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Photo-induced toxicity in the rotifer *Branchionus plicatilis* by anthracene (ANT) in absence and presence of UV irradiation and mechanisms underlying observed effects

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Anthracene, a polycyclic aromatic hydrocarbon (PAH), undergoes a series of chemical reactions by absorbing ultra-violet (UV) light, and is potentially phototoxic to aquatic organisms. The aim of this study was to investigate the effects of photo-induced toxicity of anthracene (ANT) alone or in combination with UV radiation on *Branchionus plicatilis* physiological and metabolic functions such as fecundity, reproductive cycle, age-specific survival rate, spawning rate and reproduction rate. The mechanisms underlying ANT-induced phototoxicity were examined by measuring oxidative stress parameters including reactive oxygen species (ROS) and malondialdehyde (MDA) content, activity of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione S-transferase (GST) and levels of glutathione (GSH). The results showed the following: (1) Compared with single UV-treated and single anthracene-treated groups, the two examned concentrations of anthracene (0.4 μg/L and 4 μg/L) under UV radiation significantly inhibited individual fecundity, shortened the reproductive cycle, and delayed or suspended the arrival of reproduction peak, life cycle parameters of *B. plicatilis* such as net reproduction rate (R0), life expectancy (E0), generation time (T), and intrinsic growth rate (rm).The reproductive capacity of the rotifers was lowered. (2) Under UV radiation, ANT significantly increased ROS and MDA content, leading to changes in activity of antioxidant enzymes. The activity of SOD and GPx decreased within the first 24 h while the activities of SOD, CAT, GPx, GST and GSH were reduced. GSH content was lowered after 48 h. Hence, ANT produced photo-induced toxicity on the reproductive capacity of *B. plicatilis*. Under UV radiation, ANT was more potent in disturbing antioxidant mechanisms resulting in diminished survival rate, reproduction and growth of *B. plicatilis*.

Keywords: Anthracene; *Branchionus plicatilis*; UV light; Life table; Antioxidant enzymes

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are resistant to degradation, persistent and display long-range transport to remote global areas. Currently, PAH are not restricted to areas with human activities, but are also found in remote ecosystems such as the Arctic and the deep sea [1-4]. Due to their high lipo-solubility and octanol-water partition coefficient, PAH are readily absorbed by organisms, bioaccumulate and are enriched along the food chain, eventually imposing threats to human health [5-6].

Photosensitization is one of the important mechanisms underlying PAHs' photo-induced toxicity. Several studies demonstrated that after PAH absorb light, reactive oxygen species (ROS) are generated as a result of photosensitization yielding reactive singlet oxygen which produces lipid peroxidation membrane denaturation and breakdown of macromolecules, eventually inducing damage and death to the organisms [7-9]. In addition, several PAH act similar to natural hormones, disturb normal hormone metabolism and produce endocrine disorders in organisms, adversely affecting the nervous, immune and reproductive systems, and even resulting in malignant neoplasms [10-12].

Anthracene (ANT) is optically active and undergoes a series of chemical reactions by absorbing UV, such that this chemical is potentially phototoxic to aquatic organisms [13]. Studies examining photo-induced toxicity of PAH on organisms which investigated the antioxidant reponse and danmage of ANT on biological by absorbing UV were conducted by several investigators [14].

Photosensitization causes mass production of reactive oxygen species (ROS). When the ROS production rate exceeds the removal rate by the antioxidant system of an organism, antioxidant reactions occur and biofilms can be damaged. Several studies demonstrated that ROS can also interact with other biological macromolecules such as proteins and nucleotides, resulting in damage or death of cells.

B. plicatilis are widely distributed, short-lived, fast-breeding, easy to cultivate, sensitive to exogenous toxicants, and play a key role in material circulation and energy transfer in marine ecosystems. Nevertheless, there are few studies on the photo-induced toxicity of PAHs on *B. plicatilis*. This paper used anthracene, which is the most optically active compound under UV light, as the stress factor and *B. plicatilis* as the subject, aiming at investigating the photo-induced toxicity of anthracene under exogenous UV radiation conditions on *B. plicatilis*. The possible mechanisms based on photosensitization theories were discussed. This paper filled the research gap

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in the photo-induced toxicity of anthracene on *B. plicatilis* and the toxicity mechanisms based on photosensitization theories, providing theoretical basis for future comprehensive evaluation on the photo-induced toxicity of anthracene on marine zooplankton.

RESEARCH METHOD

Lively and strong female *B. plicatilis* cultivated by the Faculty of Fishery, Ocean University of China were used in this study. They were cultivated in 1.5-L glass beakers and then placed in intelligent lighting incubators (GXZ-3008), with a temperature of 20±1°C. The light intensity was 1313.8±54.8 μW/($cm^2 \cdot s^{-1}$) and the photoperiod was 12 h: 12 h. Before the start of formal experiments, the rotifers were tamed for three months under the experimental conditions.

Light Source Setting and Experimental Set-up

The acute toxicity experiment was carried out in lighting incubators at a constant temperature. The UV radiation system mainly consists of two UV-A fluorescent tubes and one UV-B fluorescent tubes. The visible light, UV-A and UV-B intensity is 1313.8±54.8 μW/cm^2, 1869.1±25.7 μW/cm^2 and 136.1±5.2 μW/cm^2, respectively. This can effectively simulate natural sunlight.

Experimental Design

Photo-induced Toxicity Effects of Anthracene on Life Table Parameters and Reproductive Value of B. plicatilis.

Totally 420 healthy and energetic larvae just born for less than three hours were selected from previously cultivated *B. plicatilis*. During the experiment, 80% of the water in each experimental group was replaced every day in order to keep the system stable. Chlorella was given each time during water replacement to maintain the concentration of 1.0×10^6 cell/L. During the first 24 to 72 h, the survival condition of *B. plicatilis* was observed at 4-h intervals with dissecting mirrors. The first spawning time and the first birth time were recorded. After that, observations were made at 8-h intervals to record the maternal spawning number and the number of survivals. At the same time, the dead rotifers were removed and the newly born larvae were moved to another 24-well plate for cultivation. The experiment ended when the last parent died.

Photo-induced Toxicity Mechanisms of Anthracene on B. plicatilis based on Photosensitization Theories

Two different concentrations of anthracene (0.4 μg/L and 4 μg/L) were used in the experiment. After concentration accumulation, the rotifers were transferred to new culture media, further divided into groups with/without UV radiation treatment. After the pre-cultured larvae were exposed to UV and UV-free (control) radiation, anthracene concentration in each group was quantified, and there were three subgroups in parallel for each group treated with certain concentration of anthracene. The rotifer density was 100 individuals/mL. There was no feeding during the experiment. There were 10000 rotifers (about 0.2 g) for each parallel sub-group. The rotifers were collected at the 24^{th} h and 48^{th} h. With the addition of 0.86% saline solution, these rotifers were disintegrated in an ice bath with an ultrasonic cell crusher, and the operation lasted for five seconds at 10-sec intervals. They were completely fragmented after five sessions of crushing and underwent centrifugation at 2500 r/min for 10 minutes at 0°C. The supernatant was used to measure the activity of different antioxidant enzymes in the crude extraction, the total suspended particulate (TSP), ROS, MDA content, and the activity of SOD, POD, CAT, GPX, GR, GST, GSH, in the crude enzyme solution.

RESULTS AND DISCUSSION

Effect of UV radiation and Anthracene Concentrations on Life Table Parameters of B. Plicatilis

Under UV radiation, anthracene greatly affects life table parameters of *Brachionus plicatilis*, specifically illustrated as follows: (1) Life expectancy is shortened very remarkably in the anthracene-treated group under UV radiation ($P<0.01$). The suppression is notably dose-dependent. (2) The net reproduction rate in the 4 μg/L anthracene-treated group without UV radiation is greatly elevated to 7.22, but drops notably to 1.51 when the counterpart is exposed to UV radiation. (3) Compared with the control group, the generation time in the 4 μg/L anthracene-treated group under UV radiation is inhibited significantly ($P<0.01$), and the resulting generation time is just 87.5% of that in the control. (4) Compared to the control, the intrinsic growth rate in the 4 μg/L anthracene-treated group without UV radiation considerately increases ($P<0.01$). The two anthracene-treated groups under UV radiation (0.4 μg/L and 4 μg/L) show remarkably lower intrinsic growth rates ($P<0.01$). The rate decreases obviously with the increasing anthracene concentration, reaching to the minimum of 0.12/h in the 4 μg/L anthracene-treated group under UV radiation.

Table 1. Toxic effects of ANT on the life table of *B. plicatilis* under UV radiation

Condition	Expectancy e_x (h)	Net reproductive rate /R_0 (ind)	Generation Time/ T (h)	Intrinsic increasing rate /r_m (h^{-1})
TROL	272.40±3.56	6.04±0.42	93.91±2.25	0.46±0.07
UV	280.20±8.43	6.53±0.33	87.60±2.94	0.51±0.08
LANT	256.02±6.33	6.34±0.54	91.76±2.56	0.49±0.11
0.4μg/LANT	273.20±4.73	5.65±0.35	91.86±4.04	0.45±0.09
4μg/LANT	278.02±5.14	7.22±0.56^{c}	87.62±1.78	0.63±0.08**
0.4μg/L ANT vs UV	210.48±3.14**	3.09±0.24**	91.65±4.50	0.29±0.05**
4μg/L ANT vs UV	175.81±4.23**	1.51±0.13**	82.09±1.78*	0.12±0.04**

Note: Data are shown as means ± SE (n=3). Asterisks indicate statistically significant differences with respect to the values of control cultures ($P < 0.05$).

The significant changes in the rotifers' life table parameters in anthracene-treated groups under UV radiation reveal noteworthy photo-induced toxicity of anthracene on *Branchionus plicatilis*. Meanwhile, the net production rate and intrinsic growth rate are sensitive indicators of evaluating the photo-induced toxicity in *Brachionus plicatilis*. Anthracene is strongly phototoxic to *Brachionus plicatilis* and can lead to a continuous increase in ROS by photosensitization.

(1) Production of ROS causes the MDA content in *Brachionus plicatilis* to rise with time. The photo-induced effects of anthracene can significantly increase the MDA level and thus further exacerbate the degree of lipid peroxidation in the rotifers.

(2) The activity of enzymes sensitive to ROS, such as POD, SOD and GPx was affected within the first 24 h of the experiment. The activity of POD, CAT, GPx and GST and the GSH content were hindered after 48 h whereas the activity of glutathione reductase (GR) was enhanced. The effects of anthracene on the antioxidant enzymes in *Brachionus plicatilis* were much stronger when exposed to UV radiation.

CONCLUSION

This paper used anthracene as the stress factor and *Brachionus plicatilis*, critical zooplankton in marine ecosystems, as the experimental subject. The effect of photo-induced toxicity of anthracene on *Brachionus plicatilis* was investigated at physiological and biochemical, individual, and population levels and the preliminary discussion was conducted on the underlying mechanisms based on photosensitization theories.

Rise in the net reproduction rate and intrinsic growth rate was observed in the 4 μg/L anthracene-treated group compared with the control, which is probably related to excitatory effect caused by toxicity. This phenomenon is commonly noted in studies using *B. plicatilis* as an experimental subject, proving that it is normal for these rotifers to respond to stimulation under low toxicity. In our study, when the rotifers were treated with anthracene under UV radiation, the individual spawning number was reduced and the reproduction peak was delayed or suspended; the age-specific survival rate, the longest life span, the net reproduction rate and the intrinsic growth rate were also considerably lowered. Besides, the generation time was remarkably shortened and the life expectancy dropped, greatly reducing the reproductive value. These results showed serious negative impacts on the rotifers' potential growth. This is likely because the rotifers' reproductive system was damaged by anthracene under UV radiation. These changes in parameters illustrate that anthracene not only possesses strong photo-induced reproductive toxicity on *B. plicatilis*, but also has photo-induced toxicity on these rotifers at the population level. Among all reproduction and life table parameters, the net reproduction rate and the intrinsic growth rate can be used as sensitive indicators for photo-induced toxicity evaluation.

Previous research has suggested that toxicity mechanisms of PAHs are mainly based on photosensitization; In particular, ROS resulting from photosensitization can cause oxidative damages to a variety of aquatic organisms. The degree of membrane lipid peroxidation in *B. plicatilis* was notably intensified due to anthracene under irradiation conditions. This severely disturbed the ROS balance in the rotifers, leading to more remarkable changes in the antioxidant system and ultimately adverse effects on the reproduction. Hence, the photo-induced toxicity mechanisms of anthracene on *B. plicatilis* might be explained by the photosensitizing activity of anthracene and the oxidative stress induced by ROS in these rotifers.

The anthracene concentration used in the study was slightly higher than its actual concentration in the sea. The UV and visible light intensity value was adopted based on the annual average sunlight UV and visible light intensity at 12:00 noon in Zhengzhou, China. The experiments were conducted in laboratories within a short period of time such that the causal relationship can be established from the data obtained, enabling us to determine and estimate the effects of pollutants in the actual environment.

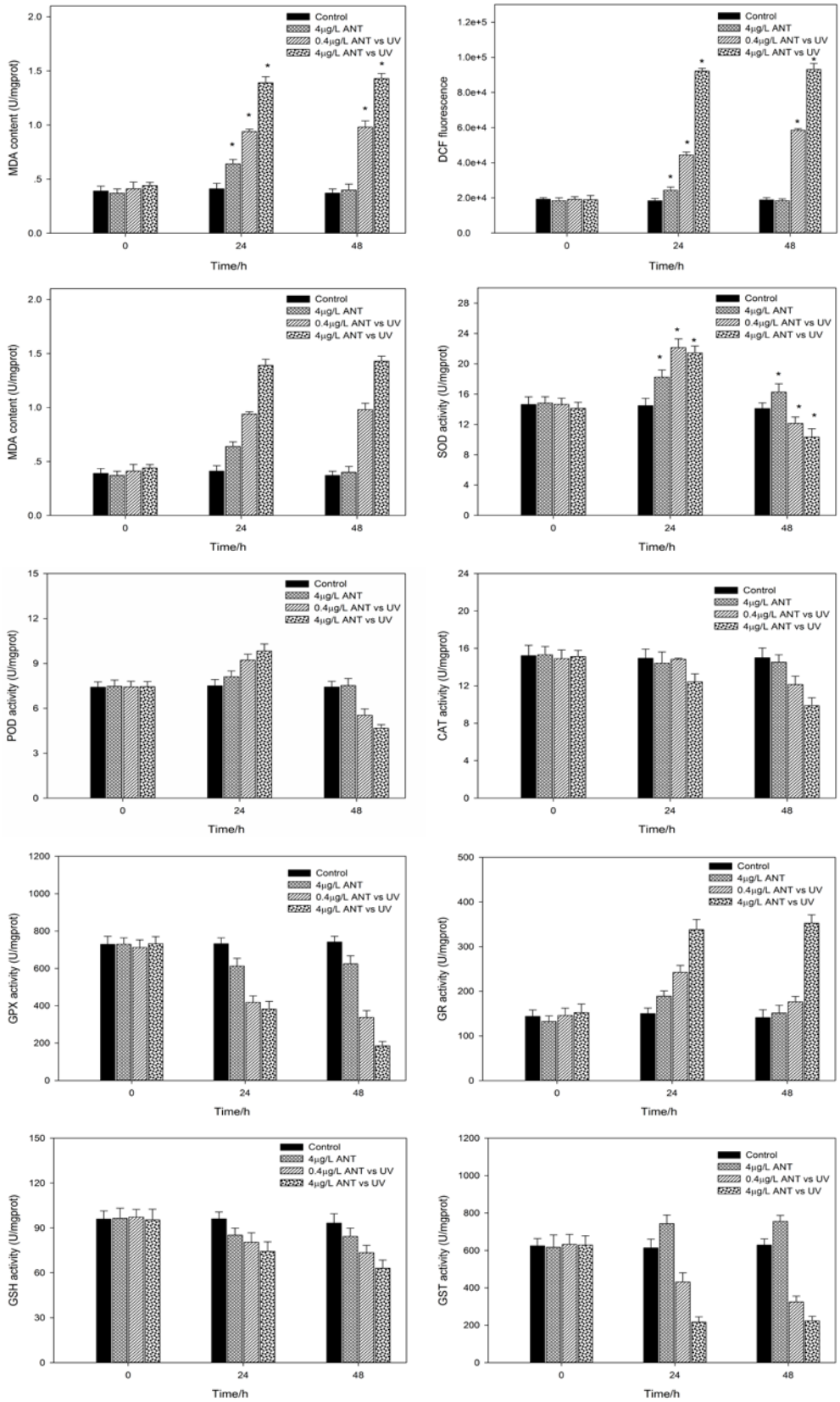

Fig. 1. Effect of different concentrations of ANT on TSP, ROS, MDA, SOD, POD, CAT, GPx, GST, GSH, GR activities in *B. plicatilis* with or without UV radiation exposure.

Note: * represents statistically significant differences between control and treatments at the $P<0.05$ level.

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