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GLOCHIDIOSIS OF SALMONID FISHES. III. COMPARATIVE SUSCEPTIBILITY TO NATURAL INFECTION WITH *MARGARITIFERA MARGARITIFERA* (L.) (PELECYPODA: MARGARITANIDAE) AND ASSOCIATED HISTOPATHOLOGY*

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ABSTRACT: The comparative susceptibility of 4 species of salmonid fishes, 30.5 to 87.0 mm in fork length, to the glochidia of the freshwater mussel (*Margaritifera margaritifera*) was determined by examination of 594 caged and 178 uncaged (native) fish for infection. Of the caged fish, 99% of the chinook salmon (*Oncorhynchus tshawytscha*), 75% of the coho salmon (*Oncorhynchus kisutch*), 88% of the cutthroat trout (*Salmo clarki*), and 95% of the steelhead trout (*Salmo gairdneri*) were infected. There was a similar relationship in infection incidence in the native fish species. Mean infection intensities in the caged and native fish were: 446 and 399 for chinook salmon, 8 and 24 for coho salmon, and 72 and 88 for steelhead trout, respectively, and 212 for caged cutthroat trout (native juvenile trout were not captured). Glochidia completed development in mussels in the Siletz River, Oregon, in 13 days at an average water temperature of 12.8 C. They were released by these mussels from 13 May to 15 June 1971. During development in fish, the parasites increased in length by 500% from an initial size of 70 to 75 μm. Encysted parasites occurred in the gill filaments, arches, rakers, and occasionally in the pseudobranchs of all fish species; but most were in the lamellae of the filaments. Initially, the cyst walls were approximately 15 μm in thickness, but as the parasites increased in size the exposed part of the wall became thinner. Up to 15 lamellae may be fused to the wall. Except for lamellae "grasped" by the parasites, blood apparently continued to flow through capillaries of the fused lamellae, but these lamellae, except the outermost ones, probably no longer functioned in respiration. Parasites encysted on the sides of gill filaments restricted blood flow by "pinching" the arterioles. Large encysted parasites on the lamellae increased the physiological dead space in the water flow. Clubbing of the filaments resulted when large parasites were located distally. These pathological changes in heavy infections may result in early death of fish by asphyxiation. In less heavy infections, the invading or exiting parasites may provide portals of entry for fungi, and delayed mortality may occur from secondary infection.

The first two papers in this series reported on the comparative susceptibility of salmonid fishes to experimental infection with *Margaritifera margaritifera* (Meyers and Millemann, 1977) and on tissue reactions of coho (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) to experimental infections (Fustish and Millemann, 1977). This paper reports the results of a study on the comparative susceptibility of salmonid fishes to natural infections with *M. margaritifera* and on development of the parasites in these fish and the associated histopathology. Some observations on relationship of temperature and development of glochidia in mussels are also reported.

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MATERIALS AND METHODS

Study area

The area was near river mile 21 on the Siletz River, a coastal stream in western Oregon. It was 365 m long, about 35 m in width, and extended from a small island to a deep pool downstream. The nearly flat stream bottom was a stable sand-gravel mixture. Water depth varied from approximately 1.0 m in the summer to at least 4.5 m during the winter, and fluctuated 0.3 m or more with the tidal cycle. Water temperature was recorded continuously from 6 March to 24 July 1971 by a thermograph placed on the stream bottom in the center of the area.

We found approximately 100,000 *M. margaritifera* adults concentrated on the bottom in rows approximately 1.0 m wide, perpendicular to the stream flow. Densities that exceeded 400 mussels/m^2 were common.

Fish

The fish, which were to be put in submersible cages, had no known previous contact with the glochidia of *M. margaritifera*. They were alevin fall chinook and coho salmon obtained from the Oregon Department of Fish and Wildlife's Bonneville and Alsea River Salmon Hatcheries, respec-

tively, and cutthroat (*Salmo clarki*) and winter steelhead trout (*Salmo gairdneri*) from the Alsea Trout Hatchery. Their mean for lengths in millimeters with ranges in parentheses were: chinook salmon 64.0 (52.5 to 77.0); coho salmon 59.0 (41.5 to 87.0); cutthroat trout 55.5 (41.5 to 73.0); and steelhead trout 51.0 (38.5 to 57.5).

Four 76 by 76 cm wood frame cages covered with hardware cloth with meshes of 3.2 mm were used to hold the fish. They were suspended approximately 1.5 m below the water surface at intervals of 4.5 m in the pool on the downstream edge of the study area.

Uncaged (native) fish in the study area at the time were alevin chinook and coho salmon, alevin and adult steelhead trout, adult cutthroat trout, prickly sculpins (*Cottus asper*), threespine sticklebacks (*Gasterosteus aculeatus*), largemouth bass (*Micropterus salmoides*), Pacific Lampreys (*Entosphenus tridentatus*), and western brook lampreys (*Lampetra richardsoni*).

Field and laboratory procedures

Adult mussels were collected from the study area biweekly from 20 February to 17 April, weekly from 24 April to 13 May, and about every 4 days from 16 May to 17 June 1971. We examined 317 mussels in the laboratory for sex determination and presence of glochidia, and 1,319 mussels in the field for presnce of eggs, early larval stages, or glochidia.

On 15 May 1971, groups of 325 chinook salmon, coho salmon, cutthroat trout, and steelhead trout were placed in the cages, one group per cage. These fish were not fed after placement in the cages. Seven live fish were taken from each cage every 2 days from 16 May through 11 June, every 4 days from 11 June through 23 June, and every 8 days thereafter until no fish remained in the cages or until 17 July when the experiment was terminated. In the laboratory, the fish were killed and 2 were put in Bouin's fixative for subsequent histological examination, and the remaining 5 examined immediately for glochidial enumeration or put in 10% neutral formalin for later enumeration. Dead fish were also removed from the cages at the sampling times and preserved in 10% neutral formalin.

Free-swimming (native) fish from the study area were killed and preserved in 10% neutral formalin for subsequent examination.

Gills were dissected from the fish and the glochidia in each holobranch counted under a microscope. Glochidia were not counted in fish with bilateral fungal infections of the holobranchs, but were counted in the uninfected gills of those with unilateral infections and the count doubled for an estimate of the total number present. These estimates are indicated in the text by the notation $2\times$ in parentheses.

Gills from caged fish were used to study the development of the parasites and for the associated histopathology. The entire gill tissue was taken for histological sectioning from: chinook salmon on exposure days 1, 5, 15, 23, 31, 35, and 39; coho salmon on days 9, 35, and 39; cutthroat trout on days 13, 15, 35, and 47; and from steelhead trout on days 5, 15, 35, and 39. Serial sections 7 to 8 μm thick were cut and stained in hematoxylin and eosin. Gill tissue from uninfected laboratory-reared fish of each species served as controls.

Because we could not determine the age of encysted parasites in the native fish, we defined arbitrarily 4 stages of parasite development to describe the infection. Recently encysted parasites were the first stage; larger parasites that measured 120 and 210 μm in cross-sectional height were the second and third stages; and the fourth stage was defined as parasites that measured 240 μm or more in length. Cross-sectional measurements were made from the hinge ligament to the ventral edge of the larval shell, and length was measured at the greatest distance perpendicular to the height measurement in lateral view. All measurements were made with an ocular micrometer.

RESULTS

Glochidia development in mussels

Eggs and glochidia were first observed in the marsupia of mussels from the study area on 30 April and 13 May 1971, respectively. Gravid mussels were not found after June 15. The average water temperature from 30 April to 13 May was 12.8 C, and from 13 May to 15 June it was 13.6 C. The average temperature for 54 days before 30 April was 8.5 C. These results agree with those of Murphy (1942) who found that *M. margaritifera* glochidia in the Truckee River, California, required 12 days to complete development at an average water temperature of 13.1 C.

The hookless, subovate glochidia of *M. margaritifera*, which are surrounded by a thin egg shell when released by the female, are smaller than those of many other freshwater mussels. The length and height of mature glochidia raged from 70 to 73 and from 75 to 80 μm. The average distance across the opened shell valves was 105 μm.

Fish susceptibilty

Including live and dead salmonid fish, 509 of 594 (86%) caged and 166 of 178 (93%) native fish were infected. On the basis of incidences and infection intensities, the fish can be ranked in order of increasing resistance to infection as: chinook salmon, cutthroat trout, steelhead trout, coho salmon (Table I, II; Fig. 1).

TABLE I. *Incidence and intensity of* Margaritifera margaritifera *infection in live caged alevin fish.*

Species	Exposure period* (days)	Incidence†	Intensity (mean No.)	Percent with infection intensities: <100	100–250	251–500	501–1,000	>1,000
Chinook salmon	39	77/78	446	36	13	19	17	15
Coho salmon	63	71/95	8	100	0	0	0	0
Cutthroat trout	55	79/90	212	65	14	9	9	3
Steelhead trout	47	76/80	72	86	10	3	0	1

* Exposure periods varied because of differential mortalities; for example, on day 40 there were no chinook salmon left in the cage.
† Numerator = number infected; denominator = number examined.

The maximum mean numbers of parasites found in any one sample of live caged chinook salmon, cutthroat trout, steelhead trout, and coho salmon were 1,094, 633, 130, and 16, respectively. They occurred in chinook salmon, coho salmon, and cutthroat trout on exposure day 17 and in steelhead trout on day 11.

Of the native chinook salmon, which ranged in fork length from 40.5 to 69.5 mm with a mean of 52.0, 32% had less than 100 parasites, 13% had 100 to 250, 25% had 251 to 500, 25% had 501 to 1,000, and 5% had more than 1,000 parasites. The highest infection intensities found in a caged and a native fish (both were dead) were 2,894 (2 ×) and 2,288 (2 ×) parasites, respectively. This is in contrast with 1,695 and 1,606 (2 ×) parasites found in a caged and a native fish that were alive and appeared healthy.

Only one native cutthroat trout was collected. It was an adult that was 185.5 mm in fork length and had 102 parasites (Table II). The highest number of parasites found in a caged fish, which was moribund, was 2,212 (2 ×); whereas, an actively swimming caged fish had 1,420 parasites.

Of the native steelhead trout, which ranged in fork length from 31.0 to 42.0 mm with a mean of 35.5, 74% had less than 100 parasites, 20% had 100 to 250, and 6% had 251 to 500 parasites. The highest infection intensities found in a caged and a native fish were 2,016 (2 ×) and 439 parasites, respectively. Both fish were alive, but the former was moribund.

Of the native coho salmon, which ranged in fork length from 30.5 to 69.5 mm and averaged 46.0, 94% had less than 100 parasites, and the rest less than 200. The highest infection intensity found was 162 parasites.

Approximately the same numbers of parasites occurred on the right and left holobranchs of live native fish. Numbers for the right and left

TABLE II. *Incidence and intensity of* Margaritifera margaritifera *infection in native fish in the Siletz River, Oregon.**

Date	Chinook salmon		Coho salmon		Cutthroat trout		Steelhead trout	
	Incidence†	Intensity‡	Incidence†	Intensity‡	Incidence†	Intensity‡	Incidence†	Intensity‡
May 22	–	–	6/8	4	–	–	–	–
24	2/2	276	1/1	–	–	–	–	–
28	1/1	–	6/6	47	–	–	–	–
30	5/5	421	2/2	42	–	–	–	–
June 1	1/1	–	7/7	56	–	–	–	–
3	3/3	385	–	–	–	–	–	–
4	59/59	362	19/21	6	–	–	35/35	118
7	1/1	–	–	–	–	–	–	–
11	–	–	–	–	1/1	–	–	–
19	2/4	100	2/5	0.4	–	–	13/16	5
Totals	74/76	399	43/50	24	1/1	–	48/51	88

* The cutthroat trout was 185.5 mm in fork length; the other species ranged in length from 30.5 to 69.5 m.
† See second footnote, Table I.
‡ Mean number.

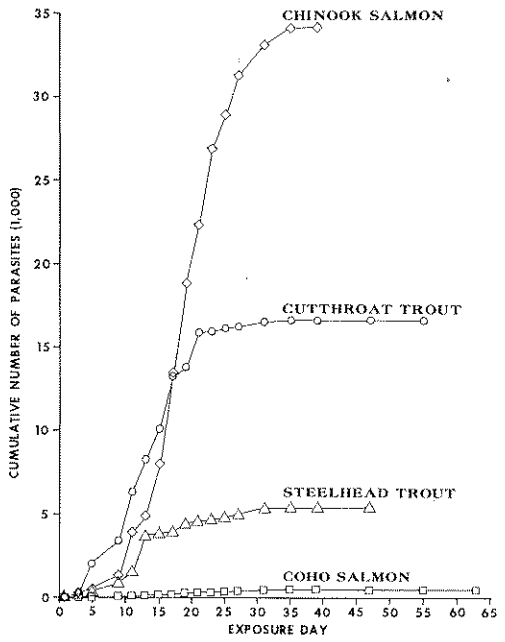


FIGURE 1. Cumulative infection levels in fish exposed continuously to *Margaritifera margaritifera*. Each point represents a sample of 5 fish.

holobranchs of chinook salmon, coho salmon, cutthroat trout, and steelhead trout were: 12,323 and 12,902; 514 and 495; 49 and 52; and 2,053 and 2,150, respectively. The same comparison was not made for caged fish because of insufficient time.

Of the caged fish, 203 chinook salmon, 199 steelhead trout, 189 cutthroat trout, and 177 coho salmon died during the study. Decomposition precluded examination of some of these fish, but of those examined for infection the following incidences and mean intensities (in parentheses) were found: chinook salmon, 103 of 105 (660); cutthroat trout 41 of 48 (309); steelhead trout, 38 of 40 (14); and coho salmon, 24 of 58 (10).

The greatest daily mortality of chinook salmon was 14 which occurred on exposure day 21. During the last 26 days, the approximate mean daily mortality was seven fish. The highest numbers of daily deaths of cutthroat trout were 16 and 8, which occurred on days 13 and 47. Less than five steelhead trout and coho salmon died during the first 31 days of exposure. Thereafter, their daily mortalities increased to 24 for steelhead trout and 9 for coho salmon, and then declined.

There was no food in the gastrointestinal tracts of 25, 46, 58 and 65% of the dead chinook salmon, cutthroat trout, steelhead trout, and coho salmon, respectively. At least one holobranch of 82% and 61% of chinook salmon and cutthroat trout was infected with fungi, but only 12% of the steelhead trout and 9% of the coho salmon were infected.

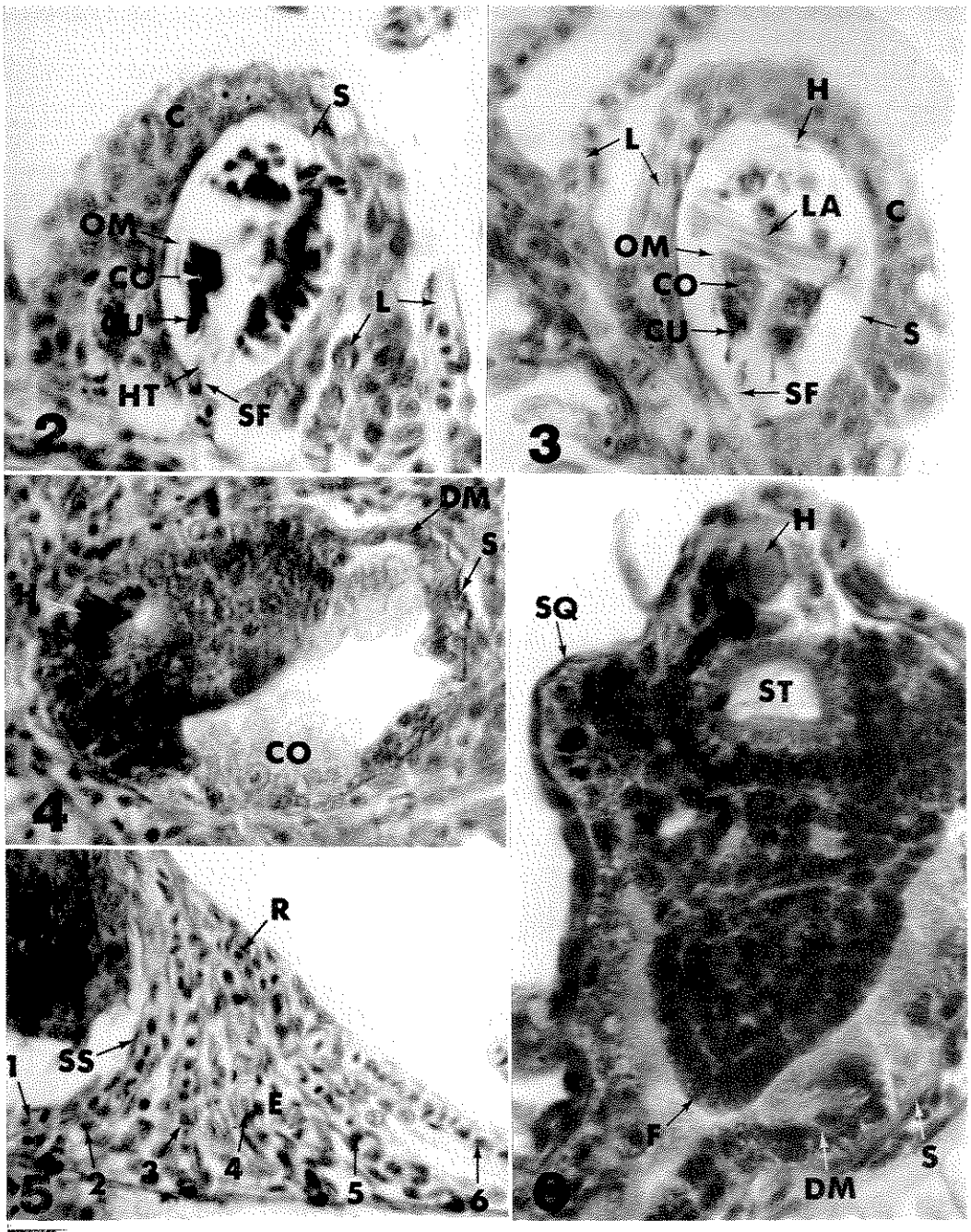
The cause of death of steelhead trout and coho salmon cannot be attributed to heavy glochidial or fungal infections. Their mortalities may have been caused by starvation or other parasites. Furthermore, 86 and 80% of the steelhead trout and coho salmon died after exposure day 31, when glochidia were no longer being released by mussels, but 78 and 48% of the chinook salmon and cutthroat trout died before day 31, probably because of their heavy parasite and secondary fungal infections. All moribound chinook salmon and cutthroat trout also had fungal infections in addition to heavy parasite infections. After exposure day 31, starvation or parasites other than glochidia may have contributed to their deaths.

The total and mean numbers of parasites in five of five native threespine sticklebacks, which averaged 63.0 mm in fork length, were 1,122 and 224, respectively. The highest infection intensity was 495 parasites in the gills of a dead fish.

Native prickly sculpins were relatively resistant to infection. Eight parasites were attached to, but not completely encysted in the gills of 3 of 11 fish, which averaged 77.0 mm in fork length. Also, 21 unattached glochidia were present in a mucus secretion between the gill filaments of these fish.

Parasite development and histopathology

We found no differences in parasite development and associated histopathology in the gills of the four species of caged and native salmonids. The well-developed hyperplasia observed by Fustish and Millemann (1977) in their parasitized coho salmon was not observed by us. Although we did not make a thorough examination of fins, we found three parasites, presumably *M. margaritifera*, but not sectioned for positive identification, between the pectoral and caudal fin rays of a heavily infected chi-



FIGURES 2–6. Cross sections of *Margaritifera margaritifera* encysted in gill filaments of chinook salmon. **2, 3.** Young parasites. × 582 and 579, respectively. **4.** Older parasite approximately 120 μm in size. Note increase in size of columnar cells of inner (glochidial) mantle layer (cf. Figs. 2, 3). × 531. **5.** Note side of cyst wall composed of 6 lamellae (1–6). × 544. **6.** Note squamous layer of wall that is several cells in thickness and covers the exposed part of the parasite. Approximately 210 μm in size. × 675. Abbreviations: C, cyst wall; CO, CU, columnar and cuboidal cells of inner mantle layer; DM, definitive mantle; E, epithelial cells; F, foot; G, glochidium; H, hinge ligament; HT, host tissue; L, lamellae; LA, glochidial adductor muscle; OM, outer mantle layer; R, red blood cells; S, shell; SF, shell flange; SQ, squamous layer of cyst wall; SS, simple squamous epithelium; ST, stomach.

nook salmon. Meyers and Millemann (1977) found *M. margaritifera* on the fins of experimentally infected coho and chinook salmon. Harms (1907, 1909) reported that *M. margaritifera* was exclusively a gill parasite of European cyprinids, but Baer (1951) stated that they were always found on the fins of cyprinids.

The term cyst in this discussion includes the parasite and the surrounding wall of host origin. Cysts occurred on gill filaments, arches, rakers, and occasionally the pseudobranchs. In coho salmon, approximately 65% were on the rakers, but in the other species they occurred more frequently on the lamellae or sides of the filament.

Initially, parasite attachment usually involved only one lamella. Parasites in lamellae are covered by stratified squamous epithelium; those on the filament sides by stratified cuboidal or squamous epithelium; and those in gill arches and rakers by stratified cuboidal epithelium. The last parasites have more compact and thicker walls than those on the filaments.

The walls, which are formed by host epidermal cells of recently encysted parasites in the filaments, are not of uniform thickness and average 15 μm (Figs. 2, 3). One lamella from each side may be fused to the wall. At this time, the parasites have not grown or begun metamorphosis into juvenile mussels. The glochidial abductor muscle, mantle, shell flanges, and enclosed host tissue are present (Figs. 2, 3). These structures were also observed by Young (1911) and Lefevre and Curtis (1912) in *Lampsilis ligamentina* encysted in fish gills. Spaces were common in the parasites in our sections where tissue had separated from the calcareous shell, probably a result of fixation.

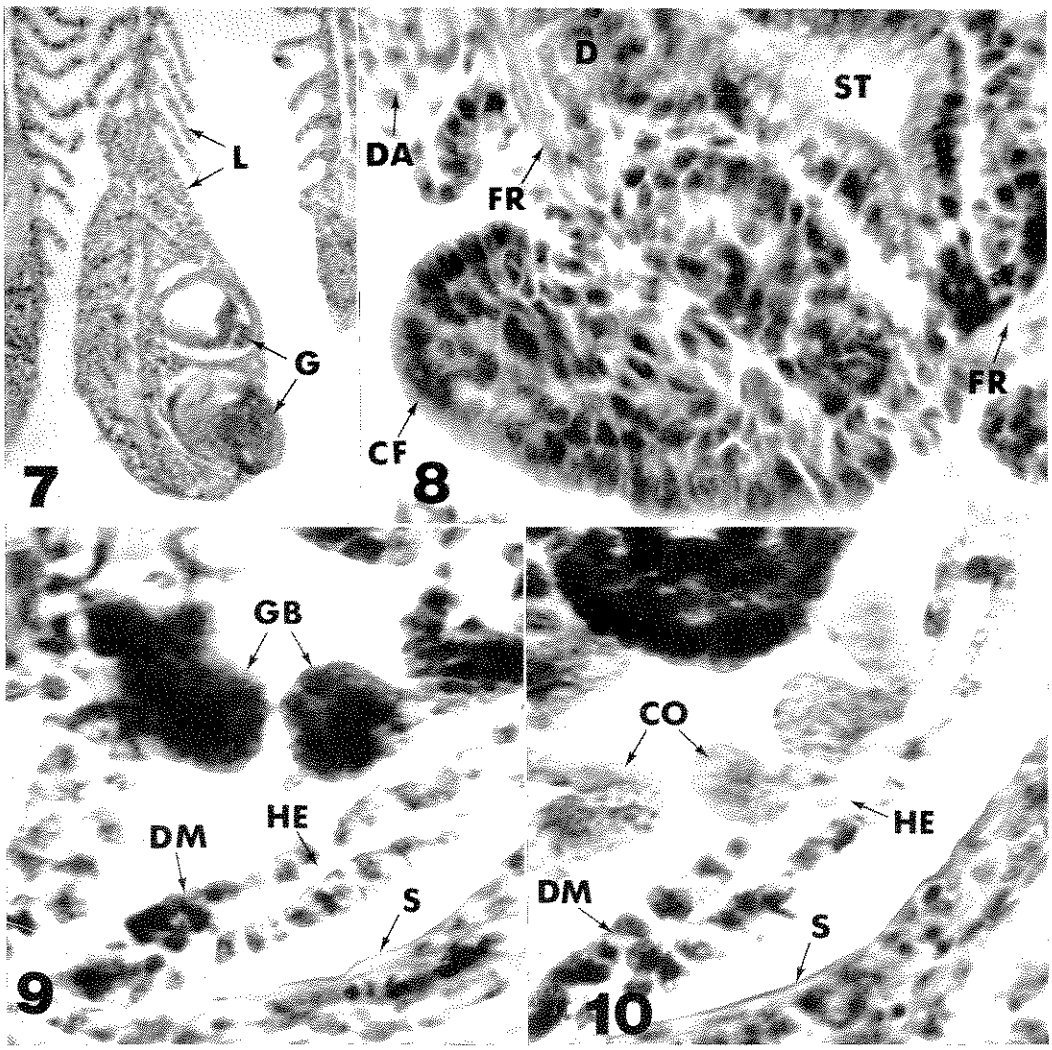
The glochidial mantle of *M. margaritifera* is composed of outer and inner layers (Figs. 2–4). The outer layer, which consists of simple squamous cells and is located next to the shell, will form the definitive mantle. The inner layer is composed of columnar and cuboidal cells, 10 by 3 and 5 μm in size, respectively.

We observed host tissue "pinched" between the two shell flanges, which are on the ventral edge of the shell. The host tissue and flanges are in contact with the cuboidal cells of the inner mantle layer. The cytoplasm of the inner layer cells is darkly basophilic and granular. Arey (1932b) speculated that granules in the inner mantle layer of *Lampsilis luteola* were associated with enzymatic digestion. The granules in *M. margaritifera* may have a similar function.

When *M. margaritifera* is about 120 μm in height, two to four lamellae from each side are usually fused with the cyst wall, and epithelial cells fill the interlamellar spaces (see Fig. 5 of a later developmental stage). Except for the lamella that is "grasped" by the parasite, those that are part of the well retain their structure. Blood apparently flowed in the capillaries of these lamellae as indicated by the presence of red blood cells in the vessels (Fig. 5). Host tissue "grasped" by the parasite and also the glochidial abductor muscle have been broken down and apparently absorbed. A prominent hinge ligament and the definitive mantle are present at this time (Fig. 4). Also, the columnar cells of the inner mantle layer have increased in size and now take a lighter basophilic stain than formerly (Fig. 4). According to Young (1911) and Arey (1932b), these cells function in the absorption and digestion of glochidial and host tissues. The cyst is covered by squamous epithelium, and the exposed part of the wall has become thinner and averages about 8 μm in thickness.

When the parasite is approximately 210 μm in height, usually five lamellae from each side are fused with the wall (Fig. 5), but parasites close to each other have fewer than five. At least the first two lamellae on each side take the shape of the nearly spherical parasite (Fig. 5). The innermost lamella is next to the parasite's shell, and only a simple squamous cell layer separates the lamellar capillary beds from the shell (Fig. 5). Blood apparently continued to flow in these capillaries because they contain red blood cells. A layer of two to three squamous cells covers the exposed part of the cyst; and the definitive foot, mantle, and anlage of the intestinal tract within the mesosomal area of the parasite are present at this time (Fig. 6).

Clubbing of the gill filament results when one or more large parasites are located terminally on the filament (Fig. 7). Lamellae on the parasitized side are fused to the cyst wall and those on the other side are also fused to each other (Fig. 7). The filament tip may bend and fuse to the wall (Fig. 7). Large cysts may also displace lamellae on adjacent filaments.



FIGURES 7–10. *Margaritifera margaritifera* encysted in gills of salmonid fish. **7.** Frontal section of chinook salmon filament. Note clubbing of lamellae. × 156. **8.** Sagittal section of early juvenile from steelhead trout. × 670. **9, 10.** Cross sections of early juveniles from coho salmon. Note increase in size and mushroom shape of columnar cells of glochidial mantle. × 680. Abbreviations: CF, ciliated foot; CO, columnar cells; D, digestice gland; DA, definitive adductor muscle; DM, definitive mantle; FR, foot retractor muscle; G, glochidium; GB, gill buds; HE, hemocoel; L, lamellae; S, shell; ST, stomach.

Parasites larger than 210 μm occurred more frequently on chinook salmon and cutthroat trout than on coho salmon and steelhead trout. Approximate lengths and widths, excluding the wall, in μm of the largest parasites from each fish species were: 270 by 160, chinook salmon; 265 by 200, coho salmon; 350 by 250, cutthroat trout; and 275 by 165, steelhead trout. Probably as a result of fixation or sectioning, the parasite body is frequently withdrawn from the shell, which usually remains attached to the wall.

The exposed part of the wall of parasites larger than 240 μm is covered by squamous epithelium, which is now only one or two cells in thickness. The rest of the wall, which may

consist of 10 to 15 fused lamellae, has decreased in thickness because of a reduction in the number of interlamellar cells.

Parasites larger than 240 μm are juveniles. A nearly complete digestive system of stomach, digestive gland, and intestine, a pair of abductor muscles, nervous system, and a ciliated foot with protractor and retractor muscles are present (Fig. 8). Also gill buds and a hemocoel which has developed within the definitive mantle may be present (Fig. 9). The columnar cells of the glochidial mantle are still present and are now mushroom-shaped (Fig. 10). Arey (1932b) found them in free-living juveniles of *Lampsilis anodontoides* and *L. luteola*.

During development our parasites increased in length by 500%. Murphy (1942) reported an increase of 660% for *M. margaritifera* in salmonid fishes in the Truckee River, California.

We found empty cysts with ruptured walls in some sections. The juvenile mussel breaks the wall with its foot, so thinning of the wall probably aids in excystation.

DISCUSSION

Our findings that chinook salmon are more susceptible than coho salmon to natural infection with *M. margaritifera* agree with those of Meyers and Millemann (1977) on the susceptibility of these species to experimental infection. In our study, cutthroat and steelhead trout were intermediate in resistance among the four species and the former was more susceptible than the latter. Meyers and Millemann could not compare their trout with their salmon because of size differences, but on the basis of 70-day mortality data, their cutthroat trout were more susceptible than steelhead trout. However, the relationship is reversed when mortalities of the two species are compared at the end of 48 hr.

The differences in susceptibility to infection of our fish could be attributed to differences in: (1) position of the cages such that each species was not exposed to equal numbers of parasites; (2) gill morphology; (3) ventilating rate; (4) behavior; (5) time of occurrence of parr-smolt metamorphosis; (6) chemical composition of gill mucus or blood; and (7) host response.

We found no evidence in support of the first four hypotheses. About the fifth hypothesis, Hoar (1951), Malikova (1957), and Evropeitseva (1957) reported that Atlantic salmon (*Salmo salar*) smolts have a lower resistance to injury and disease than parrs. Of our fish, chinook salmon was the only species that could have been undergoing parr-smolt metamorphosis, and whether or not they were could not be determined. The other species usually metamorphose at a larger size, which is attained after one or more years in fresh water. Physiological changes associated with parr-smolt metamorphosis may lower resistance of salmonids to glochidiosis, but experimental evidence for this hypothesis is lacking.

Regarding the sixth hypothesis, the nonswimming glochidia undoubtedly reach the gills passively in the ventilating current, and attachment probably results from physical contact with the gills (Murphy, 1942). Tips of adjacent hemibranchs touch during normal respiration (Hughes, 1966), which would facilitate attachment. Differences in chemical composition of gill mucus or blood of fish could affect attachment and development of the glochidia.

Finally, natural and acquired resistance of fish to glochidiosis may also have a tissue basis as suggested by the studies of Arey (1932c, d) and Fustish and Millemann (1977). Arey observed basophilic spherules, eosinophilic plastids, and eosiniphils around and in the glochidia of *L. anodontoides* in naturally resistant largemouth bass and the glochidia of *L. luteola* in largemouth bass with acquired resistance. The glochidia in both cases were either destroyed by cytolysis or sloughed by the second day of infection.

Fustish and Millemann (1977) found that previously unexposed coho salmon experimentally infected with *M. margaritifera* sloughed the parasites by 4.5 days postinfection by a well-developed hyperplastic reaction. No such reaction was seen by them in the gills of chinook salmon, which retained the parasites for 12 weeks through complete metamorphosis to juvenile mussels. The occurrence of such a well-developed tissue defense mechanism in coho salmon and its absence in chinook salmon would explain the differences in infection rates found in our caged and native fish. The degree of tissue response by our other fish species could account in part for their position in the spectrum of susceptibility to infection. Sim-

ilarly, Murphy's (1942) results with *M. margaritifera* infections in brook trout (*Salvelinus fontinalis*), Tahoe suckers (*Catostomus tahoensis*), "black minnows" (*Rhinchthys osculus robustus*), and Lahontan redsides (*Richardsonius egregius*) could be explained. He produced heavy initial infections in these fish but only a few parasites survived to complete metamorphosis.

This is the first detailed description of the metamorphosis of *M. margaritifera* and the histopathology associated with infection. Young (1911), Lefevre and Curtis (1912), and Arey (1924, 1932a, b, c, d) described the metamorphosis and histopathology of infections caused by *Lampsilis recta*, *L. ligamentia*, *L. luteola*, *L. anodontoides*, and *Unio complanatus* in the gills of freshwater fishes. Lamellar fusion is not as extensive in these infections as it is in *M. margaritifera* infection. Leferve and Curtis (1912) reported "smoothing" or obliteration of lamellae near the cysts of *L. ligamentina* in largemouth bass. Examination of sectioned tissue might have revealed fusion. Arey (1932a) stated that lamellae adjacent to *L. anodontoides* and *L. luteola* may fuse to form the basal part of the cyst, but he did not give the number of lamellae involved. The glochidia of these species, however, do not increase in size during metamorphosis as do those of *M. margaritifera* and, thus, lamellar fusion may be limited.

The walls of fully formed *M. margaritifera* cysts consist almost entirely of epithelial cells. Young (1911) found the same for *L. ligamentina*. Arey (1932a) reported that cysts of *L. luteola*, whose glochidia are about 3 times larger than *M. margaritifera*, consisted of epithelial cells and connective tissue. Apparently, larger glochidia "bite" deeper into host tissue and the cysts become embedded in the stromal layer.

Reduction or complete cessation of blood flow undoubtedly occurs in lamellae "grasped" by *M. margaritifera*. Such a reduction may be compensated for either by an increase in blood flow through other lamellae, because pathways are not fully utilized, or by shunting of blood through alternate nonrespiratory capillary beds within the filament (Randall, 1970). Although these compensatory mechanisms may be operative during infections, in heavy infections respiration might still be impaired. As *M. margaritifera* grows, adjacent lamellae are fused to the wall and frequently lamellae on the nonparasitized side are fused together. About 15 lamellae may be attached to the wall of a parasite 250 to 350 μm in length. The respiratory function of these lamellae, except perhaps for the outermost ones (e.g., No. 6, Fig. 5), probably is impeded. Because of the distance involved, it is unlikely that oxygen can diffuse into the fused lamellae. Also, the physiological dead space in the water flow over the gills, as described by Hughes (1966), probably increases near the large parasites because they displace lamellae on the attached and adjacent filaments. This would cause an increase in the volume of nonrespiratory water flow.

We could not determine if *M. margaritifera* utilizes for nutritional purposes its close association with the lamellar capillary beds. Because they grow, however, such a relationship seems likely.

We frequently found *M. margaritifera* encysted on the sides of gill filaments in the epidermal tissue that covers the afferent and efferent filamental arteries. This tissue is about 10 to 15 μm in thickness, and so the parasites undoubtedly constrict the blood flow in the arteries. Arey (1924, 1932a) reported that the hookless glochidia of *L. luteola* "cut" through the epithelium and "pinched" the filamental blood vessels. Matteson (1948) also reported that the hookless glochidia of *Elliptio complanatus* "pinched" the filamental arteries and the blood was blocked. In heavy infections, fish died from asphyxiation.

Margaritifera margaritifera in the gill arches and rakers are in the epithelium covering the structures. Because the branchial arteries are in connective tissue of the arch, blood flow cannot be impeded by "pinching" of the parasites. Also, we doubt that these parasites interfere with the water flow over the gills.

In conclusion, we believe that the respiratory function of the gills is markedly impaired in heavily infected fish. Reduction of blood flow in lamellar capillaries and filamental arteries from parasite attachment, and a decrease in functional lamellae from fusion probably represent the main stresses. However, the subtle effect of an increase in the physiological dead space caused by large cysts may also cause

respiratory stress because fish would have to pump more water over their gills to maintain an adequate blood oxygen level. If respiration is severely restricted, fish will die of asphyxiation. Murphy (1942) and Matteson (1948) stated that this was the principal cause of early death in their heavily infected fish.

We could not determine if our fish acquired the number of parasites needed to cause early death. All of the moribund and most of the dead caged chinook salmon and cutthroat trout also had external filamentous fungal infections, which probably caused or would cause their deaths. The invading or exiting parasites may have provided portals of entry for fungi or weakened the fish, making them more susceptible to fungal infection. Murphy (1942) and Matteson (1948) ascribed the delayed deaths of their less heavily infected fish to secondary fungal or bacterial infections. As stated previously, our steelhead trout and coho salmon probably died from starvation and not directly from infection.

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