

Introduction

Early development is considered an especially sensitive stage in the fish life-cycle. As a result, many regulatory agencies recognize the fish Early Life-Stage (ELS) test as an important component of environmental risk assessments. The most recent revision of EPA guideline OSCPP 850.1400 updated the list of recommended endpoints for the fish ELS to include mean time to hatch. This endpoint represents the mean elapsed time between test initiation and completed hatch for the embryos in a replicate. This endpoint is thought to be most suitable in fish ELS studies with species that exhibit hatching behavior over a longer hatch period (i.e., rainbow trout, the recommended freshwater fish species in this guideline), but the relevance of the endpoint is less understood in test species with shorter hatch periods. This review examines the control mean time to hatch for fourteen fish ELS studies conducted with fathead minnow (*Pimephales promelas*) since the implementation of OCSP 850.1400 (2017 – 2018). Using control data, correlational analyses were performed with the mean time to hatch endpoint and both embryo initiation stage and exposure temperature. This was done to explore whether there is evidence of bias in time to hatch results based on these study variables. Additionally, using full study data sets, correlational analyses were performed between the time to hatch endpoint and other endpoints that were found to demonstrate significant toxicological effects. This was done to indicate if mean time to hatch is a relevant predictor of other toxicant related effects.

Methods

This review is of fourteen fish ELS studies conducted at the Smithers Viscient Massachusetts research facility (Wareham, MA), under flow-through conditions. All fish ELS studies were initiated with fathead minnow embryos that were ≤24 hours old. Thirty (30) embryos per replicate were introduced into mesh-bottomed eggcups, which were then suspended in their respective test aquaria. Biological observations were made once daily, at roughly 24-hour intervals. The embryo stage of the exposure was run until all viable embryos had hatched. Once at least 90% of embryos had hatched, that replicate was thinned to 20 larvae, which were then released into their respective test aquaria to begin the larval stage of the exposure. Daily biological observations would continue through the larval stage. The larval stage would continue for 28 days post release, at which point all organisms were humanely terminated, and measured for total length and weight (wet or dry depending on guideline).

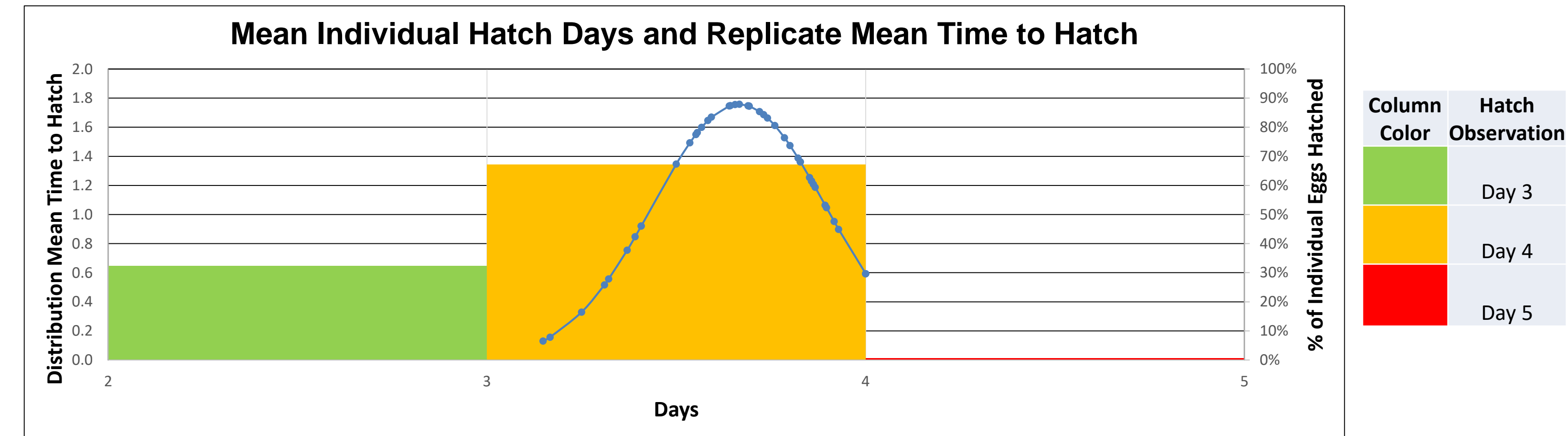


Figure 1: Curve in foreground displays the normalized distribution of mean Time to Hatch. Bars in background display percentage of embryos hatched during particular observations.

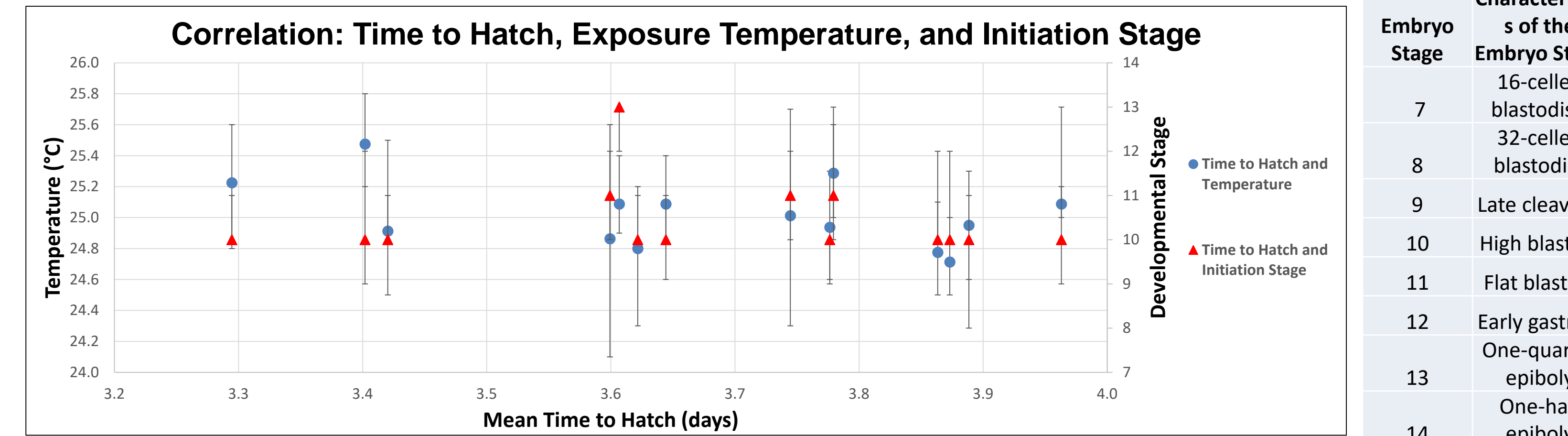


Figure 2: Mean developmental stage and mean exposure temperature compared to mean time to hatch. Bars represent developmental stage range and temperature range respectively. No significant correlation found with mean developmental stage (Pearson's correlation: -0.076, P-value: 0.814), or mean exposure temperature (Pearson's correlation: -0.472, P-value: 0.121).

Preliminary Conclusions

- Review found that mean time to hatch across the fourteen studies was 3.6 days (S.D. 0.22 days). On an individual organism basis, 31.4% of organisms hatched by test day 3, 68.0% hatched by test day 4, and 0.6% hatched by test day 5.
- Control data analyses found no significant correlation between mean time to hatch and either embryo initiation stage or exposure temperature.
- Correlational analyses on full study data sets found three examples of correlation between mean time to hatch and the total length, and a single example of correlation between time to hatch and wet weight, post-hatch success, and overall survival. Observed correlational trends between time to hatch and survival endpoints (post-hatch success and overall survival) were consistently positive, while correlational trends between time to hatch and growth endpoints (total length and wet weight) were consistently negative. These results suggest that the mean time to hatch endpoint could be predictive of toxicant related effects in the larval stage of fish ELS studies.



Figure 3: High Blastula Embryo (Stage 10). Amy Snow, August 7, 2014.



Figure 4: Fathead Minnow Larvae. Michael Patnaude, October 2, 2009.

Endpoint	Number of Studies Toxicologically Relevant	Correlated with Time to Hatch	Pearson's Correlation	P-value
Embryo Hatching Success	1	0	NA	NA
Post-Hatch Success	3	1	0.428	0.026
Overall Survival	2	1	0.453	0.015
Total Length	4	3	-0.561, -0.420, -0.559	0.004, 0.041, 0.005
Dry Weight	0	NA	NA	NA
Wet Weight	3	1	-0.509	0.011
Time to Hatch	2	NA	NA	NA

Table 1: Correlational analyses between toxicologically relevant endpoints and the time to hatch endpoint in 14 ELS studies. Survival endpoints are consistently positively correlated to time to hatch. Growth endpoints are consistently negatively correlated to time to hatch.

Future Work

- Continue collecting ELS data from future studies to improve sample size for correlational analyses.
- Extend analysis of time to hatch endpoint to cover other common ELS species (ex. Sheepshead minnow).
- Comparative analysis of ELS time to hatch data in negative and solvent control groups.

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