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Alan Blake, Chief Executive Officer
Yorktown Technologies, L.P.
Austin, Texas 78759

Dear Mr. Blake,

I write this letter in response to your questions regarding ecological risk assessment for transgenic zebrafish expressing fluorescent proteins. These fish may be marketed soon in the United States through the ornamental fish trade. I understand that the State of California has implemented a strict permit requirement for transgenic fishes, and that you have requested an exemption from the permit requirement. You may, if you choose, share this letter with the California Fish and Game Commission.

Before discussing the issues, for the sake of any other readers, I will relate that I was a co-author of the American Fisheries Society position statement on transgenic fishes. I was a member of the working group that developed Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish, adopted by the U.S. Department of Agriculture as its policy for research with transgenic fish. I was a member of a National Research Council panel that wrote *Animal Biotechnology: Scientific Concerns*, which discussed, among other issues, environmental risk issues posed by transgenic fishes. I have authored over a dozen peer-reviewed papers on ecological risk assessment regarding aquaculture biotechnology. My expertise has been sought by the U.S. Department of Agriculture, Food and Drug Administration, Chinese Ministry of Agriculture, Food and Agriculture Organization of the United Nations, and World Health Organization, and by the scientific and popular media, including *Science*, *Nature*, the *New York Times*, *Washington Post*, and many other outlets. Hence, I am not surprised that you asked my thoughts concerning transgenic zebrafish.

When considering ecological safety assessment, the threshold issue is whether the transgenic fish would give rise to a self-sustaining population. In this particular case, zebrafish are known to be a tropical species sensitive to low temperatures. Despite decades of production and use in the United States, zebrafish are not known to have established self-sustaining populations in the United States. Of particular interest, a U.S. Geological Survey fact sheet shows that the species was reported in the Westminster flood control channel near a fish farm in Westminster, Orange County, California in 1968. Apparently, the species has not persisted there. The California Department of Fish and Game will have further knowledge of whether zebrafish have persisted or become established in any waterbody in the state.

A second issue is whether the genetic modification would affect the species' invasiveness. Preliminary results reported by Gong et al. (2003) suggest no apparent difference in early viability relative to wild-type individuals, with the exception that fish expressing the green fluorescent protein appeared to exhibit a lower survival rate. The reproductive success of the transgenics approximated expectations. While preliminary, these results support the expectation

that the modification at issue would not increase invasiveness, and that, if anything, might decrease it somewhat. That is, brightly-colored fish, part of whose energy budget is devoted to producing a large amount of a novel pigment in their muscle tissues, would be expected to be removed from an ecosystem more rapidly than wild-type zebrafish. The possibility that the zebrafish might give rise to ecological impact before being removed from the ecosystem by predation or physiological stress seems remote.

In our discussion, you indicated that you might market triploid or monosex stocks of zebrafish. This would be wise from the viewpoint of protecting your investment in the development of the transgenic lines from unauthorized commercial production. It also would minimize any ecological concerns. I'll add that in the native range of zebrafish in south Asia, reproductive confinement and restrictions on commercial production – for example, requiring strict containment of fertile stocks – seem justifiable from the risk management viewpoint.

In summary, from the viewpoint of ecological risk, I have no objection to your request to the California Fish and Game Commission for permission to sell your transgenic zebrafish through commercial outlets in California. Should you or the California Fish and Game Commission want to discuss the suite of issues further, contact me at 540-231-3257 or at ehallerm@vt.edu.

Regards,

Eric M. Hallerman
Professor

Literature cited

Gong, Z., H. Wan, T.L. Tay, H. Wang, M. Chen, and T. Yan. 2003. Development of transgenic zebrafish for ornamental and bioreactor by strong expression of fluorescent proteins in the skeletal muscle. Biochemical and Biophysical Research Communications 308:58-63.