DEVELOPMENT OF *ICHTHYOPHTHIRIUS MULTIFILIIS* (CILIOPHORA) IN NAIVE AND PREVIOUSLY EXPOSED CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

By

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Bachelor of Science in Arts and Sciences

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1985

Submitted to the Faculty of the
Graduate College of
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1988

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PREFACE

The contents of this thesis, which have been written in publication format for The Journal of Protozoology, have been split into two studies. The first study, chapter I through chapter IV, covers the development of Ichthyophthirius multifiliis in the body and fin epithelium of channel catfish. The second study, chapter V through chapter VIII, covers the development of I. multifiliis in the gill epithelium of channel catfish.

I wish to express my thanks to Dr. Margaret S. Ewing for serving as my major advisor. Her support, advice and encouragement throughout this study are appreciated. I am also grateful to Dr. R. J. Miller and Dr. K. M. Kocan for serving on my advisory committee and for their helpful criticism of this manuscript. Special thanks are due to Dr. Miller for introducing me to Dr. Ewing, and to Dr. Kocan for allowing generous use of her laboratory facilities and for sharing her expertise in electron microscopy techniques.

The help of Dr. S. A. Ewing, Ms. Robin Estep-Harris and Ms. Wanda Edwards during critical times of this study is also appreciated.

To my parents, George and Ruth-Anne Dusanic, and especially to my husband, Chris, for their support, encouragement and belief in my abilities, I extend a sincere

thank you.

Supplies and computer funds were provided by the Department of Zoology, and funds for the use of electron microscope facilities were provided by the University Center for Water Research.

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CHAPTER I

INTRODUCTION

The hymenostomatid, Ichthyophthirius multifiliis Fouquet 1876, causes white spot disease in freshwater fish worldwide. This parasite invades the epithelium of the skin and gills where, as a trophont, it feeds on tissue fluids and cellular debris (16, 19). After maturation in the host epithelium or when the host dies (2) the trophont escapes and becomes a free-living tomont. Tomonts encyst on the substrate by discharging a mucoid layer that surrounds the parasite (7). Asexual reproduction within the cyst ends with the production of up to 1,000 infective stages, or theronts, which escape the cyst and swim in search of a host (11, 15). Recently Ewing et al. (1988) have suggested that reproduction by the trophont also occurs within host epithelium.

It has been shown that fish previously infected with I. multifiliis are resistant to reinfection with the parasite (1, 10, 12, 14, 20). However, few researchers have compared differences in trophont population density between naive and resistant fish (e.g., 14). On the basis of observations days 1-5 postexposure (PE), we offer evidence that trophonts are larger in naive channel catfish (Ictalurus punctatus)

and, depending upon habitat in the host, population densities are greater and reproduction by the parasite more extensive.

CHAPTER II

MATERIALS AND METHODS

Ichthyophthirius multifiliis was maintained by serial passage through channel catfish (Ictalurus punctatus) obtained as swim-up fry from the Tishomingo National Fish Hatchery and raised to mean fork length 8.82 cm in our laboratory. Moribund channel catfish infected with I. multifiliis were placed in bowls of conditioned filtered water (CFW -aerated tap water passed through an activated carbon column) to collect departing trophonts. The fish were periodically transferred to clean CFW to prevent fouling of the water. The free-living tomonts developed for 24 h to produce infective theronts for experimental exposures. A mixture of two strains, one from an ornamental fish and another from a native south-central Oklahoma fish, were used to produce resistant fish. A central Oklahoma native fish strain was used in the subsequent challenge of naive and resistant fish. Concentrations of theronts suspended in CFW were determined by counting theronts in 0.5-ml aliquots (5). All exposures were carried out at a mean temperature of 22.5 $\pm$ 2^{0}C.

Production of resistant fish. In a preliminary study, resistant fish were produced by exposing naive channel

catfish to a suspension of 30 theronts/ml CFW. Upon challenge, these fish harbored smaller trophonts than their naive counterparts, but trophont population density (number/mm^2) was not smaller. Therefore, a larger initial infective dose (360 theronts/ml) was used to produce resistant fish in the present study.

Twenty-six fingerling channel catfish were exposed to 360 theronts/ml CFW (30,000 theronts/fish) for 30 min (3). The fish were then transferred to 4-liter aquaria containing clean CFW. On the third day PE, when reproductively competent parasites are first expected to depart the host at 21^0C (8), the water was treated with malachite green (0.05 ppm, commercial Ich Cure by Kordan) to prevent reinfection. Beginning at day 5 PE the water was treated daily with copper sulfate (2 ppm day 5 PE, 1 ppm daily thereafter). In preliminary studies, channel catfish recovered from infection in approximately 16 days when reexposure was prevented, a recovery period similar to that reported for mirror carp (10). Therefore, in the present study, treatment continued daily for three weeks. Fish were fed daily beginning at day 5 PE. After recovery, they were transferred to 10-liter aquaria and fed daily.

Trophont maturation in naive and resistant catfish. Six weeks after recovery, twenty-six naive and twenty-six resistant fish were exposed to 300 theronts/ml CFW (30,000 theronts/fish) for 30 min. After 30 min the fish were transferred to 10-liter aquaria containing clean CFW, and

beginning day 3 PE the fish were treated daily with copper sulfate (1.5 ppm) to prevent reinfection. At 70 min PE, and daily, days 1-5 PE, three to six fish from each group were killed by placing them in cold (4°C) 2% (v/v) glutaraldehyde in a 0.27 M sodium cacodylate buffer (13). Gills from one side of the fish were excised (for another study), and the rest of the body was placed in 10% (v/v) formalin. Unexposed controls were sampled in the same manner at day 5 PE.

Population densities of trophonts in the body epithelium of fish sampled days 3-5 PE were estimated by enumerating trophonts in two 0.5-cm^2 sample areas with the aid of a dissecting microscope (5). Population densities in the top surface of one pectoral fin were also estimated for fish sampled days 2-5 PE by mapping all trophont positions and determining fin area using a dissecting microscope and ocular micrometer. Because Ewing et al. (4) presented evidence that I. multifiliis reproduces within epithelium, the number of trophonts occurring in each parasite locus was noted. Removal of the epithelium overlying the trophonts often was necessary to accomplish this task. Diameters of ten solitary trophonts dissected from the skin of each fish were measured using a binocular microscope and ocular micrometer. Differences in trophont size and population densities of naive and resistant fish were analyzed for statistical differences using Student's t-test (17). In tests involving percentages, arcsine, square root

transformations of proportions were performed to normalize data (18).

CHAPTER III

RESULTS

Trophont size. The mean diameters of trophonts in resistant fish (Fig. 1) were significantly smaller than those in naive fish at days 4 and 5 PE ($P=0.0001$ and $P=0.0495$, respectively).

Population densities. Population densities of trophonts in skin of body and fin varied markedly. Therefore, when naive and resistant fish were compared, body and fin population densities were analyzed separately.

Population densities: naive vs. resistant fish. In the body, the mean number of trophonts/mm^2 was greater in naive than in resistant fish except at day 5 PE, however no differences between naive and resistant fish were statistically significant (Table I). In fin, the mean number of trophonts/mm^2 was greater in naive than in resistant fish days 2-5 PE, but not significantly different (Fig. 2). In the body of resistant fish and in the fin of both naive and resistant fish trophont density increased beginning on day 3 PE even though reinfection was prevented. The greatest increase occurred in fin of naive fish between days 3 and 4 PE.

A trophont cluster is defined as two or more contiguous

trophonts at one locus in epithelium. Trophont clusters were found in the body of naive fish days 3-5 PE, and inresistant fish days 4-5 PE. No differences in mean number of trophont clusters/mm^2 body were statistically significant and no pattern over time was evident.

No clusters occurred in fin of either naive or resistant fish day 2 PE. Trophont clusters were first seen in fin of naive fish at day 3 PE, and of resistant fish at day 4 PE (Fig. 3). The mean number of trophont clusters/mm^2 was greater in naive fish than in resistant fish 3-5 days PE, significantly greater on day 4 PE ($P=0.032$).

No significant difference was found between naive and resistant fish in the mean percent of the trophont population found in clusters in the body (% trophonts in clusters). In fin, the % trophonts in clusters was greater in naive than in resistant fish days 3-5 PE, significantly greater at day 4 PE ($P=0.0006$) (Fig. 3).

Population densities: fin vs. body. In naive fish, trophont population densities and % trophonts in clusters were similar in fin and body at day 3 PE, but increased markedly in fin compared with body days 4-5 PE as reflected in the fin/body ratios (Table II). In resistant fish, trophont densities were slightly higher in fin than in the body days 3-5 PE; however only at day 5 PE was the % trophonts in clusters higher in fin than in the body (Table II). In naive fish, significant differences between fin and body occurred with regard to the trophont population density

day 5 PE (P=0.043), and the % trophonts in clusters day 4(P=0.0014). No significant differences occurred between finand body populations in resistant fish.

The proportion of naive fish that harbored clusters in the body increased from 50 to 66% from day 3 to day 5 PE, and the proportion for resistant fish increased from 0 to 60% (Table III). The proportion of naive fish that had clusters in fin increased from 50 to 100% day 3 to day 5 PE, and the proportion for resistant fish increased from 0 to 60%.

CHAPTER IV

DISCUSSION

Many researchers have demonstrated that fish previously exposed to Ichthyophthirius multifiliis became resistant to reinfection (1, 10, 12, 14, 20). The present study describes differences in trophont development between naive and resistant fish. Perhaps one consequence of host response to prior infection is a suppression of parasite growth, as observed in this study (Fig. 1) and in a preliminary study as well.

Not only did naive fish harbor significantly larger trophonts than resistant fish (Fig. 1), trophont densities in pectoral fin of naive fish were also greater (Fig. 2). In fin of both naive and resistant fish, the changes in trophont population density from days 3-5 PE were paralleled by changes in both numbers of clusters/mm^2 and the % trophonts in clusters (Fig. 3). A positive correlation has been shown between tomont size and the number of theronts produced (2, 8). The present study suggests a correlation between trophont size and the ability to reproduce in fin epithelium as well.

In body epithelium, no significant differences between naive and resistant fish were found with respect to trophont

density, cluster density, or % trophonts in clusters. This suggests that trophonts on the body of naive fish, even though significantly larger than those on resistant fish, did not produce more daughter trophonts.

Perhaps observed differences between fin and body trophont populations reflect differences in suitability of these sites for parasite reproduction. Trophont population densities and the % trophonts in clusters were markedly higher in fin than in body epithelium of naive fish days 4-5 PE, as indicated by fin/body ratios (Table II). Therefore, fin appears to be a more suitable site for reproduction in naive fish. The suitability of fin for reproduction in naive fish is also reflected in the percentage of fish harboring clusters (Table III). At day 4 PE, 100% of naive fish harbored clusters in fin whereas only 60% of these same fish harbored clusters on the body.

The ratio of the trophont population density in fin to that in body also suggests that fin is a more favorable habitat for trophonts in resistant fish (Table II). In studies of I. multifiliis infections in carp, reexposed resistant fish became infected only on the periphery of the fins (12), further evidence that fin may be a more favorable habitat for trophonts.

Even though fin seems to be a preferred habitat for trophonts in resistant fish, reexposed resistant fish appeared to suppress trophont reproduction to a greater extent in fin than in body epithelium. This is indicated by

the lower trophont population density fin/body ratios in resistant than in naive fish at days 4 and 5 PE and the even more marked reduction in fin/body ratios for the % trophonts in clusters in resistant fish (Table II). Furthermore, the percentages of resistant fish harboring clusters in body and in fin were not different.

McCallum (14) concluded, based on trophont population density comparisons of black mollies (Poecilia latapinna) that trophonts remained longer on control fish than on those with previous experimental exposure to I. multifiliis. In the present study, trophont density declined in naive fish and increased in resistant fish days 4-5 PE, suggesting that trophonts left naive fish sooner than resistant fish. McCallum (14) did not report an increase in population density over a 10-day period, but clusters might be easily mistaken for single cells in scaled fish.

Host response to I. multifiliis infection has been investigated by other researchers (9, 19). In infections in which more than one generation of parasite infected the host, epithelial proliferation was reported consistently. Eosinophils, neutrophils, and lymphocytes were found in association with the trophonts, depending upon host species and parasite location. Serum and mucus from fish infected with I. multifiliis have been found to immobilize trophonts (10, 20). Immobilization factors in serum and mucus may play a role in the decrease in trophont size and reproduction in resistant fish.

In the present study, we have provided evidence that in resistant fish parasite growth is suppressed and, depending upon habitat, reproduction as well. However, further studies are needed to determine the specific immune response to trophonts in epithelium of resistant fish.

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APPENDIX A

TABLES

Table I. Trophont population density (number/mm^2) in body epithelium (± standard error). Day PE indicates day postexposure.

DAY PE	NAIVE	RESISTANT
3	0.21 (± 0.10)*	0.03 (± 0.01)
4	0.14 (± 0.05)*	0.06 (± 0.02)*
5	0.09 (+ 0.02)*	0.10 (+ 0.04)*

*Trophont clusters observed.

Table II. Trophont population density (number/mm^2) and mean percentage of the trophont population found in clusters: ratio of quantity in fin to that in body. Day PE indicates day postexposure.

	TROPHONT POPULATION DENSITY		PERCENTAGE OF TROPHONTS IN CLUSTERS	
	RATIO OF FIN/BODY		RATIO OF FIN/BODY	
DAY PE	NAIVE	RESISTANT	NAIVE	RESISTANT
3	0.78	1.58	2.55	0.00
4	3.74	2.43	4.94	0.62
5	4.22	1.86	3.66	1.43

Table III. The percentage of naive and resistant fish harboring clusters in pectoral fin and body epithelium days 3-5 postexposure (PE).

	NAIVE		RESISTANT	
DAY PE	FIN	BODY	FIN	BODY
3	50	50	0	0
4	100	60	40	40
5	100	66	60	60

APPENDIX B

FIGURES

Figure 1. Mean length of trophonts in body epithelium.

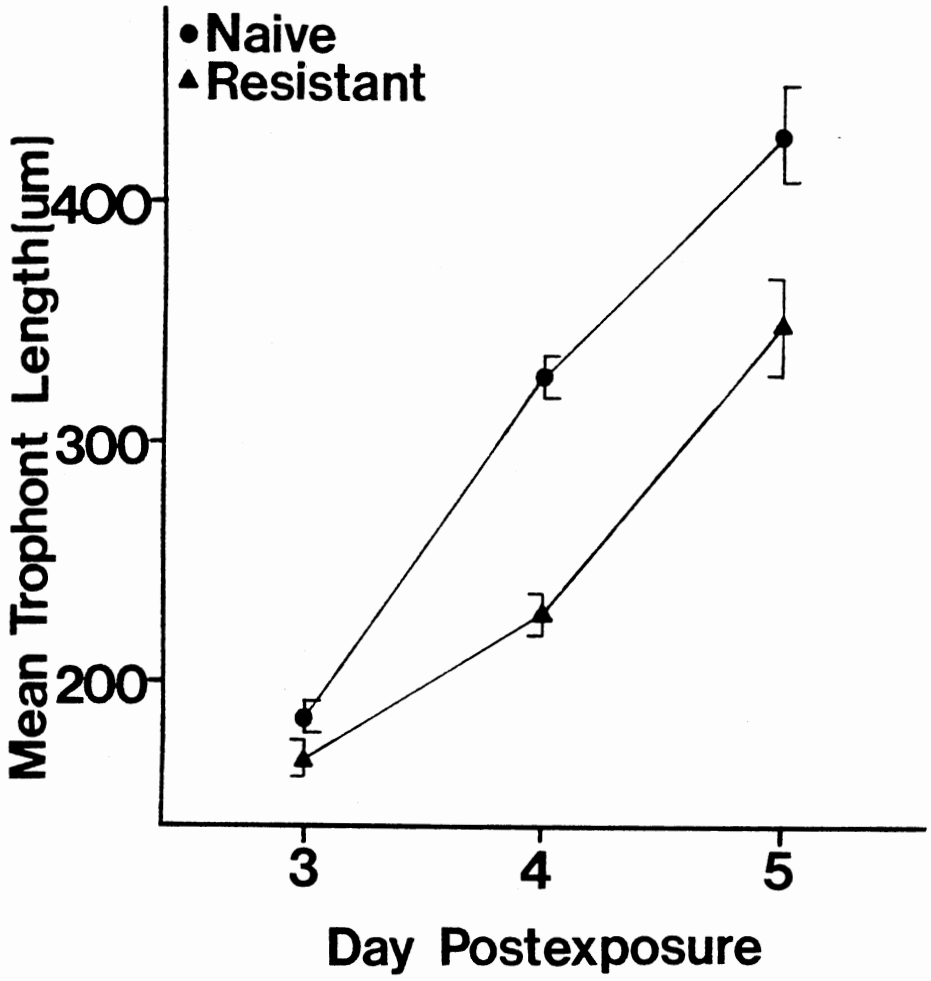

•Naive
▲Resistant
Mean Trophont Length(um)
400
300
200
3
4
5
Day Postexposure

Figure 2. Population density of trophonts in pectoral fin.

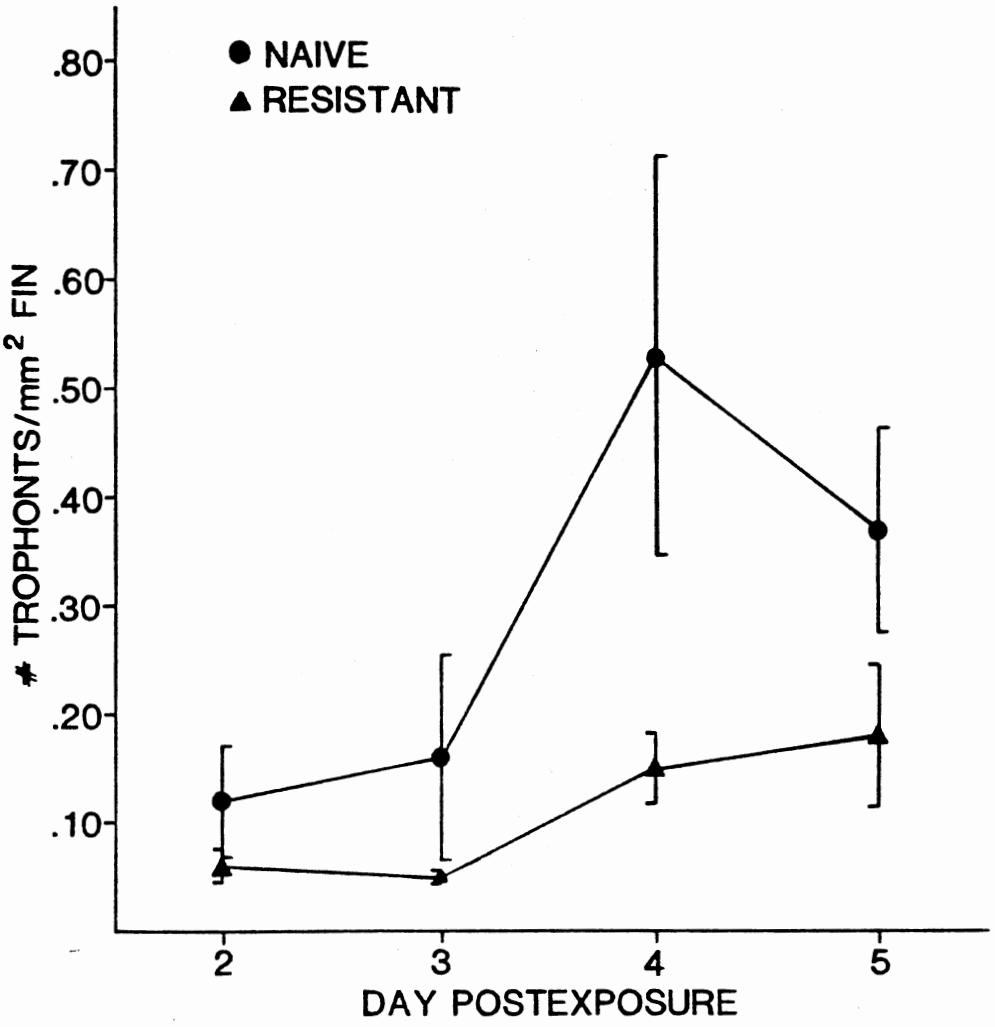
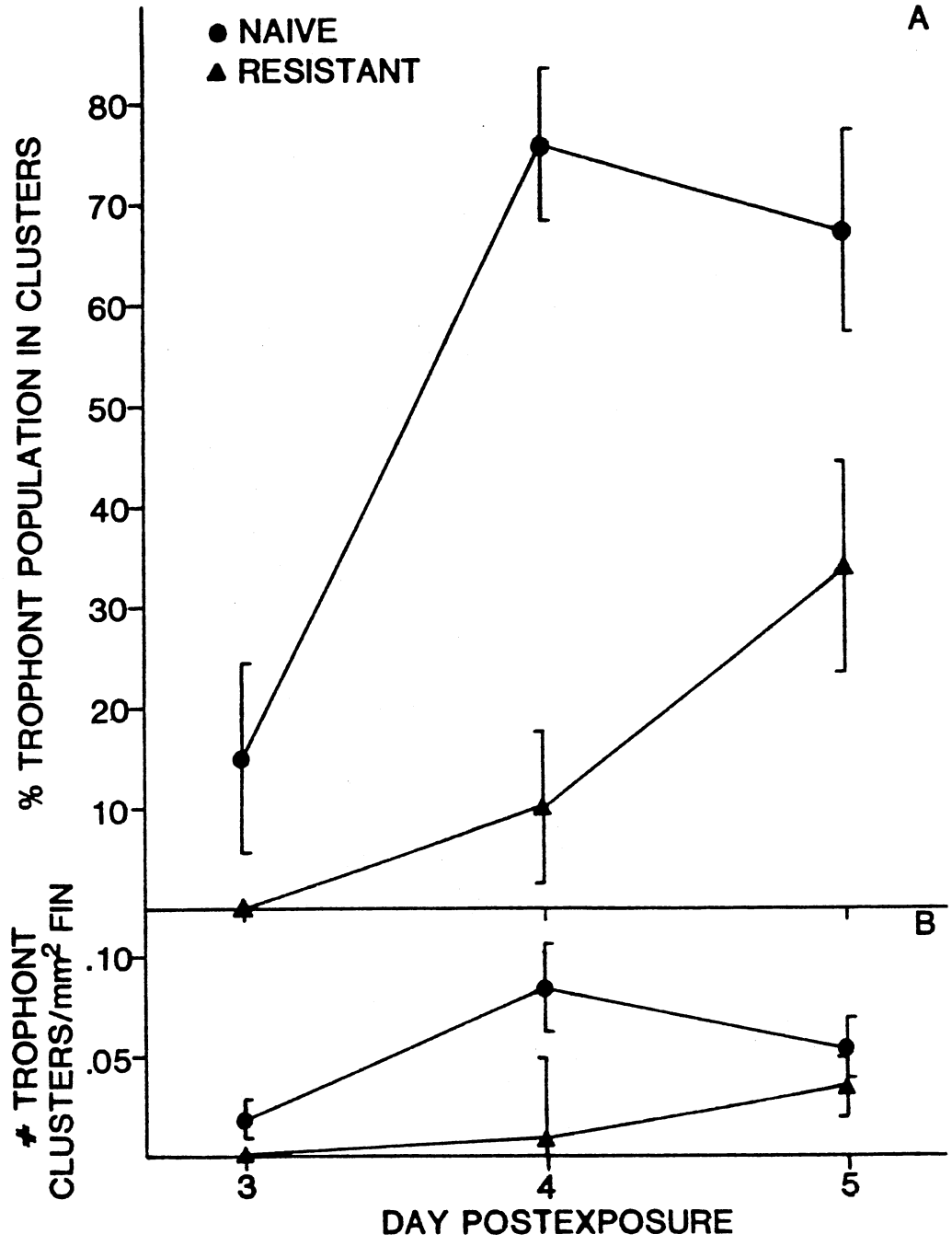

NAIVE
RESISTANT
.80
.70
.60
.50
.40
.30
.20
.10
TROPHONTS/mm² FIN
2
3
4
5
DAY POSTEXPOSURE

Figure 3. Mean percent of the trophont population that is found in clusters (A) and number of trophont clusters per mm^2 (B) in pectoral fin.


NAIVE
RESISTANT
A
% TROPHONT POPULATION IN CLUSTERS
80
70
60
50
40
30
20
10
B
TROPHONT CLUSTERS/mm² FIN
.10
.05
3
4
5
DAY POSTEXPOSURE

CHAPTER V

INTRODUCTION

Various researchers have studied the interaction between the parasite Ichthyophthirius multifiliis and fish hosts upon repeated exposure to the parasite. Many have found that fish previously exposed to I. multifiliis are resistant to reinfection (1, 15, 16, 19, 25). It also has been shown that catfish vaccinated with Tetrahymena pyriformis cilia are afforded some protection against infection with I. multifiliis (11). In the previous study (Taylor I) evidence was provided that, upon reexposure, resistant fish suppressed parasite growth. Depending upon trophont habitat within the host, reproduction was limited as well. However, no comparisons of trophont maturation within host gill epithelium have been made between naive and resistant fish. On the basis of observations 70 minutes postexposure (PE) and days 1-5 PE, evidence is offered that in gill, trophont population densities, the interaction between trophont and host epithelium, and host response to infection differed between naive and resistant fish.

CHAPTER VI

MATERIALS AND METHODS

Ichthyophthirius multifiliis was maintained by serial passage through channel catfish (Ictalurus punctatus) obtained as swim-up fry from the Tishomingo National Fish Hatchery and raised to mean fork length 8.82 cm in our laboratory. Moribund channel catfish infected with I. multifiliis were placed in bowls of conditioned filtered water (CFW -aerated tap water passed through an activated carbon column) to collect departing trophonts. The fish were periodically transferred to clean CFW to prevent fouling of the water. The free-living tomonts developed for 24 h to produce infective theronts for experimental exposures. A mixture of two strains, one from an ornamental fish and another from a native south-central Oklahoma fish, were used to produce resistant fish. A central Oklahoma native fish strain was used in the subsequent challenge of naive and resistant fish. Concentrations of theronts suspended in CFW were determined by counting theronts in 0.5-ml aliquots (6). All exposures were carried out at a mean temperature of 22.5 $\pm$ 2^{0}C.

Production of resistant fish. Thirty-six fingerling channel catfish were exposed to 360 theronts/ml CFW (30,000

theronts/fish) for 30 min (3). The fish were then transferred to 4-liter aquaria containing clean CFW. On the third day PE, when reproductively competent parasites are first expected to depart the host at 21^{0}C (9), the water was treated with malachite green (0.05 ppm, commercial Ich Cure by Kordan) to prevent reinfection. Beginning at day 5 PE the water was treated daily with copper sulfate (2 ppm day 5 PE, 1 ppm daily thereafter). In preliminary studies, channel catfish recovered from infection in approximately 16 days when reexposure was prevented, a recovery period similar to that reported for mirror carp (15). Therefore, in the present study, treatment continued daily for three weeks. Fish were fed daily beginning at day 5 PE. After recovery, they were transferred to 10-liter aquaria and fed daily.

Trophont maturation in naive and resistant catfish. Six weeks after recovery, twenty-six naive and twenty-six resistant fish were exposed to 300 theronts/ml CFW (30,000 theronts/fish) for 30 min. After 30 min the fish were transferred to 10-liter aquaria containing clean CFW, and beginning day 3 PE the fish were treated daily with copper sulfate (1.5 ppm) to prevent reinfection. At 70 min PE (day 0 PE), and daily, days 1-5 PE, three to six fish from each group were killed by placing them in cold (4^{o}C) 2% (v/v) glutaraldehyde in a 0.27 M sodium cacodylate buffer (18). Gills from one side of the fish were excised and placed in the buffered glutaraldehyde, and the rest of the body was

placed in 10% (v/v) formalin. After fixation in glutaraldehyde, the gill tissue was washed several times in the same buffer and post-fixed in 2% (w/v) osmium tetroxide in a 0.27 M cacodylate buffer. The tissue was washed several times then dehydrated through a graded series of ethanol. Propylene oxide was used as the intermediate solvent in the infiltration process using Dow Epoxy Resin (DER) 732 (18). Unexposed controls were sampled in the same manner at day 5 PE.

Cross-sections of gill filaments were cut using a Sorvall MT-5000 ultramicrotome. Thick sections (1.5 µm) were stained with Mallory's stain (20). Thin sections (70-90nm) were cut with a Diatome diamond knife, collected on 200 mesh copper grids, stained with uranyl acetate and lead citrate (23), and observed with a JEOL JEM-100cx Temscan electron microscope operated at 80kv.

For every individual fish, three thick sections, each representing a different block of gill tissue, were examined. Population densities were estimated as the number of trophonts per filament in thick sections harboring trophonts. In gill filaments harboring trophonts, the occurrence of more than one trophont per filament, staining intensity of the trophont in section, presence of cell debris near the trophont, position of individual trophonts in the filament, and the contiguity of gill epithelium and trophont were noted. The presence of inflammatory response in infected filaments was also noted, particularly the

occurrence of macrophages and granulocytes. A total of 414 trophonts were observed. Thin sections from naive fish days 3, 4 and 5 PE and from resistant fish days 2 through 5 PE were viewed by electron microscopy. Results were analyzed for statistical differences using Student's t-test (21). In tests involving percentages, arcsine, square root transformations were performed to normalize data (22).

CHAPTER VII

RESULTS

Trophont population densities. Trophont population densities (mean number of trophonts/gill filament) in gill epithelium of naive fish increased from 70 minutes PE (day 0 PE) to day 3 PE, then declined days 3-5 PE (Fig. 1). The two-fold increase from day 2 to day 3 PE in the trophont population density was statistically significant (P=0.0004). Trophont population densities in resistant fish showed little change over the sampling period. Although the population densities in resistant fish were greater on day 2 PE than on any other sample day, the only significant difference in trophont population densities in this group occurred between days 2 and 5 PE (P=0.029). Differences in trophont population densities between naive and resistant fish were significant on days 3 and 4 PE (P=0.001 and P=0.0081 respectively).

On every sampling day at least one infected gill filament in resistant fish harbored more than one trophont. Among naive fish, this was observed days 1-4 PE (Table I). No significant differences occurred between the two groups with respect to this variable. In naive fish, the pattern of change in the mean percent of infected filaments

harboring more than one trophont paralleled the pattern of change in the trophont population density. In resistant fish no similar parallel occurred. After day 2 PE, little change occurred in either parameter in resistant fish.

Trophont position in filaments. In naive fish, the mean proportions of trophonts that were near the afferent or efferent vessel increased from less than half (42%) day 0 PE to the majority (91%) day 3 PE, then declined to 50% and 78% days 4 and 5 PE respectively (Table II). Of the trophonts that were near a major vessel, the mean percent on the outer edge, or tip of the filament, increased from 8% day 0 PE to 81% day 3 PE. In resistant fish, with the exception of day 1 PE, the majority of trophonts were adjacent to a major vessel on all days PE. However, unlike the trend in naive fish there was no pattern with respect to trophonts at the outer edge of the filament adjacent to a major vessel. No differences between naive and resistant fish in the mean percent of trophonts midfilament or near a major vessel were statistically significant.

Trophont staining characteristics. The majority of trophonts in both naive and resistant fish stained with an intensity similar to host cells. Relatively light staining might indicate that a trophont was in poor condition. No significant differences were found between naive and resistant fish with regard to the mean proportion of the trophont population with light staining characteristics. In both naive and resistant fish the greatest proportion of

light staining trophonts (36% and 25%, respectively) occurred day 0 PE (70 minutes PE).

Contiguity of trophonts and gill epithelium. At all days PE, the majority of trophonts in naive fish were closely apposed by gill epithelium along more than one-half of their margins; however, only on days 2-4 PE were the majority of trophonts in resistant fish contiguous along more than one-half of their margins (Table III). At days 0, 1 and 2 PE, the mean proportion of trophonts contiguous along more than one-half of their margins was significantly greater in naive fish than in resistant fish. At days 1 and 2 PE, the majority of trophonts in naive fish were completely contiguous with host epithelium along their margins, a significant difference in comparison with trophonts in resistant fish. No significant differences between naive and resistant fish occurred at any other day PE.

Cell debris adjacent to trophonts. In naive fish, from day 0 to 3 PE, the majority of trophonts were surrounded by intact epithelium or relatively little cell debris (Table IV). Only on days 4 and 5 PE was more than a little cell debris found near trophonts in this group. In resistant fish at least 10% of trophonts were surrounded by more than a little cell debris all days PE. Naive and resistant fish differed significantly day 2 PE in the mean percent of trophonts surrounded by intact epithelium, little cell debris and more than a little cell debris. They also

differed significantly in the mean percent of trophonts surrounded by more than a little cell debris day 3 PE.

Host cell response. From days 0-4 PE, the proportion of infected gill filaments with active macrophages was greater in resistant fish than in naive fish (Fig. 2). The only significant difference between naive and resistant fish occurred when the mean percentage of filaments harboring active macrophages decreased dramatically in naive fish day 2 PE ($P=0.0005$). In both naive and resistant fish, macrophages in gill filaments became larger and highly vacuolated over the 5 day sampling period.

A different host cell type became apparent at day 1 PE in resistant fish. These cells had a characteristic nucleus in which chromatin was concentrated centrally, giving the nucleus the appearance of a "target", and the cytoplasm contained large, dark-staining granules (Fig. 3). Junctional complexes were seen at points of contact with other cells. The "target cells" were not seen in naive fish until day 3 PE (Fig. 4). The mean percent of infected filaments harboring these cells differed significantly between naive and resistant fish days 1, 2 and 3 PE ($P=0.047$, $P=0.022$, and $P=0.0013$, respectively).

Hyperplasia of infected gill filaments first appeared in naive fish at day 2 PE and in resistant fish at day 3 PE (Table V). The mean proportion of infected filaments that were hyperplastic was at least four times greater in naive fish compared to resistant fish days 4 and 5 PE,

significantly greater day 4 PE (P=0.019).

By light microscopy, granulocytes were first seen in resistant fish day 1 PE and in naive fish day 2 PE. Since a monochromatic stain was used on thick sections, granulocytes were not characterized by light microscopy. By electron microscopy, two different granulocyte types were encountered, type I and type II. Type I granulocytes contained two granule types; small, round granules and larger oval or elongate granules (Fig. 3). The type II granulocytes contained large, round granules with an electron-dense core (Fig. 5). Type I granulocytes were seen in thin sections from naive and resistant fish days 3, 4 and 5 PE, and type II granulocytes were seen in section from a resistant fish day 4 PE and a naive fish day 5 PE. In the resistant fish, the electron-dense core of the type II granulocytes was surrounded by a light area along the inner margin of the granule.

CHAPTER VIII

DISCUSSION

It has been demonstrated that upon experimental challenge, trophont population densities in fish previously exposed to I. multifiliis are generally lower than trophont population densities in naive fish (Taylor I, 19). Fish previously exposed to the parasite are refractory to reinfection (1, 15, 16, 19, 25). In the present study of gill trophont population densities, the interaction between trophont and host epithelium, and host response to infection were different in naive and resistant fish.

No evidence of dying trophonts or trophonts in poor condition was found. The loss of parasite staining intensity has been associated with the expenditure of cellular reserves during the process of invasion (8). Thus, light staining intensity might indicate a trophont in poor condition. In the present study, no significant differences were found between naive and resistant fish with regard to the mean percent of trophonts with light staining intensities.

In naive fish, the trophont population density increased from day 0 to 3 PE, whereas no significant increase occurred in resistant fish (Fig. 1). Ewing et al.

(5) have found indications of trophont reproduction in host epithelium. In that study, as parasite departure from the host increased, trophont population densities in epithelium also increased, even though host reinfection was prevented. In the present study, trophont population densities increased in pectoral fin epithelium of both groups over the 5-day sampling period (Taylor I). The trophont population density increase was greater in naive than in resistant fish, but not significantly different. Clusters, believed to be reproductive units, occurred in the fin epithelium of both groups, first appearing in naive fish day 3 PE and in resistant fish day 4 PE.

In pectoral fin, changes in the trophont population density were paralleled by changes in the mean proportion of the trophont population found in clusters in both naive and resistant fish (Taylor I). Similarly, in the present study, the changes in the trophont population density in gill epithelium of naive fish were paralleled by the changes in the mean proportion of gill filaments infected with more than one trophont. The peak in population density in naive fish occurred one day later in fin than in gill epithelium, similar to findings of Ewing et. al. (5), who reported that clusters appeared later in fin than in gill. Thus, the increase in trophont population density in naive fish may reflect reproduction in host epithelium.

The majority of trophonts in gill epithelium of naive fish had migrated by day 3 PE to a position near a major

vessel (Table II), a pattern described also by Ewing and Kocan (7). However, with the exception of day 1 PE, the majority of trophonts in resistant fish were found near a major vessel all days PE. Furthermore, the trend of movement toward the margin/tip of the filament seen in naive fish was not seen in resistant fish. Serum and mucus from fish infected with I. multifiliis have been found to immobilize trophonts (15, 25). Therefore, immobilizing substances in tissue fluids may have limited trophont migration in resistant fish.

Although trophont population densities in naive and resistant fish were very similar days 0 to 2 PE, differences existed between the two groups with regard to the interaction between trophonts and host epithelium. The vast majority of trophonts in naive fish were highly contiguous with host epithelium days 0 to 3 PE, similar to the findings of Ewing and Kocan (7). However, the majority of trophonts in resistant fish had a low degree of contiguity with host epithelium early in the sampling period and were not highly contiguous with host epithelium until day 2 PE (Table III). Even though the majority of trophonts in resistant fish were highly contiguous with host epithelium day 2 PE, the majority were not completely contiguous as were trophonts in naive fish.

Even though Ewing et al. (8) suggested that by 40 minutes PE the majority of trophonts ingested necrotic tissue damaged during invasion by the parasite, in the

present study, only a little over half of the trophonts in naive fish were surrounded by intact epithelium day 0 PE (70 min PE). Little cell debris was seen adjacent to the remaining trophonts (Table IV). In resistant fish, the majority of trophonts were not surrounded by intact epithelium day 0 PE, and 28% of the trophonts were surrounded by more than a little cell debris.

From day 0 to 4 PE, the mean proportion of infected filaments harboring active macrophages was greater in resistant than in naive fish (Fig. 2). The increase in the mean percent of infected filaments harboring macrophages days 3 to 5 PE in naive fish was paralleled by an increase in the mean percent of trophonts surrounded by necrotic tissue. The decrease in the mean proportion of infected filaments harboring active macrophages in naive fish at day 2 PE was paralleled by a decrease in the mean percent of trophonts surrounded by necrotic tissue day 2 PE.

By light microscopy, granulocytes were seen in resistant fish at day 1 PE, one day earlier than in naive fish. No information could be concerning "target cells", which were also seen in resistant fish day 1 PE, two days earlier than in naive fish.

Two types of granulocytes were found by electron microscopy. Type I granulocytes seen by electron microscopy from both naive and resistant fish were ultrastructurally similar to granulocytes in channel catfish blood identified as heterophils (neutrophils) by Cannon et al. (2). In

mirror carp with ichthyophthiriasis, neutrophils in the blood increased between the first and fifth day of infection (12), and neutrophils in gill epithelium increased between the eighth and twelveth day of infection (13).

Type II granulocytes were seen by electron microscopy day 4 PE in a resistant fish and day 5 PE in a naive fish. Granulocytes containing granules with an electron-dense core, similar to type II granulocytes, have been described as eosinophils in the kidney of carp, river bleak, and tench (17), and as eosinophilic granule cells in the gut of rainbow trout (10). In a review of fish leukocytes, Ellis (4) noted that eosinophils in fish tissues are often found in association with the surface epithelium of the gill, intestinal tract and skin. The presence of eosinophils in the blood of channel catfish has been confirmed by Williams and Warner (26) and denied by Cannon et al. (2). Hines and Spira (12) did not report finding eosinophils in the blood of mirror carp infected with Ichthyophthirius. However, eosinophils in carp with ichthyophthiriasis have been found infiltrating necrotic tissue from 24 to 40 hours postexposure (24).

Epithelial proliferation in skin and gills of infected fish has been reported by other researchers (13, 24). In infections in which reinfection was not prevented, Ventura and Paperna (24) suggested that epithelial hyperplasia interfered with the penetration of host tissue by new generations of parasites. However, severe hyperplasia of

gill filaments causes physiological dysfunction by reducing the surface area for gas diffusion, and increasing the distance for gas exchange between water and blood (14). In the present study, the lower incidence of hyperplasia in resistant fish compared to naive fish may reflect an adaptive response, resulting in less respiratory stress associated with hyperplasia of gill tissue (Table V).

Differences between naive and resistant fish occurred in nearly every parameter measured. Early in the infection, trophont population densities were similar in naive and resistant fish, but the two groups differed in contiguity of trophonts with host epithelium and amount of cell debris surrounding trophonts. Later in the infection trophont population densities were greater in naive fish than in resistant fish. Active macrophages were found in more infected filaments early in the infection in resistant fish. Cells with "target" nuclei appeared earlier in resistant fish. By light microscopy, granulocytes appeared one day earlier in resistant fish. In general, it appears that the host cell response to infection is similar in naive and resistant fish, but that, with the exception of hyperplasia, the host cell response begins later and with less intensity in naive than in resistant fish.

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APPENDIX C

TABLES

Table I. Mean percentage (± standard error) of infected filaments harboring one or more than one trophont per filament. Day 0 postexposure (PE) indicates 70 min PE.

	NAIVE		RESISTANT	
DAY PE	ONE	> ONE	ONE	> ONE
0	100	0	88 (± 7)	12 (± 7)
1	93 (± 7)	7 (± 7)	76 (±14)	24 (±14)
2	93 (± 3)	7 (± 3)	99 (± 1)	1 (± 1)
3	81 (±10)	19 (±10)	96 (± 3)	4 (± 3)
4	89 (± 6)	11 (± 6)	97 (± 3)	3 (± 3)
5	100	0	94 (+ 4)	6 (+ 4)

Table II. Position of trophonts in gill filament cross-section, mean percentage at each location (± standard error). Percentages of trophonts in secondary lamellae are not included in the table. Day 0 postexposure (PE) indicates 70 min PE.

NAIVE FISH

DAY PE	MIDFILAMENT	NEAR A MAJOR VESSEL
0	44 (±15)	42 (±13)
1	47 (±27)	47 (±26)
2	48 (± 7)	52 (± 7)
3	8 (± 2)	91 (± 2)
4	50 (± 8)	50 (± 8)
5	22 (± 6)	78 (± 6)

RESISTANT

DAY PE	MIDFILAMENT	NEAR A MAJOR VESSEL
0	30 (± 7)	70 (± 7)
1	65 (± 5)	35 (± 5)
2	40 (±15)	60 (±15)
3	28 (±11)	72 (±11)
4	38 (±19)	57 (±17)
5	32 (±18)	68 (±18)

Table III. Mean percentage (± standard error) of the trophont population highly contiguous with gill epithelium: "C>1/2" indicates the percentage of trophonts with greater than one-half of their margins contiguous with host epithelium, "C=1" indicates the percentage of trophonts completely contiguous with host epithelium. Day 0 postexposure (PE) indicates 70 min PE.

	NAIVE FISH		RESISTANT FISH	
DAY PE	C>1/2	C=1	C>1/2	C=1
0	81 (± 10)[a]	36 (± 7)	27 (± 7)	27 (± 7)
1	97 (± 3)[b]	58 (± 14)[d]	20 (± 15)	0
2	97 (± 3)[c]	86 (± 5)[e]	84 (± 5)	29 (± 8)
3	88 (± 4)	29 (± 6)	71 (± 11)	29 (± 13)
4	60 (± 7)	12 (± 8)	73 (± 15)	15 (± 7)
5	51 (± 16)	13 (± 10)	29 (± 12)	5 (± 3)

Differences between naive and resistant significant at [a]P=0.035, [b]P=0.0114, [c]P=0.033, [d]P=0.0296, [e]P=0.0018

Table IV. The mean percentage (± standard error) of trophonts adjacent to cell debris: no cell debris (0), little cell debris (+), and more than a little cell debris (++/+++). Day 0 postexposure (PE) indicates 70 min PE.

	NAIVE FISH			RESISTANT FISH		
	NECROTIC CELL ABUNDANCE			NECROTIC CELL ABUNDANCE		
DAY PE	0	+	++/+++	0	+	++/+++
0	56(± 6)	44(± 6)	0	36(±21)	37(±15)	27(±24)
1	47(±25)	53(±25)	0	72(± 6)	18(± 5)	10(± 7)
2	89(± 6)[a]	11(± 6)[b]	0[c]	41(± 6)	43(± 3)	16(± 5)
3	66(± 8)	34(± 8)	0[d]	40(± 8)	39(±14)	21(± 9)
4	60(±12)	34(± 9)	6(± 4)	58(± 5)	19(± 9)	23(± 8)
5	47(± 7)	36(± 2)	18(± 8)	23(± 6)	38(±10)	39(±12)

Difference between naive and resistant fish significant at [a]P=0.0011, [b]P=0.0077, [c]P=0.0027, [d]P=0.033.

Table V. Mean percentage of infected gill filaments with hyperplastic tissue (± standard error). Day 0 postexposure (PE) indicates 70 min PE.

DAY PE	NAIVE	RESISTANT
0	0	0
1	0	0
2	10 (± 8)	0
3	0	9 (± 6)
4	41 (± 12)*	7 (± 5)
5	53 (± 20)	13 (± 10)

*Difference between naive and resistant fish significant at P=0.019.

APPENDIX D

FIGURES

Figure 1. Mean number of trophonts per gill filament in thick sections harboring trophonts. Day 0 postexposure (PE) represents 70 minutes PE.

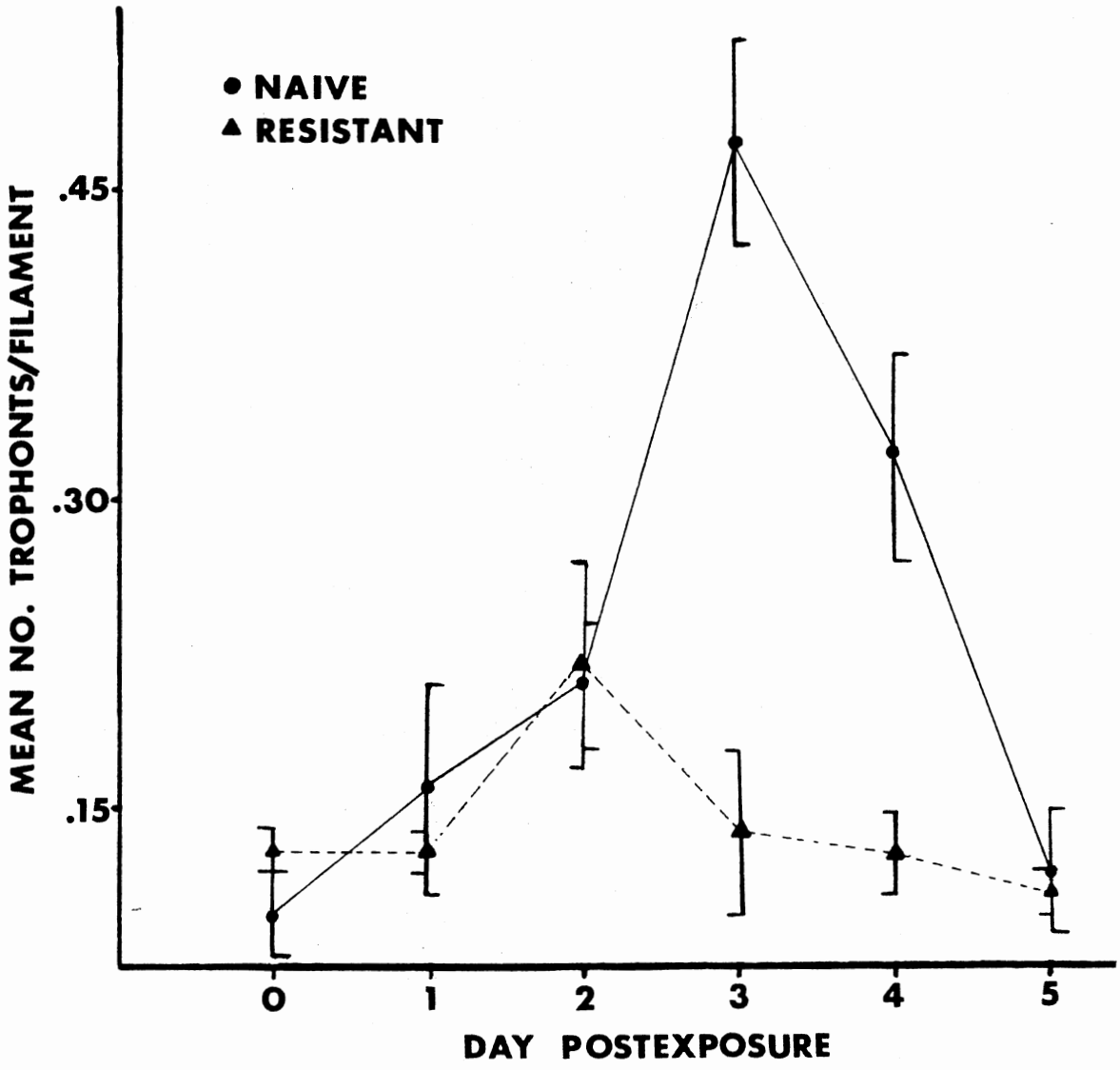

● NAIVE
▲ RESISTANT
MEAN NO. TROPHONTS/FILAMENT
.45
.30
.15
0
1
2
3
4
5
DAY POSTEXPOSURE

Figure 2. Mean percentage of infected gill filaments harboring active macrophages. Day 0 postexposure (PE) represents 70 minutes PE.

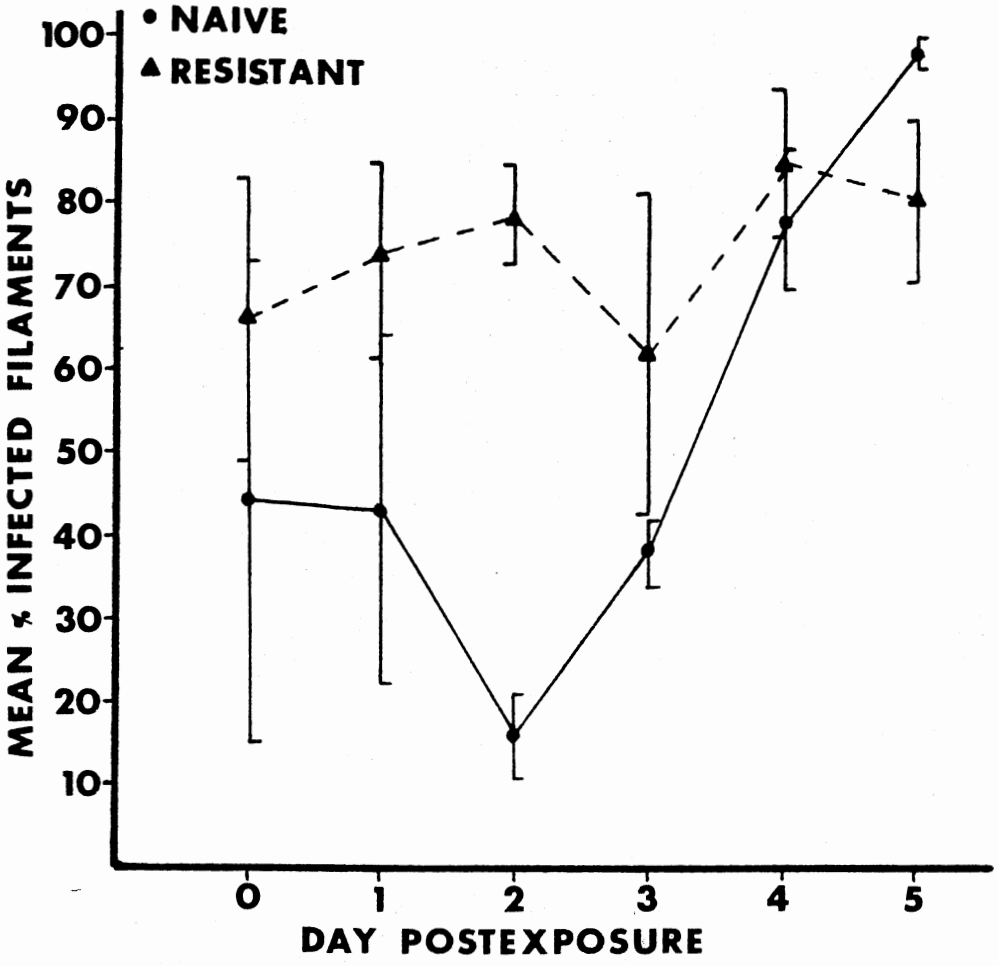

• NAIVE
▲ RESISTANT
MEAN % INFECTED FILAMENTS
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
DAY POSTEXPOSURE

Figure 3. Type I granulocyte (right) and a "target cell" (left) from naive channel catfish gill filament, 5 days postexposure. Arrows indicate junctional complexes. Transmission electron micrograph, X3,600.

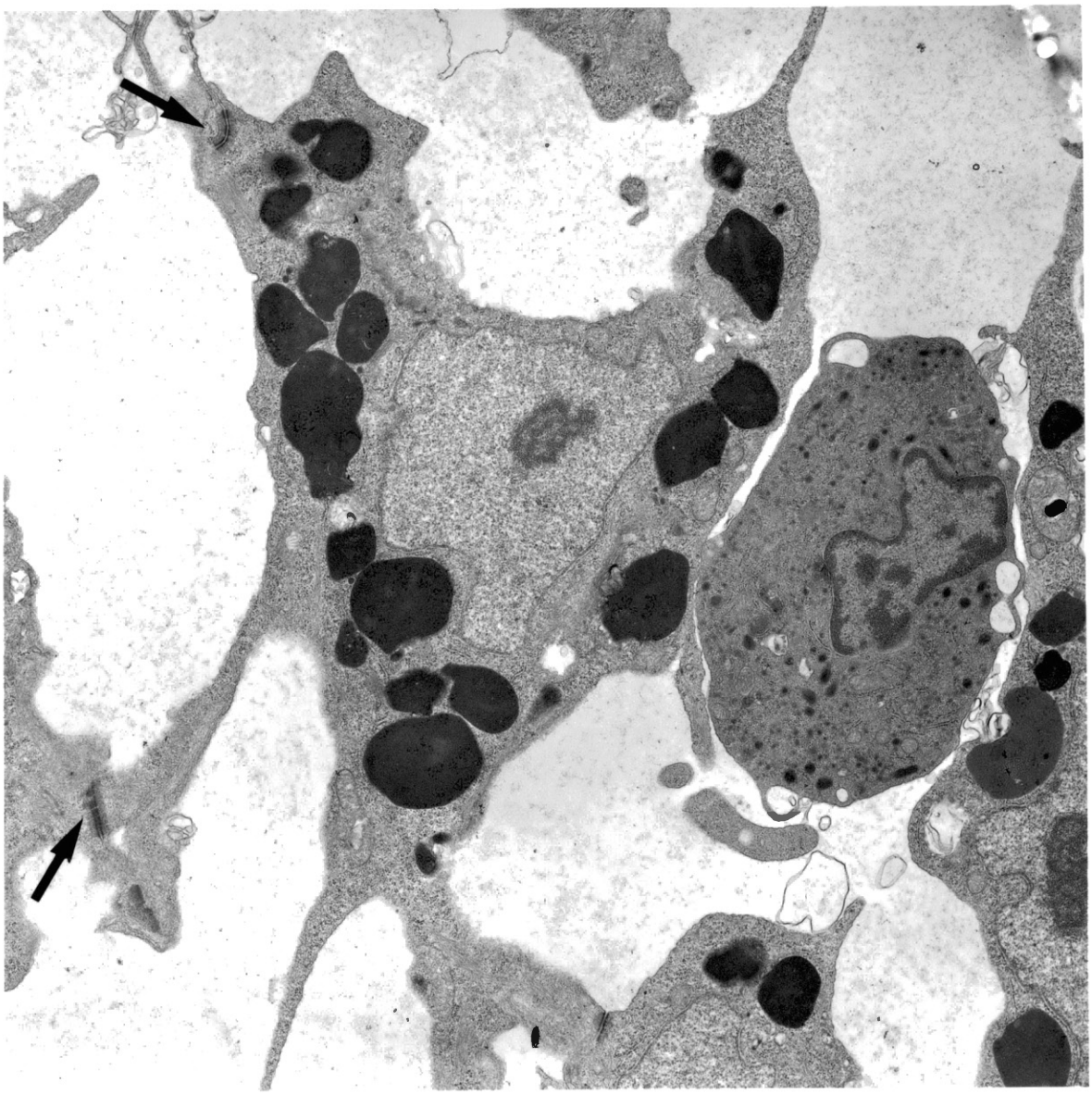


Figure 4. Mean percentage of infected gill filaments containing "target cells". Day 0 postexposure (PE) represents 70 min PE.

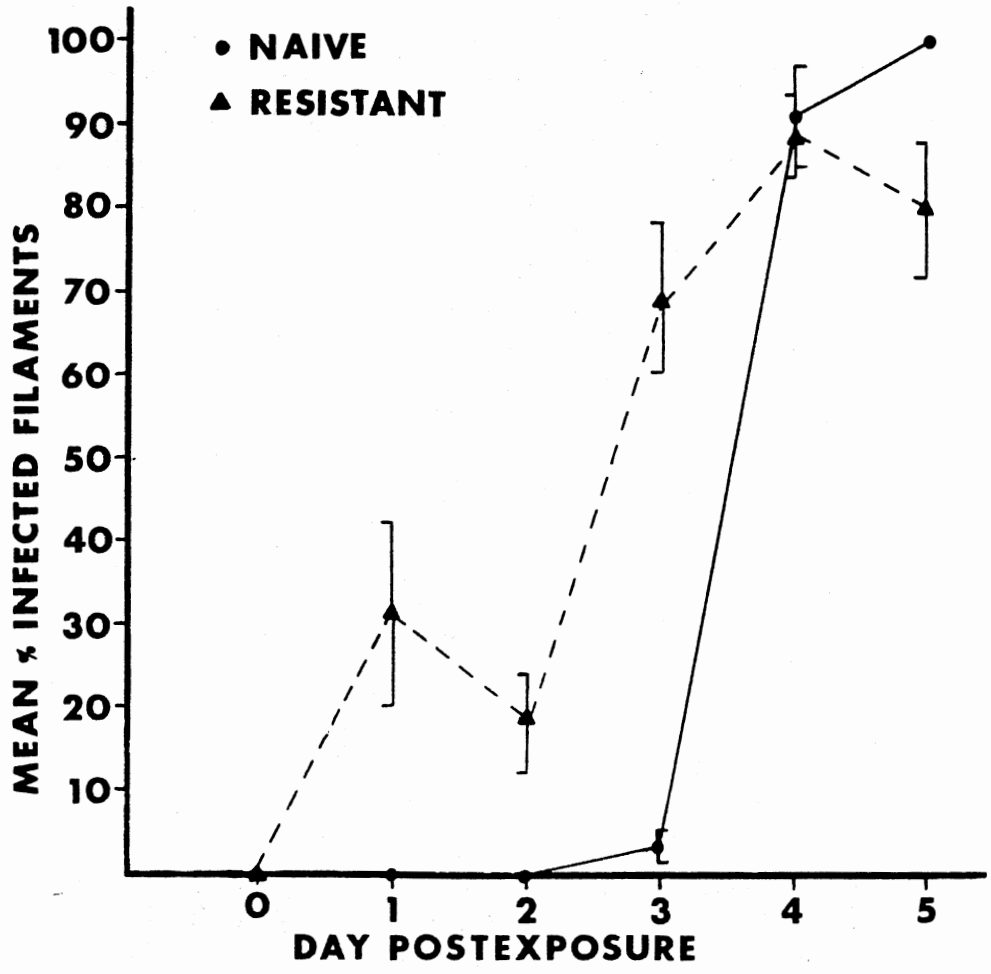
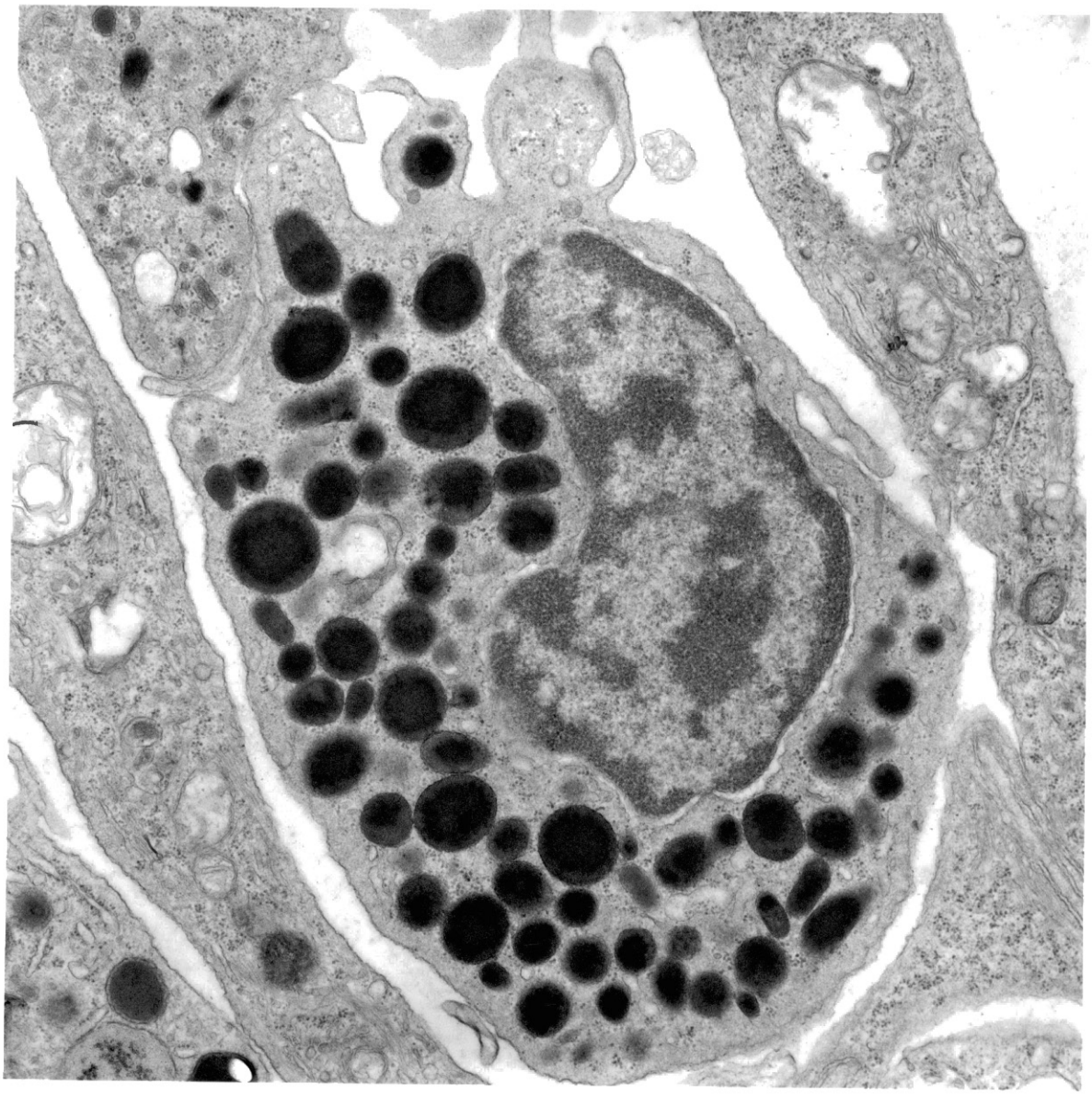

• NAIVE
▲ RESISTANT
MEAN % INFECTED FILAMENTS
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
DAY POSTEXPOSURE

Figure 5. Type II granulocyte from naive channel catfish gill filament, 5 days postexposure. Transmission electron micrograph, X10,000.



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