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Review

Environmental stressor induces morphological alterations in zooplankton

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ABSTRACT. Morphological alterations in zooplankton are induced by exposure to hazardous materials in the environment. These alterations in the body are excellent indicators of adverse effects at the (sub) individual and population levels. These changes might be undetectable within a population in the first generation; however, it was recently documented that alterations are more frequent in the subsequent generations. Because of this, we revised scientific literature that reported malformations in cladocerans, copepods, oligochaetes, and rotifers caused by diverse toxicants when organisms were exposed either in the laboratory or in natural conditions. From an environmental perspective, we focused on the importance of morphological alterations in zooplankton, the most likely causes, and their consequences. Furthermore, the present article shows that alterations of the normal morphology could be used as consistent biomarkers, but further research requires discriminating the influence of natural behavior and the consequences of exposure to toxic compounds.

Keywords: ecological indicator; alterations teratology; transgenerational effects; freshwater zooplankton; water pollution

INTRODUCTION

A morphological alteration can affect biological behavior as well as normal biochemical and physiological function. It can be observed in an organism's tissues, organs, and systems (Alvarado-Flores et al. 2015). Deformations in zooplankton can be observed due to exposure to toxic substances, although abnormalities might be observed to a certain extent in non-exposed organisms. Moreover, morphological alterations can be undetectable within a population in the short term but become evident in the subsequent generations, or vice-versa (Aránguiz-Acuña et al. 2016). Morphological alterations typically occur in insufficient proportions within a population: cadmium 42.79% (Pérez-Yañez et al. 2019), sexual hormones 0.71% (Alvarado-Flores & Rico-Martínez 2019), and vinclozolin 0.645% (Alvarado-Flores et al. 2015), such that they are sometimes imperceptible. Some morphological changes are associated with regular life cycles and arise because of high reproductive rates.

In contrast, other changes associated with a high ecological risk are often induced by exposure to toxicants. Thus, structural damage is the result of the combined effects of exposure time and concentration. Deleterious morphological alterations begin with 1) entry and intracellular dispersion of a toxicant in the organism, 2) subsequent loss of cellular homeostasis and genetic damage (transgenerational effects), and finally 3) adverse effects manifested as physically and chemically abnormal cells and deformed tissues that ultimately result in cell necrosis and mortality (Fig. 1). Varieties of morphological alterations have been documented in several zooplankton groups, both *in vitro* and *in situ* experiments. They include reduction in size, structural changes in the lorica, abnormal length, width, and shape of appendages, antennae, feet, fingers, bristles, and structures generally important for locomotion feeding and reproduction. At the cellular level, damage to the cilia of the epithelial and increased size of reproductive glands can be observed (Alvarado-Flores et al. 2015).

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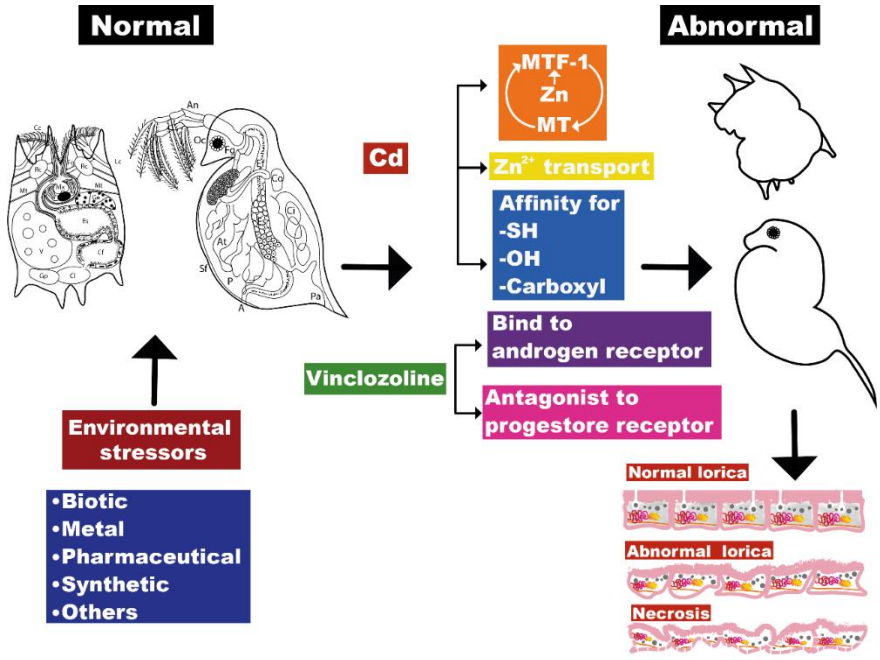

Figure 1. Conceptual model of induction of morphological alteration in zooplankton by inorganic (cadmium) and organic toxic (vinclozoline) as an example, elaborate by Pérez-Yañez, D. MTF-1: metal transcription factor-1 to MT: metallothionein proteins, SH: sulfhydryl group, OH: hydroxyl group, Zn: zinc. The drawings of zooplankton species correspond to rotifers and cladocerans. Cc: crown of cilia, Rc: trochal disc, Mt: transverse muscle, Es: stomach, Cf: flame cells, Cl: cloaca, Gp: foot gland, Oc: ocellus, An: antenna, Ef: pharynx, Co: heart, At: appendix, A: anus, Ci: intestine, P: postabdomen, and Sf: lorica.

This review aims to provide relevant information and a detailed analysis of the consequences of morphological alterations in zooplankton due to exposure to contaminants. The discussion is divided into three sections: 1) *in vitro vs. in situ* exposure, evidence of malformations, 2) significant alterations and their ecophysiology implications, and 3) plausible mechanisms underlying the development of morphological alterations in zooplankton. Finally, the authors discuss future perspectives and possible directions in the study of malformations that could serve as silent signs of future disorders in invertebrate populations and potentially pose threats at higher ecological levels.

Literature review

The manuscript revision was initially achieved with a well-organized search in academic search engines using the query terms were morphological abnormalities, cladocerans, copepods, fish embryo, rotifers, and phytoplankton, examined within all text of the publications. The information search was carried out in Google Academic. Finally, the authors analyzed and designed the conceptual model presented, and the tables included to strengthen the manuscript review.

Malformations in zooplankton are induced by xenobiotics: *in situ* and *in vitro* evidence

The terms 'abnormal morphology', 'malformations', 'arrested development' and other related terms can be frequently found in the scientific literature, but the number of publications using these terms concerning aquatic organisms seems to be limited in comparison to other research topics. As far as the authors know, a growing number of reports from studies involving both *in situ* and *in vitro* observations describe alterations of the typical morphology of fish embryos. Such studies helped generate data regarding changes in embryo development and hatchability and morphological abnormalities in juveniles exposed during early life stages. Thus, working with fish guarantees large offspring and a sufficient number of animals for toxicology studies throughout the year, with no influence by external factors (De Esch et al. 2012). Moreover, several fish species used in research exhibit a clear and transparent chorion, which allows visualization of any deviation from normal development (Henn & Braunbeck 2011). Compared to toxicity tests using juveniles and adult fish, assays of the early life stages require a lower volume for test chambers and

consequently produce lower volumes of residues (Rico-Martínez et al. 2016).

For zooplankton, which appears to offer the same or greater advantages as fish embryos given their smaller size, the number of publications describing abnormal morphology remains limited, focusing primarily on several specific groups (Fig. 2). For instance, in freshwater systems, the most-studied organism is the cladoceran *Daphnia magna*, followed by some other Daphnid species (Sarma & Nandinini, 2006, Siciliano et al. 2015, Karpowicz et al. 2020). Also, some research involving the Chydoridae and Ilyocriptidae families has been recently published. Copepods are essential indicators used in ecological risk assessment because they are naturally widespread in seawater and freshwater ecosystems (Raisuddin et al. 2007, Kwok et al. 2015). The population dynamics of the copepods is altered by the available food, for example, if they are exposed to the most dominant diatoms in the water ecosystems, which produce a variety of toxins that can alter or arrest development in this zooplankton group (Ianora et al. 2004, Vargas et al. 2006). However, to identify the causes of such abnormalities observed in animals captured in the wild, different protocols have been developed to study mechanisms and relationships using *in vitro* bioassays.

In vitro assays include both short- and long-term tests commonly used to monitor the effects of effluents and toxic compounds. Standard protocols include endpoints such as mortality (*dx*), survival (*lx*), and fecundity (*mx*), data regarding which are used to estimate effective concentrations at a given proportion, for instance, the lethal median concentration (LC_{50}) or the half-maximal effective concentration (EC_{50}) for a certain response. Thus, the EC_{50} for fecundity might differ from the EC_{50} for survival or induction of abnormalities. As analysis of these individual responses does not facilitate the elucidation of toxicity mechanisms, analyses of suites of reliable biomarkers have become common. Biomarkers provide data regarding several levels of the biological hierarchy, from molecular to physiologic to behavioral alterations (Van der Oost et al. 2003). Some researchers have included molecular probes in studies of zooplankton, using the expression of cytoskeletal proteins as housekeeping genes to normalize that of target genes (Roelofs et al. 2009). However, significant changes in gene expression could be the cause of abnormal morphology in exposed organisms (Hye-Min et al. 2017, Jun-Chul et al. 2017, Lee et al. 2017).

The most studied freshwater zooplankton species is *D. magna*, a large cladoceran that can reach up to 5 mm in size under laboratory conditions and produces large offspring. Several protocols using this cladoceran have been developed to evaluate teratogenicity (Wang et al. 2011b). The first researchers to describe the use of parthenogenetic eggs of *D. magna* were Toshiro-Otha et al. (1998). However, since their report, the number of publications describing abnormal morphology in cladocerans has not increased as rapidly as publications involving fish. Nevertheless, some reports on inducing malformations and arrested development in zooplankton using in vitro assays appear to be employed more frequently than *in situ* bioassays. For instance, it was demonstrated that metals such as mercury (Khangarot et al. 2009) and organic pollutants (Wang et al. 2011a) induce alterations in the typical morphology of the cladoceran *D. magna*. In contrast, vinclozolin promotes lorica deformations in *Brachionus calyciflorus* (Alvarado-Flores et al. 2015). Data obtained from organisms collected from unpolluted and polluted sites have demonstrated that physicochemical properties of water might be linked to the presence of abnormalities in planktonic organisms (Table 1).

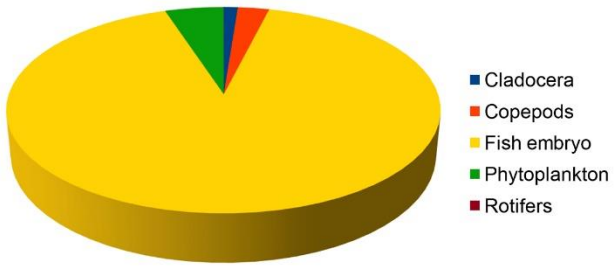

Figure 2. The relative number of publications available in Scientific Search Engines (https://link.springer.com; https://sciencedirect.com; https://www.tandfonline.com). The query terms were morphological abnormalities, cladocerans (69 articles), copepods (153 articles), fish embryo (5164 articles), rotifers (1 article), and phytoplankton (287 articles), and searched within all text of the publications.

Larval development is affected by eutrophication, which is accelerated by human activities and by phytoplankton blooms (with the respective production of various toxins, for example, short-chain α, β, γ and δ unsaturated aldehydes (Ianora et al. 2005)). These processes modify the water chemistry and induce changes in cladoceran and copepod appendages (Melo 2017).

Elmoore-Loureiro (2004) found abnormal postabdomen characteristics in *Ilyocryptus spinifer* and indicated that since these characteristics were not inherited, they might be related to brief exposure to an environmental stressor, likely nonylphenol, according to results presented by Shurin & Dodson (1997). They described antennae and postabdomen malformations in daphnids. Coelho et al. (2019) described the presence

Table 1. Reports of morphological alterations in zooplankton. CL: cladocerans, Ro: rotifers, Co: copepods, DI: diatoms, and WO: worms.

Scientific name	Morphologic alteration	Environmental stressor	Probable cause	Source
Daphnia magna (CL)	Deformed shell	Ethylene thiourea	Teratogenic effects on the formation of the carapace	Toshiro-Otha et al. (1998)
Daphnia carinata (CL)	Abnormal eggs	Mercury	Developmental arrest, abnormal ontogenesis	Khangarot et al. (2009)
Daphnia sp. (CL)	Deformed and imbalanced offspring, reduced ephippial production	Microcystine	Severe stress from cyanobacteria, an imbalanced reproductive effort to ephippia in response to stress	Shurin & Dodson (1997)
Daphnia sp. (CL)	Deformed and imbalanced offspring, reduced ephippial production	Nonylphenol (acetone as solvent)	Endocrine disrupters, direct chemical interactions with developmental processes	Shurin & Dodson (1997)
Daphnia sp. (CL)	Loss of cytoplasmic density, disorganized nucleus, lysis	Salts (potassium dichromate, zinc sulfate, and cupric sulfate)	Metal deposition on cells	Rajaretnam & Stanley (2015)
D. gessneri (CL)	Deformed shell	Uncertain environmental factor(s)	Chemical contaminants, organochlorine compounds, cadmium, iron, copper, lead, and manganese. Bacteria and viruses. Congenital	Zanata et al. (2008)
D. leavis (CL)	Deformed shell	Uncertain environmental factor(s)	Chemical contaminants, organochlorine compounds, cadmium, iron, copper, lead, and manganese. Bacteria and viruses. Congenital	Zanata et al. (2008)
D. lumholtzi (CL)	Deformed shell	Uncertain environmental factor(s)	Chemical contaminants, organochlorine compounds, cadmium, iron, copper, lead, and manganese. Bacteria and viruses. Congenital	Zanata et al. (2008)
Ilyocryptus spinifer (CL)	Deformed postabdomen	Uncertain environmental factor(s)	Domestic waste discharges, oil-derivatives leakage, organic waste from farms and slaughterhouses	Elomoor-Loureiro et al. (2004)
Daphnia galeata mendotae (CL)	Tumor (exophytic lesions)	Uncertain environmental factor(s). High trophic state	Chemical contaminants, UV radiation, viral or bacterial infections	Omair et al. (1999)
Diaphanosoma sp. (CL)	Tumor (exophytic lesions)	Uncertain environmental factor(s). High trophic state	High trophic state. Chemical contaminants, UV radiation, viral or bacterial infections	Omair et al. (1999)
Bosmina longirostris (CL)	Intestine	Uncertain environmental factor(s). Increased trophic state	Organic load, microcystins	De Melo et al. (2017)
Bosmina tubicen (CL)	Intestine	Uncertain environmental factor(s). Increased trophic state	Organic load, microcystins	De Melo et al. (2017)
Ceriodaphnia silvestrii (CL)	Intestine	Uncertain environmental factor(s). Increased trophic state	Organic load, microcystins	De Melo et al. (2017)
Chydorus pubescens (CL)	Prolapse intestine	Uncertain environmental factor(s). Increased trophic state	Organic load, microcystins	De Melo et al. (2017)
D. gessneri (CL)	Rostrum folded tail spine	Uncertain environmental factor(s). Increased trophic state	Organic load, microcystins	De Melo et al. (2017)
Daphnia magna (CL)	Transgenerational epigenetic effects, body length, and reproduction	Vinclozolin	DNA methylation	Vandegehuchte et al. (2010)
Cyclops bicuspidatus (CO)	Protrusions	Puncture wounds, external predators, parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Cyclops spp. (CO)	Protrusions	Puncture wounds, external predators, parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Acartia lilljeborgi (CO)	Intestinal prolapse, chitin rupture, extrusion of cell material and protoplasm	Uncertain environmental factor(s)	Industrial and domestic waste	De Oliveira-Dias et al. (1999)
Diaptomus spp. (CO)	Tumor in the ventral prosoma	Uncertain environmental factor(s). High trophic state	Chemical contaminants, UV radiation, viral or bacterial infections	Omair et al. (1999)

Continuation

Scientific name	Morphologic alteration	Environmental stressor	Probable cause	Source
Epischura lacustris (CO)	Tumor (exophytic lesions)	Uncertain environmental factor(s). High trophic state	Chemical contaminants, UV radiation, viral or bacterial infections	Omair et al. (1999)
Limnocalanus macrurus (CO)	Tumor (exophytic lesions)	Uncertain environmental factor(s). High trophic state	Chemical contaminants, UV radiation, viral or bacterial infections	Omair et al. (1999)
Polyphemus pediculus (CO)	Tumor (exophytic lesions)	Uncertain environmental factor(s). High trophic state	Chemical contaminants, UV radiation, viral or bacterial infections	Omair et al. (1999)
Diaptomus sicilis (CO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Diaptomus ashlandi (CO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Diaphanosoma sp. (CO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Epischura sp. (CO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Eurytemora spp. (CO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Limnocalanus spp. (CO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Mesocyclops edax (CO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Mesocyclops spp. (CO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Polyphemus sp. (CO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Stephanoceros spp. (CO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Keratella spp. (RO)	Protrusions	Puncture. Wounds, external predators, or parasites	Herniation (protrusions on the membranous intersomite region)	Omair et al. (2001)
Brachionus calyciflorus (RO)	Abnormal resting eggs	Food availability	Unbalanced food resources allocation	Gilbert (2010)
Brachionus koreanus (RO)	Growth retardation	Pharmaceuticals (acetaminophen, atenolol, carbamazepine, oxytetracycline, sulfamethoxazole, trimethoprim)	Interaction of pharmaceuticals or metabolized residues with biologically active compounds	Jae-Sung et al. (2012)
Keratella cochlearis (RO)	Deformed lorica	Chemical contaminants, UV	Intrinsic factors, accumulation of deleterious mutations.	Cieplinski et al. (2018)
Brachionus plicatilis (RO)	Changes in body, egg and spine size and distance, dorsal sinus depth, and head aperture	Coal fly ash (As, B, Mo, Se, and V)	Limited food and high pollution	Xue et al. (2017)
Brachionus calyciflorus (RO)	Accelerated or decreased offspring reproduction (hormesis) and decreased lifespan	Dimethoate	Hormone agonists and endocrine disruptors	Guo & Chen (2019)
Testudinella mucronata (RO)	Concavities in lorica	Uncertain environmental factor(s)	Food availability, predation events	Coelho et al. (2019)
Testudinella patina (RO)	Concavities in lorica	Uncertain environmental factor(s)	Food availability, predation events	Coelho et al. (2019)
Brachionus plicatilis (RO)	Higher mictic female production increased lorica size	Vertebrate hormones (HCG, GABA, G.H., estradiol)	Direct hormones stimulation, increased efficiency of nutrient assimilation, the release of other hormones	Gallardo et al. (1997)
Brachionus calyciflorus (RO)	Deformed lorica, offspring imbalance	Vinclozoline	Endocrine impairment	Alvarado-Flores et al. (2015)
Aeolosoma hemprichi (OL)	Necrosis	Cadmium	Damage to the epidermis and digestive tract	Pérez-Yañez et al. (2019)
Nitzschia palea (DI)	Deformed valves	Organic and inorganic toxicants	Teratology. Taxa prone to shape deformities	Lavoie et al. (2017)
Eunotia sp. (DI)	Deformed valves	Organic and inorganic toxicants	Teratology. Taxa prone to shape deformities	Lavoie et al. (2017)
Achnanthidium minutissimum (DI)	Deformed valves	Organic and inorganic toxicants	Teratology. Taxa prone to shape deformities	Lavoie et al. (2017)

of concavities on the lorica of *Testudinella* spp. (Rotifera: Monogononta), which could have been related to environmental factors such as pollution. Nevertheless, the authors pointed out that such a case was unlikely and that abnormal morphology resulted from predation, as evidenced by the presence of *Asplanchna* in the sampling sites.

As seen in Figure 1, the research that was focused on rotifers is still minimal. However, this group is also of high interest because it links primary producers and higher levels within food webs, and these organisms are also of high nutritional value in aquaculture (Dahms et al. 2011). Thus, it might be considered that studies of abnormal development in rotifers could help researchers anticipate further effects in fish and other organisms and thereby prevent the noxious effects of water pollution. As such, the study of morphological abnormalities in rotifers might represent an area of opportunity, as available information remains limited, and the mechanisms underlying observable effects remain unknown.

Eco-physiologic implications related to morphological alterations stated

Zooplankton develops morphological alterations due to exposure to toxic substances and environmental stressors (Table 1). Changes induced during development or the entire life cycle are generally rapid and easy to identify compared with the typical structure and shape. Morphological alterations can sometimes affect the expected behavior of the individuals, for example, inducing the production of males or the formation of unfertilized cysts, leading to infertility (Alvarado-Flores et al. 2015). Other factors that induce structural changes in zooplankton but are not induced by toxicants include energy resources, abiotic and biotic environmental factors, and biological interactions. Gilbert (2010) reported that the development of normal-appearing females (rotifer from *Brachionus* species) is favored when food quality is acceptable; otherwise, the females tend to be smaller and cannot produce cysts. Ying-Hao et al. (2017) reported that rotifers develop a stable, long, lateral posterior spine and relatively small body size in habitats with high predation pressure. Also, the previous authors reported that rotifers collected from polluted habitats exhibit smaller body sizes than specimens from unpolluted habitats.

It is essential to point out that structural, phenotypic, and genetic changes are stable in populations when necessary for survival. For example, Ya-Li et al. (2018) found three morphotypes of the rotifer *Keratella quadrata*, described that the morphotype without posterolateral spines is abnormal (5%) and not expected within the population. In contrast, the morphotype with two posterolateral spines is very common in the population and confers a survival advantage by diminishing depredation risk.

Even though the rotifers' endocrine system has not been thoroughly characterized, several authors have described changes in population growth, alterations in the life cycle, morphological deformations, molecular mechanisms, cell signaling alterations, and gene expression related to reproductive control. For example, juvenile hormone and gamma-aminobutyric acid at 0.05 and 5 mg L^{-1} increased the length and width of the lorica of the marine rotifer *Brachionus plicatilis* by 9.6%: in comparison to non-exposed organisms (Assavaaree & Hagiwara, 2011). In contrast, the hormones 20 hydroxyecdysones, triiodothyronine, and human chorionic gonadotropin reduced the size of the lorica by 3.9 to 8.2% (Gallardo et al. 1997). Moreover, follicle-stimulating hormone and luteinizing hormone-induced morphological alterations and infertility in *B. calyciflorus* (Alvarado-Flores & Rico-Martínez 2019). Studies conducted by Snell & DesRosier (2008), Stout et al. (2010), and Yang & Snell (2010) assessed the effect of progesterone in rotifers. They suggested that this hormone plays a fundamental role in sexual and asexual reproduction enhanced (resting eggs, male production, and growth intrinsic) and in hormonal control and signaling. Therefore, changes in feedback and chemical signaling induce physiological and histological changes. In addition, marine rotifers like *Brachionus* spp. is a non-conventional model species for functional genomic studies. Jae-Sung et al. (2012) reported that the marine rotifer *Brachionus koreanus* has a small genome, making this species well-suited for research, such as in epigenetic studies related to exposure to hazardous substances chemicals. Thus, rotifers and other zooplankton species have unique characteristics that can be useful in understanding the adverse effects of exposure to toxic substances. For instance, Snell (2014) suggested that rotifers are ideal model organisms for use in studies of pathways or genes involved in aging. Specifically, generational changes and associated effects on unexposed and exposed cohorts could provide relevant information on adverse events effects on a population scale. Smith & Snell (2012) conducted a 385-days study of life cycle changes and aging in the rotifer *Brachionus plicatilis*. They were able to observe over 84 generations and concluded it is a good model species.

How xenobiotic induced morphological alterations in the lorica of zooplankton?

The intake of toxicants is generally performed in zooplankters via filtration (i.e. a digestive route). Then,

the toxic substances are concentrated and absorbed in the digestive tract (Alvarado-Flores et al. 2012). The authors know that the toxicant interacts at the molecular level with the cell membrane and enters or initiates a response within the cell. Whether inside or outside of a cell, toxic substances can alter cell homeostasis, in general, whether inside or outside of a cell, by initiating an intracellular cascading response that triggers biochemical stress, oxidative stress, lipid peroxidation in the cell membrane, gene silencing, and epigenetic changes. For example, stress granule (SGs) induction has been identified in rotifers, suggesting these organisms have a rapidly acting adaptive primary response mechanism activated when exposed to a toxic substance. Such SGs have also been reported in the marine species *Brachionus manjavacas*. They are formed when the organisms are subjected to stressors such as heat, osmotic stress, and food deprivation, according to Jones et al. (2013). These authors reported that SGs are components of a response mechanism involved in gene silencing and cell signaling. When a toxic substance induces immediate damage to a cell or its offspring, especially in germline cells, abnormal cells emerge. Such damage is of greater magnitude and more-rapid action (e.g. cell necrosis induced by poisoning).

In our analysis of deleterious effects of morphological alterations, the authors suggested the following. After exposure to environmental stimuli such as biogenic compounds and hazardous chemicals, transgenerational effects are associated with equal risk and magnitude as immediate effects in zooplankton as they occur in developing germ cells involved in the formation of body and rotifers, cladocerans, ostracods, and copepods. Transgenerational effects can be devastating for the dynamic population because they affect physiology, morphology, reproduction, and life cycles. The deleterious transgenerational effects are reduction in copulation, feeding, movement; overall, they modify normal biological behavior and reproductive capacity. They are probably affecting the segregation of the germline from somatic tissues, an essential process in the development of all animals (Smith et al. 2010).

Adverse effects that result in structural changes can be observed at the cellular, tissue, and whole-animal levels. Cells undergo stress in response to exposure to a hazardous substance; this results in declines in the synthesis of digestive enzymes, muscle enzymes, and cellular communication (Jones et al. 2013). Hazardous substances that act as endocrine disruptors in zooplankton alter endocrine communication and the production of stress response proteins, such as metallothioneins, thermal shock proteins, and membrane receptors, resulting in a disruption of cell homeostasis (Snell & Marcial 2017). The underlying mechanisms have been documented by some authors in studies of zooplankton, resulting in the induction of males, reductions in sexual reproduction, infertility, and reductions in intrinsic growth rate. Moreover, there are no target effects or consequence effects; the toxic substances can bioaccumulate in cells and then metabolize and detoxify them (Alvarado-Flores et al. 2012, Hernández-Ruiz et al. 2016). However, adverse effects occur during this process that is mostly irreversible and potentially ecologically deleterious (Fig. 3). For example, reductions in cyst hatching and infertility compromise the generation of offspring and can permanently affect an ecosystem (Aránguiz-Acuña & Pérez-Portilla 2016).

Structural alterations can include tumors, lorica deformations, a fusion of the feet to the head, no lateral spine, and deformed leaflets (Table 1). These alterations are irreversible. Therefore, significant structural changes at the individual level can be devastating; however, such changes may not be significant at the population level, depending on the frequency of the morphologic alterations within the population (Alvarado-Flores et al. 2015). For example, the lorica in rotifers is a tissue that maintains turgor, functions in osmotic exchange with the medium, plays an essential role in reproductive behavior, food acquisition, and thus survival. In this sense, and according to Kleinow (1993), there are two biochemically different rotifer types: 1) hard (loricated rotifers) and 2) soft (illoricated rotifers). The lorica contains disulfide bridges (approximately 5×10^{-8} M in lyophilized material) and keratin (Yu & Cui 1997), together with lysine, glutamate, and aspartic acid bonds (Kleinow 1993). These characteristics allow metals to bioaccumulate in the lorica and thus become immobilized.

To sum up, the aquatic ecosystems are contaminated with a wide variety of toxic substances that can induce morphological alterations, summarized in Table 2 for xenobiotic compounds and their toxicological guidelines, including national and international regulations recommendations, to understand better the risk of adverse effects of a xenobiotic to aquatic systems and biota.

The continual presence of toxic substances in an ecosystem can become a severe hazard to aquatic life, and it can lead to the loss of ecologically important species. Toxicants that induce morphologic alterations in rotifers that have been characterized in laboratory experiments include cadmium, mercury, nonylphenol, ethylene thiourea, and vinclozolin. The mechanisms through which these toxicants induce morphologic alterations are briefly described below, with a general

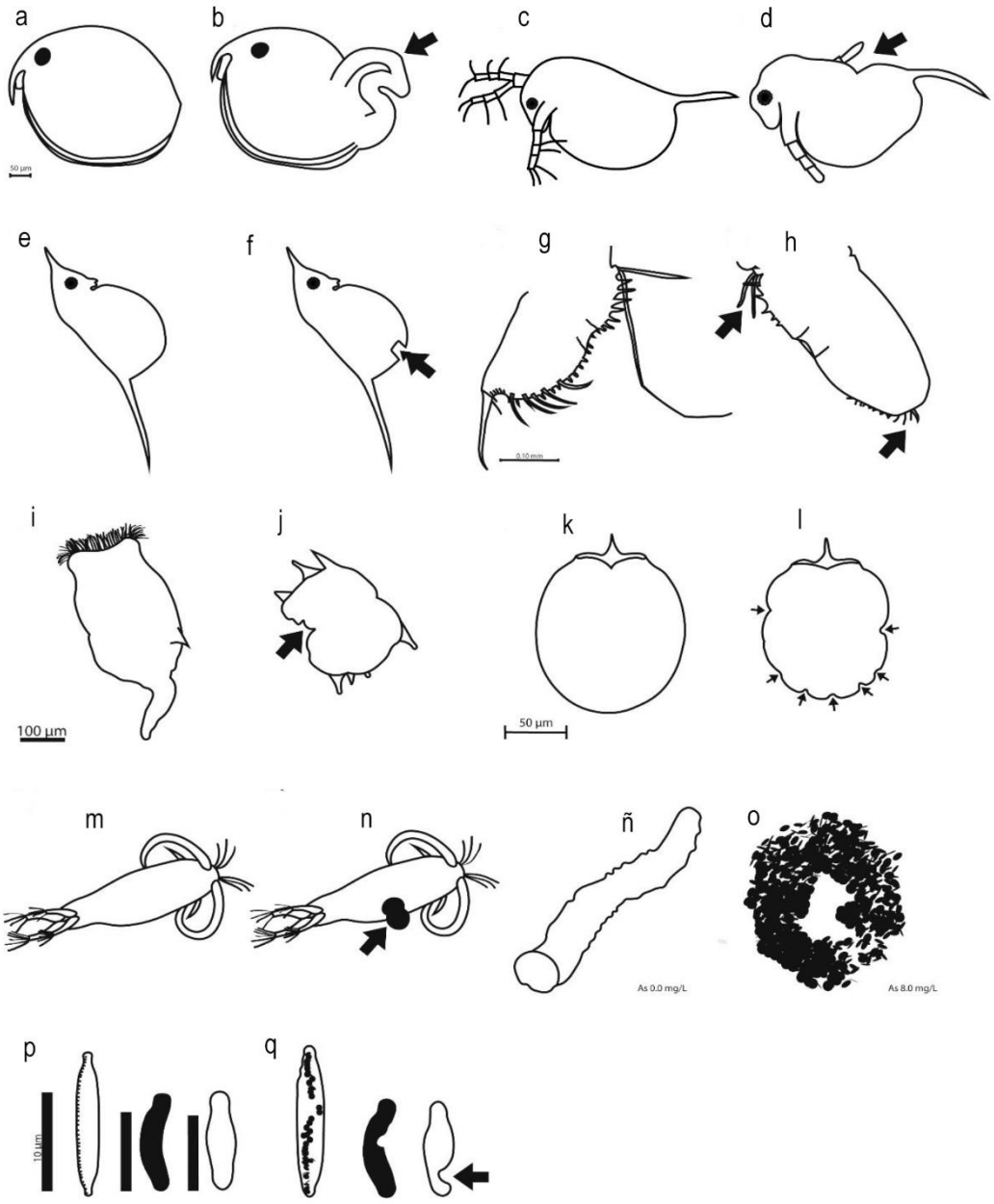

Figure 3. Deformations reported in zooplankton (see authors in Table 1) and drawn by D. Pérez-Yañez. Black arrows indicate the precise location of the deformation of the lorica. a-b) *Chydorus* (cladoceran), c-d) *Daphnia* (cladoceran), e-f) *Ceriodaphnia* (cladoceran), g-h) *Ilyocryptus* post-abdomen (cladoceran), i) *Brachionus* (rotifer), j) *Brachionus* (rotifer) k-l) *Testudinella* (rotifer), m-n) *Diaptomus* (copepod), ñ-o) *Aeolosoma* (worm), p-q) diatoms (phytoplankton).

idea on cadmium and the fungicide vinclozolin (Alvarado-Flores et al. 2015, Pérez-Yañez et al. 2019). The effects of toxicants at the cellular level derive primarily from their mimicry or similarity to other molecules essential for life; for example, lead with calcium and cadmium with zinc alters normal cellular biochemical pathways, causing severe damage (Clarkson 1993).

Finally, we describe an overview of the mechanistic of cadmium and vinclozolin to the inductor of abnormal rotifers (Alvarado-Flores et al. 2015, Pérez-Yañez et al. 2019). Cadmium (Cd^{2+}) induces the expression of genes involved in maintaining cuticle structural integrity (Roelofs et al. 2009). Once Cd^{2+} enters the stomach and is absorbed by epithelial cells, the molecules remain inside the cell. Cadmium ion (Cd^{2+}) enters the cytosol via ionic channels related to Zn^{2+}

Table 2. Xenobiotic compounds and their toxicological information from environmental regulations of hazardous substances. (Source: https://www.epa.gov/; https://www.who.int/es; https://www.acgih.org/; https://www. riskconusa.com/; https://www.hse.gov.uk/pubns/priced/eh40.pdf; http://www.fao.org/home/es/; https://www.gob.mx/ semarnat).

Xenobiotic compound(s)	Regulation guidelines
Cadmium	SEMARNAT (Mexico). Aquatic life protection: 0.2 mg L^{-1}. USEPA 1.8 µg L^{-1} freshwater. WHO 0.003, g L^{-1}
Copper	SEMARNAT (Mexico). Aquatic life protection: 4.0 mg L^{-1}. USEPA 3.1 µg L^{-1} saltwater. WHO 2.0 mg L^{-1}
Cupric sulfate	American Conference of Governmental Industrial Hygienists (ACGIH) 1 mg m^{-3} as Cr
Iron	USEPA 1 mg L^{-1} Saltwater. WHO: 0.8 mg kg^{-1} of body weight, 1-3 mg L^{-1} drinking water
Lead	SEMARNAT (Mexico). Aquatic life protection: 4.0 mg L^{-1}. USEPA 3.2-82 µg L^{-1} freshwater. WHO 10 µg L^{-1}
Manganese	WHO: 0.4 mg L^{-1} drinking water, 0.06 mg kg^{-1} of body weight
Mercury	OSHA-US Department of Labor: 0.025 to 0.1 mg m^{-3}, cutaneous, and occupational exposure limit. USEPA 0.77 - 1.4 µg L^{-1} freshwater
Potassium dichromate	American Conference of Governmental Industrial Hygienists (ACGIH) 0.05 mg m^{-3} as Cr
Zinc sulfate	No occupational exposure limits have been established. Zn Mexico: Aquatic life protection 20 mg L^{-1}; human consumption 5 mg L^{-1}.
Coal fly ash	OSHA-US Department of Labor: 15 mg m^{-3} as total dust and 5.0 mg m^{-3} as a breathable fraction
UV radiation	n.a. (not applicable)
Acetaminophen	UK EH40/2005 Workplace exposure limits: 10 mg m^{-3}
Atenolol	No occupational exposure limits have been established
Carbamazepine	No occupational exposure limits have been established
Hormones: HCG, GABA, GH, estradiol	HCG, G.H., GABA: not considered hazardous by the OSHA. Estradiol: 0.2 µg m^{-3}, skin[2]
Oxytetracycline	No occupational exposure limits have been established
Sulfamethoxazole	No occupational exposure limits have been established
Trimethoprim	No occupational exposure limits have been established
Dimethoate	No occupational exposure limits have been established
Ethylene thiourea	Health Council of the Netherlands, occupational exposure limit: 0.024 mg m^{-3}
Nonylphenol	USEPA: 28 µg L^{-1} drinking water
Organochlorine compounds	Frequently expressed as a mixture of compounds. OSHA-US Department of Labor: Hexane 1800 mg m^{-3}, Toluene 200 ppm
Vinclozolin	FAO: 0-0.01 mg kg^{-1} b.w. (body weigth)

transport (Lavoine et al. 2014). Therefore, Cd^{2+} affects all intracellular biochemical processes related to Zn^{2+}. Once Cd^{2+} is inside the cell, it affects enzymes and transcription factors dependent on Zn^{2+} (Landis & Ming-Ho 2005, Lavoine et al. 2014). Indeed, Cd^{2+} induces the transcription of genes via MAPK kinase, reportedly leading to autophagy in rotifers (Hye-Min et al. 2017, Jun-Chul et al. 2017, Lee et al. 2017). Cd^{2+} has an affinity for several radicals, including SH^-, OH^-, carboxyl, phosphate, cysteinyl, and histidyl groups, resulting in toxicity (Ramírez 2002). Cd^{2+} is a potent enzyme inhibitor with antimetabolite characteristics (a substance that replaces, inhibits, or competes with a specific metabolite) that affects the activities of enzymes due to strong binding to the SH^- groups of intracellular proteins. Cd^{2+} also competes with essential elements such as zinc, copper, iron, and calcium. Cd^{2+} displaces zinc from metallothioneins, which are proteins that protect the cellular enzyme system (Ramírez 2002). Once formed, cadmium-metallothionein complexes are more toxic than free Cd^{2+}, and these complexes allow the metal to remain longer in cells and thus bioaccumulate (Ramírez 2002, Landis & Ming-Ho 2005). Moreover, some toxicological information is acute inhalation toxicity, mucosal irritations, cough, shortness of breath, inhalation may lead to the formation of edemas in the respiratory tract. They are suspected of causing genetic defects, damaging the unborn child. It is also damaging fertility. It causes damage to organs through prolonged or repeated exposure.

Finally, vinclozolin binds the androgen receptor and functions as an antagonist to the progesterone receptor (Molina-Molina et al. 2006). Snell & DesRosiers (2008) recently demonstrated the presence of a membrane-associated progesterone receptor in rotifers, which suggests that a progesterone-like ligand plays a role in regulating reproduction (Snell 2011). Alvarado-Flores et al. (2015) suggested the alterations on *Brachionus calyciflorus* due to the mechanical action of vinclozolin at the level of endocrine systems. Vinclozolin caused skin irritation. It might cause an allergic reaction. Germ cell mutagenicity-mouse fibroblast. Limited evidence of carcinogenicity in

studies with animals. Possible human reproductive toxicity.

Future perspectives and directions on studies of lorica malformations in rotifers

Studying the morphologic changes and the frequency of their occurrence may be an important toxicological/ ecological index. The authors suggest these endpoints as a priority related to maintaining the health of ecosystems and preserving resources for future generations. They also establish a battery of species that indicate structural damage or morphology changes associated with toxicant exposure. The results of these reviews could be used to create a baseline enabling researchers to answer questions such as: which toxicants cause morphologic alterations? In addition, what is the minimum concentration necessary for causing structural damage and transgenerational effects? Additional research should focus on risk analysis, mainly related to toxicants that induce morphological alterations and transgenerational effects in aquatic species.

CONCLUSION

Morphological alterations can occur in zooplankton in response to exposure to environmentally relevant substances concentrations considered highly toxic and of global importance. Such alterations have been demonstrated whether in laboratory experiments and observed in natural water bodies. However, few studies are reporting the use of zooplankton for detailed analyses of the transgenerational consequences of morphological abnormalities caused by toxic substances. It is, therefore, a priority to study and monitor contaminated aquatic ecosystems using biological indicators such as morphology changes in zooplankton to estimate the risks to the aquatic biota associated with specific substances.

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