Moore, W. J. 1972. Physical Chemistry. 4th ed. Prentice-Hall, Inc., Inglewood Cliffs, N.J. 977 p.

Pervomaiskii, G., V. Osipyan, V. Kazhdan. L. Maslii, A. Shustrov, B. Graborskii, D. Zhogolev and I. Dunavea. 1963. Voenna-Med. Akad. in Dirova, U.S.S.R., Medl. Parazitol. Parazit. Bolez. 36(6):730-733.

Roadhouse, L. A. O. 1953. Laboratory studies on insect repellency. Can. J. Zool. 31:535-546.

Shimmin, R. K., S. F. Bayles, T. S. Spencer, R. Grothaus and W. A. Akers. 1974. Four-site

method for mosquito repellent field trials. Proceedings and Papers of the American Mosquito Control Association, Anaheim, CA, 121–123.

Smith, C. N., I. H. Gilbert, H. K. Gouck, M. C. Bowman, F. Acree, Jr. and C. H. Schmidt. 1963. Factors affecting the protection period of mosquito repellents. U.S. Dept. Agr. Tech. Bull. 1282-1-26

Bull. 1285:1-36. Stepanor, M., V. Osipyan, V. Kazhdan, L. Maslii, B. Graborskii and I. Dunavea. 1969. Leningrad Scientific Research Institute Chem. Brit. Patent 1, 151, 153.

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TRANSPORTING GAMBUSIA AFFINIS FROM ALABAMA AND LOUISIANA AND STOCKING IN NORTHEASTERN ILLINOIS

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ABSTRACT. In 1973, 1974, and 1975 the Clarke Outdoor Spraying Company mass transported *Gambusia affinis* (Baird and Girard) from Alabama and Louisiana to permanent water mosquito breeding areas in northeastern Illinois.

INTRODUCTION

The problem of resistance to insecticides coupled with the increasing expense of developing new insecticides has triggered the search for alternative control methods. The use of alternative biological control agents on a large scale may play an increasingly important role in future mosquito control operations.

The predator poeciliid topminnow, Gambusia affinis (Baird and Girard), (Figure 1), known commonly as mosquitofish, occurs as 2 subspecies: Gambusia affinis affinis and Gambusia affinis holbrooki. The natural range of G. a. affinis extends from the Gulf of Mexico through the Mississippi River Valley and its tributary waters as far north as central Illinois. G. a. holbrooki ranges from southern New Jersey along the Atlantic coast to the Florida Keys and along the Gulf of Mexico

The logistics of the operation included initial survey and planning, collecting, packaging, shipping and stocking. Economic, overwintering, and ecological factors were considered. The goal was to minimize mortality and expense.

to northwestern Florida (Dees 1961). This subspecies is unable to survive northern winters (Wascko 1950).

Introductions have expanded the range





Fig. 1. The mosquitofish, Gambusia affinis (Eddy 1969).

of mosquitofish into at least 16 northern and western states (Dees 1961). The use and effectiveness of *G. affinis* in mosquito control operations have been well documented. In Michigan Krumholz (1948) found in experiments during 2 summers that mosquitofish were 81 and 95 percent effective in controlling breeding in ponds. In Georgia ponds and swamps, Hildebrand (1925) found this species able to reduce anopheline and culicine populations by 50 and 80 percent, respectively.

G. affinis is a successful predator for the following reasons: 1. It feeds voraciously in shallow, shoreline waters where mosquito larvae develop; 2. It avoids predation by other fish in the shoreline habitat; 3. Its mouth is adapted for efficient surface feeding and use of the atmospherewater interface in oxygen deficient waters (Lewis 1970); 4. It has a rapid reproductive cycle; and 5. It tolerates temperature changes, organic pollution, and brackish water.

In 1973, 1974, and 1975 the Clarke Outdoor Spraying Company (commercial contractor) mass transported G. affinis from Alabama and Louisiana to northeastern Illinois. The goal of the operation was to minimize mortality and expense during shipment and insure establishment at appropriate sites.

METHODS

Commercial fish farmers look upon G. affinis as an undesirable "pest" which may threaten the existence of game fish species by feeding on their young fry (Myers 1965). No fish hatchery could be located that raises mosquitofish commercially. The natural habitat of canals, estuaries, and sloughs provided the source of G. affinis.

In 1973, the Clarke Outdoor Spraying Company obtained 24,000 *G. affinis* through the Deep South Fisheries of Tuscaloosa, Alabama. This company seined and packaged the fish. In 1974 and 1975, the Jefferson Parish Mosquito Control Department of Metairie, Louisi-

ana trapped G. affinis (37,800 and 40,000, respectively), while National Tropical Fish Imports of Kenner, Louisiana packaged them. In all 3 years, the fish were shipped to Illinois via commercial airline. From O'Hare International Airport, Chicago, Illinois, Clarke personnel transported the fish to the stocking sites.

The operation is described in 5 stages, developed through 3 years experience.

INITIAL SURVEY AND PLANNING. Mosquito breeding areas in each community were surveyed and appropriate sites for G. affinis chosen. These sites were typically permanent water ponds and marshes, sewage treatment lagoons, and golf course water hazards.

Once the stocking sites and total acreage were known, the exact number of mosquitofish required for a successful stocking operation was determined. A stocking rate of 300 G. affinis per acre was used (Hoy et al. 1971). The number of fish handled per day was dependent upon labor and vehicle availability. One man efficiently handled and stocked 3,000 fish (10 acres) per day.

Using the survey data, a daily stocking schedule was prepared. The communities were grouped geographically and a stocking sequence organized. The source for *G. affinis* was then confirmed and the number of fish for each day of the operation ordered.

COLLECTING TECHNIQUES. The capture methods used were seining and trapping. In each method a minimal number of fish per unit catch was desired. Fewer fish in the net or trap decreased bruising of fish epidermis. Bruising removes the protective mucous of the epidermis resulting in a breakdown of the natural defense mechanism, and the fish then become more susceptible to attack by bacteria and fungi.

G. affinis were easily observed in shallow shoreline waters cruising and feeding on the surface. In 1973, small nylon seine nets (10 x 4 ft with 1/2 in mesh) were employed by having one man on each end of the net. Approximately 10 ft from

shore, the net was lowered and moved swiftly along the bottom toward the bank. At the shoreline, the water surface adjacent to the outer edge of the net was splashed by foot to prevent fish escaping. The seine was then quickly scooped out of the water onto shore.

In 1974 and 1975, the mosquitofish were collected with a two-piece, cylindrical, wire minnow trap, Herter's Live Bait Trap (16¾ in. overall length x 8¾ in. center dia x 7 in. end dia). The trap was floated in deep water or along the shore and baited with a dry pellet-type dog food. The fish were attracted into the trap and captured. To prevent injury to the fish epidermis, 1 to 1½ in. of the trap remained above water surface.

Enough G. affinis were collected to meet the demand of the stocking schedule, previously planned. It should be noted that other undesirable fishes and aquatic animals were inadvertently collected. These were separated by hand, leaving the G. affinis.

PACKAGING TECHNIQUES. Once G. affinis were collected and on shore, the fish were carefully transferred from the net or trap into plastic bags containing 2 gal of 1 percent saline, aged tap water at the rate of 350 fish/bag. The fish length ranged in size from 1 to 2 in. with many of the females gravid. The bag and source water temperatures were equal to prevent thermal shocking. The saline tap water minimized the presence and growth of bacteria and fungi. Three hundred fifty fish/bag reduced bruising compared to higher packing rates.

The mosquitofish were then transferred into a holding tank containing the same water described above for 12 hr. This step allowed the fish to adjust to the clean water. The fish were then transferred into plastic bags containing 2 gal of the 1 percent saline water at the same rate of 350 fish/bag. A 2.0 percent aqueous solution of methyl blue dye was added at the rate of 8 oz/bag to further inhibit bacterial and fungal growth. Finally, the bags were charged with oxygen, sealed and

packed in styrofoam boxes which were then placed inside cardboard boxes (17 x 17 x 10 in) for shipping.

SHIPPING TECHNIQUES. Once the G. affinis were prepared for shipping, the boxes were sent via commercial airline to the destination. A direct flight was preferred to minimize travel time and handling. Special care was taken to keep the boxes level. Tipping will slosh the water and possibly damage the fish.

Transporting and Stocking Techniques. Once boxed, the fish were relocated in Illinois within 24 hr. Using the above packaging techniques, the fish could have been kept for 36 hr with minimal mortality. More fish/box decreases the "safe keeping time" due to the higher demand for oxygen, increased fish contact, and a higher concentration of metabolic waste.

While in transit from the airport to the stocking sites, the *G. affinis* were left sealed in the boxes. If opened, the exposure to daylight will increase swimming activity resulting in greater oxygen consumption and bruising.

At the stocking sites, the boxes were opened and the plastic bags immersed in the water. To guard against thermal shock, the temperatures of the bag and ambient water were checked. If the temperature difference was greater than 2° F, water was slowly splashed into the bag to allow equalization. At this point, the G. affinis were released into their new environment.

RESULTS AND DISCUSSION

In the 3 years of transporting and stocking mosquitofish, a 3 percent mortality rate was observed in 1975, after 25 and 70 percent in 1973 and 1974, respectively (Table 1). The mortality rates were determined at the stocking sites by estimating the percentage of dead G. affinis/bag. In 1975, fish survival approached 100 percent and the cost per individual fish transported nearly equaled the cost per survivor (Table 1). The operational

Table 1. Comparison and cost of critical factors of transporting and stocking operation by year.

	1973	1974	1975
Total fish transported Fish per box Surviving fish stocked Mortality rate Collecting and packaging cost Cost per fish transported Cost per surviving fish	24,000 500 18,000 25% \$800.00 \$ 0.05	37,800 600 11,340 70% \$423.31 \$ 0.025 \$ 0.08	40,000 350 38,800 3% \$970.00 \$ 0.045

goal of minimizing mortality and expense has been approached through trial and error experimentation, resulting in refined logistic techniques.

The noticeable errors made in 1973 and 1974 were the following: 1. Overcrowding the fish (up to 600/box) in their contaminated natural habitat water without adding methyl blue dye; 2. Placing the fish directly on the aircraft without holding in tanks; 3. Shipping the fish on an indirect flight; 4. Opening the boxes at the airport; and 5. Not having sufficient labor and vehicles for transporting and stocking.

The 1975 techniques were improved so that the only critical factor affecting the mortality rate was the number of fish packaged/box. The techniques of collecting and packaging were the most critical factors affecting the cost of the operation. The additional time and precautions taken in these stages increased the cost. The air freight and stocking costs are proportionate to the total number of fish handled.

In the overall analysis, the most advantageous combination of fish packaged/box and acceptable mortality rate has yet to be determined. Achieving 100 percent survival may outweigh the cost involved. Increasing the fish/box will decrease the number of boxes required for shipping and lower the air freight cost. Keeping all other techniques constant, the mortality rate will increase due to overcrowding in this limited microenvironment. This adjustment in procedure may prove to be more economical.

From the data compiled, it appears the most economic packaging rate is between 350 and 500 fish/box.

Overwintering and Ecological Considerations. Each spring following the 1973-74 stocking operations many sites were inspected for surviving mosquitofish. None was observed to have overwintered. Many of the stocked fish have been identified as G. a. affinis, the hardier of the 2 subspecies. This indicates that this southern population of G. a. affinis, native to warm waters of Alabama and Louisiana, is unable to adapt and acclimate to the harsh northern Illinois winters.

From the ecological standpoint, it is well that the southern population of G. a. affinis does not overwinter. The persistence of hardy G. a. affinis in nothern Illinois waters could reduce or even eliminate native fish species and other aquatic organisms due to competition for food and territory and alteration of the ecosystem by mosquitofish predation (Hurlbert et al. 1972).

The inability of southern G. a. affinis to overwinter makes this biological control agent a temporary control measure. The fish are introduced in early summer, control populations of mosquito larvae, and die and "biodegrade" in the winter, thus maintaining the ecological balance.

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Literature Cited

Dees, L. T. 1961. The mosquitofish, Gambusia affinis. U. S. Dept. of the Interior, Fish and Wildlife Service, Bureau of Commercial Fisheries. Fishery Leaflet Number 525.

Eddy, S. 1969. (Sixth Printing, 1974). How to Know the Freshwater Fishes. Wm. C.

Brown Company, Dubuque, Iowa.

Hildebrand, S. F. 1925. A study of the top minnow Gambusia holbrooki in its relation to mosquito control. U. S. Public Health Service, Bull. No. 153:1-36.

Hoy, J. B., A. G. O'Berg and E. E. Kauffman.

1971. The mosquitofish as a biological control agent against Culex tarsalis and Anopheles freeborni in Sarramento Valley rice fields. Mosquito News 31(2):146-152.

Hurlbert, S. H., J. Zedler and D. Fairbanks. 1972. Ecosystem alteration by mosquitofish (Gambusia affinis) predation. Science 172: 630-641.

Krumholz, L. A. 1948. Reproduction in the western mosquitofish, Gambusia affinis affinis (Baird and Garard), and its use in mosquito control. Ecological Monographs 18(1):1-43.

Lewis, W. M. 1970. Morphological adaptions of cyprinodontoids for inhabiting oxygen deficient waters. Copeia 1970:319-326.

Myers, G. S. 1965. Gambusia, the fish destroyer. Trop. Fish Hobby. January:31-32, 53-54.

Wascko, H. 1950. Gambusia in northwestern Ohio. Ohio Dept. of Natural Resources, Bull. No. 240:1-4.