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Avoidance Response of Juvenile *Chromis punctipinnis* to Chlorinated Seawater

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Chlorination of seawater effluents released by power plants is a subject of current concern. The actual ecological impact of chlorinated effluents is dependent upon the behavioral responses of animals inhabiting the discharge area (DINNEL et al. 1979) and the dispersal characteristics of the discharge zone. For instance, the toxicity of chlorine-produced oxidants may be mitigated by avoidance of the discharge plume. Alternatively, attraction to the effluent plume would aggravate the problem of toxicity.

Since the behavioral responses of fish to chlorinated seawater are species-dependent (MELDRIM et al. 1974), the need for site-specific information in this field is obvious. As most studies have taken place on the East coast, little data are available on the attraction/avoidance responses of non-salmonid Pacific species to chlorinated effluents (STOBER et al. 1978, DINNEL et al. 1979). In addition to the variability of response by different species, other factors associated with power plant effluents, such as temperature, have been proven to influence the behavioral responses of fish to chlorinated discharges (CRUMLEY et al. 1980).

The blacksmith, Chromis punctipinnis, is an important member of kelp and artificial harbor communities in the warm temperate regions of the Pacific coast (STEPHENS 1979). C. punctipinnis, a planktivore, is regularly observed feeding on organisms entrained in the effluent plume of the Redondo Beach Steam Generating Station in King Harbor Marina, California (STEPHENS pers. comm.). Due to its feeding habits, C. punctipinnis may be subjected to chlorinated effluents resulting from nearby power plants.

This study was undertaken to determine the behavioral responses of juvenile C. punctipinnis to chlorinated seawater. The effects of various parameters such as fish size, water temperature, acclimation temperature, and food availability on the behavioral response of C. punctipinnis to chlorinated seawater were also explored.

MATERIALS AND METHODS

Fish used in behavior tests were collected in King Harbor Marina, California by SCUBA divers using gill nets. Fish were transported directly to laboratory holding facilities and maintained in 100-L polyvinyl chloride holding tanks immersed in a temperature-

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controlled water bath. Each tank was supplied with a once-through continuous flow of filtered (5 μm) aerated harbor water. Harbor water quality parameters were measured using Orion specific ion electrodes. Dissolved oxygen levels were maintained near saturation, pH from 7.6 to 7.9, and ammonia from 0.03 to 0.06 ppm.

Fish were brought from the field collection temperature to the acclimation temperature at 2°C per day and were acclimated two weeks prior to experimentation. Fish were fed ad libitum daily with brine shrimp. The standard lengths of the fish ranged from 40 to 100 mm.

Responses of juvenile C. punctipinnis to chlorinated seawater were studied in a behavior chamber similar to the counterflow system described by DINNEL et al. (1979), but including the following modifications. Ambient seawater (33-34°/₀₀ salinity) was pumped into a temperature-regulated header tank which distributed the seawater to two small header tanks. One small header tank was supplied with unchlorinated seawater, while the test header tank received a NaOCl solution prepared by mixing 5% NaOCl (Chlorox®) with deionized water. The hypochlorite solution was delivered to the tank by a piston metering pump at a flow rate ranging from 1 to 115 mL/min. Solutions from the control and test header tanks were delivered to opposite ends of the behavior chamber at approximately five L/min and flowed toward a central drain.

The behavior chamber with replicate compartments was covered by black plastic sheeting with small slits cut at either end for direct monitoring. Light levels were maintained at 0.29-0.44 watts/m^2 at the water surface.

From three to four fish were placed in each replicate side chamber. After a one hour acclimation period, a set of control observations was recorded before chlorination commenced. The percentages of fish occurring on each side were calculated from 25 observations of fish positions taken at 15 sec intervals. The control observation set was followed by three test observation sets performed at increasing hypochlorite concentrations. Three replicate experiments of each control and test series were completed.

Chemical analyses were performed using Orion specific ion electrodes attached to an Orion microprocessor ionalyzer 901. Total residual oxidant (TRO) concentrations were measured according to the procedure described by ORION (1977) using a residual chlorine electrode (Model 97-70) sensitive to 10 ppb TRO. Dissolved oxygen levels were maintained near saturation (from 5 to 9 ppm). Ammonia concentrations were less than 0.10 ppm, and pH values were between 7.7 and 7.9. Water temperatures were monitored using a Yellow Springs Instruments telethermometer. Temperature and TRO determinations were averaged from readings taken in each of the quadrants before and after each observation set.

Replicate responses were first tested for homogeneity using a Chi-square analysis utilizing Yates' correction for continuity (SNEDECOR & COCHRAN 1976). The percentages of fish occurring in the chlorinated quadrants at various TRO concentrations were fitted to a regression line (BOGARDUS et al. 1978). The concentration of TRO which the fish significantly avoided (the level at which avoidance became statistically significant using a Chi-square determination, $p = 0.05$) and totally avoided (0% occurrence on the chlorinated side) and the accompanying standard errors were then calculated from the regression equations.

RESULTS

In the behavior chamber, schools of C. punctipinnis swam freely between the two sides of the tank before chlorination commenced. After chlorination started, the amount of time spent in the chlorinated quadrants declined until the fish remained exclusively in the non-chlorinated quadrants. At TRO concentrations slightly above their avoidance level, the fish often swam to the central drain and then quickly swam back into the non-chlorinated side of the chamber. At high concentrations of TRO, the fish did not venture into the interface area, but remained in the non-chlorinated quadrants for the rest of the experiment.

A significant relationship was demonstrated between the degree of avoidance and increasing TRO concentration ($r \geq 0.50$, $p < 0.001$). In all cases, juvenile C. punctipinnis exhibited an active avoidance response to seawater containing greater than 0.35 ppm TRO. With the exception of the fish starved prior to testing, the remainder of the fish totally avoided TRO concentrations less than or equal to 0.20 ppm.

The total TRO avoidance levels of C. punctipinnis ranging in size from 1 to 17 g wet weight were calculated (Figure 1). The weight of the fish and its TRO avoidance level were found to be directly correlated ($r = 0.82$, $p < 0.01$).

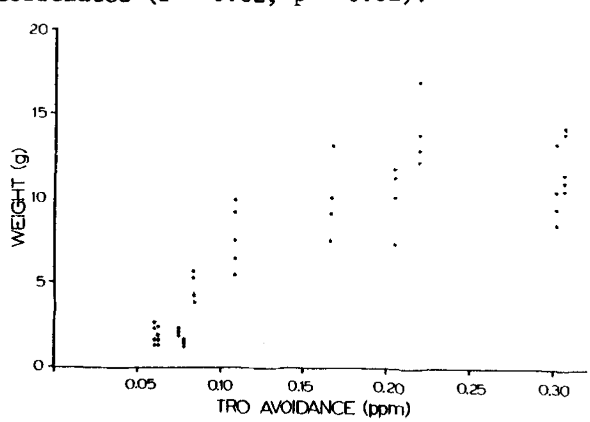

Figure 1. Total residual oxidant (TRO) avoidance by different size classes of juvenile C. punctipinnis.

Since chlorinated effluents are released at a higher than ambient temperature, the temperature of the chlorinated quadrant was elevated 3°C above that of the non-chlorinated side. Juvenile *C. punctipinnis* were very sensitive to heated seawater containing low concentrations of TRO (Table 1). Fish totally avoided 0.086 ± 0.005 ppm TRO ($\bar{X} \pm SE$) with a Δt of 3°C existing between the chlorinated and non-chlorinated quadrants. This avoidance level was about half of that found when the temperatures of all the quadrants were equal ($Z = 4.70$, $p < 0.001$).

Table 1. Total residual oxidant (TRO) avoidance by juvenile *C. punctipinnis* at 20°C and with a Δt of 3°C in the chlorinated quadrants of the behavior chamber.

Experimental Temperature	n	Significant Avoidance (ppm) $\bar{X}$	Significant Avoidance (ppm) SE	Total Avoidance (ppm) $\bar{X}$	Total Avoidance (ppm) SE
20°C	80	0.084	0.010	0.156	0.014
20/30°C	54	0.035[1]	0.004	0.086[2]	0.005

[1] $Z = 4.54$, $p < 0.001$. [2] $Z = 4.70$, $p < 0.001$.

The effects of ambient seawater temperature on the avoidance response of *C. punctipinnis* were studied (Table 2). Experimental temperatures approximating winter (15°C), summer (20°C) and discharge (24°C) water temperatures were used. Fish tested at 15 and 20°C displayed similar avoidance levels of 0.152 and 0.162 ppm TRO, while those tested at 24°C exhibited a significantly lower avoidance level of 0.132 ppm TRO (ANOVA, SNK, $p = 0.05$).

Table 2. Total residual oxidant avoidance (TRO) by *C. punctipinnis* tested at 15, 20 and 24°C. Significantly different avoidance levels ($p = 0.05$) are enclosed in brackets.

Experimental Temperature °C	n	Significant Avoidance (ppm TRO) $\bar{X}$	Significant Avoidance (ppm TRO) SE	Total Avoidance (ppm TRO) $\bar{X}$	Total Avoidance (ppm TRO) SE
15	64	0.103	0.007	0.162	0.007
20	64	0.100	0.008	0.152	0.009
24	64	0.048	0.004	0.132	0.007

The effects of acclimation to two different temperatures, 15 and 20°C, on TRO avoidance were found to be insignificant (Table 3, $p > 0.05$). Both groups of fish totally avoided TRO concentrations near 0.16 ppm.

Since effluent often acts as a food source for fish (YOUNG & MEARNS 1978), this factor was tested by adding a constant supply of brine

Table 3. Total residual oxidant (TRO) avoidance by juvenile *C. punctipinnis* acclimated and tested at 15 or 20°C.

Acclimation Temperature °C	n	Significant Avoidance (ppm TRO) $\bar{X}$	SE	Total Avoidance (ppm TRO) $\bar{X}$	SE
15	64	0.103	0.007	0.162	0.007
20	80	0.084[1]	0.010	0.156[2]	0.014

[1]Z = 1.56, not significant. [2]Z = 0.38, not significant.

shrimp to the chlorinated quadrants of the behavior tank (Table 4). Fish tolerated higher TRO concentrations before avoiding the chlorinated quadrants than when food was absent from the chlorinated areas ($Z = 2.93$, $0.005 < p < 0.010$).

Table 4. Total residual oxidant avoidance by juvenile *C. punctipinnis* with and without a constant supply of brine shrimp added to the chlorinated quadrants of the behavior chamber.

Group	n	Total Avoidance (ppm TRO) $\bar{X}$	SE
No Food	64	0.162	0.007
With Food	80	0.203[1]	0.012

[1]Z = 2.93, 0.005 < p < 0.010.

Likewise, fish which were starved for one day prior to testing remained in the chlorinated quadrants for a significantly longer time than did fish which were previously fed ($t_{38\ df} = 4.81$, $p < 0.001$). The starved fish totally avoided 0.327 ± 0.012 ppm TRO, while satiated fish exhibited an avoidance level of 0.175 ± 0.043 ppm TRO (Table 5).

Table 5. Total residual oxidant (TRO) avoidance by juvenile *C. punctipinnis* and fish starved for one day prior to testing.

Group	n	Total Avoidance (ppm TRO) $\bar{X}$	SE
Satiated	20	0.175	0.043
Starved	20	0.327[1]	0.012

[1]$t_{38\ df} = 4.81$, $p < 0.001$.

DISCUSSION

Avoidance of chlorinated seawater by juvenile _C. punctipinnis_ was statistically significant at TRO concentrations of 0.08-0.10 ppm, and avoidance was complete at 0.15-0.16 ppm. The TRO avoidance thresholds reported in this study are slightly lower than those for shiner perch and higher than those for coho salmon published by STOBER et al. (1978). While shiner perch statistically avoided TRO concentrations greater than 0.175 ppm, coho salmon avoided all TRO concentrations above 0.002 ppm.

Other biological variables (i.e. fish size, health) and environmental factors (i.e. temperature, light, salinity, heavy metals) have been found to influence the behavioral reactions of fish to chlorinated seawater. In contrast to the result obtained by MELDRIM et al. (1974) in which fish size and TRO avoidance levels were inversely related, the experiments with _C. punctipinnis_ suggest a direct relationship between size and TRO avoidance thresholds. Starvation of _C. punctipinnis_ may have elicited a searching response (EHRLICH et al. 1979) which raised the TRO avoidance threshold. Similarly, the presence of food in the chlorinated seawater flow resulted in tolerance of higher TRO levels. Thus, the availability of food in the discharge plume and in the surrounding area may modify the normal avoidance response of fish to chlorinated seawater.

Generally, higher water temperatures decrease the TRO avoidance threshold, presumably by affecting the HOCl dissociation constant (LARRICK et al. 1978). Acclimation of _C. punctipinnis_ to winter and summer water temperatures of 15°C and 20°C, respectively, did not change the TRO avoidance levels, but exposure to normal discharge plume temperatures of 23-24°C significantly lowered the avoidance threshold.

Although the LD_{50} value for chlorinated seawater has not been determined for this species, _C. punctipinnis_ significantly avoided TRO concentrations near the 96 h LD_{50} values for other Pacific fish species (THATCHER 1978) of 0.065 to 0.090 ppm TRO.

The experimental results indicate that juvenile _C. punctipinnis_ would avoid chlorinated discharge areas which ultimately would prove toxic to them. Indeed, preliminary underwater observations of an experimental power plant chlorination suggest that resident fish species, including _C. punctipinnis_, temporarily leave the discharge area when plume TRO concentrations exceeded 0.10 ppm (STEPHENS unpubl). Complementary laboratory and field studies seem necessary to elucidate the influence of such variables as elevated temperature and food availability on the behavioral responses of fish species normally associated with discharge areas.

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