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Photoperiodic Acclimation and Circadian Variations in Tolerance of Juvenile Rainbow Trout (*Salmo gairdneri*) to Zinc

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Circadian variations in the tolerance of mammals to drugs have been demonstrated repeatedly and are attributable at least in part to circadian rhythms of stress hormones (REINBERG and HALBERG 1971; MOORE EDE 1973). Comparable diel rhythms of stress hormones have been found in fish (SRIVASTAVA and MEIER 1972; SINGLEY and CHAVIN 1975, 1976). However except for a recent study (SPIELER et al. 1977) the effect of time of day on the sensitivity of fish to toxicants has been ignored.

The intent of this study was to determine if circadian variations occur in the tolerance of juvenile rainbow trout (Salmo gairdneri) to an acutely lethal concentration of zinc. Further, the influence of photoperiodic acclimation on this tolerance was examined since seasonal or photoperiodic acclimation may alter hormonal rhythms and toxic responses at a particular time of day; thus complexing the potential variations in acute lethal or sublethal aquatic toxicity data. Although SPIELER et al. (1977) noted that differing acclimation photoperiods did not shift the diel rhythm of tolerance of a fish species to formalin, ZITKO and CARSON (1977) recently reported seasonal variations in acute tolerance to zinc of juvenile salmonid fish held under constant temperature but ambient photoperiod conditions; and TERPIN et al. (1976) found changes in critical thermal maxima of fish due to photoperiodic acclimation. In mammals, shifts in photoperiod are known to alter circadian patterns of drug tolerance (REINBERG and HALBERG 1971).

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METHODS AND MATERIALS

A stock of underyearling rainbow trout obtained from Troutlodge Inc. (Washington State) was divided into two groups, each of which was maintained under identical conditions other than photoperiod. Lighting to each tank was overhead incandescent illumination regulated by timer (summer photoperiod; 16L:8D, light onset 0430 h) or photocell (natural photoperiod), and rheostat (30-min twilight). Water supply for tanks (12-15 ℓ/min/tank) and bioassays was dechlorinated Vancouver City tap water (12 $\pm$ 1°C, pH 6.4 $\pm$ 0.2, conductance 14.1 $\pm$ 4.6 µmho/cm, EDTA hardness 4.6 $\pm$ 0.9 mg $CaCO_3$/ℓ, alkalinity 3.3 $\pm$ 0.7 mg $CaCO_3$/ℓ). Fish were fed Oregon Moist Pellets with the initial ration offered 4 h after the onset of the light cycle. Feeding was withheld for 24-48 h prior to the initiation of bioassays. At the time of the bioassays fish were acclimated to their respective photoperiod for 3.5 months. At this time the natural photoperiod was 11L:13D, with lights on at 0730 h. Other conditions were as described previously (GORDON and McLEAY 1978).

At 0800 h and at 2-h intervals thereafter, throughout a 24-h period, 36 fish from each tank were transferred to three test vessels (12 fish/aquaria), each containing 45 ℓ of a solution of 10 mg zinc/ℓ (as $ZnSO_4$). The time to death of each fish and weight at death were recorded. Lighting conditions for the fish stocks remained undisturbed during the study; however all bioassays were conducted under constant illumination. The temperature, pH, dissolved oxygen content and zinc content of each solution were measured at the initiation and termination of each bioassay. Zinc was measured by atomic absorption spectrophotometry.

The time to 50% mortality (LT50 value) together with its 95% confidence limits were computed for each group of 12 fish according to LITCHFIELD (1949). The time to death values were subjected to analysis of variance and covariance. Hour, photoperiod, photoperiod-hour interaction and replicability at each hour were considered as the main effects; whereas fish weight, pH, dissolved oxygen, temperature and zinc concentration were treated as covariates.

RESULTS AND DISCUSSION

Mean ($\pm$SD) values for fish weight and water conditions as determined during the bioassays are given in Table 1. The LT50 values for each of the triplicate bioassays initiated at 2-h intervals are shown in Fig. 1.

TABLE 1

Fish weight and water conditions during bioassays

	Weight (g)	Temperature (°C)		pH		O_2 (mg/ℓ)		Zinc (mg/ℓ)	
		initial	final	initial	final	initial	final	initial	final
Natural photoperiod	4.23 (1.58)	11.75[a] (0.22)	11.32 (0.28)	6.52 (0.12)	6.44 (0.18)	10.07 (0.41)	9.15 (0.35)	10.00 (0.14)	10.00 (0.19)
Summer photoperiod	4.37 (1.72)	11.68[a] (0.20)	11.23 (0.21)	6.49 (0.14)	6.50 (0.45)	10.30 (0.23)	9.33 (0.50)	10.02 (0.07)	10.01 (0.10)

[a] mean (SD) value for 39 determinations.

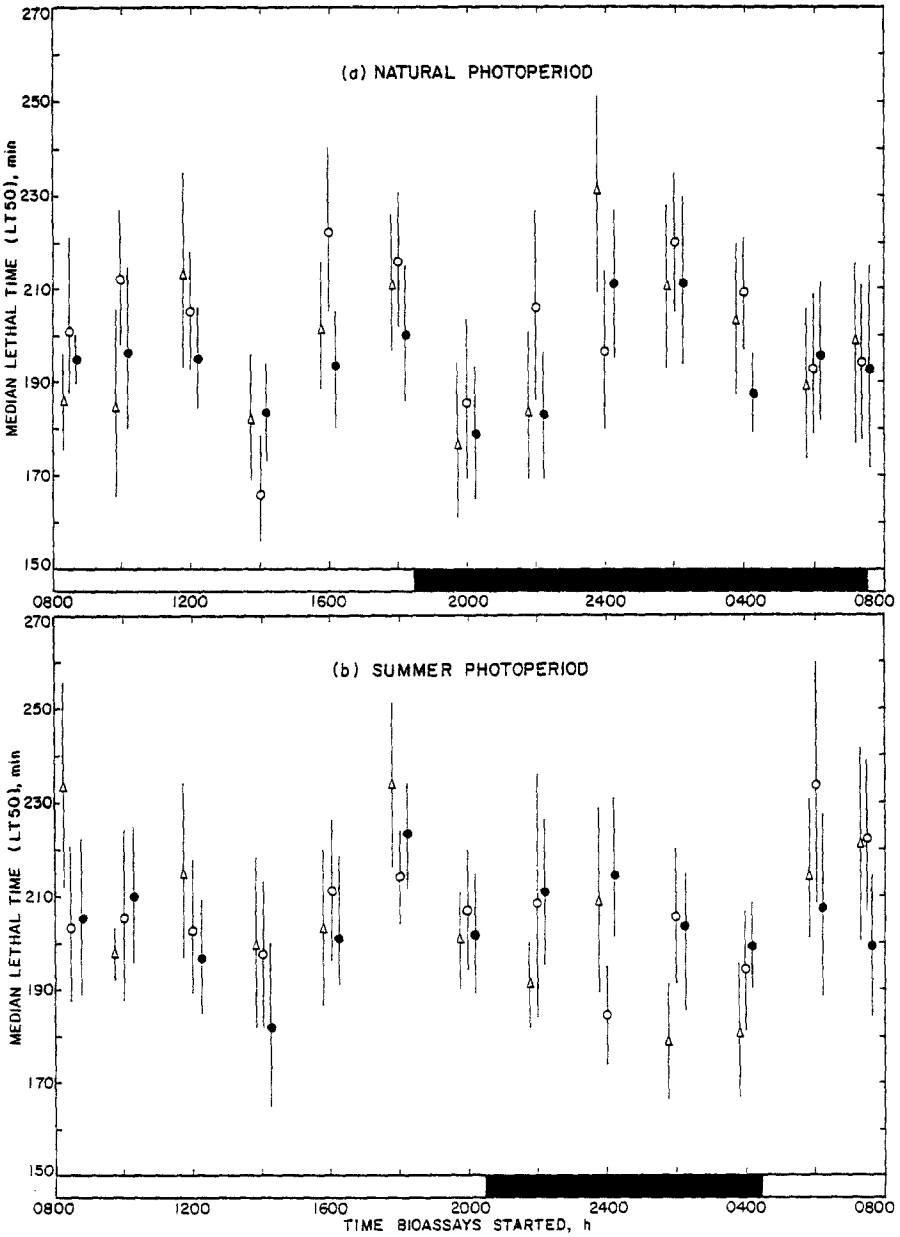

Figure 1. Variation in median lethal times for juvenile rainbow trout acclimated to either a natural (a) or a fixed summer (b) photoperiod and exposed to zinc (10 mg/ℓ) in triplicate bioassays initiated at 2-h intervals throughout a 24-h period. Confidence limits (95%) are indicated as vertical lines.

According to the analysis of variance, the variance between replicates clearly was not significant ($P>0.05$). Hour and photoperiod-hour effects were highly significant ($P<0.0001$); photoperiod by itself caused a lesser but significant ($P<0.01$) effect. These results indicate that, for both conditions of photoperiodic acclimation, the within-replicate variance was less than the hour-by-hour variance for times to death; i.e. circadian variations in tolerance to zinc were evident. Fish acclimated to a natural photoperiod showed periods of maximum tolerance at 1200, 1600-1800 and 2400-0200 h; and periods of maximum sensitivity at 1400 and 2000 h (Fig. 1a). Those acclimated to a fixed summer photoperiod showed maximum tolerances at 1800 and 0600-0800 h; and maximum sensitivities at 1400 and 0400 h (Fig. 1b). The covariates tested (pH, dissolved oxygen, temperature, zinc concentration and fish weight) did not affect the time to death data significantly.

For each fish stock the times to death at 0800 h, measured on two consecutive days at the initiation and termination of the respective tests, were similar (Fig. 1a and 1b). These results suggest that the times to death for fish acclimated to a given photoperiod are consistent if the time of day that the exposure is initiated is the same. Further, they support the findings of CAIRNS *et al.* (1975) for goldfish that a variation in lapsed time since feeding of 24-48 h does not affect their survival time in zinc.

Present findings support those of SPIELER *et al.* (1977) concerning the existence of diel differences in tolerance of fish to acutely lethal concentrations of chemicals. This study also provides new evidence that these diel variations can be affected by the photoperiodic regime to which the fish are acclimated. The specific mechanisms involved in these circadian differences in sensitivity are unknown. However diel variations in levels of circulating hormones and in the rates of drug absorption, metabolism and excretion, as documented for mammals (MOORE EDE 1973), no doubt contribute to these changes. Reports that each drug has its own individual rhythm of toxicity in mammals (MOORE EDE 1973) complicate the issue even further. Nonetheless the time of day that exposures are initiated, with regard to the photoperiodic acclimation, should be considered when designing and interpreting results from acute lethal or sublethal toxicity tests. Additional studies should be conducted to determine if cyclic variations in acute responses to zinc and other toxicants are evident in fish; and to examine the influence of photoperiodic acclimation together with the significance of such rhythms in terms of acute toxicity tests and drug administrations.

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