated earlier and would thus have been more 'ready' to spawn in a natural setting – although little is known about how egg quality might improve between ovulation and natural ovoposition, as opposed to the decrease in quality that occurs when ovoposition is delayed (egg over-ripening; Lahnsteiner 2000).

Female physiological stress did not appear to affect progeny viability at the treatment level (as discussed previously), although a trend to this effect ($P = 0.062$) was noted in multiple regression. Few studies have in fact demonstrated a direct link between

stress levels and subsequent progeny survival (reviewed in Brooks et al. 1997; but see Campbell et al. 1994; Eriksen et al. 2006) – including a comparison of moribund and healthy Weaver sockeye that differed in most of the physiological variables we measured (Patterson 2004). The effects of stress on progeny survival implied by our model are in contrast to our previously mentioned suggestion that females in late maturation can buffer the effects of stress on their progeny. Clearly, the influence of female physiological condition on egg quality warrants further investigation.

We found that maternal size did not significantly affect progeny survival, in line with some previous studies (Keckeis et al. 2000; Patterson 2004) but contrary to others (e.g. Heath et al. 1999). The nature of the relationship between maternal size (or egg size by proxy) and progeny survival remains somewhat equivocal, but varies in part as a function of the life history stage being considered (Kamler 2005). We note that our experiment did not include female-size-mediated spawning ground interactions that could have ultimately affected progeny survival, such as competition for partners or redd sites (Quinn 2005).

Progeny size

Fry families varied considerably in terms of length and mass (described together given their high correlation). Female identity remained a highly significant driver of offspring size after three weeks of exogenous feeding, emphasizing the fact that maternal influences can have persistent effects on the early life history traits (see Chapter 2). Maternal size explained at least half of the variability in offspring size, consistent with the strong allometric relationships we detected in sex trait morphology and the well-

documented correlation between egg size and progeny size (reviewed in Heath and Blouw 1998; Kamler 2005). Female size appeared to be responsible for the significant size differences we noted between offspring from fast and native arrival fish (values could not be corrected for maternal size). Indeed, progeny from the latter were smaller (Table 3.4), and closer examination revealed that native arrival females were also (non-significantly) smaller than fast fish by 6.5 cm.

As in our treatment-based analyses, we found no support for the prediction that low energy and high stress in individual mothers deleteriously affected offspring size. In fact, there was a significant positive effect of stress on progeny length, accounting for 9% of the variability in this trait. To our knowledge, studies reporting stress effects on progeny size (e.g. Campbell et al. 1994; Contreras-Sánchez et al. 1998; Eriksen et al. 2006; McCormick 2006) invariably found a negative relationship. Consequently, further research is warranted before our finding can be interpreted conclusively.

Conclusion

Subjecting migrating sockeye salmon to two different current velocities resulted in differences in energy stores and stress levels, but did not generate the differences in reproductive investments we had predicted. Although we were limited in our ability to detect treatment effects based on energetic trade-offs (*sensu* Kinnison et al. 2001, 2003), our findings supported the notion that in late maturation, sockeye have the ability to buffer reproductive traits against increased energetic demands and physiological stress (*sensu* Shreck et al. 2001). We also highlighted important inter-individual variability in progeny traits. Significant relationships were found between female attributes and

offspring size and survival, even though the exact mechanisms underlying fertilization success in individual female sockeye remain elusive. This is clearly an avenue for future research, with important implications for both aquaculture and resource management. Particular attention should be paid to the effect of ovulation and spawning timing, which can be overlooked when using artificial fertilization approaches.

REFERENCES

Beacham, T. D., R. E. Withler, and C. C. Wood. 1995. Stock identification of sockeye salmon by means of minisatellite DNA variation. North American Journal of Fisheries Management 15: 249-265.

Beacham, T. D., M. Lapointe, J. R. Candy, B. McIntosh, C. MacConnachie, A. Table, K. Kaukinen, L. Deng, K. M. Miller, and R. E. Withler. 2004. Stock identification of Fraser River sockeye salmon (*Oncorhynchus nerka*) using microsatellites and major histocompatibility complex variation. Transactions of the American Fisheries Society 133: 1106-1126.

Brooks, S., C. R. Tyler, and J. P. Sumpter. 1997. Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7: 387-416.

Campbell, P. M., T. G. Pottinger, and J. P. Sumpter. 1994. Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture 120: 151-169.

Contreras-Sánchez, W. M., C. B. Schreck, M. S. Fitzpatrick, and C. B. Pereira. 1998. Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). Biology of Reproduction 58: 439-447.

Cooke, S. J., G. T. Crossin, D. A. Patterson, K. K. English, S. G. Hinch, J. L. Young, R. F. Alexander, M. C. Healey, G. Van Der Kraak, and A. P. Farrell. 2005. Coupling non-invasive physiological assessments with telemetry to understand inter-individual variation in behaviour and survivorship of sockeye salmon: development and validation of a technique. Journal of Fish Biology 67: 1342-1358.

Cooke, S. J., and S. G. Hinch. 2005. Survival, behaviour and physiology of migrating adult late-run Fraser River sockeye: identifying the cues and causes of abnormally high mortality prior to spawning. Final Report for 2004 Research Activity, Southern Endowment Fund, Pacific Salmon Commission. Pp. 1-49.

Crossin, G. T., S. G. Hinch, A. P. Farrell, D. A. Higgs, A. G. Lotto, J. D. Oakes, and M. C. Healey. 2004. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. Journal of Fish Biology 65: 788-810.

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

Eriksen, M. S., M. Bakken, A. Espmark, B. O. Braastad, and R. Salte. 2006. Prespawning stress in farmed Atlantic salmon *Salmo salar*: maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. Journal of Fish Biology 69: 114-129.

Farrell, A. P., P. E. Gallaugher, J. Fraser, and D. Pike. 2001. Successful recovery of the physiological status of coho salmon on board a commercial gillnet vessel by means of a newly designed revival box. Canadian Journal of Fisheries and Aquatic Sciences 58: 1932.

Gable, J., and S. Cox-Rogers. 1993. Stock identification of Fraser River sockeye salmon: methodology and management application. Pacific Salmon Commission Technical Report 5: 1-36.

Garant, D., P.-M. Fontaine, S. P. Good, J. J. Dodson, and L. Bernatchez. 2002. The influence of male parental identity on growth and survival of offspring in Atlantic salmon (*Salmo salar*). Evolutionary Ecology Research 4: 537-549.

Gilbey, J., A. McLay, D. Houlihan, and E. Verspoor. 2005. Individual-level analysis of pre- and post first-feed growth and development in Atlantic salmon. Journal of Fish Biology 67: 1359-1369.

Gilhousen, P. 1990. Prespawning mortalities of sockeye salmon in the Fraser River system and possible causal factors. International Pacific Salmon Fisheries Commission Bulletin 26: 1-58.

Green, B. S., and M. I. McCormick. 2005. Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. Marine Ecology Progress Series 289: 263-272.

Groot, C., and L. Margolis. 1991. Pacific salmon life histories. University of British Columbia Press, Vancouver, Canada.

Healey, M. C., R. Lake, and S. G. Hinch. 2003. Energy expenditures during reproduction by sockeye salmon (*Oncorhynchus nerka*). Behaviour 140:161-182.

Heath, D. D., and D. M. Blouw. 1998. Are maternal effects in fish adaptive or merely physiological side effects? Pp. 178-201 *In* T. A. Mousseau and C. W. Fox, eds. Maternal effects as adaptations, Oxford University Press, Oxford, U.K.

Heath, D. D., C. W. Fox, and J. W. Heath. 1999. Maternal effects on offspring size: variation through early development of chinook salmon. Evolution 53: 1605-1611.

Hendry, A. P., and O. K. Berg. 1999. Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. Canadian Journal of Zoology 77: 1663-1675.

Hendry, A. P., A. H. Dittman, and R. W. Hardy. 2000. Proximate composition, reproductive development, and a test for trade-offs in captive sockeye salmon. Transactions of the American Fisheries Society 129: 1082-1095.

Hinch, S. G., and J. Bratty. 2000. Effects of swim speed and activity pattern on success of adult sockeye salmon migration through an area of difficult passage. Transactions of the American Fisheries Society 129: 598-606.

Hinch, S. G., and P. S. Rand. 2000. Optimal swimming speeds and forward-assisted propulsion: energy-conserving behaviours of upriver-migrating adult salmon. Canadian Journal of Fisheries and Aquatic Sciences 57: 2470-2478.

Hinch, S. G., S. J. Cooke, M. C. Healey, and A. P. Farrell. 2006. Behavioural physiology of fish migrations: salmon as a model approach. Pages 239-295 *In* K. A. Sloman, S. Balshine, and R. W. Wilson, editors. Fish physiology, Volume 24. Academic Press, London.

Hinch, S. G., E. M. Standen, M. C. Healey, and A. P. Farrell. 2002. Swimming patterns and behaviour of upriver-migrating adult pink (*Oncorhynchus gorbuscha*) and sockeye (*O. nerka*) salmon as assessed by EMG telemetry in the Fraser River, British Columbia, Canada. Hydrobiologia 483: 147-160.

Hoysak, D. J., and N. R. Liley. 2001. Fertilization dynamics in sockeye salmon and a comparison of sperm from alternative male phenotypes. Journal of Fish Biology 58: 1286-1300.

Hruska, K. A., S. G. Hinch, M. C. Healey, and A. P. Farrell. 2007. Electromyogram telemetry, nondestructive physiological biopsy, and genetic markers: linking recent techniques with behavioral observations for the study of reproductive success in sockeye salmon mating systems. American Fisheries Society Symposium 54: 17-29.

Idler, D. R., and W. A. Clemens. 1959. The energy expenditures of Fraser River sockeye salmon during the spawning migration to Chilko and Stuart Lakes. International Pacific Salmon Fisheries Commission Progress Report 6: 1-80.

Jain, K. E., and A. P. Farrell. 2003. Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*. Journal of Experimental Biology 206: 3569-3579.

Jain, K. E., I. K. Birtwell, and A. P. Farrell. 1998. Repeat swimming performance of mature sockeye salmon following a brief recovery period: A proposed measure of fish health and water quality. Canadian Journal of Zoology 76: 1488-1496.

Jensen, J. O. T., W. E. McLean, P. J. Rombough, and T. Septav, 1992. S.I.R.P. user manual. Salmonid incubation rearing programs for IBM compatible microcomputers. Canadian Technical Report of Fisheries and Aquatic Sciences: 1878.

Kamler, E. 2005. Parent-egg-progeny relationships in teleost fishes: an energetics perspective. Reviews in Fish Biology and Fisheries 15: 399-421.

Keckeis, H., E. Bauer-Nemeschkal, V. V. Menshutkin, H. L. Nemeschkal, and E. Kamler. 2000. Effects of female attributes and egg properties on offspring viability in a rheophilic cyprinid, *Chondrostoma nasus*. Canadian Journal of Fisheries and Aquatic Sciences 57: 789-796.

Kinnison, M. T., M. J. Unwin, and T. P. Quinn. 2003. Migratory costs and contemporary evolution of reproductive allocation in male chinook salmon. Journal of Evolutionary Biology 16: 1257-1269.

Kinnison, M. T., M. J. Unwin, A. P. Hendry, and T. P. Quinn. 2001. Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution 55: 1656-1667.

Kubokawa, K., T. Watanabe, M. Yoshioka, and M. Iwata. 1999. Effects of acute stress on plasma cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon during the breeding season. Aquaculture 172: 335-349.

Lahnsteiner, F. 2000. Morphological, physiological, and biochemical parameters characterizing the over-ripening of rainbow trout eggs. Fish Physiology and Biochemistry 23: 107-118.

Lahnsteiner, F., T. Weismann, and R. A. Patzner. 1999. Physiological and biochemical parameters for egg quality determination in lake trout, *Salmo trutta lacustris*. Fish Physiology and Biochemistry 20: 375-388.

Lee, C. G., A. P. Farrell, A. Lotto, M. J. MacNutt, S. G. Hinch, and M. C. Healey. 2003. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. Journal of Experimental Biology 206: 3239-3251.

Macdonald, J. S. 2000. Mortality during the migration of Fraser River sockeye salmon (*Oncorhynchus nerka*): a study of the effect of ocean and river environmental conditions in 1997. Canadian Technical Report of Fisheries and Aquatic Sciences 2315.

Magnoni, L. J., D. A. Patterson, A. P. Farrell, and J.-M.. Weber. 2006. Effects of long-distance migration on circulating lipids of sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 63: 1822-1829.

McCormick, M. I. 2006. Mothers matter: crowding leads to stressed mothers and smaller offspring in marine fish. Ecology 87: 1104-1109.

McDonald, G., and L. Milligan. 1997. Ionic, osmotic and acid-base regulation in stress. Pp. 119-144 *In* G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. B. Schreck, eds. Fish stress and health in aquaculture, Cambridge University Press, Cambridge, U.K.

McMaster, M., K. Munkittrick, and G. Van Der Kraak. 1992. Protocol for measuring circulating levels of gonadal sex steroids in fish. Canadian Technical Report of Fisheries and Aquatic Sciences 1836.

Pankhurst, N. W., and G. Van Der Kraak. 1997. Effects of stress on reproduction and growth in fish. Pp. 73-94 *In* G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. B. Schreck, eds. Fish stress and health in aquaculture, Cambridge University Press, Cambridge, U.K.

Patterson, D. A. 2004. Relating the sockeye salmon (*Oncorhynchus nerka*) spawning migration experience with offspring fitness: a study of intergenerational effects. Masters thesis, Simon Fraser University. Burnaby, Canada.

Patterson, D. A., J. S. MacDonald, S. G. Hinch, M. C. Healey, and A. P. Farrell. 2004. The effect of exercise and captivity on energy partitioning, reproductive maturation and fertilization success in adult sockeye salmon. Journal of Fish Biology 64: 1039-1059.

Pickering, A. D., and T. G. Pottinger. 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiology and Biochemistry 7: 253-258.

Portz, D. E., C. M. Woodley, and J. J. J. Cech. 2006. Stress-associated impacts of short-term holding on fishes. Reviews in Fish Biology and Fisheries 16: 125-170.

Postlethwaite, E., and D. McDonald. 1995. Mechanisms of Na^+ and Cl^- regulation in freshwater-adapted rainbow trout (*Oncorhynchus mykiss*) during exercise and stress. Journal of Experimental Biology 198: 295-304.

Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. UBC Press, Vancouver, BC.

Rand, P. S., and S. G. Hinch. 1998. Swim speeds and energy use of upriver-migrating sockeye salmon (*Oncorhynchus nerka*): Simulating metabolic power and assessing risk of energy depletion. Canadian Journal of Fisheries and Aquatic Sciences 55: 1832-1841.

Rand, P. S., S. G. Hinch, J. Morrison, M. G. G. Foreman, M. J. MacNutt, J. S. MacDonald, M. C. Healey, A. P. Farrell, and D. A. Higgs. 2006. Effects of river discharge, temperature, and future climates on energetics and mortality of adult migrating Fraser River sockeye salmon. Transactions of the American Fisheries Society 135: 655-667.

Rideout, R. M., E. A. Trippel, and M. K. Litvak. 2004. Paternal effects on haddock early life history traits. Journal of Fish Biology 64: 695-701.

Schreck, C. B., W. Contreras-Sanchez, and M. S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197: 3-24.

Shrimpton, J. M., D. A. Patterson, J. G. Richards, S. J. Cooke, P. M. Schulte, S. G. Hinch, and A. P. Farrell. 2005. Ionoregulatory changes in different populations of maturing sockeye salmon *Oncorhynchus nerka* during ocean and river migration. Journal of Experimental Biology 208: 4069-4078.

Standen, E. M., S. G. Hinch, and P. S. Rand. 2004. Influence of river speed on path selection by migrating adult sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 61: 905-912.

Standen, E. M., S. G. Hinch, M. C. Healey, and A. P. Farrell. 2002. Energetic costs of migration through the Fraser River Canyon, British Columbia, in adult pink (*Oncorhynchus gorbuscha*) and sockeye (*Oncorhynchus nerka*) salmon as assessed by EMG telemetry. Canadian Journal of Fisheries and Aquatic Sciences 59: 1809-1818.

Tyler, C. R., and J. P. Sumpter. 1996. Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6: 287-318.

Wedemeyer, G. A., B. A. Barton, and D. J. McLeay. 1990. Stress and acclimation. Pp. 451-489 *In* C. B. Schreck and P. B. Moyle, eds. Methods for Fish Biology, American Fisheries Society, Bethesda, MD.

Wendelaar Bonga, S. E. 1997. The stress response in fish. Physiological Reviews 77: 591-625.

Yamamoto, T., and U. G. Reinhardt. 2003. Paternal effect on otolith size at emergence in masu salmon. Journal of Fish Biology 62: 1226-1231.

CHAPTER 4

CONCLUSIONS

This thesis consisted of experiments intended to bridge the gap between two disparate research efforts: those focusing on the fate of adult salmon migrating upriver versus those examining the fate of eggs and emergent fry. Both my experiments centered on the artificial fertilization of sockeye salmon from Weaver Creek. Chapter 2 investigated the role of adults in shaping juvenile traits; namely survival, size, and for the first time, burst swimming ability, as well as the magnitude and persistence of these influences. Chapter 3 evaluated the role of migration difficulty on reproductive attributes and subsequent progeny traits, by experimentally subjecting migrating adults to different current speeds while they completed sexual maturation.

My first set of conclusions broadly relates to the notion that individual spawners have important influences on the early life history of their progeny. Two aspects of such influences were particularly evident in this thesis: the importance of both of maternal and paternal contributions, and the temporal persistence of these influences. Both are briefly summarized below.

By rearing half sibs separately, I was able to compare the importance of maternal versus paternal influences on progeny traits such as survival, size, incubation time, and burst swimming ability. As expected, maternal influences largely dominated. Chapter 3 showed that female size, energy levels, and physiological status were all drivers of the observed offspring variability, although future research will be required to corroborate the relative importance of the latter two. In particular, the factors driving embryo viability

in sockeye warrant further attention, given that female condition does not appear to be a straightforward predictor of this trait (Patterson 2004; Chapter 3 of this thesis).

Paternal influences were found to be significant for several traits, a factor that has traditionally been overlooked in this type of study (Rideout et al. 2004). Particularly interesting was the detection of a male influence on egg incubation times, a little-documented effect which could be further investigated under different temperatures and dissolved oxygen concentrations, the primary drivers of incubation time (Quinn 2005). Even so, it is important to point out that the overall magnitude of male contributions was small (except for survival in Chapter 2, which was somewhat equivocal due to possible tank effects). In traits where significant male effects were noted, the proportion of variance explained was generally in the vicinity of 10% (Chapter 3). Given these estimates, I suggest that future studies should be designed to account for paternal influences, yet focus on maternal contributions as the more ecologically relevant source of variance. Such studies could circumvent the analytical issue of staggered ovulation dates by conducting several sets of crosses (with different males) as newly ovulated females became available, an approach which would still allow for quantifying male contributions. In this regard, my attempt to fertilize all 2006 females with the same males was probably unwarranted, resulting in a considerable sample size reduction because milt was not successfully preserved in the days between sets of fertilizations.

In both chapters, parental influences were detected in offspring size several weeks after fry had begun feeding independently. These results confirmed the ecological relevance of studying reproductive output in Pacific salmon: parental influences on offspring (irrespective of the underlying causes) are not rapidly 'outgrown', but rather

persist through crucial early life history stages, thus potentially contributing to shaping population dynamics and genetic diversity. This interpretation implicitly assumes that size hierarchies would persist similarly in the wild, and that being the case, that larger fry would have a fitness advantage over smaller ones. I believe this is a safe assumption to make, as many studies have directly or indirectly supported the notion that 'bigger is better' (e.g. Einum and Fleming 2000; Green and McCormick 2006). Surprisingly few actually tested the mechanisms assumed to be behind the increased fitness of larger juveniles (e.g. reduced susceptibility to predation, Taylor and McPhail 1985). In the present thesis, I did not directly test whether larger fry had higher fitness. Nonetheless, I found that fry burst swimming ability (Chapter 2), presumably a trait under substantial selective pressure in the wild, was very weakly related to size. This result calls for caution when interpreting size differences observed the lab, and suggests that attempts should be made to directly assess the biological relevance of parental effects in a natural setting – for example, whether emergent sockeye fry at either end of the size spectrum (i.e. a difference of approximately 50 mg and 3 mm; Chapter 3) actually have differential survival potential in terms of food acquisition or predator avoidance.

My second set of conclusions relates to Chapter 3 findings on the effects of migratory experience on reproductive development and subsequent progeny traits. Using experimental treatments, I did not find support for the hypothesis that salmon divert resources away from reproductive investments when faced with relatively adverse conditions. The lack of treatment effects was equivocal, however, because my ability to experimentally generate energetic trade-offs was limited by the advanced maturational state of fish. Energetic depletion and stress could also have played important roles in

reproductive success and progeny quality via effects on spawning ground behaviour. By conducting artificial fertilizations, I effectively eliminated differences that might have arisen from such behaviours – an aspect that is currently being investigated by a colleague (K. Hruska, Ph.D. dissertation, University of British Columbia, unpublished data).

My results suggested that in late maturation, reproductive mechanisms are relatively buffered against changes in energetic and physiological status. Adverse conditions encountered during this period may thus be a bigger threat to survival and/or the capacity to spawn. This would be concurrent with the observation that some fish suffer pre-spawn mortality (PSM, death after spawning ground arrival but without spawning), including >15% of wild Weaver Creek sockeye in 2005 and 2006 (R. Stitt, Fisheries and Oceans Canada, pers. comm.) – yet remarkably, gametes from these fish (dead or dying) frequently produce viable progeny when artificially fertilized (Patterson 2004).

The hypothesis that salmon experience trade-offs in energetic allocations to swimming, reproduction, and other functions during migration is conceptually appealing – particularly because migrants are not feeding, which should facilitate the observation of such processes. As a result, studies are increasingly mentioning individual-level energetic trade-offs as a theoretical possibility or as a documented occurrence (e.g. Crossin et al. 2004; Keefer et al. 2004; Fraser and Bernatchez 2005), nearly all citing Kinnison et al. (2001, 2003). To my knowledge, the latter studies are the only ones to have empirically demonstrated energetic trade-offs whereby experimentally increasing migration difficulty decreased allocations to reproductive traits. Surprisingly, these effects were noted

between groups migrating 17 or 100 km, a negligible difference in Fraser River terms. Clearly, additional research is needed to substantiate the extent and magnitude of putative energetic trade-offs in individual migrants. Particular attention should be paid to the sub-organismal mechanisms potentially underlying these trade-offs, for example the effects of exhaustive swimming on the rate of Vtg uptake by maturing eggs.

In Chapter 3, I used current speeds/travel distances as indicators of 'migratory difficulty', yet these are only some elements to be considered in our broader understanding of how migrants are affected by in-river conditions. Owing to climate change, Pacific salmon will soon face conditions to which they have not adapted (Rand et al. 2006). Understanding how Fraser River sockeye will fare under these conditions, such as their ability to reach spawning grounds (Crossin et al., in review) or to complete normal reproductive development, will be essential to ensure healthy populations in the years to come.

REFERENCES

Crossin, G. T., S. G. Hinch, A. P. Farrell, D. A. Higgs, A. G. Lotto, J. D. Oakes, and M. C. Healey. 2004. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. Journal of Fish Biology 65: 788-810.

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

Fraser, D. J., and L. Bernatchez. 2005. Adaptive migratory divergence among sympatric brook charr populations. Evolution 59: 611-624.

Green, B. S., and M. I. McCormick. 2005. Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. Marine Ecology Progress Series 289: 263-272.

Keefer, M. L., C. A. Peerya, T. C. Bjornna, M. A. Jepsona, and L. Stuehrenbergb. 2004. Hydrosystem, dam, and reservoir passage rates of adult chinook salmon and steelhead in the Columbia and Snake rivers. Transactions of the American Fisheries Society 133: 1413-1439.

Kinnison, M. T., M. J. Unwin, A. P. Hendry, and T. P. Quinn. 2001. Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution 55: 1656-1667.

Kinnison, M. T., M. J. Unwin, and T. P. Quinn. 2003. Migratory costs and contemporary evolution of reproductive allocation in male chinook salmon. Journal of Evolutionary Biology 16: 1257-1269.

Patterson, D. A. 2004. Relating the sockeye salmon (*Oncorhynchus nerka*) spawning migration experience with offspring fitness: a study of intergenerational effects. Masters thesis, Simon Fraser University. Burnaby, Canada.

Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. UBC Press, Vancouver.

Rideout, R. M., E. A. Trippel, and M. K. Litvak. 2004. Paternal effects on haddock early life history traits. Journal of Fish Biology 64: 695-701.

Taylor, E. B., J. D. McPhail. 1985. Burst swimming and size-related predation of newly emerged coho salmon *Oncorhynchus kisutch*. Transactions of the American Fisheries Society 114: 546-551.