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Discerning adaptive divergence within an endangered conservation unit – Gulf of Maine Atlantic salmon

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ABSTRACT

Background: Conservation units for endangered or threatened species are often defined based on close genetic affinities. Seven populations of one such unit, the Gulf of Maine's Distinct Population Segment (GOM DPS) of endangered Atlantic salmon (*Salmo salar*), are listed as part of one, genetically cohesive conservation unit. All populations within GOM DPS are managed through a single captive rearing and supplementation conservation programme.

Hypothesis: Significant adaptive trait variation exists among component populations within this unit.

Time and location: Craig Brook National Fish Hatchery (2002–2003), a captive-rearing and supplementation facility in East Orland, Maine that manages the endangered, distinct population segment.

Analytical methods: A common-environment rearing design assessed within and among population variation in fitness-related traits of adults (somatic vs. reproductive investment) and offspring (time-to-hatch, mass, and growth).

Results: Significant heritable variation in fitness-related traits is evident within the GOM DPS. Salmon populations differ in ovarian-to-somatic tissue investment, time-to-hatch, and larval mass.

Conclusions: Although the Gulf of Maine DPS of Atlantic salmon is defined based on close genetic affinities of component populations, those populations show considerable variation in fitness-related traits. Such variation indicates the presence of important adaptive diversity that should be monitored and conserved within the larger conservation unit. We also demonstrate how potential adaptive variation can be assayed within the confines of an active endangered species programme.

Keywords: captive rearing, common-garden experiment, Distinct Population Segment, egg size, larval development, reproductive investment, supplemental breeding.

INTRODUCTION

Anthropogenic influences such as over-harvesting, habitat degradation, and invasive species introductions have reduced the population size or range of many species (Vitousek, 1994; Sala *et al.*, 2000; Fiumera *et al.*, 2004). As a result, captive and supplemental breeding schemes have become

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an important component of many species conservation programmes. These programmes are generally charged both with limiting further demographic declines and preserving adaptive genetic variation. They can have either positive or negative effects on fitness and population persistence (Avise and Hamrick, 1996; Araki *et al.*, 2007). Unfortunately, because of the time-critical nature of conservation biology, many such programmes are initiated with limited information to guide the effective preservation of genetic diversity and its use for recovery (Snyder *et al.*, 1996; Williams and Hoffman, 2009).

Conservation units for most organisms seek to preserve phylogenetic lineages with presumed adaptive significance relative to other populations or lineages within the species range (Moritz, 1995; Clardige *et al.*, 1997; Crandall *et al.*, 2000). Neutral molecular markers have been a primary means to assess lineage affinities within species and to estimate the risk of loss of genetic variation under demographic declines and inbreeding (Pereira and Wajntal, 1999; Schreiber and von Hegel, 1999; Fiumera *et al.*, 2000; Consuegra *et al.*, 2005; Cordes *et al.*, 2005; King *et al.*, 2005; Verspoor *et al.*, 2005; Lage and Kornfield, 2006). However, such markers provide, at best, indirect insights into the structure or fate of adaptive diversity that is more closely linked to fitness (Reed and Frankham, 2001; Wang *et al.*, 2002). Failure to recognize adaptive population variation within broader conservation lineages may impair recovery, or even hasten extinction, if management actions unwittingly compromise such adaptations.

Obtaining data on adaptive trait variation poses its own significant challenges, especially for endangered and threatened species. Common approaches for assessing heritable trait variation, such as reciprocal translocations or common-garden experiments, may often be impractical or even present further threats to extinction (e.g. hybridization). That said, we believe captive or supplemental breeding programmes for endangered or threatened species often offer the controlled environmental conditions needed to provide an initial assessment of potential adaptive trait variation among component populations of larger conservation units. Here, we consider evidence for adaptive trait variation among river-specific populations (or demes) nested within an actual endangered conservation unit, the Gulf of Maine Distinct Population Segment (GOM DPS) of Atlantic salmon (*Salmo salar*).

Atlantic salmon populations at the edge and core of the species distribution have experienced marked declines in the last 30–50 years (Friedland *et al.*, 2003; Jonsson and Jonsson, 2004; Consuegra *et al.*, 2005; Jokikokko and Jutila, 2005; Boylan and Adams, 2006). The data we consider were collected within the confines of a conservation breeding programme, taking advantage of the largely common-garden conditions this programme affords. In particular, we considered two primary character groups that are presumably closely linked to fitness, reproductive investment strategies (somatic vs. reproductive tissue, egg size vs. egg number), and patterns of embryonic and larval development (time-to-hatch, alevin size at age, and specific growth rates) (Kinnison *et al.*, 1998; Hendry and Stearns, 2004). In addition to discussing our findings with respect to population adaptation in general, we also consider the relevance of our findings for conservation and recovery strategies of these endangered fish.

METHODS AND MATERIALS

Study system

Since 2000, Atlantic salmon broodstocks from six rivers in the GOM DPS have been maintained through supplemental breeding at the United States Fish and Wildlife Service's Craig Brook National Fish Hatchery (CBNFH) in East Orland, Maine. The six rivers in this

programme are all in small coastal watersheds (130–500 square miles) and include the Machias, Narraguagus, Sheepscot, East Machias, Dennys, and Pleasant rivers. River-specific parents are currently used to produce fry that are in turn stocked back into each of the respective rivers. The parental broodstock are captured as parr (mostly one year of age) from each river and transported to CBNFH where they are reared for at least two years to maturity. During this period these fish undergo approximately 99.9% of their growth (collected at approximately 3–4 g and mature at 3–4 kg) in identical tanks on the same water supply, water flow, diet, and light regimen. Because CBNFH holds environmental variables constant across rivers, it provides an approximate common-garden, or common-environment, experiment that can be used to assess potential heritable trait variation among these endangered populations (Garcia de Leaniz *et al.*, 2007).

Salmon broodstock from the geographically proximal, and much larger (watershed ~2380 square miles) Penobscot River GOM DPS (74FR29344, 2009), are captured as returning sea-run adults and transported to CBNFH. The wild rearing of these adults obviates direct comparisons with the captive-reared adults from the other DPS rivers. However, at the hatchery eggs from these wild adults are stripped and fertilized in a similar fashion to the above populations. As such, we were able to rear Penobscot salmon eggs and larvae in the same fashion as the original GOM DPS populations, facilitating a comparison of juvenile traits.

The river-specific broodstock programme for the GOM DPS was initially instituted as a conservative approach to preserve unknown local adaptations. However, these endangered populations have fallen to severely small sizes in the wild, with less than 160 total adult returns in 2009 (USASAC, 2009). Inevitably, this increases risks of drift and inbreeding (Lande, 1988; Gilligan *et al.*, 1997; Frankham *et al.*, 2002). Some degree of outbreeding could theoretically offset these problems, but carries a risk of disrupting local adaptation (Frankham *et al.*, 2002; Allendorf and Luikart, 2007). This risk would depend on the extent of local adaptation within the DPS and the ability of managers to use adaptively similar sources. Knowledge of the extent and patterns of adaptive divergence are thus essential to inform conservation strategies.

Data collection

Midorbital-hypural length (MHL), total ovarian mass, egg size, and egg number were recorded from 714 female salmon spawned in November of 2002 and 2003 as part of normal propagation activities at CBNFH. Female salmon were anaesthetized (tricaine methanesulphonate) and digitally photographed immediately before spawning. Photographs were taken at a fixed focal length, and a length standard (10 cm) was included in each digital photo for scale. The MHL was then estimated from each digital photograph as the distance (cm) between a point at the centre of each individual's eye to a point at the posterior edge of the caudal peduncle flexure.

Eggs were extracted from females by manual pressure or injection of oxygen into the body cavity. Eggs were then fertilized, water-hardened (i.e. hydrated to their full size), and placed in isolated chambers in a heath tray incubator. In 2002, we relied on hatchery water temperature records for early temperature data and in 2003 we placed Hobofi temperature loggers (Onset Corporation, Bourne, MA) in the incubation water at spawning for each population to track development in degree days for each family. Egg size, egg number, and total ovarian mass data were collected when eggs reached the eyed (~250 degree days) developmental stage (February 2002, 2003).

To collect reproductive (ovarian) data, the total mass of eggs for each female was transferred into a colander, drained of excess water, and weighed (g). In total, 200 eggs were sub-sampled from this total mass and weighed to the nearest 0.01 g to estimate each female's mean egg size. Total ovarian mass was simply divided by mean egg size to estimate total egg number (Kinnison *et al.*, 1998). Due to a very small population size, the egg contributions of some Pleasant River females were split into as many as three aliquots for fertilization by different males. The ovarian mass for each of the aliquots derived from a given female was summed to obtain the full female contribution and the mean egg size calculated for the total ovarian mass. Summed ovarian mass was divided by mean egg size to estimate the total egg number for each Pleasant River female.

To collect embryonic and alevin (larval) development data, 30 eggs from each of 30 families per population were sampled at the 'eyed' development stage for experimental rearing. The families were sampled proportionately across the spawning dates within each population. Eggs were transported to the University of Maine, where they were placed in porous cups positioned randomly in Heath trays and reared under a common environment (water flow = 12 $L \cdot min^{-1}$; temperature = 4.5–5.0°C). A few families were injured during transport (mostly from the Sheepscot and Penobscot populations), and were eliminated from further sampling. Again, temperature regimes were monitored using Hobo temperature loggers to calculate degree days.

As hatching approached, families were examined four times daily (every 4–7 h) to record the number of eggs hatched and to remove mortalities. Hatching times were measured based on the date and time when a family first passed 50% hatch. Five alevins were sampled at this time and preserved (in 10% buffered formalin). Hatching samples then served as time zero for consideration of alevin development patterns. Alevin samples were collected at two additional standardized times following hatch for each family: 90 degree days from hatch (~50% yolk absorption) and 275 degree days from hatch (nearing 100% absorption). Yolk tissue was dissected from each alevin and the yolk and body were then independently dried (95°C for more than 24 h) and weighed (Kinnison *et al.*, 1998).

Due to logistical uncertainties surrounding CBNFH temperature records during the earliest period of egg rearing in the 2002 brood year, we could not use the 2002 eggs to evaluate degree days to hatching. However, we were able to use that year's larvae to assess subsequent larval development, which began with hatching at the University of Maine. The egg development portion of our study is determined for families created in 2003, when detailed temperature logger data were available beginning at spawning.

Data analysis

All traits were log-transformed (log10) to account for allometric relationships as described in Kinnison *et al.* (2003), unless otherwise indicated. All statistical analyses were performed using SPSS v.8.0 software. A sequential Bonferroni adjustment (Rice, 1989; Jastrebski and Robinson, 2004) was employed as a conservative control for multiple comparisons.

Reproductive versus somatic investment

Total ovarian mass was analysed relative to midorbital-hypural length (MHL) to quantify relative ovarian versus somatic investment. The effects on ovarian mass of population (fixed), body length (covariate), age (fixed = years in hatchery), and the population-by-length interaction were analysed using analysis of covariance (ANCOVA). The interaction

between age and length was not included because there was little overlap in length among ages, which would violate the assumptions of ANCOVA. Multiple pairwise ANCOVAs of the same form were used to perform contrasts between the ovarian mass to length slopes among all populations to identify which populations likely contribute to divergence. Similarly, contrasts among estimated marginal population means of ovarian mass were used to assess whether populations differ in mean ovarian mass at a grand mean body size (mean size of covariate – MHL – among ALL populations) of 52 cm, after taking into account their respective ovarian mass to body size slopes.

To determine whether variation in size-dependent ovarian investment (relative to body size) *within* populations is better explained by size-dependent variation in egg size or egg number, we obtained studentized residuals from the fitted model of ovarian mass, egg size or egg number on age and body length for each population. We then performed linear regression analyses on residual ovarian mass relative to both residual egg number and residual egg size for each population. Similarly, to determine if *among*-population variation in size-dependent ovarian mass is better explained by population differences in size-related egg size or egg number, we obtained the estimated marginal mean ovarian mass, egg size, and egg number from the above full ANCOVA models. The interaction was not significant for egg size, and was thus removed. Linear regression analyses of marginal mean ovarian mass estimates were performed on marginal mean egg number and egg size.

Egg size versus egg number within ovarian investment

The above analyses consider patterns of investment into ovarian tissue relative to body size (and how egg size and number within and among demes contribute to such size-dependent investment). Another level of reproductive trade-off concerns variation among populations in how they apportion a given ovarian investment between egg number or size, irrespective of fish size. Variation in the trade-off between egg number and size *within* a given total ovarian investment was assessed by performing an ANCOVA with either egg number or egg size as the dependent variable and ovarian mass as the covariate, with population and age again as fixed factors. Because ovarian mass is defined as the product of egg number and mean egg size, variation in the relationship between egg size and ovarian mass, and between egg number and ovarian mass, represent reciprocal analyses of the same egg size–number trade-off. Pairwise population contrasts were again run to identify particular cases of population divergence. The estimated marginal means of egg size and egg number were obtained to depict population variation in egg size and egg number for the calculated standard ovarian mass of 692.5 g.

Juvenile development

Egg size influences early development (Hutchings, 1991; Einum and Fleming, 2000). Hence, the ANCOVAs of population effects on hatch time incorporated egg size as a covariate. Similarly, because yolk provides the only food source for the developing alevin (i.e. maternal effect), egg size was again included as a covariate. Each of the three temporal samples of alevin dry body mass (0, 90, and 275 degree days) was analysed separately. In addition, we calculated and compared growth rate [(ln mass final – ln mass initial × 100)/time] of dry body mass for each family over three inclusive time intervals: early growth (0–90 degree days), late growth (91–275 degree days), and overall growth (0–275 degree days). To determine

if growth rates vary among populations, we performed ANCOVAs with growth rate as the dependent variable, population as the fixed factor, and available yolk (dry) at the onset of each growth period as a covariate.

RESULTS

Reproductive trade-offs

Variation in total ovarian mass relative to length

Populations differed in their ovarian mass to body length relationships (Fig. 1). For example, multiple contrasts with Bonferroni adjustment showed that the Sheepscot population differed significantly from the Narraguagus ($P < 0.001$), East Machias ($P < 0.001$), and Pleasant ($P = 0.016$) populations, but not from the Machias ($P = 0.118$) and Dennys ($P = 0.127$) populations in the population-by-length interaction. After taking into account such slope differences, some populations still differed in their estimated marginal mean ovarian mass at the grand mean body length of 52 cm. For example, the Sheepscot population's ovarian mass differed significantly from that of the Narraguagus ($P < 0.001$), East Machias ($P < 0.001$), and Pleasant ($P = 0.003$) populations, but not from the Machias ($P = 0.069$) and Dennys ($P = 0.087$) populations. Contrasts for all population differences at estimated marginal means (irrespective of slope differences) are shown for each population in Table 1. It should be noted that the slope and intercept estimates of the Pleasant River population were extreme relative to the other populations, but were statistically different from only one other population, the Sheepscot (Table 1). This is likely due to the smaller sample sizes for the Pleasant River population.

Residuals analyses indicated that egg number explained more of the size-dependent variation in ovarian mass at a given body size than egg size *within* each of our study populations (Table 2). However, egg number and egg size explained nearly equal variation in *among*-population patterns of size-dependent ovarian mass (Table 2).

Egg size and number allocation within an ovarian mass

Age was not significant ($P = 0.242$) in our ANCOVAs of the effects of egg size on total ovarian investment. Likewise, there was no population-by-ovarian mass interaction ($P = 0.642$), indicating that populations show a similar proportional scaling of egg size and number with total ovarian investment. However, there was evidence that populations differed significantly in their relative egg size to ovarian mass relationships ($P = 0.021$). Examination of estimated marginal means (Table 3) suggests that the greatest absolute difference (about a 6.6% difference in egg size or number relative to ovarian mass) is found between the Narraguagus and Pleasant river populations, with most of the remaining populations different from the Pleasant by approximately 3%. Statistical comparisons of estimated marginal mean egg size and number contrasts of focal populations against remaining populations suggest, however, that only two populations differ significantly in intercepts from one another at the grand mean ovarian mass of 692.5 g (Table 3). Specifically, the Machias population's egg number relative to ovarian mass is significantly less than that of the Narraguagus ($P < 0.001$), with the opposite pattern for egg size.

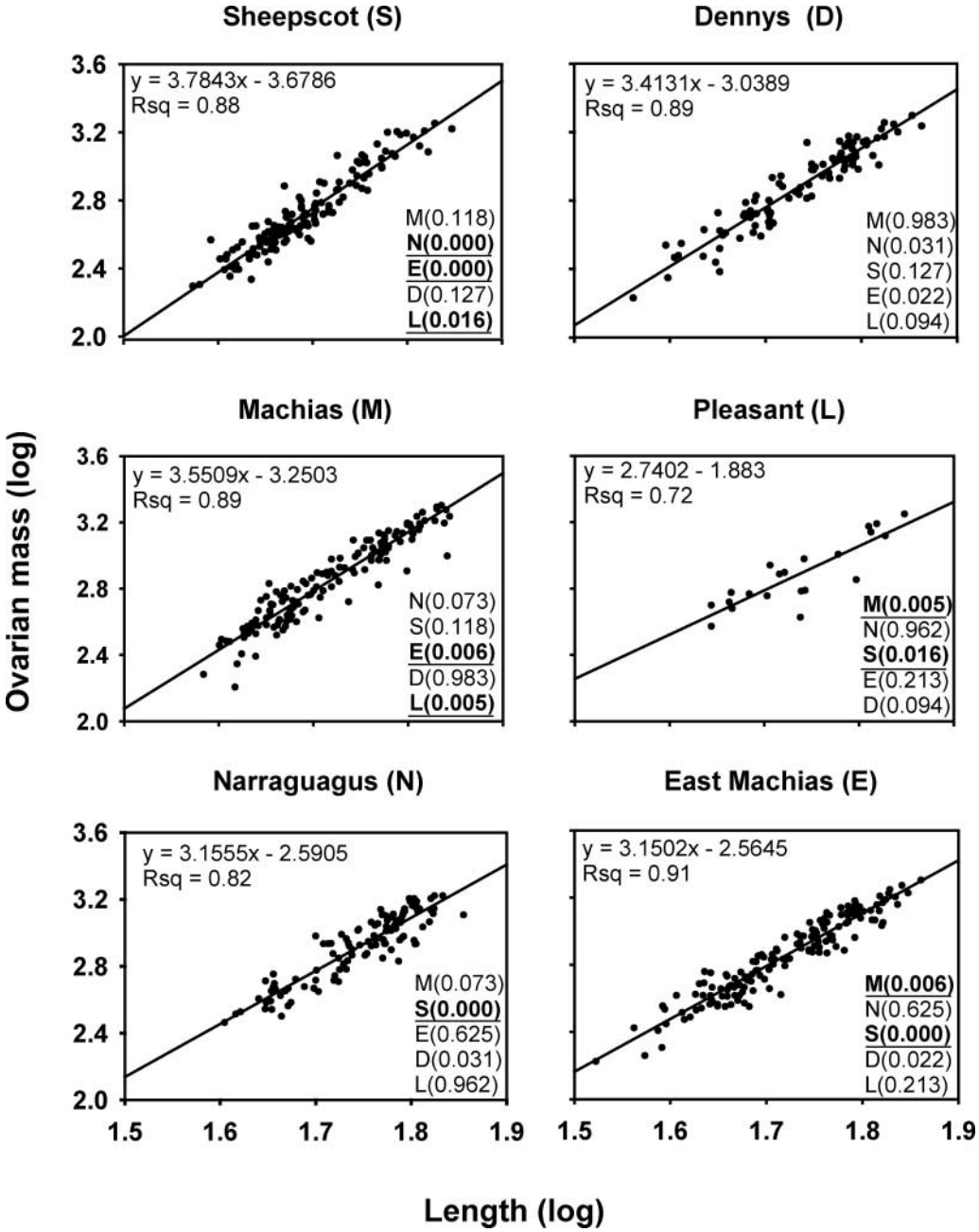

Fig. 1. Ovarian mass-by-length relationship of GOM DPS salmon populations. Significant population contrasts of pairwise slopes are supplied for each population with *P*-values in parentheses. Significant contrasts following sequential Bonferroni adjustment of the overall alpha (0.05) are shown in **bold** and are underlined. The regression equation and R^2 value are included for each population.

Table 1. Population contrasts of ovarian mass (g) at a grand mean length of 52.52 cm

Population (*n*)	EMM	95% confidence intervals		Population contrasts (*P*-value)
		Lower	Upper	
Machias (140)	740.26	718.50	762.68	N (0.024), S (0.069), E (0.016), D (0.934), L (0.032)
Narraguagus (112)	694.26	669.86	719.54	M (0.024), **S (0.000)**, E (0.756), D (0.046), L (0.404)
Sheepscot (139)	699.23	675.88	723.39	M (0.069), **N (0.000)**, **E (0.000)**, D (0.087), **L (0.003)**
East Machias (95)	734.96	714.58	755.92	M (0.016), N (0.756), **S (0.000)**, D (0.042), L (0.289)
Dennys (95)	688.37	663.87	713.76	M (0.934), N (0.046), S (0.087), E (0.042), L (0.042)
Pleasant (21)	774.69	714.29	840.19	M (0.032), N (0.404), **S (0.003)**, E (0.289), D (0.042)

Note: Estimated marginal means (EMM) are anti-logged estimates taken from the ANCOVA model in Fig. 1. Significant pairwise *P*-values (in parentheses), following sequential Bonferroni adjustment to original alpha (0.05), are in **bold** and underlined. M = Machias, N = Narraguagus, S = Sheepscot, E = East Machias, D = Dennys, L = Pleasant.

Table 2. Egg number and egg size contributions to within- and among-population variation in total ovarian mass

Population (*n*)	Zero-order correlation	
	Estimated egg number	Mean egg size
Machias (140)	0.8724	0.0912
Narraguagus (112)	0.8284	0.0826
Sheepscot (139)	0.8520	–0.0788
East Machias (155)	0.7857	0.1410
Dennys (95)	0.8361	–0.0191
Pleasant (21)	0.8658	0.5964
Among populations	0.8584	0.8425

Note: Within-population variation described by river-specific correlations (*r*-value) of studentized residuals for egg size (log) or egg number (log) regressed on the residuals for ovarian mass (log). Among-population variation described by correlation of estimated marginal mean egg number or egg size on marginal mean ovarian mass (at approximately 52 cm fork length). All correlations are significant at $P < 0.001$.

Table 3. Divergence in estimated marginal mean egg size and number at an overall ovarian mass of 692.5 g

Population (*n*)	EMM	95% confidence intervals		% difference	Population contrasts (*P*-value)
		Lower	Upper		
Estimated number of eggs					
Machias (140)	4152	4074.24	4232.10	1.65	**N (0.000)**, S (0.343), E (0.295), D (0.177), L (0.536)
Narraguagus (112)	4354	4265.76	4445.95	6.61	**M (0.000)**, S (0.016), E (0.013), D (0.069), L (0.020)
Sheepscot (139)	4205	4123.25	4288.64	2.94	M (0.343), N (0.016), E (0.954), D (0.634), L (0.277)
East Machias (155)	4208	4133.70	4284.17	3.01	M (0.295), N (0.013), S (0.954), D (0.657), L (0.258)
Dennys (95)	4235	4141.82	4330.51	3.67	M (0.177), N (0.069), S (0.634), E (0.657), L (0.189)
Pleasant (21)	4085	3886.17	4294.21	0.00	M (0.536), N (0.020), S (0.277), E (0.258), D (0.189)
Mean egg size (g)					
Machias (140)	0.1668	0.1637	0.1700	4.89	**N (0.000)**, S (0.341), E (0.292), D (0.177), L (0.545)
Narraguagus (112)	0.1590	0.1558	0.1623	0.00	**M (0.000)**, S (0.016), E (0.013), D (0.068), L (0.021)
Sheepscot (139)	0.1647	0.1615	0.1680	3.57	M (0.341), N (0.016), E (0.953), D (0.636), L (0.282)
East Machias (155)	0.1646	0.1616	0.1675	3.49	M (0.292), N (0.013), S (0.953), D (0.661), L (0.263)
Dennys (95)	0.1635	0.1599	0.1672	2.84	M (0.177), N (0.068), S (0.636), E (0.661), L (0.194)
Pleasant (21)	0.1695	0.1612	0.1782	6.58	M (0.545), N (0.021), S (0.282), E (0.263), D (0.194)

Note: Percent difference is calculated relative to the smallest population value. Significant pairwise *P*-values (in parentheses), following sequential Bonferroni adjustment to original alpha (0.05), are in **bold** and underlined. M = Machias, N = Narraguagus, S = Sheepscot, E = East Machias, D = Dennys, L = Pleasant.

Juvenile development

Hatch time

ANCOVA suggests that populations share a common slope relationship between egg size and hatch time (population-by-egg size interaction: $P = 0.071$). Both population ($P < 0.001$) and egg size ($P = 0.001$), nonetheless, had a significant influence on hatch time. Contrasts of estimated marginal means (at an egg size of 0.16 g) demonstrated which populations differed most in hatch time (Table 4). For example, the Narraguagus population required the fewest degree days to hatch (390.5), whereas the East Machias required the most degree days (407.4).

Table 4. Differences in time to hatching at a grand mean egg size of 0.16 g among seven Maine Atlantic salmon populations

Population (*n*)	EMM	95% confidence intervals		Contrasts
		Lower	Upper	
Machias (30)	403.93	399.40	408.46	**N (0.000)**, S (0.076), E (0.379), D (0.239), L (0.429), P (0.012)
Narraguagus (33)	390.51	386.19	394.83	**M (0.000)**, **S (0.014)**, **E (0.000)**, **D (0.002)**, **L (0.002)**, P (0.309)
Sheepscot (30)	398.14	393.58	402.70	M (0.076), **N (0.014)**, **E (0.009)**, D (0.545), L (0.395), P (0.300)
East Machias (29)	407.36	402.74	411.99	M (0.379), **N (0.000)**, **S (0.009)**, D (0.041), L (0.116), **P (0.001)**
Dennys (30)	400.19	395.66	404.72	M (0.239), **N (0.002)**, S (0.545), E (0.041), L (0.760), P (0.124)
Pleasant (27)	401.09	395.91	406.27	M (0.429), **N (0.002)**, S (0.395), E (0.116), D (0.760), P (0.097)
Penobscot (17)	394.14	388.00	400.27	M (0.012), N (0.309), S (0.300), **E (0.001)**, D (0.124), L (0.097)

Note: Contrasts of the estimated marginal means (EMM) are displayed for each river against all other rivers. The *P*-values are presented in parentheses, and **bold**, underlined font indicates significant differences after a sequential Bonferroni correction of the original 0.05 alpha level.

Alevin body mass

Populations did not differ in their alevin body mass to egg size slopes at the first (population-by-egg size interaction: $P = 0.696$) or second (population-by-egg size interaction: $P = 0.670$) alevin sampling intervals (Fig. 2), and all populations were thus assumed to share a common within-groups slope (slope 1 = 0.720; slope 2 = 0.677). Estimated marginal means differed among many of the rivers, often in a consistent fashion at the first and second samples (Fig. 2). For example, the Machias population tended to have the largest body mass and the Dennys and Penobscot populations tended to have the smallest body mass for a given initial egg size.

The slope of the body mass to egg size relationship did vary among populations at the third alevin sample (population-by-egg size interaction: $P = 0.027$). Pairwise comparisons demonstrate that this is largely due to contrasts associated with the Sheepscot population (Penobscot vs. Sheepcot and East Machias vs. Sheepscot), which had the smallest sample size (16 vs. 20–30 for the other populations) among our populations (Fig. 3). When the Sheepscot was removed from the analysis, the population-by-egg size interaction was no longer significant ($P = 0.127$), and there were again differences in the intercepts among populations consistent with earlier sampling intervals.

Specific growth rates

ANCOVA demonstrated no significant population-by-yolk mass interaction for the early interval growth rate ($P = 0.344$). Indeed, yolk mass did not explain significant variation in early growth rate ($P = 0.128$). Still, populations did differ significantly in specific growth rate

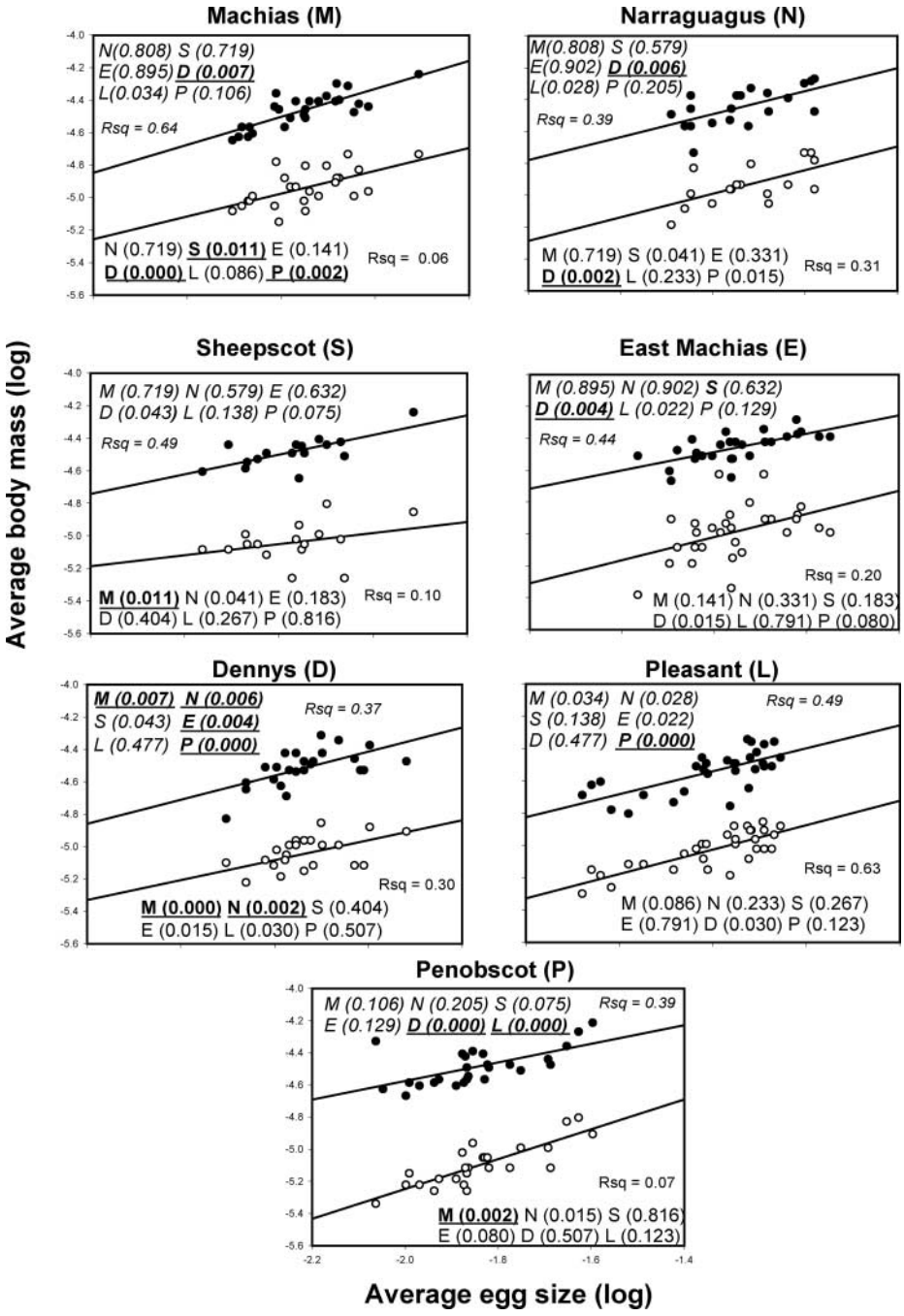

Fig. 2. Differences in alevin dry body mass at sample 1 (open circles, normal font) and sample 2 (solid circles, italic font) when taking into account variation due to egg size (covariate). Contrasts of estimated marginal means at a grand mean egg size of 0.16 g are provided for each population against all other populations. The *P*-values for each contrast are provided in parentheses, and **bold**, underlined font indicates a significant difference following a sequential Bonferroni adjustment to the alpha level (0.05).

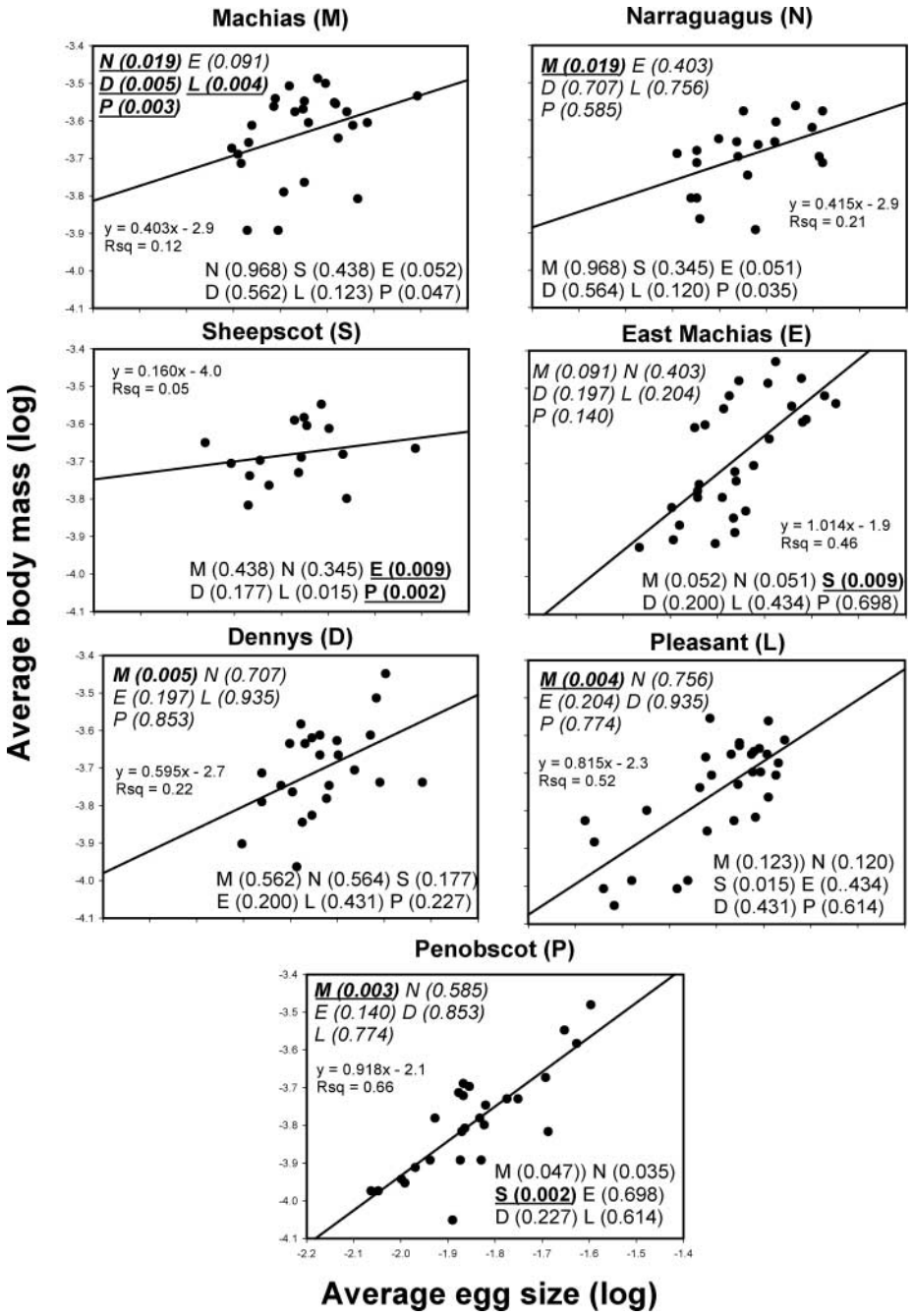

Fig. 3. Mean body mass to egg size relationships at sample 3 (275 degree days). *P*-values for population divergence are presented in parentheses. **Bold**, underlined font indicates a significant difference following a sequential Bonferroni adjustment of the alpha level (0.05). *Italics* indicate contrasts of marginal means with the Sheepscot population removed.

in this interval ($P < 0.001$; see Fig. 4). For example, the Pleasant population differed from the Sheepscot ($P = 0.004$), Dennys ($P = 0.009$), and Penobscot ($P < 0.001$) populations, but not from the East Machias ($P = 0.018$), Machias ($P = 0.763$), or Narraguagus ($P = 0.019$) populations.

Unlike the early growth interval, the interaction between river and available yolk was significant for the later growth interval ($P = 0.001$). Comparisons of population slopes (Fig. 4) showed that growth rate increased with available yolk in some populations (e.g. East Machias, Penobscot, and Pleasant) and decreased with available yolk in others (e.g. Narraguagus, Machias, and Sheepscot).

Interestingly, the growth rate effects apparent in the first and second intervals appear to cancel out (i.e. compensatory development). ANCOVA with yolk mass as the covariate suggested that there is no significant influence of either available yolk ($P = 0.114$) or population ($P = 0.759$) on growth rate over the entire growth period (from sample 1 to sample 3).

DISCUSSION

The objectives of this study were to assess variation in heritable, adaptive traits within and among seven endangered populations of Maine's anadramous Atlantic salmon. These populations are all very closely related relative to the broader diversity of the species (King *et al.*, 2000, 2001; Spidle *et al.*, 2003). First, we considered potential population divergence in trade-offs associated with reproductive investment in six of the federally listed populations within the Gulf of Maine Distinct Population Segment (GOM DPS). Second, we investigated potential population divergence in early life-history traits in the same six populations, plus the Penobscot River population, the largest remaining run of Atlantic salmon in the United States. Our results point to the potential for apparent heritable divergence among these populations in both reproductive trade-offs, and early development and life history. This indicates that different populations are likely reservoirs of locally adaptive trait variation.

Generally, life-history traits such as total reproductive investment, offspring size, and offspring number are considered closely linked to fitness, and thus adaptive (Kinnison *et al.*, 1998, 2003; Hendry *et al.*, 2001; Juanes *et al.*, 2004). For example, investment into ovarian production versus somatic mass presumably reflects a trade-off between positive benefits of investment into offspring biomass and costs associated with investment in other components of reproductive investment or mobility (Gunn and Gatehouse, 1993; Kinnison *et al.*, 2003; Ji and Wang, 2005). Additionally, early life-history traits such as timing of hatching, juvenile body size, and growth rate significantly influence offspring survival (Metcalfe and Thorpe, 1992; Sinervo *et al.*, 1992; Gilbey *et al.*, 2005) and later ontogenic development (Metcalfe and Thorpe, 1992; Einum and Fleming, 2000; Gilbey *et al.*, 2005). For example, research suggests that hatch timing and subsequent larval development rates are synchronized with optimal times for alevin to emerge from nests and transition to exogenous feeding in their natal environments, balancing feeding opportunities with risks of predation and competition (Brannon, 1987; Einum and Fleming, 2000). In the following sub-sections, we consider some of our findings in the context of life-history theory and its relevance to conservation of Atlantic salmon.

The ovarian versus somatic trade-off

Salmon can invest substantial amounts of energy into aspects of reproduction other than ovarian investment, such as migration and spawning behaviours. Translocation experiments

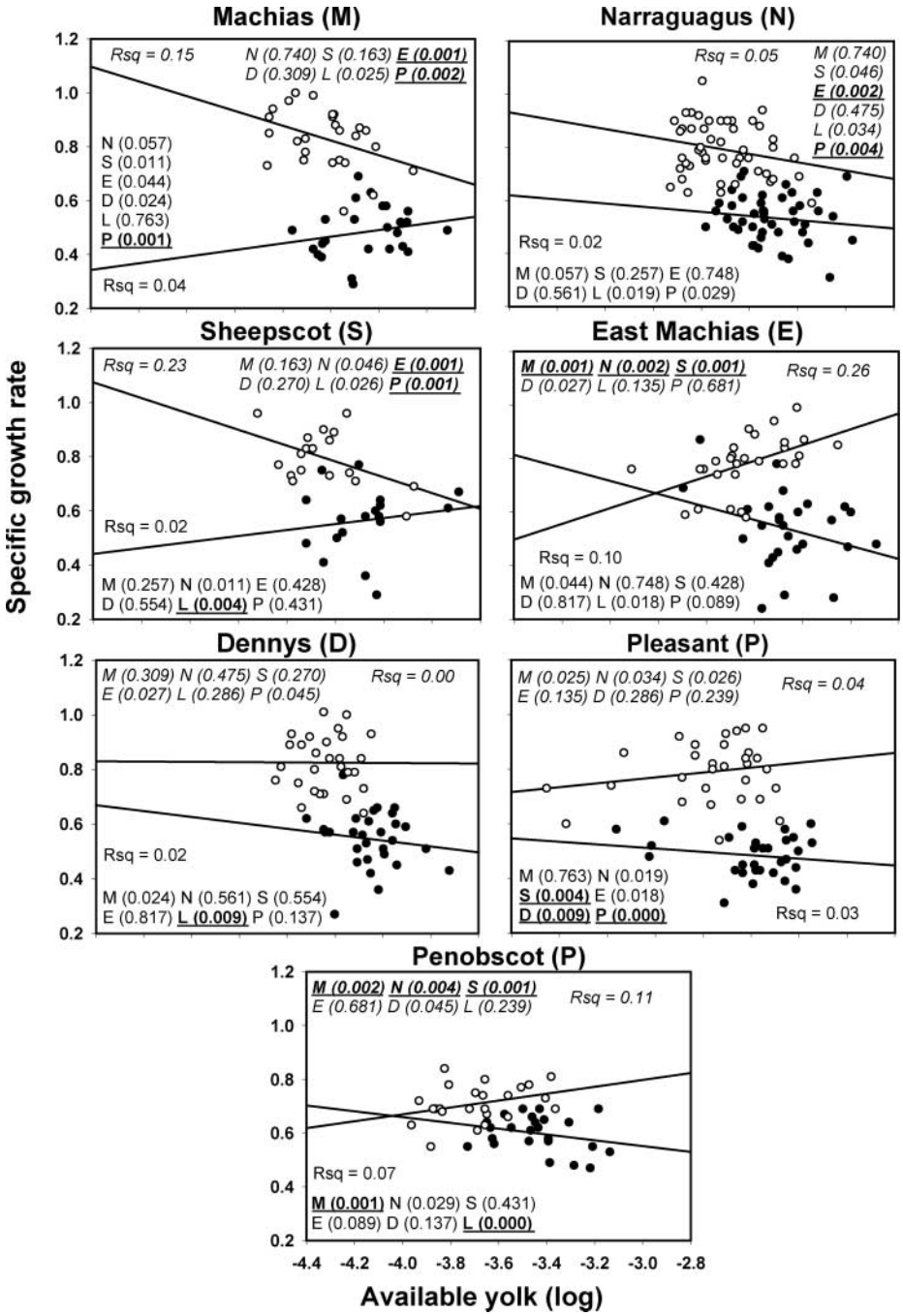

Fig. 4. Differences in early and late growth intervals among Maine's salmon populations. Early intervals are represented by solid circles and normal font. Late growth intervals are represented by open circles and *italic* font. **Bold**, underlined font indicates significant contrast differences between populations after a sequential Bonferroni adjustment on the alpha level (0.05).

have shown that migration and ovarian investment are in direct competition for resources in other salmon species (Kinnison *et al.*, 2001). They also showed that populations adapted to more difficult migrations can evolve countergradient (Conover and Schultz, 1995) allocation into ovarian mass to make up for a portion of their migratory costs, though they generally do not fully compensate for the cost of migration on total ovarian mass, perhaps due to yet another trade-off with hydrodynamic efficiency. There were no clear relationships between river length and ovarian investment in captivity for the Maine populations that would overtly correspond to such investment patterns. However, the rivers used by the populations of salmon in the GOM DPS are all relatively short. Variation in ovarian investment may thus be better explained by geographic variation in other determinants of energetic investment and reproductive success, such as local thermal conditions, intensities of mating competition, or variability in juvenile mortality rates.

Egg number and egg size trade-off

Empirical evidence for optimization of egg size exists for a range of organisms, including reptiles (Sinervo and Licht, 1991) and fish (Einum and Fleming, 2000). For example, Einum and Fleming (2000) experimentally showed that female Atlantic salmon produce eggs of a near optimum size predicted for their natal environment. Some intra-population variation in egg size is evident in the GOM DPS populations, particularly in relation to individual fish size and ovarian mass. This might appear counterintuitive to an optimal egg size model. In reality, such variation is expected as a condition-dependent variant of Smith and Fretwell's (1974) hypothesis because maternal size can have a strong influence on offspring environment through nest site selection and construction (Einum *et al.*, 2002) that causes optimal egg size to vary with body size. We found that most of the *within*-population variation in residual ovarian investment was explained by variation in residual egg number, which is consistent with the theoretical expectation that egg size is more constrained toward a local size-dependent optimum than egg number.

Egg size–offspring performance functions are expected to commonly vary among populations, leading to local divergence in the egg size–number trade-off (Smith and Fretwell, 1974; Fleming and Gross, 1990; Hutchings, 1991). Such variation might result from habitat differences in size-dependent predation risks, juvenile competition, or egg-size-related survival in the gravel. Maine salmon populations showed evidence of subtle divergence in egg size investment. For example, the Narraguagus population had the smallest egg size and largest egg number for a given ovarian mass and thus commonly differed from the Machias (with Bonferroni) and Sheepscot (without Bonferroni) populations, which tended towards the opposite extremes. Divergence in optimal egg size among populations likely explains why *among*-population variation in size-dependent ovarian investment is more equally associated with both egg number and egg size variation than was size-dependent ovarian investment in egg size and number *within* populations.

Time-to-hatch

Our results suggest that degree days required for hatching tends to increase with egg size in the same general fashion for all Maine salmon, but eggs of a given size require different numbers of degree days to hatch in different populations. Variation in embryonic development rates in salmonids are often considered adaptations to local thermal regimes that themselves influence optimal hatching or emergence timing (Kinnison *et al.*, 1998; Obedzinski and Letcher, 2004). For example, the Penobscot River is larger, runs further inland and further north

than the other six rivers. As expected, this population required fewer degree days to hatch for a given egg size, fitting with the cooler temperatures that likely prevail in such habitats (snow melt, elevation, etc.). Such an interpretation also suggests that incubation habitats in the Narraguagus might have naturally been among the coldest of the smaller coastal DPS populations.

Larval size at age and growth rate

Because the initial yolk reserves in eggs provide the resources for embryonic and alevin growth, we might expect that egg size would have a significant influence on early body mass in families (Kinnison *et al.*, 1998; Obedzinski and Letcher, 2004). This was indeed the case at all three sampling times in our analysis. Such contributions to juvenile size represent maternal effects (Heath *et al.*, 1999). However, body size at age differed among populations, even after accounting for such maternal influences. This would suggest that populations differ in the relative rates or efficiency with which egg resources are converted into alevin tissue. We did find evidence of some population differences in early and late alevin growth rates in this study, although rate differences appear to have cancelled out over the full time interval (i.e. crossing slopes in Fig. 4). Interestingly, yolk reserves explained relatively little family variation in specific growth rates (Fig. 4) in our controlled environment, which adds credence to the idea that larval growth rates in salmon are heritable and can eventually obscure maternal yolk contributions (Heath *et al.*, 1999).

Given that alevins live interstitially in the gravel before emergence, it is somewhat difficult to assess which selective factors might shape size or growth rates during that period. It could be that alevin sizes and growth rates evolve under direct selection in the gravel environment (e.g. size limitations due to gravel porosity), or indirect selection associated with competing demands on energy reserves (e.g. thermal conditions or dispersal activity) or correlated sizes at high mortality stages following emergence.

We found somewhat different patterns of population divergence than noted by Obedzinski and Letcher (2004), who considered a subset of the populations we evaluated. Contrary to our results, Obedzinski and Letcher (2004) indicate that time to hatch did not differ significantly among the GOM DPS populations. However, both studies suggest similar results for alevin size and growth rates. The inconsistency between results may result from differences in experimental design. We were able to offer greater control of hatchery origin, temperature and conditions, thus narrowing the opportunity for confounding environmental effects.

Conservation implications

Modern conservation programmes are generally charged with not only limiting demographic declines, but also preserving adaptive genetic variation and the potential for a species to persist in the wild (Crandall *et al.*, 2000; Fiumera *et al.*, 2000; Blanchet *et al.*, 2008; Williams and Hoffman, 2009). Maine's endangered Atlantic salmon are managed as seven independent populations, each with its own breeding programme (Piper and Schramm, 1995; National Research Council, 2004). The differences that we have noted in reproductive investment and early development support the contention that these populations are likely reservoirs of locally adaptive trait variation. As such, it is worth considering potential threats to that adaptation and what actions might be required to remediate those threats.

Salmon populations in the GOM DPS have shown consistent declines in the wild for nearly two decades and their effective population sizes, even in captivity, are now well below

recommendations for avoiding substantial losses of genetic variation due to drift (N_e = ~500), and are approaching levels where risks of inbreeding depression are appreciable (N_e = 50) (Frankham *et al.*, 2002). A relatedness-based mating regimen is now in place using microsatellite diversity to reduce the odds of matings between close kin (Letcher and King, 1999). However, such an approach is only likely to slow the threat of inbreeding, as inbreeding becomes inevitable in small, closed populations (Letcher and King, 1999; Frankham *et al.*, 2002). Ultimately, drift, inbreeding, and other elements of the classic vortex of extinction (Soule *et al.*, 1986) could come to compromise the fitness of these populations.

Consider the Pleasant River population as an example. This population was consistently extreme in its ovarian mass, egg number, and egg size relationships even if they were not always statistically different from those of other populations (perhaps due to sample size limitations). This might represent evidence that the Pleasant population is unique among Atlantic salmon in Maine. However, this population fell to critically small sizes faster than the other DPS populations (National Research Council, 2004; USASAC, 2006), and the much higher correlation between residual egg size and residual ovarian mass that we found for the Pleasant River population (Table 2), compared with all others, is somewhat troubling. That correlation can be interpreted as evidence that egg size may now be less strongly optimized in that population.

Genetic rehabilitation through population mixing, however, can be a risky undertaking if such mixing disrupts remaining local adaptation. Studies like ours can provide data on trait variation that might be used to guide rehabilitation in ways that minimize adaptive conflicts of outbreeding. In the case of the Pleasant River population, fish from the Machias appear most similar for the traits we have studied and might thus be the best source for rehabilitation. Of course, the ultimate costs and benefits of outbreeding strategies should ideally be evaluated under controlled conditions in the wild, well in advance of their acute conservation needs.

Closing comments

The use of heritable trait divergence to initially delineate conservation units may often not be possible, but conservation breeding programmes themselves may often provide a means to subsequently assess and monitor adaptive variation. We believe this should be a priority of restoration programmes, and not just an optional element. As adaptive trait information comes to light, it may often supersede indirect insights from neutral markers and improve evolutionary management. The research that we present here on endangered salmon in Maine attests that adaptive trait variation may often exist within previously defined conservation units, and shows that assessment of such trait variation can be readily incorporated as part of a comprehensive genetic management programme.

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