Ecotoxicology and Environmental Safety 161 (2018) 45–56

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Review

The use of freshwater planarians in environmental toxicology studies: Advantages and potential

Jui-Pin Wu[a], Mei-Hui Li[b,*]

[a] Graduate Institute of Environmental Engineering, National Taiwan University, Taipei, Taiwan
[b] Environmental Toxicology Lab, Department of Geography, National Taiwan University, Taipei, Taiwan

ARTICLE INFO

Keywords:
Animal model
Environmental pollutant
Freshwater planarian
Invertebrate
Toxicology

ABSTRACT

Regarding the humane use of animals in scientific research, invertebrates are often recommended in toxicological studies. "Freshwater planarians" refers to numerous free-living freshwater members of the Class "Turbellaria" of the phylum Platyhelminthes. This group of invertebrates has received extensive attention from biologists for many years because of their unique biological characteristics, such as the primitive form of the central nervous system and notable capability to regenerate tissues. Using freshwater planarians as test animals in chemical toxicity studies has grown in popularity since the 1960s. Results from various toxicological experiments have collectively suggested that freshwater planarians can serve as not only alternative models for chemical toxicity screenings in laboratories but also as potential bioindicators for the quality of freshwater environments. However, thus far, no standardized battery of tests for conducting toxicological studies that includes freshwater planarians has been proposed. This paper comprehensively reviews the toxicological information obtained from chemically exposed planarians and proposes practical factors for consideration in toxicity experiments with freshwater planarians as test organisms.

1. Introduction

Following the guidelines on the humane use of animals in scientific research, also known as the three Rs (replace, reduce, and refine) (Russell and Burch, 1959), non-mammalian organisms are often recommended as surrogates for mammals in toxicological studies (Clay, 1996). Among such organisms, invertebrate species usually receive the most attention because they not only appear to be useful models for linking the sub-organismal effects of toxicants to changes at the population and community levels but also raise fewer societal concerns than do vertebrates (Lagadic and Caquet, 1998). The relatively high sensitivity of invertebrates to environmental chemicals makes them promising screening tools for predicting the acute toxicities of pollutants in relation to mammals (Calleja et al., 1994; Neuhauser et al., 1985, 1986). Moreover, because of their unique biological characteristics, such as relatively simple body structures and systems, shorter life cycles, and diverse reproductive strategies, invertebrates are useful experimental organisms to study biological and toxicological questions that are more difficult or time consuming to investigate by using mammalian systems. As the basic knowledge of the biology and physiology of invertebrate species is sufficient to assess the effects of a given chemical and to evaluate differences between invertebrates and vertebrates, replacing vertebrate animals with invertebrates in toxicity testing can likely yield more insightful results (Lagadic and Caquet, 1998).

Freshwater planarians are invertebrates that have received extensive attention from scientists over the preceding three decades (Fig. 1). Although planarian studies can be traced back to the early 1900s or before, the number of published works began to increase markedly in the 1950s. After 2000, the number of planarian-related publications increased substantially, with more than 50 papers having been published every year since. The number of planarian-related works is still increasing. In addition, since 2000, many review articles and books proposing the development of freshwater planarians as testing models in various scientific fields, such as aging (Oviedo et al., 2008a); pharmacology and drug abuse (Pagán, 2017; Pagan et al., 2009; Raffa and Rawls, 2009); human diseases (Lemieux and Warren, 2012; Prokai et al., 2013); ciliary assembly and motility (King and Patel-King, 2016); chemical toxicity, teratogenicity and tumorigenicity (Hagstrom et al., 2016a); neurotoxicology (Hagstrom et al., 2015, 2016a); carcinogenicity (Stevens et al., 2017) and stem cell biology and regenerative medicine (Gentile et al., 2011; Saló and Agata, 2012; Sheiman and Kreshchenko, 2015; Simanov et al., 2012), have been published. Such works indicate that the usefulness of freshwater

* Corresponding author.
E-mail address: meihuili@ntu.edu.tw (M.-H. Li).

https://doi.org/10.1016/j.ecoenv.2018.05.057
Received 9 March 2018; Received in revised form 19 May 2018; Accepted 22 May 2018

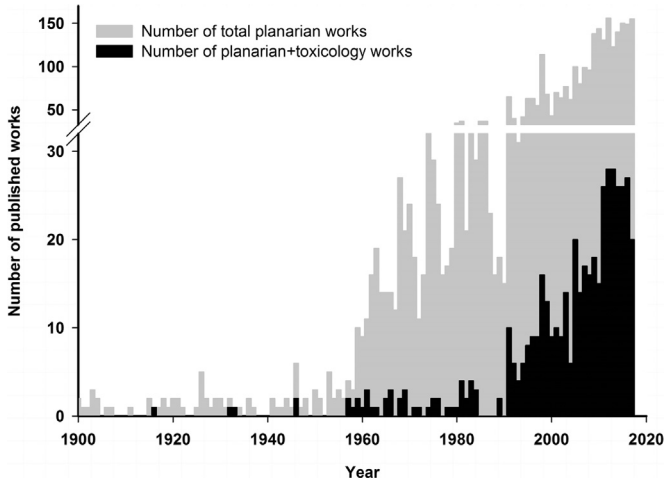

Fig. 1. Temporal changes in the number of journal articles reporting freshwater planarian biology (gray bars) and specific toxic effects on planarians (black bars) (1900–2017). The literature search was conducted using Web of Science with the following keywords: ((((planarian) OR flatworm) NOT marine) NOT parasitic) for the number of journal articles, and (((((planarian) OR flatworm) NOT marine) NOT parasitic) AND (toxic OR effect)) for the toxic effects.

planarians in biological research has been widely recognized by scientists in various fields.

Use of freshwater planarians as test animals in the field of toxicology started to become more common in the 1960s (Fig. 1). The literature search was conducted using Web of Science with the following keywords: (((((planarian) OR flatworm) NOT marine) NOT parasitic) AND (toxic OR effect)). The number of toxicological studies using freshwater planarians increased in the 1990s. Using freshwater planarians in toxicological studies appears to be promising. Freshwater planarians are often used to examine the toxicities of xenobiotic chemicals, especially those generated from human activities. Moreover, freshwater planarians have been suggested as invertebrate bioindicators for the quality of freshwater environments (Knakievicz, 2014).

Experimental results on the sensitivity and toxicological responses of freshwater planarians to environmental pollutants have increased over the preceding three decades. This paper comprehensively reviews these results. The first section of this review paper addresses the systematics and general biology of freshwater planarians to provide readers with background knowledge regarding these unique animals. In the second section, some critical advantages of using planarians in toxicological studies are addressed, and planarian-derived experimental systems are introduced. In the third section, studies are reviewed to provide information regarding planarians' sensitivity and toxicological responses to various classes of environmental pollutants. In the final section, recommendations for developing toxicological methods involving freshwater planarians for toxicological studies are discussed.

2. Systematics and biology of freshwater planarians

Freshwater planarian is the common name used for a wide range of freshwater and free-living members of the traditional taxon "Turbellaria" in Platyhelminthes, especially those placed in the order Tricladida (Ehlers, 1984; Noreña et al., 2015; Schockaert et al., 2008). The scheme of the current phylogenetic relationships of major taxa in Platyhelminthes, including turbellarians, is available from the turbellarian taxonomic database (http://turbellaria.umaine.edu) and some published works (Noreña et al., 2015; Schockaert et al., 2008). Thus far, at least 15 species of freshwater planarians have been used as test animals in toxicological studies, as listed in Table 1 alongside their current taxonomical status. Notably, the systematics of some species has been revised on the basis of new evidence; for example, *Girardia* and *Schmidtea* were considered subgenera of *Dugesia* until 1991, when they were elevated to independent genera (Vries and Sluys, 1991). To prevent misunderstandings regarding species identification in laboratory model systems, especially in comparative studies, authors and researchers prefer to use all available updated scientific names in their works to enable readers to understand linkages between old and new names.

Freshwater planarians exhibit worldwide distribution, are a part of the benthos, and inhabit floating vegetation in unpolluted freshwater environments (Noreña et al., 2015; Schockaert et al., 2008). Their role in the food web is that of a predator of other aquatic invertebrates in their surrounding habitat. The body structure of freshwater planarians is simple but has some unique biological characteristics. They are acoelomates; the free space unoccupied by organs (e.g., intestines and reproductive organs) is filled with the connective tissue or parenchyma. In addition to serving as a matrix to maintain organization, the parenchyma in planarians houses stem cells or neoblasts and is crucial for tissue regeneration (Sheiman and Kreshchenko, 2015). Freshwater planarians have an obvious bilobed neuronal cluster located in the head section. This neuronal cluster, or cephalic ganglion, fulfills the criteria for the definition of the brain in animals (Sarnat and Netsky, 2002). Thus, the central nervous system present in vertebrates may have evolved from its ancestor in planarians (Sarnat and Netsky, 2002); however, this idea was not supported by phylogenetic data (Northcutt, 2012). In addition, free-living freshwater planarians are hermaphrodites, and their reproduction can be either sexual or asexual. For a complete introduction of the bodily structure and physiology of turbellarians, see Noreña et al. (2015).

3. Using freshwater planarians in toxicological studies

3.1. Advantages of using freshwater planarians in toxicological studies

3.1.1. Being an representative invertebrate in aquatic environments

Many freshwater invertebrate models, such as annelids (Famme and Knudsen, 1985), arthropods (Gélinas et al., 2013), and bivalves (Faria et al., 2009), have been developed to assess the toxicity and ecological impact of environmental chemicals. In the food web, many of these aquatic invertebrates are decomposers or primary consumers that feed on organic debris or algae. By contrast, freshwater planarians are predators that assume the role of secondary consumers that mainly feed on other aquatic invertebrates. Collecting toxicity data from organisms located in multiple nodes of the food web is critical to ecotoxicologists and national environmental protection agencies for the comprehensive assessment of the impact and risk of environmental chemicals on the aquatic ecosystem.

3.1.2. Ease of acquisition

For research purposes, freshwater planarians can be easily acquired either commercially or collected from the wild. In the wild, they can be collected in bulk from the benthos (under rocks or fallen trunks) of unpolluted freshwater areas or by setting a trap with the fresh liver as bait (Noreña et al., 2015). Freshwater planarians are slow-moving animals with negligible resistance to salinity, rendering their long distance and transoceanic dispersal unlikely (Knakievicz, 2014). Therefore, each species of freshwater planarian generally has restricted geographical distribution. Predictably but notably, selections of planarian species by researchers for related studies have exhibited geographical dependence (Table 1). In other words, planarian researchers often use species that are native to areas in which they are located for easy acquisition.

3.1.3. Low maintenance cost

Freshwater planarians can be cultured easily (Noreña et al., 2015). Once in the laboratory, they can be stored in dechlorinated tap water, spring water, or reconstituted freshwater. Compared with established