CONTINUOUS PRODUCTION OF *GAMBUSIA AFFINIS*

Craig Downs and Charles Beesley

Contra Costa Mosquito Abatement District
1330 Concord Avenue, Concord, California 94520

ABSTRACT

Methodology, as well as production figures, for a continuous production culture system for *Gambusia affinis* was investigated. Preliminary work indicated that fish grown at 25°C grew as fast as those grown at 30°C and survival was better at 25°C. Fry grown at a density of .43 fry/l grew faster than fry at a density of .85 fry/l although total length gain per system was greater at .85 fry/l.

In continuous production trials fry were raised to 5 months of age. During this time gravid females in the population were induced to spawn at 3,4 and 5 months. Number of males mature, females gravid, females that dropped fry and number of fry dropped were recorded. Fry produced per female that dropped was calculated. Results showed a marked increase in all categories at 5 months.

INTRODUCTION.–California has experienced a chronic shortage of mosquitofish, *Gambusia affinis*. Traditionally fish are seined and collected from local reservoirs of waterways such as sloughs. Consequently, there has been a progression of methods used to produce large numbers of these fish. Outdoor methods to date include overwintering ponds (Coykendall, 1977), raceways for artificial production (Reynolds 1977), and small shallow ponds for spawning fish (Johnson 1976). More recently, geothermal ponds have been utilized (Cheyne 1981). However, all three of these methods are subject to environmental stress such as drought, contamination and predation and there is a limited availability of geothermal sites. A fourth, more ideal alternative would be to produce fish in an intensive culture system which has both temperature and water quality control. Drazba and Gall (1980) reported on the feasibility of intensive culture of mosquitofish under confined space. This last alternative was selected as the method of choice and experiments were conducted to investigate the feasibility of continuous production of fish year-round at Contra Costa Mosquito Abatement District.

Continuous production in this report is defined as the year-round production of fry, which can only be accomplished if a constant supply of gravid females is available. In order to achieve this, each experiment had three phases: (1) holding populations of adults, (2) spawning chambers, and (3) growth of fry. The limitations of these phases would be relative to the carrying capacity of the system which is defined as "the animal load that a system can hold" (Spotte 1979). Initial work by Drazba (1980), indicated optimal fry growth around 1 fry/ liter, which was the basis for the following growth and production studies.

Basic growth parameters such as temperature and fish density for optimum production have been suggested by Cech (1981) and Drazba (1980) respectively. As both were deduced by small scale experiments, we chose to re-test these parameters in our production system. Based on preliminary results from our density and temperature work, we designed the following approach to achieving a continuous production system. Initial fry would be obtained by bringing gravid females into the lab and inducing them to spawn by isolating them and elevating the temperature. Fry would be separated into four groups and grown-out. At maturity these fry would be thinned out to mate. Gravid females would then be isolated and induced to spawn at predetermined intervals. Fry produced would be subsequently grown-out in the lab, space permitting, or removed to stock ponds. Some fry would be retained to replace aging broodstock as needed.

MATERIALS AND METHODS.–The District converted a small storage building into an aquaculture laboratory for production of fish. Within the building there are two independent, recirculating aquatic systems as previously reported (Beesley 1981). Each system is comprised of four tanks (7′ x 3′ x 18″) connected to a sump (22″ x 22″ x 30″), a 55-gallon cylindrical carboy biofilter and a 10-gallon water heater which is in turn controlled by a thermoregulator. Water is pumped from the sump to the biofilter, gravity fed to the tanks and returns to the sump by gravity overflow. The biofilter is composed of 4 layers and types of rock, a 6″ bottom layer of California Gold Rock, a 6″ layer of dolomite, a 6″ layer of pea gravel and a 6″ top layer of aquarium gravel. Both systems are fully independent of each other.

In all trials the initial fry were obtained by bringing gravid females into the lab, placing them in 4″ by 4″ strawberry baskets lined with 1/8″ or 8x8 mesh window screen and elevating the temperature to 30°C. This induced 90% of the females to drop fry within 7 days (unpublished data). Fish were fed twice daily with Tetramin® flake (ad libitum) and supplemented with 4 g of *Artemia nauplii* weekly. Photoperiod was maintained at 16:8 light-dark by fluorescent lights on an automatic timer. All measurements were made in total length.

The two densities tested were .85 fry/l and .43 fry/l; trials were conducted at 25°C for 31 days. Two replicates of each density were tested in each system. The two temperatures tested were 25°C and 30°C; trials were conducted for 8 weeks at .24 fry/l. Three replicates of each temperature chosen were tested in each system.

Two consecutive continuous production tests were conducted for five months at 25°C in one system. Each trial consisted of 1800 fry obtained from a mass spawn of wild caught gravid females. Four tanks were initially stocked with 450 fry each (1 fry/liter) for fry grow-out. Populations were thinned out at eight weeks to 120 fish/tank (.3 fish/l) for the duration of the test. Sexually mature males and females were counted and spawning tests were run at 3, 4 and 5 months. Spawning tests were conducted by transferring the gravid females to the other system at 30°C and isolating females in individual chambers for two weeks. Numbers of females that spawned were noted and fry were counted. Females were then returned to the original tank and system at 25°C. This procedure was repeated at four and five months, although mature males were not recorded at four months.

RESULTS AND DISCUSSION.--Growth rate was significantly greater (F.05) at .43 fry/l than .85 fry/l (Table 1), while survival exceeded 90% at both densities. Drazba and Gall (1980) reported the highest rate of growth for fry occurred at a density of .8 fry/l, which probably reflects differences in system designs including rate of flow, biofilter size, and raceways or tanks. Regardless of the differences in optimal density, both studies indicate that maximal biomass gain/system was achieved at the higher density. In other words, although the growth/fry was slower at .8 fry/1, total length gain for all fish was greater: 210 vs. 114. Length gain is calculated by multiplying the total number of fish times average gain/day/fish. Consequently, from a mass-rearing standpoint the number of fish being reared would be more important than the density/liter provided mortality is not high.

As seen in Table 2, there was no significant difference in growth rate (F. 05) at 25°C vs. 30°C while there was significant difference (F.01) in survival (F.01). Mortality was predominant at the higher temperature and was observed to occur primarily in young males between 4-6 weeks of age in both trials.

The results of continuous production trials are seen in Table 3 and all gravid females were isolated for spawning. There was a constant increase in gravid females and mature

Table 1. Average growth in *Gambusia* fry, measured in total length (mm) at two densities (25 C; 31 days).

Fry per liter	Total number fry	Initial length	Final length	Length gain	Gain/day/fish	Total length gain	Survival %
.85	1400	10.1	14.7	4.6	.15	210	92
.43	600	10.1	16.0	5.9+	.19	114	96

+ indicates a significant difference at the 5% confidence level.

Table 2. Average growth in *Gambusia* fry, measured in total length (mm) at two temperatures (.24 fry/l; 8 weeks).

Temperature C	Total number fry	Initial length	Final length	Total length gain	Gain/day/fish	Total/gain/day	Survival %
25	300	7.0	25.9	18.9	.34	102	97
30	300	7.0	25.1	18.1	.32	97	70++

++ indicates a significant difference at the 1% confidence level.

Table 3. Continuous production of *Gambusia affinis* (25° C; .3 fish/l).

Trial	Age (months)	Number fish	Number mature males	Number gravid females	Number females dropped	Number fry dropped	Fry/female
1	3	480	17	44	9	54	6.0
	4	452	--	30	6	60	10.0
	5	443	122	137	110	836	7.6
2	3	480	14	56	7	64	9.1
	4	464	--	46	22	125	5.7
	5	458	108	69	52	634	12.2

Table 4. Maturation and reproduction of *Gambusia affinis* in a continuous production system, an average of two trials.

Age (months)	% gravid females	% mature males	% gravid females spawned	Number fry/female spawned	Number fry produced
3	10	3	16	7.38	59
4	8	---	37	6.61	92
5	23	26	70	9.07	735

males with time. There was no apparent difference between trials in numbers of mature males over time but there was a substantial difference in number of gravid females at the 5 month mark, 137 in trial 1 vs. 69 in trial 2. Nevertheless, in both trials the percentage of gravid females that dropped fry was more directly associated with time. This continuum was also seen in the increase in total number of fry dropped. A more complete picture is provided in Table 4 which is a summation of both trials. Increased egg development by females was evident by comparing the percentage of sexually mature males and females to the percent which spawned. Although the number of fry/female dropped slightly between months four and five, the total number of fry produced continued with time, with a marked increase the fifth month (1470 fry). This could be associated with the increase in number of mature males at five months (26%), but this could also be due to the increase in gonadotrophic activity as exhibited by the marked increase in gravid females (23%).

The fecundities attained (Table 4) were lower than those realized by Busack (Gall, personal communication), for *Gambusia* of the same age in isolated aquaria, 22.8 fry/female at 2.5 months of age and much less than wild females induced to spawn in this system, 39.3 fry/female of unknown age (unpublished data). The higher fecundities were probably due to the larger size of the females used by Busack, and the older age of fish used in our unpublished data. Although fry were observed once from fish two months old our data does not support the contention that early maturity and reproduction at 60 days (Busack 1981) is to be expected in a closed recirculating system used for continuous production.

CONCLUSIONS.–Based upon the results, intensive culture of *Gambusia affinis* is feasible. Success would be predicated on a continuous supply of gravid females and production of adequate numbers of fry. The number of fry per female may not be as critical to overall success as the total number of fry produced as seen in Table 4, and the total length gain for the system used appears to be more important than actual gain/day/fish as seen in Table 1.

Within the limits of a small scale system there are two possibilities for manipulating mass production. One would be to have a reliable source of gravid females, induce them to spawn, and transplant fry for appropriate uses. Brood stock would have to be replaced when fry production declined. During the colder months, brood stock would best be replaced by females which had matured under artificial conditions, as those brought in from the field are not likely to spawn within a desired period of time. The other method would be continuous production. In this method the supply of gravid females is produced by raising fry to maturity. Thereafter, excess fry obtained from this brood stock can be removed to alternate sites for appropriate use whether field stocking or over the counter distribution to the public. There are two principle differences between these two methods, in the former the brood stock is maintained outside the lab and all the fry are transplanted, while in the latter the brood stock is maintained within the system and a small percentage of fry are periodically retained and raised to maturity to produce the next brood stock. The first method is limited by availability of gravid females in the colder months. With continuous production a year-round supply of fish is more realistic and production would be a function of the size and efficiency of the system.

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