North Pacific Anadromous Fish Commission
Technical Report No. 9: 104-106, 2013

Exposure to Elevated Temperature During Early Development Affects Sexual Development in *Oncorhynchus mykiss*

Kathleen S. Cole[1], **David L. G. Noakes**[2], **Neil Thompson**[3], **Michael Blouin**[3], **Bruce Morrison**[4] and **Carl B. Schreck**[5]

[1]*Department of Zoology, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA*
[2]*Department of Fisheries and Wildlife and Oregon Hatchery Research Center, Oregon State University, Corvallis, Oregon 97331, USA*
[3]*Department of Zoology, Oregon State University, Corvallis, Oregon 97331, USA*
[4]*Lake Ontario Management Unit, Ontario Ministry of Natural Resources, 41 Hatchery Lane, RR#4, Picton, Ontario K0K 2T0, Canada*
[5]*Oregon Cooperative Fish and Wildlife Research Unit, and Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon 97331, USA*

Keywords: alternate life history, sexual phenotype, climate change, wild, hatchery, steelhead

Conditions experienced during early development can have a significant impact on phenotypic variation and subsequent life history options, particularly among species that normally express multiple phenotypes. In *Oncorhynchus mykiss*, known as steelhead and rainbow trout in anadromous and freshwater resident forms respectively, the expression of one or the other of these two forms is determined in part by environmental conditions and in part by genetic architecture (Thrower et al. 2004, Hecht et al. 2012, 2013). However, our understanding of how environmental changes can affect the biology and life history of species having diverse life histories is limited. We do know that sexual development and sexual phenotype among some fishes can be strongly influenced by environmental factors (Ospina-Alvarez and Piferrer 2008; Wedekind et al. 2012). Among salmonids, *O. nerka* (sockeye salmon) show altered sex ratios following embryonic exposure to elevated temperature (Craig et al. 1996; Azuma et al. 2004). Similar studies of *O. mykiss* have had mixed results, from no apparent effect (Van den Hurk and Lambert 1982; Baroiller et al. 1999), to partial effects dependent on the type of temperature application treatment and parental lineage (Magerhans et al. 2009). Current projections are for continued warming of aquatic environments associated with climate change (Meehl et al. 2007), a phenomenon that is likely to have significant impact on temperate fishes occupying temperature-sensitive freshwater habitats. We undertook a comparative study of wild and hatchery sourced *O. mykiss,* using a number of steelhead pairs as a parental source of embryos, to evaluate to what extent genotype (stock source and parental line) and differing temperatures may interact to influence phenotypic sex.

The experiment was conducted at the Oregon Hatchery Research Center (OHRC) located on Fall Creek. Wild and hatchery stocks of *O. mykiss* were collected from returning mature steelhead at the OHRC fish ladder on Fall Creek. Family lines were established by single-pair matings of adults following standard procedures (Noakes and Corrarino 2010) to create five wild family lines and four hatchery family lines. The resulting embryos of these matings resulted in five wild and four hatchery families. These sibling groups were held at either ambient or elevated (+5°C above ambient) temperatures (two replicates for each experimental condition) for a total of 36 (9 families by two temperatures by two replicates) experimental groups. The time of exposure to elevated temperature extended from the beginning of the experimental period at fertilization until swim-up, after which all fish were maintained at ambient temperatures until termination of the experiment. At the end of the experiment all surviving fish were humanely euthanized and processed for taking length/weight data and DNA tissue samples for ongoing studies. In addition, gonadal tissue was removed from 16 fish from each experimental group and processed histologically to determine phenotypic sex.

Assessment of phenotypic sex was based on microscopic examination of histologically-processed gonadal tissue, which was characterized as either ovariform or testiform based on features of gonadal architecture and gonial cell type (Fig. 1), using previously described techniques and criteria (Cole 2010).

Data were analyzed using a mixed generalized linear model with a binomial family distribution. The model assessed was y = source + thermal regime + random (treatment replicate number nested in source replicate number). The 'glme' function in the R statistical package, lme4, was used (Bates et al. 2012) and fixed effects generalized linear models were also assessed. In comparing the phenotypic responses of sibling embryos collected from multiple families (spawning pairs) from both hatchery and wild stocks, our data analysis took into account the difference in experimental group sizes resulting from differential mortality.

Based on the fish analyzed histologically, the frequency of occurrence of phenotypic females among heated groups was significantly less than expected (i.e., 50%) and also significantly less than the frequency of occurrence of phenotypic females among ambient temperature groups.

All correspondence should be addressed to K. Cole.
e-mail: colek@hawaii.edu

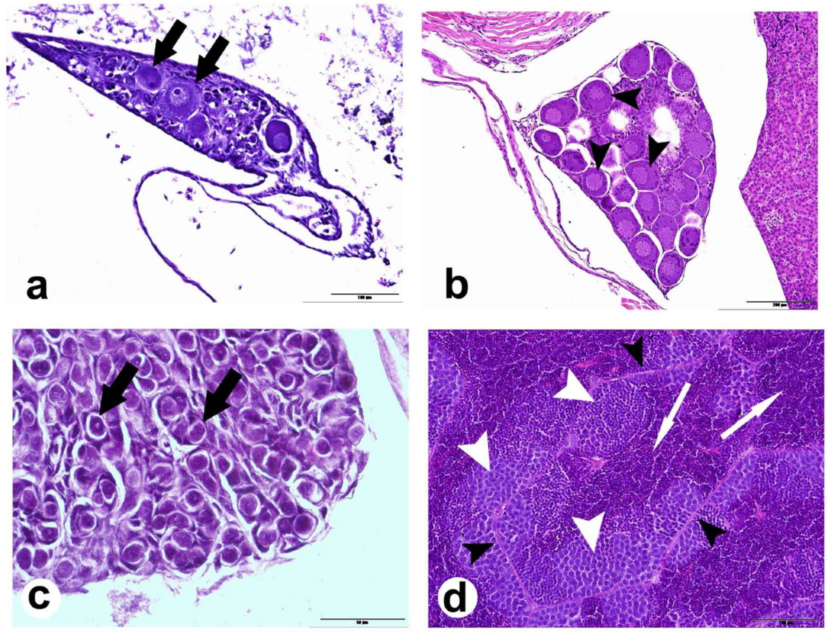

Fig. 1. Gonadal morphology of experimental *Oncorhynchus mykiss*. a. Early-stage ovary showing chromatin nucleolus stage oocytes (black arrow). Scale bar is 100 μm. b. Differentiated immature ovary with perinucleolar stage oocytes (black arrowhead). Scale bar is 200 μm. c. Early stage testis showing cord and cluster organization of spermatogonia and primary spermatocytes (black arrow) Scale bar is 50 μm. d. Mature (precocious) testis with seminiferous lobules made up primarily of spermatocysts (white arrowhead) containing same-stage germ cells (mostly spermatocytes) surrounding a central lumen filled with free spermatozoa (white arrow) and bounded peripherally by connective tissue (black arrowheads). Scale bar is 100 μm.

These findings indicate that increased temperature exposure during early development influences sexual development in *O. mykiss*. The increased temperature (+5°C above ambient) applied here is within the currently predicted range for temperature increases that could be experienced by the end of this century, taking in to account assumptions of greatest surface air temperature warming occurring over land and at higher northern latitudes (Meehl et al. 2007). Moreover, the exposure time was relatively short, lasting only from fertilization to swim-up, before significant gonad development starts to occur. Longer term exposure to elevated temperatures, for example throughout the full period of early gonad development, may result in an even greater male bias in the resulting phenotypic sex ratio.

The prospect of altered sex ratios in *O. mykiss* in response to increased climatic temperatures associated with a reduction in numbers of phenotypic females has significant implications on a number of levels. Both population management plans and conservation strategies are usually developed on an assumption of balanced sex ratios. A significant reduction in either sex is likely to have significant negative impacts on population sustainability. Of greater critical concern, a persistent loss of females brings into question the ability of a species to survive. The objectives of hatchery managers, conservation biologists and fisheries harvesters are quite different, but it is vitally important for all to understand both the mechanisms that regulate development and life history in this species, and the environmental factors that may significantly influence the outcomes of altered developmental patterns.

REFERENCES

Azuma T., K. Takeda, T. Doi, K. Muto, M. Akutsu, M. Sawada, and S. Adachi. 2003. The influence of temperature on sex determination in sockeye salmon *Oncorhynchus nerka*. Aquaculture 234: 461-473.

Baroiller, J.F., Y. Guigeun, and A. Fostier. 1999. Endocrine and environmental aspects of sex differentiation in fish. Cell. Mol. Life Sci. 55: 910-931.

Bates, D., M. Maechler, and B. Bolker. 2012. Linear mixed-effects models using S4 classes. R-Statistical Package 'lme4'.

Cole, K.S. 2010. Gonad morphology in hermaphroditic gobies. *In*: Reproduction and sexuality in marine fishes. *Edited by* K.S. Cole. Univ. Cal. Press, Berkeley. pp. 117-162.

Craig, J.K., Foote C.J., and Wood C.C. 1996. Evidence for temperature-dependent sex determination in sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 53: 141-147.

Hecht, B.C., F.P. Thrower, M.C. Hale, M.R. Miller, and K.M. Nichols. 2012. Genetic architecture of migration-related traits in rainbow and steelhead trout, *Oncorhynchus mykiss*. G3 Genes, Genomes, Genetics 2: 1113-1127.

Hecht, B.C., N.R. Campbell, D.E. Holecek, and S.R. Narum. 2013. Genome-wide association reveals genetic basis for the propensity to migrate in wild populations of rainbow and steelhead trout. Mol. Ecol. 22: 3061-3076.

Magerhans, M., and G. Hörstgen-Schwark. 2010. Selection experiments to alter the sex ratio in rainbow trout (*Oncorhynchus mykiss*) by means of temperature treatment. Aquaculture 306: 63-67.

Meehl, G.A., T.F. Stocker, W.D. Collins, P. Friedlingstein, A.T. Gaye, J.M. Gregory, A. Kitoh, I.G. Watterson, A.J. Weaver, and Z-C Zhao. 2007. Global climate projections. *In* Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. *Edited by* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, and H.L. Miller. Cambridge Univ. Press, New York. pp. 747-845.

Noakes, D.L.G., and C. Corrarino. 2010. The Oregon Hatchery Research Center: a research laboratory in a natural setting. World Aquaculture 41: 33-37.

Ospina-Alvarez, N., and F. Piferrer. 2008. Temperature-dependent sex determination in fish revisited: prevalence, a single sex ratio response pattern, and possible effects of climate change. PloS ONE 3: e2387.

Thrower, F.P., J.J. Hard, and J.E. Joyce. 2004. Genetic architecture of growth and early life-history transitions in anadromous and derived freshwater populations of steelhead. J. Fish Biol. 65(Suppl A): 286-307.

Van den Hurk, R., and J.G.D. Lambert. 1982. Temperature and steroid effects on gonadal sex differentiation in rainbow trout. *In:* Proceedings of the international symposium on the reproductive physiology of fish. *Edited by* C.J.J. Richter, and H.J.T. Goos. 2-6 August, 1982, Pudoc, Wageningen. pp. 69-72.

Wedekind C., G. Evanno, T. Szekely. Jr, M. Pompini, O. Darbellay, J. Guthruf. 2012. Persistent unequal sex ratio in a population of grayling (Salmonidae) and possible role of temperature increase. Cons. Biol. 27: 229-234.