Lifelong and carry-over effects of early captive exposure in a recovery program for Atlantic salmon (*Salmo salar*)

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Abstract

A full life cycle understanding of how different captive rearing strategies affect wild fitness is needed for many species of conservation concern. Over the life cycle of endangered Atlantic salmon, we measured effects on wild fitness resulting from two widely applied conservation captive rearing strategies. One strategy releases juveniles before the onset of feeding (reduced exposure) and the other after 5 months of captive feeding (extended exposure). Fish were released into the wild and monitored 1–3 years later as seaward migrating juveniles. A sample of migrating fish from both rearing strategies was held captive in the ocean until mature, and artificially bred to monitor offspring viability. Extended early captive exposure resulted in smaller size-at-stage throughout life, shorter generation time and reduced offspring viability. Altering early captive exposure by 5 months generated long-term and transgenerational effects on fitness and life history traits that likely accelerate domestication effects and hence provide insight into effective recovery strategy design.

Introduction

Recovery programs involving captive rearing and release of wild-origin individuals are increasingly used to lower extinction risk or to restore self-sustaining populations (Snyder *et al.*, 1996; Allendorf & Luikart, 2007). These programs can provide single generation demographic boosts to wild populations (Fraser, 2008), but many fall short of sustained recovery. This is due, in part, to domestication effects resulting in captive exposed individuals being inferior to wild conspecifics in measures of fitness in the wild (Price, 1999; Frankham, 2008; Fraser, 2008). Even relatively brief exposure to captivity, perhaps especially during early life, can induce plasticity in certain traits with concomitant effects on individual fitness (de Mestral *et al.*, 2013; Jonsson & Jonsson, 2014).

Recent studies across taxa show that wild fitness generally decreases with the number of generations and duration in captivity (Araki, Cooper & Blouin, 2007; Frankham, 2008; Kosten, Kimb & Lee, 2012; Milot *et al.*, 2012; Christie, Ford & Blouin, 2014). The ontogenetic timing at which captive exposure occurs is important, specifically, that earlier captive exposure can reduce wild fitness more than exposure at later life stages (Roberts *et al.*, 2014). Overall, these fit-

ness effects can be lessened with captive exposure enrichment or quality (Laviola *et al.*, 2008; Näslund *et al.*, 2012; Hyvarinen & Rodewald, 2013). Captive rearing can also generate transgenerational effects on fitness. For example, Araki, Cooper & Blouin (2009) found lower offspring survival for parents hatched in the wild which descended from individuals hatched in captivity. Similarly, Evans *et al.* (2014) reported offspring survival in Atlantic salmon was positively related to early wild residence time experienced by captive-hatched parents. The improving understanding of effects of captive exposure is not yet resulting in widespread improvements in recovery program success, especially in fish populations which have been the subject of recovery programs for decades.

Here, we hypothesized that longer captive exposure early in life would result in successive lifelong and transgenerational net reduction of wild fitness compared to strategies maximizing wild exposure early in life (*sensu* Frankham, 2008; Fraser, 2008; Araki *et al.*, 2009; Christie *et al.*, 2014). We tested this hypothesis by investigating a long-running recovery program for Atlantic salmon *Salmo salar* from the Inner Bay of Fundy (IBoF) in Atlantic Canada, which releases juveniles of common genetic background at different life stages to the Upper Salmon River (USR) in Fundy

National Park (FNP). By monitoring the same individuals at successive stages in a common environment over a complete life cycle (from release into the wild through to the offspring they produce), we isolate observed effects to alterations in the early life rearing environment. We contribute to the understanding of the directional effects of captive exposure length, quality and timing on wild fitness to help managers improve the fitness of animals released into the wild for population recovery. Where populations are not self-sustaining, programs must necessarily produce individuals fit for the wild environment using temporary captivity. Thus, captive rearing strategy is critical to program success.

In the FNP recovery program, fish hatched in captivity are reared under two widely applied strategies (Fraser, 2008). Fish are either released into the wild at the 'fry' stage in spring before the onset of feeding (hereafter termed 'reduced captive exposure' fish, abbreviated as RCE) or are fed in captivity for 5 months and released in autumn as 'parr' (hereafter termed 'extended captive exposure' fish, abbreviated as ECE). Temporarily rearing wild individuals in captivity to provide enhanced survival or size at a certain life stage has been widely adopted in fisheries management, however long-term effects on population sustainability are increasingly documented as negative (Fraser, 2008). To determine lifelong effects of RCE and ECE, we collected measures of fitness at successive life stages and into a subsequent generation. We discuss the potential mechanisms underlying our observed effects, and offer considerations for effective recovery program design.

Materials and methods

Population recovery program history

Fifty rivers draining into the IBoF constitute the freshwater habitat for the endangered IBoF Atlantic salmon meta-population [Department of Fisheries and Oceans (DFO) 2010]. The Canadian DFO considers poor marine survival (from seaward migrating juveniles termed 'smolts', to the adult stage) as the factor most limiting recovery (DFO 2010). Causes of low marine survival are unknown but are suspected to include environmental shifts, interactions with farmed salmon, fisheries and Allee effects (DFO 2010). IBoF population persistence currently depends on a DFO captive breeding recovery program (DFO 2008).

There are two salmon rivers in FNP: Point Wolfe River (PWR) and USR (Fig. 1). Fully native salmon stocks do not likely exist in either; both were vacant for extended periods while blocked by logging dams near their mouths in the 1930s (Hutchings, 2003). Access to the 9 km of salmon habitat in USR resumed during the early 1960s. Returning adults were observed up to the 1990s, all confirmed to be strays from other nearby IBoF Rivers (M. Dadswell, unpubl. data; Fraser *et al.*, 2007). Wild adult returns have been effectively absent since 2003 (FNP 2010, unpublished data).

The last migrating, wild-produced smolts in the two rivers were collected from 2002 to 2005 for use as future broodstock. Genetic analyses showed low levels of diversity in these remnant collections, thus nearby Big Salmon River (BSR) (Fig. 1) stock was used to supplement PWR/USR broodstock to augment genetic variation (O'Reilly, 2004, unpubl. data) because (1) located 30 km west of FNP, it was the nearest IBoF stock and (2) it had been periodically released historically into the vacant PWR (Hutchings, 2003). Releases of BSR-origin RCE and ECE fish resumed annually in 2006 in USR.

Study design

We hypothesized that longer captive exposure would result in lifelong and transgenerational net reduction of wild fitness compared to strategies maximizing wild exposure early in life. We tested our hypothesis using three project 'phases' (Fig. 2). In Phase 1, we compared two released year classes of RCE and ECE fish that were sampled years after release at the smolt life stage. With current marine life stage survival being effectively zero for USR smolts, the natural marine environment was unsuitable for producing offspring from migrating smolts. In Phase 2, we collected a sample of RCE and ECE smolts and reared them to maturity in Bay of Fundy marine net pens for 16 months to coincide with their natural marine migration stage in the Bay of Fundy. In Phase 3, we artificially bred a subset of fish to compare the effect of extended versus RCE for parents on their offspring viability. In summary, smolts resulting from the release of RCE and ECE fish to USR in 2008 and 2009 (Table 1) were the focus of Phase 1. In Phases 2 and 3 (Fig. 2), we used USR smolts collected in 2010 which were individuals released in 2008 and 2009 (Table 1).

Phase 1: Effects of early exposure on older juvenile (smolt) phenotype

Juvenile salmon were created from artificial breeding and incubated in untreated ground water flow-through troughs at the DFO Mactaquac Biodiversity Facility in New Brunswick, Canada. All fish were released in the upper reaches of USR (Fig. 1). RCE juveniles were released in spring, before the onset of external feeding while some were randomly retained and reared for five additional months in tanks at the Mactaquac facility thus becoming ECE fish. Notable rearing condition differences during ECE included shelter from predation, consumption of commercial diet (www.coreyaqua.ca) and elevated water temperatures, compared to conditions in the wild. ECE fish were marked at release by removing the adipose fin. Adipose fin clipping is widespread in fisheries management and is generally accepted to have no effect on behaviour or development (Vander Haegen *et al.*, 2005).

Samples of the 2008 and 2009 releases were captured as migrating smolts from 2009 to 2012 using a rotary screw trap (Flanagan, Jones & O'Reilly, 2006). With effectively zero mature fish to spawn in the wild (FNP 2010, unpublished data), all USR smolts resulted from juvenile releases. Trapping occurred from late April until mid-June each year beginning and ending with consecutive zero-catch days, thus
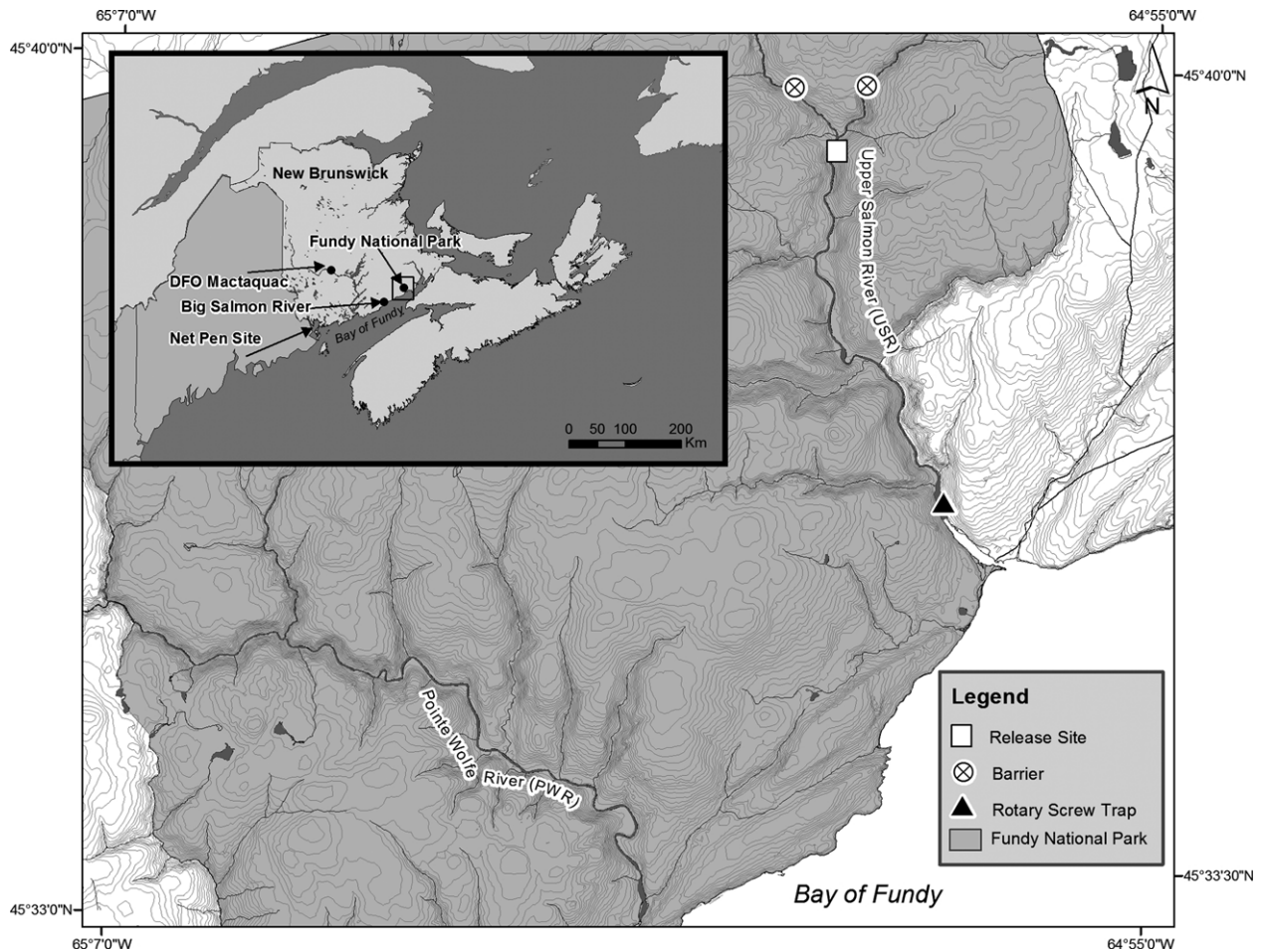
Figure 1 Map of the study area in context of Atlantic Canada. Upper Salmon River and Point Wolfe River in Fundy National Park. Big Salmon River, marine rearing site in the Bay of Fundy and the Department of Fisheries and Oceans (DFO) Mactaquac Biodiversity Facility, New Brunswick, Canada.

trapping was assumed to sample the entire migration. Smolts were collected daily and measured onsite. A mark–recapture experiment estimated that capture efficiency for migrating smolts was 10.5% in 2008 (FNP 2008, unpublished data), a value similar to longer running experiments on the nearby BSR using an identical trap (Flanagan *et al.*, 2006).

Smolts migrated at ages 1, 2 or 3 years, but juveniles are released annually for the FNP recovery program. To track only releases from 2008 and 2009, scale ages were determined from samples taken from captured smolts in 2009–2012. We also recorded weight, migration date and rearing strategy for each smolt (reduced vs. extended exposure, by the presence or absence of adipose fin respectively).

Statistical model structure is found in the Supporting Information Table S1. Phase 1 examined effects of captive rearing strategy on smolt age, seasonal migration timing and weight using analyses of variance (ANOVAs) in a general linear model (GLM). A nested ANOVA was carried out to determine whether rearing strategy affected migration day (i.e. the Julian day in which the fish was captured). Nesting

smolt age in release year allowed us to compare migrating smolts of the same age from the same release year to determine if migration day depended on rearing strategy (RCE or ECE). Median migration day (MMD) was the day at which 50% of the entire migration had occurred and is presented in the Supporting Information Fig. S1. All statistical analyses were carried out using the Minitab 16® Software package (Minitab Inc., State College, PA, USA) except R which was used for the embryo analyses in Phase 3. Assumptions of parametric statistics were checked, and $\alpha = 0.05$.

Phase 2: Effects of early exposure on post-smolt growth, survival and maturation

In May 2010, 1446 smolts (RCE and ECE fish, Table 1) were captured from USR, sampled as described above, and inserted with a 2.5×12.5 mm, 125 Hz individually coded passive integrated transponder (PIT) tag (BIOMARK Inc., Boise, ID, USA) in the dorsal musculature on the day of capture. This group included 18 smolts which were of age 3,
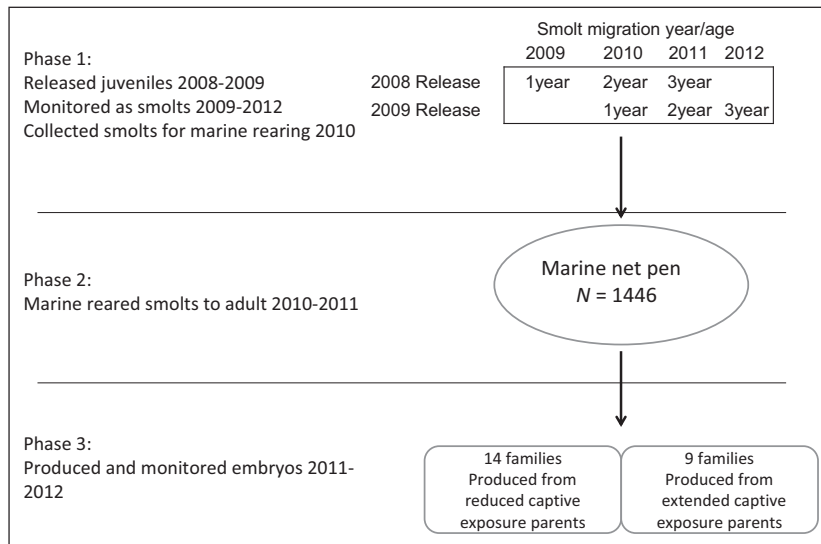
Figure 2 Outline of project phases across a salmon life cycle. Phase 1: 2008 and 2009 juvenile releases captured as migrating smolts from 2009 to 2012. Phase 2: 2010 smolts captured and reared in marine net pens between May 2010 and September 2011. Phase 3: embryos created from parents which had either reduced or extended early captive exposure. Embryos monitored for 5 months between November 2011 and March 2012.

Table 1 Number of Atlantic salmon released in 2008 and 2009 by rearing strategy into the Upper Salmon River

| Release group | Total released | Total survival to smolt (%) | Age structure of surviving smolts sampled (%) | | | No. collected in 2010 |
			Age 1	Age 2	Age 3	
2008 Reduced	280 000	3	10	88	2	627
2008 Extended	25 000	13	82	18	0	59
2009 Reduced	158 000	2	9	85	6	41
2009 Extended	29 000	33	85	15	0	701

Estimates (based on 10.5% capture rate) of survival from time of release to smolt and proportional age structure of smolts resulting from each release group. Number of smolts collected in 2010 from each rearing strategy.

and thus from releases of 2007 otherwise not considered here. All smolts were transported to customized marine net pens operated by the aquaculture industry in the Bay of Fundy, *c.* 160 km west of FNP (Fig. 1). Daily sorting of captures by size and release strategy allowed us to distribute similar proportions of RCE and ECE and ages to four net pen blocks so that each block approximately represented the 2010 migration.

A square array of four $3.6 \times 7.3 \times 1.8$ m deep rectangular marine net pens were divided at midpoints to yield eight 3.6 m^2 $\times$ 1.8 m deep compartments (rearing blocks). While in marine pens, fish were provided commercial salmon diet (www.coreyaqua.ca) by constant trickling during daylight hours via automatic feeders. A census of each block was carried out six times across 16 months of marine rearing. At each census, all fish were transferred from the four stocked

blocks to the four vacant blocks to allow cleaning of netting. Pen dividers were removed in April 2011 and fish were reared in two larger groups. Censuses recorded counts by rearing strategy, by noting adipose fin presence and were used to observe survival between RCE and ECE fish during marine rearing. At 5 and 16 months, at the end of summer growing seasons, we obtained lengths, weights and tag identification in addition to count data. Due to unexpected PIT tag loss, only those fish retaining tags were included in growth rate and final weight analyses. The RCE:ECE ratio for fish without tags was similar to the group which retained their tags so no tag retention-by-rearing strategy bias was expected.

Phase 2 weight gain was analysed using specific growth rate (SGR) which is calculated by [(log final mass − log initial mass)/time$_{months}$], while controlling for rearing block. Final weight was analysed to determine whether RCE or ECE fish had differing size at the final census. Phase 2 survival was analysed by calculating the survival odds ratio (survival odds of RCE ÷ survival odds of ECE) using counts collected at 1, 3, 5 and 16 months of marine rearing. Odds ratios were tested for the entire marine rearing period (month 1–end) using a generalized linear model and logit link in binary logistic regression routine (Saloniemi *et al.*, 2004). To determine whether survival was dependent on sampling time period (i.e. survival from month 1 to end or month 5 to end), we included a factor for census. The census × rearing strategy interaction term was significant, meaning that the survival odds ratio changed through time in marine net pens. The model was broken down *post hoc* and survival by rearing strategy was analysed for four independent periods of Phase 2, including months 1–3, month 1–end, month 3–end and month 5–end. These analyses used

count data from each census, not individual tag identities. This allowed us to include all surviving fish that had shed their tags.

After 16 months, we removed all fish from the marine net pens. We transported the first 100 RCE and 100 ECE tagged fish back to the Mactaquac facility for Phase 3. At the onset of spawning in November 2011, and using the retained group of 200 individuals for Phase 3, the maturation odds ratio of the groups (RCE:ECE) was also calculated. Significance of maturity odds ratio was tested as above for survival count data.

Phase 3: Effects of early exposure on next-generation offspring viability

In late November 2011, mature individuals were artificially spawned within each group. Families were generated from 12 full-sibling pairs and two half-sibling pairs (14 females, 12 males; 2 males used twice) of RCE parents and nine full-sibling pairs of ECE parents (9 males, 9 females). Individual families were then held in separate containers in untreated ground water for 2 h, after which two replicates of 300 eggs each were extracted to incubation baskets. All baskets of fertilized eggs were dipped in 1% Ovadine™ (Western Chemical, Ferndale, WA, USA) disinfectant solution with untreated water for 10 min (as required by regulation) and placed at random in a single, indoor, continuous flow ground water incubation trough.

Prior to fertilization, we digitally photographed each female's eggs. Using 1.47v ImageJ (National Institutes of Health 2008), we digitally measured the diameter across 12 random eggs from each female, these were averaged to obtain mean female egg size. Fecundity was manually counted from the same photos.

Mean fecundity and egg size were compared between rearing strategies using a GLM, while controlling for female length. Opaque or otherwise damaged embryos were considered unviable and were removed and recorded weekly. Embryos were monitored until the end of March 2012. The mean proportion of viable embryos remaining at the end of monitoring was recorded across the two replicates per family. Both replicates of one ECE family had zero survival by 4 weeks post spawning and were conservatively removed from viability analyses. Using R, a GLM with a binomial error distribution was conducted to analyse embryo viability in relation to rearing strategy. Initial models found that female size and egg size did not significantly affect embryo viability. We therefore proceeded with a simplified model including rearing strategy as a fixed effect and family as a random effect, to control for variation among replicates within families.

Results

Phase 1: Effects of early exposure on older juvenile (smolt) phenotype

An estimated 2–3% of released RCE fish and 13–33% ECE fish survived to migrate as smolts (Table 1). ECE resulted in significantly younger smolt age ($F_{1,2557} = 2192.4$,

$P < 0.001$). Proportions of smolt ages for each release year (Table 1) consistently demonstrated that c. 85% of RCE smolts migrated at age 2, whereas almost the same proportion of ECE smolts migrated at age 1 (Fig. 3a). Seasonally, both rearing strategies migrated at similar times when controlling for smolt age and release year ($F_{1,2543} = 2.14$, $P = 0.144$) (Supporting Information Fig. S1).

The interaction of captive rearing strategy and release year had a significant effect on smolt weight ($F_{1,2543} = 30.16$, $P < 0.001$). Thus, a one-way ANOVA for each release year was carried out, which revealed that smolts of both rearing strategies were significantly larger from 2008 than 2009 release years (Fig. 3b). However, within release years, the consistent difference in ages resulted in the older RCE smolts being significantly heavier than ECE smolts for the 2008 release at 34 g (SE ± 0.288 g) versus 24 g (SE ± 0.452 g) ($F_{1,1185} = 310.87$, $P < 0.001$) and for the 2009 release at 33 g (SE ± 0.391 g) versus 20 g (SE ± 0.217 g) ($F_{1,1357} = 821.96$, $P < 0.001$).

Phase 2: Effects of early exposure on post-smolt growth, survival and maturation

Through Phase 2, RCE resulted in heavier adults, but RCE and ECE smolts had no significant difference in maturation rate despite varying in age. By the end of Phase 2, analysis of a subsample of 131 RCE and 266 ECE fish that retained tags revealed that captive exposure duration affected SGR ($F_{1,396} = 89.54$, $P = 0.002$). RCE fish had a lower SGR than ECE fish (0.102 SE ± 0.000852 vs. 0.113 SE ± 0.000662) (Fig. 3). Despite slower growth rate, mean final weight for RCE fish was 1238 g (SE ± 28.7 g) and was significantly ($F_{1,396} = 45.24$, $P < 0.001$) higher than ECE fish at 991 g (SE ± 16.7 g) (Fig. 3). Overall Phase 2 survival (month 0–end) was 24% for RCE fish and 44% for ECE fish [RCE–ECE survival odds ratio: 0.40 (95% CI 0.32–0.51), $G = 63.58$, $P < 0.001$]. However, the survival odds ratio depended significantly on the interaction of rearing strategy and census period analysed ($G = 351.75$, $P < 0.001$), thus the model was broken down *post hoc*. The survival odds (RCE:ECE) were 0.21 (95% CI 0.17–0.26) from months 1 to 3 ($G = 201.91$, $P < 0.001$), 1.45 (95% CI 1.04–2.01) from months 3 to 16 ($G = 4.99$, $P = 0.027$) and 1.51 (95% CI 1.07–2.12) from months 5 to 16 ($G = 5.68$, $P = 0.019$). Count data across censuses of Phase 2 are presented in Supporting Information Fig. S2.

Of the subset of 200 individuals retained for assessment, and excluding 12 post-collection mortalities (6 RCE, 6 ECE), 31/94 (34%) of RCE fish and 41/94 (44%) of ECE fish matured. The odds ratio of maturation (RCE:ECE) was 0.67 (95% CI 0.37–1.20) and was not significant ($G = 1.817$, $P = 0.178$).

Phase 3: Effects of early exposure on next-generation offspring viability

Total fecundity was not significantly affected by rearing strategy including when controlling for female length (GLM, $F_{1,21} = 0.34$, $P = 0.564$). Mean fecundity for RCE and ECE females was 1950 (SE ± 182) and 1980 (SE ± 162) respec-
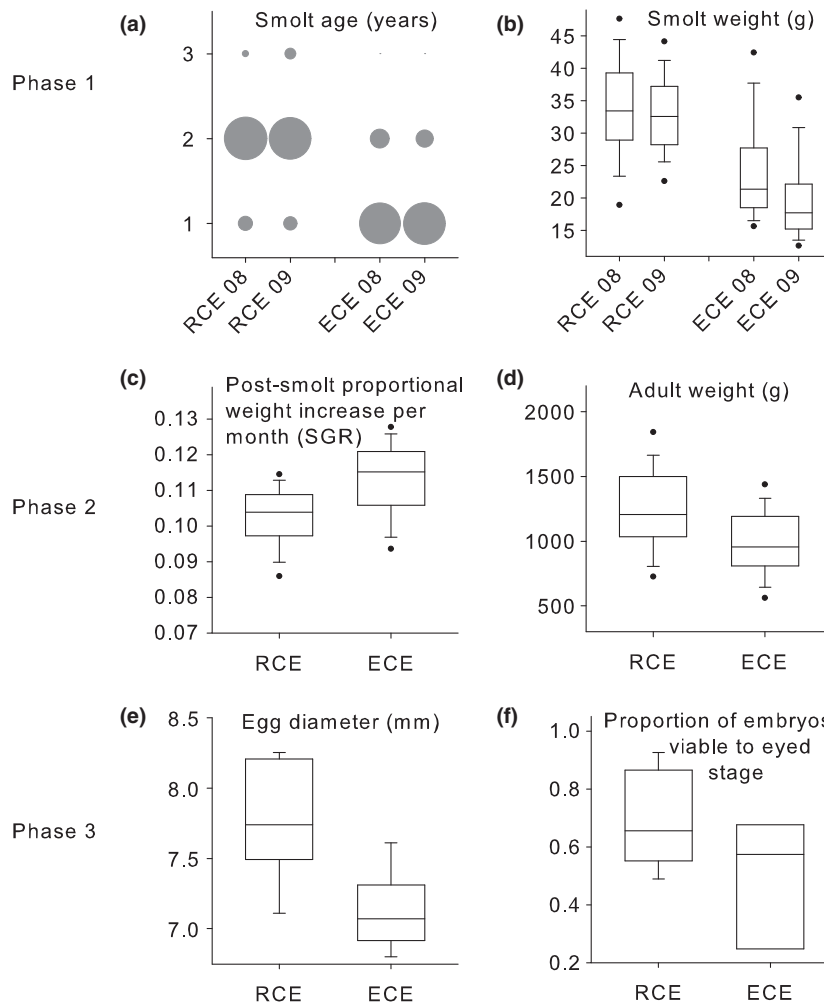
Figure 3 Key results shown by project phase for reduced captive exposure (RCE) and extended captive exposure (ECE) rearing strategies. All panels present significant results with tolerance of Type 1 error at 0.05. Proportions of smolts ages produced by each rearing strategy (a), smolt weight (b), post-smolt growth rate in marine pens (c), adult weight after marine rearing (d), egg diameter (e) and proportion of viable embryos 5 months post-fertilization (f). In e, outer quartile indicators not shown as sample size were not large enough for their calculation for ECE parent crosses.

tively. Controlling for female length, egg size was significantly (GLM, $F_{1,21} = 22.33$, $P < 0.001$) higher for RCE at 7.765 mm (SE ± 0.107) versus ECE at 7.127 mm (SE ± 0.085) females (Fig. 3).

The mean proportion of viable embryos from RCE and ECE parents was significantly different (likelihood ratio test, $\chi^2_{(1)} = 5.743$, $P = 0.017$); families derived from RCE parents had higher survival (0.693, range: 0.407–0.963) than families derived from ECE parents (0.493, range: 0.030–0.780) (Fig. 3). Due to similar fecundity, RCE parents therefore produced significantly more viable offspring at 5 months post-fertilization than ECE parents.

Discussion

We present evidence that varying exposure to captive conditions during early life induced a lifelong divergence in important phenotypic traits for fitness that carried over into

the next generation. We found that extending captive exposure resulted in (1) younger and therefore smaller migrating juveniles, (2) smaller adults after rearing in a captive marine environment, (3) substantially less wild exposure (after accounting for rearing strategy differences), (4) a shorter generation time and (5) smaller and less viable offspring in the next generation, compared to fish with reduced captive rearing. Our findings should be relevant to population recovery managers considering trade-offs between captive rearing regimes, the maintenance of wild fitness at different life stages and the boosting of population size.

Effects of early exposure on older juvenile phenotype

The RCE smolts migrated predominantly at age 2 resulting in larger body sizes, while smaller ECE smolts migrated as smaller smolts year earlier. These trends are consistent with

recent works on other salmon populations (Skilbrei *et al.*, 2010; de Mestral *et al.*, 2013; Jonsson & Jonsson, 2014). They are notable because a larger smolt body size is positively associated with higher marine survival in wild Atlantic salmon populations (Lundqvist *et al.*, 1994; Antonsson, Heidarsson & Snorrason, 2010). Being released into the wild 5 months earlier resulted in RCE juveniles spending 17 months more time in the wild before migrating as smolts, compared to ECE counterparts (24 months in the river for most RCE smolts and 7 months for most ECE smolts). ECE smolts were younger, smaller and had more captive exposure than RCE smolts. These effects are related, however both may independently reduce marine survival in the wild (Farmer, 1992; Jonsson, Jonsson & Hansen, 2003; Vøllestad, Peterson & Quinn, 2004).

Varying captive exposure duration resulted in different release-to-smolt survival estimates (2% and 3% for RCE fish and 13% and 33% for ECE fish). We suggest that survival to smolt stage favouring the ECE group does not necessarily reflect improved fitness due to their shorter wild residence time noted above. This is discussed further below for the effect on generation time for ECE fish.

Smolts from ECE and RCE groups migrated at similar times. Even when comparing smolts of different ages and release years, MMD for each cohort varied by no more than 2 days. This is in contrast to some studies finding that significantly smaller smolts (such as ECE in our case) in other populations migrated later (Skilbrei *et al.*, 2010). Non-significant differences in Julian migration day may be due to the small physical size of USR or alternatively, salmonid migration timing has been shown to be a population-specific trait (Kallio-Nyberg & Ikonen, 1992; Pascual & Quinn, 1994) and we considered only one population.

Effects of early exposure on post-smolt growth, survival and maturation

Bay of Fundy marine pens provided commercial feed and deliberate protection from predators. This was a necessary experimental limitation to provide mature adults for Phase 3 given negligible marine survival in the wild.

Older, larger, RCE smolts produced heavier adults when grown in marine pens. As smaller and younger smolts, ECE fish grew faster, matured similarly, and overall survived better across Phase 2 than RCE smolts. We measured growth rates only on fish which survived to the end of Phase 2. We acknowledge that growth rate could have affected mortality differently for either release strategy, however we suggest below that Phase 2 survival was not likely reflective of wild fitness trends. We suggest that the faster growth rate of ECE fish in the marine net pens could be attributed to their smaller size via allometric growth (Jonsson & Jonsson, 2007), but it is likely due, at least in part, to previous experience with feeding in captivity during early life, which is known to be important for salmonid learning and behavioural development (Kihslinger & Nevitt, 2006; Salvanes *et al.*, 2013).

About 24% of RCE fish survived the entire marine rearing phase compared to 44% for ECE fish. However, this was due mostly to the very poor survival within the first 3 months for RCE fish. After 3 months marine rearing, survival favoured RCE fish. Our observed high mortality during July for RCE fish is similar to July peaks in mortality in wild salmon smolts reared in marine pens by Dempson *et al.* (1999). In that study, failed smolt syndrome (McCarthy *et al.*, 1996) was attributed to the increased mortality as wild fish did not recognize or adjust to commercial feed. Our RCE smolts had no experience consuming commercial feed (while ECE smolts did) thus it is reasonable to suspect failed smolt syndrome contributed to their increased mortality early in Phase 2. Conversely, higher overall survival in Phase 2 for the ECE group was likely an effect of rearing in a familiar captive environment rather than an indication of improved wild fitness.

We found that the proportion of mature fish was lower in RCE than ECE fish, although the differences were not significant. This is notable as most RCE fish were a full year older than most ECE fish. The ECE smolts grew at a faster rate, and matured similarly to the older RCE smolts, following long-presented findings that faster early growth rates result in earlier maturity in salmonids (Alm, 1959). For recovery programs spanning multiple generations of release-collect-spawn-release actions such as IBoF salmon, the consequence of inducing earlier age at maturation is a reduction in generation time and thus an increase in the number of generations of captive exposure during the recovery process. This increases the expected adaptation to captivity and associated compromises to fitness (Araki *et al.*, 2007; Frankham, 2008; Fraser, 2008).

Effects of early exposure on next-generation offspring viability

We found transgenerational effects resulting from differing early life captive exposure, as RCE fish produced significantly larger and more viable embryos than their ECE counterparts. Jonsson, Jonsson & Fleming (1996) showed that growth rate during early life was negatively related to future egg size, evidenced by hatchery reared smolts growing faster, but having smaller eggs after maturing in the wild than counterparts reared entirely in the wild. In that study, producing larger eggs was suggested to be a response to wild fish experiencing less favourable growing conditions early in life. Similarly in our work, due to improved early growing conditions in captivity, ECE fish were assumed to grow faster than RCE fish in pre-smolt life stages. We showed that ECE fish grew faster in post-smolt stages and, as expected, produced smaller and less viable embryos. This implies a survival advantage in the wild for offspring of RCE parents as more viable, larger eggs produce more and larger fish after the onset of feeding, which is related to higher survival and a competitive advantage over smaller fish (Einum & Fleming, 2000; Burton *et al.*, 2013). In a complimentary study manipulating wild exposure, Evans *et al.* (2014) found that captive broodstock with extended wild exposure produced better surviving offspring in the wild than counterparts with reduced wild exposure. Our observations suggest decreasing exposure to captive conditions early in life would

increase the size, and therefore survival, of offspring produced in the wild in the next generation, an important consideration for those attempting to re-establish self-sustaining populations.

Captive rearing/conservation program implications

By experimentally monitoring commonly applied supplementation strategies in fish, our work supports established findings on the effects of early growth and captive exposure. By following cohorts for an entire life cycle, we reveal that differences in early captive exposure can generate cascading effects through later life stages that can carry over to impede fitness in the next generation and ultimately, population recovery. Our results support past recommendations to (1) use captive rearing only to avoid grave consequences such as local extirpations, (2) minimize captive exposure duration, and (3) maintain 'normal' phenotypic and life history trajectories of populations during the captive rearing process (Frankham, 2008; Fraser, 2008).

With rates of development and mortality being high, if not maximal, early in life within many highly fecund species (Cunjak & Therrien, 1998; Williams & Hoffman, 2009; Kennedy, Crozier & Allen, 2012), the earliest life stages predominantly shape the raw material available to produce the next generation. Indeed, a growing field of study shows that significant cognitive development occurs at very early salmonid life stages (Kihslinger & Nevitt, 2006; Salvanes *et al.*, 2013), making these stages most sensitive to environmental manipulations (Roberts *et al.*, 2014). The implication is that managers should prefer wild environments shaping a population at these early stages when wild fitness is the goal. This could be achieved by releasing earliest possible life stages into the wild to minimize early experience to captivity and to produce individuals which are more shaped by, and therefore more fit for, life in the wild. In our study, RCE improved measures of individual fitness later in life and their offspring's viability. In contrast, ECE induced divergence from wild phenotypes but interestingly, likely contributed to their improved 'captive fitness' as they survived better while reared in a captive marine environment later in life.

Recovery program managers must release individuals which are also capable of producing an adequately fit generation to have a sustained effect. Nevertheless, we acknowledge that the reality of some situations may preclude having the earliest possible life stages exposed into the wild; in those cases, the early captive rearing environment should include enriched or naturalized elements specific to the life stage to minimize wild fitness loss (see Johnsson, Brockmark & Naslund, 2014). Taken together, our general recommendation to managers of population recovery programs which require captive rearing would be to keep captive exposure as brief, naturalized and as late in the animal's life as possible. For IBoF salmon, a recommended design would intervene with captive support only during the marine (juvenile subadult) life stage and return adults to the river coinciding with their natural re-entry to freshwater. This way, only the recovery limiting later life stages are artificially manipulated while reproduction and the earliest life stages – those experiencing intense selection – occur in the wild, free from both the demonstrated and yet to be completely understood effects of captivity. Due to the current inability for IBoF salmon to effectively complete its marine life stage in the wild and the need for collaboration discussed in Gross (1998), private industry was engaged to rear wild-caught fish during their natural marine life stage. To our knowledge, this is the first published account of rearing wild endangered fish populations in a commercial marine setting to contribute to conservation. Experimental marine captive rearing methods employed here could theoretically achieve the recommended design, but additional study is required to determine the feasibility of this approach as a sustainable model.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Cumulative proportions of smolts migrating from 2008 to 2009 releases.
Figure S2. Total post-smolt counts by release stage during six standard censuses of net pen blocks conducted in June 2010 (1), July 2010 (2), August 2010 (3), October 2010 (4), July 2011 (5) and September 2011 (6). RCE (closed circles), ECE (open circles).
Table S1. Summary of applied models throughout Phases 1–3.