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Toxic Effects of Residual Chlorine on Larvae of *Hydropsyche pellucidula* (Trichoptera, Hydropsychidae): A Proposal of Biological Indicator

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Elemental chlorine (Cl_2) and other chlorine compounds, such as chloramines and hypochlorites, have been used as important bactericides in desinfection of drinking waters for many years. Residual chlorine concentrations in municipal water supplies are normally between 0.5 and 2 mg/L (Boyd 1982). In water at pH values above 5, elemental chlorine and hypochlorite compounds react rapidly, resulting in the formation of hypochlorous acid (HOCl) in equilibrium with hypoclorite ions (OCl^-) (Alabaster and Lloyd 1980). Although the chemical basis of chlorine toxicity to organisms is not well understood, it has been suggested that sulphydryl (—SH) groups of enzymes within cells may be irreversibly oxidized and enzymatic activity thereby destroyed (Green and Stumpf 1946). Nowadays it is thought that the most toxic chemical species in chlorine toxicity to aquatic life is hypochlorous acid. Nevertheless, up to the present, very little is known about the toxicity of residual chlorine on aquatic insects that comprise the macroinvertebrate communities of freshwater ecosystems.

This study examines the toxic effects of municipal chlorinated waters on larvae of *Hydropsyche pellucidula* (Curtis), a common insect species in macrobenthic communities of rivers and streams in the Iberian Peninsula and other European countries, evaluating implications on their life cycles. Field and laboratory studies were performed in order to reach these goals.

MATERIALS AND METHODS

Larvae of *H. pellucidula* were hand-collected from macrobenthic riffle communities 300 m upstream and 200 m downstream from Navallar plant, which is located in the middle Río Manzanares (Madrid, central Spain). This municipal plant generates hydroelectric power using chlorinated waters and is known to release sporadic chlorinated discharges into the river (Montes et al. 1987). Specimens were preserved in 2% formalin and examined using a binocular microscope. The dry weight of larvae was obtained in an oven at 60 °C for 24 hr.

On the other hand, last instar larvae of *H. pellucidula* were hand-collected from chlorine unpolluted areas of the Río Duratón (Segovia, northern Spain). In the laboratory, they were randomly distributed into two glass aquaria each with a volume of 10 L dechlorinated tap water. Sodium thiosulfate pentahydrate ($Na_2S_2O_35H_2O$) was used as the best dechlorinating agent (Boyd 1982). Twelve

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larvae per aquarium were acclimatized to water quality conditions for 4 d prior to the chlorine test and were not fed then nor during test. Chamber environmental temperature and natural photoperiod (12 hr) were utilized, and water oxygenation and turbulence were produced by air pumps. All animals built their retreat and capture nets in the early hours of their acclimatization.

At the start of the chlorine test, most of the water of both aquaria was carefully exchanged for dechlorinated and chlorinated tap waters, respectively. Hardness, alkalinity, conductivity, pH, water temperature, chloride and dissolved oxygen were analysed using standardized methods described by APHA (1980). Residual chlorine was measured using an Orion-USA model 97-70 specific ion electrode. This specific ion electrode was calibrated according to Orion Research (1983). Larvae were exposed to chlorinated tap water for 72 hr. Toxic effects were checked by comparing larvae in the chlorine aquarium with those in control aquarium.

In order to determine whether toxic effects of chlorinated waters may interfere with the life cycles of these larvae, organisms were placed after chlorine testing into a maintenance aquarium with dechlorinated tap water, sand and stones, until their emergence as imagoes or their death. This aquarium was divided into two equal compartments by means of a middle glass and lateral nylon meshes. In this way, control larvae and surviving animals exposed to chlorine were able to be held without mixing in the same water-quality conditions. They were fed with freshwater algae, decaying leaves and Tetra-Min feed (see Lawrence 1981). Imagines were identified in accordance with Malicky (1983).

RESULTS AND DISCUSSION

Thirty-seven larvae of *H. pellucidula* (average dry weight 7.2±1.3 mg) were collected downstream from Navallar plant. Twenty-three animals exhibited altered tracheal gills, and 11 had also their anal papillae protruded and darkened. Gill processes were reduced in number, the tracheal gills being simplified to a single short stub in some cases. The damage to anal papillae started at the distal end of each one. In contrast, fifty-two larvae (average dry weight 6.8±1.6 mg) collected upstream from Navallar plant had branched tracheal gills with numerous filaments and anal papillae without darkening.

Physicochemical parameters analysed during laboratory studies are presented in Table 1. All values of these parameters were within water quality criteria for aquatic organisms (USEPA 1986), except residual chlorine with an initial concentration of 1.73 mg/L, about 1.12 mg HOCl/L (see Alabaster and Lloyd 1980). Compared with animals in the control aquaria, larvae exposed to chlorinated tap water exhibited four main sublethal effects (Figure 1): (1) 91.7% of larvae migrated from their retreat and capture nets, (2) 100% of larvae protruded their anal papillae, (3) 75% of larvae had darkened anal papillae, and (4) 100% of larvae had damaged tracheal gills on their thoracic and abdominal segments. In addition, two larvae (average dry weight 9.3±0.5 mg) were dead at the end of the chlorine test. After about 5 wk in the maintenance aquarium, ten control larvae had successfully completed their life cycles to adulthood. In contrast, only one of all surviving larvae exposed to chlorinated tap waters completed the life cycle; eight larvae (average dry weight 10.1±0.9 mg) died without reaching the pupal stage.

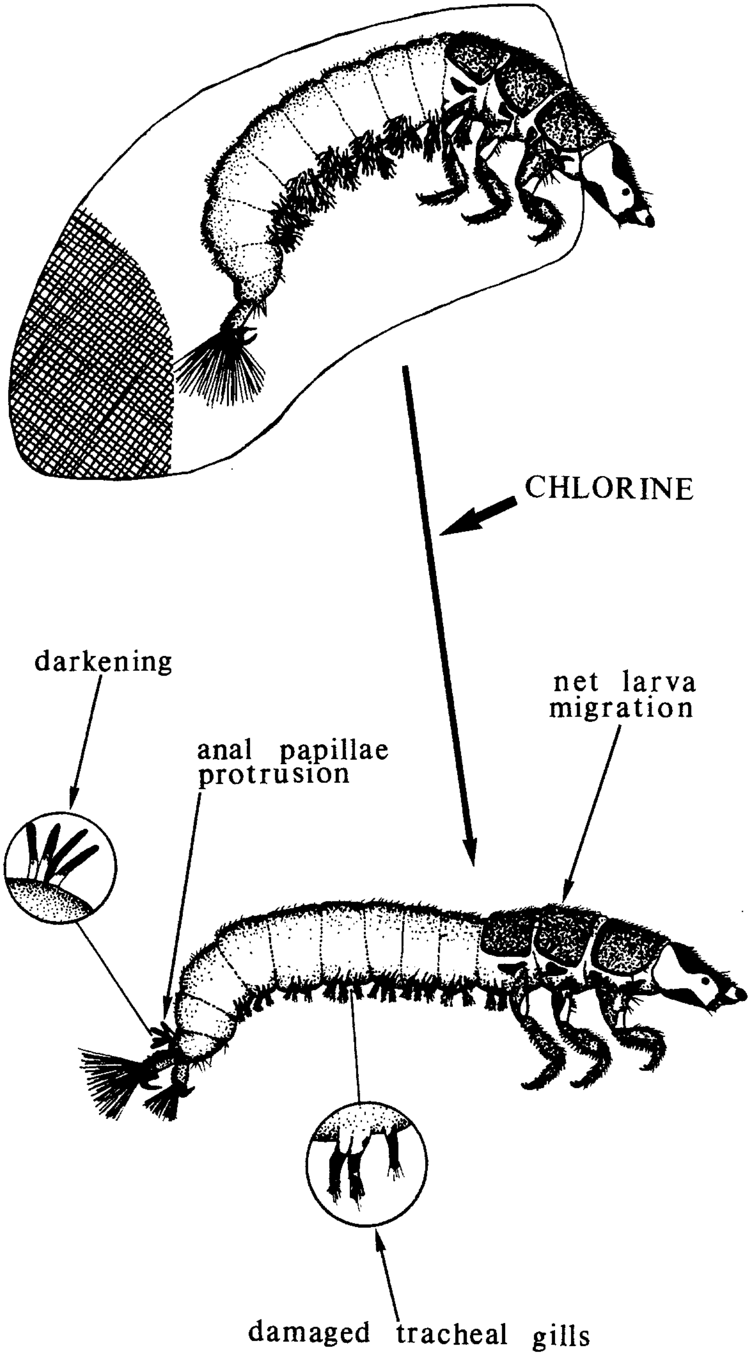

Figure 1. Toxic effects in larvae of *Hydropsyche pellucidula* (Curtis) exposed to chlorinated tap waters during laboratory studies.

Table 1. Values of water parameters analysed in control, chlorine and maintenance aquaria; b.d. = below detection (< 10 μg/L).

	Control	Chlorine	Maintenance
Alkalinity (mg CaCO3/L)	36.8	37.0	48.1
Hardness (mg CaCO3/L)	25.1	25.6	37.9
Conductivity (μmhos/cm)	50.0	50.0	95.0
Chloride (mg/L)	2.1	2.4	7.8
Residual chlorine (mg/L)	b.d.	1.73	b.d.
pH	7.5	7.5	7.7
Water temperature °C	15.5	15.5	17.2
Dissolved oxygen (mg/L)	9.0	9.2	10.1

Field and laboratory studies have demonstrated that chlorinated waters generate specific toxic effects on larvae of *H. pellucidula*. In addition, these sublethal effects may inhibit the normal development of larvae into their imago stages.

The net migration of larvae exposed to residual chlorine may be interpreted as a useful adaptation in running waters to escape from unfavorable environmental conditions. The anal papillae protrusion may be physiologically interpreted as an action to increase the elimination of residual chlorine. In this connection, it has been reported that anal papillae may function as important ion regulatory organs in several species of caddisflies and flies (Copeland 1964; Nuske and Wichard 1972), being able to be voluntarily protruded and retracted by muscular action (Wichard 1976). Moreover, the residual chlorine in waters around protruded anal papillae might generate biochemical changes in the cellular tissues of these ion regulatory organs, resulting in darkening. These same sublethal effects have been observed in larvae of *H. pellucidula, H. lobata, H. exocellata* and *H. bulbifera* exposed to sodium fluoride (Camargo 1989).

On the other hand, the residual chlorine is also responsible for corrosive injury to tracheal gills, resulting in a destruction of gill functions. In this respect, Simpson (1980) observed a reduction in the number of tracheal gills in larvae of *Phasganophora capitata* and *Cheumatopsyche sp.* living below chlorinated discharges, and Cohen and Valenzuela (1977) found an extensive destruction of gill lamellae in mosquito fish (*Gambusia affinis*) exposed to chlorine, corresponding mortality to gill damage as a probable result of blood hypoxia.

The normal development of larvae of *H. pellucidula* to adulthood would be mainly inhibited by the destruction of tracheal gills because these immature animals could not obtain sufficient oxygen. In this connection, Becker (1987) found that the physiological efficiency of larvae of *H. pellucidula* decreased rapidly with falling oxygen concentration, so that development into an imago was no longer possible below 85% oxygen saturation.

It is concluded that sublethal effects, mainly darkened anal papillae and damaged tracheal gills, generated by municipal chlorinated waters on larvae of *Hydropsyche pellucidula* may be used as biological indicators for detecting chlorine pollution in freshwater ecosystems. These toxic effects may be expected to be found in other *Hydropsyche* species exposed to residual chlorine.

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