



Original Paper

Laboratory Fish Strains and Species of Fish Suitable for Water Quality Research

PETRESCU-MAG Ioan Valentin^{1,2}, Ioan Gheorghe OROIAN*¹,
Romica TOMESCU³, EȘANU Valentin Ovidiu⁴

¹University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, Mănăștur no. 3-5, 400372, Cluj - Napoca, Romania

²SC Bioflux SRL, Ceahlau St., no. 54, 400488 Cluj-Napoca, Romania

³ICAS Bucuresti, Eroilor Bd., Voluntari, Bucharest Romania

⁴University of Liege, Agro-Bio Tech Gembloux, 2, Passage des Deportes, B-5030, Gembloux, Belgium

Received 2 March 2014; received and revised form 16 March 2014; accepted 20 March 2014

Available online 31 March 2014

Abstract

The paper approaches the recent issue of using fish species, strains or varieties as bioindicator/model organisms in studies of aquatic ecotoxicology. Both from ecological effects and species extrapolation perspectives, fish tests are an important component of pollutants screening and testing programs. The fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias latipes*), and zebrafish (*Danio rerio*), are particularly used as model organisms for most testing programs, but the guppy fish (*Poecilia reticulata*), rainbow trout (*Oncorhynchus mykiss*) and the common carp (*Cyprinus carpio*) are serious candidates for future research programs. Many other fish species are currently tested for suitability in ecotoxicological research. For the detection of heavy metals, fluorescent new zebrafish strains have been produced and maintained in laboratories.

Keywords: aquatic environment, ecotoxicology, model organisms, guppy, zebrafish, medaka, fathead minnow.

1. Introduction

Man, in his approach to identify various water pollutants and their concentration in environment, developed different techniques, tools and equipments for analysis of water quality.

A special category of "tools" used in ecotoxicology and other branches of the environmental sciences are the so called "model organisms" and/or "bioindicators".

They may be aquatic or terrestrial, animal or vegetal, vertebrate or invertebrate animals, superior or inferior plants, microorganisms, fungi and so on. Fish are the main representatives of the aquatic vertebrates, therefore, some of them are very useful model organisms in aquatic ecotoxicology studies.

2. Research on endocrine-disrupting chemicals

Endocrine-disrupting chemicals (EDCs) or endocrine disruptors, particularly those affecting the hypothalamic-pituitary-gonadal axis of vertebrates, have become a focus of regulatory screening and testing worldwide. Tiny fish species, predominantly the fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias latipes*), and zebrafish (*Danio rerio*), are used as such model organisms for most of these testing programs [1].

* Corresponding author.
Tel.: +40 264 596384; Fax: +40 264 592 793
e-mail: neluoroian@gmail.com

However, other small fish species such as the guppy (*Poecilia reticulata*), as well as more traditional large fish models such as the rainbow trout (*Oncorhynchus mykiss*) and the common carp (*Cyprinus carpio*), also proved useful in the EDCs screening research. Many other fish species are currently tested for suitability in ecotoxicological research. Fish are appropriate models for testing EDCs, not only in terms of existing ecological impacts, but also from the perspective of extrapolation to other species [1].

3.Zebrafish: an ideal bioindicator for identifying heavy metals from water

The latest research in molecular genetics have allowed the production of fluorescent zebrafish lines [3]. They are the cheapest, but also among the most accurate tool for identification of pollutants in water [3]. If the genetically engineered organisms often been fought by environmental forces, these fluorescent fish of different colors, bearing the generic name Glo-fish were first produced in

ecological purpose, namely that of water quality testing.

Each color indicates the presence of a heavy metal in water. Fluorescence is due to fluorescent proteins encoded by genes transferred from some species of jellyfish and sea anemones (table 1). Impact studies on short and long term show the absence of any ecological risk if the fish get in nature [12]. Fluorescence does not give them an increased fitness but, instead, is a selective disadvantage, which leads to the disappearance of individuals or populations reached accidentally in nature. On bioethical issues, fluorescent animals do not suffer, the fluorescence being a neutral in this regard. There are no side effects on predatory species caused by ingestion of fluorescent proteins, and this fact is logic: no predator of jellyfish affected by fluorescent proteins was reported so far. Fluorescent proteins derived mainly from a few donor species: *Aequorea victoria* and *Discosoma* spp [3].

The fluorescent zebrafish is also present on Romanian market [8].

Table 1. How the fluorescent proteins were produced

Fluorescent proteins	Donnor species	Color of the fish	How the color was produced
GFP (Green Fluorescent Protein)	<i>Aequorea victoria</i>	Green	Cloning cADN
YFP (Yellow Fluorescent Protein)	<i>Aequorea victoria</i>	Yellow	Cloning cADN of the GFP followed by site directed mutagenesis
CFP (Cyan Fluorescent Protein)	<i>Aequorea victoria</i>	Blue-cyan	-II-
BFP (Blue Fluorescent Protein)	<i>Aequorea victoria</i>	Blue	-II-
RFP (Red Fluorescent Protein)	<i>Discosoma spp.</i>	Red	Cloning cADN
RFP+YFP	<i>Discosoma spp.</i> , <i>Aequorea victoria</i>	Orange	Hybridization of the red strain with the yellow one

4.The guppy fish and detection of sublethal concentrations of EDCs

In the last decades, there is considerable interest in computer-assisted image analysis of biological images for the assessment of endocrine disrupting amplitude [2]. Image textures are defined as complex visual patterns composed of entities or elements having characteristic brightness, colors, slopes, sizes etc. Thus, texture can be regarded as a similarity grouping in an image [4, 10]. Digital analysis of orange skin surfaces at the exterior of guppy fish, using special methods and programs, allowed the researchers to evaluate the degree of

endocrine disruption in this fish exposed to sublethal concentrations [6, 11] (see Fig.1). Red coloration of fish skin is determined by deposition of carotenoids (in the wild) and red pteridine pigments (here) within the skin [5]. Testosterone is a key regulator of pteridine biosynthesis and influences the production and distribution of pigment within the skin [9]. The EDCs affects the testosterone production and, indirectly, the red surfaces on the fish skin.

Another reason to consider *Poecilia reticulata* one of the best model organisms is its easy reproduction and precocity [7].

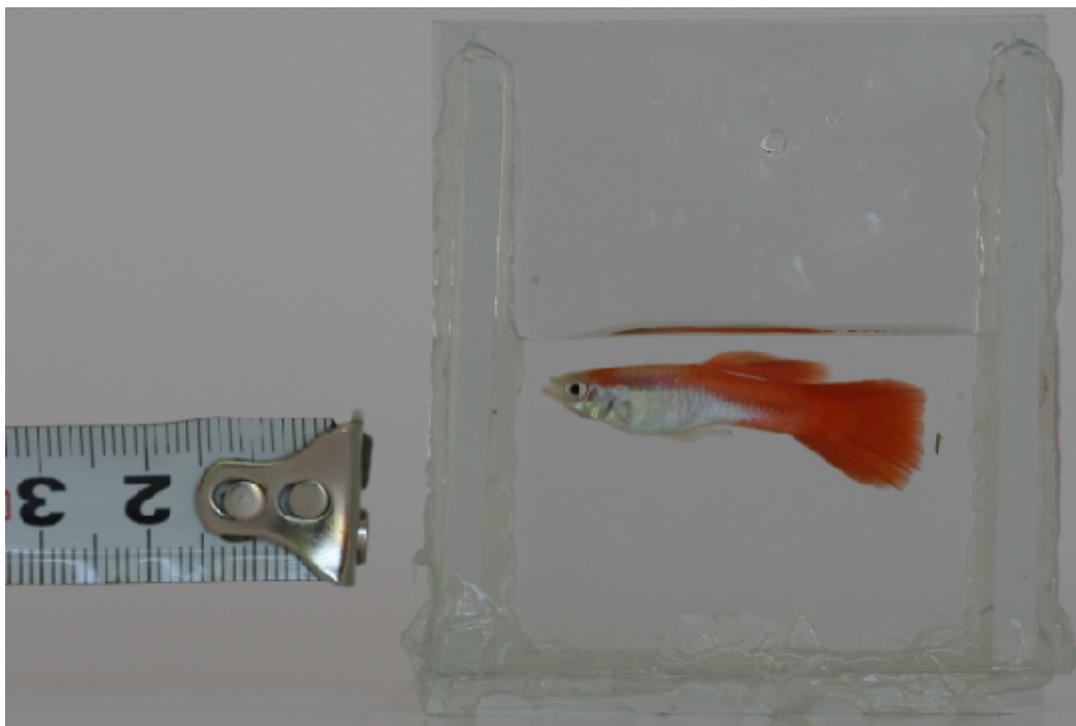


Figure 1. Red Blond variety of guppy fish during orange surfaces analysis

5. Conclusions

Both from ecological effects and species extrapolation perspectives, fish tests are an important component of pollutants screening and testing programs. The fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias latipes*), and zebrafish (*Danio rerio*), are particularly used as model organisms for most testing programs, but the guppy fish (*Poecilia reticulata*), rainbow trout (*Oncorhynchus mykiss*) and the common carp (*Cyprinus carpio*) are serious candidates for future research programs. In our opinion, the guppy is at least as much important as the first three species are, due to the fact it is extremely sensitive to sublethal concentrations of EDCs. Such sublethal concentrations of EDCs can be statistically quantified in terms of red areas on the skin, number of spots on the skin, and so on.

References

- [1] Ankley, G. T., Johnson, R. D., 2004, Small fish models for identifying and assessing the effects of endocrine-disrupting chemicals. *Inst Lab Anim Res J* 45:469-483.
- [2] Baatrup, E., Junge, M., 2001, Antiandrogenic perspectives disrupt sexual characteristics in the adult male guppy (*Poecilia reticulata*). *Environ Health Perspect* 109(10): 1063-1070.
- [3] Gong, Z., Wan, H., Tay, T. L., Wang, H., Chen, M., Yan, T., 2003, Development of transgenic fish for ornamental and bioreactor by strong expression of fluorescent proteins in the skeletal muscle. *Biochem Biophys Res Com* 308: 58–63.
- [4] Materka, A., Strzelecki, M., 1998, Texture analysis methods - a review. Technical University of Lodz, Institute of Electronics, Poland: COST B11 Report.
- [5] Miller, E. G., Karlslake, E. B., Masanoff, J. R., Park, J. P., Sammons, A. J., Watson, L. C., Newaj-Fyzul, A., Petrescu-Mag, I. V., Breden, F., Allen, T. C., Bourne G. R., 2010, Poeciliid livebearing fish polymorphisms: providing answers to questions of color, sex, mate acquisition, and personality. In: G.R. Bourne and C.M. Bourne (eds.), *The CEIBA Reader: an introduction to the people, ecosystems, plants, animals and cuisine of CEIBA Biological Center, Guyana*. St. Louis, MO, Yerfdog Publishing. Chapter 4.21.
- [6] Oroian, I. G., Petrescu-Mag, I. V., Păsărin, L., 2013, Long term exposure to sublethal concentrations of vanadium affects the fertility but not the secondary sexual traits of the guppy fish. *Metal Int* 18(5), 280-284.
- [7] Petrescu-Mag, I. V., 2008 Biophysiological characterization of *Poecilia reticulata* and its particularities. *ABAH Bioflux*, 1-56. [In Romanian]
- [8] Petrescu-Mag, R. M., Păsărin, B., Şonea, C. G., Petrescu-Mag, I. V., 2013, Customer preferences and trends for aquarium fish in Transylvania (Romania). *North-West J Zool* 9(1), 166-171.

- [9] Rodd, F. H., Hughes, K. A., Grether, G. F., Baril, C. T., 2002 A possible non-sexual origin of mate preference: are male guppies mimicking fruit? Proc R Soc Lond B 269:475-481.
- [10] Rosenfeld, A., Kak, A., 1982, Digital Picture
- [11] Țălu, Ș., Petrescu-Mag, I. V., Păsărin, B., 2012, Investigation on acute toxicity of lindane in guppies, *Poecilia reticulata* Peters, 1859. Poec Res 2(1):9-14.
- [12] Van Eenennaam, A. L., Olin, P. G., 2006, Careful risk assessment needed to evaluate transgenic fish. California Agriculture 60(3):126-131.