This Nile tilapia is the preferred eating size by many farmers in northeastern Thailand, usually barbequed or made into soup. (Photo by D.C. Little)

Population Control in Farmed Tilapias

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Breeding at a small size in tilapia diverts energy from growth into reproduction (territorial/courtship behavior and the metabolic cost of gamete production). Further, the progeny produced by the stocked fish compete for available space and food resources, thereby inhibiting the growth of the stocked fish, especially in ponds, where space and food quickly become limiting. Even in many parts of Southeast Asia where marketable size of tilapia is small (80–100 g), recruitment results in harvests that include 10–20% of small, largely unmarketable, fish. In African countries and urban areas in Asia where the premium market size for fresh fish is much larger, this figure can be as high as 50%.

Sexual maturity in tilapias can occur at very small sizes (as low as 15–20 g) but this is independent upon management practices for fingerlings. Fry that have been nursed at high densities for considerable periods of time, prior to stocking, may be stunted but sexually mature and will thus spawn at a smaller size. It is likely that the deleterious effect of reproductive effort on the growth of the stocked fish is very dependent on their age at stocking and will be felt most during the initial period of breeding when fry production is at its highest.

Under the majority of culture practices, there is little doubt that reduction or elimination of reproduction in culture ponds is likely to result in significantly improved yields of marketable fish.

Two common methods by which tilapia farmers maximize available resources are to keep the small unmarketable fish (recruits) at harvest for use as alternative foods (such as fermented fish or fish paste) or for distribution to other farmers for restocking. Repeated stocking of this nature will, however, quickly result in widespread degradation of stocks through inbreeding depression, further reducing yields. This practice of restocking may well have been a major contributory factor in the rapid deterioration in the performance of some tilapia stocks such as has occurred with the early introductions of *Oreochromis mossambicus* into Asia. This practice is not recommended on a long-term basis.

The existing methods for control of reproduction in tilapia are shown in the Box. Clearly, an elegant and effective solution to the problem of uncontrolled reproduction would be the culture of monosex male progenies which would combine the benefits of population control with the faster growth of males.

Other technologies for control of reproduction in tilapia

INTERMITTENT HARVEST

Parents are removed by seining and are restocked in another pond, or water is drained to remove fry. This method is largely impractical in most farming operations due to the continuous and asynchronous breeding cycle of tilapia in tropical climates. This technique needs to be performed regularly and is thus time consuming, labor intensive and does not exclude energy wasted in reproduction.

MANUAL SEXING

Sexes can be separated before they reach sexual maturity and the males restocked (as male tilapia grow significantly faster than female). However, it is difficult for even the most skilled workers to achieve greater than 90% accuracy in sexing and so breeding and reproduction is rarely completely controlled. In addition this method is inevitably inefficient and wasteful as the females are normally discarded.

PREDATORS

The stocking of piscivorous fish in growout ponds to cull tilapia fry and fingerlings has been used with varying degrees of success. However, there is no universal recipe for the control of reproduction by these means, the methodologies necessarily varying on a case by case basis according to optimal predator/prey stocking rates and availability, survival and control of the predators themselves.

HIGH DENSITY EFFECT

It has been reported that reproduction is considerably reduced when tilapia are stocked at very high densities (>10 $kg \cdot m^{-3}$). Such high densities can only be achieved in intensive tank culture systems or in cages with supplementary feeding.

CAGE CULTURE

Fry production can be prevented by stocking tilapia in net cages. This is provided that the mesh size is 2.5 cm or greater so that any spawned eggs are lost. It is important to minimize fouling which can provide a substrate for spawning. This system prevents recruitment rather than stopping breeding so losses to reproductive effort will still exert a negative influence on growth.

DELAYED SEXUAL MATURITY

It has been suggested that sexual maturity in some species of tilapia can be delayed by growing them in high salinities (25 ppt). However, in saline intolerant species this is likely to be accompanied by an increased susceptibility to disease and reduced growth and would thus be impractical in the majority of culture situations. A sustainable and more beneficial approach could be the development of late maturing strains through selection.

STERILITY

The sterilization of some salmonids by induction of triploidy to effectively minimize the deleterious effects of sexual maturation is now widespread in temperate developed countries. Triploidy has been successfully induced in a number of tilapia species by application of heat, cold or pressure shocks to fertilized eggs. However, induction of triploidy on a large scale is impractical due to the small individual clutch size of female tilapia. In addition, there is no evidence to suggest that growth performance of triploid tilapia is superior to that of diploids.

HYBRIDIZATION

It has been reported by many authors that 95-100% male progeny can be produced in a number of interspecific hybrid crosses, most consistently in (female x male) *O. niloticus* x *O. hornorum* and *O. niloticus* x *O. aureus*. Indeed this system is successfully used in some intensive and semi-intensive systems particularly in the Israel tilapia culture industry. However, widespread adoption of this technology would result in the introgression of tilapia species with deleterious implications for the conservation of tilapia genetic resources.

Hormonal Sex Reversal

Given the significant disadvantages of hybridization, the best available technology for large-scale production of all-male tilapia is through mass androgen induced sex reversal. First described barely twenty years ago, this technique in which sexually undifferentiated fry are masculinized by the incorporation of a steroid hormone in the feed, is yet to become routine practice in either small-scale or large tilapia hatcheries. The technique is only commonplace in Taiwan and Israel, countries with well developed and intensive tilapia culture industries. Elsewhere the technique seems to remain in the laboratory or the preserve or large companies.

Undoubtedly, lack of infrastructure and logistical factors have hampered the introduction of the technique under some circumstances. Under commercial conditions, sex reversal has often failed to achieve the degree of reliability and 'use friendliness' that is required for widespread acceptance.

The major problems of hormonal sex reversal relate to the difficulty of mass producing sexually undifferentiated *Oreochromis* fry and to the effectiveness of the subsequent hormone treatment.

Currently 'swim-up' fry suitable for commercial sex reversal are removed after natural incubation by broodfish stocked in earthen ponds. In Israel, fry are completely harvested 18–20 days after stocking of broodfish. In Taiwan, fry are 'partially' removed over a longer period by 'eye-seining' and dipnetting the perimeter of earthen ponds. Both methods have their problems in terms of efficiency but do provide sufficient fry of approximately the same age and size (<12–13 mm total length) to make hormonal treatment worthwhile. Intensive techniques using ponds, *hapas*-in-ponds, or concrete tanks, developed at the Asian Institute of Technology (AIT), can make mass production of fry possible under a wider range of conditions.

The primary objective of treatment is to produce all-male fry. In practice this generally means production of sufficiently high proportions of male or sterile fish to prevent breeding at all or at least to reduce recruitment to an insignificant level. Some breeding can occur as a few viable females may remain. If sex reversal is ineffective, i.e., produces less than 96% males, the usefulness of the technique becomes questionable. A recent experiment demonstrated that recruits could form up to 33% of the harvest biomass with as little as 5% of the stocked fish being female.

A further major objective during the period of treatment is to maximize survival of fry during the period when *Oreochromis* are particularly cannibalistic (10–30 days after first breeding).

The main factors affecting the efficacy of hormonal treatment and the important 'predisposing' factors are given in Fig. 1. Many of these factors are interactive and together may explain the poor success described by some workers under both laboratory and field conditions. The 'critical' levels are still the focus of research both at the AIT and at Auburn University, where much of the early work on sex reversal was done.

Sexual differentiation is known to occur in *Oreochromis* fry at or around 17–19 days after hatching. Research suggests that successful hormonal sex reversal requires the level of male hormone to be raised before this time and to remain high during the sensitive period of early sexual development. Thus, methods of fry production that harvest fry at the earliest possible age, permitting early feeding of fry, provide a greater assurance that the intake of hormone during the critical period will be adequate. Moreover, methods producing fry of greater or uncertain age and variable size also tend to suffer from greater cannibalism-related mortality.

The intake of hormone by the fry may be influenced by many factors.

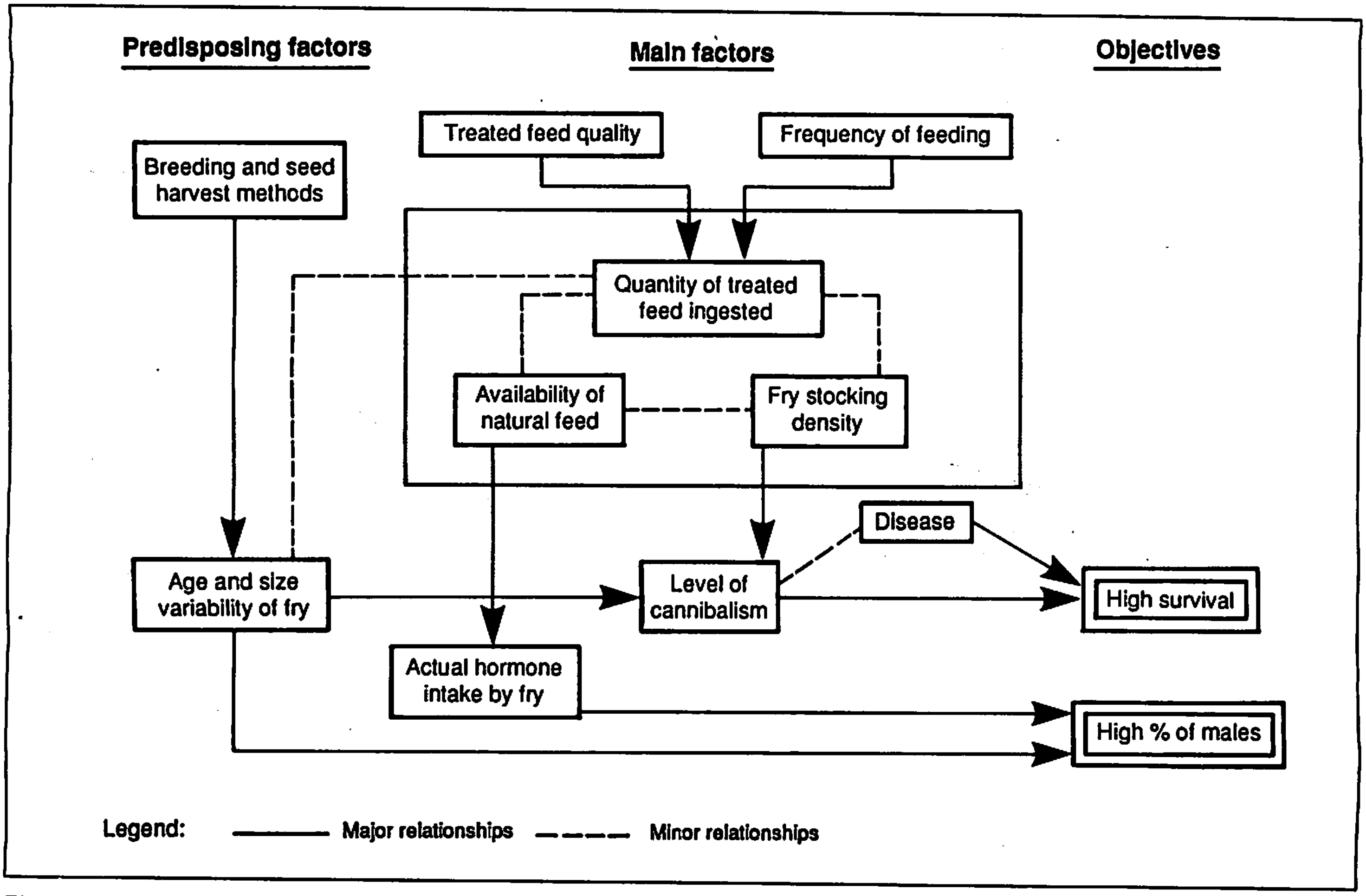


Fig. 1. ***Factors affecting the efficacy of hormonal sex reversal of tilapia.***

Same size, same age, same sex. These 30-day old O. niloticus *fry have been hormonally sex reversed in nylon* hapas. (Photo by D.C. Little)

The quality of the treated feed given to the fish can be affected by the composition of the raw ingredients and the method of preparation and storage. The hormone level incorporated into the feed that has given good results varies from 35 to 60 $mg{\cdot}kg^{-1}$ of feed. Such small amounts of hormone are sometimes difficult to estimate under field conditions but may be stored as a concentrated stock solution after mixing with alcohol (0.5 $g{\cdot}l^{-1}$). The volume of alcohol required to ensure homogeneous mixing of the hormone with the feed is in the order of 240 $mg{\cdot}kg^{-1}$ of feed; any less may result in uneven distribution of the hormone-alcohol mix and poor results. After mixing, the feed should be spread out on shallow trays and sun-dried or dried in an oven at 80°C for a period of two hours to evaporate off the ethanol. Both stock solutions and treated feed, the latter after sealing in a plastic bag, should then be refrigerated. Hormone or feed-mixed-with-hormone should not be exposed to ambient tropical temperatures.

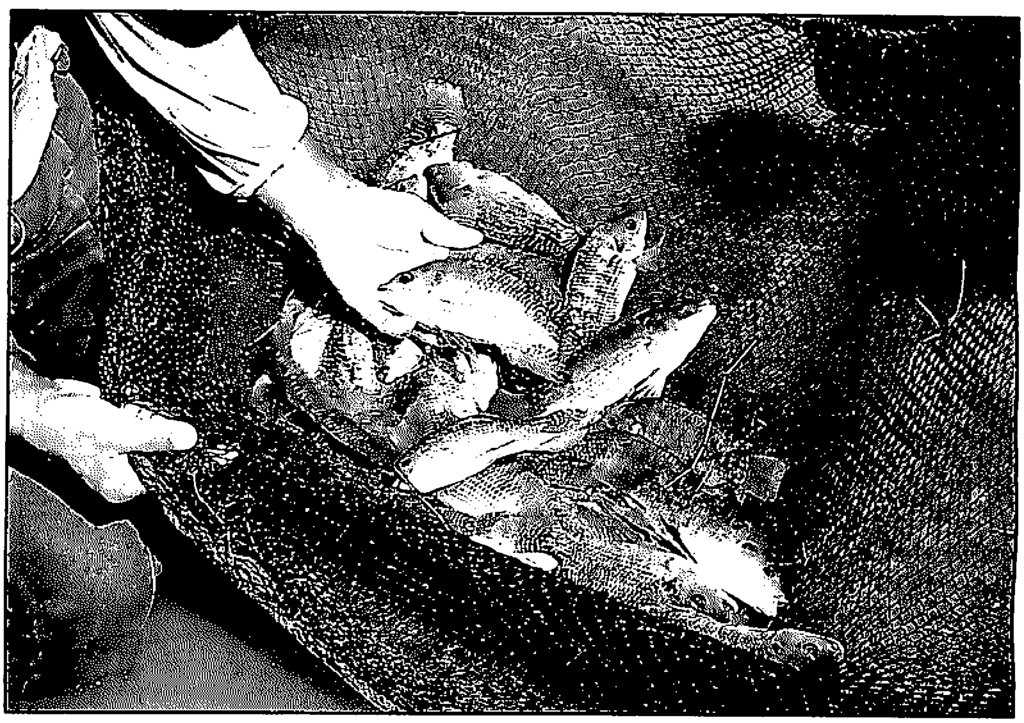
Palatability and particle size will both affect the actual intake of treated feed by first-feeding fry. Feed should be of a quality suitable for optimum growth. Trout and eel starter diets have been recommended as suitable but are often unavailable in developing countries. A sieved low-grade marine fish meal has been found to be satisfactory. In systems with no natural feed available, vitamin C (ascorbic acid) is added at 5 $g{\cdot}kg^{-1}$.

Frequency of feeding will also affect the actual intake of feed; a minimum of five times daily is recommended. *Ad libitum* feeding, or feeding to body weight in excess of 20% per day is recommended in the initial stages of treatment (first 10 days). This may be reduced to 15% for the subsequent period of sex reversal (20 days). Some workers now recommend that the period of treatment may be reduced to as little as 21 days.

Environmental factors also affect the actual intake of feed and success of treatment. It used to be thought that sex reversal was only possible in water low in natural feed but fry are now routinely treated in plankton-rich *hapas* and tanks. Poor water quality, low temperature (<22°C) and exposure to disease tend to reduce appetite, growth and effectiveness of sex reversal.

Stocking density during treatment should be high to ensure that the relative amount of natural food available is limited (12 fry per liter of water). Poor sex reversal may result if the actual intake of treated feed is low when natural food is abundant, especially if the feed offered is of low appetency. This may be balanced to some extent by the use of a higher hormone dose in green water systems (60 $mg{\cdot}kg^{-1}$ feed) compared to clear water systems (40 $mg{\cdot}kg^{-1}$ feed).

Under some conditions, opportunity for hormone treatment may be limited by accessibility to hormone, a suitable feed, or alcohol. The quality of alcohol required is the subject of some interest



All-male culture produces larger, more uniform-sized fish and absence of recruits allows total control of the production cycle. (Photo by G. Mair)

Harvest from ponds stocked at different sex ratios: L-R 100%, 95% and 50% male. The monosex male fish were 80% larger than the mixed sex (50% male) fish. Significant recruitment has occurred in ponds stocked with 95% males. (Photo by G. Mair)

as this may represent one of the major costs. Food grade (95% pure) ethanol is known to be suitable but the usefulness of lower grade, locally distilled liquors should be investigated. Lack of reliable electricity and/or refrigeration to keep hormone and feeds in good condition has also caused problems and the period of time that unrefrigerated feed and/or hormone maintain efficiency under tropical conditions should be researched.

Earlier fears of a risk to human health from hormone residues in sex reversed fish are unfounded; studies have shown that the levels of steroids in fish treated with hormone were actually lower than in untreated fish within comparatively short periods after cessation of treatment. Exposure to the hormone during preparation and feeding appear to be an insignificant risk if normal laboratory precautions are observed. However, the widespread distribution of hormone or ready prepared hormone-treated feed for "do-it-yourself" sex reversal could lead to abuse of the hormone. Apart from the increased risk of poisoning, poorly advised farmers may attempt to utilize the analogic properties of the androgens by applying the hormone-treated feed as a growth promoter. This will increase the levels of hormone in the environment and may result in the marketing of fish retaining unacceptably high levels of hormone. Such practices should be actively discouraged.

Generally, logistic problems may be of secondary importance to those of management in constraining the dissemination of sex reversal by direct hormone treatment of fry. Where tilapia are currently produced in small-scale or mini-hatcheries, the practicality or desirability of hormone treatment should be carefully considered. Evidence from the Philippines suggests that hormone treatment is not suited to smaller-scale operations. Economies of scale are very evident for the technology and it is likely that larger hatcheries with their greater resources will dominate once the virtues of the produce are more widely appreciated. In Thailand where small-scale tilapia seed production is not competitive with carp production, large producers already dominate. The introduction of all-male fry using a franchise nursing system in which fry are produced centrally by large hatcheries and then nursed and marketed locally by small operators is a possibility. In the Philippines, where small and large hatcheries coexist, wider acceptance of hormone-treated sex reversal could have negative effects on the small hatcheries. The daily and frequent feeding schedule required during treatment may effectively prevent government agencies from sustainable and predictable production of all-male fry if working schedules are inflexible or staff poorly motivated. Any wide scale introduction of hormone-treated fry could therefore change the structure of tilapia seed production, bringing about a centralization of fry production in large-scale hatcheries in the commercial sector.

Monosex culture in conventional fertilized earthen ponds with supplementary feeding produces fish of larger individual size. On average the final size of hormone-treated fish exceeds that of untreated male and female fish by around 16 and 30%, respectively.

An Alternative Approach?

The perceived and potential problems of the hormonal sex reversal technique has stimulated research on alternative methods for the generation of monosex male progenies. Studies at the University of Wales, Swansea, UK, have concentrated on elucidating sex determining mechanisms in commercially important tilapia species. Based on the hypothesis of monofactorial sex determination with homogametic female (XX) and heterogametic male (XY), a model was proposed for the generation of monosex male progeny by genetic manipulation of sex determination in *O. niloticus*. This model (Fig. 2) is similar to that outlined for

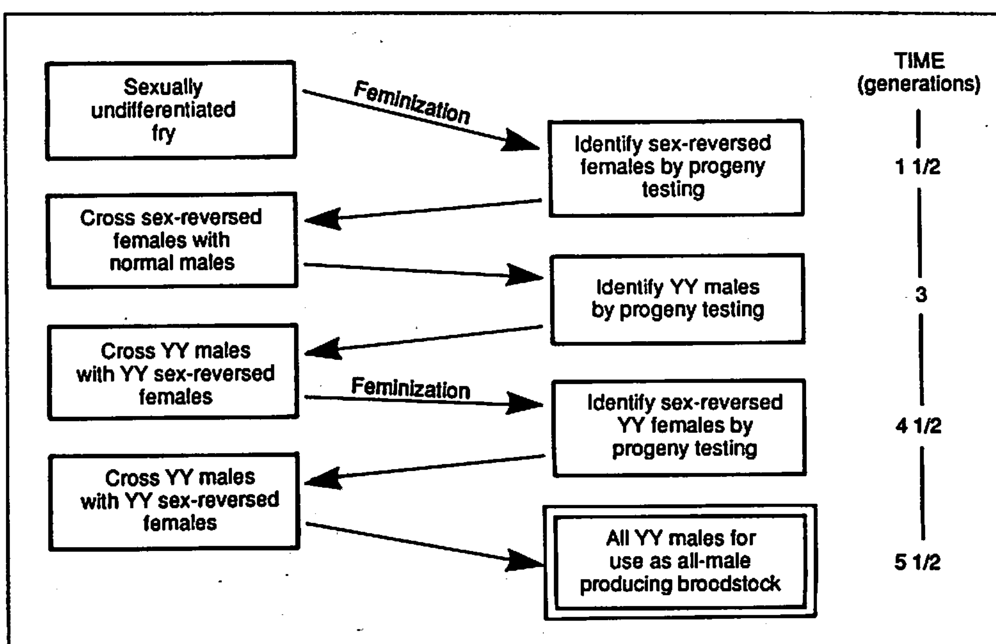


Fig. 2. *Schematic diagram depicting model for large-scale production of YY males.*

Table 1. Potential advantages and disadvantages of technique for generation of all-male tilapia by genetic manipulation of sex determination relative to problems of hormonal sex reversal.

Advantages	*Disadvantages*
Progeny are genetically all-male	Still in developmental stages
Potential to produce 100% male progeny	Initial phase is complex and time consuming
Potential that no reproduction will occur	May not be applicable to all species or strains
Applicable to most fry production systems	Estrogen used in initial phase is toxic
Not labor intensive after initial phase	Requires isolation of broodstock from contamination
No consumer resistance	Problematic to integrate into selective breeding programs
No centralization of fry production	

O. mossambicus by Pandian and Varadaraj (*Naga*, July 1990, p. 3) and an alternative model and somewhat simpler model was proposed for species with homogametic males such as *O. aureus*. The technique for *O. niloticus* is based on the production of large numbers of "supermales" of the novel genotype "YY" which should yield all male progeny when crossed with normal females.

Although still in its developmental stages, the use of genetic manipulation to generate all-male producing broodstock could provide answers to the constraints on hormonal sex reversal outlined above. The method could potentially produce large numbers of all-male producing broodstock which could be utilized in existing fry production operations (with the important proviso that contamination by normal male broodstock is prevented) to generate genetically all-male progeny. These may be superior in performance to hormone-treated fish which constitute approximately 50% female genotypes (little is known about the relative performance of these sex reversed males). Preliminary growth trials suggest that all (genetic) male progeny produced in this way could grow 20–40% faster than phenotypically mixed sex progeny.

Studies at the University of Wales, Swansea; the University of Maduria, India; and by a number of Chinese researchers (who actually pioneered this work in *O. mossambicus* as early as 1975) have shown that sex reversed females (Δ ♀♀) and YY "supermales" are both viable in *O. niloticus* and *O. mossambicus*. In most cases these produced the sex ratios predicted by the theory of monofactorial sex determination, although some YY male *O. niloticus* did not give the predicted 100% male progeny (only 95–99%). There have, as yet, been no reports of successful sex reversal of YY males to female, an important step in the mass production of YY males.

The common custom of stocking breeders at sex ratios of 1:4 (♂:♀) or lower would mean that large numbers of monosex fry could be produced from relatively small numbers of YY males.

Due to the time necessary to generate YY males in new stocks (four generations) it is more difficult to integrate this technique into selective breeding programs but this would be possible. Selection could be performed in the production of YY males, (indeed this may be necessary to remove supermales which do not give 100% male progeny) and the resulting males crossed with the current generation of selected females.

Table 1 shows a comparison of the major potential advantages and disadvantages of all-male production by genetic manipulation of sex determination relative to the problems of hormonal sex reversal.

This technology could be applied and extended to the industry at two levels. It is likely that the complexity and time scale of the operation to generate all-male producing broodstock would necessitate this being performed largely in research institutions and government hatcheries. However, once produced in large numbers, these YY-male broodstock could be kept on-station to generate large numbers of all-male fry or the breeders could be distributed to both small and large regional hatchery operators who could then produce the all-male fry for distribution to growout operators. It is thus possible, provided the premium on "supermale" broodstock is not too high, that this method of monosex fry production could be used within a decentralized system of commercial sector tilapia hatcheries, both large and small.

There is little doubt that the tilapia culture industries will change and evolve in response to developing technologies and market pressures. Given the potential benefits of monosex fish culture using either of the methodologies described above, it is likely that this will become increasingly important. Market preference for larger and more uniform sized fish together with an overall improvement and intensification of farming operations, will speed the introduction of monosex production. Hatchery development will be both affected by the introduced technology and, in turn, developments within this sector of the industry will affect the adoption of the improved fish by growout farmers.

Further Reading

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