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# Application of Planarian Brain Regeneration: Detection of Water Pollution

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## Abstract

Due to over industrialisation, the environmental pollution problem is becoming increasingly serious, especially in aquatic ecosystems. Compared with traditional physical and chemical detection methods, the use of biological indicators has become popular. The freshwater planarian *Dugesia japonica* is distributed extensively in aquatic ecosystems and has been applied to the area of environmental toxicology for its high chemical sensitivity. Moreover, *D. japonica* also has a powerful regenerative capability in which the injured planarian can regenerate a new brain in 5 days and complete an adult individual remodelling in 14 days. Therefore, it has been used as a new model organism in the field of neuro-regeneration toxicology. In our past study, *D. japonica* can be used as a biological indicator to detect water pollution. This can provide basic data for the detection of water pollution and provide a warning system in regard to aquatic ecosystems.

## Keywords

Planarian, Brain Regeneration, Aquatic Ecosystem, Detection, Neuro-Regeneration Toxicology

## 1. Introduction

Throughout the ages, water has always played an irreplaceable role in human daily life and ecosystem balance [1]. However, at this stage, due to the sustained and rapid development of the economy, environmental pollution has become increasingly serious, especially water environmental pollution [2]. Thus, monitoring and observing the water environment has become increasingly important. Since the commercial instruments of available water quality detection are limited to the data at specific points and the price is very expensive [3], indicator organisms may be considered as available water quality detection. Because of its many

advantages, such as easy to breed, strong regeneration ability and short regeneration time, the planarian *Dugesia japonica* has become an important indicator organism in the process of water pollution detection. Planarians are distributed all over the world, and their regeneration ability is very strong [4]. Most live in clean and oxygen-rich water environments. Most planarians in streams hide under rocks, resting during the day, and moving at night (**Figure 1**). Their response to changes in water quality is very rapid. This method reflects changes in water quality according to the regeneration of planarians in different water environments; therefore, they can be used as indicator organisms for water quality detection. Physiological changes of aquatic animals directly reflect information regarding the water environment [5].

Currently, environmental problems are becoming increasingly serious, and we are giving increasing attention to environmental protection. Now, our government has begun to implement new strategies to protect the environment and strictly control environmental quality. Water quality monitoring is used to monitor the types, concentrations and patterns of pollutants in water bodies. Its scope is very wide, covering water sources that have not been polluted and those that have been polluted. As a water quality testing industry that started to operate to maintain the water environment, it has been highly valued by the majority of government departments and administrators. In recent years, the amount of wastewater discharged through different channels has increased year by year, which has also led to an increase in the overall wastewater discharge year by year. Therefore, in the face of the substantially increasing wastewater discharge every year, the state should take this problem seriously and take more stringent measures to monitor and manage wastewater discharge.

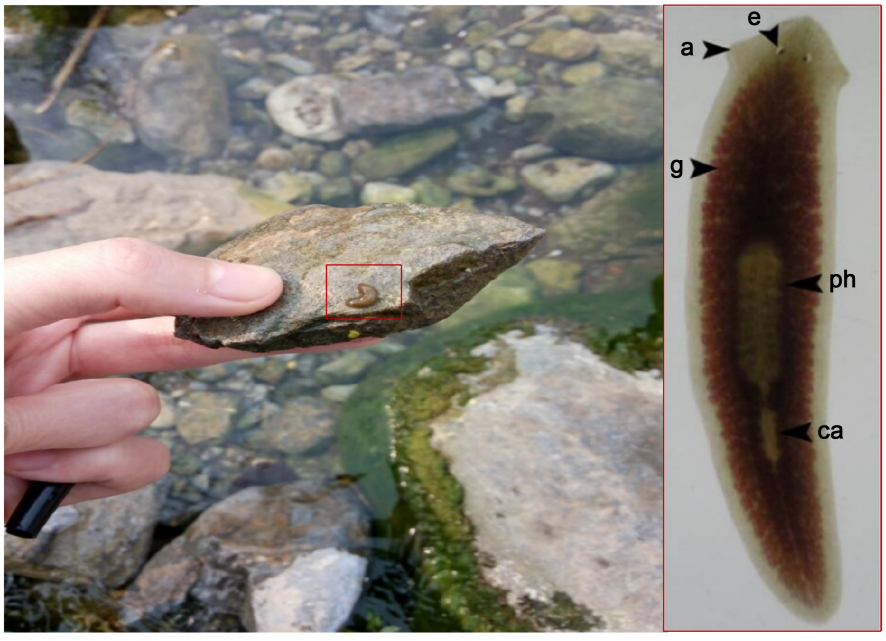


**Figure 1.** A planarian morphologyns [4] (a: ear process; e: eye point; g: intestine; ph: pharynx; and ca: mating device).

With the increasing scale of the water quality monitoring industry, the development of water quality monitoring technology is also accelerating. However, it is still falling behind. In addition, physical and chemical monitoring technologies still account for a large proportion of water quality monitoring technologies, including the ion selective electrode method, electrochemical method, chemical method, plasma emission spectrometry (ICP-AES) method, etc. However, during actual monitoring, traditional monitoring technology focuses more on the pollutants, such as monitoring the pH value, chromaticity, turbidity, chemical oxygen demand (COD) and other aspects of the water body. These parameters can accurately reflect the physical and chemical characteristics of the effluent environment, but it is difficult to reflect the harm of effluent environmental pollution to organisms. For example, many pollutants combine in the water environment and interact with each other, which will have some impact on organisms. However, it is difficult to effectively verify traditional physical and chemical monitoring technology. Biological monitoring technology can rely on the changes in individual organisms or biota to understand the serious impact of pollutants [6]; therefore, biological monitoring technology has been developed.

Biological monitoring by aquatic animals has the advantages of objectivity, continuity and intuition [7]. Although it cannot ultimately show the types and concentrations of pollutants contained in these tested water samples, it can comprehensively reflect the severity of pollution and its harm to organisms. This method can neutralize the disadvantage in which it is difficult to detect and evaluate the effects caused by the long-term mixing of various harmful substances in the physical and chemical monitoring process [5]. The asexual reproduction of planarians is a kind of reproduction mode under the conditions of rich nutrients, a superior living environment and good physiological conditions. They are very sensitive to changes in water quality in the process of dividing and regeneration. Therefore, when the water quality is polluted, the planarian response will be very sensitive. At this time, their asexual reproduction and growth speed will also change correspondingly due to the degree of water pollution. Planarians can reproduce asexually, and they have strong regeneration ability and wide distribution and are easy to collect and raise. Therefore, it is feasible to use planarians as indicator organisms for water quality monitoring. Here, we will introduce the planaria advantages in detection of water environment.

## 2. Water Pollution

The pollution of rivers and lakes does great harm to the environment, the whole ecosystem and human health. Generally, there are many reasons for water pollution, most of which come from industrial wastewater, urban sewage and chemical substances used in agricultural production. These generally pollute the water source in two main ways, namely, point source pollution and nonpoint source pollution. Water quality pollution caused by point source pollution includes industrial wastewater and urban wastewater. In agriculture, the pollution route is

nonpoint source pollution, and the pollution route is extensive [8].

### 2.1. Agricultural Pollution

In the process of agricultural production, to make crops thrive, many chemicals, such as fertilizers, are used. These fertilizers contain a large number of harmful chemicals, such as phosphorus and sulfur [9]. When these harmful substances accumulate to a certain extent, they will gradually infiltrate into the ground, causing soil problems and threatening the water safety of the human. Moreover, in agricultural production, water sources are often used for irrigation. If polluted water is used for irrigation, it will not only cause irreversible damage to crops, with the passage of time, it will also lead to groundwater pollution [2].

### 2.2. Industrial Pollution

In today's new era, world's economy is developing rapidly. The rapid development of enterprises is bound to produce a large amount of wastewater in the process of industrial production. At the same time, to save money, some enterprises will choose to discharge the untreated wastewater generated in the production process into streams or rivers. All of these factors will lead to serious pollution of the aquatic environment. The wastewater discharged without permission contains many toxic chemicals, which will eventually pollute the water environment. These substances will affect organisms in the aquatic environment. For example, if Japan discharges nuclear sewage from Fukushima into the sea, the radioactive chemicals in the sewage will spread to the nearby sea areas. When nuclear sewage from Fukushima is discharged into the sea, it will seriously endanger fisheries [10]. It will also pose a threat to our daily life. Some heavy metals contained in industrial wastewater will affect our lives if they enter the human body. For example, lead will greatly increase the prevalence of cancer and cardiovascular disease [11], and excessive copper increases the risk of neurological defects and liver disease [12]. This is bound to affect the sustainable development of the world.

### 2.3. Domestic Pollution

In our daily lives, some people have never been aware of protecting the aquatic environment. In our lives, a large amount of domestic garbage and domestic wastewater will be produced, which will lead to water environmental pollution. At present, our country still chooses to landfill or incinerate the domestic waste generated in daily life. The hazards caused by landfilled waste will exist for a long time, which is bound to affect groundwater sources. At the same time, we also choose to directly discharge the wastewater generated in daily life. If we discharge the wastewater for a long time, it will eventually affect the water resources. For example, in daily life, to make clothes cleaner, a large number of detergents containing phosphorus will be used, which will lead to the enrichment of nutrients in surface water and eutrophication of water [13].

## 3. Planarians

### 3.1. Basic Characteristics of Planarians

Planarians are small and very soft. They are willow shaped. Their backs are mostly brown, very close to the colour of stones. Their ventral colours are relatively light, and they crawl in the water. The head of the planarian is a triangle, the black spot on the back of the head is the eye point, the ear process is on both sides, and the pharynx is in the middle of the abdomen. Planarian water environmental requirements are high; most live in a cool environment with a slow stream flow, avoid strong light, and have adapted to a weak light environment [14]. On the 7th day after the removal of its head or tail, without consuming any food and only in a clean water environment, the planarian can complete the regeneration of all organs and tissues, including a completely functional central nervous system (**Figure 2**).

The commonly used planarian in the laboratory is the Japanese delta planarian. It is widely distributed all over the world. Most live in clean and unpolluted water and like shade. For example, they live in streams and lakes hidden under rocks [15]. Because freshwater planarians live in clean and clear water environments for a long time and are particularly sensitive to toxic teratogenic factors in the environment, they are excellent experimental materials for water pollution detection and adult neurodevelopmental toxicology research. In 2005, acting as a water quality detection animal for the first time, freshwater planarians successfully evaluated the biological safety of toxic factors in a river in Porto Alegre, Brazil. The role of planarians in the biosafety assessment of aquatic environments has aroused the interest of a large number of scientific researchers. Freshwater planarians have an extraordinary ability to regenerate. *D. japonica* also has a powerful regenerative capability in which the injured planarian can regenerate a new brain in 5 days and complete an adult individual remodelling in 14 days; therefore, it can realize the adult study of neurodevelopment (**Figure 3**).

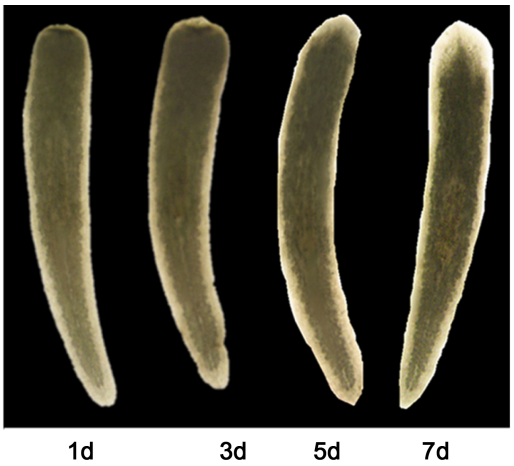


**Figure 2.** Head regeneration for the 1st, 3rd, 5th and 7th day after amputation (d: day).

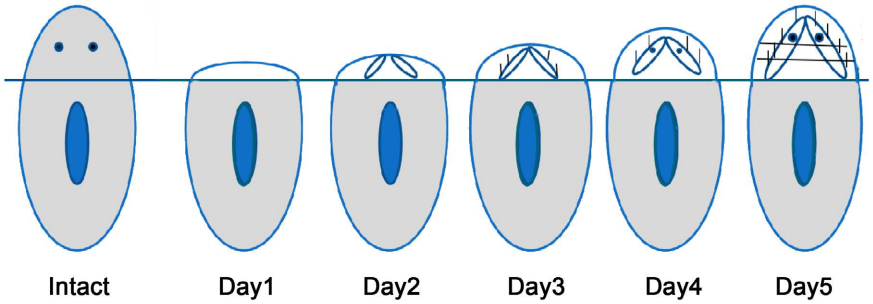


**Figure 3.** Central nervous system regeneration after planarian head resection.

### 3.2. The Reason Why Planarians Have Strong Regeneration Ability

Regeneration is one of the greatest mysteries in biology, and it involves replacing missing organs, attachments, or large body areas. Almost all representative species of the major phyla have a regenerative capacity [16].

Planarians, as representative organisms of Platyhelminthes, have attracted much attention due to its extraordinary regenerative ability. Planarians are one of the lowest-grade animals with a centralized head [17] and they are flatworms with amazing regeneration ability. Planarian regeneration includes the generation of new tissue on the wound surface through cell proliferation (bud formation) and the remodelling of the original tissue to restore symmetry and proportion (morphological axis) [18].

The commonly used planarian species in the laboratory is the Japanese delta planarian. When the planarian is cleaved, cells near the wound quickly proliferate, divide, and transform into various functional cells [19]. When fission occurs, the true planarian splits itself into two, transforming into a head and a tail, and each part regenerates all missing body structures. The regeneration of the missing part after fission is mediated by the presence of adult stem cells or newborn cells [20]. When fission occurs and the planarian is cut (**Figure 4**), it is able to regenerate a complete individual in a short time without leaving any scars on the body. Therefore, the planarian has been a model to study the mechanisms of animal regeneration.

The strong regenerative ability of planarians is due to the large number of adult stem cells or newborn cells [21]. The strong regenerative ability of planarians is derived from their large newborn cells [22], that is, adult undifferentiated cells, which are the only type of stem cells with the potential for proliferation and differentiation in planarians. This is the basis of tissue renewal, injury repair and regeneration [23]. They have the ability to proliferate and divide and can differentiate into different types of cells in the body of planarians. The powerful ability of such cells enables them to repair or replace damaged or dead organs and tissues in organisms [24] and ultimately become intact individuals. This characteristic of planarians has attracted the attention of researchers, and they are widely used in cell differentiation and dedifferentiation and chromosomes. [25], which has certain scientific research value. Therefore, planaria is a

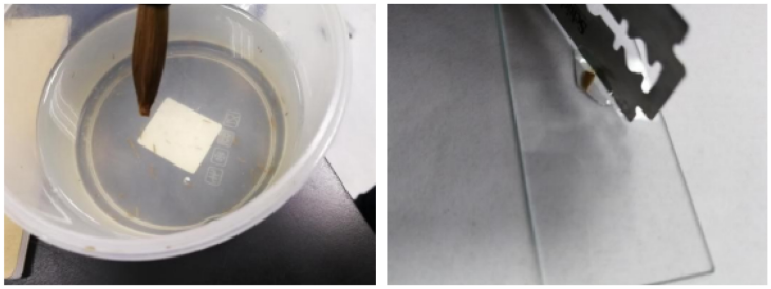

**Figure 4.** Schematic diagram of cutting planarian.

good model to study the regulation of stem cell differentiation *in vivo* [19].

## 4. Monitoring Technology by Using Planarian Regeneration Ability

### 4.1. Principle and Significance of Biological Monitoring Technology

#### 4.1.1. Principle

Biological monitoring is the use of communities, populations or individual organisms to monitor and assess environmental pollution. It is monitored according to the development and reproduction of living individual organisms and biological systems, as well as the number of populations, communities and changes in ecosystems [8]. This monitoring method has many advantages. It can monitor and evaluate the pollution degree of the living environment of the organism by measuring some indicators in regard to the organism.

In the field of ecological nature, some organisms and water environments are closely related and affect each other. However, if the content of pollutants in water is too high, it will directly harm the growth and development of organisms. The establishment of a biological monitoring method is based on the theory of environmental biology. Biological monitoring technology relies on biological reactions to make a specific and in-depth analysis of the diseases of different biota in the water environment and their sensitivity to water bodies to measure the status and degree of water pollution and clarify the types of water pollution [2].

#### 4.1.2. Significance

Biological monitoring can provide a timely understanding of the comprehensive toxic effects of pollutants and potential environmental risks. By mastering the quality of the aquatic environment, environmental problems that cannot be addressed by general physical and chemical monitoring can be identified. The use of indicator organisms for monitoring indirectly indicates that biological monitoring methods are practical, comprehensive, timely and irreplaceable [9].

### 4.2. Effects of Toxic Factors on Water Detection in Planarian

#### 4.2.1. Heavy Metal Ion

Natural heavy metals exist in the Earth's crust [10]. However, they have obvious

toxicity, cause serious harm to organisms and they also pollute the ecosystem [26]. Heavy metal wastewater is the most serious industrial wastewater in the process of industrial production. This pollution is harmful to the aquatic environment, the human body and the whole ecosystem. The heavy metals in the produced wastewater cannot be decomposed or degraded by various conventional water treatment methods. Therefore, they can only change their physicochemical state and transfer their existing position. Heavy metals have toxic effects on the regeneration characteristics, stem cells and genetic material of freshwater planarians. Guecheva *et al*. [12] studied the toxic effect of copper sulfate on freshwater planarians. Their results showed that the higher the concentration of copper ions was, the stronger the genotoxicity to planarian, and it could inhibit the repair of DNA damage caused by methyl methanesulfonate. Therefore, planarian can be used as an indicator organism to evaluate the pollution degree of the aquatic environment and its harm to organisms. At the same time, it can also be the best choice for testing the toxicity of heavy metals in water.

#### 4.2.2. Pesticides

As a compound, pesticides are widely used to control harmful insects, eliminate weeds and make crops thrive [13]. There are several ways for pesticides to pollute the water environment: directly using pesticides in the water environment; discharging rainwater polluted by pesticides or herbicides into the influent environment; and washing or integrating residual pesticides adhered to crops or soil into the water environment when it rains. These methods will lead to a high concentration of pesticides in the water environment, which will lead to the death of a large number of aquatic organisms. As an excellent species for water quality monitoring, freshwater planarians are very sensitive to pesticide residues in water; therefore, it is possible to use planarians as indicator organisms to study the biological toxicity of pesticides. This biological detection technology is based on the influence of harmful toxic factors in pesticides during the regeneration of planarian. The toxicity of pesticides to planarians and the pollution degree of the aquatic environment were analysed by comparing the regeneration speed after cutting.

At present, the pesticides used mainly include organochlorine, organophosphorus and other types. Best *et al*. [27] studied the toxic effects of organochlorine pesticides on freshwater planarians. When the planarian was placed into a chemical reagent for testing, it was found that with the increase in the concentration of these reagents, the cycle from cutting to death of the planarian was shortened, and its mortality also increased significantly. These studies showed that planarian and other indicator organisms can detect and evaluate the pollution degree of the aquatic environment caused by harmful toxic factors in pesticides, as well as the toxicological aspects of organisms.

## 5. Challenges and Future Prospects

Currently, with the continuous development of the economy and technology,

world's environmental problems are becoming increasingly serious. Moreover, due to the modern people's indifference to environmental protection, the pollution problem is more serious. Water environmental pollution is also very serious. However, with the improvement of national quality and the strong support of the state, the government has begun to consider the issue of environmental protection. The water quality testing industry is gradually rising. However, as an emerging industry, although its scale has been continuously expanded, its performance still lags behind that of foreign high-quality brands. Moreover, there are still some problems with traditional water quality monitoring methods. At this time, biological monitoring technology has come into being, and the use of indicator organisms for water quality detection is in line with the requirements of the public. Among them, it is feasible to monitor water pollution by using the regeneration ability of planarians [28] [29] [30] [31]. The past research showed *D. japonica* was a wonderful biological indicator to detect water pollution [32] [33] [34] [35]. This can provide basic data for the detection of water pollution and provide a warning system in regard to aquatic ecosystems.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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