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GENETIC IDENTIFICATION AND STATUS OF TILAPIA REGIONAL STRAINS IN SOUTHERN CALIFORNIA

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Abstract

Microsatellite DNA marker studies of 224 tilapia from 12 locations (4 from the wild in Africa, 4 feral, 4 in culture) were studied to determine species composition of fish present in southern California waters. Genetic similarities among tilapias sampled and relationships to a global tilapia genetic database were compared by unweighted pair-group method arithmetic (UPGMA) dendrograms and principal coordinate analysis. Tilapia in commercial, closed system aquaculture and a Colorado River tilapia population were almost identical genetically and similar to a reference population of *Oreochromis niloticus* (the GIFT strain of relatively recent African origin [Macaranas et al. 1995]). This *niloticus* group was found to be significantly distinct and separate from a second large grouping that included all other California tilapia samples plus *Oreochromis mossambicus* reference samples from two locations in Africa.

Morphometric and meristic measurements of tilapias cannot be used to identify tilapia species in California and Latin names are useless outside of the wild in Africa because the species have been so manipulated (McAndrew and Majumdar 1983). It is recommended that North American aquaculturists adopt a system of regional strains (code names) defined by DNA microsatellite marker frequencies, and that a regional strain registry of tilapias based upon DNA markers be established. We propose to create two southern California regional strains: "California Mozambique" and "California Nile", named in reference to their genetic similarity to *O. mossambicus* and *O. niloticus* reference samples at specific microsatellite loci. The "Nile" and "Mozambique" components of the names are indicative of genetic affinities without implying an exclusive taxonomic composition. We suggest that to qualify as a regional strain the fish should exist in the wild and not merely under cultivation. Other terms, e.g. "breed", "variety", "cultivar" or "strain" (without the "regional" qualifier) are available for purely domestic populations of tilapia. California regional strains are characterized as:

(1) <u>CALIFORNIA MOZAMBIQUE (marker alleles from loci Os64 and Os7r):</u> A California regional strain found in the Salton Sea, in drainage creeks/agricultural ditches inputting into the Salton Sea in Niland, CA, and in aquaculture in two of the southern California farms sampled, having a small number of genes from *Oreochromis urolepis hornorum*;

(2) <u>CALIFORNIA NILE (marker alleles from loci Os25 & Os 64):</u> A California regional strain found in the Colorado River near Blythe, CA and in closed system culture at one southern California farm sampled.

Southern California tilapias were not imported directly to California as native genetic resources from Africa but arrived from third or fourth party sources. Tilapias were imported primarily for aquatic pest control, not aquaculture development. A review of Fish and Game and museum records and the relevant literature showed founder populations of southern California tilapias were small in number, and were introgressed and hybridized before importation or escape to the State. However, average

heterozygosities of southern California tilapias were as high as found at microsatellite loci in natural populations of other fish. The low heterozygosity of one farm sample (0.21) indicated the probability that a serious "bottleneck" and/or inbreeding had occurred (all other sample heterozygosities ranged 0.34-0.72). As a result of the small sizes of founder stocks, uncontrolled hybridization, introgression, and isolation, tilapia populations in southern California appear to be unique from other tilapia populations so far examined.

Oreochromis niloticus is present in southern California in closed system aquaculture by legal permit from California Department of Fish and Game. This sample was nearly identical to a wild population in the Colorado River near Blythe, CA. *O. niloticus* alleles were found at a very high confidence level in these two samples which closely matched *niloticus* alleles found in our global reference. We suggest that the proposed "California Nile" regional strain was *O. niloticus* with little or no introgression from other species. Distinctive microsatellite DNA markers of *O. niloticus* were not found in any other Californian naturalized or farmed tilapia populations. The other tilapia samples (including the Salton Sea tilapias) were closely grouped in principal coordinate space and also in the UPGMA dendrogram developed, justifying their designation as a second regional strain. This strain was sufficiently similar to the *O. mossambicus* reference samples to justify its designation as "California Mozambique", without excluding the possibility that a small number of genes from *O. urolepis hornorum* are also present at low frequencies.

There is justified concern that a relaxation of controls on tilapia species regulations in California will allow importations of new diseases across California's state and international borders threatening its growing tilapia aquaculture industry. It is recommended that a cautious approach be taken to allowing any new importations of *Oreochromis niloticus* to California due to the: (1) significant risks in importing new tilapia diseases and parasites, especially *Streptococcus* (California's ban on live catfish imports is a significant successful example in this regard); (2) concerns about importations of new, exotic species and their potential negative impacts on the State's native aquatic biodiversity and its public investments in aquatic species recovery programs; (3) fact that the genetic diversity of the tilapia species already present and permitted legally in the State may be adequate to sustain and further expand the State's tilapia aquaculture industry into the future if planned, scientific, genetic improvement programs and investments were made; (4) fact that significant tilapia genetic resources (e.g. the "California Nile" regional strain) are already present in the state in the Colorado river, and that further "bioprospecting" and DNA marker studies may document additional promising populations that already exist.

Introduction

The global crisis in capture fisheries will require a rapid expansion of aquaculture. Out of the 200 commercial fish stocks being tapped globally, one-quarter are overfished and 38% more are fully exploited (FAO 1995). About 1 billion people rely upon fish as their main source of animal protein, and this number of "fish eaters" is increasing due to population growth and rising incomes, primarily in Asia. While recovery programs for reclaiming the bounty of marine fish stocks are imperative for global food security, farm-raised fish must help fill the growing gap between aquafoods supply and demand. The tilapias are prime candidates due to their ease of farming and favorable product characteristics (Pullin 1985, 1986, 1991).

From the 1950's to mid-1970's tilapias were exported from their native ranges in sub-Saharan Africa to Asia and onwards throughout the tropical world to over 50 nations for aquaculture development, aquatic weed and insect control. Some have likened this widespread seeding of the world's warmwater ecosystems as a "tilapia craze" similar to the carp "craze" in the late 19th century (Cardone, in press).

The most widely spread tilapia was the Mozambique tilapia (*Oreochromis mossambicus*, once known as the "Java" tilapia). After being stocked nearly everywhere environmentally suitable, in the mid-1970's this species seriously deteriorated in its naturalized environments, and small sized, poor quality fish lost consumer acceptance (Pullin 1988, 1991). In addition, scientific studies showed the Mozambique tilapia negatively impacted many aquatic ecosystems due to its aggressive, broad-spectrum omnivorous feeding habits and precocious breeding behavior. The feeding niche of the Mozambique tilapia was found to be extremely plastic - tilapia even became a predator in some locales (on milkfish fry, Lobel 1980). As a result of these undesirable traits, the Mozambique tilapia has been replaced in nearly all of the major tilapia producing countries by the Nile tilapia, *Oreochromis niloticus*.

The global consensus that the Nile tilapia (*Oreochromis niloticus*) is the best species for aquaculture development is due to at least three important commercial characteristics: {1} its faster growth rate to larger maximum sizes than other species; {2} its larger size at first reproduction; {3} its grazing feeding habits, and lower position on the aquatic food web, making it cheaper to grow, and with less environmental impacts (Moreau et al. 1986; Pauly et al. 1988; Pullin 1988, 1991; Pullin and Capili 1988; Dempster et al. 1993). However, *O. mossambicus* is not everywhere a pest. In the impoverished Asian nations of post-WW II (esp. Indonesia), and today in modern Sri Lanka, the fish have been thought to have saved millions of poor people from starvation and protein malnorishment (DeSilva and Senaratne 1988).

The largest tilapia producing nations are in Asia. China is the world's largest tilapia producer (about 160,000 tons), followed by the Philippines (63,000 tons), Indonesia (60,000 tons), and Egypt (22,000 tons) (FAO 1995). At a recent fisheries conference in

Sri Lanka, tilapia was named the "Fish of the Decade" because of the rapid worldwide growth in market demands for the fish.

A growing market demand also exists for tilapia in the USA, with the fish being named in seafood circles as the "new white fish" (Engle, this volume). Increasingly, tilapia are being viewed as a replacement for cod and hake which are in short supply. In 1994, for the first time, tilapia consumption in the USA surpassed trout consumption (30,000 versus 27,500 tons). Tilapia is now the most frequently requested fish in the US restaurant trade (American Tilapia Association [ATA] 1995), and new markets carrying the fish report rapid acceptance. For example, a fish market at a Publix superstore in Palm Harbor, FL reported that tilapia had "almost overnight become the leading seller in the department". Publix offered customers fresh 3-5 oz tilapia fillets at a retail price of $6.49 per pound. Repeat sales were extremely high and customer satisfaction with the product was exceptional (M. Ednoff, Florida Department of Agriculture, personal communication). The culinary characteristics of the fish match almost perfectly the desires of the US consumer, e.g. a white flesh, boneless, relatively odorless, with a very mild flavor.

US domestic consumption of tilapia reached an all time high of 58 million pounds live weight in 1995 (ATA 1996). It has been estimated that current demand for tilapia in the US is 76 million pounds, and that demand will grow at 25% per year for the next 10 years (North American Fish Farmers Cooperative 1995). Most tilapia are marketed live at premium prices to Asian-American markets throughout North America. In California in 1995, for example, farm gate prices held above $2 per pound live whole fish in these markets for most of the year (Engle, this volume; W. Engler, Pacific Aquafarms, Inc., personal communication). Processed tilapia accounted for only 25% of total sales in 1995 (ATA 1996). The ATA (1996) reported higher prices in 1995 for all three categories of frozen, fresh fillet, and frozen fillet tilapia, indicating increased consumer acceptance and familiarity with tilapia. However, most of the increased domestic consumption of tilapia comes from imported fish, not domestic production, and US imports of tilapia are rising.

The USA imports over three times the amount of tilapia it grows. Tilapia imports increased from 16 million tons in 1993 to 24.5 million tons in 1994 (ATA 1996). The major importers were (in order by value): Taiwan, Thailand, Costa Rica, Indonesia, and Columbia. The ATA (1996) listed 19 nations exporting tilapia to the US in 1995. Foreign producers dominated the fresh fillet market (Costa Rica and Columbia) and the frozen fillet market (Taiwan and Indonesia). The ATA (1995) reported that consumer trends were clearly towards frozen fillets - that market increased 400% from 1993 to 1994 - while fresh fillets increased just over 50% and whole frozen fish just 10% over the same period. Tilapia imports now contribute a measurable share of the large US trade deficit in seafood products. In 1995, tilapia were the third largest imported aquaculture product to the US after shrimp and salmon (ATA 1996). While the growth in domestic tilapia consumption is breathtaking, percentage growth in tilapia production in the USA is slower. The main reason for the loss of domestic market share to foreign competition is the high costs of tilapia production in the USA.

Tilapia are being produced in nearly every state using thermal structures, greenhouses, waste heat, and geothermal waters. Domestic tilapia production increased over 300% from 1991 to 1995 (from 5 to 15 million pounds live weight), with production expected to reach 19 million pounds by end 1996 (ATA 1996). Most US production (11.9 of 15.0 million pounds in 1995) was from ponds and tanks in western (California, Arizona, Idaho) and southern (Texas, Mississippi, Alabama, Florida) states (ATA 1996). California is the largest domestic tilapia producer (ATA 1996).

For the domestic producer, niche markets offer the higher prices needed to profit because of the high costs of production. Penetration of mass markets in the USA is small, with tilapia remaining primarily a specialty item in niche markets (Engle, this volume). The largest niche markets are live markets in Asian ethnic areas of California, New York, and Toronto. Projections are that the largest growth in tilapia markets will be where global population is concentrated - on the Pacific Rim - and that significant expansion of the market could occur in California if persons of Hispanic heritage increase their growing consumption of tilapia. In the USA, projections are that tilapia will exceed catfish consumption early in the next century when the demand could exceed 200,000 tons (M. Ednoff, Florida Department of Agriculture, personal communication).

Systematics of the Tilapias

In the 1970's there was general confusion and debate among fish biologists over the systematics of the tilapias. Confusion was due to publication of a proposed new classification system which split the tilapias into mouthbrooders and substrate spawners, and created *Sarotherodon* as a distinct, mouthbrooding genus (Trewavas 1973). In 1982, Trewavas again revised the tilapias, splitting the mouthbrooding tilapias into two genera, *Sarotherodon* and *Oreochromis* (Trewavas 1982). This revision was not accepted by some fish biologists (the "lumpers")

Taxonomic confusion was especially apparent among applied workers, especially aquaculture scientists and farmers in the Americas. They had witnessed over a short time a relatively new farmed species with a difficult and largely unrecognized name, which, until the 1970's, was a single genus, *Tilapia*, change, in a short period of time, from *Tilapia* to *Sarotherodon*, then to *Oreochromis*. Fortunately, there have been no major taxonomic revisions of the tilapias since 1982. Presently tilapias are classified scientifically as:

{1} *Oreochromis* (the maternal mouthbrooders);
{2} *Sarotherodon* (the paternal or biparental mouthbrooders);
{3} *Tilapia* (the substrate spawners).

These taxonomic standards are now recognized widely in the international scientific literature since the publication of a large, comprehensive monograph on the tilapias (Trewavas 1983). The scientific tilapia classification scheme has been shown to be inclusive but yet distinctive (Pullin 1988). For their common name, however, all tilapias may be referred to colloquially as "tilapias", with a small 't' and no italics (Trewavas 1982).

Unfortunately, due to the confusion in the 1970's, plus the classic scientific arguments made by taxonomic "lumpers" versus "splitters", the taxonomy of Trewavas (1982) has not accepted by the American Fisheries Society (AFS). AFS scientific publications still refer to all of the tilapias as belonging to a single genus of *Tilapia* (Robins et al. 1991). In this chapter (and this volume) all authors follow the international standards of Trewavas (1982, 1983).

The most important tilapias in aquaculture are the maternal mouthbrooders (Schoenen 1982; Pullin 1985): the Nile tilapia (*O. niloticus*), Mozambique tilapia (*O. mossambicus*), and blue tilapia (*O. aureus*). Of secondary (localized) importance are *Sarotherodon galilaeus* and *S. melanotheron* (principally in West African lagoons), and red tilapia hybrids (especially *O. mossambicus* hybrids with *O. aureus, O. niloticus* and *O. urolepis hornorum*).

Background Review of Tilapias in Southern California

Studies of both southern California and Arizona tilapias have been conducted using systematic (taxonomic) nomenclature based upon external color and appearance, distinctive spawning behaviors, and morphometric and meristic measurements (Minckley 1973; Barrett 1983; Lopez and Ulmer 1983). Barrett (1983) completed an extensive morphometric and meristic study of tilapias in the lower Colorado River basin. He reported that *Oreochromis mossambicus* and *Oreochromis urolepis hornorum* have hybridized to the extent that they are essentially one reproducing population. He also reported that *Oreochromis aureus* (the blue tilapia) was the "dominant cichlid in Arizona" and that "recent evidence indicated that *Oreochromis niloticus* (the Nile tilapia) genes were also present". However, taxonomic studies of introduced tilapias using external characteristics cannot resolve tilapia species differences because of the "supraspecies" nature of the tilapias.

The concept of the "supraspecies" is where a widely distributed species is "falling apart" (Pullin 1988). In the wild in Africa, tilapias have a wide distribution considered ancient. Due to the "recent" drying of the continent, tilapia populations with wide distributions have become isolated. These developed as distinct populations, evolving unique genetic markers. These newly isolated populations do not freely interbreed in the wild unless brought together by some major environmental events (such as floods, earthquakes, etc.). Recognition of the level of differentiation necessary to assign a given population to a new species is a matter of expert opinion - the classic "lumpers" versus "splitters" debate (Pullin 1988).

In aquaculture settings, or anywhere in the tropical aquatic environment where large scale introductions of exotic tilapias occur, there is a risk of interbreeding and hybridization among populations that may be genetically distinct but reproductively compatible due to their relatively recent divergence. Where a mixture of tilapia species have been stocked, reproductively viable hybrids have resulted, and the use of external morphometric characterizations of these hybrids to determine species mixes is fruitless (Wohlfarth and Hulata 1983; Pullin 1988).

Tilapia Transmission into Southern California

California state regulations permit aquaculture of only three tilapia species (*Oreochromis mossambicus, Oreochromis urolepis hornorum, Tilapia zillii*), and restrict tilapia aquaculture to the southern part of the State below the Tehacapi Mountains in the counties of San Bernardino, Los Angeles, Orange, Riverside, San Diego and Imperial. Other tilapia species used widely for aquaculture worldwide, especially the Nile and blue tilapias (*Oreochromis niloticus* and *Oreochromis aureus*) are illegal. However, the California Department of Fish and Game (CDGF) has issued a permit to Solar Aquafarms, Inc. (Sun City, CA) to grow Nile tilapia in an indoor recirculating system where all fish are marketed as processed products (e.g. no live fish sales are permitted).

Zill's Tilapia (*Tilapia zillii*) in Southern California

Tilapia zillii are native to a large swath of north central sub-Saharan Africa from Senegal in West Africa through northern Zaire and the Sudan, and north into the Nile River basin and Asia Minor (Figure 1). The distribution extends south to the central African rainforest and around Kisangani, Zaire where it meets the distribution of another closely related substrate spawner, *Tilapia rendalli*. It is believed that *T. zillii* and *T. rendalli* are a supraspecies complex that until recent time were the same species. When the 'drying' of Africa occurred and they became separated into a northern form (*T. zillii*) and a southern form (*T. rendalli*) (Trewavas 1983) (Figure 1).

T. zillii are noted for their hardiness, having wide temperature (7-42°C) and salinity tolerances (upwards of 45 ppt) (Chervinski 1971). During a sampling in the southern Salton Sea (near Obsidian Butte on March 6, 1995), one of us (BCP) sampled *T. zillii* that had already spawned; the water temperature that day was 15°C and salinity 43 ppt.

T. zillii was imported to southern California due to its ability to feed on nuisance aquatic weeds and other macrophytes which were clogging irrigation canals. It was hoped *T. zillii* could be a biological control agent to offset the high costs of mechanical and chemical controls (Hauser 1975a,b). *T. zillii* was first imported to California (University of California Davis and University of California Riverside [UCR]) in the 1960's from the Arizona Cooperative Fishery Unit (ACFU), University of Arizona, but these fish died out (Legner and Pelsue 1977). Another permit was issued on October 15, 1971 to import 150 *T. zillii* from the ACFU to the Division of Biological Control, UCR.

Legner and Fisher (1980) reported the *T. zillii* in Arizona originated from just 3 male/female pairs imported from Israel in 1965. Hauser (1977) found that at water temperatures below 16°C *T. zillii* became lethargic and lost equilibrium and were extremely vulnerable to predators and disease. In Imperial County irrigation canals, mortalities were observed in December-January and the fish were frequently stressed by low temperature from mid-December to March (Hauser 1974, 1977). Survival of the fish in southern California occurred only in areas where warm seepage water or geothermal waters created thermal refugia for overwintering.

For aquaculture purposes *T. zillii* is a poor candidate because of its high fecundity and high spawning periodicity; its slow overall growth rate to a small maximum size; and its narrow temperature optimum for good growth. However, Hauser (1975c) reported an unusually fast growth rate of *T. zillii* from a California irrigation ditch (the highest ever recorded; see Pauly et al. 1988). Two year old fish reached 380-709 g. This report is suspect, however, since all other reports of growth are much slower (Pullin 1986). Fecundity in *T. zillii* is 10-20 times higher than mouthbrooding tilapias. Lowe-McConnell (1955) reported that 10 mixed sex *T. zillii* of just 2.6-4.8 cm total length produced 9,000 progeny in 9 months. Platt and Hauser (1978) found that at water temperatures less than 20°C, feeding rates on macrophytes and growth rates of *T. zillii* approached zero. Hauser (1977) concluded that due to its thermophilic nature, *T. zillii* would not be self-sustaining in southern California irrigation ditches and canals without thermal refugia, and that any effective biological control of aquatic weeds in irrigation canals would require expensive annual restocking.

T. zillii have been implicated in the decline of the desert pupfish in the Salton Sea area (Schoenherr 1988). In addition, *T. zillii* have been collected from southern California coastal waters near a power plant off Huntington Beach, and in Upper Newport Bay in Orange County (Knaggs 1976). No known recent marine collections have been made. Cardone (in press) states that *T. zillii* has "not established breeding populations in coastal marine and estuarine waters".

In conclusion, *Tilapia zillii* populations in southern California have a restricted genetic basis (the progeny of just 3 reproductive pairs were imported to Arizona and distributed to California), presenting severe genetic bottlenecks and restricting the adaptability of the species. The route of the fish to southern California is summarized in Figure 2. The species has poor environmental compatibility with California: it is of no use as a cost-effective biological control agent, and it has no economic value to aquaculture, capture or recreational fisheries. *Tilapia zillii* can adapt to natural and man-made thermal refugia; it impacts aquatic vegetation during warm months of the year; and its populations reportedly conflict with restoration programs for threatened, native, desert fishes. It is predicted that introduced populations of *Tilapia zillii* will decline in California and will eventually disappear altogether. It is recommended that the decline of *Tilapia zillii* be studied for the insights it may give to the eradication of other nuisance exotic aquatic species; and that studies be made on cost-effective, ecological approaches to hasten its demise and rapid eradication from California waters.

The Mozambique Tilapia (*Oreochromis mossambicus*) in Southern California

The Mozambique tilapia is native to the eastward-flowing rivers of East Central Africa. In the northern part of its range it is present below Kapachera Falls in the lower Shire River in Southern Malawi, the lower Zambezi, and in Mozambique in all coastal rivers down the southeastern African coast to Algoa Bay, South Africa (Figure 3).

The Mozambique tilapia and the common carp are the two most widely spread exotic aquatic animals in the World. From the 1930's to the mid 1970's the Mozambique tilapia was spread throughout the world by fisheries biologists and managers for the control of nuisance aquatic weeds and insect pests, and for aquaculture and fisheries development. As stated earlier, from the early 1980's to the present, however, a wholescale conversion of species occurred in the major tilapia farming nations worldwide, with the Mozambique tilapia being replaced by the Nile tilapia (*Oreochromis niloticus*) (Moreau et al. 1986; Pauly et al. 1988; Pullin 1988). A similar trend has occurred among tilapia producers in the USA. California remains one of the only major tilapia farming areas of the world to produce significant quantities of Mozambique tilapia (Indonesia and Sri Lanka are others).

The Mozambique tilapia is more widely farmed in Asia and elsewhere than in its genetic home of Africa. The route *O. mossambicus* first took out of Africa to Asia is unknown. Tilapia were "discovered" in Asia - first in Java, Indonesia in 1938 - and were hailed as a miracle. For decades, their genetic heritage in Africa was forgotten and they were called the "Java tilapia". During WW II the tilapia served as invaluable protein food for the poorest of poor ravaged by the Japanese occupation of Indonesia. Dr. Schuster, a Dutch fisheries biologist working in Dutch occupied Indonesia, reported a fish farmer in Java found 5 tilapia (2 females and 3 males) in a coastal brackishwater pond (Schuster 1952). After WW II, Schuster returned to Indonesia (he was interned as a prisoner of war by the Japanese) and found that *O. mossambicus* had spread throughout Java.

Hawaii was the first state to receive tilapia from Asia. In 1951, 60 "small" Mozambique tilapia were sent to Honolulu from Singapore (Brock 1960). The Singapore tilapia is of unknown origin but likely resulted from wartime traffic back and forth across the Straits of Malacca between Singapore and Indonesia. Dr. Roger S.V. Pullin believes the Indonesian tilapia came from an aquarist in Singapore who imported the fish from Africa and released them to Indonesia in 1938 (R. Pullin, ICLARM, Philippines, personal communication). Whatever their origin in Singapore, only 14 of the Mozambique tilapia survived transit to Hawaii from Singapore (unknown numbers of males and females) (Brock 1960). These fish were bred and "the resultant offspring were used for stocking purposes" in Hawaii and elsewhere in the Pacific (Brock 1960; Lobel 1980).

Because of the unknown origin and numbers of the founder stocks sent from Africa to Asia, Pullin (1988) has raised the specter that *all* Asian *O. mossambicus* populations could be derived from the 5 fish found by Schuster in Java. And Asia, not Africa, is the origin of all *Oreochromis mossambicus* in the USA.

After establishment in Hawaii, tilapia were sent from Hawaii first to the Steinhart Aquarium in San Francisco, then to the New York Aquarium in multiple shipments in 1953-55. Fish were on public display from July 1954-June 1955 at Steinhart. The Steinhart Aquarium also shipped *O. mossambicus* to Auburn University in 1953 (Keely 1957). Mr. C. Coates at the New York Aquarium produced *O. mossambicus* during this time, and shipped fish back to Hawaii to Dr. Vernon Brock via the Steinhart Aquarium. It appears that in the early establishment of the tilapias in the USA, that the public aquariums were as active as government fisheries biologists in seeding the tilapias around the USA (Tom Tucker, Steinhart Aquarium, California Academy of Sciences, personal communications).

Arizona imported its *Oreochromis mossambicus* stock from Hawaii early 1961. By October 1961, more than 27,000 progeny of the original Hawaii fish were produced. These were stocked into the Gila Bend Canal. In January 1963, an unknown number of this stock was also released into the Yuma Canal (Barrett 1983). In 1962 in Arizona, McConnell (1966) began experiments with the "Malacca hybrids" importing a second stock of Mozambique tilapia from the Tishomingo, Oklahoma National Fish Hatchery, and also bringing an unknown number of *Oreochromis urolepis hornorum* from Malacca, Malaysia (Hickling 1960). Reproductively viable hybrids of these two species were released widely to canals, dams, ponds and reservoirs in Arizona from 1965-1981 (Barrett 1983).

Interestingly, the "Tishomingo strain" of *O. mossambicus* (that eventually ended up in California via Arizona) appears to have originated in California as its first port of entry into the continental USA! The fish were imported to Tishomingo from Auburn University. Auburn originally obtained its Mozambique tilapia from California (the Steinhart Aquarium). The early route of penetration of *O. mossambicus* into USA and California is summarized in Figure 4.

Hickling (1960) pioneered the "all male hybrid progeny" cross of a male *Oreochromis urolepis hornorum* (then called the Zanzibar tilapia) and a female *O. mossambicus* ("Java tilapia") which gained worldwide attention. In December 1963, the California Department of Fish and Game (CDFG) obtained "8 males of the Zanzibar strain, plus 50 male and female fingerlings, and 6 adult males of the Java strain" from the Arizona Cooperative Fishery Unit (ACFU) for experimental use at the CDFG station in Chino, CA (Outdoor California 1964). The fish were authorized for use as a biological control of aquatic weeds and insects in irrigation systems of Southern California on November 5, 1971, and the Mozambique tilapia and hybrids were officially stocked in southern California thereafter.

By 1968, tilapias had been found in some 15 miles of irrigation canals (the Araz Drain and Reservation Main Drain) near Bard in Imperial County, CA (Hoover and St. Amant 1970). Since tilapia were not officially stocked into southern California waters until 1971 (Pelzman 1973), these tilapias likely represented movements into California from 1961-63 stockings of Arizona tilapias in irrigation canals connected to California.

In a separate development, in January 1964, Mozambique tilapia were discovered illegally in 0.25 acre pond at a private tropical fish farm near the Hot Mineral Spa (Niland, CA) (St. Amant 1965). The pond was poisoned with rotenone and over 5,000 tilapia removed by the CDFG. However, in June 1965, CDFG workers observed a breeding population had reestablished in a drainage ditch choked with emergent macrophytes that was connected to the Salton Sea.

The Mozambique tilapia has penetrated California's coastal marine waters but does not appear to have formed large populations. Horn (1988) reported that they are "now part of the Upper Newport Bay fauna"; however, Horn collected no tilapia in a year-long (1978-79) study. Tilapia were, however, observed to build nests at the mouth of San Gabriel River in other years. During this study, one of us (BCP) interviewed fishers at Warmwater Beach off the Encino Power Plant in Carlsbad, CA, who reported catching tilapia. Apparently, where thermal refugia exist along the southern California coast, the Mozambique tilapia have been able to penetrate seawater. The biology and environmental impacts of these marine tilapia populations are unknown: if these populations are fully saline; if spawning occurs in freshwater or if fish are simply flushed to the coastal areas during the rainy season and then adapt to marine conditions.

In conclusion, the taxonomic status of Mozambique tilapia (*Oreochromis mossambicus*) in California was established through at least three routes (Figure 4): (1) an illegal "aquarium route" and establishment in the Salton Sea; (2) an invasion from Arizona through transit in irrigation canals, ditches and contiguous water bodies; and (3) deliberate stocking of both the Mozambique tilapia and its hybrids with the Wami River tilapia by CDFG. The most bizarre aspect of one of the Mozambique tilapia founder stocks was that one population of *Oreochromis mossambicus* actually traveled from Singapore to Hawaii to California to Alabama to Oklahoma to Arizona and back to California, and then was used for large scale breeding/stocking purposes!

Small founder stocks, resulting genetic bottlenecks and introgression with *Oreochromis urolepis hornorum* put into question the nature of *Oreochromis mossambicus* as a distinct species in southern California in doubt. As well, there are questions if it currently exists there at all in an unmixed state. Our proposal to better define a California Mozambique regional strain using DNA markers would seem to reflect the history and observed genetic affinities of the *mossambicus*-like Californian tilapia populations reasonably well.

Like most tilapias, the domestication of a pure line Mozambique tilapia from Africa has not been accomplished. Lombard (1960) examined the culture performance of some *Oreochromis mossambicus* strains in Africa and found a wide diversity of growth to larger maximum sizes than reported in the modern tilapia aquaculture literature. Caulton (in Pullin and Lowe-McConnell 1982, p. 333-334) stated that *Oreochromis mossambicus* from the lower/middle Zambezi had a much different appearance than those found elsewhere in the world, and that fish from South Africa performed well in culture. One of us (BCP) has also made similar observations of wild *O. mossambicus* populations in the lower Shire and Zambezi Rivers in east Africa.

The Wami River Tilapia (*Oreochromis urolepis hornorum*)

The Wami River tilapia originates from the Wami River, in eastern Tanzania. *Oreochromis urolepis* has been found in four coastal locations in Tanzania, but the Wami River subspecies, *Oreochromis urolepis hornorum*, is known only from the Wami River and Zanzibar Island (Trewavas 1983). The Wami River tilapia, however, is not native to Zanzibar. An unknown number of fish were imported from the Tanzanian mainland in 1918 by Dr. W.M. Aders for experimental fish culture (Talbot and Newell 1957). It is unknown if the Wami River subspecies were mixed with a native Zanzibar tilapia or with other tilapias imported from the African mainland (such as *Oreochromis mossambicus*!) before its worldwide distribution.

The Wami River tilapia is famous in aquaculture not for its role as a distinct species grown for its own merits, but as the male parental stock mated with female *Oreochromis mossambicus* to produce "all male" hybrid progeny (Hickling 1960). Because of the precocious breeding behavior of the Mozambique tilapia leading to poor growth and stunting in ponds, this development excited worldwide interest. As a result, the Wami River tilapia was exported worldwide for hybridization with the Mozambique tilapia.

Hickling (1960) reported receiving only 36 fingerling Wami River tilapia from Zanzibar at the Fish Culture Research Station, Malacca, Malaysia. Therefore, Hickling's, and the experiments of all other workers worldwide, may have been affected by the small size of the original founder stocks and the fact that the founder stock of the Wami River tilapia came not from its place of origin but from a fish farm in an exotic location that may have already been hybridized and introgressed!

McConnell (1965, 1966) began experimentation with the "Malacca hybrid" at the ACFU by importing in 1962 an unknown number of Wami River tilapia (*O. u. hornorum*) from Malaysia. In Arizona, there were few controls taken to prevent mixing of parental stocks with the reproductively-viable hybrid progeny. As a result, uncontrolled hybridization and backcrosses occurred (Barrett 1983). Stated Barrett (1983, p. 5), "Both parental species and reciprocal hybrids were introduced into ponds to assess their survivorship and angler susceptibility". The route of transfers of O. u. hornorum into California is summarized in Figure 5.

There is also confusion about the status of the Wami River tilapia in brackish and marine waters in southern California. Legner and Pelsue (1977) refer to large reproducing populations of "*Tilapia hornorum*" in Coyote Creek, Los Angeles due to continuous power plant waste heat discharge into the creek at 85°F year round. He states, "Daily tidal washes of the site favor the persistence of this fish over the other two species (e.g. *O. mossambicus* and *T. zillii*) which are less interhaline".

Talbot and Newell (1957) reported *O. u. hornorum* grew and reproduced in 33-39 ppt water. One aquaculture company in southern California has selected tilapias from the Salton Sea for broodstock and reports persistence of a grayish-brownish tilapia fitting the external characterization of *O. u. hornorum* according to Trewavas (1983). However, much more biochemical DNA work on these fish needs to be done before confirmation that any *O. u. hornorum* persists in southern California. Strangely, the Wami River tilapia itself still has unknown aquaculture potential since nowhere in the world is it grown as a pure species for its own merits.

DNA Marker Technology to Identify Tilapias in Southern California

Genetic investigation of tilapias was based upon use of "microsatellite" loci. These loci consist of variable numbers of tandemly-arranged repeats of simple base sequences (VNTRs). There are a number of reasons why microsatellite polymorphisms have become the markers of choice for fisheries genetic studies (Wright and Bentzen 1994):

(1) the level of polymorphisms at microsatellite loci is usually very high - heterozygosities approach 100% at some loci - allowing for identification of families in hatcheries and high resolution population and species identifications;

(2) the alleles at each locus are inherited in strictly co-dominant Mendelian fashion (except in cases of high mutation rates) which facilitates analyses of hybrid populations at the second and subsequent generations;

(3) the scoring procedure is numerical and gene frequency information can be stored, compared, and exchanged among researchers working on different populations and species in different laboratories without ambiguity;

(4) numerical scoring permits semi-automated data collection on automated sequencing equipment, giving rise to attractive economies of scale;

(5) microsatellite systems developed for one species frequently work for other closely related species, greatly reducing effort required to adapt procedures to new problems and resolving the composition of hybrid species;

(6) microsatellite polymorphism has been recommended by an FAO Working Group as the preferred genetic identification system for a global program on conservation and identification of breeds of domestic livestock (Barker 1994). If adopted technologies developed (including statistical and data processing analyses) for terrestrial and aquatic species will be compatible and will develop rapidly.

Other DNA-level polymorphic systems in use in fisheries and aquaculture research are restriction fragment length polymorphisms (RFLP) in nonrepetitive (e.g. single copy) nuclear DNA, multi-locus minisatellite DNA fingerprinting, randomly amplified polymorphic DNA (RAPD), and mitochondrial DNA RFLP and sequence polymorphisms.

None of these systems combines all of the favorable features of microsatellites (Wright and Bentzen 1994). Protein polymorphisms (mainly isozymes or allozymes) have also been used to mark or identify genotypes in aquaculture and for breed comparison and genetic stock identification (GSI). GSI is the analysis of the composition of mixed stocks; that is, stocks composed of mixtures of fish of different origins (Millar 1987; Brodziak et al. 1992).

The laboratory practice and underlying molecular biology of DNA microsatellite polymorphism in fish has been comprehensively reviewed (Franck et al. 1991; Wright and Bentzen 1994). The basis of the polymorphism is variation in the number of copies of tandemly-repeated sequences of 2 to 6 bases (GCGCGC..., ATAATAATA..., ATAGATAGATAG..., etc.). Changes in the copy number are thought to occur during meiosis through slipped-strand mispairing or slippage of DNA during replication (Schlotter and Tautz 1992). VNTRs with 2 base-pair repeats, called dinucleotide microsatellites, have been used in this study.

Sample Collection, Preservation and Processing

Twelve different tilapia samples existing either in the wild in Africa, feral in California, or grown commercially in California were obtained (Table 1 and Figure 6). Two hundred and twenty-four fish were analyzed at the Marine Gene Probe Laboratory, Dalhousie University, Halifax, Nova Scotia, Canada.

The California fish samples were obtained either by use of a gill net, beach seines or hand nets from lakes, creeks, ditches, tanks and ponds. Fresh fish were wrapped individually in foil and put in ziplock bags on ice in the field, then frozen at -20°C upon return to the laboratory. Fish were defrosted and 0.5-1.0 g pieces of muscle tissue without skin from the mid-dorsal area above the lateral line were taken and put into 1 ml of 95% ethanol (completely denatured) in 2.0 ml screwcap Eppendof microcentrifuge tubes. DNA was extracted from muscle tissue according to the procedures of Ruzzante et al. (1996). Development of the *Oreochromis* probes and the protocols for laboratory analysis of the microsatellites are described by Brooker (Brooker et al. 1994) and modified at Dalhousie University (Ambali 1996; Ambali et al. 1996).

Genetic Characterization Using Microsatellite Alleles

Average heterozygosities in many of the samples (Table 2) were as high as found at microsatellite loci in natural populations of other fish (Wright and Bentzen 1994; Ambali et al. 1996; Herbinger et al. 1996). However, the low heterozygosity of one Solar Aquafarms, Inc. sample (reported to be *O. mossambicus* when collected) indicates a strong probability that a serious "bottleneck" or inbreeding has occurred there (0.21 versus a range of 0.34-0.72 for other samples).

Overall genetic similarities among samples and relationships to reference species are illustrated by a UPGMA dendrogram (Saitu and Nei 1987) (Figure 7). Relationships among populations shown in Figure 7 are as likely to be affected by mixing and hybridizations as by evolutionary processes leading to divergence. This dendrogram implies no evolutionary relationships but gives a visual impression of the way populations are grouped (Nei's distance adjusted for small sample size; Nei et al. 1983).

The hypothesis of the likelihood the pattern shown in Figure 7 arose by chance was tested by bootstrap resampling of the 4 microsatellite loci (Felsenstein 1985). If a given element of the pattern arises in 90% or more of the random bootstrap samples it can be considered to be a real property of the populations and highly significant. It also indicates that the relationships are not highly dependent on only one of the 4 loci.

Proposed "California Nile" Regional Strain

Ninety-nine percent of bootstrap re-samplings of the 4 loci separated the Solar Aquafarms "*niloticus*" and the Colorado River tilapia populations from the remainder of the data. An equally large (97%) of re-samplings grouped these two populations together with the GIFT reference population of *Oreochromis niloticus.* The existence of this clear-cut *niloticus* grouping shows that *O. niloticus* is present in both cultured and feral populations in California.

Proposed "California Mozambique" Regional Strain

This group of samples includes the Solar Aquafarms *mossambicus* and all other California samples except those assigned to the *niloticus* regional strain. These California samples are separated from the other data in 97% of the bootstrap samples with no statistically significant internal structure. In this regional strain the Salton Sea and Niland ditch samples do however consistently group together during bootstrap re-sampling (79%).

The UPGMA dendrogram (Figure 7) summarizes the relationships among Southern California tilapias for a single highly derived variable, genetic distance. This approach, which is appropriate for the study of evolutionary processes where gene pools (e.g. species) are assumed to diverge independently after speciation, is obviously inconsistent with the known history of the Californian tilapias as described above. A more interesting question (for the possible future exploitation of aquaculture genetic resources) is whether California has experienced introductions and hybridizations of tilapia strains that separated from each other so long ago that their African evolutionary phylogenies at the species level are no longer relevant.

Principal coordinate analysis, or multi-dimensional scaling, has been successfully employed in analogous situations to trace the evolution, movement and mixing of human populations (Cavalli-Sforza et al. 1994). Principal coordinate analysis is especially useful in the resolution of mixtures of populations with differing characteristics. Figure 8 represents a principal coordinate analysis of an Euclidean distance matrix derived from the allele frequencies. The total number of alleles at the 4 loci was 113, representing 109 degrees of freedom, yielding large discriminating power. The three coordinate axes in Figure 8 represent 69% of the total genetic variation among populations, calculated as the cumulative sum of positive eigenvalues of the distance matrix (Podani 1993). The first, second and third principal coordinate comprise 43% and 14% and 12%, respectively, of the total variance among groups.

The first two axes clearly separate the Californian samples into *O. mossambicus* and *O. niloticus* groups (i.e. Californian regional strains) (Figure 8). The third coordinate appears to separate all the Californian samples from all the recent African samples of both species), emphasizing the uniqueness of the North American tilapia.

The complicated history of *Oreochromis mossambicus* (Figure 4) goes a long way towards explaining why the proposed "California Mozambique" regional strain has close genetic affinities with *O. mossambicus* samples recently obtained from Africa, but is nevertheless easily distinguished from them (Figure 8). The Salton Sea tilapia group has a close affinity to other *O. mossambicus*-like California samples and show no special affinity to the *O. u. hornorum* reference sample (Figures 7 and 8).

Conclusions

As a result of these microsatellite DNA frequency analyses we conclude that *Oreochromis niloticus* is already present in Southern California in culture at Solar Aquafarms, Inc.(by legal permit from California DFG) and in the wild (collected from the Colorado River near Blythe, CA). We are proposing that for aquaculture and conservation purposes they be considered together as a "California Nile" regional strain. The wild fish in this regional strain are likely escapees from tilapia aquaculture farms in Arizona since *O. niloticus* is a legal species there. Genetic confirmation that *Oreochromis niloticus* is present near Blythe in the California region of the Colorado River suggests that the species and its hybrids may be distributed in irrigation and drainage canals elsewhere in the eastern part of the State.

Presumed hybrids of *Oreochromis mossambicus* x *Oreochromis urolepis hornorum* were stocked extensively in California to the point that it is *a priori* unlikely that pure lines of *Oreochromis urolepis hornorum* (the Wami River tilapia) or *O. mossambicus* exist as distinct species. Nevertheless, no strong indications of *O. u. hornorum* were found in the samples we examined, but the lack of historical knowledge of global movements and research in Africa on the *O. urolepis* stocks make interpretations of this data difficult. It is possible that hybridization or introgression between *O. mossambicus* and *O. u. hornorum* does not persist in natural populations after release into natural water bodies. It is also possible that chromosome segments carrying *hornorum* microsatellite markers are relatively quickly eliminated by selection. Further research is needed to decide whether some such biological barriers to gene flow exist or whether the apparent absence of *hornorum* markers in California is merely a population or genetic sampling phenomenon peculiar to the present study.

Recommendations

There is alarm that the poorly regulated movements of tilapias in the USA are leading to the spread of new and dangerous aquatic diseases, especially the rapid and sudden spread of *Streptococcus* disease in tilapia farms. *Streptococcus* has been reported in Texas, North Dakota, Costa Rica, and Indonesia due to uncontrolled importations, over-intensification, and poor handling. It is significant that this disease has not reported to be a major problem in tilapia aquaculture in southern California to date.

There is justified concern that a relaxation of controls on tilapia species regulations in California will allow importations of new diseases across California's state and international borders threatening its growing tilapia aquaculture industry. It is recommended that a cautious approach be taken to allowing any new importations of *Oreochromis niloticus* to California due to:

(1) the significant risks to importing new tilapia diseases and parasites, especially *Streptococcus* (California's ban on live catfish imports is a significant successful example in this regard);

(2) the concerns about importations of new, exotic species and their potential negative impacts on the State's native aquatic biodiversity and its public investments in aquatic species recovery programs;

(3) the fact that the *niloticus* genes already in the State may be adequate to sustain and further expand the State's tilapia aquaculture industry into the future if planned, scientific, genetic improvement programs and investments were made;

(4) the fact that other, significant, undocumented tilapia genetic resources may already be present in the State and that further "bioprospecting" and DNA marker studies may document promising populations that already exist.

While divergence from parental material has certainly occurred in California, DNA marker data in this study indicates that a highly heterozygous population with strong affinities to *Oreochromis mossambicus* exists in the Salton Sea. We propose that this lacustrine population be grouped with certain other cultured and feral populations and named as a "California Mozambique" regional strain. There is a second regional strain with close affinities to *O. niloticus*. These findings are promising since other, distinguishable tilapia populations or population groups may occur elsewhere in southern California that could be of use to aquaculture breeding programs.

It is recommended, however, that the CDFG continue to allow importations of new founder stocks of *Oreochromis mossambicus* for tilapia genetic improvement programs, and that these stocks be imported directly from Africa to the southern

California aquaculture industry under strict quarantine. It is also recommended that "biological prospecting" be done in selected, isolated locations in southern California to determine if other distinct regional strains of *Oreochromis* (possibly of hybrid origin, possibly containing *O. urolepis hornorum*) exist as a potential resource for aquaculture.

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Figure Legends

Figure 1. Map of the distribution of *Tilapia zillii* and *T. rendalli* in the wild in Africa (from Pullin 1988).

Figure 2. Map of the distribution of *Oreochromis mossambicus* in the wild in Africa (From Pullin 1988).

Figure 3. Trace of the route of *Tilapia zillii* from its unknown origins in sub-Saharan Africa to California. Dates on top of the arrows are the year of transfer and numbers below are the number of fish shipped.

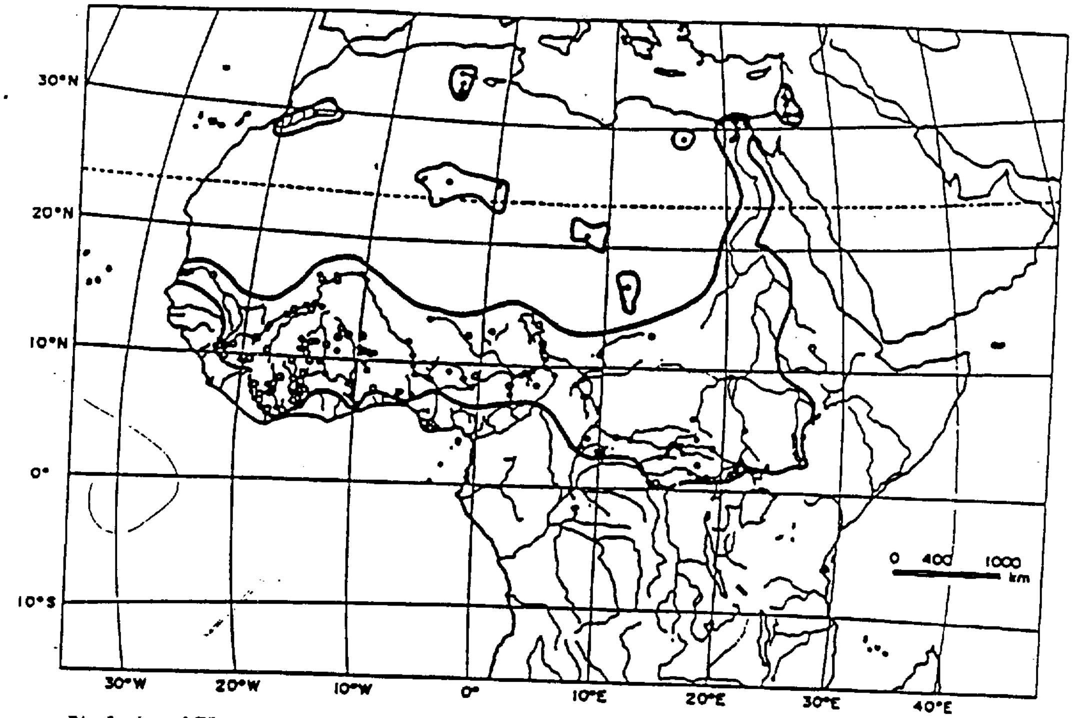
Figure 4. Trace of the route of *Oreochromis mossambicus* from unknown origins in sub-Saharan Africa to Asia then to Hawaii. One route from Hawaii before 1953 was promulgated by the aquariums (Steinhart Aquarium), and a second (in 1961) was a direct transfer from Hawaii to Arizona. Dates of transfers are on top or on the left of the arrows and numbers of fish transferred are below or to the right as taken from literature sources and/or from interviews conducted duuring these studies.

Figure 5. Trace of the route of *Oreochromis urolepis hornorum* from Tanzania to California from literature sources and interviews conducted during these studies. Labels are as in Figures 3 and 4.

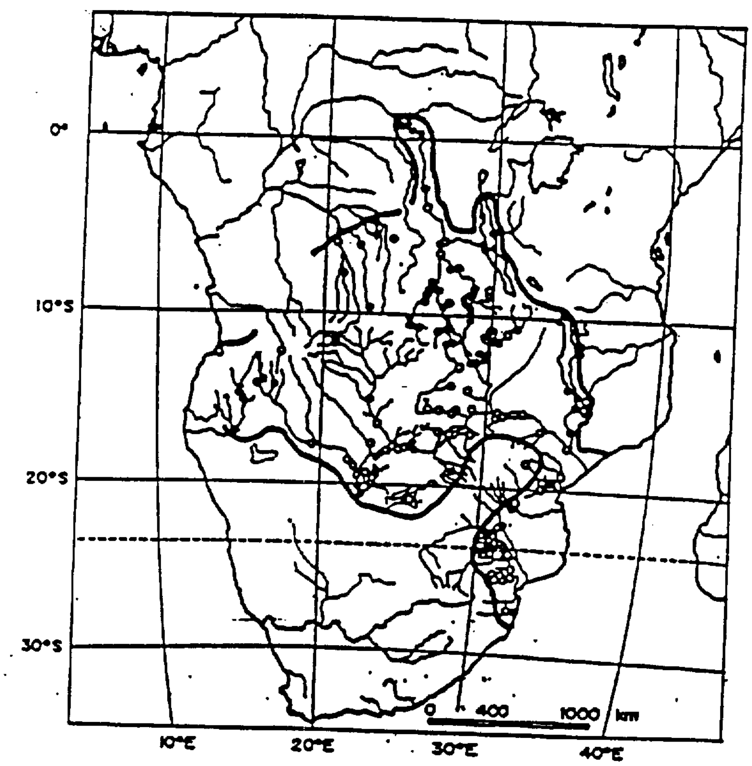
Figure 6. Map of California showing locations of sampling of southern California tilapias.

Figure 7. UPGMA (unweighted pair-group method arthimetic) dendrogram (Saitu and Nei 1987). Sample designations correspond to those in Table 1. Bootstrap frequencies are shown as numbers associated with the branches. The proposed "California Mozambique" regional strain comprises samples 1, 2, 4, 5, 6 and 8, a grouping that, together with the recent African *O. mossambicus*, is separated from the other samples by 97% of the bootstrap re-samplings. The proposed "California Nile" regional strain comprises samples 3 and 7, which grouped with the ICLARM GIFT *O. niloticus* reference sample (Macaranas et al. 1995), 97% of the time.

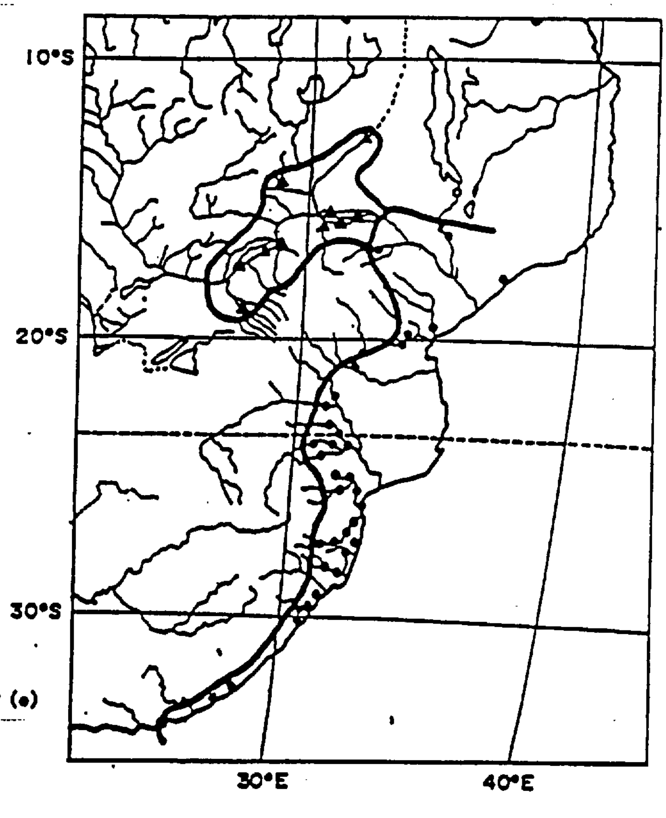
Figure 8. Principal coordinate plot (Cavalli-Sforza et al. 1994) derived from 113 alleles at 4 loci. The three principal coordinates account for 69% of the total variance among groups. The members of each regional strain are linked by minimum spanning trees for ease of visual identification. In this figure the third coordinate separates the recent African samples (9, 10, 11, 12) from all of the California samples.



Distribution of *Tilapia zillii*

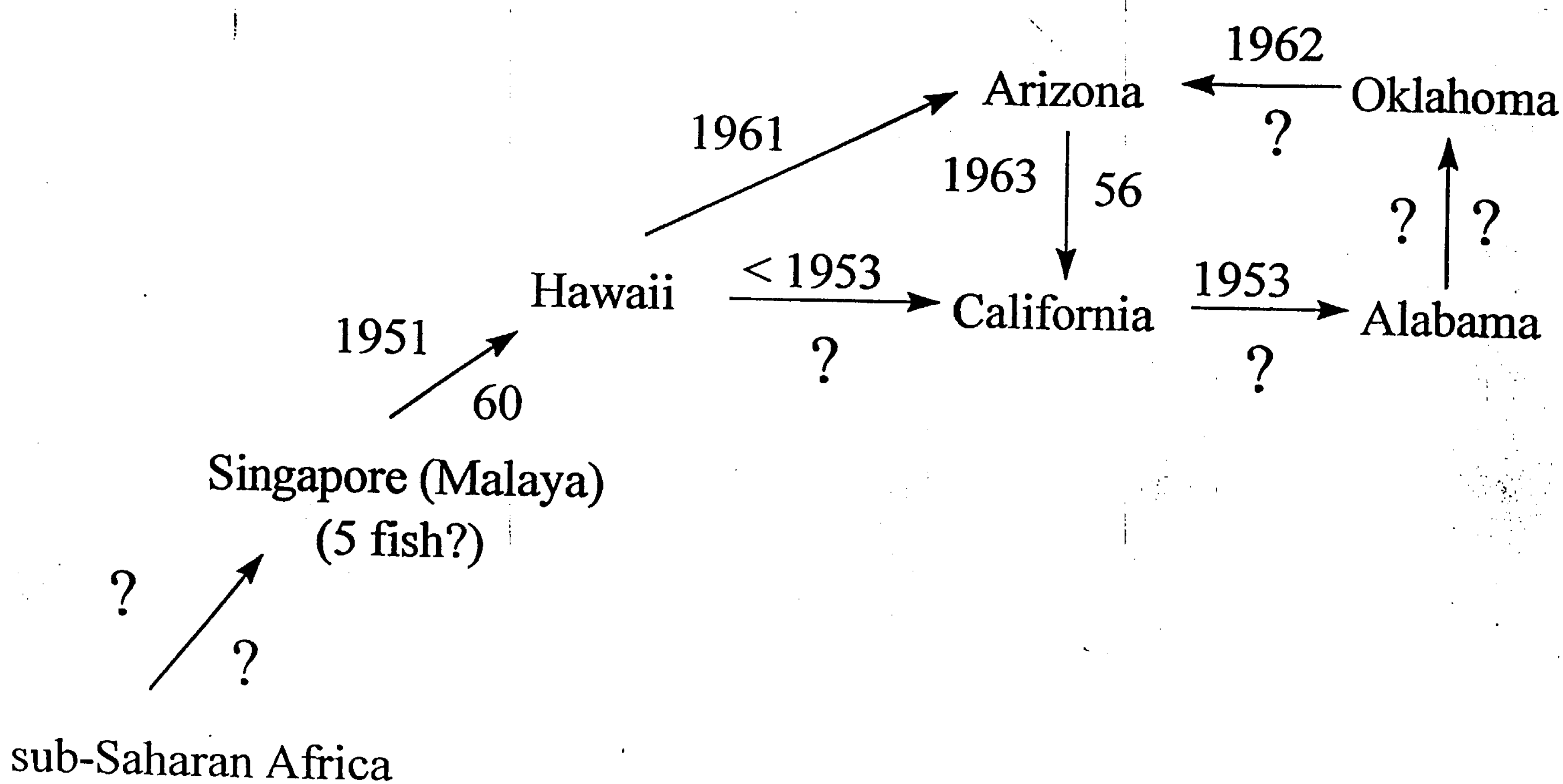


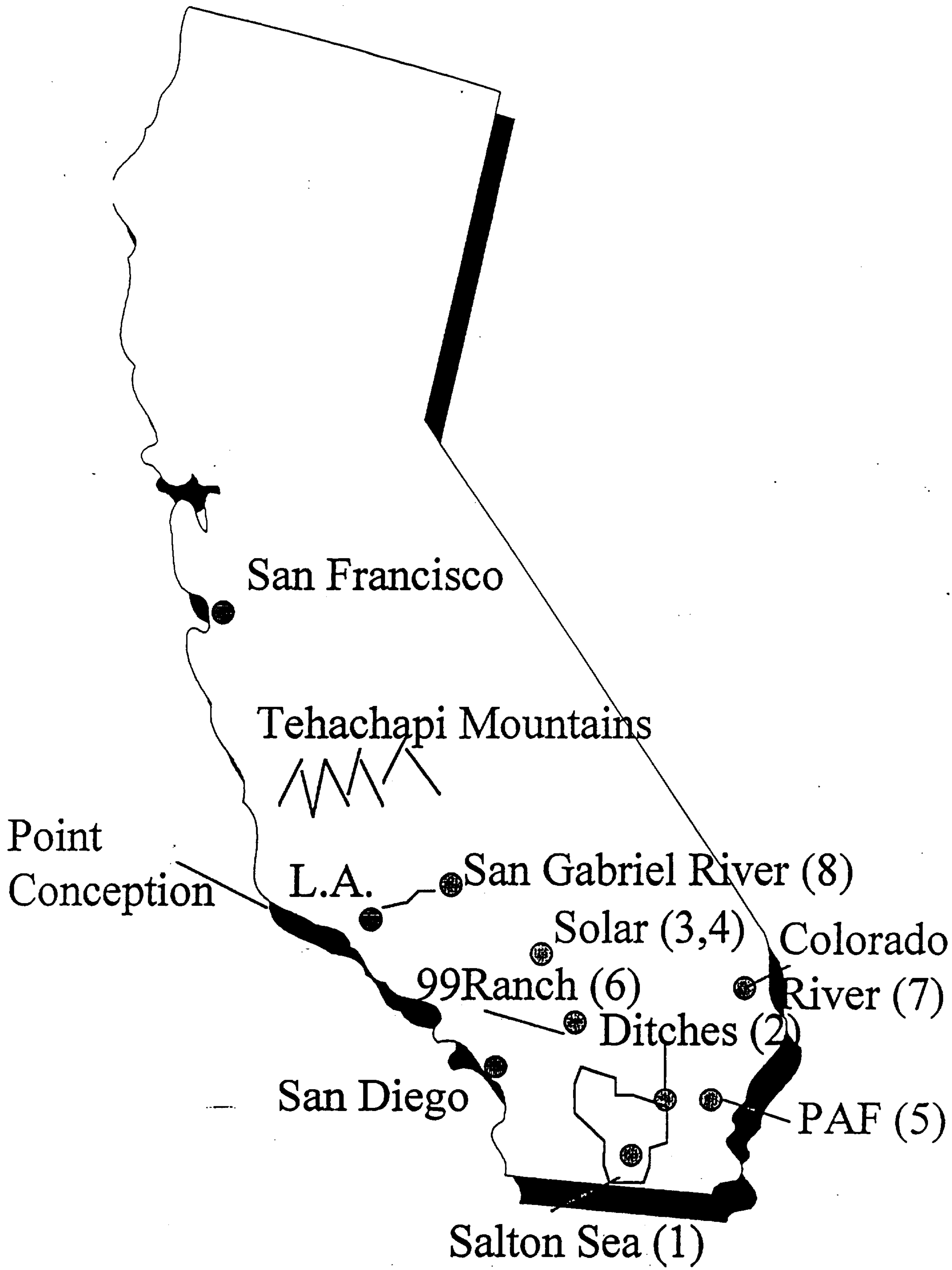
Distribution of *Tilapia rendalli*

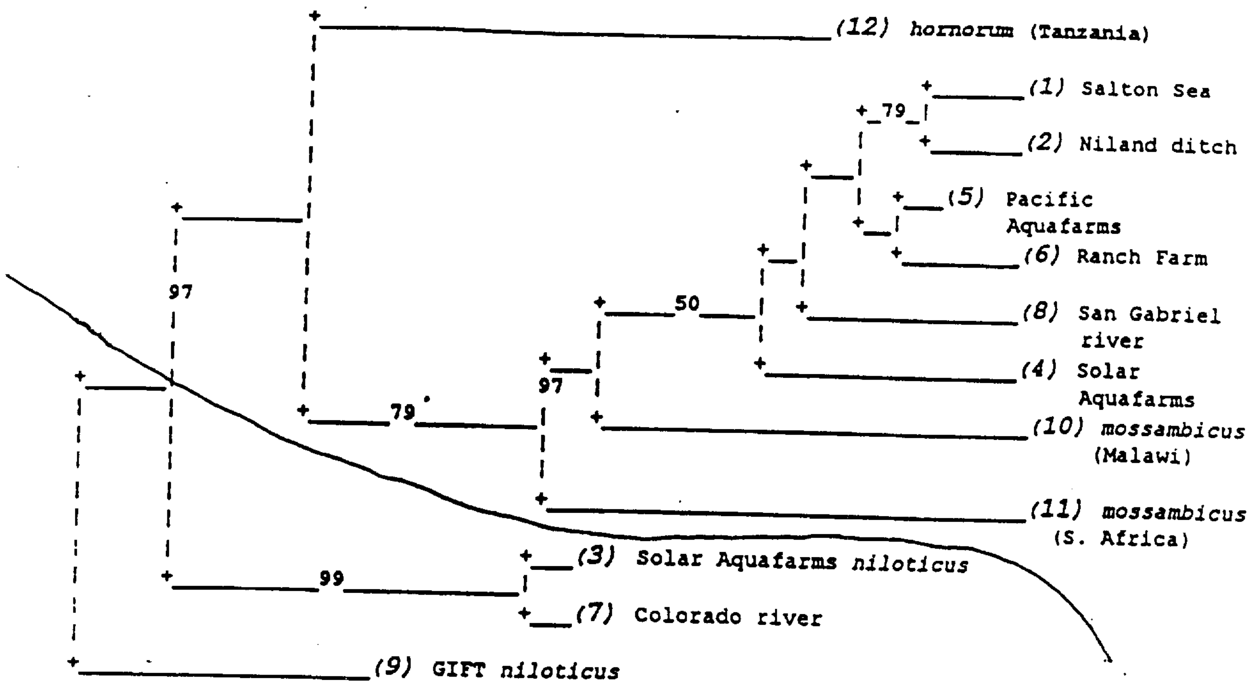


Distribution of *Oreochromis mossambicus* (●) and *O. mortimeri* (▲).

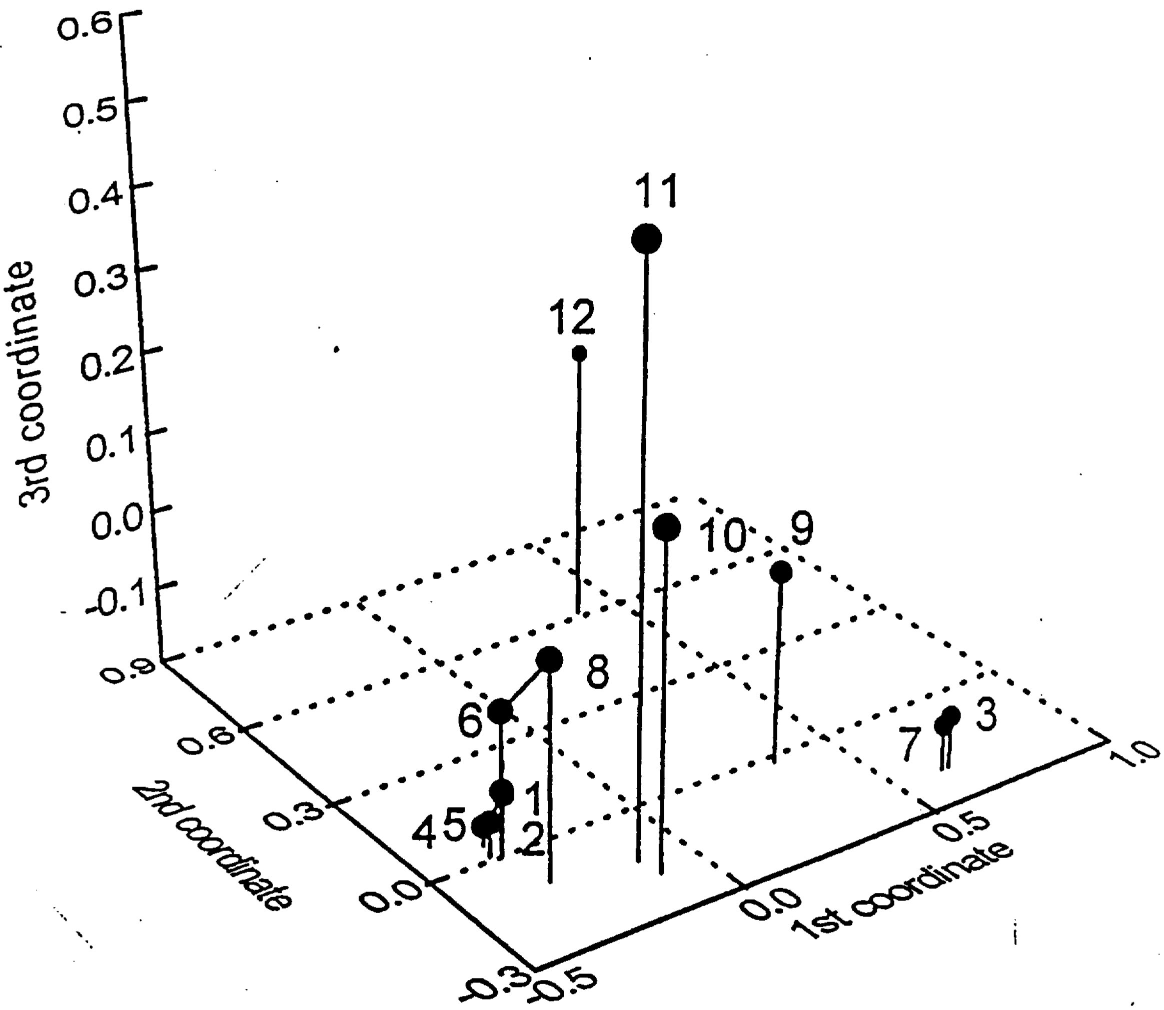
<u>Oreochromis</u> <u>mossambicus</u>




San Francisco
Tehachapi Mountains
Point Conception
L.A.
San Gabriel River (8)
Solar (3,4)
Colorado River (7)
99Ranch (6)
Ditches (2)
San Diego
PAF (5)
Salton Sea (1)



This evidence shows an evolutionary tree. The groups split.
Perhaps this is not a good model.
The nile tilapia may not be niloticus but rather an amalgamation
we can now call "CA nile"
Same may be true for "CA mossambicus."



98 alleles
94 bits of genetic info
segregated into 4 microsattelite groups

Table 1. Tilapia samples collected in southern California. Reference numbers used to identify these samples are also shown in Figures 6-8.

(1) Salton Sea (Southern Area near Linsey and Lack Roads) (8 fish)
(2) Three Different Outlet Ditches Draining to Salton Sea from Tilapia Farms, Niland (10, 21, 10 fish each; 41 fish total)
(3) *O. niloticus* (Solar Aquafarms, Sun City; 10 fish))
(4) Solar Aquafarms (10 fish)
(5) Pacific Aquafarms, Niland (10 fish)
(6) 99 Ranch Tilapia Farm, Industry (20 fish)
(7) Colorado River, Blythe (8th Street and Lovekin) (20 fish)
(8) San Gabriel River, Los Angeles (20 fish)
(9) *O. niloticus* (GIFT, ICLARM, Philippines; 30 fish)
(10) *O. mossambicus* (Malawi, Africa; 36 fish)
(11) *O. mossambicus* (South Africa; 10 fish)
(12) *O. urolepis hornorum* (Tanzania; 7 fish and Stirling University; 2 fish)

Table 2. Average DNA microsatellite heterozygosities and standard errors for tilapias sampled from southern California.

(1) Salton Sea	0.717125 ≅ 0.068979
(2) Niland ditch	0.644391 ≅ 0.117101
(4) Solar Aquafarms	0.210526 ≅ 0.136589
(5) Pacific Aquafarms	0.533239 ≅ 0.188768
(7) Colorado river	0.346651 ≅ 0.162966
(10) Malawi *O. mossambicus*	0.677372 ≅ 0.060142
(11) South Africa mossambicus	0.189474

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CALIFORNIA DEPARTMENT OF FISH AND GAME

SAMPLER:______________________________
DATE:________ WATER TEMP:______________ COUNTY:_____________
ARRIVAL TIME:________ START TIME:__________ END TIME:_________
LOCATION:____
WEATHER:____
TOTAL NO. OF ANGLERS: Boat :______ Shore:________
ROUND #:__________ SHEET #:_________

COUNTY CODES:

LA-Los Angeles
SB-San Bern.
SD-San Diego
R-Riverside
V-Ventura
OR-Orange
I-Inyo
O-Other

SPECIES CODES:

1-STRIPED BASS
2-RAINBOW TROUT
3-CHNL. CATFISH
4-L. MOUTH BASS
5-SHAD
6-BLK. BULLHEAD
7-WHT. CRAPPIE
8-YLW. BULLHEAD
9-BASS
10-ANY

ANGLER START TIME	ANGLER END TIME	AGE 1)<16 2)16-55 3)>55	CO. CODE	GENDER		TARGET SPECIES CODE	FISH CAUGHT		FISH KEPT		COMPLETE TRIP		BOAT OR SHORE		LICENSE DISPLAYED		# OF TROUT WITHOUT ADIPOSE FIN	ANGLER IDENTIFICATION NOTES
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GENERAL NOTES:____

The California State Tilapia Growers Group comprises most of the aquaculture facilities raising Tilapia as a business in California. Because of the report generated for California Game and Fish Department by Dr. Barry Costa-Pierce and Dr. Roger Doyle, it is timely that we submit a statement regarding our position on the current regulation governing Tilapia culture and transportation within the state.

First, we concur with the study's findings that *Tilapia nilotica* (California Nile) is confined to one specific farm in California (Solar Aquafarms) and apparently in the Colorado River bordering the state. In compliance with California State regulations, the Salton Sea Area Growers have not introduced an additional exotic species into the region.

Second, we concur with the study that the stocks of fish used for commercial aquaculture in California are primarily of *Tilapia Mossambica* genetics. No contamination of Nilotica DNA was discovered amongst Tilapia reared commercially in the study. As the Mossambica was introduced to California in the 1950's, we feel that it is an established and accepted species.

Third, we have the following concerns with regard to allowing *Tilapia nilotica* into the California live market:

1. The approval would open the doors to the spread of unwanted diseases from other countries and states.

2. Studies show Nile Tilapia tolerate cool enough conditions to inhabit some California waters. Other studies recognize the aggressive nature of Nile Tilapia and conclude that the species competes directly with preferred sport fishes such as large mouth bass for forage and spawning territory.

3. Nile Tilapia are recognized as faster growing, more efficiently converting fish. Allowing these fish into the live market would pose a serious disadvantage to California growers presently not allowed to grow Nile Tilapia.

Fourth, if a new species of fish is to be condoned by California Game and Fish, then the California Tilapia Growers request a "Grace Period" whereby we would be allocated a period of ten (10) years to acquire new broodstock, build inventories of Nilotica genetics, test performance and breeding technologies, change farming methodologies and grow for sale the new species, before the sale of Nile Tilapia would be legal.

The following commercial Tilapia Aqua-farms agree with the aforementioned conclusions and recommend that the current regulations banning *Tilapia nilotica* for sale in California as a live fish product be maintained.

Pacific Aqua Farms - Bill Engler

Fred F. Bartlett - Imperial Fish Farm

1/13/97 OCEAN RICH INC - [illegible]

DONNA Farrell

14 Aquaculture Farm [illegible]

1/14/97 H&T Aquatics [illegible]

S.S. Vont fish farm [illegible]

Dashun Fisheries Inc Alex Ma

WESTERN AQUATIC ENT, INC [illegible]

CA. Desert Fish Farm, Inc. - [illegible]