A phototaxis inhibition assay using barnacle larvae

Article in Environmental Toxicology and Water Quality · January 1997
DOI: 10.1002/(SICI)1098-2256(1997)12:3<231::AID-TOX5>3.0.CO;2-9
A Phototaxis Inhibition Assay Using Barnacle Larvae

R. S. S. Wu,¹ P. K. S. Lam,¹ B. S. Zhou²

¹ Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong
² Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China

Received 9 April 1996; revised 20 June 1996; accepted 14 July 1996

ABSTRACT: The effects of sublethal concentrations of phenol and cadmium on the phototactic responses of the stage II nauplii of the barnacle Balanus amphitrite were investigated. Increased toxicant concentrations caused a reduction in phototactic responses. Balanus amphitrite nauplii exposed to nominal phenol concentrations of 100 ppm and higher for 1–12 h failed to exhibit phototactic responses, while longer exposure times of 24 and 48 h reduced the lowest observable effect concentration (LOECs) to 80 and 60 ppm, respectively. For cadmium, the LOECs, based on nominal concentrations, for B. amphitrite following 1, 6, 12, 24, and 48 h exposures were 20, 4.5, 4.0, 1, and 0.75 ppm, respectively. The LOECs can be significantly reduced by increasing the duration of exposure to the toxicants. A good relationship exists between the phototactic response and toxicant concentration as well as exposure time. Results of this study indicate that the toxicant-induced reduction in phototactic responses of barnacle larvae can be used in a sensitive, rapid screening test for ecotoxicological assessments. © 1997 by John Wiley & Sons, Inc. Environ Toxicol Water Qual 12: 231–236, 1997

Keywords: phototactic response; phototaxis; larvae; screening test; barnacle; phenol; cadmium

INTRODUCTION

Many zooplankton (meroplankton or holoplankton) exhibit behavioral responses to light (see reviews by Thorson, 1964; Lampert, 1989; Svane and Young, 1989). These phototactic responses often have an important adaptive significance in optimizing feeding efficiency (e.g., Stearns and Forward, 1984), predator avoidance (e.g., Forward and Hettler, 1992), and larval dispersal and settlement (e.g., Thorson, 1964; Svane and Young, 1989; Dirnberger, 1993; Hurlbut, 1993). Accordingly, individual species or various larval stages of the same species may show differential responses to light of different wavelength (McFarland, 1986). This important orientative ability, integrating responses at nervous, muscular, and energetic levels, clearly has an important influence on the individual’s fitness, and ultimately the survival of the species. Provided that the organisms play a significant role in the functioning of their aquatic system, the impairment of such a phototactic response will have an indirect effect on the ecosystem as a whole. Indeed, the phototactic response of aquatic organisms, including Daphnia magna, Artemia salina, and Aedes aegypti (a mosquito larva), has been employed by some workers in developing sublethal bioassays for a number of xenobiotics (e.g., Saunders et al., 1985; Dojmi Di Delupis and Rotondo, 1988; Goodrich and Lech, 1990; Dojmi Di Delupis et al., 1992). Similarly, the phototactic behavior of the larvae of the barnacle, Balanus improvisus, had been used to assess the toxicity of copper (Lang et al., 1981). Here, we report upon the changes in the
Table I. Nominal toxicant concentrations used in the phototaxis inhibition assay of different exposure durations

<table>
<thead>
<tr>
<th>Exposure Duration (hours)</th>
<th>Cadmium</th>
<th>Phenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0, 10, 20, 30, 40</td>
<td>0, 40, 60, 80, 100</td>
</tr>
<tr>
<td>6</td>
<td>0, 3, 3.5, 4, 4.5</td>
<td>0, 40, 60, 80, 100</td>
</tr>
<tr>
<td>12</td>
<td>0, 3, 3.5, 4, 4.5</td>
<td>0, 40, 60, 80, 100</td>
</tr>
<tr>
<td>24</td>
<td>0, 0.25, 0.5, 0.75, 1</td>
<td>0, 40, 60, 80, 100</td>
</tr>
<tr>
<td>48</td>
<td>0, 0.25, 0.5, 0.75, 1</td>
<td>0, 40, 60, 80, 100</td>
</tr>
</tbody>
</table>

light-induced orientative ability of the Stage II nauplii of the barnacle *Balanus amphitrite* under the influence of two reference toxicants: phenol and cadmium. This paper examines the possibility of exploiting the sensitivity of the phototactic response of this marine larva to toxicants in a sensitive, rapid screening bioassay for ecotoxicological assessments.

**MATERIALS AND METHODS**

All tests were conducted using seawater filtered through a glass-fiber (Whatman GF/F) (temperature: 20 ± 1°C; salinity: 32 ± 1‰; pH: 7.8; dissolved oxygen: 7 ± 1 mg/L).

**Test Organisms**

The barnacle *B. amphitrite* Darwin inhabits the intertidal to sublittoral fringe of circumtropical and subtropical shores (Foster, 1982). Specimens were collected from Ma Lui Siu Ferry Pier in the New Territories of Hong Kong, kept in aerated seawater, and brought back to the laboratory. The animals were dissected, and adult brood sacs containing mature nauplii removed. The brood sacs were suspended in seawater to allow the nauplii to hatch. Stage II nauplii actively swimming toward a light source were collected using a pipette, and used for subsequent experimentation.

**Range-Finding (LC50) Tests**

Analytical grade cadmium (CdCl2) and phenol were used as reference toxicants. Sublethal phototaxis inhibition bioassays involving the two toxicants were preceded by an acute range-finding test. For each of the range-finding tests, Stage II nauplii were exposed to a range of toxicant concentrations in 25 mL beakers. There were three replicates per concentration for each toxicant. In each beaker, the number of swimming, nonmotile (with vibrating appendages) and dead animals were recorded after a 24 and a 48 h exposure to nominal cadmium concentrations of 0, 0.1, 0.15, 0.25, 0.50, 0.75, 1.0, 1.2, 1.5, 1.8, 2.1, 2.4, and 2.7 ppm, and nominal phenol concentrations of 0, 40, 60, 80, 100, 120, 140, 160, 180, 200, 240, and 270 ppm. LC50 values were estimated for each test by probit analysis following Finney (1980). Based on the results of these tests, the range of toxicant concentrations to be used for subsequent sublethal bioassays were determined (Table I). Sublethal cadmium concentrations for exposure periods of 1, 6, and 12 h were also determined following the same procedure outlined above (Table I).

**Phototaxis Inhibition Assay**

The phototaxis inhibition assay was carried out in an experimental chamber, a rectangular (40 x 40 x 150 mm) plexiglass container with a single transparent window at one end (cf. Marsden, 1990). The sides and the

---

**Fig. 1.** Percentage mortality of *B. amphitrite* nauplii exposed to cadmium and phenol for 24 and 48 h. The vertical lines are ± 1 SE.
other end of the chamber were opaque and nonreflective. The chamber was divided (by an engraved line on the bottom) into five equal-sized, nonpartitioned sections: S1, S2, S3, S4, and S5. S1 occupied the window end of the chamber, S2 was located at 30–60 mm from the window, S3 at 60–90 mm, and so on. An artificial light source (a 6.5 V, 2.75 A tungsten bulb in a MEIJI microscope lamp) was placed at 10 cm in front of the transparent window. Light intensity in seawater in each of the sections (S1–S5) was determined using a luminance meter (TOPCON BM5). The intensities measured at S1, S2, S3, S4, and S5 were 1.07, 0.47, 0.26, 0.13, and 0.06 (× 10⁻³ Cd m⁻²), respectively. The barnacle larvae were preexposed to the toxicants for 1, 6, 12, 24, and 48 h separately before their phototactic behavior was examined. Nominal toxicant concentrations used are given in Table I. At least 60 experimental animals were used for each set of observations, and the number of test organisms in each section were recorded at the end of the exposure period. There were three replicates per concentration per exposure period for each toxicant. All observations were carried out in a darkroom at 20°C.

**Statistical Analysis**

When the test organisms exhibit a positive phototactic response, the distribution of the animals should skew toward the sections with lower light intensities and deviate significantly from an even distribution (Marsden, 1990). Following this principle, the no observable effect concentration (NOEC) could be defined as the highest toxicant concentration at which the distribution of the test organisms showed a significant deviation from an even distribution, while the lowest observable effect concentration (LOEC) would correspond to the lowest concentration at which no significant deviation from an even distribution was detected (cf. Marsden, 1990). In this study, however, we used the distribution pattern of the test organisms at zero toxicant concentration as the control and defined the NOEC as the highest toxicant concentration at which no significant deviation from the control distribution pattern was detected, while the lowest toxicant concentration at which a significant deviation from the control was detected was taken as the LOEC. In principle, the procedure suggested here
should be more sensitive and thus will be more effective in minimizing Type II error, which is of greater importance in ecotoxicological assessments. All comparisons between “test” and “reference” distributions were carried out using chi-square goodness-of-fit tests (Zar, 1984).

RESULTS

Results of the range-finding tests for 24 and 48 h exposure periods are given in Fig. 1. For B. amphitrite, the LC₅₀ values for cadmium under 24 and 48 h exposures were 1.6 and 0.8 ppm, respectively, while those for phenol under 24 and 48 h exposures were 230 and 175 ppm, respectively. Under sublethal concentrations, increased toxicant concentrations caused a reduction in phototactic responses. As examples, the distribution patterns of barnacle larvae in response to different light intensities under a 24 h exposure to cadmium and phenol are shown in Figs 2 and 3, respectively. Balanus amphitrite nauplii exposed to phenol concentrations of 100 ppm and higher for 1–12 h failed to exhibit phototactic responses giving LOECs of 100 ppm, while longer exposure times of 24 and 48 h reduced the LOECs to 80 and 60 ppm, respectively (Fig. 4). For cadmium, the LOECs for B. amphitrite following 1, 6, 12, 24, and 48 h exposures were 20, 4.5, 4.0, 1, and 0.75 ppm respectively (Fig. 4).

DISCUSSION

The phototactic response of B. amphitrite was sensitive to low concentrations of cadmium and phenol. In general, deviation of the distribution pattern of the test organisms from the control becomes more pronounced as the toxicant concentration increases, indicating that the reduction in phototactic response is dose dependent. Moreover, the LOECs for cadmium were significantly reduced by gradually increasing the exposure time from 1 to 48 h, while such a relationship between critical toxicant concentrations (NOECs and LOECs) and exposure durations was only apparent for phenol when the exposure time was longer than 12 h. Notwithstanding, an exposure time of, say, 24 h, is still relatively short when compared with the normal 48 or 96 h LC₅₀ and LT₅₀.
PHOTOTAXIS INHIBITION ASSAY 235

supply of test organisms. The positive phototaxis of B. amphitrite nauplii is likely to be an adaptive response that allows these animals to move to areas of high primary productivity or food abundance. An impairment of such a response could thus have a major deleterious impact on the survival (fitness) of individual larva. The physiological basis of phototaxis is at least partly neurological (Marsden, 1990, and references therein) and involves high energy expenditure, thus any chemicals that have an effect on the nervous system or cellular respiration should be amendable to testing by this method. Indeed, this may be the reason why the phototaxis inhibition assay outlined here appears to have a higher level of success with cadmium than phenol. Specifically, cadmium is a highly toxic metal that has a high affinity for -SH groups (Varma and Katz, 1978). Cadmium can compete and displace zinc in a number of Zn-containing metalloenzymes by irreversibly binding to active sites, thus interfering with normal metabolism (Forstner and Wittmann, 1981). Phenols, on the other hand, are known to be uncouplers of oxidative phosphorylation (Corbett, 1974). Further investigations should be carried out to examine the details of the toxicity mechanisms for the two toxicants.

Results of the present testing procedure were highly reproducible as indicated by the low variability between replicates (cf standard deviations in Figs. 2 and 3). Indeed, graphs in Fig. 4 are based on two sets of data obtained from two replicate experiments which yielded identical results. This test is extremely simple and sensitive, and can be completed within a short time, making it suitable for use as a standard routine bioassay. It should also be pointed out that this phototaxis inhibition assay can readily be deployed in situ when required, and has an additional advantage in that it can easily be adapted to ecological equivalents in many geographic regions.

We thank Patrick Lai for his assistance in this work. We are grateful to Douglas Holdway and an anonymous referee for useful comments on the manuscript. The authors acknowledge the support of a research grant from the City University of Hong Kong.

REFERENCES


