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How *Daphnia* (Cladocera) Assays may be used as Bioindicators of Health Effects?

Antonietta Siciliano*, **Renato Gesuele, Giovanni Pagano and Marco Guida**

Department of Biology, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy

***Corresponding auhtor:** Antonietta Siciliano, Department of Biology, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy, Tel: +390812531111 ; E-mail: totasiciliano_1985@libero.it

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Abstract

Cladoceran daphnids are among the most widely chosen aquatic invertebrates for ecotoxicology, since early 1900's. The use *Daphnia* bioassays in monitoring water quality is due a number of reasons, such as daphnid sensitivity to chemicals and their easy culturing, and because they are important members of aquatic food chains. The genome sequence accessibility of *Daphnia pulex* (first crustacean to have a sequenced genome) has expanded to explore broad-ranging biological disciplines, including host-parasite interactions, and evolution, physiology, microbiology, molecular biology, pathology, and genetics.

Genetic screening allows identify genes underlying that function within a biological process and/or pathway of interest for humans. This review attempts to relate the use of *Daphnia* model in the prospect of human health effects. To date this research is still in its infancy but there are good approaches that could be satisfactory to find this relationship.

Keywords: *Daphnia* spp.; Water fleas; Ecotoxicology; Genetic effects

Introduction

Daphnia spp., commonly called water fleas, are small planktonic crustaceans (1-5 mm long), suborder of Cladocera, that are ubiquitous in freshwater aquatic environments [1].

They occupy a key position in the aquatic food chain as the intermediate link between primary and secondary productivity [2], and they also serve as model species in environmental toxicology because of their high sensitivity to water quality [3].

Water fleas are capable of either clonal or sexual reproduction. Under favorable conditions, *Daphnia* reproduces by parthenogenesis in which clonal offspring are produced by females asexually [4] (Figure 1).

Induced by environmental changes, some females can produce haploid eggs that need fertilization by males. The fertilized eggs remain in a dormant state being enclosed by several protective membranes, the ephippium, and can survive strict conditions for many decades before hatching [5-7].

Standardized testing procedures using the crustacean have been adopted by various environmental organizations, including the US Environmental Protection Agency, American Society for Testing and Materials, International Standardization Organization and Organization for Economic Cooperation and Development [8-11].

Thus, like other invertebrate organisms, daphnids have become cornerstones of systems biology research for alternative testing and methodologies, prompting their use for prescreening tests before vertebrate testing, for both practical and ethical reasons [12].

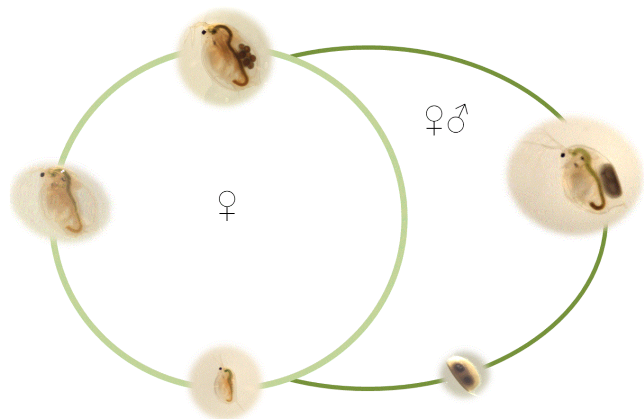

Figure 1: The reproductive cycle of *Daphnia*.

In genetic screening, the use of *Daphnia* may facilitate the understanding the complex regulation of genes and cellular and molecular processes that respond to environmental perturbations.

Relevance of *Daphnia* in classical and current ecotoxicology studies

At the beginning of the last century, Ernest Warren introduced *Daphnia*, particularly *D.magna* Straus, as a model to study the toxicity of sodium chloride as environmental stressor, laying the ground what is now called "ecotoxicology" [13].

The extensive literature on the use of *Daphnia*, primarily *D.magna* and *D.pulex*, as bio-indicators is due to the fact that in acute toxicity are considered as high sensitivity analytical tools to screening toxicity of common environmental chemicals and monitoring of effluents and contaminated waters [14-18].

Furthermore, the ease of culturing the crustaceans *Daphnia*, its well-studied biology, and its attributes of short development time and large brood size make this one of the oldest organism models for toxicological studies [19-21].

In "traditional" ecotoxicity test, growth, reproduction, immobility and mortality of *Daphnia* are all well studied and can be used as quantifiable parameters of its health; dose-response data can aid in predicting the consequences of exposure at other dose levels and life stages, in other species, or in susceptible individuals.

Various contaminants, including the "emerging pollutants", such as Endocrine Disrupting Chemicals (EDCs) [22], Pharmaceutical and Personal Care Products (PPCPs), heavy metals and organophosphates [23] are widely present in environment and can perturb the ecological pyramid, as well as humans. Thus, monitoring some of these could help to protect the natural environment and human health.

For example, heavy metals , pollutants whose persistence in environment was well confirmed by numerous studies, were analyzed on *D. magna* to evaluate toxicity by mortality, brood size, body length, and movement [24], and half maximal Effective Concentration (EC50) [25], a useful tool to define inherent toxicity of tested chemicals.

Several studies showed that low concentrations of heavy metals (μg/L), i.e., cadmium, induced mortality and decreased the survival, feeding rate and production of the offspring [26-28], while others [29] evaluated the toxicity of copper, lead, and zinc mixtures to *Ceriodaphnia dubia* and *D. carinata* assuming that individual chemicals usually underestimate the overall mixture exposure effect and that the combination of contaminant at lower concentrations may elicit additive and synergistic effects.

Significant correlation of *D. magna* immobilization was observed when grew the level of insecticide pollution in stream and river waters [30,31], acute and chronic effect resulted in exposure of *Daphnia* to the "green chemicals" ionic liquids [32,33] and Rare Earth Elements (REEs) [34,35].

Regarding EDCs, beyond the traditional toxicity outcomes, different endpoints were considered i.e., offspring sex ratio [1] and reductions in steroid hormone biotransformation/elimination [36].

In the last decade, *D. magna* was also used as an aquatic model to investigate the effects of nanoparticles and coating agents [37-40]. In studies of nanotoxicology, integrated datasets were obtained from daphnids and from other organisms, including human cells. Geiser et al. (2012) reported on the potential toxic effects of Ag and CeO2 particles of nanoscale and larger size, by comparing their effects in *D. magna*, *Cyprius carpio* (a freshwater fish), human hepatocyte and intestinal cell lines, and fish hepatocytes; the results showed similar biological response ranking across species. This interspecies comparison suggested a commonality in toxicity of these particle types across taxa and, thus, cross-species extrapolations were suggested for future nanoparticle toxicity testing [41].

Daphnids have also been used extensively to analyze the effects and modes of action for a variety of pharmacological agents singly [42,43] or in mixture [44-48]. Since pharmaceuticals are designed to target specific pathways in humans or animals, when introduced into the environment they may affect the same or comparable pathways in mammals and invertebrates having the drug target homologies [49,50].

Gunnarrsson et al. [51] found that *D. pulex* had orthologs to 61% of human drug targets conserved and predicted homology (49%) with humans between the serotonin transporter [51,52].

A study by Furuhagen et al. [53] reported on the impact of drug target conservation on the toxicity of miconazole (an anti-fungal drug), promethazine (anti-histamine), levonorgestrel (synthetic steroid hormone) on *D. magna*. This report highlighted the relevance of considering drug target conservation in environmental risk assessment of pharmaceuticals.

Related to genomic studies, Heckmann et al. [54] suggested that the potential mechanism of toxicity in Daphnia, after chronic exposure of ibuprofen (a non-steroidal anti-inflammatory drug) was similar to the mode of action in mammals and like in these this drug inhibited eicosanoid biosynthesis [54].

Although mammals and crustaceans have different routes of exposure, target organs and toxic mechanisms, *Daphnia* could be a valid test model species prior to mammalian testing [55]. Moreover, this model organism could aid in predicting pharmacokinetics and pharmacodynamics in human.

Environmental sanitation

Several microorganisms can result in significant effects on humans and on health, both positive, such as commensal bacteria of the intestinal flora, and adverse, such as pathogenic bacteria. Therefore, by demonstrating that daphnids can counteract the virulence of a pathogenic microorganism or, otherwise, affect microbial concentration might imply a role of daphnids in improving human health.

Several surveillance studies were carried out on *Daphnia* spp. offering potential for reducing microbial pathogen that could control the abundance of these in natural waters.

Connelly et al. [56] showed that *Daphnia pulicaria* could act on the natural control of the density, viability, and infectivity of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts (zoonotic parasites) under artificial conditions. Repeated ingestion and excretion of both of these protozoan cysts by *D. pulicaria* resulted in the degradation of their walls and in loss of infectivity in nearly all cases [56].

Another study investigated the ability of *Daphnia carinata* to reduce populations of *Campylobacter jejuni* (a human pathogenic bacterium) in simulated natural aqueous conditions after a 72-hr exposure [57]. The plankton predation caused the death of the bacteria reducing *C. jejuni* population by 2 logs compared to the control (without the presence of *D. carinata*).

Daphnia rapidly led to depletion of Avian influenza virus [58], a potential source for the emergence of human influenza pandemics. The authors suggested that the reduction of viral population and the absence of virus viability could prevent viral transmission, though not excluding the vector potential.

In controlling pathogen in nature, Ramirez et al. [59] demonstrated the role of cladocerans, particularly *Moina macrocopa* and *D. pulex* reducing *Vibrio cholerae* densities better than rotifers.

However, other authors reported that not all bacteria are digested by filter-feeders [60]. These experiments were carried out on *D. ambigua*, and demonstrated that in general coccoidal bacteria after digestion survived, unlike rod shaped. And viable gut-passage might be another mechanism of surviving cladoceran grazing.

Study of human pathogen virulence

Daphnia was a model host to study interaction with bacterial pathogens [61] but almost all of these studies focused on species-specific parasites.

Two studies reported on the interaction of *Daphnia* with environmental human pathogens. Le Coadic et al. [62] tested the virulence of several other environmental pathogenic bacteria. When *D. magna* were exposed to various concentrations of *Pseudomonas entomophila*, *Pseudomonas aeruginosa*, or *Photorhabdus asymbiotica* (Gram negative pathogenic bacteria), arapid dose-dependent death of crustaceans was observed, while *Klebsiella pneumoniae* (enteric bacterium) failed to cause this effect. The authors concluded that D. magna was sensitive to virulence traits similar to those described in other host models and that their simple use make *Daphnia* a powerful model to analyze coevolution of hosts and pathogens in a natural context [62].

Another study revealed that *Bacillus cereus* is toxic to *D. magna* and that expression of *B. cereus* hemolysin II in *B. subtilis* made it pathogenic for *D. magna* [63].

Hemolysin II induced cell lysis forming pores in intestinal cell membrane, this mechanism was accompanied by a decline of mitochondrial transmembrane potential in the intestinal cells as it occurs for lysed cultured human cells [63,64].

Physiology

Daphnia have been reported to have a myogenic heart [65], which responds to many cardio-active drugs that affect heart rate and rhythm in humans [66].

In order to understand the role of toxins in human diseases, e.g. lactose intolerance as related to systemic symptoms and arrhythmia [67,68], Campbell et al. [69] tested the effects of lactose on *Daphnia* heart, assuming similar effects to those observed in humans. In this study, were tested also the effects of caffeine (1-10 mM), β-adrenergic antagonist propranolol (100 μM), adrenaline (10-1000 μM), isoproteronol (100 μM) and carbachol (100 μM) to establish the validity of model system. Lactose, at concentrations found in dairy products, caused a dramatic decrease in *Daphnia* heart rate and induced severe arrhythmia. The authors concluded emphasizing the role of *Daphnia* as a unique model system in biology and medicine.

Ouabain, metaproterenol and metoprolol (cardio-active drugs) exerted analogous effects to those observed on humans probably due to the presence of Na, K-ATPase in crustacean heart [70], while Verapamil caused the acceleration of the heart beat rate at low concentration, in contrast to human heart [66].

Podosinovikova et al. (80) investigated the influence of cholinergic ligands, atropine (antagonist) and carbamylcholine (agonist) on the *Daphnia* cardiac rhythm and found that these drugs resulted in opposite influence on the heart beat rate [71].

Other results indicated that Shilajit (a mineral-rich complex organic compound used in Ayurvedic medicine) had a negative chronotropic effect on the *Daphnia* heart at low concentrations and a positive chronotropic effect to arrhythmia at higher concentrations. This effect could be due to mimicking of adrenaline- and noradrenaline-like effect or a change in Ca^{2+} level [72].

Daphnia heart is not similar to mammalian heart, thus its changes in heart rate cannot predict similar changes in mammalian heart under the same conditions. Nevertheless, the similarity of the response to many substances can be exploited to provide interesting information about the mechanisms of drug action on a metabolic process.

In addition to comparing *Daphnia* and humans, heavy metal-induced production of Reactive Oxygen Species (ROS) as well as lipid peroxidation in invertebrates ad vertebrates [73-76].

The versatility of *Daphnia* as a biological model has led to studies of oxidative stress, involved in the aging process and in several chronic diseases. Another study reported on sucralose-induced alterations in Acetylcholinesterase (AChE), the enzyme that degrades the neurotransmitter acetylcholine, and oxidative status [77]. In humans, AChE activity generally increases with age; furthermore, the elevated AChE activity affect to neurodegenerative diseases. A type of cholinesterase (ChE) purified from *Daphnia* was found to be more similar to a type of human pseudocholinesterase (PChe), whereas PChE is more like a scavenger to prevent anticholinesterases. Indeed, sucralose may induce neurological and oxidative mechanisms on other animals [77].

D. magna was used for the initial evaluation of the toxicity and efficacy of reactivators of phosphorylated acetylcholinesterase (oximes) for human use in the treatment of organophosphate poisoning [78].

A new type of antioxidant, SkQ, a derivative of plastoquinone was found to decelerate three models of progeria, a rare genetic condition of accelerated aging. Skulachev et al. [79] studied the effects of SkQ1 on mice, *Drosophila* and *Daphnia*, and found a decrease in age-related mortality [79]. These data might foster human studies, where SkQ might inhibit the development of diseases such as osteoporosis, cataract, and retinopathy [79].

In other two studies, the dopamine neurotoxin was applied to *D. magna* and resulted in a decrease in movement, thus indicating a role for dopamine in *Daphnia* movement. It was suggested to use *D. magna* as a simple and informative test object for the modeling of dopaminergic transmission deficiency and for the primary screening of various substances aiming at the pharmacological correction of dopamine transmission disturbances [80]. Dopamine was also shown to play an important role in vertebrate locomotor function and loss of dopaminergic neurons in the substantia nigra pars compacta leads to motor symptoms including rigidity, bradykinesia, and tremors which are hallmark features of Parkinson's Disease [81].

The antioxidant activity of three hydrophilic thiol compounds was tested on *D. magna* for comparative evaluation of the antioxidant action of water-soluble preparations in vivo [82].

Genetic studies

Among traditional model systems, genetic studies have utilized non-human primates, mice, *Drosophila* and, most recently, *Danio rerio* (zebrafish). These organisms played an important role in yielding results of cancer [83], genetic and *immunologic research [84]* in view of translational applications to humans.

Drug discovery research has successfully used *Caenorhabditis elegans* [85] and *Drosophila melanogaster* [86]. Expanding the inventory of whole-genome sequences has fueled the identification and development of new model systems, for the potential study of human diseases [87]. In part, efforts to introduce new model specie to the standard mammalian experimental are motivated by the fact that some traditional animal models more closely related to humans present significant obstacles including slow generation time, high cost, and difficulty in measuring phenotypes.

For example, Maxwell et al. [88] with an orthologue detection algorithm on 2,727 human disease genes derived from OMIM (Online Mendelian Inheritance in Man), indentified 1,810 ortholog clusters in *D. pulex*, corresponding to a percentage of observed disease gene orthologs 66.4%. This value is higher compared to that found for *C. elegans* and *D. melanogaster*, other two invertebrates that have long been valid model organisms in biomedical research.

The biomarker concept was initially applied in medical research as an indicator of disease in humans [89] became very appealing in environmental context [90].

In 2007, the Daphnia Genomics Consortium (DGC) sequenced the *D. pulex* genome (200Mb with 31,907 genes), which allowed to conduct related genomic and proteomic research [91]. Among arthropods, *Daphnia* gave closest homology and best matches to human genes (http://wfleabase.org/). The first draft of genome sequence of *D. magna* was released in March 2015. Thanks to the recent findings in genomics, genetic information such as Expressed Sequence Tags (ESTs) is now available. Availability of the genome sequence and the research of homology will have implications to study family member's genes and/or biomarker involved in biochemical defense against toxicants.

Analyses of *Daphnia* genomes have identified, for example, 75 genes of the cytochrome P450 family, a protein family involved in xenobiotics detoxification [92] and ABC transporter superfamily, ATP-binding cassette membrane transport-proteins [93]. *Daphnia* was also suitable for the study of human hypoxic injury as a model organism supported by high homology of HIF (Hypoxia-inducible factors) related genes among *Daphnia* and human [94].

Conclusion

The shift in recent years from mammals to invertebrates for human health investigations has been encouraged, first and foremost, for ethical and practical reasons reducing the number of animals used and replacing mammals with non-mammalian species.

These findings can have implications for the water quality in terms of the potential role of Daphnia in the biocontrol of human bacterial pathogens in recreational waters and drinking water reservoirs and for studies of human diseases and of signaling pathways. The current scientific knowledge will enable us to fully achieve these goals in the not-so-distant future.

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