Dispersal of *Ichthyophthirius multifiliis* (Ciliophora)

Thomas A. Nickell and Margaret S. Ewing

Zoology Department, Oklahoma State University, Stillwater, Oklahoma 74078

Laboratory study of the fish parasite *Ichthyophthirius multifiliis* indicates that the largest numbers of tissue-dwelling trophonts leave the epithelium within 4 hr of death of the host. Three times as many departing individuals prefer light substrate as prefer dark.

INTRODUCTION

Ichthyophthirius multifiliis, Fouquet 1876, is a ciliate that parasitizes a wide variety of freshwater fish. Its life cycle includes a tissue-dwelling trophont, which departs upon maturity to become a free-living tomont, the stage that divides repeatedly to produce a new generation of infective individuals (1). Dispersal of the tomont, either at maturation of the trophont or upon death of the host, depends on differentiation of several organelles (2). Typically the tomont swims for 2-6 hr, then encysts (3). Little is known of the temporal pattern of departure by a population inhabiting a host or of reactions of the free-swimming tomonts to light. The objectives of the present study were to describe the temporal pattern of dispersal and determine the effect of light upon the pattern of encystment by the tomonts.

METHODS

Ichthyophthirius multifiliis, cultured from Oklahoma hatchery channel catfish (*Ictalurus punctatus*), was maintained by serial passage at 20 °C in a variety of laboratory fish. All fish were experimentally exposed for 15 min to infective theronts in glass dishes.

Temporal Pattern

Fifteen experimentally infected fish were pithed when trophonts were mature, i.e., at 4 or 5 d postexposure (PE). Each fish was placed in conditioned filtered water (4) in a glass bowl, 5.5-cm or 10.5-cm in diameter, depending upon size of the fish. The smallest fish used were black bullheads (3 cm in length); the largest were channel catfish (8 cm in length). Fish were randomly assigned to trials. Each fish was moved hourly for 6 hr to a clean bowl containing clean water. When tomonts had encysted in the bowl, they were preserved and stained with 1% (v/v) solution of Lugol's iodine or 1% (v/v) solution of Quensel's fixative to which was added 0.1 mg acidified methyl green/mL Quensel's fixative. Tomonts were then counted and mapped as seen with a dissecting microscope. The hypothesis of equal rates of departure in all sample intervals was tested using chi-square statistic (5).

Light Versus Dark Substrate Preference

Preliminary experiments providing departing tomonts a choice between a light substrate exposed to incident light and a dark substrate without incident light suggested a preference for well-lighted substrate, light in color. Therefore, in this series of trials five experimentally infected fish were pithed and placed in individual bowls fitted with plastic liners, each of which was one half black in color and one half light. Each bowl was fitted with a black collar to prevent incident light from entering the sides. The half of the bowl containing the black substrate was covered with black paper. Each experimental fish was placed with the head-tail axis on the border dividing light and dark areas for 6 hr. After the fish was removed, encysted tomonts were stained with Lugol's iodine (1% v/v) and counted. Results were analyzed using a chi-square statistic (5).

Light Intensity Preference

In each of four trials a pithed fish was placed in a glass bowl with black collar and

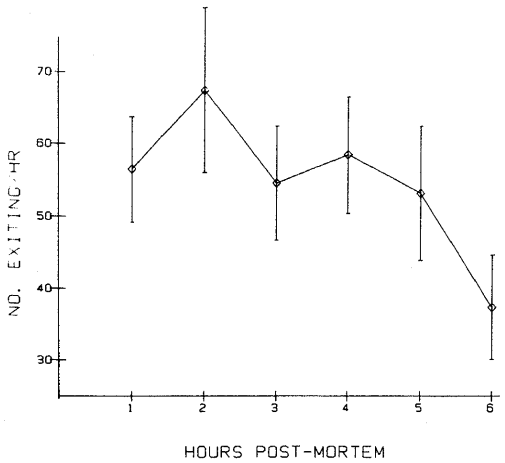

FIGURE 1. Mean number of tomonts exiting per hour. Error bars indicate ± standard error.

complete lid to prevent interference of incidental light. The bowl was placed on a ring stand 45 cm above a Sylvania cool white fluorescent light source. The bowl rested on a Kodak Projection Print Scale above a single pane of clear glass to provide a series of sectors offering ten graded intensities of light. Each fish was positioned with head between the highest and lowest intensities. Fish were removed after 4 hr and tomonts stained after encysting. The number of tomonts encysting in each intensity section was counted and analyzed using a chi-square statistic (5).

Colored Light Preference

In 12 trials tomonts were provided a choice of substrate with light from a cool white fluorescent bulb passing through red, blue, yellow, or clear glass filters. The apparatus was basically like that used in the Light Intensity Preference Experiment with the substitution of four lighted areas for the intensity gradient in that experiment. Position of the fish was varied in relation to filtered light areas. Each pithed fish remained in its bowl 4 hr; tomonts were stained after encysting. Numbers of tomonts settling in each quadrant of the bowl were compared using the chi-square statistic (5).

RESULTS AND DISCUSSION

Following death of the host, tomonts swim free and encyst. The numbers of parasites departing and encysting increased between 1 and 2 hr after death of the host and the number exiting per hour declined substantially after 5 hr (Fig. 1) though differences were not statistically significant. In the first hour after death of the host, large numbers of tomonts, were probably able to exit rapidly owing to the disturbance of the epithelium that occurred with handling and pithing. Numbers of parasites exiting then increased as the epithelium began to deteriorate until most parasites had departed.

Tomonts showed a marked photoresponse when offered a choice between an uncovered light and a covered dark substrate (Table 1). Significantly more tomonts were found in the lighted than in the dark area in every trial. Nearly three times as many tomonts were observed on light surfaces as on dark. Although photopositive behavior has not been analyzed for ciliates, it has been suggested that in *Euglena* the photopositive response initially involves random movement into a lighted zone followed by phobic reaction to encountering the margin of the adjacent dark zone (6).

TABLE 1. Light versus dark substrate preference of exiting parasite.

Number encysting	Trial 1	2	3	4	5	Total
Light substrate	12	163	127	296	113	711
Dark substrate	4	100	75	105	83	367
Chi-square	4.0[a]	15.1[a]	13.4[a]	91.0[a]	4.6[a]	

[a]Significant at $p < .05$

Tomonts nevertheless showed no significant preference for graded light intensities well within the extremes of light and total dark. The difference between numbers settling in the highest light intensity sector and in the lowest light intensity section was less than the standard deviation for the trial in three out of the four light intensity preference trials. This behavior is consistent with reports that most other ciliates react only to extremes in light intensity (7 -9).

TABLE II. Color preference of exiting parasites in twelve trials.

Light filter color	Number encysting
Blue	238
Red	351
Yellow	431
White	481
Chi-square	89.85[a]

[a]Significant at $p < .05$

Tomonts did not settle on all colored light areas with equal probability, as indicated by chi-square analysis (Table 2). Comparison of frequencies of encystment in the four quadrants suggests a preference for yellow and white filtered light. The existence of pigments that might selectively absorb specific wave lengths of light has not been demonstrated in this species.

The adaptive advantage of preference for a well lighted substrate light in color can only be suggested. The swimming tomont is pale gray to white in color and might be best camouflaged from predators against a pale background.

ACKNOWLEDGMENTS

The technical assistance of R.J. Miller and L.J. Willson is appreciated. This research was supported by the O.S.U. College of Arts and Sciences.

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