

A description of the optimal salinity to enhance husbandry of medaka (*Oryzias latipes*) eggs



Jeffrey Kramer¹ and Robert Leaf²

¹jkramer@vt.edu, Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA
²Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA



Objectives and Introduction

We determined for each of the three salinity treatments:

1. The proportion of eggs that hatch to produce larvae.
2. The temporal dynamics of hatching.
3. The causes of egg mortality.

Medaka (*Oryzias latipes*) is a widely used model organism for studies of toxicology, genetics, behavior, and evolution. However, little information is available regarding the conditions of egg-rearing that maximize the efficiency of a husbandry program for this species. The addition of salt solution is known to enhance survival of some fish larvae but it is not known if this is true for medaka or if the addition of small amounts of salinity (≤ 5 parts per thousand, ppt) may deleteriously affect the competency of eggs.

Methods

- The experiment consisted of replicated 800 ml mason jars ($n = 9$) with three salt solutions, concentration in parts per thousand (ppt): 0 ppt (control), 2.5 ppt, and 5 ppt, $n = 3$).
- Salt solutions were composed of a mix of different salts, see table below.
- ~100 viable Medaka eggs were placed in each jar.
- Jars were aerated and placed in a temperature-controlled water bath (25 to 27° C).
- Egg condition was monitored every one to three days, for ten days. Dead eggs, molded eggs, and larva were removed and counted.



Composition of salt concentrations in the three treatments.

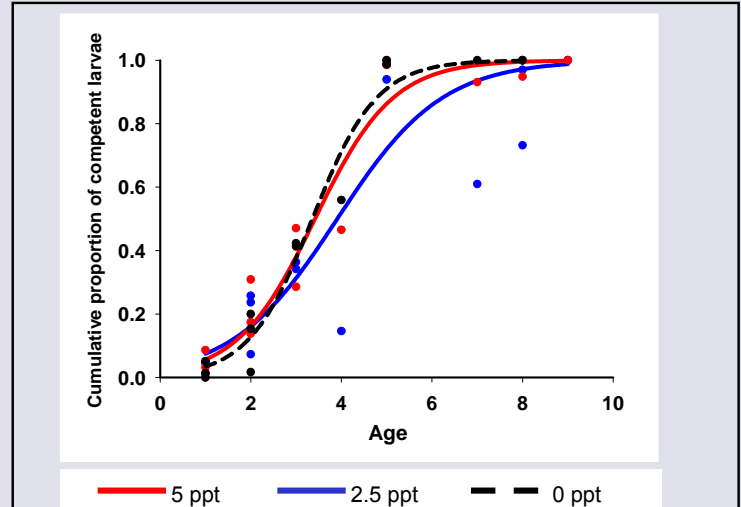
These are the recommended salt compositions for rearing eggs for freshwater teleosts (Kirchen and West 1976).

Salt (g/L)	Solution concentration (ppt)		
	0	2.5	5
NaCl	-	1.96	4.46
KCl	-	0.30	0.30
CaCl ₂ ·2H ₂ O	-	0.20	0.20
MgSO ₄ ·7H ₂ O	-	0.04	0.04

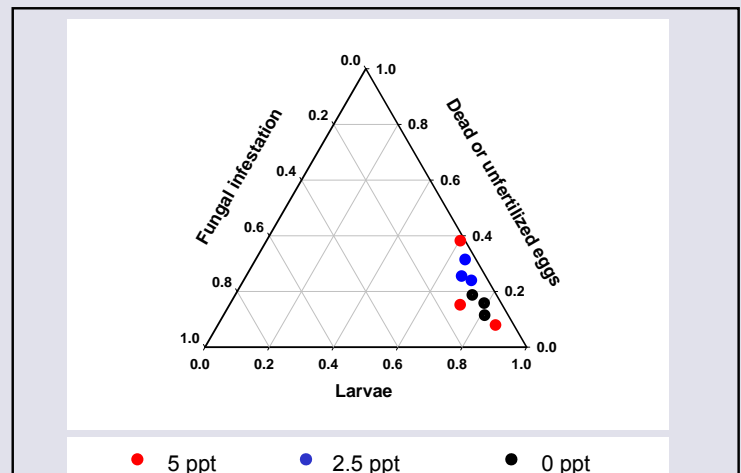
Results

We found that the mean proportion of eggs that hatch to larvae in each of the three treatments is 73% (95% confidence interval: 53 to 93%) and that there was no significant difference in mean number of larvae produced as a proportion of eggs among the treatments ($p > 0.05$) but one 5 ppt treatment did result in the greatest larvae production.

Results (continued)



We used a two parameter logistic curve to describe the cumulative number of larvae produced as a function of the number of days after eggs were collected. We found no difference in the curve parameters among the three treatments. Approximately 100% of larvae hatched after seven days in each treatment.



There was no difference in the composition of the terminal stage (larvae, fungal infestation, or dead/unfertilized) of eggs in each of the zero ppt and 2.5 ppt treatments. However, the composition of eggs in each of the terminal stages of the 5 ppt treatment were variable and were independent of the treatment effect ($\chi^2, p < 0.05$).

Recommendations and Discussion

We recommend using a dilute salt solution with a concentration of 5 ppt because total larvae production may be marginally increased by this concentration. Hatching rate is unaffected however. The application of an antifungal agent should be tested in a multifactor design with the salinity treatments to optimize rearing conditions. This work may be applicable to other fish species and provides a starting point for further experimentation.