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A correlation between two different species of fish embryos used in a freshwater qualitative pollution test

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CRINA LAURA MOȘNEANG[1], ADRIAN GROZEA[2], EUGENIA DUMITRESCU[1], FLORIN MUSELIN[3] AND ROMEO T. CRISTINA[1]

[1] *Department of Pharmacology and Pharmacy, Faculty of Veterinary Medicine, Banat's University of Agriculture and Veterinary Medicine "King Michael I of Romania" from Timișoara (BUAVMT), 119, Calea Aradului, 300645, Timișoara, Romania.*

[2] *Department of Aquaculture, Faculty of Animal Science and Biotechnology, BUAVM Timișoara, Romania*

[3] *Department of Toxicology, Faculty of Veterinary Medicine, BUAVM Timișoara, Romania.*

** Corresponding author: Romeo T. CRISTINA, DVM, PhD. rtcristina@yahoo.com*

Abstract

Pollutants of freshwater sources, through their eco-risk potential can affect the human and animal health, numerous risk-assays being imagined and applied in the last decade. In this respect our aim was to make, an initial acute eco-risk study comparing the zebrafish embryos with an of interest autochthon species, namely pikeperch embryos, to see if this species is suitable and can be introduced as reliable testing tool in temperate region's water eco-pollution studies. The ISO 15088:2007 test method, adapted by our collective to pikeperch embryos stage development was used. Accordingly, 100 zebrafish embryos (Danio rerio) and 20 as control, in parallel with 100 pikeperch embryos (Sander lucioperca) and 20 control were kept in water samples provided from a swine farm surroundings, diluted in ten different concentrations: 1X, 2X, 3X, 4X, 6X, 8X, 12X, 16X, 24X, 32X. Any behaviour changes and mortality was recorded and statistically analysed according to Kolmogorov-Smirnov normality test, where P value was significant when $P < 0.05$. Though pikeperch embryo use for testing are not a pattern yet, as zebrafish embryos, results revealed that the indigene species embryos can be successfully used in studies applicable in ecotoxicology or other biomedical domains, with comparable results to the classical one.

Keywords: acute embryo-toxicity, pikeperch, zebrafish, risk-assay.

1. Introduction

In eco-toxicology and biomedical domains zebrafish adults and embryos are successfully used, in a large palette of diagnostic and experimental models, in order to test various substances, from environmental pollutants to the active medicinal substances. From these, the acute embryo tests are helpful in providing an easily available, low-cost option, which can give enough accurate results in a limited amount of time [1-15]. Toxicity effects of diverse components in freshwaters was highlighted by the changes in anatomical and ethologic development of the zebrafish embryonic and larval stages, their eggs being increasingly used in current pollution hazard and risk assessments, because of their qualities: transparency, lack of adhesion, reduced diameter, that give them a great availability and reproducibility [16-19].

Based on the fact that, acute toxicity tests on fish embryos can be successfully used as indicators of the river's pollution, including with animal effluents, and our observations made in different fish species embryos, that revealed useful similarities between zebrafish and another species, more common and of great interest for the temperate climate, namely the

pikeperch embryos (*Sander lucioperca*), we have tried to extrapolate this bio-methodology from zebrafish to pikeperch embryos.

In addition, our study was also justified due to the large producing pikeperch countries (e.g. Czech Republic, Denmark, Hungary, Romania, Tunisia, Ukraine) [20], great scientific interests in this species, this being another reason why a comparative study it should have been made, in the aim to develop a risk assessment tool, helpful for ecotoxicology, the fish industry and/or public health domains.

2. Material and methods

Samples collection and methodology

Water samples for this test were obtained from Ciacova, Timiș County, Romania. The place was chosen due to the river Timiș placement, in the near vicinity of the swine farm.

Previously to this test we have measured after the known methodology the main physicochemical parameters, including nitrate; chloride and phosphate levels (with values within the European legislation limits) [21] (Table 1).

Table 1. Main indicators and parameters for the sampling site

Parameters		Values
Location GPS coordinates	**Long.**	45°51'65"
	Lat.	21°1'49"
Water temperature (°C)		9.4
Ambient temperature (°C)		8
Atmospheric pressure (hPa)		1012
Weather condition		Cloudy
Relative humidity (%)		63
Conductivity (µS)		730.70
Salinity (mg/L)		351.0 (0.4 ‰)
pH		7.7
Dissolved oxygen (mg/L)		9.45
Chloride (ppm)		120.0
Nitrate (mg/dm^3)		1.797
Phosphate (mg/dm^3)		0.0175

In this initial assay there were used 100 zebrafish embryos (*Danio rerio* Hamilton-Buchanan) and 20 controls in parallel with 100 pikeperch embryos (*Sander lucioperca L.*) and 20 controls.

The common test method used, was ISO 15088:2007: Water quality - Determination of the acute toxicity of waste water to zebrafish eggs (*Danio rerio*), a rapid risk-assay, this standard being reviewed and confirmed in 2011, adapted by us for the pikeperch, according to their species stage development. According to its description, this International Standard specifies a method for the determination of degrees of dilution or of concentrations as a measure of the acute toxic effect of waste water to fish eggs within 48 h. This International Standard is also applicable to treated municipal waste water and industrial effluents [22].

In both tests, using zebrafish and pikeperch embryos, there were used ten dilutions respectively: 1X, 2X, 3X, 4X, 6X, 8X, 12X, 16X, 24X, 32X. As negative control, it was used system water. Fish embryos were placed in 24-well plates (one embryo in one well) and incubated. Unlike zebrafish embryos, incubated at 26°C, pikeperch embryos were incubated at 18°C, until there were obtained 80°C cumulative degrees until fish hatching. All behavioural and developmental changes were monitored for the entire test duration and the standard critical endpoints were noted for each sample.

3. Statistics

Obtained data was analysed for normal distribution with Kolmogorov - Smirnov (K-S) normality test using Minitab Statistical Software version 16 (Minitab Inc., UK). The non-parametric test K–S test compare one or two samples with a reference probability distribution being one of the most useful for comparing two samples, as it is sensitive to differences in both location and shape of the empirical cumulative distribution functions of the two samples, the results being considered significant when $P < 0.05$.

4. Results and Discussions

In this initial assay, from day 0 post-fecundation to the standard defined endpoints: coagulated eggs, lack of heartbeat, developmental tail and larval disorders, there were observed and registered comparatively for both fish species and for all tested samples (Fig 1). Our observations revealed a great similitude between pikeperch and zebrafish as evolution of the developmental changes including here also the eggs behaviour to the tested concentrations.

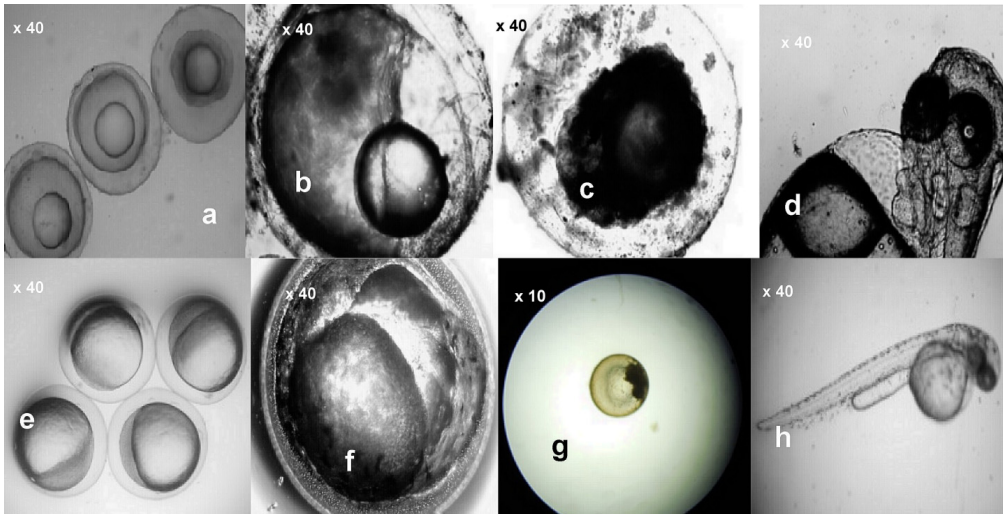

Figure 1. pikeperch vs. zebrafish: (a)/(e) - eggs on day 0 post-fecundation; (b)/(f) - undeveloped embryos; (c)/(g) coagulated eggs, (d)/(h) larvae neo-formation bodies.

Additionally to the endpoints, in both fish embryo species samples, there were observed similar mortality rates for the great concentrations: 16X, 24X and 32X (Fig 2). According to K-S normality statistical test, P value obtained was significant ($P = 0.047$) for zebrafish embryo mortality (Fig 3), while pikeperch embryo mortality had no statistical significance in our case ($P = 0.372$).

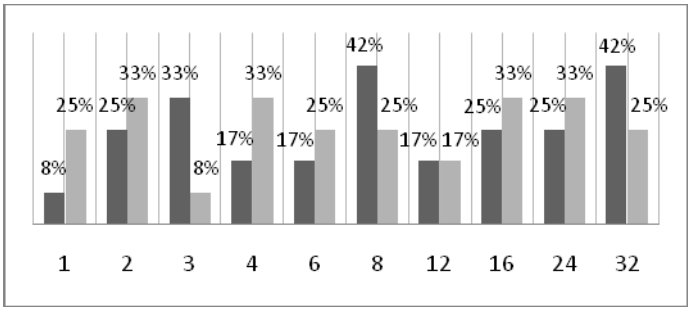

Figure 2. Mortality percentage of Pikeperch (black) and Zebrafish (gray) embryos

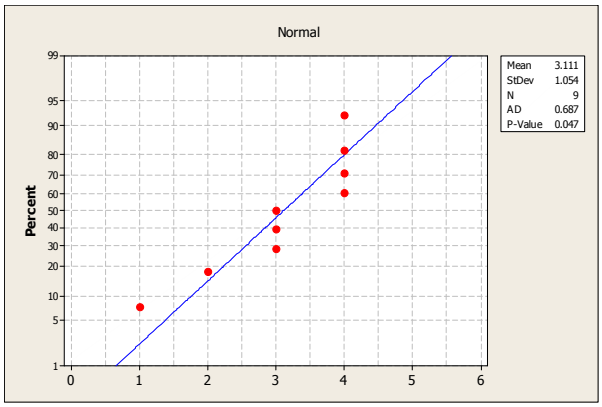

Figure 3. Statistical interpretation of zebrafish embryo sample

In the last decade, studies on zebrafish diversified much, bringing new information, especially on the fields of environmental, aquatic, and reproductive (biomarkers) toxicology [23- 25]. Among these, embryo-tests showed that they are sensitive enough to detect pollutants in wastewaters both in acute and long-term studies, more and more researchers putting the problem of using the embryo toxicity test (FET) (as alternative to the fish acute toxicity test), being a reliable tool to assess the embryo toxicity potential, affirmation that we fully agree [26-28].

In this aim, also a notable advantage of this testing methodology, is that according to the European welfare regulations, zebrafish embryo testing is categorized as an *in vitro* testing, thus not being subject to the animal protection legislation [29, 30], situation that, in our eyes, can be easily extrapolated to the pikeperch embryos and further develop the studies on this species.

Knowing that zebrafish have a very similar development to mammals becoming an accessible model in research, we supposed correctly that pikeperch embryos also can have such feature more suitable to temperate conditions, being a suitable and possible replacer for the zebrafish embryos. Comparative zebrafish vs. pikeperch studies are at the beginning, only a few communications being available in the literature until now.

In pikeperch it was yet established using same methodology as for zebrafish, that nitrogenous compounds have an impact in the development of fishes, the early life-stage forms being most susceptible [31, 32]. From other previous studies made, it was highlighted until now, that pikeperch is rather susceptible to nitrite in comparison with zebrafish [33]. Also bio substances accumulation in pikeperch tissues appreciating their impact in case of consumption by humans was established by some authors using zebrafish methodology [34-36].

5. Conclusions

Although pikeperch embryos use are not yet a pattern, like zebrafish embryos with large applications, this species embryos have been shown to be successfully used in comparative studies. In our opinion pikeperch embryos can be considered fully experimental model organisms and a risk assessment tool, helpful for ecotoxicology, the fish industry and/or public health domains.

In our opinion the main advantages of implementing a 48 hours acute toxicity risk-assay for pikeperch embryos could be: high numbers of embryos that can be used with low costs, efficiency results revealed in a short period of time, fast and precise response to the freshwater and eco-environments pollutants, medicinal active substances or heavy metals.

This eco-toxicity method could be used as an alternative assay in the fish industry for the pikeperch breed in the recirculating water systems.

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