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Effects of Three Generations of Captive Breeding and Rearing on Survival, Growth, and Other Phenotypic Traits in Inner Bay of Fundy Atlantic Salmon (*Salmo salar*)

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Live Gene Bank populations of Stewiacke River and Big Salmon River inner Bay of Fundy Atlantic Salmon (*Salmo salar*) have now undergone approximately three generations of captive breeding and rearing. Several characteristics of the Stewiacke Live Gene Bank program, including the maintenance of pedigree and other information on nearly all salmon spawned each year, make possible the monitoring of several key traits through time and the testing of some of the possible effects of the general Live Gene Bank program (and specific management strategies employed within), on several indicators of offspring performance.

A moderate amount of intra-year (or intra-generation) variation was observed for nearly all of the approximately 15 traits studied, and differences between years were often significant. The timing of some of the larger among-year differences observed often overlapped with either changes in measurement methodologies used or captive rearing conditions employed. However, we observed very few trends or directional changes in measured traits, potentially indicative of either plastic responses to directional environmental (captive or wild) changes through time or adaptation to captive conditions.

Our assessment of the overall general impact of the Live Gene Bank program on possible indicators of fitness was limited to testing for differences in performance traits across salmon that had experienced between 2.0 and 3.25 ancestral generations of captive breeding and rearing. Even so, survival in the wild from release at Age 0+ to recapture at Age 1+ appeared to decrease with increasing numbers of program generations, and this relationship was significant in one of four spawning years assessed. Results of investigations into some of the possible effects of individual variables, each associated with specific management strategies employed, on different metrics of offspring performance were mixed. Parental and maternal early juvenile rearing environments did not appear to influence offspring survival in the wild from release at Age 0+ to recapture at Age 1+ or Age 2+, approximately 15 and 27 months later, respectively, but it did appear to impact size (length and weight) of Age 1+ parr in the wild. We did not detect any effect of (1) parent family size in the wild, (2) parent Mean Kinship, (3) pedigree inbreeding (F), or (4) offspring expected heterozygosity, on any offspring performance trait measured. Female (but not male) parent age did have a significant (positive) impact on nearly all metrics of offspring performance assessed, and only some of this effect appears to be due to increasing egg size.

INTRODUCTION

The Atlantic Salmon (*Salmo salar*) (inner Bay of Fundy (iBoF)) Live Gene Banking program (LGB), recently described in the companion document to this publication (O'Reilly et al. 2018), began in 1998 when some of the last wild-origin native iBoF juveniles were collected from the Big Salmon (BSR) and Stewiacke (STW) rivers, and were brought into DFO's (Fisheries and Oceans Canada's) biodiversity facilities for captive breeding and rearing. As of 2015, some adults in the Live Gene Banking program will have experienced three cycles or generations of Captive Breeding and Rearing (CBR), and associated offspring produced that year (as of 2017) will be well into their fourth generation; the proportion of program adults experiencing at least three generations of CBR is expected to increase through to 2019 or 2020.

Well controlled, highly replicated experiments on model organisms, including invertebrate and vertebrate species, demonstrate that animals reared and bred in captivity for multiple generations adapt to captive conditions and experience cumulative genetic change that appears to reduce fitness in the wild (Woodworth et al. 2002; McPhee, 2003; Montgomery et al. 2010; Malo et al. 2010). Moreover, a number of recent studies involving several species of Pacific salmon have reported large reductions in wild fitness after only one or two cycles of captive breeding and rearing (Araki et al. 2007a, b, and 2009; Theriault et al. 2011; Christie et al. 2012). Finally, studies carried out on iBoF LGB salmon directly (de Mestral et al. 2013; Wilke et al. 2014; N. Wilke, pers. comm.; I. Fleming, pers. comm.) reported environmental and/or selective effects of a single generation of CBR on several phenotypic traits studied, including egg size, parr size, smolt size, and smolt run timing. It is becoming increasingly apparent that genetic adaptation to captivity (and associated loss of fitness) is a more serious threat to future population restoration than previously recognized (Frankham 2008). Genetic management programs for endangered species intended for reintroduction into the wild need to be altered to explicitly address adaptation to captivity if future restoration efforts are to be successful (Montgomery et al. 2010).

A number of modifications to conservation programs have been proposed to minimize rates of adaptation to captivity and loss of fitness over the years, many of which were recently reviewed by Williams and Hoffman (2009). These authors grouped the observed approaches into four main categories:

1. minimizing selection (Sh2) for captivity via (a) the use of breeding programs (Equalization of Family Size or EFS and Minimization of Mean Kinship or MMK) or (b) naturalization of the rearing environment;
2. fragmentation of populations;
3. immigration of individuals from the wild into captive populations; and
4. minimizing the number of generations spent in captivity (early return of populations to the wild, or delaying reproduction and cryopreservation of gametes).

Many of the above measures have been employed, to some extent, in one or more of the region's iBoF LGB programs. For example, in the STW iBoF LGB program, spawners are selected and paired using Ranked Mean Kinship (RMK) breeding protocols (O'Reilly et al. 2018), designed to minimize average Mean Kinship (MK) in a population (Ivy and Lacy 2012). Theoretical (Ballou and Lacy 1995; Fernandez et al. 2001) and empirical studies (Lacy et al. 2013; Willoughby et al. 2015) demonstrate that MMK programs are highly effective at minimizing loss of genetic variation and accumulation of inbreeding. It has also been suggested that MMK breeding protocols are expected to slow or even stop adaptation to captivity (Lacy 2009). Although there are some indications that rates of evolutionary change in traits measured

may indeed be reduced under MMK compared to random mating (reviewed in Frankham 2008), there is little empirical evidence to date to indicate that fitness loss in the wild is also minimized (Williams and Hoffman 2009). Furthermore, after a few generations, once founder contributions are equalized, MMK is expected to approximate EFS (Montgomery et al. 2010; Frankham 2008), and EFS is expected to relax selection against deleterious alleles, which could lower fitness in the wild (Rodriquez-Ramilo et al. 2005). Theoretical analyses suggest that relaxation of selection under EFS (or MMK after a few generations) will only occur in very large populations or over many generations (Fernandez and Caballero 2001; Theodorou and Couvet 2003). These results have since been corroborated by empirical studies involving highly replicated lines of *Drosophila melanogaster* (Common Fruit Fly) maintained in captivity across 38 generations; no difference was observed between individuals maintained under EFS versus under no management (free contributions) in (1) egg-to-adult viability, (2) mating success and (3) global fitness (Rodriguez-Ramilo et al. 2005). These authors did, however, report higher heritability for sternopleural bristle number and an increased number of microsatellite alleles (both measures of within-population genetic diversity), as expected, under EFS. The effects of MMK strategies (again, functionally similar to EFS after a few generations) on indicators of fitness have also been investigated directly in one vertebrate species, the White-footed Mouse (*Peromyscus leucopus*), where replicated lines were maintained in captivity across ten generations (Malo et al. 2010). Again, no negative effects of MMK breeding protocols on the two traits measured (sperm quality and fertility) were detected; rates of decline were similar compared to random mating. However, little information on the possible effects (positive or negative) of MMK breeding protocols on fitness is available for salmonids, a highly fecund group of organisms that normally experiences very high early juvenile mortality in the wild and potentially intense natural selection against maladaptive genes or gene combinations (Waples 1999).

Population ecologists have also expressed concerns over the use of MMK ranking and selection strategies under specific conditions, where breeders (originally born in captivity and released into natural river habitat) are obtained from the wild after a period of natural selection. For example, if large numbers of captive-bred individuals from multiple families are released into the wild, exposure to natural selection may be expected to reduce the size of less fit families relative to more fit families. Subsequent collections of later stage individuals from released groups would be expected to consist of fewer representatives of the remaining smaller less fit families and more representatives of the larger more fit families. On average, individuals belonging to these smaller families would be expected to exhibit lower MK values, and individuals from larger families, higher MK values. In theory, subsequent program-driven preferential spawning of low-MK individuals obtained from the wild (relevant here, see below) may then increase the frequency of genes associated with reduced survival in the wild, thereby decreasing the average individual fitness of salmon in the wild over time.

Given the broad theoretical concerns over the long-term application of MMK and/or EFS on fitness in the wild, information from additional species, particularly under-represented vertebrate taxa with different life histories and fecundities (such as Atlantic Salmon), would be useful in further assessing their possible effects.

Another common recommendation to reduce rates of adaptation to captivity (reviewed by Williams and Hoffman 2009), is the naturalization of rearing environments. In the iBoF LGB program, less emphasis has been placed on modifying or naturalizing the hatchery environment. Instead, the majority of offspring produced each year are transferred to native river habitat at a very early stage (approximately 1.5 months post-hatch), where they reside for one to two years before a subsample is collected and returned to the hatchery. During this period, mortality, including possible selection-mediated mortality, can be quite high (Waples 1999);

individuals exposed to wild river conditions experience predation, competition for scarce resources, natural pathogen regimes, etc. These individuals are referred to here as “wild-exposed” salmon. A small number of post-hatch fry (5–10 per family) are also retained in the hatchery for exclusive captive rearing through to maturity; individuals so managed are referred to as “captive-reared” salmon (see Figure 1 for a schematic depicting these two groups). Although some of these captive-reared salmon were spawned in the production of the next generation of LGB salmon, the ratio of wild-exposed to captive-reared salmon spawned in a given year has been steadily increasing, and the STW LGB program is now in the process of shifting towards the spawning of wild-exposed salmon only; captive-reared salmon are to be maintained only for contingency purposes.

In addition to potentially minimizing rates of adaptation to captivity over time, early exposure of LGB parents (as juveniles) to natural river conditions may benefit the iBoF recovery program through several additional mechanisms. It has long been known that early exposure of organisms to certain environmental conditions can bring about non-genetic changes to the genome (including methylation of DNA (Deoxyribonucleic Acid)) that can affect gene transcription and ultimately the phenotype; this is indeed a likely basis of at least some of the phenotypic plasticity observed across a range of taxa studied. Surprisingly, a growing body of research, involving a wide range of studied species, is now indicating that some of these environmentally-induced non-genetic changes may be passed from generation to generation (reviewed in Bossdorf et al. 2008). If these intergenerational effects influence the offspring phenotype in a direction that increases survival when reared under conditions similar to that experienced by their parents during the same period of development, early exposure of a modest number of LGB parents (dozens) to natural river conditions may increase the survival of large numbers of offspring (hundreds of thousands) released into similar wild river environments, potentially benefiting supplementation and/or restoration efforts. Indeed, direct exposure of iBoF LGB BSR parents to native river conditions as juveniles has been shown to increase, by a factor of two, offspring survival in the wild when compared to offspring of genetically similar parents reared exclusively in captivity their entire lives (Evans et al. 2014). However, more research is needed to determine the generality of these findings and the extent to which this management strategy can be expected to benefit offspring of similarly reared parents in other conservation programs. Additionally, insight into the likely mechanisms behind these transgenerational effects (i.e., maternal egg provisioning, epigenetic, or genetic) would be useful in assessing the long-term impact of this conservation strategy and how best to employ it in restoring wild self-sustaining populations.

Currently, STW Atlantic Salmon are spawned exclusively in their fifth year (at Age 4 relative to their hatch year, or Age 5 relative to their brood year or fertilization year) for the LGB program, in an attempt to minimize the number of cycles of captive breeding and rearing, the top (most likely to be effective) recommendation for minimizing adaptation to captivity (Williams and Hoffman 2009). In other words, offspring of STW salmon that are spawned in their fifth year only are managed according to the above “captive-rearing” and “wild-exposure” regimes (Figure 2), where representatives are recovered, genotyped, pedigreed and spawned in the production of the next generation of LGB program salmon. Many of these same salmon are also spawned in their fourth year (at Age 3 relative to their hatch year, or Age 4 relative to their brood year or fertilization year), but the offspring produced by these individuals are released throughout the STW River for supplementation purposes and none are recycled back into the LGB program. There are several additional implications of this conservation measure. First, Atlantic Salmon in general (Scott and Crossman 1998), and iBoF salmon in particular (Verspoor et al. 2002), often spawn at Age 3 (relative to their hatch year), after approximately two years (post-hatch) in freshwater and one year at sea. In the current STW LGB program, selection for maturation at this earlier age has been relaxed; individuals are spawned in the production of the next

generation of LGB salmon in their fifth year (at Age 4 relative to their hatch year, Age 5 relative to their brood or fertilization year). Second, in humans the incidence of genetic abnormalities, disease, and birth defects appears to increase with maternal (Hook 1981; Heffner 2004) and paternal (Kong et al. 2012; McIntosh et al. 1995) age; a similar association between parent age and genetic mutations or offspring defects may exist in other less well studied taxa, including Atlantic Salmon. Moreover, in Atlantic Salmon early offspring survival is strongly influenced by egg size and egg quality (Einum and Fleming 2000), which, in salmonids, is influenced by parent size (Hendry and Day 2003), which also generally increases with maternal parent age. Information on effects of parent age, particularly in the context of egg size, on survival in the wild would be useful in evaluating some of the potential consequences of delaying reproduction in STW LGB salmon until the fifth year.

It is generally recognized that inbreeding and associated inbreeding depression represent a serious threat to small populations (Frankham and Ralls 1998). When evaluated using well-controlled experimental designs, often carried out in captivity, researchers nearly always report some negative impacts of inbreeding on offspring performance (reviewed in Wang et al. 2002). Moreover, amounts of inbreeding depression associated with a given level of inbreeding are often higher when assessed in the wild where conditions are likely to be more stringent (Ryman 1970; Thrower and Hard 2009). Finally, recent research involving large, well-controlled and highly replicated experimental designs have detected inbreeding depression (lower sperm motility and fertility) at very low levels of inbreeding (F=0.03; Malo et al. 2010), further highlighting the risk this phenomenon poses to long-term conservation programs involving closed finite populations, where some accumulation of inbreeding is unavoidable.

Possible effects of inbreeding and outbreeding have recently been assessed in iBoF Atlantic Salmon (Houde et al. 2011; Rollinson et al. 2014). In these experiments, crosses were carried out within and between salmon obtained from the Great Village (GRV), Economy (ECO), and STW rivers, all from the Minas Basin side of the iBoF. Intentional crosses between known siblings (inbred crosses) were also carried out. Next, offspring from all six within- and between-population crosses, as well as offspring of the intentional inbred crosses, were released into all three native river environments. Juveniles were sampled one and two years later via electrofishing, and relative survival of different cross types were assessed from recapture information.

Even though survival was assessed in the wild, the experiment was well controlled (offspring from each population and hybrid cross were reared in all three river environments in a reciprocal translocation design), and multiple crosses were carried for each cross type; however, results were mixed. Houde et al. (2011), which assessed results from a single brood year, reported little evidence of inbreeding depression (reduced survival) from intentional full- and half-sibling crosses, little evidence of heterosis[1] in between-population crosses, and a trend towards possible outbreeding in one hybrid population cross. On the other hand, Rollinson et al. (2014) assessed results across two brood years (including the year analyzed by Houde et al. 2011) and reported some evidence of inbreeding and outbreeding depression, results that varied by year, but more consistent negative effects of intentional full- or half-sib inbreeding than outbreeding. Further information from additional years, and crosses involving STW and other more divergent iBoF river populations, would be useful in assessing effects of existing levels of inbreeding and expected benefits of outbreeding.

[1] Heterosis, hybrid vigor, or outbreeding enhancement, is the improved or increased function of any biological quality in a hybrid offspring.

Natural environments are often very heterogeneous, fluctuating in space and time, and this variability can have large effects on the survival and reproductive success of organisms living within them. Natural selection and evolution have provided animals and plants several ways to accommodate this variability, thereby increasing their chances of surviving to successfully reproduce. Animals may respond to environmental variability in the short term (within a generation) via phenotypic plasticity (Thompson 1991), in the medium term (within a few generations) via epigenetic or other non-genetic transgenerational mechanisms (Foley et al. 2009), and in the longer term (within a few to multiple generations) genetically through genetic adaptation. Atlantic Salmon reared in the hatchery environment may be expected to change phenotypically, epigenetically, and genetically in response to these different conditions. Indeed, hatchery-associated changes have been observed in egg size (Heath et al. 2003), behaviour (de Mestral and Herbinger 2013), body shape (Von Cramon-Taubadel et al. 2005), body colouration (Janhunen et al. 2009) and physiological condition (Berejikian et al. 2005). Important phenotypic traits known to be influenced by the hatchery environment and that are suspected of impacting survival and reproductive success in the wild should be monitored for change over time.

Atlantic Salmon eggs and milt from European (EU) farm salmon were imported into Maine in 1989–1994, and within a few years European genes permeated 30–50 percent of all local broodstock (Baum 1998). Juvenile hatchery farm salmon (identified from fin erosion and scale growth-ring data) collected from a New Brunswick river whose watershed was completely contained within the province exhibited multiple mitochondrial and DNA markers for EU ancestry, indicating that EU farm salmon were also being used by the aquaculture industry in Canada (O'Reilly 2006). Recently, markers for European ancestry have been detected in Atlantic Salmon juveniles collected from several rivers of the iBoF (O'Reilly et al. 2018). European (EU) and North American (NA) salmon have been reproductively isolated for approximately 1 million years (Hurst et al. 1999), and they exhibit deep genetic divergence, including chromosomal differences (Roberts 1970; Hartley 1987; Brenna-Hansen et al. 2012). It can therefore be expected that local farm salmon escapees of European origin may represent a greater threat to wild iBoF salmon relative to escapees of local origin. Several initially undetected putative European farm/iBoF hybrids (16) were unintentionally spawned in the STW LGB program, and hybrid offspring were released alongside other pure STW offspring into an isolated stretch of the Stewiacke River. The current pedigree-based wild-exposure program (Figure 2), with further details provided in O'Reilly et al. (2018), thus provides the opportunity to assess some of the possible effects of EU farm genes on early juvenile survival of STW salmon in the wild.

The objectives of this study are as follows:

1. to report on the direction and rates of change in a number of phenotypic characteristics across years and generations that might be expected to have been influenced by environmental and selective effects of captive breeding and rearing;
2. to assess the effects of multiple cumulative generations of captive breeding and rearing on offspring survival in the wild;
3. to test for possible effects of several variables associated with one or more specific management strategies or decisions, including parental rearing environment, cumulative ancestral rearing environment, Mean Kinship, parent family size in the wild, pedigree inbreeding, offspring genetic variability (expected observed family/offspring heterozygosity), cross type (outbreeding), and maternal and paternal parent age, on offspring performance in captivity and in the wild; and

4. to assess the effects of possible EU farm salmon genes, recently observed in several iBoF Atlantic Salmon populations, on early juvenile survival in wild iBoF river habitat.

The above assessments of the effects of different variables, including some associated with specific management strategies, on offspring performance in the wild are possible because of two somewhat unique aspects of the STW LGB program. First, all STW LGB salmon spawned in the production of the next generation are tissue-sampled, genotyped, and pedigreed. Detailed information on parental rearing environments, number of program generations, Mean Kinship, pedigree inbreeding, molecular genetic heterozygosity, parent length, parent age, mean family egg size, mean family fry size, etc., are maintained on nearly all program individuals. Second, a similar number of offspring (as fry) are enumerated, equalized, and released into an isolated segment of the Stewiacke River, where they reside for one to two years before recovery as Age 1+ or Age 2+ parr, at which time a subset of individuals are sampled, genotyped and pedigreed (Figure 2; see also O'Reilly et al. (2018) for more details). This allows assessment of the effects of many parental variables (such as parent age or parental rearing environment) on offspring survival and growth in wild native habitat. Very few pedigree-based, multigenerational studies of the effects of captive breeding include response data from individuals residing in truly wild environments; most involve assessments of performance in simulated wild environments (Woodworth et al. 2002; Malo et al. 2010), although there are some and they are becoming more common (Araki et al. 2007a, b; Christie et al. 2012; Hess et al. 2012; Theriault et al. 2011). However, the primary purpose of the LGB activities (equalizing family size, releasing offspring into the wild, recovery of offspring, genotyping, pedigree assignment, etc.) associated with the production of the data analyzed here is the conservation of genetic characteristics of iBoF Atlantic Salmon, including the maintenance of genetic variation but also the minimization of adaptation to captivity. As a result, many elements of the "experimental design" (sample size, numerical evenness of crosses associated with different treatments, treatment group size, etc.) in this study are not, in a statistical sense, optimal for detecting differences between groups and reporting on the magnitude of specific treatment effects on offspring performance. However, in a number of instances, we feel that sample sizes and other program elements are adequate to make several important inferences, especially in the context of Maritime Atlantic Salmon conservation, and decisions may need to be made based on the balance of probabilities.

METHODS

MONITORING CHANGES IN PHENOTYPIC TRAITS OVER TIME

Over the course of the LGB program (2000–2015), several traits were monitored at various life stages in endangered iBoF Atlantic Salmon from several stocks. A more in-depth monitoring program was established for the STW stock, which was chosen as the indicator river for the program (see Table 2 for a list of traits monitored in this group of salmon).

Adult Size (Length, Weight and Body Depth)

Each year at spawning, mature males and females were identified by their Passive Integrated Transponder (PIT) tag number or carlin tag number (2000–2001) and crossed according to a prescribed mating plan. Individual length and weight measurements, recorded to the nearest 0.1 cm and 0.01 kg, respectively, were tracked for each spawning adult. These measurements were recorded for most of the duration of the program with length data dating back to 2000 and weight data dating back to 2005. Condition factor (CF) was calculated as:

$$CF = 100(W/L^3) \qquad (1)$$

where, W = weight (g) and L = length (cm).

Since 2010, images have been captured of each spawning pair (left side of fish) using a mounted camera above a gridded platform. Using ImageJ 1.48v software (US National Institutes of Health, Bethesda, Maryland), adult images were used to measure body depth, anterior to the dorsal fin, to the nearest 0.001 mm, for each male and female spawner.

Egg Size (Area and Weight)

Since the 2013 brood year (BY), also referred to as spawning year, a subsample of eggs (20 to 30 eggs) from each cross were removed from the spawning bowl prior to the addition of milt. From the subsamples of pre-fertilized eggs, bulk weights (to the nearest 0.001 g) and images of 20 eggs per cross were obtained after blotting dry to remove excess water. Not every egg included in the egg images was included in the bulk weight estimates, and vice versa, as the subsample usually contained more than 20 eggs and the 2 were not done concurrently. Images were later processed, using ImageJ, to determine average egg area for each cross.

In the 2011-2012 BY, egg weights were not obtained and experiments were carried out to determine the optimal time and method of capturing egg area. In 2011, a subsample of eggs from each cross was photographed after water hardening (i.e. water intake and membrane hardening). The first 30 crosses were omitted from analyses due to poor image quality (eggs were imaged out of water without blotting, making their edges indistinguishable from the pooled water). The remaining 58 crosses were imaged underwater, which provided higher quality images that could be processed later using ImageJ. In the 2012 BY, eggs were imaged out of water, after blotting excess water from egg surfaces (described in the above paragraph); this method has been used to date.

Prior to 2011, rather than using images and weights to determine egg size, egg diameter was measured and subsequently used to calculate the egg area. When eggs reached the well eyed stage of development (i.e. shock; see below in *Well Eyed Stage* section), approximately 20 crosses were selected for egg diameter measurements and fecundity counts (see selection criteria below). The average egg diameter was then determined by calculating the average length (cm/10 eggs) of three replicate samples and dividing by 10 eggs.

To account for changes in the methodology, two correction factors were applied. The correction factors were based on repeated measurements of eggs taken at various developmental stages from a selection of 2015 BY crosses. Measurements were taken using the imaging method and measured egg diameter method described above. The first correction factor standardized for the methodology while the second accounted for the stage of egg development (Appendix Figures A1–A3).

Fecundity

Fecundity was estimated for each female based on the following length/count relationship:

$$\text{Fecundity} = a^{b*FL} \qquad (2)$$

where, a and b are estimated parameters from a subsample of females, and FL = fork length (cm). This relationship was adjusted yearly based on the egg counts from a subsample of crosses (average of 22 crosses/year), selected to represent the range of female spawner lengths present within the brood year. The initial fecundity of the selected crosses was determined by hand counting at the well eyed stage and correcting for previous mortality based on the records.

Starting with the 2010 BY, each cross was photographed at spawning to capture the initial fecundity. Initial images were taken of all the eggs (either pre-fertilized or after water hardening) out of the water, which resulted in poor quality images that had to be hand counted. In 2012,

each cross was photographed after water hardening. Eggs were placed in a shallow pan of water inside a light-dissipating camera tent to eliminate shadows and prevent glare. When required, eggs were split onto multiple trays to ensure they were only one layer deep, and imaged separately. These improved methods allowed images to be processed using ImageJ, which counted all the eggs automatically within the image.

Relative fecundity was used to account for the influence of female size upon fecundity and was calculated as the number of eggs/ female weight (kg).

Well Eyed Stage

When the eggs developed to the well eyed stage, they were subjected to a physical shock. This shocking process ruptures the sensitive yolk membrane of unfertilized and dead eggs, releasing the protein globulin, which precipitates when not in a salt solution within the yolk (Leitritz and Lewis 1980). The precipitate causes the eggs to turn white, making them more easily identified from living eggs and allowing their removal to prevent fungus from growing (Leitritz and Lewis 1980). Starting in 2013, a subsample of 20 “dead” eggs (i.e. white eggs) was collected from each cross and stored in Stockard’s Solution. Stockard’s Solution both preserves and clears eggs as it causes the globulin precipitate to go back into solution. Eggs were examined under a dissecting microscope to identify the proportions that were unfertilized versus those that had reached various stages of early development.

Egg mortalities for each cross have been recorded throughout the program. Since the 2010 BY, the eggs from each cross have been photographed to confirm the number of remaining eggs after removal of shocking mortalities. Imaging and processing methods followed the same procedures as described above for initial fecundity.

Percent survival, from fertilization to shock, was determined for each cross using the following formula:

$$\% \text{ Survival} = T_2/T_1*100 \qquad (3)$$

where, T_1 is the number of eggs per cross at fertilization and T_2 is the number of eggs at shock. Survival was then adjusted based on fertilization rates to determine survival of fertilized eggs from 2013–2015.

Fry Number and Size (Length and Weight)

Since the 2006 BY, after shocking, a subsample of eggs from each cross was removed and placed in individual baskets for rearing in heath units through hatch. The number of eggs per cross, and therefore baskets (maximum of 200 eggs per basket), for these equalized family groups (EQU) varied by year (Appendix Table A1) in an effort to maximize the recovery of families, increase the effective population size, and minimize rates of loss of genetic variation. The EQU baskets were randomly assigned a location within the heath units and reared through hatch until the yolk sac was 98% absorbed (typically until April or May). Mortality was tracked in order to obtain estimates of remaining fry, and images have been taken for each cross since 2010 to verify these estimates prior to release. Variance in family size was minimized again, just prior to release. This involved determining the target equalization family size (the maximum family size) for crosses, and then removal of excess individuals from larger families to achieve this number (see O’Reilly et al. (2018) for more details). Crosses were well mixed and then released into the Pembroke River above an impassable barrier (waterfall) to isolate EQU fry groups (wild-exposed LGB juveniles) from both a) juveniles released elsewhere in the STW for supplementation purposes, and b) offspring produced by released LGB adult salmon, returning

LGB salmon (released as juveniles) or (potentially) native remnant wild salmon or strays. Incidence and type of fry deformities were also tracked using the images taken prior to release.

A subsample of fry (15–20 fry) from the 2012-2013 BY was removed from each cross (half from each of the two baskets per cross) during equalization to obtain weight and length measurements. To assess possible basket effects, 15 fry from each of the two baskets per cross were sampled separately for 2014 and subsequent brood years. Within 48–72 hours of equalizing, fry were euthanized using a concentrated anaesthetic solution (MS-222). Fry were then lightly blotted to remove excess water, and a bulk weight (to the nearest 0.001 g) recorded. Next, fry were positioned on their right side, straightened (not stretched) with the caudal fin spread out, and several images taken of each family set of fry. Processing of images with ImageJ allowed for length determination of each fry (to the nearest 0.001 mm). A family-specific condition factor was calculated (see formula 1 above), using the average fry length and an approximate individual weight determined from the bulk weights (i.e. weight/# of fry).

Family-specific survival was calculated for shock to pre-release using formula 3. In this case, T1 is the number per cross at shock and the T2 is the number at pre-release.

Every year a subsample of 10–20 eggs from each cross was collected and reared communally at the Coldbrook Biodiversity Facility to the adult stage; these individuals constitute the captive-reared (CAP) component of the STW population. This group served as a backup or alternate source of families, in case their wild-exposed siblings were not recovered from Pembroke.

Parr Size

Prior to the initiation of EQU releases into the Pembroke River (2000–2007), parr were collected via electrofishing from various locations throughout the Stewiacke River. In the fall of 2008, and subsequent years, wild-exposed (WE) parr (1+ and 2+, also referred to as Age 1 and Age 2), previously released as EQU fry (2006 BY), were recaptured from the isolated stretch of the Pembroke River. Parr were captured using a backpack electrofisher and brought back to the Coldbrook Biodiversity Facility for rearing to the adult stage. Starting in 2013, parr were anesthetized using MS-222 (*Tricaine methanesulfonate*) and individually tagged using a small PIT tag (8 mm) within 6 days of their arrival. Weight (0.01 g) and length (0.1 cm) measures were recorded and a condition factor was calculated using formula 1. Each fish was photographed and a tissue sample was collected for genetic analysis, which was performed by the Aquatic Biotechnology Laboratory, located at the Bedford Institute of Oceanography, Dartmouth, Nova Scotia. Microsatellite genotype information obtained was used to assign individuals to families via exclusion-based parentage analysis. In previous years, parr were reared at Coldbrook for up to a year and a half before tagging and tissue sampling, making growth in the wild indistinguishable from growth in the hatchery environment.

Parentage assignment results were used to determine the age of WE juveniles (by identifying the cross of origin and the year the cross was carried out) and allowed for family-specific growth rates (FSG) to be calculated for their period of exposure to a wild environment as:

$$FSG = (\ln L_{T2} - \ln L_{T1}) * 100/t \qquad (4)$$

where, ln = natural log, L = length (cm), T1 = time 1, T2 = time 2, and t = time or # days between T1 and T2. Additional calculations were carried out for family-specific growth rates substituting weight (g) for length in the above formula.

Family size at capture was used as a proxy for survival in the wild (from release to recapture) using Formula 3. It should be noted, however, that this should be considered an approximate indicator of survival because although variance in family size at the time of release was minimized, and most (>80%) of all families released in a given year were identically sized, high

mortality prior to release for a subset of crosses resulted in some families (typically less than 10%) consisting of fewer individuals. For many analyses, family size was standardized to the number of individuals released. For brood years when exact numbers of EQU fry releases were not known, 2006–2008 (Appendix Table A1), the initial EQU number collected per family (Appendix Table A1) was adjusted by the average survival from shock to pre-release, and subsequently used as the T1 in Formula 3.

Smolt

Smolt from the STW River system, originating directly from juvenile WE or supplementation releases, or as offspring of a) program adult releases, b) program adult returns (released as juveniles), or c) remnant wild adult salmon, were originally collected using a rotary screw trap (also known as a smolt wheel) located near the mouth of the Stewiacke River. In 2009, the rotary screw trap was replaced with a fyke net that was deployed on the Pembroke River (below the waterfall) to capture migrating WE individuals from the EQU fry distributions. Smolt collected are mostly approximately Age 2, therefore the majority of the 2009 collections would likely be from the 2006 BY. This was the first BY for EQU distributions to the Pembroke. Smolt collections from the Pembroke fyke net have diminished in recent years as resource availability and weather has limited the duration and timing of active fishing.

Statistical Analysis (Trait Monitoring)

One-way analyses of variance (ANOVA) was used to test for year effects on each monitored trait. All data were tested for normality. ANOVA tests are robust to violations of normality, particularly when datasets are large. However, when sample sizes were small, or the data highly skewed, non-parametric Kruskal-Wallis (KW) tests were performed. When the assumption of homogeneity of variances was violated, Welch's ANOVA ($ANOVA_W$) was used, as it allows for unequal variances.

While it may be useful to determine variations from year to year, this was not the focus of these analyses. The main goal was instead to determine the magnitude and direction of any trends within the data over the years. Linear (LR) and quadratic regressions were used to determine overarching trends for each variable over time. When the data violated the assumptions of normality or equal variance, bootstrapping of the residuals using 10,000 iterations was applied, which is not subject to those assumptions. Bias corrected bootstrapped values were reported along with 95% confidence limits (CI_{LL}=lower limit, CI_{UL}=upper limit). Significance of the bootstrapped slope was determined by looking at the 95% confidence limits; if zero fell within the limits then the slope was not significantly different from zero.

Two-sample t-tests were used to determine differences between groups, such as gender or methodology. Although t-tests are fairly robust to violations of certain data assumptions, the Aspin-Welch unequal variance test ($T\text{-}test_{AW}$) was used when the variances of the two groups were not equal and the paired t-test ($T\text{-}test_P$) was employed when the samples were not independent. If data were not normally distributed, or there were small sample sizes, the Wilcoxon Rank-Sum Test (WRS) was used to test for differences in medians. Pearson correlation coefficients (r) were used to evaluate the association between two variables; when the normality assumption was violated the Spearman Rank Correlation was used (r_S).

The covariate effect of program generation was analyzed for some variables with data ranging over the available years of the LGB program (2000–2015) (see below section on the Number of program generations for description of how program generation was determined). Generations with a sample size of 1 were removed prior to analysis. To ensure that there were no interactions between year and program generation on any tested variable, general linear models

(GLM) were used. If no interaction was detected, *p*-values from ANOVAs (one way, Welch's, or Kruskal-Wallis when appropriate) were reported for the effect of program generation. Planned post-hoc comparisons were conducted to compare the following: founders (G_0) to the first generation (G_1), G_1 to the second generation (G_2), G_0 to the last generation (G_n), G_1 to G_n, and G_2 to G_n. Post-hoc analyses were completed with Tukey-Kramer (TK) multiple comparison tests, Games-Howell (GH) when variances were unequal, or with Kruskal-Wallis multiple comparison Z-value test (KW_z) with non-normal data.

Averages are reported ± one standard deviation throughout. A critical value of α=0.05 was used for all statistical analyses. Data plots were constructed using the graphing program SigmaPlot 11.0 (Systat Software Inc., San Jose, California), and statistical analyses were performed with the NCSS 2004 and NCSS 11 (NCSS, Kaysville, Utah) statistical packages and the RStudio 1.0.44 (2009–2016 RStudio Inc., Boston, Massachusetts) statistical package.

TESTING FOR POSSIBLE EFFECTS OF CAPTIVE BREEDING AND REARING ON OFFSPRING PERFORMANCE IN CAPTIVITY AND IN THE WILD

As mentioned above, a suite of phenotypic, environmental and genetic information is maintained on nearly all STW LGB salmon, including actual male and female parents spawned in the production of the next generation STW LGB salmon. This information, including number of cycles of captive breeding and rearing, early parent rearing environment, Mean Kinship values, etc. (see below for details) was then used as predictor variables to explore possible effects of one or more aspects of the iBoF LGB program on one or more measures of offspring performance.

Predictor Variables

All of the predictor variables analyzed here, including early parental rearing environment, cumulative early parental and grandparental rearing environment (across the parents and grandparents), number of program generations, average parent family size in the wild, average parental Mean Kinship, maternal Mean Kinship, offspring pedigree inbreeding, offspring expected observed heterozygosity, population cross type (outbred versus inbred), paternal parent age at spawning, maternal parent age at spawning, and presence of European farm salmon ancestry, are ultimately based on parentage assignment and associated pedigree linkages. Estimates of pedigree inbreeding will also be strongly influenced by original kinship assignment results. Parentage and kinship assignment methods, and likely accuracy of the latter, have been described in O'Reilly et al. (2018), the companion document to this publication.

Parental Early Juvenile Rearing Environments

The parental rearing environment of a given family (offspring as eggs, fry, or parr) refers to the early juvenile rearing environment (late-stage Age 0+ fry to, generally speaking, Age 1+ or Age 2+ parr) experienced by one or both of its two immediate (direct) parents. The two early juvenile parental rearing environments possible are (1) captive, and (2) wild. A parent reared in the captive environment during this period is identified as "captive-reared", and a parent released into the wild before being captured as "wild-exposed". Since each family has both a maternal and paternal parent, there are four specific parent cross types with respect to their early environment:

1. wild-exposed maternal/wild-exposed paternal (WExWE);
2. wild-exposed maternal/captive-reared paternal (WExCAP);
3. captive-reared maternal/wild-exposed paternal (CAPxWE); and

4. captive-reared maternal/captive-reared paternal (CAPxCAP).

In order to test for maternal effects (and to increase sample size), we also grouped (a) WExWE and WExCAP together to create the general wild-exposed maternal parent cross type (WE female), and (b) CAPxWE and CAPxCAP to create the general captive-reared maternal parent cross type (CAP female).

Cumulative Parental and Grandparental Early Juvenile Rearing Environments

We also investigated possible cumulative effects of early (juvenile) exposure of parents and grandparents to captive versus wild environments on offspring growth and survival in the wild, though we did not distinguish between maternal and paternal (or grandpaternal and grandmaternal) effects. In other words, we looked for a possible association between cumulative ancestral early (Age 0+ fry to Age 1+ or Age 2+ parr) exposure to captivity (going back two generations) and offspring performance in the wild. Experimental families of fry released into Pembroke whose parents and grandparents were all wild-exposed were identified as exhibiting no (0) ancestral early captive rearing, while those whose parents and grandparents were all captive-reared were identified as exhibiting complete (1.0) ancestral early captive rearing. Because grandparents pass one quarter of their genes (including potentially epigenetically modified genes) on to their grandoffspring, and parents one half of their genes on to their immediate offspring, all intermediate levels of ancestral captive rearing were quantified as follows. Families descended from two captive-reared grandparents, two wild-exposed grandparents, and two wild-exposed parents, were considered to exhibit a level of cumulative ancestral captive rearing of 0.250 [(0.25+0.25)/2]; the sum of cumulative ancestral captive rearing contributed by the grandparents and parents is divided by 2 so that the metric ranges between 0 and 1.0. If three grandparents and one parent were captive-reared, and all other ancestors (one parent and one grandparent) going back two generations were wild exposed, a given family was considered to exhibit a level of cumulative ancestral captive rearing of 0.625 [(0.25+0.25+0.25+0.5))/2].

Number of Program Generations

The number of program generations (generations of captive breeding and rearing) experienced by a particular family (offspring of a particular cross) was obtained from pedigree information, and was taken to be the average number of generations of CBR experienced by each of its two parents, plus 1. For example, a family produced by the spawning of a G0-generation individual (a population founder) and a G1 salmon (the direct offspring of two G0 parents) experienced a total of 1.5 cycles of CBR [(0+1)/2)+1].

Mean Kinship

In the early years of the iBoF LGB program, the MK value of a candidate spawner was taken to be the average kinship coefficient between that individual and all other living members of the population, including itself, and was calculated using pedigree information and formula 5.5 from Ballou and Lacy (1995) [see Figure 36 with associated text in O'Reilly et al. (2018), a companion document to this publication]. Beginning in the year 2005, it was noticed that large families with little relatedness to other families were being prioritized very low, with, in some instances, no or few representatives being selected for spawning at all. This unintended outcome of early MMK breeding protocols was observed in other conservation programs involving similarly fecund Pacific salmon species (O'Reilly and Kozfkay 2014) and in simulation-based comparisons of the expected efficiency of several alternate breeding algorithms (Ivy and Lacy 2012). In response to this observation, MK values in the LGB program (from approximately 2005 to 2012) were calculated assuming one representative per full-sib family. Therefore, in this analysis of the possible effects of MK prioritization of parents on offspring performance in the

wild, MK values were similarly calculated (assuming one individual per family). Specifically, the MK predictor variable against which offspring/family performance was being tested was taken to be the average of the two parents' MK values so calculated (average parental Mean Kinship), though we also tested for possible effects of the MK value of the sole female parent (maternal Mean Kinship). Note that although STW salmon were selected for spawning using Ranked Mean Kinship from 2013 on (where family size was incorporated into calculations of MK values), the offspring/families involved in this study were produced in years when MK values were calculated as described immediately above and salmon selected using Mean Kinship Assist (O'Reilly and Harvie 2010; O'Reilly and Kozfkay 2014; O'Reilly et al. 2018 for more details). It should be noted that Ranked MK-based breeding programs also mitigate the impact of very large family size on MK values and the low prioritization of all representatives of larger families. Although many representatives of large families are prioritized low for spawning under this breeding program, some are more highly prioritized by the algorithm, with the ranking of at least one representative reflecting, to an increasing degree, relatedness among families. In order to minimize possible confounding effects of parental rearing environment on assessments of MK effects, analyses included only families (offspring) produced by two wild-exposed parents.

Average Parent Family Size in the Wild

Each year, representatives of STW LGB families are sampled/collected from the wild after residing in native river habitat for, generally speaking, one to two years (O'Reilly et al. 2018). Recovered families vary considerably in numerical size, from 1 to 17 in any given year (unpublished data). This variation in family size reflects a) numbers of fry released (but to a minimal extent in the period assessed), b) chance sampling effects, c) differential emigration out of the release/recapture site, and d) survival in the wild. Sample collections obtained 1 or 2 years after release may be expected to contain more representatives of these larger (potentially higher surviving) families relative to their numerically smaller sized counterparts. In the absence of pedigree information, representatives from these larger numerically dominant families would be spawned more frequently than those from smaller families. If selection intensities were directional and strong, and variance in family size in sample collections obtained driven primarily by natural selection, this might be expected to minimize loss of fitness in the wild in the next generation. However, breeding programs that favour the selection and spawning of individuals from small families relative to large families (including EFS, MKA, and MMK), after a period of exposure to *native wild river conditions*, could minimize the between-family component of natural selection to wild conditions, possibly reducing some of the potential fitness benefits of this management strategy. Here, we test for possible immediate effects of, specifically, parent family size in the wild (and hence spawner selection regimes that incorporate family size information) on offspring performance in the wild. Specifically, the predictor variable used in the associated analyses is the mean of the family size of the male and female parents of a given cross (family) in electrofishing collections carried out on the Pembroke at Age 1, approximately 15 months after release into the wild. This information will be compared with offspring performance in the wild in the same stretch of river (described in more detail below). Note that by definition, this analysis only includes families/offspring produced by the spawning of two wild-exposed parents.

Offspring or Family Pedigree Inbreeding

Pedigree inbreeding was calculated using kinship coefficients obtained from the STW pedigree (see O'Reilly et al. 2018 for details). The level of pedigree inbreeding expected in the actual offspring/family for which trait performance (see below) was being assessed was used as the predictor variable. Also, note that in order to minimize possible confounding effects of parental rearing environment on performance, analyses included only families (offspring) produced by two wild-exposed parents.

Offspring Expected Observed Heterozygosity

Offspring or family expected observed heterozygosity was calculated from the multilocus genotypes exhibited by its male and female parents, assuming Mendelian inheritance and independent assortment. For example, consider the two hypothetical parents (Parent 1 and Parent 2) and their respective multilocus genotypes.

Table 1. Calculation of the Family Expected Observed Heterozygosity using a 2 loci example.

Parent	Locus 1, Allele 1	Locus 1, Allele 2	Locus 2, Allele 1	Locus 2, Allele 2
Parent 1	222	222	333	337
Parent 2	222	226	361	365
Family expected observed heterozygosity (single locus)	50%		100%	
Family expected observed heterozygosity (multilocus)	75%			

At Locus 1, all offspring will inherit a single 222 allele from Parent 1; half of these will inherit a second 222 allele from Parent 2 and the other half a 226 allele. The expected observed heterozygosity in the offspring (family) at this locus is 50%. At Locus 2, the 2 alleles observed in each of the 2 parents are different, and no 1 allele is common to both parents, so the expected observed heterozygosity in the offspring (family) at this locus is 100%. The average expected observed heterozygosity across the 2 loci is 75% [(50 + 100)/2]. All estimates of family expected observed heterozygosity used here were based on the same 12-locus set of microsatellites (see Table 20 in O'Reilly et al. 2018). Values so obtained were used as predictor variables in tests for associations between family expected observed heterozygosity and offspring survival.

Population Cross Type (Outbreeding), North Minas Basin x Stewiacke

Beginning in 2007, iBoF salmon from GRV, Debert (DEB) and ECO rivers (referred to collectively in this analysis as NMB salmon) were intentionally interbred with STW River salmon both to 1) capture genetic variation from wild-origin salmon collected from these three rivers, and 2) potentially minimize inbreeding and to increase allelic diversity in the STW LGB population. In order to test for possible heterosis effects in the offspring of these hybrid crosses (and, therefore, the presence of ancestral inbreeding and inbreeding depression in the STW population), we compared the performance of offspring of these outbred NMBxSTW crosses with that of offspring of crosses involving STW ancestry only, referred to here as inbred STWxSTW. Since the number of hybrid crosses for which offspring trait information was available in any one year was too small (1 to 10 depending on the year and trait studied) to test for differences between reciprocal cross types, crosses where the NMB parent was female and the STW parent male (NMBxSTW) were combined with crosses where the STW parent was female and the NMB parent male (STWxNMB) into the one cross type, identified as outbred NMBxSTW here. The F1 hybrid families were produced in each of the years 2007, 2008 and 2009, but the timing of initiation of different trait monitoring activities constrained which offspring performance traits could be assessed in any given year (described in more detail below). Note that crosses exhibiting any known European ancestry were excluded from these analyses.

Population Cross Type (Outbreeding), Gaspereau x Stewiacke

In 2013, 16 experimental hybrid crosses were carried out between STW and moderately divergent Gaspereau River (GAK) salmon (see O'Reilly et al. 2018 for information on phenotypic and genetic differences between these two groups). These crosses were performed because of concerns over the true origins of GRV and ECO salmon involved in other hybrid crosses studied here and elsewhere (Houde et al. 2011; Rollinson et al. 2014) and the expectation for heterosis. If ECO and GRV salmon were recent strays from the larger STW population, the scope for increases in genome-wide heterozygosity or the masking of deleterious recessive alleles from the mixing of genomes of these and remaining STW LGB salmon was very limited. The 16 outbred GAKxSTW hybrid families created are referred to as "outbred GAKxSTW", and were produced by spawning each of 8 different GAK female salmon once with 1 of 8 different STW males, and each of 8 different STW female salmon once with 1 of 8 different GAK males. Offspring of these 16 outbred crosses were reared, from fertilization on, alongside 16 paired GAKxGAK LGB (referred to here as inbred GAKxGAK) and 75 paired STWxSTW LGB (referred to here as inbred STWxSTW) crosses. In creation of both these latter 2 sets of crosses, each female was spawned once with each male, in a series of paired spawnings, as described above for outbred GAKxSTW crosses. The number of hybrid crosses was again too small to analyze the 2 reciprocal cross types separately. Note that crosses exhibiting any known European ancestry were excluded from these analyses.

Paternal and Maternal Parent Age at Spawning

In the years 2008–2013 (the relevant brood years studied in this analysis), male and female spawners ranged in age from three to eight, though most were four years of age; in all associated analyses, parent or spawner age is based on the brood year or fertilization year of these individuals, and not the year of hatching. From 2013 on, in an effort to reduce the number of cycles of captive breeding and rearing, reproduction of STW salmon (for the LGB program) was delayed until Age 5 (no salmon were spawned for the LGB program in later years). In order to investigate possible immediate impacts of this management decision, we tested for possible effects of male and female parent age (in the brood years prior to 2013) on family/offspring survival in the wild. Since age is positively correlated with female body size at spawning, and female body size with egg size, we also assessed the effects of egg size on survival, and interactions between parent age and egg size in assessments of offspring survival. Parent age was determined by assigning offspring to known parental crosses, noting the year the relevant cross was carried out, and consulting records to determine the age of the parent at that time. See above for details on how egg size was assessed. Since we were investigating possible effects of female and male parent age separately, the predictor variables in these series of analyses were, specifically, female or male parent age at spawning (female or male age when a given cross or family, for which survival was being assessed, was produced).

European Ancestry

In 1999, three parr collected from the STW River as part of the G0 founder group exhibited multiple markers for European (EU) ancestry (O'Reilly et al. 2018). These three G0 founder salmon are suspected of being EU farm/STW F1 hybrids, exhibiting 50% EU farm salmon ancestry. Each undetected EU F1/STW hybrid was then inadvertently spawned with other pure STW LGB salmon to create F2, exhibiting 25% EU farm ancestry. In 2009, one such individual was spawned to produce an F3-generation EU/STW hybrid family exhibiting 12.5% EU farm genes; in 2010 to 2012, 16 F3-generation EU farm /STW salmon were spawned to create 16 F4-generation EU/STW hybrid families, exhibiting 6.25% EU genes. In 2010–2013, as part of standard LGB operations (described above) a subset of these 17 EU farm/STW hybrid families were enumerated and released (as fry) into an isolated segment of the Pembroke River

(a tributary of the STW) alongside similarly managed STW-origin (STWxSTW) families, allowing assessment of the possible impacts of a relatively small percentage of EU farm ancestry on offspring survival in the wild.

Response Variables

We looked for possible effects of the above predictor variables, each associated with one or more elements of STW LGB management, on one or more response variables (indicators of offspring performance). Information associated with each response variable was obtained primarily from the STW WE LGB families, subsets of individual LGB crosses destined for release into Pembroke as Age 0+ fry for later capture as Age 1+ or Age 2+ parr for additional captive rearing, and subsequent recycling back into the LGB program as possible spawners.

These response variables were:

a. family mean length (mm) (the average across individuals within a family) or average family mean length (the average of family means within a treatment group) as fry at Age 0+ (pre-release);

b. individual or family mean length (cm) (the average across individuals within a family) or average family mean length (the average of family means within a treatment group) as parr at Age 1+ (post-capture from the wild);

c. individual or family mean length (cm) of adult females at Age 4+ (age of adults based on the brood year or fertilization year);

d. individual or family mean length (cm) of adult males at Age 4+ (age of adults based on the brood year or fertilization year);

e. family mean weight (g) (the average across individuals within a family) or average family mean weight (the average of family means within a treatment group) as Age 0+ fry (pre-release);

f. individual or family mean weight;

g. (the average across individuals within a family) or average family mean weight (the average of family means within a treatment group) of parr at Age 1+ (post-capture from the wild);

h. individual or family mean weight (kg) of adult females at Age 4+ (age of adults based on the brood year or fertilization year);

i. individual or family mean weight (kg) of adult males at Age 4+ (age of adults based on the brood year or fertilization year);

j. family percent deformities at Age 0+ (fry, pre-release) (percentage of individuals within a family exhibiting deformities) or average family percent deformities (the average percent deformities across families within a treatment) at Age 0+ (fry, pre-release);

k. average family incidence of deformities at Age 0+ (also equivalent to the proportion of sampled families exhibiting any deformities);

l. standardized family size in the wild at capture at Age 1+ (family size at Age 1+ standardized to the number of fry released from the same family at Age 0+, discussed below);

m. family percent survival in captivity from shock (at the egg stage, mid development) to pre-release as Age 0+ fry;

n. family percent survival in captivity from shock (at the egg stage, mid development) to tagging (at approximately Age 4);

o. family percent survival in the wild from release as Age 0+ fry to capture as Age 1+ parr;

p. family percent survival in the wild from release as Age 0+ fry to capture as Age 2+ parr;

q. family percent survival in the wild from Age 1+ parr to Age 2+ parr;

r. percentage (or proportion) of increasingly later year class groups (Age 0+ pre-release fry then Age 1+ post-capture parr, then Age 2+ post capture parr) comprised of representatives of the different cross or groups types; and

s. family specific growth rate (length) from release as Age 0+ fry to capture as Age 1+ parr.

Information was also collected on egg size, an important covariant when assessing the effects of predictor variables like female spawner age on offspring survival in the wild. Details on how egg size (area), fry, parr and adult length and weight were obtained is given in the above methods section on *Monitoring Changes in Phenotypic Traits Over Time*.

Estimates of survival in captivity from shock at Time 1 (at the egg stage, mid development) to pre-release as fry at Age 0+ (Time 2) were based on counts of all individuals in the experiment at both points in time and, therefore, directly reflect survival over the respective time interval; estimates of survival in captivity from shock at Time 1 (at the egg stage, mid development) to tagging at Age 4+ were based on counts of all individuals in the experiment at Time 1, but only a sample of remaining individuals at Time 2 (only a portion of captive-reared salmon were reared through to Age 4+, and only a fraction of these were tissue sampled and genotyped). Estimates of survival in the wild from release as Age 0+ fry to either Age 1+ or Age 2+ parr were based on complete counts at Time 1 but also on samples (electrofishing-based) of all remaining offspring from that cohort in native river habitat at Time 2. Estimates of survival in the wild from Age 1+ to Age 2+ were based on electrofishing samples of all remaining offspring at Time 1 and Time 2.It is important to note that although we are comparing relative survival across families (or between treatment groups), one or both of (1) random sampling effects and (2) differential emigration out of the research site, may also have contributed to numbers of individuals observed in sample collections and hence estimates of survival reported. More details about how survival estimates were carried out in captivity and in the wild during these different developmental periods are provided above in the section on *Monitoring Changes in Phenotypic Traits Over Time*.

Additional details on egg and fry rearing conditions, enumeration of eggs post-shock, enumeration of fry pre-release, changes in rearing conditions across years, Pembroke habitat location and conditions, and electrofishing methodology can be found in O'Reilly et al. (2018).

Offspring family size in the wild as parr at Age 1+ was standardized to slight differences in the number of fry actually released ($FSW_{age1.std}$) across families as follows:

$$FSW_{age1.std} = (MFS_{age0}/RFS_{age0})*FSW_{age1.obs}$$

where MFS_{age0} = the Maximum Family Size at release (Age 0+), RFS_{age0} = Released Family Size (the number of fry actually released from a given family at Age 0+), and $FSW_{age1.obs}$ = Family Size in the Wild at Age 1+ observed in electrofishing collections.

When sample sizes permitted, we attempted to minimize the effects of possible confounding variables on the response variable (offspring performance) being assessed by restricting families included in the analyses to like-types with regards to other possibly important predictor variables. For example, in analyses of the effects of pedigree inbreeding on both survival in the wild to Age 1+ and length in the wild at Age 1+ (Figures 46 and 47), we included only those

families produced by two WE parents (minimizing early juvenile parental rearing environment effects on inbreeding results). Which groups/families types were included in a particular analysis is indicated in the corresponding figure legend.

Statistical Analyses (Experimental Data)

For all datasets involving continuous data, normality was assessed using the Shapiro-Wilk test, and homogeneity of variances using the Brown-Forsythe test. When all assumptions were met, differences between groups were tested using analysis of variance (ANOVA). When variances were not homogeneous but data were normally distributed, Welch's ANOVA was used. When data were not normally distributed, data were first rank-transformed, and then analyzed using ANOVA; datasets that employed rank-transformed ANOVAs were further analyzed using the Kruskal-Wallis nonparametric test. Unbalanced, fixed-effects, nested ANOVA was calculated using the regression method, with family nested within parental/maternal rearing environment. When ANOVA, Welch's ANOVA, and nested ANOVA analyses involved multiple groups, the Games-Howell test was used to determine pairs that differed significantly. Standardized sample effect size η^2, the proportion of the total variance accounted for by the treatments, and its 95% confidence interval, were calculated using the formulas of Shieh (2013) for ANOVA, Welch's ANOVA, and regression. Kendall's tau was first converted to r^2 using the formula of Walker (2003). Incidence of fry deformities is a categorical response variable, taking one of two values (presence or absence), and was analyzed using binary logistic regression. For proportion data we used the G-test of goodness of fit with Williams' correction (Sokal and Rohlf 1981).

All data analyses were generated using Microsoft Excel 2010 and the Real Statistics Resource Pack Software, Release 4.3, Excel add-on (Zaiontz 2015).

RESULTS AND DISCUSSION

TRAIT MONITORING RESULTS

Size at Stage

Spawning Adults

No significant difference in length (Table 3) between mature spawning males and females was detected ($T\text{-test}_{AW}$; p=0.6103) and the average length of all spawning adults was 48.25 ± 10.23 cm. Adult length started at a minimum of 39.86 ± 4.47 cm in 2000 and increased to a maximum of 54.82 ± 10.36 cm in 2005, before levelling off around 46.04 ± 9.10 cm between 2008 and 2015. Differences in weight associated with gonad development between the sexes caused a significant increase in the weight (T-test; p=0.0123) of females (1.65 ± 1.08 kg) compared to males (1.52 ± 1.18 kg). Male and female weight (Table 3) was highly variable and ranged from 0.05 to 6.95 kg and 0.26 to 10 kg, respectively. Condition factor (Table 3) was significantly higher ($T\text{-test}_{AW}$; p=0.0000) in females (1.37 ± 0.19) compared to male spawners (1.22 ± 0.18) due to the added egg weight.

To eliminate age-related differences, body characteristics (length, weight, condition factor, and body depth) of Age 4 spawning adults were compared (Figure 3) over all the years of the LGB program. In 2014, the program shifted to focus on Age 5 spawning adults; these are included in Figure 3 simply to maintain yearly continuity, but were excluded from statistical analyses. There was a significant difference ($T\text{-test}_{AW}$; p<0.0001) between gender for all of the body characteristics compared (length, weight, CF, and body depth), with females typically exhibiting slightly larger body sizes. The average length for Age 4 spawning females was 41.89 ± 4.97 cm while males were 40.74 ± 6.77 cm, and ranged from a maximum of 47.57 ± 6.21 cm and

49.74 ± 7.21 cm in 2005 to a minimum of 38.24 ± 4.29 cm and 36.51 ± 7.04 cm in 2011 for females and males, respectively. Weight also peaked in 2005 (female, 1.50 ± 0.51 kg; male, 1.61 ± 0.68 kg), with lows in 2011 (female, 0.76 ± 0.27 kg; male, 0.63 ± 0.32 kg), and averaged 1.05 ± 0.48 kg for females and 0.85 ± 0.45 kg for males. Condition factor (Figure 3) had a narrow range around the average of 1.33 ± 0.20 and 1.22 ± 0.14 for females and males, respectively. As expected, Age 4 females (average of 9.85 ± 1.20 cm) had larger body depths compared to males (8.91 ± 1.64 cm) of the same age.

No yearly trends were detected in the length of Age 4 spawners for either gender. The bootstrapped slope (mB) of the linear regression for Age 4 females (m_B=0.0095) was not significantly different from zero as determined by the 95% confidence limits (CI_{LL}=-0.0667, CI_{UL}=0.0846). Similarly, linear regression (LR) indicated no trend for Age 4 males (m=-0.0559, *p*=0.3374). The weights of Age 4 females (m_B=-0.0525, CI_{LL}=-0.0708, CI_{UL}=-0.0334) and males (LR; m=-0.0292, *p*=0.0264) had significant decreases over the years (Figure 3). In 2005, Age 4 female and male spawning adults were on average 1.5 and 1.98 times heavier, respectively, than their counterparts in 2007–2013. Linear regression detected no trends in Age 4 spawner weight over the years 2007–2013 (female, m=-0.0127, *p*=0.3088; male, m=0.0259, *p*=0.0636). There was no change in the condition factor (Figure 3) of Age 4 spawning adults (LR; female, m=-0.0051, *p*=0.2185; male, m=-0.0063, *p*=0.1322) over the years. Condition factor was not affected by the 2005 year as both factors influencing CF (length and weight) increased proportionally. Although limited by the number of available years, a significant increase in female (LR; m=0.1752, *p*=0.0302) and male (LR; m=0.2991, *p*=0.0484) body depth was detected (Figure 3).

To account for differences in rearing environments, Age 4 spawners were separated into wild-origin (WO), wild-exposed (WE), and captive-reared (CAP) groups for analysis (Figure 4). Wild-exposed (WE) females were significantly larger than WE males with an average length of 40.98 ± 4.39 cm ($T\text{-test}_{AW}$; p=0.0220), weight of 0.92 ± 0.42 kg (T-test; p=0.0001), and CF of 1.29 ± 0.15 (T-test; *p*=0.0000). Linear regression did not detect any trends throughout the years for female length (m=-0.2360, *p*=0.1054) and CF (m=0.0019, *p*=0.7317), however female weight presented a significant decrease (m_B=-0.0385, CI_{LL}=-0.0698, CI_{UL}=-0.0042). WE males had an average length of 40.31 ± 6.10 cm, weight of 0.76 ± 0.36 kg, and CF of 1.19 ± 0.12 and did present significant decreases over time (length: m=-0.6209, *p*=0.0056; weight: m=-0.0582, *p*=0.0046; CF: m=-0.0163, *p*=0.0233). There was no difference detected in the length of WO females compared to WE females (T-testAW; *p*=0.1014); however, WO males were significantly longer than WE male spawners (T-test; *p*=0.0101; Figure 4).

A significant difference in size was detected between genders ($T\text{-test}_{AW}$; *p*<0.0009) for Age 4 CAP adults; however, both sexes were consistently larger than their WE counterparts ($T\text{-test}_{AW}$; *p*<0.0015) in length, weight and CF. The CAP adults were more variable over the years (Figure 4). The CAP females had an average length of 43.76 ± 5.56 cm ranging from 40.71 ± 4.83 to 47.57 ± 6.21 cm while the yearly range for WE females was less than 5 cm. The CAP males were smaller than CAP females at 41.67 ± 7.97 cm but just as variable, ranging from 35.74 ± 7.14 cm to 49.74 ± 7.22 cm, a 14 cm difference while WE males differed by 6.7 cm. The average weights for CAP males and females (Age 4) were 0.93 ± 0.52 kg and 1.28 ± 0.47 kg, respectively, and the average CF was 1.26 ± 0.16 and 1.40 ± 0.25, respectively. No significant trend was detected with linear regression in CAP adult length (female: m=0.1193, *p*=0.3254; male: m=-0.3323, *p*=0.0912), weight (female: m=-0.0184, *p*=0.1785; male: m=-0.0091, *p*=0.6128), or CF (female: m=0.0069, *p*=0.3393; male: m=0.0010, *p*=0.8489) for either gender over the duration of the LGB program (Figure 4).

Adult length measurements were available over the entire program and thus were used as a proxy to assess generational effects on spawning adult size (Figure 5). Males and females from

three groups were analyzed separately: all Age 4 spawning adults, WE, and CAP salmon. The WE and CAP groups start at G_1, therefore wild-origin (WO) fish were included in both analyses to have a more complete dataset that includes the initial generation (i.e. G_0). There was no interaction between year and program generation for any of the analyses (GLM; p=1.0000). There was a significant effect of program generation on both female ($ANOVA_W$; p=0.0137) and male (ANOVA; p=0.0000) Age 4 spawners (Figure 5). Planned comparisons showed significant differences between G_0 vs G_1 (GH; p=0.0200) and G_1 vs G_2 (GH; p=0.0300) in female length (G_0=41.29 ± 3.41 cm, G_1=42.63 ± 5.74 cm, G_2=40.96 ± 5.06 cm), and G_1 vs G_2 (TK; p<0.05) in male length (G_1=41.91 ± 7.29 cm, G_2=38.99 ± 6.60 cm). There was, however, no difference between the first 2 generations and the last generation of females (G_3=40.70 ± 5.47 cm) or males (G_0=40.87 ± 5.85 cm, $G_{2.75}$=42.63 ± 9.03 cm). The overall trend was no change over generation for either gender as the slope of the linear regressions were not significantly different from zero (female, m_B=0.2288, CI_{LL}=-0.1198, CI_{UL}=0.5664; male, m=-0.3768, p=0.2184).

No effect of program generation was detected for either gender ($ANOVA_W$; p>0.0620) in the WO/WE group and average lengths were 40.99 ± 4.39 cm and 39.70 ± 6.10 cm for females and males, respectively (Figure 5). The WO/CAP group had larger variability over the generations and a significant generational effect was detected for both females ($ANOVA_W$; p=0.0000) and males ($ANOVA_W$; p=0.0012). First-generation CAP females (G_1=43.62 ± 5.88 cm) were significantly larger (GH; p=0.0000) than WO females (G_0=41.29 ± 3.41 cm). No other differences were detected, however a significant increase over program generation ($G_{2.5}$=48.62 ± 1.76 cm) was observed (m_B=1.7903, CI_{LL}=1.3009, CI_{UL}=2.2806). Planned comparisons did not detect any significant differences between generations of WO/CAP males (G_0=40.87 ± 5.85 cm, G_1=43.17 ± 7.85 cm, G_2=39.63 ± 6.85 cm, $G_{2.75}$=46.77 ± 4.39 cm) and no trend was observed (m_B=0.2851, CI_{LL}=-0.5100, CI_{UL}=1.0848; Figure 5).

Eggs

Egg weight measurements have only been available for the last three years (Figure 6) and have averaged 0.101 ± 0.014 g, ranging from 0.0338 to 0.1467g. Welch's ANOVA detected a significant effect of year (p=0.0330). This was most likely due to sample size and unequal variances, as a bootstrapped regression analysis found that the slope (m_B=0.0008) was not significantly different from zero (CI_{LL}=-0.0012, CI_{UL}=0.0027).

Egg weight is the best measure of egg size; however, egg weight data availability was limited. Egg area was highly correlated (r=0.9479, p=0.0000) to egg weight when comparing family-specific results from the 2013–2015 BY (Figure 7), and should be a good proxy for egg size.

Although correction factors were applied, the egg area results processed from images were still slightly higher ($T\text{-test}_{AW}$; p=0.0004) than those originating from ruler measurements, indicating that the corrections may not have compensated thoroughly for the differences (Figure 8). A significant effect of year was detected ($ANOVA_W$; p=0.0000); however, this effect may be due to variance in sample size. When analyzing images, every cross was processed (approximately 100 crosses per year), compared to a select number of crosses (approximately 20 crosses per year) from all previous years. To account for this, subsamples of crosses were selected from 2011–2015 using the same selection criteria as 2002–2009. No significant yearly differences ($ANOVA_W$; p=0.0673) were detected when analyzing the selected crosses from the 2000-2015 BY. Egg area was on average 25.64 ± 2.95 mm^2 and ranged from 23.71 ± 3.51 mm^2 to 26.54 ± 1.66 mm^2. Similarly, no trend was found when analyzing the slope of the regression (m=0.0988, p=0.0597) of selected samples over the years (Figure 9), and the slight increase was probably due to remaining differences between the 2 methods.

Egg area from selected samples, using the same correction methods as above, was plotted over the generations of the program in Figure 10. The program generation was determined by

adding one to the average generations of both parents. There was no effect of year (p=0.9616) and no interaction between year and generation (p=1.0000), using a two-factor GLM. There was an effect of generation, using Welch's ANOVA for unequal variances, on corrected egg area (p=0.0202); however, all planned comparisons (i.e. G_1 vs G_2, G_2 vs G_3, G_1 vs $G_{3.875}$, and G_2 vs $G_{3.875}$) were not significant (GH; p>0.9900). The average egg area across all generations was 25.67 ± 3.44 mm^2 and ranged from 23.59 ± 4.68 to 30.73 ± 3.26 mm^2. The slope of the regression was not significantly different from zero (m=0.1790, p=0.4814); however, from the third (26.54 ± 3.21 mm^2) to the fourth (29.52 mm^2) generations, there does seem to be a steady increase occurring along with a reduction of variance. It is likely these changes are due to the more accurate imaging method for determining egg area that began in 2011; all crosses from generations 2.75 to 4, with the exception of two, are from the 2011–2015 time period; however, it will be important to continue monitoring.

Fry

Size (length and weight) and CF of fry just prior to release into the wild was on average 26.52 ± 1.39 mm, 0.137 ± 0.028 g, and 0.724 ± 0.073, respectively. Welch's ANOVA detected a significant effect of year (p=0.000) for each parameter (Figure 11). When analyzing yearly trends, there was no significant change over the available years in the length of fry (m_B=0.1162, CI_{LL}=-0.0080, CI_{UL}=0.2427), but there were decreases in weight (m_B=-0.0046, CI_{LL}=-0.0068, CI_{UL}=-0.0023) and CF (LR; m=-0.0316, p=0.0000).Fry size was correlated to egg size; egg area and egg weight showed higher correlations to fry weight (egg area: r=0.6277, p=0.0000; egg weight: r=0.6223, p=0.0000) than to fry length (egg area: r=0.5681, p=0.0000; egg weight: r=0.5925, p=0.0000). Yearly data was limited and caution should be noted when analyzing yearly patterns, since the inclusion of additional years may drastically change the outcome.

Parr

Wild-exposed parr collected from the Pembroke River are predominately Age 1 or Age 2 parr, averaging 78% and 19% of brood year collections, respectively (2006–2012; Figure 14). Availability of parr measurements immediately after capture has resulted in two brood years for each age class: 2011-2012 for Age 1, and 2010-2011 for Age 2 (Figure 12). The average size of Age 1 parr was 8.94 ± 0.94 cm and 7.60 ± 2.53 g, while Age 2 parr were significantly larger at 11.02 ± 0.85 cm (T-test; p=0.0000) and 14.28 ± 3.52 g ($T\text{-test}_{AW}$; p=0.0000). Condition factor was not affected by an additional year in the wild; no significant difference in CF (T-test; p=0.0738) was detected between Age 1 (1.03 ± 0.09) and Age 2 (1.05 ± 0.08) counterparts. There was a significant effect of year ($ANOVA_W$; p<0.0048) on the size and CF of both age classes of parr, where all fish caught in 2014 (2012 BY for Age 1, 2011 BY for Age 2) were smaller then fish caught in 2013 regardless of age (Figure 12). Although it is too early to indicate trends in the size of LGB parr after exposure to the wild, it is a starting point for future analyses.

Growth (Specific Growth Rates)

Due to the time lag and limited availability of size information collected from fry upon release into the wild (2012–2015 BY) and WE parr at time of recapture (2011–2012 BY), there is only one brood year where specific growth rate in the wild can be calculated. Age 1 WE parr collected from the Pembroke River in 2014 are offspring of the 2012 BY and can be compared to fry released in 2013 (Table 2) to calculate a growth rate for 'release to Age 1'. Age 2 WE parr collected the same year, 2014, would result from 2011 BY and can be compared to Age 1 parr captured in 2013 to provide a growth rate for 'Age 1 to Age 2' (Figure 13). Growth rates were more than 3 times higher for the 'release to Age 1' time period (average FSG of 0.396 ± 0.007 d^{-1} and 0.434 ± 0.024 d^{-1} for length and weight, respectively) compared to parr from 'Age 1 to Age 2' (average FSG of 0.046 ± 0.022 d^{-1} for length and 0.133 ± 0.069 d^{-1} for

weight) (Figure 13). Variability was also smaller from 'release to Age 1' with ranges of 0.032 d^{-1} and 0.113 d^{-1} for length and weight, respectively, compared to 0.091 d^{-1} and 0.289 d^{-1}, respectively, for parr from 'Age 1 to Age 2'.

There was a positive correlation between the size of fry and the size of Age 1 parr (length: r=0.3843, *p*=0.0003; weight: r_S=0.3901, *p*=0.0003). As expected, the growth rates for weight were highly correlated to length for both growth periods, 'release to Age 1' (r=0.8735, *p*=0.0000) and 'Age 1 to Age 2' (r_S=0.9255, *p*=0.0000). Growth rates for length and weight during 'release to Age 1' were compared to the corresponding family-specific measurement for the initial size of fry and the final size of Age 1 parr. There was a negative correlation between the FSG for length (r=-0.4328, *p*=0.0000) and weight (r=-0.5678, *p*=0.0000) with fry size, indicating that smaller fish demonstrated larger growth rates during the time period. A positive correlation was detected between FSG for length (r=0.6746, *p*=0.0000) and weight (r_S=0.4505, *p*=0.0000) with parr size, showing that larger parr resulted from higher growth rates. The same pattern was observed when comparing 'Age 1 to Age 2' growth rates to Age 1 (length: r_S=-0.6030, *p*=0.0000; weight: r_S=-0.6934, *p*=0.0000) and Age 2 parr (length: r_S=0.5405, *p*=0.0003; weight: r_S=0.5591, *p*=0.0002) measurements.

Sex Ratios

With the exception of the first 2 years of collections, fish obtained from the wild were predominately made up of Age 1 parr (Figure 14), averaging 80% of each collection and ranging from 54% to 95%. The 2012 BY is only represented by Age 1 parr because older parr were collected in 2015, and genotyped in 2016, after these analyses were carried out. Age 2 parr made up an additional 20% (2002–2011) of collections and ranged from 5–42%.

Figure 15 (Panel A) depicts the gender breakdown of Age 1 and Age 2 WE parr for each brood year. Although the number of parr collected varied throughout the years, the Age 1 parr sex ratio was approximately 1:1 (Table 4), and there was no difference (T-$test_P$; *p*=0.1109) between the number of females and males. There was a significant difference (T-$test_P$; *p*=0.0009) in the number of females and males for Age 2 parr (Figure 15, Panel A), however, with males occurring at a higher level. The ratio of females to males for Age 2 parr ranged from 1:2 in 2004 to 1:7 in 2009 (Table 4). When plotting this data by capture year, rather than brood year, the trends are very similar, only slightly shifted (Appendix Figure A4), as Age 2 parr from 1 BY (e.g. 2009) would be collected (e.g. 2012) along with Age 1 parr from the following BY (e.g. 2010).

Smolt collections, made up of predominately Age 2 individuals, were highly variable over the program, ranging from 0 to 185 fish (Figure 15, Panel B). In a paired t-test, a statistically significant difference was detected (*p*=0.0417) when comparing the numbers of each gender for smolt. Figure 15 (Panel B) indicates a pattern of typically higher numbers of female Age 2 smolt, an average of 3:1, in contrast to the higher male proportions collected as Age 2 parr (Table 4).

The number of tagged CAP individuals has varied over the LGB program. When numbers were large, release of non-target salmon (individuals not needed for broodstock purposes) and mortalities made it difficult to tabulate gender and maturation statistics (Figure 15, Panel C). Those CAP salmon that remained and survived demonstrated an approximate 1:1 ratio of females to males (Figure 15, Panel C), with no difference between gender over the years (WRS; *p*=0.2777), similar to Age 1 WE parr (Table 4).

Maturation

The average age of maturation (as adults) for WE parr caught at (1) Age 1, and (2) any age, was 4.21 ± 0.41 for both age groups and there was no significant difference (T-test; *p*=0.8575)

between the two groups (Figure 16). With the exception of 2006, the average age of maturation for WE parr increased from 4.03 ± 0.17 in 2002 to 4.49 ± 0.50 in 2005 and then decreased again over the next few years (Figure 16). The results show a stronger fit (r^2=0.1057) for a quadratic regression model rather than a bootstrapped linear model (r^2_B=0.0085). Captive-reared fish matured at a significantly younger age (T-test$_{AW}$; p=0.000) than WE parr, with an average age of 4.08 ± 0.27. The average age of CAP fish decreased from 4.19 to 4.0 between 2001 and 2011 (Figure 16), and a significant declining trend was detected over the years (m_B=-0.0140, CI_{LL}=-0.0173, CI_{UL}=-0.0107).

No differences (T-test; p>0.9215) were detected between WE parr caught at Age 1 and WE parr caught at any age when comparing the average percent of individuals (female, male, and both genders combined) per family that matured at Age 4 versus at any age. The number of females, males, and unknown gender fish are presented for CAP and WE parr at Age 1 in Figure 17. The average percent of family maturation for each gender, separately, only includes individuals whose gender was eventually determined; however, gender combined columns (i.e. both) also take into consideration individuals whose gender was never known, which can cause the results to be lower (i.e. percentage of a larger total number).

The average percent of family maturation at Age 4 for CAP females, males, and both combined was significantly higher than WE counterparts (T-test$_{AW}$; p<0.0038). However, the average percent of individuals that matured at any age was not different between the two rearing groups (T-test; p=0.6571). Gender differences indicate a significantly higher percent of CAP males matured at Age 4 than CAP females (T-test$_{AW}$; p=0.0000), while a significantly higher percent of WE females matured at Age 4 than WE males (T-test$_{AW}$; p=0.0000; Figure 17).

No yearly differences were detected for WE individuals that matured at any age (KW; p=0.1474). There was an effect of year (KW; p=0.0000) for the average percent of WE females, males, and both combined, that matured at Age 4 per family (Figure 17). No linear trends were detected for WE males (m_B=0.7390, CI_{LL}=-0.5546, CI_{UL}=1.9795), and both genders combined (m_B=0.9105, CI_{LL}=-0.0816, CI_{UL}=1.8799); however a significant increase over time was detected for WE females maturing at Age 4 (m_B=2.3113, CI_{LL}=1.2590, CI_{UL}=3.3085). The CAP-reared fish (female, male, and both combined) that matured at Age 4, and any age, had a significant effect of year (KW; p=0.0000). No trend was detected for CAP individuals maturing at any age (m_B=0.1624, CI_{LL}=-0.0279, CI_{UL}=0.3524); however, there were significant increases over the years for CAP females (m_B=2.1549, CI_{LL}=1.5678, CI_{UL}=2.7259), males (m_B=0.8771, CI_{LL}=0.3509, CI_{UL}=1.3774) and both genders combined (m_B=2.3367, CI_{LL}=1.8732, CI_{UL}=2.7959) that matured at Age 4 (Figure 17).

Survival

Fecundity

Fecundity results from all 3 methods and for all ages of female spawners are depicted in Panel A of Figure 18. Using a paired t-test, no significant difference was detected between fecundity counts and estimates (p=0.7585); however, there was a difference between images and estimates (p=0.0147). This difference may have been due to sample size, as the average difference was only 68.42 eggs and the 2 methods follow each other tightly throughout the last 6 years (r=0.8828, p=0.0000; Figure 18, Panel A). Counts were not compared to images as they are simply a subset originating from the ImageJ results. There was a significant effect of year on all 3 methods (ANOVA$_W$; p=0.0000), which was expected considering the large variation in fecundity; the minimum and maximum fecundity from the count method was 395 and 14,081 eggs, respectively. The average fecundity from counts was 3,524 ± 2,348 eggs and ranged from 1,445 ± 461 eggs in 2001 to 5,164 ± 3,337 eggs in 2007 (Figure 18, Panel A).

To compensate for age, Panel B of Figure 18 depicts fecundity for Age 4 spawners over the years. here was no difference between fecundity estimates and counts (T-test$_P$; p=0.8450) or images (T-test$_P$; p=0.4063). There was no effect of year on fecundity counts (ANOVA; p=0.23847), which averaged 1,832 ± 802 eggs; however, there was an effect of year on images (ANOVA$_W$; p=0.0009) and estimates (ANOVA$_W$; p=0.0000). Linear regression detected no significant trends for fecundity counts (m=-2.7776, p=0.8686) nor estimates (m=6.9748, p=0.1669). Fecundity from images was not analyzed for yearly trends as availability was limited to four years (Figure 18, panel B).

Relative fecundity, eggs per unit weight of female, standardized the data by female size and reduced variability (Figure 18, Panel C). Once again, there were no differences between the various methods (T-test$_P$; p>0.8300). No effect of year was detected on relative fecundity using count data (ANOVA; p=0.6167); however, Welch's ANOVA found significant yearly differences for estimates (p=0.0000) and images (p=0.0006). Relative fecundities from counts averaged 1,796 ± 587 eggs/kg across all years (Figure 18, Panel C). Linear regression analysis detected no yearly trends for relative fecundity from counts (m=0.3360, p=0.9793) and estimates (m= 6.9316, p=0.0689). A significant yearly decline was detected for relative fecundity from images (m_B=-37.2357, CI_{LL}=-60.1966, CI_{UL}=-12.0529); however, caution interpreting this result is required given the limited years of available data (Figure 18, Panel C).

Fecundity and relative fecundity, determined by counts and estimates, were both plotted over program generation (Figure 19). Single observations were removed prior to analysis. No interaction was detected between year and generation (GLM*; p*=1.0000) for any response variable. There was no effect of program generation on fecundity (ANOVA; p=0.1805) or relative fecundity (ANOVA; p=0.3067) based on counts. Differences were detected for fecundity (ANOVA$_W$; p=0.0000) and relative fecundity (ANOVA$_W$; p=0.0000) from estimates. Fecundity estimates (Figure 19, Panel A) in G_1 (3,121 ± 1,879 eggs) and G_2 (3,338 ± 2,086 eggs) were not different from each other (GH; p=0.9500) or from G_4 (3,464 ± 926 eggs; GH; p=1.0000), but were significantly larger than G_3 (2,368 ± 1,208 eggs; GH; p=0.0000).Bootstrapped linear regression detected a decreasing trend in fecundity estimates over the generations (m_B=-275.4284, CI_{LL}=-349.9040, CI_{UL}=-199.3774). No trend for relative fecundity from estimates (LR; m=-13.9878, p=0.4390) was detected over the generations (Figure 19, Panel B). Games-Howell post hoc tests found that relative fecundities from estimates for G_1 (1,512 ± 213 eggs) was significantly lower than G_2 and G_3, 1,783 ± 333 and 1,886 ± 488 eggs, respectively, with p values of 0.0100 and 0.0000, respectively. The G_1 and G_2 were not different from the last generation, $G_{3.875}$ (GH; p>0.2100), which had 1,716 ± 152 eggs.

Survival Rates

A paired t-test detected a significant difference (p=0.0001) between the 2 methods, mortality records and images, for the percent survival from 'fertilization to shock' (2010–2015; Figure 20). Kruskal-Wallis ANOVA on the ranks detected yearly differences in survival for both records (p=0.0000) and images (p=0.0000). Survival averaged 84.8 ± 18.5% for images while records averaged 84.8 ± 20.5% for the same time period (2010–2015). In 2003, there was a significant fungal outbreak that caused unusually poor survival (29.2 ± 27.4%); therefore, that year was excluded from the following analyses. The average survival from records for 2000 to 2015 (without 2003) was 79.8 ± 23.7% and ranged from a minimum of 51.0 ± 30.1% to a maximum of 92.0 ± 16.1% (Figure 20). Throughout the years, there was a significant increasing trend in survival from records (m_B=1.4143, CI_{LL}=1.1697, CI_{UL}=1.6625). The most significant increases in survival were the first three years of the program and the last two years (Figure 20). There was no significant difference in survival (ANOVA; p=0.1611), and no trend (m_B=0.0090, CI_{LL}=-0.3108, CI_{UL}=0.3379), when looking across 2002–2013, and the average for the time period was 82.4 ± 20.5%.

Average fertilization rates for 2013, 2014, and 2015 were 85.4 ± 17.0, 86.5 ± 15.0, and 89.7 ± 13.9%, respectively, which did not differ significantly (ANOVA; p=0.1571) over the available years (Appendix Figure A5). Using the fertilization rates, mortalities were corrected to account for unfertilized eggs and accurately reflect survival (Figure 20). No significant difference was detected between the 2 methods ($T\text{-test}_P$; p=0.9969) for the corrected survival rates. Although a limited number of years were available, no effect of year was detected for corrected records (KW; p=0.4447) and corrected images (KW; p=0.1720). After correcting for fertilization success, the average survival rate for 'fertilization to shock', 2013–2015, was 99.7 ± 1.6% for both records and images alike, significantly higher than the uncorrected survival rates for the time period (WRS; p=0.0000).

Survival from 'shock to pre-release' (Figure 21) was equally high with an average of 96.6 ± 6.8%. There was no trend over the years (m_B=0.0868, CI_{LL}=-0.0405, CI_{UL}=0.2173); however, some fluctuation between years did cause a significant year effect (KW; *p*=0.0000). The survival rates for this time period are derived from the equalized family groups (EQU), where the initial number of eggs taken from each cross increased from 110 to 400 between 2008 and 2015 (Figure 21). The lowest survival, 87.0 ± 13.0%, occurred in 2011 when the EQU number rose to 400 eggs and were reared in containers within a trough instead of baskets in the heath units. Survival rebounded to 97.5 ± 3.2% the following year when eggs were reared within the heath units using 2 baskets per cross. Highest survival was in 2008 (98.9 ± 3.0%), with the lowest EQU number, and 2015 (98.9 ± 2.7%) when a filtration system was installed that reduced the sediment in the water (Figure 21).

Figure 22 plots survival for the 2 time periods over program generation. After single observations were removed, no interaction was detected between year and program generation for either time period (GLM; *p*=1.0000). There was a significant effect of program generation detected for survival from 'fertilization to shock' (KW; *p*=0.0000). The G_1 was significantly lower than G_2, G_3, and G_4 (KW_Z; z>4.1444), while G_2 was not different from G_3 (KW_Z; z=0.3083) but was different from G_4 (KW_Z; z=2.0068). A significant increasing trend in survival, from 63.03 ± 32.39% to 92.11 ± 13.03% (Figure 22), was detected over the generations for the 'fertilization to shock' time period (m_B=9.2486, CI_{LL}=7.9761, CI_{UL}=10.5474). Survival from 'shock to pre-release' had a significant effect of program generation (KW; *p*=0.0000; Figure 22). Differences were found between both $G_{1.5}$ (KW_Z; z=2.7732) and G_2 (KW_Z; z=6.3987) compared to G_3. No significant linear trend was detected over the program for survival from 'shock to pre-release' (m_B=-0.2214, CI_{LL}=-0.7438, CI_{UL}=0.3071).

Table 5 reports the average family size at capture and the average percent family recovery (as a proxy for survival) for various ages of WE parr. Although the numbers are quite low, there was on average 4 times more Age 1 WE parr captured per family (1.40 fish) than Age 2 (0.36 fish). A clear increasing trend was detected in the total number of parr captured (i.e. any age) per family over brood year (m_B=0.2344, CI_{LL}=0.2000, CI_{UL}=0.2677). An effect of year (KW; *p*=0.0000) was detected for the percent survival in the wild of WE individuals from 'release to recapture' as Age 1 parr (Table 5; Figure 23). The yearly trend was a significant increase over time (m_B=0.1002, CI_{LL}=0.0628, CI_{UL}=0.1379). Survival of WE Age 1 parr ranged from a minimum of 0.64 ± 0.79% in 2008 to a maximum of 1.71 ± 1.27% in 2010 (Table 5; Figure 23). Recapture was used as a proxy for survival, which would be affected by the number of fish that were targeted, and captured, on the day of electrofishing (Appendix Table A1). The number of collected parr for each BY has doubled, from approximately 200 in 2006–2008 to approximately 400 in 2010–2012 (Table 5).

Fry Deformities

Deformities (Appendix Figure A6) occurred in approximately 54% of all families over the last 6 years (2010–2015), ranging from a minimum of 43% in 2010 to a maximum of 72% in 2011 (Figure 24). There was a significant effect of year (KW; p=0.0000) on the rate of deformities. The rate of deformities from all families, between 2010 and 2015, averaged 0.56 ± 1.90% and ranged from a maximum of 0.91 ± 2.98% in 2010 to a minimum of 0.28 ± 0.56% in 2015 (Figure 24). Although the average rates of deformity have been consistently low for all years, a significant decreasing trend was detected (m_B=-0.1121, CI_{LL}=-0.1909, CI_{UL}=-0.0067).

TRAIT MONITORING DISCUSSION

Size at Stage

Female spawners were generally larger than male counterparts, particularly in weight, condition factor, and body depth, which could be attributed to larger gonad development. Captive-reared individuals were significantly larger, and more variable, in size at spawning than WE individuals, an advantage of their additional year of consistent feeding and familiarity to dry feed pellets. Other than a few exceptions, which can be explained by a limited availability of years (body depth) or highly influential years due to smaller sample sizes (WE male size), there was typically no significant trend over time for the size of spawning adults.

Spawner length analyzed over program generation supports the recommendation, reviewed by Williams and Hoffman (2009), to increase the naturalization of rearing habitats in order to minimize rates of adaptation to captivity. There was no effect of generation on the length of WO/WE spawners, while WO/CAP females showed an increasing trend over program generation and CAP G_1 were significantly larger than WO G_0 individuals.

Although other studies have found significant changes in parr size and egg size (de Mestral et al. 2013; Wilke et al. 2014; N. Wilke, pers. comm.; I. Fleming, pers. com.), the results of this study did not completely agree. Egg weight, although limited in the number of years, did not have a significant trend, but was highly correlated to egg area. There was no significant effect of year or program generation on egg area through the LGB program. Although no overall trend in egg area was detected throughout, an increasing trend, along with a reduction in variability, from G_3 to G_4 was emerging. These changes correspond to the change in methodology (use of ImageJ) and may be a result of the more accurate method used; however, it will be important to continue monitoring. Fry length showed no trend over the four years, while weight and CF decreased slightly. Data availability for fry and WE parr size are limited and should be interpreted with caution; however, it is a starting point for future years and should continue to be monitored.

Growth

Growth rate data was limited; therefore, no yearly trends could be analyzed for changes throughout the LGB program. Results showed that WE parr growth was significantly larger in the first year in the wild compared to the second year, and small fish grew faster and larger parr resulted from faster growth rates.

Age 1 WE parr and CAP fish both had 1:1 sex ratios over the years, while there was more Age 2 male WE parr and more female Age 2 smolt.

The CAP fish matured at a younger age compared to WE individuals, and a higher percent of CAP fish matured at Age 4 than did their WE counterparts. This may be explained by their larger size, caused by increased years of consistent feeding in a captive environment, initiating

earlier maturation. The CAP individuals may be subjected to higher rates of adaptation to captivity as there was a significant decreasing trend in the average age of maturation over the years with a corresponding increasing trend in the percent of CAP fish maturing at Age 4. No trend in the percent of fish maturing at Age 4 was observed for WE males, and both genders combined; however, an increase over the years was detected in WE females. All fish, whether CAP or WE, did mature as there was no difference between the two groups and no trend over the years for the percent of individuals that matured at any age.

Survival

Fecundity models have most likely estimated fecundity successfully over the years as no difference was detected between the various methods used. Two variables that could affect fecundity are female size and egg size; since no trends were detected in those variables, it was not unexpected to see that fecundity, and relative fecundity, likewise did not show any trends over the years. There was also no effect of program generation on fecundity, and relative fecundity, when analyzing count data. Increased sample size, and therefore an increase in power to detect small differences, led to a significant effect of program generation on fecundity, and relative fecundity, using estimates. There was a significant decreasing trend over generation for fecundity estimates; however, the first two generations did not differ significantly from the last generation, and no trend was detected in relative fecundity estimates over generation. Variation in fecundity seems to be decreasing over the generations. One contributing factor may be a decrease in the variability of female size in the last few years; however, methodology has also become more accurate with the use of ImageJ.

Survival from ‘fertilization to shock’ has increased significantly over the years, particularly the first 3 years and the last 3 years of the program. An exception was in 2003, when a fungus outbreak caused unusually poor survival. Fertilization success was 87.17±15.4% from 2013–2015 and after correction of unfertilized eggs, survival from ‘fertilization to shock’ was >99%. Since the fertilization success indicates that most of the mortality recorded during this time period is actually unfertilized eggs, the increase in survival over the years could indirectly be considered an increase in fertilization success. There was a similar increasing trend over program generation for survival from ‘fertilization to shock’; G_1 was significantly lower than all other generations and G_2 was significantly different from G_4. This may be due to improving spawning and rearing techniques; however, it may also point to a selection for success in a captive environment.

There was no trend detected for survival from ‘shock to pre-release’ over the years as it remained very high, with an average >96%. The yearly fluctuations that were evident are explained by changes in rearing practices over the years. Although differences were detected between some generations, there was no trend in survival from ‘shock to pre-release’ over program generation. Survival in the wild (from ‘release to recapture’) exhibited an increasing trend over the years; however, the number of individuals collected from the wild has also increased with year (approximately doubling from 2006–2012), which could be greatly influencing the results.

EXPERIMENTAL RESEARCH

Exclusion-based Parentage Assignment

Many of the findings in this study were based on parentage assignment results and, in theory, could have been impacted by genotyping errors and/or mutations, and the resulting potential failure to correctly match offspring to their true parents. All offspring were tested against all biologically possible pairs of male and female LGB parents spawned using exclusion methods.

In Figure 25, we provide an example of exclusion results for the 198 CAP and 410 WE salmon (for a total of 608 candidate offspring) produced in 2010 tested against all known biologically plausible crosses. Comparisons between offspring and the first matching parental pair (that exhibited the most compatible loci, or the fewest mismatching loci) and the second-best matching parental pair (that exhibited the next most compatible loci, or the second-fewest mismatching loci) are presented. A total of 548 offspring were compatible with the first (best) matching parental pair at 100 percent of all loci (10, 11 or 12) assayed in all three individuals. A further 58 offspring were compatible with the first (best) matching parental pair at 91.67% (11 of 12) of loci assayed, and only 2 offspring were compatible with the first (best) parental pair at 83.33% (10 of 12) of loci assayed. Nearly all of the *second-best matching parental pairs* for these 608 offspring exhibited markedly fewer compatible loci (50 percent or less). This large discontinuity between the first and second-best matching parental pair (see Figure 25) indicates that it is highly unlikely that one or two genotyping errors in a given triad led to the failure to identify the true parental pair while incorrectly assigning an offspring to the next best (but incorrect) parental pair.

Effect of the Early Parental Rearing Environment on Offspring Performance in the Wild

We did not detect consistent (across years) or statistically significant effects of the early juvenile rearing environment of the parents (captive-reared vs wild-exposed) on early offspring survival in the wild, from release at Age 0+ to Age 1+ (approximately 15 months after releases). In 2009 and 2012, average percent survival was highest for offspring of wild-exposed females (WE) x captive-reared males (CAP) (WExCAP), but in 2010 and 2011, survival was highest for offspring of CAPxCAP and CAPxWE parents, respectively (Figure 26). The only parent treatment group for which offspring survival was not higher than all others in at least 1 within-year comparison was WExWE. In fact, in all years, offspring survival in this group was similar to the lowest performing group (typically CAPxCAP, except in 2010). Additionally, many differences between pairs of treatment groups were modest (often within 25%) and, when larger, 1 or more treatment groups was often represented by relatively few families (e.g., WExCAP [7], CAPxWE [5], and CAPxCAP [2], in the year 2011). Finally, no differences among the four parental rearing group types in any of the four within-year comparisons were statistically significant (Table 6). Results were similar when combining WExWE and WExCAP, and CAPxWE and CAPxCAP groups to produce the 2 maternal groups, WE female and CAP female (Figure 27). Although offspring sample sizes (number of families and total numbers of individuals) associated with the contrasted parent group types were larger, patterns observed were again not consistent across years; offspring survival was higher for the WE female parent group in 2009 and 2012, but higher for the CAP female parent group in 2010 and 2011. Additionally, once again, no significant differences between treatment groups within any of the four spawning years assessed were detected (Table 6), despite these larger sample sizes.

These results were different from those reported by Evans et al. (2014) in similar comparisons of early juvenile survival (in wild native river habitat) of offspring produced by wild-exposed BSR LGB vs captive-reared BSR LGB parents. Offspring of wild-exposed parents exhibited significantly higher survival (up to a two-fold increase) compared to offspring of captive-reared parents, and offspring of parents that spent two years in wild river conditions as juveniles exhibited higher survival compared to offspring of parents that spent one year in wild river conditions in their study (Evans et al. 2014).

There are several possible reasons for the observed divergence in results (failure to detect parental rearing environment effects on offspring survival in the wild). First, Evans et al. (2014) included only 2 cross types in their experiment, wild-exposed females x wild-exposed males,

and captive-reared females x captive-reared males, equivalent to our WExWE and CAPxCAP cross types, Figure 26. This aspect of their design increased the magnitude of potential parental environment effects on offspring survival across all of their compared groups, as both parents associated with the contrasted cross types studied were exposed to a given environment as juveniles. A second possible reason for some of the differences observed between these 2 studies is that the average number of offspring recovered per family was very low in our study (2.5–4.5, depending on the year), compared to their study (approximately 12) for Age 1+ offspring sampled from the wild. This demonstrates an important disadvantage of carrying out research coinciding with conservation activities (implementation of the LGB program). Average family size at the time of capture, despite similar sampling effort (electrofishing and genotyping of offspring) was much lower here because the set of offspring analyzed in this study was produced by over 100 crosses; this many crosses was necessary to maintain large effective population size, a requirement of the conservation program. The greater number of parent group types here (4) versus in their study (2) (also a function of the different program objectives) also impacted offspring group size, which may also have contributed to differential abilities to detect differences between the 2 studies. However, even with the current experimental design (number of crosses, number of treatment groups, and number of offspring sampled at Age 1+) we did indeed detect both (a) statistically significant differences between treatment groups for some predictor (independent) variables, and (b) consistent patterns of effects across years for these same variables (discussed below). In other words, we do expect to be able to detect moderate-to-large differences in survival when they do exist. Additionally, it is also possible that female parent age (which appeared to have a large effect on offspring survival across all years, discussed further below) may have impacted our results, depending on how adults of various ages were distributed across parent types. However, as discussed further below, at least some portion of the observed female parent age effect appears to be associated with egg size, and egg size was not found to be a significant covariate in analyses of the effect of parental rearing environment presented here (results not shown).

Potential biological reasons for observed differences between studies are as follows. First, it is possible that mortality associated with variance in family size on the BSR is more selection-mediated, resulting in greater genetic differences between sampled wild-exposed and captive-reared BSR parents compared to analogous sets of STW parents, which in turn may have conferred greater survival advantages to offspring of BSR wild-exposed x wild-exposed parents in their study. It is also possible that differences in the rearing environments at Mactaquac (for BSR salmon) and Coldbrook (for STW salmon) throughout the life cycle of captive-reared parents, and/or during the early or mid-to-late portions of the life cycle of wild-exposed parents, could have impacted the results. If, for example, environmental conditions experienced by captive-reared salmon at one of the two facilities were less divergent from those experienced by wild-exposed salmon, the scope for differential offspring performance (via either genetic or epigenetic mechanisms) might be reduced.

It is perhaps worth noting that although differences in offspring survival between parent group types here were not (overall) consistent or statistically significant, the average percent offspring survival for WExWE groups was higher than CAPxCAP groups in three of four within-year comparisons (Figure 26). Were we to have assessed parental rearing effects for a single brood year (as done in Evans et al. 2014), either in 2009, 2011 or 2012, and compared WExWE and CAPxCAP groups only, the directionality of differences reported across the two studies would have been the same (WExWE>CAPxCAP). Also, the approximate magnitude of differences observed (approximately 10–20%) would have been similar between the 2 studies, if comparisons in Evans et al. were restricted to similar offspring (sampled at Age 1+) and parent groups (their WE1 crosses, involving parents that spent approximately 1 year in the wild); though differences here would not have been statistically significant. If trends across both

studies are considered, a general conclusion of this research would be that offspring of WExWE crosses may exhibit higher survival than offspring of CAPxCAP crosses, and that spawning of exclusively WE parents may increase offspring survival relative to the spawning of exclusively CAP parents. However, here, in all 4 within-year comparisons, offspring of WExCAP crosses appeared to exhibit higher survival compared to offspring of WExWE crosses (often by as much as approximately 50%), though differences were not significant

Effects of the early juvenile rearing environment of the parents (WExWE vs WExCAP vs CAPxWE vs CAPxCAP) (Figure 28), or early juvenile rearing environment of the maternal parent (WE female vs CAP female) (Figure 29), on offspring survival in the wild from release at Age 0+ to Age 2+ (approximately 27 months after releases) were different than observed at Age 1+ (for overlapping spawning years, 2009–2011), though some general similarities in the relative ranking of different parent groups were observed. For example, offspring survival to both Age 1+ and Age 2+ was higher for WExWE relative to CAPxCAP parental groups in 2009 and 2011, but the opposite was true in 2010 for both offspring groups. Overall, results were perhaps the most divergent for the two offspring group types (Age 1+ vs Age 2+) when the number of families associated with at least one parent group type was very low (e.g., in the spawning year 2011). The relative ranking of maternal groups was similar for the Age + and Age 2+ offspring groups in 2009 and 2010 when the number of families involved was high, but not in 2011, when the number of families in the CAP female maternal group was low (7). No consistent patterns were observed across spawning years within the Age 2+ offspring group, and no differences between groups within a given brood year comparison were significant (Table 6). Note that results in this analysis are further complicated by the fact that percent survival to Age 2 also reflects possible differences in timing of smolt out-migration (Age 2 vs Age 3) between groups, and will not be discussed further.

The early juvenile rearing environment experienced by maternal and paternal parents may, at least in some years, impact offspring size (especially length) in the wild at Age 1+. Because length data of wild-exposed parr was only available from the year 2013 on, size data for offspring of wild-exposed and captive-reared parents exist only for 2011 and 2012 spawning years. In 2011, average family mean body length of offspring at Age 1+ was similar across all four parent group types (Figure 30, see also Figure 31), and differences were not significant in ANOVA (Table 6) and unbalanced fixed effects ANOVA (Table 7), though it should be noted that the number of families in the CAPxWE (5) and WExWE (2) groups were very limited. In 2012, where the numbers of families in CAPxWE and CAPxCAP groups were higher, the length of offspring at Age 1+ was more divergent across groups, a consistent trend across groups reflecting the amount of wild exposure was observed, with (WExWE > WExCAP > CAPxWE > CAPxCAP) (Figure 30, see also Figure 31), and differences were significant (ANOVA, $p = 0.0167$, Table 6 and unbalanced fixed effects ANOVA, $p = 0.0044$, Table 7). Of the six possible pairwise group comparisons within this analysis, only WExWE vs CAPxCAP, involving the most divergent parent cross types, was significant (p=0.0185, Table 8); p-values for WExWE vs CAPxWE and WExCAP vs CAPxCAP, both involving different maternal parent types, were 0.0995 and 0.0845, respectively (Table 8).

When WExWE + WExCAP, and CAPxWE + CAPxCAP groups are combined to produce the WE female and CAP female groups, respectively, patterns across years are consistent (WE female > CAP female) (Figure 32); differences in 2011 are not significant (p= 0.1718), but differences in 2012 were highly significant (p=0.0007) (Table 6).

Overall, similar patterns were observed across parent group types for the weight response variable. In the 2011 spawning year, average family mean body weight of offspring at Age 1+ was similar across all four parent group types (Figure 33, see also Figure 34), and differences were not significant in ANOVA (Table 6) and unbalanced fixed effects ANOVA (Table 7). In

2012, a consistent trend across parent group types reflecting the amount of wild exposure was observed, with WExWE > WExCAP > CAPxWE > CAPxCAP (Figure 33 and Figure 34), and overall among-group differences were significant in ANOVA (p=0.0146, Table 6) and unbalanced fixed effects ANOVA (p=0.0032, Table 7). Of the six possible pairwise group comparisons within this analysis, WExWE vs CAPxCAP (p=0.0217) and WExWE vs CAPxWE (p=0.0310) were significantly different (Table 8). When comparing across years, the relative ranking of average family mean body weight across parent environment types was the same, with the exception of WExCAP and CAPxWE groups in 2011, which were very similar.

When WExWE + WExCAP, and CAPxWE + CAPxCAP groups are combined to produce the WE female and CAP female groups, respectively, patterns across years were consistent (WE female > CAP female) (Figure 35); differences in 2011 were not significant (p= 0.4842), but differences in 2012 were highly significant (p=0.0004) (Table 6). Offspring egg size was not observed to be a significant covariate with parental rearing environment in any size comparison. Evans et al. (2014) did not detect any size differences between wild-exposed and captive-reared offspring groups analyzed.

Effects of Cumulative Ancestral Early Rearing Environment on Offspring Performance in the Wild

If trans-generational effects of exposure of ancestors (parents and grandparents) as early juveniles to captivity were cumulative, we might expect descendants of both parents and grandparents that were all reared in captivity from pre-release to Age 1+ to perform much worse in the wild than descendants of both parents and grandparents that were all reared in the wild during this same period, with descendants of parents and grandparents reared under a mixture of captive and wild conditions (some reared in captivity, others in the wild) exhibiting intermediate levels of performance.

We either detected no association between the extent of cumulative ancestral early juvenile exposure to captivity on offspring survival in the wild from release to Age 1+ (years 2009, 2010, and 2012) (Figure 36, Table 9), or a possible positive relationship in year 2011 (Figure 36, Table 9, p=0.0237), with descendants (offspring) exhibiting more cumulative early captive rearing appearing to show higher percent survival in the wild to Age 1+ (Figure 36). Offspring egg size was found not to be a significant covariate in this analysis.

No association was observed between cumulative ancestral captive rearing and offspring survival in the wild from release to Age 2+ for the spawning years 2009–2011 (Table 9), though there was a trend towards lower survival with increased cumulative captive rearing in 2011 (Table 9, p=0.0599) (see also Figure 37). However, since Age 2+ parr are collected in later summer/early fall, any differences in survival that were observed could reflect both variation in mortality and out-migration as smolt earlier in the spring.

We also tested for a possible association between cumulative ancestral early juvenile exposure to captivity and family (offspring or descendant) mean length in the wild at Age 1+ (Figure 38). Overall, we find little evidence for an association in the two years tested; mean family length appeared to increase slightly with the amount of ancestral captive rearing in 2011 but appeared to decrease as ancestral captive rearing increased in 2012 (Figure 38), though neither relationship was statistically significant, with p-values of 0.4249 and 0.0551, respectively (Table 10).

Effect of Number of Program Generations on Offspring Performance in the Wild

Immediately above, we tested for possible cumulative effects of ancestral captive rearing environments (from release as fry at Age 0+ to Age 1+ or Age 2+) on offspring performance in

the wild. In that analysis, we attempted to isolate only the effects of the early juvenile rearing environment of ancestors (parents and grandparents), from several weeks post-hatch to Age 1+ or Age 2+, on offspring performance. Here, we investigate the effects of an increasing number of program generations (or complete cycles of captive breeding and rearing), with each cycle including the combined effects of

a. one round of captive or artificial breeding;
b. rearing of all from fertilization to post-hatch in captivity; then
c. a mixture of captive rearing (for a portion of the population) and wild exposure (for the remainder of the population) from release to Age 1+ or Age 2+; and then
d. further captive rearing of all from Age 1+ or Age 2+ to maturity as adults, right through until spawning.

The number of program generations experienced by offspring/families compared are not all whole numbers (e.g., 2.5) because many of the crosses that produced them were between male and female parents exhibiting a different number of program generations (e.g., 1.0 and 2.0). Program generations experienced by offspring included in this study begin at 2.0 because survival data for offspring in the wild were not available for spawning years 2000–2008; program generation 1.0 offspring, direct descendants of G0 founders, were produced in these earlier years.

In general, the number of program generations was not strongly correlated with survival in the wild, but some possible associations were observed. No statistically significant relationship between survival, release to Age 1+, and program generations was observed in spawning years 2009, 2011 and 2012 (Table 9), though survival mostly appeared to decline with increasing program generations (Figure 39). In 2010, however, survival was negatively correlated with increasing number of program generations (p=0.0242).

de Mestral et al. (2013), in a study of the environmental and selective effects of the iBoF LGB program on multiple phenotypic traits (smolt run day, smolt length, weight, and condition factor) in BSR smolt in the wild, did not find that trait values varied consistently with increasing numbers of program generations. In fact, the average trait value of G2 generation program smolt was often intermediate between G1 smolt and wild-origin smolt reared under common native river conditions (de Mestral et al. 2013). Similarly, early juvenile growth rates of G1 and G2+ (a mixture of G2 and G3) BSR LGB salmon analyzed by Wilke et al. (2014) were nearly indistinguishable, though both were highly divergent from offspring of wild G0 generation salmon, when reared in common captive environments. Although divergence in phenotypic traits between offspring of wild and captive G1 salmon are often quite large (Araki et al. 2007b), reflecting the combined maternal (egg size or resource partitioning) and genetic responses to these different environments, further genetically based trait divergence is likely to accumulate more slowly, and could be more difficult to detect. Captive populations of *Drosophila melanogaster* maintained at 100 and 250 individuals across 50 generations exhibit very different levels of mean fitness when reared under stringent environmental conditions compared to truly wild flies, but divergence in mean fitness between populations maintained in captivity between two and five generations is very modest (see Figure 3 in Woodworth et al. 2002).

It should also be noted that because early survival information in the wild in this study was only available from 2009 on (discussed above), the total number of program generations across which differences in survival could be compared was very limited (1.0 to 1.25 generations, Figure 39). Differences in survival between offspring of G0 and G2+ generation salmon (juveniles exhibiting 1.0 and 3.0+ program generations of captive breeding and rearing), more

relevant to assessments of cumulative impacts of the STW LGB program to date, might have been greater.

Effect of Parent Family Size in the Wild on Offspring Performance in the Wild

In principle, variation in family size observed in STW River wild-exposed parr collected at Age 1+ (O'Reilly et al. 2018) may be partially or largely driven by selection-mediated mortality in the wild. Small and large families observed in yearly sample collections of wild-exposed parr may be genetically and qualitatively quite different, with individuals from small families being less fit than individuals from large families. In our current STW LGB program (which is based on Ranked Mean Kinship breeding protocols), individuals in small families are assigned lower Mean Kinship (MK) values than individuals in large families (at least in initial MK calculations; for details see O'Reilly et al. 2018; Ivy and Lacy 2012), and are prioritized higher for spawning. This could increase the frequency of less fit genes, lowering the fitness of the entire population.

We found no relationship between average parent family size (the average of the male and female parents' family sizes) at Age 1+ in the wild and offspring family size at Age 1+ in the same wild habitat. In 2010, 2011 and 2012, standardized offspring family size of parents from small families (0–1.5) ranged considerably, from small (0) to large (16), and did not appear to be quantitatively different than parents from large family sizes (>1.5) (Figure 40). Indeed, standardized offspring family size was not associated with average parent family size in any of years 2010 to 2012 (p=0.2522–0.6489, Table 10).

Effect of Mean Kinship on Offspring Performance in Captivity and in the Wild

We also looked for possible effects of prioritizing low-MK individuals on fitness directly, by testing for associations between average parent MK values (the average of the male and female parents of a given family/offspring) and

a. family/offspring survival in the wild from release to Age 1+;
b. family/offspring survival in the wild from release to Age 2+;
c. family/offspring survival in the wild from Age 1+ to Age 2+; and
d. family/offspring survival in captivity from the egg stage at shock to tagging/genotyping at Age 4.

Survival data were included for families produced by two wild-exposed parents only, including for (d) above, to control for possible effects of parental rearing environments on results.

No association between average parental MK values and any of the above metrics of family/offspring survival (in any year, in any environment) was observed (Figures 41–44, Table 9), except one. The MK values were positively associated with percent survival, release to Age 1+, for the set of families produced in the year 2010 (Figure 41) (p=0.0018, Table 9). In other words, families produced in 2010 that exhibited higher MK values appeared to exhibit higher survival than families exhibiting lower MK values. However, note that of the four spawning years tested (2009–2012), a significant association (though negative) was also observed between survival in the wild from release at Age 0+ to Age 1+ and the number of program generations for the set of wild-exposed families produced in 2010 (Figure 39, Table 9); families with more program generations exhibited lower survival than families with fewer program generations that year. No association, positive or negative, was observed in any other year. Family MK values are expected to increase with a higher number of program generations due to additional co-ancestry between spawner pairs. In other words, at least some of this apparent association between MK and survival in 2010 may have been driven by increasing

numbers of program generations, which may have impacted offspring survival this one year. Indeed, in 2010, MK values do appear to increase with program generations, particularly from 2.25 to 3.0 generations, though the relationship is not statistically significant (p=0.1082, data not shown). No association between maternal MK values and family/offspring survival from shock to tagging (approximately Age 4) in captivity was observed, for any of the sets of families produced in the years 2007–2012 (Figure 45, Table 9).

Malo et al. (2010) compared several indicators of fitness (sperm production, fertility, and resistance to osmotic stress) in White-Footed Mice maintained across a much longer period (10 generations) under 3 different breeding regimes (minimization of Mean Kinship, random mating, and selection for docility), and did not find any negative effect of minimization of mean kinship breeding protocols on fitness at the end of the study. In their experimental design, program generations did not confound assessments of Mean Kinship effects; comparisons were made between separate Mean Kinship versus random mating lines, both reared through to the tenth generation.

Our results on the effects of (1) parent family size in the wild and (2) parent MK values on offspring performance in the wild, however, do not indicate that selection during wild exposure is not important, and that the effects of MMK breeding programs do not need to be monitored going forward. It is possible that selection intensities are relatively modest, and that their effects of variation in family size low compared to stochastic influences, as observed elsewhere across a range of other taxa studied (Snyder and Ellner 2018). If selection is weak but consistent and directional, MMK breeding programs and the preferential spawning of low MK salmon could still represent a long-term risk to the population, although it should also be understood that going forward, MMK will approximate EFS.

Effect of Pedigree Inbreeding on Offspring Performance in Captivity and in the Wild

No association was found between the amount of pedigree inbreeding (F) calculated for families and any metric of family/offspring performance, including

a. survival in the wild from release at Age 0+ to Age 1+;
b. family mean length at capture at Age 1+;
c. family mean weight at capture at Age 1+;
d. average family incidence of deformities as age 0+ fry in captivity, and;
e. percent deformities as Age 0+ fry in captivity, in any spawning year dataset assessed (Figures 46–50, Tables 9, 10, and 11).

In the year 2015 (one of six years assessed), there may have been a slight (non-significant) trend towards increasing incidence of deformities with increased inbreeding (p=0.1451), but it should be noted that levels of inbreeding in this set of families was very low (generally 0.002–0.006) (Figures 46–50 here; see also Figure 51 in O'Reilly et al. 2018). In addition to overall low levels of pedigree inbreeding and limited ranges in values of F across which performance was assessed, several other factors may have contributed to the reported lack of any association between these metrics. First, in all years, when even moderate levels of inbreeding were observed, very few (one to a few) families exhibited these higher values (Figures 46–50; see also O'Reilly et al. 2018), contributing to very unbalanced experimental designs. Second, estimates of F used here consider only pedigree information from the G-1 generation forward; relatedness due to the co-occurrence of common G-2 or earlier generation ancestors was not included. Third, kinship reconstructions of first-order relatedness amongst G0 salmon was not

expected to be completely accurate (especially when family sizes were small) (see O'Reilly et al. 2018 for more details), resulting in somewhat imprecise estimates of even pedigree inbreeding (*F*) values used here. Finally, other aspects of this dataset (including mean family size of wild-exposed offspring at Age 1+ parr and uneven treatment group sizes), were not optimal for the detection of small differences between treatment groups (discussed above).

Effect of Expected Observed Heterozygosity on Offspring Performance in Captivity and in the Wild

Given the limited pedigree depth upon which calculations of F above are based, and expected inaccuracies of some G0 kinship assignments and their possible impacts on some estimates of pedigree F, we also tested for associations between another possible indicator of inbreeding and offspring performance. Multilocus microsatellite genotypes (molecular genetic information) from male and female parents were used to calculate the expected observed molecular genetic heterozygosity of their offspring (see *Method* section for more details). Offspring or family expected observed heterozygosity was then compared with three metrics of family performance, (a) survival in the wild from release at Age 0+ to Age 1+ for the spawning years 2009–2012, (b) average family incidence of deformities of Age 0+ fry in captivity for the spawning years 2010–2015, and c) family percent deformities of Age 0+ fry in captivity also for the spawning years 2010–2015. No statistically significant association between family/offspring observed expected heterozygosity and family/offspring performance was observed for any trait in any spawning year (Figures 51–53, Tables 9 and 11). Although p-values for the comparison between observed expected heterozygosity and family percent deformities (Figure 43) approached significance (0.0762, Table 9) in the 2014 spawning year comparison, it should be noted that there were a large number of pairwise comparisons involving this performance metric (6).

Effect of Cross Type on Offspring Performance in Captivity and in the Wild

In 2007 to 2009, a small number of crosses were carried out between salmon obtained from the ECO, GRV and DEB rivers (small North Minas Basin drainages potentially harbouring residual populations of salmon) and STW River salmon in an attempt to preserve genes from these nearby iBoF sources and possibly minimize the extent of inbreeding in the STW LGB population. Some information on offspring performance (survival in the wild from release at Age 0+ to Age 1+, length of males and females at Age 4, and weight of males and females at Age 4) is available for these outbred crosses (Outbred) and for non-outbred or potentially inbred STW crosses (Inbred STW) to assess some of the effects of this action on STW LGB salmon (Figures 54–58). Outbred families (N=10) produced in 2009 (the only year information is available for the relevant offspring trait) appear to exhibit approximately 2 times greater survival in the wild from release at Age 0+ to Age 1+ compared to inbred STW families (N=91) (Figure 54), and this difference is significant (p=0.0368, Table 6). This possible heterosis could be due to the mixing of genes from these potentially different populations in the F1 hybrids. However, it should also be noted that (a) the number of outbred crosses was small, (b) these results could be driven by just two or three high performing STW or NMB parents involved in the Outbred crosses (Figure 54, lower panel) and (c) large potentially confounding parental effects have been noted by Houde et al. (2010) in their analyses of inbreeding versus outbreeding effects involving STW, GRV and ECO salmon populations. Additionally, Houde et al. (2011) did not observe increased survival of offspring of STWxECO or STWxGRV crosses over STWxSTW crosses, though in the second year of the same study (Part II), Rollinson et al. (2014) may have. Clearly, possible benefits of further outcrossing in this population need to be investigated, especially since levels of inbreeding in the STW LGB population are expected to increase in the near future (O'Reilly et al. 2018). However, it should also be understood that nearly all available

NMB Atlantic Salmon have already been interbred with STW River salmon; Gaspereau River salmon are the only remaining Minas Basin salmon available to introgress into the STW gene pool (discussed below).

Outbred male and female salmon produced in the years 2007, 2008 and 2009 generally appeared to exhibit decreased family mean length or weight compared to inbred STW salmon at Age 4 (Figures 55–58), but sometimes the opposite pattern was observed, and no statistically significant differences between were observed (Table 6).

In the year 2013, 16 crosses were carried out between salmon from the genetically (O'Reilly et al. 2018) and phenotypically divergent Gaspereau River population and STW LGB salmon. Offspring from these crosses were reared alongside 16 pure Gaspereau River and 75 pure Stewiacke River crosses, and the following traits monitored:

a. survival from shock to release as fry at Age 0+;
b. average family mean fry (Age 0+) length;
c. average family mean fry (Age 0+) weight; and
d. average family percent deformities of Age 0+ fry.

Outbred hybrid crosses (GAKxSTW, referred to as Outbred) exhibited slightly lower average shock to pre-release survival compared to the two pure treatment groups (GAKxGAK and STWxSTW, referred to as Inbred GAK and Inbred STW, respectively), though differences were not significant (Figure 59, p=0.1099, Table 6). Data from these three sets of families (cross types) on survival in the wild from release as fry at Age 0+ to Age 1+ was not available at the time these analyses were carried out. However, preliminary analysis conducted since then suggest that hybrid families do not exhibit higher survival than non-hybrid families in the wild and that differences across all three cross types are modest and non-significant (de Mestral, pers. comm). Average family mean fry weight and length across these three groups were very similar (Figures 60 and 61), and differences observed were not significant (Table 6). Average percent deformities were many times higher (10X) in the outbred group compared to the two inbred groups (Figure 62), though levels were low (below 2%) for all, and differences were, perhaps surprisingly, non-significant (Table 6).

As mentioned previously, GAK and STW salmon exhibit a moderate degree of genetic divergence, and potentially important life history differences. We do not recommend introgression of GAK genes into the STW LGB population at this time, but outbreeding (introgression of non-native genes) should be considered if evidence of biologically meaningful levels of inbreeding depression are observed in the future. STW (and GAK) populations should continue to be monitored for possible signs of inbreeding depression. Levels of known (pedigree) inbreeding across STW families created during standard LGB operations has been increasing from 2000 to 2015 (see Figure 51 in O'Reilly et al. 2018), and is expected to increase further in the near future. This will create additional challenges for detecting inbreeding effects by looking for correlations between pedigree F and offspring performance, using standard LGB trait data only. Therefore, consideration should be given to carrying out additional experimental outcrosses between STW River salmon and individuals from other source populations known to be at least moderately reproductively isolated (and genetically divergent) from STW salmon.

Effect of Maternal and Paternal Age on Offspring Performance in Captivity and in the Wild

The current conservation program for the STW LGB population includes the delaying of reproduction to year five (to Age 5, where age is based on the brood year or fertilization year,

Age 4 where age is based on the year of hatch). The STW River salmon may also be spawned in their fourth year, but associated offspring are released for supplementation purposes only and are essentially lost to the LGB program (these crosses, and the offspring produced, do not impact allele or genotype frequencies in the STW LGB population). In order to assess possible negative effects of delaying reproduction on the STW LGB population, some of which may have had a genetic basis and could possibly be passed from generation to generation, we tested for associations between paternal and maternal age and several measures of offspring performance, including average family mean length of Age 0+ fry, average family mean weight of Age 0+ fry, average family mean length of Age 1+ parr, average family mean weight of Age 1+ parr, average family percent survival from shock to pre-release fry at Age 0+, and average family percent survival from release as Age 0+ fry to capture as Age 1+ parr. Effects of egg size, as a possible important covariate in assessing parent age effects on fry length at Age 0+ was also investigated.

Male parent age at spawning in general does not appear to be associated with any measure of offspring performance. Average family mean length and weight of Age 0+ fry and Age 1+ parr produced by male parents of varying ages (and in particular those produced by Age 4 vs Age 5 male parents, where averages were based on large numbers of families) were similar, with most error bars overlapping (Figures 63–66). Additionally, when slight differences were observed, directional trends in length or weight with increasing age were usually absent (Figure 63, length at Age 0+, spawning year 2012; Figure 64, weight at Age 0+, spawning year 2012; Figure 65, length at Age 1+, spawning years 2011 and 2012; Figure 66, weight at Age 1+, 2012). All associations between paternal age and length or weight of fry at Age 0+ were non-significant once egg area effects were taken into account (Table 12). Similarly, paternal age was not found to be associated with length or weight of parr at Age 1+ (Table 10). Shock to pre-release survival at Age 0+ appeared to increase with male parent age, at least from Age 4 to Age 5 (where the number of families involved was generally quite large) in spawning year datasets 2008, 2009, 2011, but appeared to decreased with age in 2010, 2012, 2013 (Figure 67); age was only correlated (positively) with shock to pre-release survival in the 2009 dataset (p=0.0451, Table 9). Similar results were observed for comparisons between male parent age and survival in the wild from release as fry at Age 0+ to Age1+. No consistent trend was observed between survival and male parent age across any one spawner year dataset (Figure 68), and survival was correlated with age (positively) only in the 2010 dataset only (p=0.0401, Table 9). Average percent survival was higher for Age 5 compared to Age 4 male parents (both consistently represented by large numbers of families) in 2009, 2010, and 2011, but not in 2012, where the opposite trend was observed but where differences were comparatively large; differences in release to Age 1+ survival between Age 4 and Age 5 parents was very modest in 2009 and 2011 (Figure 68).

On the other hand, maternal parent age at spawning appeared to have a large and consistent effect on multiple measures of offspring performance. Average family mean length and weight of Age 0+ fry generally increased with maternal parent age in the two spawning years for which data were available (Figures 69 and 70), and the overall association between both response variables and female age, independent of egg size, was significant in the 2012 comparisons (p=0.0300 and p=0.0023, respectively, Table 12). Average family mean length and weight of Age 1+ parr also appeared to generally increase with maternal parent age (Figures 71 and 72), particular between ages 4 and 5, each represented by large numbers of families, and associations in 2012 were again significant (p=0.0388) or highly significant (p=0.0041), respectively, for these two response variables (Table 10).

Average percent survival from shock to pre-release as Age 0+ fry also appeared to generally increase with female parent age in all six spawning years investigated (2008–2013), at least to

Age 5 (Figure 73); trends were more variable from Age 6 on, when the number of families associated with a given parent age was greatly reduced. This apparent association between shock to pre-release survival and female parent age was significant in 2009 (p=0.0409) and highly significant in 2011 (p=0.0030), but not in the remaining years (Table 9).

Average percent survival from release at Age 0+ to Age 1+ in the wild similarly appeared to generally increase with female parent age, particularly across ages 4 and 5 (when the number of families associated with a given female parent age was high), for all four years investigated (2009–2012); trends after female parent Age 5 were again variable but survival appeared to generally increase further with female parent age, at least until Age 6 (Figure 74). Associations between female parent age and survival in the wild from release to Age 1+ were highly significant (p<0.01) for all four spawning year datasets (Table 9). Egg area was observed to be strongly associated with survival to Age 1+, with larger eggs exhibiting higher survival than small eggs in both years investigated (2011 and 2012, Figure 75); this association was highly significant in both years (p<0.0001, Table 9).

In summary, we do not see any indicators of potential concerns with the delay of spawning of STW salmon for LGB purposes (where offspring are intended to be recycled back into the program), at least until Age 5 (for male or female salmon). Indeed, based on several metrics, offspring survival through to at least Age 1+ might be expected to increase with female parent age, in part due to associated increases in egg size. In fact, these results, and the known relationship between female age and fecundity, provide important insight into potentially efficient ways of markedly increasing smolt production in this river, a key recommendation of the 15-year iBoF LGB review (DFO 2018). Many non-targeted STW adult salmon (salmon not identified by Ranked MK breeding programs as important broodstock) are currently either released post-maturation but before spawning or immediately after spawning at Age 4. If more non-targeted Age 4 females were retained and spawned again at Age 5 for supplementation purposes, the resulting larger number of offspring (due to both an increase in the number of female parents but also increases in female body size and hence individual fecundity) and expected increases in offspring survival (both in captivity from shock to pre-release and in the wild from release to at least Age 1+) could result in a multiplicative increase in the number of late-stage juveniles and, potentially, out-migrating smolt. If this increase in smolt numbers is sufficiently large, this might lead to the possible return of a handful of adults to the STW in the years ahead, providing the potential for some selection to current marine conditions.

Earlier, we discussed how many experimental design elements in this study (including mean family size at capture at Age 1+, and the unevenness of sample sizes of contrasted treatment groups) were driven by the primary objective of associated activities, the implementation of the STW LGB program. These limitations were expected to impact, to some degree, our ability to detect differences and to estimate the magnitude of effect of some predictor (independent) variables on response (dependent) variables like early juvenile survival in the wild. These findings on the effects of female age (and egg size) on offspring performance also indicate that effects of moderate size (when they exist) can be detected given the LGB program-driven experimental design used here to test the effects of all other predictor variables discussed above (e.g. parental rearing environment, MK values, etc.) on many response metrics analyzed here.

Effect of European Ancestry on Offspring Performance in the Wild

European/STW hybrid families (referred to as EU hybrid) produced in each of the four spawning years (brood years) assessed (2009 2012) appear to exhibit lower average percent survival in the wild from release as fry at Age 0+ to Age 1+ (15 months after release) compared to pure STW families (referred to as STW) that experienced a similar number of program generations

(Figure 76), though differences in the 2012 spawning year comparison were very small, and differences in any single spawning year comparison were non-significant (Table 6). Similar results were observed in analyses based on Age 2+ parr (Figure 77); average percent survival in the wild from release at Age 0+ to Age 2+ was lower for EU hybrid families compared to STW families (Figure 77), though differences for any single spawning year comparison were non-significant (Table 6). Since Age 2+ parr were sampled via electrofishing in September, several months after some Age 2+ individuals would have been expected to out-migrate as smolt, differential results in this comparison possibly reflect both variations in mortality and emigration rates as Age 2+ smolt. Still, it is interesting to note that in all seven comparisons of Age 1+ or Age 2+ salmon where differences reflect, at least in part, variation in mortality in the wild, average percent survival of European/STW hybrid families (or average percent recovery of families) was lower than that of comparable STW families. Additionally, if results at Age 2+ do largely reflect differences in out-migration rates between EU hybrid and STW salmon, this would also be of concern, since age of smoltification in iBoF salmon may be adaptive, and introgression of EU farm genes may be altering this trait.

We also compared the relative proportion of European/STW hybrid offspring (EU hybrid, black portion of bars) to pure STW offspring (STW, grey portion of bars) over time, from release as fry at Age 0+ to Age 1+ to Age 2+, by spawning year (individually) and across all spawning years (Figure 78). Offspring across all families within a cross type were combined. Information for Age 2+ parr in 2012 was not available at the time these analyses were carried out. In the 2009, 2010 and 2011 spawning year comparisons, the relative proportion of EU hybrid to STW salmon appeared to decline from release at Age 0+ to Age 1+. Whether or not differences were statistically significant was not tested in the 2009 comparison because of null values for the EU hybrid 1+ group (no EU hybrid offspring were observed in the collection, despite the release of large numbers of Age 0 EU hybrid fry), but differences in proportions were significant in the 2010 and 2011 comparisons (p=0.0155 and 0.0424, respectively, Table 13). When all spawning years were combined, the proportion of EU hybrid to STW salmon appeared to decline from release at Age 0+ to Age 1+, and differences were significant (p=0.0085, Table 13). Similar results were observed when comparing relative proportions of EU hybrid to STW offspring at Age 0+ vs Age 2+; proportions of EU hybrid to STW observed in collections appeared to decline over time for all three spawning year comparisons for which data were available (2009, 2010, and 2011). Again, whether or not differences were significant were not tested in the 2009 and 2010 comparisons because of null values for the respective EU hybrid 2+ groups, though proportions across the 2011 spawning year comparison were not significant. When data from all spawning years were combined, the proportion of EU hybrid to STW offspring appeared to decline between release at Age 0+ and Age 2+, and differences were significant (p=0.0460, Table 13). When combining data across all spawning year comparisons (increasing the number of families included in the analysis), a consistent pattern of decreasing proportions of EU hybrid to STW offspring from Age 0 to Age 1 to Age 2 was observed (Figure 78, All Years). This result would be expected if the effects of EU genes on survival were cumulative, at least to Age 2+. However, as discussed above, the latter half (Age 1+ to Age 2+) of this apparent declining trend in the proportion of EU hybrid to STW offspring may have been influenced, at least in part, by the differential age of outmigration of smolt between these two cross types, with more offspring of EU hybrid salmon leaving the release-recapture site as Age 2+ smolt, several months before the relevant sampling collections occurred. Note that age of smoltification is strongly influenced by specific growth rates (Bailey et al. 1980; Metcalfe et al. 1988), and that Age 1+ EU hybrid offspring appear to be larger than STW salmon at this same age (Figure 79), though we did not test whether differences were statistically significant.

Although the number of families exhibiting EU farm genes included in this study was modest (17), and spread out over four spawning year collections of families, it should be noted that the

number (and proportion) of STW LGB families potentially impacted by EU farm genes increased across generations in this population, and that nearly five percent of all 2016 candidate spawners may have exhibited some level of EU farm ancestry (based on high stringency criteria, see Table 95 in O'Reilly et al. 2018). Moreover, EU farm ancestry may be even more prevalent in other iBoF LGB populations (including the BSR), though percent EU ancestry within individuals (at least in the STW LGB population) may be declining through time (O'Reilly et al. 2018).

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TABLES

Table 2. List of traits monitored in Stewiacke River Atlantic Salmon for the Inner Bay of Fundy Live Gene Bank Program. Dashes (-) denote empty or blank cells; WE=Wild-exposed; CAP=Captive Reared.

Group or Life Stage	Trait	Method	Years Available	Corresponding Brood Year
Spawning Adult	Length	Individual	2000–2015	2000–2015
	Weight	Individual	2005, 2007–2015	2005, 2007–2015
	Body Depth	Images	2010–2015	2010–2015
Egg	Weight	Bulk weights	2013–2015	2013–2015
	Area	Measured (select crosses)	2002–2009	2002–2009
		Images	2011–2015	2011–2015
	Initial Fecundity	Counts (select crosses)	2000–2015	2000–2015
		Estimates	2000–2015	2000–2015
		Images	2010–2015	2010–2015
	% fertilization at Shock	-	2014–2016	2013–2015
	Eggs remaining after Shock	Records	2001–2016	2000–2015
		Images	2011–2016	2010–2015
Equalized Fry	Number Remaining	Records	2009–2016	2008–2015
		Images	2011–2016	2010–2015
	Deformities	Images	2011–2016	2010–2015
	Length	Individual	2013–2016	2012–2015
	Weight	Bulk weights	2013–2016	2012–2015
WE Parr	Length	Individual	2013–2014	2011–2012 (Age 2) 2010–2011 (Age 3)
	Weight	Individual	2013–2014	2011–2012 (Age 2) 2010–2011 (Age 3)
Smolt	Gender/Maturation	-	2004–2015	2001–2012 (Age 3)
CAP offspring as Adults	Various (Length, weight, body depth, maturation, etc.)	Individual	2004–2015 2005–2015	2000–2011 (Age 4) 2000–2010 (Age 5)
WE offspring as Adults	Various (Length, weight, body depth, maturation, etc.)	Individual	2006–2015 2007–2015	2002–2011 (Age 4) 2002–2010 (Age 5)

Table 3. Body characteristics (length, weight, and condition factor) of all spawning Stewiacke River adult Atlantic Salmon from the Live Gene Bank Program for each brood year. Weight and condition factor were not recorded in 2000–2004. Dashes (-) denote empty or blank cells; SD=Standard Deviation.

Sex and Year -	Length (cm) Average	Length (cm) SD	Length (cm) Minimum	Length (cm) Maximum	Weight (kg) Average	Weight (kg) SD	Weight (kg) Minimum	Weight (kg) Maximum	Condition Factor Average	Condition Factor SD	Condition Factor Minimum	Condition Factor Maximum
FEMALE	-	-	-	-	-	-	-	-	-	-	-	-
2000	40.76	3.01	33.00	50.00	-	-	-	-	-	-	-	-
2001	45.91	5.80	33.80	63.00	-	-	-	-	-	-	-	-
2002	50.74	6.51	38.10	65.00	-	-	-	-	-	-	-	-
2003	50.91	8.00	35.60	78.00	-	-	-	-	-	-	-	-
2004	51.90	10.19	30.00	76.00	-	-	-	-	-	-	-	-
2005	51.30	7.57	28.40	72.20	1.97	0.97	0.54	6.20	1.39	0.25	0.83	2.79
2006	52.07	10.35	35.00	80.90	2.04	0.46	1.54	2.72	1.21	0.24	0.89	1.75
2007	51.71	11.62	31.80	85.00	2.24	1.90	0.30	10.00	1.36	0.26	0.93	3.00
2008	44.96	9.19	29.60	71.80	1.39	1.02	0.26	5.40	1.33	0.20	0.56	2.41
2009	47.85	9.31	31.80	78.00	1.69	1.10	0.40	7.53	1.38	0.17	0.96	2.12
2010	46.52	8.74	30.90	71.50	1.60	1.09	0.40	5.32	1.41	0.15	0.95	1.90
2011	42.86	8.63	25.40	66.00	1.19	0.83	0.31	3.75	1.34	0.17	0.94	2.21
2012	47.58	9.23	32.40	75.00	1.66	1.21	0.40	5.80	1.35	0.23	0.58	2.72
2013	45.53	6.74	32.80	70.00	1.38	0.74	0.44	4.98	1.35	0.15	0.88	1.64
2014	48.87	5.19	38.80	64.60	1.62	0.54	0.69	3.46	1.34	0.11	1.14	1.61
2015	47.47	5.55	32.80	59.00	1.61	0.52	0.52	2.73	1.45	0.13	0.82	1.82
MALE	-	-	-	-	-	-	-	-	-	-	-	-
2000	38.95	5.42	15.50	51.00	-	-	-	-	-	-	-	-
2001	46.32	7.43	25.00	62.50	-	-	-	-	-	-	-	-
2002	51.67	7.14	29.70	71.00	-	-	-	-	-	-	-	-
2003	49.91	7.51	30.00	69.00	-	-	-	-	-	-	-	-
2004	56.50	10.84	27.20	80.00	-	-	-	-	-	-	-	-
2005	58.34	11.53	33.00	81.00	2.67	1.47	0.58	6.82	1.20	0.25	0.65	2.70
2006	52.94	13.79	26.90	85.40	1.65	0.50	0.78	2.12	1.09	0.20	0.91	1.46
2007	48.05	11.63	22.00	85.60	1.49	1.24	0.22	7.54	1.15	0.19	0.54	1.80
2008	45.51	12.08	20.10	74.90	1.43	1.19	0.12	4.92	1.25	0.16	0.94	2.09
2009	46.50	13.05	18.70	91.90	1.53	1.29	0.08	7.43	1.28	0.15	0.96	1.71
2010	47.03	11.08	22.10	75.60	1.47	1.07	0.16	4.76	1.22	0.12	0.60	1.53
2011	41.36	10.49	17.00	72.80	1.00	0.84	0.05	4.34	1.18	0.12	0.83	1.50
2012	46.65	10.46	25.60	81.20	1.46	1.23	0.19	6.95	1.24	0.15	0.88	2.21
2013	44.19	6.24	20.80	58.60	1.07	0.40	0.11	2.20	1.19	0.15	0.60	1.52
2014	45.97	8.92	25.40	72.10	1.32	0.86	0.22	5.33	1.23	0.11	1.00	1.66
2015	45.87	7.22	31.10	64.50	1.33	0.57	0.42	3.09	1.33	0.25	1.07	2.98

Table 4. Gender ratios (Females / Males) of wild-exposed parr (Age 1 and Age 2) and smolt, and captive-reared Atlantic Salmon from the Stewiacke River Live Gene Bank Program. Dashes (-) denote empty or blank cells.

Brood Year	Female (F)	Male (M)	Sex Unknown	Ratio (F/M)
a) Wild-exposed Parr, Age 1				
2000	0	0	0	-
2001	2	6	0	0.33
2002	66	71	10	0.93
2003	36	39	0	0.92
2004	64	70	2	0.91
2005	43	39	2	1.10
2006	60	70	0	0.86
2007	64	79	1	0.81
2008	57	39	0	1.46
2009	106	108	1	0.98
2010	149	173	8	0.86
2011	156	175	43	0.89
2012	0	0	354	-
b) Wild-exposed Parr, Age 2				
2000	2	5	0	0.40
2001	22	41	2	0.54
2002	8	37	0	0.22
2003	2	13	1	0.15
2004	14	24	0	0.58
2005	0	4	0	-
2006	9	22	1	0.41
2007	7	43	0	0.16
2008	22	51	1	0.43
2009	8	54	0	0.15
2010	9	32	1	0.28
2011	0	0	69	-
2012	0	0	0	-
c) Smolt				
2000	0	0	0	-
2001	0	4	0	-
2002	18	28	2	0.64
2003	10	4	0	2.50
2004	127	57	1	2.23
2005	70	0	0	-
2006	133	39	2	3.41
2007	128	22	0	5.82
2008	4	1	0	4.00
2009	9	0	0	-
2010	0	0	0	-
2011	0	0	0	-
2012	0	0	0	-
d) Captive Reared Salmon				
2000	91	81	248	1.12
2001	95	47	190	2.02

Brood Year	Female (F)	Male (M)	Sex Unknown	Ratio (F/M)
2002	150	122	26	1.23
2003	117	119	165	0.98
2004	40	49	0	0.82
2005	89	77	1	1.16
2006	49	35	0	1.40
2007	104	96	0	1.08
2008	93	102	2	0.91
2009	103	97	0	1.06
2010	93	102	3	0.91
2011	61	73	6	0.84
2012	0	0	128	-

Table 5. Average (± one standard deviation) family size at fry release and at capture, and the percent family recovery (as a proxy for survival in the wild) for wild-exposed Atlantic Salmon parr collected from Pembroke River and reported by brood year. Dashes (-) denote empty or blank cells.

Brood Year	Family Size at Fry Release	Family Size at Capture			Percent Family Recovery/Survival (%)			Parr Collected		
		Age 1 Parr	Age 2 Parr	Any Age	Release to Age 1	Release to Age 2	Age 1 to Age 2	Age 1	Age 2	Total
2000	-	0	0.04 ± 0.22	0.10 ± 0.37	-	-	-	0	7	17
2001	-	0.07 ± 0.34	0.57 ± 1.03	0.69 ± 1.22	-	-	50.00 ± 54.77	8	65	79
2002	-	1.28 ± 2.92	0.39 ± 0.95	1.70 ± 3.48	-	-	63.82 ± 91.77	147	45	195
2003	-	0.56 ± 1.30	0.12 ± 0.55	0.68 ± 1.57	-	-	22.62 ± 74.34	75	16	91
2004	-	0.77 ± 1.48	0.21 ± 0.63	0.99 ± 1.81	-	-	24.34 ± 66.25	136	38	176
2005	-	0.58 ± 1.99	0.03 ± 0.16	0.60 ± 1.99	-	-	6.25 ± 24.59	84	4	88
2006	110*	0.75 ± 1.08	0.18 ± 0.46	0.97 ± 1.26	0.70 ± 1.02**	0.17 ± 0.43**	18.72 ± 40.38	130	32	168
2007	120*	1.06 ± 1.11	0.37 ± 0.56	1.57 ± 1.34	0.91 ± 0.96**	0.32 ± 0.48**	31.86 ± 49.55	144	50	215
2008	137 ± 33	0.92 ± 1.15	0.71 ± 0.99	1.70 ± 1.87	0.64 ± 0.79	0.49 ± 0.69	55.18 ± 67.17	96	74	178
2009	146 ± 15	2.09 ± 2.42	0.60 ± 0.91	2.71 ± 2.85	1.42 ± 1.64	0.41 ± 0.63	41.32 ± 70.45	215	62	279
2010	173 ± 24	2.95 ± 2.27	0.38 ± 0.65	3.37 ± 2.49	1.71 ± 1.27	0.21 ± 0.37	16.35 ± 34.15	330	42	377
2011	328 ± 77	3.78 ± 3.01	0.70 ± 0.95	-	1.16 ± 0.87	0.22 ± 0.37	23.51 ± 45.79	374	69	453
2012	325 ± 102	3.40 ± 3.05	-	-	1.03 ± 0.84	-	-	354	0	354
2013	367 ± 42	-	-	-	-	-	-	-	-	-
2014	349 ± 30	-	-	-	-	-	-	-	-	-
2015	359 ± 46	-	-	-	-	-	-	-	-	-

* Family size at release was not known; therefore the initial EQU number collected per family at shock was adjusted by the average survival (96.6 %) from 'shock to pre-release'.

** Calculated using the estimated family size at fry release for T1 of Formula 3.

Table 6. Test statistics for ANOVA and Welch's ANOVA, including p-value, effect size eta-squared, and the 95% confidence interval for eta-squared for each analysis year.

Independent Variable	Dependent Variable	Figure Number	Year	P-Value	Eta-Squared (η^2)	95% Confidence Interval for η^2
Par.R.Env.	Surv,0+ to 1+	26	2009	0.9111	0.0061	0–0.0302
Par.R.Env.	Surv,0+ to 1+	26	2010	0.7244	0.0128	0–0.0555
Par.R.Env.	Surv,0+ to 1+	26	2011	0.2244	0.0548	0–0.1471
Par.R.Env.	Surv,0+ to 1+	26	2012	0.5345	0.0297	0–0.1039
M.Par.R.Env.	Surv,0+ to 1+	27	2009	0.9704	<0.0001	0–0.0037
M.Par.R.Env.	Surv,0+ to 1+	27	2010	0.8411	0.0004	0–0.0342
M.Par.R.Env.	Surv,0+ to 1+	27	2011	0.2770	0.0149	0–0.1035
M.Par.R.Env.	Surv,0+ to 1+	27	2012	0.8149	0.0007	0–0.0509
Par.R.Env.	Surv,0+ to 2+	28	2009	0.5072	0.0263	0–0.0915
Par.R.Env.	Surv,0+ to 2+	28	2010	0.0878	0.0619	0–0.1474
Par.R.Env.	Surv,0+ to 2+	28	2011	0.5775	0.0249	0–0.0912
M.Par.R.Env.	Surv,0+ to 2+	29	2009	0.9413	<0.0001	0–0.0184
M.Par.R.Env.	Surv,0+ to 2+	29	2010	0.1838	0.0169	0–0.0933
M.Par.R.Env.	Surv,0+ to 2+	29	2011	0.2057	0.0199	0–0.1136
M.Par.R.Env.	Fam Len, 1+	32	2011	0.1718	0.0282	0–0.1392
M.Par.R.Env.	Fam Len, 1+	32	2012	0.0007	0.1692	0.0337–0.3276
Par.R.Env.	Fam Len, 1+	30	2011	0.6796	0.0230	0–0.0991
Par.R.Env.	Fam Len, 1+	30	2012	0.0167	0.1684	0.0055–0.3147
Par.R.Env.	Fam Wei, 1+	33	2011	0.9356	0.0057	0–0.0284
Par.R.Env.	Fam Wei, 1+	33	2012	0.0146	0.1735	0.0079–0.3201
M.Par.R.Env.	Fam Wei, 1+	35	2011	0.4842	0.0072	0–0.0892
M.Par.R.Env.	Fam Wei, 1+	35	2012	0.0004	0.1805	0.0401–0.3390
Crs,out.inb,NMB	Surv,0+ to 1+	54	2009	0.0368	0.0433	0–0.1423
Crs,out.inb,NMB	Fam LenF,4+	55	2007	0.4704	0.0330	0–0.2880
Crs,out.inb,NMB	Fam LenF,4+	55	2008	0.5071	0.0159	0–0.1868
Crs,out.inb,NMB	Fam LenF,4+	55	2009	0.7561	0.0022	0–0.0911
Crs,out.inb,NMB	Fam LenM,4+	56	2007	0.8680	0.0009	0–0.0950
Crs,out.inb,NMB	Fam LenM,4+	56	2008	0.0936	0.2165	0–0.5177
Crs,out.inb,NMB	Fam LenM,4+	56	2009	0.4212	0.0176	0–0.1647
Crs,out.inb,NMB	Fam WeiF,4+	57	2007	0.2430	0.0841	0–0.3623
Crs,out.inb,NMB	Fam WeiF,4+	57	2008	0.4136	0.0240	0–0.2062
Crs,out.inb,NMB	Fam WeiF,4+	57	2009	0.5762	0.0070	0–0.1178
Crs,out.inb,NMB	Fam WeiM,4+	58	2007	0.9566	0.0001	0–0.0334
Crs,out.inb,NMB	Fam WeiM,4+	58	2008	0.4692	0.0445	0–0.3439
Crs,out.inb,NMB	Fam WeiM,4+	58	2009	0.4902	0.0133	0–0.1561
Crs,out.inb,GAK	Surv,Shk to 0+	59	2013	0.1099	0.0416	0–0.1260
Crs,out.inb,GAK	Fam Len, 0+	60	2013	0.8685	0.0027	0–0.0322
Crs,out.inb,GAK	Fam Wei, 0+	61	2013	0.2105	0.0295	0–0.1053
Crs,out.inb,GAK	Perc. Deform.	62	2013	0.2990	0.0230	0–0.0928
Crs,EUHyb,STW	Surv,0+ to 1+	76	2009	0.1354	0.1080	0–0.3649
Crs,EUHyb,STW	Surv,0+ to 1+	76	2010	0.1084	0.0836	0–0.2934
Crs,EUHyb,STW	Surv,0+ to 1+	76	2011	0.2180	0.0210	0–0.1221
Crs,EUHyb,STW	Surv,0+ to 1+	76	2012	0.8296	0.0011	0–0.0798
Crs,EUHyb,STW	Surv,0+ to 2+	77	2009	0.3659	0.0411	0–0.2756
Crs,EUHyb,STW	Surv,0+ to 2+	77	2010	0.3127	0.0340	0–0.2189
Crs,EUHyb,STW	Surv,0+ to 2+	77	2011	0.7752	0.0011	0–0.0575

Par.R.Env. = parental rearing environment or the early juvenile parental rearing environment experienced by a family's maternal and paternal parents
M.Par.R.Env. = maternal parental rearing environment or the early juvenile rearing environment experienced by a family's maternal parent
Surv,0+ to 1+ = average family percent survival in the wild from release at Age 0+ to Age 1+
Fam Len, 1+ = average family mean length at Age 1+
Fam Wei, 1+ = average family mean weight at Aage 1+
Crs,out.inb,NMB = cross type, Outbred (NMBxSTW) versus Inbred STW (STWxSTW)
Fam LenF,4+ = average family mean length at Age 4+, females only
Fam LenM,4+ = average family mean length at Age 4+, males only

Fam WeiF,4+ = average family mean weight at Age 4+, females only
Fam WeiM,4+ = average family mean weight at Age 4+, males only
Crs,out.inb,GAK= cross type, Outbred (STWxGAK) vs Inbred GAK (GAKxGAK) vs Inbred STW (STWxSTW)
Surv,Shk to 0+ = average family percent survival in captivity, shock (egg stage) to pre-release at Age 0+
Fam Len, 0+ = average family mean length at Age 0+
Fam Wei, 0+ = average family mean weight at Age 0+
Perc. Deform. = average family percent deformities
Crs,EUHyb,STW = cross type, EUxSTW hybrid vs STWxSTW
Surv,0+ to 2+ = average family percent survival in the wild from release at Age 0+ to Age 2+

Table 7. Test statistics for unbalanced, fixed-effects ANOVA, including p-values for the rearing environment group effect and the family within rearing environment subgroup effect for each analysis year.

Independent Variable (Predictor)	Dependent Variable (Response)	Figure Number	Year	Rearing Environment P-Value	Family Within Rearing Environment P-Value
Par.R.Env.	Ind. Len, 1+	31	2011	0.7300	<0.0001
Par.R.Env.	Ind. Len, 1+	31	2012	0.0044	<0.0001
Par.R.Env.	Ind. Wei, 1+	34	2011	0.9351	<0.0001
Par.R.Env.	Ind. Wei, 1+	34	2012	0.0032	<0.0001

Par.R.Env. = parental rearing environment or the early juvenile parental rearing environment experienced by a family's maternal and paternal parents
Ind. Len, 1+ = length of individual parr by family at Age 1+
Ind. Wei, 1+ = weight of individual parr by family at Age 1+

Table 8. Test statistics for Games-Howell post-hoc tests for ANOVA, Welch's ANOVA, and unbalanced, fixed-effects ANOVA, including groups compared, p-values, and the 95% confidence interval for the difference between means for the 2012 analysis year.

Independent Variable (Predictor)	Dependent Variable (Response)	Figure Number	Group 1	Group 2	P-Value	95% Confidence Interval for Means
Par.R.Env.	Fam Len, 1+	30	WExWE	WExCAP	0.9441	-0.3149–0.4805
Par.R.Env.	Fam Len, 1+	30	WExWE	CAPxWE	0.0995	-0.0460–0.7119
Par.R.Env.	Fam Len, 1+	30	WExWE	CAPxCAP	0.0185	0.0612–0.8163
Par.R.Env.	Fam Len, 1+	30	WExCAP	CAPxWE	0.3103	-0.1427–0.6431
Par.R.Env.	Fam Len, 1+	30	WExCAP	CAPxCAP	0.0845	-0.0365–0.7474
Par.R.Env.	Fam Len, 1+	30	CAPxWE	CAPxCAP	0.8459	-0.2737–0.4843
Par.R.Env.	Fam Len, 1+	31	WExWE	WExCAP	0.7587	-0.1468–0.3265
Par.R.Env.	Fam Len, 1+	31	WExWE	CAPxWE	0.0182	0.0441–0.6321
Par.R.Env.	Fam Len, 1+	31	WExWE	CAPxCAP	0.0040	0.1343–0.8684
Par.R.Env.	Fam Len, 1+	31	WExCAP	CAPxWE	0.1450	-0.0550–0.5515
Par.R.Env.	Fam Len, 1+	31	WExCAP	CAPxCAP	0.0262	0.0374–0.7856
Par.R.Env.	Fam Len, 1+	31	CAPxWE	CAPxCAP	0.7111	-0.2447–0.5713
Par.R.Env.	Fam Wei, 1+	33	WExWE	WExCAP	0.8464	-0.0006–0.0011
Par.R.Env.	Fam Wei, 1+	33	WExWE	CAPxWE	0.0310	0.0001–0.0016
Par.R.Env.	Fam Wei, 1+	33	WExWE	CAPxCAP	0.0217	0.0001–0.0018
Par.R.Env.	Fam Wei, 1+	33	WExCAP	CAPxWE	0.2410	-0.0003–0.0014
Par.R.Env.	Fam Wei, 1+	33	WExCAP	CAPxCAP	0.1617	-0.0002–0.0016
Par.R.Env.	Fam Wei, 1+	33	CAPxWE	CAPxCAP	0.9752	-0.0007–0.0009
Par.R.Env.	Fam Wei, 1+	34	WExWE	WExCAP	0.4300	-0.0002–0.0008
Par.R.Env.	Fam Wei, 1+	34	WExWE	CAPxWE	0.0101	0.0002–0.0015
Par.R.Env.	Fam Wei, 1+	34	WExWE	CAPxCAP	0.0103	0.0002–0.0018
Par.R.Env.	Fam Wei, 1+	34	WExCAP	CAPxWE	0.2083	-0.0002–0.0012
Par.R.Env.	Fam Wei, 1+	34	WExCAP	CAPxCAP	0.1220	-0.0001–0.0015
Par.R.Env.	Fam Wei, 1+	34	CAPxWE	CAPxCAP	0.9391	-0.0007–0.0011

Par.R.Env. = parental rearing environment or the early juvenile parental rearing environment
Fam Len, 1+ = average family mean length at Age 1+
Fam Wei, 1+ = average family mean weight at Age 1+

Table 9. Test statistics for Kendall's coefficient of rank correlation analysis, including tau, p-value, effect size eta-squared, and the 95% confidence interval for eta-squared for each analysis year.

Independent Variable (Predictor)	Dependent Variable (Response)	Figure Number	Year	Tau	P-Value	Eta-Squared (η^2)	95% Confidence Interval for η^2
Amt.J.Cap.R	Surv,0+ to 1+	36	2009	0.41	0.1599	0.3627	0–0.6486
Amt.J.Cap.R	Surv,0+ to 1+	36	2010	0.12	0.4239	0.0347	0–0.2273
Amt.J.Cap.R	Surv,0+ to 1+	36	2011	0.23	0.0237	0.1300	0.0094–0.3012
Amt.J.Cap.R	Surv,0+ to 1+	36	2012	0.04	0.7146	0.0032	0–0.0831
Amt.J.Cap.R	Surv,0+ to 2+	37	2009	0.11	0.7389	0.0302	0–0.3705
Amt.J.Cap.R	Surv,0+ to 2+	37	2010	0.12	0.4530	0.0377	0–0.2328
Amt.J.Cap.R	Surv,0+ to 2+	37	2011	-0.21	0.0599	0.1036	0.0025–0.2688
Prog.gen.	Surv,0+ to 1+	39	2009	-0.05	0.5308	0.0065	0–0.0753
Prog.gen.	Surv,0+ to 1+	39	2010	-0.17	0.0242	0.0707	0.0061–0.1788
Prog.gen.	Surv,0+ to 1+	39	2011	-0.16	0.0626	0.0648	0.0006–0.1876
Prog.gen.	Surv,0+ to 1+	39	2012	0.08	0.3540	0.0170	0–0.1119
Avg.Par.MK	Surv,0+ to 1+	41	2009	0.07	0.5526	0.0134	0–0.1641
Avg.Par.MK	Surv,0+ to 1+	41	2010	0.27	0.0018	0.1698	0.0379–0.3227
Avg.Par.MK	Surv,0+ to 1+	41	2011	0.07	0.3918	0.0129	0–0.1103
Avg.Par.MK	Surv,0+ to 1+	41	2012	0.11	0.3089	0.0313	0–0.1845
Avg.Par.MK	Surv,0+ to 2+	42	2009	-0.005	0.9730	<0.0001	0–0.0149
Avg.Par.MK	Surv,0+ to 2+	42	2010	0.02	0.8742	0.0006	0–0.0505
Avg.Par.MK	Surv,0+ to 2+	42	2011	0.12	0.1858	0.0351	0–0.1553
Avg.Par.MK	Surv,1+ to 2+	43	2009	-0.18	0.2758	0.0750	0–0.3147
Avg.Par.MK	Surv,1+ to 2+	43	2010	-0.04	0.6802	0.0043	0–0.0895
Avg.Par.MK	Surv,1+ to 2+	43	2011	0.13	0.1773	0.0423	0–0.1782
Avg.Par.MK	Surv,egg to 4+	44	2007	0.01	0.9430	0.0002	0–0.0521
Avg.Par.MK	Surv,egg to 4+	44	2008	0.06	0.5378	0.0099	0–0.1157
Avg.Par.MK	Surv,egg to 4+	44	2009	0.04	0.7680	0.0035	0–0.1232
Avg.Par.MK	Surv,egg to 4+	44	2010	0.01	0.8794	0.0005	0–0.0474
Avg.Par.MK	Surv,egg to 4+	44	2011	-0.13	0.1760	0.0382	0–0.1606
Avg.Par.MK	Surv,egg to 4+	44	2012	0.05	0.7053	0.0051	0–0.1184
M.Par.Mk	Surv,egg to 4+	45	2007	-0.04	0.7421	0.0030	0–0.1170
M.Par.Mk	Surv,egg to 4+	45	2008	0.004	0.9685	<0.0001	0–0.0103
M.Par.Mk	Surv,egg to 4+	45	2009	-0.07	0.4692	0.0129	0–0.1627
M.Par.Mk	Surv,egg to 4+	45	2010	0.01	0.8981	0.0003	0–0.0408
M.Par.Mk	Surv,egg to 4+	45	2011	-0.14	0.1099	0.0486	0–0.1773
M.Par.Mk	Surv,egg to 4+	45	2012	-0.03	0.7283	0.0029	0–0.1050
F (offspring)	Surv,0+ to 1+	46	2009	0.06	0.6840	0.0088	0–0.0820
F (offspring)	Surv,0+ to 1+	46	2010	-0.06	0.5667	0.0081	0–0.0726
F (offspring)	Surv,0+ to 1+	46	2011	-0.06	0.5328	0.0093	0–0.0894
F (offspring)	Surv,0+ to 1+	46	2012	0.05	0.6728	0.0069	0–0.0860
F (offspring)	Perc. Deform.	50	2010	0.004	0.9632	<0.0001	0–0.0128
F (offspring)	Perc. Deform.	50	2011	0.02	0.7817	0.0015	0–0.0572
F (offspring)	Perc. Deform.	50	2012	0.06	0.5601	0.0075	0–0.0877
F (offspring)	Perc. Deform.	50	2013	-0.07	0.4416	0.0128	0–0.1031
F (offspring)	Perc. Deform.	50	2014	0.04	0.7162	0.0032	0–0.0739
F (offspring)	Perc. Deform.	50	2015	-0.07	0.3961	0.0137	0–0.0941
H (offspring)	Surv,0+ to 1+	51	2009	-0.18	0.1754	0.0784	0.0050–0.2021
H (offspring)	Surv,0+ to 1+	51	2010	0.14	0.1218	0.0498	0.0002–0.1510
H (offspring)	Surv,0+ to 1+	51	2011	0.07	0.4253	0.0127	0–0.0983
H (offspring)	Surv,0+ to 1+	51	2012	0.03	0.8115	0.0020	0–0.0640
H (offspring)	Perc. Deform.	53	2010	-0.02	0.8158	0.0008	0–0.0418

Independent Variable (Predictor)	Dependent Variable (Response)	Figure Number	Year	Tau	P-Value	Eta-Squared (η^2)	95% Confidence Interval for η^2
H (offspring)	Perc. Deform.	53	2011	-0.08	0.3520	0.0145	0–0.1017
H (offspring)	Perc. Deform.	53	2012	0.09	0.3143	0.0204	0–0.1191
H (offspring)	Perc. Deform.	53	2013	0.02	0.8590	0.0007	0–0.0508
H (offspring)	Perc. Deform.	53	2014	0.16	0.0762	0.0610	0–0.1905
H (offspring)	Perc. Deform.	53	2015	-0.11	0.1776	0.0301	0–0.1279
Age, paternal	Surv,Shk to 0+	67	2008	-0.01	0.9428	0.0001	0–0.0243
Age, paternal	Surv,Shk to 0+	67	2009	0.18	0.0451	0.0798	0.0064–0.2002
Age, paternal	Surv,Shk to 0+	67	2010	-0.03	0.7212	0.0019	0–0.0503
Age, paternal	Surv,Shk to 0+	67	2011	0.14	0.1084	0.0500	0–0.1663
Age, paternal	Surv,Shk to 0+	67	2012	-0.09	0.3324	0.0191	0–0.1166
Age, paternal	Surv,Shk to 0+	67	2013	0.01	0.9042	0.0003	0–0.0415
Age, paternal	Surv,0+ to 1+	68	2009	-0.07	0.4387	0.0107	0–0.0869
Age, paternal	Surv,0+ to 1+	68	2010	0.16	0.0401	0.0636	0.0040–0.1691
Age, paternal	Surv,0+ to 1+	68	2011	-0.005	0.9584	<0.0001	0–0.0177
Age, paternal	Surv,0+ to 1+	68	2012	-0.17	0.0651	0.0709	0.0011–0.2004
Age, maternal	Surv,Shk to 0+	73	2008	0.12	0.2112	0.0325	0–0.1309
Age, maternal	Surv,Shk to 0+	73	2009	0.19	0.0409	0.0871	0.0088–0.2096
Age, maternal	Surv,Shk to 0+	73	2010	-0.04	0.6098	0.0040	0–0.0600
Age, maternal	Surv,Shk to 0+	73	2011	0.27	0.0030	0.1716	0.0459–0.3142
Age, maternal	Surv,Shk to 0+	73	2012	-0.03	0.7268	0.0025	0–0.0672
Age, maternal	Surv,Shk to 0+	73	2013	0.11	0.2726	0.0275	0–0.1340
Age, maternal	Surv,0+ to 1+	74	2009	0.38	<0.0001	0.3224	0.1697–0.4525
Age, maternal	Surv,0+ to 1+	74	2010	0.36	<0.0001	0.2867	0.1488–0.4110
Age, maternal	Surv,0+ to 1+	74	2011	0.43	<0.0001	0.3875	0.2218–0.5164
Age, maternal	Surv,0+ to 1+	74	2012	0.24	0.0087	0.1377	0.0248–0.2820
Egg area	Surv,0+ to 1+	75	2011	0.44	<0.0001	0.4106	0.1745–0.5743
Egg area	Surv,0+ to 1+	75	2012	0.33	<0.0001	0.2454	0.0904–0.3940

Amt.J.Cap.R.= amount of cumulative early juvenile captive rearing experienced by a given family's immediate parents and grandparents
Surv,0+ to 1+ = average family percent survival in the wild from release at Age 0+ to Age 1+
Prog.gen.= number of program generations, or generations of captive breeding and rearing
Avg.Par.MK = average parental Mean Kinship
Surv,0+ to 2+ = average family percent survival in the wild from release at Age 0+ to Age 2+
Surv,1+ to 2+ = average family percent survival in the wild from Age 1+ to Age 2+
Surv,egg to 4+ = average family percent survival in captivity from shock (egg stage) to Age 4+; adult age is based on the individual's brood or fertilization year
M.Par.Mk = Mean Kinship of the maternal parent
F (offspring)= pedigree inbreeding for offspring/family
Perc. Deform. = average family percent deformities
H (offspring) = offspring Exp.Obs.Mol. Gen. Heterozygosity, or the expected observed heterozygosity in the offspring/family based on the molecular genetic data for the parents
Age, paternal = age of paternal parent at spawning
Surv,Shk to 0+ = average family percent survival in captivity, shock (egg stage) to pre-release at Age 0+
Age, maternal = age of maternal parent at spawning based on the individuals brood or fertilization year
Egg area = egg area, mm^2

Table 10. Test statistics for linear regression analyses, including slope, p-value, effect size eta-squared, and the 95% confidence interval for eta-squared for each analysis year.

Independent Variable (Predictor)	Dependent Variable (Response)	Figure Number	Year	Slope	P-Value	Eta-Squared (η^2)	95% Confidence Interval for η^2
Amt.J.Cap.R.	Length, 1+	38	2011	0.46	0.4249	0.0139	0–0.1373
Amt.J.Cap.R.	Length, 1+	38	2012	-0.68	0.0551	0.0677	0–0.2208
Fam size, par	Fam size, off	40	2010	0.4814	0.2522	0.2509	0–0.6158
Fam size, par	Fam size, off	40	2011	-0.1733	0.6481	0.0370	0–0.4188
Fam size, par	Fam size, off	40	2012	0.2540	0.6489	0.0058	0–0.1298
F (offspring)	Length, 1+	47	2011	-4.18	0.6260	0.0043	0–0.0917
F (offspring)	Length, 1+	47	2012	12.03	0.6266	0.0886	0–0.5499
F (offspring)	Weight, 1+	48	2011	-0.02	0.4194	0.3371	0–0.7035
F (offspring)	Weight, 1+	48	2012	0.02	0.6883	0.0612	0–0.5231
Age, paternal	Fam Len, 1+	65	2011	-0.11	0.3239	0.4571	0–0.7523
Age, paternal	Fam Len, 1+	65	2012	-0.06	0.4356	0.2116	0–0.6284
Age, paternal	Fam Wei, 1+	66	2011	-0.0004	0.1783	0.6752	0–0.8432
Age, paternal	Fam Wei, 1+	66	2012	-0.0001	0.4210	0.2238	0–0.6348
Age, maternal	Fam Len, 1+	71	2011	0.42	0.1420	0.9511	0–0.9716
Age, maternal	Fam Len, 1+	71	2012	0.16	0.0388	0.8055	0–0.9017
Age, maternal	Fam Wei, 1+	72	2011	0.001	0.1911	0.9125	0–0.9504
Age, maternal	Fam Wei, 1+	72	2012	0.0004	0.0041	0.1215	0.0131–0.2739

Amt.J.Cap.R.= amount of cumulative early juvenile captive rearing experienced by a given family's immediate parents and grandparents
Length, 1+ = family mean length of wild-exposed parr at Age 1+
Weight, 1+ = family mean weight of wild-exposed parr at Age 1+
Fam size, par = average parental family size at Age 1+, or the average of a family's maternal and paternal family size in the wild at Age 1+
Fam size, off = std. offspring family size at Age 1+ or offspring family size in the wild at age 1+ standardized to slight differences in numbers of individuals released across families
F (offspring) = pedigree inbreeding for offspring/family
Fam Len, 1+ = average family mean length at Age 1+
Fam Wei, 1+ = average family mean weight at Age 1+

Table 11. Tests statistics for binary logistic regression analyses, including p-values, for each analysis year.

Independent Variable (Predictor)	Dependent Variable (Response)	Figure Number	Year	P-Value
F (offspring)	Incid. deform.	49	2010	0.8170
F (offspring)	Incid. deform.	49	2011	0.7058
F (offspring)	Incid. deform.	49	2012	0.8849
F (offspring)	Incid. deform.	49	2013	0.4409
F (offspring)	Incid. deform.	49	2014	0.7908
F (offspring)	Incid. deform.	49	2015	0.1451
H (offspring)	Incid. deform.	52	2010	0.5675
H (offspring)	Incid. deform.	52	2011	0.7650
H (offspring)	Incid. deform.	52	2012	0.5701
H (offspring)	Incid. deform.	52	2013	0.8989
H (offspring)	Incid. deform.	52	2014	0.2866
H (offspring)	Incid. deform.	52	2015	0.4861

F (offspring) = pedigree inbreeding for offspring/family
Incid. deform. = incidence of deformities (observed = 1.0 or not = 0) in a given family, averaged across families exhibiting a given level of inbreeding
H (offspring) = offspring Exp. Obs. Mol. Gen. Heterozygosity, or the expected observed heterozygosity in the offspring/family based on the molecular genetic data for the parents

Table 12. Test statistics for multiple linear regression analyses with egg area as a covariate, including p-values for spawner age and egg area, overall p-value, slope, effect size eta-squared, and the 95% confidence interval for eta-squared for each analysis year.

Independent Variable (Predictor)	Dependent Variable (Response) [non-egg area variable]	Figure Number	Year	Age P-Value	Egg Area P-Value	Overall P-Value	Slope	Eta-Squared (η^2)	95% Confidence Interval for η^2
Age, paternal	Fam Len, 0+	63	2012	0.7310	<0.0001	<0.0001	-0.04	0.6764	0.5394–0.7506
Age, paternal	Fam Len, 0+	63	2013	0.8368	<0.0001	<0.0001	-0.05	0.4000	0.2167–0.5254
Age, paternal	Fam Wei, 0+	64	2012	0.7162	<0.0001	<0.0001	0.0006	0.8001	0.7085–0.8465
Age, paternal	Fam Wei, 0+	64	2013	0.9481	<0.0001	<0.0001	-0.0003	0.4744	0.2951–0.5881
Age, maternal	Fam Len, 0+	69	2012	0.0300	<0.0001	<0.0001	0.26	0.6968	0.5665–0.7665
Age, maternal	Fam Len, 0+	69	2013	0.9487	<0.0001	<0.0001	-0.02	0.3997	0.2163–0.5251
Age, maternal	Fam Wei, 0+	70	2012	0.0023	<0.0001	<0.0001	0.006	0.8244	0.7429–0.8652
Age, maternal	Fam Wei, 0+	70	2013	0.4451	<0.0001	<0.0001	0.003	0.4786	0.2998–0.5916

Age, paternal = age of paternal parent
Age, maternal = age of maternal parent
Fam Len, 0+ = average family mean length at Age 0+
Fam Wei, 0+ = average family mean weight at Age 0+
Adult or parent age used is based on the individual's brood or fertilization year

Table 13. Test statistics for G-tests for goodness of fit including groups compared, p-value, Cramer's V statistic, and the 95% confidence interval for Cramer's V for each analysis year and for all years combined. Lineage = EU hybrid (F2–F4 EU x STW) versus STW (pure Stewiacke).

Independent Variable (Predictor)	Dependent Variable (Response)	Figure Number	Group 1	Group 2	Year	P-Value	Cramer's V	95% Confidence Interval for V
Lineage	Proportion EU hybrid	78	Fry	Age 1+	2009	n/a	n/a	n/a
Lineage	Proportion EU hybrid	78	Fry	Age 1+	2010	0.0155	0.2777	0.0483–0.5025
Lineage	Proportion EU hybrid	78	Fry	Age 1+	2011	0.0424	0.1215	0–0.2389
Lineage	Proportion EU hybrid	78	Fry	Age 1+	2012	0.9592	0.0038	0–0.0737
Lineage	Proportion EU hybrid	78	Fry	Age 1+	All	0.0085	0.1086	0.0274–0.1894
Lineage	Proportion EU hybrid	78	Fry	Age 2+	2009	n/a	n/a	n/a
Lineage	Proportion EU hybrid	78	Fry	Age 2+	2010	n/a	n/a	n/a
Lineage	Proportion EU hybrid	78	Fry	Age 2+	2011	0.5210	0.0890	0–0.3594
Lineage	Proportion EU hybrid	78	Fry	Age 2+	All	0.0460	0.2178	0–0.4316

FIGURES

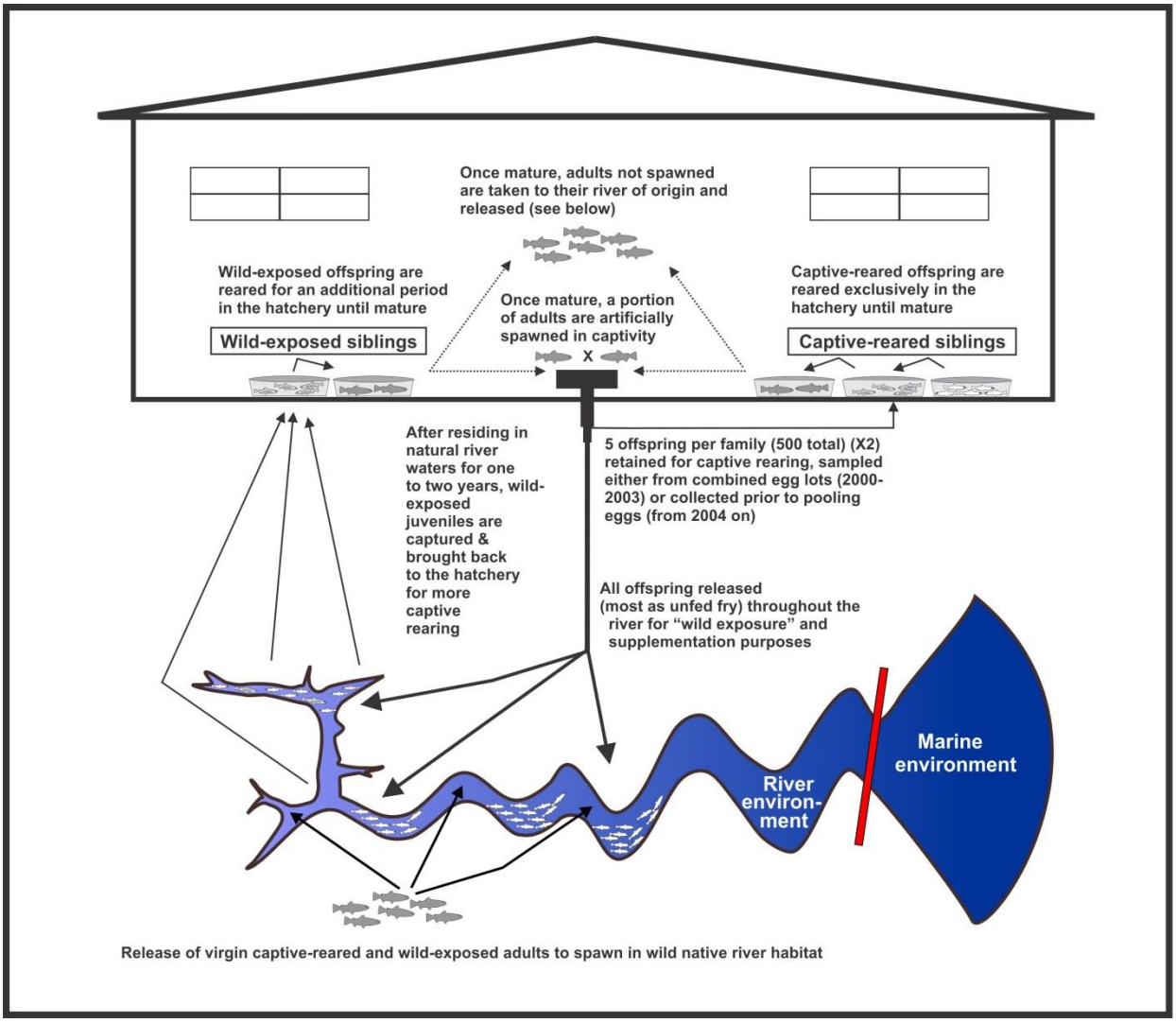


Figure 1. Schematic of the original Stewiacke River inner Bay of Fundy Live Gene Banking program (2000–2005). First, the original founders were crossed according to breeding protocols described within. Two sets of five eggs were then taken from each family, and the first set combined to form Group A and the second set Group B. Both sets of families were reared communally exclusively in captivity through to maturity as adults. Individuals so managed are referred to as 'captive-reared' salmon. Nearly all remaining offspring were released throughout the river as unfed fry. After one to two years of exposure to native river conditions, several hundred were caught via electrofishing and brought back to the hatchery for captive rearing through to maturity as adults; all juveniles not captured lived their remaining lives in river habitat or out-migrated into waters of the Bay of Fundy/Gulf of Maine (few were expected to return given current marine conditions). These electrofished 'wild-exposed' juveniles were then reared alongside their exclusively captive-reared siblings, during which time all or most individuals from both groups were genotyped and pedigreed. At or around spawning time, a portion of mature adults (captive-reared and wild-exposed) were released into native river habitat to potentially free spawn in the wild, while the remaining were artificially spawned in the production of the next generation of Stewiacke River inner Bay of Fundy Atlantic Salmon.

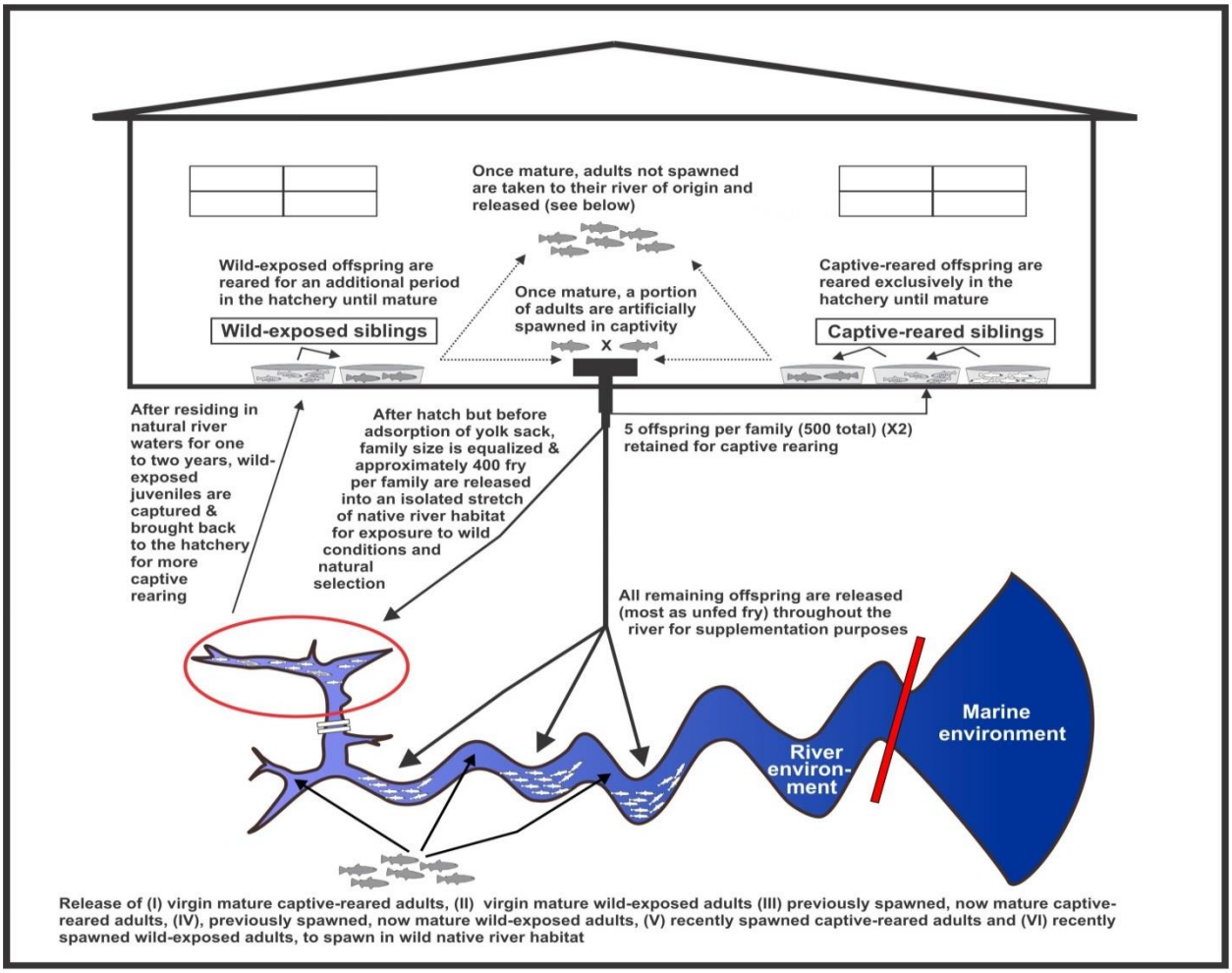


Figure 2. Schematic of the present-day Stewiacke River inner Bay of Fundy Live Gene Banking program (2006+). Atlantic Salmon are crossed according to a minimization of mean kinship breeding protocol described within. Two sets of five eggs are then taken from each family, and the first set combined to form Group A and the second set Group B. Both sets of families are reared communally exclusively in captivity through to maturity as adults. Individuals so managed are referred to as 'captive-reared' salmon. Four hundred eggs from each family are also taken and reared in isolation (by family) until post-hatch, after which all surviving fry are enumerated, variance in family size minimized by removing individuals from larger families (see text for more information), and all combined and mixed prior to release into Pembroke River, an isolated tributary of the Stewiacke River system. After one to two years of exposure to native river conditions, several hundred are caught via electrofishing and brought back to the hatchery for captive rearing through to maturity as adults. These electrofished 'wild-exposed' juveniles are then reared alongside their exclusively captive-reared siblings, during which time all or most individuals from both groups are genotyped and pedigreed. All remaining offspring are released throughout the river as unfed fry for supplementation purposes; after one to two years, most surviving offspring out-migrate to Bay of Fundy/Gulf of Maine (few are expected to return given current marine conditions). At or around spawning time, a portion of mature adults (captive-reared and wild-exposed) are released into native river habitat to potentially free-spawn in the wild, while the remaining are artificially spawned in the production of the next generation of Stewiacke inner Bay of Fundy Atlantic Salmon.

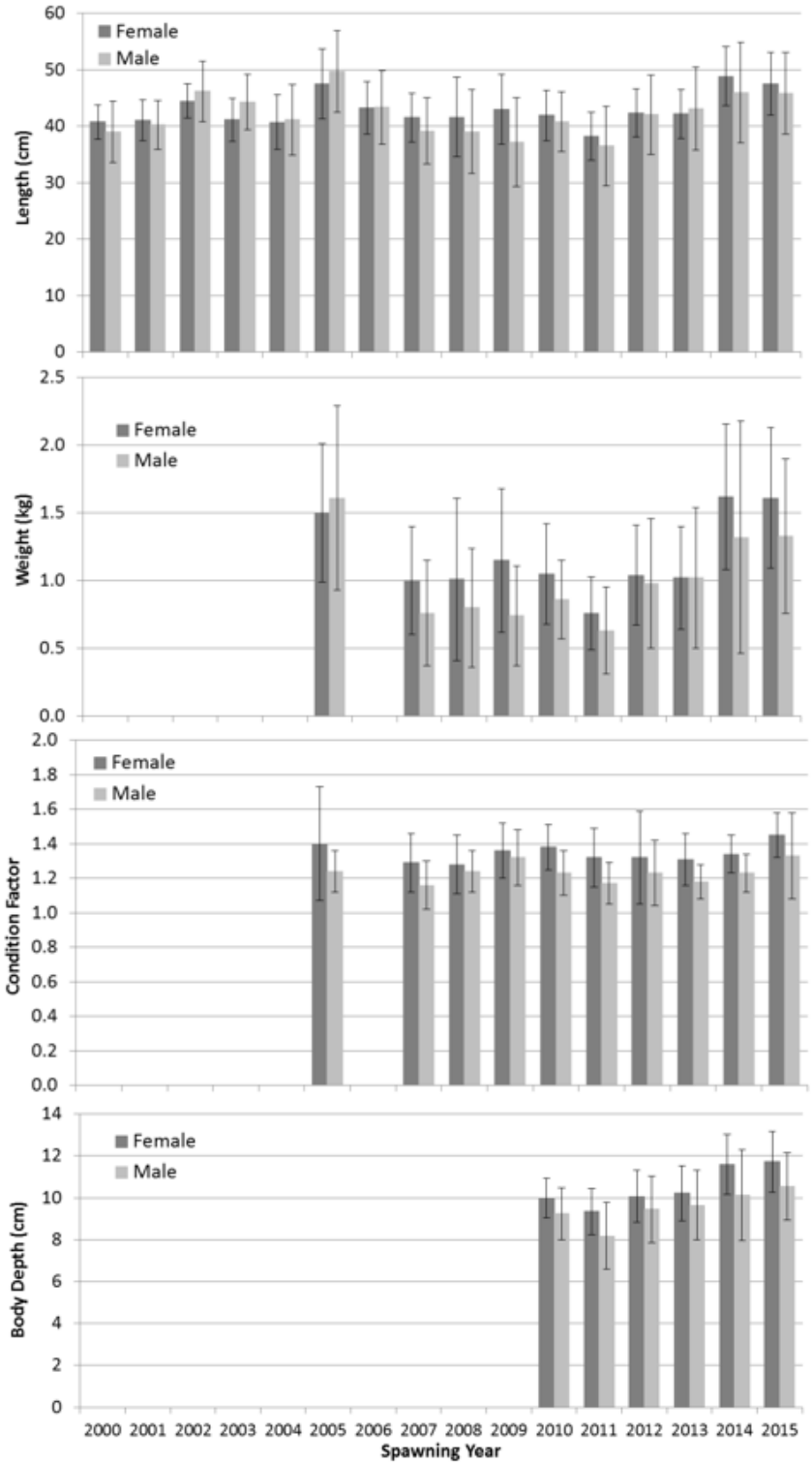


Figure 3. Average (+/- standard deviation) body characteristics at spawning for all Age 4 (2000–2013) and Age 5 (2014–2015) adult Atlantic Salmon. In 2014, the Live Gene Bank program changed to spawning salmon exclusively at Age 5; in previous years salmon were mostly spawned at Age 4. Adult age used here is based on the brood or fertilization year of individuals.

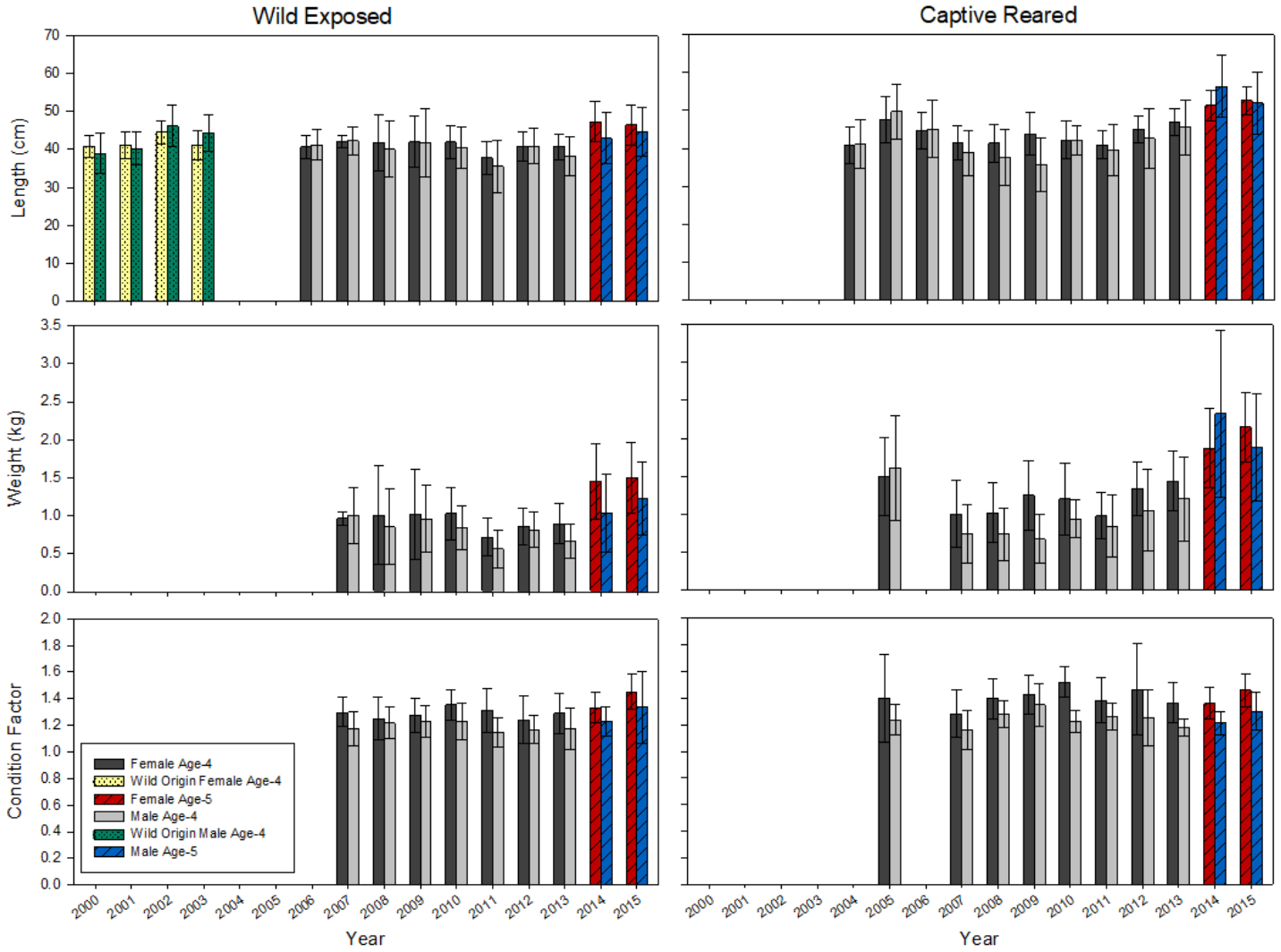


Figure 4. Average (± standard deviation) body characteristics at spawning for wild-origin, wild-exposed, and captive-reared adult Atlantic Salmon at Age 4 (2000–2013) and Age 5 (2014–2015). In 2014 the Live Gene Bank program shifted from predominately spawning Age 4 adults to one that focused on Age 5 only. Adult age used here is based on the brood or fertilization year of individuals.

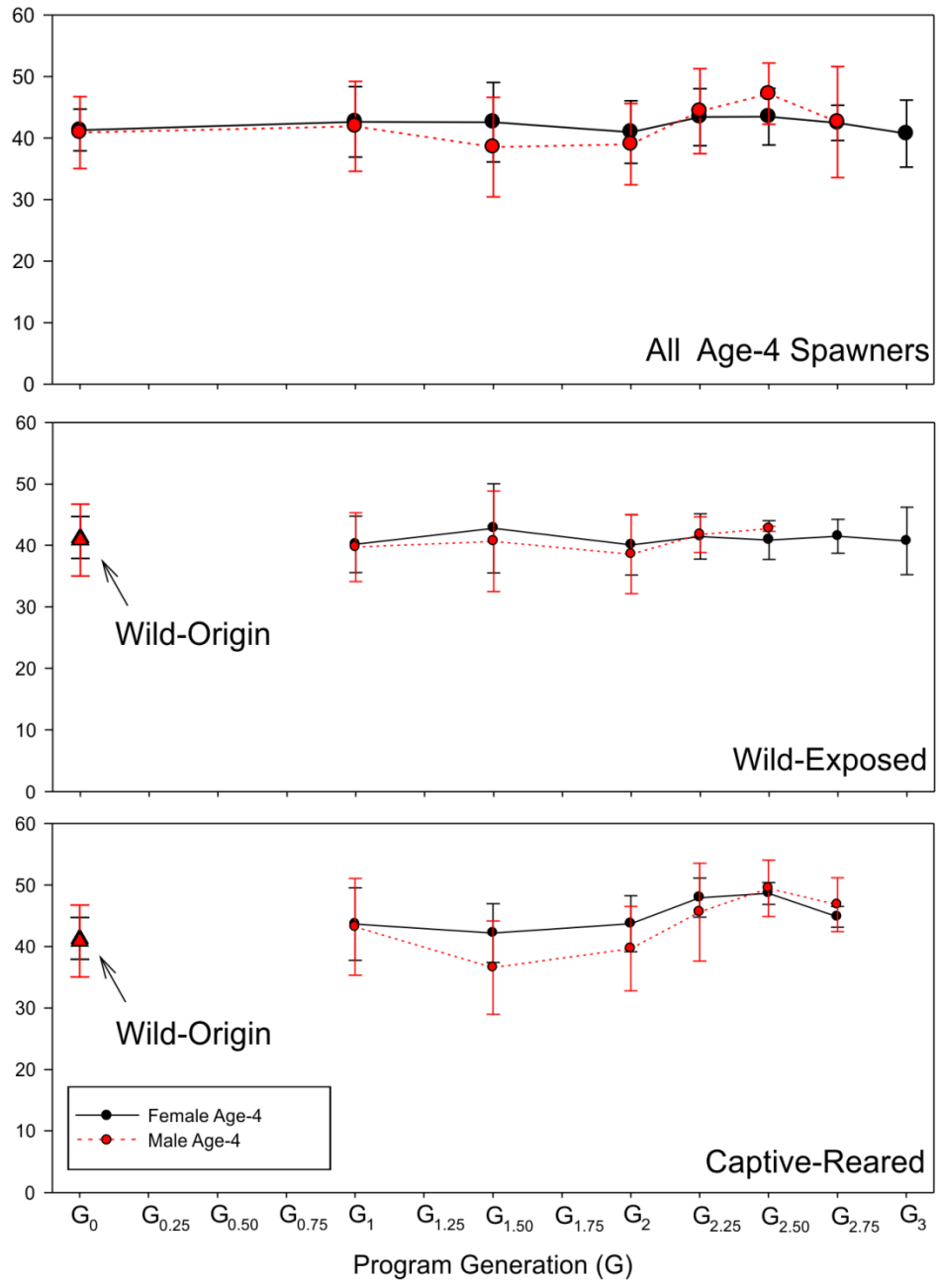


Figure 5. Average (± standard deviation) fork length at spawning for all spawners, wild-origin, wild-exposed, and captive-reared adult Atlantic Salmon at Age 4 over program generation of the Live Gene Bank program. Adult age used here is based on the brood or fertilization year of individuals.

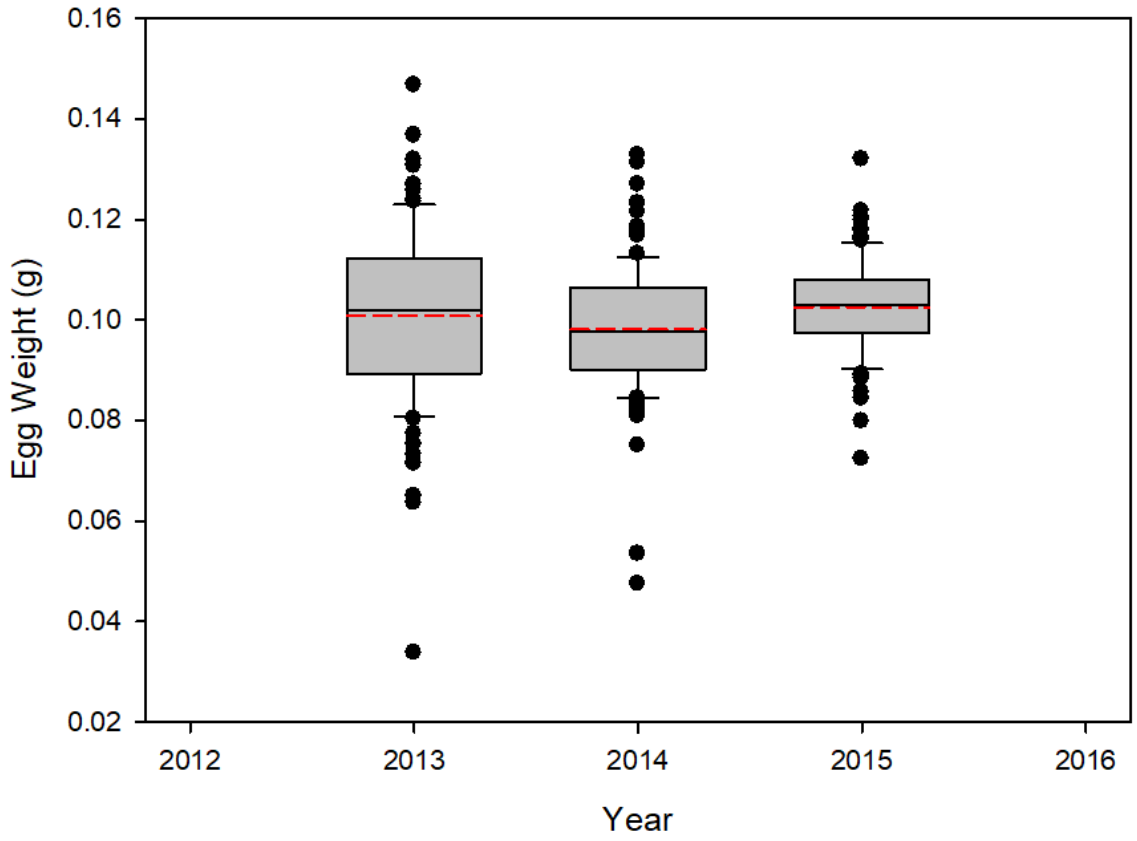


Figure 6. Average egg weights (g) for Atlantic Salmon families within each brood year of the Live Gene Bank program. The box plots represent the 10th, 25th, 75th, 90th percentiles while the solid black line represents the median and the red dashed line is the mean.

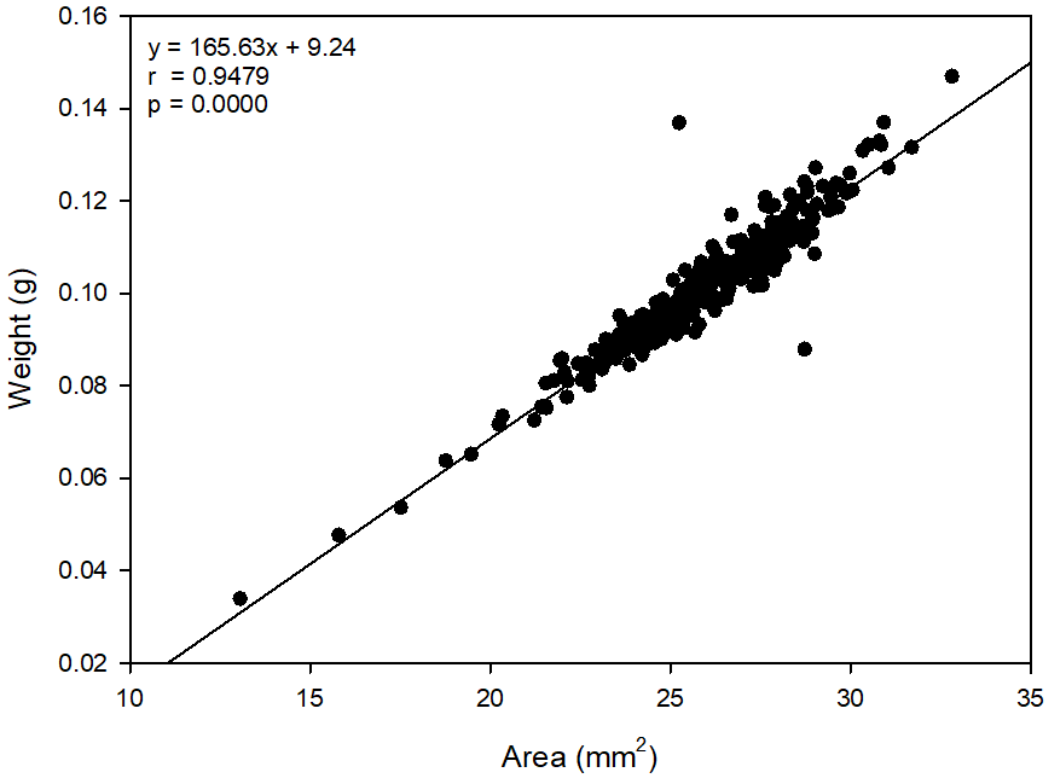


Figure 7. Family-specific correlation of egg weight (g) and egg area (mm^2) for Atlantic Salmon families in brood years 2013–2015 of the Live Gene Bank program.

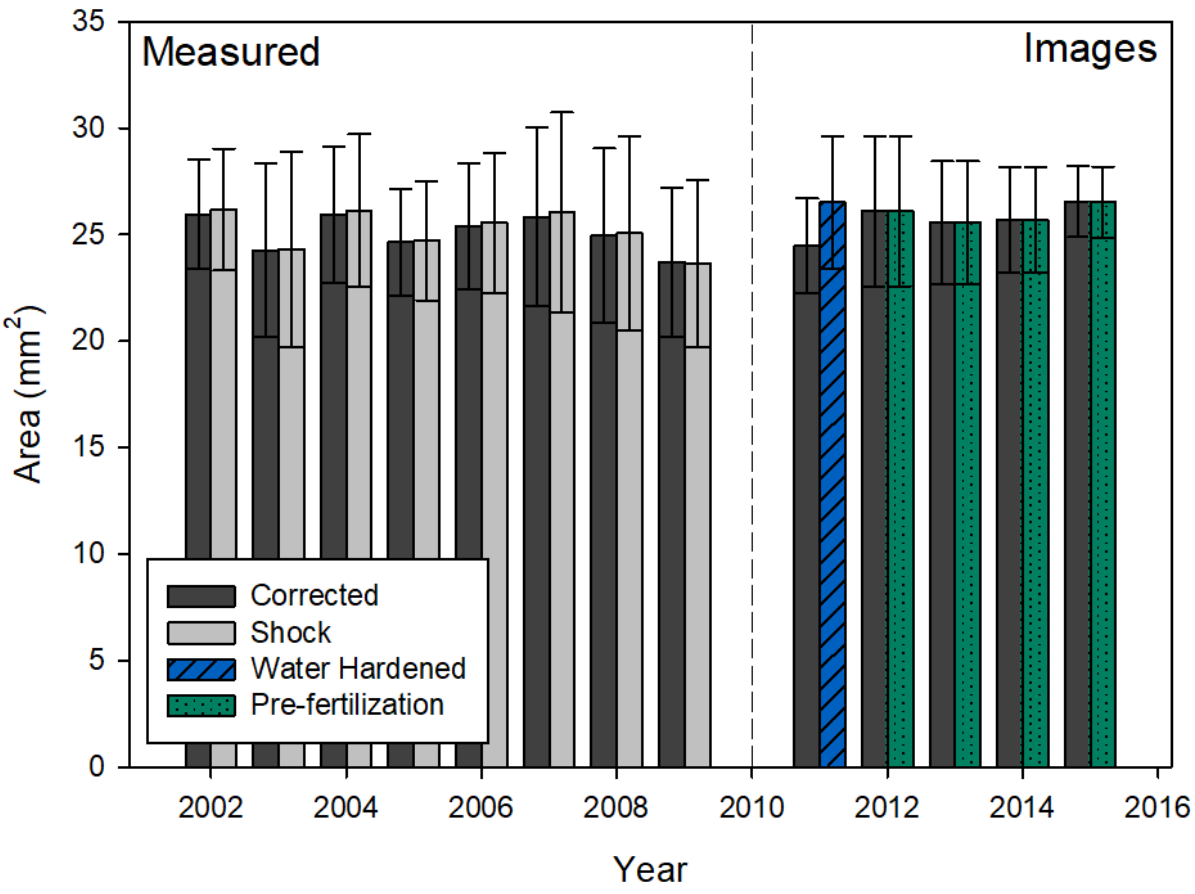


Figure 8. Average (± standard deviation) egg area (mm^2) for Atlantic Salmon eggs from prescribed mating pairs within the Live Gene Bank program. Prior to 2010, egg area was calculated from average egg diameters measured from approximately 20 selected families at the well eyed stage (i.e. shock). No egg area/diameters were recorded for 2010. After 2010 egg area was calculated from images of all families using the ImageJ program; images were taken of pre-fertilized eggs in all years with the exception of 2011 where they were photographed after water hardening. Two correction factors (see Appendix Figures A1–A3) were applied, first to standardize between the two methods used and second to standardize the egg areas to a pre-fertilization stage, indicated by the dark bars.

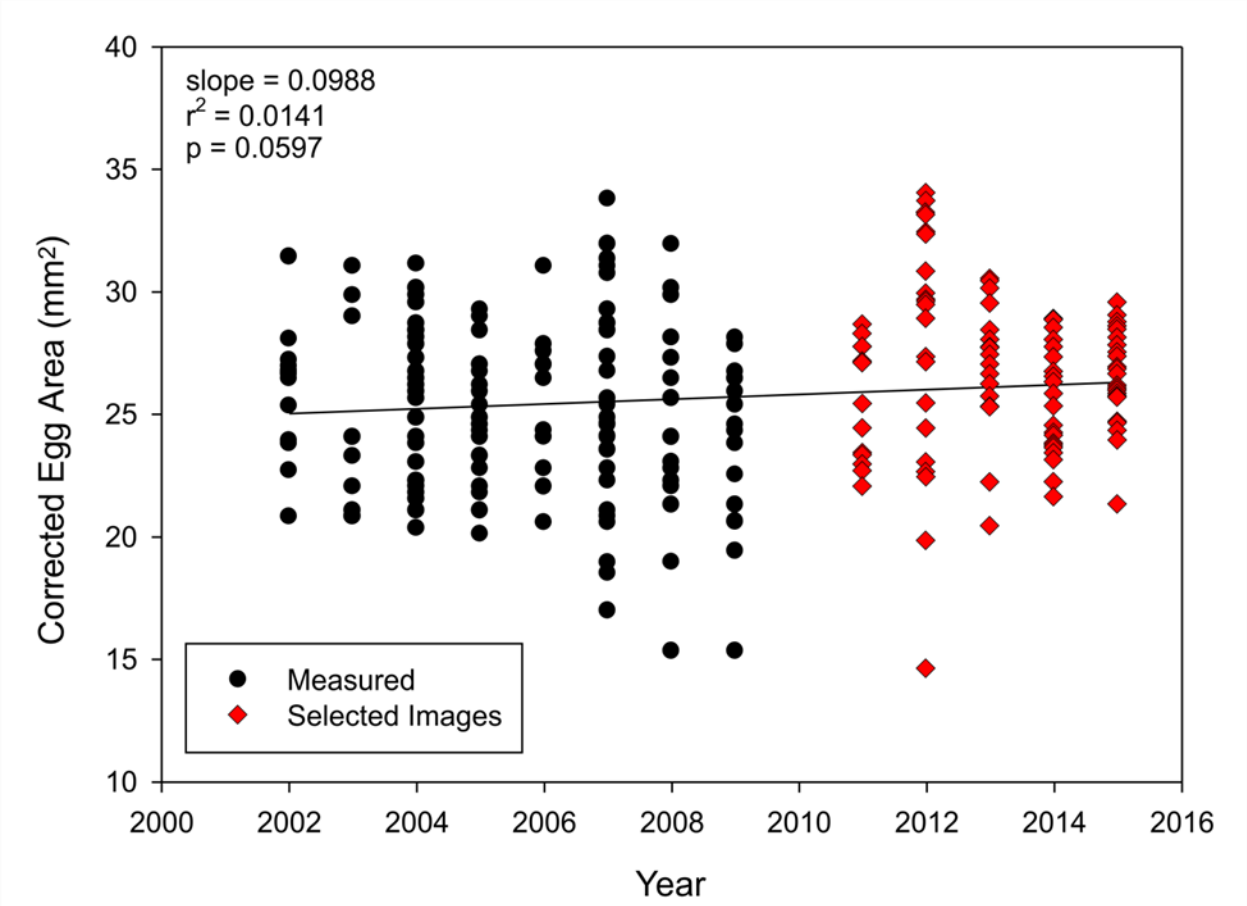


Figure 9. Regression of egg area from Atlantic Salmon families on the years of the Live Gene Bank program, corrected for the method and standardized to a pre-fertilization stage. From 2002–2009, egg area was calculated from average egg diameters measured from approximately 20 selected families at the well eyed stage (i.e. shock); after 2010, egg area was calculated from images of all families using the ImageJ program. For years where all crosses were analyzed (2011–2015), only the data for approximately 20 selected crosses are presented, using the same selection criteria as the previous years.

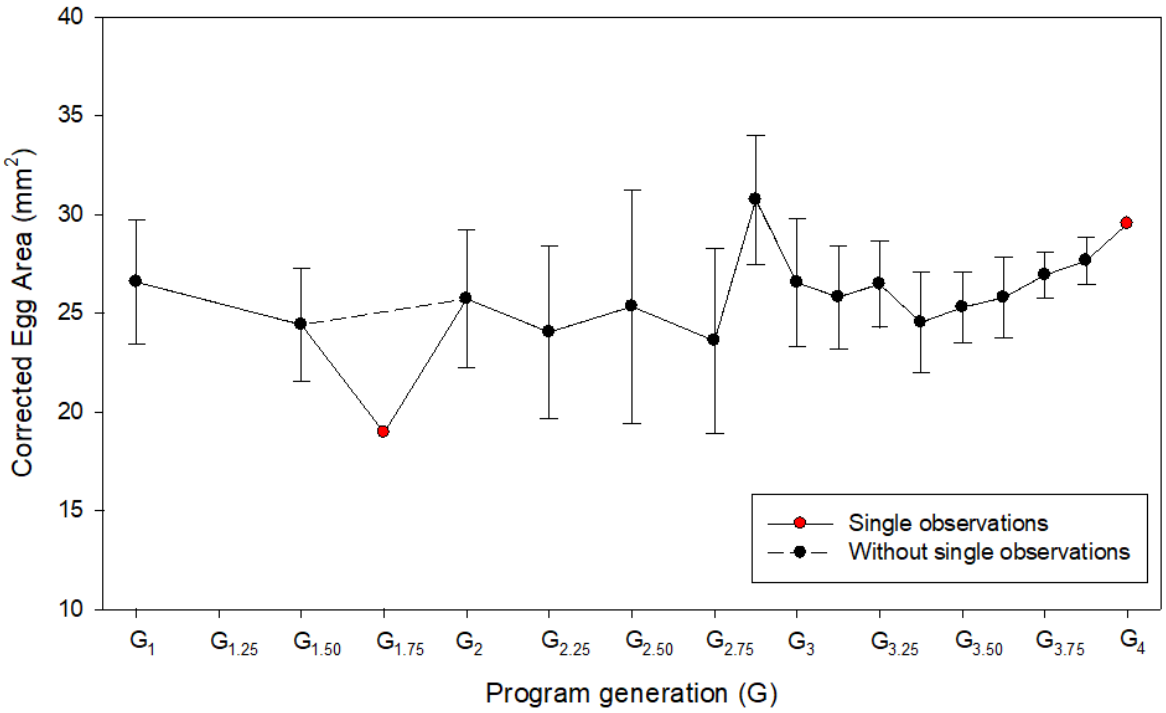


Figure 10. Average (± standard deviation) corrected egg area (mm^2) for Atlantic Salmon families throughout the generations of the Live Gene Bank program. Correction factors were applied (see Appendix) to standardize for method and stage of egg development.

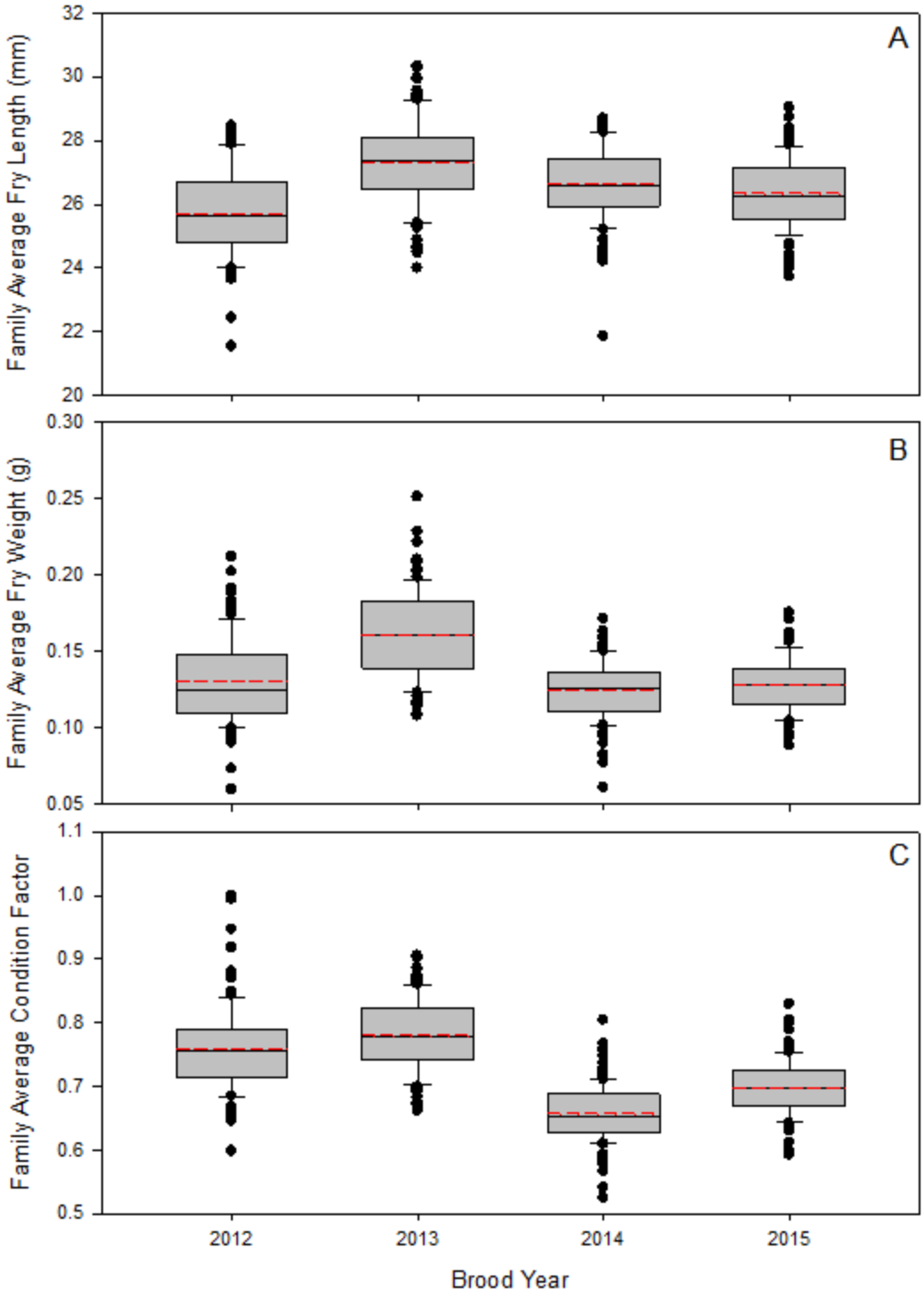


Figure 11. Family average (15–20 fry per family) body characteristics, A) length, B) weight, and C) condition factor, of Atlantic Salmon fry from families within the Live Gene Bank program. Box plots indicate median (solid black line), mean (dashed red line) and 10th, 25th, 75th, 90th percentiles.

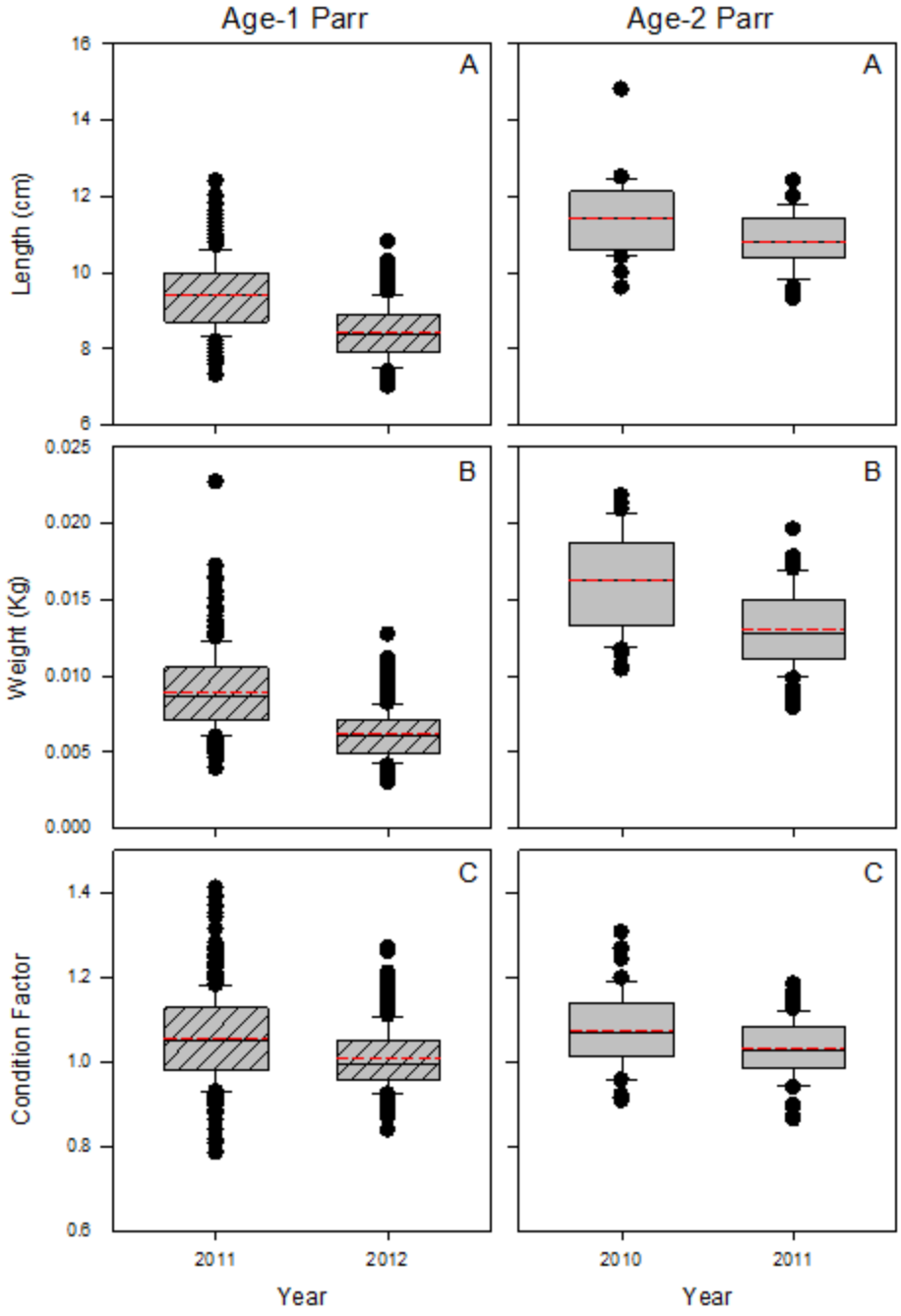


Figure 12. Family average of Live Gene Bank offspring body characteristics, A) length, B) weight, and C) condition factor, for wild-exposed Atlantic Salmon parr captured at Age 1 and Age 2 on the Pembroke River and measured within 6 days of capture. Box plots indicate median (solid black line), mean (dashed red line) and 10th, 25th, 75th, 90th percentiles.

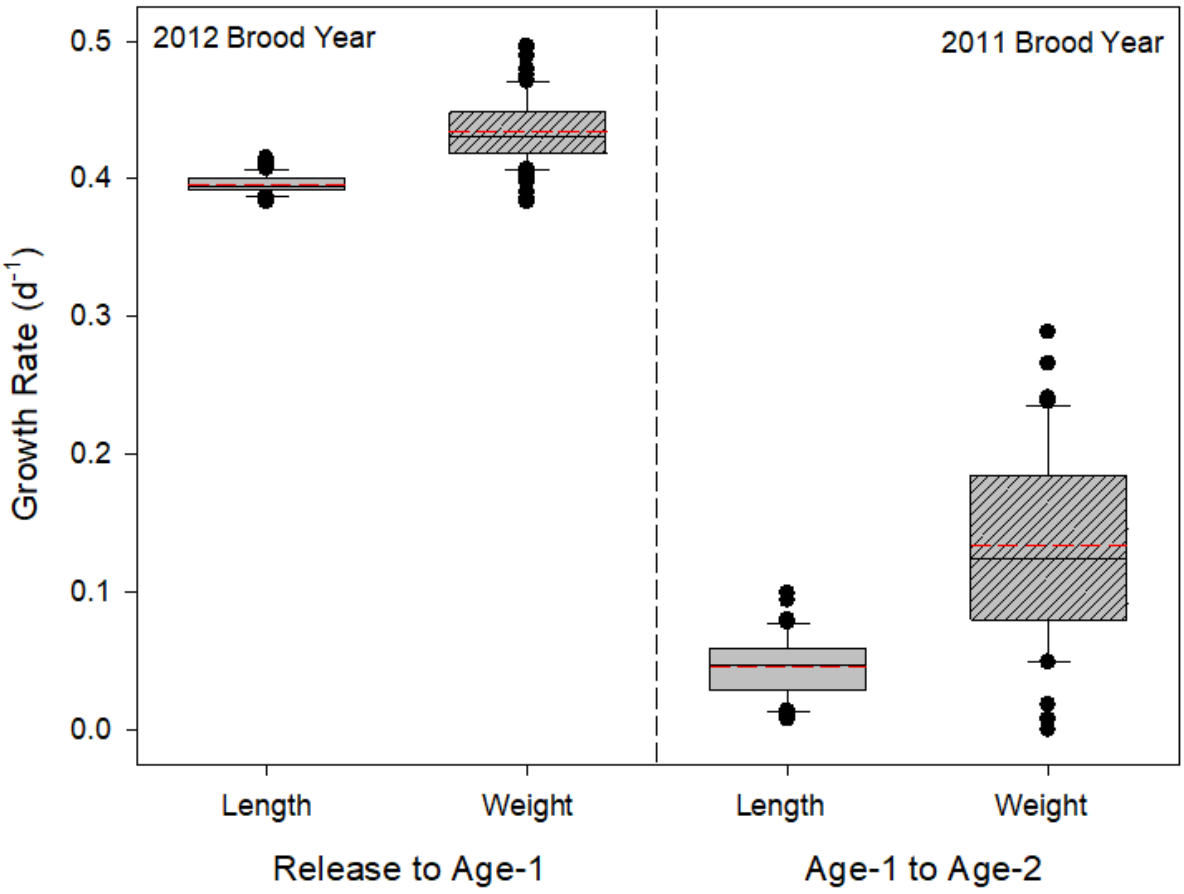


Figure 13. Growth rates (length and weight) for wild-exposed Atlantic Salmon parr captured from the Pembroke River at Age 1 and Age 2. Measuring of fry at release and parr at capture began in 2013, which corresponds to the 2012 brood year (BY) for fry and 2011 BY for Age 1 parr. Due to the time lag of various life stages (i.e. Age 1 parr captured in 2014 are 2012 BY) and available data, only one year was available for each time period. Growth rates for parr between 'Age 1 and Age 2' were determined by comparing parr captured as Age 2, in 2014, to family-specific counterparts captured as Age 1, in 2013; 'release to Age 2' was not possible due to a lack of fry measurement for the 2011 BY. Box plots indicate median (solid black line), mean (dashed red line) and 10th, 25th, 75th, 90th percentiles.

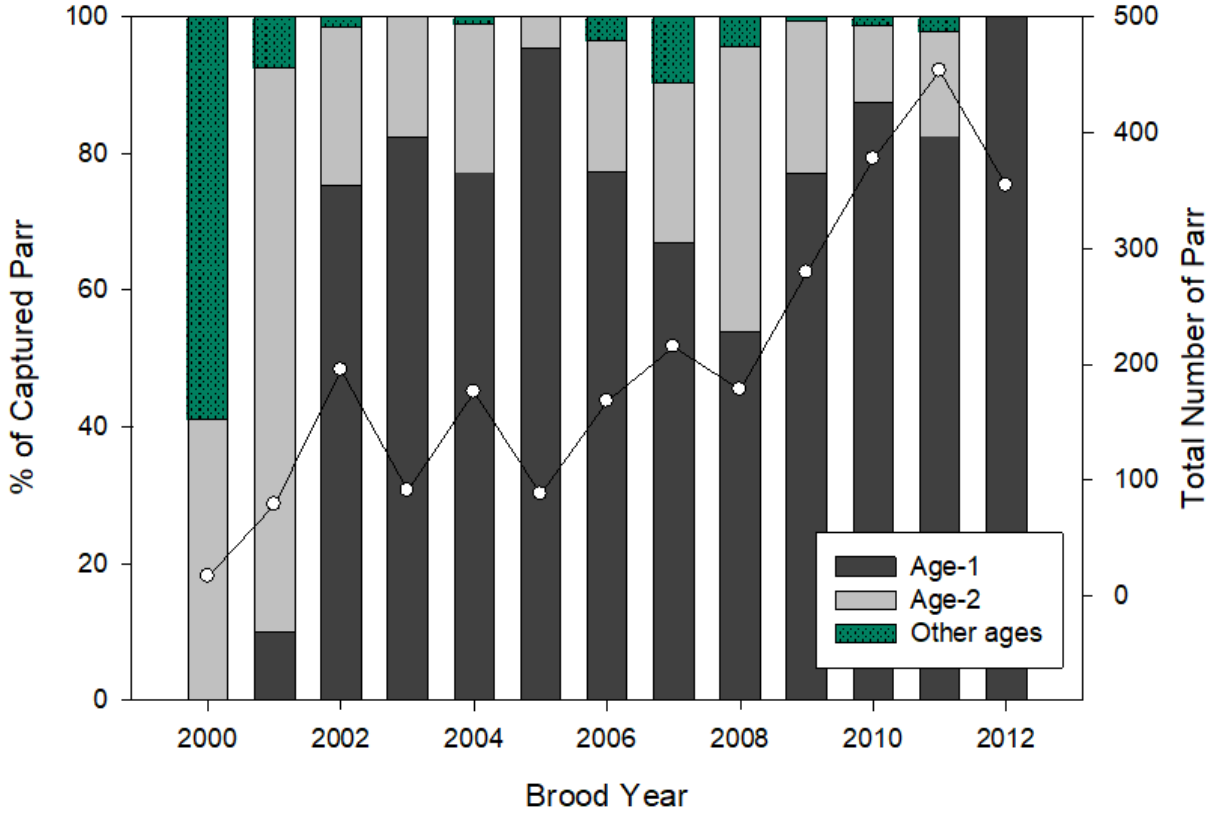


Figure 14. Percent of Age 1 and Age 2 Atlantic Salmon parr collected from the wild for each brood year of the Live Gene Bank (LGB) program, with the total number of parr (open circles) captured from each brood year superimposed. In later years, parr came from the isolated section of the Pembroke River (seeded with fry from the LGB program); however prior to 2006, parr were collected from various locations throughout the Stewiacke River system. Due to the time lag in development, only Age 1 wild-exposed parr numbers are available for the 2012 brood year.

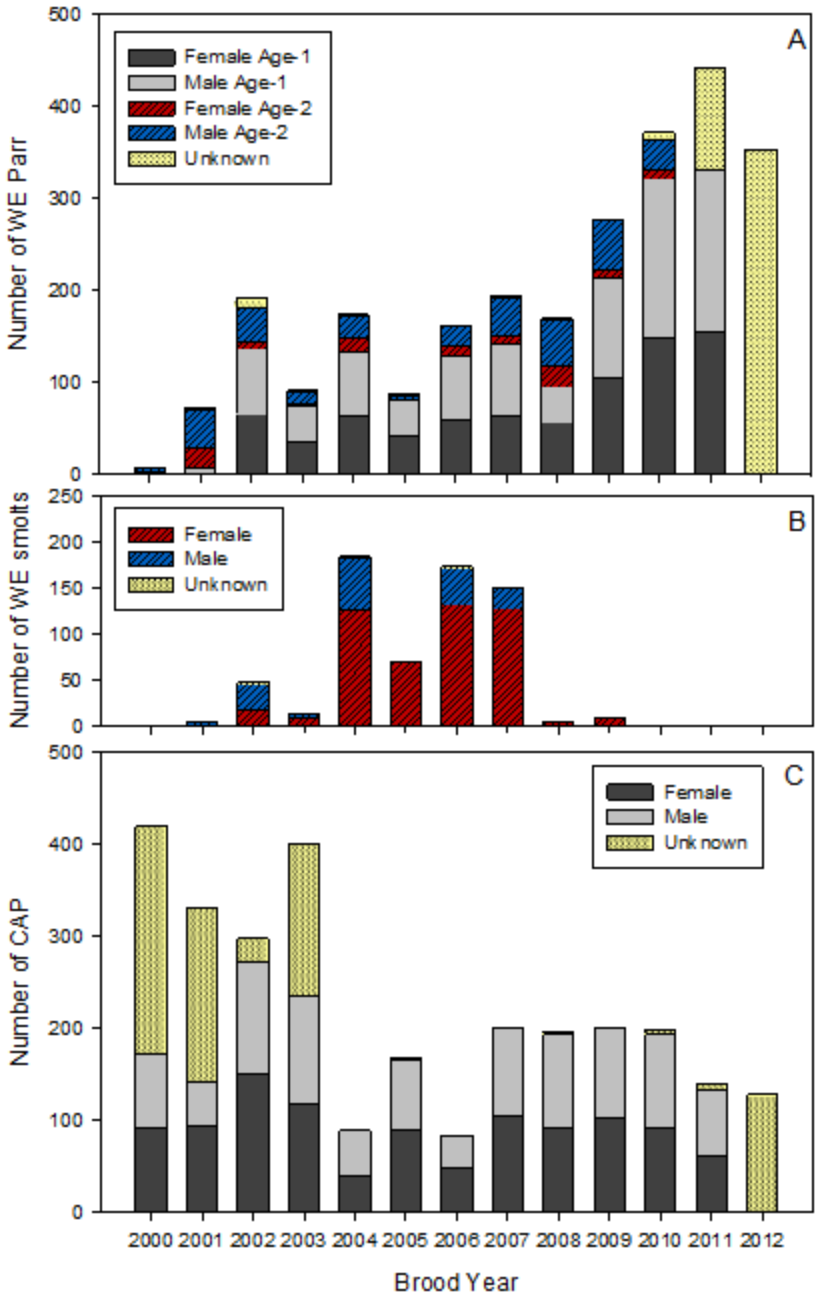


Figure 15. Gender breakdown of A) Age 1 and Age 2 wild-exposed (WE) Atlantic Salmon parr collected from the wild, B) WE smolts (predominately Age 2), and C) captive-reared (CAP) individuals for each brood year of the Live gene Bank program. In later years, WE individuals came from the isolated section of the Pembroke River (seeded with WE fry from the program); however prior to 2008 and 2009, WE parr and smolts, respectively, were collected from various locations throughout the Stewiacke River system.

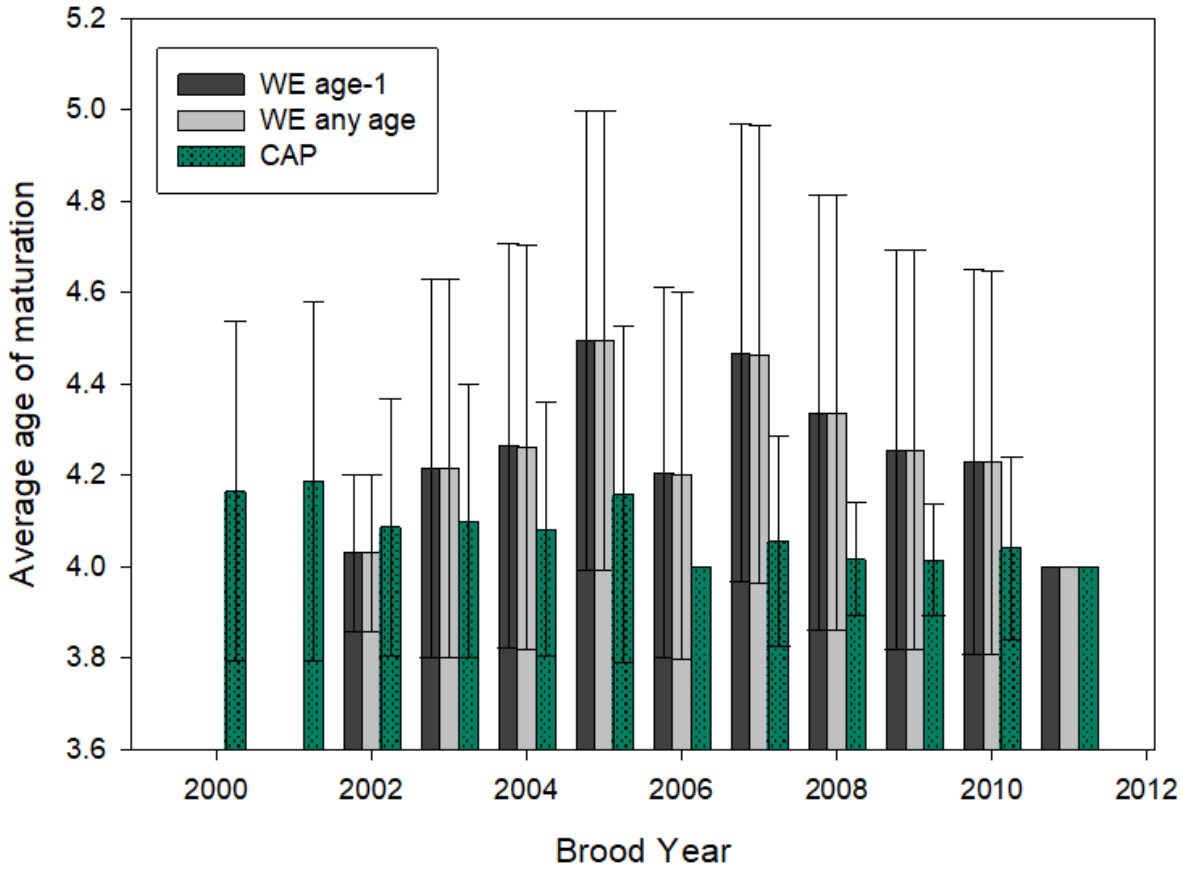


Figure 16. The average (± standard deviation) age of maturation of captive-reared (CAP) Atlantic Salmon and wild-exposed (WE) parr collected at various ages for the Live Gene Bank program. Due to the time lag in development, offspring from the 2011 brood year would only be Age 4 in 2015 (i.e. the last year of analysis); therefore only those fish are included, while others are yet to mature. Those individuals that matured as precocious parr were not included in the average age of maturation. Adult age is based on the brood or fertilization year. Age of maturation is relative to the brood or fertilization year.

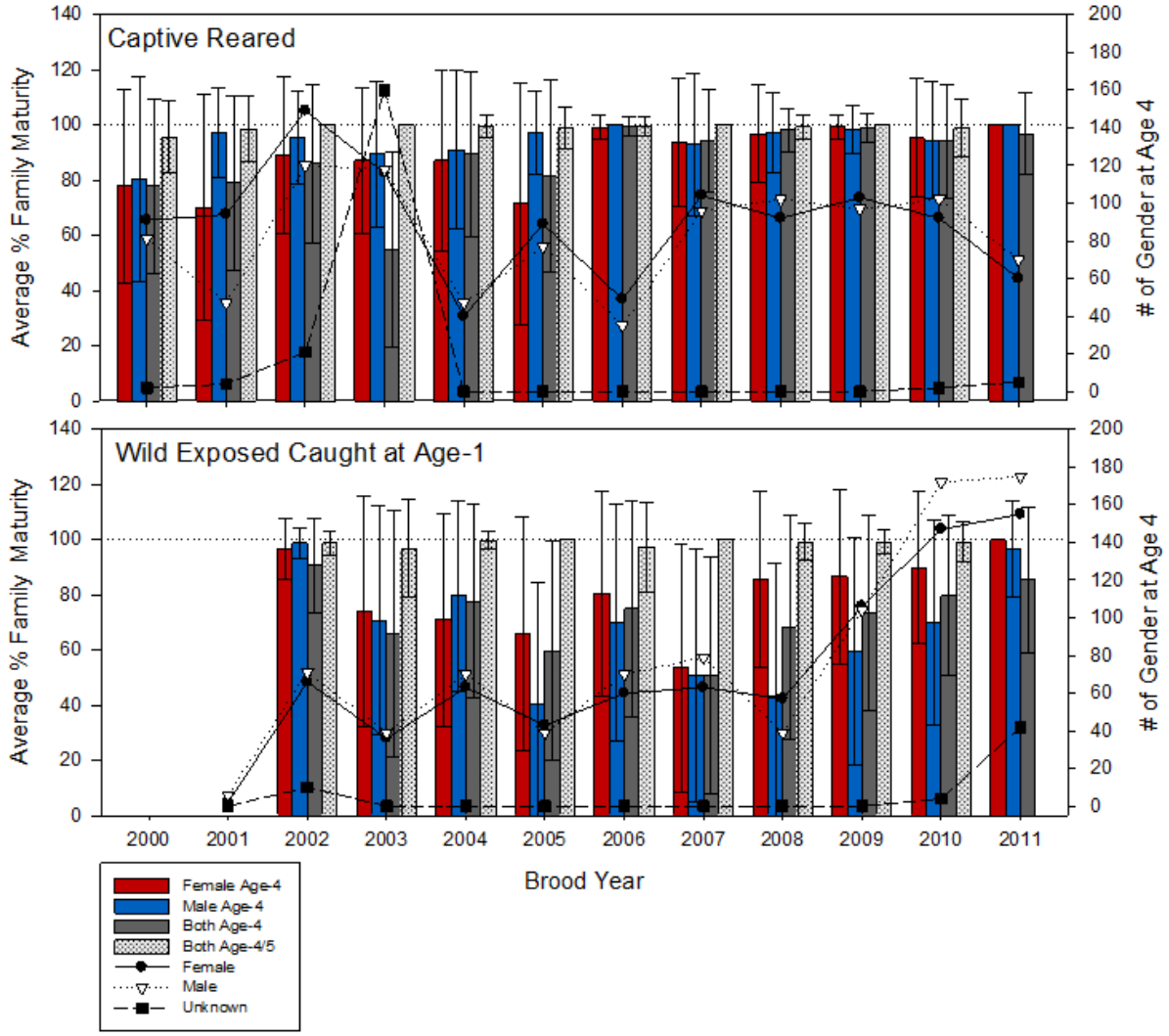


Figure 17. The yearly average (± standard deviation) of the percent of Live Gene Bank captive-reared (CAP) and wild-exposed (WE) Atlantic Salmon parr collected at Age 1 that matured at Age 4, and cumulatively for Age 4 and Age 5, per family. Male and female percentages only include individuals whose gender was eventually known, however gender-combined columns (i.e. Both) also take into consideration individuals whose gender was unknown, which can cause them to be lower. The number of male, female, and unknown individuals for each brood year is superimposed. Due to the time lag in development, only fish that matured in their fourth year are available for the 2011 brood year, and a higher number of unknown fish are recorded. The horizontal dotted line indicates the 100% average family maturity level. Adult age used here is based on the brood or fertilization year of individuals.

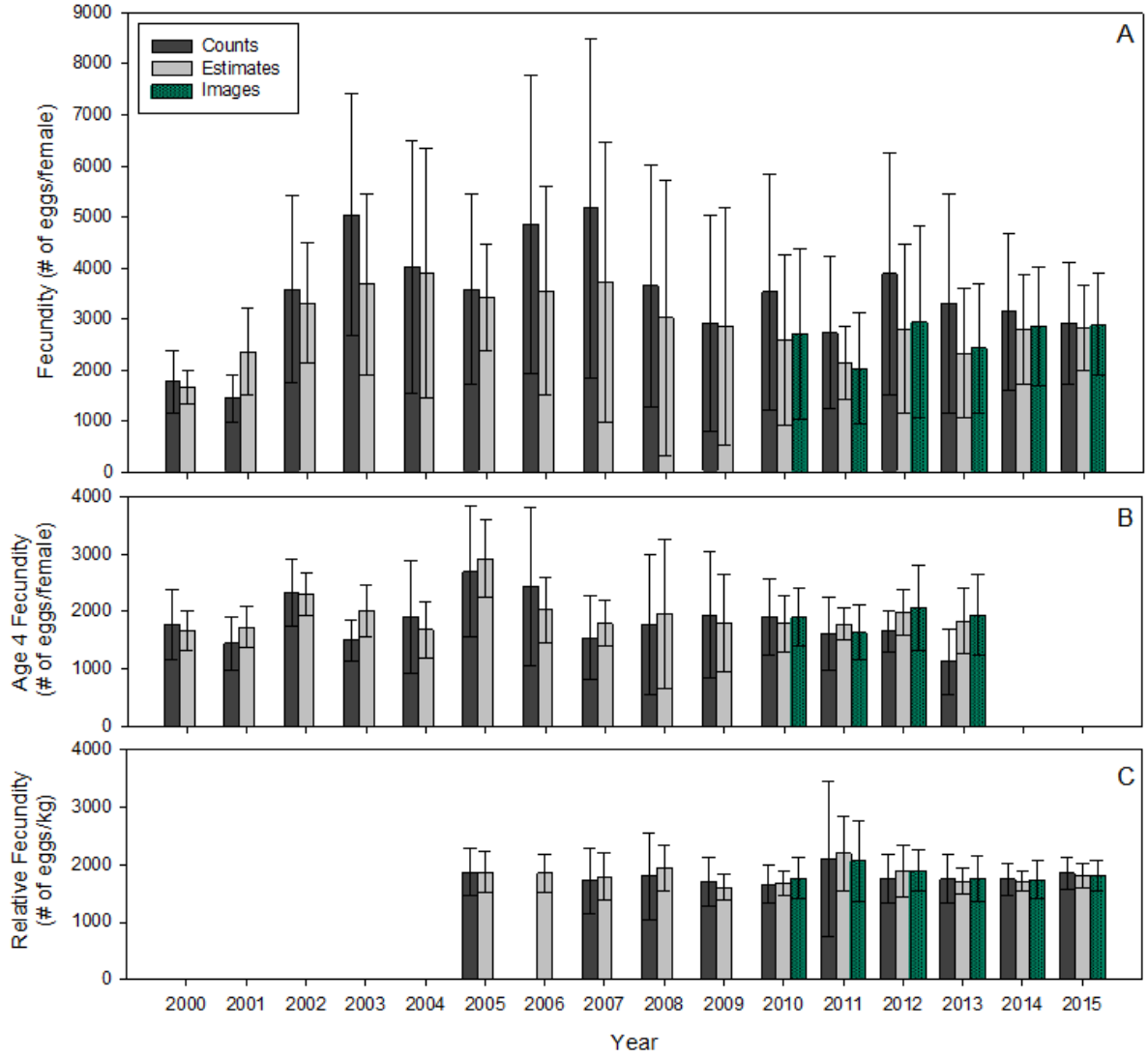


Figure 18. Average fecundity (± 1 standard deviation) of Live Gene Bank (LGB) program Atlantic Salmon females determined from three different methods (counts, estimates, and images) for A) all females and B) Age 4 females. Relative fecundity, the number of eggs per unit weight of the female, is depicted for all three methods in Panel C. Each year, from 2000–2009, a selection of families (approximately 20 crosses) were chosen and hand counted (i.e. counts) in order to determine an equation to estimate the fecundity for all crosses. After 2009, images of each cross were taken and processed using ImageJ to determine the fecundity for each family. Using the same selection criteria as previous years, approximately 20 crosses were chosen for each year from 2010–2015 to represent the "counts" and create an estimated equation to determine estimates for comparison across the entire LGB program. Adult age used here is based on the brood or fertilization year of individuals.

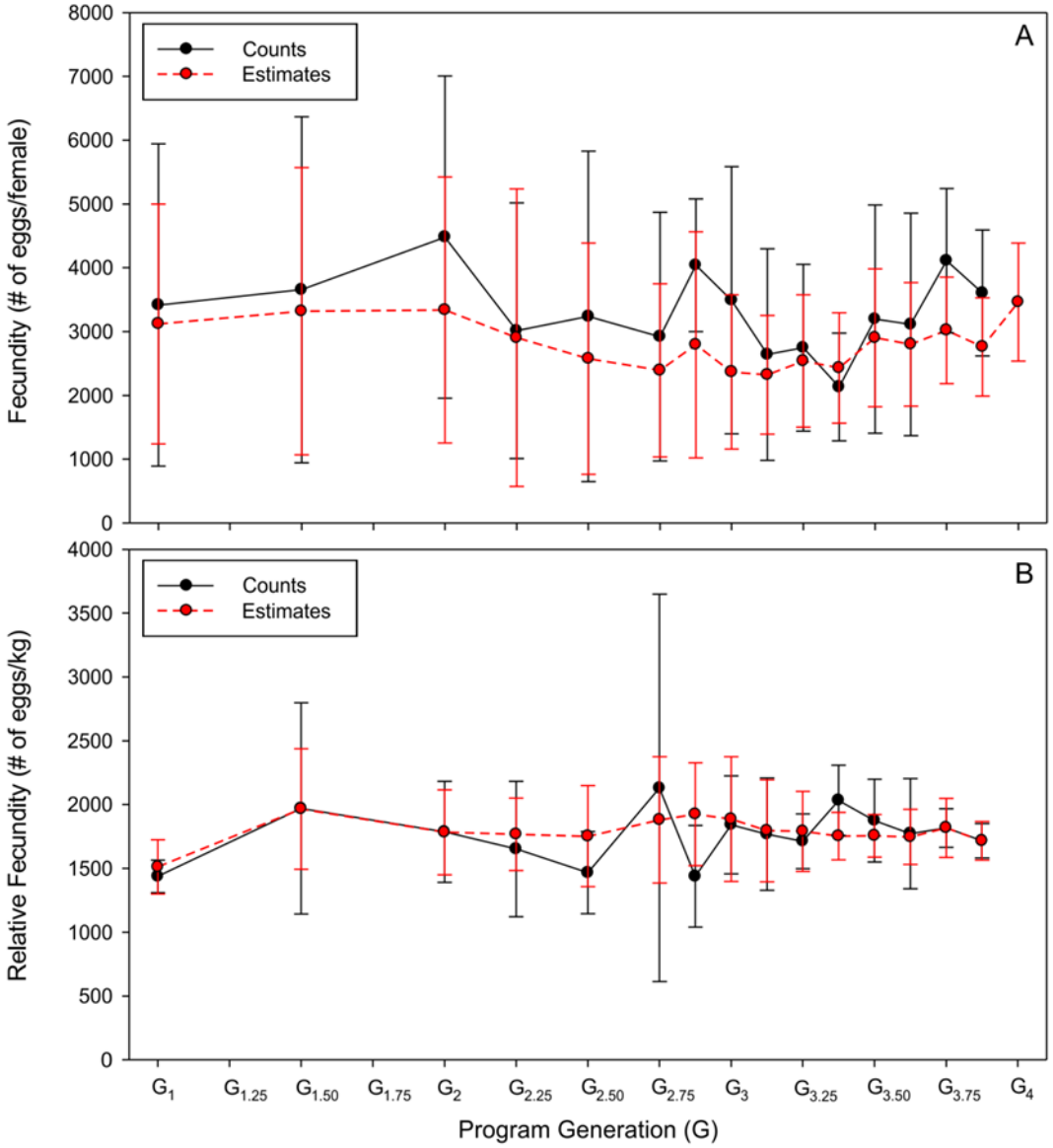


Figure 19. Average fecundity (panel A) and relative fecundity (Panel B), determined from counts and estimates for Atlantic Salmon females, throughout the generations of the Live Gene Bank program. Each year, from 2000–2009, a selection of families (approximately 20 crosses) were chosen and hand counted (i.e. counts) in order to determine an equation to estimate the fecundity for all crosses. After 2009, images of each cross were taken and processed using ImageJ to determine the fecundity for each family. Using the same selection criteria as previous years, approximately 20 crosses were chosen for each year from 2010–2015 to represent the "counts" and create an estimated equation to determine estimates for comparison across the entire LGB program. Single observations were removed and error bars represent one standard deviation.

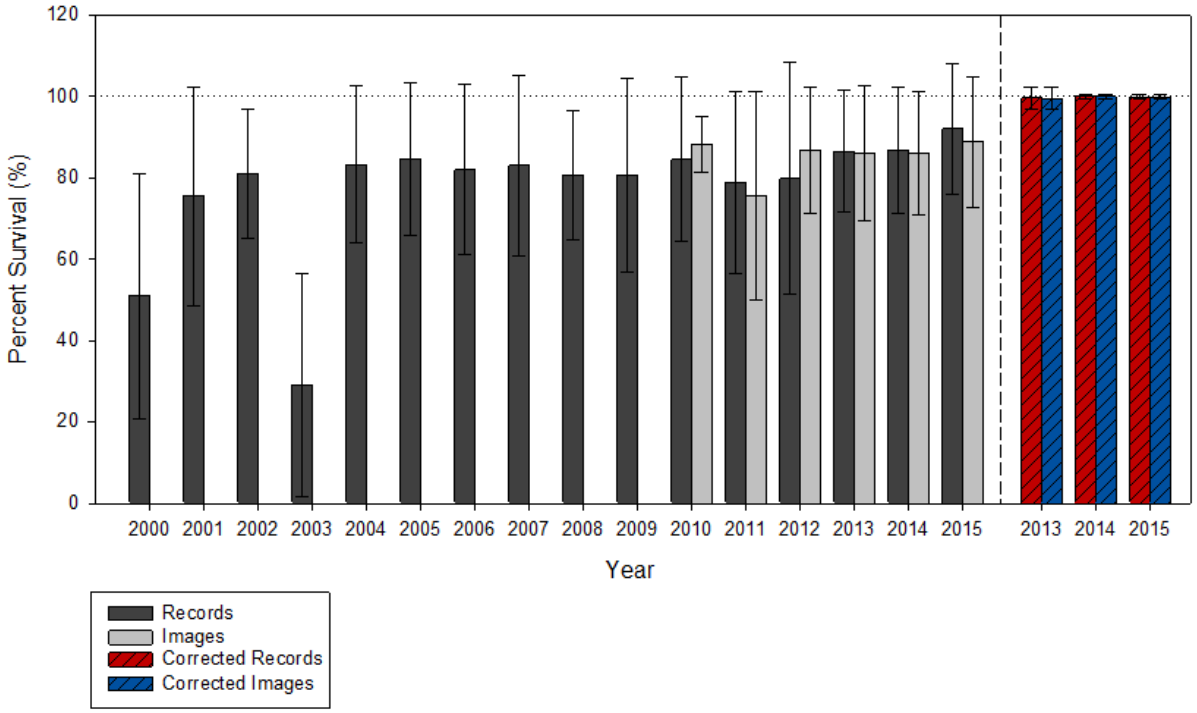


Figure 20. Average family survival (%) of Atlantic Salmon eggs from the Live Gene Bank program from fertilization (November) to the well eyed stage, also referred to as shock (approximately February). Results were determined from mortality records for the time period and recently through images taken at spawning and after shock. The proportion of "dead" eggs that were actually unfertilized were determined using a subsample (20 eggs) taken at shock and cleared with Stockard's Solution to record development. Mortality records were then corrected to compensate for unfertilized eggs that were initially identified as dead eggs. Dotted line indicates 100% survival and error bars represent one standard deviation. The vertical dashed line indicates the beginning of corrected values.

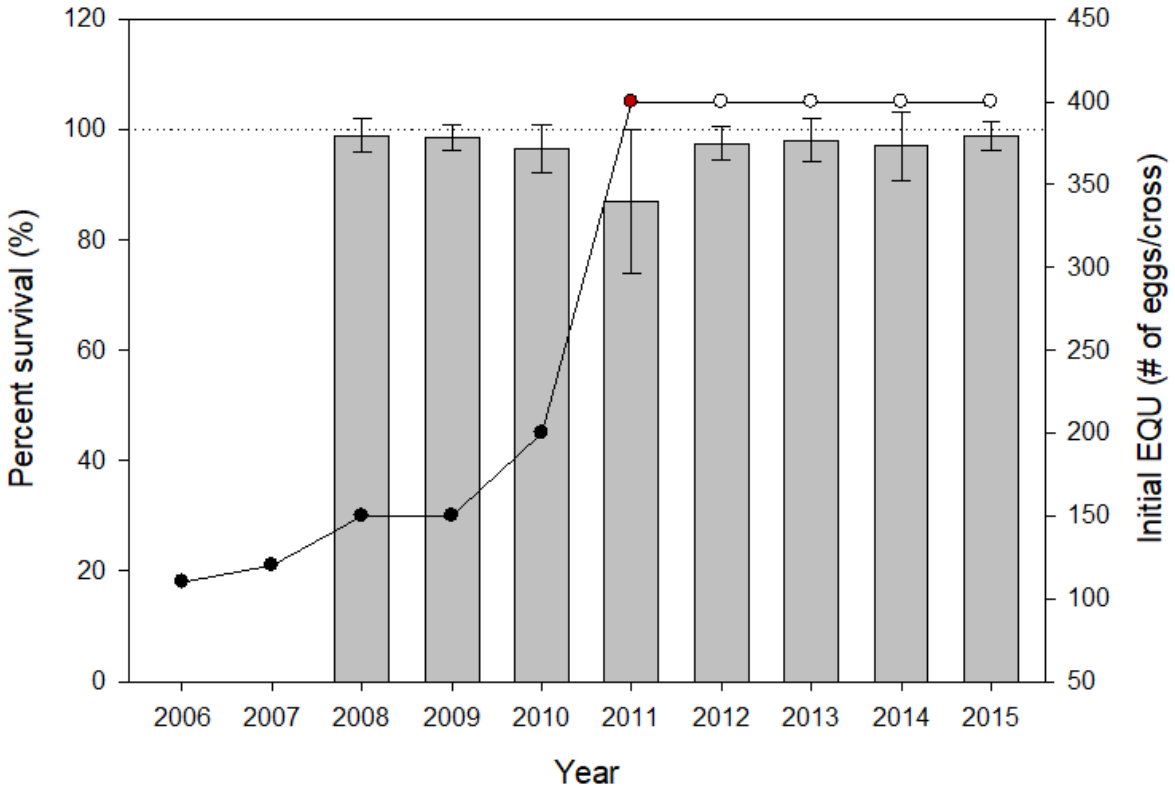


Figure 21. Average family survival (%) of Atlantic Salmon eggs and fry from the Live Gene bank program from the well eyed stage (February), also referred to as shock, to pre-release (approximately May). Mortality records for this time period were determined from the Equalized Family Groups (EQUs), where the initial number of eggs for each family was collected at shock (indicated by the circles) and reared separately through hatch. Just prior to release in the spring, the remaining number of unfed fry for each family was determined through imaging. The EQU groups were initially reared in one cup placed randomly in a Heath Unit (solid black circles). In 2011 the number was too large for one cup so separate containers were placed in a trough (solid red circle). Returning to the cup method for later years, two cups were used (200 eggs/cup) and placed randomly in Heath units (open circles). Dotted line indicates 100% survival and error bars represent one standard deviation.

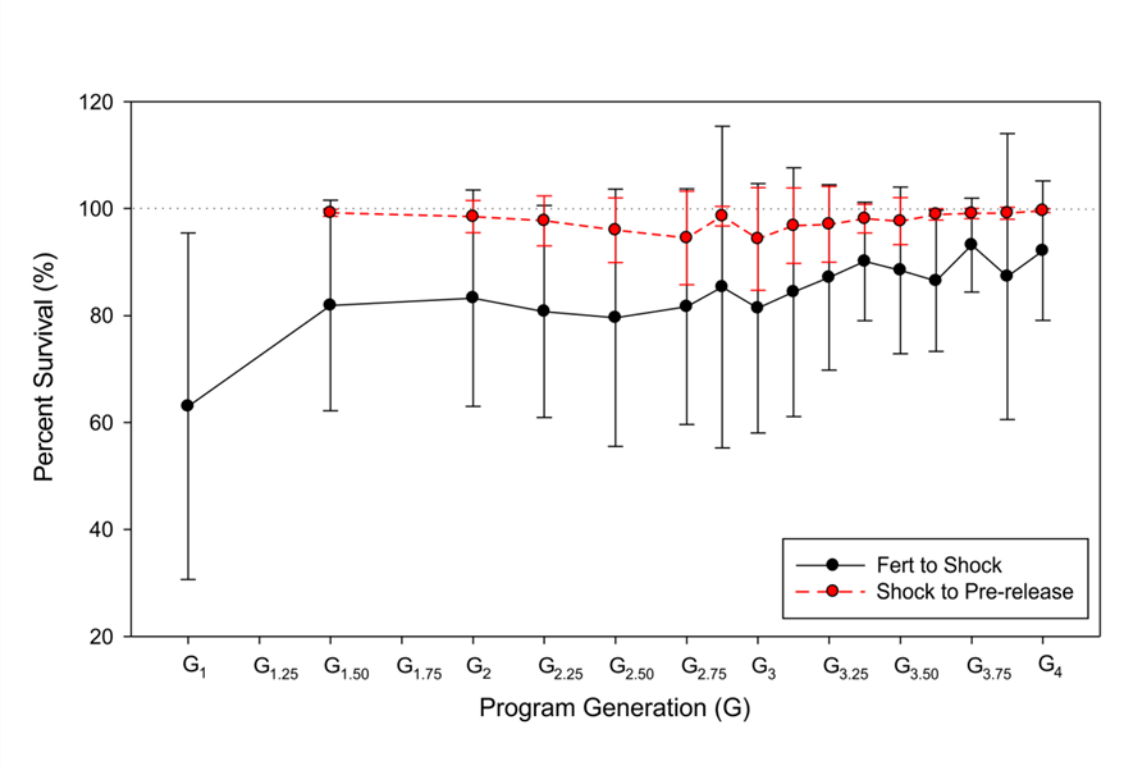


Figure 22. Average family survival (%) of Atlantic Salmon egg and fry for two time periods, 'fertilization to well eyed stage (i.e. shock)' and 'shock to pre-release', depicted over the Live Gene Bank program generations. Dotted line indicates 100% survival and error bars represent one standard deviation.

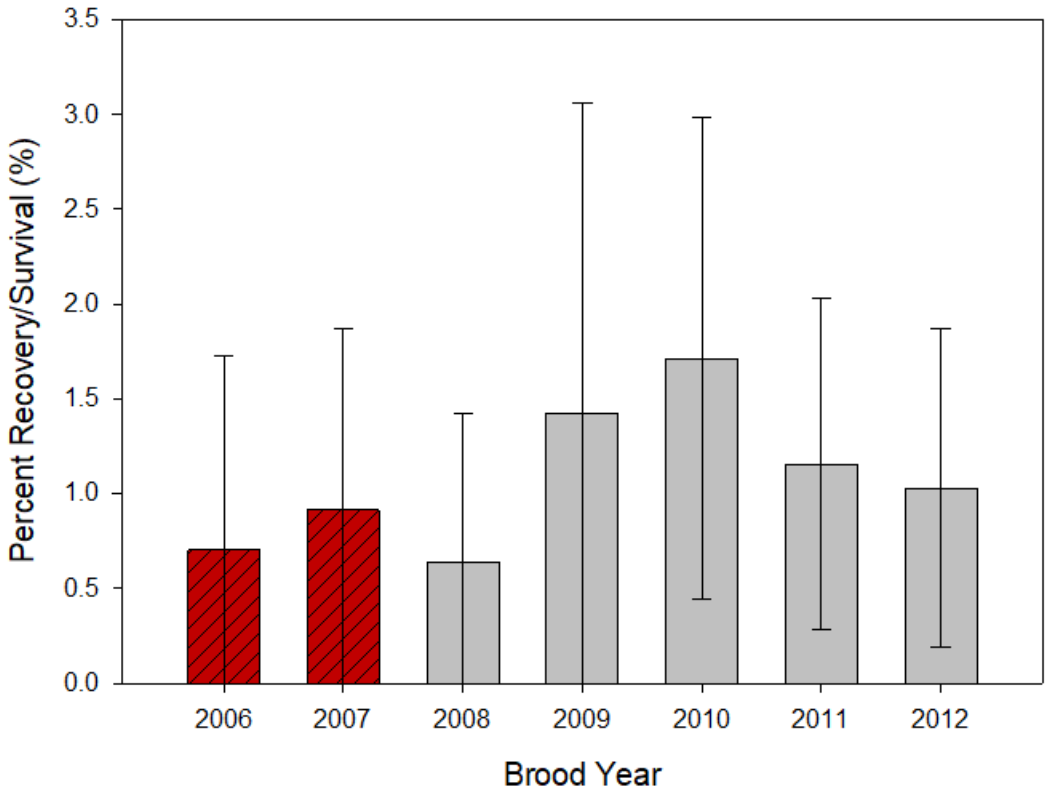


Figure 23. Average family survival (± standard deviation) of Atlantic Salmon fry from the Live Gene Bank program from release to the wild to recapture as wild-exposed Age 1 parr, 2006–2012 (red and grey bars). Just prior to release in the spring, the remaining number of unfed fry for each family was determined through imaging and when necessary equalized a second time before being released into an isolated stretch of the Pembroke River. When the exact numbers released for each family were not known (2006–2008) the initial equalized (EQU) basket number was corrected by the average survival from 'shock to pre-release' (red hatched bars). The number recaptured was used as a proxy for survival in the wild.

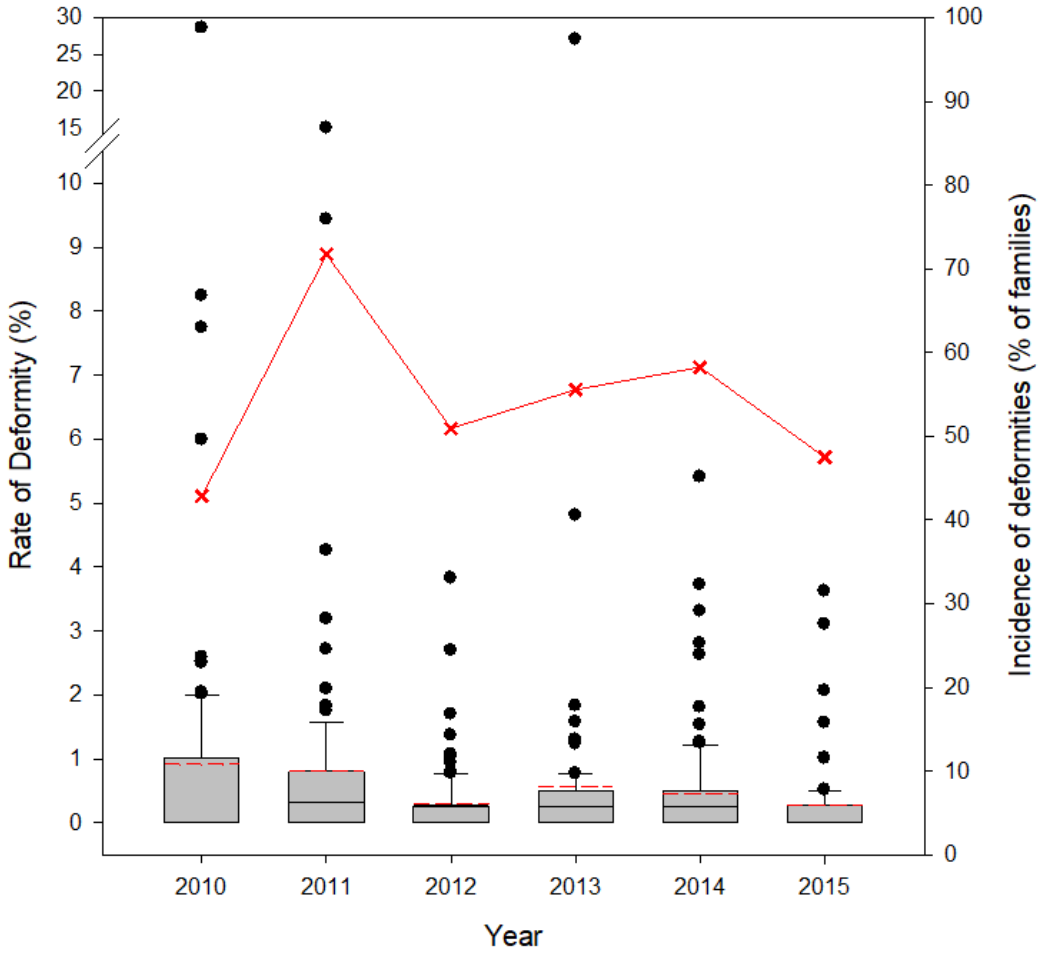


Figure 24. Rate of Live Gene Bank Atlantic Salmon offspring deformity within each family (i.e. conjoined, skeletal deformities, blind, or underdeveloped) taken from images of equalized groups prior to release. The incidence of family deformity is superimposed (connected red crosses). Box plots indicate median (solid black line), mean (dashed red line) and 10th, 25th, 75th, 90th percentiles.

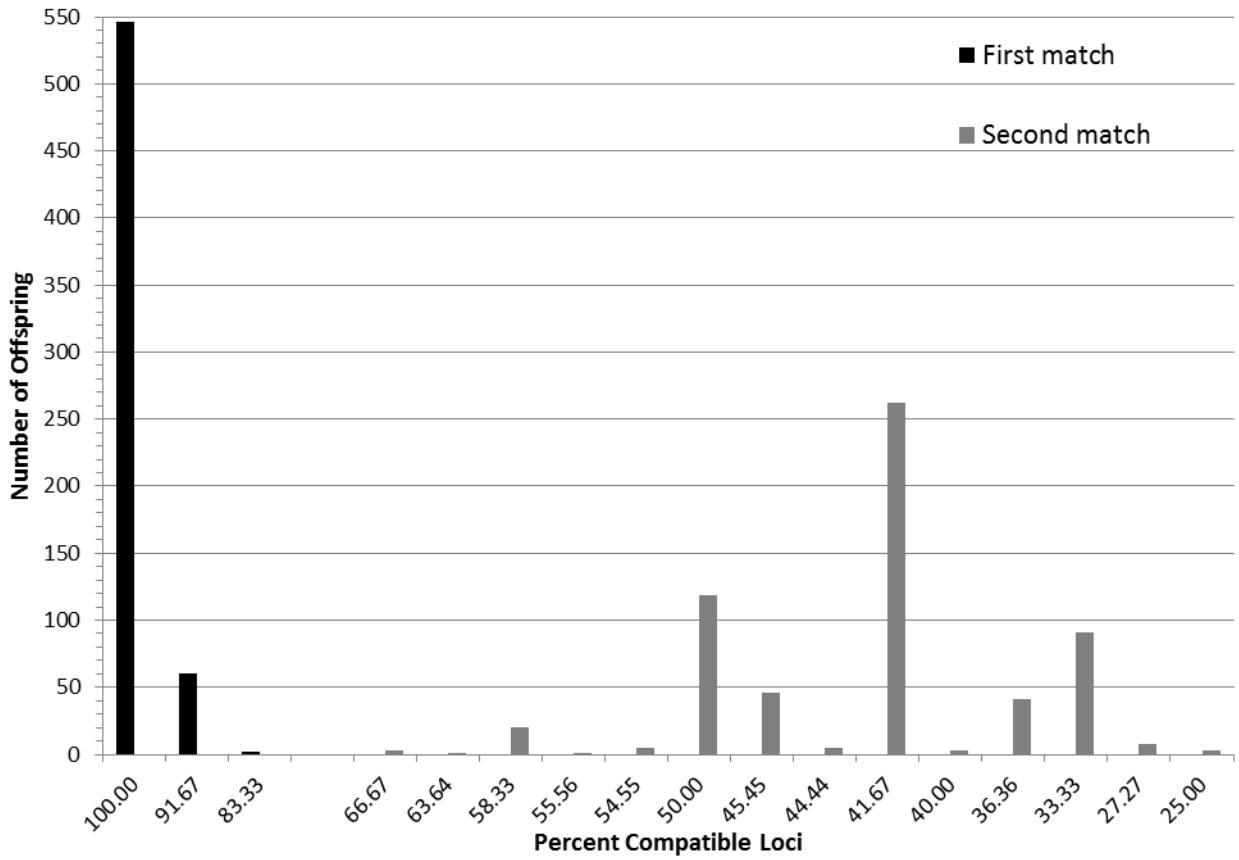


Figure 25. Exclusion-based parentage assignment results for 608 wild-exposed and captive-reared offspring produced in 2010, tested against all biologically possible sets of known LGB crosses. Candidate offspring and the best or first matching candidate parental pair tested were almost always (606 of 608) compatible at 100 or 91.67% of the 10–12 loci common to all three individuals (black bars). Candidate offspring were compatible with the second best matching parental pair at a much reduced percentage of loci assayed, generally 50% or less (grey bars).

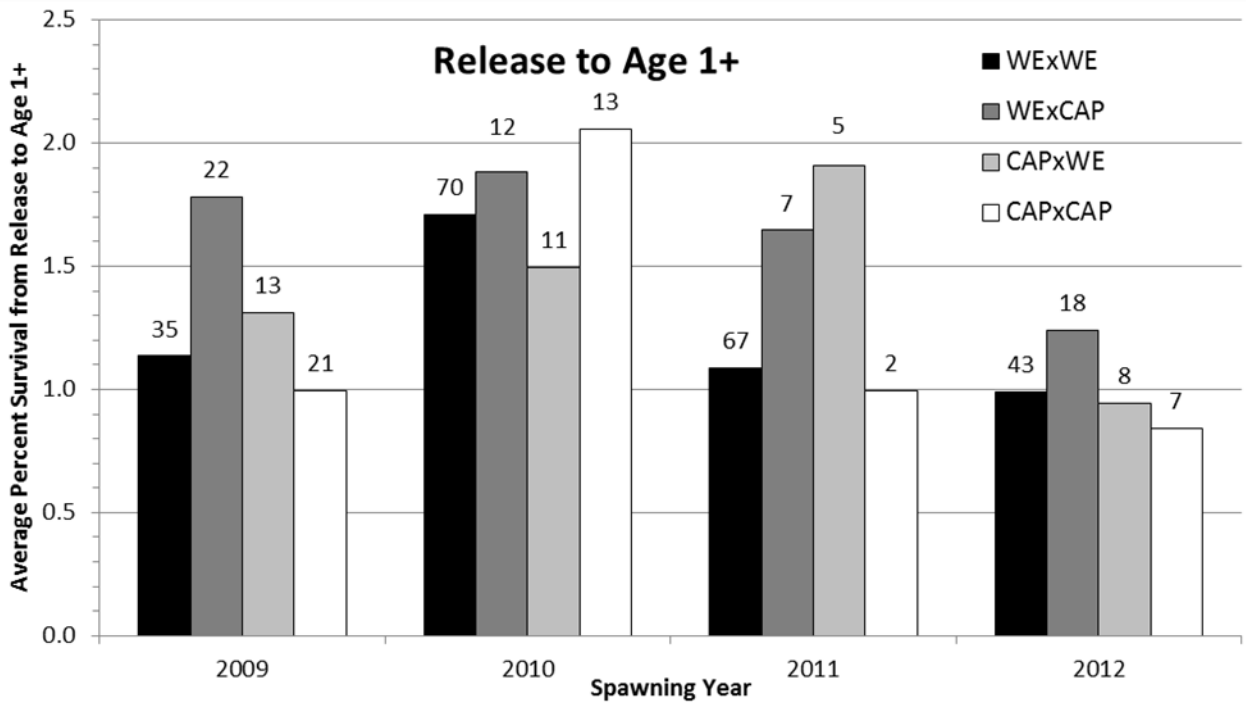


Figure 26. Average family percent survival from release as Age 0+ fry to capture as Age 1+ parr for four parental rearing environment types, where one or both parents were wild-exposed (WE) or captive-reared (CAP); female parent is specified first, followed by the male in all four parent type designations. Results are given for spawning years 2009–2012. Sample sizes (number of families) for each group are indicated above their respective bars.

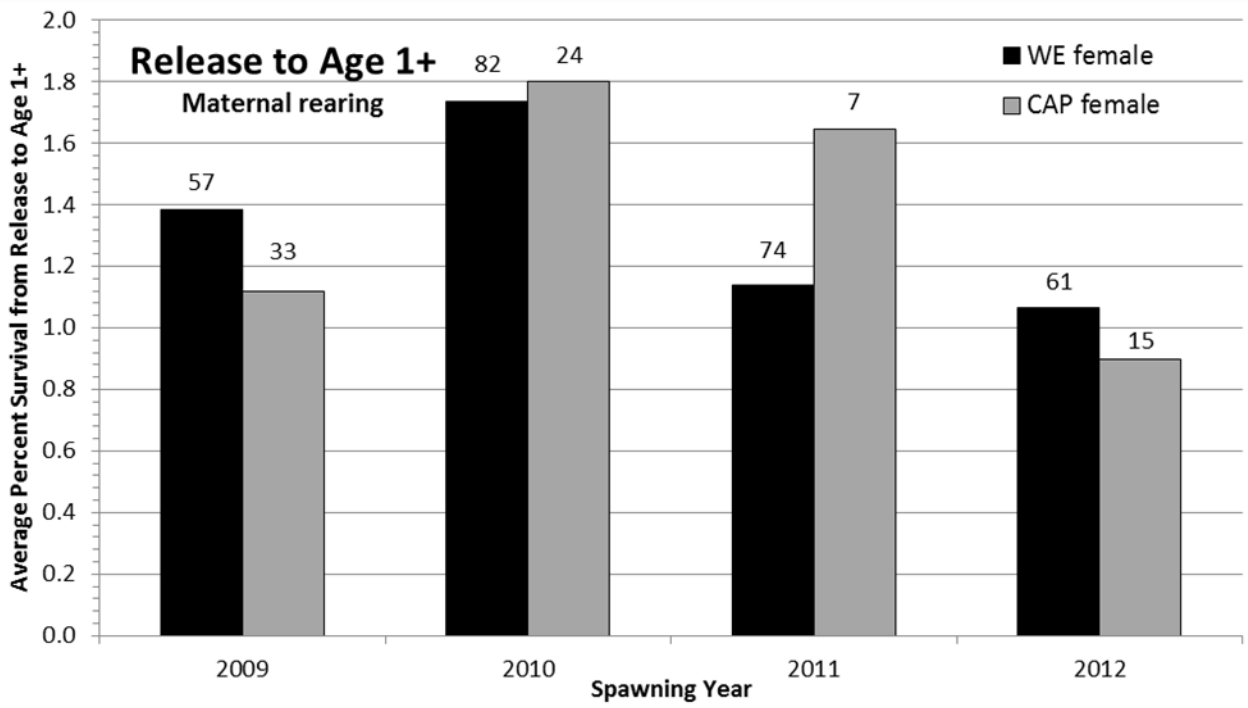


Figure 27. Average family percent survival from release as Age 0+ fry to capture as Age 1+ parr for two maternal parent rearing environment types, wild-exposed (WE) female and captive-reared (CAP) female, for spawning years 2009–2012. Sample sizes (number of families) for each group are indicated above their respective bars.

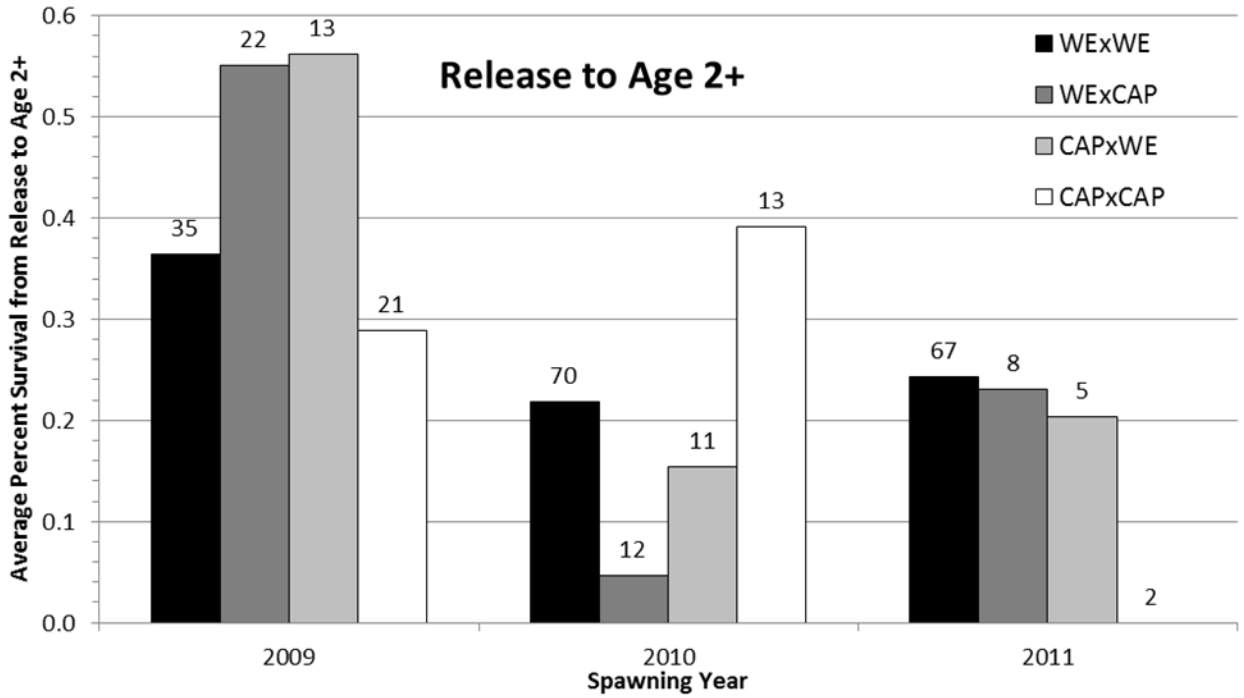


Figure 28. Average family percent survival from release as Age 0+ fry to capture as Age 2+ parr for four parental rearing environment types, where one or both parents were wild-exposed (WE) or captive-reared (CAP); female parent is specified first followed by male parent in all four parent type designations. Results are given for spawning years 2009–2011. Sample sizes (number of families) for each group are indicated above their respective bars.

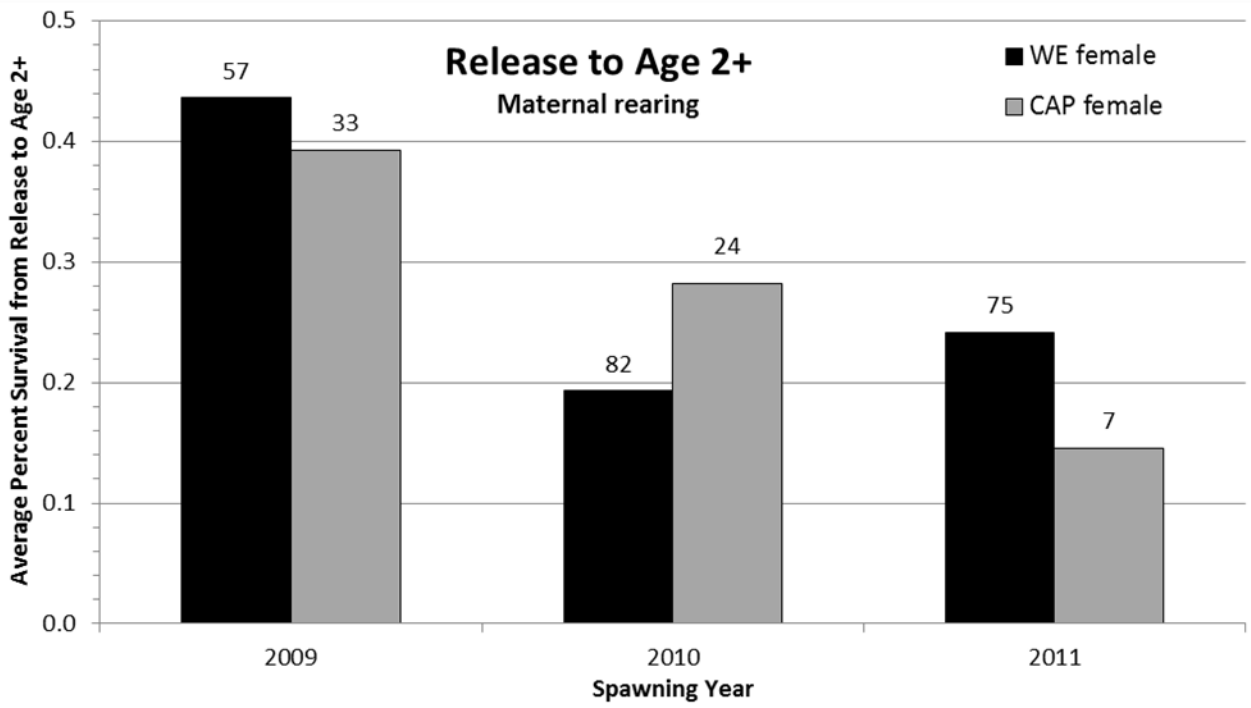


Figure 29. Average family percent survival from release as Age 0+ fry to capture as Age 2+ parr for two maternal parent environment types, wild-exposed (WE) female and captive-reared (CAP) female, for spawning years 2009–2011. Sample sizes (number of families) for each group are indicated above their respective bars.

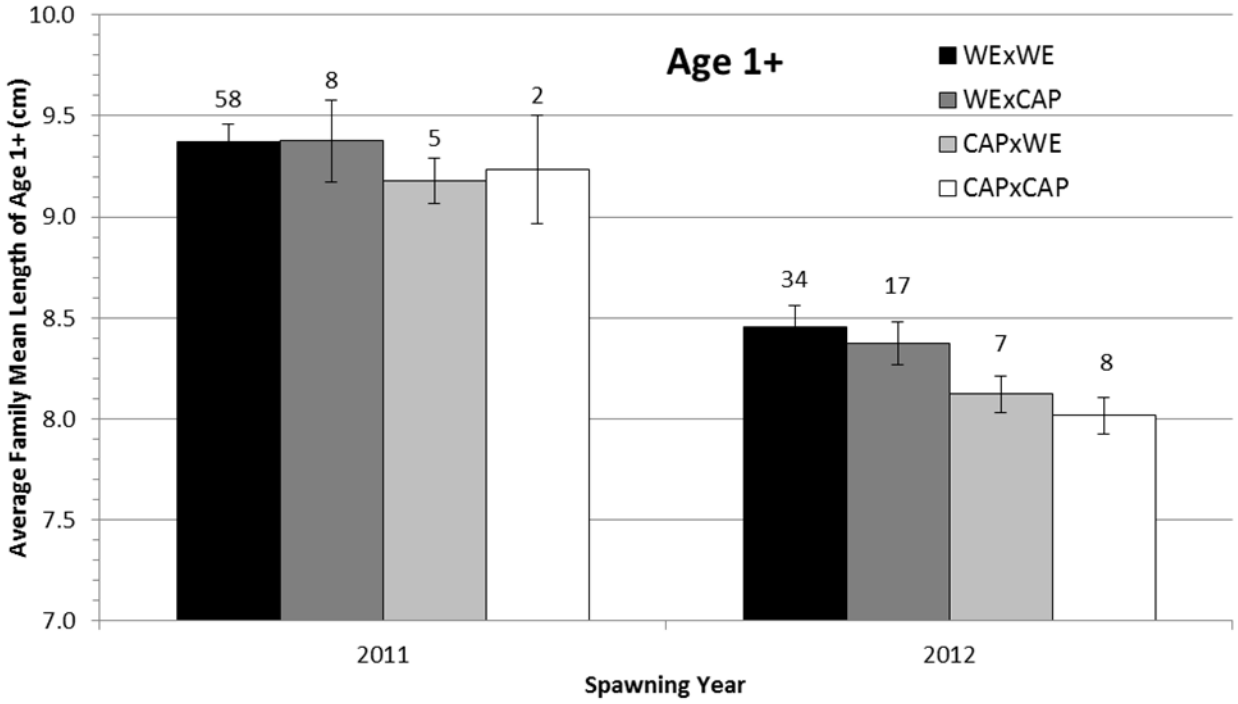


Figure 30. Average family mean length (cm) of parr captured at Age 1+ for four parental rearing environment types, where one or both parents were wild-exposed (WE) or captive-reared (CAP); female parent type is specified first, followed by the male parent type in all four parent type designations. Results are given for spawning years 2011 and 2012. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error.

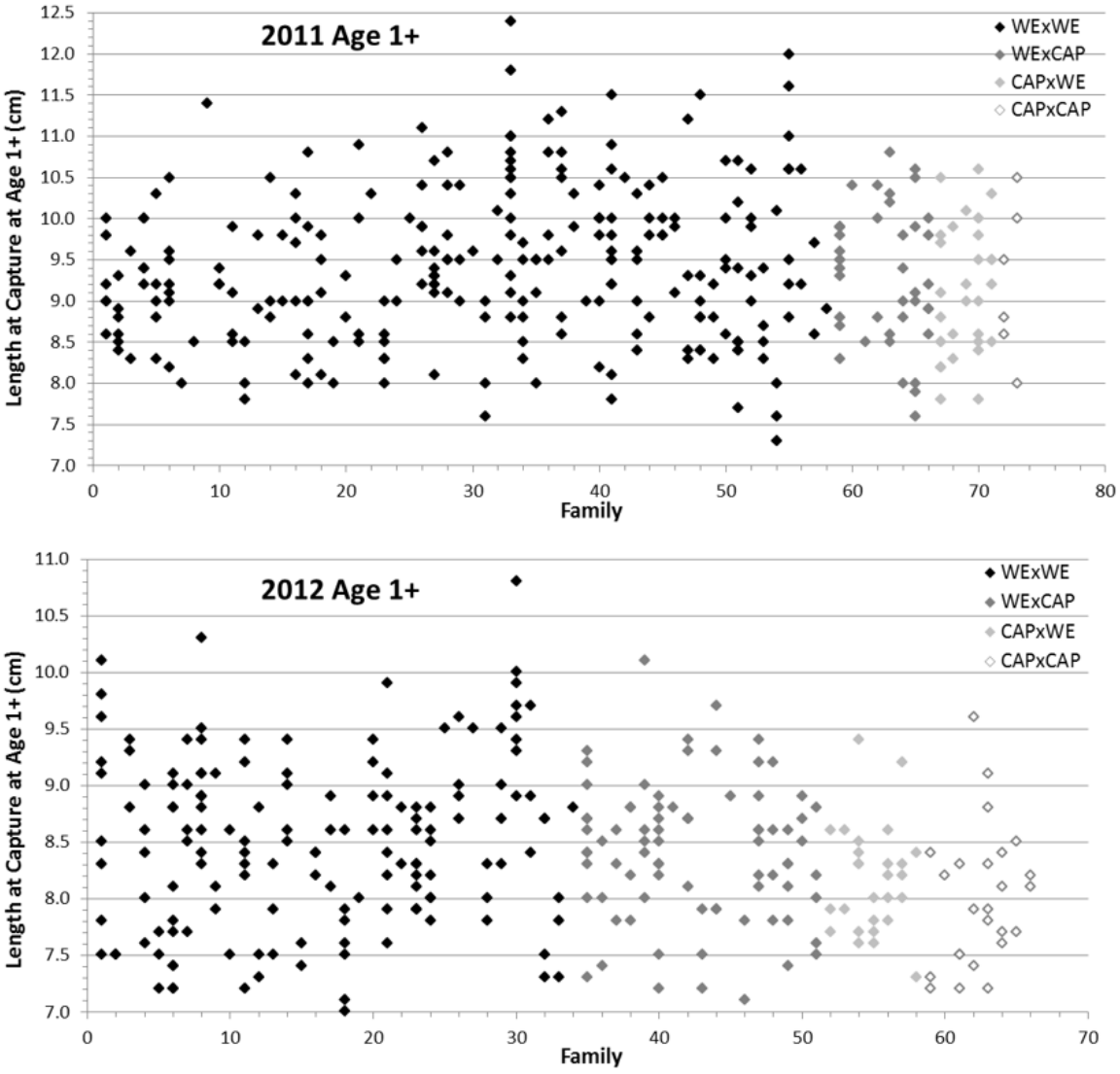


Figure 31. Length (cm) of individual parr captured at Age 1+ by family for four parental rearing environment types, where one or both parents were wild-exposed (WE) or captive-reared (CAP); female parent is specified first followed by male parent in all four parent type designations. Results are given for spawning years 2011–2012.

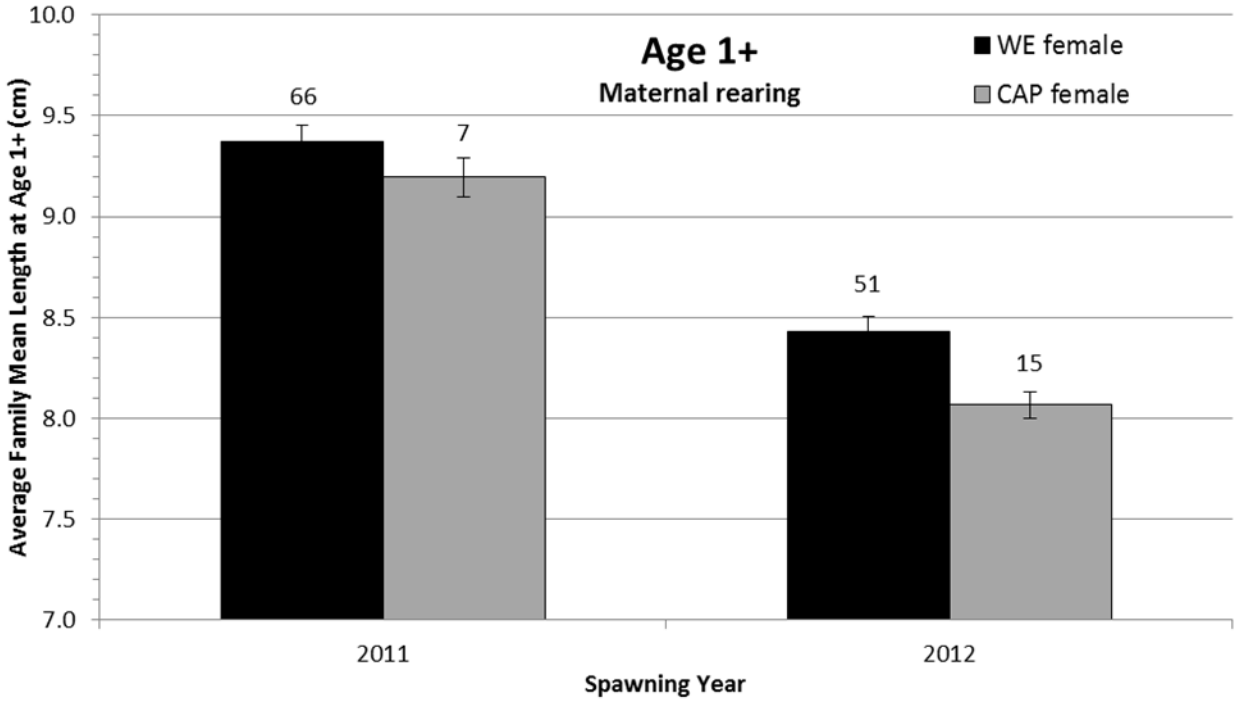


Figure 32. Average family mean length (cm) of parr captured at Age 1+ for two maternal parent rearing environment types, wild-exposed (WE) female and captive-reared (CAP) female, for the years 2011 and 2012. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error.

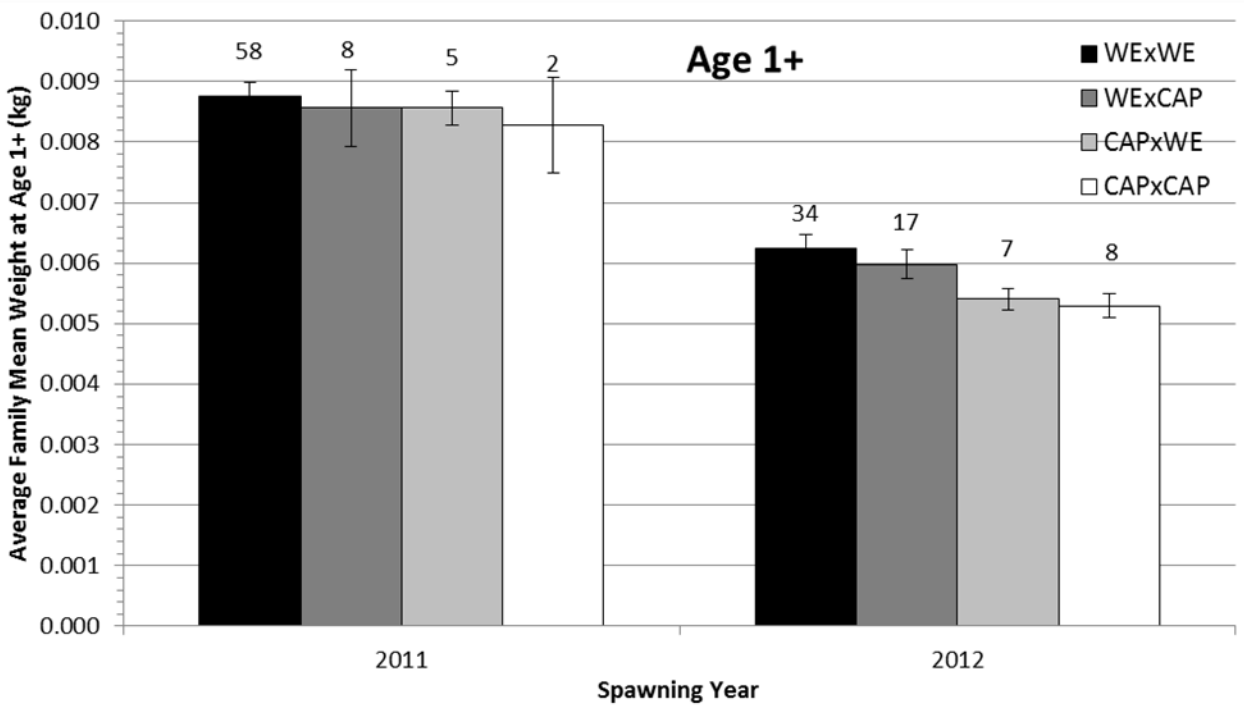


Figure 33. Average family mean weight (kg) of parr captured at Age 1+ for four parental rearing environment types, where one or both parents were wild-exposed (WE) or captive-reared (CAP); female parent type is specified first, followed by the male parent type in all four parent type designations. Results are given for the spawning years 2011–2012. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error.

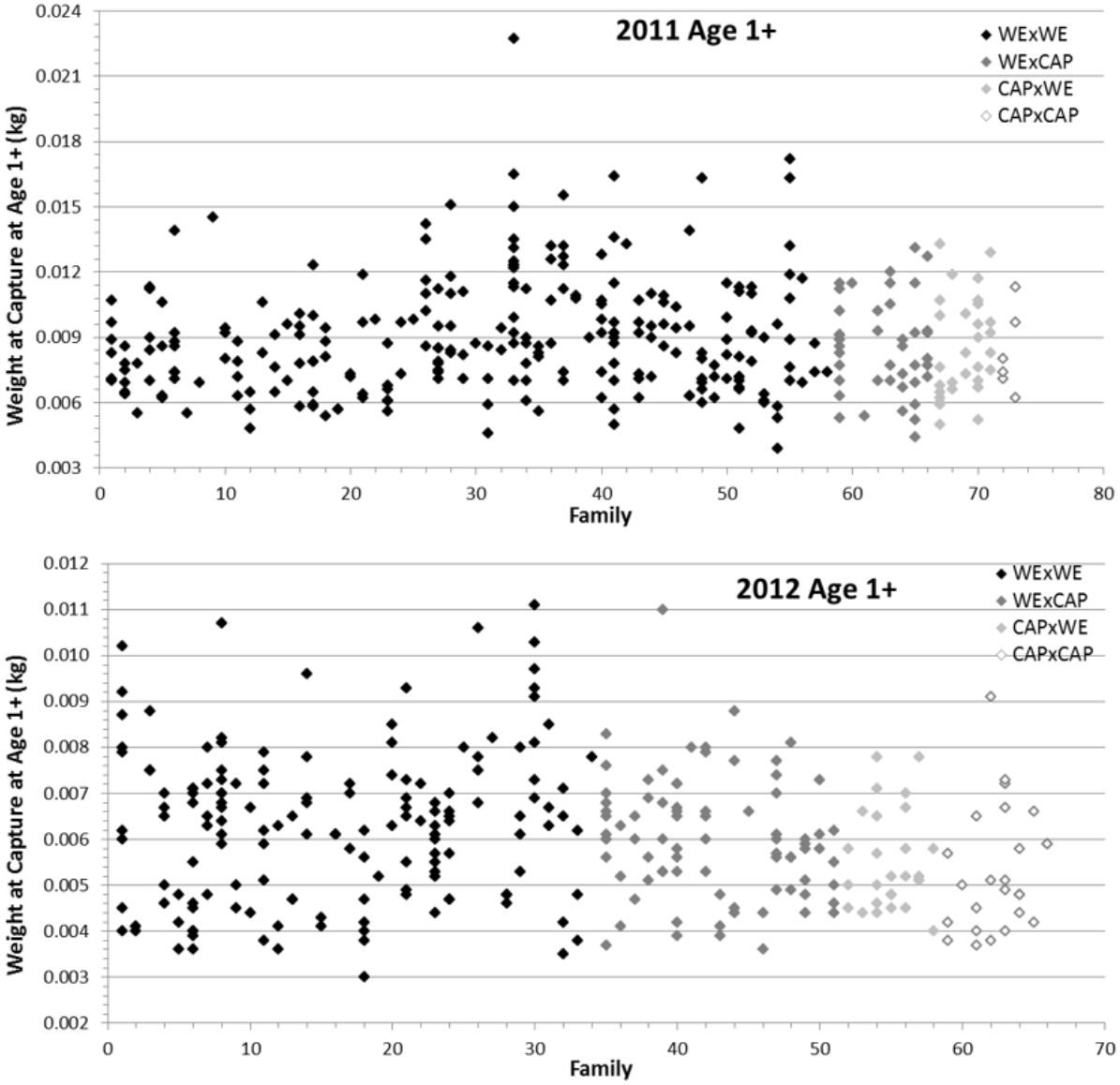


Figure 34. Weight (kg) of individual parr captured at Age 1+ by family for four parental rearing environment types, where one or both parents were wild-exposed (WE) or captive-reared (CAP); female parent type is specified first, followed by the male parent type in all four parent type designations. Results are given for spawning years 2011–2012.

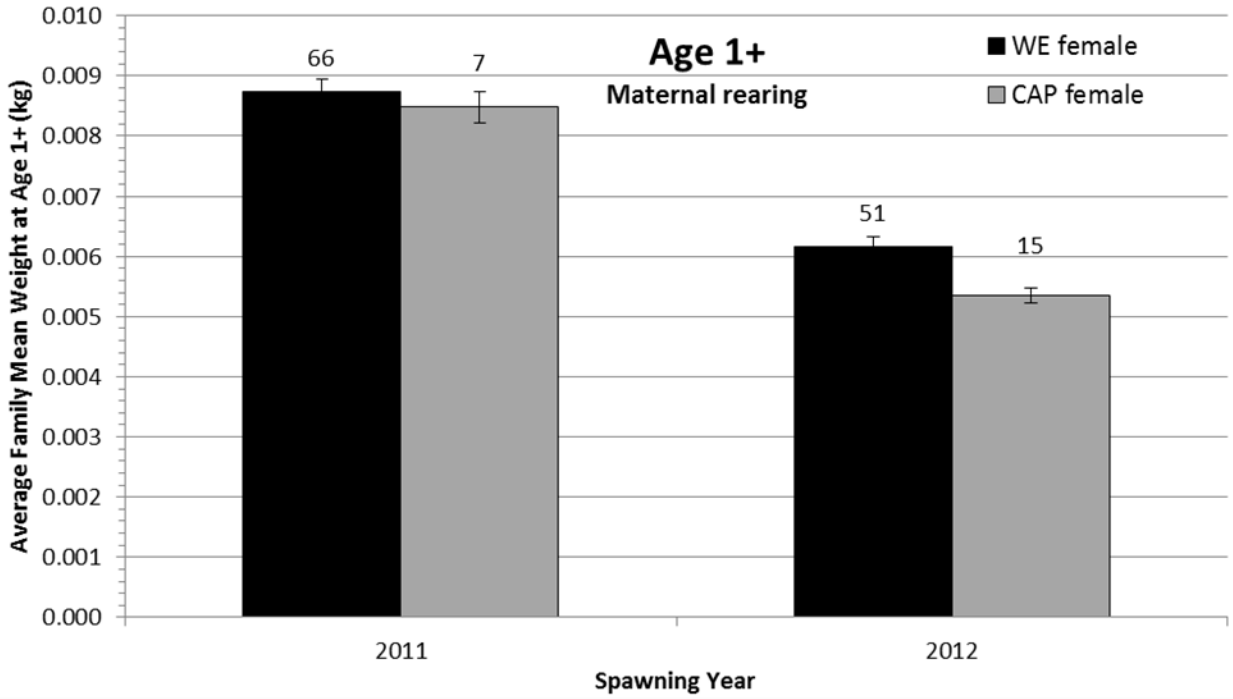


Figure 35. Average family mean weight (kg) of parr captured at Age 1+ for two maternal parent rearing environment types, wild-exposed (WE) female and captive-reared (CAP) female, for the spawning years 2011–2012. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error.

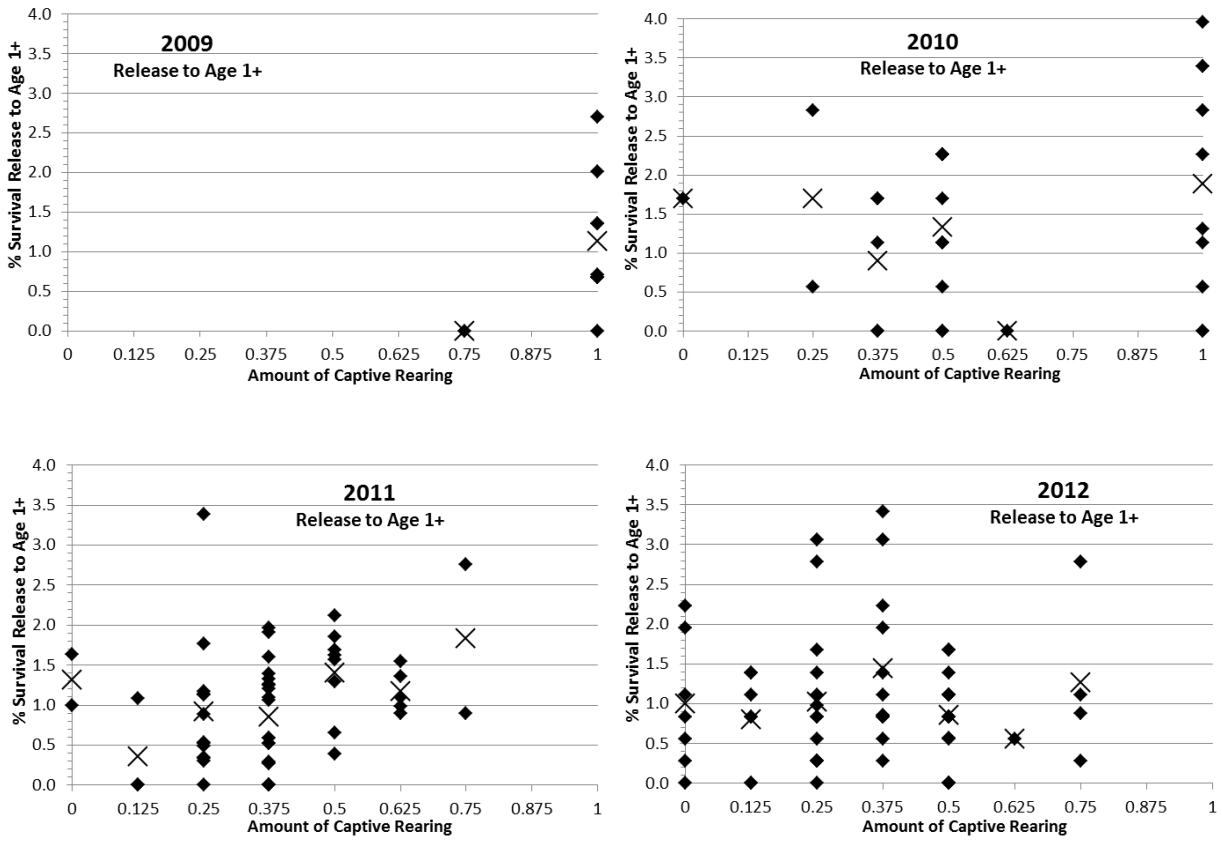


Figure 36. Family percent survival from release as Age 0+ fry to capture as Age 1+ parr for varying levels of cumulative early (juvenile) captive rearing (across the parents and grandparents). Results are given for spawning years 2009–2012. Average percent recovery at each level of captive rearing is indicated by a large X.

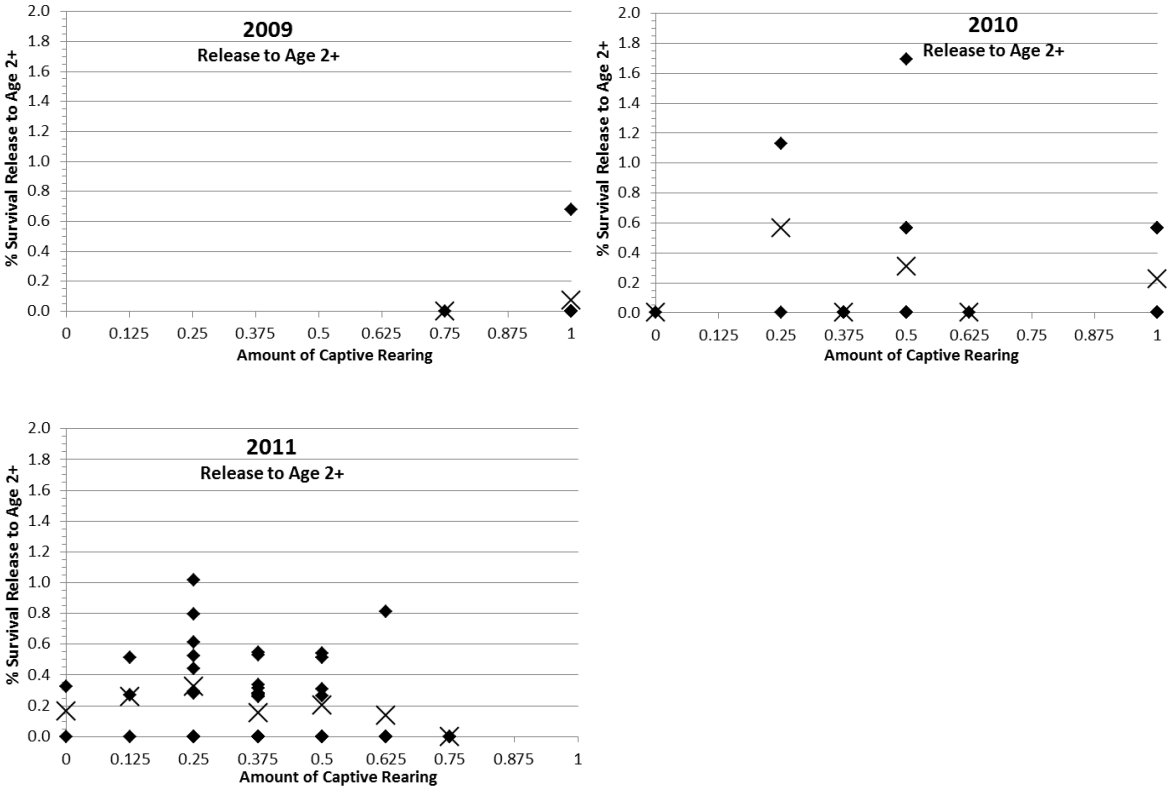


Figure 37. Family percent survival from release as Age 0+ fry to capture as Age 2+ parr for varying levels of cumulative early (juvenile) captive rearing (across the parents and grandparents). Results are given for spawning years 2009–2011. Average percent recovery at each level of early juvenile captive rearing is indicated by a large X.

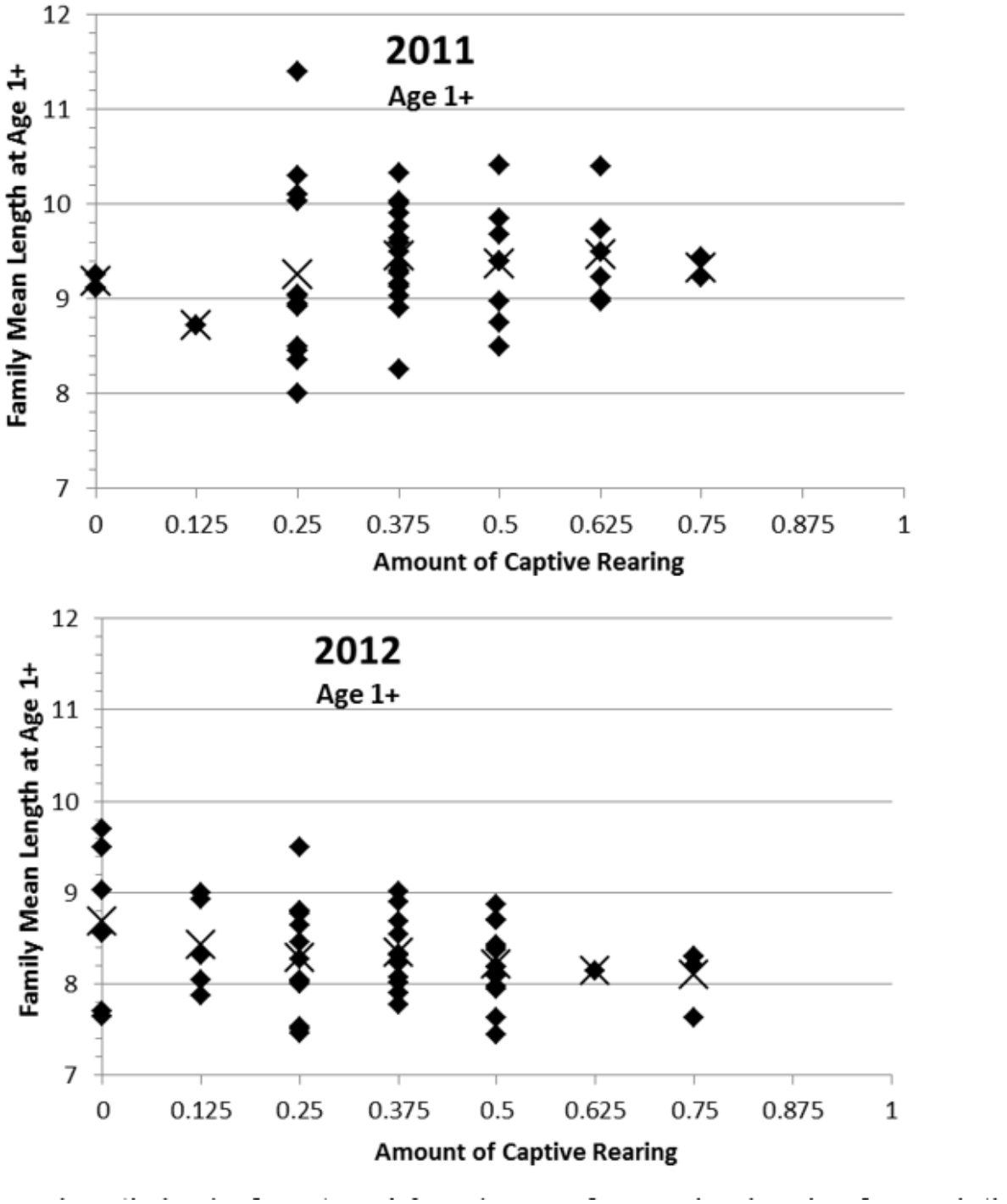


Figure 38. Family mean length (cm) of captured Age 1+ parr for varying levels of cumulative early (juvenile) captive rearing (across the parents and grandparents) for the spawning years 2011–2012. Average family mean length at each level of captive rearing is indicated by a large X.

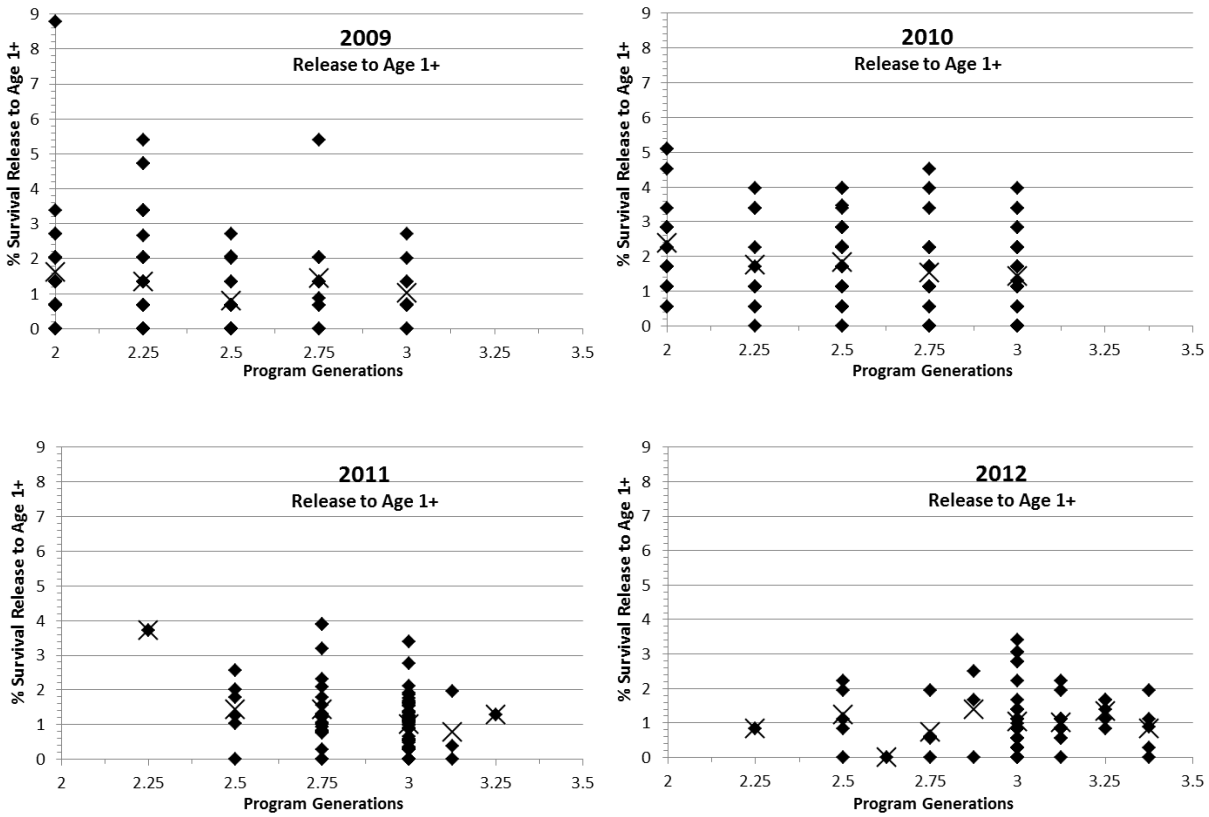


Figure 39. Family percent survival from release as Age 0+ fry to capture as Age 1+ parr for families exhibiting different numbers of program generations for the spawning years 2009–2012. Average percent recovery for different program generations is indicated by a large X.

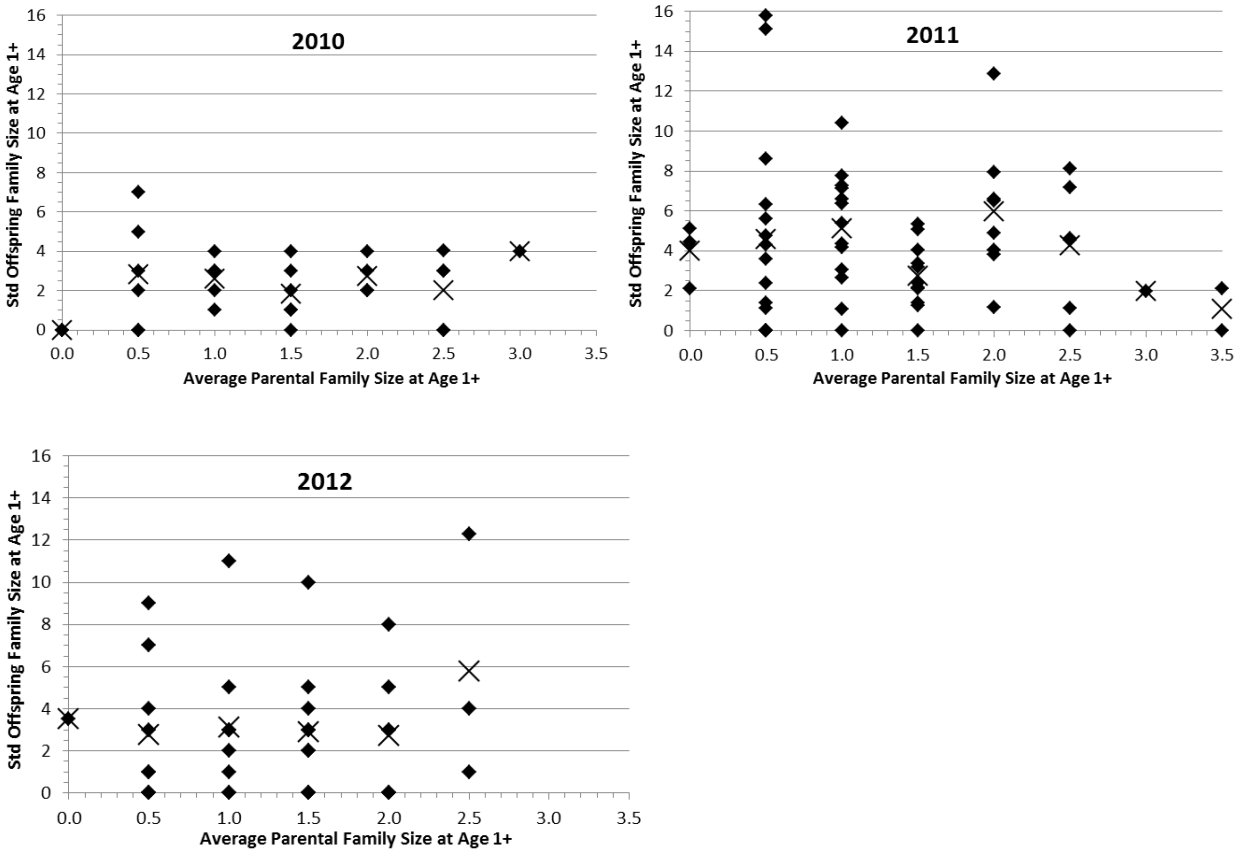


Figure 40. Standardized offspring family size in the wild at capture at Age 1+ versus average parental family size in the wild at capture at Age 1+ for the spawning years 2010–2012. Average offspring family size at each level of parental family size is indicated by a large X. Results include data from offspring of wild-exposed parents only.

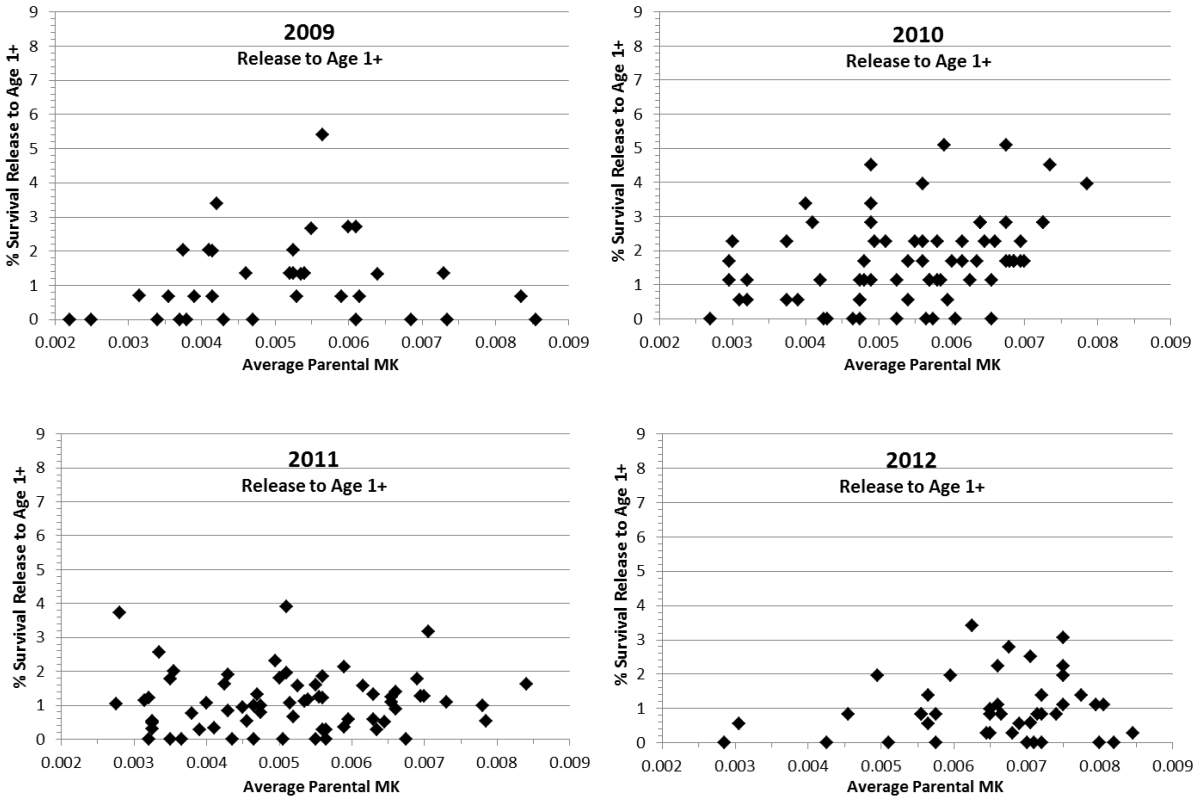


Figure 41. Family percent survival from release as Age 0+ fry to capture as Age 1+ parr versus the family's average parental mean kinship (MK), for the spawning years 2009–2012. Results include data from offspring of two wild-exposed parents only.

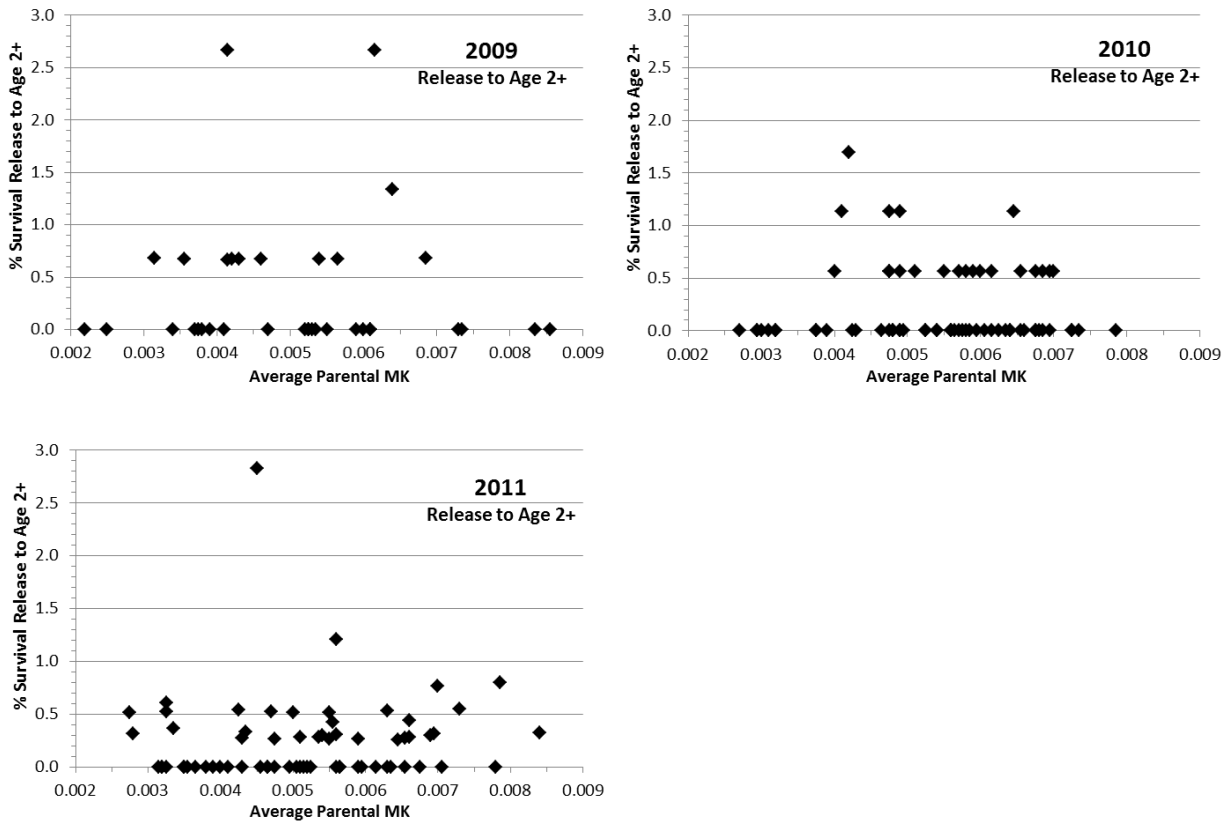


Figure 42. Family percent survival from release as Age 0+ fry to capture as Age 2+ parr versus the family's average parental mean kinship (MK), for the spawning years 2009–2012. Results include data from offspring of two wild-exposed parents only.

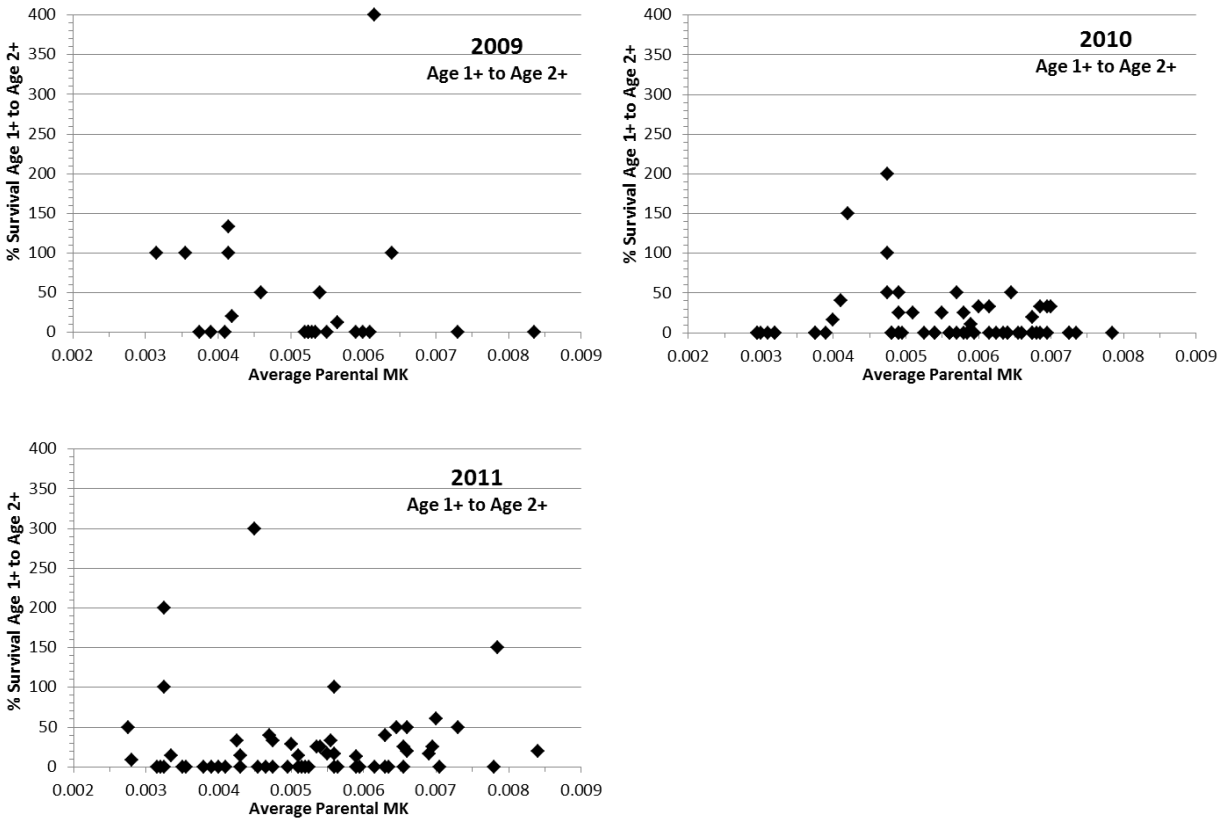


Figure 43. Family percent survival from capture as Age 1+ parr to capture as Age 2+ parr versus the family's average parental mean kinship (MK), for spawning years 2009–2011. Results include data from offspring of two wild-exposed parents only.

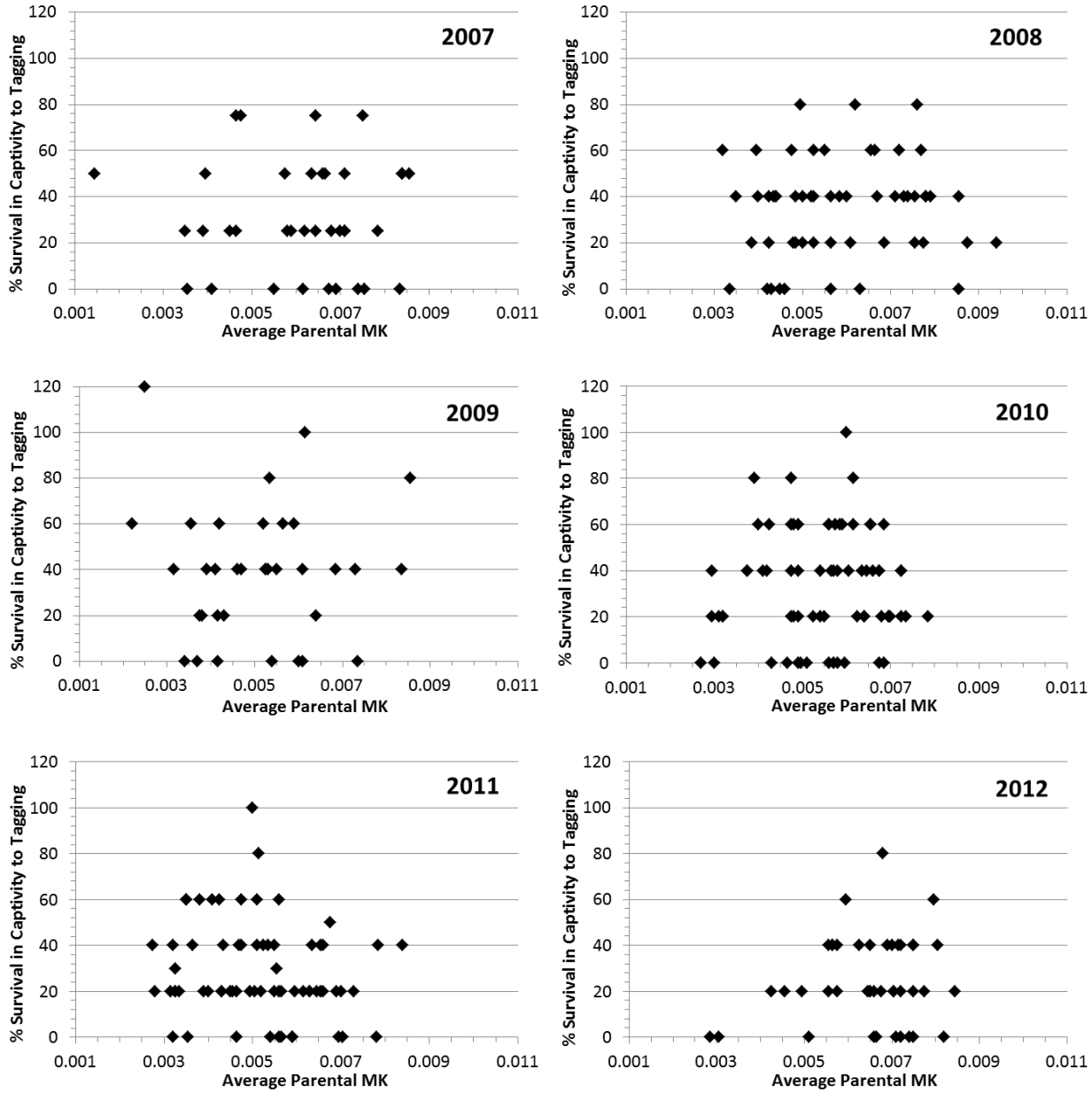


Figure 44. Family percent survival in captivity from shock (at the egg stage, mid-development) to tagging (approximately Age 4) versus the family's average parental mean kinship (MK) for spawning years 2007–2012. Results include data from offspring of two wild-exposed parents only.

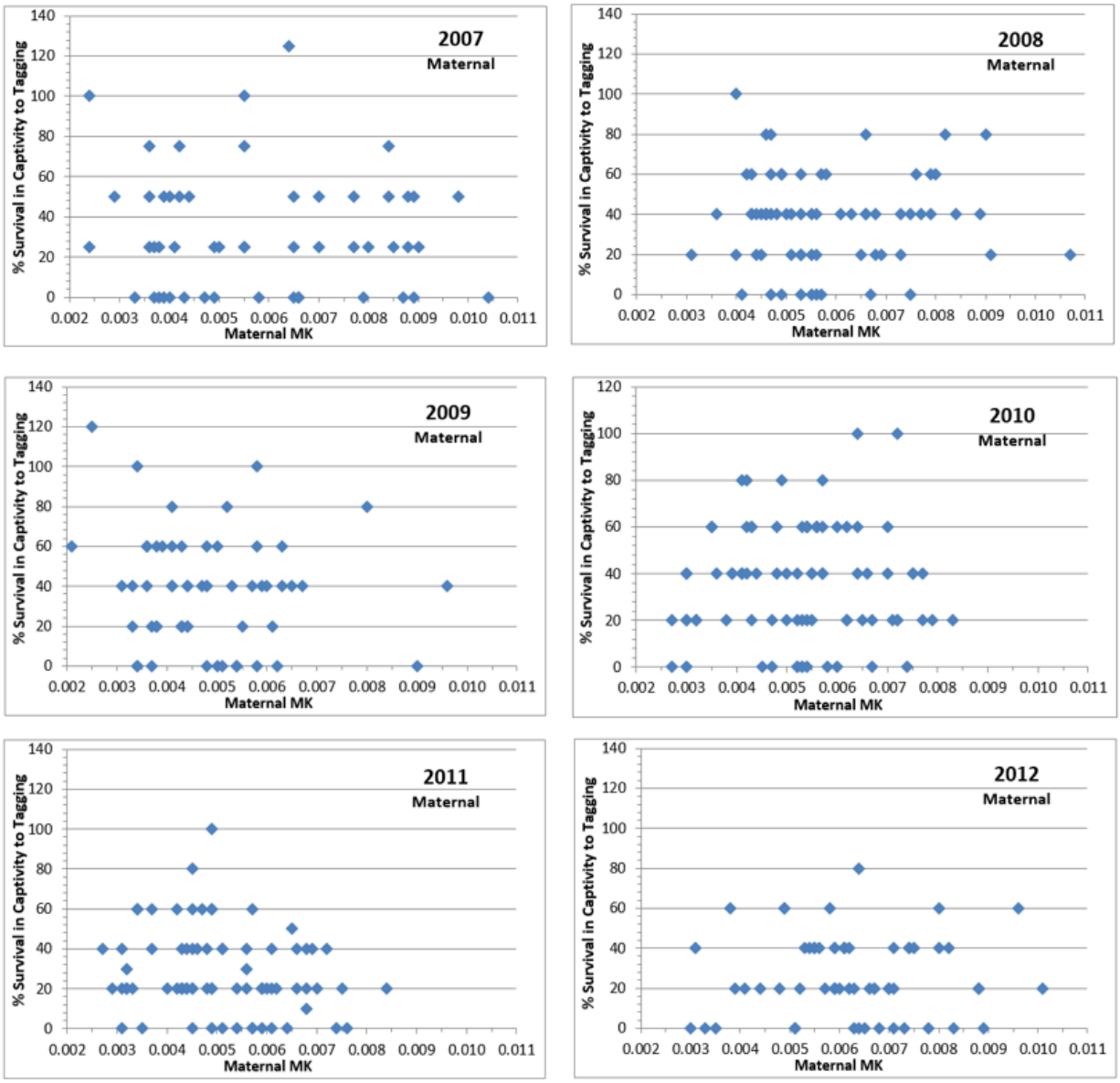


Figure 45. Family percent survival in captivity from shock (at the egg stage, mid-development) to tagging (approximately Age 4) versus the family's maternal mean kinship (MK) for spawning years 2007–2012. Results include data from offspring of two wild-exposed parents only.

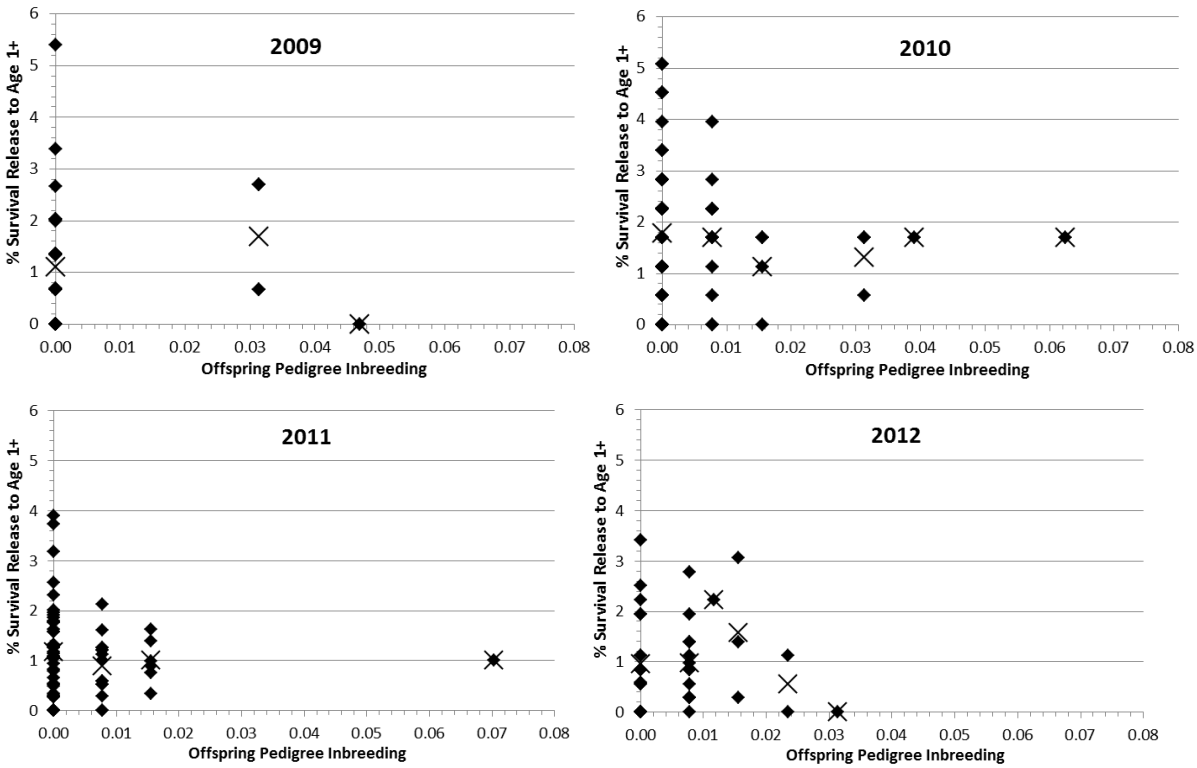


Figure 46. Family percent survival from release as Age 0+ fry to capture as Age 1+ parr versus level of offspring pedigree inbreeding, for the spawning years 2009–2012. Results include data from offspring of two wild-exposed parents only. Average percent recovery across families for a given level of inbreeding is indicated by a large X.

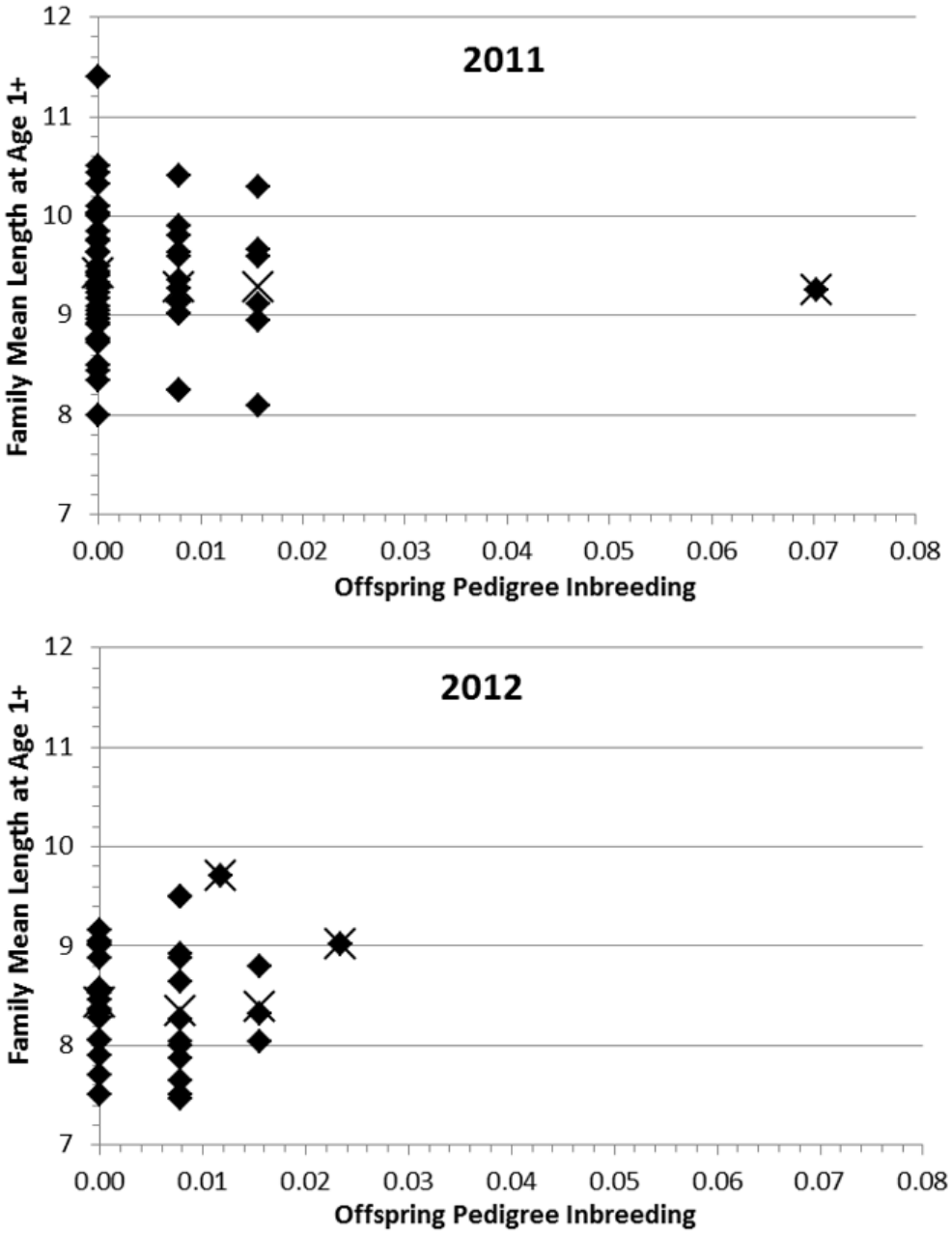


Figure 47. Family mean length (cm) at capture as Age 1+ parr versus offspring pedigree inbreeding, for the spawning years 2011–2012. Results include data from offspring of two wild-exposed parents only. Average family mean length at each level of offspring pedigree inbreeding is indicated by a large X.

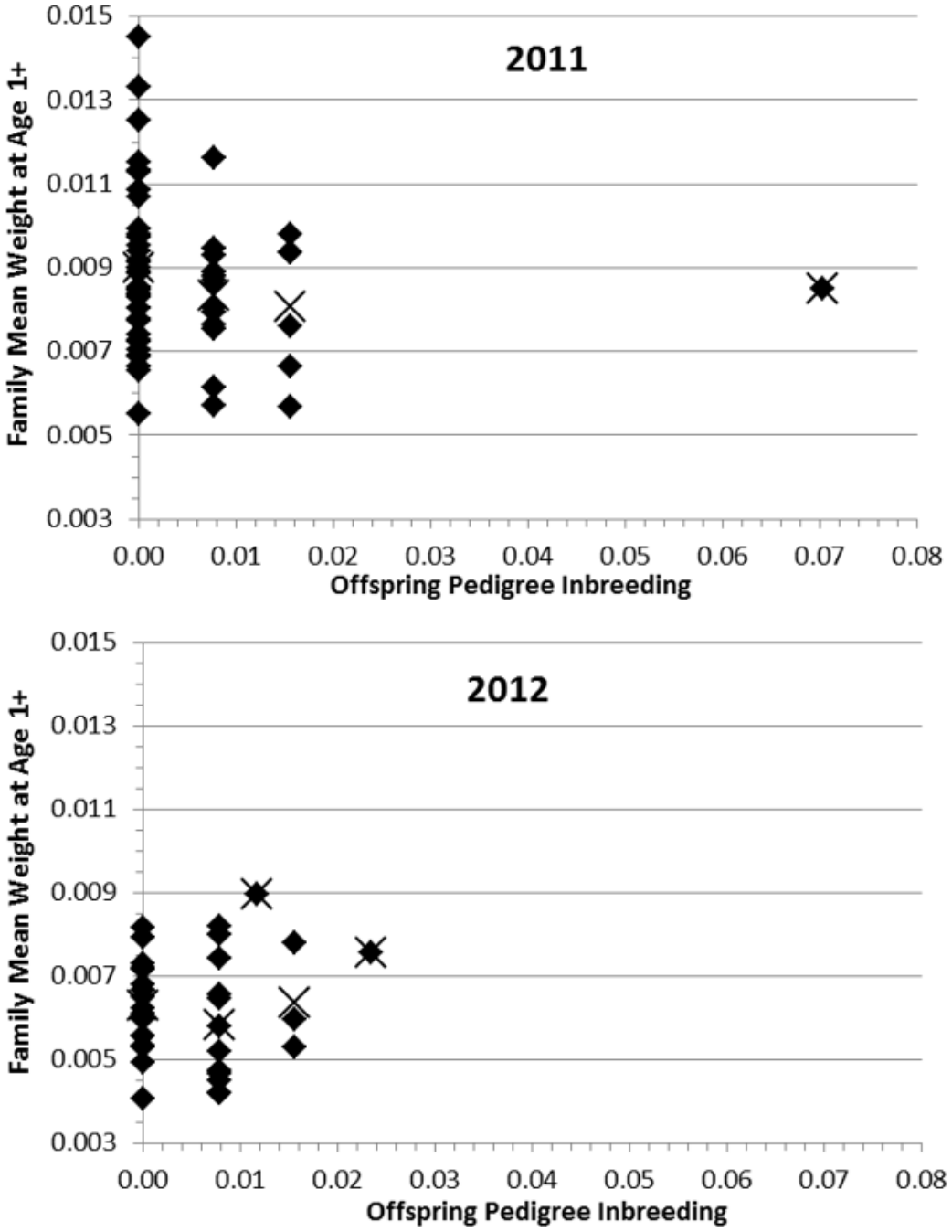


Figure 48. Family mean weight (kg) at capture as Age 1+ parr versus offspring pedigree inbreeding for spawning years 2011–2012. Results include data from offspring of two wild-exposed parents only. Average family mean weight at each level of offspring inbreeding is indicated by a large X.

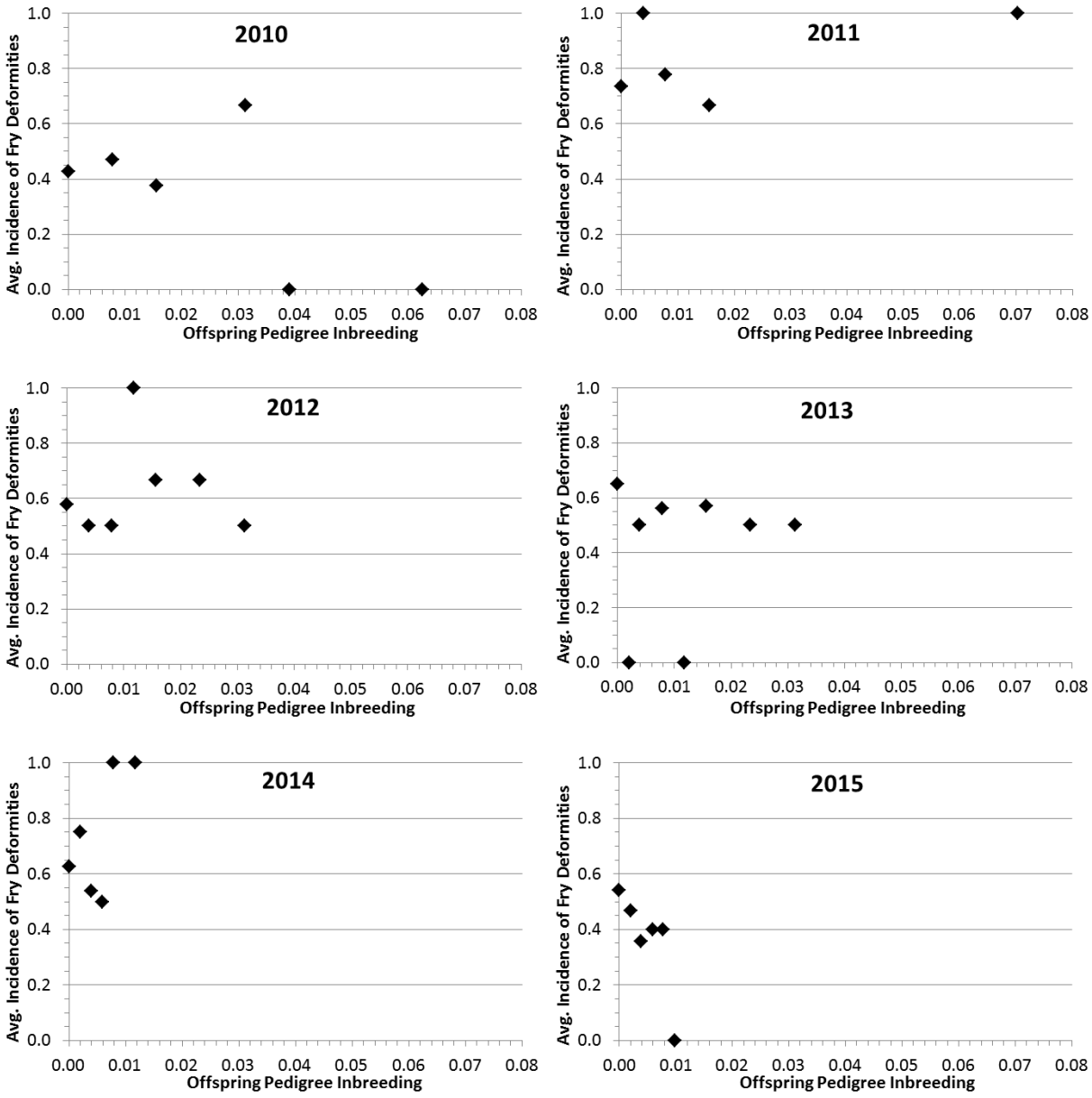


Figure 49. Average family incidence of deformities at the Age 0+ fry stage versus offspring pedigree inbreeding, for the spawning years 2010–2015. Results include data from offspring of captive-reared and wild-exposed parents.

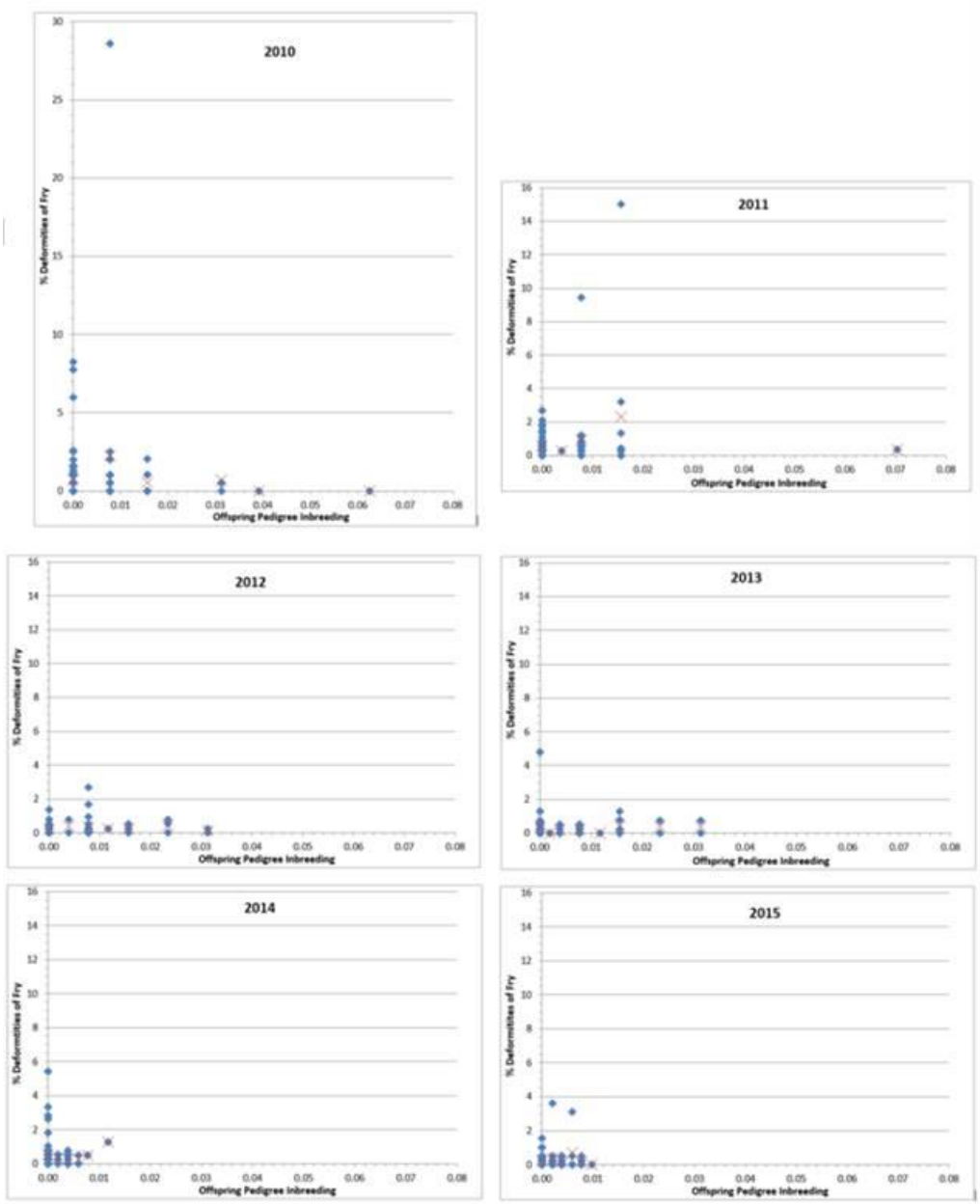


Figure 50. Family percent deformities at the Age 0 fry stage versus offspring pedigree inbreeding for spawning years 2010–2015. Results include data from offspring of wild-exposed and captive-reared parents. Average percent deformities across families at each level of inbreeding is indicated by an X.

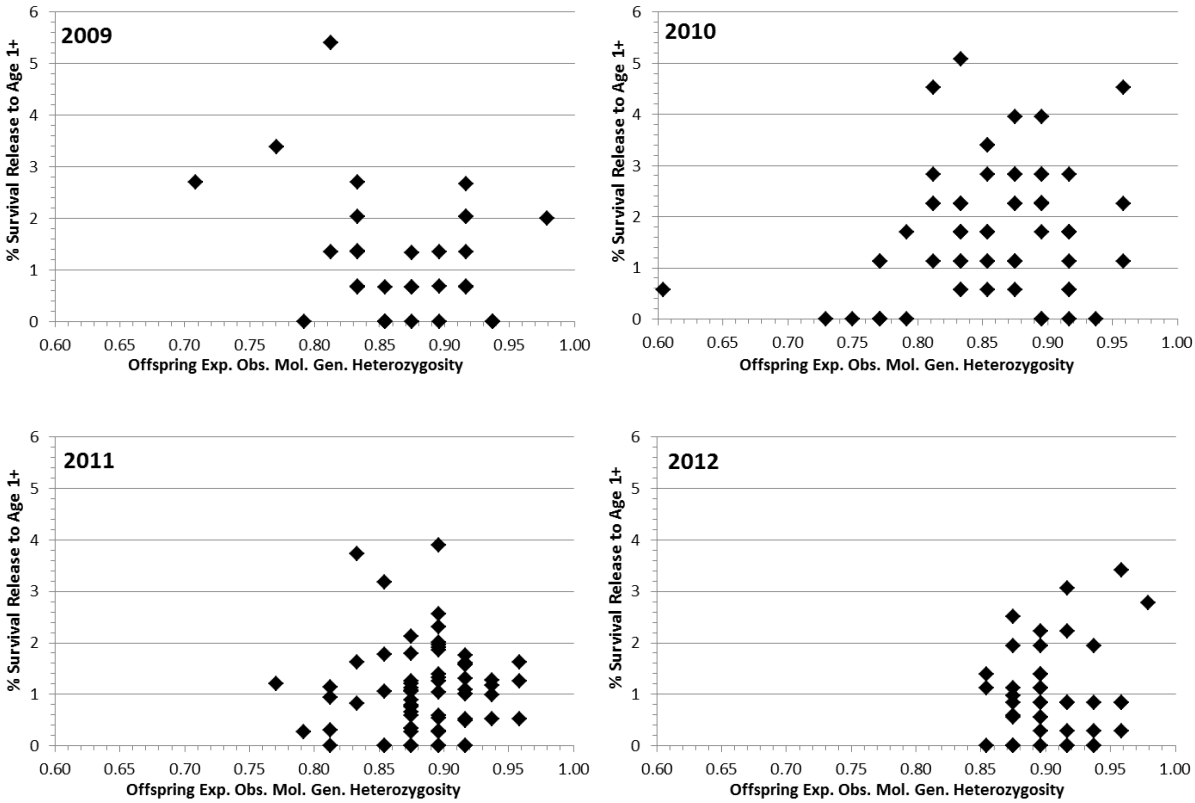


Figure 51. Family percent survival from release as Age 0+ fry to capture as Age 1+ parr versus offspring expected observed heterozygosity, for the spawning years 2009–2012. Results include data from offspring of two wild-exposed parents only.

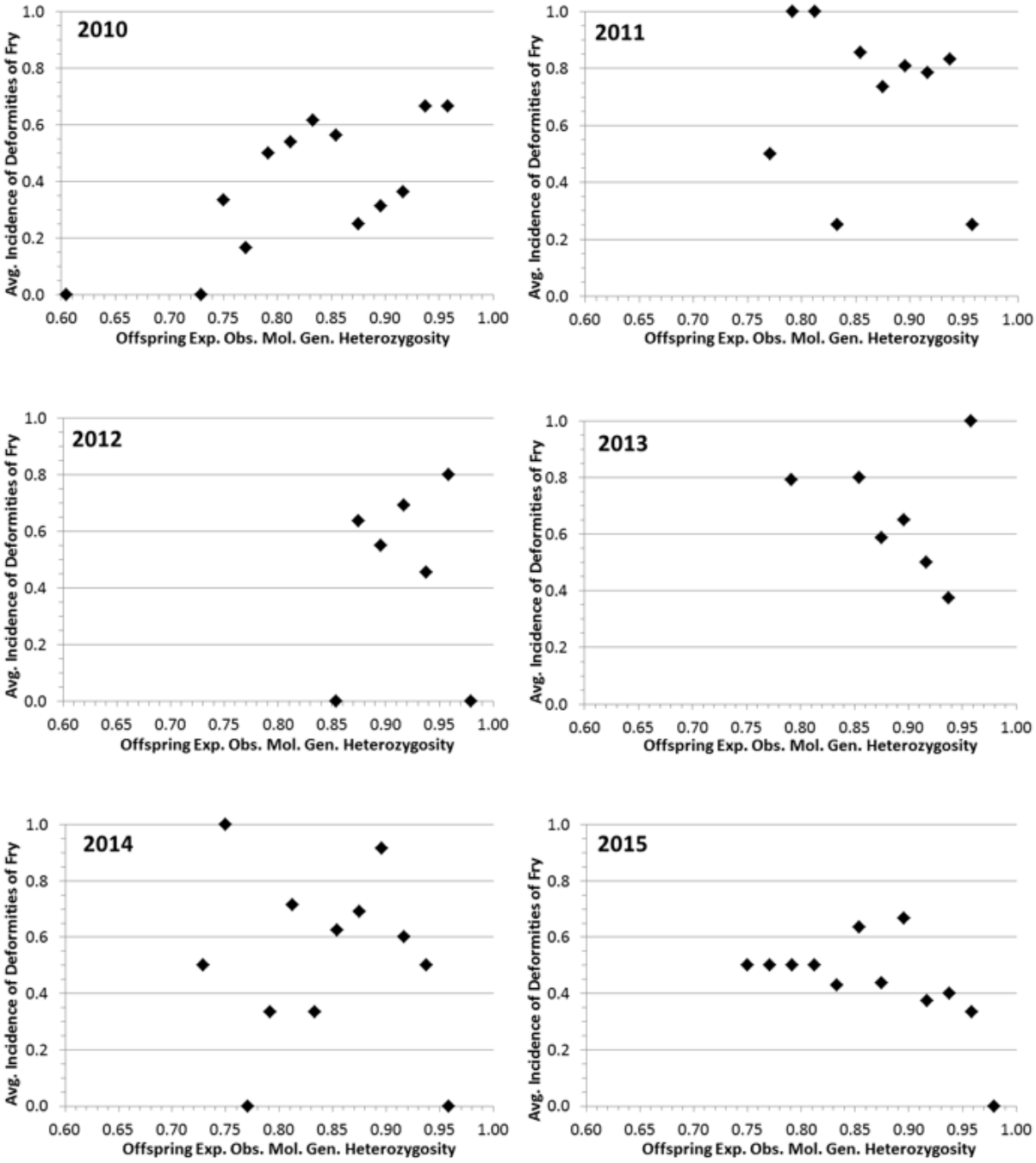


Figure 52. Average family incidence of deformities of Age 0+ fry versus the offspring expected observed heterozygosity, for the spawning years 2010–2015. Results include data from offspring of captive-reared and wild-exposed parents.

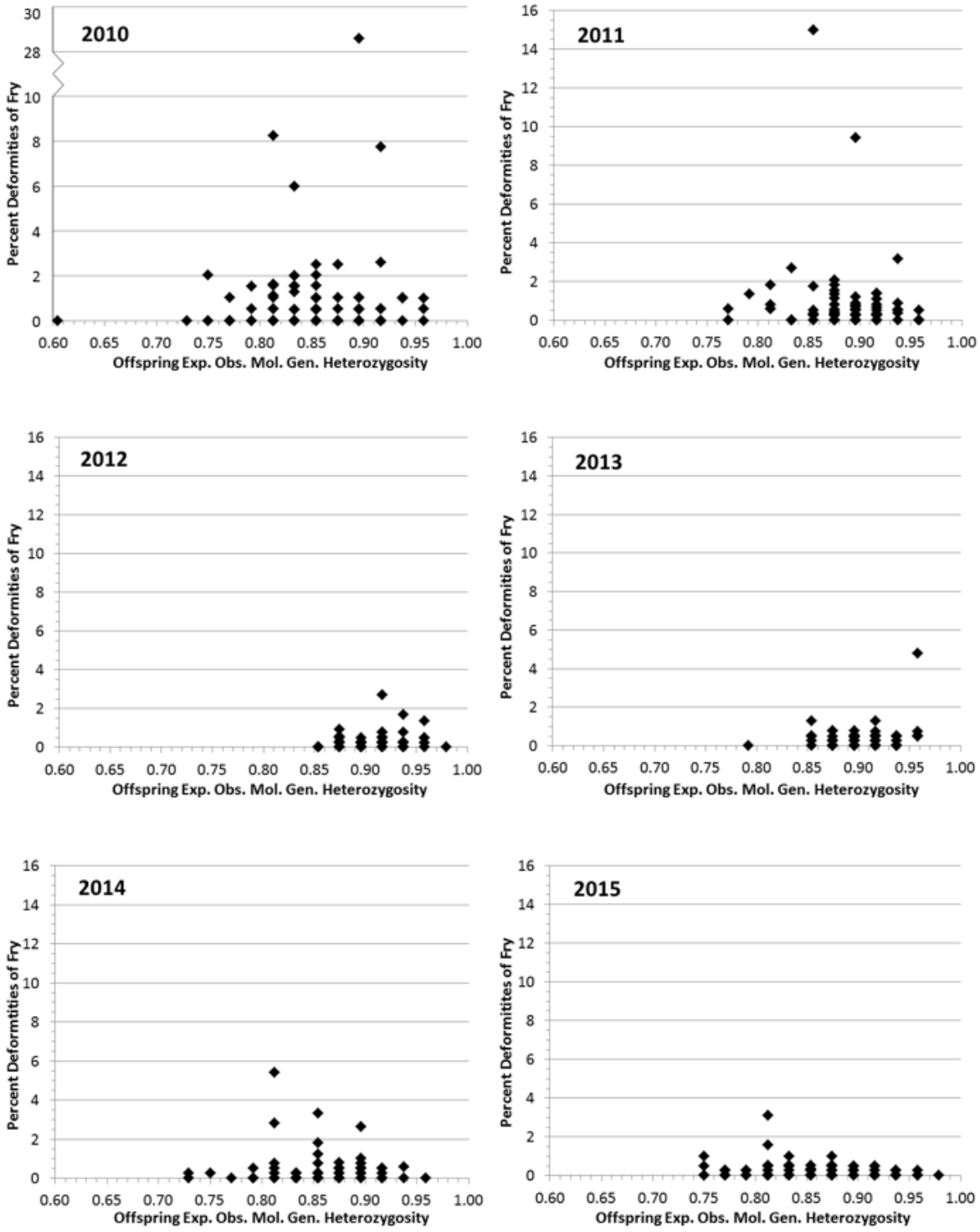


Figure 53. Family percent deformities of Age 0+ fry versus offspring expected observed heterozygosity, for spawning years 2010–2015. Results include data from offspring of wild-exposed and captive-reared parents.

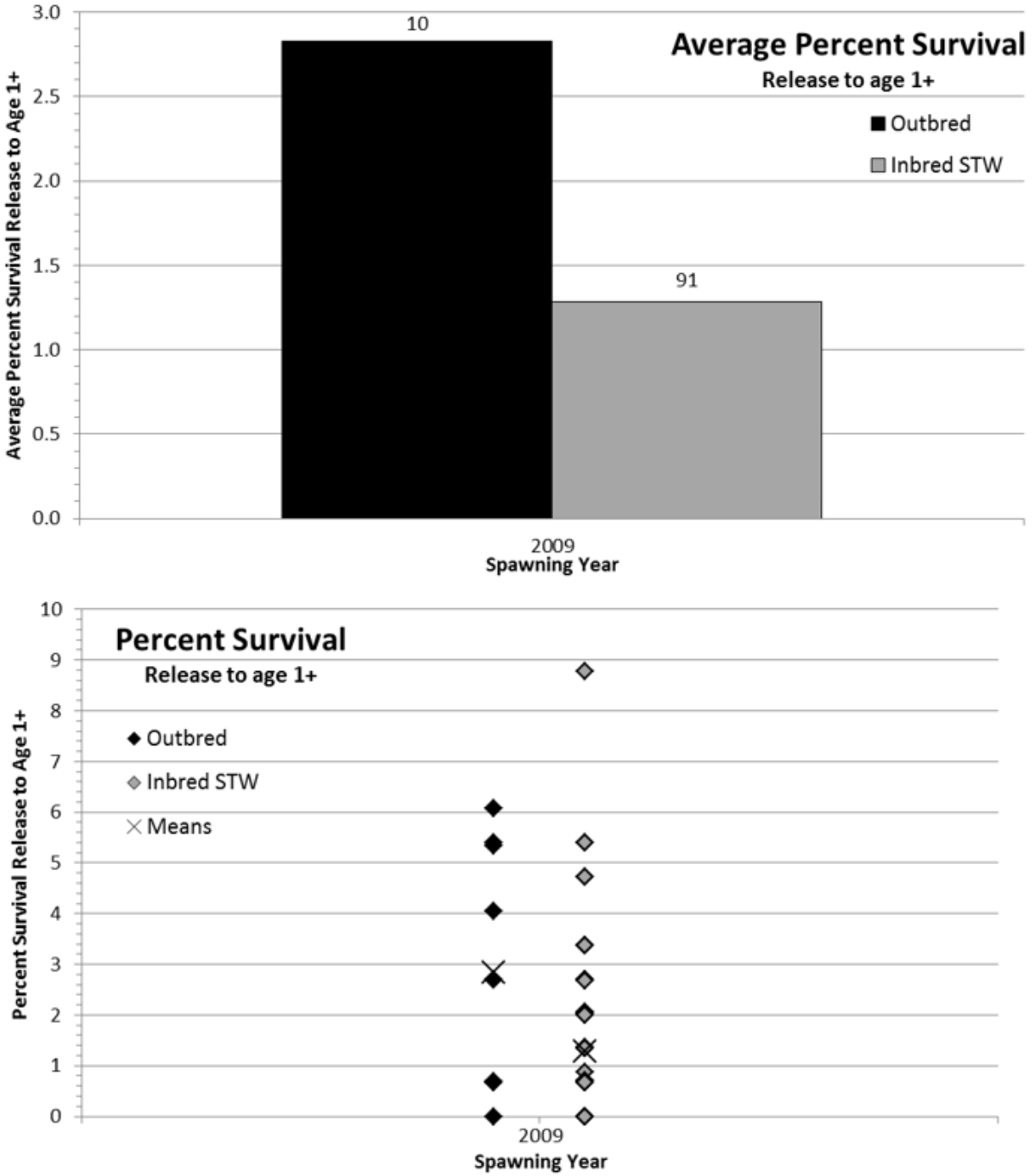


Figure 54. Average (upper panel) and individual (lower panel) family percent survival from release as Age 0+ fry to capture as Age 1+ parr for two parental cross types, North Minas Basin X Stewiacke (outbred) and Stewiacke X Stewiacke (inbred STW), for the spawning year 2009. Sample sizes (number of families) for each group are indicated above their respective bars.

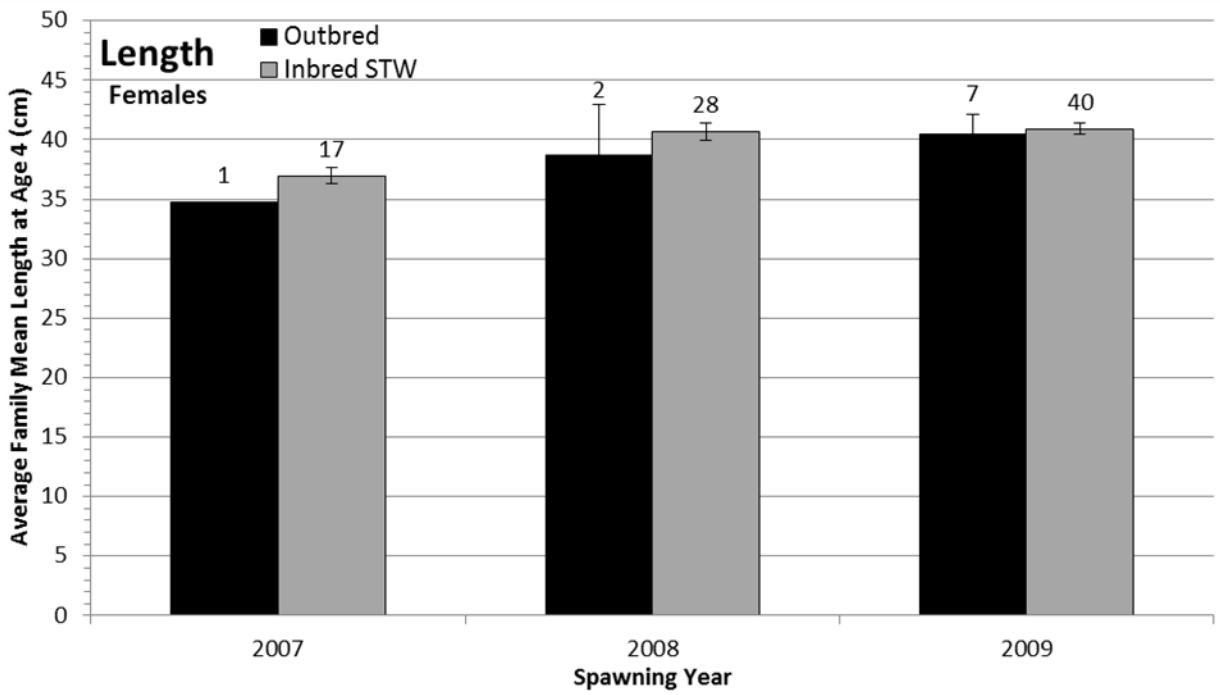


Figure 55. Average family mean length (cm) at Age 4+, females only, for two parental cross types, North Minas Basin X Stewiacke (outbred) and Stewiacke X Stewiacke (inbred STW), for the spawning years 2007–2009. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Adult age used here is based on the brood or fertilization year of individuals.

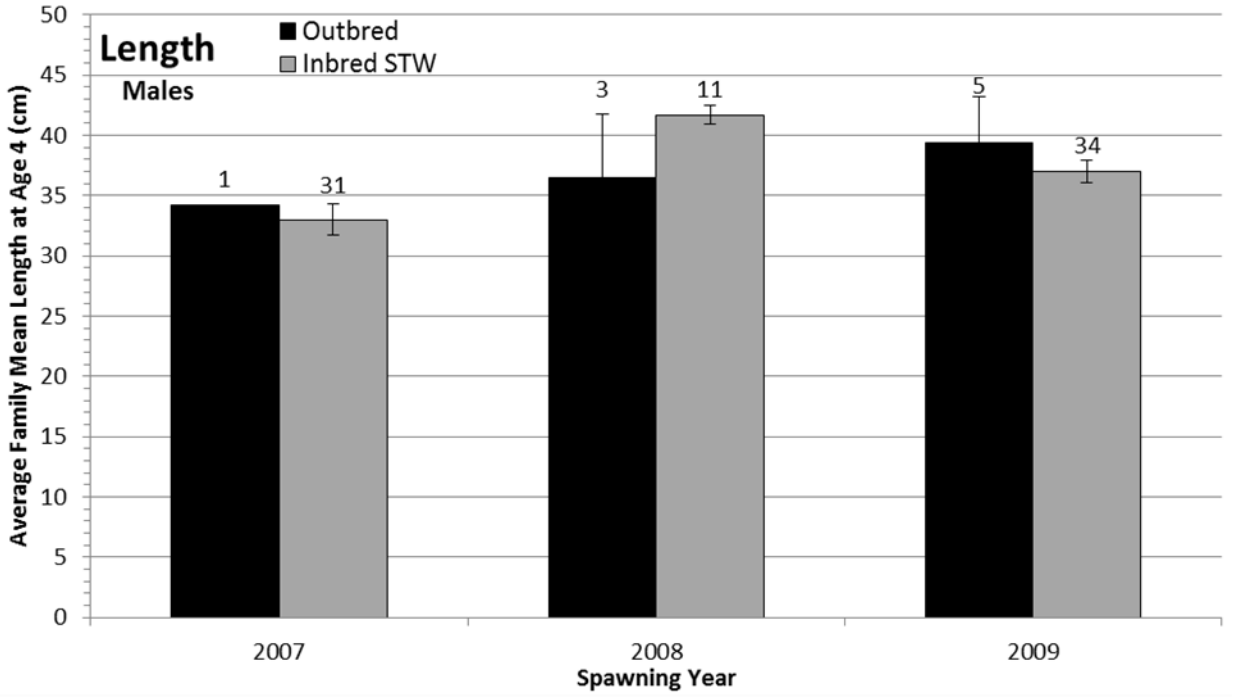


Figure 56. Average family mean length (cm) at Age 4+, males only, for two parental cross types, North Minas Basin X Stewiacke (outbred) and Stewiacke X Stewiacke (inbred STW), for the spawning years 2007–2009. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Adult age used here is based on the brood or fertilization year of individuals.

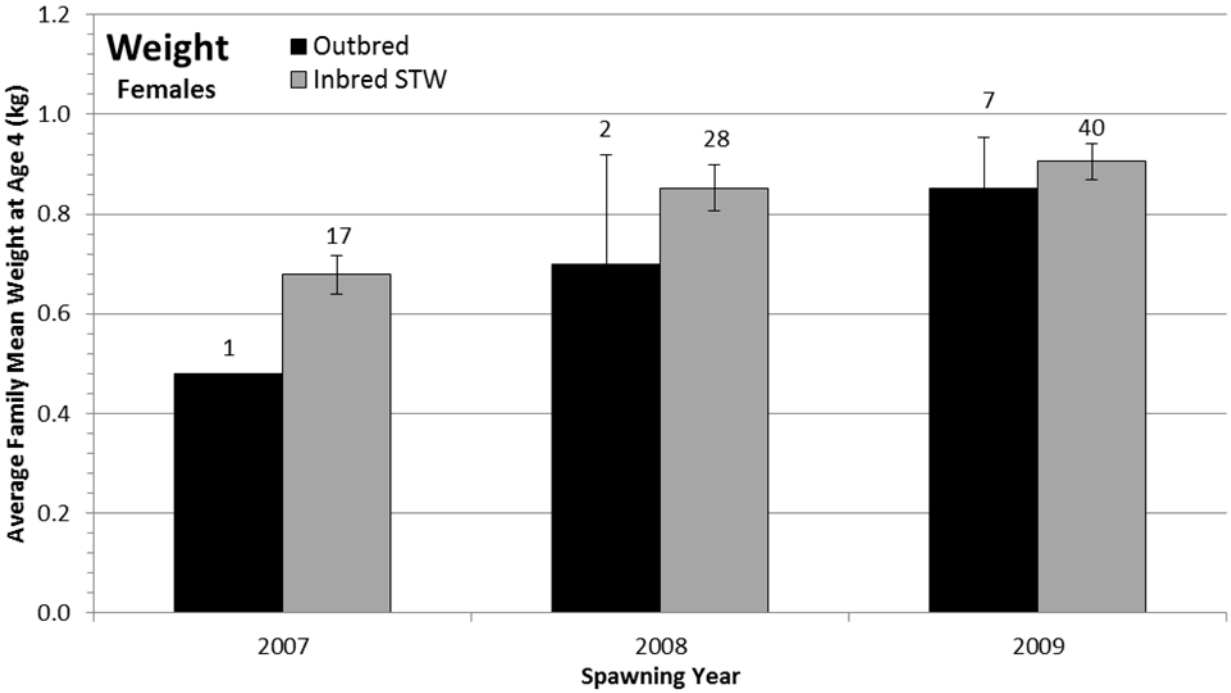


Figure 57. Average family mean weight (kg) at Age 4+, females only, for two parental cross types, North Minas Basin X Stewiacke (outbred) and Stewiacke X Stewiacke (inbred STW), for the spawning years 2007–2009. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Adult age used here is based on the brood or fertilization year of individuals.

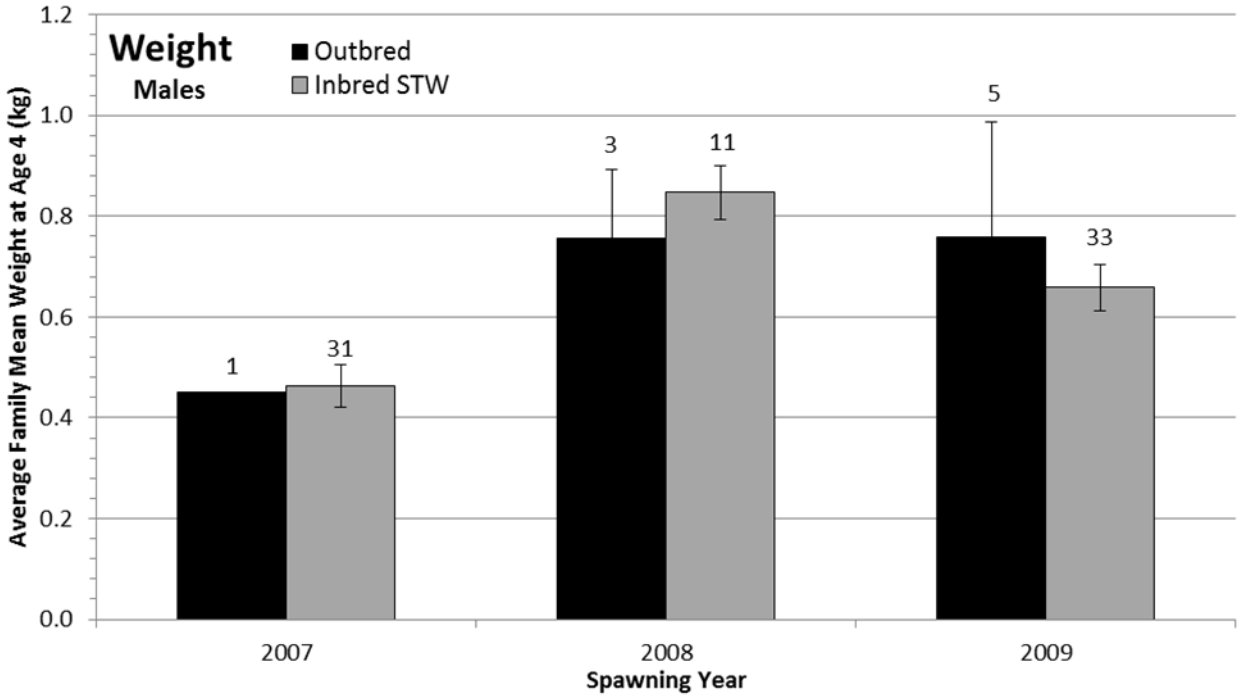


Figure 58. Average family mean weight (kg) at Age 4+, males only, for two parental cross types, North Minas Basin X Stewiacke (outbred) and Stewiacke X Stewiacke (inbred STW), for the spawning years 2007–2009. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Adult age used here is based on the brood or fertilization year of individuals.

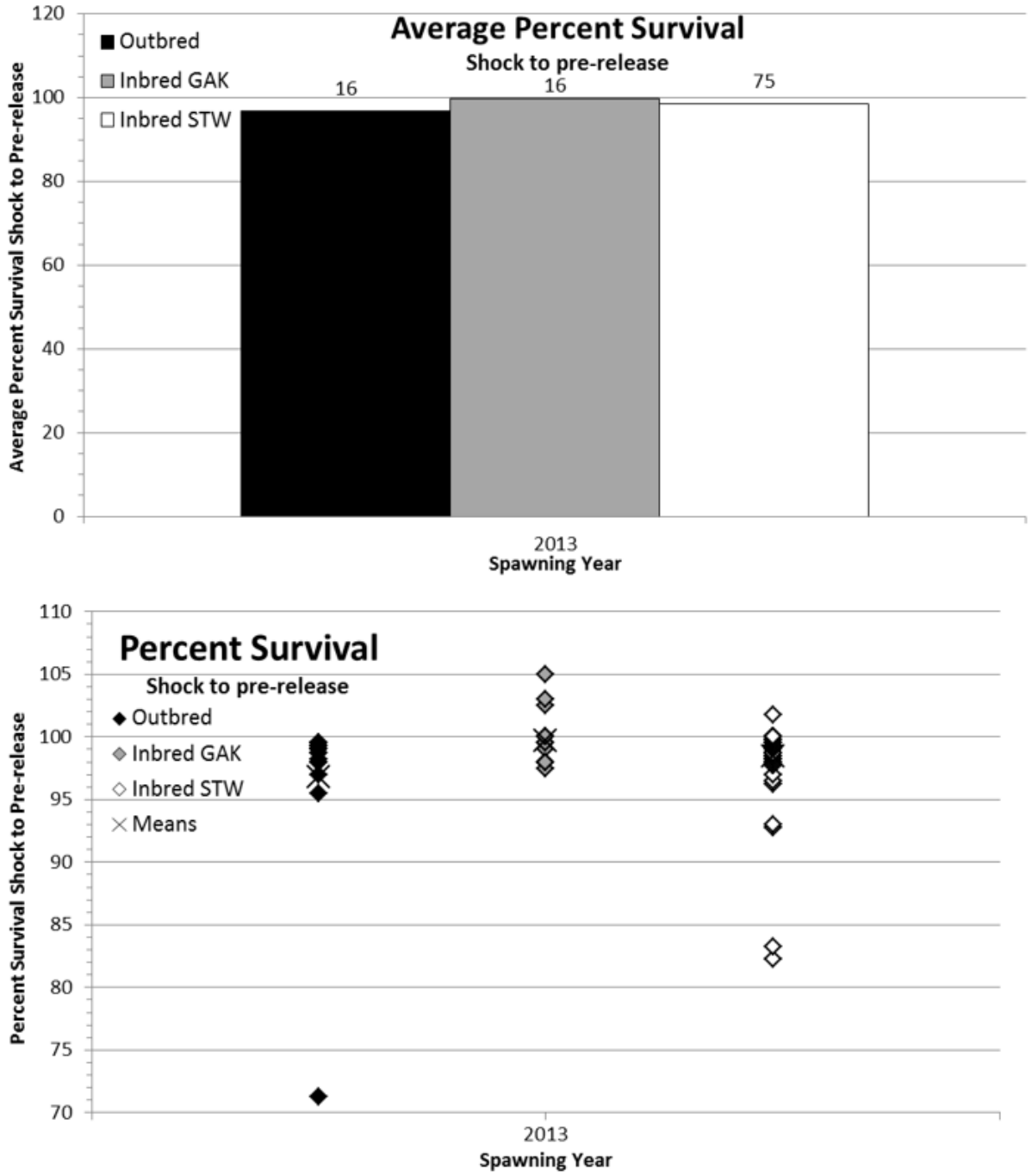


Figure 59. Average (upper) and individual (lower) family percent survival, from shock (at the egg stage, mid-development) to pre-release as Age 0+ fry, for three parental cross types, Gaspereau X Stewiacke (outbred), Gaspereau X Gaspereau (Inbred GAK), and Stewiacke X Stewiacke (Inbred STW) for the spawning year 2013. Sample sizes (number of families) for each group are indicated above their respective bars.

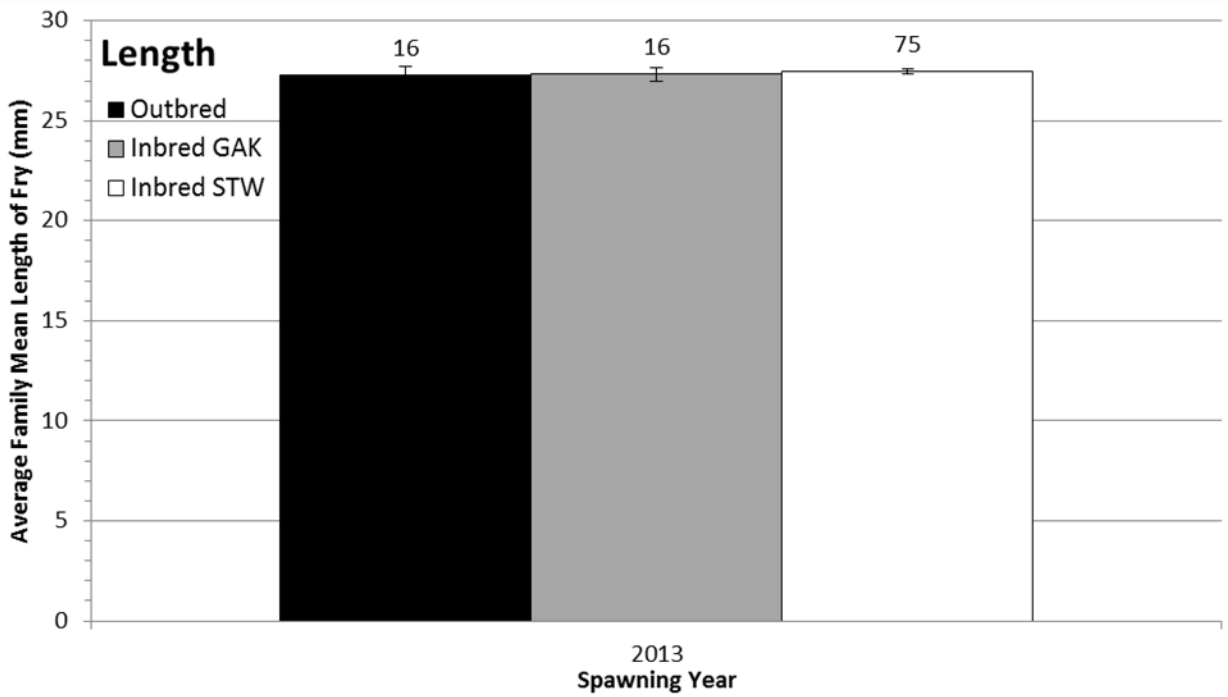


Figure 60. Average family mean length (mm) of Age 0+ fry for three parental cross types, Gaspereau X Stewiacke (outbred), Gaspereau X Gaspereau (Inbred GAK), and Stewiacke X Stewiacke (Inbred STW) for the spawning year 2013. Sample sizes (number of families) for each group are indicated above their respective bars.

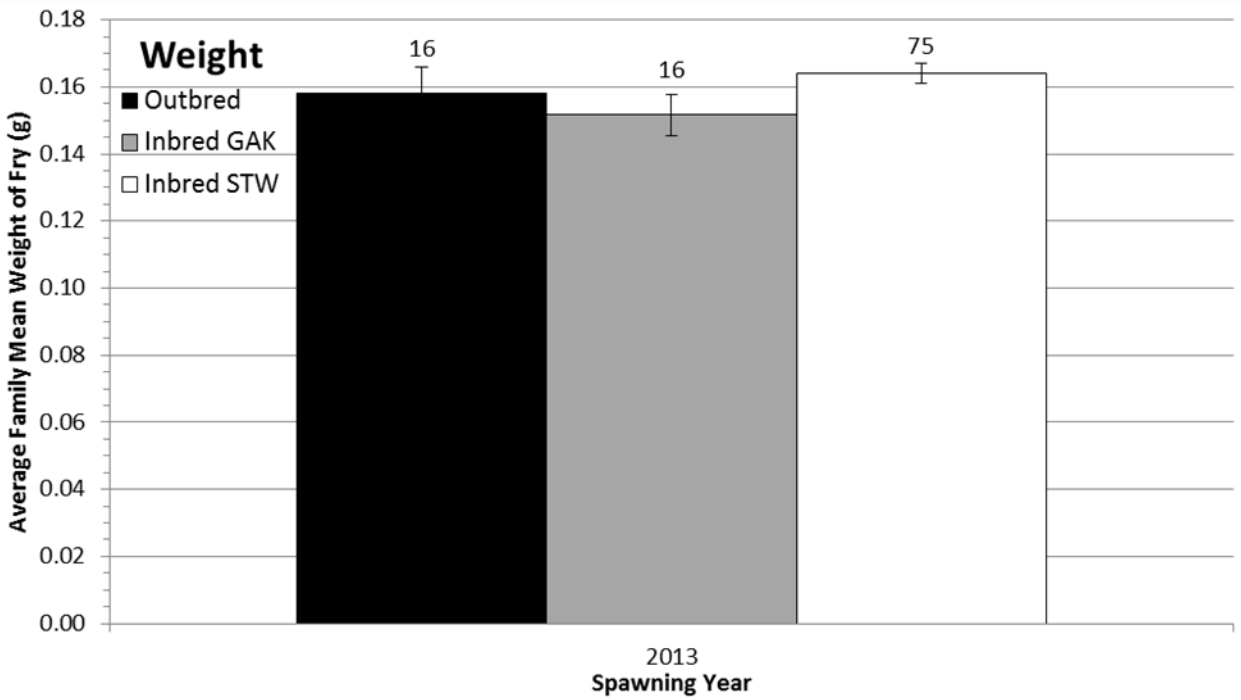


Figure 61. Average family mean weight (g) of Age 0+ fry for three parental cross types, Gaspereau X Stewiacke (outbred), Gaspereau X Gaspereau (Inbred GAK), and Stewiacke X Stewiacke (Inbred STW) for the spawning year 2013. Sample sizes (number of families) for each group are indicated above their respective bars.

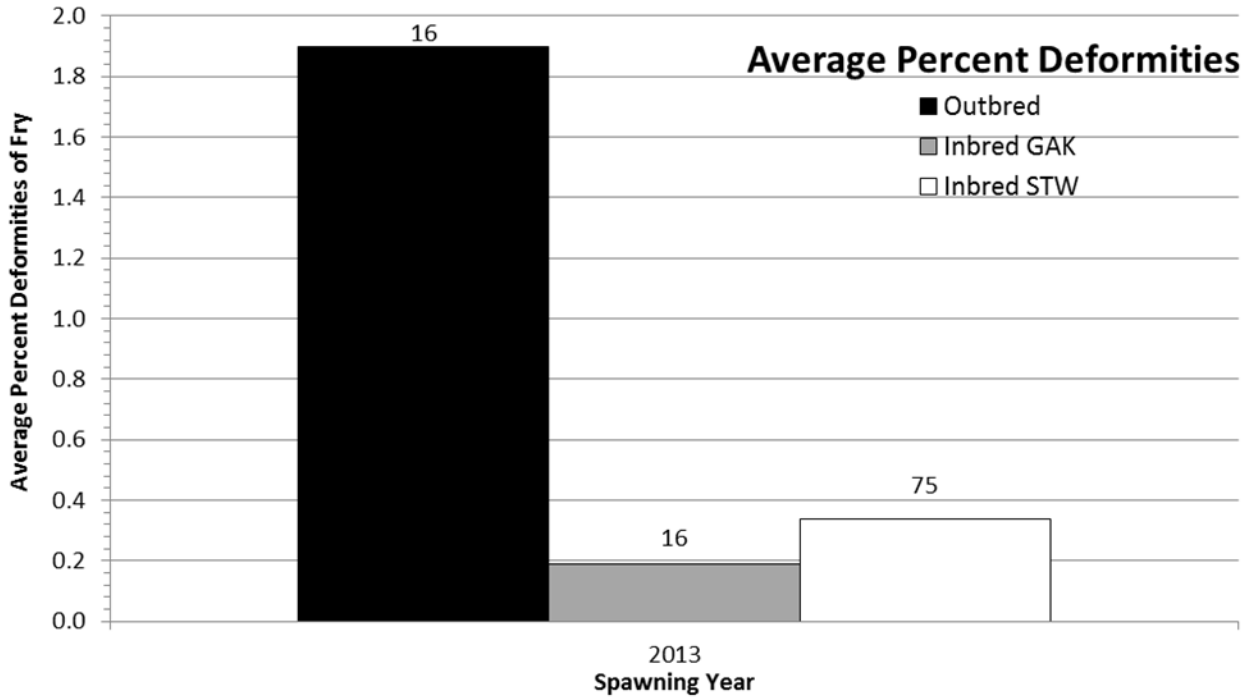


Figure 62. Average family percent deformities of Age 0+ fry for three parental cross types, Gaspereau X Stewiacke (outbred), Gaspereau X Gaspereau (Inbred GAK), and Stewiacke X Stewiacke (Inbred STW) for the spawning year 2013. Sample sizes (number of families) for each group are indicated above their respective bars.

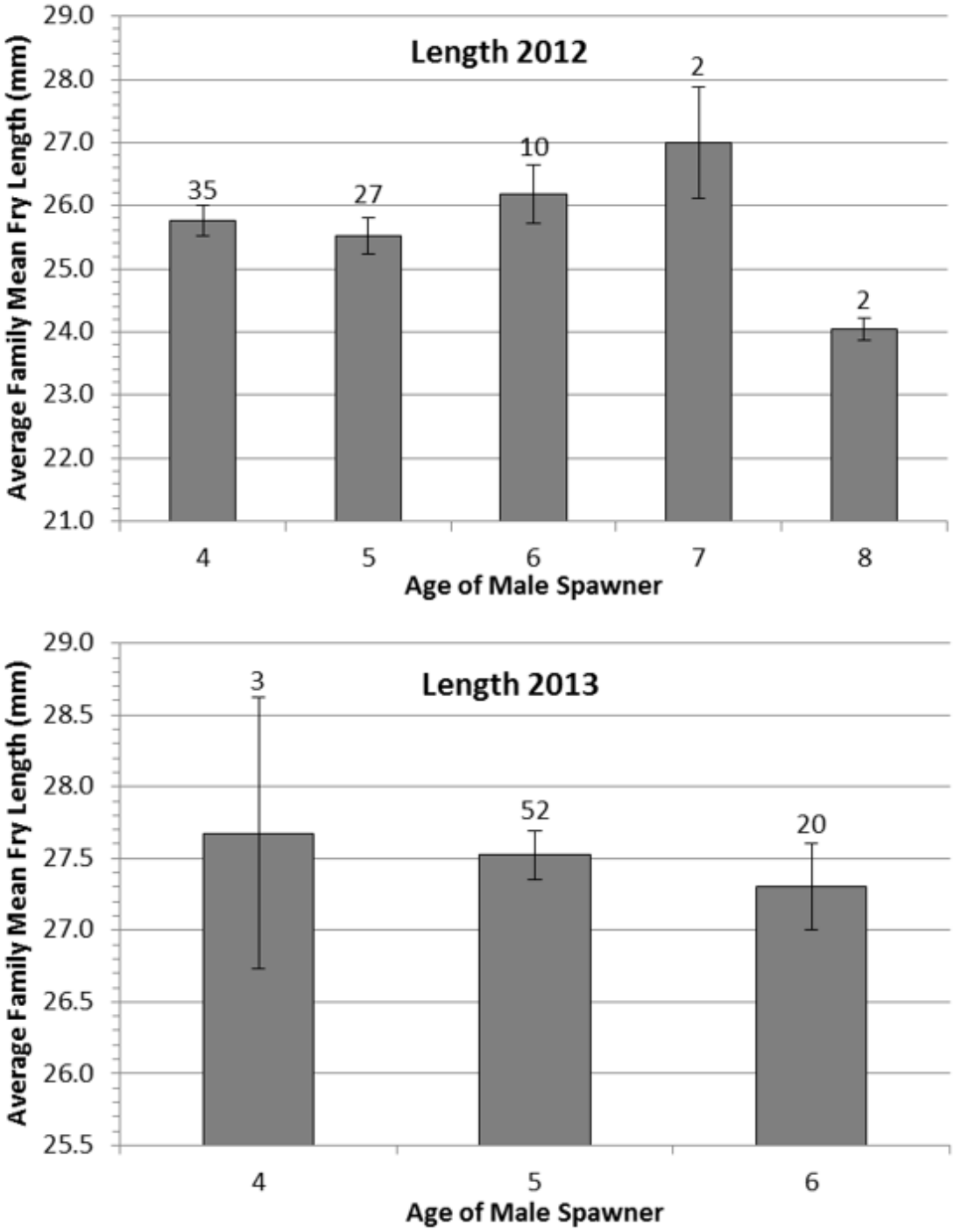
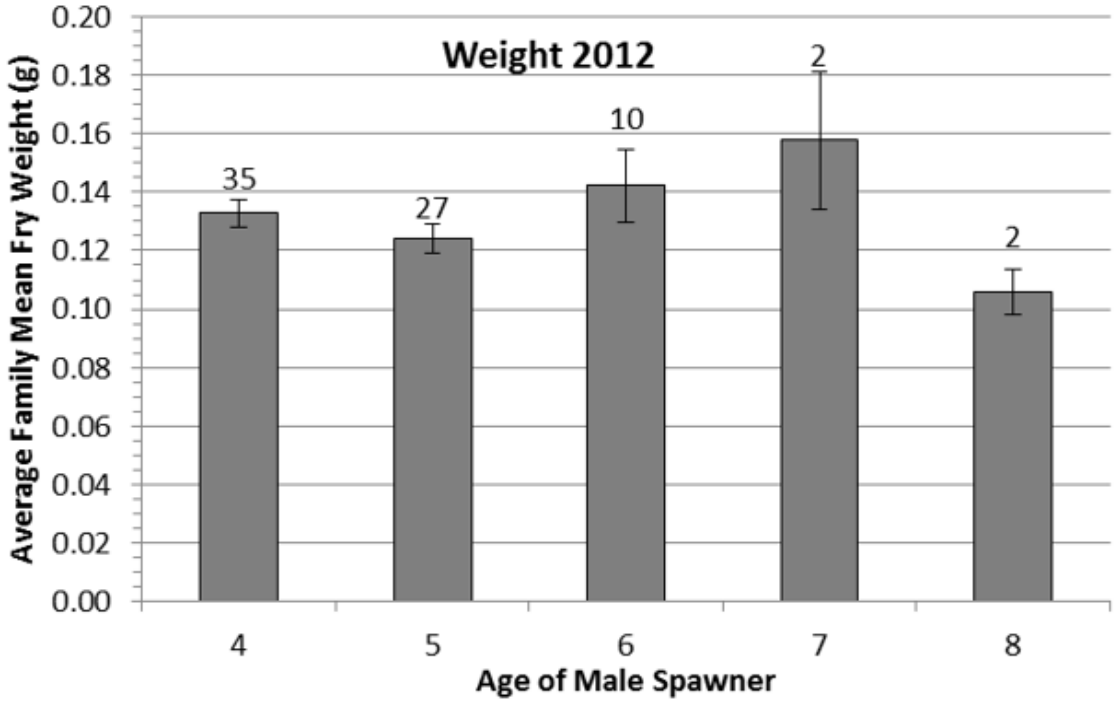


Figure 63. Average family mean length (mm) of Age 0+ fry versus age of the paternal parent at spawning, for spawning years 2012–2013. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Spawner age used here is based on the brood or fertilization year of individuals.



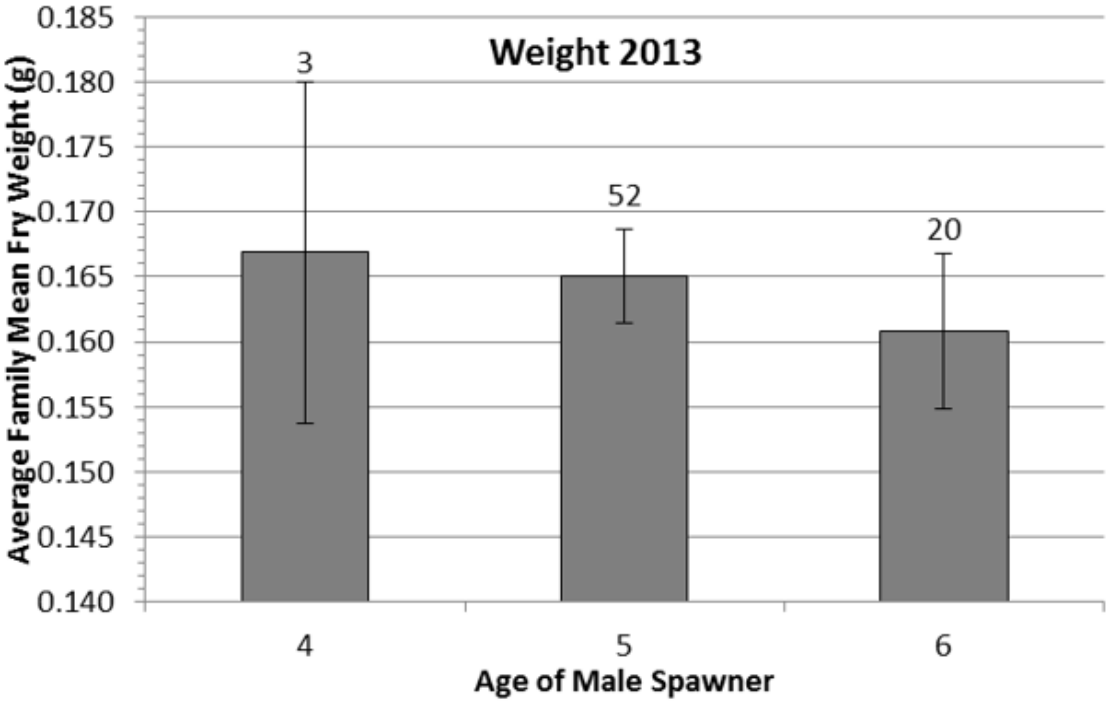


Figure 64. Average family mean weight (g) of age 0+ fry versus age of the paternal parent at spawning, for spawning years 2012–2013. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Spawner age used here is based on the brood or fertilization year of individuals.

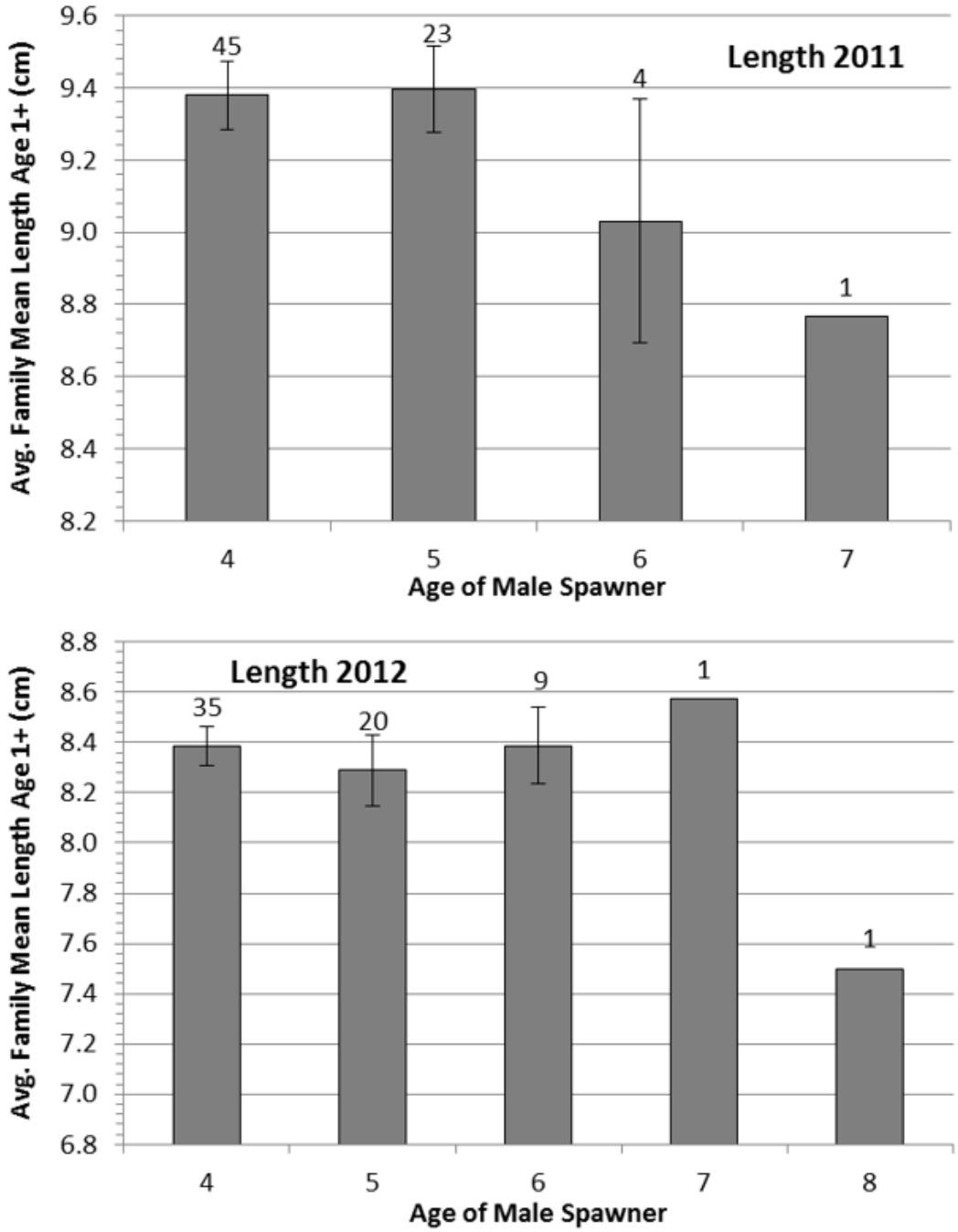


Figure 65. Average family mean length (cm) of Age 1+ parr versus paternal parent age at spawning, for spawning years 2011–2012. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Spawner age used here is based on the brood or fertilization year of individuals.

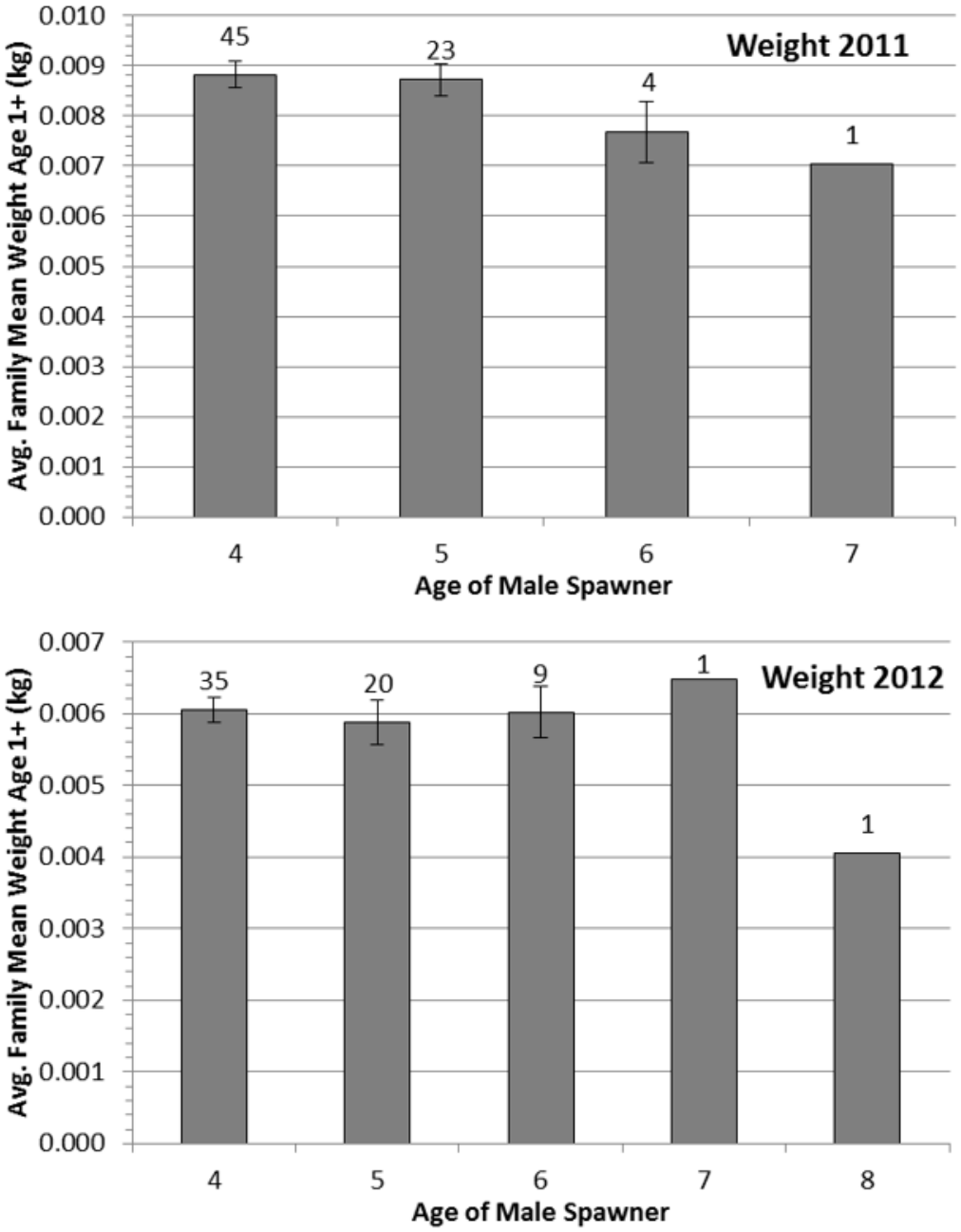


Figure 66. Average family mean weight (kg) of Age 1+ parr versus paternal parent age at spawning, for spawning years 2011–2012. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Spawner age used here is based on the brood or fertilization year of individuals.

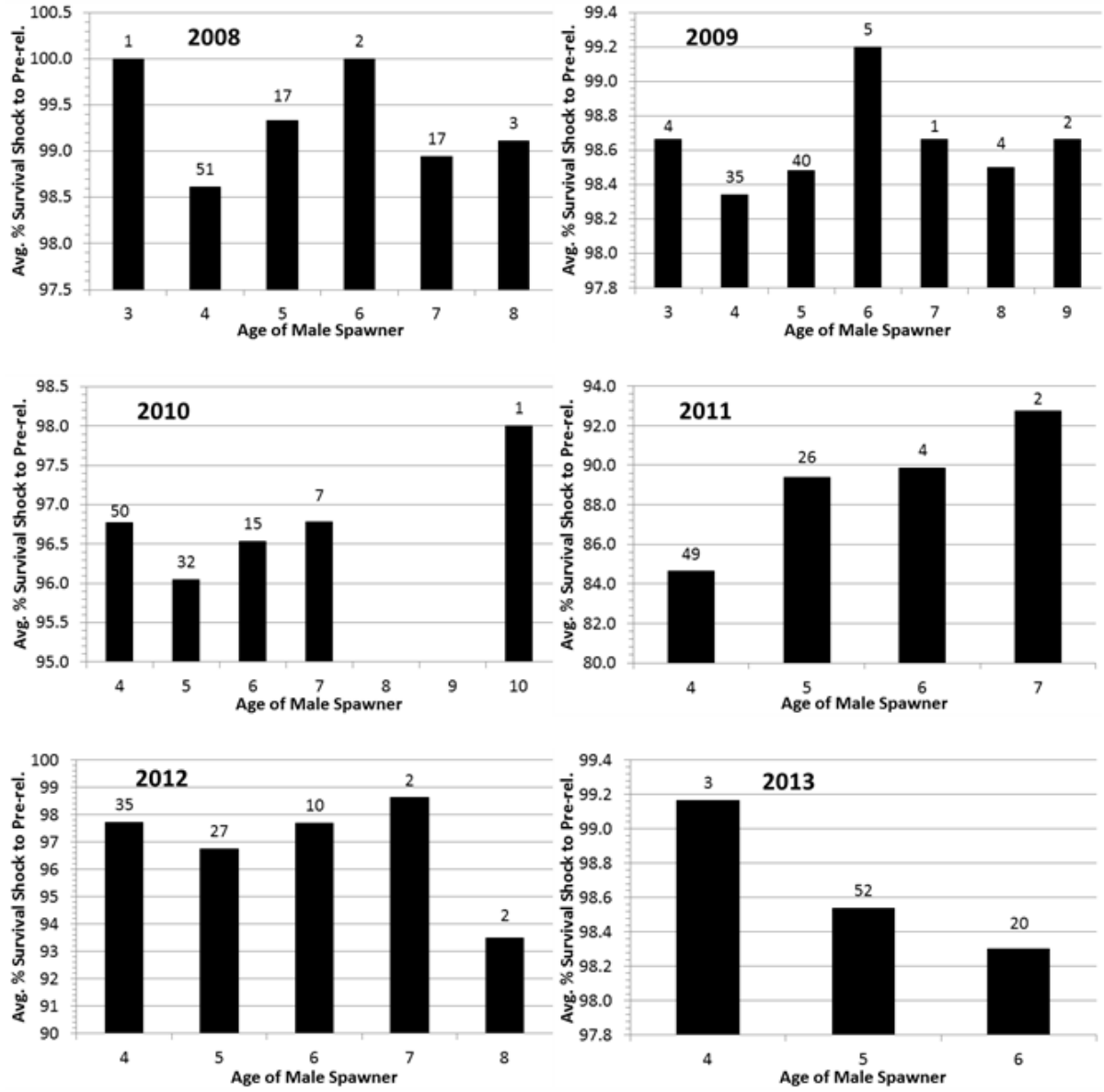


Figure 67. Average family percent survival from shock (at the egg stage, mid-development) to pre-release as Age 0+ fry versus paternal parent age at spawning, for spawning years 2008–2013. Sample sizes (number of families) for each group are indicated above their respective bars. Spawner age used here is based on the brood or fertilization year of individuals.

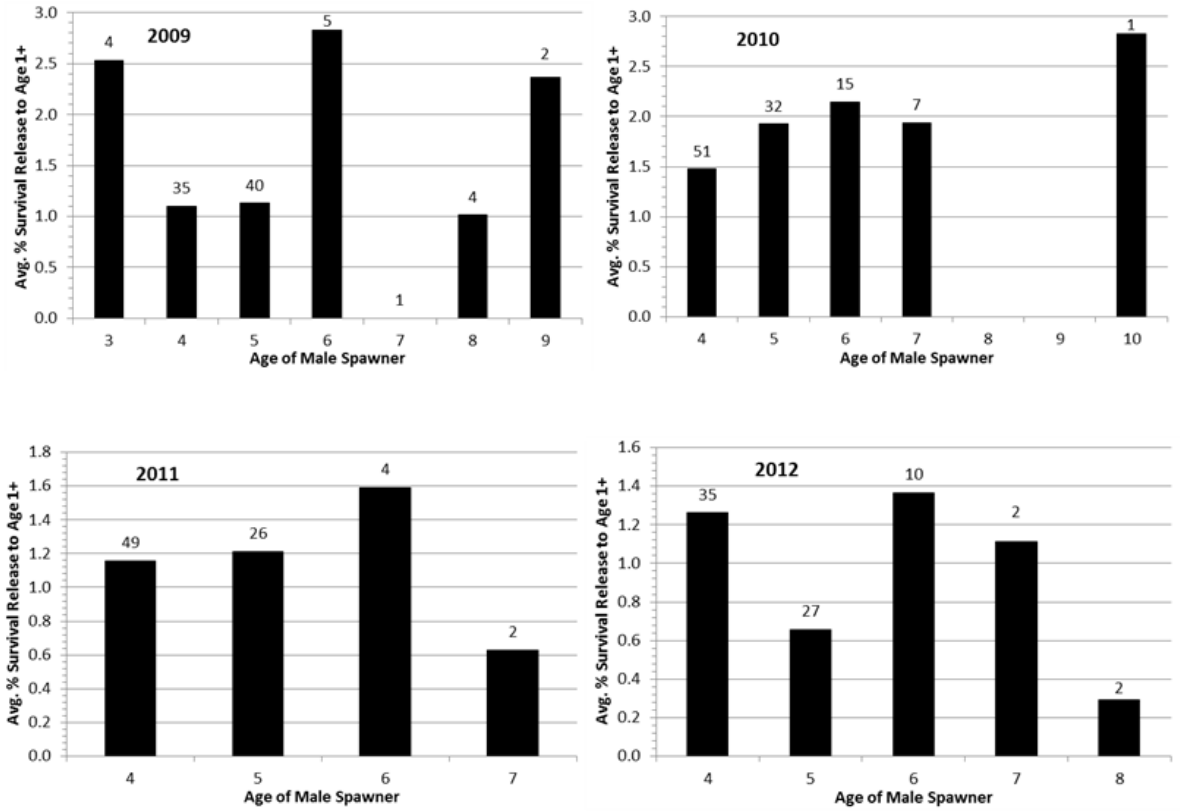


Figure 68. Average family percent survival from release as Age 0+ fry to capture as Age 1+ parr versus the paternal parent age at spawning, for spawning years 2009–2012. Sample sizes (number of families) for each group are indicated above their respective bars. Spawner age used here is based on the brood or fertilization year of individuals.

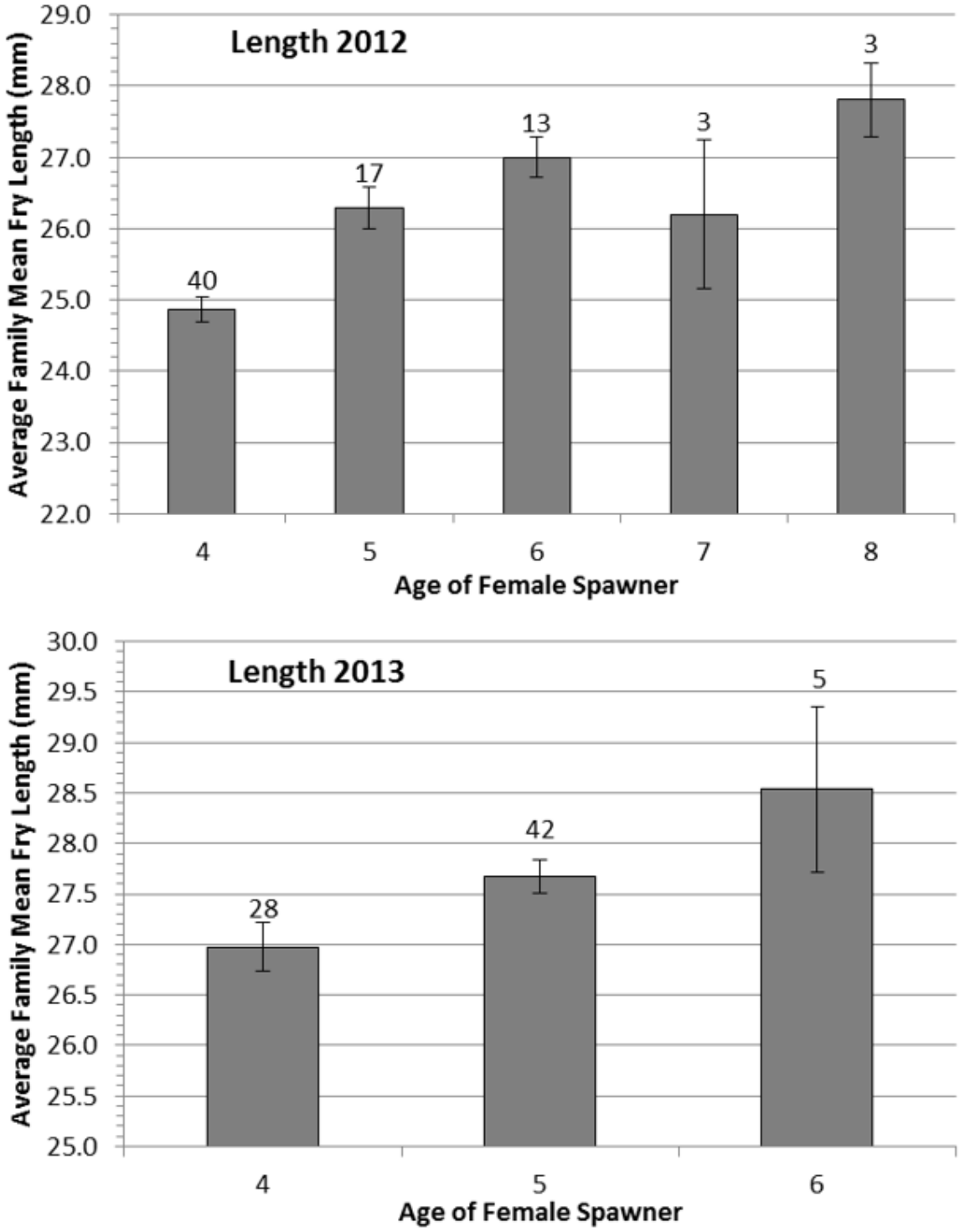


Figure 69. Average family mean length (mm) of Age 0+ fry versus maternal parent age at spawning, for spawning years 2012–2013. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Spawner age used here is based on the brood or fertilization year of individuals.

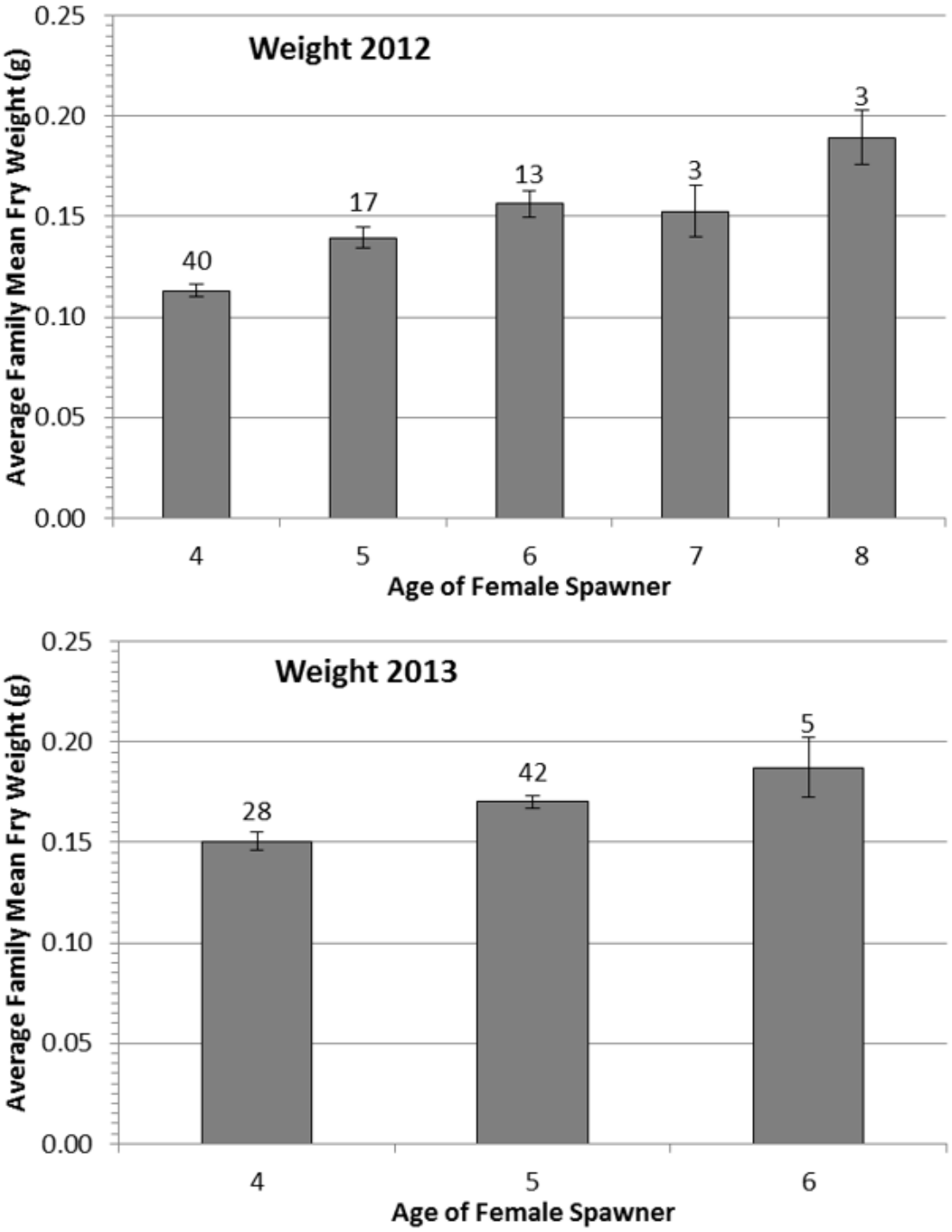


Figure 70. Average family mean weight (g) of Age 0+ fry versus maternal parent age at spawning, for spawning years 2012–2013. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Spawner age used here is based on the brood or fertilization year of individuals.

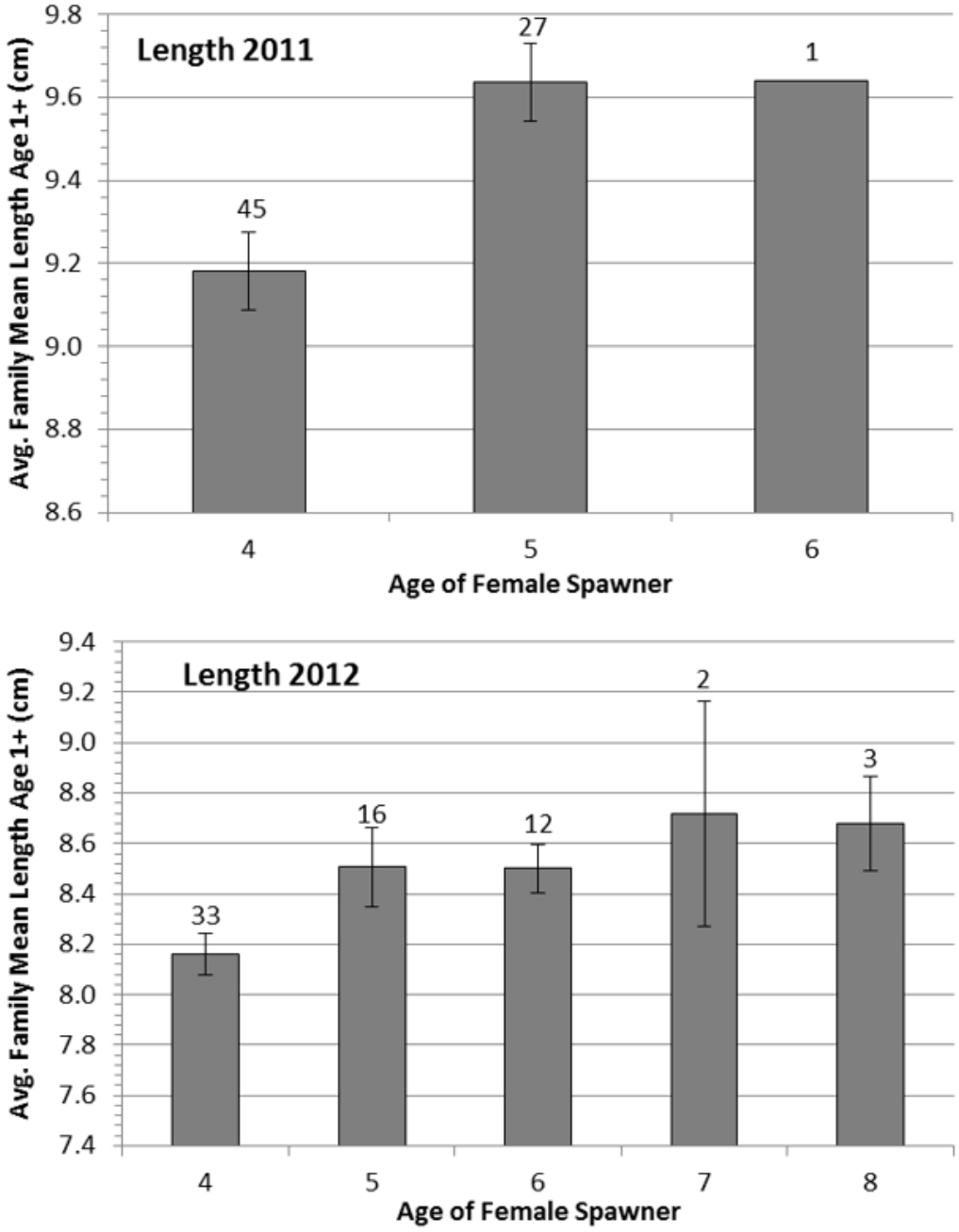


Figure 71. Average family mean length (cm) of Age 1+ parr versus maternal parent age at spawning, for spawning years 2011–2012. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Spawner age used here is based on the brood or fertilization year of individuals.

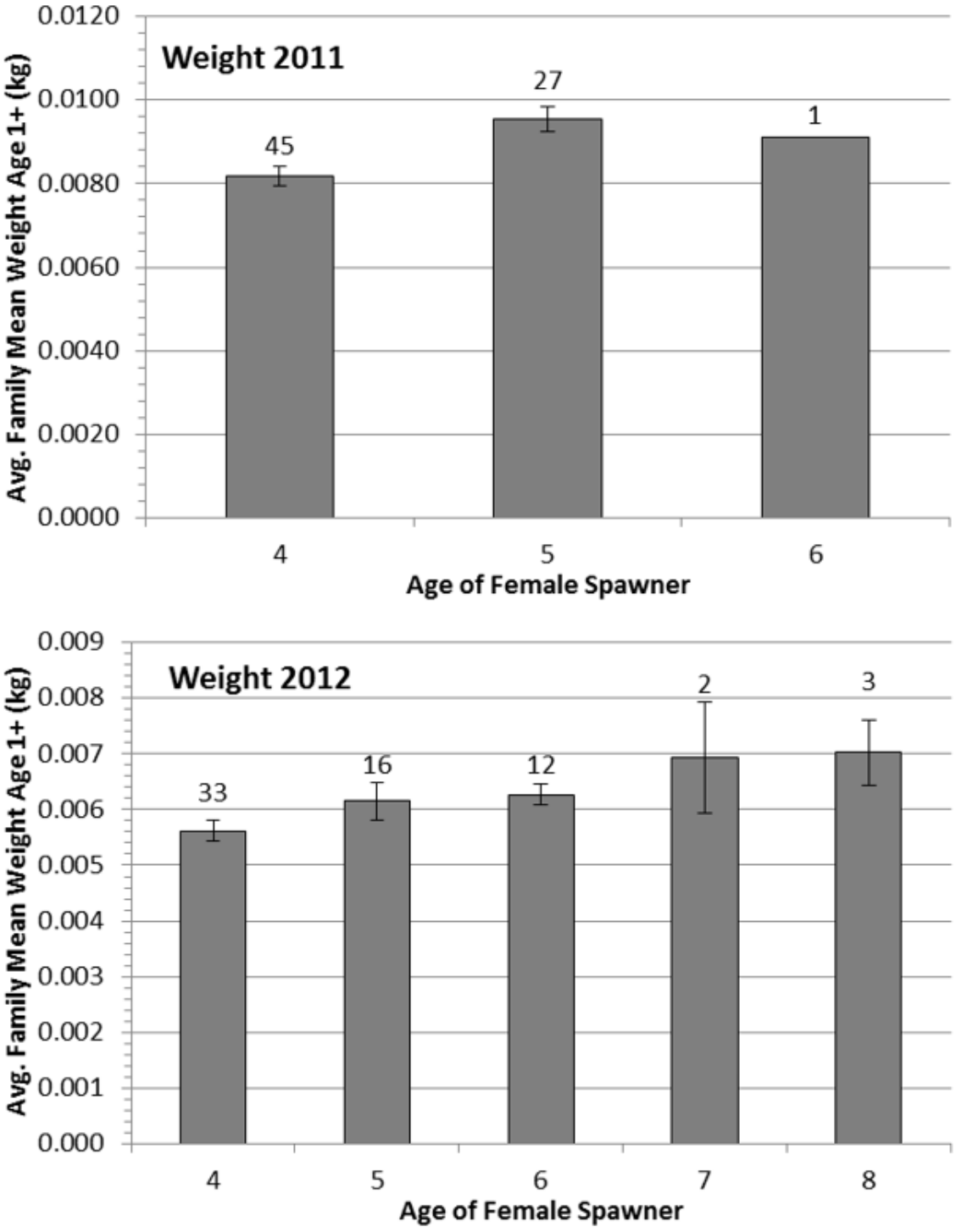


Figure 72. Average family mean weight (kg) of Age 1+ parr versus maternal parent age at spawning, for spawning years 2011–2012. Sample sizes (number of families) for each group are indicated above their respective bars. Error bars represent one standard error. Spawner age used here is based on the brood or fertilization year of individuals.

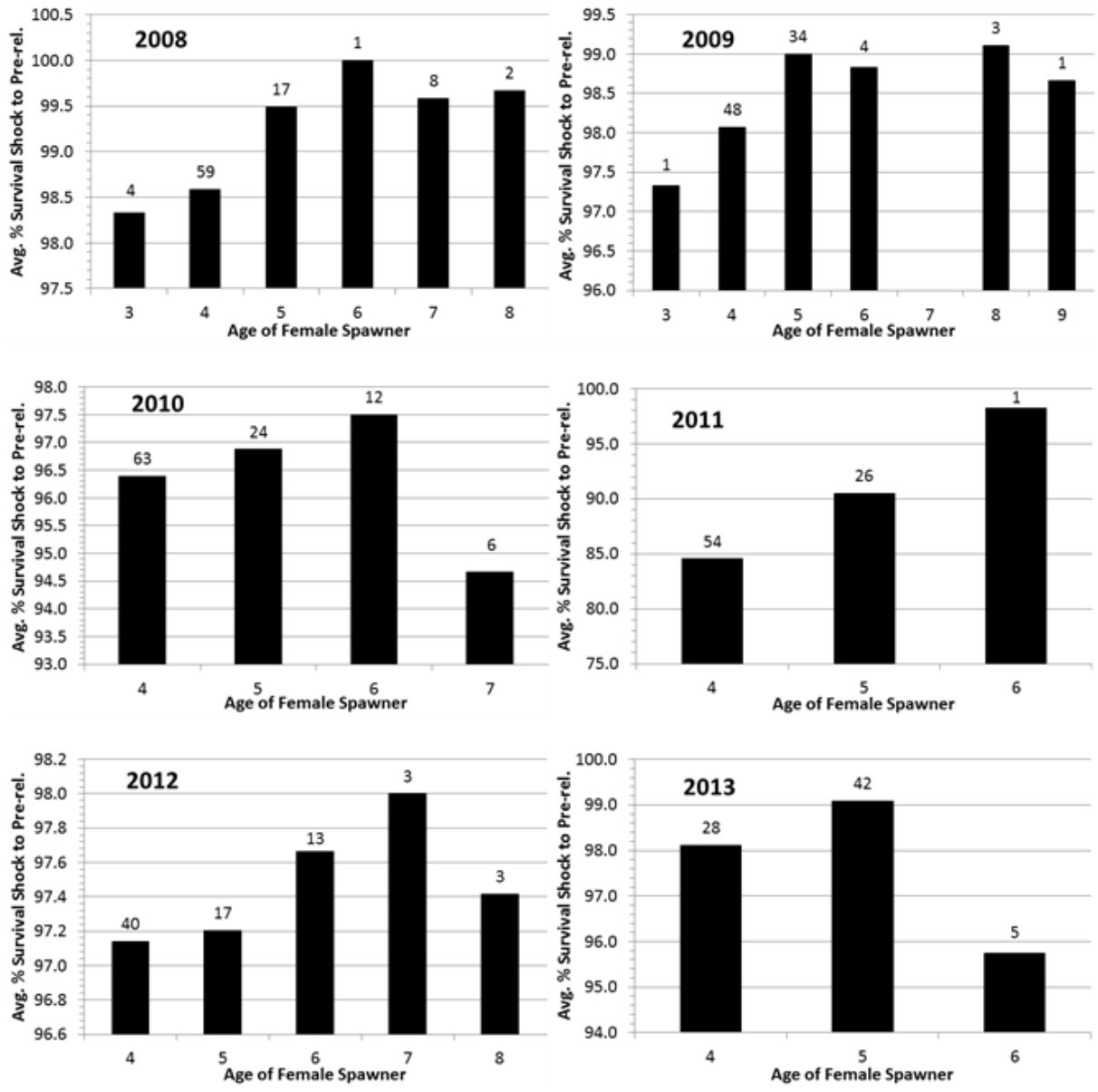


Figure 73. Average family percent survival from shock (at the egg stage, mid-development) to pre-release as Age 0+ fry versus maternal parent age at spawning, for spawning years 2008–2013. Sample sizes (number of families) for each group are indicated above their respective bars. Spawner age used here is based on the brood or fertilization year of individuals.

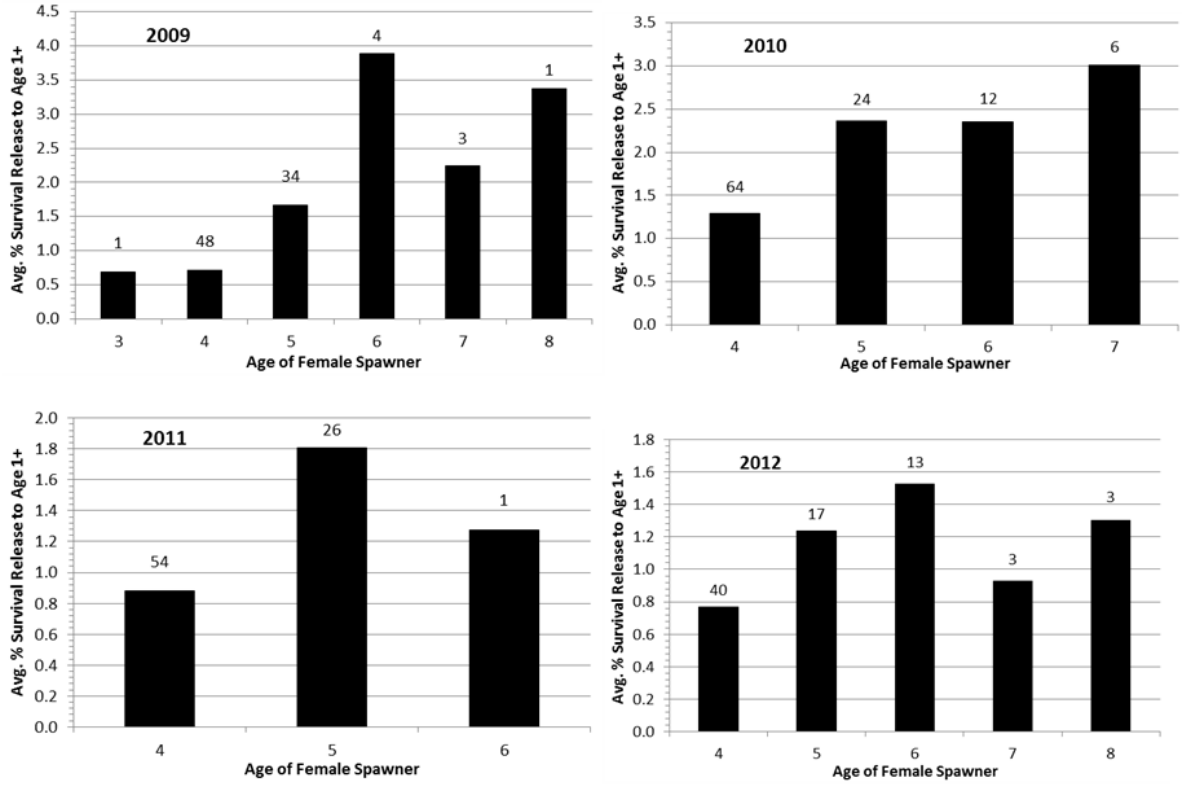


Figure 74. Average family percent survival from release as Age 0+ fry to capture as Age 1+ parr versus maternal parent age at spawning, for spawning years 2009–2012. Sample sizes (number of families) for each group are indicated above their respective bars. Spawner age used here is based on the brood or fertilization year of individuals.

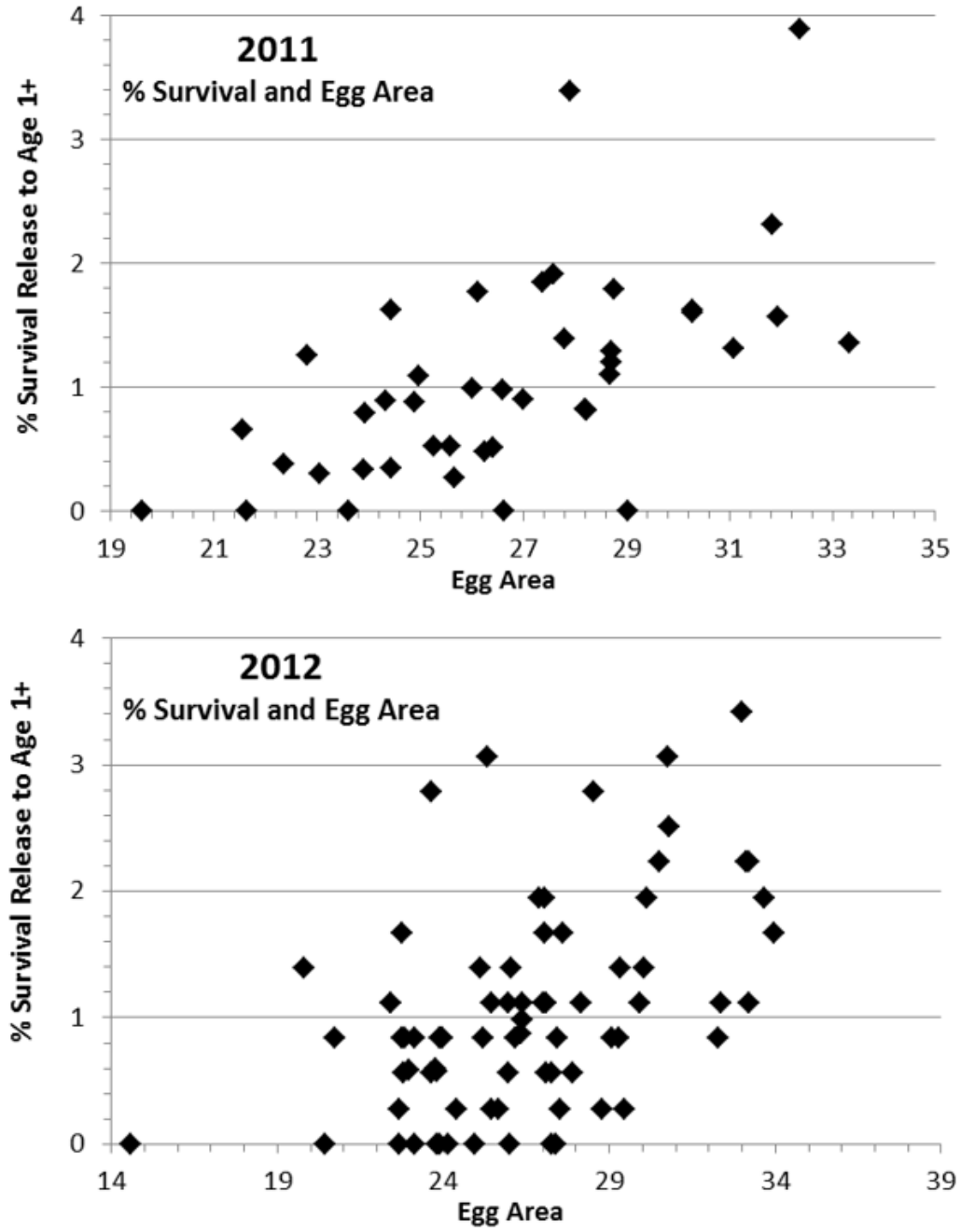


Figure 75. Family percent survival from release as Age 0+ fry to capture as Age 1+ parr versus the average family egg area (mm^2), for spawning years 2011–2012.

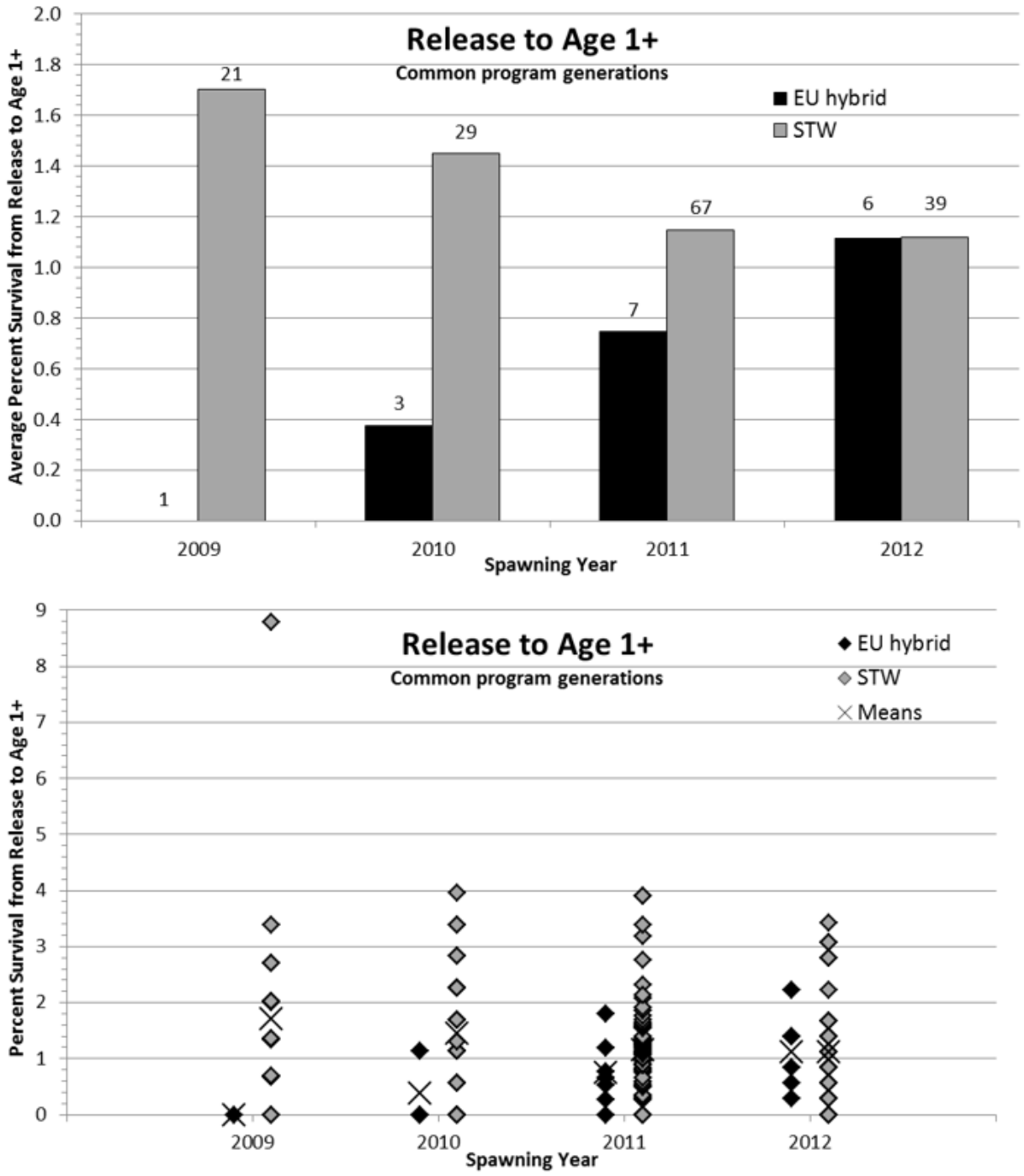


Figure 76. Average (upper panel) and individual (lower panel) family percent survival from release as Age 0+ fry to capture as Age 1+ parr, for European farm/Stewiacke hybrids (EU hybrid) and pure Stewiacke (STW) lineage salmon for the spawning years 2009–2012. Only those Stewiacke families exhibiting a similar number of program generations as the European farm/Stewiacke hybrids families compared in a given year were included. Sample sizes (number of families) for each group are indicated above their respective bars.

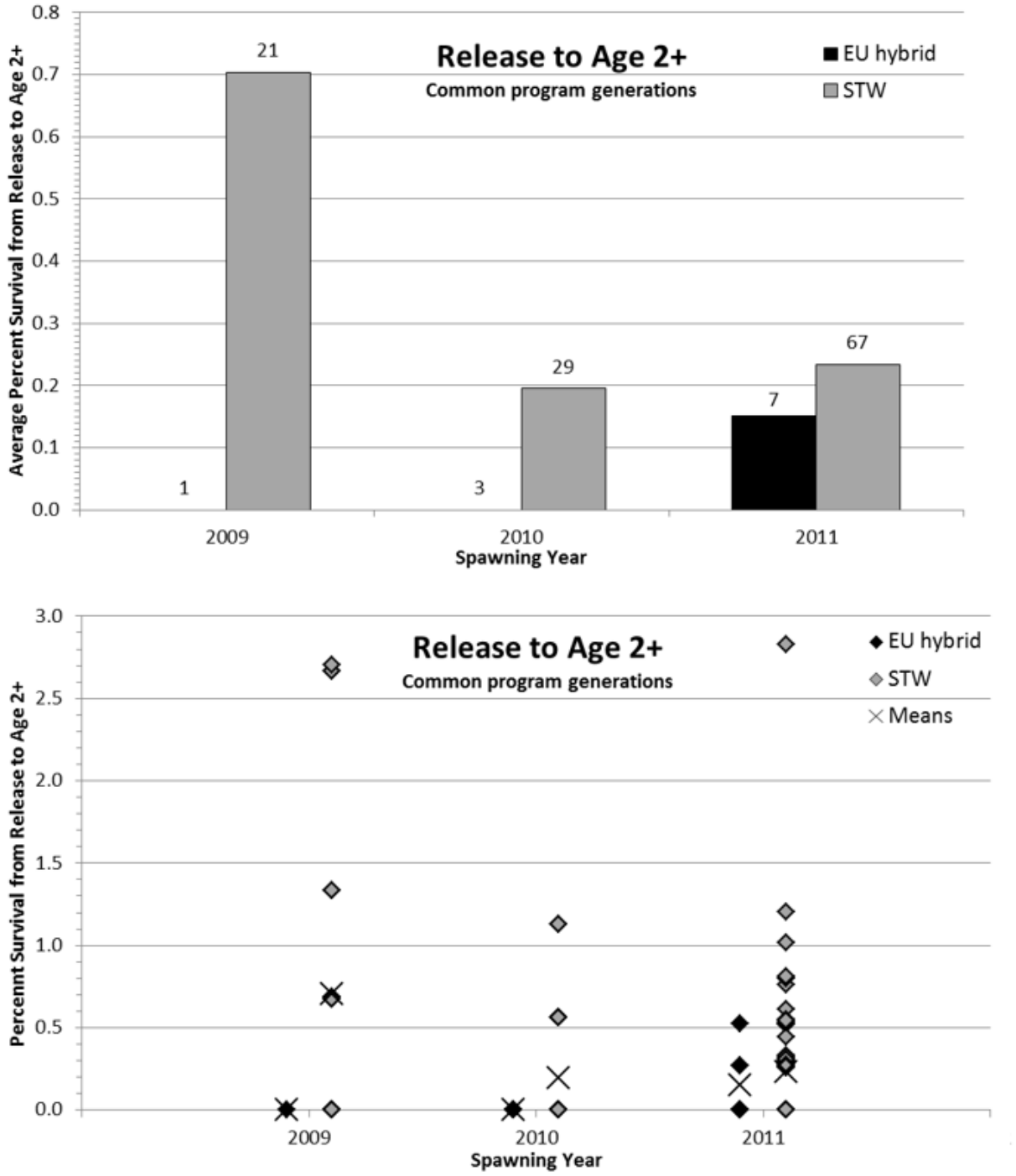


Figure 77. Average (upper panel) and individual (lower panel) family percent survival from release as Age 0+ fry to capture as Age 2+ parr, for European farm/Stewiacke hybrids (EU hybrid) and pure Stewiacke (STW) lineage salmon for the spawning years 2009–2012. Only those Stewiacke families exhibiting a similar number of program generations as the European farm/Stewiacke hybrids families compared in a given year were included. Sample sizes (number of families) for each group are indicated above their respective bars.

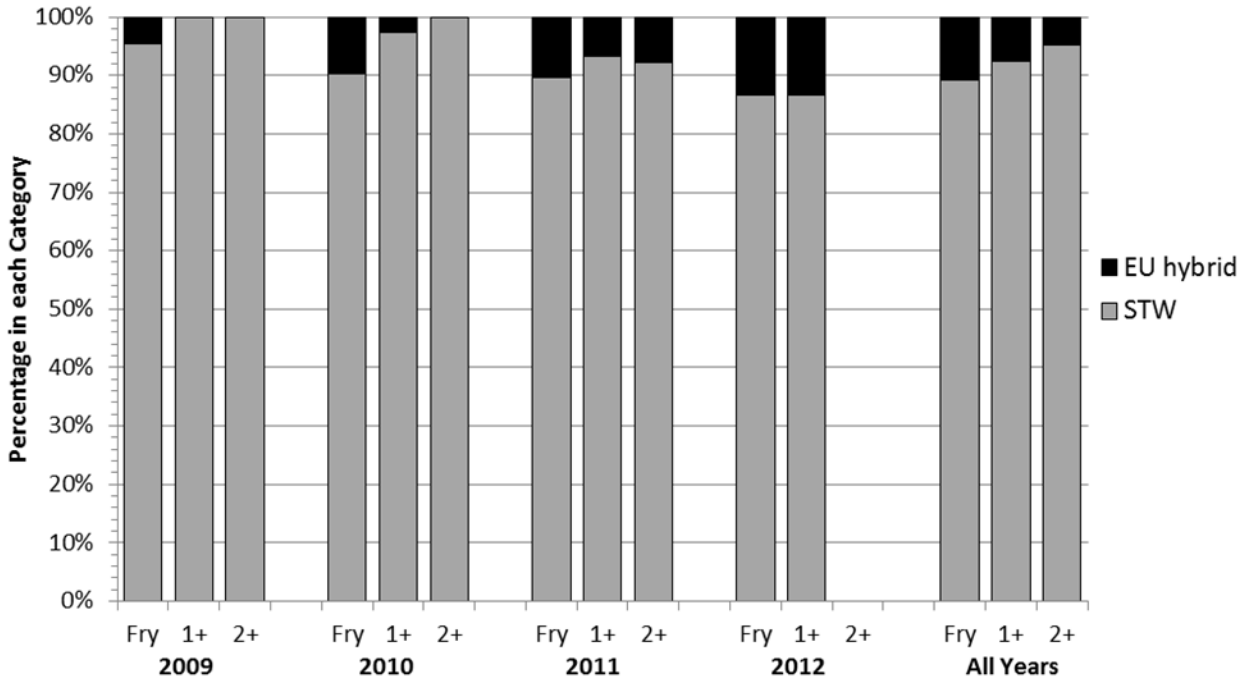


Figure 78. Percentage Age 0+ released fry (Fry), captured Age 1+ parr (1+), and captured Age 2+ parr (2+) groups comprised of European farm/Stewiacke hybrid (EU hybrid) and pure Stewiacke lineage (STW) salmon, for the spawning years 2009–2012 (and all four spawning years combined). Only those Stewiacke families exhibiting a similar number of program generations as the European farm/Stewiacke hybrid families compared in a given year were included. Data for Age 2+ parr for the 2012 spawning year were not yet available when these analyses were carried out.

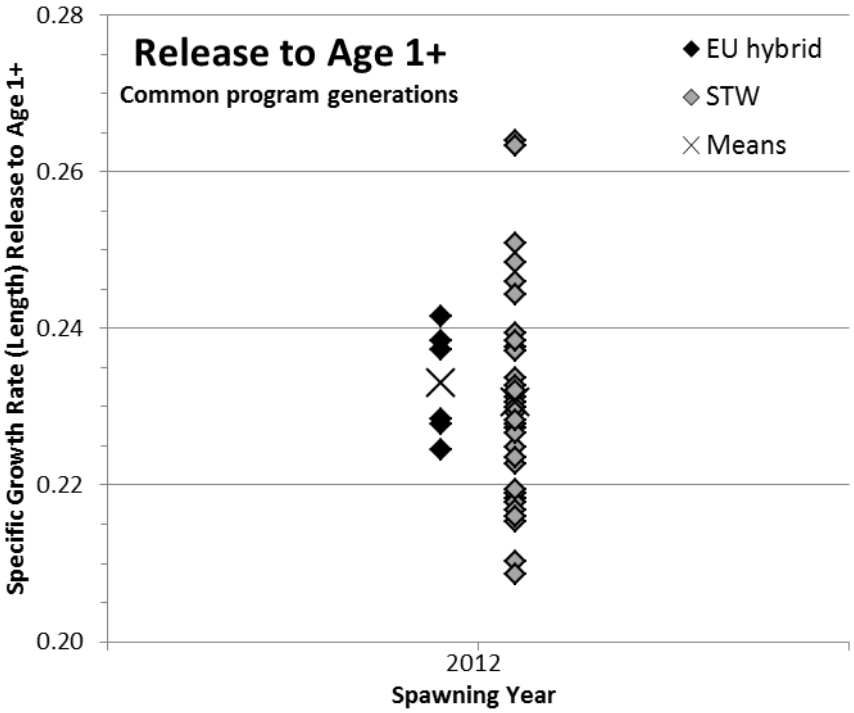


Figure 79. Family specific growth rate (length) from release as Age 0+ fry to capture as Age 1+ parr for European farm/Stewiacke hybrids (EU hybrid) and pure Stewiacke (STW) salmon for the spawning year 2012. Only those Stewiacke families exhibiting a similar number of program generations as the European farm/Stewiacke hybrids (EU hybrid) families compared in a given year were included.

APPENDIX

Table A1. Egg and fry numbers for Equalized Family Groups reared at Coldbrook Biodiversity Facility and released into an isolated stretch of the Pembroke River, Stewiacke River system, and later collected as wild-exposed parr for the Live Gene Bank Program

Spawning Year	Eggs Per Family	Number of Baskets	Equalized as Fry (Y/N)	Number to Equalize to	EQU Fry Released	Collection Year (1+ parr)	Total Parr Collected (1+ and 2+)**	Target Number	Number of Days Electro-fishing	Number of Teams Electro-fishing
2006	110	1	N	NA	18,721*	2008	148	150	1	1
2007	120	1	N	NA	16,508*	2009	177	150	1	1
2008	150	1	N	NA	14,083	2010	158	150	1	1
2009	150	1	N	NA	30,150	2011	390	400	1	2
2010	200	1	N	NA	19,081	2012	455	400	1	2
2011	400	2	N	NA	32,784	2013	460	400	1	2
2012	400	2	Y	359	33,822	2014	531	500	1	2
2013	400	2	Y	376	39,665	2015	NA	NA	NA	NA
2014	400	2	Y	356	35,927	2016	NA	NA	NA	NA
2015	400	2	N	NA	38,496	2017	NA	NA	NA	NA

*Exact release numbers were not available therefore totals here are the total number of eggs collected from all the families at shock. Average survival for the time period was 96.6%.
**Total parr collected may not match with data found in other sections as this number includes mortalities prior to tagging, untagged fish, non-genotyped fish, and a few Age 0+ and trout that may have been mistakenly collected. Other sections report only genotyped individuals.
NA indicates not applicable (no equalization done).

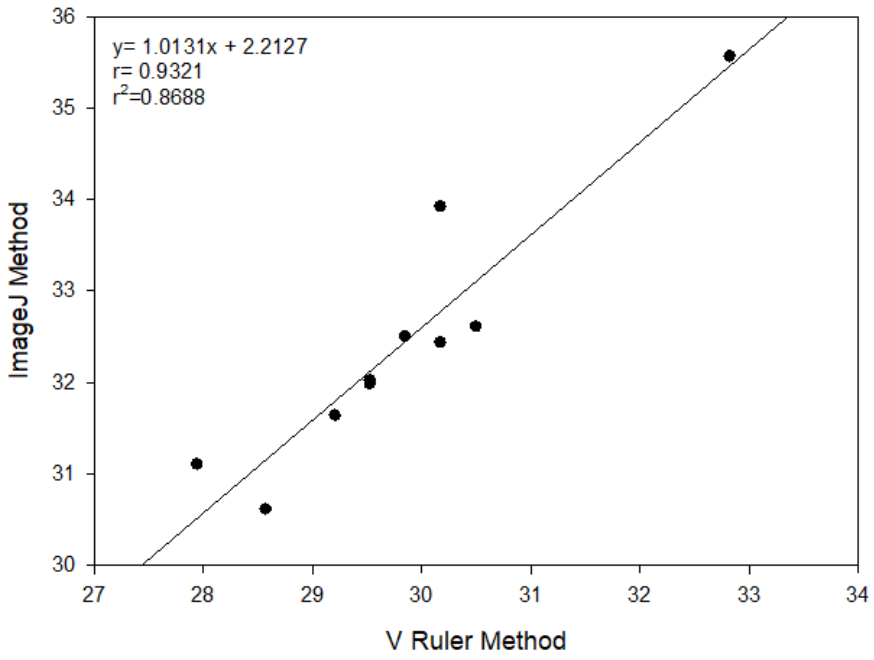


Figure A1. Family-specific regression and correlation of Atlantic Salmon egg areas determined from the two methodologies used throughout the Live Gene Bank program to calculate egg area (V ruler versus images). At the well eyed stage, also referred to as shock (approximately 3 months after spawning), 3 replicates of 10 eggs each were measured for a total diameter using a specialized V-shaped ruler. The average egg radius was determined and the area was calculated and compared to egg areas determined from images of the same 30 eggs using the ImageJ software. The equation was used to correct data collected in 2002–2009 from eggs measured at shock with the V-ruler method.

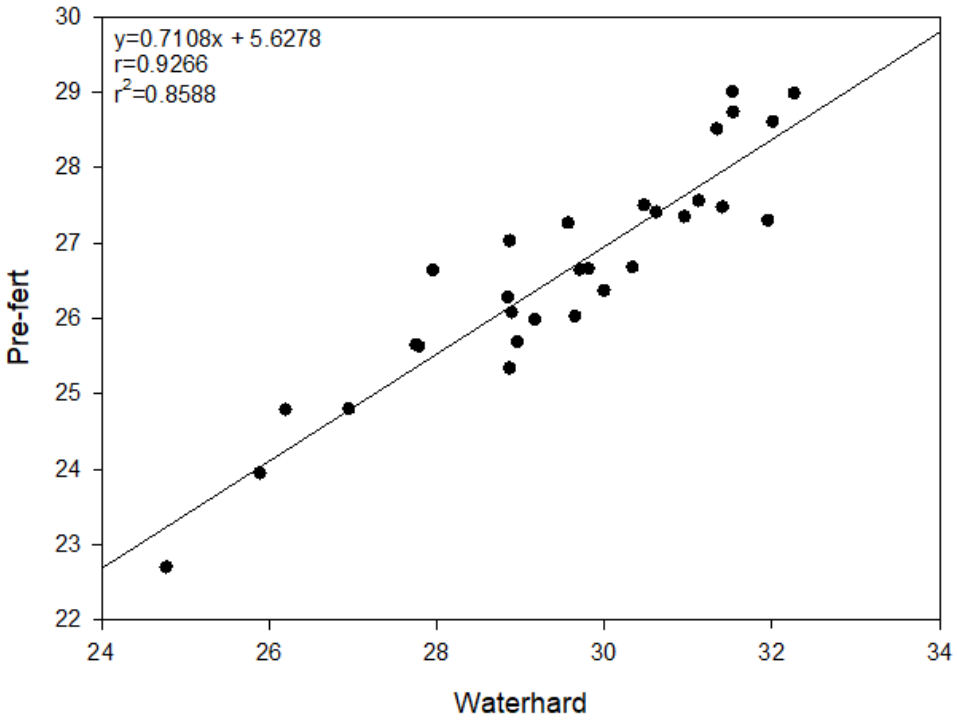


Figure A2. Family-specific regression and correlation of Live Gene Bank Atlantic Salmon egg areas calculated from images taken at pre-fertilization and after water hardening. Equation was used to correct data collected in 2011 from images taken after water hardening and standardize to a pre-fertilization stage.

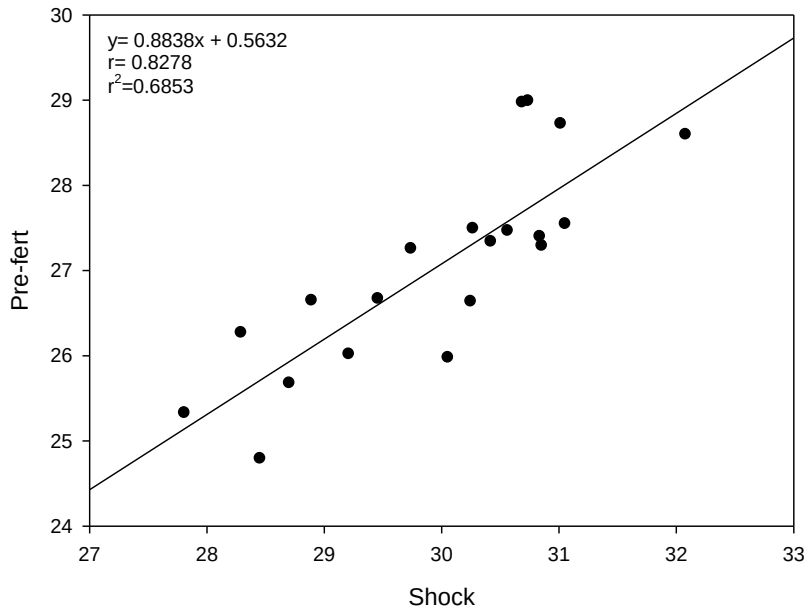


Figure A3. Family-specific regression and correlation of Live Gene Bank Atlantic Salmon egg areas calculated from images taken at pre-fertilization and at the well eyed stage, also referred to as shock (approximately 3 months after spawning). Equation was used to correct data collected in 2002 – 2009 from eggs measured at shock and standardize to a pre-fertilization stage.

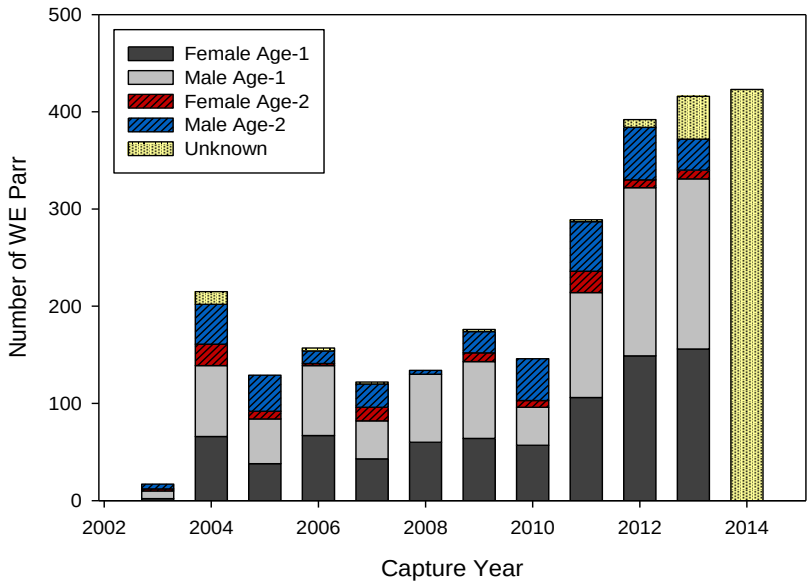


Figure A4. Gender breakdown of Age 1 and Age 2 wild-exposed (WE) Live Gene Bank (LGB) Atlantic Salmon parr collected from the wild and plotted by capture year (rather than brood year). In later years, WE individuals came from the isolated section of the Pembroke River (seeded with WE fry from the LGB program); however prior to 2008, WE parr were collected from various locations throughout the Stewiacke river system.

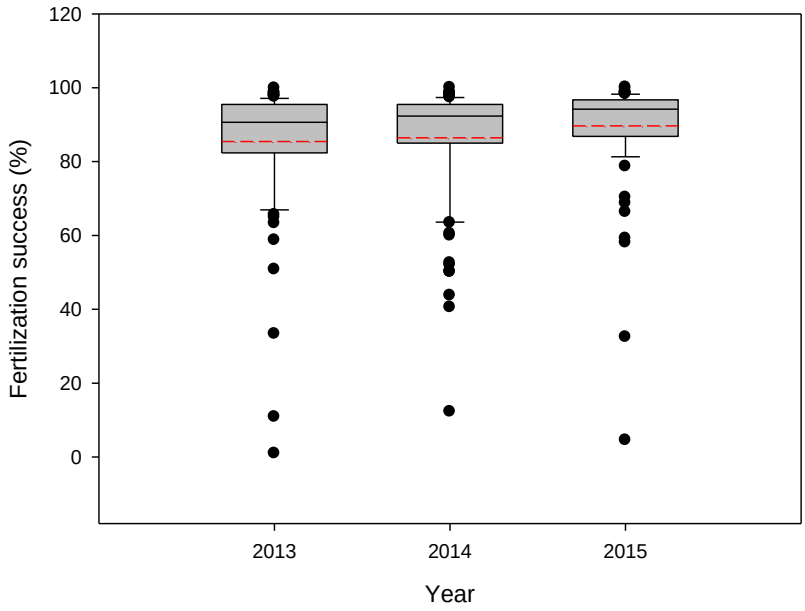


Figure A5. Average percent fertilization rate for Live Gene bank families taken from subsamples (20 eggs) of Atlantic Salmon egg mortalities at the well eyed stage (i.e. shock) and cleared in Stockard's solution. The box plots represent the 10th, 25th, 75th, 90th percentiles while the solid black line represents the median and the red dashed line is the mean.

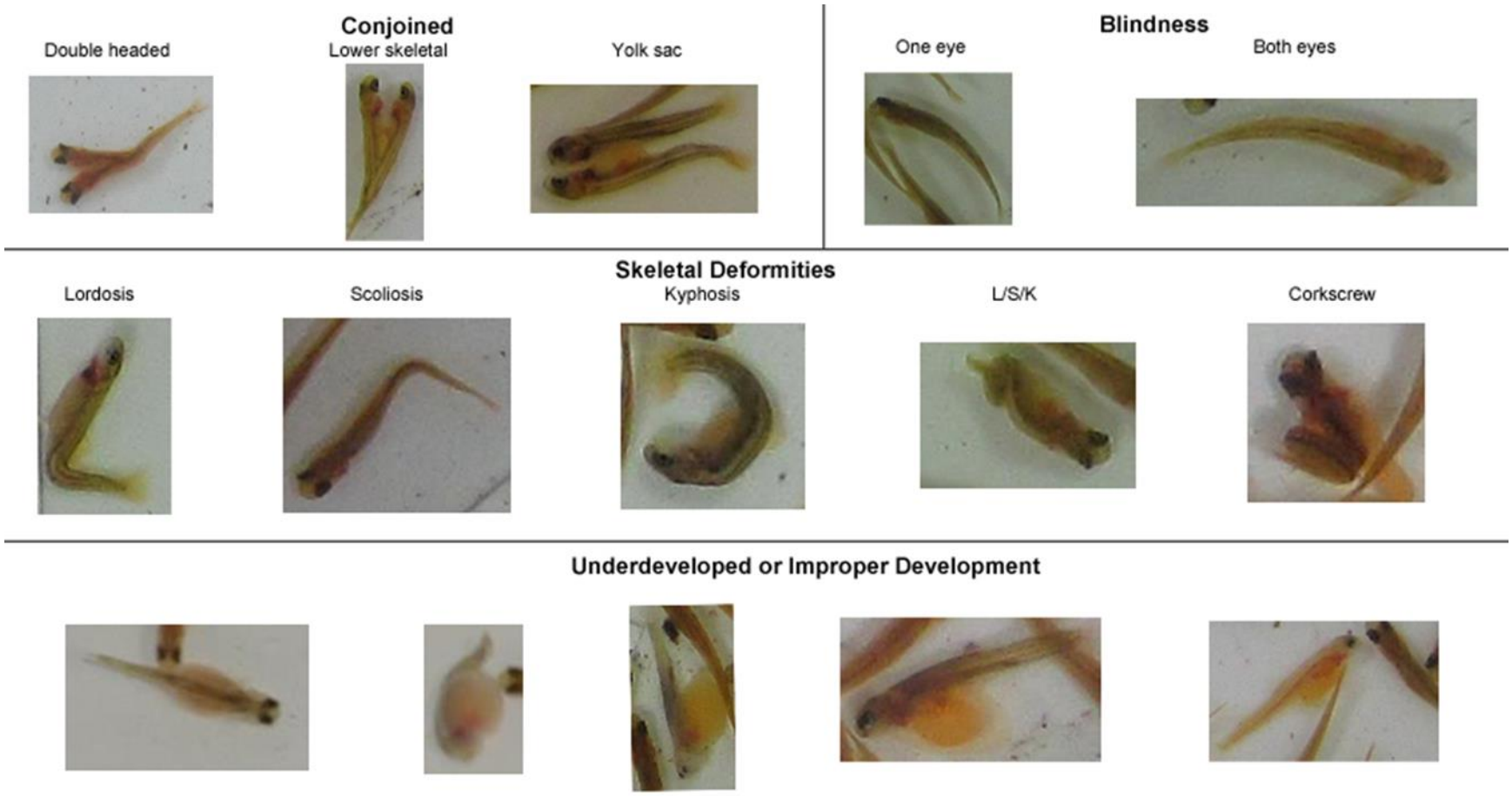


Figure A6. Legend of various types of deformities observed in Live Gene Bank Atlantic Salmon fry prior to release in the spring.