A review of the biology of *Gambusia affinis* and *G. holbrooki*

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Abstract

Eastern and Western *Gambusia* (i.e., *Gambusia holbrooki* and *G. affinis*, respectively) are considered together here because these two fish species are very closely related, similar in appearance, similar in biology and often confused. Widely divergent attitudes have developed with regard to these fish with some viewing them as being highly beneficial to humans through controlling mosquitoes and the diseases they harbor, and others expressing concern about the negative impacts that these fish may have on other species with which they interact. Because of the widespread distribution, high levels of abundance, ease of capture and captive maintenance, and divergent attitudes, a very large and diffuse literature has developed with regard to these species. In fact, few fish species have been studied as much as or more than these two species combined. There has, however, been no comprehensive review of their biology published to date. As it is not possible to provide a comprehensive review of *Gambusia* biology in one reasonably sized document, I provide here a review of aspects of their biology at the level of species and individual. In another review I focused instead on the levels of population and species communities and consider the impacts that these fish have on mosquitoes and other organisms (Pyke, unpublished). As would be expected of such widespread and abundant species, *Gambusia affinis* and *G. holbrooki* are clearly very tolerant, adaptable and variable in

their biology, at both an individual and population level. Both individuals and populations can tolerate, and often thrive within, a wide range of conditions and the abilities of individuals to do this are enhanced if they have time to acclimate to any changes. Populations can adapt through genetic or evolutionary changes in response to conditions that vary in space or time, and there is significant genetic variation within and between populations.

Introduction

The Eastern and Western *Gambusia* (i.e., *Gambusia holbrooki* and *G. affinis*, respectively) are considered together here because these two fish species are very closely related, similar in appearance, similar in biology and often confused (see below). Except where there are apparent differences between them, they are referred to collectively as *Gambusia*.

Divergent attitudes have developed with regard to *Gambusia*. Some have viewed these two fish species as being highly beneficial to humans through efforts to control mosquitoes and the diseases they harbor (Washino, 1969; Sholdt et al., 1972; Green and Imber, 1977). Along with this view has gone the common name 'Mosquito Fish', collectively applied to both species (Seale, 1917; Jordan et al., 1930). Relatively recently, on the other hand, others have expressed concern about the negative impacts that these fish may have on other species with which they interact and the common name 'Plague Minnow' has sometimes been used in this context (Ehmann, 1997; Pyke and White, 2000).

These two fish species are extremely widespread and abundant, and are relatively easy to maintain in captivity. Having been successfully introduced from North America to most of the warmer parts of the world, they are now collectively the most widely distributed freshwater fish in the world and are present and widespread on all continents except Antarctica (Krumholz, 1948; Lloyd, 1984; Lloyd and Tomasov, 1985; Lloyd, 1986). In habitats where they occur *Gambusia* are generally common to abundant fish (Vooren, 1972; Berra et al., 1975; Webb and Joss, 1997). Methods for keeping and breeding them in captivity are well known and readily implemented (Robison et al., 1983; Abshier et al., 1991; Coykendall et al., 1991; Beidler, 1995).

Because of the widespread distribution, high levels of abundance, ease of captive maintenance and divergent attitudes, a very large and diffuse literature has developed with regard to *Gambusia*. Included in my bibliographic database are about 1800 documents that relate to *Gambusia*, representing about 400 different journals, many book chapters and reports, and at least 50 University theses. Most journals occur just once in this database and *Copeia*, the most frequent, accounts for only about 5% of journal articles (i.e., 86/1666).

Despite divergent attitudes towards these two species and the extensive literature concerning them, there has so far been no comprehensive review of their biology (Lloyd et al., 1986). Such a review should increase our understanding of the biology of these species, the reasons for their very successful spread throughout the world, their effectiveness as mosquito control agents, any impacts they may have on other animal species, and their management. It should also be comprehensive, identify gaps and limitations in our knowledge, and be reasonably easy to revise whenever additional information becomes available (Pyke, 2001). This paper provides such a review.

It is not possible, however, to provide a comprehensive review of *Gambusia* in one document. Given the extensive literature and wide array of issues and topics regarding these fish, a single review that tried to include everything would be excessively large. In another document I have focused on the reasons for the very successful spread of these fish throughout the world, their effectiveness as mosquito control agents, any impacts they may have on other animal species, and aspects of their general biology that are relevant to these issues (Pyke, unpublished). My goal in the present document is therefore to review those aspects of the general biology of *Gambusia* that are not directly relevant to the above-mentioned issues and hence not included in my other review. The present review therefore omits a number of topics including distribution, habitat-use, abundance, and population biology. It also omits consideration of possible impacts with other aquatic species.

Examination of the literature regarding *Gambusia* will always remain incomplete, but I assume that the documents that I have viewed represent an

unbiased sample of the relevant literature, and permit a comprehensive and accurate review. Tracking down this literature has proven both tedious and time consuming, and many documents have been difficult or impossible to obtain through available library resources. I have so far been able to view about 700 of the relevant documents in my bibliography and obtain abstracts from about 400 more, but this still leaves about 700 documents for which I know little more than the title. In addition, keeping this review to reasonable size has meant that citations have had to be restricted to those most relevant. As a result of these two factors, my present review cites just 263 documents. My complete bibliography is available upon request.

This review was developed using the strategy recommended in Pyke (2001) and illustrated in Pyke and White (2001), Pyke (2002) and Pyke and Read (2002, 2003). I have divided my review into sections based on the level at which observations regarding the species are focused (i.e., species, individual, population) and how these observations are made. Community-level observations are considered in my other review. Within each of these sections there are also sub-sections that reflect the extent of knowledge about these species.

Biology at the species level

Species names, appearance and differentiation

The etymology of the generic name *Gambusia* belies any impacts, positive or negative, that fish species in this genus may have had, and conveys nothing of their appearance or biology. In introducing this genus in 1851, Poey (1854) modified a provincial Cuban word Gambusino, meaning nothing, a joke or farce, and added that 'one says to fish for Gambusinos when one catches nothing' (Jordan and Gilbert, 1882; Jordan and Evermann, 1896; Kuntz, 1913). *Gambusia* is also a Latin word meaning nothing or frustration (Gall et al., 1980). Fish of this genus were initially viewed as being 'of no importance' and 'worthless' (Kuntz, 1913).

On the other hand, a variety of common names have been adopted for *Gambusia affinis* and *G. holbrooki*, reflecting their appearance, behavior, taxonomic status and impacts on other organisms. The initial common name of Top Minnow for *Gambusia affinis* (which included *G. holbrooki* at the time) reflected its minnow-like appearance and its habit of swimming near the surface (Hildebrand, 1919b), though it was also often referred to simply by its generic name *Gambusia* (Smith, 1912). After about 1905, when both *Gambusia affinis* and *G. holbrooki* began to be introduced into waters for control of mosquito larvae, they became increasingly known (collectively) as the Mosquito Fish (Seale, 1917), though the names Top Minnow and *Gambusia* have continued to be used (Grant, 1978). Because of concern regarding deleterious impacts of these fish on other organisms including other fish, frogs and aquatic invertebrates, some people have recently begun to use the name Plague Minnow (Ehmann, 1997; Pyke and White, 2000; Richard, 2002). With the elevation in 1988 of these two fish from the sub-species level, the names Eastern and Western *Gambusia* have been adopted by some authors (Meffe, 1989).

Gambusia are relatively small, non-descript fish. They generally range in size from about 1 to 5 cm, occasionally reaching up to about 6 cm (Kuntz, 1913; Hildebrand, 1919b; Wu et al., 1974; Vondracek et al., 1988). They are generally silver in color, sometimes with a greenish hue, with scattered gray and black markings (Lake, 1959). They have a single dorsal fin, with multiple fin rays, and a large rounded caudal fin (Grant, 1978).

Through differences or changes in the degree of melanism in the skin, the general darkness in appearance of *Gambusia* varies both between individuals and populations, and also within individuals in accordance with their environment. There is sometimes noticeable variation in degree of melanistic color within and between populations of *Gambusia* (Sumner and Douderoff, 1938; Snelson et al., 1986). In addition, *Gambusia* are apparently able to change in appearance in accordance with the darkness of their surrounding conditions, through the aggregation and desegregation of melanin pigment in the chromomatophores (Sumner, 1934, 1935a, b). Individuals that have been kept for 7 weeks or longer in black tanks are darker in appearance than individuals kept in white tanks (Sumner, 1934, 1935a, b). If these individuals are transferred into a container of the opposite color to

the tanks they have been in, they change in color with the dark fish becoming lighter and the light fish becoming darker, but remain distinguishable for several days from other fish that have not been given a different background color (Sumner, 1934, 1935a, b).

Male *Gambusia* have an external organ called a gonopodium, which is the anal fin, modified during development to provide a tube for the passage of sperm into the urogenital opening of the female during mating (Constantz, 1989). Some of these developmental modifications permit the male to swing the gonopodium forward, so that it points anteriorily and slightly laterally and can penetrate the posteriorly opening female urogenital opening (Peden, 1975; Howell et al., 1980). The gonopodial tip is equipped with barbs and spines that serve as holdfast devices during its brief insertion into the female urogenital opening (Rosen and Gordon, 1953).

Gambusia have internal and external morphology that reflects their diet and foraging behavior. They have strong, conical teeth and a relatively short esophagus and intestine, typical traits of predatory fish (Odum and Caldwell, 1955; Rosen and Mendelson, 1960; Meffe and Snelson, 1989). They have a small, dorsally oriented mouth and a dorso-ventrally flattened head (Lewis, 1970), which fit their habit of foraging at and near the water surface (see below). They have no cephalic canals (Rosen and Mendelson, 1960) and, hence, have relatively poor ability to detect water disturbances or vibrations (Walker, 1987; Helfman et al., 1997). They presumably rely more on sight to detect prey, predators and other physical objects (Lanzing and Wright, 1982).

The two species can be distinguished on the basis of differences in external morphology, chromosome morphology and genetic makeup. They differ in the number of dorsal fin rays, with *G. affinis* having seven and *G. holbrooki* having eight (Grant, 1978). Both species have 24 chromosome pairs, but a differing sex determining mechanism (Roberts, 1965; Black and Howell, 1979; Angus, 1989). *G. affinis* shows chromosomal heteromorphy with a WZ-ZZ sex determining mechanism, while in *G. holbrooki* there is no chromosomal heteromorphy and the mechanism is XX-XY (Chen and Ebeling, 1968; Black and Howell, 1979; Howell et al., 1980; Angus, 1989).

Earlier estimates of chromosome number for these two fish species were incorrectly low (Geiser, 1924). *G. affinis* and *G. holbrooki* show differing patterns of genetic allele frequencies and an abrupt differentiation in local genetic constituence between them (Wooten et al., 1988).

Age/sex categories

Four age/sex categories, namely adult males, adult females, immature males and others, can be distinguished amongst free-swimming fish on the basis of externally visible characteristics. Individuals with gonopodia (external insemination organs) that are not fully developed (see below) are considered immature males (Stearns, 1983; Meffe, 1992). Individuals that lack a gonopodium and have a dark, peritoneal spot on the side of the body above the gonoduct are mature females (Peden, 1973; Howell et al., 1980). In addition, yolked eggs about 1 mm in diameter can, in some cases, be seen in the abdomen of a pregnant female when viewed against a strong light (Stearns, 1983). Mature but sterile males can also be recognized on the basis of a relatively forward position of the anal fin, but without the development of this fin into a gonopodium (Geiser, 1924).

In some cases it may be possible to assign individuals to an age/sex category on the basis of just their size, but variability in rates of growth and development generally make this difficult (Yan, 1987; Zulian et al., 1993). Because males born relatively late in the breeding season mature more slowly and at a larger size than males born earlier, mature males sampled late in the season may, for example, be markedly smaller than males that are still maturing (Hughes, 1985c). An individual that is the size of an immature male but lacks a developing gonopodium, is more likely to be an immature female than a late developing male.

Taxonomy and systematics

The taxonomy of the two species *G. affinis* and *G. holbrooki* has changed considerably over the years. *Gambusia affinis* and a synonymous species, *G. patruelis*, were described in 1853 from two locations in Texas (Baird and Girard, 1853). *Gambusia holbrooki* was described a year later by

Agassiz on the basis of specimens from Charleston, South Carolina, and again by Girard in 1859 (Jordan and Gilbert, 1882; Jordan and Evermann, 1896). Subsequently these three species were considered to be a single species *G. affinis* (Jordan and Gilbert, 1882; Jordan, 1885) with *G. patruelis* and *G. affinis* being considered to be a single sub-species *G. affinis affinis* and *G. holbrooki* to be a second sub-species *G. affinis holbrooki* (Hildebrand, 1927; Collier, 1936; Hubbs, 1957a; Rivas, 1963). In 1979, these two subspecies were recognized as semi-species (Black and Howell, 1979), and finally in 1988 they were considered separate species, *Gambusia affinis* and *G. holbrooki* (Wooten et al., 1988; Smith et al., 1989). This is the present situation.

Because of the changing taxonomic relationships between *G. affinis* and *G. holbrooki*, and because of complex patterns of translocation of these two species throughout the world, it is often difficult to be certain as to whether a study or project has involved *G. affinis* or *G. holbrooki* or both species (Krumholz, 1948; Lloyd et al., 1986). The *Gambusia* in Australia were not, for example, identified as *G. holbrooki* until 1981 (Milton and Arthington, 1983; Lloyd and Tomasov, 1985) and almost all published articles prior to 1985 refer to them as *G. affinis* (Arthington and Milton, 1983; Lloyd, 1984). However, some confusion regarding their species identity has continued through to at least the early 1990s with some published articles continuing to refer to Australian *Gambusia* as *G. affinis* (Koehn and O'Connor, 1990; Hughes et al., 1991). A similar situation has occurred in Europe (Vargas and de Sostoa, 1996). In addition, despite the current general recognition of *G. affinis* and *G. holbrooki* as distinct species, some recent studies still refer to them as subspecies of *G. affinis* (Komak and Crossland, 2000).

Hybridization and sub-specific variation

These two fish species have been found to hybridize with other members of the genus *Gambusia* including *G. georgei* (Hubbs and Peden, 1969), *G. nobilis* and *G. heterochir* (Hubbs, 1957a, b; Hubbs and Delco, 1960; Yardley and Hubbs, 1976). The prevalence of such hybridization is, however, presently unknown.

Gambusia show patterns of geographic variation in terms of population genetics, morphology, physiological processes, physiological tolerances, biochemical characters and behavior, with the degree of dissimilarity between populations increasing with either the geographic distance between them or with the degree of habitat difference between the sites where they occur. Differences between populations have, for example, been found in terms of annual lipid cycle, enzyme activity levels, protein concentration, body size, weight and shape, a number of life-history parameters, resistance to insecticide and other toxins, temperature tolerance and tolerance of high salinity (Otto, 1973; Angus, 1983; Stearns, 1983; Meffe and Snelson, 1993a; Fuernwein et al., 2004). Langerhans et al. (2004) found that *Gambusia* from populations where predatory fish were present were able to swim faster than fish from predator-free populations and exhibited morphological differences that were expected to be associated with higher swimming speed. Genetic differences in terms of allele frequencies have been found between different populations of *Gambusia* (Smith et al., 1983; Kennedy et al., 1985; McClenaghan et al., 1985; Hughes et al., 1991). The pattern of genetic heterogeneity has been found to be correlated with environmental variables such as water temperature and flow characteristics (Smith et al., 1983; Liu et al., 1985) and the presence in the water of contaminants such as organochloride pesticides (Hughes et al., 1991) and radionuclides (Theodorakis and Bickham, 2004). In other cases little association with habitat has been found (McClenaghan et al., 1985; Zimmerman et al., 1987). In general, neighboring *Gambusia* populations have been found to be genetically more similar to each other than more distantly separated populations (McClenaghan et al., 1985), though sometimes there are significant genetic differences over short distances (Stearns and Sage, 1980).

Patterns of genetic variation within *Gambusia* populations are, however, not necessarily constant. In some cases the pattern of genetic variation within a population has been found to change over time, especially if conditions change (Smith et al., 1983; McClenaghan et al., 1985; Congdon, 1989), while in other cases allele frequencies have

displayed temporal stability across years (McCle-naghan et al., 1985).

These patterns of variation may involve evolutionary changes within *Gambusia* populations or flexible responses by individual animals. Variation in physical traits of the fish such as morphology, physiology or biochemistry may be due to evolution, brought about by differential selection pressures, or to the effects of different circumstances on individual animals (Zimmerman et al., 1987). On the other hand, the spatial and temporal patterns of genetic variation are believed to be brought about through a combination of selection, inbreeding, genetic drift and genetic exchange between populations (Yardley and Hubbs, 1976; Smith et al., 1983; McClenaghan et al., 1985). Artificial selection experiments have shown that some traits can be selected for and, hence, must have a genetic component. These traits include body weight, growth rate, age at maturity and size of first brood (Busack, 1983; Busack and Gall, 1983).

Despite the noticeable geographic variation within *Gambusia*, no sub-species of either *G. affinis* or *G. holbrooki* have been formalized with taxonomic status and a Latin name.

Habitat preference

Gambusia are able to utilize a very wide range of habitats across a very broad range of landscapes and environments and are able to tolerate, and at times thrive, under significantly different physical, chemical and biological characteristics (Rees, 1958; Lloyd et al., 1986; Hubbs, 2000). However, this section focuses on habitat preference while patterns of habitat-use are reviewed elsewhere (Pyke, unpublished).

Observations and experiments indicate that *Gambusia* prefer the habitats in which they are generally found over other available habitats. They prefer, for example, water temperatures around 31–35 °C to other temperatures. Winkler (1979), for example, observed that *Gambusia* exhibited daytime migrations between relatively deep and shallow water and attributed this to temperature changes in the water with the fish having a preference for 31 °C. Maglio and Rosen (1969) observed diurnal variation in the two-dimensional distribution of *Gambusia* across a pond with variable depth and temporal variation in the pattern of sun and shade. In addition, laboratory choice experiments indicated that *Gambusia* prefer water temperatures around 31–35 °C to both higher and lower temperatures. Cherry et al. (1976) found, for example, that *G. holbrooki* (based on geographic location) preferred a water temperature of about 35 °C and had an upper temperature avoidance limit of 39 °C, regardless of whether they were acclimated at 30 or 36 °C. Similarly, Winkler (1979) found that *G. affinis*, acclimatized to temperatures above, below and at 31 °C, have a preferred temperature of 31 °C. It is not clear, however, whether these observations demonstrate a genuine difference between *G. affinis* and *G. holbrooki* in preferred temperature as the studies differed in methodology and involved single populations of each species (Cherry et al., 1976; Winkler, 1979). *Gambusia* have also been found to prefer calm water over turbulent water, areas without any aquatic vegetation over areas with just a floating layer of aquatic vegetation, and areas with submerged natural vegetation or submerged plastic model vegetation over areas that either lack aquatic vegetation or have only a floating layer (Casterlin and Reynolds, 1977).

Gambusia prefer water that is shallow (i.e., about 8–15 cm deep), but not too shallow. Its preferences with regard to substrate are less clear. In laboratory experiments Casterlin and Reynolds (1977) observed a preference for dark over light substrate. However, Maglio and Rosen (1969) found that substrate preference depended on the sex and reproductive status of the fish tested and on the topography of the underlying substrate. They found that, when water depth is constant at about 7.6 cm and fish have a choice between black and white substrate, non-pregnant females and females in early stages of pregnancy show a high preference for the black substrate, but this preference declines as pregnancy advances, while newborn fish show a low preference for the black substrate. They also found that when the experimental aquarium was tilted, the fish generally choose the deeper end when this end had a depth of 7.6 or 15.2 cm regardless of the substrate. Miura et al. (1979) found that Gambusia preferred pond areas that were < 20 cm deep to equally available areas that were about 35–60 cm deep, regardless of whether

or not emergent aquatic vegetation was present. Habitat preference in *Gambusia* may also depend on individual age and size. Adults are generally observed in shallow, open water while immature fish tend to aggregate in shallow, densely vegetated areas (Miura et al., 1979).

The nature and extent of any diurnal or seasonal variation in patterns of habitat use by *Gambusia* are also unclear. *Gambusia* can be observed swimming near the water surface both during the day and at night, though they are often more conspicuous at night (Pyke, personal observation). Foraging by *Gambusia* has been reported to occur throughout the day with peaks in the early morning and at dusk (see below). Much lower densities of *Gambusia* are observed during the winter than during the warmer months of the year, but it is unclear how much of this is due to reduced population sizes in the winter versus sheltering behavior that makes them less observable. Such sheltering behavior may include retreating to relatively deep pond areas or burrowing in the mud. *Gambusia* have sometimes, for example, been observed immobile and scattered over the bottoms of deeper pools (Winkler, 1979), and, by burrowing into the mud, they may be able to survive the icing over of some ponds or other inimical conditions (Lloyd, 1984).

Biology of individual animals

Physiology

Gambusia are able to store fats or lipids in ways that give them considerable flexibility in terms of survival and reproduction (Reznick and Braun, 1987; Meffe and Snelson, 1993b). *Gambusia* have been found to show different patterns of lipid storage and utilization, depending on how cold it is during the winter, with winter survival making use of relatively more of the lipid supply in relatively cold areas (Meffe and Snelson, 1993b). *Gambusia*, in populations from northern USA where winters are relatively severe, deposited large lipid stores in autumn and late summer and used some of these stores as an energy source to survive the winter and to produce the first clutches in the spring (Reznick and Braun, 1987; Meffe and Snelson, 1993b). Further to the south in South

Carolina where winters are milder, the amount of lipid stored in somatic tissue was highest during the non-reproductive autumn/winter months and lowest during the March–September reproductive period, when large amounts of lipids shifted to the ovaries, eggs and developing embryos, while the reproductive tissues showed the reverse pattern (Meffe and Snelson, 1993b). In addition, individual *Gambusia* can delay maturation or cease breeding before the end of the breeding season, storing fat instead for subsequent use to enhance either winter survival or reproduction at the onset of the next breeding season (Reznick and Braun, 1987).

Gambusia physiology has also been investigated with regard to metabolic rate and provision of maternal resources to developing embryos. *Gambusia* are generally considered to have yolk dependent (i.e., lecithotrophic) embryos rather than embryos that receive a continuous supply of maternal nutrients via a blood supply connection with the mother (i.e., matrotrophic) (Reznick and Miles, 1989; Howe, 1995). However, Wourms (1981) pointed out that a constant dry weight of the embryo during gestation suggests that the developing embryo gets about 30–40% of its resources from the mother. In addition, Marsh-Matthews et al. (2001) found experimental evidence of maternal-to-embryo nutrient transfer in *Gambusia geiseri*, suggesting the same is probably true for *G. affinis* and *G. holbrooki*. Finally, metabolic rate for stationary *Gambusia* has been found to increase with increasing temperature (from 10 to 35 °C) and with reduced level of dissolved oxygen (Cech et al., 1980; Cech et al., 1985), but it remains relatively constant as salinity varies from 0 to 20 ppt (Akin and Neill, 2003).

Growth, development and morphological relationships

Fertilization in *Gambusia* is internal and young develop inside the mother until they are born as small free-swimming fish (Wourms, 1981). Birth of a clutch of young usually lasts from 1 to 3 h, but may extend to up to 1–2 days (Smith, 1912; Hildebrand, 1919b; Herms and Gray, 1940).

Growth and development of *Gambusia* can be divided into three phases. The first or gestation

phase involves eggs and embryos from the time of fertilization to the time when they are born. During the second or immature phase the fish change from immatures to adults, and the final phase is the period when the fish are adult.

The durations of these phases show considerable variation amongst individuals and from one time of year to another. The gestation period is usually 22–25 days (Krumholz, 1948; Lloyd et al., 1986), but can extend from 15 to 50 days depending on water temperature, season and locality (Gall et al., 1980; Reznick, 1981; Milton and Arthington, 1983; Fernandez-Delgado, 1989). For males the immature phase has lasted 18 days to 8 weeks in most studies (Lloyd et al., 1986; Meffe, 1992), but can also range up to about 8 months (Trendall, 1982). For females it has lasted about 18 days to 10 weeks in most studies (Lloyd et al., 1986; Vondracek et al., 1988; Meffe, 1992), and up to about 8 months if water temperature is low (Hildebrand, 1919a; Vondracek et al., 1988). In addition, Hughes (1985c) showed that males born early in the breeding season matured rapidly at small body size, while males born later in the breeding season delayed maturation and achieved larger size.

Developmental changes occur within at least the first two of these phases. When the ovaries of pregnant females have been dissected, a series of sequential embryonic stages have been observed, though the number of recognized stages has varied from three to six to ten (Peden, 1973; Meffe, 1987; Brown-Peterson and Peterson, 1990; Galat and Robertson, 1992). Soon after fertilization eyes form in the developing embryos, and the presence of these eyes is sometimes used to distinguish embryos from unfertilized eggs (Peden, 1973; Brown-Peterson and Peterson, 1990). From about the time of birth there are visible differences between the internal male and female reproductive systems that permit the sexing of young, as well as older developmental stages (Kuntz, 1913; Seale, 1917; Geiser, 1924; Hildebrand, 1927). In males, the gonopodia begin to form during the immature phase and reproductive maturity is considered to have been reached when these gonopodia are fully developed (Trendall, 1982; Busack and Gall, 1983; Meffe, 1992). At this stage the hooks at the ends

of two of the rays (i.e., 4p and 5) are free of opaque tissue, giving the apex of gonopodium a distinctly pointed appearance (Busack and Gall, 1983). There are also changes that occur within the male testes during the development of spermatogonia (Kuntz, 1913; Geiser, 1924; Hildebrand, 1927; Harrington, 1974). As female immatures age, they develop both mature, yolked eggs within their ovaries and a dark, periproctal spot, which stimulates males to attempt copulation and hence apparently indicates they are reproductively mature (Stearns, 1983). Females are assumed to have reached reproductive maturity when either one of these changes is observed (Stearns, 1983). No developmental changes during the adult phase for females have so far been reported.

Gambusia grow, both in size and weight, through each of these phases. Eggs range in diameter from about 0.2 to 1.0 mm when immature and reach about 1.4–1.8 mm when mature (Vargas and de Sostoa, 1996). Embryos are initially about 2.2 mm in diameter (Vargas and de Sostoa, 1996). As embryos they increase in snout-anus length from an initial 1.6 to 2.1 mm (Geiser, 1924; Wu et al., 1974) to a length of about 6–8 mm at birth (Rees, 1958; Meffe, 1990). During this gestation period, however, embryo weight remains relatively constant (Wourms, 1981), with early-eyed embryos having a dry weight of about 1.1–2.4 mg (Stearns, 1983), and the dry weight of newborn young being about 0.6–2.8 mg (Stearns and Sage, 1980; Meffe, 19871990). In males, gonopodia begin to form and can be recognized on the basis of anal fin structure at an average snout-anus length of about 15 mm (Ham, 1981), with reproductive maturity being reached at an average snout-anus length of about 17–20 mm, ranging from about 13 to about 23 mm (Hildebrand, 1919b; Trendall, 1982; Merrick and Schmida, 1984; Meffe, 1992). In females, reproductive maturity is reached at an average standard length of about 17–20 mm, ranging from about 13 to 24 mm (Hildebrand, 1927; Trendall, 1982; Meffe, 1992). Consistent with this, the smallest pregnant *Gambusia* are generally about 20 mm in length (Ham, 1981), but have been recorded as small as 16 mm (C. Hubbs, personal observation).

Males and females, therefore, generally mature at about the same body length. As immatures,

both male and female *Gambusia* grow at a roughly constant rate of about 1–2 mm per week until they reach maturity (Seale, 1917; Sokolov, 1936; Schoenherr, 1981; Trendall, 1982; Stearns, 1983). Males and females generally mature at about the same age. During this phase they also increase in weight as they grow and develop from a weight (i.e., wet weight) of about 3.8 mg when newborn, to adult weights of about 0.2–1.0 g for females and about 0.13–0.20 g for males (Ryder, 1882; Brown and Fox, 1966; Lewis, 1970). During this phase females increase in weight faster than males with females being heavier on average than males of the same length (Vargas and de Sostoa, 1996). As adults, males grow very little while females continue to grow (Johnson, 1976; Hughes, 1986; Zulian et al., 1993; Vargas and de Sostoa, 1996). Mature females are consequently generally larger than mature males with snout-anus lengths rarely exceeding about 3.5 cm for males and about 6 cm for females (Hildebrand, 1919b; Vondracek et al., 1988).

This variation in duration of the different phases of development may be due, at least in part, to the effects that various physical, chemical and biological factors have on the rates of development and growth. Growth rate in *Gambusia* has, for example, been found to be influenced by water temperature, salinity, diet, rate of food consumption, and density of conspecifics. The rate of development has been found to vary with temperature and conspecific density. In all cases the rates of growth and development either increase or decrease together (see below). Development rate and phase duration are necessarily inversely related.

Growth and developmental rates in *Gambusia* are greater, and age at maturity less, at 25–30 °C than at other temperatures. Meffe (1992) found that *Gambusia* grew faster and matured at a younger age and smaller size when kept at a relatively high 32 °C as compared with fish kept at a more normal 25 °C. Wurtsbaugh and Cech (1983) found that growth rate at a food ration level equivalent to 20% dry body weight/day peaked at 25 °C. Meffe and Snelson (1989) found that temperature and salinity interact to affect growth with maximum growth occurring at a temperature of 25 °C and salinity of 5 ppt. Snelson (1989) found that growth rate increases with

increasing temperature up to about 25–30 °C and then declines. Vondracek et al. (1988) found that growth rate at 20 °C was much lower than at 25 or 30 °C, and that ages at maturity for male and female *Gambusia* were greater at 20 °C than at 25 or 30 °C. Vargas and Sostoa (1996) found that, on the Iberian peninsula in Spain, *Gambusia* grew during the warmer months and not through the winter.

Growth rate in *Gambusia* depends on salinity but in no consistent fashion. Stearns and Sage (1980) found that *Gambusia affinis* from Texas grew more slowly in fresh water rather than in brackish (i.e., 10 ppt) water, while Zimmerer (1983) obtained the opposite result for *Gambusia* from southern Florida (therefore *G. holbrooki*). Meffe and Snelson (1989) found that, at 25 °C, maximal growth rate was obtained at a salinity of 5 ppt.

Growth rate in *Gambusia* also varies with diet. Meffe and Crump (1987) reported that cannabilistic *Gambusia* grew faster than *Gambusia* that were raised on various other diets. Wurtsbaugh and Cech (1983) found that growth rates of *Gambusia* fed on *Artemia nauplii* were higher than those that were fed tubifex worms. Reddy and Shakuntala (1979) found that, with a mixed diet of tubifex worms and mosquito larvae, the rate of growth for juvenile *Gambusia* increased with an increasing proportion of worms.

Growth rate in *Gambusia* increases with food level or ration (Shakuntala and Reddy, 1977; Wurtsbaugh and Cech, 1983; Vondracek et al., 1988). Trendall (1983) found that somatic growth rate peaked at intermediate food levels, while fecundity and brood mass continued to increase with increasing food level.

Rates of both growth and development in *Gambusia* generally decrease with increasing fish density. Stearns (1983) found that female *Gambusia* subjected to crowding in the first 10 days of life reached reproductive maturity at about 80 days, whereas those kept on their own took about 40 days. Zulian et al. (1993) found that both age at maturity and body length at maturity were higher for males grown in groups compared with males grown individually, but they found no effect of group size on female growth rate. Hughes (1985c) attributed slower growth rates of males later in the breeding season to the effects of increasing fish density through the season.

Size differences that develop among individuals during one phase of development may or may not persist during subsequent growth and development. The ultimate size attained by male *Gambusia* has, for example, been found to increase with increasing size at maturity for individuals grown in groups and, hence, maturing at a relatively large size, but male ultimate size is thought to be unrelated to size at maturity for individuals grown singly and, hence, maturing at a relatively small size (Zulian et al., 1993).

Body weight and various other size-related measurements increase with body length in *Gambusia*. Body weight, for example, increases in proportion to the snout-anus length raised to about the power 3.5 with no difference between males and females (Liu et al., 1985; Brown-Peterson and Peterson, 1990). In addition, total gut length and mouth gape size increased linearly with increases in snout-anus length (Booth, 1980).

Little is known about the underlying physiological mechanisms that control growth and development in *Gambusia*, though experimental work indicates that the normal pattern of sexual development in *Gambusia* is under the partial control of sex hormones. Turner (1941a, b, 1942b) found that the normal patterns were disrupted by either castration or exposing individuals to the androgenic hormone ethinyl testosterone.

Reproduction

Both male and female *Gambusia* have annual reproductive cycles with a distinct breeding season that, in most locations, extends from about mid-spring until mid-autumn with a peak in summer. Mature males generally show an annual testicular cycle, with a period of spermatogenesis from about mid-spring to mid-autumn and a period of testicular quiescence during the remainder of the year (Fraile et al., 1992, 1993; Fraile et al., 1994). Spermatozoa are released from the testis during almost the entire spermatogenesis period (Self, 1940; Fraile et al., 1992). Mature females show an essentially identical annual cycle in terms of reproduction, with ovarian maturation beginning in early-spring and oogenesis occurring throughout the period from mid-spring to mid-autumn (Self, 1940; Brown-Peterson and Peterson, 1990). Pregnant females are also generally found throughout this latter period (Hildebrand, 1919b; Hughes, 1985b; Brown-Peterson and Peterson, 1990). During the non-reproductive months ovaries represent a negligible amount of total body weight and contain a very small amount of lipids (Meffe and Snelson, 1993a). Reproductive activity apparently peaks during early- to mid-summer in most areas with both maximum testicular volume and maximum percentage of pregnant females occurring at this time (Hughes, 1985b; Fraile et al., 1992).

However, the breeding season for *Gambusia* sometimes varies from one location to another within a region as an apparent function of local water temperature and might be expected to vary geographically as well. In early-spring almost all females in thermally heated water were, for example, pregnant while no females from a nearby site with relatively cold water were pregnant. Despite the relatively warm and constant water temperatures at the thermal site, pregnant females were rarely found there during the period from mid-spring to mid-summer (Galat and Robertson, 1992). Similarly, for a pond in winter that was heated by thermal effluent, Ferens and Murphy (1974) found that the percentage of females greater than 30 mm in length bearing eyed-embryos was linearly related to the water temperature and that no females from non-heated areas were carrying eyed-embryos. Similarly the breeding season in thermally heated pools in Utah started earlier and finished later than that in unheated pools (Rees, 1958). In regions where there is a summer dry period and a winter wet period and where winter temperatures are generally mild, *Gambusia* might breed mostly during the winter.

Experiments using *G. affinis* collected in Spain (Fraile et al., 1994), Texas (Medlen, 1951) and Japan (Koya et al., 2004) have shown that temperature, photoperiod and reproductive state are all important in determining (in a proximal sense) reproduction in *Gambusia*. Mild temperature (i.e., 20 °C) at the end of the quiescence period induced spermatocyte formation independent of the photoperiod, but both mild temperature and relatively long photoperiod (i.e., 16L:8D) were required for meiosis and spermiogenesis (Fraile et al., 1994). However, mild temperature and long photoperiod had no effect on spermatogenesis in the late phase of testicular quiescence (Fraile et al., 1993).

Medlen (1951) found little or no evidence of reproduction amongst female *Gambusia* if the water temperature was less than about 16 °C regardless of other conditions and also that females under an extended light regime became pregnant earlier than other females. He concluded that both photoperiod and water temperature regulate reproduction in *Gambusia*, with photoperiod playing a relatively minor role. Koya et al. (2004) found that parturition intervals became shorter at higher temperatures under constant photoperiod, and shorter under longer day-length periods at a constant temperature. They concluded that temperature primarily influences the rate of embryonic development while photoperiod primarily affects the progress of vitellogenesis (timing of fertilization after parturition) and has little effect on embryonic development. *Gambusia* kept in aquaria may produce young during the winter months (Self, 1940). The reproductive responses to these and other factors could, of course, change evolutionarily and, hence, lead to different reproductive patterns in different regions.

Female *Gambusia* that are pregnant with a litter of young do not usually become pregnant again until after the first litter is born, Therefore, superfetation, or the presence of more than one litter of developing embryos in the same animal at the same time, does not usually occur in *Gambusia* (Turner, 1937; Scrimshaw, 1944; Trendall and Johnson, 1981; Reznick and Miles, 1989). This is best indicated by the observations that females with developing embryos may also have maturing unfertilized eggs but not fertilized eggs (Vondracek et al., 1988; Vargas and de Sostoa, 1996), and there is usually a pause of several days to 2 weeks between the birth of one litter and fertilization of the next (Turner, 1937; Reznick and Miles, 1989). However, that *Gambusia* may occasionally have superfetation is suggested by the observation that there may sometimes be a range of developmental stages of embryos present in the ovary (Scrimshaw, 1944; Nelson, 1983), though this could reflect variation within a litter rather than different litters.

Female *Gambusia* can store viable male sperm and have considerable flexibility in terms of when their eggs are fertilized. They may retain viable sperm in their oviducts for several months after their last mating and may produce multiple broods using sperm from a single mating (Hildebrand, 1919b; Krumholz, 1948). They can, in particular, retain viable sperm over the non-reproductive period (Haynes, 1993). This makes it very difficult to know when mating has occurred and it is not clear whether *Gambusia* mate just once during a breeding season, or more often (Turner, 1937). It is likely that retained sperm are nourished within the lining of the ovary (Hoar, 1969).

Male *Gambusia* are apparently capable of successful mating any time during the spermatogenesis period which extends through the breeding season. Spermatozoa are released from the testis during almost this entire period, but those formed at its end remain in the testis during the quiescent period and are released at the beginning of the nest spermatogenesis period (Self, 1940; Fraile et al., 1992).

Gambusia females may have multiple broods over a single breeding season, with older and larger females having more broods during the breeding season than younger females. That this should be possible is suggested by the short durations of both gestation and pre-fertilization periods relative to the length of the breeding season. With a gestation period as short as 2–3 weeks and as little as about a week between birth of one litter and fertilization of the next, it is theoretically possible that *Gambusia* females will reproduce about every 3–4 weeks (Turner, 1937). If maintained over an entire breeding season of about 6–7 months, this could result in up to about nine broods per female per season. Laboratory studies have in fact observed intervals between broods as low as 28–30 days (Turner, 1937; Trendall, 1982). Six consecutive broods during one breeding season have been observed on a number of occasions (Hildebrand, 1919a; Milton and Arthington, 1983), and the maximum observed number of broods per female per season has been nine (Milton and Arthington, 1983). Both Krumholz (1948) and Maglio and Rosen (1969) found that females that are breeding in their second season had 4–5 broods, whereas smaller young-of-the-year females generally produced no more than two broods.

It is not clear how many broods a single *Gambusia* female can and does have during her lifetime. According to Minckley (1973), a single female may produce up to about five broods in her

lifetime. However, if a female that had nine broods in one season (as described above) and was able to breed again in the following season, then she would have produced 10 or more broods over her lifetime. There could, however, be a tradeoff between reproduction and survival so that such theoretical lifetime fecundity is rarely if ever achieved.

That *Gambusia* populations are capable of having multiple generations within the one breeding season is suggested by the short durations of both gestation and pre-adult periods relative to the length of the breeding season. With a gestation period of 2–3 weeks and 1–2 months between when an individual is born and when it is reproductively mature (see above), it is possible for there to be up to 3–4 successive generations during a single breeding season with about 2–3 months between each generation. Rees (1958) reported that there are 3–4 generations per breeding season in normal unheated ponds in Utah and even more in thermally heated ponds.

Clutch size in *Gambusia* varies considerably. Average clutch sizes have ranged from 5 to over 100 (Krumholz, 1948; Hoy and Reed, 1970; Brown-Peterson and Peterson, 1990), while minimum clutch sizes have ranged from 1 to 84 (Smith, 1912; Gall et al., 1980) and maximum clutch sizes have ranged from 10 to 375 (Krumholz, 1948; McDowall, 1978; Trendall, 1982).

A number of factors have been found to influence clutch size in female *Gambusia*, including age at first reproduction, whether early or late in the breeding season, level of available food, female body weight or length, and geographic location. Females that breed for the first time in their second breeding season, having been born near the end of the previous breeding season, have larger clutches than females that breed in the same season in which they were born and are only about 1–2 months old (Krumholz, 1948; Maglio and Rosen, 1969; Haynes and Cashner, 1995). Because of this, clutches are generally larger at the beginning of the breeding season than later on (Wu et al., 1974; Hughes, 1985b; Meffe and Snelson, 1993a). Clutch size increases with increasing level of available food (Gall et al., 1980; Meffe, 1986) and with increasing female body length or weight (Busack and Gall, 1983; Milton and Arthington, 1983; Vondracek et al., 1988; Brown-Peterson and Peterson, 1990).

Schoenherr (1981) found a linear increase in clutch size with female body length, while others found that it was exponential (Wu et al., 1974; Hughes, 1985b; Kennedy et al., 1986). Clutch size has been found to increase linearly with body weight and to depend on geographic location (Wu et al., 1974; Vargas and de Sostoa, 1996). Clutch size has so far been found to be unaffected by water temperature (Vondracek et al., 1988).

Females invest relatively more body resources into reproduction than males. Female gonad weight has sometimes, for example, reached almost 25% of total weight, whereas male gonad weight has rarely reached 4% (Vargas and de Sostoa, 1996).

Courtship and mating

The courtship/mating in *Gambusia* involves three distinct phases: association, courtship and mating. During the association phase males and females tend to move together, males often congregate around a female and males are generally aggressive towards one another, with the larger individual in a group becoming dominant and chasing others away from the vicinity of the female (Itzkowitz, 1971; Hughes, 1985a; Bisazza and Marin, 1991). This association occurs mostly in late spring and early summer; at other times males and females are more dispersed (Maglio and Rosen, 1969; Martin, 1975). Courtship usually involves the female and the dominant male of her attendant group, and may include one or more matings (Bisazza and Marin, 1988). Courtship and mating in *Gambusia* also peak in late spring and early summer (Maglio and Rosen, 1969; Martin, 1975).

Courtship in *Gambusia* involves sexually aggressive approaches to the female by the male and acceptance or rejection on the part of the female. The overt courtship behavior of the males may include the gonopodial swing, when the gonopodium is erected but copulation is not attempted, chasing a female without erecting the gonopodium, and attempted copulation with erect gonopodium (Rosen and Tucker, 1961; Haynes, 1993). Before copulation occurs, the female will usually either allow the male to approach her or she may approach and follow

the male (Bisazza and Marin, 1991). She may also exhibit 'arching behavior', which is thought to signal sexual receptivity (Bisazza and Marin, 1991). She may also simply swim away (Rosen and Tucker, 1961). Whether or not copulation occurs appears to be under the control of the female.

Copulation attempts by males are generally frequent but unsuccessful, and if they are successful copulation itself is very brief. Attempted copulations typically occur at a rate of about once per minute (Martin, 1975; Bisazza and Marin, 1991) and involve very rapid thrusts of the male gonopodia towards the female urogenital opening (Haynes, 1993). Copulation involves only a very brief contact of the gonopodial tip with the oviductal opening (Seal, 1911; Collier, 1936) and in the closely related *G. heterochir* takes on average only 0.9 s (Warburton et al., 1957). Though the speed with which attempted copulation occurs makes it difficult to distinguish successful and unsuccessful copulation attempts (Haynes, 1993), it is nonetheless apparent that the males often miss their mark (Seal, 1911).

Male courtship behavior is influenced both by the presence of reproductively mature females and water temperature. Experiments with artificial models of females have shown that the dark anal spots on the females, that indicate female maturity, attract the males and facilitate orientation when the gonopodium is thrust at the females urogenital opening (Peden, 1973). In addition, male sexual activity, as measured by chases/time, attempted copulations/time and average pursuit distance, decreases if the water temperature is experimentally lowered, with an apparent threshold temperature of 10 °C, below which no sexual activity occurs (Haynes, 1993).

Female *Gambusia* may sometimes exhibit mate choice, but males are unlikely to ever do so. Female *Gambusia* have sometimes been found to exhibit a mate preference for relatively large males (Hughes, 1985a) and other times to show no size-based mate preference (Bisazza and Marin, 1991). Similar experiments, as well as experiments involving one male and two female fish placed together in the same aquarium, have shown that male *Gambusia affinis* discriminate poorly between conspecific females and females of another species with which this species is known to hybridize (e.g.,

G. heterochir) in comparison to how well they discriminate when the heterospecific female is from a species with which hyrbridization does not occur (e.g., *G. hurtadoi* and *G. geiseri*) (Hubbs and Delco, 1960, 1962; Peden, 1975). They therefore seem unlikely to discriminate between different females within their own species. Furthermore, given the relatively small amounts of time and other resources invested in reproduction by males, males would not be expected to show significant mating preference amongst different females (Andersson, 1994). On the other hand, the aggressive behavior amongst males may limit any choices in mates available to the females.

The extent to which there is reproductive isolation between *G. affinis* and *G. holbrooki* through courtship behavior is unclear. Since *G. affinis* males do not discriminate between conspecific females and females of *G. heterochir*, they are unlikely to discriminate with regard to *G. holbrooki* females (Hubbs and Delco, 1960, 1962; Peden, 1975). Any discrimination and mate choice must be carried out by the females. There are differences in male courtship behavior between *G. affinis* and *G. holbrooki* (Itzkowitz, 1971; Martin, 1975; Hughes, 1985a; Bisazza and Marin, 1991; Haynes, 1993), but it is not known whether female *Gambusia* discriminate on the basis of this behavior.

Foraging, Diet, Food Consumption, Digestion and Food Conversion

Gambusia often obtain necessary food and oxygen at or near the water surface. Though *Gambusia* can survive in water with very little dissolved oxygen (see below), their survival as dissolved oxygen in the general water column approaches zero depends on their ability to gain access to the surface of the water (Homski et al., 1994), where they either take in water that is relatively oxygen-rich at the atmosphere-water interface (Lewis, 1970) or gulp air from the atmosphere (Odum and Caldwell, 1955; Sjogren, 1972). *Gambusia* usually forage near the water surface and often take food items from the surface itself (Hildebrand, 1919b; Vooren, 1972). Their dorsally oriented mouth and dorso-ventrally flattened head facilitate these uses of the surface water layer (Lewis, 1970; Lloyd, 1984).

Gambusia show both diurnal and seasonal foraging patterns. They forage throughout the day (Belk and Lydeard, 1994), with analysis of stomach contents suggesting two diurnal peaks in feeding activity, one in the morning and another around dusk (Hess and Tarzwell, 1942; Walters and Legner, 1980). Average food consumption and presumably time spent foraging by individual *Gambusia* also varies seasonally, with a peak in summer and a trough in winter (Morton et al., 1988). Morton et al. (1988) found that in winter most *Gambusia* stomachs were empty, suggesting that most individuals were not feeding at all at the time, while a few stomachs were full, suggesting that these few individuals were feeding extensively.

Gambusia in the wild have diets that include an exceptionally wide array of different food types and may vary considerably from one time and place to another (Rees, 1958; Miura et al., 1979; Blanco et al., 2004). Based on analysis of stomach contents, these include insects and spiders that either land or fall on the water, crustaceans, worms, molluscs, larvae and pupae of other aquatic invertebrates, algae and other plant material, smaller fish of both its own and other species and diatoms (Crivelli and Boy, 1987; Arthington, 1989a; Arthington and Marshall, 1999; Garcia-Berthou, 1999). Based on analysis of faecal samples, wild *Gambusia* feed on crustaceans, molluscs, diatoms and algae (Bay and Anderson, 1966). Direct observations of *Gambusia* feeding are not generally reported but wild *Gambusia* have been observed attacking the tails of other larger fish, removing pieces of their fins and other body parts (Lloyd et al., 1986; Arthington, 1989b). Analysis of stomach contents also indicates that the diet of *Gambusia* may vary with time of day and with feeding location (Maglio and Rosen, 1969).

Based on the analysis of stomach contents, it is apparent, in particular, that wild *Gambusia* often include mosquito larvae in their diet, but also that mosquito larvae typically constitute a small proportion of the food eaten (Sokolov and Chvaliova, 1936; Washino, 1968; Harrington and Harrington, 1982). Only occasionally mosquito larvae have been found to constitute a large proportion of the observed diet (Harrington and Harrington, 1961; Morton et al., 1988; Lounibos and Frank, 1994).

Most amphibians have aquatic eggs and larvae, yet reports of these food types being included in the diets of *Gambusia* in the wild are conspicuous by their general absence. Being soft bodied and without hard body parts, the eggs and tadpoles of amphibians rapidly disintegrate once ingested by a fish, and it not surprising that there are no reports of tadpoles from *Gambusia* stomach contents (Reynolds, 1995). There are also very few reports of wild *Gambusia* observed feeding on frog eggs or tadpoles (Ghate and Padhye, 1988). Both laboratory experiments and circumstantial evidence suggest, however, that the extent of these reports greatly underestimates the importance of amphibian eggs and larvae in the diet of *Gambusia* (see below).

Comparisons of stomach contents of wild caught *Gambusia* with samples of available prey show biases towards or away from certain prey types, and these biases are generally interpreted as indications of feeding preferences (Hess and Tarzwell, 1942; Farley, 1980). Predation by *Gambusia* has, for example, been found to be biased towards small versus large tadpoles (Blyth, 1994), chironomid larvae and ceratopogonid larvae versus other types of invertebrate prey (Hess and Tarzwell, 1942; Farley, 1980), one kind of mosquito (*Culicini*) rather than another (*Anopheles*) (Hess and Tarzwell, 1942), late versus early instars of *Anopheles* mosquitoes (Hess and Tarzwell, 1942), and *Anopheles* pupae versus larvae (Hess and Tarzwell, 1942). In addition, large *Gambusia* tend to eat different and often larger prey than do smaller fish (Hess and Tarzwell, 1942; Farley, 1980).

These biases could, however, be the result of differences in terms of where different prey are distributed coupled with biases in terms of where the *Gambusia* choose to feed (Maglio and Rosen, 1969), or differences in the abilities of the prey to hide from or escape the *Gambusia* (Hess and Tarzwell, 1942), or variation in the ability of *Gambusia* to catch different prey. *Gambusia* are, for example, less able to catch food in running versus still water, and their predatory efficiency declines as water speed increases (Reddy and Pandian, 1974). *Gambusia* are also more likely to attack moving than stationary prey. Herms and Gray (1940) found that, when mosquito larvae and pupae are placed in the same water as *Gambusia*, the first items to be eaten by the *Gambusia* are the larvae, and they suggest that the stationary nature of the pupae may make them less susceptible to

predation than the moving larvae. Similarly Hildebrand (1919b) found that *Gambusia* eat live mosquito larvae in preference to dead larvae and suggested that movement by a larva greatly increases its likelihood of being eaten immediately. Similarly *Gambusia* have been observed to swim past stationary mosquito larvae while any that begin to move are quickly eaten (Hildebrand, 1919a; Rees, 1958).

That *Gambusia* have wide and flexible diets is also indicated by the range of foods that have been used to maintain them in captivity. In these situations they are usually fed some type of commercial fish food (Meffe, 1992; Zulian et al., 1993; Fraile et al., 1994) but they will also eat a wide variety of other food types such as smaller or minced *Gambusia*, including a female's own young, other fish and fish eggs, mosquito larvae and pupae, *Drosophila* adults and pupae, corixid larvae, odonate larvae, *Daphnia*, copepods, back swimmers, brine shrimp, oligochaete worms, boiled egg, bread soaked in beaten egg and dried, finely chopped snails and liver (Seale, 1917; Geiser, 1924; Dionne, 1985; Meffe and Crump, 1987; Murdoch and Bence, 1987). Captive *Gambusia* will, in particular, eat the eggs and tadpoles of amphibians if provided with them (Lawler, 1989; Gamradt and Kats, 1996; Komak and Crossland, 2000).

Gambusia, especially if well fed and not hungry, show dietary preferences in choice experiments with respect to both prey size and prey identity. Consistent with the expectations arising from optimal foraging theory (Pyke, 1984), starved *Gambusia* feed indiscriminately (Bence and Murdoch, 1986).

When the alternative prey are relatively small invertebrates, *Gambusia* show a preferred prey size which increases with increases in the size of the *Gambusia*. Bence and Murdoch (1986) found, for example, that, when feeding on *Daphnia*, *Gambusia* have a preferred prey size which increases with increasing size of the *Gambusia*. Similarly, Homski et al. (1994) found that *Gambusia* prefer one mosquito instar level over others and that the preferred instar increases with size of the *Gambusia*. Similarly Wurtsbaugh et al. (1980) found that larger *Gambusia* prefer to eat larger mosquito larvae (*Culex tarsalis*). Consistent with this, Booth (1980), working with relatively large

fish and small prey, found that *Gambusia* preferred larger prey.

When *Gambusia* are provided with alternative foods of different types, they generally exhibit some preference amongst them. *Gambusia* generally eat invertebrate prey in preference to tadpoles. Lawler et al. (1999) found, for example, that *Gambusia* attack mosquito (*Culex*) larvae, *Daphnia* and corixid larvae in preference to tadpoles. Reynolds (1995) found that *Gambusia* eat mosquito larvae and *Daphnia* in preference to tadpoles, and Hubbs (1991) reported that *Gambusia* seemed to prefer larval *Drosophila* over adults and *Drosophila* over Tetramin fish food. *Gambusia* will sometimes eat mosquito larvae in preference to other invertebrate prey, but other times the reverse is true. Murdoch and Bence (1987) found, for example, that *Gambusia* prefer mosquito larvae over back-swimmers (*Notonecta* sp.), and Seale (1917) found that *Gambusia* prefer to eat mosquito larvae rather than corixid larvae. However, Reddy and Shakuntala (1979) found that *Gambusia* prefer worms (*Tubifex tubifex*) over mosquito larvae (*Culex fatigans*). Rees (1958) reported that they ate small crustaceans before eating any mosquito larvae, and Miura et al. (1979) found that they selected against mosquito larvae as food when a variety of other aquatic organisms was available.

When *Gambusia* have been provided with different species of prey from the one prey type, they have sometimes exhibited a prey preference and sometimes have not. Rajasekharan and Chowdaiah (1972) found, for example, that *Gambusia* eat one mosquito species (*Aedes aegypti*) in preference to another (*Culex fatigans*) and Komak and Crossland (2000) found that *Gambusia* prey on tadpoles of one frog species (*Limnodynastes ornatus*) in preference to tadpoles of another frog species (*Bufo marinus*). However, Pyke and White (2000) did not observe any preferences when *Gambusia* were provided with choices of different tadpole species.

In choosing from among alternative foods, *Gambusia* may prefer food types that yield relatively high rates of energy gain, while avoiding prey that contain toxic or use repellent chemicals. Bence and Murdoch (1986) found, for example, that the sizes of *Daphnia* preferred by *Gambusia* of different sizes were the most profitable in terms of food yield per unit time. Kerfoot (1982) found that

release of hydrogen peroxide by small notonectids acts or helps to repel predation by *Gambusia*. The relatively high toxicity of tadpoles of *Bufo marinus* may explain why they are less preferred by *Gambusia* than tadpoles of another frog species (Komak and Crossland, 2000). That *Gambusia* generally prefer invertebrate prey to tadpoles may similarly be due to the relatively higher toxicity of tadpole tissue to this fish.

Growth and survival of *Gambusia* depend on its diet, with mixed diets possibly yielding the highest rates. Growth and survival have, for example, been found to be relatively low when *Gambusia* feeds solely on mosquito larvae (Reddy and Pandian, 1972; Reddy and Shakuntala, 1979; Murdoch and Bence, 1987). Growth of *Gambusia* is better on a diet of tubifex worms than on a diet of just mosquito larvae (Reddy and Pandian, 1972; Reddy and Shakuntala, 1979), and higher still on a diet of shrimp (Wurtsbaugh and Cech, 1983). Reddy and Pandian (1972) found that *Gambusia* have a relatively high mortality rate when fed just mosquito larvae, especially with *Culex* sp. rather than *Anopheles* sp. and that *Gambusia* exhibited better survival and growth when fed a mixed diet of tubifex worms and mosquito larvae than when fed either worms or mosquito larvae alone. Vondracek et al. (1988) found, similarly, that *Gambusia* had a higher growth rate when fed both tubifex worms and commercial fish flake rather than just worms.

Experiments have shown that the rate of food consumption by *Gambusia* depends on water temperature and salinity, the sex and body size of the fish, available prey size, food abundance, and the hunger level of the fish (as determined by how long it has been since the fish last had food), with increased consumption generally reflecting increased food requirements relative to food availability. Food consumption, for example, increases with increasing water temperature (Reddy, 1975), female *Gambusia* eat more than males of the same size (Reddy, 1975; Shakuntala, 1977), food consumption increases with increasing body size (Hess and Tarzwell, 1942; Maglio and Rosen, 1969; Shakuntala and Reddy, 1977; Homski et al., 1994), and the number of food items consumed per unit time decreases with increasing prey size (Homski et al., 1994). In addition, hungry fish eat more than less-hungry fish (Dionne, 1985), and

food consumption increases with increase in the level of food abundance (Shakuntala and Reddy, 1977).

Experiments have also shown that the rate of food consumption depends on what kind or kinds of prey are available, in ways generally consistent with apparent feeding preferences as discussed above. When *Gambusia* are provided with both mosquito larvae (*Culex fatigans*) and worms (*Tubifex tubifex*), their rates of food consumption (weight/fish/day) increase as the proportion of worms increases from 0 to 100%, reflecting a feeding preference for the worms (Reddy and Shakuntala, 1979). Komak and Crossland (2000) found that *Gambusia* show relatively high levels of predation on early-stage tadpoles of the frog *Bufo marinus* and lower predation on both eggs and later-stage tadpoles of this species, and explained this on the basis of the eggs being imbedded in a thick gelatinous material, making predation on them difficult, and the tadpoles increasing in toxicity as they age. When *Gambusia* and mosquito larvae have been offered together, consumption of the mosquito larvae by the fish has sometimes seemed high (Seale, 1917; Hildebrand, 1919a, b) and other times seemed more modest (Reddy and Shakuntala, 1979). This may reflect the apparently ambivalent responses of *Gambusia* to mosquito larvae in prey preference experiments.

In *Gambusia* small prey are digested faster than larger prey and the rate of digestion increases with increasing water temperature. At about 20 °C small fish larvae (< 5 mm total length) are digested beyond the point of recognition within 2–3 h, while larger larvae do not reach this stage until at least four hours after ingestion (Ivantsoff and Aarn, 1999). At 30 °C digestion of mosquito larvae begins about two hours after ingestion and complete discharge has occurred by 3–4 h, but at 17–23 °C discharge occurs after 5–6 h (Sokolov and Chvaliova, 1936).

The rate at which food consumed by *Gambusia* is converted into fish biomass depends on the level of food supply, but not on the age or size of the fish. When oligochaete worms were provided as food for just two hours per day, the rate of conversion was about 8%, but it increased to about 14% when unlimited food was supplied (Shakuntala and Reddy, 1977). At both levels of food supply the conversion rate

did not depend on fish age (Shakuntala and Reddy, 1977).

Other behavior

Gambusia exhibits both social and anti-social behavior. It is well-known for its aggressive behavior, both intra- and inter-specific, towards other fish (Courtenay and Meffe, 1989). This aggressive behavior includes biting and physical pushing or thrusting (Caldwell and Caldwell, 1962; Meffe et al., 1983; Meffe, 1985; Lloyd et al., 1986), and as a result, dominance hierarchies are established with larger fish generally dominant over small fish (Caldwell and Caldwell, 1962; Bisazza and Marin, 1991; Mills et al., 2004). This aggression is not usually site specific and territories are rarely defended (Itzkowitz, 1971; Winkler, 1979). On the other hand, *Gambusia* is often found in aggregations or in schools that move together, and during these times individuals are clearly tolerant of other individuals in close proximity (Rees, 1958; Maglio and Rosen, 1969; Al-Daham et al., 1977).

Physical and chemical tolerances

Changes in the physical and/or chemical environment may influence the likelihood of mortality for individual *Gambusia* with the effects of such changes depending on the rate and duration of change, the initial environment and how long it was in this state, and the final state and how long it has been this way. These effects may also depend on individual traits such as physiology and genetic background. Congdon (1994a), for example, found genetically based differences in salt tolerance. Aspects of the environment that have been considered include salinity, water temperature, dissolved oxygen and a variety of chemical substances.

Salinity has been the best studied of the various physical and chemical factors and studies of it have demonstrated several of the above effects. Firstly, if *Gambusia* are transferred abruptly from freshwater to partly saline water, mortality increases with both the salinity of the new water and the duration of exposure to it. Congdon (1994a) found, for example, that mortality after transfer from freshwater to a salinity of 17 ppt was 16% after 1 day, 46% after 2 days and 67% after 3 days. In addition, Al-Daham

and Bhattit (1977) found that 90% or more *Gambusia* survived for 6 days after transfer from fresh water to water with salinity either 10 ppt or 20 ppt, but all *Gambusia* were dead within 24 h after transfer to 31 ppt salt water (i.e., 75% sea water). Similarly, Nordlie and Mirandi (1996) found 100% survival over 14 days when *Gambusia* were transferred from freshwater to salinities of up to 6 ppt, and that survival over this period decreased to 60% when the new salinity was 10 ppt and further decreased to 37% at 12 ppt. Ahuja (1964) found that survival was 50% over about seven weeks, after transfer from freshwater to a salinity of 10 ppt, and that survival decreased to about 5% at 16 ppt. Similarly, Chervinski (1983) found that survival over 1–2 days was about 90% when *Gambusia* were transferred from freshwater to a salinity of 20 ppt, and that this decreased to 0% at salinities of 24 ppt and 28 ppt. Congdon (1994a) (see above) found 67% survival over 3 days in *Gambusia* transferred from freshwater to a salinity of 17 ppt and 0% survival if transferred to 30 ppt. It is difficult, however, to compare these studies because of their different durations, differences in body size of the fish used (Congdon, 1994a) and other differences in experimental protocol.

Secondly, survival of *Gambusia* in water with relatively high salinity is increased if the fish are subjected to slow increases in salinity, allowing for gradual adaptation to the new conditions. Chervinski (1983), for example, found that after gradual adaptation survival by *Gambusia* over 7 days was 100% at 20 ppt (compared with 90% for abrupt transition), 80% at 36 ppt and 65% at either 36 or 40 ppt (compared with 0% for abrupt transitions to these salinities).

Survival by *Gambusia* at relatively high water temperatures also shows the effects of initial and final conditions. Survival by *Gambusia* in fresh water at increasing temperatures is generally high until an upper lethal temperature is reached at which rapid mortality occurs (Otto, 1973; Cherry et al., 1976). This upper lethal temperature increases with increases in the temperature to which the *Gambusia* are acclimated beforehand. Otto (1973) found, for example, that the upper lethal temperature increased from about 32 °C to about 43 °C as the acclimation temperature

increased from 5 to 35 °C. Similarly, Cherry et al. (1976) found that, when *Gambusia* are acclimated at either 30 or 36 °C, the upper lethal temperature is about 38 °C.

Survival of *Gambusia* at relatively low water temperatures shows similar effects. In the wild, *Gambusia* sometimes survive reasonably cold winters and have even been observed swimming around beneath the ice on some ponds in water that must have been only a few degrees Celsius (Krumholz, 1944). Laboratory experiments have been consistent with these observations, with Ham (1981) reporting that *Gambusia* survived a water temperature of only 1 °C for brief periods. Glover (1979) reported that *Gambusia* survived temperatures as low as 3 °C without signs of stress for at least 24 h, and Otto (1973) reporting that fish acclimated to low water temperature showed reasonable survival at temperatures below 5 °C. On the other hand, they sometimes do not always survive the winter in the wild (Rees, 1934; Maglio and Rosen, 1969; Rees et al., 1969) and have been observed to die under ice in some locations (Rees et al., 1969; Duryea et al., 1996). The reasons for these differences in survival are unknown.

Experiments have also shown that *Gambusia* can survive increasingly low concentrations of dissolved oxygen, but only if the duration of exposure decreases or they are able to gulp air at the water surface. *Gambusia*, that did not gulp air at the water surface, have been found, for example, to show 100% survival if the oxygen concentration was 1.3 mg/l (Odum and Caldwell, 1955), to survive a little less well at a concentration of 1.0 mg/l (Homski et al., 1994), and to be able to survive only briefly at a concentration of 0.5 mg/l (Glover and Sim, 1978). However, in situations where *Gambusia* are able to gulp air at the water surface, they have been found to survive concentrations of dissolved oxygen as low as 0.28 mg/l (Odum and Caldwell, 1955).

Unusually high levels of radiation can also affect *Gambusia* mortality. Blaylock (1969) found, for example, that *Gambusia* females that were exposed to radiation at the rate of about 11 rads/day showed high levels of dead embryos and abnormally developed young.

In summary, whether through basic abilities, physiological adaptation to conditions, or genetic changes at the population level, *Gambusia* are able to survive a very wide range of physical conditions. They may survive water temperatures ranging from about 1 to about 40 °C., salinities from that of freshwater to that of sea-water, and concentrations of dissolved oxygen as low as about 0.28 mg/l.

The effects of various chemical substances on *Gambusia* survival have also been relatively well studied. This has mostly been the result of concern that agricultural use of chemicals such as herbicides and insecticides might reduce *Gambusia* densities and hence their effectiveness as mosquito control agents (Lewallen, 1959; Mulla, 1961; Mulla et al., 1963; Davey et al., 1976). There has also, however, been relatively recent interest in using chemical and other means to control or eliminate *Gambusia* in certain situations (Hubbs and Brodrick, 1963).

Gambusia may suffer relatively high mortality when exposed to a variety of chemical substances, with mortality increasing with increases in the concentration of the substance and, in some cases, being high at extremely small concentrations. Ahmed et al. (1970) found, for example, that mortality over 24 h in *Gambusia* increases with increases in concentration of methyl parathion with 100% mortality occurring at only 0.3 ppm. Increases in mortality with increasing concentration were also found by Ferguson and Boyd (1964). Some insecticides result in 50% mortality in *Gambusia* at concentrations that are measured in parts per billion (Culley and Ferguson, 1969). Darwazeh and Mulla (1974) found high mortality in *Gambusia* exposed to some herbicides at 10 ppb. Pollard (1973) found rapid die off in *Gambusia* in water aerated with air that had passed over a 'pest strip' containing an insecticide. Reed and Bryant (1975) found that *Gambusia* densities in rice fields were reduced if the fields were sprayed with the insecticide parathion.

Some chemical substances are more toxic to *Gambusia* than others. Darwazeh and Mulla (1974) found, for example, complete mortality in *Gambusia* exposed for 48 h to the mosquito larvacide "Chloropyrifos" at a concentration of 5 ppm, while the same concentration of the larvacide "Abate" did not result in any mortality over this period. Similar results have been obtained in other studies (Lewallen and Brydon, 1958; Lewallen, 1959; Mulla and Isaak, 1961; Mulla et al., 1963).

Gambusia from populations that have had a long history of exposure to toxic chemicals such as herbicides, insecticides or industrial pollutants show less mortality when experimentally exposed to these chemicals than *Gambusia* from other relatively unaffected populations (Boyd and Ferguson, 1964). Culley and Ferguson (1969) found, for example, that *Gambusia* taken from a site with a history of application of various insecticides, including DDT and parathion, were relatively resistant to these insecticides in comparison with fish taken from a site that was distant from any use of these chemicals. Similar results have been obtained in a number of other studies (Ferguson and Boyd, 1964; Ferguson, 1966; Ferguson et al., 1966; Burke and Ferguson, 1969). These differences between populations may result from either genetic differentiation between them or differences in physiological adaptation on the part of the fish, or both (Lloyd, 1984).

Gambusia may also show behavioral, histological, physiological or biochemical effects of acclimation to different water temperatures. Hammill et al. (2004) found, for example, that the sustained swimming speeds by fish at different temperatures depended on the long-term temperature to which they were acclimated and that this acclimation effect was associated with changes in muscle structure and function.

Gambusia may also show similar effects of exposure to chemical contaminants in the water and some of these effects have been used as biomarkers of contamination. Capps et al. (2004) found, for example, that *Gambusia* exposed to ammonium perchlorate developed macrophage aggregates, a sign of immunotoxic effect, in the trunk kidney. Castro et al. (2004) found that post-exposure feeding inhibition and the enzymes acetylcholinesterase, lactate dehydrogenase and glutathione S-transferases acted as biomarkers of acid mine contamination.

Chemical substances can also induce abnormal development in *Gambusia*. Turner (1941a, 1942a), for example, found that ethynyl testosterone induced precocious gonopodial development in immature males and some gonopodial development in young females. Howell et al. (1980) found, similarly, that effluent from a paper mill was associated with abnormal expression of secondary sex characters. Toft and Guillette (2005) found

that a lower sperm count and disrupted sexual behavior in fish exposed for one month to water from a lake with antiandrogenic and estrogenic contamination compared to those from reference uncontaminated sites.

Just as *Gambusia* are relatively tolerant of a wide range in the physical properties of their water habitat, they are also, compared with other fish, highly resistant to the effects of toxins and adverse conditions (Cherry et al., 1976; Lloyd, 1984; Lloyd et al., 1986; Lloyd, 1989b). Most fish are killed, for example, by a rotenone concentration of about 0.5 ppm, but *Gambusia* apparently survive this concentration with little mortality (Bonham, 1946). Based on the times taken for 50% and 90% of individuals to gasp at the water surface under different rotenone concentrations, *Gambusia* is only about half as sensitive to rotenone as *Neochanna diversus* (Willis and Ling, 2000). *Gambusia* are more tolerant to ''Dursban'', an organic phosphorus insecticide, than the fish species *Lepomis cyanellus* and *Notemigonus crysoleucas* (Ferguson et al., 1966). Henry et al. (2004) found that *Gambusia* did not die after exposure to a voltage gradient of 16 V/cm, whereas half the individuals of most other species died at much lower gradients. Saiki et al. (2004) found that female *Gambusia* can accumulate high concentrations of selenium in their body tissues and yet maintain a very high rate of survival of newborn young.

Dispersal, movement patterns and orientation

Gambusia are non-migratory and individuals usually move about locally within relatively small areas, with some individuals occasionally dispersing over longer distances. *Gambusia* do not, for example, show regular seasonal pattern of movements between different habitats or regions (Marsden and Gehrke, 1996). They may, however, move about locally as a function of variation in conditions and time of day. Winkler (1979) found, for example, that *Gambusia* changed location during the day, in ways possibly reflecting spatial and temporal variation in water temperature, and at night, *Gambusia* were immobile and scattered over the bottoms of the deeper ponds. They may also disperse widely, especially when its habitat is disturbed, such as by a flood (Moore, 1973; Congdon, 1994b) or an influx of artificially heated water (Brown, 1985, 1987). In

this case, however, there may be genetic, sex, age and physical differences between those individuals that disperse and those that do not (Brown, 1985, 1987; Robbins et al., 1987; Congdon, 1994b). Except when undergoing dispersal, individual *Gambusia* remain resident in relatively small areas. Booth (1980), for example, followed marked fish that moved, over the course of 1–2 h, up to 30 m along the edge of a water body, but never venturing more than about 2 m from the shore.

Gambusia sometimes move about in cohesive groups or schools, especially during the early part of the breeding season (Maglio and Rosen, 1969), when they are in relatively large water bodies (Rees, 1958), or when they have just been introduced into a new water body (Sumner, 1934, 1935a). Sometimes these schools contain reasonable numbers of both sexes; other times they may involve mostly females (Bisazza and Marin, 1991).

Discussion

Few fish species have been studied more than *Gambusia affinis* and *G. holbrooki*. The computerized bibliographic database Zoological Records includes, for the period 1978–2004, a total of 895 citations with these scientific species names (including *G. patruelis*, see above) in the title, abstract or list of keywords. This number is exceeded for only a few very well-studied fish species such as the rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) for which there are, respectively, 1824 and 2180 citations that include the scientific species name. It is similar to the 844 citations that include the scientific name for the goldfish (*Carassius auratus*).

The Zoological Records database provides an incomplete but very useful list of relevant citations for *Gambusia affinis* and *G. holbrooki*. My own bibliographic database includes about 500 publications that are not included in the list of 895 produced from Zoological Records. This, of course, is hardly surprising, since the search via Zoological Records does not include publications where reference is made to a particular species in the main text, but not more prominently in the title, abstract or keywords. However, in the case of *Gambusia*, a computerized search of Zoological Records very quickly provides a list of publications corresponding to about 64% of my much

larger list, which has taken a long time to develop. A similar situation seems likely to apply to other species. It is also impossible, as the present review demonstrates, for anyone to read and incorporate every relevant publication for very well studied species such as *Gambusia*.

As would be expected of such widespread and abundant species, *Gambusia affinis* and *G. holbrooki* are clearly very tolerant, adaptable and variable in their biology, at both an individual and population level. Both individuals and populations can tolerate, and often thrive within, a wide range of conditions and the abilities of individuals to do this are enhanced if they have time to acclimate to any changes. Populations can adapt through genetic or evolutionary changes in response to conditions that vary in space or time and there is significant genetic variation within and between populations.

Though the extensive literature in relation to *Gambusia affinis* and *G. holbrooki* has provided a great deal of information about these two species, our knowledge and understanding of the biology of these species is, as you would expect, incomplete. At the levels of the species and individual some information gaps are identified above, but these are not major. Much larger gaps are identified when one looks at the level of populations and species communities (Pyke, unpublished data).

References

Abshier, S.E., Coykendall, R.L. and Kauffman, E.E. (1991) A portable cage aquaculture system for the supplemental production of mosquitofish. *Proc. Paper Annu. Conf. Calif. Mosq. Vector Control Assoc.* **59**, 89–93.

Ahmed, W., Washino, R.K. and Gieke, P.A. (1970) Further biological and chemical studies on *Gambusia affinis* (Baird and Girard) in California. *Proc. Calif. Mosq. Contr. Assoc.* **38**, 95–97.

Ahuja, S.K. (1964) Salinity tolerance of *Gambusia affinis*. *Ind J. Exp. Biol.* **2**, 9–11.

Akin, S. and Neill, W.H. (2003) Routine metabolism of mosquitofish (*Gambusia affinis*) at three different salinities. *Texas J. Sci.* **55**, 255–226.

Al-Daham, N.K. and Bhatti, M.N. (1977) Salinity tolerance of *Gambusia affinis* (Baird and Girard) and *Heteropneustes fossilis* (Bloch). *J. Fish Biol.* **11**, 309–313.

Al-Daham, N.K., Huq, M.F. and Sharma, K.P. (1977) Notes on the ecology of fishes of the genus *Aphanius* and *Gambusia affinis* in southern Iraq. *Freshwater Biol.* **7**, 245–251.

Andersson, M.B. (1994) *Sexual Selection*. Princeton University Press, Princeton, NJ, 624 pp.

Angus, R.A. (1983) Phenol tolerance in populations of mosquitofish from polluted and nonpolluted waters. *Trans. Amer. Fish. Soc.* **112**, 794–799.

Angus, R.A. (1989) A genetic overview of Poeciliid fishes. *In*: Meffe, G.K. and Snelson F.F. (eds.), *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, NJ, pp. 51–68.

Arthington, A.H. (1989a) Diet of *Gambusia affinis holbrooki*, *Xiphorus helleri*, *X. maculatus* and *Poecilia reticulata* (Pisces: Peociliidae) in streams of southeastern Queensland, Australia. *Asian Fish. Sci.* **2**, 193–212.

Arthington, A.H. (1989b) Impacts of introduced and translocated freshwater fishes in Australia. *In*: De Silva, S.S. (ed.), *Proceedings of Workshop on Introduction of Exotic Aquatic Organisms in Asia. Asian Fisheries Special Publication 3*. Asian Fisheries Society, Manilla, Philippines, pp. 7–20.

Arthington, A.H. and Marshall, C.J. (1999) Diet of the exotic Mosquitofish, *Gambusia holbrooki*, in an Australian Lake and potential for competition with indigenous fish species. *Asian Fish. Sci.* **12**, 1–16.

Arthington, A.H. and Milton, D.A. (1983) Effects of urban development and habitat alterations on the distribution and abundance of native and exotic freshwater fish in the Brisbane region, Queensland. *Austr. J. Ecol.* **8**, 87–101.

Baird, S.F. and Girard, C. (1853) Descriptions of new species of fishes collected by Mr. John H. Clark, on the U.S. and Mexican boundary survey, under Lt. Col. Jas. D. Graham. *Proc. Acad. Nat. Sci. Philadelphia* **6**, 387–390.

Bay, E.C. and Anderson, L.D. (1966) Studies with the mosquitofish *Gambusia affinis* as a chironomid control. *Ann. Entomol. Soc. Am.* **59**, 150–153.

Beidler, E.J. (1995) Biocontrol from a mosquito control director's point of view. *J. Am. Mosq. Contr. Assoc.* **11**, 258–259.

Belk, M.C. and Lydeard, C. (1994) Effect of *Gambusia holbrooki* on a similar-sized, syntopic poeciliid *Heterandria formosa*: competitor or predator? *Copeia* **1994**, 296–302.

Bence, J.R. and Murdoch, W.W. (1986) Prey size selection by the mosquitofish: relation to optimal diet theory. *Ecology* **67**, 324–326.

Berra, T.M., Moore, R. and Reynolds, L.F. (1975) The freshwater fishes of the Laloki River System of New Guinea. *Copeia* **3**, 316–326.

Bisazza, A. and Marin, G. (1988) Sexual selection and sexual dimorphism in the poeciliid fish *Gambusia affinis holbrooki* Grd. *Monitore zool. ital. (NS)* **22**, 530–531.

Bisazza, A. and Marin, G. (1991) Male size and female mate choice in the eastern mosquitofish. *Copeia* **1991**, 728–733.

Black, D.A. and Howell, W.M. (1979) The North American mosquitofish, *Gambusia affinis*: a unique case in sex chromosome evolution. *Copeia* **1979**, 509–513.

Blanco, S., Romo, S. and Villena, M. (2004) Experimental study on the diet of mosquitofish (*Gambusia holbrooki*) under different ecological conditions in a shallow lake. *Intl Rev. Hydrobiol.* **89**, 250–262.

Blaylock, B.G. (1969) The fecundity of a *Gambusia affinis affinis* population exposed to chronic environmental radiation. *Rad. Res.* **37**, 108–117.

Blyth, B. (1994) Predation by *Gambusia holbrooki* on anuran larvae at the RGC wetlands centre, Capel, Western Australia. RGC Wetlands Centre Technical Report No 22. Curtin University of Technology, Western Australia.

Bonham, K. (1946) Management of a small fish pond in Texas. *J. Wildlife Manag.* **10**, 1–4.

Booth, D. J. (1980) Investigations into the extent and mechanisms of prey-size selection by the fish species *Pseudomugil signifer* Kner and *Gambusia affinis* (Baird and Girard). B.Sc. (Hons) Thesis, University of Sydney, Sydney.

Boyd, C.E. and Ferguson, D.E. (1964) Spectrum of cross-resistance to insecticides in mosquito fish, *Gambusia affinis*. *Mosquito News* **24**, 19–21.

Brown-Peterson, N. and Peterson, M.S. (1990) Comparative life history of female mosquitofish, *Gambusia affinis*, in tidal freshwater and oligohaline habitats. *Environ. Biol. Fish.* **27**, 33–41.

Brown, C.J.D. and Fox, A.C. (1966) Mosquito fish in a Montana pond. *Copeia* **1966**, 614–616.

Brown, K.L. (1985) Demographic and genetic characteristics of dispersal in mosquito fish. *Copeia* **1985**, 597–612.

Brown, K.L. (1987) Colonization by mosquitofish of a Great Plains River Basin. *Copeia* **1987**, 336–351.

Burke, W.D. and Ferguson, D.E. (1969) Toxicities of four insecticides to resistant and susceptible mosquitofish in static and flowing solutions. *Mosq. News* **29**, 96–101.

Busack, C.A. (1983) Four generations of selection for high 56-day weight in the mosquitofish (*Gambusia affinis*). *Aquaculture* **33**, 83–87.

Busack, C.A. and Gall, G.A.E. (1983) An initial description of the quantitative genetics of growth and reproduction in the mosquitofish, *Gambusia affinis*. *Aquaculture* **32**, 123–140.

Caldwell, M.C. and Caldwell, D.K. (1962) Monarchistic dominance in small groups of captive male mosquitofish, *Gambusia affinis patruelis*. *Bull. South. Calif. Acad. Sci.* **61**, 37–43.

Capps, T., Mukhi, S., Rinchard, J.J., Theodorakis, C.W., Blazer, V.S. and Patino, R. (2004) Exposure to perchlorate induces the formation of macrophage aggregates in the trunk kidney of zebrafish and mosquitofish. *J. Aqua. Anim. Health* **16**, 145–151.

Casterlin, M.E. and Reynolds, W.W. (1977) Aspects of habitat selection in the mosquitofish *Gambusia affinis*. *Hydrobiologia* **55**, 125–127.

360

Castro, B.B., Sobral, O., Guilhermino, L. and Ribeiro, R. (2004) An in situ bioassay integrating individual and biochemical responses using small fish species. *Ecotoxicology* **13**, 667–681.

Cech, J.J., Massingill, M.J., Vondracek, B. and Linden, A.L. (1985) Respiratory metabolism of mosquitofish, *Gambusia affinis*: effects of temperature, dissolved oxygen, and sex difference. *Environ. Biol. Fish.* **13**, 297–307.

Cech, J.J., Massingill, M.J. and Wragg, T.E. (1980) The food demands of mosquitofish, *Gambusia affinis*. *Proc. Calif. Mosq. Vector Contr. Assoc.* **48**, 45–47.

Chen, T.R. and Ebeling, A.W. (1968) Karyological evidence of female heterogamety in the mosquitofish, *Gambusia affinis. Copeia* **1968**, 70–75.

Cherry, D.S., Guthrie, R.K., Rodgers, J.H., Cairns, J. and Dickson, K.L. (1976) Responses of mosquitofish (*G. affinis*) to ash effluent and thermal stress. *Trans. Amer. Fish. Soc.* **105**, 686–694.

Chervinski, J. (1983) Salinity tolerance of the mosquitofish, *Gambusia affinis* (Baird & Girard). *J. Fish Biol.* **22**, 9–11.

Collier, A. (1936) The mechanism of internal fertilisation in *Gambusia. Copeia* **1936**, 45–53.

Congdon, C.B. (1989) The role of natural selection, gene flow and genetic drift in population subdivision of *Gambusia affinis. In*: Pollard, D.A. (ed.), *Proceedings of the Australian Society for Fish Biology's Workshop on Introduced and Translocated Fishes and their Ecological Effects, Magnetic Island, Townsville, August 1989 Bureau of Rural Resources Proceedings No 8*. Australian Government Publishing Service, Canberra, pp. 123.

Congdon, B.C. (1994) Salinity-related fitness differences amongst GPI genotypes in the mosquitofish *Gambusia holbrooki* (Poeciliidae: Teleostei). *Biol. J. Linnean Soc.* **53**, 343–352.

Congdon, B.C. (1994) Characteristics of dispersal in the eastern mosquitofish, *Gambusia affinis. J. Fish Biol.* **45**, 943–952.

Constantz, G.D. (1989) Reproductive biology of Poeciliid fishes. *In*: Meffe, G.K. and Snelson F.F. (eds.), *Ecology & Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, New York, pp. 33–50.

Courtenay, W.R. and Meffe, G.K. (1989) Small fish in strange places: A review of introduced poeciliids. *In*: Meffe, G.K. and Snelson F.F. (eds.), *Ecology and Evolution of Livebearing Fishes*. Prentice Hall, New York, pp. 319–332.

Coykendall, R.L., Kauffman, E.E. and Abshier, S.E. (1991) A versatile cage culture system for the mosquitofish, Gambusia affinis (Baird and Girard). *Proc. Paper Ann. Conf. Calif. Mosq. Vector Contr. Assoc. Inc.* **58**, 107–110.

Crivelli, A.J. and Boy, V. (1987) The diet of the mosquitofish (*Gambusia affinis* (Baird and Girard) Poeciliidae) in Mediterranean France. *Rev. Ecol.* **42**, 421–435.

Culley, D.D. and Ferguson, D.E. (1969) Patterns of insecticide resistance in the mosquitofish *Gambusia affinis. J. Fish. Res. Board Can* **26**, 2395–2401.

Darwazeh, H.A. and Mulla, M.S. (1974) Toxicity of herbicides and mosquito larvicides to the mosquito fish *Gambusia affinis. Mosq. News* **34**, 214–219.

Davey, R.B., Meisch, M.V. and Carter, F.L. (1976) Toxicity of five ricefield pesticides to the mosquito-fish, *Gambusia affinis*, and green sunfish, *Lepomis cyanellus*, under laboratory and

field conditions in Arkansas, USA. *Environ. Entomol.* **5**, 1053–1056.

Dionne, M. (1985) Cannibalism, food availability and reproduction in the mosquitofish (*Gambusia affinis*); a laboratory experiment. *Amer. Nat.* **126**, 16–23.

Duryea, R., Donnelly, J., Guthrie, D., O'malley, C., Romanowski, M. and Schmidt, R. (1996) *Gambusia affinis* effectiveness in New Jersey mosquito control. *Eighty-Third Annual Meeting of the New Jersey Mosquito Control Association*, pp. 95–102.

Ehmann, H. (1997) *Threatened Frogs of New South Wales: Habitats, Status and Conservation.* Frog and Tadpole Study Group of NSW, Sydney.

Farley, D.G. (1980) Prey selection by the mosquitofish, *Gambusia affinis* (Baird and Girard) on selected non-target organisms in Fresno County rice fields. *Proc. Calif. Mosq. Vector Contr. Assoc.* **48**, 51–54.

Ferens, M.C. and Murphy, T.M. (1974) Effects of thermal effluents on populations of mosquitofish. *In*: Gibbons, J.W. and Sharitz R.R. (eds.), *Thermal Ecology, AEC Symposium Series 32*. Technical Information Center, United States Atomic Energy Commission, Augusta, Georgia, pp. 237–245.

Ferguson, D.E. (1966) Toxicity of Dursban to three species of fish. *Mosq. News* **26**, 80–82.

Ferguson, D.E. and Boyd, C.E. (1964) Apparent resistance to methyl parathion in mosquito-fish (*Gambusia affinis*). *Copeia* **1964**, 706.

Ferguson, D.E., Ludke, J.L. and Murphy, G.G. (1966) Dynamics of endrin uptake and release by resistant and susceptible strains of mosquitofish. *Trans. Amer. Fish. Soc.* **95**, 335–344.

Fernandez-Delgado, C. (1989) Life-history patterns of the mosquitofish, *Gambusia affinis*, in the estuary of the Guadalquivir river of south-west Spain. *Freshwater Biol.* **22**, 395–404.

Fraile, B., Saez, C.A., Vicentini, M.P.D.M. and Paniagua, R. (1992) The testicular cycle of *Gambusia affinis holbrooki* (Teleostei: Poeciliidae). *J. Zool.* **228**, 115–126.

Fraile, B., Saez, C.A., Vicentini, M.P.D.M. and Paniagua, R. (1993) Effects of photoperiod on spermatogenesis in *Gambusia affinis holbrooki* (Teleostei: Poeciliidae) during the period of testicular quiescence. *J. Zool.* **230**, 651–658.

Fraile, B., Saez, F.J., Vicentini, C.A., Gonzalez, A.D., Miguel, P. and Paniagua, R. (1994) Effects of temperature and photoperiod on the *Gambusia affinis holbrooki* testis during the spermatogenesis period. *Copeia* **1994**, 216–221.

Fuernwein, H., Nemeschkal, H. and Kratochvil, H. (2004) Ecological differentiation in *Gambusia holbrooki* Girard, 1859 - a morpho-metric analysis of isolated populations influenced by habitat and climate. *Zeitschrift fuer Fischkunde* **7**, 43–53.

Galat, D.L. and Robertson, B. (1992) Response of endangered *Poeciliopsis occidentalis sonoriensis* in the Rio Yaqui drainage, Arizona, to introduced *Gambusia affinis. Environ. Biol. Fish.* **33**, 249–264.

Gall, G.A.E., Cech, J.J., Garcia, R., Resh, V.H. and Washino, R.K. (1980) Mosquitofish – an established predator. *Calif. Agricul.* **34**, 21–22.

Gamradt, S.C. and Kats, L.B. (1996) Effect of introduced crayfish and mosquitofish on California newts. *Conserv. Biol.* **10**, 1155–1162.

Garcia-Berthou, E. (1999) Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *J. Fish Biol.* **55**, 135–147.

Geiser, S.W. (1924) Sex-ratios and spermatogenesis in the top-minnow, *Gambusia holbrooki* Grd. *Biol. Bull.* **47**, 175–207.

Ghate, H.V. and Padhye, A.D. (1988) Predation of *Microhyla* tadpoles by *Gambusia*. *J. Bombay Nat. Hist. Soc.* **85**, 200–201.

Glover, C.J.M. (1979) Studies on Central Australian fishes: further observations and records. Part I. *South Austr. Nat.* **53**, 58–62.

Glover, C.J.M. and Sim, T.C. (1978) Studies on Central Australian fishes: a progress report. *South Austr. Nat.* **52**, 35–44.

Grant, E.M. (1978) *Guide to Fishes*. Department of Primary Industry, Brisbane.

Green, M.V. and Imber, C.F. (1977) Applicability of *Gambusia affinis* to urban mosquito problems in Burlington County, New Jersey. *Mosquito News* **37**, 383–385.

Ham, R. (1981) *The Ecology of Six Native and Two Introduced Fish Species in the Enoggera Creek system, South-East Queensland*. B.Sc.(Hons) Griffith University, Brisbane, 157 pp.

Hammill, E., Wilson, R.S. and Johnston, I.A. (2004) Sustained swimming performance and muscle structure are altered by thermal acclimation in male mosquitofish. *J. Therm. Biol.* **29**, 251–257.

Harrington, R.W. (1974) Sex determination and differentiation in fishes. *In*: Schreck, C.B. (ed.), *Control of Sex in Fishes, Sea Grant Extension Division*. Virginia Polytechnic Institute & State University, Blacksburg, VA, pp. 4–12.

Harrington, R.W. and Harrington, E.S. (1961) Food selection among fishes invading a high subtropical salt marsh: from onset of flooding through the progress of a mosquito brood. *Ecology* **42**, 646–666.

Harrington, R.W. and Harrington, E.S. (1982) Effects on fishes and their forage organisms of impounding a Florida salt marsh to prevent breeding by salt marsh mosquitoes. *Bull. Marine Sci.* **32**, 523–531.

Haynes, J.L. (1993) Annual reestablishment of mosquitofish populations in Nebraska. *Copeia* **1993**, 232–235.

Haynes, J.L. and Cashner, R.C. (1995) Life history and population dynamics of the western mosquitofish: a comparison of natural and introduced populations. *J. Fish Biol.* **46**, 1026–1041.

Helfman, G.S., Collette, B.B. and Facey, D.E. (1997) *The Diversity of Fishes*. Blackwell Science, Malden, MA, 512 pp.

Henry, T.B., Grizzle, J.M., Johnston, C.E. and Osborne, J.A. (2004) Susceptibility of ten fish species to electroshock-induced mortality. *Trans. Amer. Fish. Soc.* **133**, 649–654.

Herms, W.B. and Gray, H.F. (1940) *Mosquito Control*. The Commonwealth Fund, New York.

Hess, A.D. and Tarzwell, C.M. (1942) The feeding habits of *Gambusia affinis affinis*, with special reference to the malaria mosquito, *Anopheles quadrimaculatus*. *Amer. J. Hygiene* **35**, 142–151.

Hildebrand, S.F. (1919) Fishes in relation to mosquito control in ponds. *Rep. United States Comm. Fish. App.* **15**, 1–15.

Hildebrand, S.F. (1919) Notes on the life history of the minnows *Gambusia affinis* and *Yprinodon variegatus*. *Ann. Rep. United States Comm. Fish. App.* **6**, 3–15.

Hildebrand, S.F. (1927) Sex ratio in *Gambusia*. *Biol. Bull.* **53**, 390–404.

Hoar, W.S. (1969) Reproduction. *In*: Hoar, W.S. and Randall D.J. (eds.), *Fish Physiology*. Academic Press, New York, pp. 1–72.

Homski, D., Goren, M. and Gasith, A. (1994) Comparative evaluation of the larvivorous fish *Gambusia affinis* and *Aphanius dispar* as mosquito control agents. *Hydrobiologia* **284**, 137–146.

Howe, E.H.I. (1995) Studies in the biology and reproductive characteristics of *Pseudomugil signifer*. PhD Dissertation, University of Technology/Sydney, Sydney, NSW, 273 pp.

Howell, W.M., Black, D.A. and Bortone, S.A. (1980) Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis holbrooki*: evidence for environmentally induced masculinization. *Copeia* **1980**, 676–681.

Hoy, J.B. and Reed, D.E. (1970) Biological control of *Culex tarsalis* in a California rice field. *Mosq. News* **30**, 222–230.

Hubbs, C. (1957a) *Gambusia heterochir* a new poeciliid fish from Texas, with an account of its hybridization with *Gambusia affinis*. *Tulane Stud. Zool.* **5**, 3–16.

Hubbs, C. (1957) Population analysis of a hybrid swarm between *Gambusia affinis* and *G. heterochir*. *Evolution* **13**, 236–246.

Hubbs, C. (1991) Intrageneric "cannibalism" in *Gambusia*. *Southwestern Nat.* **36**, 153–157.

Hubbs, C. (2000) Survival of *Gambusia affinis* in a hostile environment. *Southwestern Nat.* **45**, 521–522.

Hubbs, C. and Brodrick, H.J. (1963) Current abundance of *Gambusia gaigei*, an endangered fish species. *Southwestern Nat.* **8**, 46–48.

Hubbs, C. and Delco, E.A. (1960) Mate preference in males of four species of gambusiine fishes. *Evolution* **14**, 145–152.

Hubbs, C. and Delco, E.A. (1962) Courtship preferences of *Gambusia affinis* associated with the sympatry of the parental populations. *Copeia* **1962**, 396–400.

Hubbs, C. and Peden, E. (1969) *Gambusia georgei* sp. nov. from San Marcos, Texas. *Copeia* **1969**, 357–364.

Hughes, A.L. (1985) Male size, mating success, and mating strategy in the mosquitofish *Gambusia affinis* (Poecilidae). *Behav. Ecol. Sociobiol.* **17**, 271–278.

Hughes, A.L. (1985) Seasonal changes in fecundity and size at first reproduction in an Indiana population of the mosquitofish *Gambusia affinis*. *Amer. Midland Nat.* **114**, 30–36.

Hughes, A.L. (1985) Seasonal trends in body size of adult mosquitofish, *Gambusia affinis*, with evidence for their social control. *Environ. Biol. Fish.* **14**, 251–258.

Hughes, A.L. (1986) Growth of adult mosquitofish *Gambusia affinis* in the laboratory. *Copeia* **1986**, 534–536.

Hughes, J.M., Harrison, D.A. and Arthur, J.M. (1991) Genetic variation at the Pgi locus in the Mosquitofish, *Gambusia affinis* (Poeciliidae) and a possible effect on susceptibility to an insecticide. *Biol. J. Linnean Soc.* **44**, 153–167.

Itzkowitz, M. (1971) Preliminary study of the social behavior of male *Gambusia affinis* (Baird and Girard) (Pisces: Poecilidae) in aquaria. *Chesapeake Sci.* **12**, 219–224.

Ivantsoff, W. and Aarn, A. (1999) Detection of predation on Australian fishes by *Gambusia holbrooki*. *Marine Freshwater Res.* **50**, 467–468.

Johnson, C.R. (1976) Observations on growth, breeding and fry survival of *Gambusia affinis affinis* under artificial rearing conditions. *Proc. Calif. Mosq. Vector Contr. Assoc.* **44**, 48–51.

Jordan, D.S. (1885) *A Catalogue of the Fishes Known to Inhabit the Waters of North America North of the Tropic of Cancer with Notes on the Species Discovered in 1883 and 1884.* Government Printing Office, Washington, D.C.

Jordan, D.S. and Evermann, B.W. (1896) *The Fishes of North and Middle America. A Descriptive Catalogue of the Species of Fish-Like Vertebrates Found in the Waters of North America, North of the Isthmus of Panama Part I. Bulletin of the United States National Museum No. 47.* Government Printing Office, Washington, D.C.

Jordan, D.S., Evermann, B.W. and Clark, H.W. (1930) *Report of the United States Commissioner of Fisheries for the Fiscal Year 1928 with Appendixes. Part II.* United States Government Printing Office, Washington, D.C.

Jordan, D.S. and Gilbert, C.H. (1882) *Synopsis of the fishes of North America. Bulletins of the United States National Museum. Volume III (No. 16).* Government Printing Office, Washington, D.C.

Kennedy, P., Kennedy, M. and Smith, M.H. (1985) Microgeographic genetic organization of populations of largemouth bass and two other species in a reservoir. *Copeia* **1985**, 118–125.

Kennedy, P.K., Kennedy, M.L., Zimmerman, E.G., Chesser, R.K. and Smith, M.H. (1986) Biochemical genetics of mosquitofish. V. Perturbation effects on genetic organization of populations. *Copeia* **1986**, 937–945.

Kerfoot, W.C. (1982) A question of taste: Crypsis and warning coloration in freshwater zooplankton communities. *Ecology* **63**, 538–553.

Koehn, J.D. and O'connor, W.G. (1990) Threats to Victorian native freshwater fish. *Victor. Nat.* **1**, 5–12.

Komak, S. and Crossland, M.R. (2000) An assessment of the introduced mosquitofish (*Gambusia affinis holbrooki*) as a predator of eggs, hatchlings and tadpoles of native and non-native anurans. *Wildlife Res.* **27**, 185–189.

Koya, Y., Ishikawa, S. and Sawaguchi, S. (2004) Effects of temperature and photoperiod on ovarian cycle in the mosquitofish, *Gambusia affinis. Jpn J. Ichthyol.* **51**, 43–50.

Krumholz, L.A. (1944) Northward acclimatization of the western mosquitofish, *Gambusia affinis affinis. Copeia* **1944**, 82–85.

Krumholz, L.A. (1948) Reproduction in the Western Mosquitofish, *Gambusia affinis affinis* (Baird & Girard), and its use in mosquito control. *Ecol. Monogr.* **18**, 1–43.

Kuntz, A. (1913) Notes on the habits, morphology of the reproductive organs, and embryology of the viviparous fish (Gambusia affinis). *Bull. U.S. Bureau Fish.* **33**, 177–190.

Lake, J.S. (1959) The freshwater fishes of New South Wales. *New South Wales State Fish. Res. Bull.* **5**, pp. 1–20.

Langerhans, R.B., Layman, C.A., Shokrollahi, A.M. and Dewitt, T.J. (2004) Predator-driven phenotypic diversification in *Gambusia affinis. Evolution* **58**, 2305–2318.

Lanzing, W.J.R. and Wright, R.G. (1982) The ultrastructure of the eye of the mosquitofish *Gambusia affinis. Cell Tissue Res.* **223**, 431–443.

Lawler, S.P. (1989) Behavioural responses to predators and predation risk in four species of larval anurans. *Anim. Behav.* **38**, 1039–1047.

Lawler, S.P., Dritz, D., Strange, T. and Holyoak, M. (1999) Effects of introduced mosquitofish and bullfrogs on the threatened California Red-legged Frog. *Conserv. Biol.* **13**, 613–622.

Lewallen, L.L. (1959) Toxicity of several organophosphorus insecticides to *Gambusia affinis* (Baird and Girard) in laboratory tests. *Mosq. News* **19**, 1–2.

Lewallen, L.L. and Brydon, H.W. (1958) Field tests with organophosphate granular insecticide against mosquito larvae in Lake County, California. *Mosq. News* **18**, 21–22.

Lewis, W.M. (1970) Morphological adaptations of cyprinodontoids for inhabiting oxygen deficient waters. *Copeia* **1970**, 319–326.

Liu, E.H., Smith, M.H., Godt, M.W., Chesser, R.K., Lethco, A.K. and Henzler, D.J. (1985) Enzyme levels in natural mosquitofish populations. *Physiol. Zool.* **58**, 242–252.

Lloyd, L. (1984) Exotic fish – Useful additions or "animal weeds"? *Fishes of Sahul* **1**, 31–34 & 39–42.

Lloyd, L. (1986) An alternative to insect control by 'mosquitofish', *Gambusia affinis. Arbovirus Research in Australia* **1986**, 156–163.

Lloyd, L. (1989b) Native fishes as alternatives to the exotic fish, *Gambusia*, for insect control. *In*: Pollard, D.A. (ed.), *Aust. Soc. for Fish Biology Workshop. Introduced and Translocated Fishes and their Ecological Effects. Magnetic Island 24–25 August 1989.* Department of Primary Industries and Energy Bureau of Rural Resources Proceedings No. 8 ed. Magnetic Island: Department of Primary Industries and Energy Bureau of Rural Resources, pp. 115–122.

Lloyd, L.N., Arthington, A.H. and Milton, D.A. (1986) The mosquitofish – a valuable mosquito-control agent or a pest?. *In*: Kitching, R.L. (ed.), *The Ecology of Exotic Animals and Plants Some Australian Case Histories.* John Wiley & Sons, Brisbane, pp. 6–25.

Lloyd, L.N. and Tomasov, J.F. (1985) Taxonomic status of the Mosquitofish, *Gambusia affinis* (Poeciliidae), in Australia. *Aust. J. Freshwater Res.* **36**, 447–451.

Lounibos, L.P. and Frank, J.H. (1994) Biological control of mosquitoes. *In*: Rosen, D., Bennett, F.D. and Capinera J.L. (eds.), *Pest Management in the Subtropics.* Intercept, Andover, pp. 395–407.

Maglio, V.J. and Rosen, D.E. (1969) Changing preferences for substrate colour by reproductively active mosquito fish, *Gambusia affinis* (Baird and Girard) (Poeciliidae: Atheriniformes). *Amer. Museum Novitates* **2397**, 1–39.

Marsden, T.J. and Gehrke, P.C. (1996) Fish passage in the Hawkesbury-Nepean river system. *In*: Gehrke, P.C. and Harris J.H. (eds.), *Fish and Fisheries of the Hawkesbury-Nepean River System.* NSW Fisheries Research Institute, Sydney, pp. 200–218.

Marsh-Matthews, E., Skierkowski, P. and Demarais, A. (2001) Direct evidence for mother-to-embryo transfer of nutrients in the livebearing fish *Gambusia geiseri. Copeia* **2001**, 1–6.

Martin, R.G. (1975) Sexual and aggressive behavior, density and social structure in a natural population of Mosquitofish, *Gambusia affinis holbrooki. Copeia* **1975**, 445–453.

Mcclenaghan, L.R., Smith, M.H. and Smith, M.W. (1985) Biochemical genetics of mosquitofish. IV. Changes of allele frequencies through time and space. *Evolution* **39**, 451–460.

Mcdowall, R.M. (1978) *New Zealand Freshwater Fishes: A Guide and Natural History*. Heinemann Educational Books (NZ), Auckland.

Medlen, A.B. (1951) Preliminary observations on the effects of temperature and light upon reproduction in *Gambusia affinis*. *Copeia* **1951**, 148–152.

Meffe, G.K. (1985) Predation and species replacement in American Southwestern fishes: a case study. *Southwestern Nat.* **30**, 173–187.

Meffe, G.K. (1986) Cannibalism, food availability, and reproduction in mosquitofish: a critique. *American Nat.* **127**, 897–901.

Meffe, G.K. (1987) Embryo size variation in mosquitofish: optimality vs plasticity in propagule size. *Copeia* **1987**, 762–768.

Meffe, G.K. (1989) List of accepted common names of poeciliid fishes. *In*: Meffe, G.K. and Snelson F.F. (eds.), *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, NJ, pp. 369–371.

Meffe, G.K. (1990) Offspring size variation in eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae) from contrasting thermal environments. *Copeia* **1990**, 10–18.

Meffe, G.K. (1992) Plasticity of life-history characters in eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae) in response to thermal stress. *Copeia* **1992**, 94–102.

Meffe, G.K. and Crump, M.L. (1987) Possible growth and reproductive benefits of cannibalism in the mosquitofish. *American Nat.* **129**, 203–212.

Meffe, G.K., Hendrickson, D.A., Minckley, W.L. and Rinne, J.N. (1983) Factors resulting in decline of the endangered sonoran topminnow (Atheriniformes: Poeciliidae) in the United States. *Biol. Conserv.* **25**, 135–159.

Meffe, G.K. and Snelson, F.F. (1989) An ecological overview of Poeciliid fishes. *In*: Meffe, G.K. and Snelson F.F. (eds.), *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, NJ, pp. 13–31.

Meffe, G.K. and Snelson, F.F. (1993a) Annual lipid cycle in eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae) from South Carolina. *Copeia* **3**, 596–604.

Meffe, G.K. and Snelson, F.F. (1993b) Lipid dynamics during reproduction in two livebearing fishes, *Gambusia holbrooki* and *Poecilia latipinna*. *Can. J. Fish. Aquat. Sci.* **50**, 2185–2191.

Merrick, J.R. and Schmida, G.E. (1984) *Australian Freshwater Fishes Biology and Management*. Griffin Press, Netley, South Australia, 409 pp.

Mills, M.D., Rader, R.B. and Belk, M.C. (2004) Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. *Oecologia* **141**, 713–721.

Milton, D.A. and Arthington, A.H. (1983) Reproductive biology of *Gambusia affinis holbrooki* Baird and Girard, *Xiphophorus helleri* (Gunther) and *X. maculatus* (Heckel) (Pisces; Poeciliidae) in Queensland, Australia. *J. Fish Biol.* **23**, 23–41.

Minckley, W.L. (1973) *Fishes of Arizona*. Arizona Game and Fish Department, Phoenix.

Miura, T., Takashi, R.M. and Stewart, R.J. (1979) Habitat and food selection by the mosquitofish *Gambusia affinis*. *Proc. Calif. Mosq. Vector Contr. Assoc.* **47**, 46–50.

Moore, V.A. (1973) *Gambusia affinis* (Mosquito destroying fish). *Hunter Nat. Hist.* **5**, 14–18.

Morton, R.M., Beumer, J.P. and Pollock, B.R. (1988) Fishes of a subtropical Australian saltmarsh and their predation upon mosquitoes. *Environ. Biol. Fish.* **21**, 185–194.

Mulla, M.S. (1961) Mosquito control investigations with emphasis on the integration of chemical and biological control in mosquito abatement. *Proc. Calif. Mosq. Contr. Assoc.* **29**, 101–105.

Mulla, M.S. and Isaak, L.W. (1961) Field studies on the toxicity of insecticides to the mosquito fish, *Gambusia affinis*. *J. Econ. Entomol.* **54**, 1237–1242.

Mulla, M.S., Isaak, L.W. and Axelrod, H. (1963) Field studies on the effects of insecticides on some aquatic wildlife species. *J. Econ. Entomol.* **56**, 184–188.

Murdoch, W.W. and Bence, J. (1987) General predators and unstable prey populations. *In*: Kerfoot, W.C. and Sih, A. (eds.), *Predation. Direct and Indirect Impacts on Aquatic Communities*. University Press of New England, pp. 1–30.

Nelson, J.S. (1983) The tropical fish fauna in Cave and Basin Hotsprings drainage, Banff National Park. *Can Field-Nat.* **97**, 255–261.

Nordlie, F.G. and Mirandi, A. (1996) Salinity relationships in freshwater populations of eastern mosquitofish. *J. Fish Biol.* **49**, 1226–1232.

Odum, H.T. and Caldwell, D.K. (1955) Fish respiration in the natural oxygen gradient of an anaerobic spring in Florida. *Copeia* **1955**, 104–106.

Otto, R.G. (1973) Temperature tolerance of the mosquitofish, *Gambusia affinis* (Baird and Girard). *J. Fish Biol.* **5**, 575–585.

Peden, A.E. (1973) Variation in anal spot expression of gambusiin females and its effect on male courtship. *Copeia* **1973**, 250–263.

Peden, A.E. (1975) Differences in copulatory behavior as partial isolating mechanisms in the poeciliid fish *Gambusia*. *Can. J. Zool.* **53**, 1290–1296.

Poey, F. (1854) Los Guajacones, peces de aqua dulce. *Memorias de Cuba* **I**, 382.

Pollard, D. A. (1973) A note on the toxicity of "pest strips" containing the organophosphate insecticide Dichlorvos (DDVP) to the mosquito fish, *Gambusia affinis*. *Austr. Soc. Limnol. Bull.* **5**, 21–24.

Pyke, G.H. (1984) Optimal foraging theory: A critical review. *Ann. Rev. Ecol. Syst.* **15**, 523–575.

Pyke, G.H. (2001) A strategy for reviewing the biology of animals. *Austr. Zool.* **31**, 482–491.

Pyke, G.H. (2002) A review of the biology of the Southern Bell Frog *Litoria raniformis* (Anura: Hylidae). *Austr. Zool.* **32**, 32–48.

Pyke, G.H. and Read, D.A. (2002) Hastings River Mouse (*Pseudomys oralis*): a biological review. *Austr. Mammal.* **24**, 151–176.

Pyke, G.H. and Read, D.G. (2003) Updating biological reviews: the Hastings River Mouse *Pseudomys oralis* as a case history. *Austr. Mammal.* **25**, 211–213.

Pyke, G.H. and White, A.W. (2000) Factors influencing predation on eggs and tadpoles of the endangered Green and Golden Bell Frog (*Litoria aurea*) by the introduced Plague Minnow (*Gambusia holbrooki*). *Austr. Zool.* **31**, 496–505.

Pyke, G.H. and White, A.W. (2001) A Review of the Biology of the Green and Golden Bell Frog (*Litoria aurea*). *Austr. Zool.* **31**, 563–598.

Rajasekharan, P.T. and Chowdaiah, B.N. (1972) Selective feeding behavior of *Gambusia affinis*. *Oecologia* **11**, 79–81.

Reddy, S.R. (1975) Effect of water temperature on the predatory efficiency of *Gambusia affinis*. *Experientia* **31**, 801–802.

Reddy, S.R. and Pandian, T.J. (1972) Heavy mortality of *Gambusia affinis* reared on diets restricted to mosquito larvae. *Mosq. News* **32**, 108–110.

Reddy, S.R. and Pandian, T.J. (1974) Effect of running water on the predatory efficiency of the larvivorous fish, *Gambusia affinis*.. *Oecologia* **16**, 253–256.

Reddy, S.R. and Shakuntala, K. (1979) Comparative studies on the food intake, growth and food conversion of two larvivorous fishes. *Proc. Ind. Acad. Sci.* **88**, 425–432.

Reed, D.E. and Bryant, T.J. (1975) Fish population studies in Fresno County rice fields. *Proc. Ann. Conf. Amer. Mosq. Contr. Assoc.* **43**, 139–141.

Rees, B.E. (1958) Attributes of the mosquito fish in relation to mosquito control. *Proc. Calif. Mosq. Contr. Assoc.* **26**, 71–75.

Rees, D.M. (1934) Notes on mosquitofish in Utah, *Gambusia affinis* (Baird and Girard). *Copeia* **1934**, 157–159.

Rees, D.M., Brown, D.N. and Winget, R.N. (1969) Mosquito larvae control with *Gambusia* and *Lucania* fish in relation to water depth and vegetation. *Proc. Ann. Conf. Calif. Mosq. Contr. Assoc.* **37**, 110–114.

Reynolds S.J. (1995) The impact of introduced mosquitofish (*Gambusia holbrooki*) on the mortality of premetamorphic anurans. B.Sc. (Hons) Thesis, University of Western Australia, Perth, Western Australia.

Reznick, D. (1981) 'Grandfather Effects': The genetics of interpopulation differences in offspring size in the mosquito fish. *Evolution* **35**, 941–953.

Reznick, D.N. and Braun, B. (1987) Fat cycling in the mosquitofish (*Gambusia affinis*): fat storage as a reproductive adaptation?. *Oecologia* **73**, 401–413.

Reznick, D.N. and Miles, D.B. (1989) Poeciliid life history patterns. *In*: Meffe, G.K. and Snelson F.F. (eds.), *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, NJ, pp. 373–377.

Richard, J. (2002) An observation of predation of a metamorph common eastern froglet (*Crinia signifera*) by the plague minnow (*Gambusia holbrooki*). *Herpetofauna* **32**, 71–72.

Rivas, L.R. (1963) Subgenera and species groups in the poecillid fish genus *Gambusia* Poey. *Copeia* **1963**, 331–347.

Robbins, L.W., Hartman, G.D. and Smith, M.H. (1987) Dispersal, reproductive strategies and the maintenance of genetic variability in mosquitofish (*Gambusia affinis*). *Copeia* **1987**, 156–164.

Roberts, F.L. (1965) A chromosome study of *Gambusia affinis holbrooki*. *Copeia* **1965**, 238–239.

Robison, W.R., Newton, S.H. and Meisch, M.V. (1983) Mosquitofish production in monoculture and polyculture ponds. *Proc. Ark. Acad. Sci.* **37**, 101–103.

Rosen, D.E. and Gordon, M. (1953) Functional anatomy and evolution of male genitalia in poeciliid fishes. *Zoologica* **38**, 1–47.

Rosen, D.E. and Mendelson, J.R. (1960) The sensory canals of the head in poeciliid fishes (Cyprinodontiformes), with reference to dentitional types. *Copeia* **1960**, 203–210.

Rosen, D.E. and Tucker, A. (1961) Evolution and secondary sexual characters and sexual behavior patterns in a family of viviparous fishes (Cyprinodontiformes: Poeciliidae). *Copeia* **1961**, 201–212.

Ryder, J.A. (1882) Structure and ovarian incubation of *Gambusia patruelis*, a top-minnow. *Amer. Nat.* **16**, 109–118.

Saiki, M.K., Martin, B.A. and May, T.W. (2004) Reproductive status of western mosquitofish inhabiting selenium-contaminated waters in the grassland water district, Merced County, California. *Arch. Environ. Contam. Toxicol.* **47**, 363–369.

Schoenherr, A.A. (1981) The role of competition in the displacement of native fishes by introduced species. *In*: Naiman, R.J. and Soltz D.L. (eds.), *Fishes in North American Deserts*. Wiley Interscience, New York, pp. 173–203.

Scrimshaw, N.S. (1944) Superfetation in poeciliid fishes. *Copeia* **1944**, 180–183.

Seal, W.P. (1911) Breeding habits of the viviparous fishes *Gambusia holbrooki* and *Heterandria formosa*. *Proc. Biol. Soc. Washington* **24**, 91–96.

Seale, A. (1917) The mosquitofish *Gambusia affinis* in the Philippines. *Philippine J. Sci.* **12**, 177–187.

Self, J.T. (1940) Notes on the sex cycle of *Gambusia affinis affinis*, and its habits and relation to mosquito control. *Amer. Midland Nat.* **23**, 393–397.

Shakuntala, K. (1977) Effect of feeding level on the rate and efficiency of food conversion in the cyprinodon fish *Gambusia affinis*. *Ceylon J. Sci.* **12**, 177–184.

Shakuntala, K. and Reddy, S.R. (1977) Influence of body weight/age on the food intake, growth and conversion efficiency of *Gambusia affinis*. *Hydrobiologia* **55**, 65–69.

Sholdt, L.L., Ehrhardt, D.A. and Michael, A.G. (1972) Guide to the use of mosquito fish, *Gambusia affinis*, for mosquito control. Navy Environment and Preventative Medicine, Norfolk, VA, Unit No. 2, Norfolk, VA.

Sjogren, J.D. (1972) Minimum oxygen thresholds of *Gambusia affinis* and *Poecilia reticulata*. *Proc. Ann. Conf. Calif. Mosq. Contr. Assoc.* **40**, 124–126.

Smith, H.M. (1912) The prolificness of *Gambusia*. *Science* **36**, 224.

Smith, M.H., Scribner, K.T., Hernandez, J.D. and Wooten, M.C. (1989) Demographic, spatial, and temporal genetic variation in *Gambusia*. *In*: Meffe, G.K. and Snelson F.F. (eds.), *Ecology & Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, NJ, pp. 235–257.

Smith, M.W., Smith, M.H. and Chesser, R.K. (1983) Biochemical genetics of mosquitofish. I. Environmental correlates and temporal and spatial heterogeneity of allele frequencies within a river drainage. *Copeia* **1983**, 182–193.

Snelson, F.F. (1989) Reproductive biology of Poeciliid fishes. *In*: Meffe, G.K. and Snelson F.F. (eds.), *Ecology & Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, New York.

Snelson, F.F., Smith, R.E. and Bolt, M.R. (1986) A melanistic female mosquitofish, *Gambusia affinis holbrooki*. *Amer. Midland Nat.* **115**, 413–415.

Sokolov, N.P. (1936) L'acclimatisation du *Gambusia patruelis* en Asie centrale. *Revista di malariologia* **15**, 325–344.

Sokolov, N.P. and Chvaliova, M.A. (1936) Nutrition of *Gambusia affinis* on the rice fields of turkestan. *J. Anim. Ecol.* **5**, 390–395.

Stearns, S.C. (1983) The evolution of life-history traits in mosquitofish since their introduction to Hawaii in 1905: rates of evolution, heritabilities, and developmental plasticity. *Amer. Zool.* **23**, 65–75.

Stearns, S.C. and Sage, R.D. (1980) Maladaption in a marginal population of the mosquito fish *Gambusia affinis*. *Evolution* **34**, 65–75.

Sumner, F.B. (1934) Does "protective coloration" protect? – results of some experiments with fishes and birds. *Proc. Natl Acad. Sci.* **20**(10): 559–564.

Sumner, F.B. (1935a) Evidence for the protective value of changeable coloration in fishes. *Amer. Nat.* **69**, 245–266.

Sumner, F.B. (1935b) Studies of protective color change. III. Experiments with fishes both as predators and prey. *Proc. Natl Acad. Sci.* **21**(6): 345–353.

Sumner, F.B. and Douderoff, P. (1938) Some effects of light intensity and shade of background upon the melanin content of *Gambusia*. *Proc. Natl Acad. Sci.* **24**, 459–466.

Theodorakis, C.W. and Bickham, J.W. (2004) Molecular characterization of contaminant-indicative RAPD markers. *Ecotoxicology* **13**, 303–309.

Toft, G. and Guillette, L.J. (2005) Decreased sperm count and sexual behavior in mosquitofish exposed to water from a pesticide-contaminated lake. *Ecotoxicol. Environm. Safety* **60**, 15–20.

Trendall, J.T. (1982) Covariation of life history traits in the mosquitofish, *G. affinis*. *Amer. Nat.* **119**, 774–783.

Trendall, J.T. (1983) Life history variation among experimental populations of the mosquitofish, *G. affinis*. *Copeia* **1983**, 953–963.

Trendall, J.T. and Johnson, M.S. (1981) Identification by anatomy and gel electrophoresis of *Phalloceros caudimaculatus* (Poeciliidae), previously mistaken for *Gambusia affinis holbrooki* (Poeciliidae). *Austr. J. Marine Freshwater Res.* **32**, 993–996.

Turner, C.L. (1937) Reproductive cycle and superfoetation in poeciliid fishes. *Biol. Bull.* **72**, 145–164.

Turner, C.L. (1941) Gonopodial characteristics produced in the anal fins of females of *Gambusia affinis* by treatment with ethynyl testosterone. *Biol. Bull.* **80**, 371–383.

Turner, C.L. (1941) Morphogenesis of the gonopodium in *Gambusia affinis affinis*. *J. Morphol.* **69**, 161–185.

Turner, C.L. (1942) Morphogenesis of the gonopodial suspensorium in *Gambusia affinis* and the induction of male suspensorial characters in the female by androgenic hormones. *J. Exp. Zool.* **91**, 167–193.

Turner, C.L. (1942) A quantitative study of the effects of different concentrations of ethynyl testosterone and methyl testosterone in the production of gonopodia in females of *Gambusia affinis*. *Physiol. Zool.* **15**, 263–280.

Vargas, M.J. and De Sostoa, A. (1996) Life history of *Gambusia holbrooki* (Pisces, Poeciliidae) in the Ebro delta (NE Iberian peninsula). *Hydrobiologia* **341**, 215–224.

Vondracek, B., Wurtsbaugh, W.A. and Cech, J.J. (1988) Growth and reproduction of the mosquitofish, *Gambusia affinis*, in relation to temperature and ration level: consequences for life history. *Environm. Biol. Fish.* **21**, 45–57.

Vooren, C.M. (1972) Ecological aspects of the introduction of fish species into natural habitats in Europe, with special reference to the Netherlands. *J. Fish Biol.* **4**, 565–583.

Walker, W.F. (1987) *Functional Anatomy of the Vertebrates. An Evolutionary Perspective.* Saunders College Publishing, Philadelphia, PA, 781 pp.

Walters, L.L. and Legner, E.F. (1980) Impact of desert pupfish *Cyprinodon macularius* and *Gambusia affinis affinis* on the fauna in pond ecosystems. *Hilgardia* **48**, 1–18.

Warburton, B., Hubbs, C. and Hagen, D.W. (1957) Reproductive behavior of *Gambusia heterochir*. *Copeia* **1957**, 299–300.

Washino, R.K. (1968) Predator prey studies in relation to an integrated mosquito control program: A progress report. *Proc. Ann. Conf. Amer. Mosq. Contr. Assoc.* **36**, 33–34.

Washino, R.K. (1969) Progress in biological control of mosquitoes – invertebrate and vertebrate predators. *Proc. Calif. Mosq. Contr. Assoc.* **37**, 16–19.

Webb, C. and Joss, J. (1997) Does predation by the fish *Gambusia holbrooki* (Atheriniformes: Poeciliidae) contribute to declining frog populations?. *Austr. Zool.* **30**, 316–323.

Willis, K. and Ling, N. (2000) Sensitivities of mosquitofish and black mudfish to a piscicide: could rotenone be used to control mosquitofish in New Zealand wetlands? *New Zealand J. Zool.* **27**, 85–91.

Winkler, P. (1979) Thermal preference of *Gambusia affinis affinis* as determined under field and laboratory conditions. *Copeia* **1979**, 60–64.

Wooten, M.C., Scribner, K.T. and Smith, M.H. (1988) Genetic variability and systematics of *Gambusia* in the southeastern United States. *Copeia* **1988**, 283–289.

Wourms, J.P. (1981) Viviparity: the maternal-fetal relationship in fishes. *Amer. Zool.* **21**, 473–515.

Wu, Y.C., Hoy, J.B. and Anderson, J.R. (1974) The relationship between length, weight, and brood size of the mosquitofish, *Gambusia affinis* (Baird & Girard) (Cyprinodontes: Poecilidae). *Calif. Vector Views* **21**, 29–43.

Wurtsbaugh, W.A. and Cech, J.J. (1983) Growth and activity of juvenile mosquitofish: temperature and ration effects. *Trans. Amer. Fish. Soc.* **112**, 653–660.

Wurtsbaugh, W.A., Cech, J.J. and Compton, J. (1980) Effect of fish size on prey selection in *Gambusia affinis*. *Proc.Ann. Conf. Amer. Mosq. Contr. Assoc.* **48**, 48–51.

Yan, H.Y. (1987) Size at maturity in male *Gambusia heterochir*. *J. Fish Biol.* **30**, 731–741.

Yardley, D. and Hubbs, E. (1976) An electrophoretic study of two species of mosquitofish with notes on genetic subdivision. *Copeia* **1976**, 117–120.

Zimmerer, E.J. (1983) Effect of salinity on the size-hierarchy effect in *Poecilia latipinna*, *P. reticulata* and *Gambusia affinis*. *Copeia* **1983**, 243–245.

Zimmerman, E.G., Liu, E.H., Smith, M.H. and Wooten, M.C. (1987) Microhabitat variation in enzyme activities in the mosquitofish, *Gambusia affinis*. *Can. J. Zool.* **66**, 515–521.

Zulian, E., Bisazza, A. and Marin, G. (1993) Determinants of size in male eastern mosquitofish (*Gambusia holbrooki*): inheritance and plasticity of a sexual selected character. *Bol. di Zool.* **60**, 317–322.