Rapid Evolution of Egg Size in Captive Salmon

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Captive breeding and release programs, widely used to supplement populations of declining species, minimize juvenile mortality to achieve rapid population growth. However, raising animals in benign environments may promote traits that are adaptive in captivity but maladaptive in nature. In chinook salmon, hatchery rearing relaxes natural selection favoring large eggs, allowing fecundity selection to drive exceptionally rapid evolution of small eggs. Trends toward small eggs are also evident in natural populations heavily supplemented by hatcheries, but not in minimally supplemented populations. Unintentional selection in captivity can lead to rapid changes in critical life-history traits that may reduce the success of supplementation or reintroduction programs.

The use of captive breeding programs is widespread, and increased use of such programs for species preservation has been recommended (1, 2). However, there is considerable debate over the wisdom of "ex situ" conservation because of the cost, disease risk, and possible genetic impacts (1–6). One potential genetic impact associated with captive breeding is unintentional selection (3, 4, 7). Although the possibility of unintentional selection in the captive environment is often suggested (4, 7), there is little empirical evidence. Most studies have been based on phenotypic differences between captive- and wild-bred individuals and do not differentiate between acclimation or behavioral conditioning, and true genetic evolutionary responses (8). Because mortality is generally low in well-managed captive populations, the potential for large selection-driven effects is thought to be low (8, 9). However, this reduction in mortality may itself drive the evolution of traits that increase fecundity at the expense of offspring survival under nonmanaged conditions.

In salmon, ex situ conservation takes the form of "supplemental" breeding (8, 10, 11); returning adult salmon are artificially spawned and their offspring reared in hatcheries until they are released back into the river as fry. The number of released hatchery-reared salmon fry averaged over 550 million per year from 1987 to 1996 in British Columbia, Canada (12). Although the practice of supplemental breeding is expected to have less impact than captive breeding programs on the genetic architecture of the population (8, 10), there is

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Fig. 1. Relationships between egg mass and (**A**) early juvenile survival and (**B**) relative fecundity in chinook salmon; each point represents a full-sib family from a single dam. (A) Egg mass was measured as the mean mass of 20 to 50 unfertilized eggs taken from 3-year-old dams at the time of spawning. Each family was separately incubated in vertical stack incubation trays, and all families were spawned over a 7-day period. Early survival was measured by counting eggs at fertilization and documenting mortality to the onset of exogenous feeding. All relationships were significant at the $P <$ 0.01 level or higher (nonlinear regression analysis). (B) Relative fecundity was calculated as the total number of eggs produced divided by the total body mass of the dam (aged 3 and 4 years); this transformation assumes a linear relationship between female body mass and egg number, which was the case in all years examined (linear regression coefficient equal to or greater than those for power function, exponential, and log-linear curves). Examination of residuals showed no bias, and leverage was <0.10 for all points in all years. Relationships between relative fecundity and egg size were significant at the $P <$ 0.0001 level (regression analysis). The study population was founded in 1985 with fertilized eggs from the Robertson Creek hatchery and has been maintained with <200 parental fish per generation at the YIAL facilities.
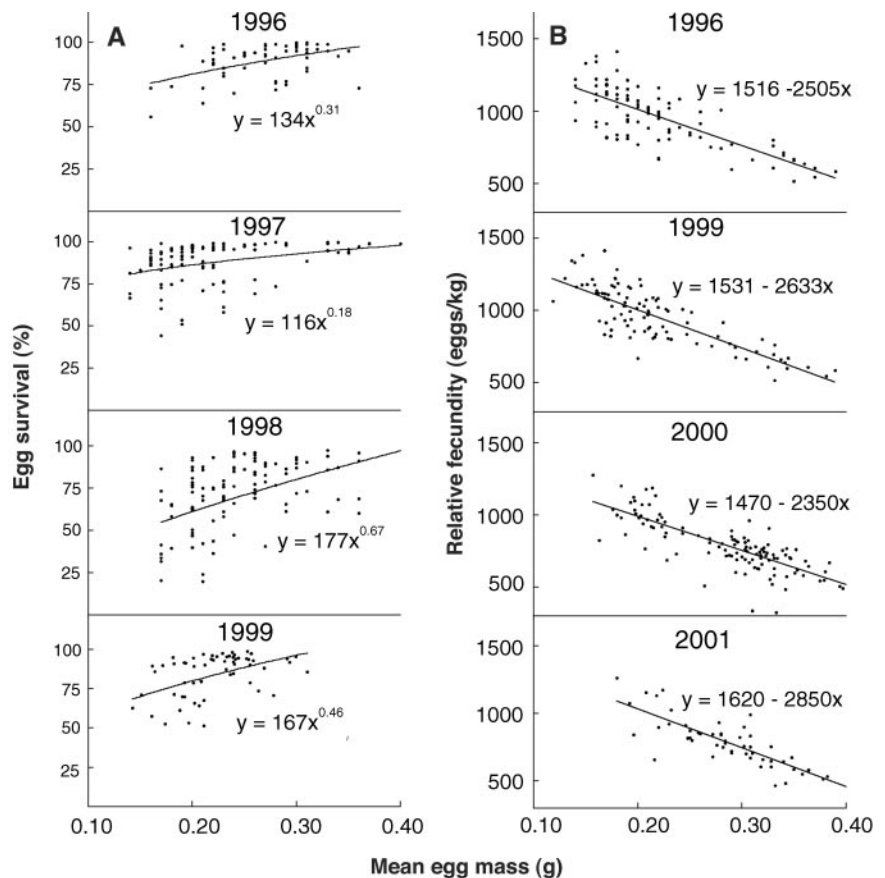
still considerable controversy (*3*, *8*, *11*), and a number of studies have documented phenotypic differences between captive- and wild-reared salmon in common garden experiments (*8*). Concerns over the potential for unintentional selection in salmon hatcheries arise because of inherent differences between the hatchery and natural environments in factors that influence fry survival (*8*).

The consequences of variation in egg size for offspring growth and survival (*13*, *14*), and the nature of the egg size–egg number trade-off (*13*, *15–21*), have been extensively studied. Although the factors that affect optimal offspring size are diverse, it is generally apparent that larger offspring typically have substantial fitness advantages over small offspring, but that females producing small offspring usually have higher fecundity. Thus, equilibrium offspring size is at least partially due to a balance between selection for high survival (large offspring) and high maternal fecundity (small offspring) (*13*, *20*). A change in either the shape of the relationship between egg size and offspring survival or the amount of selection on fecundity will change the equilibrium point and result in a selection differential acting on offspring size. In salmon, the selective advantage of large eggs has mostly been documented during the fry stage (*22*, *23*); however, recent work has shown that large egg size provides a survival advantage during the egg stage as well (*21*).

In a population of chinook salmon, *Oncorhynchus tshawytscha*, reared under standard aquaculture conditions at Yellow Island Aquaculture Limited (YIAL), a commercial salmon farm in British Columbia, Canada, we found a positive relationship between egg size and early survival in all 4 years for which the relationship was quantified (Fig. 1), with little change in curve shape across years. We also found a highly significant ($P < 0.0001$) negative linear relationship between egg size

and relative fecundity in all 5 years in which we tested for a relationship (Fig. 1) (*24*). The product of the two functional relationships (survival × fecundity) was used to estimate maternal fitness as a function of egg size during the early life stages (when offspring survival is most sensitive to variation in egg size) (*13*, *21–23*, *25*) under hatchery rearing conditions (Fig. 2). The resulting curve shows the typical topology for fitness relationships (Fig. 2). The calculated equilibrium egg size (0.16 g) (Fig. 2) is considerably smaller than the mean egg size observed in this captive population near founding (1988) (0.27 g) and is slightly smaller than the mean size of eggs in this population at the end of our sample period (0.20 g) (Fig. 2). Thus, YIAL females produce eggs that are larger than the egg size that maximizes maternal fitness; therefore, selection should favor smaller eggs within the hatchery environment. Assuming that the initial egg mass of 0.27 g was the preselection optimal egg size, and that the egg mass–fecundity relationship did not change over the course of the study (*26*), we estimate that the observed decline in egg size (0.27 to 0.20 g) represented a ~6% decrease in maternal fitness (fecundity × survival) and a 24% decrease in egg survival if the evolved YIAL fish were to be reintroduced into the wild (*26*).

We found high heritabilities (h^2) for both egg mass and for the residuals of the egg mass–mother mass regression [mother-daughter regression for heritability (*27*) calculated with 50 dams and 109 daughters at YIAL; $h^2_{(egg\ mass)} = 0.26 \pm 0.12$; $P < 0.05$; $h^2_{(residuals)} = 0.39 \pm 0.15$; $P < 0.05$]. The high additive genetic variance and the large selection differential (Fig. 2) indicate that the YIAL population should be rapidly evolving toward smaller eggs. Census data support this prediction (Fig. 3A); egg size decreased dramatically between 1988 and 2001 with no

change in female body mass (fig. S1). The change in egg size (response) generates a selection intensity (*i*) estimate of −1.04, a value in the top 3% of reported selection intensities in natural populations (*28*). The rate of evolution estimated with our time series data for egg size ($-21.92 \pm 6.1 \times 10^3$
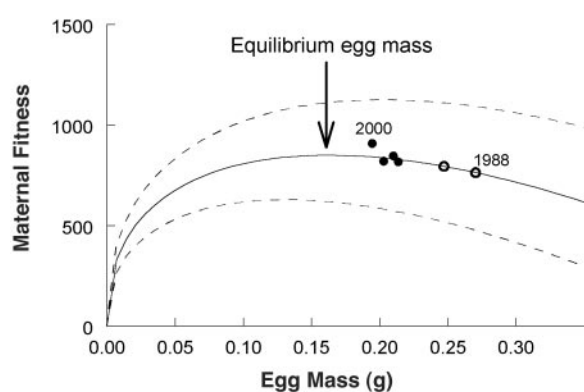

Fig. 3. Change in egg size over time for (**A**) the captive population (YIAL) and (**B**) four river populations of chinook salmon on Vancouver Island, British Columbia, Canada. (A) Mean egg mass ± 1 SE (g) in the captive YIAL chinook salmon population. Mean egg mass estimates were made with most (>80%) of 3-year-old females spawned. The decline in egg mass was highly significant (regression analysis; $P < 0.001$), whereas there was no significant trend in body mass over the same period (regression analysis; $P > 0.15$) (fig. S1). (B) Mean egg volume (cm³) for four populations of chinook salmon on Vancouver Island. Supplementation effort was quantified as the number of females spawned in the hatchery divided by the total number of adult females returning to the system, averaged over years for which we have egg size data. Mean supplementation efforts were as follows: Robertson Creek, 28%; Quinsam River, 43%; Nanaimo River, 16%; and Nahmint River, 4%. The fitted regression lines are for the two populations (Robertson Creek and Quinsam River) that show significant decreases in egg size ($P < 0.01$). There was no significant decrease in egg size detected in the Nahmint or Nanaimo data. There was no significant trend in mean female length (in mm; postorbital to hypural plate) for any of the populations with data ($P > 0.20$) (fig. S2).

Fig. 2. Relationship between egg mass and maternal fitness for the YIAL captive chinook salmon [fitness = 2505(egg weight)$^{0.41}$ + 4450(egg weight)$^{1.41}$]. This relationship was generated as the product of early survival and relative fecundity (both as functions of egg mass) (Fig. 1). The coefficients of the maternal fitness equation are calculated from the mean curve fit parameters for the survival and fecundity relationships. The 95% confidence limits (dashed lines) were estimated by using the variance in the independent estimates of the empirical relationships of egg mass versus survival and fecundity. The identified equilibrium egg mass was estimated by setting the first-order derivative of the maternal fitness relationship to zero and solving for egg mass. We plotted mean egg mass versus maternal fitness for the 4 years for which we have survival and fecundity data (1996, 1997, 1999, and 2000) with solid circles; the 2 years for which we have only egg size data (1988 and 1994) are indicated with open circles and were placed on the derived maternal fitness curve.
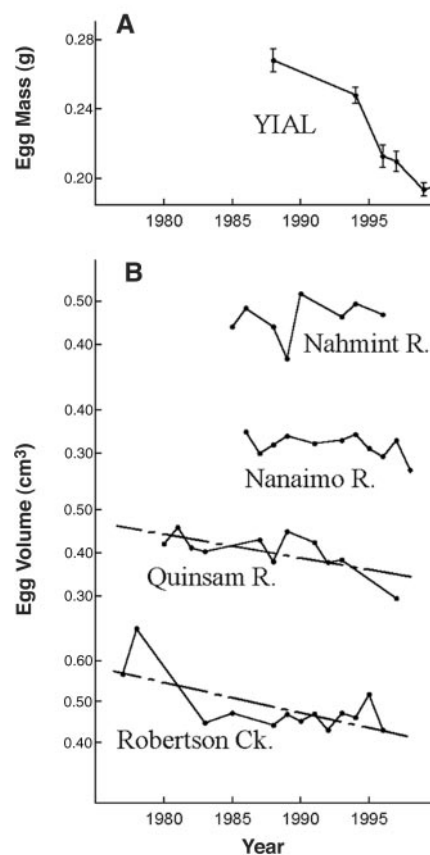
darwins, and -1.20 ± 0.47 haldanes) (29) is substantially higher than most reported values (29) for contemporary evolution studies. Because we still observe a survival advantage for large eggs and high overall survival (73 to 89% mean early survival) (Fig. 1), the observed rapid evolution toward small egg size probably reflects intense selection on female fecundity and reduced selection for large egg size, with small egg size evolving as a correlated response to selection on fecundity—YIAL mean fecundity increased by ~10% (from 3590 ± 101 to 3960 ± 92 eggs) from 1996 to 2000 (fecundity data are not available for earlier years or for our field populations). The possibility that the observed change in egg size was environmentally driven is discounted by the results of a reciprocal hybridization study involving YIAL and wild stock (Big Qualicum River); the mean egg size from the hybrid offspring was about midway between the parental egg sizes (wild egg size = 0.271 ± 0.006 g; YIAL egg size = 0.208 ± 0.005 g; reciprocal hybrid egg sizes = 0.229 ± 0.008 g and 0.234 ± 0.006 g). Furthermore, inbreeding effects on egg size have been shown to be present but very small in the YIAL population (30).

Does the observed rapid evolution of egg size at YIAL have implications for wild populations of salmon? Government hatchery records suggest that captive breeding may also drive the evolution of small egg size in some supplemented wild populations of salmon. We examined mean egg size over 20 years in four river populations of salmon subjected to variable levels of supplementation. We found significant declines in egg size in two of these populations, both of which experienced significant supplementation (43% and 28%), whereas two populations that receive lower supplementation (16% and 4%) showed, at most, weak trends toward decreasing egg size (no significant decrease in egg size) (Fig. 3B). There was no consistent decrease in female body size during this 20-year period (fig. S2); therefore, the observed declines in egg size are not due to reductions in female body size over the same time period. These data indicate that unintentional selection resulting in small egg size is potentially a serious concern for the long-term success of salmonid supplementation efforts, but the effect could be minimized through modified breeding practices.

The high rate of evolutionary change in egg size observed in this study demonstrates that captive breeding and supplementation programs can lead to rapid change in critical life-history traits simply by rearing animals in a benign environment and thus relaxing selection normally experienced in nature. In commercial captive rearing (e.g., aquaculture and agriculture), the long-term consequences of relaxed selection may be minimal, but for threatened or endangered species, maladaptive traits that evolve in captive breeding programs may have large negative effects when animals are released back to nature. In chinook salmon, the combination of high fecundity and captive conditions resulted in an evolutionary response as fast as, or faster than, any observed in previous studies. Similar evolutionary change is likely under captive breeding conditions for other species and could seriously limit the success of conservation efforts.

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Modulating Sphingolipid Biosynthetic Pathway Rescues Photoreceptor Degeneration

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Mutations in proteins of the *Drosophila* phototransduction cascade, a prototypic guanine nucleotide–binding protein–coupled receptor signaling system, lead to retinal degeneration and have been used as models to understand human degenerative disorders. Here, modulating the sphingolipid biosynthetic pathway rescued retinal degeneration in *Drosophila* mutants. Targeted expression of *Drosophila* neutral ceramidase rescued retinal degeneration in arrestin and phospholipase C mutants. Decreasing flux through the de novo sphingolipid biosynthetic pathway also suppressed degeneration in these mutants. Both genetic backgrounds modulated the endocytic machinery because they suppressed defects in a dynamin mutant. Suppression of degeneration in arrestin mutant flies expressing ceramidase correlated with a decrease in ceramide levels. Thus, enzymes of sphingolipid metabolism may be suitable targets in the therapeutic management of retinal degeneration.

Sphingolipids are integral components of eukaryotic cell membranes and also a rich source of second messengers for several signal transduction cascades. Sphingolipid metabolism generates and interconverts various metabolites including ceramide, sphingosine, and sphingosine 1-phosphate, which are second messengers in diverse signaling pathways that affect cell cycle, apoptosis, and angiogenesis, among others (1, 2). Serine palmitoyl-CoA transferase (SPT) catalyzes the rate-limiting first step in the de novo biosynthesis of sphingolipids including ceramide (fig. S1). Ceramidases hydrolyze ceramide to sphingosine, and neutral or alkaline ceramidase is proposed to function in signaling (3). Mutant analyses in yeast have