Faculty of Science
Department of Biological Sciences

NUS National University of Singapore

Sept. 30, 2003

Mr. Alan Blake
Chief Executive Officer
Yorktown Technologies, L.P.

Dear Mr. Blake:

At your request, I wish to communicate some important information regarding the possible environmental risk posed by fluorescent transgenic ornamental fish. Throughout most of my scientific career, I have been involved in transgenic fish research. In 1988, I joined Professor Hew Choy Leong's group in Toronto, where I was involved in the growth hormone transgenic salmon project. This work ultimately led to Aqua Bounty's AquAdvantage™ Salmon, which is currently under review by the United States Food and Drug Administration.

In 1995, I moved to Singapore, and decided to apply transgenic technology to ornamental fish, something that had not been done previously. Our goal was to insert fluorescent protein genes, originally isolated from jelly fish and several other coral reef species, into ornamental fish by using zebrafish and medaka fish as models. In 1999, we successfully generated green fluorescent transgenic zebrafish, and we subsequently obtained red, yellow, and orange fluorescent transgenic fish.

Because of my work with the transgenic salmon, I was very much aware of public concern relating to transgenic food fish in aquaculture and related environmental safety issues. Due to this awareness, from the beginning, I have carefully monitored the fitness of fluorescent transgenic fish in comparison with that of the wild type fish of the same species.

As correctly pointed out by Dr. William Muir in his letter to you, six factors appear to be most important for evaluation of the potential risk of transgenic fish in the environment: juvenile and adult viability, age at sexual maturity, fecundity, fertility and mating success. Over the past few years, our research has covered essentially all of the six factors. The following is a brief summary of our conclusions.

Viability. In general, transgenic fish showed lower survival rates, particularly the green fluorescent transgenic fish. Part of the data has been published in Reference 1, noted below. We usually conducted the survival rate tests based on survival from hatched fry to spawning adult, and thus the survival rates we observed incorporated both juvenile and adult viability. When fish were maintained under good conditions, both transgenic fish and wild type fish had comparable survival rates. However, when the fish were cultured under slightly adverse conditions, the green fluorescent transgenic fish were usually more susceptible to disease, thus causing higher mortality rates. In terms of growth rate, there is no significant difference in the first few months. However,

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the wild type fish generally ultimately grow to a bigger size than the transgenic fish. This may reflect the additional burden of expressing fluorescent proteins.

Age at sexual maturity. We did not notice any difference between transgenic fish and wild type fish with respect to their age at sexual maturity.

Fecundity. We found that transgenic females produced less than 50% the number of eggs produced by wild type females.

Fertility. The fertility of transgenic males is less than 90% that of wild type.

Mating success. By using two different tank designs, we have also carried out dichotomous choice tests by the standard approach used to test guppy fish mating choice (reference 2 below), and none of the tests indicated that the fluorescent transgenic fish have an advantage in mating selection.

In conclusion, fluorescent transgenic fish do not have any apparent fitness advantage over wild type fish of the same species. To the contrary, because fluorescence carries additional burdens in biosynthesis, energy distribution, and predator avoidance, fluorescent fish are likely to have reduced fitness. Thus, we can confidently conclude that they would be expected to pose no greater risk to the environment than would be expected from a wild type zebra fish, particularly in areas where they are non-native.

I hope that this information is useful to you.

Best regards,

Zhiyuan Gong

References:

1. Gong, Z., H. Wan, T.L. Tay, H. Wang, M. Chen and T. Yan (2003) Development of transgenic fish for ornamental and bioreactor by strong expression of fluorescent proteins in the skeletal muscle. *Biochem. Biophys. Res Comm.* 388:58-63.
2. A.E. Houde, Sex, color, and mate choice in guppies. Princeton University Press, Princeton, New Jersey, 1997.