

SENSITIVITY TO MECHANICAL SHOCK IN ATLANTIC SALMON EGGS DURING
THEIR FIRST SIX HOURS AFTER FERTILIZATION

William F. Krise
National Biological Service
Research and Development Laboratory
Rural Delivery #4, Box 63
Wellsboro, PA 16901
717-724-3322, Extension 231, Fax: 717-724-2525, Krisecol@epix.net

Abstract

Atlantic salmon eggs from six domestic female and six sea-run kelts were tested for mechanical shock sensitivity from 0.5 to 6.0 hours after fertilization using force generated by dropping eggs from measured heights. Estimates of drop height and force causing 10% (LC10) and 25% (LC25) mortality were used to compare sensitivity to shock relative to handling encountered in the process of collecting, disinfecting, and transporting of eggs to incubation facilities. There were no significant differences in LC10 and LC25 estimates among sample times through 6 hours post-fertilization. Estimates of shock causing 10% mortality ranged from drop heights of 23.5 to 26.9 cm or force of 5.2 to 6.0×10^3 ergs for domestic eggs and 17.5 to 38.5 cm or 3.5 to 7.7×10^3 ergs for kelt eggs. LC25 estimates of drop height and force were 46.1 to 60.3 cm and 10.2 to 13.4×10^3 ergs for domestic stock and 43.7 to 65.1 cm and 8.8 to 13.1×10^3 ergs for kelt eggs. Variability in shock sensitivity among females was high, and similar to differences in 24-hour mortality found in eggs transported for incubation.

Introduction

Atlantic salmon (*Salmo salar*) egg mortality has increased for eggs collected, fertilized, and then transported to incubation facilities located several hours travel from the fertilization site. Once fertilized, eggs require handling and receive shocks from procedures like disinfection, packing, transport, unpacking, a second disinfection, and placement into incubators. Once all procedures are completed and eggs are transported to incubation facilities, significant mortality occurs the first day after fertilization. The mortality rates are highly variable among eggs from individual females. The purpose of this and a related study is to determine the major causes of egg mortality during transport in Atlantic salmon.

In general, salmonid eggs are thought to withstand shock from routine handling for the first 48 hours after fertilization (Piper et al. 1982). We determined the effects of mechanical shock to eggs during the course of typical transport times used in the Atlantic salmon egg program, apart from possible effects of transport shock. We also tested two types of Atlantic salmon broodstock (domestic stock and reconditioned wild adults, or kelts) for variability between groups and among individuals. The methods used were intended to simulate shock to eggs from water poured over eggs during disinfection or packing procedures. The mechanical shock administered by dropping eggs was to approximate the effect of pouring eggs in water from a similar height (Jensen and Alderdice 1983). We use mechanical shock treatments similar to those of Jensen and Alderdice (1983, 1989) to test differences in shock sensitivity of eggs during the first 6 hours after fertilization.

Methods and Materials

Eggs were collected from six individuals of domestic strain Atlantic salmon (Cronin National Salmon Station, Sunderland, Massachusetts) and six kelts (North Attleboro National Fish Hatchery, North Attleboro, Massachusetts). After fertilization in 10° C water, samples of 20 to 45 eggs were placed into 500 ml jars to serve as controls and for the set of 30-minute sample jars. One set, a negative control, was placed into coolers and not handled further; the other control set was handled the same as those given mechanical shock, but not shocked. At each sampling period thereafter (1, 2, 4, and 6 hours post-fertilization) one control group of eggs was placed into jars and three test (mechanical shock) groups were also placed into separate jars. A pre-test showed that drop heights of 10, 40, and 90 cm produced low, medium, and high egg mortality, leading to selection of those heights for the tests.

To start a test, eggs were gently removed from sample jars, and placed into 6-cm-diameter petri dishes. After all water was drained, dishes were either dropped the appropriate height (experimental groups) or placed back into jars without further handling (controls) as in Jensen and Alderdice (1983, 1989). After a single shock challenge, eggs were transferred back to the water-filled sample jars and placed into coolers for storage at 6-8° C until the end of the test. Two hours after completion of the final mechanical shock, water was drained from jars and eggs placed either into Davidson's fixative (six parts ethanol, four parts formalin, two parts acetic acid and six parts distilled water; for the domestic broodstock eggs) or 10% formalin (kelt eggs). Eggs fixed in Davidson's fixative were examined under 7X magnification to determine mortality because fixed dead eggs returned to amber color. Dead eggs in formalin were easily identified without magnification.

Statistical analysis included estimation of force or drop height causing 10 and 25% mortality (LC10 and LC25) using estimates generated through Tablecurve 2D software (Jandel Scientific, San Rafael, California). Force, or energy transferred to the eggs, in 1×10^3 ergs, was calculated as in Jensen and Alderdice (1989). One-way analysis of variance (ANOVA) was used to determine if there were differences in shock sensitivity among the sample times and two-way ANOVA to determine differences between broodstock groups. All statistical tests were conducted at the $p=0.05$ level of significance.

Results

Estimates of 10 and 25% mortality were similar for both domestic and kelt eggs and are shown as LC10 and LC25 as cm drop height, as force estimated to cause mortality, and as 95% confidence intervals of percent mortality at LC10 and LC25 (Table 1). In all cases, variability among estimates was high, because eggs from certain individual females were much more sensitive to shocks than others (Table 1). Force associated with egg mortality estimates (Table 1) was similar among broodstock types and sample times through the 6-hour sample period. Force calculations ranged between 3.5 and 7.7×10^3 ergs for all LC10 estimates, with most estimates between 5.0 and 6.0×10^3 ergs. Force estimates for 25% egg mortality ranged from 8.8 to 13.4×10^3 ergs.

Table 1. Drop height (cm) estimates of 10% (LC10) and 25% (LC25) Atlantic salmon egg mortality (95% confidence intervals) and corresponding force (10^3 ergs) from 0.5 to 6.0 hours after fertilization of mortality for LC10 and LC25.

Broodstock	Hours	LC10 (cm)	Force (10^3 ergs)	Mortality 95% CI	LC25 (cm)	Force (10^3 ergs)	Mortality 95% CI
Domestic	0.5	26.9	6.0	(4.8 to 15.2%)	59.2	13.1	(15.9 to 34.1%)
	1.0	26.9	6.0	(4.8 to 15.2%)	59.2	13.1	(15.9 to 34.1%)
	2.0	23.5	5.2	(4.5 to 15.5%)	56.2	12.5	(16.7 to 33.3%)
	4.0	25.3	5.6	(5.5 to 14.5%)	60.3	13.4	(18.6 to 31.4%)
	6.0	25.2	5.6	(-1.8 to 21.8%)	46.1	10.2	(15.1 to 34.9%)
Kelt	0.5	26.5	5.3	(-1.1 to 21.1%)	53.6	10.8	(13.5 to 36.5%)
	1.0	38.5	7.7	(-0.3 to 20.3%)	65.1	13.1	(-31.3 to 81.3%)
	2.0	17.5	3.5	(-0.3 to 20.3%)	43.7	8.8	(10.7 to 39.3%)
	4.0	25.6	5.1	(1.0 to 19.0%)	52.5	10.6	(13.5 to 36.5%)
	6.0	27.6	5.5	(-9.4 to 29.4%)	48.4	9.7	(3.5 to 46.5%)

Estimates of 10 and 25% mortality in domestic broodstock were nearly the same over 6 hours post-fertilization. Variability in egg mortality among the six individuals ranged from 5 to 20%. More variability in estimates occurred with kelts than domestics; however, mortality rates were similar in the two groups.

Discussion

Atlantic salmon egg mortality measured in this study, and at the water temperatures experienced, considers egg development stages which were limited to elevation of the blastodisc, before the first cell division, or before the two-celled stage (Battle 1944). Sampling times for Atlantic salmon eggs in this study approximated those used for handling and transport in the current Connecticut River program. The timing of mechanical shock to eggs during this period did not appear to affect egg mortality. Coho salmon (*Oncorhynchus kisutch*) appeared slightly more sensitive to mechanical shock than Atlantic salmon, with a median shock limit of 31.1 cm decreasing to 16.4 cm at 8 hours after fertilization (Jensen and Alderdice 1983). Coho salmon eggs used in the Jensen and Alderdice (1983) study were less sensitive to shock during the first hour after fertilization, as median shock sensitivities ranged from 383.4 to 38.3 cm.

Jensen and Alderdice (1989) tested shock sensitivity of six salmonid species: coho, chinook (*O. tshawytscha*), sockeye (*O. nerka*), chum (*O. keta*), pink salmon (*O. gorbuscha*), and steelhead (*O. mykiss*); they also determined the LC10 and LC50 values for these groups through the incubation period. The LC10 values for Atlantic salmon eggs in our study are comparable to those from inactivated fertilized eggs and stage 1 eggs (before the first cell division), both sensitive periods for eggs of the six species listed above. LC10 shock sensitivity (in drop height) of inactivated fertilized eggs for these species ranged from 12.1 to 41.7 cm. Values for Atlantic salmon were within this range for domestics (26.9 cm) and kelts (26.5 cm). Estimates of force transferred to cause 10% mortality in Atlantic salmon eggs one half hour after fertilization (5.3 to 6.0×10^3 ergs) were similar to those of Pacific salmonids (3.9 to 7.0×10^3 ergs; Jensen and Alderdice 1989), with the exception of a lower estimate (1.2×10^3 ergs) for sockeye salmon. Sensitivity of eggs shocked between 1 and 6 hours compared favorably with eggs from Jensen and Alderdice

(1989) stage 1, or mounded single cell cytoplasm. Of the Pacific salmonids, only sockeye had a shock sensitivity close to Atlantic salmon (23.0 cm versus 23.5 to 38.5 cm). All other salmonids were somewhat more sensitive to shock during this period than Atlantic salmon, with steelhead being most sensitive (LC10 of 8.2 cm) and chinook least sensitive (18.2 cm). Jensen and Alderdice (1989) found breakage in coho and steelhead eggs at lower force (1.2 to 1.9 x 10³ ergs) than that for Atlantic salmon which was LC10 from 3.5 to 7.7 x 10³ ergs. Other Pacific salmonid species had similar force estimates at LC10 (Jensen and Alderdice 1989). Considering the variability in design of tests run with Atlantic salmon, these estimates are probably not different.

Variability in LC estimates was high in our Atlantic salmon study because samples were not pooled, but rather tested as individual female groups. Similar variability is present in 24-hour egg mortality data among Atlantic salmon egg groups (W. F. Krise, unpublished data). Jensen and Alderdice (1983) subjected coho salmon eggs to mechanical shock and found that breakage force of eggs was similar between 0 and 100 cm whether dropped or poured. The current program for Atlantic salmon egg incubation in northeastern states usually requires handling and transport of eggs for 4 hours or more. Most egg mortality probably occurs during handling procedures, because handling includes pouring eggs on several occasions for disinfection, packing, or unpacking. Further research is underway to determine benefits of delayed fertilization after completion of transport. Additional research should be conducted to determine (1) effects of transport after fertilization, (2) average estimates of shock sensitivity using replicate egg groups pooled from several adults, and (3) development of reduced shock handling procedures.

References

- Battle, HI 1944 The embryology of the Atlantic salmon (*Salmo salar*, Linnaeus). Canadian Journal of Research 22:105-125.
- Jensen, JOT, and Alderdice, DF 1983 Changes in mechanical shock sensitivity of coho salmon (*Oncorhynchus kisutch*) eggs during incubation. Aquaculture 32:303-312.
- Jensen, JOT, and Alderdice, DF 1989 Comparison of mechanical shock sensitivity of eggs of five Pacific salmon (*Oncorhynchus*) species and steelhead trout (*Salmo gairdneri*). Aquaculture 78:163-181.
- Piper, RG, McElwain, IB, Orme, LE, McCraren, JP, Fowler, LG, and Leonard, JR 1982 Fish Hatchery Management. United States Department of the Interior, Fish and Wildlife Service, Washington, DC.