FISHES IN CALIFORNIA MOSQUITO CONTROL

By

THE BIOLOGICAL CONTROL COMMITTEE

CALIFORNIA MOSQUITO & VECTOR CONTROL ASSOCIATION

Edited By

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CALIFORNIA MOSQUITO AND
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PREFACE

This publication presents introductory, technical, and reference material concerning the selection, use and culture of fishes for mosquito control. It is directed at the technician involved in mosquito control to provide a complete overview of the fishes associated with California mosquito control. Information is presented in a way that both the novice to mosquito control and the seasoned veteran can appreciate. Most of the text focuses on the mosquitofish, *Gambusia affinis* (Baird & Girard); because it is the primary fish cultured, used and distributed for mosquito control in California. The first California introduction occurred in April, 1922 when 590 mosquitofish were transported from Austin, Texas to a lily pond at Sutter's Fort, Sacramento, by the California State Board of Health. The species became well established in California, largely through the efforts of Louva G. Lenert and Edward Stuart of the mosquito control program, California State Board of Health. In 1923, they established some 25 hatcheries from Redding to Santa Ana, from which mosquitofish were distributed to 24 counties during antimalarial and mosquito control work.

This second edition is a result of the California Mosquito and Vector Control Association Biological Control Committee's charge to revise the first edition, which was edited by Robert L. Coykendall, in 1980. Much of the material in the first edition is still relevant and remains. New material and updated text are included where appropriate. Information on parasites and pathogens infecting the mosquitofish is greatly expanded into a separate section. Two new fishes, the Inland Silverside and the Green Sunfish are included in the Fishes' section. An expanded references section is included for the professional who desires more in-depth information. A section on the products and services associated with the use and culture of fish is listed in appendix B. In addition, useful mathematical equivalents (appendix D) and a recommended reading list (appendix A) have been included.

As you read this publication, you will notice differences in styles and formats between sections. Many people have contributed to this publication and the editor has not attempted to standardize sections. If there are questions about a particular section, refer to the listing of contributors in appendix C and direct them to the appropriate person.

Craig W. Downs
October 1991

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ACKNOWLEDGEMENTS

This publication is the result of a cooperative effort by many individuals of the California Mosquito Control Association. I would like to thank the members of the Biological Control Committee, particularly the following members that authored and revised sections of the manuscript. Robert L. Coykendall, Sutter-Yuba MAD, was the first edition editor and author of the sections on the sticklebacks, mosquitofish, all the operational techniques sections except the new section on parasites and pathogens, and the aquaculture techniques sections. He also revised the second edition sections on guppies, transportation equipment and methods, and anesthetics and chemical transport agents. Kaaren J. Hiscox, Sierra College, Nevada City, CA, authored the first edition sections on the goldfish, annual killifishes, pupfishes, and guppies. David G. Farley, Fresno MVCD, was the first edition author of the minnows and carp section and revised the second edition sections on goldfish and mosquitofish applications. Gary T. Reynolds, Orange County VCD, was the first edition author of the tilapia section and revised the second edition sections on tilapia and incidental production. Stan A. Wright, Sacramento-Yolo MVCD, authored the section on parasites and pathogens. Arthur E. Colwell, Lake County MAD, authored the second edition section on the inland silverside and revised the section on pupfishes. Vicki L. Kramer, Contra Costa MAD, authored the section on green sunfish and revised the section on sticklebacks. Werner P. Schon, Sacramento-Yolo MVCD, contributed revisions to the extensive culture section. The sections on annual killifishes, minnows and carp, mosquitofish, aquatic and holding systems, and intensive culture were revised by this editor.

A special thanks goes to Vicki L. Kramer and Robert L. Coykendall for their invaluable editorial comments. Also, I would like to extend my appreciation to Robert Coykendall, Arthur Colwell, and Susan Bauer for assisting with the references and literature cited section.

Acknowledgment for most of the fish illustrations goes to Ruth A. Willson. Debbie Dritz provided the mosquitofish illustrations. Illustrations of the green sunfish, the inland silverside, the tui chub, the hitch, the splittail, and the Sacramento blackfish were reproduced, with permission, from Moyle 1976.

Section 238.5 of Title 14, California Administrative Code governing stocking of aquaculture products reads as follows: "No person shall stock aquaculture products in this state except in accordance with the following general terms and conditions:

(a) All aquaculture products stocked under these provisions must be legally reared or possessed by an aquaculturist registered in this state. No person shall stock aquaculture products which are parasitized, diseased, or of an unauthorized species.

(b) Live aquaculture products shipped to Inyo or Mono counties must be certified by the department as disease and parasite-free before being stocked in waters in those counties.

(c) A registered aquaculturist producing or possessing rainbow trout *Oncorhynchus mykiss*, largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, redear sunfish *Lepomis microlophus*, Sacramento perch *Archoplites interruptus*, channel catfish *Ictalurus punctatus*, blue catfish *Ictalurus furcatus*, and white catfish *Ictalurus catus*, may stock these species under the following terms and conditions.

(1) Only publicly owned lakes covered by a cooperative agreement between the department and the lake operator and privately owned reservoirs, lakes, and ponds in the following counties or portions thereof may be stocked without a stocking permit: Alameda, Butte, Colusa, Contra Costa, Glenn, Imperial, Kern, except in the Kern River drainage above Democrat Dam; Kings Lake, except in the Eel River drainage; Los Angeles, Merced, Napa, Orange, Riverside, Sacramento, San Benito, San Bernardino, San Diego, San Joaquin, Santa Barbara, Solano, Stanislaus, Sutter, Tehama, Ventura, Yolo, Yuba, those portions of Almador, Calaveras, El Dorado, Mariposa, Nevada, Placer, and Tuolumne west of Highway 49; Fresno west of the Sierra and Sequoia National Forest boundary; and Tulare west of the Sequoia National Forest and Sequoia National Park boundaries.

(d) Except for those species listed in Section 236.5(c) when planted into those specific areas and waters covered in Section 238.5(c)(1), no person shall stock aquatic plants and animals except as follows:

(1) Each stocking of fish shall require a separate Private Stocking Permit (FG 749) issued by the department. A copy of this permit shall accompany all shipments. However, with the exception of Inyo and Mono counties, a copy of the same permit (FG 749) may be used for additional consignments of the same species when stocked in the same water, until cancelled by the department.

(2) Application for the private stocking permit shall be made to the regional manager of the Fish and Game region in which the fish are to be stocked. An application will be supplied to each applicant upon request.

(3) No person shall stock any species of fish in any water in which the stocking of such fish is contrary to the fisheries management programs of the department for that water or drainage, or in any water from which such fish might escape to other waters where such fish are not already present. All applicants will be advised upon request of the said departmental fisheries management programs.

(4) Permittee shall notify the regional office of the department not less than 10 days in advance of stocking in order to make arrangements for inspection. Such inspection may be waived at the discretion of the department. If, upon inspection, diseased or parasitized fish or unauthorized species are found by the department to be present, they shall be disposed of by the permittee as directed by the department. The department may require that the expense of any inspection made necessary by the provisions of these regulations be borne by the permittee.

(5) A stocking permit may be cancelled or suspended by the department upon conviction of a violation of these regulations by a court or competent jurisdiction. Cancellation or suspension may be appealed to the commission.

(6) A stocking permit is valid only when signed by the applicant.

(e) A registered aquaculturist selling and transporting aquatic plants and animals for the purpose of stocking in this state shall retain copies of documents required by Section 15005(b) of the Fish and Game Code for a period of three years following stocking of the fish. The documents shall be shown upon written demand by the director of the department. The information contained in the documents is confidential except that such information may be disclosed in accordance with a proper judicial order in cases or actions instituted for enforcement of this section or for prosecution of violations of this section.

(f) Except for Inyo, Mono, San Bernardino, Riverside, and Imperial counties, mosquitofish *Gambusia affinis*, may be planted for purposes of mosquito control without obtaining a permit otherwise required by these regulations. In Inyo and Mono counties and in public waters of San Bernardino, Riverside, and Imperial counties, mosquitofish may not be planted without the written concurrence of the department.

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Figure 1. A composite diagram of a hypothetical fish.

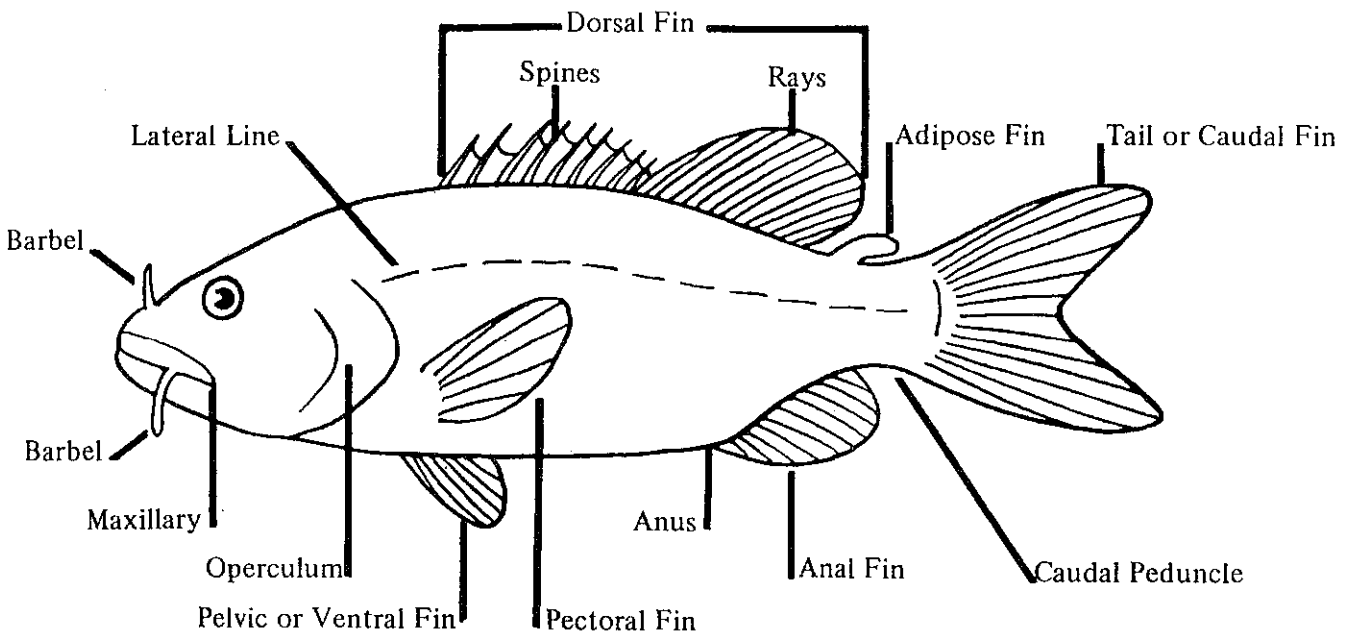
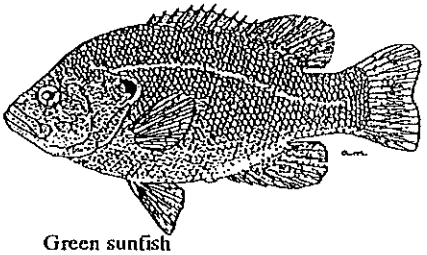
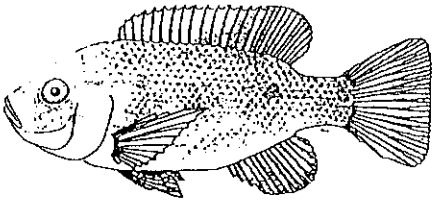


Figure 2. Fishes In California Mosquito Control

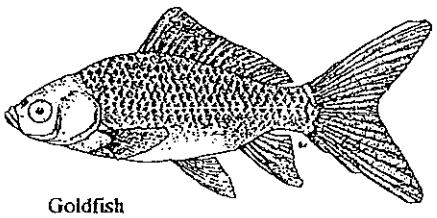
Inland silverside



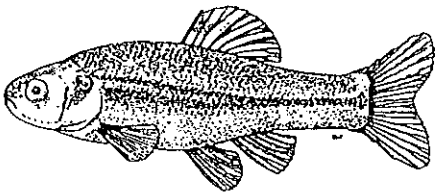
Green sunfish



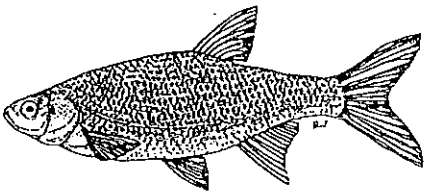
Mozambique mouthbrooder



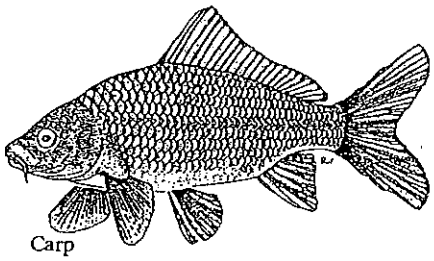
Goldfish



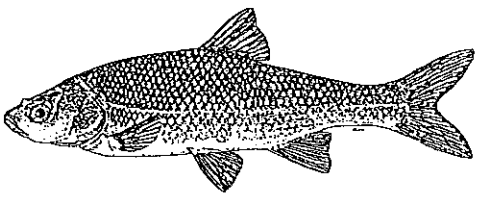
Fathead minnow



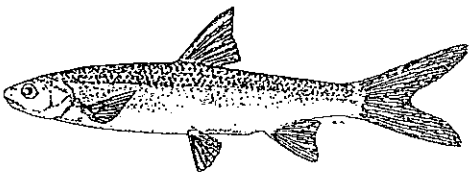
Golden shiner



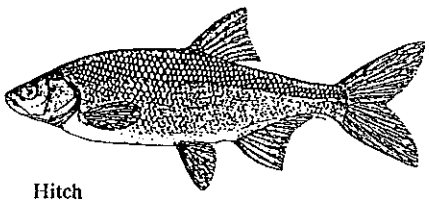
Carp



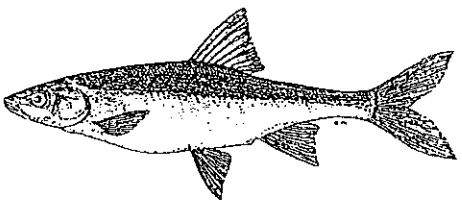
Tui Chub



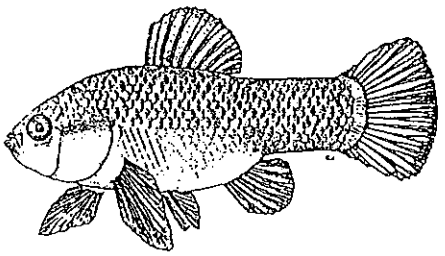
Sacramento splittail



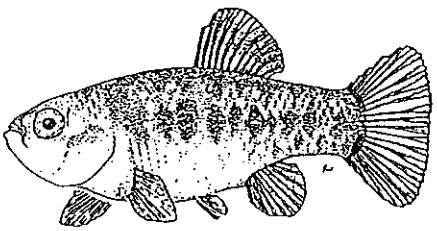
Hitch



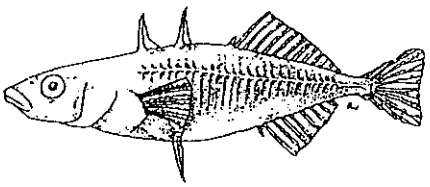
Sacramento blackfish



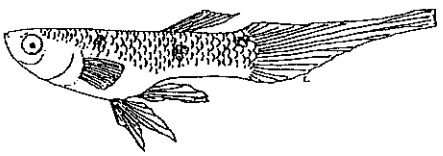
Desert pupfish, male



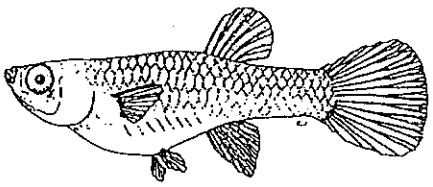
Desert pupfish, female



Threespine stickleback

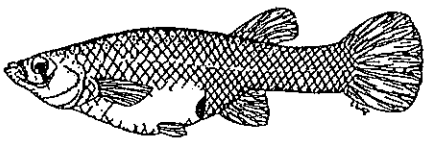


Guppy, male



Guppy, female

Mosquitofish, male



Mosquitofish, female

THE FISHES

ATHERINIDAE FAMILY

INLAND SILVERSIDES

Identification. The inland silverside, *Menidia beryllina* (Cope), is a small (to 15cm TL) elongate moderately compressed fish. A silver lateral band extends from the pectoral fins to the caudal fin. The two dorsal fins are widely separated and the caudal fin is forked. The inland silverside has a pale greenish dorsum and is pale or translucent ventrally. There are two rows of pigment spots on the bottom of the caudal peduncle between the anal fin base and the caudal fin base. The mouth is terminal and oblique. The head is flattened on top and the eyes are large.

Names. *Menidia* is reportedly derived from an old Italian word for small silvery fish (Moyle 1976). *Beryllina* is from the Latin "beryllinus", meaning green-colored. This species has also been known as the Mississippi silverside, the tidewater silverside, and *Menidia audens*.

Distribution. The inland silverside is native to the lower Mississippi Valley. Its range now extends along the Atlantic coast from Massachusetts to Veracruz, Mexico, and in associated freshwater streams and rivers (Buettner 1983). During the 1960s silversides were introduced into California in Lake, Alameda, and Santa Clara counties. Silversides now occur throughout the Sacramento and San Joaquin delta systems and in most connecting lakes.

Life History. A silverside grows rapidly during its first summer, but growth is very slow during the winter. A silverside reaches sexual maturity at ca. 50 mm SL (Wurtsbaugh and Li 1985). Usually the fish spawns and dies during its second summer. A few silversides may survive an additional year. Spawning has been reported from April to September when the water temperature is between 17°C and 34°C (Moyle 1976). Spawning takes place among aquatic macrophytes in shallow water (less than 65 cm deep). Schools of male fish form near the rooted aquatic plants. When a school of females passes nearby, males may approach, follow and spawn with a female as she dives into the vegetation. A female only deposits 10 to 20 eggs at a time (Fisher 1973), but can spawn repeatedly during the summer and may lay up to 15,000 eggs (McGinnis 1984). The larval fish hatch in four to thirty days, depending on temperature (Moyle 1976).

Silversides can occur in fresh water and in brackish water approaching the salinity of seawater (Hubbs et al. 1971). They can survive relatively high temperature, low dissolved oxygen concentration, high turbidity, and moderate organic pollution. Silversides can become very abundant in some areas of reservoirs, streams, and estuaries. Even in deep lakes, silversides typically form schools near the water surface.

Inland silversides are primarily planktivorous and feed on large cladocerans and copepods (Elston and Bachen 1976; Wurtsbaugh and Li 1985). Cech and Moyle (1983) indicated that inland silversides could be considered for rice field mosquito control, but noted their fragility to handling minimizes the likelihood of easy culture of these fish. Middaugh et al. (1985) reported some culture methods and found that silversides readily consumed mosquito larvae in the laboratory. In brackish water field test plots in Florida the fish successfully controlled mosquitoes (Middaugh et al. 1985). However in wild rice plots in California, silversides did not reproduce well and did not significantly reduce mosquito populations (Kramer et al. 1987).

In large lakes it has been suggested that silversides can control populations of nuisance midges (Cook 1981). However these insects typically constitute only a small percentage of the diet (Jacobs et al. 1979), and silversides cannot be depended upon to control pestiferous dipterans. These delicate fish can experience significant mortality during transport and stocking operations.

Status. The inland silverside is a species which was introduced into California and spread into many new areas naturally and through legal and illegal introductions. Silversides can become extremely abundant and compete with some native fishes and with planktivorous stages of sport fishes (Li et al. 1976) so their range should not be extended (Cech and Moyle 1983). The California Department of Fish and Game should be consulted regarding the status of inland silversides in localized areas.

CENTRARCHIDAE FAMILY

GREEN SUNFISH

The green sunfish, *Lepomis cyanellus* Rafinesque, is one of 30 species belonging to the Centrarchidae family. Many of these species are important gamefish, inhabiting warm ponds, lakes and slow-moving streams. The Centrarchidae evolved in North American waters but the family now has worldwide distribution.

Identification. Green sunfish are dark olive on the back becoming lighter on the sides. The breast and bellies are yellow orange, and the dorsal and anal fins have a large dark blotch on the rear of the soft-rayed portion. Green sunfish have large mouths, short stiff opercular flaps, and long slender gill rakers. Their dorsal fins have 9 to 10 spines, 10 to 12 rays; anal fins have 3 spines, 8 to 9 rays; pelvic fins have 1 spine, 5 rays; and the lateral line has 45 to 50 scales. Green sunfish are small, seldom exceeding 15 cm SL.

Names. *Lepomis* means scaled cheek, since the scales present on the operculum were once considered to be a significant distinguishing feature (Moyle 1976). *Cyanellus* means green.

Distribution. Green sunfish, introduced into California in 1891, can be found throughout the state. The only region where they seem to be absent is the Klamath River system.

Life History. Green sunfish prefer to live in small, warm streams and ponds, and the shallow weedy areas of lakes and reservoirs. They can survive high temperatures (over 36°C), low oxygen levels (less than 3 ppm), and high alkalinities (up to 2000 mg/l), (McCarraher 1972). Green sunfish are quick to colonize new, disturbed areas, and are rarely found in habitats with more than three or four other species of fish. They coexist with mosquitofish in some habitats, but will prey on mosquitofish fry.

Green sunfish reach reproductive maturity at the beginning of their third year. Spawning activity occurs in the summer, usually after the water temperatures exceed 19°C. The males first congregate in shallow water, and then build nests on the gravel bottom, generally under overhanging bushes or other cover. The males defend their nests against other males, and court and spawn with the females. The eggs adhere to the nest and are guarded by the male until they hatch, usually in about five to seven days.

Status. Small green sunfish feed primarily on crustaceans and aquatic insect larvae, whereas the larger sunfish (> 8 cm SL) prey on large aquatic insects, crayfish, and small fish. The mosquito control potential of green sunfish was evaluated by Davey and Meisch (1977) against *Psorophora columbiae* (Dyar and Knab) in Arkansas rice fields. They found that green sunfish stocked at 480 fish/acre reduced larval numbers by about 85%. Higher stocking rates did not produce greater reduction of mosquito larvae. When stocked with mosquitofish (500 mosquitofish and 120 sunfish/acre), the larval reduction was greater (96.7%) than with either species alone.

Blaustein (1986) found that the impact of green sunfish on mosquito larvae in California rice fields depended on such factors as the abundance of alternative prey, the abundance of green sunfish, the size of green sunfish, the species of mosquito, and the presence of mosquitofish. When alternative prey (such as ostracods, cladocerans and chironomids) densities were high, sunfish did not reduce mosquito populations. When alternative prey densities were low, immature green sunfish were effective predators of *Culex tarsalis* but not of *Anopheles freeborni*, except when sunfish densities were very high. Large green sunfish did not reduce numbers of either species of mosquito. Green sunfish can enhance the control potential of mosquitofish against *An. freeborni* because, when green sunfish are present in the borrow pit, mosquitofish are more likely to inhabit the rice stand, where mosquito larvae are numerous. Green sunfish do, however, prey on mosquitofish and can drastically reduce their numbers.

CICHLIDAE FAMILY

TILAPIA

Identification. Cichlids are a colorful group of spiny-rayed fish with a perch-like appearance. They characteristically have a long spiny dorsal fin, a single nostril on each side of the snout, with an interrupted lateral line in either two or three parts. *Tilapia* taxonomy can be very confusing to the lay person. Older publications on *Tilapia* culture may refer to all closely related species in the genera *Tilapia, Sarotherodon*, and *Oreochromis* as "*Tilapia.*" Also, the fish presently known as *Oreochromis mossambicus* (Peters) may be found in previous literature as *Tilapia mossambica* or *Sarotherodon mossambicus*. This is further complicated when several species introduced into the same area may hybridize. It may be necessary to use biological or physical characters or even behavioral patterns to distinguish *Tilapia* from other cichlids. Examples of these include; color and size of eggs, shape of the nest, color patterns when breeding, size of the fish, and other features only determined by actual dissection or measurement.

Names. The name *Tilapia* (pronounced: Ti-lay'pee-a) originated from the African word "thape," which means "fish" (Moyle 1976). A beneficial trait for this group is the extended parental care given to their eggs and young fry. The three genera in the *Tilapia* grouping are classified by their distinct type of parental care. Substrate spawners, such as *T. zilli* (true *Tilapia*), lay their eggs and attach them to the substrate. Mouthbrooders, after laying their eggs, collect and brood them in their mouths. Mouthbrooders are further subdivided into groups where only the female mouth broods the eggs, *Oreochromis*, and groups where either the male or the female mouth broods the eggs, *Sarotherodon*.

Distribution. An estimated 600 species are native to Africa, Asia, Central and South America. Most species occur in tropical areas, yet several species have been introduced and thrived in the southwestern United States, namely Arizona, California, and Texas. The California Department of Fish and Game is attempting to limit the introduction of *Tilapia* to the waters south of the Tehachapi Range in southern California.

Life History. Spawning activity begins when water temperatures reach 20°C (68°F). The males of both *O. mossambicus* and *T. zilli* begin the breeding process by digging a small crater in sand or mud. Although under artificial conditions they have spawned with only a wooden or plastic substrate. Apparently both species can live and reproduce in fresh and brackish water. *T. zilli* is reported to reproduce at salinities up to 29 °/oo and survive in salinities of 45 °/oo. Once a nest is prepared by a male, the female deposits her eggs in the depression. *O. mossambicus* is a true mouthbrooder. There are conflicting reports whether the eggs are fertilized in the nest or in the female's mouth, but in either event, the female picks the eggs up in her mouth shortly after they are laid and carries them until they hatch. The time required for hatching depends on the species and temperature but is usually within five to ten days. Egg production varies considerably in relation to the size of the female, but is reported as 200 to 500 eggs for *O. mossambicus* and from 1,000 to 5,000 eggs for *T. zilli*. During optimum temperatures of 22°C (71.6°F), *Tilapia* have been shown to produce successive broods with intervals of only 23 days. Thus, with a high reproductive potential such as this we can see why these fish tend to overpopulate a pond. However, one factor that can reduce or decimate a *Tilapia* population is low water temperature. Temperature limitations of *O. mossambicus* and *T. zilli* appear to be 12°C (53.6°F) and 7°C (44.6°F) respectively. Though they may survive lower temperatures than this for short periods of time, it is generally considered that exposure to these temperatures for extended periods is fatal. Once the eggs hatch, they become free-swimming in three to five days. The young fry are protected by the parents for an additional week. During this time the youngsters often swim back into the parent's mouth for protection. *T. zilli* is not a true mouthbrooder but a substrate spawner. The male and female remain together at the nest following oviposition and fertilization to protect the eggs and keep them clean. Soon after hatching, the young fry are picked up in the mouth of one parent and transferred to small pits previously constructed

around the perimeter of the main nest. When these fry move away or are swept away from the pits by water currents the parent quickly return them. This close association between parents and young lasts for about a week. Then the young may continue to group together in shallow water for a short time before leaving the parents. Most of the *Tilapia* species are monogamous, unlike the *Sarotherodon* and *Oreochromis* species.

Status. Because of a need for control of aquatic weeds in southern California irrigation systems, *T. zilli, O. mossambicus*, and *O. homorum* were authorized for such use on November 5, 1971. It was thought that these species could not survive California winters and, therefore, would not pose a threat to game fish. *O. mossambicus* and *O. homorum* remain in only a few irrigation drains where warm ground water and agricultural or industrial flows maintain sufficient water temperature to allow their survival. *T. zilli*, however, can overwinter and reproduce in irrigation systems in Imperial, Coachella, and Palo Verde Valleys. This fish could conceivably populate a variety of waters in central and northern California and has been designated a prohibited species in these areas. It may be able to overpopulate and compete directly or indirectly with game fish or native nongame fishes with possible detrimental effects. Also, it would be undesirable in California's rice fields, where its predominantly herbivorous nature might make it unpopular with rice growers. *T. zilli* and *O. mossambicus* have shown significant mosquito and chironomid midge predation in some habitats in addition to their well-known reduction of aquatic weed growth. It is possible that these species could perform a desirable biological control function in certain habitats. However, before any attempt to stock *Tilapia*, or any other exotic fish for that matter, a stocking permit must be obtained from the California Department of Fish and Game. Its authorities will carefully consider requests to stock these fish in southern California waters.

CYPRINIDAE FAMILY

GOLDFISH

Identification. Wild populations of goldfish, *Carassius auratus* (Linnaeus), usually lack the bright colors of aquarium stock and tend to be olive on the back, have a white belly, and retain the silvery or bronze coloration of domesticated fish only on the sides. Goldfish closely resemble their relative, the carp (*Cyprinus carpio* Linnaeus). Both are heavy-bodied with a long dorsal fin, serrated spines along the leading edges of the dorsal and anal fins, a terminal mouth and large cycloid scales. Goldfish lack the barbels on the upper jaw which distinguish them from true carps.

Names. The generic name *Carassius* is the Latinized version of the common name for the European Crucian carp (*Carassius carassis*). The word *auratus* is from the Latin 'Aureus' or golden (Neilson et al. 1951). Pronunciation: Car-ass'-e-us are-a te'us.

Distribution. The original range of the goldfish was from eastern Europe to southern China. They were domesticated in China around the 10th century A.D., first as a food source and later as an ornamental fish. Their distribution is now worldwide. In California, goldfish have become established in many lower and mid-elevation lakes, rivers, sloughs, and canals. Most introductions have occurred as a result of releasing pet fish into the wild.

Life History. Goldfish may spawn in their second year and spawn for six to seven years or more thereafter. Before spawning, the fish become extremely active, the males chasing the females - at first aimlessly, then more purposefully - bumping their abdomens to facilitate the release of eggs. As the eggs are released, often up to 4,000 in number, they are fertilized by the males. The eggs sink and adhere to aquatic plants, hatching in 8 to 9 days at 18°C (65°F). Temperatures of 15-23°C (59-74°F) are required for successful spawning of goldfish. Goldfish can survive temperatures of 0-41°C (32-106°F) (Moyle 1976).

Goldfish are adapted for feeding on phytoplankton, but they also consume zooplankton and aquatic insects. Mosquito control workers have reported that goldfish can keep a well balanced garden pool free of culicine larvae. However, many incidences have been observed in which goldfish have failed to control *Culex spp.* larvae in ornamental ponds. Davey et al. (1974) found only a 0.3% reduction in the numbers of *Psorophora confinnis* larvae in rice plots stocked with goldfish.

Due to their morphology and feeding behavior which favors a diet of phytoplankton and associated zooplankton, goldfish are undependable as mosquito control agents.

Status. Goldfish, although currently prohibited as a bait minnow, are found in many of the warm waters of California.

CYPRINIDAE FAMILY

MINNOWS AND CARP

Identification. Minnows are those fishes belonging to the family Cyprinidae. Approximately 23 species of minnows exist in California of which 15 are native. Minnows differ in appearance to some extent, but generally they have silvery, elongate bodies, often with a dark stripe running down the side. The caudal fin (tail) is typically forked. Scales are cycloid and generally evenly distributed over the body but absent from the head. Minnows lack fin spines; however, carp and goldfish have hardened fin rays which closely resemble spines. Minnows also lack the teeth in the mouth but have well-developed pharyngeal teeth.

Eight species of minnows commonly found in California are cited in the literature as possible mosquito biological control agents (Chidester 1916; Washino 1968; Davey et al. 1974; Zewadski 1975; Cech and Moyle 1983). The eight species; fathead minnows (*Pimephales promelas*), golden shiners (*Notemigonus crysoleucas*), carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), tui chub (*Gila bicolor*), Splittail (*Pogonichthys macrolepidotus*), hitch (*Lavinia exilicauda*), and Sacramento blackfish (*Orthodon microlepidotus*), may be of use in mosquito control in a limited number of habitats. For specific identification and more complete information, see Moyle (1976) and the preceding section on goldfish.

Names. The family name Cyprinidae is probably derived from Cyprus, the island home of Venus, and has indirect reference to the reproductive capabilities of the member species. The common name "minnow" is an Old English word of possible Latin origin and is used in America to denote all cyprinids.

Distribution. Of the eight minnow species implicated as mosquito control agents, four are native (tui chub, splittail, hitch, and Sacramento blackfish), two have been introduced into California from the Eastern United States (fathead minnows and golden shiners) and two where introduced from Asia (carp and goldfish). All eight species can be found in suitable waters within the state.

Life History. Fathead minnows are very adaptable, but seem to do best in small intermittent streams and ponds (Moyle 1976). They can survive in highly alkaline water and water with low dissolved oxygen, high turbidity, and high organic pollution.

Fathead minnows spawn from May through August. Generally water temperatures of 64°F (17.8^{o}C) are required before spawning is initiated. Females can contain anywhere from 600 to 2300 eggs which develop at different rates, allowing each female to spawn several times during the season. Egg deposition generally takes place on the underside of submerged objects. Males select and guard each nesting site in which several females may spawn.

Fathead minnows are primarily bottom and mid-water feeders, so it appears they would be good mosquito control agents only in shallow water habitats. Their preferred feeds are algae, diatoms, organic matter, and small invertebrates; but they probably feed on whatever is most abundant in the area. Davey et. al. (1974) found that fathead minnows stocked at 0.03 fish per square foot (0.3 fish per square meter) in rice plots gave 77.8% control of *Psorophora confinnis* within 48 hours after stocking. Fathead minnows would probably work best in small shallow ponds with little algae growth.

Golden shiners prefer quiet, densely vegetated waters, but even do well in stagnant areas with little dissolved oxygen. They are a schooling fish which roam widely in a body of water.

The spawning season for golden shiners in California extends from May to August. Water temperatures of 60° to 80°F (15.6^{o} to 26.7 ^{o}C) are required before spawning can take place (McKechnie 1966). The female scatters her adhesive eggs over submerged vegetation and bottom debris, where they are fertilized by males. Eggs hatch in approximately four days.

The diet of young golden shiners is composed primarily of algae and microcrustaceans. Older fish feed mainly on the water surface or in mid-water and eat young fish, insect larvae and adults, plankton, algae, etc. Dobie et. al. (1956) found that insects composed approximately 35% of the diet of adult golden shiners, and Davey et. al. (1974) found that golden shiners reduced the population of *Psorophora confinnis* in small ponds by 74% when applied at a rate of 0.03 fish per square foot (0.3 fish per square meter). The Illinois Natural History Survey Report (Zewadski 1975) recommended the use of golden shiners

in mosquito control. The golden shiner may hold promise in controlling mosquitoes in selected small, shallow, weedy habitats.

Carp are hardy fish which can withstand adverse conditions such as high turbidity, low dissolved oxygen concentrations, and temperature extremes. Their hardiness has made them one of the most abundant and widespread fishes in the United States. Carp prefer rich, shallow-water environments with silty bottoms and good aquatic weed growth.

Carp spawn at water temperatures of 65°F (18.3^{o}C) and above. Spawning takes place in shallow water. Females broadcast their eggs in batches of about 500, and may lay 50,000 to 2,000,000 eggs during the season. The eggs adhere to the substrate where they are fertilized and lay unguarded until they hatch in about four days. The young stay in shallow, weedy areas until they are approximately four inches (10.2 cm) in length.

Young carp feed primarily on zooplankton and phytoplankton. As they grow, their diet changes to primarily bottom-dwelling insects such as chironomids. Washino (1968) indicated that *Culex tarsalis* larvae were found in small numbers in the gut contents of carp from California rice fields. Davey et. al. (1974) found that carp gave 97.3% control of *Psorophora confinnis* in rice plots within 48 hours when stocked at 0.03 fish per square foot (0.3 fish per square meter). It would seem that the tendency of the carp to muddy the water by disturbing the silty bottom in search of food would be more of a detriment to mosquitoes than their predatory behavior. The muddy water produces much less food for mosquito larvae and renders a body of water less attractive as an oviposition site. This behavior of the carp as well as the associated uprooting of plants also makes the carp a detriment to the aesthetics of most ponds. Rice farmers dislike carp because they believe they uproot rice seedlings; some farmers have screened their field drains to exclude them.

Tui chub are a relatively hardy species. They prefer the weedy shallows of lakes and ponds. Colder winter months are spent in the deeper waters, presumably on the bottom in a semi-dormant state (Moyle 1976).

Tui chub spawn at water temperatures above 46°F (8°C) and a larger female (28 cm TL) can spawn ca. 25,000 eggs (Bond 1948).

Although tui chub are omnivorous and will consume mosquito larvae their tendency to concentrate on invertebrates associated with the bottom or with aquatic plants may limit their effectiveness as a mosquito control agent.

The splittail is a large cyprinid (up to 40 cm FL) which is very tolerant of brackish waters (up to 12 ppt.). They spawn between March and mid-May and their fecundity ranges to 185,000 eggs (Moyle et al. 1982).

The splittail, like the tui chub, may be limited in effectiveness as a mosquito control agent, due to its preference for bottom dwelling invertebrates.

At first glance hitch look like large golden shiners (over 35 cm TL). They are found in warm, quiet waters of low-elevation lakes, ponds, sloughs, and slow-moving stretches of rivers.

Hitch spawn from March through July in nature and are quite prolific breeders. Schools of hitch migrate up into small streams or into the shallow water of a lake or pond. They spawn on fine to medium gravel in water temperatures of 57° to 64°F (14° to 18°C)(Murphy 1948).

Preliminary results on the effectiveness of hitch on controlling mosquitoes in white rice fields was not promising. Numbers of mosquito larvae in experimental rice paddies stocked with hitch were not suppressed and in fact where similar to numbers in control paddies (Cech and Linden 1986). Additional investigations with various fish stocking rates and age classes of hitch should be conducted before recommending this species as a mosquito control agent.

Sacramento blackfish are another large member (up to 50 cm SL) of the Cyprinidae (minnow family). Spawning occurs between April and July, at water temperatures ranging from 54° to 75°F (12° to 24°C)(Moyle 1976). It has a high fecundity, up to 371,500 eggs (Moyle 1982).

Although the adult blackfish is primarily a herbivorous filter feeder their young-of-the-year are more selective and feed more on invertebrates and small cladocerans. Experiments indicate that blackfish exert significantly less predatory control on mosquitoes in experimental white rice paddies than do mosquitofish (Cech and Linden 1985, 1986). This seems to be due to their quick growth rate and consequent shift to a filtering mode of feeding.

While many of the cyprinids have demonstrated limited potential for mosquito control to date, at least one minnow species (carp) has shown potential in controlling midges. Carp stocked at 400 pounds per acre have provided control of midges in secondary wastewater holding ponds in Santa Rosa, California (R. Keith, pers. comm.). Similarly, other adult minnows may be successful in midge control due to their bottom feeding habits.

Before stocking minnows for aquatic insect control your regional Department of Fish & Game should be contacted for necessary permits .

Status. Many species of native and introduced minnows may play a role in mosquito control. The

extensive use of minnows is probably not as effective as extensive use of mosquitofish because of the very adaptive nature of this live-bearing fish. However, minnows may work in selected sources. The mosquito control worker should determine the extent of the natural control of mosquitoes by existing native minnows in a source before altering the environment by the introduction of additional cyprinid or poeciliid fishes. Native California minnows should certainly receive additional consideration to determine if they could be advantageously employed as mosquito control agents.

CYPRINODONTIDAE FAMILY

ANNUAL KILLIFISHES

Identification. Annual killifishes are members of the family Cyprinodontidae, the egglaying toothed carps, whose eggs are desiccation resistant. Often there is an obligatory dry incubation period. Most mosquito control research in California has been done with the South American genus *Cynolebias*, specifically *Cynolebias bellottii* Steindachner, *C. nigripinnis* Regan, *C. whitei*, and *C. alexanderi*. The first two species have shown the most promise and will be emphasized here. The males of both species are deep bodied with a bass-like conformation. *Cynolebias bellottii* males range from 1.5 to 4 inches (3.5 - 10 cm) in total length, whereas *C. nigripinnis* males are generally smaller, 1 to 2.5 inches (2.5 - 7.5 cm) TL. The male *C. bellottii* is turquoise blue with a bold black vertical eyeline and iridescent silvery blue spots or "pearls" on the flanks and fins. In the male *C. nigripinnis*, the background color is a velvety black setting off the many "mother-of-pearl" spots heavily flecking the body and sides. Females of both species are smaller than males, averaging 1.5 inches (3.5 cm) TL. They are wider than males when viewed from above and often resemble tadpoles when heavy with eggs. They are grey in color, mottled with brown spots, and completely lack the iridescent "pearls" of the males. Female *C. nigripinnis* and *C. bellottii* are virtually indistinguishable in the field.

Names. The generic name *Cynolebias* (Sy' no-lee" be-ass) comes from the Greek "Kyon", meaning dog, and "lebias", meaning small fish. They are, therefore, the small dog-like fish. Commonly, fish in this genus are referred to as the pearlfishes due to the iridescent silvery blue spots which fleck the males.

The Argentine pearlfish, *Cynolebias bellottii* (bel-lot' ee-eye) was named after Dr. Bellottii of Italy. The female of this species was originally described as a separate species: *C. maculatus* Steindachner. This was due to the great sexual dimorphism in the species, even extending to differing fin ray counts (Regan 1912).

The black pearlfish, *C. nigripinnis* (nig' ri-pin' is) is named for the male's velvety black body color as *nigri-* means black in Latin. Its laterally flattened shape and slow drifting swimming motion are reflected in the suffix pinnis, from the Latin *pinnis*, or feather.

Distribution. *Cynolebias* are found naturally in South America where they inhabit temporary pools and slow streams. *Cynolebias bellottii* is found in southern Brazil, Uruguay and northern and central Argentina (lat. 30° - 40° S)(Bay 1966). Most specimens are from the La Plata Basin in Argentina near Buenos Aires. *Cynolebias nigripinnis* is more prevalent in Uruguay, especially in ponds near the Parana River and in the Province of Santa Fe (Terceira 1974).

Both species are common in the aquarium trade in the United States and Europe. *Cynolebias nigripinnis*, being easier to spawn than *C. bellottii*, is more readily available in the United States.

Life History. *Cynolebias* inhabit temporary waters such as vernal pools or slow moving intermittent streams. They survive the dry periods as desiccation resistant eggs. During the rainy season, when the habitat is flooded, the eggs hatch and develop rapidly, in six to eight weeks, into sexually mature fish. They can therefore complete their lifecycle before the next dry season begins. Annual killifish are quite large when they hatch and are able to bypass the infusorial feeding stage necessary for the fry of most egglayers (Bay 1966).

Research has shown that a dry embryonation is not obligatory for the production of viable *C. nigripinnis* fry, but that this improves the hatching percentage (Hiscox et al. 1974). The length of time that eggs can be dry without loss of viability varies from species to species, but most *Cynoloebias* eggs remain viable for about five months. Thereafter the hatching rate declines so that after two years of storage, few fry will emerge. In natural situations, the eggs will last longer, as evidenced by the "contamination" of ponds at the University of California, Riverside (UCR) by *C. bellottii* (Walters 1976). This occurred following a raking and cleaning of the ponds that uncovered *C. bellottii* eggs several years after the ponds were no longer used for rearing them.

The adult fish are referred to as "divers" because they completely bury themselves in the substrate when spawning. Other annual fish, by contrast, scatter their eggs on the surface or plough a small trough. This deep burial helps protect the eggs from extremes of temperature and humidity. The eggs are laid singly so that there is little chance of fungus from an infertile egg contaminating another egg.

The adults usually live just one year in the wild due to the drying out of their habitat. They have, however, survived up to five years in aquaria. They are found primarily in temporary waters because they are apparently unable to compete with livebearers. It is axiomatic among collectors that if livebearers are found, no annual fish will be present. This opinion was demonstrated when mosquitofish were accidentally introduced into a test plot containing *C. whitei* and the *C. whitei* disappeared (Hiscox 1974).

Cynolebias whitei, C. nigripinnis, and *C. bellottii* have been able to survive the summer in Butte County, California rice fields. However, during the three years the fish were introduced into the fields, they did not reproduce. The objective of the study was to establish a cycle in the rice fields so that the fish would be present to feed on mosquito larvae without yearly introductions, as is necessary with mosquitofish. It is not clear why reproduction did not occur. Perhaps the heavy clay soils were inconducive to egg laying, embryonation, or hatching.

Cynolebias whitei and *C. nigripinnis* were able to overwinter in Butte County outdoor tanks, even when ice formed on the water surface. *Cynolebias bellottii* has reproduced in outdoor pools at UCR, and *C. nigripinnis* and *C. whitei* have been easily mass produced in aquaria.

A breeding colony of *C. nigripinnis* was established at the University of California, Davis in 1981 (Speas 1981). It was determined that eggs (1.5 mm in diameter) incubated at 24°C for ten weeks in peat moss had a 95% hatching rate. Hatching occurred within 35 hours following flooding. Fry, measuring 4-4.5 mm, required food of a minute size, *Artemia* nauplii being to large. Preliminary work indicated that the fry were sensitive to high salinities, such as those encountered in brackish waters of California during low runoff years. Fry began to exhibit sexual dimorphism by four weeks of age and reached sexual maturity by six weeks of age. Spawning occurred while male and female fish were completely submerged in the substrate; fecundity averaged 78 eggs per female per week under ideal conditions of 18-22°C in fresh water. Adults measured 5-6.5 inches, withstood temperatures as low as 7°C and lived for approximately 9 months in captivity. Eventually, annual killifish may find a niche in California mosquito control, possibly in rice fields or vernal pools.

Status. Annual killifishes are not currently approved for field introduction in the United States. Preliminary work towards an eventual introduction permit was done by the Butte County Mosquito Abatement District. This information is on file at the District office and with the California Department of Fish and Game.

CYPRINODONTIDAE FAMILY

PUPFISH

Identification. Pupfish are small killifish (family Cyprinodontidae) of the genus *Cyprinodon* found in the deserts of the American Southwest. Pupfish are small (less than 3 inches or 7.5 cm in total length), deep-bodied, chunky fish with relatively large scales. The toothed mouth is angled upward and the caudal (tail) fin is rounded or square. Male pupfish have deeper bodies and, during the breeding season, are more brightly colored (e.g. blue) than the olive-brown females. To accurately separate the various species of *Cyprinodon* in California, see Moyle (1976), and Soltz and Naiman (1978).

Names. The generic name *Cyprinodon* means carp with teeth. Pupfish refers to the "playful" territorial/breeding behavior of these fish.

Distribution. Pupfish are most successful in extreme habitats where other species of fish cannot survive. In southern California, some species of pupfish live in isolated desert springs where temperatures and salinities vary greatly. Pupfish also occur in the drains around the Salton Sea area. There are several California sanctuaries for pupfish (e.g., in Anza-Borrego State Park, Owens Valley Native Fish Sanctuary, and Death Valley National Monument). Research colonies of *C. macularius, C. radiosus, C. nevadensis amargosae, C. nevadensis nevadensis, C. salinus* and *C. milleri* have been maintained under permits at various times at Butte County MAD, Lake County MAD, and at the University of California (Davis and Riverside).

Life History. Pupfish are egglayers which breed throughout the summer if water temperatures exceed 20°C (68°F). Females lay 50 to 800 eggs per season. Aquaria specimens often lay over 100 eggs in a single breeding session. Males defend territories during the breeding season while females and young fish forage in loose schools. Males join these schools during the winter. Schools are generally composed of fish of similar size and age (Moyle 1976). In some hot spring pools, sexual maturity may be reached within one month after hatching, and there may be as many as ten generations per year (McGinnis 1984).

Pupfish are generalistic feeders, and will consume aquatic insects (including chironomid and ceratopogonid midge larvae) and other small invertebrates. Mosquitoes are readily eaten so pupfish have been considered for mosquito control. Kennedy (1916) reported that pupfish (*C. radiosus*) controlled mosquitoes in the Owens Valley. Mosquitoes became a problem there shortly after piscivorous bass reduced the pupfish density.

Pupfish (*C. macularius*) sometimes feed more extensively upon *Anopheles* than *Culex* mosquitoes (Danielson 1968). However, *C. macularius* reduced *Culex* mosquito larvae by more than 99% in small ponds (Legner and Medved 1974). Because *C. macularius* is not piscivorous, it might be preferred for mosquito control in areas where *Gambusia affinis* feeding on young native fishes could be a problem (Walters and Legner 1980).

Some pupfish can be effective in preying upon mosquito larvae even in floating algal mats or heavy growths of emergent vegetation (Legner et al. 1975). *Cyprinodon nevadensis* controlled mosquitoes in 210-L (55 gallon) tanks of wastewater containing dense growths of Sago pondweed (Castleberry and Cech 1988).

Pupfish can readily be bred in captivity, and may be able to adapt to wild habitats which are lethal to mosquitofish and other mosquito eating fishes. Pupfish can tolerate waters having high (43°C) temperatures, low (2°C) temperatures (Feldmeth 1981), low (0.4 ppm) oxygen concentrations (Moyle 1976), high (130 ppt) salinities (Soltz and Naiman 1978),and high (45ppm) boron concentrations (Colwell 1981). Some pupfish can survive and reproduce in rice fields (Walters and Legner 1980). Pupfish may reach densities greater than that of most other fish species in California (McGinnis 1984). However, in habitats where other species of fish flourish, pupfish populations may be reduced or eliminated by predation or competition.

Status. Several species of pupfish (including *C. macularius*, and *C. radiosus*) are on the Federal endangered species list and may not be taken without permission. As these can closely resemble unprotected species great care should ne taken when obtaining pupfish. Some other species may soon appear on Federal or State threatened and endangered species lists due to declines of pupfish populations. *Cyprinodon nevadensis calidae* has become extinct due to habitat alteration. The California Department of Fish and Game should be contacted prior to collecting any pupfish.

GASTEROSTEIDAE FAMILY

STICKLEBACKS

There are five species of sticklebacks recognized in North America. Only one of these species, the three-spine stickleback, *Gasterosteus aculeatus* L., occurs in California, and it is represented by three subspecies, *G. a. aculeatus* (Northern threespine stickleback), *G. a. microcephalus* (West Coast threespine stickleback), and *G. a. williamsoni* (Unarmored threespine stickleback). The unarmored stickleback is currently endangered (Leach and Fisk 1972), and is thus protected by California state law.

Identification. The stickleback body is elongated, compressed, and tapers rearward to a narrow caudal peduncle. Teeth are present in both jaws, but are absent from the tongue and palate. The sides of the body may be covered with vertical bony plates, but other areas are free of any scales or armoring. The first three rays (rarely two or four) of the dorsal fin form a series of sharp detached spines, hence the common name.

Names. "Gaster" translates to "belly", "osteus" means "bone", and "aculeatus" refers to the spined condition.

Distribution. In California, threespine sticklebacks are common in coastal waters from the Mexican border to Oregon, and they are widely distributed in Central Valley streams and reservoirs.

Life History. The threespine stickleback is tolerant of high salinities and has both freshwater and anadromous populations. They are quiet water fish and prefer to live among aquatic vegetation; marine populations generally stay close to shore. Sticklebacks are often found coexisting with mosquitofish, although they may prey on mosquitofish fry. Sticklebacks are ideal prey for avian and piscine predators because of their shallow water habits, slow movements, and small size (usually < 6 cm TL).

Sticklebacks generally complete their life cycle in one year. In California, most of the breeding occurs from March to July. At the onset of the breeding season, the male moves away from the school, sets up a territory and undergoes a marked color change (the iris of the eye becomes a highly iridescent blue-green, the body turns blue or greenish-blue, and the ventral surfaces become reddish). The male stickleback then constructs a nest by gathering materials, primarily vegetable matter, and pasting the materials together with a sticky substance secreted by his kidneys. The male creates a tunnel in the nest where the female deposits 50 to 300 eggs. The male guards the eggs, which hatch after about one week, and subsequently guards the fry.

Status. Sticklebacks feed primarily on crustacea and insects, but will consume algae during periods of food shortage. In a detailed study of the stickleback diet, Hynes (1950) reported that 40 food items were consumed by the 1581 fish examined. These included chironomid larvae and pupae, cladocerans, copepods, ostracods, and aquatic oligochaetes. In an earlier study, Markley (1940) found that the fish consumed, in addition to the above items, chironomid adults, coleopteran larvae, and algae. Mosquitoes were not found in the guts of sticklebacks in either study.

Hubbs (1919) however believed that sticklebacks were well suited as mosquito predators because of observations made in streams, pools and marshes throughout California. He found that mosquito larvae were absent in pools and marshes with abundant sticklebacks, but numerous in adjacent waters which were devoid of the fish. Hubbs noted that the stickleback could be a useful ally to mosquito control because (1) its size and mouth structure limits it to prey upon smaller insects such as mosquito larvae, (2) it feeds throughout the water column, (3) it quietly lies in the water waiting for the movement of suitable prey species, (4) it is armored and is able to withstand competition and harassment from other larger fishes, (5) it is widely distributed, (6) it can live and breed in small bodies of water that could become infested with mosquitoes, (7) it can withstand great water temperature extremes, (8) its stocks are abundant enough to provide an ample supply for the stocking of new habitats, and (9) it is hardy enough to tolerate transportation in open buckets for long distances.

POECILIIDAE FAMILY

GUPPIES

Identification. The Guppy, *Poecilia reticulata* Peters (pronunciation: Pee-sill'ee-uh re-tik'you-lay"ta), is a small tropical fish somewhat similar in shape to, but smaller in size than the mosquitofish, *Gambusia affinis*. Both species are members of the livebearer family, Poeciliidae. They are further characterized by unusually large scales and superior mouths which reflect their surface feeding habits.

The anal fin of the male guppy, like that of the mosquitofish, is greatly elongated and serves as an intromittent organ to internally transfer sperm to the female which subsequently bears live young. The slender male, with a maximum length of about 28 mm (1.1 in.), is significantly smaller than the adult female. Growth in the male ceases shortly after reaching sexual maturity. Females, however, grow throughout their life-span and can attain lengths of approximately 48 mm (1.9 in.). Pregnant females are much more robust than males and typically exhibit a large dark "gravid spot" just ahead of the anal fin. Female guppies superficially resemble female mosquitofish, but lack the three transverse rows of dark spots characteristic of the mosquitofish. Also, in the female guppy, insertion of the dorsal fin is directly above or precedes the anal fin; whereas in the female mosquitofish, the dorsal fin is positioned behind the anal fin.

The color of the wild female guppy is gray with shadings towards blue, green or yellow depending on its surroundings. In contrast, the colorful male has large dark spots (ocelli) on a multicolored, iridescent body. In addition, males commonly possess colorful trailing dorsal and caudal fins. Male guppies are generally smaller than mosquitofish males, which are colored a uniform gray or olive. Some mosquitofish, although rarely seen in California, may possess irregular dark spots on the body.

Names. The guppy was name *Girardinus guppyi* after the Reverend Robert Lechmere Guppy who discovered it in Trinidad in 1866. When it was realized that the fish had earlier been described by Peters in 1859, the name was changed to *Lebistes reticulatus* (Peters 1859). Guppy, however, remained as the name in common usage. When Rosen and Bailey (1963) reevaluated the Poeciliidae, the name was revised to *Poecilia reticulata* Peters. Poecilia comes from the Greek word Poikilos, meaning variegated. In Latin, reticulatus means netlike or resembling a web. (Neilson et al. 1951). Guppies are also known as "Barbados millions" due to the phenomenal populations attained in many habitats.

Distribution. The guppy was originally described from Venezuela where it occurs naturally in coastal rivers and streams east to Guyana, the Netherlands Antilles, Barbados and neighboring island areas. Guppies were originally brought into the European aquarium trade in 1908 and the United States in 1912, and have been thoroughly domesticated by aquarists for over 70 years. They are widely used as laboratory animals for research in genetics, physiology, and toxicology.

This fish has been introduced into many tropical and semitropical regions for mosquito control. A partial list of introductions includes: Argentina, Borneo, Burma, Hawaii, Japan, Malaysia, Singapore, Sri Lanka, Tahiti, Taiwan and Thailand. In some areas, including Taiwan, Thailand, and Burma, the guppy has provided good control of *Culex pipiens fatigans* in highly polluted sites. These sources included barnwash ponds, run-off ditches from poultry watering troughs, and ground pools receiving domestic waste (Bay and Self 1970).

Guppies are often introduced into similarly polluted waters in California for control of *Cx. stigmatosoma* and *Cx. pipiens* larvae. Permanent populations of guppies have been established at the sewage treatment plants at Chico, Oroville, the White Slough facility near Lodi and the University of California at Davis.

Habitat. Guppies can often survive in highly polluted waters that are lethal to other fishes, including the hardy mosquitofish. They have been found in untreated sewage ponds in Thailand, Taiwan, and Burma (Bay and Self 1970). In American sewage treatment facilities, guppies often thrive in many secondary treatment ponds containing high levels of organic waste and metal ion pollution. At one facility, where copper sulfate was applied for algae control, the guppies survived despite a copper concentration of 12 ppm in the water; they accumulated up to 4 ppm in their tissues (Bay and Self 1970).

Water quality encountered in natural or many man-made habitats rarely limits survival in guppies. Aquarists indicate that guppies prefer acid water, but these fish are also known to thrive in alkaline waters. Guppies can tolerate a wide range of salinity, from fresh to brackish waters.

Water temperature is recognized as the most important limiting factor for guppies in temperate regions of North America. They survive extremes of 11 to 38°C (52 to 100°F), but reproduce and thrive at temperatures between 20 and 30°C (68 to 90°F). If kept warm enough, guppies will usually produce young throughout the year; thus suggesting a reproductive cycle largely independent of photoperiod influence. Technicians involved with guppies have reported that dissolved oxygen levels in some waters that are too low to sustain other fishes, including the mosquitofish, are often tolerated by guppies.

Introduced guppies are usually confined to polluted habitats because they often don't survive in natural watersheds containing native piscivorous fishes. Bay and Self (1970) reported this to be the case in Southeast Asia. In California, guppies and mosquitofish aren't known to naturally coexist. Where environmental conditions permit mosquitofish survival, coexisting populations of guppies will eventually be eliminated.

Life History. Courtship behavior in guppies is fairly elaborate and can be observed almost constantly during warm weather. Successful matings are less frequent, however, and must usually be preceded by a slow circular swimming pattern of the female to be successful. The male inseminates the female by actually inserting its gonopodium into the genitalia of the female. This unique organ can be swung to either side for mating and once properly aligned, it is locked into a forward position by the pelvic fin on one side or the other. Together these fins form a tube for the transfer of sperm packets. There are hooks at the distal end of the gonopodium to help maintain contact with the female during copulation.

Once inseminated, the female stores viable sperm and may have up to eight broods resulting from a single mating. A female may also mate repeatedly; thus any one brood may represent numerous fathers. At the optimum water temperature of 80°F (27°C), 24 days are required after mating for the birth of the first brood of fry. Successive broods will usually be produced at approximately 28-day intervals. The first brood of a small adult female will generally consist of about 6 to 12 fry, with later broods ranging from 60 to 80 fry depending primarily on the size of the female. Occasional broods of 100 to 200 fry have been observed. If mature males are present, an immature female guppy can be inseminated at eight days of age; however, her first fry will not be born until she attains 10 to 12 weeks of age. Males are not capable of mating until the gonopodium becomes fully developed at three to four weeks of age (Emmens 1970).

The female guppy retains the fertilized eggs in her urogenital tract until they hatch. The fry are then forcefully ejected, drop to the bottom for a brief resting period and then surface for air to initially fill their swim bladders. Once the swim bladder is expanded, the fry are able to swim normally and seek shelter from their occasionally cannibalistic elders.

Under similar regimes, guppies have been observed to attain greater population densities than mosquitofish. Cannibalism rates in guppy populations appear to be much less pronounced than observed in mosquitofish populations, and this may help explain the guppy's more rapid population growth. Guppy populations are usually comprised of smaller fish on the average than mosquitofish, which may be able to penetrate denser vegetation. Although guppies can live up to seven years in captivity, a one to two year life-span in the wild is the norm.

Guppies are omnivorous and opportunistic feeders. They feed predominantly on insects and crustaceans, but will consume other organisms and occasionally their own fry in addition to minor amounts of plant material. Through their consumption of eggs of other fishes, guppies are reported to have harmed fish populations on the island of Mauritius (Burton and Burton 1969). Guppies will feed voraciously on mosquito eggs, larvae, and pupae and they are therefore effective mosquito control agents. They will also feed on human feces which speeds up the natural purification process in raw sewage ponds (Sasa et al. 1964). De Maria and Pellegrino (1966) found that the guppies will feed on the blood flukes, *Schistosoma mansoni* and *S. japonicum*. These are the causative agents of schistosomiasis or bilharziasis, a severe disease which occurs in the tropics. Schistosomiasis affects 180-200 million people worldwide and is on the increase. Guppies appear to have potential for interrupting the life cycle of this parasite because they feed on the cercariae of the flukes after they emerge from the intermediate molluscan host, but before they infect vertebrate hosts.

Ongoing research studies being conducted at U.C. Davis (D. Castleberry and J. Cech, personnel communication) have clearly demonstrated that guppies are effective mosquito predators in municipal wastewaters. Because the guppy population growth rate often exceeds that of other fishes, comparative levels

of predation upon larvae are often unexcelled due to the sheer numbers of hungry guppies present in a given body of water. Their studies also demonstrated that even greater predation levels were achieved by stocking both guppies and mosquitofish in simulated weedy wastewater habitats.

Status. Guppies have been introduced into many waters in California as mosquito control agents and by the careless release of aquarium stocks. They appear to have been able to establish themselves and overwinter only in warm environments. The guppies' inability to survive temperatures below 11°C (52°F) prevent them from surviving in most natural northern California waters.

Current California statutes prohibit unauthorized stockings of guppies; however, they may be planted by permit in closed bodies of water where there will be no possible interaction with other fishes. Thus, they are often successfully used in municipal wastewater lagoons, and dairy and poultry farm sumps, where mosquitofish have previously been unable to survive because of low dissolved oxygen levels and pollution.

Technicians must contact their regional office of the California Department of Fish and Game prior to obtaining or using guppies in their agency's biological control program.

POECILIIDAE FAMILY

MOSQUITOFISH

Identification. The mosquitofish, *Gambusia affinis affinis* (Baird and Girard) and *G. a. holbrooki* (Girard) are almost identical in color, form, and size. Their color is usually olive or dull silver, and is darkest on the head, lightest on the belly, but this character tends to vary with habitat. In just moments, mosquitofish can adjust their body pigmentation to more closely match a new background color. Individuals living in ditches and drains are usually pale, and those in dark colored water of swamps are dark green, with a distinct purple bar below the eye. There is frequently a fine dark line along the sides, and sometimes a dark blotch below the eye; a dark purplish blotch is usually present on the side above the vent in the female. The dorsal fin has 2 or 3 transverse rows of fine black spots. The anal fin in the female is dark edged. The caudal fin has 3 or 4 irregular transverse rows of dark spots and the other fins are dusky. The fins are small and the scales large in proportion to the size of the fish. Dark pigment outlines each scale.

There are a few differences between the subspecies. *Gambusia affinis holbrooki* has 8 rays in the dorsal fin, and the third ray of the gonopodium (male sexual organ) shows a deep split when examined under the microscope (Innes 1966). *Gambusia affinis affinis* has only 7 rays in the dorsal fin, and the split in the third ray of the gonopodium is lacking.

The sexes differ in form and structure. The female has a deeper body than the male. Females, whose maximum size depends on the fertility of the water in which they live, grow throughout life and may reach a length of 2 1/2 inches (64 mm). Males grow little after the gonopodium is formed and do not exceed 1 1/2 inches (38 mm). The female has a small, rounded anal fin and the male has the third, fourth, and fifth anal fin rays united and lengthened to form the gonopodium.

Names. According to Jordan and Evermann (1896), the generic name *Gambusia* has its derivation in the provincial Cuban word Gambusino, which signifies 'nothing' in a scoffing sense. The species name *affinis* means 'related', apparently referring to the relationship existing between this fish and *G. holbrooki* (which is now recognized as *G. a. holbrooki*). Pronunciation: Gam-bew'see-a aff'in-iss.

Distribution. *Gambusia affinis affinis* is native to central North America from southern Illinois to Alabama and Texas, while *G. a. holbrooki* is native to the Atlantic coast from New Jersey to Florida. Successful introductions of mosquitofish have been reported from most of the American states. Mosquitofish have also been established in Canada, Central and South America, as well as islands of the Pacific Ocean and the Caribbean Sea.

Mosquitofish were introduced into Europe in 1921 through shipments of *G. a. holbrooki* to Italy and Spain. It has become established in many Asian and African nations; so the mosquitofish can truly be considered a cosmopolitan species.

There have been too many introductions to list each individually, but detailed distribution information can be located in the work of Krumholtz (1948) and Gerberich and Laird (1966). Recent fish stock assessments have revealed that there have been numerous unrecorded mosquitofish introductions elsewhere throughout the world. Several authors have stated that *G. affinis* enjoys a wider distribution than any other freshwater fish.

Feeding Habits.- Mosquitofish are opportunistic feeders. Over fifty types of plant and animal life are recorded as having been consumed by this fish. A small portion of their diet may include organisms such as algae, desmids, and diatoms. Animal prey includes copepods, chironomid larvae, nematodes, and other planktonic life, in addition to mosquito larvae. Larger mosquitofish are cannibalistic and will readily prey upon their newborn young and possibly other smaller fish. Mosquitofish can not utilize food at temperatures below $5^{o}C$ ($41^{o}F$); at these low temperatures, they move to deeper water or to the mud-water interface and become inactive (Krumholz 1948).

The consumption of mosquito larvae by mosquitofish has been reported by many workers. Rees (1958) found that prefed fish 29-41 mm (1.1-1.6 inches) in length consumed 15-49 fourth instar *Aedes* larvae in a thirty minute period. In the same period, starved fish 38-42 mm (1.5-1.7 inches) in length consumed 49-65 larvae. The maximum number of larvae consumed by a single fish in this study was 168 in an eight hour period. In addition, he reported fish as small

as 6.9 mm (0.3 inches) consuming third instar *Aedes* larvae. Rees also demonstrated rapid digestion of mosquitofish larvae; they can devour large numbers of larvae and completely digest them in roughly eight hours.

Hess and Tarzwell (1942) reported foraging rates for mosquitofish in a reservoir study. The value of the foraging ratio was dependent upon various ecological factors, including the mosquito species composition, relative density, and stage of development of the food organisms. Greater preference was shown for culicine larvae over anopheline larvae and, as mosquito densities increased, the number of fish and the number foraging increased. The foraging ratio for *Anopheles* was 1 when the larval density was 2 per square foot (21.5 per square meter). The maximum foraging ratio was over 14 larvae when the larval density was 17 per square foot (183 per square meter).

Male mosquitofish ate about half as much food as females of the same size. As the female grew, food consumption increased. Large females were the most effective predators on *Anopheles* larvae.

In mosquitofish food size selection is positively correlated with fish size. They actively select among prey size classes and will choose the largest prey they can successfully capture (Bence and Murdoch 1986, Wurtsbaugh, et al. 1980).

Washino and Hokama (1967) studied the feeding pattern of mosquitofish in a rice field habitat. Marked seasonal changes involving aquatic plants and food organisms occurred throughout the summer. Food consumed by mosquitofish consisted primarily of crustaceans, principally Cladocera, followed by chironomid larvae. Mosquitofish also fed on terrestrial insects such as aphids, muscoid flies, and Cicadellidae.

Culicine larvae were consumed in July in low numbers with peak predation occurring in August, although the larval peak was observed in June. Peak predation on anophelines occurred two weeks after larval populations peaked in July. Other organisms in the environment also displayed peak population levels seasonally. This suggests availability of a wide variety of food organisms in early summer. Food availability tends to decrease in late summer, apparently intensifying predation on mosquito larvae.

Growth and Population Dynamics.- Hubbs (1971) documented the existence of pre- and post-reproductive periods for both male and female mosquitofish. Both sexes are considered pre-reproductive until they attain a length of 26-28 mm (1.1 inches). When the male's gonopodium (modified anal fin) is completely formed, sexual maturity is reached and overall growth virtually ceases. However, females in this study reached 44-45 mm (1.8 inches) in their first year and grew continually throughout their adult life.

Krumholz (1948) found that mosquitofish populations from several ponds had different mean lengths and associated these size differences with variations in pond fertilities. Goodyear et al. (1972) offered further corroboration for this association and also postulated that a direct correlation existed between mosquitofish production and a pond's net and gross primary productivity, as measured by the level of organic nitrogen present.

Hubbs (1971) and Johnson (1976) correlated greater mosquitofish growth with increased diurnal fluctuations in summer water temperatures. Coykendall (1977a) measured population growth rates and found that the fish biomass increased roughly one percent per day in central California fish ponds stocked at 50 pounds of fish per acre (56 kg per hectare). They were fed supplemental rations equivalent to 5-10% of their biomass daily during the six month summer production study.

Hoy and Grady (1971) made estimates of final population densities for mosquitofish stocked in rice fields. Ten weeks after stocking at 100 fish per acre (247 fish per hectare) the population grew to an estimated 5,509-6,291 mature females per acre (13,613-15,545 fish per hectare), as estimated by removal trapping techniques. In another study of fish density increase in rice fields, Reed and Bryant (1974) arrived at an estimate of 4,868 mature females per acre (12,029 fish per hectare) when the original stocking thirteen or fourteen weeks earlier had been 100 mature females per acre (247 fish per hectare). Norland and Bowman (1976) conducted a rice field stocking experiment and reached the conclusion that fish populations may peak and stabilize at lower levels because of limitations in food supply and related rice culture management.

Coykendall (1974) and Johnson (1976) both discussed the importance of planktonic algae to the growth and survival of newborn mosquitofish. This algae form serves as food for the young and inhibits cannibalism by the sight feeding adults.

Habitat. Emerick (1941) made the first adaptation of mosquitofish in California to highly organic waters at the Calistoga sewage disposal plant. He first introduced fish into the clearest of the six ponds. Then every six months he transferred fish from pond to pond until they eventually entered the septic tank and survived.

Odum and Caldwell (1955) in Florida studied aerial respiration in topminnows. Water devoid of dissolved

oxygen emanating from a limestone aquifer flowed downstream, absorbing oxygen by diffusion and photosynthesis of algae. Total dissolved oxygen ranged from 0.05 to 3.6 ppm with a pH of 7.2 to 7.7. *G.a. holbrooki* were observing breaking the water surface along with other fishes where the dissolved oxygen was less than 0.3 ppm. The mosquitofish were observed pumping water over their gills in stagnant areas. This was not the means of respiration since definite gulping of air was observed. When prevented from gulping, fish drowned quickly. Fish kept out of the water twenty-one minutes were alive and demonstrated normal behavior when returned to water. Dissection of fish from springs revealed a closed swim bladder, a short esophagus and short intestinal opening, and the presence of pseudobranches which were presumably being used for surface respiration. Odum and Caldwell also noted that oxygen levels ranged from 0.00 to 0.81 ppm.

Healthy mosquitofish populations have been observed in California sewage oxidation ponds where dissolved oxygen profiles have ranged from 35 ppm at 1 inch (2-3 cm) depths to 0 ppm at 39.4 inches (1 meter). Fish frightened by surface disturbances would readily dive to these oxygen deficient depths for short periods of time without apparent harm (Coykendall, unpublished data).

Mosquitofish have an extremely wide range of temperature tolerance. Otto (1973) estimated cold-acclimated fish stocks in Utah could tolerate water temperatures down to 0.5°C (32.9°F), while warm-acclimated stocks from an Arizona hot springs were observed living in water having a temperature in excess of 42°C (107°F). In California populations, similar minimum and maximum temperatures tolerances have been observed in field work; however, most fish surviving these extremes have been young mature females (Coykendall, unpublished data).

McHugh et al. (1964) studied the ecology of mosquitofish in log ponds in Oregon. Mosquitofish from various sources were planted in holding ponds which produced the breeding stock for log ponds. Mosquitofish were efficient in scores of ponds, although failures were observed in a few ponds that were highly polluted. Although floating mats of aquatic vegetation offered protection to larvae, the mosquitofish fed voraciously among floating bark and in the shallows atop partially submerged bark. Removal of excessive flotage is accomplished by concentrating the flotage to a portion of the shore with a log boom and removing the masses with a continuous belt loader. Ponds providing good support for mosquitofish contained a chemical oxygen demand (COD) of 40.4 to 121.0 ppm. Apparently a COD of 150 ppm is the upper limit for successful reproduction at water temperatures of 25°C to 30 °C (77°F to 86°F). A COD of 200 ppm is probably near the maximum limit for survival. In the Oregon log ponds studied, pH levels ranged from 4.9 to 9.5. As observed by McHugh, mosquitofish form small schools in log ponds and swim in formation; in ponds that are too polluted for survival they swim singly and erratically at the water surface, then appear to lose equilibrium and die.

According to McHugh, sticklebacks in Oregon are apparently in forceful competition with mosquitofish in sand dune lakes, ponds, and freshwater areas adjacent to salt marshes, and do not allow mosquitofish to survive. Stickleback predation on the young mosquitofish was presumed to be the principal factor.

California workers have observed and experimented with mosquitofish in numerous diversified ecosystems. In brackish salt marsh waters, mosquitofish do well and are used to stock similar areas. However, high mortality may occur when mosquitofish are quickly transferred from brackish water to fresh water or the reverse. Reproducing mosquitofish populations have been observed in the thermal coolant waters of a California power generation plant where salinities were 15 ppt (ca. 43% seawater concentration).

Aquatic habitats that contain a mixture of sewage effluent, dairy drainage, and potable waters support and produce the greatest numbers and most vigorous fish in some localities. In other localities, drain ditches of pasture and alfalfa grazed by livestock, natural sloughs, shallow streams, and fresh water drains containing intermittent stands of aquatic vegetation produced high densities of fish. Such habitats are utilized as major sources of fish for planting purposes.

Mosquitofish survive well in summer in the presence of shade. Survival has been observed as being somewhat poor in winter in less than 18 inches of water. Fish have been successfully transferred from a pasture and dairy drain environment to plastic lined holding pond habitats. Reproduction occurs, but individuals of the succeeding generation may be smaller. Occasionally winter freezing of holding pond water to a depth of 1/8 inch (3 mm) usually causes little mortality.

Reproduction. Dees (1961) described the life history of mosquitofish as follows: "*Gambusia* are ovoviviparous: that is, a female produces eggs that hatch within her body. The male fertilizes the eggs in the female by using the gonopodium to deposit sperm in her genital tract. A female is able to produce several broods of young after being fertilized once.

Fertilized eggs hatch in 21 to 28 days. Within several hours a female expels from a few to several hundred live young. About three-eights of an inch (10 mm) long at birth, they are well developed and completely formed. They have prominent black eyes and are often uniformly yellowish. The fins are dusky, and the caudal fin usually has a cross series of dots. At birth, they are able to swim. Growth of mosquitofish is rapid, depending on the food supply and to a lesser extent on the water temperature."

Studies of *G. a. affinis* collected from eight ponds in northeastern and central Illinois reveal much about their breeding habits (Krumholz 1948). Males matured at smaller sizes and at slightly earlier ages than females. Some females in two ponds became sexually mature for the first time early in their second summer of life when they were eight to ten months old and 1 to 2 inches (25-50 mm) long. They reproduced for ten to fifteen weeks, usually bearing three to four broods. The offspring of their first and possibly their second brood became sexually mature during their first summer of life when they were four to six weeks old and about 1 inch (25 mm) long. They reproduced for four to ten weeks, generally bearing only one or two broods. However, Krumholtz found that newly-established fish populations in ponds having high fertility resulted in parent stock release of four to five broods and their offspring had at least three broods of young.

Krumholz states: "In the first brood, males and females were in almost equal numbers, but in succeeding broods the female gradually outnumbered the males. The average number of young in a brood decreased as the age of the mother increased. Some metabolic factor, coincident with the approaching end of the reproductive season may control the number of young."

In the same study, Krumholz found that unlike many other fishes, mosquitofish display a definite period of sterile senility following reproduction. Reproduction efficiency of the female also declines with age, with the broods becoming smaller as the reproductive period progresses. According to Krumholz, females that bear young at early maturity become senile more quickly than those that do not reproduce until their second season. His studies disclosed that reproduction is affected by (a) rate of growth, (b) maximum age and size attained by male and female fish, (c) size and age at first maturity, (d) the duration of the reproductive period, (e) the period of gestation, (f) the fecundity of the female, (g) the period of senility, (h) the sex ratio, and (i) the differential death rate. Although males and females are born in approximately equal numbers, more females are encountered in the environment since females are hardier and longer lived. Krumholz attributed the decrease in size of successive broods to deterioration in natural conditions including water temperature, chemical composition of water, water depth, availability of food, changes in population pressure, and physiological changes in the fish.

Hubbs (1971) and Sawara (1974) concluded that the limiting factor for reproduction is probably day length (photoperiod) rather than water temperature. Sawara presumed the "critical day length" to be 12.5-13 hours. Photoperiod is a function of latitude, but Sawara discovered that the reproductive season may not always coincide with this "critical day length".

Water temperatures limiting the reproductive cycle haven't been identified as racial differences exist among stocks from different locales. Also, daily water temperature fluctuations may influence reproduction.

Hubbs (1971) found that the interbrood interval averaged 35 days and that 25°C (77°F) water temperatures were optimal in his study. Females of the same study produced second broods in 60 days in waters of 20°C (68°F) and in 24 days in waters of 30°C (86°F). Therefore, in the early spring and late fall, broods may be produced every two months, while in midsummer broods can be liberated as often as every three weeks.

Pesticide and Chemical Tolerance.- Numerous laboratory and field observations on the effect of toxic materials on fishes have been reported by various workers. Mulla et al. (1961, 1963, 1966) reviewed the literature relating to the effects of organochlorine and organophosphorus insecticides on various fishes, including the genus *Gambusia.* He reported that the response of a fish to toxicants is influenced by many factors, including physical characteristics of toxicants, substrates and water, in addition to biology, physiology, and ecology of the test organism. Chemical stability of compounds, and the degradation products in organisms or the environment, also influence the effect of toxicants on organisms in the field. The environment is complex, requiring extensive work associated with each factor. Selective materials which are effective against mosquito larvae without harming fish populations are the ideal insecticides. Nonpersistent materials should be used where introduction of fish is contemplated after treatment for mosquito larvae. The value of this integrated approach to chemical and biological control is well illustrated here.

Drawing further from Mulla's summary of the insecticides in current use for mosquito control, Malathion is more toxic to fish than parathion

causing 48% to 70% loss of caged fish in field tests at 0.5 lb/acre (0.56 kg/hectare). Fenthion at the rate of 0.1 lb/acre (0.11 kg/hectare) exhibited little or no toxicity to mosquitofish, but at the rate of 0.4 lb/acre (0.45 kg/hectare) produced 18% mortality in 48 hours. Parathion at 0.1 lb/acre (0.11 kg/hectare) against caged fish resulted in 18% and 30% mortality, while methyl parathion manifested no mortality. However, parathion applied at 0.1 lb/acre (0.11 kg/hectare) to free swimming mosquitofish resulted in no mortality. Apparently ethyl analogues of organophosphates display greater toxicity because of their greater stability, low hydrolysis rate, and other factors. Free swimming mosquitofish were also found with the ability to accumulate and store parathion.

Ferguson et al. (1966) and Washino et al. (1972) tested mosquitofish against chlorpyrifos in various environments and found little or no mortality at field applied rates. Hurlburt et al. (1970) found caged mosquitofish reproduction and survival were not adversely affected, even when the application rates were as high as one pound per acre (1.12 kg/hectare).

Johnson (1977a) found that fenthion and temephos did not affect mosquitofish at recommended field rates; however, methyl parathion, chlorpyrifos, and malathion depressed fish activity temporarily. Malathion at 500 ppb caused 100% mortality in laboratory tests.

Two insect growth regulators, Dimilin and methoprene, didn't cause any mortality or loss of orientation in mosquitofish exposed to concentrations ranging up to 200 ppb; however, thermal tolerances were reduced in male fish at 10 ppb and in female fish at 100 ppb (Johnson 1977b).

Some herbicides used aquatically at normal field applied rates can be very toxic to fish and other aquatic organisms. Copper sulfate pentahydrate is commonly used as an algicide in fish ponds; unfortunately, application rates found to be nontoxic in one area may prove to be highly toxic in other areas. Many environmental factors probably contribute to this variation in toxicity.

Simazine, diquat, endothall compounds, chelated copper formulations, and potassium permanganate have been safely employed by fish farmers.

For specific information regarding the toxicities of various chemicals to mosquitofish, see the listing of the results of acute toxicity bioassays provided on the following page.

Vector Control Usage and Efficacy. Ecological data and field observations on mosquitofish are widely scattered in the literature. Some of the more pertinent observations, experimental, scientific, or casual, together with personal and largely unpublished experiences of California workers are summarized as follows: Hildebrand (1919), after extensive experiments and observations aptly stated, "*Gambusia affinis* is especially suited to anti-mosquito work because: (a) it seeks its food at the surface, (b) it is very prolific, (c) it gives birth to living young, therefore requiring no special environment for depositing and hatching eggs, (d) it lives and thrives under a large variety of conditions and frequents areas especially suitable for the support of mosquito larvae, (e) it lives and multiplies in ponds stocked with predaceous fishes, provided it has very shallow water for refuge."

With regard to actual use, many workers have measured the efficacy of mosquitofish under a variety of conditions. Where stock ponds, ditches, natural streams, lakes, ornamental garden pools, and animal troughs are free of excessive vegetation and algae, mosquitofish will usually provide effective control of mosquito larvae. Important mosquito sources of this nature usually require little attention other than routine inspections as long as mosquitofish are present.

In the presence of aquatic vegetation, the feeding efficiency of mosquitofish may be reduced somewhat depending upon the type and density of vegetation, extent of matting and related factors. Overhanging carpets of Bermuda grass (*Cynodon dactylon*), matted stands of parrot's feather (*Myriophyllum brasiliense*), yellow waterweed (*Jussiaea californica*), pond weed (*Potamogeton diversifolius*), milfoil (Myriophyllum spp.), and bladderworts (*Utriuclaria* spp.) have been reported as affording good protection to larvae and creating barriers against penetration by mosquitofish seeking food.

Hildebrand (1919) and Emerick (1941) observed that *Anopheles* larvae are not affected by mosquitofish in the presence of dense algal mats. In streams having extensive lily growth, both observers have commented on the numbers of anopheline larvae surviving within individual lily cups and protected from abundant mosquitofish present in the surrounding waters. Emerick also commented that fish ponds, troughs, and other devices located under mulberry, eucalyptus, and walnut trees would not support topminnows when leaves of these plants were present in the water.

In studies undertaken in 1921 and 1925 in ponds affording good protection for mosquito larvae, Hildebrand (1925) found that anopheline larvae and pupae were reduced 57.8% by well established mosquitofish populations. Culicine larvae and pupae were reduced by 80.8%.

Table 1. Acute toxicity of various chemicals to mosquitofish.

Chemical	Exposure (hours)	Temp. (°F)	LC_{50},LD_{50} (ppm)	Mortality (%) at Concentration (ppm)		Reference
Herbicides:						
copper sulfate	24	75	-	89%	7	Sjogren 1972
copper sulfate	72	80	10+	-	-	Ahmed 1973
Cutrine-plus™	24	-	290	-	-	Leung et al. 1983
Cutrine-plus™	96	-	170	-	-	Leung et al. 1983
diquat	24	-	723	-	-	Leung et al. 1983
diquat	96	-	289	-	-	Leung et al. 1983
diuron	72	80	10+	-	-	Ahmed 1973
endothal	72	80	10+	-	-	Ahmed 1973
2,4-D ester	24	68	7	-	-	Hansen 1972
Insecticides:						
b. sphaericus	24	-		0	520 spores/ml	Mulligan et al. 1978
b. thuringiensis						
var. israelensis	336	-	-	0	$1x10^7$spores/ml	Garcia et al. 1980
H-14	24	80	-	0	100,000[b]	Tietze et al. 1991
chlorpyrifos	24	68	4	-	-	Hansen 1972
fenoxycarb	24	80	1.05[b]			Tietze et al. 1991
fenthion	24	80	2.94[b]			Tietze et al. 1991
GB-1111™	24	80	593.4[b]	-	-	Tietze et al. 1991
malathion	24	80	12.68[b]			Tietze et al. 1991
malathion	48	80	3.44[b]			Tietze et al. 1991
malathion	24	70	-	40	0.05	Lewallen 1959
malathion	24	68	2	-	-	Hansen 1972
methoprene	24	80		0	30,000[b]	Tietze et al. 1991
methyl parathion	72	72	-	0	6	Ahmed et al. 1970
naled	24	80	3.5[b]	-	-	Tietze et al. 1991
naled	24	70	-	3	0.03	Lewallen 1959
parathion	24	70	-	33	0.004	Lewallen 1959
parathion	24	72	-	0	0.05	Ahmed et al. 1970
parathion	24	72	-	100	0.3	Ahmed et al. 1970
parathion	24	-	0.02-2	-	-	Kynard 1974
resmethrin	24	80	6.93[b]	-	-	Tietze et al. 1991
resmethrin	48	80	5.24[b]	-	-	Tietze et al. 1991
resmethrin	24	-	9.85	-	-	Pennick-Bio Corp. 1986
resmethrin	96	-	2.96	-	-	Pennick-Bio Corp. 1986
temephos	24	80	5.60	-	-	Tietze et al. 1991
Other Chemicals[a]:						
potassium permanganate	96	70-75	12	100	18	Wallen et al. 1957
sodium chloride	96	68-73	17,550	100	21,000	Wallen et al. 1957

[a] Wallen et al. 1957 collected data on the toxicity of 86 pure chemicals; see this reference for other chemical compounds not included above.
[b] 3 - 5 day old mosquitofish.

Krumholz (1948) during a two year study in Michigan observed a reduction of anophelines from 80.9% to 94.8% in ponds having natural growth of aquatic vegetation. Sokolov (1936) found 90% of anopheline larvae eliminated in rice field experiments as compared to control ponds. However, Valle (1928) noted mosquitofish were only 20% effective where heavy aquatic vegetation was the prime factor.

Horsfall (1942) in rice field experiments with mosquitofish indicated .1 acre plots without fish contained 6.2 anopheline larvae per square foot, plots with one mosquitofish per plot had a seasonal total of 2.2 larvae per square foot, and those with two fish had 1.2 larvae per square foot. Larval densities were thus reduced approximately 6 to 1 by the addition of fish. Fish were more efficient before the rice began to lodge. The protection afforded by the older rice reduced the efficiency of the mosquitofish.

More recent tests of mosquitofish stockings in California white rice fields by several researchers (Hoy and Reed 1970; Hoy et al. 1971; Hoy et al. 1972; Norland and Bowman 1976) have been conducted which further demonstrate its usefulness as a biological control agent for both culicine and anopheline mosquito larvae.

However in wild rice fields, Kramer et al. (1987,1988) found that mosquitofish did not significantly control mosquitoes at release rates of 0.5 or 1.5 lbs/acre during a 1986 study, but that in 1987, the fish did significantly reduce numbers of mosquito larvae at release rates of both 1 and 3 lbs/acre. In the 1987 study, fish numbers in the minnow traps were much higher than in the 1986 study. The larger mosquitofish population in 1987 may have been due to a more abundant prey population, a larger proportion of pregnant females released into the fields and an earlier (by 10 days) fish release date. The authors conclude that fish populations must be monitored post-release to assess their control potential

Mosquitofish have also proven their effectiveness in the control of mosquito production in Sacramento Valley drainage and roadside ditches, agricultural seeps, vernal ponds, and railroad borrow pits (Armstrong 1977a,b,c).

Some irrigated pasture and seasonally flooded wildfowl refuge areas have been stocked with mosquitofish and the results of past and present field studies support their use in these habitats; although in some situations physical modifications to the land is necessary to sustain and enhance mosquito predation (Sutter-Yuba MAD, unpublished).

Ecological Considerations. Bay (1965) also substantiated the importance of species introduction, precautions, and consequences: "There is a universal reluctance among biologists, naturalists, and sportsmen to see any new species introduced into an area, for fear that it will succeed too well and become a pest. These fears are based on such mistakes of the past as the introductions of the gypsy moth, the English sparrow, the European starling, and the carp into the USA. Similar cases of unwanted introductions can be cited throughout the world, and they have been perhaps the most common in aquatic environments. For this reason, it is well for those responsible for mosquito control to work as closely as possible with those entrusted with the management of freshwater resources. Carefully supervised ecological surveys of would-be larvivore release sites, followed by restricted, in-the-field larvivore evaluations, can benefit both mosquito control and fisheries staff. If the larvivore proves to be an effective mosquito predator without threatening existing fisheries, the sportsman can pursue his pleasure in greater comfort. If, on the other hand, it should transpire that a candidate larvivore has disadvantages that outweigh its mosquito control potential, careful documentation of the situation is of value to all parties concerned.

Ecological surveys for predator evaluation should be begun at least one year prior to actual fish releases and should include not only a survey of the target mosquito larvae, but also, as far as possible, a survey of the indigenous mosquito predators, fish that may be endangered, and potential predators of the fish to be introduced. Taking into account what is known of the candidate fish's life requirements, measurements of water quality should also be made, both to predict if the fish will survive and to provide documentation on which to base specific recommendations for its use. Aside from the extremes of temperature, salinity, and pollution, and the need of some species for specific breeding sites, little is actually known of the critical requirements for most fish. After all is said and done, the ability of a fish to survive and reproduce in a new environment can be determined only by releasing it into that environment. Likewise, its release is the only reliable way to evaluate its mosquito controlling ability. Obviously, it would be both unrealistic and impracticable to require a thorough ecological survey of each individual site into which it is desired to introduce a new species. Instead, selected release sites representative of those in the proposed area of introduction should be carefully chosen for study, additional releases in the area being dependent on the results.

Whenever possible, trial introduction sites should be confined to closed water systems of manageable size

that will permit close surveillance of predator establishment and efficiency. This precaution is also important to facilitate early eradication of the species introduced, should it show signs of becoming a pest." Although dense aquatic vegetation, floatage, and debris, as well as other factors, have allowed mosquito development in the presence of abundant mosquitofish, this approach to biological control should not be discredited. Bay (1967) evaluated the status of mosquitofish as follows: "*Gambusia* has not always lived up to its mosquito control expectations, however, and in a number of reported instances, it has even been described as detrimental to indigenous larvivore populations. Some of the habitats in which *Gambusia* has failed to give adequate mosquito control are those which are too cold, too plant infested, polluted, lacking in protection from their natural enemies, or simply too extensive or temporary for the fish to achieve mosquito controlling densities.

In short, *Gambusia* introductions into these situations were usually ill conceived, without taking into account either the ecological requirements of the fish or the conditions which the environment had to offer. Such mismatches of fish with situation have at times tarnished the otherwise excellent reputation of *Gambusia* as the number one naturalistic control of mosquitoes; but from the immediate and practical standpoint, number one it remains."

Hence, in the adaptation of mosquitofish to various ecosystems, fish management must be practiced. Removal or control of aquatic vegetation is sometimes necessary to assure effective mosquito control. Also, acclimatization of fish and water quality control practices may need to be considered. A sound working knowledge of fish ecology and behavior, plus considerable ingenuity, experimentation and careful evaluation of results are necessary for success in using fish as a mosquito control measure.

OPERATIONAL TECHNIQUES

AQUARIA & HOLDING SYSTEMS

Mosquitofish may be reared in aquaria without difficulty at water temperatures between 21°C and 29°C (50-86°F). Live foods are preferred but any processed feed formulated for fish will provide adequate nutrition.

In aquaria or other crowded conditions, mosquitofish display cannibalistic tendencies. The adult fish will feed upon the young fish unless they are protected. This may be accomplished by providing sufficient plant growth or other harborage in which the young may hide or by separating the older fish from the young.

Breeding traps especially designed for livebearers provide some measure of protection for the very young from other larger fish and are a necessity if aquarium breeding is desired.

Zanco (1933) reported having kept an individual *G. holbrooki* in an aquarium to an age of at least five years. Krumholz (1948) kept individual mosquitofish under similar conditions for nearly four years. He stated that such longevity is probably attributed to the fact that the life-span of many animals may be considerably prolonged under conditions of semistarvation

Gall (1983) reported that the optimum conditions for the growth and reproduction of mosquitofish were 25°C (86°F), a photoperiod of 16:8 L:D, and a loading density of 1 to 1.5 fish per liter. The system maintained a pH of 8.5, dissolved oxygen at 8 ppm; NO_2 at 0.03 ppm and NO_3 at 4 ppm.

Mosquitofish and other fishes can be kept in outdoor holding tanks in relatively large numbers with the use of aeration. Electric agitators manufactured for the food and bait fish industry are useful, but small mesh basket screens must be attached to these units to prevent the fish from encountering the paddles or blades. Be sure to adequately ground the unit to help eliminate any shock hazard as the agitator motor housing may get wet at times. In addition, unplug all agitators and other electrical equipment before working in a holding tank.

Holding tanks can be constructed of several materials. Fiberglass is strong, inert, easy to clean and will weather slowly. Concrete is also very good, but is heavier and quite porous. Interior sealants may be used to reduce leaching and prevent pathogens and parasites from occupying cracks and pores. Wooden tanks are practical if adequately sealed from all water contact. Plywood tanks that are covered both inside and out with plastic resin make good holding tanks, although their life-span will generally be shorter than that of either fiberglass or concrete. Stainless steel, although expensive and hard to work, is suitable for holding tanks. Other metals, such as iron, aluminum, and galvanized steel shouldn't be used unless one can seal the metal from exposure. Certain metals like copper, zinc, and lead may prove to be very toxic to fishes and should be avoided for use where long term fish exposure is planned.

Holding tanks should have bottom drains for complete emptying. They should also have overflow drains in the event that water is to be either periodically or constantly replenished. Water levels can be held constant through the use of standpipe drains or water control float valves. All excess water should be removed from near the bottom of the tank to avoid fish metabolite accumulations. PVC plastic pipe or other plastic materials are usually ideal for all holding tank plumbing needs.

Some planktonic algae which commonly grow in mosquitofish holding tanks may be merely unsightly, but filamentous algal forms are detrimental to both fish movement and collection by operational personnel. Excessive algae blooms may be controlled by several different methods. Many commercially available water dyes are quite effective. Compatible algicides can be also be used. Be sure to follow instructions and read precautions when adding any chemical to a fish culture system. Shading of the water is also helpful in reducing algae blooms. Finally, frequent water replacement will remove excess algae.

Filtration units are often used to mechanically remove suspended wastes or to biologically convert harmful waste metabolites, such as urea. Biological filtration units should be separate from, rather than inside the holding tank. This is necessary because commonly used antibiotics or medicines applied to the waters of the holding tank may kill the useful bacteria in an inboard biological filtration unit. In such instances, it is not unusual to lose both fish and bacteria because of rapid buildups of toxic nitrogenous compounds with subsequent loss of most of the normally available dissolved oxygen.

Outboard biological filtration units receive water from the holding tank by means of airlift or electric pumps. The filtered water is returned to the holding tank through pumping or by gravity flow. Spotte (1979) and Wheaton (1977) provide detailed information and instructions for the design and operation of biological, chemical and mechanical filtration equipment for closed, recirculated aquatic culture systems.

CAPTURE EQUIPMENT AND METHODS

Hand Nets. Fish habitats differ with respect to physical configuration and vegetation, thus appropriate equipment and techniques should be selected for each collection site in order to maximize fish catch per unit effort. Puddles, weed choked drains, and other small shallow bodies of water usually prohibit the use of seines; therefore, scap, bait, or skimmer hand nets are usually employed in these smaller sites. The purpose of these shallow-draft hand nets is to allow rapid skimming of the surface waters. Small but significant numbers of fish can be recovered in this manner from sites that are temporal because of draining, seepage, or evaporation. Scap nets should be constructed such that the support framework is light and presents little resistance to ordinary skimming movements of the water. The handle should be fairly long to reduce unnecessary stooping by the operator. Care should also be taken to ensure that sharp pieces of wire mesh and other potentially injurious projections are trimmed from the netting and framework.

Hand nets suitable for transferring all but very young mosquitofish usually have 1/16 inch (1.5 mm) Ace knotless nylon mesh bags with steel or aluminum support hoops. Handles are generally wood or aluminum tubing in lengths from 18 inches (46 cm) to 36 inches (91 cm). Several manufacturers will custom make hand nets in sizes specified by the purchaser. One particular hand net configuration which works very well has a 1/8 inch mesh bag measuring 14 inches (36 cm) square by 8 inches (20 cm) deep. The hoop is galvanized 1/4 inch (6 cm) diameter steel wire and the handle is aluminum tubing 24 inches (61 cm) in length.

In the larger hand net sizes, several pounds of fish can be transferred in one dipping. However, if the hoop is unduly flexed the wire may fatigue and eventually break. One should either limit the quantity of fish being transferred or simply grasp the hoop at both sides to prevent unnecessary flexing. Newborn fish or fry may be handled with ordinary fine mesh nylon tropical fish nets. Select only those nets having stiffer wire hoops or handles as weaker units are too easily deformed. All hand nets will last much longer if a narrow strip of cloth or plastic is wrapped or sewn around the hoop as a guard against abrasion.

Seines. A seine used to capture mosquitofish is simply a long net having lead weights attached along a line to its bottom edge and floats strung along a corresponding line on its top edge. This construction enables the seine to be pulled by the two lines, with associated poles or bridles at each end, through the water so that fish are either held against the seine or herded ahead of this physical barrier. Once the seine ends are retrieved to the shoreline, the fish are usually trapped between the netting and the bank.

Mosquitofish and minnow seines are usually quite similar or identical in their manufacture. Nylon netting in 1/8 to 1/4 inch (3 to 6 mm) mesh weave is used, although 1/8 or 3/16 inch (3 or 5 mm) mesh is most commonly used in mosquitofish seines. The float- and leadlines are braided nylon rope; however, the rope diameter selected depends largely on the length and the weight of the seine. Floats are generally constructed of molded or sponge plastic and their size and spacing usually correspond to the amount of buoyancy desired for a particular seine configuration. Netting manufacturers supply finished seines with the proper weighting and buoyancy so they "bag" easily as they are towed through the water. Mosquitofish seines in lengths greater than forty feet (12.2 m) should be ordered with extra heavy duty nylon float and leadlines because of the added weight and stresses that will be encountered.

Poles attached to the ends of the lines must be sturdy enough to withstand the forces involved. The standard depths of mosquitofish seines are either four or six feet (1.2 or 1.8 m). Seines shallower or deeper than this are either inefficient or too cumbersome to use. Two able-bodied workers can tow seines six feet (1.8 m) deep by 3/16 inch (5 mm) mesh in lengths up to eighty feet (24.4 m). Because of the additional weight, longer seines than this often require more manpower at both ends.

Different fish sources may require slight variations in seining technique to maximize the harvest of catchable fish. The traditional method of seining is to tow the seine through the water by having workers at each end of the seine walking abreast at the same rate while holding onto the two seine poles. One improvement in this basic technique which usually results in the capture of more mosquitofish and less debris is that of allowing one seiner to proceed well ahead of the other so that an acute angle is formed by the seine in the direction of travel. This slanting of the seine permits the

seiners to maintain constant tension on the floatline and thus keep it on the surface and in a straight line. This technique generally promotes a larger harvest because the herded fish are less likely to swim around the forward seiner. Less mud and debris is picked up because the floatline is kept straight and taut which prevents the leadline from 'digging in' at the center of the seine. In addition, the seine is moved at an angle which tends to move debris off to one side rather than collect it.

If the water is shallow enough to permit wading, one person can tow from the shoreline and the other from a point offshore slightly less distant than the overall length of the seine. It is important to hold the seine poles correctly. To do so, one should grasp the pole so the leadline precedes the floatline to the extent that the netting 'bags' at its midpoint when it is pulled through the water. If the seine isn't 'bagged' properly the netting may get caught underneath and behind the leadline which may force fish beneath the seine when towing commences. It's important to maintain enough tension on the floatline to keep the top of the seine at or above the water surface throughout its length or fish may escape over it. Seine towing rates need not be rapid but should be kept at a fairly uniform speed. If one seiner is pulling the seine from a position offshore, they should maintain a position even with or ahead of the seiner onshore, funneling the fish into the seining area. If the source is narrow enough to stretch the seine completely across it, the seiners may proceed at just enough speed to herd the fish towards the capture point.

Rope bridles may be attached to one or both ends of the seine to aid in towing, especially if a pond is too deep to wade in or its banks too steep or high to walk along comfortably. A single rope may be connected to the outboard seine pole near its midpoint. A heavy lead sinker attached to the bottom of this pole at the leadline tie point can serve to maintain the seine in an upright position as it is towed by a single rope tied around the pole just above its center. A shorter length of rope may be tied to the nearshore seine pole at its top and bottom in a simple loop to permit the seiner to move further up the bank or perhaps on top of a pond levee. From this higher elevation, the nearshore seiner may be on more solid ground to conduct his towing while still maintaining proper pole position by manipulation of the rope looped around him. Floating cradles may also be constructed to hold the outboard seine pole in a vertical position; although this may just add to the gear the seiners have to carry to a fishing site.

When the seiners reach the shoreline where they intend to terminate the run, they should concentrate on maintaining the floatline above the water surface. This is especially critical because the fish soon realize they are trapped and usually try to go through or jump over the top of the netting. When bringing the seine shoreward, it is best to keep one seiner stationary at the shoreline and quickly pivot the other seiner around him in a wide arc until this puller also reaches the bank. Once both seiners are in shallow water very near the shoreline, they should work the floatline inshore at a constant rate while periodically bringing in just enough excess leadline so the fish can't escape underneath the seine. If the leadline is kept overly taut as the seine is being retrieved into the increasingly shallow water it may dig in and pick up undesirable debris from the bottom of the pond. When the floatline of the seine is retrieved to a point just a few feet from shore, one must raise the floatline just clear of the water surface and continue retrieving the leadline by skimming it along the mud bottom to the water's edge in order to capture most of the fish hiding there. Both seiners now pull back on their lines and raise the top and bottom edges of the seine well clear of the water. A third person in the water near the center of the seine should lift the leadline and floatline at this time to prevent any sagging there. Seiners shouldn't attempt to lift the entire body of the seine and the captured fish clear of the water nor should they concentrate them into too confining an area. This may unduly stress the fish, especially if the catch is large and the weather is very warm.

Once the fish are enclosed along the length of the seine one seiner should slowly work the fish towards the other seiner by raising his end of the netting and working inward. When the fish are adequately concentrated they may be easily taken from the seine. A third person may remove them with a hand net to the transfer buckets.

Motorized fishing craft may be employed to tow a seine. This operation takes skill and practice, but may be necessary in very large, deep impoundments such as some sewage stabilization ponds. Stable boats in lengths ranging from 10 to 14 feet (3 to 4.3 m) equipped with outboard motors having around ten horsepower are usually preferred for towing long seines. Special bridle assemblies should be constructed so that the boat can be steered with a seine in tow.

Traps. Trapping as a means to collect or sample fish stocks is a universal practice with almost an infinite variety of trap configurations in use today. Many traps used to collect mosquitofish are small in size and aren't employed to gather large numbers of

fish. However, some simple traps that consist of screened barricades placed completely across a ditch or stream are capable of congregating many fish if positioned below the outfall of a sizeable fish source such as a stocked rice field.

Portable traps range in size from those suitable for sampling a few minnows to large box traps capable of holding tens of thousands of mosquitofish. The simplest configuration in portable traps is a screened box or cylinder with a small one-way or small entry portal. This portal or gap is usually cone-shaped or in the form of a vertical V-slot. Traps may have single or multiple entry gaps at various locations. Most box traps have entrances at opposite ends and are open at the top or have a removable screened lid which permits easy access to captured fish.

Traps, being a passive capture technique, must depend on the fish coming to them in contrast to active pursuit methods such as seining. Traps can be modified to improve their effectiveness. Elongated wings may be fixed to the entry portal to guide fish from open waters towards the gap. Likewise, leaders extending perpendicularly away from the gap also serve to help guide fish into the trap. The V-shaped entry wings may be duplicated internally to make it more difficult for captured fish to find their way outside the trap.

Construction materials used in forming the framework of traps include: steel, wood, aluminum, wire, glass and various plastics. Hardware cloth can be obtained in many different mesh sizes to suit the need of the builder. Any materials selected should be resistant to the effects of prolonged exposure to water and weathering. If not, most can be protected by sealants and paints.

Trapping mosquitofish requires some knowledge of their behavior. Most mosquitofish prefer shallow shoreline areas; therefore catches will generally be greater if traps are concentrated in these habitats. If the trap has two opposing entry gaps, the trap should be placed such that if an imaginary line is drawn through these two openings, it will be parallel to the shoreline. Traps should probably not be wholly submerged as it is possible for the fish to suffocate in submerged traps under certain circumstances. In some nutrient-rich waters, there can be normally occurring diurnal fluctuations in dissolved oxygen with deficits so low that mosquitofish, when prevented from reaching the surface, may suffocate. Leaving some portion of the trap exposed to the air will usually alleviate the problem. Traps need not be left in the field for long periods of time as studies have shown that mosquitofish activity levels are at their peak at midday and trap catches correspond with their activity. Very few fish will enter a trap at night because the fish do not forage at that time to any great extent.

Traps should be placed out of public view to reduce vandalism and theft. Bird predators will enter and prey upon fish in traps if they are not covered with a screened lid or closure of some type. Snakes, frogs, and turtles will feed upon trapped fish if permitted to enter a trap. Other species of fish entering a trap may also prey extensively upon captured mosquitofish. Reducing the gap width may aid in excluding possible predators from mosquitofish traps.

Crowding and Grading Devices. In large holding tanks, it is usually easier to capture fish by first concentrating them at one end of the tank. Large rigidly-framed screens of hardware cloth or plastic screening make ideal crowding devices, especially when they conform exactly to the width and depth of the tank. When using these movable barriers, move the screen slowly and always leave enough space at the end for the concentrated fish to respire. Don't attempt to hold large numbers of fish in these crowded areas for long periods of time as waste metabolites accumulate and the resultant reduction in dissolved oxygen may rapidly induce severe stress and subsequent mortality. These movable barriers may be constructed with various mesh sizes; thus, fish stocks in holding tanks may be selectively graded.

Another type of grading device is manufactured especially for aquaculturists and consists of a floating chamber with metal bar grating which forms the bottom and ends of the container. The bars are uniformly spaced so fish above a certain size are retained while those smaller swim through into a holding tank or pond. When using these graders, avoid overloading the unit as fish stress will result if too many fish are held in the grader for extended periods. Aquaculture suppliers custom manufacture graders with any bar spacing specified by the purchaser.

Transfer Containers. Many different types of buckets or pails are use to transfer fishes. Translucent or white poly buckets in the 4 or 5 gallon (15.1 or 18.9 l) sizes have become increasingly popular. These plastic containers are used extensively by food suppliers to the restaurant trade and are usually locally available at very reasonable costs. It is possible to carry up to 25 pounds (11.4 kg.) of fish for short periods of time in one of these buckets. With mosquitofish, it is possible to carry this amount of fish in the bucket with no water as long as the transfer period does not exceed a couple of minutes. For longer periods, one should probably

reduce the load by one-third and add fresh, oxygenated water. When one notices the bucketed fish are becoming stressed for oxygen and are crowding themselves at the water surface, every effort should be made to either replace the water with fresh water or complete the transfer as rapidly as possible. Cooler source and transport water temperatures will usually allow one to extend the transfer period while warmer temperatures usually shorten this period. Cooler water can dissolve more oxygen than warmer water and the fishes metabolic rates are lower at lower temperatures. In the summer, high temperatures could decrease transfer times to the extent that less fish and more water must be bucketed to ensure survival.

Scales and Other Measuring Equipment. Field collection, transportation or distribution of fish usually necessitates the use of some sort of measuring device so fish quantities can be accurately determined. Particular applications will require equipment and techniques that provide appropriate levels of speed, accuracy, convenience, and economy. At this time, fisheries workers most often employ weight measurements to quantify fish stocks, although actual fish enumeration is sometimes performed. In the past, most vector control workers in California used common kitchen strainers in several sizes to enumerate fish. The worker would simply dip a strainer-full of fish, count or estimate the number of fish within and multiply this figure by the total number of strainers filled and thereby arrive at the total number of fish collected. Unfortunately, individual fish sizes and sex ratios can vary throughout the season to the extent that one may have from 200 to 2000 fish in a given strainer; thus the worker should actually count at least one strainer-full from each source every trip or estimation errors will be too easily compounded. This simple system, however, is still the least expensive and may be suitable for those workers using comparatively few fish in their programs.

A much more accurate method is that of using biomass (live weight) measurements exclusively. One should select a scale on the basis of its intended application. Most fisheries technicians use three-revolution biologist's, milk or dairy scales, plus buckets to weigh larger numbers of fish. Dairy scales can be purchased in several appropriate weight capacities ranging from 25 to 60 pounds (11.3 to 27.2 kg). For rough field work, translucent plastic buckets can be marked along their sides at even weight intervals which correspond to known weights of fish; thus a displacement system of sorts is created thereby reducing the time spent weighing with scales.

Balances are generally not used in the field as they are too fragile and slow to operate. They may be put to better use in laboratories where individual fish measurements are more often made.

TRANSPORTATION EQUIPMENT AND METHODS

Mosquitofish and other small fishes present some rather unique problems in their safe transport. Conventional live hauling systems employed by state and federal fisheries departments are usually designed for larger "clean" fishes; that is, those fishes that haven't been fed intentionally for 24 to 48 hours prior to transportation. This procedure is very beneficial to fishes because the amount of digestive matter that the stressed fish would normally release by defecation and regurgitation into the transport water is substantially reduced. If the transport water becomes contaminated with this organic matter, which has a high demand for dissolved oxygen, it may be very difficult to maintain enough oxygen in the transport water to sustain the fish for the required travel time.

Unfortunately, it usually isn't convenient or practical to starve large numbers of mosquitofish at remote collection sites prior to transportation. Consequently, some altered procedures have been adopted in order to transport these "dirty" mosquitofish.

The quantity of fish that can be transported safely is basically dependent upon the condition of the fish, physical handling and water quality. If the fish aren't greatly stressed in their capture, loading and movement, resultant survival rates will usually be quite good. Conditions that produce stress are: rough handling of fish in nets and seines, slow transfer of fish from the seine to the tank, temperature shocks, and transport tank water sloshing. Relevant factors which influence the capacity of any live-hauling system are listed as follows:

1) water volume capacity of the transport tank
2) tank water temperature and any changes enroute
3) temperature of the water at the fish source
4) ambient air temperatures encountered enroute
5) condition, sex and size of the fish collected
6) seining and other handling methods used
7) dissolved oxygen content of the transport water maintained enroute, and other chemical changes to water quality
8) cleanliness of transport water maintained enroute
9) total time or distance involved in transport
10) functional capacity and reliability of the aeration system
11) quality of roads driven
12) the time of year, with respect to all environmental factors

With so many important elements involved, the best way to arrive at a safe hauling capacity is to thoroughly test each transport system by starting with a relatively small number of fish and slowly increase loading each trip until early signs of stress or mortality are observed. Male mosquitofish will usually begin to suffer first, so by keeping a close watch on male mortality one is likely to gain some measure of induced stress. Generally speaking, most well designed transportation systems will be capable of handling fish loads ranging from 0.5 to 1.5 lbs of fish per gallon (60 to 159 grams per liter) of transport water. For very short hauling distances or with "clean" fish, it may be possible to increase these figures a bit; but one should do so with extreme care.

Tanks. Numerous types of containers can be used to transport mosquitofish and other small fishes. It is crucial that any tank used be rigid enough to withstand the water pressures involved, be relatively nontoxic to fishes, and be watertight. Tank configuration is also important if one is to maximize hauling capacities. Unless pure oxygenation is used, a tank should be obtained or built with as great a surface area to volume ratio as is practicable. This factor aids in the exchange of gases at the air-water interface and thereby supplements aeration. In other words, a long, wide rectangular tank would be able to support more fish than would a barrel or drum of equal volume if supplemental aeration wasn't provided. Any tank used should be filled to near capacity to provide as much volume as possible and to minimize enroute sloshing or surging. Interior baffles may be inserted to reduce sloshing; but should be removable to facilitate fish access and emptying.

Transport tanks can be constructed of aluminum, steel, plywood, plastic or fiberglass. Rectangular tanks are often built of resin coated plywood because of simple construction and efficient space utilization. Bait fish dealers most often employ treated plywood or fiberglass tanks which are equipped with recessed lids. Usually two opposing lids are fastened with hinges to a fixed center support which is both rigid and wide enough to accommodate aeration gear, such as agitators or air line. The lids are recessed to permit water that is sloshed outside to flow back into the tank instead of being spilled. Tanks made of 1/2 to 1 inch (13 to 25 mm) plywood in

exterior or marine grades should receive plastic resin or epoxy coatings on all surfaces. White exterior finishes provide added solar insulation with resultant cooler tank water temperatures; while interior finishes of dark colors seem to impart a pacifying effect on mosquitofish. Exposed tank edges should be reinforced with lengths of aluminum or mild steel angle. Metal covered runners can be placed on the bottom of the tank to facilitate loading and unloading. These runners will elevate the tank just enough so a bottom drain can be installed.

Commercially manufactured bait fish tanks of molded fiberglass are now available from several firms and are provided in various sizes and configurations. Some of these tanks feature double walls with interposed insulation to help them maintain constant water temperatures.

Agitators. The most common aeration method used to commercially transport small bait fishes is that of water agitation. An agitator is simply a 12-volt, direct current motor suspended above the tank with a partially submerged paddle which revolves at several thousand rpm. The paddle is isolated from the fish by means of a cylindrical basket formed of metal or plastic screening. The spinning paddle creates a disturbed mixing zone for air diffusion to take place and the aerated water is then propelled away from the paddles through the basket screening and around the tank. Agitation works quite well for mosquitofish if special care is taken to ensure that the basket mesh is fine enough to keep small fish from entering and being chopped up by the whirling paddle. Plastic window screening wrapped around an agitator basket will eliminate this problem.

Another method of using this system is to distribute all fish to be transported in covered, perforated plastic or metal buckets immersed in the agitated tank water. This can speed up removal of known quantities of fish from the tank at each delivery point or stocking location.

Multiple agitator installations are preferred for larger tanks; however, they do consume a lot of electrical energy. Dual charging and storage battery systems will reduce the possibility of having a stalled vehicle and dead fish because of a discharged battery.

Another type of agitator, instead of having a simple paddle, consists of a hollow metal tube with an enclosed shaft which is connected to what looks like a tiny squirrel-cage fan blade. This fan is enclosed in metal screening and is wholly submerged in the tank water. In operation, the rotating fan creates suction in the tube it is connected to and air is drawn from the atmosphere and spread into the tank by the fan. These units appear to work well and may be slightly better for use with mosquitofish because they don't create as much current flow that often fatigues the fish.

Agitators are presently manufactured for the fish farming industry for use in transport vehicles as described above; but they are also available in a variety of 110-volt, alternating current models for holding tank installations.

Compressed Air.- A basic way of aerating water to maintain satisfactory levels of dissolved oxygen is to simply pump air into the water by means of an air compressor, tubing and a weighted bubbler or diffuser. Electrically operated air pumps can be easily adapted for vehicular use. Some automotive fuel pumps will adequately serve as air pumps and can be powered directly from a truck's 12-volt electrical system. For very small transport systems this type of aeration source may suffice. These pumps are manufactured to pump liquid fuel so it is advisable to obtain one whose seals and diaphragms will also adequately pump air. It is advisable to periodically inspect and lubricate these pumps to keep their rubber parts from becoming weather checked and leaky. Silicone lubricants will protect the seals and help keep them pliable.

Vibrator aquarium pumps may also be used in conjunction with a mobile power inverter unit which converts the truck's 13.8-volt, direct current to 110-volt, alternate (square-wave or sinusoidal) current. It is important that only vibrator type air pumps be used with square-wave power inverters because many motor driven pumps require sine-wave alternating current. It is also crucially important that the power inverter selected has a high enough reserve wattage rating to satisfy the power demands of the pump or pumps.

Bubblers or diffusers should be chosen on the basis of their size, configuration and porosity with respect to their employment in a transport tank. Aeration is best achieved when a diffuser emits many tiny bubbles rather than when it discharges few, but large bubbles. Gentle aeration is more desirable for use with mosquitofish than is vigorous aeration because currents generated by the excessive bubbling can quickly fatigue the fish with subsequent high mortality. Several ganged bubblers may be spread about to provide more even aeration throughout the tank. These individual bubblers can be connected to the air supply through the use of a manifold. New elongated or linear diffusers in plastic, rubber or nylon are now available. They emit tiny bubbles throughout their length, that results in gentle even aeration of the tank water. Inline aquarium valves

may be used to control air flow from the pump or to bleed off excess air.

Compressed Oxygen. Pure, compressed oxygen is finding ever increasing use in the transport of large numbers of live fishes. It is already a common practice in the southern United States to employ compressed or liquid oxygen aeration systems in very large transport vehicles. Undoubtedly, this is the most direct way of maintaining high dissolved oxygen levels in transport waters. It is also one of the most reliable methods used in fish transportation.

Unfortunately, it also has the potential of being an extremely hazardous technique because the source of oxygen is highly pressurized. If a vessel or its valve was to rupture accidentally, the transport vehicle as well as its occupants would most likely be destroyed. Special safety precautions must be followed to prevent an accident.

Basically, a compressed oxygen delivery system consists of a compressed oxygen vessel (cylinder), a medical (or any suitable low flow) oxygen regulator, connecting tubing and a weighted diffuser. In operation, sufficient oxygenation takes place with very gentle bubbling to maintain or even surpass normal saturation levels of dissolved oxygen for a given water temperature. The strong currents typically generated with compressed air or agitation equipment are virtually eliminated; thus mosquitofish don't tire as quickly.

In fact, fish stressed during seining and showing signs of lethargy as they are placed into an oxygen enriched transport tank are often observed to be much more vigorous after several hours of transportation. Pure oxygen aeration is clearly superior to other systems when high densities of fish are to be transported long distances in hot weather. Oxygen use is quite economical as one 750-gallon (2839-liter) bottle can typically supply oxygen for 24 to 48 hours depending upon the flow rate selected. Industrial or medical oxygen may be used and compressed gas dealers will either lease their bottles or refill privately-owned cylinders.

The only disadvantage of pure oxygen systems is their potential danger. One should not attempt to build this type of aeration system without expert advice. Compressed oxygen together with any petroleum or oil-based fuel is unbelievably combustible. Mere contact with animal, vegetable and mineral oils, fuels or greases can result in explosive spontaneous combustion. Oxygen enriched atmospheres also support the rapid combustion of materials one would normally consider to have a low flammability potential. Careless smokers have had their cigarettes turn instantly into torches in their mouth and their clothing burned away just as easily.

Cleaning of any oxygen equipment regulators and connectors should be done only with a absolutely clean, dry cloth or facial tissue which will be discarded after a single use. Any lubrication of oxygen equipment is strictly prohibited and highly dangerous. Technicians shouldn't even touch the exposed inlet gland of a regulator with bare hands because enough oily secretions may be present to support spontaneous combustion when the regulator is connected to a high pressure vessel and its valve is opened.

Oil contaminated regulators should be returned to the manufacturer where they may, at the discretion of the firm, be rebuilt. Fisheries technicians should not attempt to decontaminate any regulators themselves. Local compressed gas dealers are also very reluctant to perform any decontamination work, but may provide addresses of the regulator companies along with names of firm representatives.

Handling or utilizing oxygen equipment requires strict adherence to the following safety precautions:

1) Allow only fully trained personnel to operate, adjust or maintain oxygen equipment.
2) Store compressed oxygen vessels (cylinders) in a sheltered upright position and secure them with a chain.
3) Never place or store any oils or fuels near oxygen equipment.
4) Don't smoke or permit any form of combustion near oxygen equipment.
5) Secure compressed oxygen vessels securely so they can't shift enroute.
6) Protect the attached regulator from falling or moving objects. A cage may be constructed especially for this purpose.
7) Never transport, carry or move a cylinder without its protective cap in place.
8) Treat regulators with special care and keep them scrupulously clean.
9) Don't refuel vehicles until the oxygen system is temporarily turned off.
10) Don't allow heavy objects to rest upon aeration tubing.
11) Always leave some pressure in a depleted vessel. An open valve on an empty vessel can permit entry of moisturized air which will cause rust and thus greatly shorten its life.
12) Shelter filled vessels from direct sunlight or high ambient temperatures. Excessive pressure can develop in hot vessels which may exceed the tolerance limit for the integral pressure relief disks. If they rupture all oxygen will be released immediately with much force and noise.

13) All pressure vessels must receive periodic hydrostatic inspection. Any vessels that have been exposed to intense heat, as in a fire, should be either rejected or sent to a hydrostatics inspection facility for an unscheduled quality test.
14) Oxygen systems must be constructed and maintained in accordance with all applicable state and federal statutes.

Despite the seemingly negative picture oxygen systems impart with all the foregoing cautionary statements, more and more agencies have adopted them because of their simplicity, capacity and reliability. Numerous commercial aquaculture supply houses now offer complete pure oxygen diffuser systems and can provide installation, use and maintenance information to the customer.

Recirculation Sprayers. The state and federal agencies most often employ gasoline or electrically powered water pumps that collect water from a screened off segment of the transport tank and return it as a spray against the internal ceiling and upper sides of the tank. Atmospheric air is thus mixed with the water and this aerated water trickles back down the inside walls of the tank. This system of aeration works very well with larger, fast swimming fishes; however, in actual experiences with unstarved mosquitofish, the pump collection intakes had to be heavily filtered to screen out detritus and the small or weak fish that invariably plugged up the intake screens. These large filters tended to become saturated with various organic materials and without frequent backflushing and cleaning the spray volumes were soon reduced to an unsatisfactory level. Another drawback was that the small pump engines have not been too reliable. Electric motors might be preferable; further experimentation is needed, however, to verify this suggestion.

Another related system that is infrequently used in fish transport is that of injecting atmospheric air into the water through the action of a venturi device. Water pumped from the tank as described above is routed through a special venturi unit and its atmospherically aerated discharge is returned to the tank. This system works well with salmonids and other fish which prefer to orient themselves against a strong water current. Mosquitofish are comparatively weak swimmers so these devices tend to fatigue them unless the discharge is sprayed against the tank's ceiling, as with the foregoing system.

The same sort of pump filtration problems encountered with a recirculation spray system would also play an important role in the success of this aeration method. Venturi aeration systems are presently being used by fish farmers in holding tanks and in the emergency aeration of small ponds.

ANESTHETICS AND OTHER TRANSPORT AGENTS

Ordinary block ice has been the traditional pacifying agent used in mosquitofish transportation. It is usually added in bulk to the tank water during hot weather to reduce the water temperature. It may also be added enroute to bring down temperatures that have increased during actual hauling.

Ice serves two functions. First, it lowers the metabolic rate of the fishes, and second, the dissolved oxygen saturation point may be increased somewhat, which permits more oxygen to be diffused. Ice is often available enroute and can be placed in a secured perforated bucket or in some other device to limit its movement inside the tank. Heavy blocks of ice if freed could crush fish and damage aeration equipment.

It is generally more desirable to transport mosquitofish in cooler (<80°F) waters rather than in warmer waters (>85°F). Slowly melting blocks of ice change tank water temperatures slowly, thereby reducing the likelihood of temperature shock. When transferring cooled fishes from the tank at the final destination, avoid stocking them into waters having greatly different temperatures. A technician may have to search out deeper receiving waters in order to secure somewhat cooler temperatures. If much warmer receiving waters are anticipated and the trip duration is relatively lengthy; it may be practical to curtail the use of ice early on and let the tank water slowly warm enroute, thus minimizing temperature differences at stocking.

The second most common material added to fish transport water is regular salt. Sodium chloride, as common table or rock salt form affords some anesthetic value and its use reduces slime production and "hardens" the skin of the fish when dissolved in transport water. (Dupree and Huner 1984). The well known antiseptic (cauterizing) action of salt help heal minor injuries to the fishes skin; thus secondary infections so common in mosquitofish are lessened. The ideal concentration for mosquitofish appears to be around 0.2 - 0.3%.

Another commonly employed "skin hardening" chemical is calcium chloride, which is used primarily in situations where inadequate water hardness is encountered. Chemical agents, such as "tris" [Tris (hydroxymethyl) -1,3,propanediol] buffer used at 5-10 g/gallon or sodium bicarbonate dissolved into the tank water at one part per thousand can help reduce upward pH shifts which may increase ammonia toxicity.

Antifoam compounds, available from most aquaculturists supply firms aid in gaseous exchange at the water surface by reducing surface foam and scum. Care must be exercised when purchasing antifoam chemicals, as many used in agricultural herbicide and insecticide applications are toxic to fish. The newest chemical transport agents are pre-packaged commercial mixtures of ammonia reducers, antibiotics, pH buffers and skin protectants. Although most of these products aren't registered for food fish use, they can be used legally and economically to afford added protection to transported mosquitofish.

Tranquilizing Chemicals.- Chemical tranquilizing agents or anesthetics haven't been thoroughly investigated specifically for mosquitofish; however, in one unpublished study, four chemicals were statically tested over a 4-hour period with promising results (Sjogren, pers. comm.). Unfortunately, only two of these compounds (MS-222^{TM}, and quinaldine) are presently registered and sold through aquacultural suppliers. MS-222^{TM} (Tricaine methanesulfonate) in 70°F tank water at a concentration of 35-45 ppm and quinaldine at 12-20 ppm did increase respiration rates somewhat in mosquitofish, but imparted demonstrable anesthesia as well. For MS-222^{TM} to be effective, buffering of the tank water to pH 7-8 is required (Dupree and Huner, 1984).

Other proprietary tranquilizers are now commercially available for non-food fish use and may be worthy of investigation as mosquitofish anesthetics. Lastly, carbonic acid (bottled soda water) has been employed as a cheap substitute anesthetic, but is tricky to use correctly as carbonation varies from brand to brand and carbon dioxide concentrations in the transport water are not easily stabilized at narcotizing levels.

MOSQUITOFISH APPLICATIONS

Types of Sources Stocked. Mosquitofish have been used in a wide variety of sources to control many different species of mosquito. Sources into which mosquitofish have been successfully introduced include: containers, creeks, dairy ponds, ditches (irrigation, roadside, etc.), drains, duck clubs, flood control basins, flood control channels, gutters, marshes, ponds (golf course, industrial, natural, ornamental, sewage, etc.), rice fields, sloughs, stand pipes, storm drains, sumps, swimming pools, tail ditches (pasture, alfalfa, etc.), vegetable gardens, water troughs, etc.

Mosquitofish are hardy creatures which can withstand wide variations in temperature, dissolved oxygen, light intensity, turbidity, and other water quality parameters. Their small size renders them subject to predation by larger fish and piscivorous birds. This must be taken into account when deciding whether or not to stock fish. Conversely, their hardiness and predatory efficiency make them effective predators or competitors of other species. Careful evaluation must be made to insure that mosquitofish are not introduced into areas where their presence might be a detriment to rare, endangered, or sensitive species.

Stocking Rates For Mosquito Control. There are probably as many different but appropriate stocking rates as there are types of mosquito sources. Exact stocking rate figures are impractical, if not impossible to itemize because a rate that works well in one particular mosquito source may be either too low or too high for an identical source in a different California locale. In addition, when one takes into account variations in weather conditions and other environmental factors, appropriate stocking rates may change for the same source from one year to the next.

Technicians must consider the species of mosquito they are intending to control with fish. Mosquitofish applied in one source that produces two species of mosquito may not provide the same level of control for both species. Therefore, it is usually necessary, in such instances, to stock for the mosquito species requiring the greatest number of fish even if this means one might be overstocking with regard to the other species present. Another factor that must be considered is whether or not the stocking is for immediate larval control or for the eventual control of mosquito populations peaking later in the summer. Immediate control stocking rates are usually much higher than the rates required for eventual control. When stocking for eventual control, one generally assumes that the fish will reproduce in that source to the extent that subsequent production of fish will coincide with the production of mosquitoes; thus initial stocking rates can be significantly lower. When an attempt is made to coordinate the fish population in a source with a mosquito breeding period peaking later in the summer, one must give the fish adequate time to reproduce, so that enough fish are produced to provide the desired control. It is therefore crucial that the fish be stocked sufficiently early.

When stocking for perennial control in permanent bodies of water, enough fish must be applied to ensure survival and reproduction. Biologically speaking, it is possible to stock too few fish to guarantee survival of that population. Conversely, it is also undesirable to overstock a source because the habitat may not be able to support that quantity of fish for an extended period of time. Stresses due to over-crowding of the fish may result in disease and mortality.

Stocking rate studies for mosquitofish in rice fields have been going on for many years. However, research concerning optimum stocking rates in other mosquito sources is generally lacking. Hoy and Reed (1970) found no significant differences in control in rice paddies stocked at 200 and 1000 fish per acre (0.4 to 2.0 pounds per acre - 500 adult female mosquitofish equal approximately one pound in most California field situations). Further research (Hoy and Reed 1971) indicated no significant differences in control in fields stocked at 100 and 200 fish per acre (0.2 to 0.4 pounds per acre).

Most mosquito control agencies in California currently stock rice fields at rates of 0.2 to 0.6 pounds per acre. The lower stocking rates may require supplemental pesticide applications early in the season, but maintain good control in July and August.

Stocking date is an important factor when mosquitofish are used. Farley and Younce (1977) found that stocking fish 15 to 25 days after rice seeding gave the best population growth and mosquito control when compared with earlier and later stocking dates. Fish stocked too early find little food in the newly flooded fields, and may leave the fields through the

outlet boxes. Stocking fields more than 30 days after seeding requires higher stocking rates to achieve adequate fish populations later in the season.

The method of stocking fish in rice fields is also important in subsequent mosquito control. Stocking in one spot within a field is the most economical method of planting fish; however, it has been found that mosquitofish do not adequately distribute themselves throughout the field when stocked by this method (Farley and Younce 1978). Stocking fish in every paddy would give the best season-long distribution, but this method is time-consuming. A good compromise is to divide the total field allotment of fish into three or four equally spaced areas of the field beginning with the second paddy from the inlet.

When stocking fish, water temperatures in the fish holding tank and the source to be stocked should ideally be the same. Thermal shock caused by rapid temperature changes can be lethal to fish, though mosquitofish show a remarkable ability to withstand temperature shocks that would kill other fish species. Stocking should never be done when the temperature of the source nears 100°F (38°C). Care must also be taken that no herbicides or other chemicals harmful to the fish are present in the source at the time of stocking.

Some basic guidelines regarding stocking rates are essential. The following table lists selected stocking rates currently employed throughout California, and was established by technicians in several locales to control various mosquito species in different source habitats. These recommended rates are intended solely to provide the technician with baseline information. Actual experience with individual sources should result in the development of optimal stocking rates.

Table 2. Typical mosquitofish stocking rates for various mosquitoes in selected habitats.

Mosquito Species	Common Habitat	Stocking Rate[a] lbs/A,(kg/ha)	Stocking Date[b]
Aedes			
dorsalis	tidal marshes, pastures	0.5(0.56)	April-June
melanimon	pastures, sloughs	0.5(0.56)	April-June
nigromaculis	pastures	0.5(0.56)	April-June
vexans	woodland ponds, sloughs	0.5-1.0(0.56-1.12)	Summer months
Anopheles			
freeborni	rice fields	0.2-0.5(0.22-0.56)	April-June
	drains, woodland ponds	0.5(0.56)	April-July
Culex			
erythrothorax	marshes, ponds	0.5(0.56)	April-Sept
pipiens	stagnant waters	0.5-1.5(0.56-1.68)	April-Sept
stigmatosoma	polluted waters	0.5-1.5(0.56-1.68)	April-Sept
tarsalis	most habitats	0.5-1.5(0.56-1.68)	April-Sept
	rice fields	0.2-0.6(0.22-0.67)	April-May
Culiseta			
incidens	shaded cool waters	0.5(0.56)	March-June
inornata	sunlit cool waters	0.5(0.56)	Nov-April

[a]Where a wide range of stocking rates is given, the amount stocked depends upon the date and severity of the mosquito problem. Stocking at later dates and/or stocking for immediate control necessitates higher stocking rates.

[b]The range of stocking dates is variable as different locales within California can have the same mosquito species breeding at different times.

PARASITES AND PATHOGENS

Introduction. The prodigious diversity of parasitic forms is overwhelmingly evident with even the smallest of samples. The more diverse the environmental conditions, the greater the number of forms that can fashion a favorable existence for themselves. The remarkably variable environment of the host body and the complexity of the relationship between host and parasite has led to some of the most extensive radiations in the evolution of extant life forms. The parasites and pathogens of mosquitofish are no exception. The mosquitofish, *Gambusia affinis* (Baird and Girard), is host to an impressive array of specific parasites and pathogenic microorganisms, along with the normal complement of nonspecific opportunistic parasites. In the mosquitofish it is evident that parasite-host relationships have a long and complex history which has stabilized itself through natural ecological and energetic feedback systems.

In natural environments, parasite-host relationships are frequently stable. That is, the hosts may suffer little or no ill effects from infestation of the parasite. In most cases, this is a factor of host exposure to numbers of the parasite or pathogen. In the natural environment, a host species may not come into contact with large numbers of any one parasite or pathogen species and therefore will suffer few or no pathogenic effects. In other situations, the host and parasite have existed together so long that they have coevolved a balanced relationship where the parasite can exist in relative large numbers in the host without the host suffering any severe pathogenic effects. In these situations, however, if the parasite numbers become too great, the balance between host health and illness becomes fine. Any further stress to this host will cause illness even if the effects are not directly caused by the infecting parasite.

In artificial environments, such as that which fish are subjected to in normal aquaculture practices, the fine balance which existed between host and parasite is disrupted. Because of the confined character of most aquaculture facilities, fish will be exposed to abnormally high concentrations of parasites and pathogens. Equally important, the infected fish will more frequently contact healthy fish, thereby increasing the exposure time for infection. Secondarily, the added stress of handling, crowding, poor water quality, and unstable water temperatures will further interfere with the balance of host and parasite, producing rapid spread of disease, severe pathogenic effects and ultimately enormous die-off. There are many parasitic organisms which are opportunistic and will only get a successful hold on a host when it becomes weakened or sick. These types generally do not coexist with the host, and in most cases, are the ultimate cause of host mortality.

Bacterial and protozoan infections generally do not follow the normal parasite-host pattern. The reproductive time of microorganisms is very short relative to that of their host. Therefore, when fish are exposed to bacteria, their defense lies in the ability to resist infection. In natural populations of healthy fish, exposure to bacteria and protozoan pathogens is generally low, and resistance to those contacted is fairly easy for a healthy immune system. In the confined environment of an aquacultural system, fish are repeatedly exposed to the pathogen over a very short period of time. The natural immune system of the fish is overwhelmed and in most cases fish succumb to the infections and die. The mosquitofish is known to be host to at least twenty-six species of helminthes and innumerable varieties of fungi, protozoans and bacteria. This great diversity of parasites in mosquitofish has much to do with their choice of habitat and position in the food web. The mosquitofish are omnivorous, eating a vast array of larval and adult insects and most varieties of microcrustaceans. In turn, mosquitofish are eaten by a large group of piscivorous organisms including larger fish, turtles and most importantly, a significant number of aquatic birds. This place in the ecological food web puts them in an optimum position to be an intermediate host for a variety of parasites using piscivorous animals as definitive hosts.

Most pathogenic diseases, including parasitic infections, demonstrate their presence through some external morphological signal. These signs are characteristic of the specific disease agent and can manifest in behavioral changes, or morphological degradation of infected fish. Reading the signals in a fish population can help in determining the extent of the epizootic, and the effectiveness of the control method. It is the fishery biologists' task to recognize the early signs of infection, and take

appropriate action to maintain the health of the population.

Monogenetic Trematodes. Monogenea are microscopic flatworms which specifically parasitize freshwater and marine fishes. This group of worms include only one host within their life cycle, a characteristic implied by their name. All monogenes attach to their host externally, either on the epidermis, fin tissue, or on gill lamellae. Their location on the host is often a characteristic of the particular parasite species, and is sometimes specific enough to be diagnostic. These parasites feed on fish mucus, epithelial cells and, occasionally, directly upon blood. In small numbers on fish, they usually cause little or no harm. However, with larger populations, their destruction of the protective mucus coat or the interference of gill operation can reduce fish health and increase susceptibility to other infections.

Fish parasitized with monogenetic trematodes demonstrate some characteristic behaviors which can be used to diagnose the infection. In the early stages of infection, fish will be seen resting on the bottom of pools along the shoreline. Fish with more advanced numbers of gill flukes will be seen gulping air at the surface. With large numbers of epithelial flukes, fish may be seen scraping themselves on rocks. In the final stages of infection, mucus can be seen on the gills or skin with ulcerations and hemorrhages. Gills with heavy infections will produce streams of mucus and blood.

All species of monogenetic trematodes are equipped with sets of attachment hooks and anchors called haptors. These haptors are characteristically positioned in a posterior body region called the opithohaptor. Anteriorly, there are two or four lobed glands, a sucking pharynx, and occasionally, eyespots.

Two major families of monogenetic trematodes are found infecting mosquitofish in natural and artificial environments. One genus, *Gyrodactylus* Nordmann, in the family Gyrodactylidae, is found exclusively on the epidermis and fins of mosquitofish. Three genera, *Dactylogyrus* Diesing, *Cleidodiscus* Mueller and *Urocleidus* Mueller, in the family Dactylogyridae, are all found infecting the gill lamellae.

The major difference between the two families has to do with their mode of reproduction. Dactylogyridae is oviparous, producing and releasing thousands of eggs into the environment. Gyrodactylidae is viviparous, giving birth to live young. One Gyrodactylidae egg produces four identical embryos, one inside the other in a Chinese box fashion. This type of reproduction is referred to as serial polyembryony. The fourth embryo of each series produces a new egg, which again develops four embryos. Both these modes of reproduction reflect the natural history of the parasite and are the most effective means of reproduction and dispersion for that life style.

The genus *Gyrodactylus* is a very important ectoparasite on North American fishes, however, it has received little attention relative to that in other countries which practice aquaculture. Because of this, only 31 species are presently described from North American fishes (Hoffman 1967). There are likely many more species yet to be described as *Gyrodactylus* is very host specific, each species parasitizing only one species of fish. One species has been described from mosquitofish, *G. gambusiae* (Rogers and Wellborn 1965), and will be the representative for this description and discussion.

The type specimen of *Gyrodactylus gambusiae* was collected from a mosquitofish in the Welaka National Fish Hatchery in Florida. It fits the basic morphology of most *Gyrodactylus* in that it has a bilobed anterior end with a structure known as a head organ in each lobe. Posteriorly, it contains one pair of large anchors in its haptor separated by a cross bar. At the margin of the haptor are sixteen small hooklets. Internally, a bifurcated intestine is clearly distinguished. Often centrally located is the ovary which is lobed and with at least one embryo. The testes are located just anterior to the ovary. Also visible is the male copulatory organ, the cirrus, consisting of a row of minute spines. Unlike other monogene genera, *Gyrodactylus* has no eye spots. Differences among the genera are determined by variation in size and shape of the above structures.

Infestation of mosquitofish with *Gyrodactylus* is strongly associated with environmental conditions and seasonal influences. Oxygen depletion seems to be the most common impetus for an infestation. Occasional body contact associated with overcrowding acts as a contagion in spreading the parasite among the fish population. *Gyrodactylus* is most frequently encountered in the late winter and early spring after environmental conditions have been the hardest on fishes. Because *Gyrodactylus* is generally host specific, mosquitofish found with an infestation are likely being parasitized by either *G. gambusiae* or *G. elegans,* which is less specific. Any infection level of fifteen or more worms per fish should be considered life threatening to the fish.

The second family of monogenetic trematodes, *Dactylogyridae,* contains at least three genera which have been identified infesting mosquitofish. Each type feeds exclusively on gill lamellae and are

often found together on the same fish.

The most common of the three is *Dactylogyrus,* which can be easily recognized in two ways. First, *Dactylogyrus* have four easily distinguishable eye spots at the anterior end of their body, which is always four lobed. Second, the opithohaptor, at the extreme posterior of the fluke, contains one set of anchors with one connecting rod or cuticular bar between them. Around the margin of the opithohaptor are sixteen equally spaced hooklets. A nonspecific parasitizer of many types of fish is *Dactylogyrus anchoratus* (Dujardin, Wagener). This species is commonly collected on most pond and stream inhabiting fishes, including the mosquitofish.

The life cycle of *Dactylogyrus anchoratus* is one of the simplest among monogenetic trematodes. As noted by Bychowsky (1961), during the warmer summer months (at 24 - 28°C), there exists a long period of egg deposition when eggs fall to the bottom of the pond or stream and eventually hatch. At low temperatures (4 - 8°C), the eggs do not hatch and remain on the bottom until the following spring. With optimum summer temperatures, the free swimming larvae will emerge in 4-6 days. The larvae come into contact with the fish when passing with water across the gill lamellae. Here the fluke grows to adulthood and will remain throughout its life. The fluke is capable of independent movement throughout the gill rakes of the fish; grasping tissue with its hooklets and anchors. The fluke will extend its body outward, touch down in a new area, then draw its posterior end with it. The opithohaptor opens and closes very much like a hand, and can securely grasp the tissue. Using its strong sucking pharynx, it rasps tissue from the gill lamellae thereby exposing the capillaries just below the surface. Blood is then sucked into the mouth along with cells from the gill tissue. Ulcerations on the gills soon develop and a thick mucus is liberated in response to the irritation caused by the flukes. The adults die in the fall and are completely absent during the cold winter months.

According to Bychowsky (1961), the life span of adult *Dactylogyrus* ranges from ten to twenty-five days during optimum temperatures. During a twenty-four hour period, an adult may deposit between four and ten eggs per hour. The average adult will deposit approximately 3,000 eggs during its life span. Eggs deposited early in the season quickly emerge as larvae, reach adulthood, and can be depositing their own eggs within a week. Such fecundity rates can quickly lead to epizootics in the fishery pond environment. Again, infection levels of over fifteen per fish are considered detrimental.

Another common Dactylogyridae trematode infecting mosquitofish is *Cleidodiscus sp..* This fluke can be distinguished from *Dactylogyrus sp.* by inspection of the opithohaptor and counting the number of hooklets and anchors. *Cleidodiscus sp.* has two sets of anchors and two cuticular bars while the opithohaptor contains seven pairs of hooklets. The head region of *Cleidodiscus sp.* has four eyespots with both sets being roughly similar in size. The anterior set is quite large and obvious, while the posterior set in most specimens, is slightly smaller. The head is four lobed, with a gland in each lobe. The head is followed directly by a large muscular pharynx. Intestinal caecae are obvious on both lateral margins. In most living specimens, the testes and ovaries are apparent. Most of the body region is filled with small spherical bodies called vitellaria. Posteriorly, muscles controlling the opithohaptor movement are visible. The anchor consists of a point, a shaft and a root. The root in *Cleidodiscus sp.* is bifurcated into outer and inner branches. Between the outer and the inner branch floats the cuticular bar. The bar is not attached to the anchors.

As with *Dactylogyrus, Cleidodiscus* moves freely about the gill lamellae. However, this fluke seems to prefer the outer margins of the lamellae. It can often be seen attached at the distal-most portion of the lamellae, waving about and contracting back and forth. Most *Cleidodiscus* adults are larger than *Dactylogyrus,* being usually wider than longer.

The life cycle of *Cleidodiscus* is very similar to that of *Dactylogyrus.* However, it seems to be more common in the early spring and less frequent in the late fall. In contrast, Dactylogyrus appears to emerge later in the spring and persist longer into the fall.

The third genus of monogenetic trematodes commonly associated with the mosquitofish is *Urocleidus* (Mueller). The structure of this fluke is very similar to that of *Cleidodiscus.* The eyes, intestine, gonads, vitellaria and haptorial bars are all the same. This fluke is equipped with two pairs of anchors and seven pairs of opithohaptoral hooks, just as with *Cleidodiscus.* The differences lie in the arrangement of the hooklets within the opithohaptor, and in the shape and size of the anchors. *Urocleidus* has all its hooklets bunched at the proximal region of the opithohaptor rather than evenly spaced throughout the margin. The anchors are much larger, more robust, more deeply curved, and with less bifurcation at the proximal end. Another distinction between the two flukes is the eye spots. Both genera have two pairs in the head region, but *Urocleidus* has the posterior pair much larger than

the anterior pair, just opposite of *Cleidodiscus*. The vitellaria bodies in *Urocleidus* are well developed and occur in lateral bands. The anchors are slightly dissimilar in size. One species, *Urocleidus seculus* Mizelle and Arcadi, was first reported from mosquitofish in California in 1945. The life cycle of *Urocleidus* is much the same as *Cleidodiscus* and *Dactylogyrus*.

Regardless of the species, the treatment is the same. In large ponds, an infestation is very difficult to treat. The most cost-efficient method consists of adding fish-tolerant levels of sodium chloride to the infected ponds. This, however, can destroy the planktonic food supply or stress young fish. In smaller tanks, or before incoming fish are added to established healthy populations, baths of potassium permanganate (0.2% for five minutes) have proven very successful. Another effective agent is Masoten at 20 g/l for five minutes (Stickney 1986).

Digenetic Trematodes. This group of trematodes contain two or sometimes three hosts within their life cycle. However, there is a great deal of variation in the number of stages and the duration of the life cycle. Generally, the fish or another vertebrate is the primary host, while a gastropod mollusc is an intermediate host. In some cases, the fish may be the intermediate host while a larger piscivorous vertebrate is the definitive host.

In most digene life cycles, an early developmental stage called the redia may develop in the snail host, while in other varieties, this stage can be skipped altogether and a sporocyst may develop directly into the cercaria.

In my collections, the mosquitofish were only an intermediate host as all digenetic specimens were preadults with undeveloped reproductive organs. However, depending upon the size of the mosquitofish it may be used as the definitive host. Each of the trematodes discussed here use the snail to develop the redia, and the definitive host is often an aquatic bird. Generally, the trematode in the fish host is found encysted in organ or muscle tissue. On occasion, the preadult worm is found free in the coelom or in specific organs. The trematode reaches maturity and reproduction occurs in the definitive host. The eggs of the adult worm generally exit the body with excreta, fall to the bottom of the pond and are eaten by the snail (either an operculate or non-operculate snail can be a primary or intermediate host in this cycle) where they develop into the larval miracidium. The miracidium produces sporocysts or rediae, which encyst in the snail's intestine and later develop into cercariae. The cercariae eventually leave the snail and swim free, find a suitable substrate to encyst on such as plants or another intermediate host, and become metacercariae. The metacercariae are the infective stage for the definitive host, which are consumed to become infective.

Fishes infected with metacercariae often have small black or white raised spots on their epithelium. In heavy infections, the black color may spread to all regions of the body. This color change is a reaction of the fish to the parasite and may not always occur. Generally, fishes with heavy cyst infections will become listless and may be found resting on the bottom. Feeding behavior is abnormal or absent and reproductive behavior is lost.

The morphology of digenetic trematodes is important in identification, but difficult to use for a basis of classification. Closely related trematodes may have drastically different morphologies, which is more related to specific host adaptations required in parasitic life styles, than to phylogeny. All digenetic trematodes fit into a set of morphological characteristics which define the order. Each of the stages in the life cycle have specific morphologies which are diagnostic for species identification.

The cercaria are equipped with an anterior mouth and centrally located ventral sucker. Some species contain two distinct eye spots just below the mouth. Around the mouth is a strong oral sucker which is connected to a pharynx and esophagus that separates into a two-branched intestine. Posteriorly, there exists an excretory vesicle with an outside exit. Within the egg shaped body there exists the undeveloped genitalia along with a variety of glands. A robust swimming tail extends the body length posteriorly. In some species, this tail is bifurcated into caudal rami.

The adult trematode has more of an elongated shape, generally three times as long as wide. As with the cercaria, the anterior end sports a large oral sucker followed by a prepharynx, pharynx, esophagus and a bifurcated intestine. At the terminal end, a large excretory vesicle empties into an excretory pore. In most specimens, the ovary and uterus are visible along with the large posteriorly located testes. A long vas efferens leads to the cirrus which is located anterior to the ventral sucker and only visible in adults. The remaining portions of the body are filled with vitellaria bodies.

The first digenetic trematode to be considered here is *Diplostomulum scheuringi* Hushes, which is frequently found in the coelom of mosquitofish where it exists as an unencysted metacercaria. In other fishes, it has be found encysted in the brain or eyes (Haderlie 1954). The body is elongate and rounded at

the ends, measuring 1.5 mm. Anteriorly, a small oral sucker is present. The ventral sucker is very small and poorly differentiated. There is a very short prepharynx followed by a short pharynx. The esophagus is long and narrow and is followed by a long bifurcated intestine. A holdfast organ is centrally located in the body. A mass of cells which represent the reproductive organs are located in the posterior region. These organs are not fully developed in the life stage that infects the fish. At the extreme posterior end is a small excretory pore (Hoffman 1967).

For *D. scheuringi* to become an adult, the infected mosquitofish must be consumed by the proper definitive host. The definitive host is as yet undiscovered, but aquatic snakes, snapping turtles and various piscivorous birds have been investigated. It is likely that the definitive host is an aquatic bird or a larger carnivorous fish. The snail host for *D. scheuringi* is *Helisoma spp.* which belongs to the family Planorbidae.

A second species of digenetic trematode commonly collected from mosquitofish is *Postodiplostomum minimum* MacCallum. It is found in the mesenteries, organs, muscles and occasionally in the coelom. It is a nonspecific parasite which exists as a encysted or nonencysted metacercaria in many species of freshwater fishes across a wide geographical area. The fish is an intermediate host, along with the snail. The parasite is transferred to the definitive host, a heron or loon, when an infective fish is eaten.

The body of *P. minimum* is elongate, narrower toward the anterior, and broad at the posterior end. At the tip of the anterior end is a large oral sucker. Following the oral sucker are the prepharynx, pharynx, esophagus and two intestinal branches. In some specimens, rudimentary gonads are visible below the centrally located ventral sucker. At the posterior-most region is located an excretory bursa and pore (Hoffman 1967). The intermediate host used by *P. minimum* are members of the snail family Physidae.

The traditional control of digenetic trematodes consists of action against the snail using any one of the many regulated molluscicides. A second method, which assumes the aquatic bird is the definitive host, consists of setting netting or screens over the ponds to keep birds and their feces out of the fish ponds. Both of these methods attempt to break the life cycle of the trematode before it can infect the fish. At present, there is no known chemical means of treatment for the trematode infected fish.

Nematoda. The nematodes, or roundworms, are common practitioners of both free-living and parasitic life styles. Most, however, seem to have evolved a parasitic approach. The life cycle of parasitic nematodes is generally quite complex and can include two or three hosts. The nematodes found parasitizing mosquitofish have two intermediate hosts and a definitive host. The eggs hatch in the mud and a free-living stage emerges which feed on detritus or suspended particulate matter. This stage is eaten by a crustacean or other arthropod which is used by the nematode as the first intermediate host. Copepods are most frequently used for this stage of development which culminates in the first infective stage. The copepod is then eaten by the second intermediate host, this time the mosquitofish. In the fish, the nematode generally encysts in viscera, muscles, or mesenteries where it waits to be consumed by the definitive host, a piscivorous bird. In the bird host, the nematode develops into a mature adult and resides either in the intestine or the proventriculum. Eggs may hatch either in the intestine or wait until they reach a suitable substrate in water; both exit the definitive host with the feces.

It is generally believed that the encysted nematode parasite in the fish causes little damage. However, some detriment has been noted in cultured fish populations. Depending upon the number of cysts or the size of the cysts within the fish, a problem with pressure narcosis of organs can develop (R. Hendrick, personal communication). Fish heavily parasitized with nematode cysts often forgo reproduction due to the energy costs of maintaining the parasite population. Finally, differences in behavior are often associated with encysted fishes. Fish with nematode cysts are frequently found on the surface of their water habitat, and generally do not effectively avoid predation compared with nonencysted fish. This behavior may be a complication of pressure applied to the air sac, which can create disorientation. In any event, the behavior change certainly increases the bird's likelihood of consuming infected fish and thereby contracting the infective parasite. Such behavioral modification would certainly be selected in the evolution of parasite transfer from intermediate host to definitive host.

A commonly encountered nematode cyst in mosquitofish is that of the species *Eustrongylides wenrichi*. This nematode is always found in the peritoneal cavity of the mosquitofish, usually located ventrally or laterally. In the early stages of infection, the nematode is quite small and located in the ventral region of the body cavity. As the worm grows, it shifts its position to the pleural region of the visceral cavity. In the latest stage of nematode development, the cyst may protrude as much as six

millimeters from the body wall of the fish. The worm is bright red in color and is coiled into a tight knot. A thick mesentery-like mucus coat covers the worm, creating the cyst. The worm will go through several growth molts during its development in the mosquitofish. In most cases, only one cyst will develop in the fish, on rare occasions two have been found, both in the same stage of development.

The advanced stage of development of *E. wenrichi* in mosquitofish ranges in length from 5.0 cm to 12.0 cm. The diameter generally remains constant at about 0.5 cm. The cuticle is covered with numerous transverse striations. The head region contains cephalic papillae in two circles of six papillae each. The width of the outer circle is approximately 0.25 mm and the inner circle is about 0.15 mm. In the center of the two rings of papillae is a cleft-shaped oral cavity. Following the mouth is a distinct esophagus, which ends after several millimeters. Posteriorly, the worm terminates with a large cloacal orifice. A nerve ring demarcates the separation of oral cavity and esophagus.

The life cycle of *Eustrongylides* has not been determined. According to Barus et al. (1978), adult *Eustrongylides* have been found in the proventriculum of fish-eating birds. In studies of Palaearctic species, Karmanova (1965) found that there are two intermediate hosts; a primary oligochaete and then a secondary freshwater fish. In a study done by Modzelewski and Culley (1974), *Eustrongylides wenrichi* was found in the bullfrog, *Rana catesbeiana* after ingesting mosquitofish infected with the nematode larvae. They also observed that if the mosquitofish should die before it is ingested by the definitive host, the larval *Eustrongylides* will exit the body. This can be speculated to be an effort to become ingested again by a suitable host (i.e., other fish or frogs which may later be eaten by the definitive host).

A second nematode reported from mosquitofish is *Camallanus oxycephalus* (Davis and Huffman 1977). This nematode is frequently found in the rectum of the mosquitofish as a first or second stage larva, and is often observed hanging out of the anus of infected fish. This suggests that the mosquitofish is an intermediate host. However, third and fourth stage larvae are also collected from mosquitofish and occasionally gravid females have been found (Davis and Huffman 1977). In this case, the mosquitofish is the definitive host for the nematode. Fourth stage larvae are often found infesting livers of the definitive host fish, while second stage larvae are found in tangled masses in the mesenteries (Hoffman 1967). It has been suggested by Davis and Huffman (1977) that *C. oxycephalus* requires at least two host fish to complete its development, using the larger fish as the definitive host. Evidently the species of fish used as a final host is not important in the development process. The first larval stage develops in several species of copepod, and likely, in other crustaceans. *Camallanus* has also been found in the intestines of amphibians and occasionally in reptiles.

The stage most encountered in mosquitofish is quite red in color, much like *Eustrongylides*. The mouth is elongated dorsoventrally in a slit-like fashion. There are no lips or papillae around the mouth. Below the mouth is a large buccal capsule equipped with two chitinous valves and a chitinous ring just below the capsule and the esophagus. The male has a ventrally directed curl in its posterior end and several pairs of papillae surrounding the anus. The female has a vulva at the mid-region of her body with a pair of uteri and a single ovary (Hoffman 1967).

The only control of nematode parasites consists of interruption of their life cycle. This is accomplished by eliminating the crustacean host or by preventing the birds from depositing feces into the cultured ponds, as mentioned above with digenetic trematodes. Currently, there is no treatment for mosquitofish already infected with nematode cysts. In most cases, if the nematode is not transferred to its definitive host within a specific time period, it will degenerate and die.

Cestoda.- The cestodes, or tapeworms, exist exclusively as parasites and are known to infect all forms of vertebrates as adults. They have a complex life cycle which can vary extensively among the different families. Most cycles usually include at least two hosts. For those infecting freshwater fishes, the cycle is similar to that of the family Pseudophyllidae. The eggs hatch in the water and release free swimming larvae called coracidia which look very much like ciliated protozoans. The coracidium is ingested by a crustacean and the onchosphere is freed within the intestine. The onchosphere is a small spheroid which penetrates the intestine wall and develops into an infective stage called the procercoid. The infected crustacean is then eaten by the fish which can function as a second intermediate host or as the definitive host depending upon its size. Generally, the procercoid penetrates the intestine of its second host and becomes encysted in various parts of the body. This stage is called the plerocercoid or sparganum and can remain encysted and inactive for long periods of time. Eventually, the second host is eaten by a larger fish which becomes the definitive host. The plerocercoid

develops into a mature adult in the intestine with its scolex securely attached to the lining. Each proglottid or worm segment develops fertilized eggs which are expelled into the water with feces.

Fish infected with tapeworms show a general reduction in activity. Often a fish may have many worms within its intestine and such a situation will reduce growth and eliminate development of reproductive gametes. These fish appear quite thin and pale and the normal sheen associated with healthy scales is lost. Certain tapeworm infections can cause a marked reduction in the life span of fishes.

There are only two cestodes, belonging to two separate families, commonly reported from mosquitofish. From the family Proteocephalidae is *Proteocephalus sp.* which is a nonspecific tapeworm of many freshwater fishes. The second is called *Bothriocephalus sp.* and comes from the family Bothriocephalidae which is considered to be one of the most dangerous to cultured fishes. A population infected with either is considered threatened, for the stress caused by the tapeworm generally reduces fecundity of females and often stops reproductive activity altogether. Infections can multiply rapidly in cultured environments where eggs are not washed away. Multiple infections are not uncommon in these situations either. In such cases, fish mortality is quite high. *Bothriocephalus* has been reported to completely destroy cultured carp populations (Bauer et al. 1969).

The cestode *Proteocephalus* is quite common in fishes and is also collected as adults from amphibians and reptiles which feed on small fishes. It has been reported from mosquitofish as both plerocercoids and as adults (Davis and Huffman 1977).

The scolex of adult *Proteocephalus* is without hooks, but has four, sometimes five, suckers arranged in a ring. The neck region is unsegmented, but the rest of the body has distinct segments, each with large marginal genital pores. The segments show distinct layers of longitudinal and circular muscle fibers. Vitellaria fill the area around the reproductive organs in each segment. In a mature adult, there is often as many as 100 segments. Each segment or proglottid contains one set of reproductive organs which continuously release fertilized eggs into the water.

The procercoid larval stage has been collected in copepods such as *Diaptomus* or *Cyclops* (Hoffman 1967). It has an elongated body region filled with vitellaria, and a knob-like posterior region with hooklets. This stage penetrates the intestinal wall. The plerocercoid consists of a long unsegmented body which is encapsulated. The plerocercoids are found encysted in various organs of small fishes. Often the gonads are heavily encysted, causing severe fibrosis that most often leads to sexual sterility (Hoffman 1967).

Bothriocephalus has a life cycle which begins with a small coracidium that hatches from the egg. The coracidium is a free swimming sphere covered with cilia and contains three small embryonic hooks. The coracidium is eaten by any one of a number of different *Cyclops* species. Inside the crustacean, the coracidium perforates the intestinal wall and enters the body cavity where it develops into the procercoid. The procercoid has an oblong body and a rounded posterior portion with three distinct hooks. Soon the crustacean is ingested by a fish which will become the definitive host for the adult *Bothriocephalus*. In the fish, *Bothriocephalus* attaches itself to the intestinal wall where it will mature and reproduce. Development of the worm in the intestine is determined by water temperature. Bauer et al. (1969) found that at optimum summer temperatures, *Bothriocephalus* can start laying eggs after about twenty days. Eggs can develop in three to five days depending upon water temperature. Incubation lasts from three to four days at water temperatures of 16-19°C and only two days at 22°C. The life span of the adult worm is about one year, but low winter temperatures are known to be fatal.

The adult worm can be recognized by its elongated arrowhead shaped scolex which contains a deep pit or bothrium on either side. Segmentation is complete and distinct, often with secondary segmentation. Proglottids overlap one another creating a serrated appearance along the margin of the tapeworm strobila. *Bothriocephalus* has a very white color and can often be viewed through the intestinal wall from the outside prior to dissection.

The best method to control tapeworm populations is through the elimination of the intermediate copepod hosts from the diet. Infected fish can be treated with di-n-butyl tin oxide (Brown 1980). Ponds that held infected fish need to be sterilized before new populations are introduced.

Acanthocephala. The acanthocephalus, or thornyhead worms, are found as adults in the intestines of most vertebrates and especially fishes, particularly those living in marine habitats. Freshwater fish are less frequently parasitized, but those that are infected suffer greatly. The adult acanthocephalus causes severe damage to the intestinal wall, their thorny proboscis producing bleeding ulcerations. The proboscis is buried deeply within the intestinal lining where it rasps away at tissue

and feeds upon blood. Generally, a fish will be infested with several worms. In some fish, the numbers of worms becomes so great as to completely occlude the alimentary canal leading to rupture of the already weakened intestinal wall.

An acanthocephalus may use two or three hosts during its life cycle. In most cases, the first intermediate host is an aquatic insect which ingests the eggs of the thornyhead worm. In some cases, crustaceans will function in this link. Next, the insect or crustacean is eaten by the fish where the worm will either develop into an adult in the intestine of the fish or will encyst in the abdominal cavity and wait for the fish to be eaten by a bird or mammal. Eggs are released by the adults in the intestines of the definitive host and enter the aquatic environment with their feces.

The male acanthocephalus is identified by the enlarged bursa copulatrix with a genital pore at the posterior region. The female worm has a linear arrangement of ovary, uterus and vagina opening at a small pore at the posterior-most region. The female tail end is rounded, while the male has a knob-like tail. Female worms are generally much larger than males. Both male and female acanthocephalus have a thorny proboscis with rows of curved hooks at their anterior end. The hooks are arranged in columns of circles with various numbers and sizes being diagnostic for species identification. The proboscis is retractable and is attached to a large central contracting ligament running the length of the worm. Just behind the head is a receptacle in which the proboscis fits when retracted. Most acanthocephalus are yellow in color, but occasionally white ones are found. The worms are compressed laterally and can reach great lengths depending upon the size of their host and the availability of intestinal space.

Two distinctly different acanthocephalus have been collected from mosquitofish. The most important being *Octospiniferoides chandleri* which uses the mosquitofish as its major definitive host. The other, *Neoechinorhynchus cylindratus* is a parasite of the largemouth bass, *Micropterus salmoides*, and is occasionally found in other fishes including *G. affinis* (Hoffman 1967).

The *Neoechinorhynchus cylindratus* infection in mosquitofish consist most frequently of a single worm, which is often too large for the intestine. Female worms up to three centimeters in length have been collected from mosquitofish. The proboscis is short and contains six hooks in each circle which spirals upward. There are three complete circles overall. The first circle of hooks are thicker and larger than those in the next more anterior circle.

Only two hosts make up the life cycle of this acanthocephalus. The intermediate host for *N. cylindratus* is the ostracod *Physocypria pustulosa*, which is widely distributed in North America. The worm is transferred to the fish when the ostracod is ingested. This ostracod can be recognized by its compressed shell, with valves of unequal height or length. The shell is smooth with the margin of the valves being tuberculate (Pennak 1978). The definitive host is most frequently a fish which lives up to five years and therefore grows to a large size. The ostracods are generally taken when the fish are young-of-year and then grow to adults with the fish. Adults have also been collected from marine fishes, frogs and turtles (Hoffman 1967). For *N. cylindratus* the mosquitofish is an incidental host, unlikely leading to reproductive success of the parasite.

Mosquitofish are the definitive host for the acanthocephalus worm *Octospiniferoides chandleri*. This worm is much smaller than *N. cylindratus* and is often collected in multiples from its host. The proboscis has three circles of hooks with eight to ten hooks in each circle (Hoffman 1967). The hooks are small and narrow with a slight inward curve. Adults exist in the alimentary tract throughout the life of the mosquitofish. Only female worms are known, as no males have been collected.

The life cycle of *O. chandleri* is quite interesting, relying both on behavior modification of the intermediate host and specific behavior characteristics of the definitive host. The eggs are eaten by either of two ostracods, *Physocypria pustulosa* or *Cypridopsis vidua*. In either ostracod, the worm develops into a cystacanth within twenty days, becoming infective on the twenty-third day. The normal behavior of uninfected ostracods is to concentrate on the bottom of pools or ponds. However, those with cystacanth larvae are behaviorally affected and become photophilic, just the opposite of uninfected ostracods (DeMont and Corkum 1982). The infected ostracods will congregate on the surface of the water, making them easy prey for the second intermediate host. Young mosquitofish feed extensively on phytoplankton and frequently take infected ostracods,becoming the second intermediate host. The definitive host is also the mosquitofish, but in its second year of life. The adult mosquitofish must eat its own infected progeny to become infected with the cystacanth larvae. This is a common behavior of adult mosquitofish, especially females. Once inside the mature fish, the worm goes through a gradual change which is divisible into four stages. Eggs produced by the adult worm exit the

fish's body with the feces. It is not uncommon for adult fish to harbor many worms. Heavy infections of *O. chandleri* eventually cause emaciation of the fishes.

The best control of this parasite in mosquitofish ponds would be to eliminate the host species of ostracod from their diet. A second method would be to interfere with the cannibalistic behavior of mosquitofish.

Control of acanthocephalus is the same as that with other helminthes mentioned. Interruption of the life cycle by eliminating the first intermediate host from the diet of the fish is the most effective means. Elimination of infected feces from fish ponds by flow through systems can also be effective.

Crustacea. One of the more notable parasites infecting mosquitofish is the vermiform crustacean *Lernaea*. This copepod parasite feeds on blood and tissue from inside the mosquitofish but most of its body protrudes on the outside, making it an ectoparasite. *Lernaea* is known to be one of the least host specific parasite genera in North America. It has been collected on most species of North American fish and on some amphibians. Due to its very general nature as a parasite, it is rarely found in high percentages on any one species of fish. However, *Lernaea* has an enormous reproductive potential, capable of producing thousands of eggs from a single female. In its normal stream habitat, most eggs are washed away by the current, to become lost, beached or eaten; few live long enough to become the infective larvae.

After the eggs have been in the water for one to three days, they hatch into free swimming six-legged larvae called a nauplii. The nauplii are plankton feeders. A metamorphosis occurs in four to sixteen days, forming the first of five copepodid stages. Mating will occur during the fourth copepodid stage. Male and female copepodids meet briefly on a fish host and cling to the gills during copulation. After mating, the male presumably dies. Females transform into the fifth copepodid or infective stage. The infective stage can then attach to any fish or amphibian. After attachment, the female undergoes a radical transformation and loses all resemblance to a copepod.

The adult overall appearance is that of a helminthes. However, vestiges of segmentation and locomotor structures can be found upon close examination. The head is fused with the thorax forming the anchor structure with which it attaches to the host. A small pore-like mouth is directed anteriorly. Two pair of horns protrude laterally from the cephalothorax. Depending upon the various species, the horns are branched in different fashions. The horns are soft, rounded and generally opaque. The neck and trunk consist of a long cylindrical tube transparent enough to easily see the slender intestine. The trunk has three segments with a reduced pair of biramose appendages below each segment. A fourth pair of appendages exists just behind the head. At the posterior-most region of the trunk exists two lobed nodules called pregenital prominences. Attached just behind the pregenital prominences are two caudal rami with a pair of conical ovisacs filled with thousands of developing eggs.

Lernaea is held secure within its hosts tissue using the pair of cephalothoracic anchors. The trunk and caudal rami protrude from the fish's tissue. The point of parasite entrance through connective and muscle tissue produces the major problem for the infected fish. The surrounding tissue is irritated, inflamed and often oozing blood. Depending upon orientation of the anchors, horns may emerge at the surface forming other severe ulcerations. These hemorrhages lead to secondary bacterial and fungal infections. *Lernaea* feeds directly on the fish fluids, thereby reducing vigor of the fish. This reduction of health permits opportunistic pathogens to take their greatest toll. Many fish infected with *Lernaea* have also been found with a large quantity of dermal tumors that have been associated by some investigators with the parasite (Reichenback-Klinke 1965).

The best approach for controlling *Lernaea* in fish hatcheries is to kill the eggs and larvae before they infect fish. There is no known treatment for infected fish. The most cost-effective method for controlling larvae consists of exposing fish to tolerant levels of salt. A concentration of 0.76% should be used for best results. According to Stickney (1986), free swimming larvae can be controlled with Bromex TM at 0.12 to 0.15 ppm of active ingredient. Ponds should be treated once a week during summer months. Dipterex TM can also be applied to ponds at concentrations of 0.2 to 0.3 ppm, once every two to three weeks.

Eumycota. The phycomycetes fungus, *Saprolegnia sp.*, is an opportunistic aquatic saprophyte and only facultatively parasitic. It is mostly found growing on dead and decaying material. Free swimming zoospores released by the growing hyphae are always present in the water. Mosquitofish which are stressed from either wounds or parasitic and bacterial infections have lower resistance to *Saprolegnia* zoospores. Once the fish becomes infected with a few zoospores, the vegetative form rapidly spreads throughout the body. Ultimately, the

fish will succumb to the infection and die. A secondary infection of *Saprolegnia* is frequently the final cause of mortality when fish are infected with parasites and bacteria.

The life cycle of *Saprolegnia* is typical for that of members in the family Phycomycilliae. The cycle can be both sexual or asexual depending on favorable or unfavorable environmental situations. Reproductive cells develop into a white hair-like mass called hyphae which protrude outside the fish's body. A larger mass of material which enters the growth substrate or fish body and absorbs nutrients is called the mycelium. The hyphae mass is different from the mycelium mass in having swollen tips and distinct cross-walls. The swollen tips become the reproductive cells.

In asexual reproduction, the swollen hyphae tips contain zoosporangia which break to liberate flagellated zoospores. Each zoospore contains a pair of flagella which allows it to swim throughout its medium. After some time, the zoospore settles on the substrate, drops off its flagella, and develops into a cellulose cyst. This cyst is a resting stage of the zoospore. After a variable period of time a new zoospore escapes from the cyst. The new zoospore has lateral rather than terminal flagella. This is the stage which will search for dead and decaying tissue upon which it can grow.

In the sexual cycle, cells at the tip of the hyphae change to become male or female sex gametes. The male gamete, called anthridi, unites with the female gamete, the oogeni, to form an oospore. The oospore may remain without germination for several months. Once the oospore germinates, hyphae and mycelia rapidly grow directly from the oospore. The oospore contains from five to ten zygotes which develop into independent fungal masses.

Fish with early infections of *Saprolegnia* can be recognized by the appearance of a white fuzzy mass in small regions of the body. In many fish, the infection begins on the fins, especially the caudal fin, and then moves toward the central region of the body. As the infection moves, all tissue is consumed by the advancing fungus. Eventually, the entire body is consumed with hyphae and mycelia, the final location being the gill space and mouth.

According to Post (1983), the main reason for *Saprolegnia* infection in fish is physical stress. An important means for such stress to occur is through the physical damage caused by excessive handling and seining. The protective mucus coating of fish is often rubbed off and wounds into epithelial tissue can occur. Such breakdowns in this physical barrier allow the zoospores or oospores to take hold on the exposed or raw tissue. Once infection occurs, hyphae and mycelia will spread to healthy tissue. Eventually, all epithelial and muscle tissue are assimilated by the growing fungi.

Stress caused by low temperature (6 to 8 ^{o}C) or high and low pH have been demonstrated to stimulate *Saprolegnia* outbreaks in cultured fishes (Post 1983). It has also been observed that infections of helminthes, protozoans, or bacteria in cultured fishes most often lead to infections of *Saprolegnia* (Brown 1980).

There are no treatments for fish infected with saprophytic fungi. Control methods consist of preventative actions to reduce abrasion of the fishes' epithelium. Using proper seines (and only when absolutely necessary) and avoiding overcrowding, often prevent the greatest percentage of infection risk.

Protozoa. Protozoa are by far the most serious parasites found to be infecting fishes. They are also some of the most difficult to identify and control. They cause more disease in fish than any other known agent. There exists an exhausting number of different types of parasitic protozoans which infect fishes, most of which are not host specific. Many protozoans can be found on the surface of fishes apparently causing no harm. Others, which are characteristically nonparasitic free-swimming forms, are often collected in dangerously large numbers from gill lamellae. Various stages in protozoal development may have drastically different morphologies which can often confuse diagnosis. Due to the great diversity and lack of host specificity common among many parasitic protozoans, only a few of the most important parasites associated with mosquitofish will be discussed. There are undoubtedly numerous others which are frequently encountered.

Protozoans infecting mosquitofish can either be internal or external parasites. Several genera of each type are frequently collected from mosquitofish in cultured environments. Each will be discussed briefly, along with some diagnostic features and suggestions for control.

Among the most common of external protozoan disease parasites is *Ichthyophthirius multifiliis* which attacks mosquitofish as a ciliated free-swimming larvae. A frequently used common name for the disease is Ich or less commonly, white spot disease. The disease is seasonally oriented, due to the protozoa preferring specific water temperatures. A narrow range between 20 to 22°C allows optimum growth and development of *I. multifiliis*. Temperatures five degrees higher or lower restrict development, and beyond that the protozoan dies.

The adult parasite is a uniformly ciliated sphere

with a "U"-shaped nucleus. This stage is called the trophozoite and is found encysted under the skin of the infected mosquitofish. The cyst causes the small white spot commonly associated with the disease. In time, the cyst bursts from the tissue and the parasite is released to become a free-swimming form. Within two to six hours, the protozoa sinks to the bottom and attaches to the substrate. On the substrate, the trophozoite transforms into a cyst which will undergo a series of multiple divisions. Depending upon the size of the cyst, it may divide up to 2,000 times. Each of the divisions will develop into a free-swimming tomite. The ciliated tomite will then actively seek a proper host. Any variety of fish will serve as a suitable host. The tomite will die within twenty-four hours if it cannot locate a suitable host. Once the tomite contacts the fish, it uses a degradation enzyme called hyaluronidase to penetrate the surface tissue. Buried under the fish's epithelium, the tomite changes to the trophozoite. The trophozoite feeds upon cells and their fluids. Given proper conditions, the cycle can be as short as ten to fourteen days or as long as five weeks.

Fish infected with *I. multifiliis* will frequently display some altered behavioral characteristics. Infected mosquitofish often are found somewhat sluggish, listing on the bottom and occasionally rubbing themselves on abrasive surfaces. In most situations, a heavily infected fish will become covered with white spots. As the infection proceeds, a thick mucus coat is released, fins become bloody and glued to the sides of their body. Occasionally, a fish with high numbers of trophozoites collected from gill lamellae will show no external signs of infection.

The most economical method for prevention and control of the disease is to continuously flush fresh water through pools, ponds and raceways. Rapidly moving water will wash the infective tomites away. Never recycle or channel water from one pond to another. New recruits should be quarantined and observed for ten days before adding them to the main stock. Ponds already infected with *I. multifiliis* should be dried and disinfected before adding fish. Some chemical treatments have been successful in controlling the protozoan. Among the most commonly used is malachite green without zinc at 25 ppm on three to four day intervals; formalin at 15-25 ppm every other day; copper sulfate at 40-50 ppm; and finally potassium permanganate at 2-5 ppm (Brown 1980 and Stickney 1986).

Another frequently encountered ectoparasite of mosquitofish is *Trichodina sp.*. This protozoan is nonspecific and therefore commonly found on most species of fish. On mosquitofish, as with other fish, *Trichodina* is found covering the epidermal tissues, including skin, fins, and gills. The parasite can be seen moving rapidly across these surfaces much like a hovercraft.

Trichodina is a disc-shaped protozoan with the outer margin covered with cilia and with an inner ring of hooked teeth. In the central region of the ventral surface there exists a cone of blades which appear to rotate as the parasite moves over the surface of the fish. According to Brown (1980), the diameter of *Trichodina* is quite variable, and may represent different species. The largest collected have a diameter of 120 microns, while some of the smallest only 15 microns. Brown also noted that those species with the smaller diameter had the greatest pathogenic effect upon cultured fishes.

Infection with *Trichodina* is most serious with fry or small fishes. This protozoan rasps away at tissue with its rotating blades, creating small hemorrhages on skin and gill lamellae. Fish respond by producing copious amounts of mucus. As the disease advances, infected fish will develop a gray film over the surface of their bodies. The tissue making up the fins often becomes opaque or even white. These symptoms, however, are not diagnostic of *Trichodina* infections and may be associated with many different protozoan diseases. Fish with heavy infections behave as if they are suffering from lack of oxygen and are often observed gulping air at the surface of the pond, moving their operculum rapidly, and grouping around flowing water. Large fish may contain heavy numbers of *Trichodina* on their bodies but show no disease symptoms. There seems to be some relationship with poor water quality and manifestation of the disease symptoms.

The best treatment for fish infected with *Trichodina* is to flush the ponds or pools with fresh water. Rapidly flowing water will wash off the protozoa from skin and gill lamellae. If a constant flow of fresh water is maintained, such infections most likely will never occur. Chemical treatment for *Trichodina* is the same as that for *Ichthyophthirius*.

A frequently undetected protozoan disease occurs with infestation of an obligate ectoparasite called *Ichtyobodo sp.* (formerly known as *Costia sp.*). These protozoans generally are overlooked during investigations because of their extremely small size (about ten to twenty microns). However, once observed they are quite distinctive. Most individuals are ovoid in shape, but sometimes are more pear-shaped. Each has four flagella, two very short and two are quite long and easily visible. The

action of these flagella gives this protozoan a flickering appearance when in motion. They also move quite rapidly and are difficult to follow without a protozoan slowing agent such as polyvinyl alcohol or methyl cellulose.

Depending upon the species of *Ichtyobodo*, it may be found on the gill lamellae, skin, fins or throughout the body. This protozoa feeds on the fish's protective mucus covering and upon the epithelial tissue. Large concentrations of *Ichtyobodo* can sufficiently reduce the mucus protection to allow severe pathogenic bacterial and fungal infections. Mosquitofish with severe infections become covered with a whitish film and often exhibit a rubbing or scraping behavior. In the final stages, fish generally remain motionless on the bottom with a loss of all reproductive and normal feeding behaviors. In this weakened state, they die from other infections or malnutrition.

Control of *Ichtyobodo* can be accomplished by maintaining good water quality and a rapid flow-through system. Rapid movement of water across the gills and epidermis will wash away most of these protozoans. Brown (1980) recommends formalin-malachite green at 25 ppm for systems which are closed or cannot accommodate a flush through treatment.

The next group of protozoans are obligatory intracellular parasites of mosquitofish. Unlike the external protozoans, these are more often quite specific in host selection and organ choice. The choice of initial infection cells within the body is generally specific enough to be diagnostic. In the more advanced stages of infection, the entire body may be involved. Identification of these parasites requires a clear understanding of their complex life cycle along with recognition of each of the different stages. In most cases, the spore is used as the identifying stage in the life cycle. Spore characteristics can be observed using a standard phase contrast microscope. The number and arrangement of polar filaments within the spore specifically places these protozoans into either of two phyla, the myxospora or myxosporidians with two polar capsules and filaments, and the microspora or microsporidians with one polar capsule and filament.

According to Hoffman (1967) and Brown (1980), the life cycle of both the microspora and the myxospora begins with a spore. The spore, a binucleated bivalve, is usually released from a dead or dying host fish. Once released into the water, it eventually settles to the bottom where it can rest for extremely long periods of time, resisting alternate drying and flooding. Eventually, the spore is eaten by a healthy fish and it finds its way into the intestine. Digestive juices break down the spore walls which activates the spore to migrate to the proper cell and attach its filament. At this point, the two groups change approaches. The myxosporidians release a sporoplasm, which is essentially a cell wall with cytoplasm and DNA. The sporoplasm moves much like an amoeba and penetrates the hosts muscle and connective tissues. Once inside the host cell it commandeers its replicating abilities for its own use. The microsporidians use the spore filament as a conjugation between itself and the host cell. Through the filament passes the sporoplasm in an injection created from the internal pressure of the spore. Inside, the spores DNA uses cellular energetics to direct its own replication. Many individual nuclei are formed within a growing mass called the schizont. At this point the cycle can enter either of two phases depending upon the conditions of the host cell. If the host cell exists within a healthy environment the cycle will "park" many of the schizonts which will multiply forming the trophozoites which can spread the infection to other host cells. This is called the vegetative or multiplicative phase. The schizont may also enter the sporulation phase, which ends in the formation of spores. This phase is activated if the cells exist in a unhealthy environment, i.e., the host body is sick or dying. In this phase the schizont divides into many singly nucleated units called merozoites. These merozoites form rows of regularly spaced units in ribbons. Each merozoite divides into sporonts at which point the recombination of genes occurs. The sporonts form sporoblasts where the process of morphogenesis into the spore occurs. Thick spore membranes are formed and a coiled polar filament develops. These spores can then be released from the host into the environment where they can either enter a state of inactivity or infect another host. The spores may exist in a cyst that burst from the surface tissue to release the spores directly in the water, or the spores may exit the fish through the gills, with feces, or wait for the fish to be consumed or die and decompose.

The myxosporidian, *Myxobolus sp.*, attacks surface tissue causing a disease known as milk scale. Cells become hypertrophied with spores that produce obvious bulges along the surface of the epithelium. When the spores are ready to be released, these raised areas, filled with a milky material, break. The released spores are washed into the surrounding water. The resultant pock marks covering the surface often become infected with bacteria and fungi. The spores appear as oval bodies with two polar capsules containing tightly coiled polar filaments. The

remaining portion of the spore contains the sporoplasm which is surrounded by a thick membrane. A large iodinophile vacuole exists in the sporoplasm separating it from the similar myxosporidian, *Myxosoma* (Hoffman 1967).

The spores of *Myxosoma sp.* develop within the skeletal and cartilaginous material of their host often causing developmental malformations. Young fish suffer more severe symptoms and are more susceptible to infections, while older fish with infections show few manifestations of symptoms. The disease has not as yet been officially reported from the mosquitofish. However, the symptomatic characteristics caused by spore infections (i.e., spine curvature and head malformation) have been noted in personal collections. Identification of spores is not easy and requires confirmation from a fisheries pathologist. The spores appear quite similar to those of *Myxobolus* with the exception of the iodinophile vacuole. Spores of *Myxosoma* can exist for months in the soil of ponds or raceways and can remain viable through several dryings (Brown 1980).

There is no known control of myxosporidians once they have infected fish. The spores can be killed by drying ponds and then treating the soil with chlorine; repeating the process several times before returning fish. However, this has been found to show only limited success against some spores (Brown 1980).

Crandall and Bowser (1981) collected a microsporidian protozoan from mosquitofish in Southern California belonging to the family Pleistophoridae and the genus *Glugea sp.*. Spores of *Glugea* are oblong shaped with one polar capsule and filament. At one end there exists a large notable vacuole. The entire spore is covered with a tough protective chitinous coat which allows it to withstand desiccation for months.

According to Crandall and Bowser (1981), microsporidian spores infect mosquitofish via ingestion. Once inside, the spore penetrates the intestinal lining and enters cells. The function of the infected cells is overridden and they begin producing the parasite trophozoite in enormous quantities. Infected cells become hypertrophic in regional clusters producing distensions and enlargements of external tissues. Within these tissue enlargements are one to two cysts. The smaller cysts draw nourishment from surrounding fluids, while the larger swellings draw on nearby blood vesicles. As the infection spreads, the entire body of the fish may become covered with these lumpy swellings. Inside these pustula-like cysts exist thousands of spores floating in a white viscous fluid. When spore volume reaches a high number, the pustule bursts, spewing thousands of spores.

The infection causes direct mortality in some fish while only reducing health in most. A reduction in swimming ability and atrophy of internal organs, especially the ovaries, has been noted. Male fish are more susceptible to infection than female fish and fry generally suffer mortality while adults show some resistance to infection and symptoms.

There is no known treatment for infected fish, nor are there any methods of control once an epizootic begins. Ponds and raceways where disease has occurred should be dried and chlorinated. The use of cement bottoms rather than soil will help in washing spores away with adequate water current.

Bacteria. Bacterial infections in fish are most frequently the result of environmental perturbations. Pathogenic bacteria are always present in the water around the fish, on the surface of fish and in their intestinal tracts. When stress occurs due to poor water quality or overhandling, the fish's natural resistance is lowered. When the body is under stress the normal antibiotic and phagocytic responses are the first to be disrupted. The bacteria already present can quickly take hold and fish health deteriorates rapidly. The stresses which cause the greatest problems associated with fish disease are temperature extremes, ammonia and metal concentrations, and pH. It has also been noted that concentrations of dissolved oxygen below 5 ppm will lead directly to bacterial infections (Brown 1980). Proper nutrition is also critical in maintaining healthy infection-free populations.

It is necessary to isolate and identify the specific bacteria responsible for an infection to diagnose and select the proper antibiotics. Bacteria are often difficult to recognize without proper staining and colonization techniques. Symptoms alone tell little, for many different strains of bacteria will cause similar reactions in fish and different species of fish will respond in a variety of ways to the same bacterial infection. Therefore, it is necessary to identify the pathogen using such parameters as size, shape, mobility, and most importantly the colony characteristics and gram stain reaction.

The use of antibiotics to control bacterial diseases has led to resistance of many bacteria to the most commonly used materials. In some long established cultured fish populations, the ambient bacteria species have become resistant to all available antibiotics. Without a means of treatment, bacterial diseases will run their natural course, often decimating the population. When treating fish populations, one must be careful to alternate between

available antibiotics to avoid or postpone resistance.

One of the more frequently encountered fish bacteria is *Flexibacter columnaris*. The infection is known by a variety of names including Columnaris disease, Cottonmouth disease, tail rot or mouth fungus. The last two names refer to a common symptom, the development of a whitish, puffy material around the mouth and fins. The bacteria attacks the mucosa but can also attack areas where the fish has lost its protective mucus coat or scales. This disease is often seen after a seine operation or after excessive fish handling. Following the initial infection, the white, puffy areas become lesions. As the colony grows, these areas begin to deteriorate into open wounds which can remove entire fins and expose the skeleton. Obviously, mortality soon follows. Spread of the disease is rapid as bacteria washed from infected wounds contact other fish in the population.

Identification of *F. columnaris* is difficult, and the likelihood of encountering various strains is high. In most cases, special laboratory analysis and microscopic techniques is necessary. However, preliminary investigations can be done at the local hatchery. The bacteria is generally found in aggregates of columns or stacks. This is a reliably diagnostic character. With phase contrast and oil immersion at 100X, the individual bacteria appear as a long rod-shaped, gram-negative form. This character is similar to many other pathogenic and nonpathogenic varieties. The best approach for positive identification of *F. columnaris* is by fluorescent antibody tests which can be conducted at State Fish and Game pathology labs.

Treatment of fish populations infected with *F. columnaris* has resulted in varying degrees of success depending upon the strain and levels of resistance. For the most part, treatments with penicillin, Ampicillin or Terramycin are effective in controlling infections (Stickney 1986). Infected and dead fish should be immediately removed from ponds in order to increase the effectiveness of the antibiotic. In the event that resistance of antibiotics occurs, ponds can be treated with potassium permanganate at 2 mg/l for two hours on alternate days to get marginal control (Brown 1980).

Another stress related bacterial infection of equal concern to aquaculturists is caused by the obligate pathogen *Aeromonas hydrophila*. This gram-negative bacteria is known worldwide and will infect most, if not all, freshwater fishes. The disease for which it is responsible is commonly known as bacterial hemorrhagic septicemia or less commonly, infectious abdominal dropsy. The *Aeromonas* bacteria is not found free in the water but only on or in infected fish. It is transferred to healthy fish via contact and causes infection generally in the early spring and commonly after some stress related event. An infected fish may carry the bacteria for long periods without showing symptoms, all-the-while passing it to uninfected fish within the population. *Aeromonas* can also be transferred from one fish to another with the monogenetic trematode *Gyrodactylus*. This parasite harbors the bacteria and transfers it with its young to a new host. Clinical or nonclinical infections with *Aeromonas* are commonly a precursor to viral infections.

The symptoms of bacterial hemorrhagic septicemia are varied and often depend upon individual species reaction. However, the accumulation of fluids in the abdomen and the subsequent dropping of that region into a extended protuberance is characteristic of all infections. In the later stages of the infection, it is common to find ulcers or hemorrhages on the epidermis.

Treatment of a fish population infected with *Aeromonas* follows that of other bacterial diseases. The use of antibiotics is the most effective means where resistant strains do not occur. Those most frequently used antibiotics against *Aeromonas* are Oxytetracycline™ (also known as Terramycin) in an oral dose of 50 to 75 mg/kg body wt/day for ten days, or Chloromycetin™ and Streptomycin™ in a hyperosmotic solution after bathing fish in a hypoosmotic solution until mildly dehydrated (Brown 1980, Stickney 1986).

The bacillus, *Mycobacterium piscium*, causes a serious fish disease called Mycobacteriosis or fish tuberculosis. The disease is chronic and frequently fatal. The bacillus is nonspecific and produces the same symptoms regardless of the species of fish infected. The symptoms most diagnostic of this disease are dark red colorations under the epidermis, progressing to hemorrhages and ulcers, protrusion of the eyeballs and distension of the abdomen. These symptoms can be associated with loss of reproductive ability and loss of weight due to a refusal to feed. If infected fish are dissected, tubercles can be found on any of the organs, especially the kidney, liver and spleen. Once the infection has taken hold, there is little chance the host's immune system will be able to fight off the bacillus.

The bacterium is a slightly curved or straight bacillus which is gram-positive, nonmotile and aerobic. It is best grown on artificial medium or upon an egg based medium. Optimum growth temperatures range between 25°C to 35°C. The colonies have a characteristic smooth surface with a light yellow color.

The disease is spread with equal effectiveness by contact with infected fish either prior to the onset of symptoms or during the display of symptoms. The disease can also be spread by the ingestion of infected tissue of dead or dying fish. In viviparous fish, such as mosquitofish, the disease can be transmitted transovarially (Roberts 1978).

There is no known control or treatment of fish once the disease has taken hold of a population. In some cases, the disease will burn itself out without destroying the entire population, in other situations none survive. The outcome of a particular disease event may depend upon the virulence of the pathogen, the resistance of the host or the effectiveness of transmission.

The final bacterial pathogen considered here is *Pseudomonas fluorescens* which causes the fish disease known as hemorrhagic septicemia. This disease can be either chronic or acute. In most situations the disease advances over a relatively long period of time. It is believed by most fishery pathologists that *P. fluorescens* is an opportunistic pathogen and that the disease is closely related to environmental stresses such as pollution, temperature extremes and overcrowding (Snieszko 1970). The bacteria takes advantage of the reduced immune system which is commonly associated with environmental stress. Depending on the level of stress, the disease can progress at various speeds. Infections are not known to occur in fish populations living in optimum environmental conditions.

The most characteristic symptom of this disease is hemorrhagic skin lesions. These ulcerations form expanding bleeding pockets in the epidermis which can lead to the eventual loss of entire body regions. This symptom can be associated with many secondary symptoms such as loss of feeding behavior and, therefore, a loss of weight, the loss of normal sheen and color, and becoming generally lethargic. Once the disease progresses to the point of open skin lesions little can be done to prevent mortality, which occurs from either the hemorrhagic septicemia, or from secondary bacterial and fungal infections.

The bacteria is an aerobic, gram-negative rod very commonly found in soil and water. With microscopic examination of a smear taken from the region of hemorrhages, large numbers of cells can be found individually, or less frequently in pairs. Most strains have many polar flagella and are actively motile. Colonies can be best grown on normal nutrient media at standard temperatures. The colonies will appear as smooth rounded domes with a fluorescent yellow color.

The best treatment for infected populations is improvement of the water quality. Maintenance of normal levels of heavy metals, pH, water temperature and fish density is the best preventative. Once stress is reduced on the population, the immune system can best defend itself from this ever present pathogen. It is possible to reverse the progress of the disease in populations with early infection levels.

Viruses. Viruses cause a significant number of fish diseases, of which only a very few have been studied. At the present time no significant work has been done on viruses infecting mosquitofish. However, there is no doubt that a sizable array of varieties exist on and are pathogens to the mosquitofish. Viruses receive the blame for the many unknown or unidentified pathogens causing diseases in fishes.

The best means of prevention is to avoid the virus. Make sure that all recruited fish are isolated and observed before introducing them to the main stock. Make certain that incoming water is clear of dead or sick animals, as most pathogenic viruses do not live long outside the body of their host. Most importantly, always maintain the pond environment at the optimum levels for the given fish species to reduce the level of stress, and thus maintaining normal immune functions.

Stress. Mortality of the host occurs when it experiences environmental stress regardless of the parasite load and relative abundance of ambient pathogens. Stress is the underlying impetus for death of the individual. If the environment of the host declines, the stresses involved will likely shift the tenuous balance of existence between host and parasite in the favor of mortality. Stress effectively disrupts or alters the system established with parasites and their hosts or temporarily opens immunological doors for the single-celled pathogens. Therefore, the most important action a fishery manager can take to maintain the health of the fish population is to establish a stress free environment from the start and to maintain it throughout the life of the population. Such actions as constant monitoring of water temperature and chemistry, moderation of population density, and most importantly, the handling of individual fish as infrequently as possible, should all be of highest priority.

Table 3. Parasites and pathogens affecting mosquitofish.

Parasite or Pathogen	Control/Treatment	Host Behavioral Changes	Diagnostic Characteristics	Host Organs Affected	Parasite Description
Gyrodactylus gambusiae	- Sodium Chloride bath - Potassium Permanganate bath 2% for 15 min. - Masoten 20 g/l/5 min.	- scraping body against substrate	- ulcerations & hemorrhages on skin - excess mucus production	- epidermis - fins	- without eyes - bilobed anterior - one pair lg.anchors - sixteen hooklets
Dactylogyrus anchoratus	- Sodium Chloride bath - Potassium Permanganate bath 2% for 5 min. - Masoten 20 g/l/5 min.	- at surface gulping - excessive opercular movement	- ulcerations on gills - thick mucus excretion	- gills	- four eyes - four lobed anterior - one pair of anchors - sixteen hooklets
Cleidodiscus sp.	- Sodium Chloride bath - Potassium Permanganate bath 2% for 5 min. - Masoten 20 g/l/5 min.	- at surface gulping - excessive opercular movement	- ulcerations on gills - thick mucus excretion	- gills	- four eyes;ant.lg.;post.sm. - two pair of anchors - seven pair hooklets - fourteen hooklets
Urocleidus seculus	- Sodium Chloride bath - Potassium Permanganate bath 2% for 5 min. - Masoten 20 g/l/5 min.	- at surface gulping - excessive opercular movement	- ulcerations on gills - thick mucus excretion	- gills	- four eyes;ant.sm.;post.lg. - two pair of anchors - fourteen hooklets - all hooklets proximal
Diplostomulum scheuringi	- interuption of parasite life cycle	- reduction in feeding - reduced fecundity	- no known changes	- coelom - eyes - brain	- elongate - anterior oral sucker small - poorly differentiated - ventral sucker
Postodiplostomum minimum	- interuption of parasite life cycle w/molluscicides or w/pond screens	- no known changes	- no known changes	- mesenteries - coelom - muscles - other organs	- elongate - narrow anterior - anterior sucker large - central sucker - broad posterior
Eustrongylides wenrichi	- interuption of parasite life cycle	- some difficulty swimming - narcosis	- protruding ventrally or laterally	- peritoneal	- bright red coiled cyst - transverse striations - twelve cephalic papillae in two circles of six
Camallanus oxycephalus	- interuption of parasite life cycle	- no known changes	- no known changes	- rectum	- red color - no lips or papillae - buccal capsule - two chitinous valves
Bothriocephalus sp.	- interuption of parasite life cycle	- lethargic	- body thin, pale - reduction in fecundity	- intestines	- head an arrowhead shape - scolex w/2 bothrium - secondary segmentation - white color
Proteocephalus sp.	- interuption of parasite life cycle	- lethargic	- body thin, pale	- intestines	- scolex w/o hooks - 4-5 suckers in ring - neck unsegmented
Octospiniferoides chandleri	- interuption of parasite life cycle	- lethargic	- anal bleeding - ulcerations - emaciation	- intestines	- a proboscis with three circles of hooks - 8-10 hooks per circle
Neoechinorhynchus cylindratus	- interuption of parasite life cycle	- lethargic	- emaciation	- intestines	- a short proboscis, three circles of hooks - six hooks per circle - circles spiraling
Lernaea sp.	- kill eggs & larvae w/0.76% Sodium Chloride bath - Bromex™ 0.12-0.15 ppm - Dipterex™ 0.2-0.3 ppm	- lethargic	- parasite protrution - inflamed tissue	- blood - tissue	- head & thorax fused - cephalothorax - branched horns - body:cylindrical tube - two caudal rami
Saprolignia sp.	- None - immediately remove infected fish	- listing on water surface	- presence of hyphae on any part of body	- any external body region - initiating on fins & around mouth	- white hair-like mass - hyphae w/swollen tips - puffy-white

Table 3. Concluded.

Parasite or Pathogen	Control/Treatment	Host Behavioral Changes	Diagnostic Characteristics	Host Organs Affected	Parasite Description
Ichthyophthirius multifiliis	- flush ponds w/fresh water - Potassium Permanganate 2 - 5 ppm - Malachite Green 25 ppm - Formalin 15 - 25 ppm - Copper Sulfate 40-50 ppm	- listing on bottom - rubbing on substrate - lethargic	- white spots - excessive mucus - glued fins	- encysted under skin	- uniformly ciliated sphere - a "U" shaped nucleus
Trichodina sp.	- flush ponds - maintain rapid water movement	- gulping air - excessive opercular movement	- sm.hemorrhages on skin & gills - excessive mucus - gray film on skin	- skin - fins - gills	- disc-shaped - outer margin w/cilia - inner ring w/hooks
Ichtyobodo sp.	- flush ponds - flow through system	- rubbing & scraping - motionless on bottom	- whitish film	- skin - gills - fins - mucus	- very small - ovoid - four flagella; 2 short, 2 long
Myxobolus sp.	- flush ponds - no known treatment	- atrophy	- developmental malformations - bulges - pocks	- skin - gills - fins	- oval - two polar capsules - tightly coiled polar filaments
Glugea sp.	- flush ponds prophylacticly - no known treatment	- atrophy	- distensions - lumpy swellings	- intestinal cells - epidermal cells	- oblong - one polar capsule - one filament
Flexibacter columnaris	- Penicillin - Ampicillin - Terramycin	- lethargic	- white puffy material around mouth & fins - lesions - open wounds	- mucus - fins - scales	- gram (-) red - cells aggregated in columns or stacks
Aeromonas hydrophila	- antibiotics	- lethargic	- accumulation of fluids in abdomen - dropsy - ulcers in epidermis - emaciation	- organ tissue	- gram (-)
Mycobacterium piscium	- antibiotics	- loss feeding behavior - lethargy	- emaciation - dark red colorations under epidermis - hemorrhages - ulcers	- any organs	- gram (+) - curved - non-motile - colonies:light yellow
Pseudomonas fluorescens	- antibiotics	- loss feeding behavior - lethargy	- hemorrhagic skin lesions in expanding bleeding pockets - emaciation	- any organs	- gram (-) red - many polar flagella - motile - colonies:fluorescent yellow

INCIDENTAL PRODUCTION OF MOSQUITOFISH IN AGRICULTURAL, DOMESTIC, AND INDUSTRIAL WATER SOURCES

Generally, mosquito abatement districts can meet or supplement their mosquitofish requirements for biological control programs by harvesting fish from previously stocked impoundments often excavated for other purposes. Potential fish sources may include:

1) Agricultural Impoundments-irrigation reservoirs, field return sumps, roadside ditches, dairy and poultry wastewater lagoons, duck or farm ponds, stock watering ponds and troughs, frost protection ponds, wildfowl refuges, borrow pits, natural depressions, and warm water springs;
2) Domestic Impoundments-municipal waste treatment ponds, recreational and ornamental lakes, reservoirs, reflection pools and municipal fountains, golf course water hazards, abandoned swimming pools, and flood channels;
3) Industrial Impoundments-fire protection ponds, waste treatment ponds, power plants or process coolant ponds and ditches, and water runoff collection sumps.

These varied sites may or may not always be suitable for fish but should be considered, especially if they also serve as mosquito sources. Technicians should exercise care when contemplating the stocking of a particular source. Inappropriately stocked fish may plug up screens, pumps, sprinkler heads, and generally prove to be a nuisance to the owners/operators.

It is often possible to proceed with stocking of a source if measures are taken to avoid these problems. Pump intakes may be screened to prevent the ingestion of mosquitofish. It is usually necessary to construct a screen enclosure in the form of a large box or cylinder into which the pump intake is inserted. The greater the surface area of the enclosure, the less likely it is to be plugged with fish or other matter. Greater pumping flow rates also mandate larger screening devices.

If the drain or pump intake orifice is located in the bottom of a basin or pool, one can place a weighted screen enclosure over it. Periodic inspections should be conducted to remove excess debris and to check for tears or holes in the screening. Stainless steel or galvanized metal screening works very well for these devices.

Fishery technicians should cooperate closely with the owner/operators of these stocked impoundments. Water management is crucial to the successful employment of these types of stocked impoundments. Often arrangements can be made whereby the technician may be allowed to adjust water levels and maintain suitable water quality. Immediate water needs or pond drainage and cleaning may necessitate prompt removal and subsequent restocking of the fish. Also, the technician should be advised by the owner when chemicals or industrial wastes are discharged into a pond so biological effects may be closely monitored. If these discharges prove to be toxic to the mosquitofish present, not only will your supply of fish be lost, but the presence of decomposing fish will be immediately noticed. Dead fish will have to be seined, removed, and buried before this occurs.

Many of these ponds must be maintained in a vegetation free condition to adequately serve their primary function. In these cases, fishery technicians may work with the owners in selecting weed treatment agents and methods that will benefit both users.

Some of the most suitable fish habitats have proven to be irrigation return sumps, golf course ponds, and municipal and food process wastewater treatment impoundments, because these waters are very rich in plant nutrients. Nutrient rich (eutrophic) water promotes tremendous development of phytoplankton, zooplankton, and small aquatic insects. Eutrophic waters may become very turbid with unicellular algae "blooms". This can be both beneficial and detrimental to stocked mosquitofish. The increased turbidity serves to provide practically unlimited forage and the decreased visibility reduces cannibalism by the sight feeding adult mosquitofish on their offspring. Unfortunately, algal populations can also cause wide diurnal fluctuations in the dissolved oxygen content of these waters. During daylight hours, photosynthesis results in the release of great amounts of oxygen, but nocturnal respiration by these same plants will consume oxygen. Calm, cloudy summer weather conditions may result in algae die-offs and subsequent vegetative decomposition which may temporarily eliminate all dissolved oxygen in a pond. Also, if phytoplankton populations are permitted to become too dense, oxygen deficits are more apt to occur which may cause fish suffocation. During winter months, phytoplankton blooms may die due to cold

temperatures and inadequate sunlight. This can result in oxygen deficits which may cause fish mortality. By limiting the nutrient input (feed and/or fertilization) to a body of water, particularly in the fall and sometimes early spring months, algal blooms can be controlled so that dissolved oxygen fluctuations are not so extreme.

It is sometimes possible to obtain the cooperation of wastewater treatment facility operators so that nutrient loading is carefully monitored and controlled to levels acceptable to both users. Newer municipal treatment plants appear to provide more stable environments for fish, especially when their oxidation/stabilization lagoons are arranged in series with adequate sewage detention times to form a satisfactory gradient of organic loading from pond to pond (Fisher 1972, Fisher et al. 1972).

Additional reading is suggested in the Extensive Culture Techniques section of this publication that may directly relate to Incidental Fish Production (especially Water Supply, Aquacultural Stocking Methods, Parasite and Disease Control, Predators and Their Control, and Aquatic Weed Control).

EXTENSIVE MOSQUITOFISH CULTURE

Introduction. Numerous mosquito abatement districts have expanded their use of mosquitofish over the years. The districts located within the Sacramento-San Joaquin Valley are using millions of mosquitofish each year for the control of rice field mosquitoes. Several of these districts have created their own fish culture facilities to help them meet local needs for mosquitofish. Allied research projects are being conducted on a continuing basis to arrive at suitable, efficient methods for extensive fish production. In addition, the University of California has been given research grants to learn more about mosquitofish, their culture, biology, and use.

The primary function of extensive fish culture is to propagate large numbers of fish in specialized outdoor impoundments through the use of supplemental feeding and pond fertilization. Historically, very little information on specific aquacultural techniques was available. However, in 1970 enough interest was generated in fish farm production of mosquitofish that a few California mosquito abatement districts decided to attempt mass rearing methods. The following text is largely the result of this applied research.

Basic to any successful extensive rearing program are the following criteria.

Economic Factors. It would be ill-advised to undertake the development of an aquaculture facility without first determining proposed costs for rearing the fish. One should consider costs for the following:

1. Land acquisition or lease
2. Excavation
3. Pipe and plumbing
4. Water supply development
5. Utilities
6. Feed
7. Fertilizer
8. Herbicides, fish medications, rodenticides
9. Allied equipment (aeration equipment, buckets, feed and fertilizer storage structures, herbicide spray equipment, predator control gear, pumps, scales, seines, tanks, trucks, waders)
10. Labor (construction and routine maintenance)

It would be inappropriate to attempt to provide actual costs for the above listed criteria, as they would undoubtedly vary tremendously throughout California. Other factors which would affect costs would be the overall size of the facility, aquacultural suitability of the area selected, aquacultural materials employed, and finally, inflation.

Site Selection. Geographical location is important as local climate will determine the length of the growing season. Local climatic factors, such as wind, may also be crucially important. In flood plain areas, ponds should be constructed to avoid periodic high water encroachment.

Soil type and slope are other criteria to consider. Impervious soil types are necessary to reduce water loss through seepage. Some slight slope of the land aids in the construction of ponds, but too much increases excavation and plumbing costs. The U.S. Soil Conservation Service can provide both expert soil analyses and pond construction information and consultation.

Vandalism potential is another factor that should be taken into account. Posted perimeter fencing is a virtual necessity to exclude people as well as animals. Expensive equipment used at the fish farm should be locked up or otherwise protected from theft or vandalism. Security patrols should be conducted even during low maintenance periods. This action will offer greater protection to the fish, especially if a vandal turns off the pump or alters the water control valve settings.

Water Supply. Suitable water is of primary importance in site selection. Quantity as well as quality must be considered. Spring water, well water, surface water from streams and lakes, and municipal water supplies can be used to support fish life. Spring or well water is usually preferred to other water sources because of its freedom from chemical contaminants and biological organisms. Water volume of at least 15 gpm per acre is needed for fish culture (Flickinger 1971). Ground water sources are especially attractive in that they offer temperature stability to a pond because their temperature is roughly equivalent to the mean annual temperature. Well water quality may or may not be suitable for mosquitofish rearing as it often contains substantial amounts of toxic gases, such as carbon dioxide, and/or hydrogen sulfide. Spray aeration of this water will usually remove these gases.

Guppies and mosquitofish are remarkably tolerant of poor water quality and can often be reared in waters not suitable for other species. For example, they thrive in many sewage oxidation impoundments that will not support introduced game fish, although tilapia have survived and reproduced in at least one location (Murray 1976). Mosquitofish also tolerate wide ranges in pH, alkalinity, and total hardness. It would be practically impossible to provide actual tested limits for all these factors as toxic effects are most often attributed to complex combinations and interaction of these chemicals and physical factors. The best way to test the suitability of a particular water source for fish habitation is to conduct a bioassay by either placing caged fish into the water source or by collecting the water and conducting the test in laboratory aquaria Serial dilutions of the tested water can be used if severe toxicity is expected.

An individual contemplating rearing large numbers of fish should definitely purchase or make up a water quality test kit (see appendix C). This kit should have the necessary reagents or instruments which would permit the measurement of pH, temperature, dissolved oxygen, hardness, alkalinity, carbon dioxide, ammonia, and turbidity.

Field turbidity measurements are usually taken with a device called a Secchi disk. It is simply a circular metal disk attached to a string that has been marked or knotted at regular intervals. The upper disk surface is divided into four quadrants alternately painted black and white. One merely sinks the disk into the pond and takes a depth measurement at that point the disk disappears from view. This simple instrument allows one to monitor the density of planktonic algae in a rearing pond.

Typical ranges for chemical and physical parameters observed in mosquitofish sources of the Sacramento-San Joaquin Valley are as follows; Total alkalinity as $CaCO_3$, 50 - 250 mg/L; Ammonia, 0.1 - 1.0 mg/L; Carbon dioxide, 5 - 15 mg/L; Total hardness as $CaCO_3$, 50 - 250; Dissolved oxygen, 7 - 15 mg/L; pH, 7.5 - 10; and Secchi depth, 15 - 50 cm. Other areas in California may exhibit slight variations from the values presented here.

Pond Design and Construction.- While mosquitofish easily adapt to most inland waters of California, long term or overwinter survival in some sites may be limited. Mosquitofish require stable (stenothermal) winter temperatures and varying (eurythermal) summer temperatures for optimum production and survival.

Shallow ponds (less than one meter of three feet deep) are more easily influenced by wind currents to the extent that pond temperatures fluctuate markedly. Thus shallow ponds favor summer growth and reproduction, but may discourage overwinter survival. Conversely, ponds of greater depth (two meter or six feet) provide more stable water temperatures by virtue of their increased volume and thus promote greater overwinter survival, but may adversely affect summer production.

It is possible to design ponds that favor both summer production and winter survival. Wind is the most important factor in causing temperature changes in ponds. It does this by moving the surface water in one direction. This water is thereby cooled and replaced by warmer subsurface water, which is subsequently cooled, creating a cyclical pattern of water movement. When the water is continuously turning over, large changes in temperature may be induced, depending on ambient air temperatures. Narrow, deep ponds with high levees offer greater resistance to wind induced water cycling. Orienting the pond so its length runs perpendicular to prevailing winter winds also reduces this turning over of the water. All these design parameters favor the creation of a stenothermal environment for the fish.

In the summer, these same ponds can be made more eurythermal by simply reducing the depth of the pond so the volume is reduced to about half of what it was during the winter months. This may not be effective if wind currents, blocked by the existing levees, are prevented from turning the water. Long narrow, rectangular ponds are preferred to square or round ponds for two reasons. First, several narrow ponds present more shoreline to the fish than does a single pond of greater width. This is important because mosquitofish are strongly oriented to these shallow areas for forage, resting, and reproduction. The only drawback to narrow ponds is their lower efficiency with regard to land use. More levees have to be built which cost more and take up area otherwise available for water. The wider rectangular ponds of the river rock rip-rap design provide many of the advantages of the narrow design except that the amount of shoreline is reduced. By providing a rip-rap of 6-15 inch river rock cobble around the shoreline of this pond, many more resting and reproductive areas are provided. The holes or spaces between the rock cobble also provide harborage for the newly released broods of young.

The U.S. Soil Conservation Service can provide aid concerning pond construction, sealing and piping installations. They provide free consultation as well as literature about pond construction costs, methods, equipment, and supplies. Actual pond construction techniques are too detailed and variable with respect to geographical location to include in this manual.

Pond Sterilization. Fish farmers often sterilize ponds before filling. Methods used include hydrated or slaked lime added to the soil (Huner and Dupree 1984) or potassium permanganate applied to partially filled ponds. Pond sterilization is strongly recommended if noticeable disease problems are known to have developed in the previously harvested fish crop. The quicklime should be spread evenly over the dry pond bottom prior to filling, then the pond should be filled slowly. It is essential that enough chlorine is released upon the addition of the water to kill all harmful organisms present. A concentration of around 8 to 10 ppm, which equates to a treatment rate of 1,000 pounds per acre for most soils, will usually suffice. Chlorine test kits for swimming pool use will allow one to determine the initial chlorine concentration. Subsequent chlorine measurements will permit a technician to determine when it is safe to introduce brood fish. Even low residual chlorine levels (less than 1 ppm) can be toxic to many fishes; therefore, it is necessary to monitor the pond water until no chlorine can be detected.

Phytoplankton and Zooplankton Production. Bait and certain game fish farmers make it a practice to increase the fertility of their pond water to boost summer fish production. These nutrient-rich (eutrophic) waters stimulate the production of phytoplankton which in turn become forage for zooplankton populations. In this food chain, small fish readily prey upon zooplankton and grow rapidly. In addition, the need for artificial or supplemental feeds can be lessened somewhat. With mosquitofish the decreased clarity of the pond water due to the phytoplankton blooms helps inhibit cannibalism. Another benefit of fertilization is that it helps prevent the establishment of rooted aquatic plants through its effective shading of the pond bottom.

Successful fertilization programs usually require some initial experimentation, constant monitoring, and proper timing. Simplified, a fishery technician introduces organic and/or inorganic fertilizer to the ponds in the early spring after post-sterilization filling has been completed. With rising water temperatures and increased photostimulation, these nutrients soon foster the growth of dense planktonic or unicellular algal blooms. Follow-up applications of fertilizer are periodically made to maintain these algal blooms throughout the summer, but are discontinued in the fall before the sharp drop in water temperatures and the onset of inclement weather conditions.

Inorganic granular or liquid fertilizers used for agricultural purposes are often employed in fish culture. Many different fertilizer formulations are generally available, however, liquid fertilizers are more flexible in use and management and are usually more economic to use. All fertilizer manufacturers are required to provide composition data on their labels. For instance, a 16-20-0 (NPK) formulation would consist of 16% available nitrogen (N), 20% available phosphorous (P_2O_5), and 0% of potassium (K_2O) by weight.

Recommended dosage rates are quite variable. Most fishery workers suggest an initial application in an amount sufficient to provide 8 pounds of available nitrogen (0.25-1.0 ppm active ingredient, based on total pond volume), 8 pounds of available nitrogen (0.25-1.0 ppm), and 2 pounds of available potassium, per acre, in February or March. Secchi disk readings should be taken once a week and ponds should be fertilized with a minimal amount of organic and inorganic fertilizer to maintain 12-18 inch (30-46 cm) planktonic algae bloom. Infrequent fertilization with larger amounts of fertilizer creates drastic changes in pond chemistry. As nutrients are used up algae blooms die off lowering pH and dissolved oxygen (D.O.) levels. Often moderate (2-3 mg/L) changes in D.O. levels will not kill mosquitofish directly but will bring them to the surface which makes them more susceptible to predation. This may amount to four to eight fertilizer applications each summer. Most researchers recommend that the last application be made in late September or October. However, oxygen depletion problems associated with the subsequent organic decomposition of dense growths of algae may necessitate earlier termination of fertilizer in some areas. Only experimentation will determine what will work best at any given location.

Sport fish production studies by Boyd and Sowles (1978) have demonstrated that it may be more economical and equally productive to apply only phosphorus at dosage rates of 9 kg (P_2O_5) per hectare (8 lbs P_2O_5/acre) throughout the summer.

There are several ways of applying fertilizer to a pond. A few methods include; to hand or mechanically broadcast it over the entire water surface; to place it in a burlap sack which is suspended from an automobile inner tube and allowed to drift about the pond; to dissolve it in water and then spray or pump it into the pond; or, to build a small flat-surfaced platform and submerge it in the pond to a depth of around one foot (30 cm). The fertilizer is simply poured onto the platform and ordinary water currents will slowly dissolve and circulate it throughout the pond. Care should be taken to avoid applying fertilizer to areas where fish congregate.

It is both wasteful and dangerous to overfertilize ponds. Secchi disk measurements should never be less than 8 inches (20 cm). Excessive planktonic algae blooms may severely reduce dissolved oxygen levels during hours of darkness and overcast days to the extent that fish may suffocate. It is recommended that a D.O. measurement be taken between 6 and 8 o'clock a.m. when it can be expected to be at its lowest level. Conversely, peak D.O. readings are usually recorded in the late afternoon or shortly before dusk. If a very low reading is taken at dusk, one can usually assume that D.O. levels will reach a critical level by early morning. To prevent this from occurring, a technician may elect to flush out old pond water and replace it with fresh, aerated water, provide supplemental aeration of existing pondwater through mechanical agitation, pump/spray recirculation, or add potassium permanganate to temporarily alleviate D.O. deficits.

Even with fertilization, it is sometimes difficult to get an algal bloom started. In such situations, poultry manure or barnyard wastes can be added to stimulate algae growth. In addition, one can obtain a few buckets of "green" water from a successfully fertilized pond and use it to inoculate the barren pond.

Organic fertilization of ponds is also recommended and is often very inexpensive. Poultry manure is preferred to other types of livestock manures because it contains higher percentages of actual nutrients, is less bulky and is more easily handled and applied. Nearby chicken or turkey ranches are excellent and inexpensive sources of fertilizer.

The nutrient content and general composition of various livestock manures are extremely variable so a technician must experiment with the type of manure obtained. Typical application rates for chicken manure range from 100 to 200 lbs per surface acre (112 to 224 kg/hectare). Other livestock manures must be applied at greater rates than this because of their lower nutrient content. Some manures, especially those with large amounts of straw and other relatively insoluble materials, are not recommended unless they are finely ground prior to use. Fresh manures are preferred over old manures because nutrient content is slowly lost in aging. Some fishery researchers recommend that combinations of both inorganic and organic fertilizers be used, however, one must experiment to determine optimum application rates and mixture ratios.

Organic fertilizers are generally applied as coarsely-ground material, liquid slurries, or screened-off manure piles submerged in the corner of a pond. Livestock manures may, through various actions of the rancher, become contaminated with pesticides or medications, both of which could be harmful to aquatic organisms.

Improvements in mosquitofish production can be made by increasing the abundance and diversity of the zooplankton foraging base in the culture ponds. The preferred foods for mosquitofish are copepods, cladocerans, ostracods, insect larvae, and rotifers which appear naturally in many temporary ponds. In newly constructed ponds their presence can be insured by inoculation from established production ponds or seasonal ponds in most areas.

Inoculating the filling production ponds with phytoplankton from established overwintering mosquitofish ponds will insure a phytoplankton bloom. High liquid inorganic fertilizer (LIF) application rates at this time will not harm production as liquid fertilizers are short lived and ponds will usually stabilize after five to seven days depending on weather conditions. Partial filling of production ponds will concentrate fertilizers until planktonic algae becomes dense (8-10 inches). Then production ponds can be completely filled and refertilized. Supplemental pond fertilization should be about half of the initial fertilizer application rate.

When a phytoplankton bloom has been established, inoculate the production ponds with the desired types of zooplankton. The best sources of zooplankton are seasonal ponds and sewage oxidation ponds. These planktors can be collected by pumping them into a plankton net submerged in a container. The collected planktors can then be poured into a unoxygenated mosquitofish transport tank and returned to production ponds to be inoculated. A small sample should be returned to the lab for identification. The recommended time for zooplankton establishment is ten to fourteen days after the pond is filled. This culturing time ensures zooplankton densities of several hundred per liter.

Seasoned production ponds normally do not require zooplankton inoculation. Grass and weeds should be cut short prior to filling, this reduces the chance of filamentous algae developing and using up nutrients intended for plankton. To insure a phytoplankton bloom, "green" water, if available, should be pumped into ponds and fertilized at the same rate with chicken manure and LIF (10-34-0) as in newly constructed ponds. A period of ten to fourteen days will be sufficient for an abundant zooplankton bloom. If too much time elapses before brood fish are stocked, zooplankton will overgraze the phytoplankton. If this occurs, pump zooplankton into ponds with low numbers of zooplankton and pump in phytoplankton from ponds with a dense phytoplankton bloom.

Zooplankton samples should be taken throughout the early production cycle and planktors should be identified to the nearest taxon. Diversity and abundance should be maintained by pumping until the first broods of young are released in the production ponds. To maintain zooplankton populations throughout the rearing cycle, mosquitofish must also be fed a commercial ration once or twice per day.

The most productive ponds contain one or more of the following species; *Daphnia similis, Simocephalus vetulus, Ceriodaphnia veticulata, Moina sp., Cyclops vernalis,* Ostracoda (seed shrimps), or Chironomid larvae (midges).

Aquacultural Stocking Methods. The underlying concept for optimal fish stocking is to introduce a selected quantity of brood fish who, along with their offspring, will fully utilize a habitat while not under- or overexploiting it. The desired result is of course the production of a maximum quantity of fish in excellent condition.

This isn't an easy concept to put into practice as many factors can affect fish production and survival throughout the summer. The following are some basic factors that can strongly influence production.

1) Date of initial stocking and length of production period
2) Pond fertility level
3) Feed quality and quantity
4) General water quality
5) Aquatic competitors (bullfrog tadpoles)
6) Predators (birds, bullfrogs)
7) Parasites and diseases
8) Type and quantity of aquatic weeds

Adult mosquitofish should be stocked as early in the spring as is practicable. The receiving ponds should already have good standing populations of phyto- and zooplankton. Ideally brood fish are stocked before they become gravid, taking advantage of the abundant plankton populations to increase growth and fecundity. Male fish are so much smaller than female fish and their combined biomass is only a fraction of that represented by gravid females that special sex separation procedures are unnecessary.

Optimal stocking conditions occur at different times depending upon one's geographical location within California. In Central California lowland areas, the best stocking time seems to be the months of February or March, however, one may have to stock either earlier or later in other areas within the state.

The timing for the release of the first brood of young appears to be strongly correlated with temperature and to a lesser degree daylength (photoperiod). In Sacramento County ponds, the first brood releases have been observed for the last five years to occur during the month of April. They seem to be triggered to when surface temperatures reach $80^{o}F$ ($27^{o}C$). The photoperiod at this time is approximately 14 hours.

Subsequent broods of young are released throughout the summer and fall. The actual number of broods would depend primarily on local weather conditions, length of growing season, and age of the female when she first enters maturity. Typically fish are born until late fall and in some heated habitats until late December. In heated habitats, young may be released as early as February.

Acceptable stocking rates may vary extensively. In Sacramento Valley pond studies (Coykendall 1977a), a mosquitofish stocking rate of 50 lbs/acre (56 kg/hectare) on May 2nd provided slightly better fish yield/surface acre than did a stocking rate of 35 lbs/acre (28 kg/hectare) and much better yield than was achieved with a rate of 75 lbs./acre (84 kg/hectare). The low stocking rate of this six-month production study actually yielded the most fish (a 17-fold weight increase), however, the medium stocking rate provided the best utilization of feed and fertilization for the surface area they occupied. For this particular locale, the ideal stocking rate probably falls somewhere between the low and medium rates employed in this experiment. Other geographical locations may allow longer or shorter growth and reproduction periods, thereby requiring different stocking rates and stocking and harvest dates.

Nutrition and Feeding Rates. Most dietary rations used for rearing trout, catfish, and especially bait fish (minnows) are suitable for mosquitofish, provided they float well and are in a size and configuration that mosquitofish can easily consume. Many commercial fish rations are now available through livestock feed outlets; however, they may prove to be too expensive for extensive mosquitofish culture programs. The recommended method of selecting a ration is to consult with local fish farmers and make inquiries as to what formulations they are currently using.

Various texts provide basic information and guidelines for developing fish rations for different fish species. Unfortunately, detailed formulations complete with exact ingredients are impractical to duplicate because some of the constituents may not be locally available. It is often possible to substitute ingredients if they are nutritionally similar and are equally acceptable by mosquitofish.

Most bait fish farmers utilize local millers of livestock feeds to mix custom rations for them. Locally produced ingredients are often selected for incorporation in a diet because they are usually less expensive. Many different formulations might find equal acceptance by the fish, therefore, it is fairly likely that one could always incorporate appropriate ingredients.

Millers are often able to provide information about diet formulations because they keep records of mixes they have milled in the past for other customers. This will permit one to get acquainted with what is available and what experienced aquaculturists are using. Rations designed for extensive rearing are rarely nutritionally complete because such diets would be prohibitively expensive. Rather, the typical bait fish ration is regarded as a supplemental feed with the remaining nutrients derived from the consumption of live organisms produced in the fertilized pondwater.

Various livestock and pet feeds might find acceptance as an emergency or temporary ration when other more suitable feeds are unavailable. Examples would include crushed dog food, chicken or other poultry mash.

Local millers may be willing to customize and grind special mosquitofish rations based upon minnow feed formulations. Nutrient levels can vary somewhat but desirable levels for protein range 25% and 35% by weight. One custom mix established specifically for mosquitofish contains 30% protein, 9% fat, 11% ash and 8% crude fiber (Coykendall 1977b) . Its ingredients include wheat bran/mill run (42%), fish meal (20%), soybean meal (20%), animal or vegetable fat (1%), salt (1%), plus very small amounts of vitamin premix and an antibiotic (Terramycin, TM-50). Antibiotic is recommended only when a specific disease is being treated. Millers can obtain vitamin premixes from companies that specialize in vitamin/mineral supplements for livestock feeds. Some of these companies produce minnow culture vitamin premixes that would suffice for a mosquitofish ration.

The ingredients itemized above are milled together and the resultant mixture is ground thoroughly and screened to reduce it to a particulate size approximating that of chick mash. The liquid fat is added to the mix to cause the smaller particulates (fines) to adhere to the coarser ingredients and to make the feed float better.

Mosquitofish rations can be applied by hand or by mechanical blowers installed on vehicles. The rations are supplied daily to the fish at various rates based upon the combined live weight (biomass) of the fish in the pond. Summer feeding rates range between 5% to 10% of the fish biomass, but less feed (1-3% of the fish biomass) is provided when the water temperatures fall below 50°F (10°C) during the winter months. Some savings in feed can be made if one correlates feeding with the pond temperature and feeds less when pond temperatures are low and more when they are high.

When temperatures exceed 90°F (32°C) or drop below 40°F (4°C), food is withheld because the fish don't utilize it effectively. Optimum utilization of feed occurs when the ration is divided into smaller portions and applied several times during the day rather than in a single large feeding. This is especially necessary when fry are present in ponds as they have little energy reserves in the form of stored fat or protein.

Parasite and Disease Control. This is probably the most complex and poorly understood aspect of fish culture. The life cycles and intermediate hosts of many fish parasites are at times complex (see section on Parasites and Pathogens). Unfortunately, the fish farmer very rarely has the laboratory equipment or knowledge to begin to identify the causative organisms. Disease identification is very difficult, even for fish pathologists. Some common diseases and parasites, however, can be identified by the signs and behavior manifested in the animal, especially if one has experienced the problem and has had it professionally identified previously.

Fortunately for mosquitofish culturists, this particular fish is very hardy. Mosquitofish seem to be able to survive and reproduce with chronic low levels of infections or infestations. It is only when the fish become stressed through harsh environmental conditions, poor nutrition, or rough handling that illnesses progress to acute or lethal stages.

Medicines and chemicals for the prevention and control of fish diseases are currently undergoing close scrutiny by federal agencies. Undoubtedly, many chemicals traditionally used by culturists will soon be banned or restricted. The new guidelines and regulations that will be adopted will be primarily concerned with food fish production. Hopefully, mosquitofish will be classified or recognized as an ornamental fish, thereby possibly circumventing some of the proposed chemical restrictions.

When a fish population under culture is suspected of having some disease or substantial parasitic infestation and is showing signs of illness, one should quickly contact personnel experienced in disease identification and have them examine the affected and dead fish. The California Department of Fish and Game maintains a fish pathology laboratory and their personnel have been helpful in identifying diseases and prescribing remedies for those outbreaks.

For additional sources of diagnostic services see appendix C.

Equipment Sterilization. The incidence of disease can be minimized through proper sanitation. All equipment that comes into contact with mosquitofish should be periodically sterilized. Hand nets, seines, traps, aquaria, buckets, and even wading or hip boots may be sterilized through immersion in a chlorine solution. Household bleaches are simply added to tap water in a container suitable for dipping gear. The chlorine solution should consist of one quart per 50 gallons of water (1 liter per 200 liters of water). Items are then immersed for roughly 30 - 45 minutes. Swimming pool chlorine in liquid form is also convenient and usually very economical to use because of its much higher chlorine content. To avoid prolonged exposure to concentrated chlorine solutions and fumes, rubber gloves should be worn and immersion baths should be set up outdoors. All sanitized equipment should be thoroughly rinsed and dried following sterilization.

Fish holding and transportation tanks should be periodically sanitized by filling them with the same solution. After 30 minutes, drain the tank, rinse, and let dry before loading with fresh water and fish .

Predators and Their Control. Mosquitofish are easy prey for many different predators. Shoreline or wading birds, such as great blue herons, green herons, bitterns, egrets, and undoubtedly others can be serious predators upon cultured and wild stocks of mosquitofish. Mosquitofish expose themselves to this sort of predation through their preference for warm, calm shoreline areas and shallows. Their slow swimming capability coupled with their behavior of basking at the water surface actually enhances predation. Many wading birds are crepuscular feeders; that is, they feed during twilight hours. This can be an insidious situation because these predators can do serious damage to fish stocks during twilight periods and leave the area during the day when workers can be present to harass them. Thus, fish numbers can rapidly dwindle with no readily apparent cause. Fecal droppings and tracks along shoreline areas may be the only signs of this sort of predation. Since a large wading bird can readily consume several hundred mosquitofish in a single feeding, several birds can quickly decimate a small pond's overwintering fish population.

Overwintering fish are especially susceptible to predation because they are often very sluggish in their movements. In addition, no reproduction and much slower growth take place during the winter in unheated ponds so recruitment of new fish to the existing biomass is at a standstill.

Diving birds can also be serious mosquitofish predators. Grebe, some diving ducks and other carnivorous swimming birds often establish themselves as residents of mosquitofish ponds because of the easy pickings of bite-size fish. These birds can capture mosquitofish during cold periods by simply striking at the fish as they rest immobile on the mud bottom of culture ponds. Grebes are usually smaller than most wading birds, but are harder to spot and drive from the pond. Their typical escape behavior is to dive and secrete themselves in any vegetation growing along the shoreline. These birds are more secretive in their behavior and may stay hidden while workers are in the area.

Another category of predacious birds are those that catch their prey on the wing. Belted kingfishers, terns, and possibly other similar birds either dive into the water or fly close to the water surface and pick off the fish without actually getting wet. These birds feed during daylight hours and are intimidated by shotgun harassment. Minor damage can be exerted on mosquitofish stocks by opportunistic birds, such as crows, blackbirds, and domestic waterfowl. These species prey upon fish trapped or confined in small areas. Crows often capture fish congregated around the discharge pipes that supply ponds with water. Smaller birds like blackbirds capture mosquitofish basking in shallow inlets and confined water left in drained ponds and ditches.

Avian predators are joined by other vertebrates including bullfrogs and snakes. Bullfrogs are not serious predators but their tadpole offspring consume tremendous quantities of fish feed and can therefore reduce mosquitofish production substantially. Seining and other fish management operations can be severely hampered by the presence of large numbers of tadpoles. Snakes are not usually regarded as detrimental organisms because the small number of fish they consume is more than offset by the frogs they capture.

The red swamp crawfish often aids in controlling aquatic weeds in ponds; however, its burrowing in levees promotes erosion making levee repair necessary in a few years. Crawfish will prey upon mosquitofish as injured fish are often observed in crawfish infested waters. During seining activities, they will attack and injure netted fish while in the net themselves. All in all, crawfish should be kept out of mosquitofish ponds.

Most fishes are not compatible with mosquitofish. For example, accidentally introduced sunfish, catfish or bass will feed extensively on mosquitofish. In fact, early bass hatcheries in this state often employed mosquitofish as forage fish. As with

crawfish, extreme care should be exerted to prevent the accidental stocking of these predacious fishes in mosquitofish culture ponds. Polyculture experiments have shown that one fish, the grass carp, *Ctenopharyngodon idella* will not harm mosquitofish. Presently, this species, in the triploid form, is stockable in the waters of the Coachella and Imperial Valleys.

Aquatic insects can be predators on mosquitofish. These include, backswimmers, water tigers, water bugs, and some water beetles. Sometimes chemical control is necessitated. Whenever chemicals are used, all federal, state, and local regulations must be followed, and only those chemicals registered and approved for aquatic use should be used. Routine chemical treatments should be avoided as they also may reduce zooplankton and beneficial insect populations.

Excluding or eradicating undesirable animals can be difficult and expensive. Virtually all avian predators can be deterred by covering mosquitofish ponds with barrier screening. Several manufacturers now produce lightweight plastic mesh screening suitable for such purposes. Unfortunately, this is very costly, both in labor and materials. Field trials with these materials have shown that they can work effectively if correctly installed. Shorebirds can be excluded by perimeter fencing which covers a narrow bank around the pond and over it out to a depth too deep for wading. The fencing should be installed so that it makes contact with the ground along the shore or birds will simply crawl under it. Also, the portion of the screening that is over water must hang low enough to prevent birds from attaining access to the shoreline by flying under the netting. This type of entry can be prevented if one allows a short skirt to hang down to the water surface from the horizontal overhead screening.

An electric bird barrier has been developed that appears to be performing well. It is basically an electrified livestock fencing adaptation for use with extensive culture ponds. It consists of numerous runs of lightweight electric fence wire installed around the perimeter of a pond to deter wading birds. Additional cross-wires zig-zag over the pond to discourage diving birds. This system has been favorably reviewed by those state and federal wildlife agencies charged with the protection of migratory waterfowl. Standard battery-powered charger units and associated hardware are used in the construction of this system, which is thoroughly described in an issue of the CMVCA Biological Control Committee publication - BioBriefs (15:2).

Traditional methods used to harass other bird species don't generally work effectively on fish eating birds. Scarecrows, gunfire, noise cannons, sound producing equipment, reflective devices, or flagging have been exhaustively tested and simply don't provide long-term avoidance. Eradication of avian predators is not feasible because most of these birds are protected by federal and/or state statutes. Depredation permits can rarely be obtained from the California Department of Fish and Game, although they may issue harassment permits and provide useful information concerning the control of various predators.

Bullfrogs are best controlled by not allowing them access to the culture ponds and removing those adults that migrate into the area. Periodic frog gigging at night is helpful. Egg masses may be netted out of the ponds by observant technicians. Larger tadpoles may be removed through the use of large-mesh seines that allow mosquitofish to pass through. Rapid draining after harvest will often leave tadpoles high and dry. Poison baits are not commonly used because the mosquitofish would probably feed on them.

Crawfish can be controlled by trapping, selective seining, poisoning, and periodic pond draining. An ordinary minnow trap can be easily modified to facilitate the capture of crawfish by enlarging its entry gaps slightly to accommodate this larger animal. Large mesh seines can be drawn through a pond prior to mosquitofish seining to selectively remove large numbers of crawfish. It is unwise to attempt to seine both fish and crawfish at the same instant because netted crawfish will prey upon the captured fish and injure or consume large numbers of them. Additionally, separating the two by hand is time consuming and rough on the fish.

Aquatic Weed Control. Excessive amounts of weed growth in ponds can be detrimental to fish growth, harvest activities, and often fish survival. While moderate amounts of planktonic algae are beneficial to the fish and the pond's food web in general, noxious weeds, such as filamentous algae, frog's bit (*Elodea* spp.), pondweeds, naiads, and cattails must be kept under control.

Many factors affect weed growth. Of primary importance is pond design. Shallow ponds less than 5 feet (1.5 meters) deep or those ponds having gently sloping shorelines encourage weed growth. Water fertility is also important. Excessive fertility can also limit the usefulness of a pond for mosquitofish production. Dissolved oxygen deficits are common to overfertilized ponds. Insufficient fertility fosters the development of submerged rooted aquatic weeds which receive their sustenance from the hydrosoil. They grow well in clear water and the pond may eventually become weed-choked when they grow to the

water surface. Mosquitofish utilize moderate amounts of vegetation for shelter and food production so some plants are desirable. Unfortunately, it is difficult to limit existing weed growth to acceptable levels. Serially arranged ponds are undesirable because weed species and disease organisms can easily pass from one pond to the next with the water. Ponds should receive their water from a common header or manifold to eliminate this hazard. Ponds should also be designed with fairly steep banks and have adequate depth throughout. Shallow waters, which mosquitofish prefer for breeding areas, present problems because weeds may originate there and infest the remainder of the pond.

Potential weed problems are difficult to spot during their early growth when control measures should ideally take place. Turbid waters make direct observations even more difficult. To help solve observation difficulties it is advisable to construct a small weighted grappling hook to secure bottom samples from the center of smaller ponds. Some sort of sampling should be routinely performed throughout the year in all ponds of the facility.

When determined that a potential weed problem exists, there are a few steps to follow to achieve the desired control or eradication. First, identify the weed species correctly. Second, select the best control measure available. If a chemical remedy is proposed, be sure to choose a material that will provide control with minimal toxicity to the mosquitofish or other animals at the facility. Tests may be necessary to determine proper dosage rates. Third, follow the label and method of application recommended by the manufacturer. Local government agencies, manufacturers, their representatives, or university staff may be of assistance in determining a proper course of action.

Table 4. Herbicides used for controlling algae and aquatic weeds.

Herbicides	Trade Name	Agriculture water use ( )[a] Crop irrigation	Livestock drinking	Ornamental pond use (non-irrigation)	Fish-safe at use rates
endothall					
Na salt	Aquathol	Yes (7 days)	Yes (7 days)	Yes	Yes (3 days)[d]
amine salt	Hydrothol 191	Yes (7-25 days)	Yes (7-25 days)	Yes	No (0.3)[b]
diquat	Ortho Diquat	Yes (10 days)	Yes (10 days)	Yes	Yes
LV-4D (2,4-D)	Aqua-Kleen (granular)	No	No	Yes	Yes
copper sulfate	-	Yes	Yes	Yes	Yes & No[c]
xylene	Ortho Aquatic Weed Killer #60	Yes	Yes	Yes	No
simazine	Aquazine	Yes (12 mo.)	Yes (12 mo.)	Yes	Yes
glyphosate	Rodeo	Yes	Yes	Yes	Yes
-	Cutrine-Plus	Yes	Yes	Yes	Yes & No[c]
-	Aquashade	Yes	Yes	Yes	Yes

[a]indicates required holding period before water use.
[b]will kill fish above approximately 0.3 ppm.
[c]more hazardous to fish in low carbonate-bicarbonate water. Trout and salmon are least tolerant.
[d]do not use fish from treated water for food or feed within three days of treatment.
NOTE: Consult the label for use and restrictions. Consult California Department of Fish and Game authorities before allowing treated water to enter streams, rivers, ponds, lakes, or the sea.

INTENSIVE CULTURE TECHNIQUES

Introduction. Intensive fish culture practices usually attempt to control or at least modify environmental factors affecting the organism being reared. Culture water may be heated, filtered, sterilized and recirculated in a typical intensive system. Photoperiod (day-length) may be modified with artificial lighting. Feed requirements are met with nutritionally-complete diets. The advantages of intensive culture are that the fish are provided with optimal growing conditions, thus producing faster growing and maturing individuals during any season. Inherently the culture facility is more compact in size than equally productive extensive systems. Their disadvantages over extensive systems are they are more expensive to build and maintain and require more expertise to operate. However, such systems prove useful or even necessary in certain circumstances, such as in urban areas where the cost of land makes extensive culture prohibitive.

Intensive systems can be categorized into open or closed systems. It has always been easier to discharge waste culture water than to recycle it. A recirculated or closed water system presents some special problems. First, before the water can be reused, it must be stripped of its load of suspended solid wastes, uneaten food, and fish waste products (metabolites). Metabolites consist largely of ammonia and ammonium compounds that, if in high concentrations, can be lethal to the fish. Mechanical filters with clarifiers are used to remove suspended matter while several different methods have been used to strip nitrogenous compounds from recycled water. The most common and inexpensive technique is that of installing a biological filter within the system. Anaerobic and aerobic bacteria living on the filter media convert these metabolites to low energy nitrate compounds that are essentially nontoxic to aquatic life. At this point, the water can be sterilized, heated, aerated and reused in the culture tanks.

A typical closed or recirculated culture system would consist of the following components; a water source; a heater or heat exchanger; the culture tanks; clarifiers; mechanical, biological, and chemical filtration devices; and sterilization equipment. Associated equipment would include automatic feeders, water quality monitoring kits, grading and transporting devices and other cultural equipment. To use such a system economically, fish density must be kept at a maximum in the tank, therefore, disease free stock must be obtained initially. Because fish are stocked very densely, aseptic conditions and constant water quality and disease monitoring are critical. Table 5 shows recommended water quality parameters for finfish in general.

Disease Control. It is best to prevent disease from occurring than to rely on treatments after an outbreak has occurred. The cost of following a few guidelines that help to prevent disease outbreaks is small compared to the loss of stock and the cost of chemicals used to treat a disease. These guidelines are as follows:

1. Quarantine all fish before introducing them into a system to ensure they are disease free.

2. Regular measurement of the major water quality parameters should be a standard procedure, e.g., dissolved oxygen, temperature, pH, ammonia and nitrite.

3. Avoid over-feeding and over-stocking culture tanks. Over feeding is probably the most common cause of poor water quality.

4. Regular removal of uneaten food and dead fish is important in reducing the production of ammonia.

5. Adequate prophylactics should be used when situations require e.g., using a salt bath after netting to prevent a fungal infection in abrasions.

6. Where possible a disinfectant tank should be used for nets after use

7. Handle fish as infrequently as possible.

Information on specific diseases and treatments can be found in the section, Parasites and Pathogens.

Nutrition. Cultured fish have no other source of sustenance other than what is provided them. The diet must be carefully selected and properly administered to ensure optimal growth and utilization. Although dietary requirements of mosquitofish have yet to be determined, a variety of processed fish feeds have been used successfully. The

Table 5. Water Quality (adapted from Aquatic Eco-Systems, Apopka, Florida)

Parameter	Desirable Levels for Finfish
Alkalinity	50-200 ppm (as $CaCO_3$)
Ammonia	< 0.01 ppm (as NH_3)
BOD, COD	Keep as low as possible. These tests are a measure of how much oxygen will be required to degrade organic material present in your water.
Carbon Dioxide	< 3 ppm
Chloride (Free and Total)	<0.033 ppm
Copper	0.006 ppm (in soft water) 0.3 ppm (in hard water)
Dissolved Solids (Total)	<400 ppm
Formaldehyde	<0.5 ppm
Hardness (Total)	50-200 ppm
Iron	<1.0 ppm
Nitrate	Non-toxic end product of nitrification. Serves as a nutrient source for plants
Nitrite	0.55 ppm
Oxygen	3-8 ppm (variable with species)
Ozone	0.003 ppm
pH	6.5-8.5 (effects toxicity of other parameters)
Phosphate	Non-toxic, serves as a nutrient source for plants
Quaternary Ammonia Compounds	<1 ppm (QAC's are used in some algicides and external fish disinfectants.)
Salinity	0-40 ppt (varible with species)
Sulfide	0.002 ppm (as Hydrogen Sulfide)
Suspended Solids	< 80 ppm
Turbidity	Depends on cause of turbidity. High sediment loads can cause gill damage.

ideal food is a nutritionally complete fish ration but these are expensive and require refrigeration to prevent spoilage. The most economical strategy is to feed a floating ration formulated for juvenile or tropical fish supplemented with a live source of food, *artemia* or mosquito larvae for example. Multiple daily feedings are best, especially when feeding fry. Offer the proper size of the feed relative to the size of fish. Feed nauplii, first instars and starter feeds to fry. Larger fish prefer later instars and larger particle sizes. Adult fish will nibble on very large feed types if necessary. Only feed that amount of feed that the fish will consume in 15 minutes. Any amount more than this will sink to the bottom of the tank and decompose, contributing to poor water quality.

Reproduction. To obtain reproduction in mosquitofish year round it is necessary to provide a photoperiod and water temperature that approaches "natural reproduction" values. Natural reproduction values are the photoperiod and temperature that occur when mosquitofish reproduce in the wild. Gall (1983) reported the optimum condition for the growth and reproduction of mosquitofish to be 25°C, 16:8 L:D photoperiod, with a loading density of 1 to 1.5 fish per liter. His system maintained a pH of 8.5, 8 ppm DO, .03 ppm NO_2, and 4 ppm NO_3. Operationally, a sex ratio of 1:1 seems to be optimum for fry production, while the optimum density for reproduction is ca. 0.5 fish per liter of water (Downs et al. 1986). Mosquitofish cultured exclusively for their reproductive potential should be held at a lower density than for optimum growth. The females seem to channel their energy toward growth and not maturation of embryos at higher densities. Although a study to substantiate this observation does not exist, Henderson-Arzapalo (1980) cites that several examples exist of biochemically based population control mechanisms in fish. The actions of these inhibitory factors appear to be specific in that only members of the same or closely related species are affected (Yu & Perlmutter 1970; Pfuderer et al. 1974).

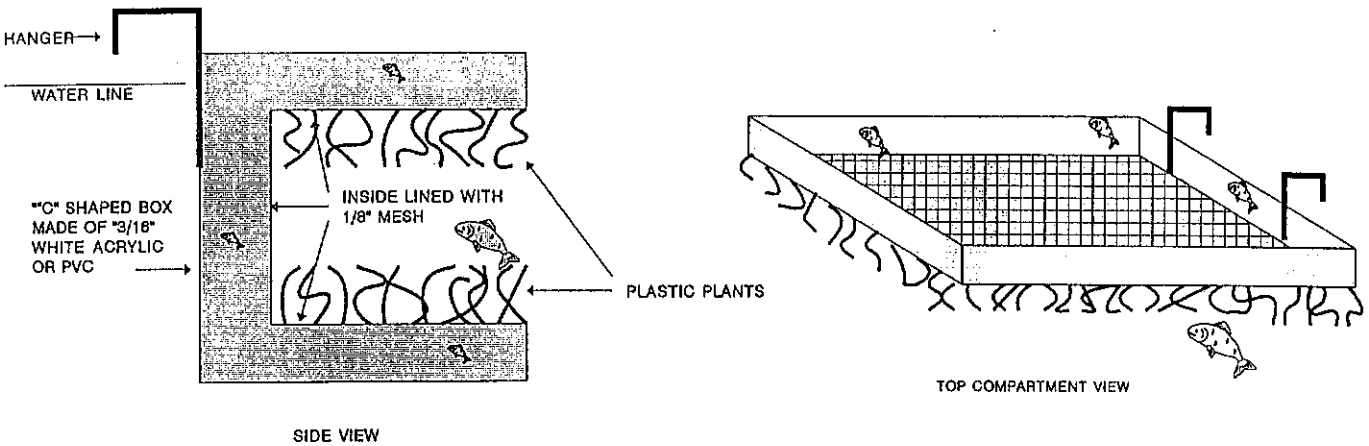
Parent-offspring cannibalism must be prevented for optimal fry production. This is done by providing gravid females access to special breeding chambers that allow the newborn to escape through protective grating into an area where no large fish reside. By using flow patterns and funneled weirs the fry can be prevented from swimming back into the breeding chambers. If females, stocked in the high densities of intensive culture, are not provided some sort of harborage in brood chambers they can reabsorb their embryos rather than release them to be cannibalized. Plastic aquarium plants or similar material will provide this necessary harborage.

One successful method of obtaining fry is to place all brood stock through a floating grader box (12/64") every two weeks. All fish retained are then transferred into breeding chambers. Females are loaded at a maximum loading density of .5 fish per square inch of breeding chamber water surface area. Brood fish are removed from breeding chambers after one week, as fry production is low after this time and brood fish mortality increases dramatically. Two factors seem to lead to the decreased fry production after the first week of isolation. First, most females that were gravid in fact release their fry, ca. 90% after one week of isolation, secondly, both the fry and adult females become more active and fry are cannibalized as they swim back into the breeding chambers (Downs, unpublished data).

In guppy culture, newborn fry are recovered by hanging trays in the brood fish tanks. Guppy fry swim up into the tray after they are born and are protected from the adults. Fry are netted daily and transferred to growout tanks. A similar method has been developed for mosquitofish fry retrieval (see figure 3). However, mosquitofish fry have a tendency to swim down (ca. 50% of the time) after birth. Therefore, a combination fry tray, a "C" shaped box structure constructed of white acrylic or PVC sheet, has been developed (see figure 3). The top tray is an open top rectangular box with 1/8" plastic mesh and plastic plants attached to the underside. The submerged bottom tray is a closed bottom rectangular box with 1/8" mesh and plastic plants attached to the top side. Gravid females seeking cover will release their fry amongst the plastic plants. Fry that swim up after birth will escape to the surface while fry that swim down after birth will escape predation in the lower compartment. The two trays are connected via a tray with 1/8" mesh on the inside. This vertical tray allows fry that swim down after birth to swim up to the top tray in a protected corridor. The dimensions of the trays can vary but should be built to maximize the surface area of the trays while not being so bulky that one person couldn't remove them for maintenance. A typical tray might be 3' x 1.5' x 4".

The floating fry tray is the simplest to build and operate. One-eighth inch opening netting, with plants attached to the underside, is attached to a rectangle of 1" PVC which floats in the water. Fry that are resting on the net are removed periodically. Although more fry are cannibalized when using this apparatus, they provide a boost in nutritive input and may serve to increase subsequent female brood sizes. Various fry retrieval designs are being investigated at the Contra Costa Mosquito Abatement District.

Figure 3. A combination fry tray.



Carrying Capacity. Carrying capacity is defined as the maximum biomass of fish that a system can safely support for an extended period of time. As long as the fish biomass is less than this capacity, and the system is operating and managed properly, mortality due to water quality deterioration should not exist. The carrying capacity is dependent on water volume, flow, temperature, oxygen content, pH, fish size and species, and accumulated metabolites. Various authors have published formulas for computing carrying capacities for certain species of fish (Hirayama 1966, Spotte 1971, Wheaton 1977, Colt 1979, Piper 1986). Basically, it is determined that the oxidizing capacity of the filter must be greater or equal to the rate of pollution or ammonia production of the system.

Particulate Filtration. One of the most difficult operations in maintaining water quality in an aquaculture system is that of solid waste removal. Fish feces, uneaten feed, algae, and bacteria all contribute to the continual amounts of solids that need to be removed. Total suspended solids (TSS) need to be removed from the culture system since they constitute a Biological Oxygen Demand (BOD), competing with the cultured fish and biological filtration process for available oxygen. In addition, the TSS readily support the growth of certain pathogenic bacteria and fungi, impede water flow, contributes to ammonia production and decreases sterilization efficiency. Methods to remove suspended matter include sedimentation and filtration processes and most successful systems will incorporate both in its TSS removal scheme. Table 6 shows typical techniques for suspended solids removal with their loading capacities and removal efficiencies.

Sedimentation. Removal of suspended material is accomplished through the use of a settling chamber (clarifier). These units promote sedimentation of the heavier suspended particles to an area within the chamber where they may accumulate and be periodically removed by siphoning, pumping or draining. Build into your design gravity flow (or siphon) with minimal turbulence into the waste collection basin. Waste should enter the basin below the water surface to avoid air bubbles attaching to the particles, allowing them to remain in suspension. Clarifiers are often employed with biological or mechanical filtration devices to trap the larger suspended wastes and prevent them from accumulating on the uppermost layers of filter media. Without a clarifier, these accumulations could develop so often that an excessive amount of time and labor would be expended in their removal (backwashing).

A clarifier should be designed and constructed with both adequate volume and flow detention or residence time to maximize sedimentation action. Internal baffle structures may be installed to lengthen residence time and speed settling. Both the inlet and outlet pipes should be at or near the water surface and at opposite ends of the clarifier.

Filtration Units.- Many designs and filter medias have been developed and successfully incorporated into culture systems. The most efficient filters incorporate granular media (GM) or porous media (PM). Various vendors offer a wide range of filter medias from sands to synthetic filter cloths

Table 6. Typical techniques applied to aquacultural systems for suspended solids removal (Chen & Malone 1990).

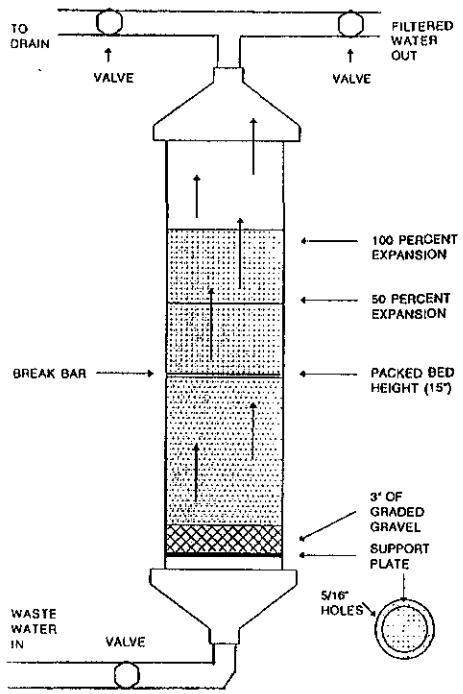
Processes	Solids Size Removed (um)	Head Loss (m)	Hydraulic Loading ($M^3/m^2/day$)	SS Removed (%)	Reference
Sedimentation	>100		24 - 94	40 - 60	EPA 1975
Settling Tank			24.2-61.3		Liao 1970
Tube Settler			30 - 90		Muir 1978
GM Filter	>20[c]	0.1 - 3	176 - 429	20 - 60	Muir 1982; EPA 1975
Rapid Sand Filter			94 - 351	67 - 91	EPA 1975
			285	70 - 90	Mayo 1975
Pressure Sand Filter		2 - 20	117 - 704	50 - 95	Muir 1982; EPA 1975
Floating Bead Filter		0.8 - 6	1935		Wimberly 1990
Screen	>75	Negligible	1 - 1000(gpm)		Huguenin & Colt 1989
Coarse Screen			587 - 1760	5 - 25	EPA 1975
Microscreen		0.01-0.1	176 - 587	17 - 50	EPA 1975
Drum Filter			840 - 2180		Ziegler Bros.[a]
Triangle Filter					Makinen et al. 1988
PM Filter	>0.1		43 - 130		Muir 1982
DE Filter	>0.1		29 - 59	>90	Muir 1982; EPA 1975
Cartridge Filter	1 - 10	ca.5	1 - 10(gpm)		Huguenin & Colt 1989
Hydrocyclones	1 - 75	14 - 35			Huguenin & Colt 1989
					Wheaton 1977
	5 - 200				Svarovsky 1977
Other	<30				
Foam Seperation			288 - 2880		Chen 1991
Ozonation			3 - 456		Keeton Fisheries[b]

[a] Zeigler Bros. Inc., Gardners, Pennsylvania 17324
[b] Keeton Fisheries Consultants, Inc., Fort Collins, Colorado
[c] The Task Committee on Design of Wastewater Filtration Facilities, 1986

(see appendix B). A very simple and efficient filtration system incorporates multiple pressurized upflow filters (see figure 4). As Malone and Burden (1988) describe, "Water flows into the bottom of the column, up through the underdrain, a 3-inch support bed of gravel, and a bed of sand. The rate at which the water flows through the sand bed controls the filter operation. During normal operation the sand bed remains packed, resting on the gravel bed. Solids entering the bed are captured, and dissolved wastes are consumed by the nitrifying bacteria attached to the sand grains. The water leaving the filter surface is clear, free of suspended solids, and contains low concentrations of dissolved waste."

Eventually, solids build up within the sand bed, gradually restricting the water flow through sections of the bed. A cleaning cycle is needed to return the filter to normal operation. During the cleaning cycle

Figure 4. A pressurized fluidized bed or upflow sand filter.



the flow is increased to expand the bed. This is simply done by restricting flow to the other upflow filters. The accumulated solids, being lighter than the sand, float to the top and exit to the waste water drain. Before the cleaning cycle is initiated, the valve to the fluidized filter is closed and the valve to the waste water drain opened. This process may be automated with automatic valves and should be conducted daily for each upflow filter during normal loading operations. For a more detailed description see Malone and Burden, 1988.

Ammonia Removal. Ammonia is the primary excretory product of fish. In its unionized form (NH_3), it is highly toxic to fish (Colt and Armstrong 1981). Therefore it is necessary to remove it from the culture system. Ammonia levels become more harmful at higher temperatures and higher pH values (see Table 7). Toxicity decreases with increasing salinity of water. Removal can best be done by biological filtration or ion exchange.

Biological Filtration.- A biological filter is a unit that provides surface area for bacteria to grow. It can consist of aggregate materials, such as, aquarium gravel, pea gravel, dolomite or oyster shell or synthetic materials such as plastic extrusions or fiberglass plates. The operational concept of the biofilter is simple. Nitrifying and denitrifying bacteria living on the filter media use and convert the high energy ammonia, urea, and associated ammonium compounds of the culture water to lower energy nitrate compounds that are essentially nontoxic to mosquitofish and most other aquatic organisms. This process is dependent upon the development of two strains of bacteria; *Nitrosomonas* and *Nitrobacter*. *Nitrosomonas* feeds on ammonia, converting it into nitrite, while *Nitrobacter* converts nitrite to non-toxic nitrate. High dissolved oxygen levels are very important for peak efficiency. It is also important when selecting a filter medium to keep in mind the rate media will clog with particulate matter and thus need to be backwashed. Meade (1985) presents a review of sublethal, chronic effects of ammonia exposure on fish and factors that can influence these effects. Flow rates for biofilters should range between 41.5 to 83 L/min. per square meter of surface area (Klontz 1981) for optimum efficiency. The proper sizing of a biofilter is also important.

External filters are preferred to those located inside the actual culture tank for several reasons. First, if fish are diseased, one can medicate the fish and simply bypass the biological filter and not harm the beneficial bacteria within the filter. Second, biofilters usually have a high biochemical oxygen

Table 7. Percent un-ionized ammonia in water at 32 to 86°F and pH 6 to 10.[a]

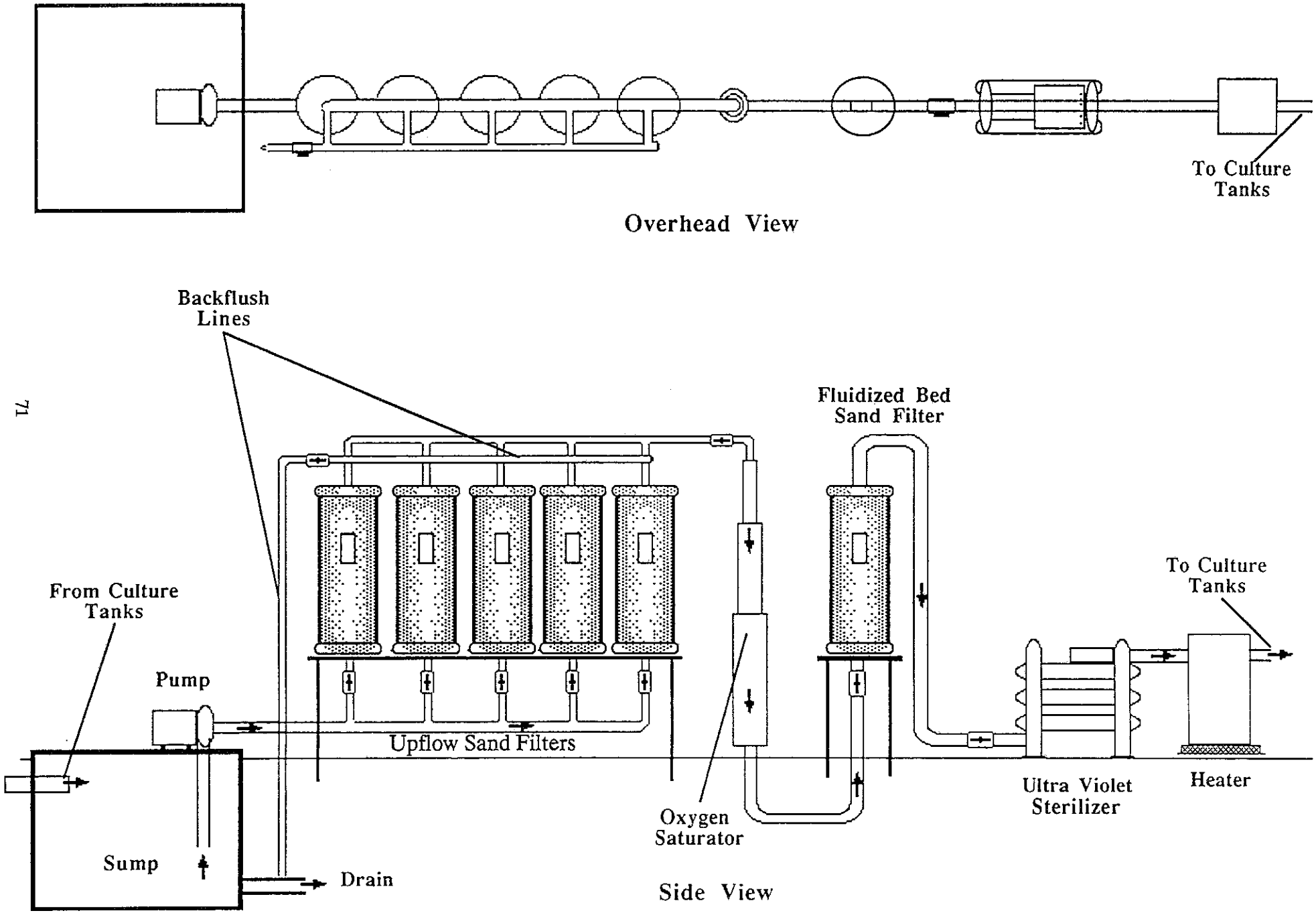
Temperature		pH				
°C	°F	6.0	7.0	8.0	9.0	10.0
0	32.0	0.008	0.08	0.82	7.64	45.3
2	35.6	0.01	0.10	0.97	8.90	49.4
4	39.2	0.01	0.12	1.14	10.3	53.5
6	42.8	0.01	0.14	1.34	11.9	57.6
8	46.4	0.02	0.16	1.57	13.7	61.4
10	50.0	0.02	0.19	1.83	15.7	65.1
12	53.6	0.02	0.22	2.13	17.9	68.5
14	57.2	0.03	0.25	2.48	20.2	71.7
16	60.8	0.03	0.29	2.87	22.8	74.7
18	64.4	0.03	0.34	3.31	25.5	77.4
20	68.0	0.04	0.40	3.82	28.4	79.9
22	71.6	0.05	0.46	4.39	31.5	82.1
24	75.2	0.05	0.53	5.03	34.6	84.1
26	78.8	0.06	0.61	5.75	37.9	85.9
28	82.4	0.07	0.70	6.56	41.2	87.5
30	86.0	0.08	0.80	7.46	44.6	89.0

[a]From K. Emerson, R.C. Russo, R.E. Lund, and R.V. Thurston. 1975 Aqueous ammonia equilibrium calculations; effect of pH and temperature. Journal of the Fisheries Research Board of Canada 32(12):2379-2383.

demand (BOD) that would be undesirable in the culture tank. Third, cleanliness and general sanitation is much more difficult to maintain with inboard units. In contrast, an outboard filter can be disconnected from the culture system for routine maintenance; therefore, fish need not be removed from the culture tank during the cleaning process. Finally, outboard biofilter bacteria can be maintained in desired quantities, though the unit isn't on line, by adding small amounts of ammonia to the filter bed at regular intervals. It is therefore possible to have backup units available just in case a problem develops in the primary biofilter. Newly installed biofilters can be easily inoculated with suitable bacteria by transferring some media from an established biofilter to a new unit or by slowly adding fish to the system. A biofilter's bacterial population will generally grow in number to process the usable nitrogenous wastes from a uniform biomass. Sudden large increases in fish biomass can render the filter ineffective. *Nitrobacter* growth is actually inhibited by high levels of ammonia. High levels of nitrite can develop before the ammonia drops to low enough levels to permit *Nitrobacter* to grow. Therefore, one should introduce new fish stocks in small increments over several weeks time so the filter's bacterial population can balance nitrogenous loading.

The fluidized bed biofilter is very efficient and is the biological filter of choice by many aquaculturists. A fluidized bed filter (Figure 4), consists of a sand bed that is continuously expanded (fluidized) by a constant flow of water. Malone and Burden (1988) state, "The turbulent environment and rapid transport of oxygen and dissolved wastes through the filter favor bacterial growth. Waste conversion rates and bacterial growth are very rapid. The sand particles increase in diameter and the bacterial layer around the sand particles thickens, while the dynamic rolling of the sands assures full utilization of the bed. During expansion, the particles bounce around in the bed, continuously striking the filter walls or other sand particles. This mildly abrasive environment continuously wears off the bacterial slim layer around the sand particles, thereby eliminating any clogging problems. The fluidized bed cannot hold solids finer or lighter than the sand particles, so sheared biomass and solids from the shedding system pass out of the filter. The bed is self-cleaning and requires no routine maintenance." This filter is very efficient at dissolved waste conversion but provides no suspended solids removal and must be used in concert with a suspended solids removal system (see figure 5).

Figure 5. A filtration system for intensive culture.



Ion Exchange. The most used media is zeolite, a naturally occurring mineral that has a high affinity for absorbing ammonia in freshwater systems, due to its vast pore structure. A major drawback of this method is zeolite eventually becomes saturated and must be regenerated by backwashing with a brine solution. This requires an added expense of time and materials, making this approach undesirable.

pH. It is very important to monitor the pH of a system. Although mosquitofish are tolerant of wide ranging pH values, other consequences of pH can harm the fish. Low pH (< 6.0) results in lower carrying capacities of hemoglobin, excess mucus production by irritated gills and increases a fish's susceptibility to disease, particularly to bacterial infections. A high pH (> 9.0) will cause destruction of gill and fin tissues. As the pH increases an increasing percentage of ammonia will be in its free, unionized form (see table 6). Free ammonia (NH_3) is much more toxic to fish than ammonium ions (NH_4).

The nitrifying bacteria in a biological filter are also sensitive to changes in pH. Optimum pH values for these bacteria are ca. 8.3. Values below 7.0 severely inhibit bacterial action.

To control pH levels, there are commercially available buffering salts to either lower or raise pH. Besides buffering salts, many aquaculturists use oyster shells or dolomite to buffer their systems. Avoid excessive plant or algae growth because this will raise the pH value by removing carbon dioxide and nitrate, both acid-forming substances.

System Design and Construction. There is no one perfect configuration for an intensive culture system. Systems are usually designed around physical constraints of the culture site or with materials readily available to the culturist. One should keep in mind that the system should be designed around any special requirements of the species being cultured, such as flowing water or spacial orientation. Mosquitofish do not have any special requirements and can be cultured in any type of culture vessel.

The two most common configurations for the actual culture vessels are rectangular raceways or circular tanks. They can be constructed of wood, metal, fiberglass, or plastic. Water normally enters a rectangular raceway at one end and exits from a standpipe at the other end. Water in a circular tank may enter at any point but should exit from a center standpipe. The drain should be recessed in the tank's bottom and should be equipped with a vertical standpipe whose height is set at the desired water level. A second but larger pipe perforated at its base is placed over this standpipe. Although more useful in a circular tank, this drain system should be used in either system. Advantages of this drain system are that fecal material and waste feed will collect between the two standpipes. It can then be flushed from the system by removing the inner standpipe. Also in the event of mechanical failure within the system, the water levels in the tanks will be maintained. Finally, water lower in dissolved oxygen is usually found at lower levels in the water column, thus removing this water will improve water quality in the culture tank.

Plumbing of the system should be installed so that most, if not all, of it is readily accessible for routine maintenance and repair. Polyvinyl chloride (PVC) pipe is popular because it is nontoxic to fish, easy to install, inexpensive and simple to modify or repair. One minor disadvantage with plastic piping is that it doesn't conduct electricity. Thus any electrical components associated with the culture system must have its own earth grounding conductors to ensure maximum safety. Heated water can be routed back to the system via CPVC plastic pipe that is manufactured to accommodate much hotter water than PVC pipe. Piping can be laid down into open channels and protected with rigid covers that allow easy access.

Heating. Heating of the culture system may be accomplished several ways. The most desirable method is to use immersion heaters. Many manufacturers offer a variety of thermostatically controlled heaters for the aquaculture industry. Their advantages are that they provide better temperature control, do not require a special heating or mixing component in the system, and heating capacities can be expanded by adding additional heating elements to the thermostat in various locations. The energy required to heat culture water with immersion heaters is generally calculated at four to twelve watts per gallon (1.3 - 2.1 watts per liter) per $9^{o}F$ ($5^{o}C$) temperature increment.

Roof-mounted solar collectors may be an appropriate addition to a conventional hot water heating system, provided unobstructed southern sky exposures are available. A closed solar heating system extends the life of the solar heating components by running only distilled water through the system. Heat exchangers are placed inside holding tanks or directly in the culture tanks. A conventional back-up heating system should be in place with any solar heating system. While solar units can't be expected to provide 100% of heating requirements throughout the year, they can substantially reduce dependency upon conventional power sources and quality units will pay for themselves through energy savings. If "free" heated water is available from, e.g. industrial sources,

an adequate back-up heating system should be available in case of emergency industrial shut-down. If possible, the back-up system should be wired so that it automatically activates when water temperature drops to a critical level. The resulting thermal homeostasis will prevent outbreaks of fish diseases associated with sudden temperature changes during these emergencies.

Another method is use of natural gas-fired swimming pool or spa heaters. One potential problem is copper toxicity. Copper is used in almost all heaters in the exchanger and heating coils because of its thermal conductance and flexibility. However, experience with mosquitofish has shown that they are not nearly as sensitive to this element as are various other species.

Lighting. Photoperiod plays an important role in the growth and reproduction cycle of fish, mosquitofish included. Proper lighting consists of the correct intensity, wavelength and duration. It is recommended that the lighting fixtures selected provide as near to natural lighting as practicable. When using fluorescent lighting, full spectrum bulbs should be used instead of cool-white units. Although little is documented in this area as far as mosquitofish are concerned, fluorescent lighting on a 15:9 light:dark regime has shown to be adequate for year-round growth and reproduction.

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APPENDIX A

RECOMMENDED READING

1. Aquaculture

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Stickney, R.R. 1979. Principles of warmwater aquaculture. John Wiley and Sons, New York. 375 p.

Wheaton, F.W. 1977. Aquaculture engineering. John Wiley and Sons, Inc., New York. 708 p.

2. Biology of Fishes

Axelrod, H. R. & W. Vorderwinkler. 1988. Encyclopedia of tropical fishes. T.F.H. Publications, Inc., New Jersey. 631 p.

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Scott, P. W. 1987. A fishkeepers guide to: livebearing fishes. Salamander Books Ltd., London. 117 p.

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3. Diseases and Parasites of Fishes

Amlacher, E. 1970. Textbook of fish diseases. (English translation T.F.H. Publications (PS-667), Jersey City, New Jersey. 302 p.

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Hoffman, G.L. and F.P. Meyer, eds. 1974. Parasites of freshwater fishes. T.F.H. Publication (PS-208), Jersey City, New Jersey. 224 p.

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4. Distribution and Identification of Fishes

Bailey, R.M. et al. 1970. A list of common and scientific names of fishes from the United State and Canada, 3rd edition. American Fisheries Society, Special Publication No. 6. Washington, D.C. 149 p.

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4. Distribution and Identification of Fishes (cont.)

McClane, A.J. 1974. Field guide to freshwater fishes of North America. Henry Holt and Co., Inc., New York. 212 p.

McGinnis, S.M. 1984. Freshwater fishes of California. University of California Press, Berkeley, California. 316 p.

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5. Laws and Regulations

California Administrative Code
Title 14 - Fish and Game Code

Section 226.7	Issuance of permits and conditions for taking inland or freshwater fishes.
Section 227	Sale of live aquaculture products
Section 238.5	Stocking of aquaculture products
Section 245	Aquaculture disease control regulations

California Department of Fish and Game. Inland Fisheries - Informational Leaflets nos. 35 & 36.

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American Public Health Association et al. 1985. Standard Methods. American Public Health Association, New York. 874 p.

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7. Water Weeds and their Control

Applied Biochemists, Inc. 1987. How to identify and control water weeds and algae. Applied Biochemists, Inc., Mequon, Wisconsin. 64 p.

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Reimer, D.N. 1984. Introduction to freshwater vegetation. AVI Publishing, Co., Inc., Westport, Connecticut. 207 p.

APPENDIX B

PRODUCTS AND SERVICES

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805-326-1473

Services: Intensive System Design, Production Process Analysis, Feasibility Studies, Financial Analysis

Aquatic Systems, Inc.
11125 Flintkote Ave., Ste. J
San Diego, CA 92121

Contact: Mike Massingill
Telephone: 619-452-5765

Services: Facility Design and Construction, Management, Marketing; Finfish and Crustacean Aquaculture

California Farm Bureau Fed.
National Affairs & Research Div.
1601 Exposition Blvd. FB-12
Sacramento, CA 95815

Contact: Ria de Grassi
Telephone: 916-924-4090

Services: Legislative/Regulatory Analysis

McCormick & Associates
1211 Spruce St.
Berkeley, CA 94709

Contact: Tom McCormick
Telephone: 415-845-9011

Services: Biological Requirements, Facility Design and Management

CONSULTING(cont.)

Montgomery Consulting
Engineers Inc, J.M.
250 N. Madison
Pasadena, CA 91101

Contact: Bill Madden
Telephone: 818-796-9141

Services: Bioengineering (ozone sterilization systems, hatchery design innovation), Waste Water Treatment Design, Water Delivery & Treatment Systems Design, Environmental Impact Studies

RI-DI Fisheries
1507 N. Cunningham Rd
LeGrand, CA 95333

Contact: Rich Michaud
Telephone: 209-382-0316

Services: Production & Marketing

Scientific Hatcheries
8152 Evelyne Circle
Huntington Beach, CA 92646

Contact: Dr. Dallas Weaver
Telephone: 714-960-4171

Services: High Density Closed Aquaculture Systems, Filters, Water Treatment, Computer Control Systems

Aquaculture & Fisheries Program
University of Calif., Davis
Davis, CA 95616

Contact: R.B. Fridley
Roger E. Garrett
Telephone: 916-752-7601

Research: Aquaculture Related
Teaching: Aquaculture Related

College of the Redwoods
Aquaculture/Fisheries Tech
7351 Tompkins Hill Rd.
Eureka, CA 95501-9302

Contact: R.J. Pierce
Telephone: 707-433-8411
ext. 6835

Teaching: Applied Skills Training Program in Aquaculture & Fisheries Technology
Outreach: Aquaculture Related Extension Service

DIAGNOSTIC SERVICE - FISH FEED

Aquaculture Chemical & Feed
Calif. Dept. of Food & Agri.
1220 N St.
Sacramento, CA 95815

DIAGNOSTIC SERVICE - FISH HEALTH & DISEASE

University of Calif.
School of Veterinary Medicine
Davis, CA 95616

Contact: Dr. Ron Hendrick
Telephone: 916-752-3411

DISEASE CONTROL - STATE & FEDERAL

Calif. Dept. of Fish & Game
Redding Regional Office
PO Box 1480
Redding, CA 96001

Contact: Mel Willis
Telephone: 916-246-6562

Calif. Dept. of Fish & Game
Fish Disease Lab.
2111 Nimbus Rd.
Rancho Cordova, CA 95670

Contact: Don Manzer
Bill Wingfield
Telephone: 916-355-0811

Calif. Dept. of Fish & Game
Mohave River Hatchery
PO Box 938
Victorville, CA 92392

Contact: Dr. Martin F. Chen
Telephone: 619-245-4076

Coleman National Fish Hatchery
Fish & Wildlife Service
Rt. 1, Box 2105
Anderson, CA 96007

Contact:
Telephone: 916-365-8622

EQUIPMENT/SUPPLIES

Algatec, Inc
250 W. Schrimpf Rd
Calipatria, CA 92233

Contact: Thomas W. Naylor
Telephone: 619-348-5244

Vitamin & Animal Feed Additives From Algae

Aquaneering
1217 Hayes Ave.
San Diego, CA 92103

Contact: Mark Francis
Telephone: 619-291-2977
FAX: 619-688-3990

Aerators/Lift Pumps, Pumps, Feeders, Net Reels, Tanks, Display, Live Haul, Hatchery

EQUIPMENT/SUPPLIES(cont.)

Aquanetics
5252 Lovelock Street
San Diego, CA 92110

Contact:
Telephone: 619-291-8444

Aeration Equipment, Chillers, Filters, Fittings, Pumps and Sterilizers

Aquatic Eco-Systems
2056 Apopka Blvd
Apopka, FL 32703

Contact:
Telephone: 407-886-3939

Aeration Equipment, Blowers, Fittings, Monitoring Instruments, Supplies and Valves

Aquatic Systems, Inc.
11125 Flintkote Ave., Ste. J
San Diego, CA 92121

Contact: Mike Massingill
Telephone: 619-452-5765

Feeders, Filters, Temperature Controllers, Fiberglass Tanks, Pumps

Aqua Vet, Div of Novalek, Inc.
2242 Davis Ct.
Hayward, CA 94545

Contact: Dr. Robert Rofen
Telephone: 415-782-4058
FAX: 415-784-0945

Airstones, Books, Chemicals, Medications, Nets, Water Test Kits, Water Conditioners, Brine Shrimp Eggs (Frozen, Live), Feeds, Vitamin Mixes

Atec
5902 Wigton
Houston, TX 77096

Contact: Ed Clark
Telephone: 713-995-0808

Oxygen System Customized For Your Operation

Due's Koi Farm
16396 S. Murphy Rd.
Escalon, CA 95320

Contact: Jean or George Due
Telephone: 209-982-1710

Vitamins For Fish

J.L. Eagar
PO Box 476
North Salt Lake, UT 84054

Contact:
Telephone: 801-292-9017

Aeration Systems, Chemicals, Feeders, Nets, Scales, Tanks, Supplies and Waders

EQUIPMENT/SUPPLIES(cont.)

Fritz Aquaculture
PO Drawer 17040
Dallas, TX 75217

Contact:
Telephone: 800-527-1323

Aeration Equipment, Blowers, Food, Medications, Monitoring Instruments, Nets, Pumps, Supplies and Valves

Hach Company
P.O. Box 389
Loveland, CO 80539

Contact:
Telephone: 800-227-4224
FAX:

Makers of Water Test Kits and Supplies

Jack Hopkins Co., Inc.
2956 B Treat Blvd.
Concord, CA 94518

Contact: Richard M. Hopkins
Telephone: 415-825-0930

Royce Instruments - Including Service & Repair
Island Science - Farm Management Software

Liquid Air
1814 Franklin St. #900
Oakland, CA 94612

Contact: Kurt Lowe
Telephone: 415-268-9800

Liquid Oxygen

Norplex, Inc.
7048 S 190th
Kent, WA 98032

Contact: Ralph Schley
Telephone: 206-251-6050
FAX: 206-251-8071

Extruded Plastic Netting - All Sizes, Poly Bags, Bird Net Orange Safety Fence

Rainbow Lifegard
PO Box 4127
El Monte, CA 91734

Contact:
Telephone: 818-443-6114

Filters, Sterilizers and Pumps

Sierra Fish Products
Rt. 5, Box 38
Red Bluff, CA 96080

Contact: Phil Mackey
Telephone: 916-597-2222

Feeders, Fiberglass Tanks (Hatching Pots, Circular Tanks)

EQUIPMENT/SUPPLIES(cont.)

VMG Industries,Inc.
858 Grand Ave.
Grand Junction, CO 81501

Contact: Bruce Marshall
Telephone: 303-242-8623

Irrigation Ditch Cleaner, Raceway Cleaner, Fish Live Cages/Tanks, Oxygen Generator & Injection Devices, Nitrogen Degassing Equipment

West Weigh Scale Co.
3990 Hicock St.
San Diego, CA 92110

Contact: Vincent Muccillo
Telephone: 619-291-8231

Scales - All Kinds, All Sizes

Whale Enterprises, Inc.
2795 Rabbittown Road
Piedmont, AL 36272

Contact:
Telephone: 205-435-8180

Nets - All Types

FEED

Associated Feed & Supply Co.
Fish Makers Feeds
5213 West Main
Turlock, CA 95380

Contact: Jon Lindskoog
Telephone: 209-667-2708
Contact: Dr. Dudley Cash
Telephone: 209-526-1710

Bait Fish Feed, Goldfish Feed, Custom Diets

Kruse, Grain & Milling, O.H.
PO Box 5000
El Monte, CA 91734

Contact: Jim Gibson
Telephone: 800-854-1781
818-575-0658

Custom Diets

Murray Elevators
Sterling H. Nelson & Sons, Inc.
PO Box 7428
Murray, UT 84107-0428

Contact: Chris Nelson
Telephone: 800-521-9092

Assorted Feeds

FEED(cont.)

Poultrymens Cooperative Assoc.
PO Box 706
Riverside, CA 92502

Contact: Ron Lowther
Telephone: 714-686-5270

Assorted Feeds, Custom Diets

Rangen Inc.
PO Box 706
Buhl, ID 83316

Contact: Bob Deisher
Dick Schwab
Telephone: 800-321-0886
208-543-6421

Assorted Feeds, Custom Diets

Star Milling Company
PO Box 728
Perris, CA 92370

Contact: Frederick L. Frost
Telephone: 714-657-3143
Orders: 800-969-2234

Assorted Feeds, Custom Diets

Western Ranchers Feeds
5935 Don Way
Carmichael, CA 95608

Contact:
Telephone: 916-485-1311

Purina Trout Chow

MISCELLANEOUS SERVICES

Aquaculture Advisor
Calif. Dept. of Food & Agriculture
1220 N St.
Sacramento, CA 95815

Contact: Lew Davis
Telephone: 916-445-4521

Aquaculture Information Center
National Agriculture Library
Room 304
Beltsville, MD 20705

Contact:
Telephone: 301-344-3704

On-Line Aquaculture Reference Database

Calif. Fish & Game Commission
1416 Ninth St.
Sacramento, CA 95814

Contact:
Telephone: 916-445-5708

MISCELLANEOUS SERVICES(cont.)

Calif. Dept. of Health Services
Environmental Mgmt. Branch
50 D St., Ste. 205
Santa Rosa, CA 95404

Contact: Douglas W. Price
Telephone: 707-576-2145

Cooperative Extension
Aquaculture Specialist
Dept. of Animal Science
University of Calif.
Davis, CA 95616

Contact:
Telephone: 916-752-7490

National Fisheries Research Lab
U.S. Fish & Wildlife Service
Dept. of Interior
PO Box 818
Lacrosse, WI 54601

Contact:
Telephone: 608-783-6451

Information on Registered Chemical Compounds

U.S. Dept. of Agriculture
Western Regional Aquaculture Center
Dept. of Animal Science
University of California, Davis
Davis, CA 95616

Contact:
Telephone: 916-752-7490

Scientific Reports & Technical Updates, Educational Sessions

NEWSLETTERS

Aquatic Farming
PO Box 1004
Niland, CA 92257

Contact: Fern Ray
Telephone: 619-359-FISH

Official newsletter of the California Aquaculture Association. Newsletter is free on request.

PERIODICALS

Aquaculture Digest
9434 Kearney Mesa Rd.
San Diego, CA 92126

Aquaculture Engineering
Elsevier Science Publishers
52 Vanderbilt Ave.
New York, NY 10017

PERIODICALS(cont.)

Aquaculture Magazine
PO Box 2329
Asheville, NC 28802

Aquatic Farming
(official Pub. of CAA)
c/o CAA
PO Box 1004

Farm Pond Harvest
Box AA, Dept. C
Monence, IL 60954

For Fish Farmers
MS Coop. Extension Ser.
MS State University
MS State, MS 39762

Journal of Applied Aquaculture
Food Products Press
10 Alice Street
Binghamton, NY 13904-1580

Journal of World Aquaculture Society
World Aquaculture Soc.
16 E. Fraternity Ln.
LA State University
Baton Rouge, LA 70803

Progressive Fish Culturist
American Fisheries Society
5410 Grosvenor Ln., #110
Bethesda, MD 20814-2199

Today's Aquaculturist
Pisces Publishing Co.
548 Naugatuck Ave,Ste 2-155
Devon, CT 06460

Transactions of the American Fisheries Society
4501 Grosvenor Ln., Ste. 110
Bethesda, MD 20814

APPENDIX C

CONTRIBUTORS

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APPENDIX D

USEFUL MATHEMATICAL EQUIVALENTS

Unit and equivalent	Unit and equivalent
Acre	Ounce (weight)
43,560 ft^2	28.35 g
4,840 yd^2	0.063 lb
4,046.8 m^2	0.96 fl oz of water
Acre-foot (A-ft)	Ounce (fluid)
43,560 ft^3	29.57 g of water
325,851 gal	29.57 mL or cc
2,718,144 lb of water	1.043 oz of water
1 surface acre covered with 1 foot of water	1/8 cup
1,233,489 L	6 tsp
Cubic foot (ft^3)	2 tbsp
28.32 L	Part per million (ppm)
28,317 mL or cc	1 mg/L of water
7.48 gal	3.78 mg/gal
1,728 in^3	3.78 g/1,000 gal of water
0.037 yd^3	0.13 oz/1,000 gal of water
62.43 lb of water	1 oz/1,000 ft^3
957.5 fl oz	28.35 g/100 ft^3
Cup	2.72 lb/A-ft of water
8 fl oz	Pint (pt)
1/2 pt	473.17 mL of water
Gallon (gal)	1/2 qt
3.75 L	16 fl oz
3,785.4 mL or cc	16.69 oz of water
128 fl oz	1/8 gal
8 pt	1.04 lb of water
4 qt	Quart (qt)
0.13 ft^3	946.34 mL or cc
133.52 oz of water	32 fl oz
8.35 lb of water	4 cups
Gram (g)	2 pt
0.035 oz	1/4 gal
1 mL or cc of water	2.09 lb
1,000 mg	Tablespoon (tbsp)
Kilogram (kg)	14.79 mL or cc
2.20 lb	3 tsp
35.27 oz	1/2 fl oz
1.0 L of water	Teaspoon (tsp)
Liter (L)	4.93 mL or cc
33.82 fl oz	1/3 tbsp
1.057 qt	1/6 fl oz
0.26 gal	Temperature
1 kg of water	$^oC = 5/9(^oF - 32)$
2.20 lb of water	$^oF = 9/5(^oC) + 32$
1/3 tbsp	

Cubic centimeter = cc
Cubic inch = in^3
Cubic yard = yd^3
Fluid ounce = fl oz
Meter = m
Milligram = mg
Milliliter = mL
Ounce = oz
Pound = lb
Square foot = ft^2
Square meter = m^2
Square yard = yd^2

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