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Use of Stains in Shrimp Mark-Recapture Experiments

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Petersen tags have been used with some success to mark the white shrimp, *Penaeus setiferus*, (Lindner and Anderson 1956) and the pink shrimp, *P. duorarum*, (Iversen and Idyll 1960). However, small shrimp, because of their general frailty, suffer excessive mortality during or shortly after the tagging process. Thus an undesirable restriction of the tagging method in shrimp population studies is that tagged populations consist only of large and robust individuals in which the recurrence of molting is minimal. In addition, shrimp agility and mobility are believed greatly impaired by tags, limiting still further the method's utility as a shrimp research tool.

On the basis of small-scale laboratory trials with the white shrimp, *P. setiferus*, Menzel (1955) reported that injection of biological stains, which concentrate and remain fast in the shrimp's gill filaments, promised a more feasible means of marking penaeid shrimp. Under contract with the U. S. Bureau of Commercial Fisheries, Dawson (1957) expanded on the staining technique and eliminated all but four vital dyes that seemed most useful for this purpose. During 3 years of experiments in which 146,500 shrimp were stained and released in the South Atlantic Ocean and Gulf of Mexico, Costello (1959) and Costello and Allen (1960) demonstrated the utility of this method for studying shrimp movements and growth. Refinements in staining techniques and increasingly efficient field procedures evolved from these experiments.

Locale and timing of experiment

Successful shrimp marking requires that experimental animals be held at temperatures and salinities most conducive to their survival. It is also important to time the staining process so it will not be undertaken while high proportions of shrimp are in a "soft-shelled" condition. A high rate of mortality will prevail if shrimp are handled and stained too soon after ecdysis.

Preferably, the sites of shrimp capture, staining, and release should be contiguous so as to maintain uniform conditions throughout the marking operation and to eliminate the need for transporting marked shrimp any great distance.

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Obtaining animals for marking

Where possible, it is suggested that an experienced commercial fisherman, especially one familiar with techniques in handling live shrimp, be contracted to obtain suitable quantities of shrimp in good condition.

Grading live shrimp

When a study of growth rates is planned, shrimp of uniform size must be released. A simple and efficient device for separating shrimp into size groups is described by Allen and Costello (1961).

Field equipment

For holding shrimp prior to marking, small floating "live cars" consisting of wood frames covered with hardware cloth are useful. Twenty-four-gallon plastic garbage cans perforated with $\frac{1}{4}$ -inch holes and supported in the water by automobile tire inner tubes serve equally well. A table with inset trays to hold shrimp just prior to marking facilitates the staining operation. The table should also be fitted with a trough in which freshly stained shrimp are carried by means of running sea water to a suitable holding tank. Other equipment such as water pumps, hose systems, etc., must be arranged to suit the particular field situation.

Stain solutions

Trypan blue (C.I. No. 477), Trypan red (C.I. No. 438), fast green (FCF, C.I. No. 42053), and Niagara sky blue (6B, C.I. No. 518) have proved to be the best dyes for marking shrimp. More than any other factor, the care with which stain solutions are prepared controls the success of shrimp staining experiments. Consider the following important points when making solutions:

- (1) Sterile distilled water is the preferred solvent.
- (2) Solutions should be filtered. Unfiltered solutions may become toxic with aging.
- (3) Stains differ according to manufacturer. Fast green manufactured by the National Aniline Co., and Trypan blue, Trypan red, and Niagara sky blue (6B) manufactured by Harleco are recommended.

Staining procedure

Shrimp are injected at the articulation of the fourth and fifth abdominal segments. Care should be taken to avoid puncturing the hindgut. One-half-cc. tuberculin syringes fitted with $\frac{1}{4}$ -inch hypodermic needles (27 or 30 gauge) are recommended for injecting shrimp of all sizes.

The quantity and strength of stain solution vary according to the type of stain and size of shrimp. For a pink shrimp 130 mm. long, 0.07 ml. of a ~~25%~~ percent solution of Trypan blue, 0.18 ml. of a ~~50%~~ percent solution of fast green (FCF), or 0.09 ml. of a 0.5 percent solution of Trypan red are optimum. Only freshly prepared solutions should be used. Shrimp can be injected at the rate of 300 to 350 per hour per man.

Stain longevity

Field tests with Trypan blue and fast green indicate that these stains remain fast over a period of at least $5\frac{1}{2}$ months. Trypan red is subject to some fading. No field tests have been conducted with Niagara sky blue (6B).

Release of marked shrimp

To eliminate from any experiment specimens that do not immediately recover from handling shock, one should not release marked shrimp for at least 4 hours following injection of the staining solution. Releases should be made in areas where undue predation by fishes at the time of release can be avoided.

Recovery of marked shrimp

Because stained shrimp are not as easily detected as tagged shrimp, greater stress must be placed on the recovery phases of mark-recapture experiments using stains as marking agents. Stained shrimp are readily recognized by trained personnel but not by commercial fishermen upon whose cooperation in recognizing and returning marked specimens the success of each experiment depends. Shrimp staining experiments have proved most successful when they have been widely publicized, when sizeable rewards have been offered for recaptures, and when carefully planned and complete coverage has been given landing ports.

Summary and discussion

The usefulness of biological stains for marking penaeid shrimps has been demonstrated. The staining method is simple, rapid, and suitable over a wide range of shrimp sizes. Successful application of the staining method in mark-recapture experiments requires: (1) careful handling of shrimp; (2) correct timing of each experiment so as to avoid too high a proportion of soft-shelled (molting) animals; and (3) injection of recommended amounts of properly prepared stain solutions. The method's disadvantages are (1) animals recaptured by commercial fishermen are not as easily recognized as tagged shrimp and (2) for purposes of growth estimation, experimental shrimp must be of uniform size when released.

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