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CADMIUM SULFIDE AND MERCURIC SULFIDE FOR MARKING SEA LAMPREY LARVAE

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Control of the sea lamprey (Petromyzon marinus) in the Great Lakes has been undertaken by chemical eradication of the larvae in tributary streams (Applegate et al., 1961). Knowledge of certain aspects of the larval stage, which might expedite control, is not complete, however. Among the incompletely understood features of the larval stage are duration of larval life, population structure, and migratory habits. Studies of these and other phases of larval life would be facilitated by an identifying mark which: (1) can be applied easily and quickly; (2) remains readily visible; (3) causes little mortality; and (4) has no effect on normal development. Subcutaneous injections of cadmium sulfide and mercuric sulfide, as described by Wigley (1952), may meet these requirements. Smith and McLain (1962) also described briefly the use of water insoluble dyes to mark sea lampreys. The present paper presents the results of one aquarium and three field studies which have helped to evaluate dye injections as a method of marking ammocoetes.

Methods and materials

The marking technique was described by Wigley (1952); the field methods employed in the capture, handling, and release of ammocoetes were developed by us. All ammocoetes used in our studies were collected from streams with a 220-volt direct-current shocker. Ammocoetes were marked with subcutaneous injections of cadmium sulfide (yellow) or mercuric sulfide (red), which are insoluble in water, formalin, or alcchol. The powdered dyes were added to equal volumes of water and injected by means of a 2.0-cc. hypodermic syringe with a sharp needle (No. 20 or 22). The ammocoete to be marked was anesthetized and placed in a shallow groove on a marking board, where it was held in place with a damp sponge. The needle was inserted between the skin and muscle and then pushed along under the skin for a distance of 1/4 to 1/2 inch in either an anterior or posterior direction (Fig. 1). The mark was produced by withdrawing the needle slowly while applying a slight pressure to the syringe plunger. The resulting mark appeared as a readily visible line of pigment, 1/32 to 1/16 inch wide and 1/4 to 1/2 inch long. Marks were located on: (1) the right or left side of the body, 1/4 to 1/2 inch posterior to the last gill opening (anterior mark), or (2) the right or left side of the body immediately anterior to the anal opening (posterior mark). Doubtless the mark could be located elsewhere.

The marking of larvae with dye injections had some faults: (1) it was very difficult to mark ammocoetes shorter than 2 inches (in the studies described, no larvae under 2 inches were marked);

Figure 1.--Marking a sea lamprey ammocoete with a subcutaneous injection of mercuric sulfide.

(2) extreme care was necessary to avoid puncturing the abdominal cavity or injecting air along with the marking solution; and (3) the suspension of dye and water rapidly clogged the needles, making frequent replacement of needles necessary.

For field operations, ammocoetes were collected, marked, and then released in streams. A crew of five men was involved; two collected larval lampreys (usually of several species, but only sea lampreys were marked) from the stream and placed them in live cages, while the other three did the marking. In marking, the ammocoetes were removed from the live cages and anesthetized with tricaine methanesulfonate (M.S. 222); sea lamprey larvae were then separated from the other species, and measured, marked, and returned to live cages. The party marked about 100 larvae per hour. After the ammocoetes had recovered from anesthesia, they were released near the locality of collection. The interval between collection and release did not exceed 4 hours.

Marks on lampreys recaptured as larvae or newly metamorphosed adults were usually detected by external examination with the unaided eye. The marks appeared as a complete or broken line of color, specks of color, or a discolored area of skin. In the aquarium study, the larvae were examined while alive (or shortly after death) by one observer. In the field studies, larvae recovered after the marking were preserved in 10-percent formalin and later examined externally by two observers; lampreys with questionable marks were cross sectioned and examined internally.

Persistence of marks and mortality in aquariums

Larvae were held from July, 1957 to July, 1960 to study persistence of marks and mortality of lampreys held in aquariums. Two lots of 50 larvae were anesthetized, measured, and marked; one lot was marked with cadmium sulfide (posterior region) and the second with mercuric sulfide (posterior region). A third lot of 50 (the control) was anesthetized and measured but was not marked. The average lengths (3.8-3.9 inches) of ammocoetes in the three lots were not significantly different. The three lots were placed in separate 10-gallon aquariums which had a 6-inch layer of sandy-silt on the bottom and a constant flow of stream water (average temperature, 48° F.). The ammocoetes were not fed. In the ensuing 3 months, the surface of the substrate in the aquariums was inspected daily for dead ammocoetes (generally larvae in distress came to the surface); subsequently, checks for dead ammocoetes were made at least once a week. Twice a year the ammocoetes were removed from the aquariums, measured, and examined.

Cadmium and mercuric sulfide marks were clearly visible on all surviving ammocoetes (Table 1) at each semiannual examination and on ammocoetes which were examined shortly after they had died; little fading of either mark was evident. In the later portion (April, 1959-July, 1960) of the study, however, the physical condition of the ammocoetes

Table 1.--Numbers of marked and unmarked sea lamprey ammocoetes surviving on successive dates after they were installed in aquariums

Date of examination		Type of dye mark		Unmarked
		Cadmium sulfide	Mercuric sulfide	
July,	1957↓	50	50	50
September,	1957	38	48	50
March,	1958	37	48	50
July,	1958	37	48	50
April,	1959	33	44	42
December,	1959	18	21	24
April,	1960	14	14	7
July,	1960	7	2	0

↓ Installation date.

in all three lots deteriorated, possibly from starvation; a loss of body pigmentation, which appeared to be associated with poor physical condition, probably made the marks more prominent.

To appraise the effect of mortality on the usefulness of the dye mark, it was necessary to distinguish between initial mortality (occurring shortly after injection of the dye and presumably due to shock or handling) and delayed mortality (occurring after initial mortality, but caused by dye marking). Initial mortality presumably can be reduced by a refinement of marking techniques, but a delayed mortality would seriously limit the usefulness of this mark.

Considerable initial mortality occurred (Table 1). This initial mortality, which took place over a 20-day period, was 24.0 percent among ammocoetes marked with cadmium sulfide (92 percent of this mortality occurred in the first week), 2.0 percent among those marked with mercuric sulfide, and nil in the control lot. The mortality of ammocoetes marked with cadmium sulfide was significantly higher than of those marked with mercuric sulfide (chi-square = 8.64) and the unmarked lot (chi-square = 11.46), but the difference between the latter two lots was not statistically significant. The high mortality in the cadmium sulfide lot, which was marked first, may have been due to inexperience in marking.

After the relatively short period of initial mortality, the dye-marked ammocoetes survived as well as the unmarked ones, strongly indicating that the mark caused no delayed mortality. There was

virtually no loss in any lot from September, 1957 to July, 1958. Mortality increased from July, 1958 to April, 1959 but the differences (between examinations or cumulative) between the three lots were not significant. A valid comparison of mortality after April, 1959 was not possible as survival in all three lots was rapidly converging on zero, presumably because of starvation.

Carp Lake River
mortality study

In 1958, a field study was conducted at the Carp Lake River, Emmet County, to determine the effect of dye injections on mortality. On July 2, 1958, 624 ammocoetes, which had been collected from a 340-foot section of stream, were marked with mercuric sulfide (167 anteriorly, 146 posteriorly) or a tail clip (311), and released in the collecting area. The tail clip (a seemingly noninjurious mark) consisted of the removal of the extreme end of the caudal fin, including about 1/32 to 1/16 inch of the apex of the trunk. To avoid a sorting effect, dye injecting and tail clipping were performed alternately in groups of 20 to 40 ammocoetes, and marking assignments were rotated periodically among the crew members. The average lengths (4.6-4.7 inches) of ammocoetes in the three lots were not significantly different. Collections with a direct-current shocker were made subsequently at the release area on July 16, August 9, and October 16, 1958.

The differences between the recovery percentages of the three lots were not significant (Table 2). Assuming that tail-clipping mortality

Table 2.--Number and percentage[1] of marked sea lamprey ammocoetes recovered at the locality of release in the Carp Lake River, Emmet County

Date of collection	Mercuric sulfide marks						Tail clips	
	Anterior		Posterior		Total			
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
July 16, 1958	3	1.8	7	4.8	10	3.2	10	3.2
August 9, 1958	6	3.6	6	4.3	12	4.0	12	4.0
October 16, 1958	4	2.5	6	4.5	10	3.4	16	5.5
Totals	13	7.8	19	13.0	32	10.2	38	12.1

[1] For each type of mark, the percentages are based on the number of marked larvae released less those collected previously.

was negligible and there was no differential migration among the three groups, it seems reasonable to conclude that, for dye-marked larvae, there was no significant mortality (initial or delayed) or loss of marks during the 3 1/2-month period after release. The apparently good physical condition of dye-marked larvae upon recovery also suggested that there was no appreciable mortality.

The mercuric sulfide marks were easily seen on all marked ammocoetes that were recovered.

Field study at the Carp Lake River

The primary objectives of this investigation were to study the migration of larvae and to make a population estimate. Other information was obtained on: (1) externally visible, concealed, and completely lost marks; (2) methods of detecting dye marks; (3) the visibility of mercuric sulfide as compared to cadmium sulfide; (4) mortality; and (5) normal development.

Between June 24 and July 2, 1958, 2,187 ammocoetes (average length, 5.0 inches) were collected, marked, and released at five stations in the Carp Lake River. The stations were located in the lower two-thirds of the stream, where lamprey density was highest. At each of four stations, the cadmium sulfide mark was applied to a different body area (anterior, right or left side; posterior, right or left side). The area and the larvae used in the Carp Lake River mortality study (see above) represented the fifth station. In 1958-62, marked and

unmarked specimens were caught during the annual downstream migration, as ammocoetes or newly metamorphosed adults, in an inclined plane trap (Applegate and Brynildson, 1952) at the mouth of the river.

Appreciable numbers of lampreys with externally visible marks were recovered during the 4-year period after release (Table 3). We suspected that some marks had been concealed by skin pigment or even completely lost. Concealed marks were defined as those which could be detected only by internal examination. To estimate the number of concealed marks, large random samples of supposedly unmarked lampreys, caught in 1958-59, 1960-61, and 1961-62, were cross sectioned and examined internally. A slicing device with 12 razor blades spaced 0.1 inch apart was used for cross sectioning; 12 transverse cuts were made in each of the two marking areas. The normal approximation formula, with a correction for finite population was used to compute an upper confidence limit on the number of concealed marks. The question of a complete loss of marks was studied by comparing the percentages of marked specimens in the yearly catches of the weir. A decline in the yearly percentages of marked specimens would indicate that a complete loss of some marks may have occurred. Supplementary data on concealed marks and complete loss were obtained by close internal and external scrutiny of marks to determine if the mark was fading or had become reduced in size.

Table 3.--Recovery of dye-marked sea lampreys at the Carp Lake River weir, Emmet County, 1958-62

Migration season[1]	Larvae			Adults		
	Total catch[2]	Number marked[3]	Percentage marked	Total catch[2]	Number marked[3]	Percentage marked
1958-59	5,365	54	1.0	4,796	87	1.8
1959-60	2,369	37	1.6	2,147	22	1.0
1960-61	663	9	1.4	8,925	55	0.6
1961-62	88	1	1.1	1,635	5	0.3

[1] A migration season extended from July of one year through June of the next year.

[2] Includes both marked and unmarked lampreys.

[3] Includes only lampreys with externally visible marks.

For specimens recovered as larvae, the marks remained externally visible and were not concealed by skin pigment. Internal examination of 2, 347, 324, and 44 supposedly unmarked larvae from the 1958-59, 1960-61, and 1961-62 weir catches revealed no (95 percent confidence) concealed marks. That there was no complete loss of some marks during the 4-year recovery period was suggested by the lack of a significant difference between percentages of marked larvae among all larvae recovered in the weir each year (Table 3). Supporting evidence that the marks persisted was the absence of progressive fading and reduction in size of the marks.

Among specimens recovered as newly metamorphosed adults, the marks were less easily seen externally in the third and fourth years after release than in the first two years. Although many marks were found by external examination, it was evident that some marks had become completely concealed. Internal examination of 1, 692, 4, 398, and 815 supposedly unmarked adults from the 1958-59, 1960-61, and 1961-62 weir catches revealed 1, 14, and 2 concealed marks. This demonstrated (at the 95 percent confidence level) that not more than 6, 36, and 7 marks respectively, were missed by the external examination.

It was uncertain if some complete loss of the mark occurred on adults, since the data were contradictory. On one hand, some complete loss was suggested by a progressive decline in the percentages of marked individuals (including the estimated number of concealed marks) among all adults caught in 1958-59 (1.9 percent), 1960-61 (1.0

percent), and 1961-62 (0.7 percent). The difference between the recovery percentages was significant (chi-square = 25.14). On the other hand, the difference between the yearly percentages of individuals bearing small or faded marks viewed internally, was not significant, indicating that the loss, if it occurred, was not gradual. Since a sudden complete loss was considered unlikely because of the granular composition of the mark, unevaluated factors such as differential mortality or collection of the larger (hence older) specimens for marking probably caused the difference in recovery percentages.

External examination is satisfactory for detecting marked ammocoetes, but is not reliable for detecting marked newly metamorphosed adults. Adults should be examined internally. Internal inspection by one observer was as fast as external inspection by two observers. Although increased precision can be expected by examining adults internally, this method may not be entirely reliable because of the possibility of a complete loss of marks.

On both larvae and adults, the mercuric sulfide mark appeared to be more easily seen externally than the cadmium sulfide mark. In a comparison of external visibility (larvae and adults combined) during the 4-year recovery period, 59.2 percent of the cadmium sulfide marks were rated as "not easily seen" in comparison to 26.2 percent for the mercuric sulfide marks. The difference in proportions was significant (chi-square = 14.12).

Brief checks for initial mortality were made at three marking stations on the day following marking. The observed initial mortality from dye-marking was 1.0 percent, a minimal figure. For specimens recovered as larvae, there was no evidence of delayed mortality during the 4-year period of recovery. The yearly percentages of marked larvae recovered in the weir were not significantly different (Table 3). For specimens recovered as adults, on the other hand, there was a significant difference in recovery rates among the 4 years of recovery, suggesting the possibility of a delayed mortality for adults. Complete loss of some marks or the selection of atypical specimens for marking are other possible explanations.

Normal development of marked larvae was suggested by the recovery of many which had metamorphosed; in addition, all recovered specimens were in good physical condition and lacked infection around the marks.

Field study at the Chocolay River

Although the purpose of this study was to determine the direction of migration, it also furnished data on persistence and visibility of the marks, initial marking mortality, and the effect of the mark on physical condition. Three study areas were set up in the Chocolay River, Marquette County. The middle area was located 60 feet from the downstream area and 100 feet from the upstream area.

During July 5-11, 1957, 461 ammocoetes were collected in the upstream area, marked with cadmium sulfide (posterior) on the left side, and released at point of capture. The process was repeated at the downstream area where 443 ammocoetes were marked on the right side. The average length of marked ammocoetes was 3.6 inches. Daily checks for initial mortality were made at the localities of release on July 5-12.

Except for the winter months, periodic collections (usually weekly) were made at the middle study area from July 11, 1957 to October 23, 1958. In addition, in 1958, collections were made at the upstream areas on March 27 and October 23-28, and at the downstream areas on June 4 and October 23-28. The study was terminated when the stream was treated with larvicide by the U. S. Bureau of Commercial Fisheries on October 30, 1958; on this date, large samples of ammocoetes killed by the larvicide were collected from all three study areas.

A total of 207 dye-marked ammocoetes were recovered during the study (16 months); 73 were recovered during July, 1957 to October, 1958 from the weekly collections in the middle study area, 114 in March to October, 1958 from collections at the localities of release, and 20 in October, 1958 after the application of a larvicide. All of the marks were readily visible and the physical condition of the ammocoetes appeared good.

The observed initial mortality was 4.0 percent, but undoubtedly this percentage is minimal because of the limited observations.

Conclusions

Sea lamprey larvae can be marked with subcutaneous injections of cadmium sulfide or mercuric sulfide at the rate of 100 per hour by three trained workers.

On specimens recovered as larvae, the marks remained externally visible for at least four years, and an external examination was shown to be a reliable method of detecting the marks. Although many marks persisted through metamorphosis, the increase in pigmentation which accompanied this change concealed some marks. It was uncertain if some complete loss of the marks occurred as a result of metamorphosis. Because of the presence of concealed marks, an internal examination was more reliable than an external examination for detecting marks on adults.

In some instances, initial mortality of marked ammocoetes was higher than desirable; this mortality can be reduced with experience and compensated for by holding marked larvae for a period of time and then releasing healthy larvae only. On the other hand, there was good evidence that no delayed mortality occurred among marked individuals while they were larvae. It could not be determined if the marks caused mortality during or after metamorphosis.

There was no evidence that the mark affected normal development.

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