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Fluorescent Pigments as Marking Agents for Aquatic Insects[1]

Practical methods for artificially marking insect populations for study in their natural environment pose many problems. To a large degree, these vary with the requirements of the investigation and information being sought. Various methods have been used for marking insects and generally can be categorized as radioactive or nonradioactive. Most methods have both desirable and objectionable characteristics. Criteria for an effective marking material should be: 1) persistent detectable character; 2) easy to apply; 3) effective for selective and mass application; 4) nontoxic; and 5) of minimal effect on behavior of organisms. Seldom are all these characteristics attained; equipment, time, effort and objectives dictate limitations.

Use of various oil paints, lacquers, and shellacs for marking insects are discussed by Munro and Saugstad (1938), Jackson (1953), Richards and Waloff (1954), and MacLeod and Donnelly (1957). These have generally been unsatisfactory because peeling often occurs.

Various radioactive materials have been used for labelling insects, most popular of which is radioactive phosphorus (P^{32}). Fredeen *et al* (1953), Ball *et al* (1963), Baldwin *et al* (1966), and Bishop and Bishop (1968) discuss their application in aquatic studies. Although these materials have many virtues, the equipment, certification of use, and possible contamination of the environment often limit their usability.

Fluorescent marking pigments applied to terrestrial insects either as a powder or with a solvent have been studied by Chang (1946), Smith and Townsend (1951), Davey (1953), Jewell (1956), and others. Turner and Gerhardt (1965) found their fluorescing properties under ultra-violet light advantageous for marking and relocating face flies. Jackson (1959) and Phinney (1966) reported the use of fluorescent pigments for marking fish.

In this study, methods of application and an evaluation of fluorescent pigments for marking aquatic insects were studied.

Materials and Methods

Glow-mark fluorescent pigments[2] were used as the marking material. Pigments can be obtained as fine powders (3-5 microns) or granules (50-350 microns) and come in four colors: red, yellow, blue and green. These pigments are slightly soluble in acetone and other ketones, but soluble in water, formaldehyde, and most aromatic hydrocarbons. The pigments fluoresce when activated by ultra-violet light.

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[2] Obtained from Scientific Marking Materials, 209 Northwest 58th Street, Seattle, Washington.

A red pigment in the form of fine powder was used as the marking agent in this study. An inexpensive, improvised fogging device, similar in design to an aspirator, was used for applying the powder. It consisted of a small jar container with ¼-inch inlet and outlet glass tubing fitted into a rubber stopper. The outlet tube was narrowed to a 2 mm opening to permit concentration of powder upon emission. The inlet tube was connected to a rubber hose, and subsequently to an air source. Initial applications were applied with an air compressor. Pressures of eight to 12 pounds per inch provided the most uniform distribution of powder and did not cause apparent abrasions or other deleterious effects to the insects. For field use, pressure from lung exhalation was found satisfactory and greatly simplified field procedures and equipment. Continuous agitation of the applicator during application resulted in more uniform emission of powder.

In addition to powder application, pigment was applied as a spray, using acetone as the solvent. This technique was discarded in favor of the former since the material solidified over the exoskeleton rendering the insect partially or completely immobile. Severe peeling was also noted in many cases after the insects were released into the water.

Techniques and effectiveness of pigments as marking agents were studied in an artificial stream, and aquaria in the laboratory and later in the field. To facilitate recovery and observation, larger species in the orders Plecoptera, Ephemeroptera, Odonata, Trichoptera and Diptera were used (Table 1).

Insects used for marking were collected and confined to 3″ x 3″ x 5″ screened containers made of 14 x 18 mesh copper screening and submerged in water. Prior to marking, the container with the insects was momentarily lifted from the water, allowing excessive water to be shed and placed over an open pan of water. The insects were then fogged and submerged several times, allowing excessive powder to be washed free. The latter is important since the water in the pan tends to trap much of the excessive powder, thus reducing stream or aquaria contamination. For field marking, it is recommended that fogging be done several feet from the water. Time required for marking a single insect or a number of insects is not appreciably different. Actual marking time usually takes 10-15 seconds, therefore, not subjecting the insects to long periods out of water.

In addition to daytime detection of marked insects, nighttime detection is also possible with the use of a high intensity, ultra-violet light. The employment of a portable power unit makes the technique practical for field use.

Discussion and Evaluation

Glow-mark fluorescent powders were found to be effective marking agents for short-term studies with many immature aquatic insects. Pigments are particularly effective and retained longer on most pubescent forms. Intersegmental membranes, wing pads, and gills demonstrated retention properties. Little or no detectable toxic effect, nor apparent respiration inhibition, was noted for the insects studied. Because of periodic molting during development, superficial marking materials are not effective for long-term studies of several weeks.

Selected insect species from the principal orders of aquatic insects were studied and evaluated (Table 1). Mayfly naiads demonstrated fair to good pigment retention with detectable pigment generally visible for one week or more. Stonefly naiads

TABLE 1. Retention Properties of Fluorescent Pigments on Selected Aquatic Insects Based on Visual Detection[1]

Species	Body Covering	Retention Time (Days)[2]	Comments
Ephemeroptera (Mayflies)			
Ephemerella doddsi Needham	Smooth	5-8	Good retention—primarily around intersegmental membranes, pronotum and wing pads.
Ephemerella grandis Eaton	Spiny, scattered pubescence	5-10	Good retention—pronotum, wing pads and outer faces of femora.
Ephemerella hecuba (Eaton)	Pubescent	5-10	Good retention—wing pads, outer faces of femora and intersegmental membranes.
Ephemerella flavilinea McDunnough	Smooth	4-8	Fair to good retention—wing pads and intersegmental membranes.
Baetis tricaudatus Dodds	Smooth	2-5	Poor to fair retention—wing pads and intersegmental membranes; nymphs too small to be practical for many field studies.
Plecoptera (Stoneflies)			
Pteronarcys californica Newport	Fine pubescence	20-35	Excellent retention—thoracic nota, wing pads and abdominal terga; large size makes them ideal for field studies.
Acroneuria sp.	Fine pubescence	18-35	Excellent retention—vertex of head, pronotum and abdominal terga; large size makes them well suited for field studies.
Isogenus sp.	Smooth	5-10	Good retention—intersegmental membranes
Alloperla sp.	Pubescent	10-21	Excellent retention—entire body; small size reduces their importance for field studies.
Odonata (Dragonflies and Damselflies)			
Coenagrion sp.	Smooth	2-6	Pigment retention fair—best retention on pronotum and wing pads.
Aeshna sp.	Fine pubescence	10-17	Excellent retention—pigment adhering to nota, wing pads and abdominal terga.
Plathemis sp.	Pubescent	<1-2	Poor retention—pigment rapidly lost although body densely pubescent.
Trichoptera (Caddisflies)			
Hydropsyche sp. (net builder)	Smooth	2-4	Poor-fair retention—pigment adhering primarily to intersegmental membranes and gills; pigment rapidly lost due to net construction and movements in intersticies of substrate; some mortality noted.

[1] Results based on laboratory studies in an artificial stream and aquaria.
[2] Retention time based on visual detection during stadium of late-instar nymphs or larvae.

TABLE 1

Species	Body Covering	Retention Time (Days)[2]	Comments
Arctopsyche sp. (net builder)	Smooth	2-4	Poor-fair retention—pigment adhering primarily to gills and intersegmental membranes; pigment lost due to net construction and movements in intersticies of bottom; some mortality noted.
Brachycentrus sp. (case builder)	case of plant material	3-6	Case has fair pigment retention—larvae mostly unexposed to pigment because of protective cases; some larvae noted leaving cases after pigment application.
Dicosmoecus sp. (case builder)	case of sand grains	7-12	Case has good pigment retention—larvae mostly unexposed to pigment because of protective cases; high incidence of larvae leaving cases after pigment application reduces effectiveness.
Diptera (Flies)			
Tipula sp.	Smooth	<1	Poor retention—pigment rapidly lost because of smooth cuticle and larval habits of living and moving in intersticies of bottom.
Atherix sp.	smooth	<1	Poor retention—pigment rapidly lost because of smooth cuticle and larval habits of living and moving in intersticies of bottom.

retained the pigment for as long as five weeks. With odonate naiads, retention proved highly variable with detectable pigment in evidence for less than one day to over two weeks for the different species studied. The dipteran larvae, *Tipula* sp. (Tipulidae) and *Atherix* sp. (Rhagionidae) showed poor pigment retention. This was probably due to their smooth cuticle and characteristic habits of living and moving in the interstices of the substrate. Trichopteran larvae generally showed poor to fair retention. Their habits, as to whether they are net builders, case builders or, in some cases, free living, greatly influenced the effectiveness of marking. The protective cases of the case-building trichopterans retained the pigment moderately well. Some mortality was noted with the net building hydropsychid larvae *Hydropsyche* and *Arctopsyche*. Numerous larvae of *Dicosmoecus* and *Brachycentrus* were observed leaving their cases after pigment application.

To effectively study insect populations, using a visible marking agent, it is important that marked individuals do not elicit abnormal responses from their unmarked cohorts or among themselves. Based on laboratory observations there was no apparent evidence of abnormal behavior for any of the species studied. Both marked and unmarked individuals of the same and/or different species were often found residing in the same proximity and behaving similarly to what would be expected under natural conditions.

Using fluorescent powders for marking aquatic insects permits a wide range of behavioral and population ecology studies both in the laboratory and in the field. Realizing the limitations of time of a superficial marking material, fluorescent pigments can be very effective for marking many aquatic insects. The studies necessarily must be short range, generally 48 hours or in some cases longer, depending on the insects used. The fluorescing properties of Glow-mark pigments under ultra-violet light enable investigators to monitor and observe the behavior of aquatic insects at night, thus permitting a more complete understanding of their daily activities with special reference to their micro-ecology, migrations, and inter- and intra-specific interactions.

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