Reprinted from PROCEEDINGS AND PAPERS OF THE FORTY-EIGHTH ANNUAL CONFERENCE OF THE CALIFORNIA MOSQUITO AND VECTOR CONTROL ASSOCIATION, INC., JANUARY 20 - 23, 1980
PUBLISHED September 1, 1980

MASS MARKING OF MOSQUITOFISH: PRELIMINARY RESULTS

Bruce Vondracek, Wayne A. Wurtsbaugh, and Joseph J. Cech, Jr.

University of California

Wildlife and Fisheries Biology, Davis, California 95616

ABSTRACT

Three marking techniques were tested to determine their applicability for mosquitofish. Tetracycline drugs and DCAF administered in the diet successfully marked laboratory-cultured mosquitofish, but exposure to direct sunlight in outdoor tanks resulted in the rapid disappearance of the marks. Preliminary data on fluorescent marks from a polystyrene pigment in a melamine-sulfonamide-formaldehyde resin forced into the dermal tissue with compressed air are more promising. At an optimal deliver pressure of 7.3 m Hg (140 p.s.i.) and spraying time of 15 sec., marking percentage is maximized and fish mortality is minimized. Mark retention time was up to 80 days in outdoor tanks at this writing. Long retention times and the availability of four colors increases the potential of this marking technique as a useful tool in field population studies of mosquitofish.

INTRODUCTION.- Although the mosquitofish (*Gambusia affinis*) has been introduced throughout California as a biological control agent for mosquitoes, many aspects of the microdistribution and population dynamics of this species are poorly understood. Accurate assessments of mosquitofish population densities, survival rates, dispersal rates, and dispersal distances are important for effective mosquito control while minimizing disturbance to local fish communities.

Marking fish in some manner (e.g. tags, fin clips) is often the most straightforward technique used in calculating population parameters (see Laird and Stott 1978). Tags are inappropriate for marking mosquitofish due to the small size of these animals and the large numbers which must be marked to obtain accurate population data. A desirable mark for mosquitofish would (1) be rapidly and easily applied to large numbers of fish, (2) not affect behavior or increase mortality rates through predation, and (3) be distinctive and retained for several months. The objective of the present study was to develop a marking technique having the above characteristics.

Three marking techniques which have been used successfully for other fishes were tested on mosquitofish. The tetracycline series of drugs was used by Weber and Ridgeway (1962, 1967) to mark skeletal structures and scales of Pacific salmonids. Tetracycline added to the diet, is incorporated into collagen fibrils of bones and scales. The deposited antibiotic is detectable by a yellow fluorescence when exposed to ultraviolet (UV) light (Weber and Ridgeway 1962). The second compound tested was 2,4 bis (N, N' di-carboxymethyl aminomethyl) fluorescein (DCAF) which is also administered in the diet. Hankin (1978) reported DCAF was incorporated into growing scales of guppies, *Poecilia reticulata* and is detected as a yellow-green fluorescence when subjected to UV light. Finally, we tested a fluorescent polystyrene pigment in a melamine-sulfonamide-formaldehyde resin which is forced into the dermal tissue with compressed air from a small sandblasting gun (Jackson 1959, Scidmore 1961, Phinney et al. 1967, Phinney and Matthews 1969). Detection of the pigment requires exposure to UV light.

METHODS. Tetracycline - - Oxytetracline and chlorotetracycline (Sigma Chemical Co.) were used to test the applicability of the tetracycline antibiotic series as a marker for mosquitofish. These chemicals, and in many cases a potentiator, were incorporated into the diet by mixing them with an aqueous slurry of a commercially available food (Basic Flake®) in a blender, then drying the slurry on foil sheets to reform a flake diet. Tetracycline was administered at three levels: 100, 250 and 500 mg tetracycline/kg of mosquitofish/day. Weber and Ridgeway (1967) state that 300 mg/kg/day fed to young salmonids resulted in good marks. Glucosamine was used as a potentiator (Weber and Ridgeway 1967) in conjunction with tetracycline at four levels 0, 25, 50 and 200 mg glucosamine/kg of mosquitofish/day.

Mosquitofish (*Gambusia affinis*) for all experiments were collected from a local stream and held in flow-through tanks, receiving a continuous flow of tap water. Temperatures of the non-chlorinated tap water supply varied seasonally between 18 and 23°C. Groups of twenty males or females of two qualitative sizes (small-ca. 15-20 mm and large-ca. 25-30 mm standard length) were used in the feeding trials. As small females exhibit the fastest growth rates and, therefore, presumably incorporate tetracycline at the highest rate, this group was also tested at various tetracycline/potentiator concentrations (Table 1). Other sex-size combinations were chosen for comparison with small females at the highest concentrations only. Each group of 20 fish was weighed in a tared beaker of water and placed in 15 1 flow-through aquaria. Each experimental group was fed at 20% initial average body weight/day in two daily feedings for 21 days.

The treated fish and a control group receiving only Basic Flake® were examined at 4, 10, 15 and 21 days after the initial feeding to assess onset and intensity of marking. Each group of fish was placed in a shallow pan (1 cm water depth) and viewed under UV light (G. E. 40W fluorescent tube, spectral emission peak 360 nm).

After 21 days each experimental group was divided in half. Ten fish were moved to outdoor 15 1 aquaria receiving direct sunlight throughout the day. After 24 hours these fish were examined and compared to the remaining experimental fish held inside the laboratory under normal fluorescent lighting.

DCAF - - 2, 4 bis (N, N' di-carboxymethyl aminomethyl) fluorescein (DCAF) was administered orally in combination with glucosamine and Basic Flake®. Two ration levels 100 and

1000 mg DCAF/kg of mosquitofish/day were fed in conjunction with 10 mg glucosamine/kg/day (Table 1). DCAF was dissolved in 0.5N NaOH and then incorporated into the flake diet in the same manner used for tetracycline. Experimental fish were fed at 20% initial average body weight/day in two daily feedings for ten days.

Fish were viewed under UV light source at 4 and 10 days after initial feeding to assess the rate of DCAF incorporation in the scales. After 10 days, half of the fish were placed outside in 15 1 aquaria. Fish were examined after one day exposure to sunlight and compared to experimental fish held inside under laboratory lighting.

Fluorescent Polystyrene Pigment - - The equipment used for the forced, external application of this powdered substance includes: (1) a low pressure spray gun and hose (Port-a-Blast, Lindberg Products Co.), (2) a compressed air tank with single stage regulator, (3) a container to hold fish during marking, and (4) the fluorescent polystyrene pigment in a melamine-sulfonamide-formaldehyde resin available from Scientific Marking Materials, Seattle, Washington.

One-hundred fish (>15 mm standard length) were used in each marking trial. The fish were placed in a 30 X 30 X 5 cm box with wood sides and plastic screen bottom (1 mm x 1 mm mesh) and top (3 x 4 mm mesh) to retain fish inside. Four delivery air pressures were tested (100, 120, 140 and 160 p.s.i.) at two time (10 and 15 sec) durations (Table 2). Immediately following pigment application fish were rinsed with water and placed into a water bath. Each group was divided in half and placed into 15 1 flow-through aquaria. A single potassium permanganate ($KMnO_4$) treatment (2 ppm) was flushed through the aquaria to retard bacterial infection.

After pigments in the external mucus coat had sloughed (3-4 days) the fish were examined under UV light. Marks clearly visible from above when the UV source was placed 45 cm above the fish were considered acceptable.

After initial inspection, treatment groups and a control group of fish were placed in outdoor glass aquaria to determine marking-induced mortality rates and the effects of sunlight on mark duration and intensity. Deaths occurring within 24 hr of pigment application were classified as "initial" mortalities. A continuing record of mortalities was mantained to determine "long term" mortality rate.

RESULTS.–Tetracycline and DCAF - - All fish fed tetracycline drugs and DCAF exhibited a visible fluorescence when exposed to UV light. Within 10 days the scales of all fish receiving DCAF in their diet were fluorescent, while 21 days was required for the tetracycline-fed mosquitofish. This fluorescence persisted in fish held inside the laboratory for at least an additional 24 hr. However, exposure to direct sunlight for one day resulted in the disappearance of fluorescence from mosquitofish fed either tetracycline or DCAF.

Fluorescent Polystyrene Pigment - - Marking success was positively correlated with delivery air pressure and time duration. The percent mosquitofish marked increased from 20% at 100 p.s.i. to approximately 60% at 160 p.s.i., based on percentage of the initial sample of 100 fish (Table 2). Initial mortality percentages were also positively correlated with delivery pressure and time duration. Percent initial mortality was very low at 100, 120, and 140 p.s.i., but increased dramatically at 160 p.s.i. (Table 2). On the other hand, extended mortality percentage appeared to be uniformly low for all delivery pressures and time durations, except for the treatment

Table 1.–Summary of experimental design of the concentration, sex and size combinations of tetracycline drugs and DCAF administered to mosquitofish in the diet.

Marking Chemical	Concentration mg/kg/d	Potentiator mg/kg/d	Size-sex	Number of fish	Mean fish Weight (g)
Tetracycline	100	25	small female	20	.342
Tetracycline	250	25	small female	20	.276
Tetracycline	500	25	small female	20	.299
Tetracycline	250	0	small female	20	.365
Tetracycline	250	50	small female	20	.355
Tetracycline	250	200	small female	20	.293
Tetracycline	250	25	large female	20	.943
Tetracycline	250	25	large male	20	.202
Tetracycline	250	25	small male	20	.124
Tetracycline-HCL	100	25	small female	20	.329
Tetracycline-HCL	250	25	small female	20	.318
Tetracycline-HCL	500	25	small female	20	.345
DCAF	100	10	small female	10	.524
DCAF	100	10	small female	10	.433
DCAF	1000	10	small female	10	.544
DCAF	100	10	mixed male	10	.209
DCAF	100	10	mixed male	10	.217
DCAF	100	10	large female	10	.904
DCAF	100	10	large female	10	1.211

Table 2.–Marking success and mortality rates of mosquitofish at different delivery air pressures and time durations with fluorescent polystyrene pigment forced into dermal tissues. Initial mortalities were recorded after 24 hr, extended mortalities after 80 days.

Delivery pressure (p.s.i.)	Time duration (sec)	Marking Success (%)	Initial Mortality (%)	Extended Mortality (%)
100	10	20	1	–*
120	10	38	0	16
140	10	28	3	3
140	15	45	4	–*
160	10	59	15	3
160	15	54	34	2
Control				20

*Extended mortality not measured.

at 120 p.s.i. The mortality percentage at 120 p.s.i. was five times higher than other treatments, but still below the number of mortalities observed in the control group (Table 2). Delivery pressures of 140 p.s.i. for 15 sec. seems to be the optimal marking protocol at this time. Marking success is near 50%, while mortality rates are low.

Mark retention studies are still underway on two groups initially marked at 100 p.s.i. for 10 sec. and 140 p.s.i. for 15 sec. These fish have retained clear and distinctive marks for 80 days at this writing, even with exposure to sunlight.

DISCUSSION. Weber and Ridgeway (1962) and Hankin (1978) using tetracycline and DCAF respectively, obtained fluorescent rings on scales of fishes. In both cases individual scales were viewed using a microscope. Marks were retained at least three months using either chemical. To allow mosquitofish life history parameters to be evaluated with minimal laboratory time, marked mosquitofish must be immediately recognized. Thus, the tetracycline and DCAF dosages in the present study were increased an average of three-fold over those used in past studies in order to mark the whole fish. Nevertheless, the fluorescent mark disappeared from *Gambusia* after one day in direct sunlight. Mosquitofish typically inhabit surface layers of shallow, warm waters. Thus, they are exposed to comparatively intense solar radiation, including some UV (Wetzel 1975) which apparently obliterates the marks produced by the tetracycline and DCAF techniques. In contrast, the preliminary results using the sprayed polystyrene pigments meet all the criteria desired for mosquitofish marks. That is, the mark is easily and rapidly applied, is not visible under visible light and is retained for a sufficient period to obtain pertinent life history data. Pribble (1976) reports that 35,000 fish can be marked per hour and that the sprayed pigment marks were retained at least two years.

The availability of 4 colors of fluorescent pigments increases the sophistication of potential mark-recapture data. For example, movements of fish simultaneously planted at different locations could be distinguished. Also the growth and mosquito predation performance of different genetic stocks could be quickly assessed.

As these preliminary data do not include replicates testing spray delivery pressures, time durations, or effects of fish size or sex, further studies should be conducted prior to wide scale field applications. Field experience should streamline the marking, handling and identification of large numbers of mosquitofish in stocked habitats. Reliable estimates of marking success and resulting mortalities should enhance the precision of mosquitofish population size calculations.

ACKNOWLEDGMENTS.–We gratefully acknowledge the loan of the spraying apparatus and pigments from R. Coykendall of the Sutter-Yuba Mosquito Abatement District. We also thank T. Wragg for technical assistance, D. Lombardo for manuscript typing, and the State of California for financial support.

REFERENCES CITED

Hankin, D. G. 1978. New fluorescent fish scale marker. Prog. Fish-Cult. 40(4):163-164.

Jackson, C. F. 1959. A technique for mass-marking fish by means of compressed air. Univ. New Hampshire. Tech. Circ. No. 17. 8 p.

Laird, L. M. and B. Stott. 1978. Marking and Tagging p. 84-100 IN: T. Bagenal (ed.) Methods for Assessment of Fish Production in Fresh Waters. IBP Handbook No. 3. Blackwell Scientific Publications, Oxford and Edinburgh.

Phinney, D. E. and S. B. Mathews. 1969. Field test of fluorescent pigment marking and fin clipping of coho salmon. J. Fish. Res. Bd. Can. 26(6):1619-1624.

Phinney, D. E., D. M. Miller and M. L. Dahlberg. 1967. Mass-marking of young salmonids with fluorescent pigments. Trans. Am. Fish. Soc. 96(2):157-162.

Pribble, J. 1976. Pressure spray marking of fish with grandular dyes. Oregon Dept. Fish. Wildl. Report, Corvallis, Oregon.

Seidmore, W. J. 1961. A test of the compressed air technique for marking fish. Minn. Dept. Conser. Div. Res. Plan. Invest. Report No. 240. 8 p.

Weber, D. D. and G. J. Ridgeway. 1962. The deposition of tetracycline drugs in bones and scales of fish and its possible use for marking. Prog. Fish-Cult. 24(4):150-155.

Weber, D. D. and G. J. Ridgeway. 1967. Marking pacific salmon with tetracycline antibiotics. J. Fish. Res. Bd. Can. 24(4):849-865.

Wetzel, R. G. 1975. Limnology. W. B. Saunders Co., Philadephia.