STUDIES ON THE MARKING OF COMMERCIAL SHRIMP WITH BIOLOGICAL STAINS

SPECIAL SCIENTIFIC REPORT–FISHERIES No. 231

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

EXPLANATORY NOTE

The series embodies results of investigations, usually of restricted scope, intended to aid or direct management or utilization practices and as guides for administrative or legislative action. It is issued in limited quantities for official use of Federal, State or cooperating agencies and in processed form for economy and to avoid delay in publication.

United States Department of the Interior, Fred A. Seaton, Secretary
Fish and Wildlife Service

STUDIES ON THE MARKING OF COMMERCIAL SHRIMP WITH BIOLOGICAL STAINS

By

C. E. Dawson
Bears Bluff Laboratories, Wadmalaw Island, S.C.
Formerly of the Institute of Marine Science
The University of Texas

This study was supported by funds made available by the Saltonstall-Kennedy Act, under a contract between the U. S. Fish and Wildlife Service and the University of Texas.

Special Scientific Report--Fisheries No. 231.

Washington, D. C.

September 1957

ABSTRACT

To determine the effectiveness of biological stains as marking agents for the commercial shrimp Penaeus setiferus, P. aztecus, and P. duorarum, experiments were conducted on captive shrimp held in storage tanks and aquariums supplied with circulating sea water. Some 26 stains were tested by immersion, injection, and feeding. Staining by immersion proved consistently unsuccessful. Distinctive and fast abnormal coloration has been induced by injection with Fast Green FCF (National Aniline), Niagara Sky Blue 6B, Trypan Blue, and Trypan Red. Feeding with mullet (Mugil cephalus) previously stained with Trypan Red also results in abnormal coloration suitable for identification. The feeding technique constitutes a satisfactory method for marking shrimp under 80 mm. in length. Whether these stains will prove equally fast under natural conditions must be determined by field experiments.

CONTENTS

STUDIES ON THE MARKING OF COMMERCIAL SHRIMP WITH BIOLOGICAL STAINS

INTRODUCTION

The role of tagging[1/] and marking as aids in determining growth, migration, fishing pressure, and mortality in a commercial-fishery population has been described by many authors (Rounsefell and Kask, 1945; Rounsefell and Everhart, 1953). With the exception of various lobster and crab investigations, tagging and marking programs have been confined to finfish.

Commercial shrimp have been tagged, using the Petersen disk, by the Fish and Wildlife Service, by the Bureau of Fisheries, and by at least one of the Gulf States. Lindner and Anderson (1956) tagged a total of 46,532 shrimp from 1935 through 1947 and reported a gross return of 15.6 percent. Data presented show that recoveries decrease with decrease in size at tagging. No recoveries were obtained from shrimp tagged at a length of 80 mm. or less, and recovery of shrimp in the 85 to 110 mm. range was less than 10 percent. The authors present no details on the maximum period of tag retention, but growth analysis based on recoveries was limited to a 60-day period. It would appear, therefore, that few recoveries were made of shrimp with more than 60 days' "out-time". McRae (1952) reported a 2.01-percent return on 1,137 shrimp tagged along the Texas coast during April, May, and June, 1951. Complete size data are not shown, but the smallest shrimp recovered was approximately 104 mm. long at time of tagging. The maximum period between tagging and recovery was 80 days.

These programs have met with some success, but a more suitable tagging or marking method is needed for shrimp under a length of 100 mm., and for use in long-term experiments on both juvenile and adult shrimp.

The tagging or marking of shrimp is complicated by their frailty and small size, and by a high shedding frequency that precludes use of the exoskeleton for either marking or tagging. The massive population and a high mortality require that large numbers be released, preferably over a short period, before a tagging or marking experiment can attain statistical validity.

Since tags may affect the normal swimming ability of shrimp and physical damage accompanying attachment may be considerable, this study was directed toward investigating the practicality of marking shrimp with biological stains. Although tags would be superior for obtaining individual growth and migration data, it is considered that field studies are directed toward the shrimp population rather than the individual. A distinctive and easily applied abnormal coloration would serve the identification requirements for population studies.

The use of biological stains for identification purposes has been tried with varying degrees of success on a number of marine and freshwater animals. Loosanoff (1937) was successful in marking starfish, Asterias, for periods up to 10 months with Nile Blue Sulfate, and Vernon (1937) marked Asterias with Neutral Red. Feder (1955) has used these stains to mark the Pacific-coast starfish, Pisaster ochraceus. Gustafson (1953) and Klawe (1954) were generally unsuccessful in their efforts to stain the marine worms Glycera and Nereis although some 14 stains were tested. Dunn and Cocker (1951) and Carranza (1953) injected fish with a number of stains with varying success. Two studies of particular interest are those of Menzel (1955) and Racek (1955). Menzel injected white shrimp, Penaeus setiferus, with Fast Green and reported that staining was plainly visible for over 60 days. Racek apparently achieved some success in marking Australian penaeid prawns by immersing them in solutions of Trypan Blue and Nile Blue Sulfate. He

1/ The term "tagging" is used to denote the application of a mechanical tag or other identification device. "Marking" refers to the use of mutilation, staining, or other means of identification not requiring the attachment or insertion of mechanical devices.

considered Nile Blue Sulfate to be most promising owing to the low staining mortality and the "long period" of stain retention.

Since certain methods have been developed which show sufficient promise to warrant large-scale field tests, the results of research conducted at the University of Texas Institute of Marine Science on the use of stains for marking shrimp are presented here.

METHODS AND MATERIALS

Storage facilities

All experiments were conducted on the dock laboratory of the University of Marine Science at Port Aransas, Texas.

Seven storage tanks with capacities ranging from 94 to 102 gallons were constructed of 3/4-inch marine plywood. Each was covered with 1/4-inch hardware cloth and divided into from two to four compartments by hardware-cloth partitions. Five aquariums and four small tanks with capacities of from 8 to 10 gallons completed the storage facilities.

Tanks and aquariums were provided with continuously circulating sea water pumped from a depth of approximately 6 feet below mean low water in Aransas Pass. Water flow averaged about 1.8 gallons per minute to each large tank and 1 gallon per minute to each aquarium and small tank. Piping for the system consisted of galvanized pipe and bronze valves. Rubber hose, glass tubing, and one length of lead pipe were used in distributing water from the main lines to the various tanks. Although the possibility of lethal contamination of the water by copper and zinc from the piping caused some initial concern, a flourishing growth of coelenterates, annelids, mollusks, other crustacea, and an occasional young fish caused this factor to be discounted. The rate of water exchange apparently precluded lethal concentration of toxic metals within the tanks.

The bottoms of several tanks were covered with about 1-1/2 inches of mud and sand in an effort to retard cannibalism. While there is some evidence of reduced cannibalism, problems of observation and counting were materially complicated by the burrowing habit of shrimp.

Salinity and temperature

Three hundred and five daily observations of salinity and temperature were made during the period from May 16, 1955, through May 31, 1956. Mean monthly tank salinities ranged from 25.9 o/oo to 38.7 o/oo and monthly means of tank water temperature range from 14.3°C. to 29.3°C.

Shrimp

In view of the fact that any tagging or marking method should be applicable to all commercial shrimp, no effort was made to separate experimental animals by species. White shrimp (Penaeus setiferus), brown shrimp (P. aztecus), and pink shrimp (P. duorarum) were used either mixed or separately, depending upon their availability. In experiments involving more than one species, no indication of specific differences in stain acceptance was apparent.

Shrimp ranged in length from 30 mm. to 175 mm. and were, for experimental purposes, divided into rough size groups of small (30 to 80 mm.), medium (80 to 120 mm.), and large (120 to 175 mm.). In general, no regular measurements were made of individual shrimp either before or during any particular experiment. Most of the shrimp fell within the medium size classification and, unless specifically stated to the contrary, medium shrimp were used in all experiments.

Shrimp were fed every other day with mullet (Mugil cephalus). Trash fish were used as food during the first 2 weeks of operations, but this practice was discontinued following an almost total mortality in one tank after feeding with Synodus foetens. The mullet, either fresh or frozen, was filleted and cut into pieces roughly 4 mm. square. These were dropped into each tank or compartment on the basis of one piece per shrimp. The size of the individual pieces was modified when feeding predominately large or small shrimp. Food supplied in this fashion is readily eaten and allows for even distribution.

Tanks were checked at least once a day

for the presence of dead individuals or evidence of shedding, and periodic counts were made of the shrimp remaining in each experiment. Abdominal and cephalothoracic exoskeletons were removed whenever found. Shedding shrimp were observed to eat the branchial cast and associated structures, and these were consequently left in the tanks. An indication of the number of shedding individuals was obtained by counting the cepholothoracic exoskeletons found in each tank. Since the entire exoskeleton as well as the newly shed shrimp may be cannibalized, this provided only a minimum figure. When bodies were found, shrimp which had died from the effects of experimental procedures or other causes were differentiated from those eaten by their fellows during or immediately after ecdysis by the presence or absence of a firm exoskeleton.

Stains

Stains were selected which complied with one or more of the following criteria: solubility in water or other nonlethal solvent, previous use as a vital stain, and a color so opposed to the natural coloration of shrimp as to be readily detected by the casual observer.

With a single exception all stains were obtained from the Hartman-Leddon Company (Harleco) or the National Aniline Division of the Allied Chemical and Dye Corporation . Wide variability in the characteristics of stains marketed by different manufacturers is common, and it was considered most practical to limit tests to material readily available from a few American producers. Stains certified by the Biological Stain Commission at Geneva, New York, were used when available.

Pertinent data on the source and quality of each stain are shown in table 1. The total dye content refers to the percentage of dye in the dry stain. The lot number is the manufacturer's identification number of the dry stain. Complete information on each stain may be found in Conn (1953).

Distilled water, filtered sea water, glycerine, mineral oil, and alcohol were used as solvents. Most of the dry stains were relatively insoluble in sea water, and it was usually necessary to first dissolve the stain in a small quantity of distilled water when preparing a sea-water solution. When a stain proved difficult to dissolve, the mixture was warmed and filtered through Whatman No. 1 filter paper. The percentage of stain solution indicated for each experiment is based on the weight of dry stain to 100 milliliters of solvent. As no advantage was found in aging solutions, stains were usually prepared just before use.

EXPERIMENTAL METHODS

The marking qualities of each stain were tested by one or more of three methods: immersion, injection, and feeding. After preliminary tests the laboratory procedure for each method was standardized as follows:

A. Immersion: The shrimp sample was placed in an aerated stain solution for a predetermined number of minutes. The shrimp were then transferred through two changes of fresh sea water at 5-minute intervals so as to remove excess stain adhering to the exterior of the animals or trapped within the branchial chamber. A final transfer was then made to an appropriate storage tank for observation.

B. Injection: A 1-cc. tuberculin syringe equipped with a No. 25 by 1/2-inch needle was most practical for laboratory use. Holding the syringe in the right hand, a shrimp was grasped with the left so that its head was pointed toward the left wrist and with its abdomen held in a flexed position by the left thumb and forefinger (fig. 1). The needle was then introduced through the articular membrane of the sixth abdominal joint slightly to the left of the middorsal line and at an angle approximating 45 degrees. The needle was inserted to a depth of from 2 to 4 millimeters until stain was visibly entering the blood-vascular system through the dorsal abdominal artery. After injection the shrimp were examined for signs of physical damage or excessive weakness. Shrimp killed or incapacitated by overinjection or obvious physical damage were replaced before transfer to final observation tanks. Volume of individual injections generally ranged from 0.01 cc. to 0.05 cc. and averaged about 0.03 cc. Injections of greater volume frequently resulted in rapid death and did not produce more vivid or durable staining. The 1/2-inch No. 25 needle

Table 1.--Stains tested

Stain	Company Identification number--Manufacturer		Lot No.	Stain Commission certificate No.	Percentage dye content
Alizarin	1027	Harleco	30		
Alizarin Red S	1034	Harleco	42	LAr-5	
Alizarin Yellow GG	36	Harleco	14		
Alizarin Yellow R	40	Harleco	11		
Alphazurin 2G	712	Harleco	1		
Bismarck Brown Y	331	Nat'l Aniline		NN-11	60
Brilliant Cresyl Blue	877	Nat'l Aniline		NV 41	60
Brilliant Vital Red	456	Harleco	12		
Chlorazol Fast Pink	353	Harleco	2		
Cresyl Violet (acetate)		Nat'l Aniline		NW 19	56
Diamond Black	299	Harleco	1		
Dianil Blue 2R	465	Harleco	3		
Erythrosin Bluish	773	Harleco	22	LEr-4	83
Fast Green Extra Bluish	691	Harleco	4		
Fast Green FCF		Harleco	7	NGf-7	97
Fast Green FCF		Nat'l Aniline		NGf-13	92
Fast Yellow	16	Harleco	5		
Janus Black	134	Nat'l Aniline	14358		
Janus Green B	133	Nat'l Aniline		NJ-18	50
Malachite Green	657	Harleco	68	LMg-8	96
Methyl Green (hydrochloride)	657	Harleco	13		
Methyl Green	684	Curtin			
Methylene Blue (chloride)	922	Harleco	80	LA-21	86
Neutral Red	825	Nat'l Aniline		NX-17	69
Niagara Sky Blue 6B	518	Harleco	3		
Nigrosin (water soluble)	865	Harleco	6	LNi-4	
Sudan IV	258	Harleco	x-10-1		85
Trypan Blue	477	Harleco	30		
Trypan Blue	477	Nat'l Aniline	15814		
Trypan Red	438	Harleco	12 & 16		
Trypan Red	438	Nat'l Aniline	14184		
Victoria Blue B	729	Harleco	15		
Wool Green S	737	Harleco	3		

permitted easy injection without excessive drip and allowed for ready control of penetration depth.

C. Feeding: Mullet, cut as for normal feeding, was placed in a stain solution for 15 to 20 minutes and agitated to ensure even staining. The solution was then decanted, and the food was washed in sea water to remove excess stain. The stained food was then dropped into the tank following normal feeding practice. After a number of stained feedings, normal food was supplied for the duration of the experiment.

Controls

The prime requisite of a shrimp stain is that it must provide a fast and easily distinguished color. Most of the experiments were designed to test staining characteristics, and regular controls were not established. Simultaneous controls were run with the more successful stains. In control experiments an equal number of untreated shrimp were held in a separate compartment in the

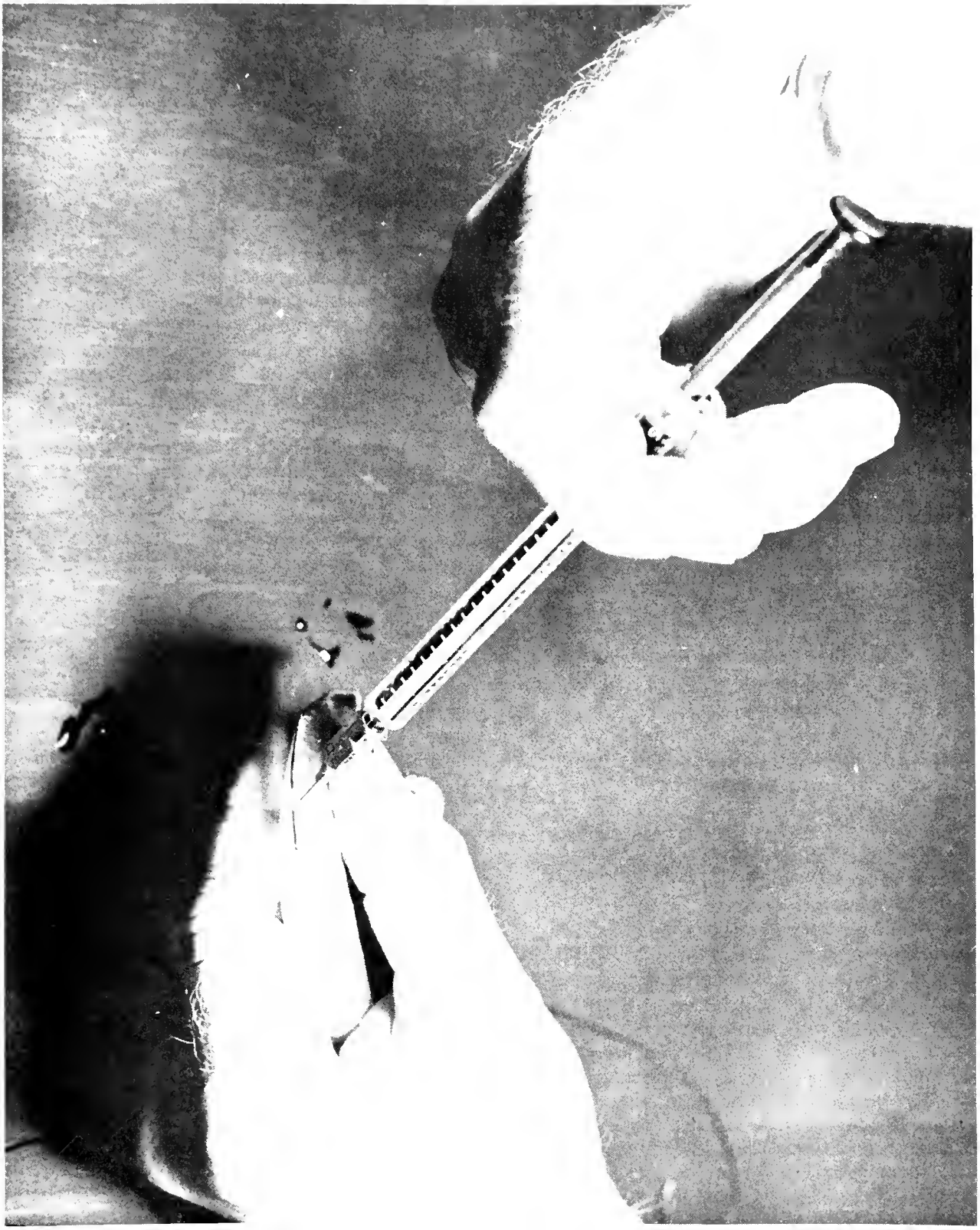

Figure 1.--Recommended method for handling the individual shrimp for injection. The needle is inserted through the articular membrane of the sixth abdominal joint.

same observation tank as the stained shrimp. A further check on experimental mortality was provided by what might be termed the natural mortality within the storage system. This is the total mortality of nonexperimental shrimp and was estimated to average about 15 percent a week.

EXPERIMENTS

Each stain, with the exception of four that proved insufficiently soluble in aqueous solution, was tested by at least one of the three staining techniques and frequently in a number of concentrations and solvents. The number of individuals used in each test ranged from 5 to 60. Of the 30 stains tested, 26 were found unsatisfactory as shrimp-marking agents. A summary of experiments with these unsuccessful stains is presented in table 2. Tests involving stains that are considered suitable shrimp-marking agents are treated in some detail below. The products of two manufacturers are compared in three of these stains.

Fast Green FCF (Harleco)

Four injection experiments were completed using this stain in concentrations of 0.75, 1.0, and 8.0 percent. Each solution was prepared with sea water. Ten shrimp injected with a 0.75-percent solution showed an immediate pale-green general coloration. Fading from the abdominal region and simultaneous concentration in the cephalothorax, especially in the branchiae, was evident within 5 hours. Coloring was pale, however, and within 3 days it was not sufficiently obvious for identification purposes.

There was an immediate general blue to blue-green coloration in 2 lots of 15 and 20 shrimp injected with the 1-percent solution. Abdominal fading was rapid, and the stain was usually concentrated in the heart and branchiae within 72 hours. Some fading was evident, but a pale-green color was retained in the branchiae of 2 shrimp remaining after 84 days. This coloration had remained fast through at least one ecdysis.

Fifteen shrimp injected with an 8 percent solution were immediately colored a deep blue-green. Abdominal fading and cephalothoracic concentration was evident within 24 hours and complete within 72 hours. Although there was some fading, the stain remained through at least one ecdysis and a pale-green color was retained by the one shrimp surviving through 160 days. Though readily apparent to a careful observer, the color was not sufficiently distinct for field use.

Ten shrimp were immersed for 15 minutes in a 0.75-percent solution made up with sea water. One shrimp died within 8 hours. There was some slight stain acceptance in the anterior digestive tract, branchiae and in irregular areas of the abdominal exoskeleton. Loss of abdominal color was complete within 24 hours. Rapid fading progressed and after 6 days only a very light green tint was detectable in 2 of the remaining 9 shrimp.

Fifteen shrimp were given three feedings stained with an 8-percent solution prepared with sea water. Stomach and digestive tract were stained a moderate to deep blue which persisted for 8 days after the final stained feeding. Thereafter, fading was relatively rapid with only a trace of abnormal coloration remaining in 13 days.

Fast Green FCF (National Aniline)

Two injection experiments were completed. Fifteen shrimp injected with a 1-percent solution made up with 10 ml. of distilled water and 90 ml. of sea water showed an immediate general blue-green coloration. One died within 18 hours. Except for a green spot at the injection site, color was rapidly concentrated in the cephalothorax. General cephalothoracic tinting and the spot at the injection site faded slowly, but coloring in the branchiae and heart remained fast for 120 days and through at least one ecdysis. The color was very distinctive and could be readily detected by even a casual observer.

Twenty shrimp were injected with a 1-percent solution made up with an unknown solvent. this solution was supplied by Dr. Winston Menzel, and was the same as that employed in his experiments (Menzel, 1955). As usual with Fast Green FCF there was rapid general green to blue-green coloration with subsequent abdominal fading

Table 2.--Summary of unsuccessful stain tests

Stain	Percent stain	Solvent[1/]	Imm.	Method feed.[2/]	Inject.	No. of shrimp	Color and area stained	Total days	Percent mortality A[3/]	Total mortality in percent	Remarks
Alizarin	20.0	SW	---	----	XXX	10	Light purple; injection site and branchiae	6	----	80	8 dead in 17 hrs.; rapid fading, no trace of color remaining after 5th day
Alizarin Red S	0.1	2.5%DW;SW	XXX	----	---	10	Light pink; general	3	----	10	10-min. immersion period; 1 died during staining; rapid fading, no trace after 48 hours.
	0.1	as above	---	----	XXX	5	Light pink; general	5	----	80	4 dead in 17 hrs. no trace remaining in 48 hrs.
	1.0	SW	---	5	---	15	Pale rust-red; anterior digest. tract and branchiae	16	6.5	33	2 undetermined mortalities; pale color unsuitable for identification.
Alizarin Yellow GG	----	----	---	----	---	--	----	--	----	--	Insufficiently soluble in aqueous solution for staining purposes.
Alizarin Yellow R	----	----	---	----	---	--	----	--	----	--	As above
Alphazurin 2G	1.0	DW	---	4	---	10	Bright blue-green; anterior digestive tract	30	20	30	Pale tinting evident in 5 at 30 days; color unsuitable for identification.
	1.0	DW	---	4	---	15	As above	55	----	33	4 undetermined mortalities; irregular fading after 15 days, no trace at 55 days.
	1.0	10%DW;SW	---	---	XXX	15	Blue-green; general, with some concentration in branchiae	7	----	13	2 dead in 17 hrs.; only 2 show trace at 7 days.

Table 2.--Summary of unsuccessful stain tests--Continued.

Stain	Percent stain	Solvent[1]	Imm.	Method feed.[2]	Inject.	No. of shrimp	Color and area stained	Total days	Percent mortality A[3]	Total mortality in percent	Remarks
Bismarck Brown Y	1.0	SW	---	----	XXX	10	Light yellow; general, some concentration in branchia	5	----	0	No trace at 72 hrs.
	0.1	2.5%DW;SW	----	----	XXX	10	As above	3	----	0	As above
	0.1	As above	XXX	----	---	10	Dark brown; branchiae and antennal scales	7	10	10	15-min. immersion; fading within 48 hrs.; color at 4 days unsuitable for identification.
	0.1	SW	XXX	----	---	10	As above	11	6.5	13	6 min. immersion; results as above
Brilliant Cresyl Blue	1.0	10%DW;SW	----	----	XXX	10	Dark blue to blue-green; branchiae, injection site and abdominal concentration	9	10	10	Solution warmed and filtered; fading rapid, no trace after 8 days.
	1.0	As above	----	9	---	16	Pale purple; general	33	----	19	2 undetermined mortalities; color unsuitable for identification after 14 days.
Brilliant Vital Red	1.0	10%Alc.;DW	----	----	XXX	5	----	0	----	100	70% alcohol used; stain flocculated in sea water; total mortality in 10 min.
	1.0	As above	----	1	---	10	Red; anterior digestive tract	5	----	10	One dead in 4 hrs. no trace after 3 days.
Chlorazol Fast Pink B	2.0	10%DW;SW	----	----	XXX	10	Purple spot at injection site	2	----	0	No trace of color remaining after 20 hrs.
	2.0	As above	----	2	---	15	Pale brown in branchiae	6	----	0	Stain acceptance by 3 shrimp only; color unsuitable for identification.
Cresyl Violet (acetate)	---	----	----	----	---	--	----	---	----	--	Insufficiently soluble solution for staining purposes.

Table 2.--Summary of unsuccessful stain tests--Continued.

Stain	Percent stain	Solvent[1/]	Imm.	Method feed.[2/]	Inject.	No. of shrimp	Color and area stained	Total days	Percent mortality A[3/]	Total mortality in percent	Remarks
Dianil Blue 2R	1.0	10%DW;SW	---	----	XXX	10	Dark to pale blue; general with concentration in branchiae and injection site	3	----	40	Solution warmed and filtered; 4 dead in 6 hrs.; no trace of color at 3 days.
	1.0	SW	---	3	---	10	Pale blue-green; Anterior digestive tract	8	----	10	1 dead after 2 feedings; color unsuitable for identification.
Erythrosin Bluish	2.0	10%DW;SW	---	----	XXX	16	Bright pink; general	14	6.5	6.5	7 undetermined mortalities; stain retention irregular and color unsuitable for identification.
	2.0	As above	---	4	---	15	Pale pink; anterior digestive tract	13	27	47	Fading complete 5 days after final stained feeding.
Fast Green Extra Bluish	1.0	10%DW;SW	---	----	XXX	15	Blue-green; general, with concentration at injection site	4	6.5	13	Fading complete in 72 hrs.
	1.0	DW	---	5	---	10	Bright blue-green; ant. digestive tract	16	10	30	Fading rapid; color unsuitable for identification in 5 days.
Fast Yellow	1.0	15%Gly.;SW	---	----	XXX	10	Pale yellow; general	4	----	0	Rapid fading; color unsuitable for identification in 48 hrs.
	1.0	10%DW;SW	---	----	XXX	15	Yellow general	8	----	13	2 dead in 8 hrs.; fading rapid; no trace remaining at 8 days.
	1.0	DW	---	5	---	10	Pale yellow in cephalothorax	16	----	10	1 dead in 18 hrs.; color unsuitable for identification
Janus Black	2.0	10%Alc.; 10%DW;SW	---	----	XXX	15	Purple-black at injection site	105	13	86	Spot at injection site was fast in shrimp remaining through 104 days; stained area not sufficiently obvious for identification. 95% alcohol used.
	<4.0	SW	---	----	XXX	10	As above; also in branchiae of one	40	50	90	Dry stain only slightly soluble in SW; rejected as above.

Table 2.--Summary of unsuccessful stain tests--Continued.

Stain	Percent stain	Solvent[1]	Imm.	Method feed.[2]	Inject.	No. of shrimp	Color and area stained	Total days	Percent mortality A[3]	Total mortality in percent	Remarks
Janus Black	<10.0	SW	---	----	XXX	10	Dark gray at injection site	4	----	10	1 dead in 3 hrs.; stain acceptance in 2 shrimp only; color unsuitable for identification
	2.0	10%Alc.; 10%DW;SW	---	1	---	10	-----	3	----	0	Food poorly stained; shrimp showed no indication of stain acceptance; 95% alcohol used.
Janus Green B	1.0	25%Gly.;SW	---	----	XXX	10	Blue-purple general	7	----	40	4 died in 87 hrs.; fading rapid and color unsuitable for identification in 6 days.
	1.0	SW	---	----	XXX	15	Blue-black; general, with branchial concentration	2	----	100	7 dead in 40 min.; total mortality in 48 hrs.
	1.0	SW	---	2	---	10	Dark blue; digestive tract	9	10	50	4 dead in 22 hrs.; stain faded rapidly after each feeding.
	0.1	SW	XXX	----	---	10	Blue-black; branchiae and exoskeleton	6	----	10	10 min. immersion period; 1 dead in 24 hrs.; rapid fading, color also lost at ecdysis.
Malachite Green	1.0	10%DW;SW	---	----	XXX	15	Dark blue; general, branchial and injection site concentrations	6	6.5	66.6	9 dead in 5 days; fading rapid; color unsuitable for identification.
	1.0	As above	---	2	---	10	Dark green; digestive tract	5	----	0	Only 1 shrimp accepted stained food; normal food readily accepted by all; staining temporary.

Table 2.--Summary of unsuccessful stain tests--Continued.

Stain	Percent stain	Solvent[1/]	Imm.	Method feed.[2/]	Inject.	No. of shrimp	Color and area stained	Total days	Percent mortality A[3/]	Total mortality in percent	Remarks
Malachite Green (hydrochloride)	---	---	---	-----	---	--	-----	--	----	----	Insufficiently soluble in aqueous solution for staining purposes.
Methyl Green	<1.0	SW	---	-----	XXX	15	Blue-green; general, injection site concentration	6	----	0	Fading evident in 2 hrs.; color unsuitable in 48 hrs.
	4.0	SW	---	-----	XXX	15	Green; as above	10	20	67	7 dead in 48 hrs.; color unsuitable in 9 days.
	0.75	SW	XXX	-----	---	10	Blue-green; cephalothorax, branchiae and ventral abdomen	3	10	10	Fading rapid; color unsuitable in 48 hrs.
	4.0	SW	---	2	---	10	Blue; digestive tract, green in branchiae	7	30	60	1 undetermined mortality; rapid fading; no evidence of staining remained 4 days after second feeding.
Methylene Blue (chloride)	1.0	10%DW;SW	---	----	XXX	15	Pale green; general, branchial concentration	2	----	6.5	1 dead in 18 hrs.; fading complete in 24 hrs.
	2.0	DW	---	3	---	15	Blue; digestive tract	11	----	33	5 dead in 5 days; fading complete in 3 days after final feeding.
Neutral Red	1.0	10%DW;SW	---	----	XXX	10	Deep red; general; branchial concentration	9	10	10	Solution warmed and filtered; fading complete in 8 days.
	1.0	As above	---	6	---	15	Pale red; digestive tract	15	10	10	Color unsuitable for identification
Nigrosin (water solution)	<1.0	10%DW;SW	---	----	XXX	10	Purple-black; general, branchial and injection site concentrations	28	----	80	Solution warmed and filtered, not completely soluble. 7 dead in 21 hrs.; color faded, unsuitable for identification.

Table 2.--Summary of unsuccessful stain tests--Continued.

Stain	Percent stain	Solvent[1/]	Imm.	Method feed.[2/]	Inject.	No. of shrimp	Color and area stained	Total days	Percent mortality A[3/]	Total mortality in percent	Remarks
Nigrosin (water solution)	<1.0	As above	---	----	XXX	11	As above	1	----	82	9 dead in 30 min.; remainder very weak.
	<1.0	As above	---	3	---	10	Black; digestive tract	13	----	0	Color pale, unsuitable for identification
Nile Blue Sulfate	1.0	SW	---	----	XXX	15	Blue; general branchial concentration	3	----	73	Injections averaged 0.18 cc. 11 dead in 24 hrs.; no color in remainder after 43 hrs.
	0.1	1.6%DW;SW	---	----	XXX	13	As above	2	7	14	Irregular stain retention; color unsuitable for identification.
	0.1	As above	XXX	----	---	20	Dark blue; margins of appendages, branchiae	4	----	0	5 min. immersion period; fading rapid, complete in 3 days.
	0.1	As above	XXX	----	---	20	As above	5	----	10	2 groups of 10 shrimp immersed for periods of 10 and 20 min.; complete fading in 4 days.
	0.1	As above	---	3	---	10	Blue; digestive tract	7	----	10	Poor food acceptance; stain fades rapidly on termination of stained feedings.
Sudan IV	2.5	MO	---	----	XXX	25	Pale red; general, branchial and injection site concentration	78	24	80	12 dead in 8 days, 1 escaped; stain fast in remainder. Color too pale for ready identification.
Victoria Blue B	1.8	9.1%Gly.;SW	---	----	XXX	7	Blue; general	1	----	86	6 dead in 15 min.; remainder very weak.
	1.0	DW	---	4	---	10	Blue; digestive tract	10	----	40	4 dead in 8 days; poor food acceptance; fading complete in 48 hrs. after final stained feeding.

Table 2.--Summary of unsuccessful stain tests--Continued.

Stain	Percent stain	Solvent[1/]	Imm.	Method feed.[2/]	Inject.	No. of shrimp	Color and area stained	Total days	Percent mortality A[3/]	Total mortality in percent	Remarks
Wool Green S	1.0	10%DW;SW	---	----	XXX	15	Blue-green general	43	13	26	1 undetermined mortality; gradual fading; color unsuitable for identification at 43 days.
	1.0	As above	---	4	---	10	Dark green; digestive tract	37	10	40	3 dead in 5 days; gradual fading; color unsuitable for identification at 37 days.

1/ DW = Distilled water; SW = Sea water; Alc. = Ethyl alcohol; Gly. = Glycerine

2/ The figure in the feeding column indicates the number of stained feedings given

3/ A = Mortality known to have accompanied ecdysis and cannibalism.

within 3 days. A green spot at the injection site was retained for varying periods up to 53 days. The green staining of the branchiae and cephalothorax showed some gradual fading, but it was retained through at least one ecdysis and remained very evident in the four shrimp surviving after 84 days.

Niagara Sky Blue 6B

Fourteen shrimp were injected with a 1-percent solution made up with 10 ml. of distilled water and 90 ml. of sea water. Immediate coloration ranged from an overall medium to a blue spot at the injection site; all showed some branchial concentration. Three shrimp died within 4 days. Gradual abdominal fading was evident, and staining was confined to a blue coloring of the branchiae and heart in 37 days. After 103 days, three surviving shrimp showed well-defined dark-blue coloration of these areas. In a similar test on 30 shrimp, the results were generally the same. Three shrimp died in 3 days; cannibalism was high and only four shrimp survived after 52 days.

Twenty-five shrimp were given 6 feedings stained with a 1-percent solution as above. By the third feeding there was a dark-blue coloration of the stomach and digestive tract, and several showed a poorly defined pale blue-green in the branchial region. One shrimp showed a bright blue-green staining of the branchiae which was retained until termination of the test 26 days after the initial stained feeding. Examination of casts showed that much of the branchial coloration was lost at ecdysis.

Trypan Blue (Harleco)

Ten shrimp were injected with a 1-percent solution prepared with 5 ml. of distilled water and 95 ml. of sea water. This resulted in pale-blue coloring of the branchiae and at the injection site; several showed an overall bluish tint. This initial staining was not spectacular and at first appeared to be insufficiently vivid for identification purposes. However, within 24 hours a dark-blue concentration was evident in the branchiae. Although there was some tendency to fade to a dark blue-grey in about 30 days, this color remained fast and was readily observed in two individuals surviving through 141 days. Staining remained through at least two shedding cycles.

Among 20 shrimp given injections averaging 0.05 cc. of a similar solution, several showed signs of weakness and one died within 8 hours. The remainder recovered and showed a moderate to dark blue in the injection area; branchial and abdominal tinting occurred in several. There was a dark-blue to blue-green concentration in the branchiae within 55 hours with simultaneous fading from other areas. Of the three shrimp remaining through 179 days, all retained dark-blue branchiae and two showed a bluish tint at the injection site.

Of 15 shrimp injected with a 1-percent solution made up with 10 ml. of distilled water and 90 ml. of sea water, 3 died within 5 minutes and 5 within 24 hours. This is presumed to have resulted from too great an injection. Owing to the initial absence of bright coloration, there is a tendency to overinject when using this stain. A general dark-blue branchial concentration resulted within 24 hours, and this remained distinct in the two shrimp surviving through 220 days.

Food acceptance was poor among 10 shrimp given three feedings stained with a 1-percent solution prepared with distilled water. The digestive tract retained a pale bluish tint unsatisfactory for identification purposes.

Five shrimp were immersed for 15 minutes in a 0.4-percent solution made up with 10 ml. of distilled water and 990 ml. of sea water. This resulted in an indistinct tinting of the cephalothorax, but no evidence of abnormal coloration could be seen after 24 hours.

Trypan Blue (National Aniline)

Twenty-six shrimp were given injections averaging 0.06 cc. of a 1-percent solution made up with 10 ml. of distilled water and 90 ml. of sea water. This stain was less soluble in sea water than the Harleco product, and the solution was filtered before use. Immediate coloration was detectable only at the injection site, in the dorsal abdominal artery, and in a pale tinting of the branchiae. Within 24 hours there was a general branchial concentration, and several

shrimp showed blue coloration of the abdomen and injection site. The abdominal color faded slowly but remained distinct in some for over 60 days. The blue to blue-gray branchial staining was retained for the duration of the 120-day experiment.

Thirty additional shrimp were injected with a 1-percent solution as above. Initial staining was similar to that detailed in the preceding test. Cannibalism accompanying ecdysis was high, and only 7 individuals remained after 52 days.

Fifteen shrimp given 5 feedings stained with a similar solution showed a temporary blue coloration of the digestive tract. All evidence of staining disappeared within 3 days after the final stained feeding.

Ten shrimp, divided into 3 lots, were immersed for periods of 7, 5, and 3 minutes in a 1-percent solution prepared with 25 ml. of distilled water and 275 ml. of sea water. Two of 3 shrimp immersed for 7 minutes were dead at completion of the staining period. In the remainder, abnormal coloration was confined to a slight bluish tinting of the branchiae and anterior margins of the antennal scales. Normal coloration was attained within 48 hours.

Trypan Red (Harleco)

Ten shrimp were injected with a 1-percent solution made up with 5 ml. of glycerine and 95 ml. of sea water. A slow diffusion resulted in a general pink color with bright-red concentrations in the branchiae and injection area. Rapid abdominal fading followed, but distinctive branchial coloration remained fast in 4 shrimp surviving 245 days.

Two lots of 10 and 15 individuals were injected with a 1-percent solution prepared with 10 ml. of distilled water and 90 ml. of sea water. A mortality of 18 shrimp within 72 hours was attributed to excessive injection volumes. Staining was essentially the same as in the preceding test, but the general coloration was persistent, together with the branchial concentration, in the 3 shrimp remaining through 124 days.

Ten shrimp injected with a 1-percent solution made up with 5 ml. of distilled water and 95 ml. of sea water showed an immediate general pink coloring and definite branchial concentration. The general coloration persisted in several individuals for at least 70 days, and a bright-red branchial staining was very evident in 4 shrimp which survived 234 days.

Eleven shrimp injected with a 1-percent solution prepared with 10 ml. of distilled water and 90 ml. of sea water showed similar coloration on injection. Abdominal staining faded within 16 days, but the red branchial concentration was fast in the one shrimp remaining after 220 days.

Eleven shrimp were given injections, averaging 0.03 cc., of a 1-percent solution prepared as above but warmed to facilitate dissolving of the dry stain. Abnormal coloration was essentially similar to that in the previous experiment. On the thirty-ninth day two shrimp showed loss of equilibrium and rested on their side rather than in the normal position. When stimulated, both showed normal swimming ability but lost balance shortly after coming to rest. Both were cannibalized within 7 days. Five shrimp surviving 46 days retained abnormal branchial color as previously described.

Thirty-three shrimp given injections, averaging 0.05 cc., of a 1-percent solution prepared with sea water showed pink abdominal and red branchial colorations. A high mortality resulted in the loss of 25 individuals within 10 days. Abdominal fading was slow but the branchial color was fast in the remainder and was evident in the one shrimp surviving 175 days.

Ten shrimp were given four feedings, stained with a 1-percent solution made up with 10 ml. of distilled water and 90 ml. of sea water, at 4-, 2-, and 4-day intervals. Red branchial concentration was apparent after the third feeding, and several showed diffuse red staining of the entire cephalothorax. Branchial color was retained through at least three shedding periods and was very evident in the one individual surviving 215 days after the final stained feeding.

Fifteen shrimp given three feedings stained with a 2-percent solution prepared with

distilled water all showed red branchial coloring before the final stained feeding and several had accepted various degrees of abdominal staining. Three died within 7 days. There was irregular retention of abdominal coloring but the bright-red branchial concentration showed no indication of fading through 79 days following the final stained feeding. An identical test on 11 large shrimp produced similar results. Three individuals surviving 65 days after the final stained feeding all showed bright-red branchiae.

Twenty-five shrimp were given three feedings stained with a 2-percent solution, as above, which had been warmed and filtered. With the exception of two individuals which had apparently not accepted stained food, all showed red branchial coloring before the final stained feeding. Of 7 shrimp surviving 183 days, 6 showed distinctly red or pink branchiae and one showed no evidence of abnormal coloring.

Fifty shrimp were given feedings stained with a 1-percent solution prepared with 45 ml. of distilled water and 5 ml. of sea water. The majority showed red to pink branchial coloring after three feedings and several showed general pink coloring. After the fifth feeding, the 20 most deeply stained individuals were isolated and subsequently given normal food; those remaining were given four additional stained feedings. Since no difference could be detected in intensity of coloring, the two groups were again combined 24 days after the final stained feeding. Seven shrimp remaining after 127 days all showed bright-red branchiae. Since cannibalism was high, it was impossible to determine differences in mortality between the five-feeding and nine-feeding groups.

Fifteen small shrimp were given eight feedings stained as for the preceding experiment. The majority showed red branchial concentration following the third feeding and this held fast in the six individuals surviving 127 days after the final stained feeding.

Immersion tests on two lots of 10 shrimp in 0.2-percent and 1-percent solutions were unsuccessful. In general all abnormal color faded within 3 days. One shrimp accepted some red coloration in the branchiae, and this remained fast for the 17-day duration of the experiment. Several fruitless attempts were made to duplicate branchial staining following immersion.

Trypan Red (National Aniline)

Twenty-five shrimp injected with a filtered 1-percent solution made up with 10 ml. of distilled water and 90 ml. of sea water showed an immediate pale-red branchial concentration and several showed general pink coloration. Initially this did not appear as bright as that produced by the Harleco product but it intensified to a bright red within 8 days. Eventually, all but one showed a diffuse pink coloring of the entire cephalothorax, and abdominal staining was retained when present. There was a high rate of cannibalism following ecdysis, but a bright-red color remained in the branchiae of four shrimp surviving 79 days.

The majority of 50 shrimp showed a bright-red branchial color after two feedings stained with a 2-percent solution made up with distilled water. This abnormal coloring was evident in all after five feedings. Cannibalism was great, but the branchial color remained fast in 7 individuals through 7 days following the final stained feeding.

Sixty shrimp were given five feedings stained with a 2-percent solution as above except that it was warmed to facilitate dissolving of the dry stain. Various degrees of abnormal coloring from a pale-pink tinting of the branchiae to a deep-red general coloration were observed following the third stained feeding. Six shrimp, all with the general coloration, died within 5 days, and on the fifth day two individuals with similar color exhibited a loss of equilibrium as noted previously. One of these died within 3 days, while the other survived 27 days and shed once without recovering normal balance. Again cannibalism was high, and after 58 days the 10 individuals remaining all retained bright-red branchiae.

Of 25 small shrimp given two feedings stained as above, all but 4, which had apparently not eaten, showed bright-red branchiae. Two died within 6 days. There was no general staining, and no loss of equilibrium was observed.

All nine shrimp surviving 43 days exhibited fast branchial coloring.

Two lots of approximately 35 small shrimp were given three feedings stained with a 1-percent solution prepared with distilled water. All showed bright-red branchiae after the final stained feeding, and this color remained fast in all individuals surviving the 11-day test.

MORTALITY

The available mortality data for each test with these more successful stains are presented in table 3. Mortality data on simultaneous staining and control experiments are summarized in table 4. Mortality A refers to deaths known to have been accompanied by ecdysis and cannibalism. Mortality B indicates the percent mortality known to have occurred without concurrent ecdysis and may be ascribed to experimental procedure, cannibalism, or other unknown causes. Shrimp listed as "undetermined" were completely cannibalized, and cause of death could not be ascertained. It was observed that isolated stained and unstained shrimp, when held for extended periods, could succumb 2 or 3 days after ecdysis. In such cases the exoskeleton did not attain normal firmness, indicating a deficiency of some unknown component in the environment. In group experiments such mortality could not be recognized and, owing to the absence of casts, these deaths would be included under Mortality B.

DISCUSSION

In 11 experiments with 8 stains, the marking of shrimp by immersion was consistently unsuccessful. Though temporary coloration was attained in several cases, fading was always rapid and was usually complete within 5 days. One instance of more permanent marking, using Trypan Red, could not be duplicated in subsequent trials. Immersion marking with Nile Blue Sulfate or Trypan Blue, suggested by Racek (1955), was unsatisfactory. Racek gives no data on the source of his stains, and differences in stain characteristics may account for contrasting results. The possibility of differential stain acceptance between the Australian and American shrimp cannot be wholly discounted, however.

Injection with Fast Green FCF, Niagara Sky Blue 6B, Trypan Blue, or Trypan Red has resulted in fast abnormal colorations, and these show potential value as shrimp-marking agents. Injection is a rapid and efficient method of marking medium and large shrimp, and can be used under most field conditions. Although tuberculin syringes were used in laboratory studies, automatic constant-volume syringes are indicated for most efficient field operation.

Fast Green FCF was originally suggested as a shrimp-marking agent by Menzel (1955), but he published no data on the source or concentration of the stain used. Menzel later reported (personal correspondence) that the stain was a National Aniline product in a 1-percent solution, but unfortunately no information was available on the solvent. A sample of this solution was kindly supplied by Dr. Menzel for inclusion in the present experiments.

Injection with Fast Green FCF results in an immediate bright-green to blue-green general coloration. Abdominal fading is rapid, and this, together with simultaneous green branchial concentration, is usually complete within 72 hours. Menzel reported branchial concentration following ecdysis, but in the present experiments this occurred either with or without shedding.

One-percent solutions proved most satisfactory, as less concentrated solutions did not provide a fast stain, and no advantage was found in using more concentrated solutions. Experimental injection volumes ranged from 0.015 cc. to 0.2 cc. per shrimp, but 0.03 cc. was found adequate for medium shrimp.

Mortality within the 24-hour period following injection average 4 percent with this stain, and no difference was observed in initial mortality between injections of the National Aniline and Harleco products. It was difficult to arrive at the experimental mortality in view of the prevalence of cannibalism and deaths from unknown causes. Reference to table 4 will show the maximum difference in total mortality of control and stained animals to be 15 percent over 84 days.

Comparison of staining characteristics shows the National Aniline product to be superior to that of Harleco for present purposes. Although

Table 3.--Summary of available mortality data on experiments with Niagara Sky Blue 6B, Fast Green FCF, Trypan Blue, and Trypan Red

Stain	No. of shrimp	Method[1] Imm.	F.	Inj.	Total days	Percent mortality A[2]	Percent mortality B[3]	Total mortality in percent	Remarks[4]
Fast Green FCF (H)	15	--	--	XX	44	87	13	100	1-percent solution in SW.
	10	XX	--	--	7	--	10	10	0.75-percent solution in SW; 15 min. immersion.
	10	--	--	XX	4	--	--	0	0.75-percent solution in SW.
	15	--	--	XX	160	21	43	93	1-percent solution in SW; 1 lost, 4 undetermined.
	15	--	3	--	22	47	47	93	8-percent solution in SW.
	20	--	--	XX	84	25	35	90	1-percent solution in SW; 6 undetermined.
Fast Green FCF (N. A.)	15	--	--	XX	120	5	20	90	1-percent solution in 10%DW, 90%SW; 13 undetermined.
	20	--	--	XX	84	15	10	80	Menzel's 1-percent; 11 undetermined.
Niagara Sky Blue 6B	14	--	--	XX	103	36	29	79	1-percent solution in 10%DW, 90%SW; 2 undetermined.
	25	--	6	--	37	32	28	76	1-percent as above; 4 undetermined.
	30	--	--	XX	52	47	23	87	1-percent as above; 5 undetermined.
Trypan Blue (H)	10	--	--	XX	141	60	10	70	1-percent solution in 5%DW, 95%SW; 1 lost.
	5	XX	--	--	2	--	--	0	0.4-percent solution in 1%DW, 99%SW; 15 min. period.
	20	--	--	XX	179	15	25	85	1-percent solution in 10%DW, 90%SW; 9 undetermined.
	15	--	--	XX	220	20	60	87	1-percent solution as above; 1 undetermined.
	10	--	3	--	23	--	20	20	1-percent solution in DW.

Table 3.--Summary of available mortality data on experiments with Niagara Sky Blue 6B, Fast Green FCF, Trypan Blue, and Trypan Red--Continued.

Stain	No. of shrimp	Method[1] Imm.	F.	Inj.	Total days	Percent mortality A[2]	Percent mortality B[3]	Total mortality in percent	Remarks[4]
Trypan Blue (N.A.)	26	--	--	XX	120	4	12	73	1-percent solution in 10%DW, 90%SW; 15 undetermined.
	15	--	5	--	14	--	--	6.5	1-percent solution as above; 1 undetermined.
	30	--	--	XX	52	47	--	77	1-percent solution as above; 9 undetermined.
	10	XX	--	--	3	--	20	20	1-percent solution in 8.5%DW, 91.5%SW.
Trypan Red (H)	10	--	--	XX	245	--	10	60	1-percent solution in 5%Gly., 95%SW; 5 undetermined.
	25	--	--	XX	124	12	76	88	1-percent solution in 10%DW, 90%SW.
	10	--	--	XX	234	30	10	60	1-percent solution in 5%DW, 95%SW; 2 undetermined.
	11	--	--	XX	220	--	--	91	1-percent solution in 10%DW, 90%SW; 10 undetermined.
	10	--	4	--	223	20	60	90	1-percent solution as above; 1 undetermined.
	11	--	--	XX	46	18	9	36	1-percent solution as above; 1 undetermined.
	15	--	3	--	83	40	13	66	2-percent solution in DW; 2 undetermined.
	11	--	3	--	69	30	40	70	Stain as above.
	25	--	3	--	183	16	40	72	Stain as above, filtered; 4 undetermined.
	33	--	--	XX	175	3	85	91	1-percent solution in SW; 2 undetermined.
	50	--	9	--	127	34	16	86	1-percent solution in 10%SW, 90%DW; 18 undetermined.
	15	--	8	--	127	6.5	26	60	1-percent solution as above; 4 undetermined.
	10	XX	--	--	4	---	--	0	5 min. period in 0.2% solution; 5%DW, 95%SW.

Table 3.--Summary of available mortality data on experiments with Niagara Sky Blue 6B, Fast Green FCF, Trypan Blue, and Trypan Red--Continued.

Stain	No. of shrimp	Method 1/ Imm.	F.	Inj.	Total days	Percent mortality A 2/	Percent mortality B 3/	Total mortality in percent	Remarks 4/
Trypan Red (H)	10	XX	--	--	17	40	--	40	4 min. period in 1-percent solution; 10%DW, 90%SW.
Trypan Red (N.A.)	50	--	5	--	60	28	13	87	2-percent solution in DW; 1 lost; 27 undetermined.
	25	--	--	XX	79	28	8	84	1-percent solution in 10%DW, 90%SW; 12 undetermined.
	60	--	5	--	58	28	28	83	2-percent solution in DW; warmed; 16 undetermined.
	25	--	2	--	43	4	8	64	1-percent solution in DW; 13 undetermined.
	35	--	3	--	11	14	--	48	1-percent solution as above; 10 undetermined.
	35	--	3	--	11	3	6	37	Stain as above; 9 undetermined.

1/ Imm.-immersion; F-feeding (figure in feeding column indicates number of stained feedings); Inj.-injection; (H)-Harleco; (N.A.)-National Aniline; Gly.-glycerine; DW-distilled water; SW-sea water.

2/ Mortality known to have accompanied ecdysis and cannibalism.

3/ Mortality known to have occurred without ecdysis and cannibalism.

4/ Complete cannibalism.

Table 4.--Summary of mortality data on simultaneous staining and control experiments

Stain	No. of shrimp	Method Imm.	Method F.	Method Inj.	Total days	Percent mortality A	Percent mortality B	Total mortality in percent	Remarks[1/]
Fast Green FCF	20	--	--	XX	84	15	10	80	11 undetermined; Menzel's solution.
	20	--	--	XX	84	25	35	90	6 undetermined; 1 percent Harleco, SW.
Control	20	--	--	--	84	10	5	75	12 undetermined.
Trypan Blue	30	--	--	XX	52	47	--	77	9 undetermined; 1 percent Nat'l. Aniline; 10%DW, 90%SW.
Niagara Sky Blue 6B	30	--	--	XX	52	47	23	87	5 undetermined; 1-percent solution; 10%DW, 90%SW.
Control	30	--	--	--	52	53	3	83	8 undetermined.
Trypan Red	60	--	XX	--	58	28	28	83	16 undetermined; 2 percent Nat'l. Aniline in DW.
Control	60	--	--	--	58	23	10	77	26 undetermined.

1/ DW = Distilled water; SW = Sea water.

both have proved relatively fast and can be distinguished for more than 120 days, the National Aniline stain results in a much more vivid coloration.

Niagara Sky Blue 6B produced distinctive abnormal coloration when injected in a 1-percent solution prepared with sea water and a small quantity of distilled water. The resultant blue branchial concentration is fast for more than 100 days. With this stain, the injection quantity is critical, and if injection volumes exceed 0.03 cc. with medium shrimp initial mortality may be high. Initial mortality excepted, available data indicate no significant mortality increase over that of uninjected shrimp.

Injection of Trypan Blue, either National Aniline or Harleco, results in distinctive blue branchial coloring which is easily identifiable over a period of at least 220 days.

Immediate coloration ranges from a general pale-blue tint to a slight bluish staining of the branchiae. Gradual branchial concentration results in a dark-blue staining of these structures within 48 hours. Subsequently the branchiae may attain a bluish-gray coloration, but this change is slight and does not serve to complicate the differentiation of stained and normal shrimp. Owing to the inconspicuous nature of the initial coloring there is a definite tendency to overinject with this stain. Excessive injection volumes result in high initial mortality, and experience has shown that injections should not exceed 0.04 cc. of a 1-percent solution with medium shrimp.

Racek (1955) abandoned Trypan Blue injections owing to excessive mortality. With the exception of high mortality in one test following overinjection, no excessive mortality was ascribed to Trypan Blue in the present study. In a 52-day

controlled experiment (table 4), total mortality of injected shrimp was 6 percent less than that of the controls.

If a blue marking agent is desired, the choice is about equally divided between Trypan Blue and Niagara Sky Blue 6B. Trypan Blue appears to cause slightly less mortality, but abdominal coloring associated with Niagara Sky Blue 6B is retained for several weeks and might prove an asset in short-term marking programs.

Injection with Trypan Red, National Aniline or Harleco, results in distinctive red branchial coloring which has remained fast for 245 days and through at least two ecdyses. Injection usually results in an immediate general pink coloration which is followed by branchial concentration within 24 hours and subsequent variable abdominal fading.

A 1-percent solution prepared with one part of distilled water and nine parts of sea water has proved most satisfactory. Glycerine was used in one experiment in place of the distilled water, but this offered no advantage. Overinjection causes a high mortality which may continue for several days, and losses of equilibrium occurring more than a month after staining can probably be attributed to excessive injections. Individual injections should cease as soon as a general pink coloration is observed, and should not exceed a volume of 0.03 cc. with medium shrimp.

With the exception of deaths attributed to overinjection, total mortalities have been low considering the long holding periods. There is little doubt that some deaths have resulted from the suspected environmental deficiency as previously noted.

A number of stains tested by the feeding technique produced various degrees of temporary abnormal coloration, but only Trypan Red caused lasting and distinct coloring suitable for field identification. Feedings stained with Trypan Red resulted in a bright-red branchial color which remained fast for more than 233 days and through a minimum of three ecdyses. Marking was successful in all sizes of shrimp from 30 mm. through 175 mm.

Excessive feeding, indicated by various degrees of pink to red general staining, results in high mortality with deaths frequently preceded by loss of equilibrium. With controlled staining, however, mortality compares favorably with that of normal shrimp. Staining of individual shrimp usually accomplished in one or two feedings over a like number of days. Since aggressiveness and appetite vary with the individual, it is impossible to stain a group of shrimp simply on the basis of a predetermined number of stained feedings. Before each stained feeding, shrimp should be examined for the presence of the characteristic bright-red branchiae, and individuals so colored should receive no further stained food. With a minimum of experience it is soon possible to separate properly marked shrimp with facility.

Staining is equally pronounced with solution concentrations of 1 percent and 2 percent, but the 1-percent solution is favored as a further check against overstaining. Since no advantage was found in using sea-water solutions, distilled water is the preferred solvent. Although differences were inconsistent, the Harleco stain appeared to produce a slightly brighter color than the National Aniline product.

All of the so-called fast stains described above exhibit a slight tendency to fade, but in every case the remaining color is quite distinct over the period indicated. Whether stained shrimp will retain abnormal coloration over similar periods under natural conditions cannot be ascertained without field testing.

Under the conditions of the experiments, captive shrimp did not exhibit normal growth. Stained and normal shrimp held for extended periods showed little observable size increment even after several shedding cycles. Under conditions of normal growth, it is doubtful whether a 30- or 40-mm. shrimp could retain sufficient stain to enable identification as an adult.

Despite these questionable factors, the use of the recommended stains and procedures offers a new and useful tool for shrimp fishery investigations. Marking by injection is a rapid method applicable to either sea or shore-based operations. The feeding technique, though probably restricted to use at shore stations,

would be most applicable to the marking of small shrimp. The number of individuals marked by this method at any one time would be limited only by the quantity of available shrimp and capacity of storage facilities.

At present, only three colors, red, green, and blue, are available for use as shrimp-marking agents. Simultaneous use of identical colors on groups of shrimp of like size in neighboring areas could lead to complete confusion of results. It is strongly urged that all shrimp-marking experiments be subject to judicious and cooperative planning by the various fishery-research agencies prior to commencement of field operations.

ACKNOWLEDGMENTS

The author is indebted to Dr. Gordon Gunter, Director, Gulf Coast Research Laboratory, and the late Mr. William Herzog for valuable suggestions and assistance during the early phases of this study. The writer is also grateful for the courtesies extended by members of the Gulf Fishery Investigations, U. S. Fish and Wildlife Service, Galveston, Texas.

SUMMARY

1. Results of tests on the marking of commercial shrimp, Penaeus setiferus, P. aztecus, and P. duorarum, with biological stains are presented.

2. Experiments were conducted on captive shrimp held in storage tanks and aquariums supplied with circulating sea water.

3. Ninety-nine tests involving more than 26 stains were conducted during the period from June 1955 through June 1956.

4. Shrimp ranging in size from 30 mm. to 175 mm. were subject to one or more of three staining methods; immersion, injection, and feeding.

5. The immersion technique has been consistently unsuccessful with the stains used.

6. Fast Green FCF (National Aniline), Niagara Sky Blue 6B, Trypan Red, and Trypan Blue have proved effective with the injection technique. Each of these stains has remained fast for more than 100 days and through at least one shedding period. Trypan Red and Trypan Blue have been retained for over 200 days.

7. Trypan Red is the only fast marking agent found among stains administered by the feeding method. It is effective throughout the experimental size ranges, and is considered satisfactory for marking shrimp under a length of 80 mm. Distinctive coloration was retained for more than 233 days and through at least three ecdyses.

8. A high incidence of cannibalism among captive shrimp makes determination of experimental mortality difficult. Evidence from control experiments indicates that when recommended procedures are followed there is little difference between mortalities of marked and normal shrimp.

9. Whether these stains will remain fast for extended periods under natural conditions can only be determined by field tests.

10. Since only three colors, red, blue, and green, have been found satisfactory as marking agents, the value of these marking techniques in a field program will depend largely upon judicious and cooperative planning by the various shrimp investigations.

REFERENCES

Carranza, J.
1953. Metodos para marcar peces de pequeno tamano de utilidad en estudio de problaciones. Ann. Escuela National Ciencias Biol. 7(1-4):111-128.

Conn, H. J.
1953. Biological stains. 6th edn., 367 pp. Biotech Publications, Geneva, N.Y.

Dunn, A., and C.M. Cocker
1951. Notes on marking live fish with biological stains. Copeia 1951 (1):28-31.

Feder, H. M.
1955. The use of vital stains in marking Pacific coast starfish. California Fish & Game 41(3):245-246.

Gustafson, A. H.
1953. Preliminary investigations in marking marine worms. Res. Bull. Maine Dept. Sea and Shore Fish. 9:1-8.

Klawe, W. L.
1954. A method for marking marine worms. Science 120(3109):187-188.

Lindner, M. J., and W. W. Anderson
1956. Growth, migrations, spawning and size distribution of shrimp Penaeus setiferus. Fish. Bull. U.S. Fish and Wildlife Service, 106:555-645.

Loosanoff, V. L.
1937. Use of Nile Blue Sulfate in marking starfish. Science 85(2208):412.

McRae, E.D., Jr.
1952. Progress report on the shrimp investigation. Ann. Rept. Mar. Lab. Texas Game Fish. Comm., Sept.1, 1950, to Aug. 31, 1951, processed.

Menzel, R. W.
1955. Marking of shrimp. Science 121(3143):446.

Racek, A. A.
1955. Penaeid prawn fisheries of Australia with special reference to New South Wales. Indo-Pacific Fisheries Council (FAO) 6th Session, Tokyo, Sept. 30 - Oct. 14, 1955. Sym. 3, 18 pp., processed.

Rounsefell, G. A., and W. H. Everhart.
1953. Fishery science - its methods and applications. 444 pp., John Wiley & Sons, New York.

Rounsefell, G. A., and J. L. Kask.
1945. How to mark fish. Trans. Amer. Fish. Soc. 73:320-363.

Vernon, A. A.
1937. Starfish stains. Science 86(2220):64.

INT.-DUP. SEC., WASH., D.C. 24460