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Geographical Distribution and Host-Parasite Relationships of *Plistophora ovariae* (Microsporida, Nosematidae) in *Notemigonus crysoleucas*

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Abstract

Plistophora ovariae undergoes schizogony and sporogony in developing ova of the golden shiner. Destruction of the ova greatly reduces fecundity and causes spawning failures. The incidence and intensity of infection is described in 49 commercial fish farms from 12 states. The parasite was found in fish from 45 of 49 sources and in a farm pond and creek from Oklahoma. Overall incidence of infection was 48% of 2759 fish. No significant difference was found in the incidence of disease from fish propagated by either intensive or extensive culture methods. There was a significant difference in incidence of infection with host age. Incidence increased from 30% in age class 0 to 75% in age class 4. Intensity of infection decreased with age and varied with season; it was greatest in May and June. Thus, the maximum number of spores and infected ova occurred during the spawning season of the host. Infected fish were generally larger and heavier than uninfected fish. Reduced egg production (partial parasitic castration) allowed nutrients and energy to be used for faster growth.

Introduction

The purpose of this study was to determine the incidence and intensity of *P. ovariae* in golden shiners from a large number of fish farms which propagate them for the bait minnow market. Ponds in golden shiner production comprised approximately 84% of the 26,500 acres in bait minnow culture in the United States in 1969. Meyer⁵ regarded *P. ovariae* as a potential threat to the bait minnow industry, and Malone⁴ expressed the opinion that this parasite "has cost the industry more in lost production than any other single problem."

The literature on this parasite is not extensive, but is unique in that most studies on the microsporida of fish occur only in the form of the species description. A prominent exception is the report on *Glugea hertwigi* from the smelt, *Osmerus mordax*, and *O. eperlanus* (Delisle³). In addition, Summerfelt and Warner⁸ described *P. ovariae* from five sources in five states, described monthly variation in a single Illinois source and compared growth of infected and uninfected shiners. Wilhelm¹⁰ described aspects of schizogony and sporogony.

Tucker⁶ surveyed the incidence in nine minnow farms from five Arkansas counties. Summerfelt and Warner⁷ described the incidence and intensity of infection in 24 minnow farms, reviewed aspects of its pathogenicity and transmission, and described effects of fixation and staining on spore dimensions as well as the influence of age and geographical origin of the host on spore dimensions. Meyer⁸ described seasonal fluctuations in the incidence of infection.

The microsporidian *Plistophora ovariae* Summerfelt undergoes schizogony and sporogony within developing ova of the golden shiner, *Notemigonus crysoleucas* (Mitchell). Many infected ova undergo atresia and disintegration. Lesions in the germinative tissue consist of densely packed areas of cellular debris, collagen fibers, fibroblasts, macrophages and a prodigious number of spores. In heavy

infections, a major volume of the ovary is occupied by this stroma (Summerfelt and Warner,⁸ Fig. 3-8). There is no cyst formation.

This report is a summary of a considerable expanded survey of the incidence and intensity of the parasite in commercial minnow farms. In the first survey (ibid.) 472 fish were collected from 24 sources, while the present study includes those plus 2287 additional fish from some of the same, and from 25 additional sources. In total, samples were taken from one or more ponds from farms which had 14,600 acres in shiner production, or about 65% of all the acres in shiner culture. Body length, body weight, ovary weight, and the condition of the infected and uninfected fish are compared. Incidence and intensity are related to age, month of capture and method of propagation of the host.

Methods

A list was assembled of 295 minnow producers from 16 states. From this list, samples were obtained from 49 producers in the 12 states where fish culture is most prevalent. Sampling was from August, 1968 through June, 1969. Fish were obtained either by personally contacting producers, or by mail. Specimens were fixed in Bouin's solution. Age of the fish was provided by the producers for 2296 of the 2759 fish sampled.

Total body length and total ovary weights were recorded from the preserved fish. Ovaries were dehydrated in an alcohol series, cleared in toluene and embedded in Paraplast. Two slides of one ovary from each fish were prepared, each

slide had 3 to 5 sections. One slide was stained with Heidenhain's iron hematoxylin and eosin; the other was stained with Mallory's aniline blue collagen stain (Biological Stain Commission).² The latter was highly differential for primary and secondary yolk, nucleoli, follicular epithelium, and the zona radiata of developing oocytes. It was also excellent for differentiation of schizonts, sporoblasts and spores from the cytoplasm and yolk. The intensity of infection was equated with the percentage of the ovary infected. This was estimated from the area occupied by infected but intact eggs, atretic eggs, and the area of the parasitic lesion observed in 7 micron thick histological sections.

Results

Geographical Distribution

Forty-eight percent of 2763 female golden shiners from 51 sources, including 2 wild sources in Oklahoma, were infected with *P. ovariae* (Table 1). The parasite is very tissue specific and has not been found in male shiners.⁸ The widespread distribution is apparently related to the

frequent exchanges of fish among producers, and because a few farms, which have been in production for many years, provided infected brood stock for many of the newer producers.

Four samples were examined which were without the parasite. Small sample size may have limited discovery of the parasite if the incidence was very low;

one source was represented by only 12 specimens, while two others provided 20 and 25 specimens, respectively. However, one source (Code 18) was sampled four times and the parasite was not seen in histological sections of 161 fish or in wet mounts of ovaries from 25 additional fish. Fish from this source are valuable

for research on transmission of the parasite and as a potential brood stock for new fish farms. This source may have been negative because it is resistant, it has not been previously exposed, or because the environment is not conducive to reproduction or transmission of the parasite.

TABLE 1. Incidence and intensity of infection of *Plistophora ovariae* in the golden shiner from 49 commercial farms and 2 wild sources.

Sample code no.	Sample size	Incidence (% with infection)	Intensity (% ovary affected)	Sample code no.	Sample size	Incidence (% with infection)	Intensity (% ovary affected)
<i>Alabama</i>				<i>Louisiana</i>			
12	36	61	36	26	115	61	24
17	22	45	26	35	135	44	42
13	8	25	48	<i>Mississippi</i>			
14	31	23	13	34	53	62	21
22	31	19	48	47	35	37	36
32	12	0	0	24	60	32	35
<i>Arkansas</i>				33	25	0	0
49	15	87	45	<i>Missouri</i>			
1	62	79	39	39	203	57	25
2	88	75	65	11	131	56	37
16	256	70	44	9	195	51	34
45	22	68	41	7	89	45	48
43	23	61	34	51	25	28	53
36	20	55	51	<i>North Carolina</i>			
46	25	48	32	27	65	45	26
42	32	38	41	<i>Oklahoma</i>			
40	47	36	47	4	2	100	40
44	38	34	52	5	23	100	58
21	30	27	44	8	31	87	39
41	49	27	34	3	25	52	49
23	76	18	51	37*	2	100	—
50	44	11	26	25*	6	33	—
48	31	3	36	<i>Tennessee</i>			
18	161	0	0	38	66	73	18
<i>California</i>				19	15	53	40
28	67	31	30	<i>Texas</i>			
29	20	0	0	30	92	88	24
<i>Kansas</i>				15	18	6	13
6	10	70	44	31	34	3	24
10	20	70	62	<i>Kentucky</i>			
20	52	50	18	52	50	18	18

*Samples from a creek and farm pond in Payne County, Oklahoma.

There were no obvious geographical foci or clinical variations in the incidence or intensity of infection (Table 1). In the four states sampled most frequently, i.e. Alabama, Arkansas, Missouri, and Oklahoma, the variations of the sample parameters both among and between the states were very large. The highest and lowest levels of infection were geographically scattered in kaleidoscope fashion. There was no trend or pattern to the variation among the states.

Relationship to Method of Culture

The golden shiner is propagated either by the extensive or wild spawning practice, or by the intensive or egg transfer method. In the former case, fish spawn on vegetation, and fry and adults remain together for a month or more until harvest or thinning. In the intensive method of culture aquatic plants are eliminated from the spawning ponds by draining, sometimes supplemented with grading. Mats of Spanish moss are provided on which the fish spawn and then are transferred to ponds in which hatching occurs, therefore, eliminating contact between the newly hatched fry and the brood stock.

In the present survey, the method of propagation was known for 2154 fish, of which 421 were produced by the extensive method of culture. The balance, 1733 fish, were from sources using the intensive method of culture. Thirty-three percent of the fish from extensive culture and 32% of the fish from intensive culture were infected. This difference was not statistically significant at the 5% level. In the first survey,⁸ the percentages were 37 and 50% for extensive and intensive culture, respectively, which was a significant difference ($X^2 < P_{.05}$). However, incidence of infection varies with age of the host. A comparison of incidence of infection between intensive versus extensive culture was made for age class 0, 1 and 2 (Table 2). No comparative data was available for age classes 3 to 6. Although the incidence was higher for fish living under extensive culture for each age class, 0, 1, and 2, respectively, the differences were not significant (5% level).

Relationships to Age of Host

Thirty percent of 185 fish hatched in spring of 1968 were infected with *P. ovariae* when sampled at the end of the

TABLE 2. Incidence of infection of *P. ovariae* in the golden shiner as related to age and method of propagation.

Age class	Stratified by method of propagation					Combined data		
	Intensive		Extensive		Chi-square ¹	Total no. exam.	% infect.	Chi-square ²
No. exam.	% infect.	No. exam.	% infect.					
0	109	28	76	33	0.4	185	30	
1	546	38	125	49	2.9	671	40	39.4
2	239	62	79	70	1.4	318	65	
0, 1, 2	894	43	280	50	2.4	1174	45	
3	176	61						
4	47	75						
5	43	33						
6	23	35						

¹ Null hypothesis is that the ratios of infected fish were the same for both methods of culture analyzed separately for each year class, where $X^2_{.05, 1 \text{ d.f.}} = 3.8$.

² Null hypothesis is that the ratios of infected fish were the same for each age class, where $X^2_{.01, 3 \text{ d.f.}} = 9.2$.

first summer of life (Table 2). Young-of-the-year (0 age class) had a lower incidence than any other age class. The incidence increased through the second year of life; it was 30, 40, and 65% in age classes 0, 1, and 2, respectively. The incidence in age classes 0, 1, and 2 were significantly different ($X^2 = 39.4$ where $X^2_{0.01, 2 d.f.} = 9.2$). Much of this difference was attributable to age class 2. Incidence of infection in age classes 3 and 4 were high, 61 and 75%, respectively, but the level was only 33 and 35% in age classes 5 and 6. Selective mortality of infected fish could result in the lower incidence in age classes 5 and 6 although there is no evidence to support this inference.

Fry probably receive the most intense exposure to spores immediately after hatching when they commence feeding. During this time they feed on animal life, or on vegetation, debris and on the mats on which they hatched. Spores, expelled during spawning, would be very abundant on spawning beds at that time. Spawning apparently is the only period when large numbers of spores are released from the host.

The viability of spores in the environment, and the time required from exposure to demonstration of patent infections are unknown. Six sources were sampled twice, one in August (1968) and again in May (1969) (Table 3). All chi-square values, where the expected

values were computed using the hypothesis of equal ratios of infected fish in both years, indicated no significant differences ($P > .05$) between the frequency of infection in the nine month period between August and May. An increase in incidence was expected, as incidence increased from 40 to 65% between age classes 1 and 2 (Table 2). Apparently, incidence does not increase until after spawning, when the spores are available for transmission.

Effects on Fecundity and Growth

Intensity of infection was used as an index of the effect on fecundity. The intensity was estimated as the percentage of the area of a section which contained infected ova or areas containing masses of spores and ova undergoing atresia.

The intensity of infection (Table 4) averaged 37% (range 14 to 61%) of ovarian sections of 1315 infected fish. Loss in fecundity was obviously greater than 37% of any single batch of maturing ova. There always exists a sizeable number of smaller ova, generally only lightly infected, which do not mature in a given spawning season. This results in probable under estimation of loss of fecundity because even where all mature ova were destroyed or heavily infected, a large area of immature ova (development stages 1, 2 and 3) were present. The intensity in the present survey was 8% less than the average intensity in our first survey. This difference is related to the fact that 27% of the total sample in this survey was comprised of fish collected in November and December when intensity of infection was lower. Only 10% of the fish in the first survey were collected in November and December.

The mean ovarian weight from infected fish, stratified by month of collection and age, were larger than the mean ovarian weight of uninfected fish in 13 of 24 comparisons (Table 4). All 7 of the comparisons where the mean weight of infected ovaries was significantly larger than the uninfected ovaries occurred in post-spawning fish collected August, September, and November. Eight of 11 comparisons where the mean weight of the uninfected ovary was larger occurred

TABLE 3. Comparison of the incidence of infection of *P. ovariae* in six populations sampled two successive years.

Year class	1968		1969		X**
	No.	%	No.	%	
63	32	34.4	32	34.8	0.00
65	51	76.5	23	65.2	0.27
66+67	75	54.7	9	66.7	0.22
67	33	54.6	32	68.8	0.53
67	38	55.3	28	46.4	0.28
67	42	45.2	44	63.6	1.33
	271	55.0	159	57.9	0.15

*Computed X² values were all non-significant (5% level).

TABLE 4. Incidence and intensity of infection, body size and weight, K factor, and gonadal weight of golden shiners with and without *Plistophora ovariae*.

Month of age	Number of fish [Ⓛ]	% ovary affected	KTL factor [Ⓜ]		Body length (mm)		Body weight (g)		Ovary weight (g)	
			inf.	uninf.	inf.	uninf.	inf.	uninf.	inf.	uninf.
1968										
August										
0 [Ⓛ]	156 (38)	50	1.00**	.86	98	79	11.9**	4.7	.43**	.14
1	359 (48)	42	1.04	1.02	118**	105	21.5**	15.4	.73	.64
2	65 (63)	42	1.13	.97	128	136	25.8	25.3	.65**	2.09
4	26 (73)	25	.91	.94	129	126	19.8	18.7	.33*	1.17
5	32 (34)	29	1.04**	.99	138	134	28.0*	23.6	1.06	1.03
Sept.										
0	26 (08)	48	1.31**	.94	113**	87	19.2**	6.4	.70**	.10
1	62 (52)	46	1.19*	.83	94**	87	10.4	5.7	.14**	.07
3	52 (50)	18	1.03	1.03	132**	111	25.9**	15.9	.82**	.45
Nov.										
0	162 (27)	29	.82	.81	99**	89	8.0**	6.4	.24**	.18
1	146 (55)	26	.92	.91	116**	99	14.8**	9.6	.51**	.34
2	127 (68)	26	1.04*	1.01	128	126	22.9	20.8	.92	.82
3	75 (79)	21	1.05	1.01	130	127	24.0	21.2	.73*	.57
Dec.										
1	126 (41)	25	.82	.89	98	96	11.4	9.8	.28	.24
3	28 (68)	14	1.09	1.04	113	115	15.9	15.9	.48	.49
1969										
May										
1	313 (16)	47	.91	.91	90	89	7.4	6.9	.60	.67
2	210 (60)	35	1.04	1.04	134**	117	26.4**	19.6	2.11	1.57
3	43 (40)	42	1.09	1.09	150**	129	37.6**	24.3	2.17	1.71
4	37 (62)	19	1.08	1.10	133	129	25.5	24.0	1.62*	2.02
6	23 (35)	42	.87	.87	142*	136	25.1*	21.8	1.15	1.25
16	10 (60)	60	1.29	1.21	187	189	84.1	83.7	6.64	8.59
June										
1	138 (55)	61	1.06	1.09	93	93	9.5	9.5	.55*	.88
2	44 (64)	42	1.00	.99	121*	109	19.9	14.8	1.12	1.55
3	23 (44)	40	1.05	1.11	138	132	23.7*	19.9	1.11	1.26
5	11 (27)	30	.97	.93	130	133	21.5	22.2	.92	.95

[Ⓛ] Figure in () indicates incidence of infection (% of sample with infection).

[Ⓜ] KTL = $W \cdot 10^3 / L^3$, where W = total length in millimeters.

[Ⓝ] Fish 0 age were in the first summer of life, 1 in second summer, etc.

* Indicates difference between the two means is significant at 5% level, ** at 1% level.

in May and June. In prespawning and spawning fish (May and June collections), the parasite reduces the abundance of ripe eggs, thereby accounting for the smaller ovarian weights. However, after spawning, the dense stroma of connective tissue of the infected ovary contributes to a larger ovarian mass than the non-infected ovary. These findings support the identical conclusion of the first survey and help to explain why Summerfelt⁷ found a lower ovarian weight in infected fish. Seventy-two percent of his samples were of pre-spawning or spawning fish.

Plistophorosis did not cause inflammation, or metaplasia. The host reaction to the infection was evidenced by the abundance of macrophages and extensive fibrosis, but there was no cyst formation or isolation of the lesions to specific regions of the ovary.

Mean body lengths of infected fish, stratified by month of collection, were larger than the corresponding mean lengths of uninfected fish in 19 out of 24 comparisons (Table 4). Ten of these 19 means were significantly larger than the corresponding body lengths of uninfected fish. There was no case where the mean body length of uninfected fish was significantly larger than the body length of infected fish. Similar comparisons of monthly means of body weight indicated that in 21 of 24 comparisons infected fish were heavier than uninfected fish. Eleven of these means were significantly heavier than the mean weight of the corresponding group of uninfected fish. In no case was the mean of uninfected fish larger than infected fish. The effect of parasitism was much greater in the first summer of life (age class 0) than the other years. This effect was especially pronounced in the age class 0 fish, for example, in August and September, infected fish were 2.5 and 3.0 times larger than the uninfected fish.

The better growth response in parasitized fish is surprising because the material and energy for spore formation is obviously at the expense of the host. Perhaps the lesions may influence production of sex steroids, or affect the estrogen-gonadotropin interrelationships. Or, because ova are infected early and most

disintegrate before reaching maturity, reduced fecundity may reduce the need for lipoproteins and phospholipids for yolk formation. Both male and female fish usually show reduced growth with the onset of sexual maturity, usually explained as a result of greater diversion of energies into production of gametes.¹

Fourteen of 24 comparisons of monthly means of the condition factor were larger for the infected fish, means in five comparisons were equal (Table 4). Five of the 24 comparisons were significant. Apparently the K factor is not a sensitive index of the parasite influence on the host.

Relationships to Transmission

The parasite remains endemic in a population month after month,⁷ and from one year to the next (Table 3). There is no evidence for spontaneous disappearance. The mode of transmission of *P. ovariae* is unknown but may occur directly from an infected egg to the progeny (i.e., transovarian), or peroral. They are not mutually exclusive possibilities. Shiners do acquire the infection early in life, as 30% of young-of-the-year fish (age class 0, Table 2) were infected by August. Incidence of infection increases with age through the third year (Table 2), indicating continued peroral transmission or the appearance of latent infections. Two previous experimental efforts at transmission by feeding spores to adult fish were unsuccessful.^{7,10}

A frequency distribution of the number of samples with specific incidence of infection (Figure 1) indicates a pond population may have a few to many infected fish, with a central tendency between 30 and 70%. Similarly, the percentage of ovary affected reaches a peak between 31 and 40%. There was a nonsignificant ($P > .05$) positive correlation ($r = .08$) between incidence and intensity of infection; using incidence and intensity in samples for 44 sources. Autoinfection, where spores germinate in the ovary where they were formed, or repeated schizogonic cycles could cause a progressive increase in intensity thereby making high levels of intensity in specimens in populations where the incidence may be very low.

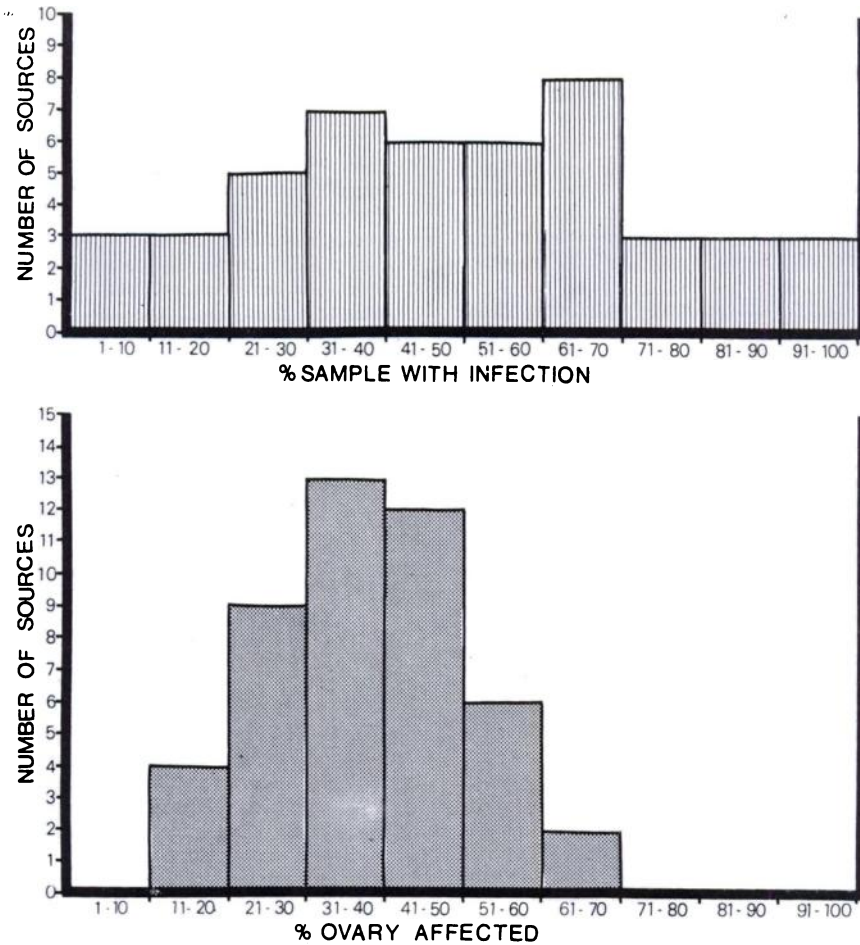


FIGURE 1. Frequency of infection (upper) and percentage of ovary affected by the infection (lower).

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