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QUANTITATIVE GENETIC VARIATION IN THE FISH, TILAPIA (OREOCHROMIS
MOSSAMBICUS)

University of Hawaii

Ph.D. 1984

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QUANTITATIVE GENETIC VARIATION
IN THE FISH, TILAPIA
(Oreochromis mossambicus)

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN BIOMEDICAL SCIENCES (GENETICS)

AUGUST 1984

BY

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ACKNOWLEDGEMENTS

I would like to express my most profound gratitude to Dr. Ming-Pi Mi for his guidance, genius, and patience during my years at the University of Hawaii - every student should have such a mentor.

My sincerest thanks are due to Dr. Phil Helfrich, Director of the Hawaii Institute of Marine Biology and to Mr. James Makasiale, Director, and Dr. Michael Hamnett of the Pacific Islands Development Program, East West Center for without their sponsorship this study would not have been possible.

For their reliable assistance and needed humor during the experimental stages of this study, I thank Messrs. Steve Shimoda and Lloyd Watarai of the Hawaii Institute of Marine Biology.

To Mr. Filemon Quiaoit of the Data Resources Program, Cancer Center of Hawaii I offer my deepest appreciation and esteemed respect for his assistance with the computers during my data analysis. In addition, I thank the friendly staff at the Data Resources Program for allowing me to use the facilities.

I also extend my gratitude to Dr. James Brock, Veterinarian with the Aquaculture Development Program for his helpful recommendations and care in keeping my fishes alive.

Lastly, a special thanks to my family whose support and faith has meant a great deal to me.

ABSTRACT

This study investigated the genetic variation of economic traits in seawater cage cultured O. mossambicus in Hawaii. A hierarchical experimental design was implemented consisting of 31 sires and 61 dams. Twenty-nine paternal half-sib families, 61 full-sib families, and 22 replicated families were analyzed. The body measurements of weight, total length, head size, and height size were recorded monthly from 1- to 5- months of age. At the end of the fifth month all fish were sacrificed and measured for the additional traits of drawnweight, intestinal length, and gill surface area. Males and females were distinguished in the 3- to 5- month old data.

In spite of past evidence suggesting a depauperate gene pool for O. mossambicus in Hawaii, the results in this study reveal genetic variation in the population. Significant differences among sires and among dams within sire were found in both males and females for weight at 4- months of age and in males only at 5- months of age for weight, drawnweight, and total length. The estimated narrow-sense heritabilities derived from the sire component of variance for the above traits were in the intermediate range with values from 0.31 to 0.39. The genetic correlations between the male traits ranged from 0.87 to unity. These findings suggest that growth rate of

0. mossambicus in Hawaii has the potential to be improved by genetic selection techniques.

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I. INTRODUCTION

Tilapias have always been regarded as an important food fish since the beginning of recorded history. The earliest example of its culture in ponds came from an Egyptian tomb frieze dating around 2500 B.C. (Hickling, 1963). Scholars also speculate that the fish Saint Peter caught in the Sea of Galilee and those which Christ fed the multitudes were tilapia (Bardach, Ryther, and McLarney, 1972). More recently, however, tilapias have been referred to as "miracle fish" in hopes their culture would make significant contributions towards alleviating malnourishment in today's developing tropical countries. Its reputation as an easily convertible and accessible source of animal protein comes from its circumtropical distribution, high yield potential, general hardiness, excellent table quality, ease of breeding, and resistance to diseases. In addition, tilapias can utilize a wide spectrum of food materials and tolerate a wide range of salinities. The United Nations Food and Agriculture Organization has endorsed tilapia as a priority aquaculture species requiring further research and development (FAO, 1980). Pacific Island leaders have also recognized the potential of this fish and have given it priority status as a strategy to increase the supply of aquatic protein in and exports from the Pacific region (Pacific Islands Conference Standing Committee, 1981).

In spite of these qualities and the exigencies of world policy makers, world production of tilapias was a disappointingly low 415,965 metric tons for 1981 (FAO, 1983). This represents about 5 percent of the world's total production from inland waters. In comparison, the same annual production for carp and other cyprinids was 706,197 metric tons. The FAO statistics are often unable to differentiate tilapia catches by species, but when classification was possible, the most important species in this group was O. mossambicus at 36,499 metric tons in 1981. The next in importance was O. niloticus at only 8,174 metric tons.

The world market for tilapia is still expanding within producing countries and in areas previously unfamiliar with this fish. In Africa, about 31 countries are involved with tilapia production for purely domestic consumption (Balarin and Hatton, 1979). In Latin America, tilapia is the leading cultured fish that is being produced in every country except Chile, Bolivia, Argentina, Uruguay, and Venezuela. It is often the only major source of animal protein accessible to local inhabitants (Inter-American Development Bank, 1977). In the United States, there are only 11 known commercial farms, but a preliminary economic feasibility report indicates the potential of a 10 million pound mainland market (Shang and Macauley, 1981). The largest production

and consumption of tilapia occurs in Southeast Asia (e.g. Indonesia, Thailand, Philippines, and Malaysia). The most dramatic and probably unique example of this fish's upcoming status is in Taiwan where 13,000 tons were produced in 1974 (Chen, 1976) compared to the present level of over 80,000 tons (Liao and Chen, 1983). It is also noteworthy that the history of Taiwanese tilapia culture is less than 40 years old.

The main reason world tilapia yields are low in most countries and have not fulfilled expectations as a panacea in food programs is because there are problems associated with its husbandry. The primary obstacle to its culture is the sexually precocious nature of this fish. Although its breeding potential is great, uncontrolled reproduction rapidly leads to an overpopulation of stunted fish too small for market. Therefore, the main research efforts on tilapia have tended to concentrate on methods of reproductive control rather than investigating other economic traits such as fast growth rate. The methods employed for curbing excessive reproduction have been reviewed by Balarin and Hatton (1979).

The first of the five tilapia species introduced into Hawaii was Oreochromis mossambicus from Singapore in 1951 (Maciolek, 1984). The Department of Planning and Economic Development for the State of Hawaii has recognized the commercial importance of this established

exotic and has been promoting tilapia culture as part of its diversified agriculture program. However, the same over-reproduction problem mentioned above is encountered. A novel genetics approach to solving this problem is to examine the feasibility of genetic selection for fast growth rate such that the fish reaches marketable size before reaching sexual maturity. In other fishes, the potential success of genetic selection, based upon heritability estimates, has been reported for the channel catfish (Reagan et al., 1976; El-Ibiary and Joyce, 1978) and salmonids (Von Limbach, 1970; Gall and Gross, 1978a,b; Refstie and Steine, 1978; Gunnes and Gjedrem, 1978).

The cage culture of tilapia is a relatively new practice that is on the increase in developing tropical countries. A comprehensive review of the subject has been published by Coche (1982). The concept of cage culture in a seawater environment is somewhat unique because tilapia are usually considered a freshwater fish traditionally cultured in freshwater ponds (Bardach, Ryther, and McLarney, 1972). The euryhaline character of O. mossambicus, presumably due to their marine ancestry (Kirk, 1972), has never been fully exploited even though they are known to successfully spawn and naturally inhabit seawater environments (Brock, 1954; Knaggs, 1977; Borgstrom, 1978; Whitfield and Blaber, 1979; Lobel, 1980). The need to explore this species in more saline areas

(e.g. estuaries and coastal waters) has become urgent in tropical countries because of the increasing competition of ponds with agriculture for suitable lands. The importance of seawater cage culture of tilapia is currently being demonstrated in research projects in the Philippines, Taiwan, the Ivory Coast, Puerto Rico, and Costa Rica (Coche, 1982; Kuo, Lee, and Huang, 1983; Liao and Chang, 1983).

The excessive reproduction problem in the husbandry of tilapia and the diminishing freshwater resources available for its culture are important economic considerations and obstacles to the future farming of this fish. The idea of genetic selection for fast growth rate in tilapia such that the fish reaches marketable size before reaching sexual maturity has never been fully explored in O. mossambicus or any other tilapia species. Hence, this study represents the first attempt to investigate the genetic variation of seawater cage cultured O. mossambicus for the potential of future genetic manipulations.

II. BACKGROUND INFORMATION

TAXONOMY

In past literature, the fish in this study has been referred to as both Tilapia mossambica and Sarotherodon mossambicus. However, the generic grouping of Tilapiini species has been undergoing a number of recent classification changes at the British Museum of Natural History (Trewavas, 1982a,b). The current nomenclature is to retain the generic name based on the type of brooding: Tilapia for substrate brooders, Sarotherodon for paternal mouthbrooders, and Oreochromis for maternal mouthbrooders. To be consistent with the updated taxonomy, this study will use the proper name of Oreochromis mossambicus. For general reference, this Cichlid group will be referred to as tilapia (pl. tilapias).

BREEDING CYCLE

In the maternal mouthbrooding species Oreochromis mossambicus, the males dig a distinctive territorial nest in the pond bottom, attracts a female, and undergoes a prolonged and complex courtship. The ripe female then lays several hundred eggs in small, successive batches each of which in turn is fertilized by the male. The female collects the eggs in her mouth after each batch

is fertilized, incubates them, and in 3 to 5 days hatching occurs (Bardach, Ryther, and McLarney, 1972). The larvae remain in her mouth for protection against predators until two weeks after the yolk sacs are absorbed. The young, free-swimming fry can mature as early as 2 to 3 months old and breed every 3 to 6 weeks as long as the water is warm and food available.

During the incubation period the female usually does not feed because the eggs require constant churning in her mouth for surface cleaning and aeration. In addition, since these eggs are very yolky, lack of churning will cause the heavy lipids to settle to the lower pole and disrupt embryo development (Fryer and Iles, 1972).

DISTRIBUTION

Among the 700 species of the Cichlidae family, the most important group are the tilapias which are mainly indigenous to Africa. The tilapia species in this study, Oreochromis mossambicus, has a natural range in southeast Africa from Kenya, Tanzania, Mozambique, to South Africa (Philippart and Ruwet, 1982). However, 20th century transplantations by man have established this species within a circumtropical distribution.

The first evidence of tilapias outside of Africa

occurred in Asia in 1939, when five O. mossambicus specimens were collected from a small lagoon on the south coast of Java. Records indicate that two females were found bearing eggs and fry in their mouths (Atz, 1954). How and when these fish left Africa and arrived in Indonesia is still a mystery. It is speculated they were imported as exotic aquarium fish (Ling, 1977). Most significant is that these five founders are responsible for the present O. mossambicus populations in the Asia/Pacific region.

During World War II, the production of these five founders increasingly replaced the traditional Indonesian practice of milkfish (Chanos chanos) culture that was deteriorating under the Japanese occupation. Tilapia were eventually spread throughout Indonesia and became a highly regarded component of the local diet. Towards the end of the war, retreating Japanese introduced tilapia to Singapore where, as a highly successful colonizing species, it spread and established itself throughout southeast Asia. It now inhabits virtually every kind of aquatic niche - ponds, ditches, canals, reservoirs, and ricefields. Practically every country in this region now considers tilapia an important food fish.

In the 1950's and 60's, there were deliberate official attempts to introduce O. mossambicus to Oceania from such countries as Java, Singapore, the Philippines,

and Malaysia (Van Pel, 1955; Devambe, 1964; Balarin and Hatton, 1979). Its establishment has been successful, but has generated mixed reactions. For example, in Papua New Guinea tilapia is a major fisheries and in Fiji and Guam it is a minor one. However, in the Cook Islands, the Fanning Atoll, and the Republic of Kiribati it is considered a nuisance in the wild and there have been deliberate attempts at eradication.

In 1951, the Hawaii Division of Fish and Game imported 14 O. mossambicus from Singapore and successfully introduced this exotic to all the major islands of Hawaii (Maciolek, 1984). The rationale for this introduction was unclear (Lobel, 1980). However, in 1955, tilapia were tested for use as a skipjack bait (Hida, Harada, and King, 1962). Currently tilapia is considered a "trash" fish among Hawaiian commercial and sports fishermen.

TILAPIA GENETICS

The investigation of genetic variation within species of tilapia has received very little attention. Tave and Smitherman (1980) of Auburn University have estimated heritabilities for weight and length of Oreochromis niloticus at 45 and 90 days. Their results from sib analysis produced heritability estimates not significantly different from zero. It was suggested that

the original foundation stock for the Auburn University population was very small and that almost a decade of subsequent inbreeding had narrowed any existing genetic base.

In a conference abstract whose papers are currently in press, Bondari et al. (1983) of the University of Georgia reported results obtained from one generation of high and low selection for body weight and total length in Oreochromis aureus. Results indicate the responses were assymmetrical with the high line consisting of heavier and longer fish after 40 weeks of growth.

In a Russian study (Chan, 1971) cited by Kirpichnikov (1981), the results from a realized heritability estimate for weight in O. mossambicus was found to range from 0.12-0.32 and 0.12-0.29 in females and males, respectively.

The existence of additive genetic variation is suggested from two studies practicing negative selection. In Lake George, Uganda, Gwahaba (1973) found that 20 years of intense overfishing of a natural population of O. niloticus resulted in a reduction of the mean weight of the species. He attributes this phenomenon to partially negative selection pressures as well as to a possibly deteriorating environment.

Silliman (1975) later conducted a three year study of culling or selecting out larger individuals from an

experimental population of O. mossambicus and found growth to be significantly depressed when compared to a contemporary, unselected experimental population of O. mossambicus.

Electrophoretic variation in O. mossambicus in Hawaii has been studied by Malecha (1968). His experimental population was similar to the one in this study in that specimens were collected from four locations on Oahu ranging from freshwater to full-strength seawater habitats. By the use of starch gel electrophoresis, serum esterase and transferrin were found to be monomorphic. In contrast, Oreochromis macrochir and the substrate spawning Tilapia melanopleura collected from the Nuuanu and Wahiawa Reservoirs, respectively, were found to be polymorphic. Malecha speculated that since the monomorphic esterase and transferrin loci of O. mossambicus were similar to some phenotypes of O. macrochir, that O. mossambicus probably had become monomorphic owing to the effects of intense genetic drift.

No other electrophoretic studies have been conducted on O. mossambicus outside of Hawaii to compare if mono- or polymorphisms also exist in other populations. O. mossambicus from Malaysia (Chen and Tsuyuki, 1970) and O. mossambicus probably from Thailand (Hines and Yashouv, 1970; Herzberg, 1978) have been electrophoretically examined for the purpose of distinguishing them from other

tilapia species. It is not possible to draw a conclusion concerning polymorphisms in the proteins or enzymes investigated, because these studies were investigating between-species electrophoretic variation rather than within-species variation.

Electrophoretic markers in other tilapia species have been used for solving taxonomic problems and investigating genetic variation in natural fish populations (Hines, Yashouv, and Wilamovski, 1971; Basasibwaki, 1975; Avtalion, Prugenin, and Rothbard, 1975; Avtalion and Mires, 1976; Avtalion et al., 1976; Kornfield et al., 1979). Screening for electrophoretic markers also has an applied value in selective breeding programs for breed identification and efficient experimental designs that reduce the number of experimental units by the mixing of genetic fish groups (Moav et al., 1976). There are currently no visual or morphological markers available in tilapia for this purpose.

The problem of excessive reproduction mentioned earlier has channeled most tilapia research efforts into interspecific hybridizations to produce all-male progenies for aquaculture production. This approach was first developed by Hickling (1960). The males are preferred over females because of their markedly faster growth rate and larger size (Lowe-McConnell, 1982). About 30 species are known to have formed 114 inter-hybrid crosses in the wild

or in the laboratory (Schwartz, 1983). Skewed sex ratios from many of these crosses have prompted research to identify the mechanism for sex determination which is not predictable from any Mendelian system. Wohlfarth and Hulata (1981) state that not a single case of a heteromorphic pair of chromosomes, which might be regarded as sex chromosomes, has been detected in tilapias. Thus the sex determining mechanism appears to be genetic rather than cytological. Many investigators (Hickling, 1960; Chen, 1969; Jalabert, Kammacher, and Lessent, 1971; Avtalion and Hammerman, 1978; Hammerman and Avtalion, 1979; Wohlfarth and Hulata, 1981; Chen, 1983) have constructed genetic models to explain these skewed sex ratios. These ranged from non-homologous chromosomes carrying sex determining factors to one pair of sex chromosomes with one sex determining locus consisting of a series of multiple alleles. Unfortunately, none of the models are yet able to offer satisfactory explanations.

III. MATERIALS AND METHODS

COLLECTION OF BREEDING STOCK

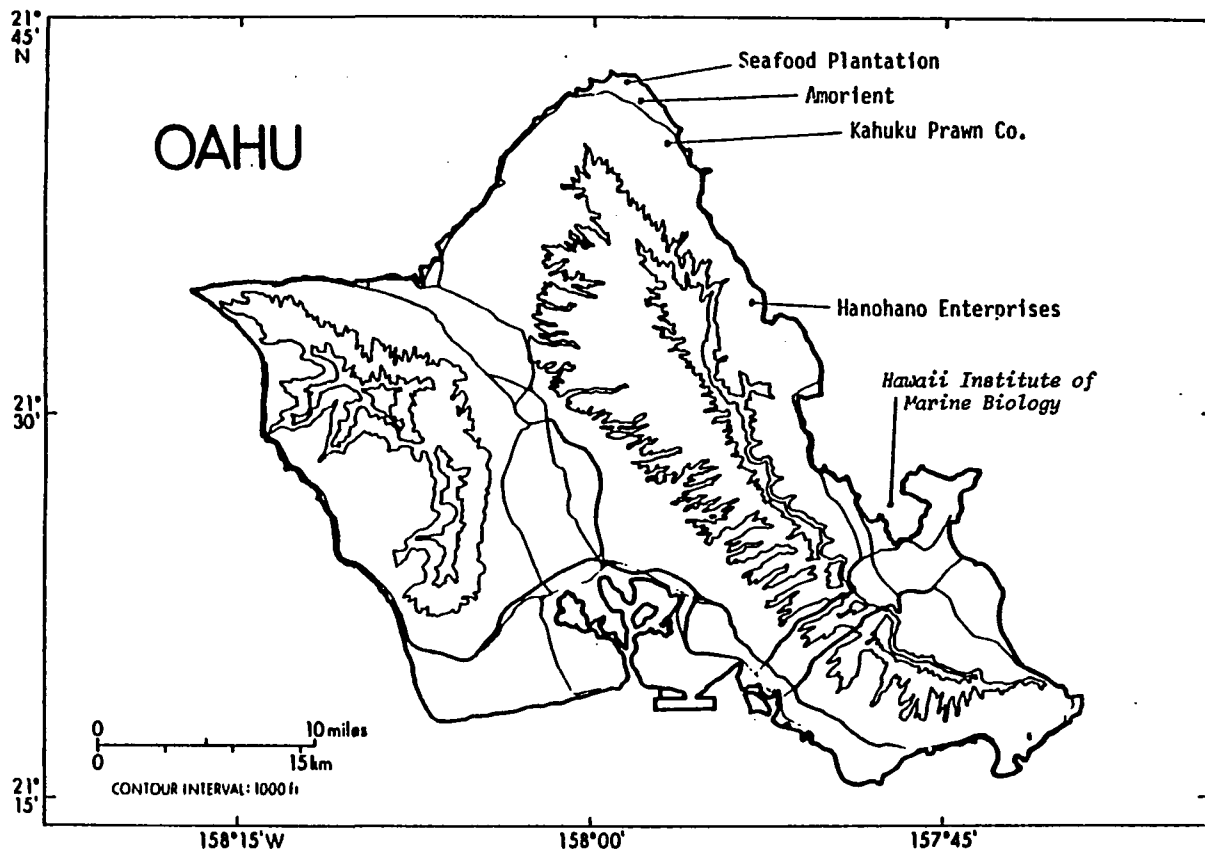
Adult Oreochromis mossambicus breeding stock were collected by using overnight fish traps at four commercial aquaculture farms on the northshore area of Oahu (Figure 1). More specifically, the collection areas were:

1. Freshwater drainage ditches of the Kahuku Prawn Company,
2. A freshwater stream at Hanohano Enterprises in Punuluu,
3. Brackish and freshwater drainage ditches at the Kahuku Seafood Plantation, and
4. Brackishwater drainage ditches and freshwater prawn ponds at Amorient in Kahuku.

The collected fish were immediately transported in oxygenated containers to the Hawaii Institute of Marine Biology (HIMB) on Coconut Island in Kaneohe Bay. These fish were then stocked into fiberglass tanks having the same salinity as their origin and separated on the basis of their sex and site of collection. During several weeks of captivity the fish were maintained on a commercial salmon feed (Silver Cup Trout Feed).

The fish were slowly acclimated to full strength seawater (34 ppt). This process took five to seven days.

FIGURE 1
COLLECTION SITES



Preliminary studies showed that when the transition took less than five days osmotic stress resulted in a very high mortality rate.

SPAWNING

The adults were fin clipped for origin identification before being transferred to outdoor floating cages in the seawater lagoon fronting the laboratory facility. For each cage, 2 to 5 sexually mature females were randomly assigned to one breeding male.

The floating cages were constructed of 1/4 inch galvanized hardware cloth with dimensions of 102cm x 91cm x 61cm (L x H x W). These were floated by two styrofoam buoys. A bottom substrate of fiberglass material was used to prevent eggs from falling through the cage during the mating process.

The cages were visually examined 2 to 3 times per week for mouthbrooding females. A brooding female was usually recognized as being solitary, having darkened eyes, flared operculums, distended mouth, lips, and lower jaw, plus exhibiting a light-yellowish body coloration. These brooding characteristics were similar to those described by Lanzing and Bower (1979) in Australia.

Once a brooding female was identified in the mating cage, she was quickly hand netted and transferred

to an indoor flow-through 75 liter fiberglass tank. Brooding females were transferred because if left in the cage they were occasionally observed with abrasions on their sides, presumably from the aggressive behavior of the male, the other females, or a combination of the two. In the transfer process, the mouth of the brooding female had to be held shut, otherwise, the eggs would be spat out and abandoned.

After about 5 to 7 days of solitary brooding, the female was separated from her fry. The fry usually had small yolk sacs, but were free-swimming. Past experience demonstrated that allowing brooding to continue until yolk sacs were fully absorbed invited maternal cannibalism in the confines of aquaria.

The matings in this study were accomplished in a 12 week period, from mid-May to mid-August. However, 95 percent of the females spawned in a 7 week interval (May 26 - July 15).

The final hierarchical mating scheme consisted of 31 males mated to 61 females for the analysis of 29 paternal half-sib families and 61 full-sib families. Also, 22 families were replicated.

STOCKING

After the fry absorbed their yolk sacs, family

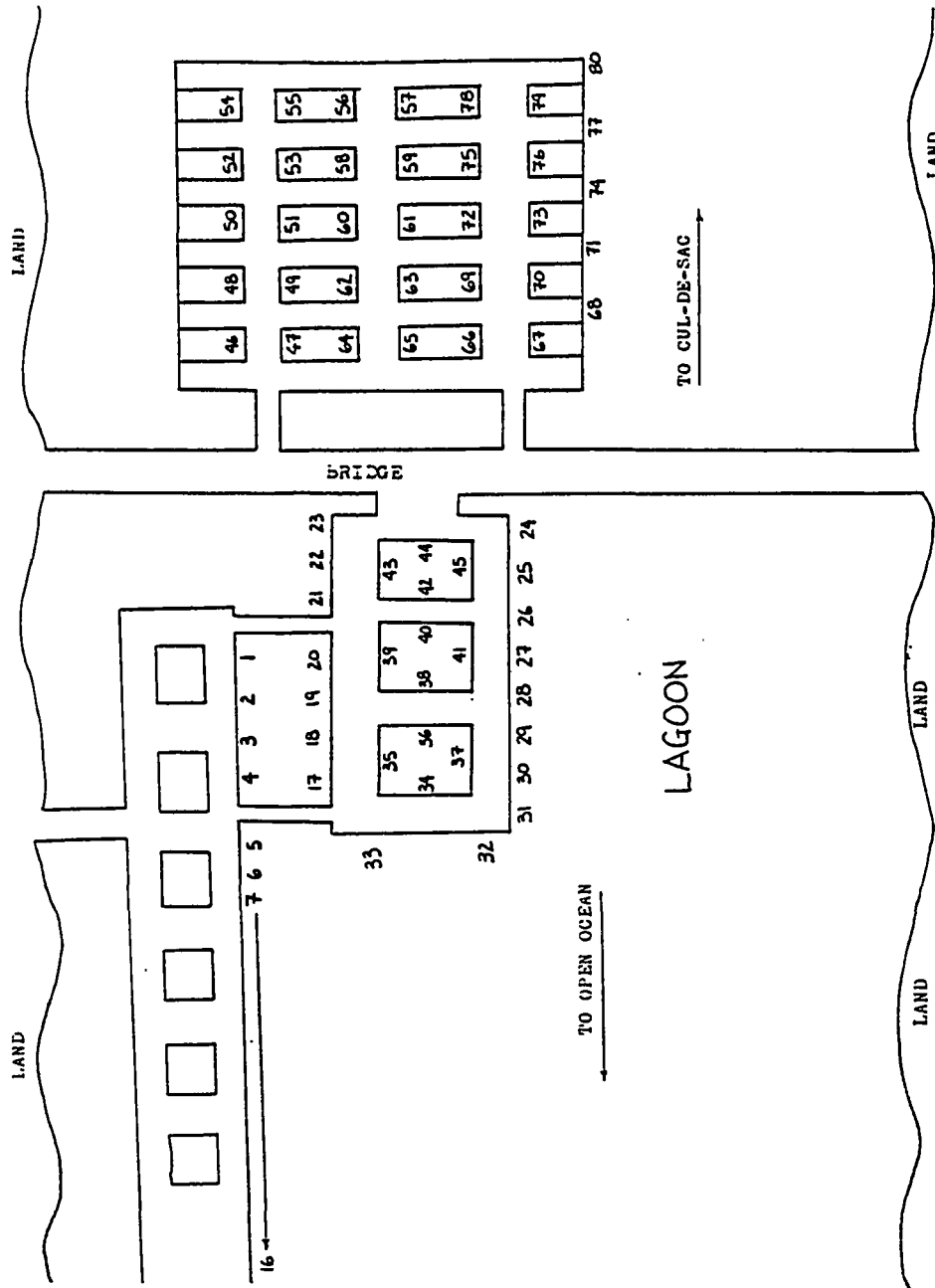
groups were randomly transferred from the indoor aquaria to outdoor floating cages. The cages previously used for mating were modified with an inside liner of fine mesh netting to prevent the small fry from escaping. A total of 80 cages were available for stocking. The number of full-sibs in a family ranged from approximately 100-900 fry depending upon size of female and the number of eggs or fry lost during handling.

Each family was raised in a cage for two months. At the end of the second month, 25 fish were randomly chosen while the remainder were culled. Each cage, thereafter, contained a family size of 25 or less fish that were raised to the end of the fifth month and then sacrificed. Figure 2 outlines the experimental site with each number representing a cage.

TAGGING INDIVIDUAL FISH

Fish were individually tagged to record their growth rate from the third to fourth to fifth month. Since three month old fish were too small for commercial tagging guns, a method developed by Hauser and Legner (1976) was initially adopted. This used a tag made of 10mm wide plastic embossing tape that had a number punched on it by a hand-held label maker (e.g. "Dymo-gun"). The tags were attached to the fish with monofilament fishing line

FIGURE 2 -- EXPERIMENTAL SITE



through the dorsal musculature near the posterior base of the dorsal fin. These tags were applied to the first 2 families, but proved unsatisfactory in that their stiff edges caused wounds in a small percentage of the fish. Therefore, another method was improvised consisting of three 1/8 inch diameter colored beads strung in combination and attached with monofilament fishing line. The color combinations provided each fish with an individual identification. This technique was highly satisfactory and produced no apparent physical trauma to the fish.

FEEDING

Feeding was done twice a day throughout the experiment. During the first 2 months of growth the fish were fed commercially prepared salmon fry pellets in quantity. At 3- to 4- months of age, feed was given to each cage at a rate of 10 percent of the total fish weight in a cage. Adjustments of feeding rates were made every two weeks by linear extrapolation of fish growth. By the fifth month, the size of the fishes necessitated using a larger sized floating catfish pellet which were fed to each cage at a rate of 5 percent of the total fish weight in a cage.

DATA COLLECTION

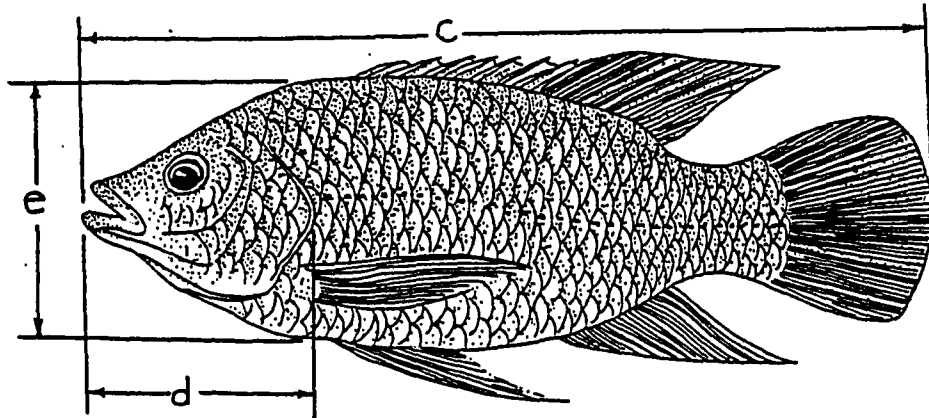
The metric characters in this study were measured at age 35, 65, 95, 125, and 155 days. However, for descriptive purposes these ages were respectively referred to as 1, 2, 3, 4, and 5 months. In the 1- and 2- month old data, 30 to 35 fish were randomly selected from each family for measurement. Whereas in the 3- to 5- month old data all fish were measured. The following variables were measured for each fish at the specified ages:

	MONTH				
	1	2	3	4	5
a. total body weight	x	x	x	x	x
b. drawn body weight					x
c. total body length	x	x	x	x	x
d. head	x	x	x	x	x
e. height	x	x	x	x	x
f. intestinal length					x
g. gill surface area					x

Total length (c) was measured from the tip of the snout to the end of the tail, head size (d) was from the tip of the snout to the end of the operculum, and height size (e) was from dorsal to ventral sides of the body.

These measurements of O. mossambicus are illustrated in Figure 3:

FIGURE 3



(illustration from Ling, 1977)

Measurements of observed traits were conducted as follows:

Total body weight and drawn body weight:

Monthly total body weight was measured in grams by a top loading electronic digital balance. Drawn body weight was a form of carcass quality, measured on the fifth month after the removal of scales, gills, and guts (Pirsch, 1982).

Total body length, head, and height:

A lateral view of each fish was photographed monthly using Kodak Plus-X black and white film. A

ruler was included in each photograph's background to later standardize measurements. The photographic set-up consisted of a tripod-mounted Nikon camera with a macro lens, and dual strobe lights. The film was immediately developed at HIMB in the event subjects had to be re-photographed. Body measurements were subsequently made from the negatives by a novel method using a Hewlett-Packard 9874A digitizer and Hewlett-Packard 2649G intelligent graphics terminal. In a preliminary study (Appendix 1), the new digitizer method developed for this work proved comparable to the traditional method of taking fish length measurements by a ruler. The digitizer method had the advantage of creating less handling stress on the fish, minimizing data entry errors, and providing a permanent record on film.

Intestinal length:

All fish were sacrificed at 5- months of age. The intestine was dissected out by cutting the connection to the stomach and anus. It was then placed on a wet plexiglass plate to prevent stretching or shrinking while being measured by hand with a ruler.

Gill surface area:

The four gills on the right side of all sacrificed 5 month old fish were dissected out and photographed. A ruler was included in each photograph's background to later standardize measurements. The Hewlett-Packard digitizer measured gill surface area from the photographic negative by calculating the area of trapezoids formed by the coordinate axes of the gills (Davis et al., 1981).

ANALYSIS OF DATA

All analyses were conducted on a Hewlett-Packard 3000 series III computer using several generalized computer programs for statistical analysis available at the Cancer Research Center of Hawaii (Mi, Onizuka, and Wong, 1977).

The data set in this study was checked for digitizing, keypunching, and formatting errors before data analysis. A simple regression was calculated treating weight as the independent variable separately regressed upon the traits of total length, head size, height size, drawnweight, intestinal length, and gill surface area for all month classes. The 99 percent confidence interval was estimated for each predicted \hat{Y} following Snedecor and Cochran (1967). Thus, for each observed weight, the dependent variable was checked for whether it was inside or outside of the regression's confidence band. If a dependent variable had an outlier value, then its record was re-examined for error.

For 3- to 5- month old fish, each sex was adjusted on a within sire basis for the effects of water temperature, photoperiod, and cage density (number of fish per cage). Data were adjusted on a within sire basis to preserve any genetic variation in the sire components. The least squares model was:

$$Y_i = U + b_1(X_1 - \bar{X}_1) + b_2(X_2 - \bar{X}_2) + b_3(X_3 - \bar{X}_3) + e_i$$

where:

U = least-squares mean;

b_1 = partial regression coefficient of trait studied on water temperature;

b_2 = partial regression coefficient of trait studied on photoperiod;

b_3 = partial regression coefficient of trait studied on cage density;

\bar{X}_1 = average monthly water temperature;

\bar{X}_2 = average monthly photoperiod;

\bar{X}_3 = average monthly cage density;

e_i = random effect.

For 1- and 2- month old fish, sex was undistinguishable and fish per cage densities were not counted. Therefore, the above statistical model for combined sexes was applied without the effects of cage density.

ESTIMATION OF VARIANCE COMPONENTS

For the 3- to 5- month old fish, a 3-stage nested ANOVA was used to estimate the variance components:

$$Y_{ijkl} = \mu + S_i + D_{ij} + R_{ijk} + e_{ijkl}$$

where:

μ = least-squares mean;

S_i = effect of i th sire;

D_{ij} = effect of j th dam mated to the i th sire;

R_{ijk} = effect of k th replicate nested within the j th dam mated to the i th sire;

e_{ijkl} = random effect.

The expected mean squares model was as follows:

SOURCE	df	EMS
Between sire	$N_3 - N_4$	$\sigma_w^2 + K4\sigma_r^2 + K5\sigma_D^2 + K6\sigma_S^2$
Between dam/sire	$N_2 - N_3$	$\sigma_w^2 + K2\sigma_r^2 + K3\sigma_D^2$
Between rep/dam/sire	$N_1 - N_2$	$\sigma_w^2 + K1\sigma_r^2$
Residual	$N_0 - N_1$	σ_w^2

The degrees of freedom in this analysis can be calculated according to Gower (1962), where:

N_0 = total number of individuals;

N_1 = number of replications;

N_2 = number of dams;

N_3 = number of sire;

N_4 = 1

The K coefficients or the coefficients of the variance components of the mean square expectations were calculated according to the method by Gates and Shiue

(1962). In general, the K coefficient of a S-stage hierarchical classification can be expressed as:

$$K_{ij} = \frac{\sum_{l=i}^{s-1} n_l^2 \left[\frac{1}{n_l} - \frac{1}{n_{l-1}} \right]}{d_i} \quad (j \geq i)$$

where:

n_i = totals used in deriving the i th source of variation;

d_i = degrees of freedom for the i th source of variation;

s = number of subclasses.

In this unbalanced design, an approximate F-test was used to evaluate the level of significance by re-calculating the mean squares using constant K coefficients. The method developed by Tietjen and Moore (1968) could not be employed to test the components of variance because the experiment was incompletely nested with only one-third of the families replicated.

For the 1- and 2- month old fish, a 2-stage nested ANOVA without the replicate was required. Degrees of freedom, K coefficients, and expected mean squares were calculated accordingly.

The 3-stage nested ANOVA was also applied to replicated family data only. This data set consisted of 22 replicated families or about one-third of the main data set. The ratio of the estimated replicate to dam variance components was used to partition out the replicate effect

from the dam variance component in the main dataset.

The sire component, which was derived from the means of half-sib families, estimated the phenotypic covariance of half-sibs. The dam component, which was derived from the differences between dam groups, estimated the covariance of full-sibs minus the covariance of the sire half-sib groups, because the sire effect was removed in the analysis of variance. The residual or within component contains the remainder of the genetic and environmental variance. The causal component relationships are tabulated according to Falconer (1981):

$$\begin{aligned}\sigma_s^2 &= \frac{1}{4} V_A \\ \sigma_D^2 &= \frac{1}{4} V_A + \frac{1}{4} V_D + V_{EC} \\ \sigma_w^2 &= \frac{1}{2} V_A + \frac{3}{4} V_D + V_{EW}\end{aligned}$$

where:

- V_A = additive genetic variance;
- V_D = dominance genetic variance;
- V_{EC} = common environmental variance;
- V_{EW} = random environmental variance.

The epistatic or interaction components (e.g. additive x additive, additive x dominance) were assumed to be zero. Also there were no practical methods for its partition (Jacquard, 1983).

The additive genetic ($V(G)$), maternal ($V(M)$), and environmental ($V(E)$) variance components were estimated according to Hazel, Baker, and Reinmiller (1943):

$$V(G) = 4\sigma_s^2$$

$$V(M) = \sigma_D^2 - \sigma_s^2$$

$$V(E) = \sigma_w^2 - 2\sigma_s^2 + \sigma_{\text{Replicate}}^2$$

The narrow-sense heritability or the ratio of the additive genetic to total phenotypic variance was estimated from the sire component of variance according to Falconer (1981):

$$h^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_D^2 + \sigma_{\text{Replicate}}^2 + \sigma_w^2}$$

This estimation remains unbiased from non-additive genetic influences (e.g. dominance genetic variance) and is the chief determinant for predicting the response of a population to selection.

The genetic correlation was also derived from the sire component of variance according to Falconer (1981):

$$r_g = \frac{\text{COV}_{S_1, S_2}}{\sqrt{\sigma_{S_1}^2 \times \sigma_{S_2}^2}}$$

IV. RESULTS AND DISCUSSION

The data for this study were checked for digitizing, keypunching, and formatting errors prior to the start of analysis. Over 2500 records were examined. Of these, less than 3 percent were found subject to error.

At 1- and 2- months of age, the sample sizes are relatively large with the body measurements more than doubling during this one-month period of growth. Table 1 shows the means and standard deviations for weight, total length, head and height size.

At the 3- to 5- month ages, it is interesting to note the marked dimorphism in size when the experimental population is sexed. For example, the males are about twice as heavy as the females at 5 months of age. Also, the growth rate appears to level off with time. Table 2 shows the same traits measured at later ages and separated by sex. The sample sizes have been notably reduced because of culling all but 25 fish per family at 3 months of age.

The 5- month of age panel of Table 2 includes additional traits which were measured when the fish were sacrificed: drawnweight, intestinal length, and gill surface area. The drawnweight is a form of carcass quality. The intestine and gills were measured in this study because of their important metabolic and

TABLE 1
 DESCRIPTIVE STATISTICS OF TRAITS AT
 1- AND 2- MONTHS OF AGE

<u>VARIABLE</u>	1 Month			2 Months		
	<u>N</u>	<u>MEAN</u>	<u>S.D.</u>	<u>N</u>	<u>MEAN</u>	<u>S.D.</u>
Weight (g)	1806	0.7	0.7	1491	6.0	3.6
Total Length (mm)	1805	32.0	9.0	1491	66.5	13.2
Height (mm)	1805	9.5	3.0	1491	20.5	4.3
Head (mm)	1805	8.8	2.3	1491	17.7	3.3

TABLE 2
DESCRIPTIVE STATISTICS OF TRAITS AT
3- TO 5- MONTHS OF AGE

VARIABLE	3 Months			4 Months			5 Months		
	N	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.
MALES:									
Weight (g)	969	17.3	8.6	954	36.7	14.8	946	57.1	21.2
Total Length (mm)	969	96.2	16.8	954	124.9	18.4	946	145.3	19.9
Height (mm)	969	29.5	5.1	954	38.4	5.7	946	44.5	6.4
Head (mm)	969	24.8	4.0	954	31.9	4.5	946	37.2	5.0
33 Drawn Weight (g)	-	-	-	-	-	-	943	50.1	18.8
Gills (mm ²)	-	-	-	-	-	-	914	465.7	119.5
Intestine (mm)	-	-	-	-	-	-	944	817.1	248.5
FEMALES:									
Weight (g)	731	13.6	5.6	725	23.3	8.0	719	31.7	10.8
Total Length (mm)	731	88.1	12.7	725	106.1	13.1	719	117.8	13.9
Height (mm)	731	27.2	4.1	725	32.6	4.0	719	36.0	4.5
Head (mm)	731	22.6	3.0	725	27.0	3.0	719	30.0	3.4
Drawn Weight (g)	-	-	-	-	-	-	716	27.4	9.5
Gills (mm ²)	-	-	-	-	-	-	692	321.7	79.7
Intestine (mm)	-	-	-	-	-	-	716	586.7	175.6

osmoregulatory functions (Smith, 1982; Gilles-Baillien and Gilles, 1983) in the culture of seawater tilapia. The coefficient of variation (standard deviation/mean) in these traits between males and females were approximately the same.

MATING SCHEME

The mating scheme of this study is shown in Appendix 2, which describes the farm origins of the adult breeders and the birthdates, cage locations, and cage densities of their families. The pairwise matings of male and female breeders from four farm locations was tested for independence or random distribution after introduction into the cages by the analysis of a 4 x 4 contingency table (Table 3). The Chi-square value was 11.81 with 9 df ($p \sim 0.25$) indicating no significant difference between the observed and expected distributions, hence, a fairly random distribution.

The progeny of sires from the four farm locations showed no significant difference in size from each other. This suggests a homogenous population rather than the existence of separate O. mossambicus farm populations.

CAGE DENSITY AND FISH MORTALITY

TABLE 3

CONTINGENCY TABLE OF MALE AND FEMALE BREEDERS
CLASSIFIED ACCORDING TO FARM LOCATION

MALES	F E M A L E S				TOTALS
	P	H	L	S	
P: Observed	9	4	12	3	28
Expected	9.26	5.61	10.66	2.81	28.34
H: Observed	5	1	7	1	14
Expected	4.63	2.81	5.33	1.40	14.17
L: Observed	6	6	4	1	17
Expected	5.64	3.42	6.49	1.71	17.26
S: Observed	0	1	0	1	2
Expected	0.60	0.37	0.70	0.18	1.85
TOTALS:					
Observed	20	12	23	6	61
Expected	20.13	12.21	23.18	6.10	61.62

$$\chi^2 = 11.81 \text{ with } 9 \text{ d.f., n.s.}$$

FARMS:

H = Hanohano Enterprises

P = Seafood Plantation

L = Amorient

S = Kahuku Prawn Co.

The total number of fish per cage for each family at 5 months of age are listed under the density column in Appendix 2. The lack of uniformity in this variable was exemplified in sire number 9 where the two half-sib families have respective cage densities of 25 and 4 fish.

There are several reasons for differing cage densities. In the first 2 months of age when hundreds of fry occupied a cage, mortality was often high due to the invasion of small, predatory, nocturnal crabs. Daily morning checks to remove crabs and securely fasten covers on cages proved a tedious battle until the fish grew large enough to escape their predators. Otherwise, crabs may have decimated entire fish cage populations.

Diseases and parasites during the early months of growth were also of paramount concern. In addition to marine monogenetic trematodes (Neobenedia spp.), copepods (Caligus spp.), and leeches (Piscicola spp.), fry appeared susceptible to bacterial septicemia (e.g. Vibriosis).

The area where this experiment was conducted was simultaneously being used by other HIMB investigators and Waikiki Aquarium staff to cage their fish specimens or collections. Such an accumulation of assorted fishes could have created a reservoir of parasites and diseases.

Preliminary studies as well as research conducted by Kaneko (1983) revealed a range of treatments and prophylactics. The entire experimental population was

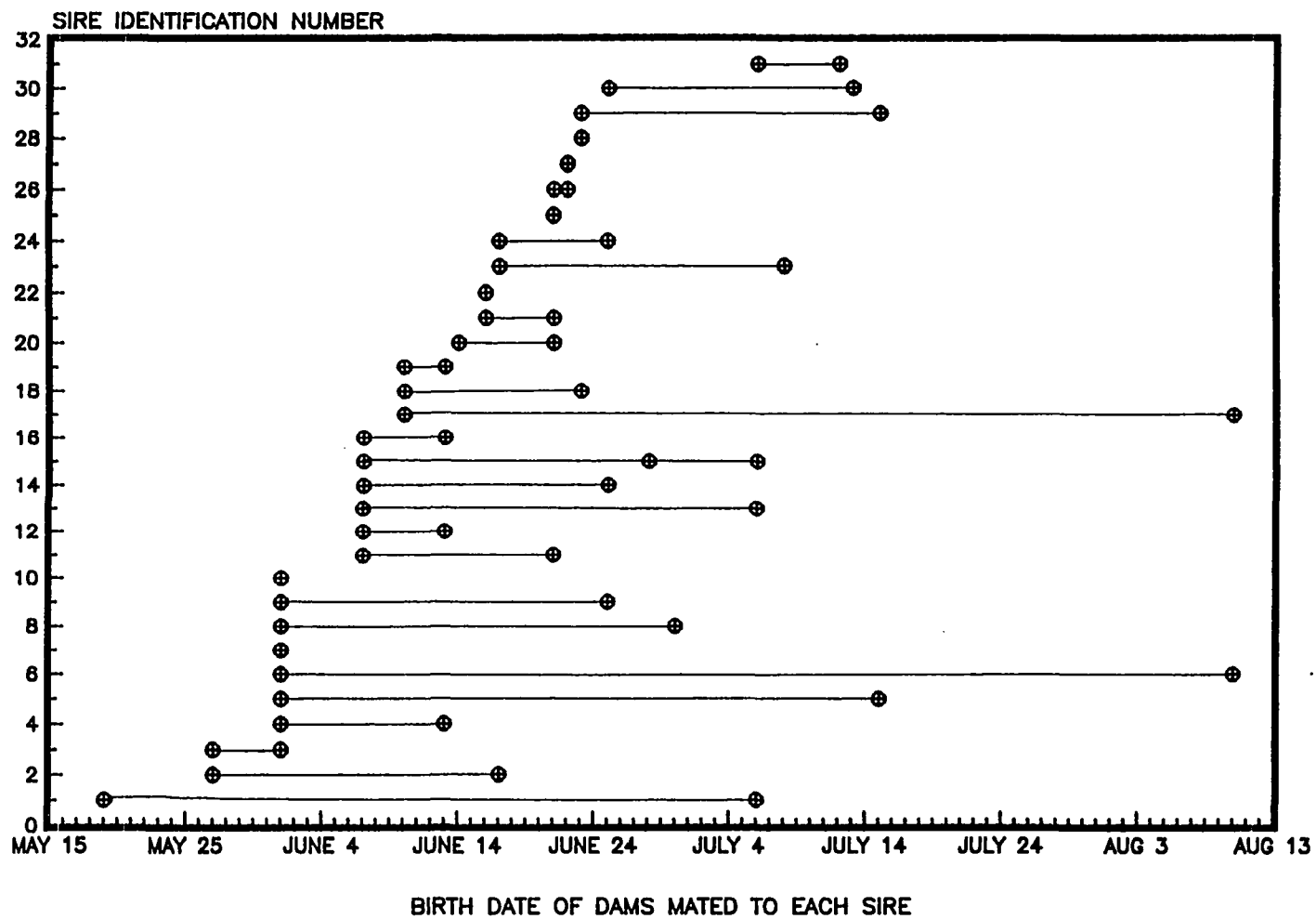
subjected to freshwater and antibiotic furacin dip treatments every 2-4 weeks. Tetracycline or chloramphenicol antibiotics were also added into pelleted diets as necessary. The residual or long-term effects that the illnesses and/or treatments had upon fish growth in this study are unknown. However, the survival or average cage density appeared to stabilize around the third month and was generally maintained through the fifth month. Since there was no evidence that these deaths were selectively based upon certain genotypes, it was assumed that all mortality was random within the population.

ADJUSTMENT OF DATA

Although every effort was made to standardize the environmental conditions in this study, a definite time effect existed due to the lack of synchronous birth dates among families. This is illustrated by the distribution of dams within sires graphed in Figure 4. The sires were chronologically rearranged from Appendix 2 by birth date for easier visualization. In this graph, sire 6 is the most obvious example showing the first half-sib family born on June 1st and the second half-sib family on August 10th - a difference of 70 days.

As a consequence, the variance between dams within sires was inflated due to environmental time differences

FIGURE 4
DAM DISTRIBUTION WITHIN SIRES



created by the differing birth dates of the respective families. To reduce this variance the adjustment of environmental effects was necessary. Adjustments were made on a within sire basis to preserve the genetic variation represented in the variation of sires.

Daily water temperatures and photoperiods were recorded at HIMB. Figure 5 shows the daily fluctuations in water temperature, ranging from a low of 70 degrees Fahrenheit to a high of 82 degrees Fahrenheit. Figure 6 shows the variation in photoperiods ranging from 11 to over 13 hours of light per day. It is evident that water temperature is subject to more fluctuation than photoperiod on a day to day basis. The water temperature and photoperiod means and standard deviations of the experimental fish populations for 1- and 2- months as well as 3- to 5- months of age are respectively shown in Tables 4 and 5. These tables show water temperature and photoperiod generally decreasing over the course of this study. Hence, data adjustment was made for water temperature, photoperiod, as well as on the stocking density of fish cages.

In using the multiple regression model for adjustment, one of the assumptions is that there be no exact relationship between the independent variables in the model. If there is collinearity, the estimated regression parameters would be quite imprecise as

FIGURE 5
DAILY WATER TEMPERATURE

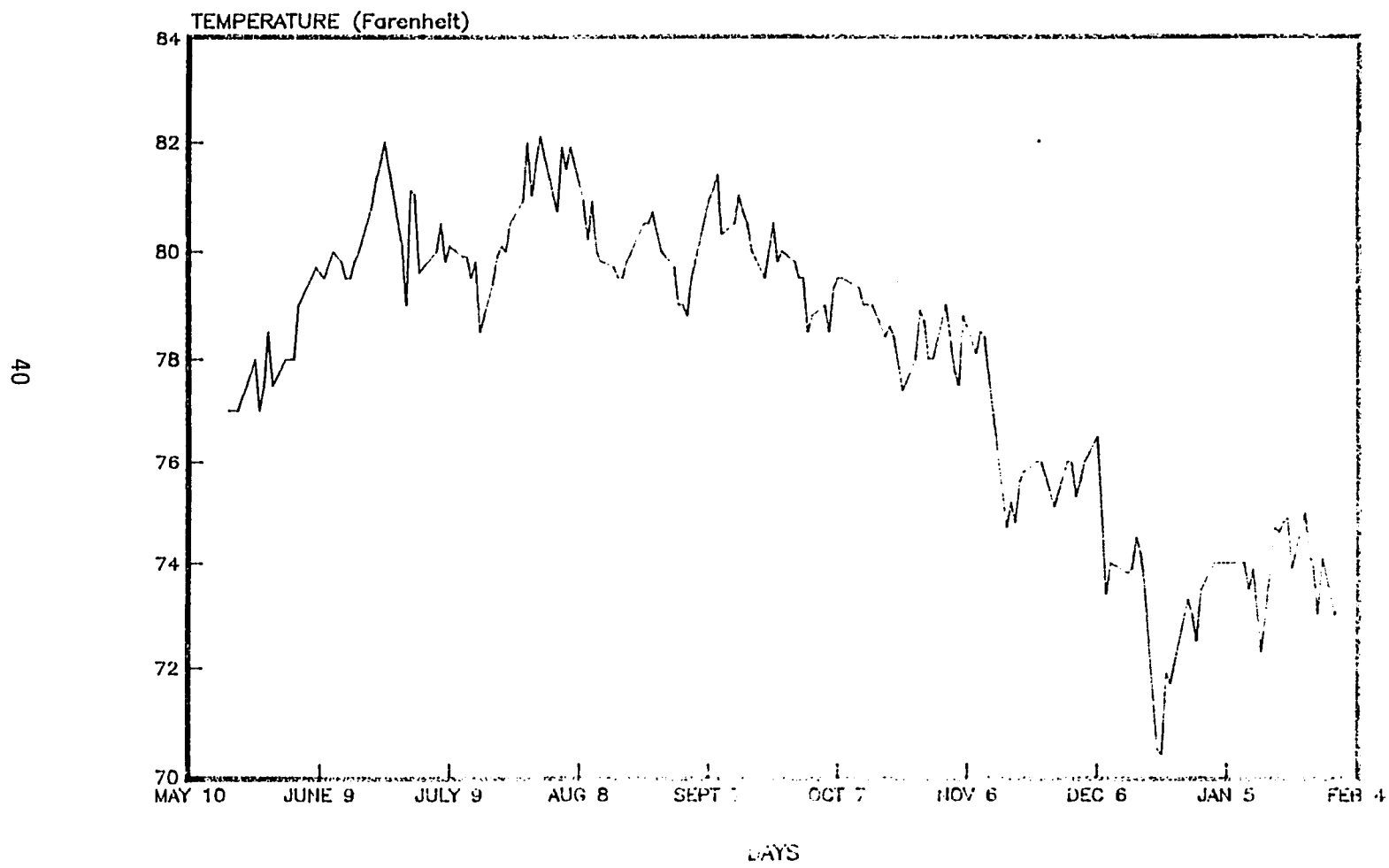


FIGURE 6
DAILY PHOTOPERIOD

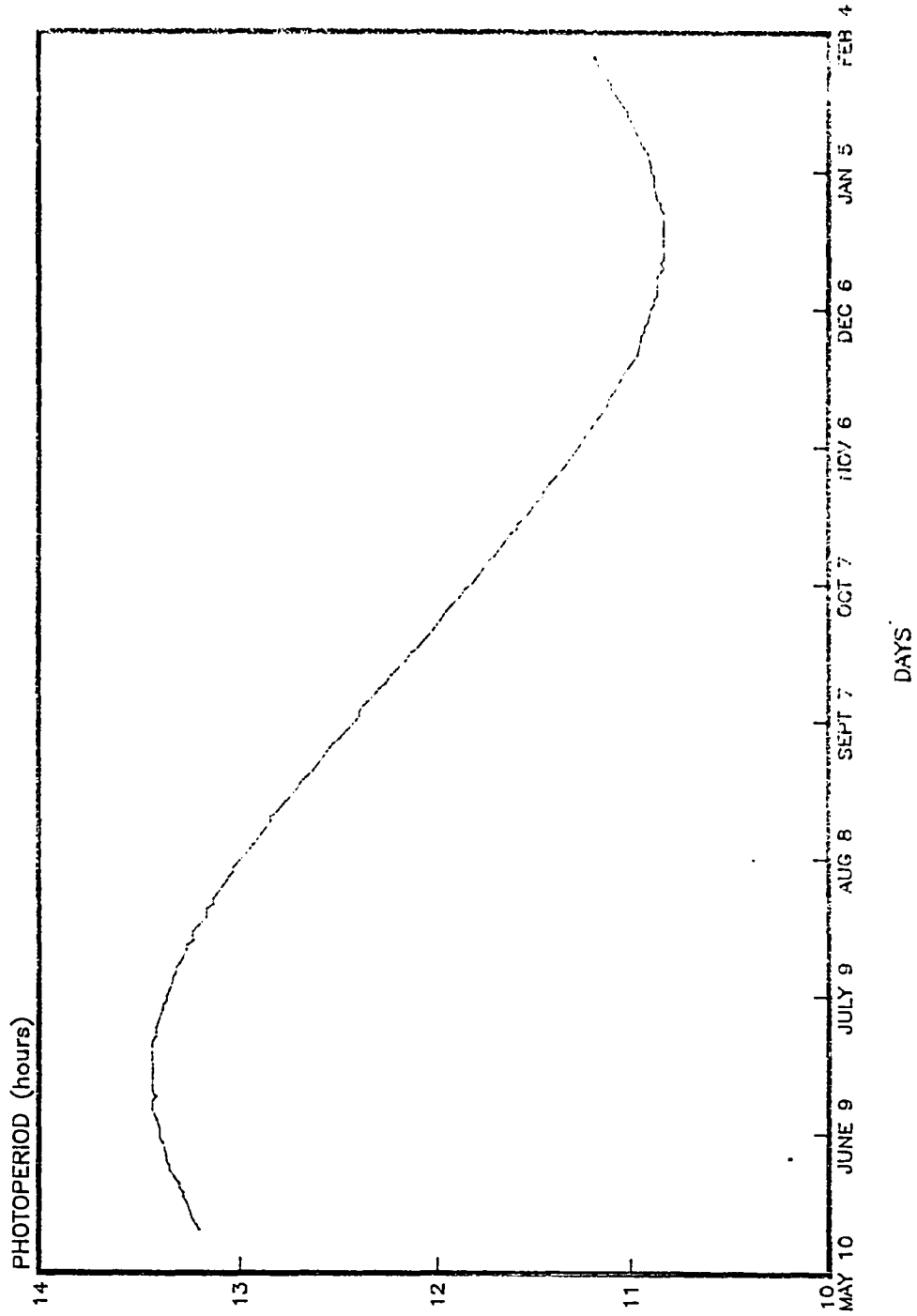


TABLE 4

DESCRIPTIVE STATISTICS OF ENVIRONMENTAL EFFECTS
AT 1- AND 2- MONTHS OF AGE

VARIABLE	1 Month			2 Months		
	N	MEAN	S.D.	N	MEAN	S.D.
Water Temperature (°F)	1477	80.1	0.3	1477	80.4	0.3
Photoperiod (hours)	1477	13.3	0.2	1477	13.0	0.3

TABLE 5

DESCRIPTIVE STATISTICS OF ENVIRONMENTAL EFFECTS
AT 3- TO 5- MONTHS OF AGE

VARIABLE	3 Months			4 Months			5 Months		
	N	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.
MALES:									
Water Temperature (°F)	971	80.0	0.5	971	79.1	1.1	971	77.4	1.4
Photoperiod (hours)	971	12.5	0.3	971	11.9	0.3	971	11.3	0.2
Density (fish/cage)	971	22.1	4.2	971	21.6	4.5	971	21.4	4.6
FEMALES:									
Water Temperature (°F)	733	80.0	0.5	733	79.1	1.1	733	77.4	1.4
Photoperiod (hours)	733	12.5	0.3	733	11.9	0.3	733	11.3	0.2
Density (fish/cage)	733	22.2	4.2	733	21.8	4.5	733	21.7	4.5

expressed in the form of very high standard errors for the estimated regression parameter. This indicates one of the highly correlated independent variables should be dropped from the analysis (Pindyck and Rubinfeld, 1981). Since this experimental design is unbalanced with respect to time, the correlations between environmental effects from monthly family means are correspondingly low. Thus collinearity is not a concern. When simulataneously incorporated into the multiple regression model, the regression coefficients of water temperature, photoperiod, and density did not have unduly large standard errors (Appendix 3a,b). There is no bias due to misspecification of the model. Furthermore, a F-test of the additional sum of squares from the simultaneous inclusion of temperature, photoperiod, and density over that of a one or two independent variable model proved to be a significantly better regression model. The coefficient of determination of the three independent variables was approximately 15 percent.

All first order interaction effects were found not to be significant. In addition, the effects of water temperature, photoperiod, and density were judged to be linear because their second degree terms in the regression model were calculated, tested, and found to be insignificant. This is illustrated by the average water temperature graphs (Figures 7 to 11) and average

photoperiod graphs (Figures 12 to 16) for each family at 1- to 5- months of age. None of these graphs are curvilinear. Although there were 61 families, less than that number are shown because some families were born on the same day and, thus, share the same average values. Growth was adjusted to a water temperature of 80 degrees Fahrenheit, a photoperiod of 12 hours of light per day, and a density of 20 fish per cage.

The least squares adjustment factors for the environmental effects of water temperature, photoperiod, and density have been tabulated in Appendix 3a for 1- and 2- month old data and 3b for 3- to 5- month old data. It is interesting to note that the significant regression coefficients of water temperature, photoperiod, and density in all traits in every month class have slopes that are respectively positive, negative, and negative. The positive slope of water temperature implies that growth was promoted by increased water temperature for increased metabolic activity. Lower cage density very likely led to reduced conspecific interaction or competition, hence, more growth. The negative growth effect of lengthened photoperiod was possibly created by oxygen depletion due to prolonged photosynthetic activity of the various micro and macro algal communities growing on the cages.

In addition to the effects of water temperature,

FIGURE 7
AVERAGE TEMPERATURE OF EACH FAMILY
1st MONTH GROWTH

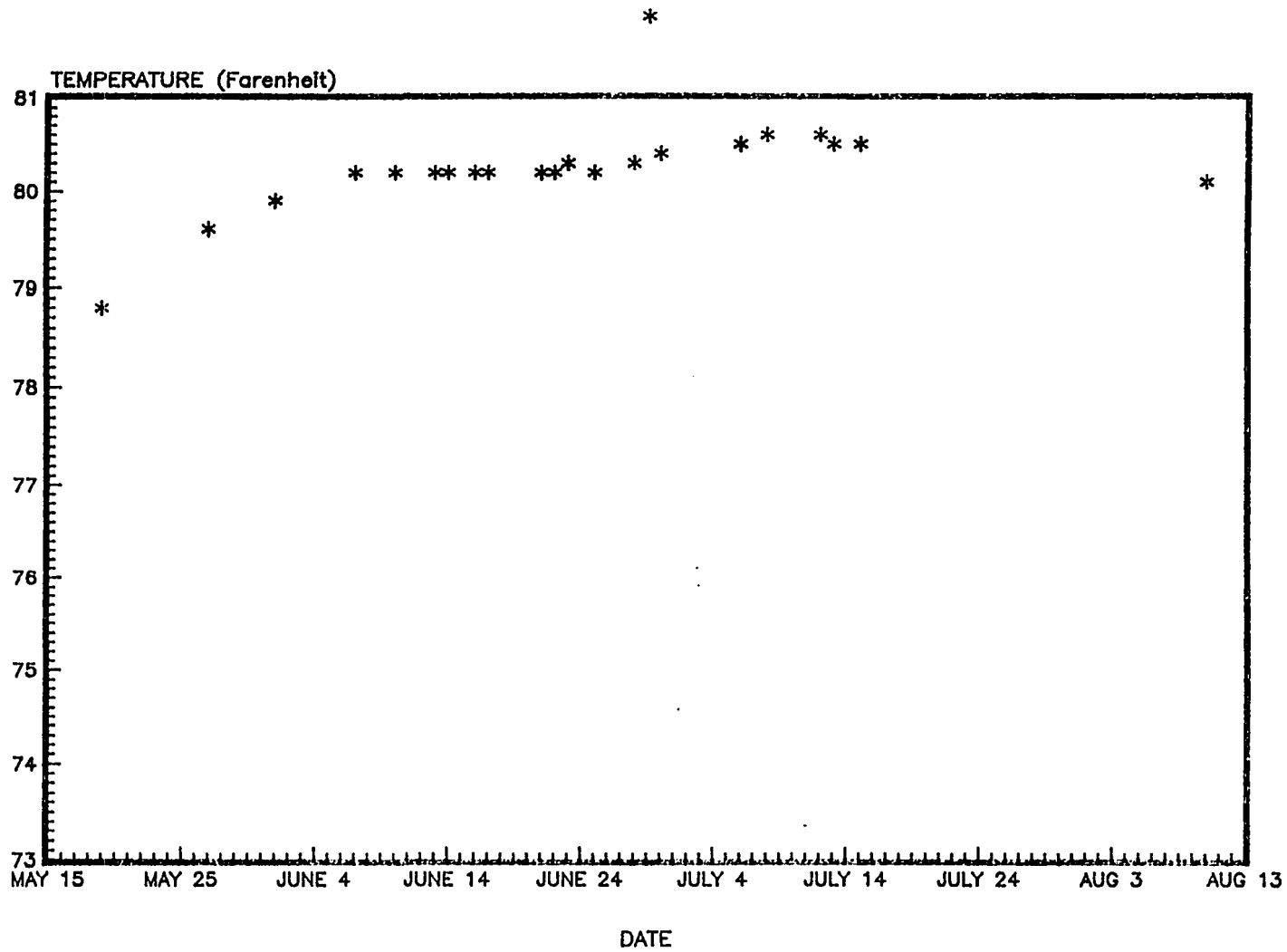


FIGURE 8
AVERAGE TEMPERATURE OF EACH FAMILY

2nd MONTH GROWTH

*

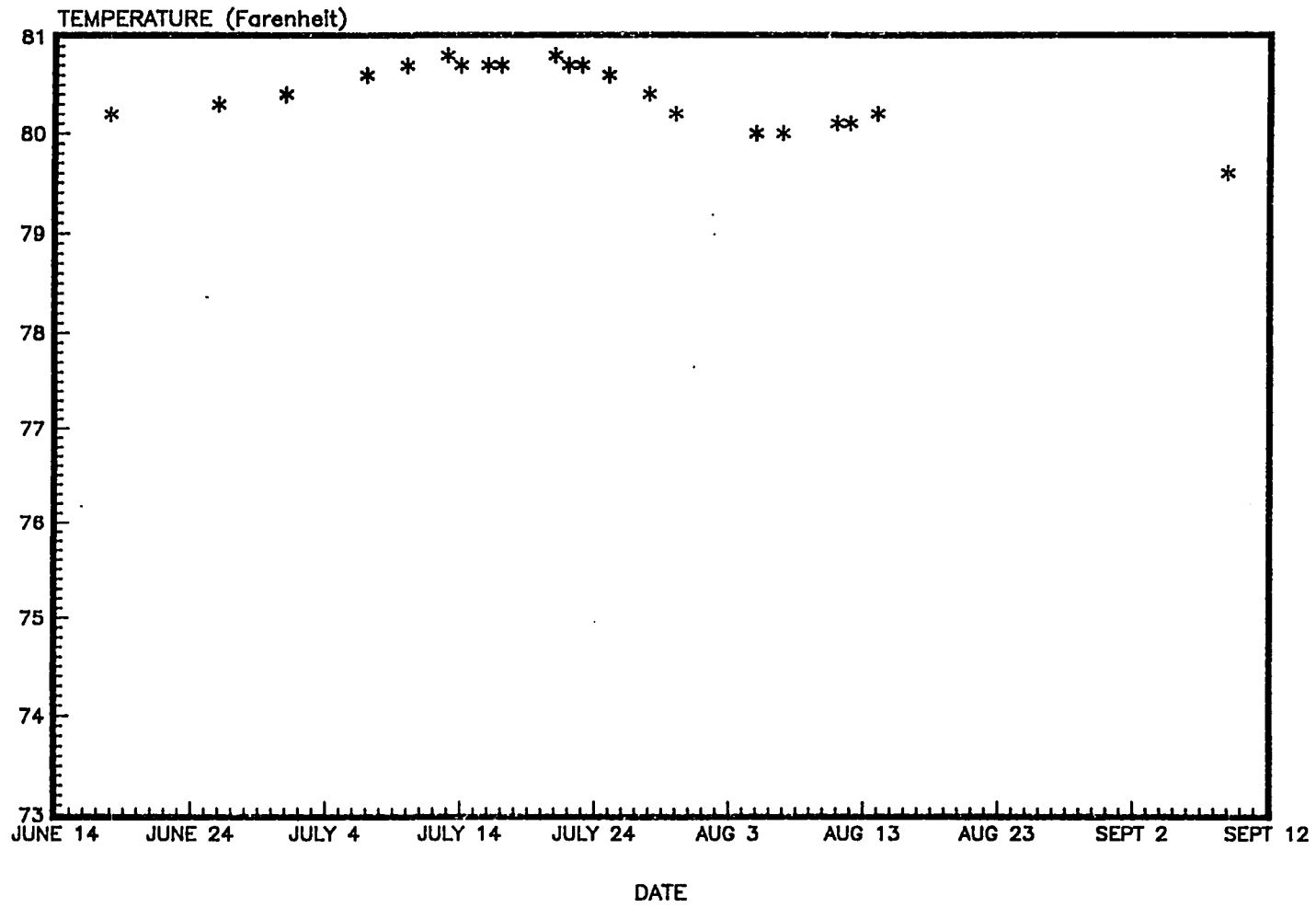


FIGURE 9
AVERAGE TEMPERATURE OF EACH FAMILY

3rd MONTH GROWTH

*

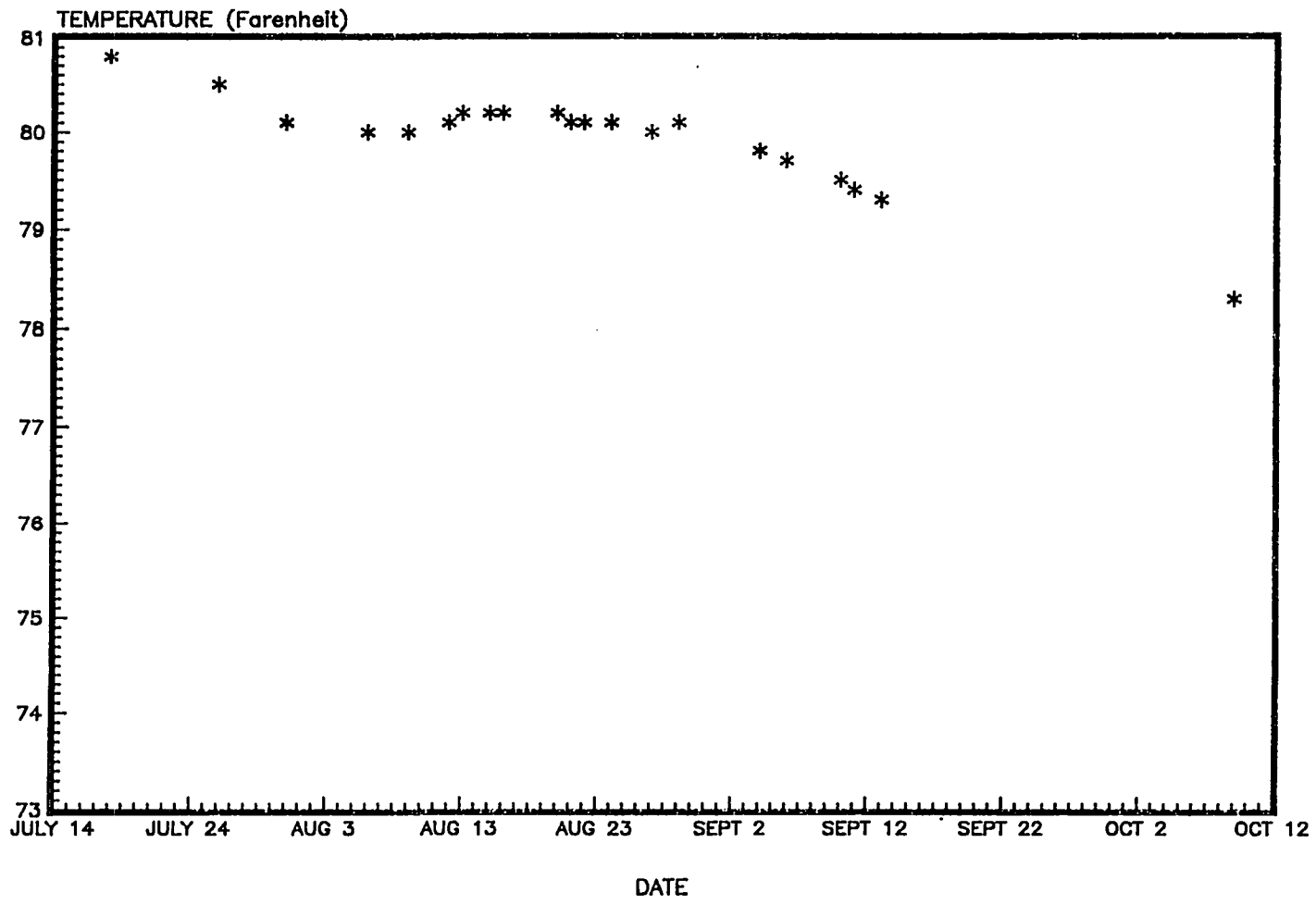


FIGURE 10
AVERAGE TEMPERATURE OF EACH FAMILY

4th MONTH GROWTH

*

49

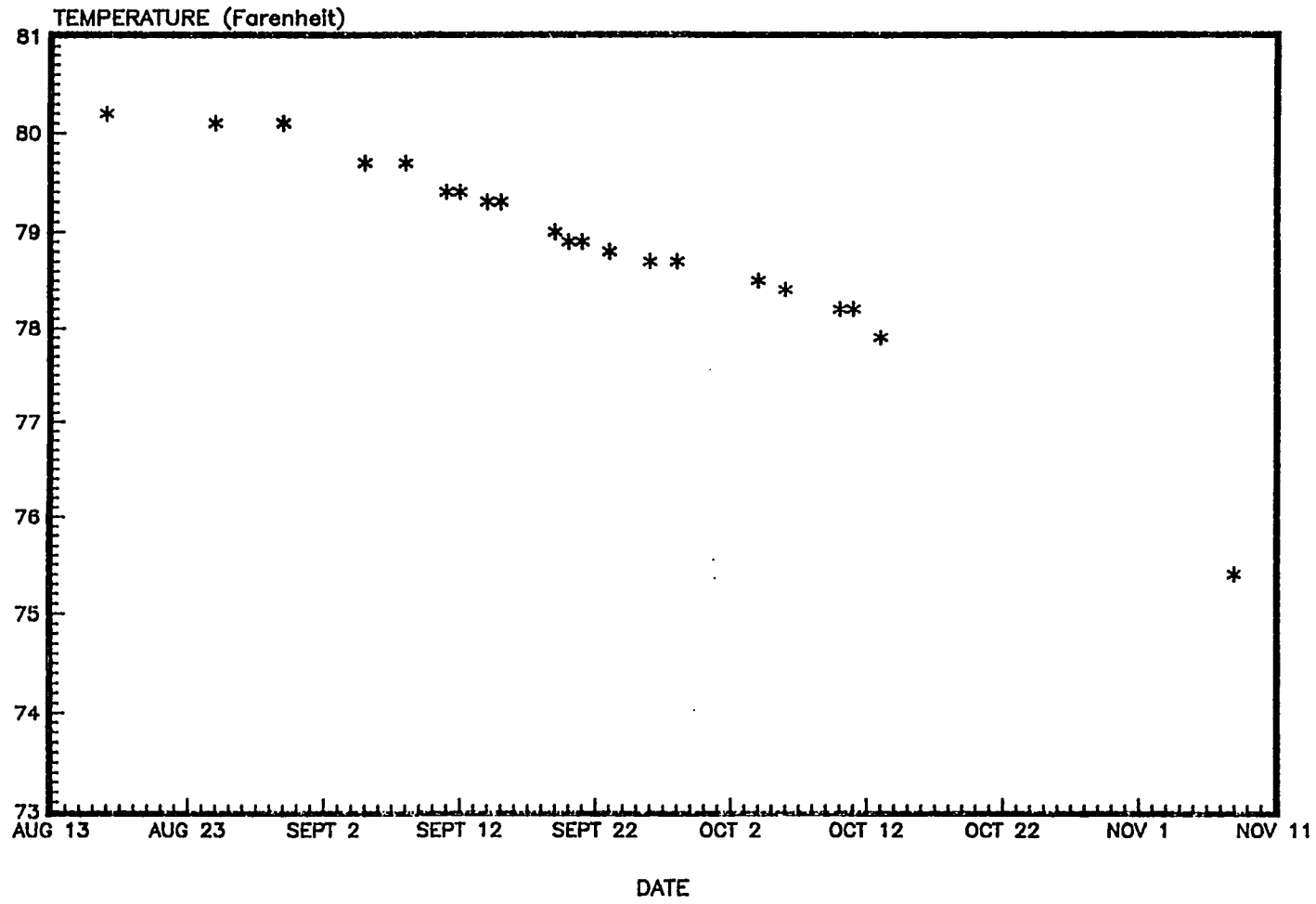


FIGURE 11
AVERAGE TEMPERATURE OF EACH FAMILY

5th MONTH GROWTH

*

50

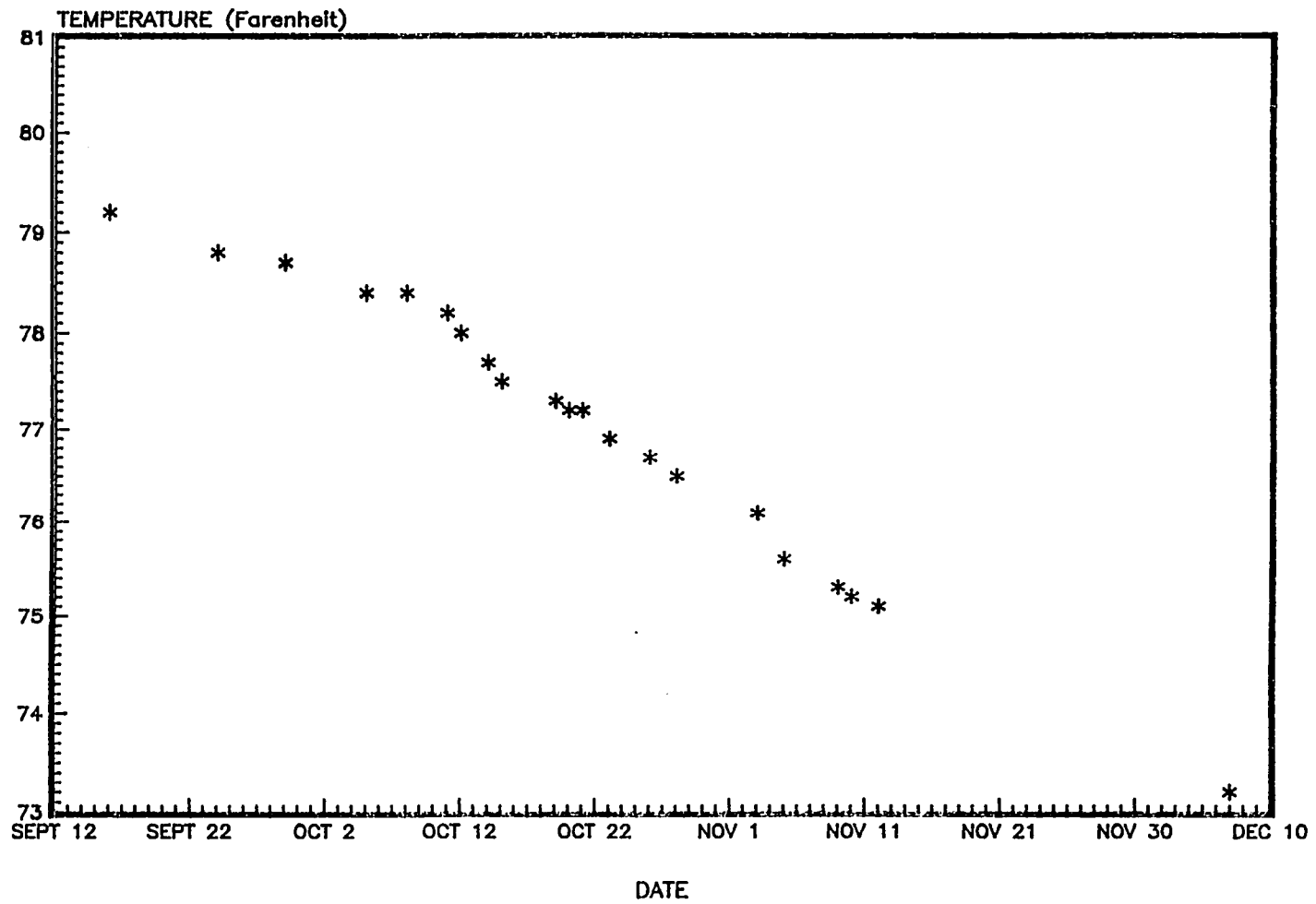


FIGURE 12
AVERAGE PHOTOPERIOD OF EACH FAMILY

1st MONTH GROWTH

△

51

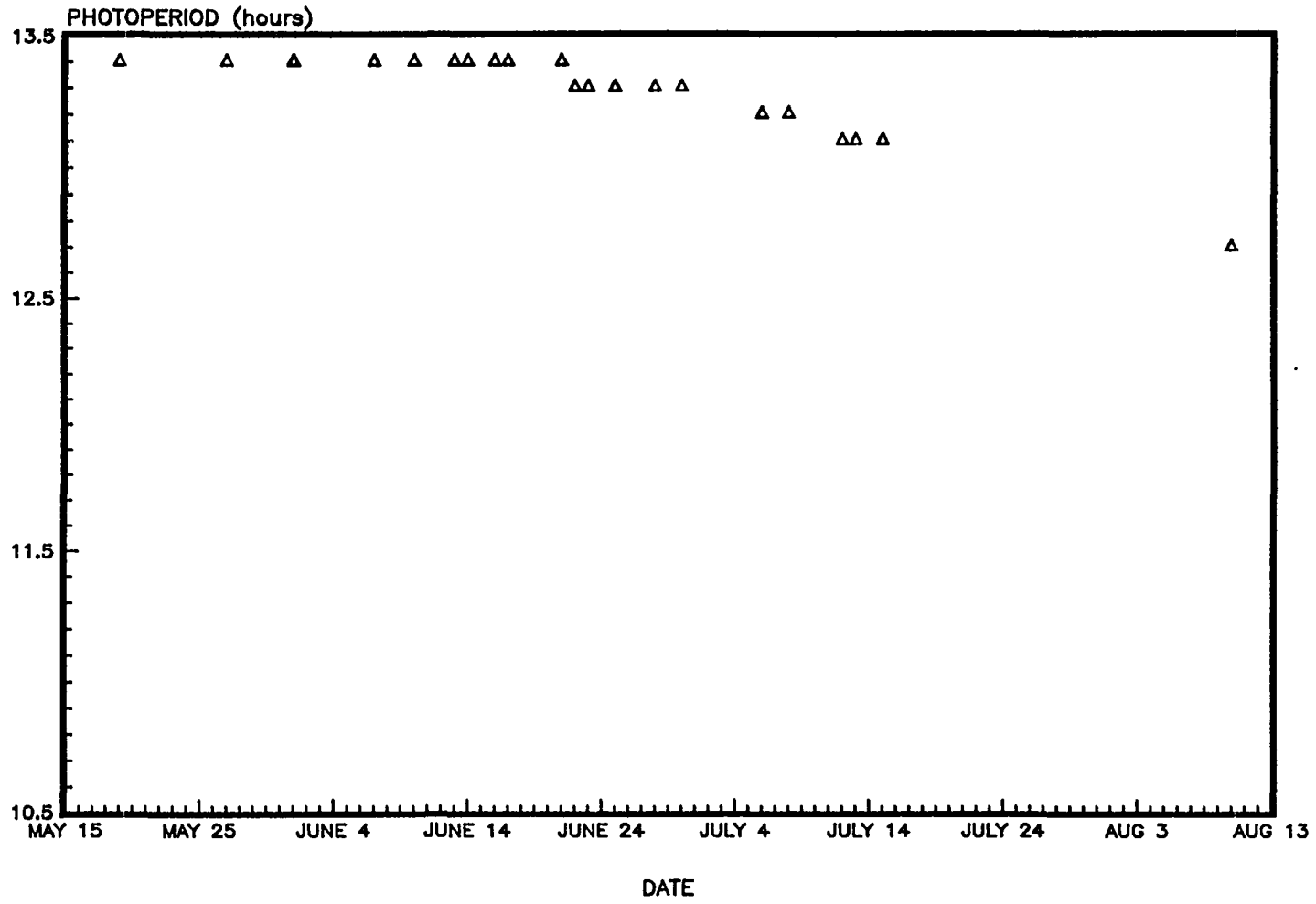


FIGURE 13
AVERAGE PHOTOPERIOD OF EACH FAMILY

2nd MONTH GROWTH

△

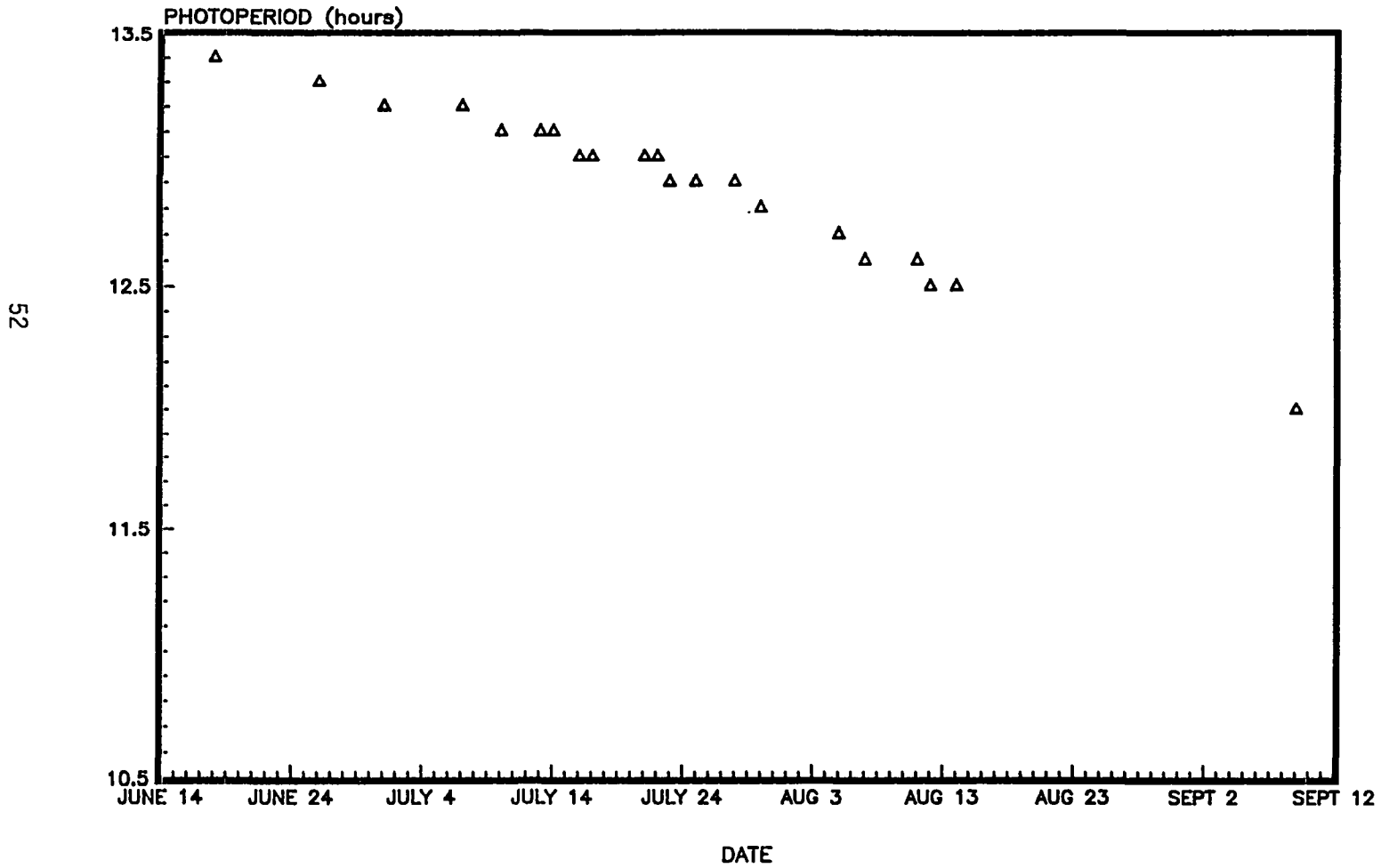


FIGURE 14
AVERAGE PHOTOPERIOD OF EACH FAMILY

3rd MONTH GROWTH

△

53

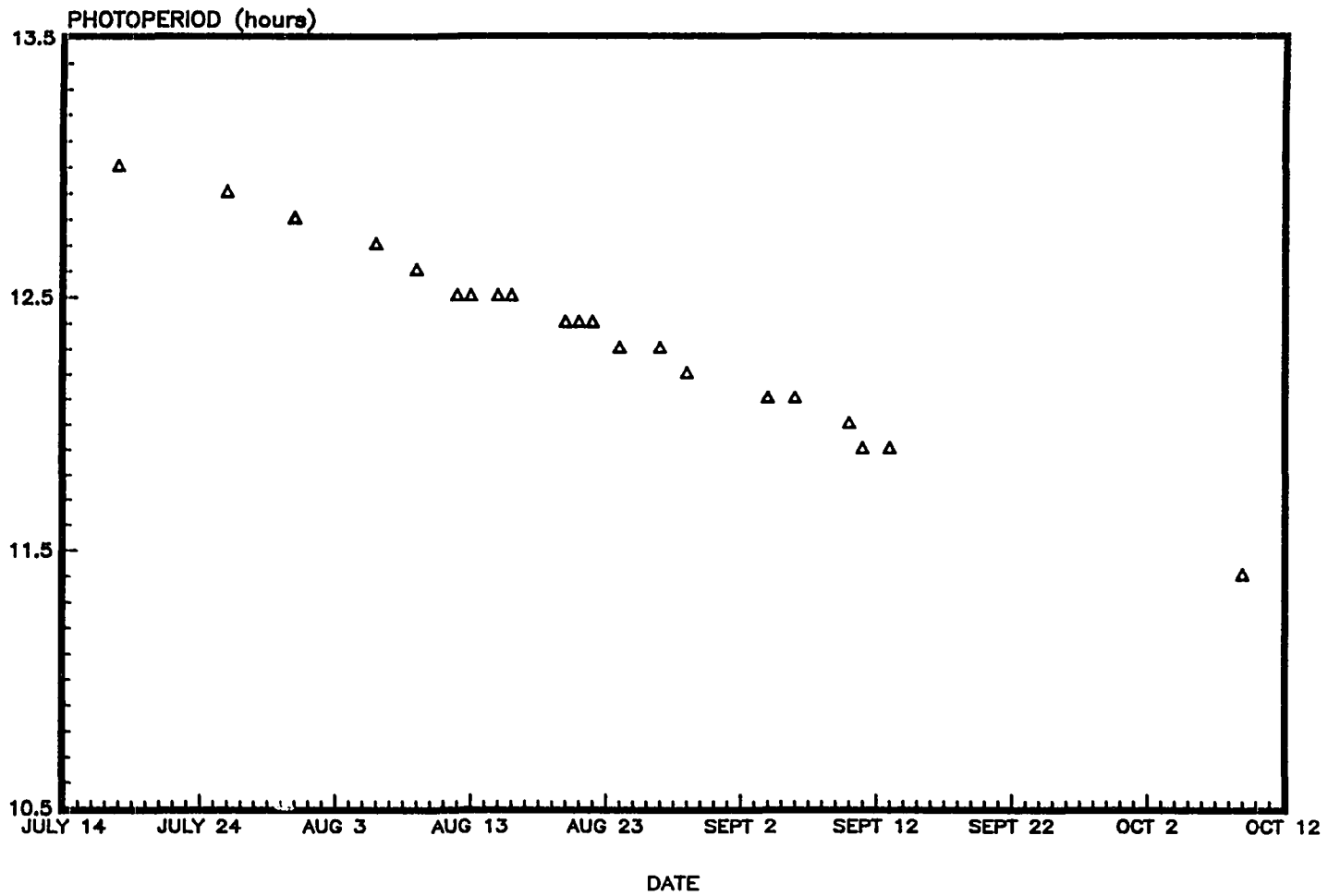
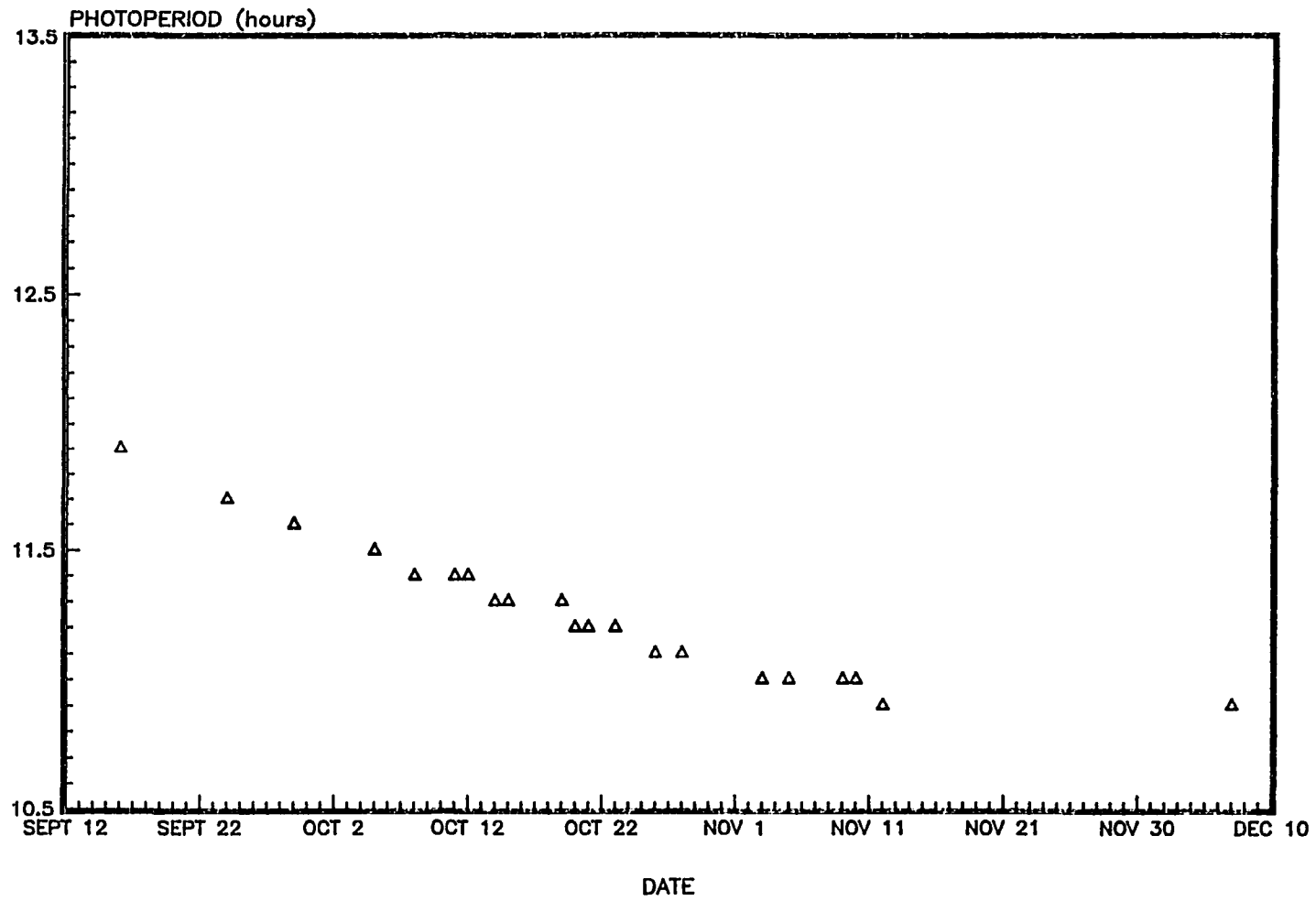


FIGURE 16
AVERAGE PHOTOPERIOD OF EACH FAMILY

5th MONTH GROWTH

△

55



photoperiod, and density there were probably other factors involved in this study. For example, the influence of micro-environmental differences from the location of cages at the experimental site could not be estimated because of the confounding of sires and dams with cage location. The sex composition in a family was thought to have an important social interaction effect, but when tested in the statistical model was found to be insignificant. Other factors influencing body measurements probably existed in this study but remain unidentified.

GENETIC ANALYSIS

The mean squares for 1- and 2- month old traits are shown in Table 6 after data were adjusted for water temperature and photoperiod. Density was not considered. Difference among dams within sires were highly significant for all traits ($p < 0.01$), but differences among sires were not significant. For the 1- month old fish measurements, the sire mean squares are smaller than the dam mean squares suggesting a zero sire component of variance. This is reflected in the variance components estimated in Table 7. In 2- month old fish measurements a sire component was recorded, but is not statistically significant. To follow the change in the components of variance between 1- and 2- months of age, Table 8 shows the additive genetic,

TABLE 6

ANOVA OF TRAITS AT 1- AND 2- MONTHS OF AGE

<u>SOURCE</u>	<u>df</u>	<u>1 Month</u>	<u>2 Months</u>
WEIGHT:			
Sire	30	5.52	134.05
Dam/Sire	30	6.58 **	83.74 **
Residual	1416	0.22	7.01
TOTAL LENGTH:			
Sire	30	853.50	1760.01
Dam/Sire	30	890.88 **	968.19 **
Residual	1416	33.54	102.26
HEAD:			
Sire	30	55.47	100.18
Dam/Sire	30	59.31 **	55.59 **
Residual	1416	2.25	6.61
HEIGHT:			
Sire	30	88.39	176.94
Dam/Sire	30	97.57 **	103.17 **
Residual	1416	3.88	11.13

*p < 0.05

**p < 0.01

K COEFFICIENTS

K1 = 22.06

K2 = 26.28

K3 = 47.56

TABLE 7
 VARIANCE COMPONENTS FOR TRAITS
 AT 1- AND 2- MONTHS OF AGE

<u>VARIANCE COMPONENT</u>	<u>WEIGHT</u>	<u>LENGTH</u>	<u>HEAD</u>	<u>HEIGHT</u>
1 Month:				
σ_s^2	0.00	0.00	0.00	0.00
σ_D^2	0.29	38.87	2.59	4.25
σ_w^2	0.22	33.54	2.25	3.88
2 Months:				
σ_s^2	0.75	13.17	0.74	1.18
σ_D^2	3.48	39.26	2.22	4.17
σ_w^2	7.01	102.26	6.61	11.13

σ_s^2 = Sire Component
 σ_D^2 = Dam Component
 σ_w^2 = Within Progeny

TABLE 8
 PERCENTAGE OF GENETIC, MATERNAL, AND ENVIRONMENTAL VARIATION
 RELATIVE TO PHENOTYPIC VARIATION AT 1- AND 2- MONTHS OF AGE

<u>VARIANCE</u>	<u>WEIGHT</u>	<u>TOTAL LENGTH</u>	<u>HEAD</u>	<u>HEIGHT</u>
1 Month:				
V(G)	0.0	0.0	0.0	0.0
V(M)	56.9	53.7	53.5	52.3
V(E)	43.1	46.3	46.5	47.7
2 Months:				
V(G)	26.7	34.1	30.9	28.6
V(M)	24.3	16.9	15.5	18.1
V(E)	49.0	49.1	53.6	53.2

V(G) = Additive Genetic Variance
 V(M) = Maternal Variance
 V(E) = Environmental Variance

maternal, and environmental components of variance as percentages. The percentage of genetic variance exhibits a marked increase with time while the maternal variance percentage decreases. Although differences among sires were not significant, the potential trend corresponds with Kirpichnikov (1982) who states that early environmental variation (e.g. maternal variation) is very high at early stages of a fish's life, but decreases thereafter with a 2 to 3 times concomitant increase in the heritability of weight and size. Since differences among sires were not significant, the heritabilities and genetic correlations were not estimated.

The ANOVA for weight at 3- to 5- months of age after adjustment of data is shown in Table 9. The mean square values for the males was notably higher than the females thus justifying the separate analysis by sex. The ANOVA table for weight is quite representative of the other traits of total length, head, height, drawnweight, intestinal length, and gill surface area (Tables 10 to 13). In general, the difference among dams within sires were significant for all the traits through time, but differences among sires were not significant. However, where sire differences occurred, especially at the fifth month, the differences among dams within sires were not significant. There was no instance when both the among sire and among dam within sire differences were

TABLE 9

ANOVA FOR WEIGHT AT 3- TO 5- MONTHS OF AGE

<u>SOURCE</u>	<u>df</u>	<u>3 Months</u>	<u>4 Months</u>	<u>5 Months</u>
MALES:				
Sire	30	568.58	1566.43	3247.73
Dam/Sire	29	357.64 **	900.58 *	1789.86
Rep/Dam/Sire	22	65.35 **	374.57 **	1273.99 **
Residual	863	32.41	108.22	247.72
FEMALES:				
Sire	30	116.61	345.21	651.33 **
Dam/Sire	29	77.80 **	196.03 *	249.90
Rep/Dam/Sire	22	16.80	94.18 **	254.96 **
Residual	635	15.89	37.07	73.02

*p < 0.05

**p < 0.01

K COEFFICIENTS

	<u>Males</u>	<u>Females</u>
K1 =	10.80	8.93
K2 =	10.73	8.20
K3 =	14.60	11.27
K4 =	12.78	9.09
K5 =	16.76	12.48
K6 =	30.33	22.91

TABLE 10
ANVOA FOR TOTAL LENGTH AT 3- TO 5- MONTHS OF AGE

<u>SOURCE</u>	<u>df</u>	<u>3 Months</u>	<u>4 Months</u>	<u>5 Months</u>
MALES:				
Sire	30	1884.37	2018.20	2393.67
Dam/Sire	29	1208.48 **	1299.01 **	1373.23
Rep/Dam/Sire	22	196.47	427.74 **	804.73 **
Residual	863	136.46	187.45	252.80
FEMALES:				
Sire	30	479.79	759.11	963.31 *
Dam/Sire	29	465.79 **	504.18 *	410.85
Rep/Dam/Sire	22	103.93	232.65 **	318.46 **
Residual	635	84.42	100.49	128.67

*p < 0.05
**p < 0.01

<u>K COEFFICIENTS</u>	
<u>Males</u>	<u>Females</u>
K1 = 10.80	8.93
K2 = 10.73	8.20
K3 = 14.60	11.27
K4 = 12.78	9.09
K5 = 16.76	12.48
K6 = 30.33	22.91

TABLE 11

ANOVA FOR HEIGHT SIZE AT 3- TO 5- MONTHS OF AGE

<u>SOURCE</u>	<u>df</u>	<u>3 Months</u>	<u>4 Months</u>	<u>5 Months</u>
MALES:				
Sire	30	167.14	164.14	238.53
Dam/Sire	29	111.19 **	117.93 *	117.50
Rep/Dam/Sire	22	24.49 **	54.99 **	108.88 **
Residual	863	13.18	19.08	27.05
FEMALES:				
Sire	30	118.88	67.61	91.18 *
Dam/Sire	29	102.70 **	50.14	43.30
Rep/Dam/Sire	22	10.74	26.79 **	40.52 **
Residual	635	9.07	10.29	13.66

*p < 0.05

**p < 0.01

K COEFFICIENTS

	<u>Males</u>	<u>Females</u>
K1 =	10.80	8.93
K2 =	10.73	8.20
K3 =	14.60	11.27
K4 =	12.78	9.09
K5 =	16.76	12.48
K6 =	30.33	22.91

TABLE 12
ANOVA FOR HEAD SIZE AT 3- TO 5- MONTHS OF AGE

<u>SOURCE</u>	<u>df</u>	<u>3 Months</u>	<u>4 Months</u>	<u>5 Months</u>
MALES:				
Sire	30	106.29	112.78	150.05 *
Dam/Sire	29	67.39 **	70.63 **	65.28
Rep/Dam/Sire	22	12.46	24.42 **	49.65 **
Residual	863	8.36	12.45	16.54
FEMALES:				
Sire	30	26.16	38.60	52.61 *
Dam/Sire	29	25.87 **	25.94	20.70
Rep/Dam/Sire	22	7.54	15.68 **	18.33 **
Residual	635	5.20	5.71	7.65

*p < 0.05

**p < 0.01

K COEFFICIENTS

	<u>Males</u>	<u>Females</u>
K1 =	10.80	8.93
K2 =	10.73	8.20
K3 =	14.60	11.27
K4 =	12.78	9.09
K5 =	16.76	12.48
K6 =	30.33	22.91

TABLE 13

ANOVA FOR DRAWNWEIGHT, INTESTINAL LENGTH, AND GILL SURFACE AREA
AT 5- MONTHS OF AGE

<u>SOURCE</u>	<u>df</u>	<u>Drawnweight</u>	<u>df</u>	<u>Intestine</u>	<u>df</u>	<u>Gills</u>
MALES:						
Sire	30	2335.26	30	448324.00 *	30	151317.00 **
Dam/Sire	29	1399.59	29	191067.00	28	45161.60
Rep/Dam/Sire	22	988.97 **	22	152479.00 **	20	55022.20 **
Residual	861	199.63	861	30597.70	835	8299.95
FEMALES:						
Sire	30	466.16 *	30	164632.00 **	30	36890.10
Dam/Sire	29	198.89	29	59123.00	28	18628.90 *
Rep/Dam/Sire	22	199.50 **	22	42159.50 **	20	9183.41 **
Residual	634	56.58	634	16335.30	613	3845.59

*p < 0.05

**p < 0.01

	K COEFFICIENTS (Drawnweight)		K COEFFICIENTS (Intestine)		K COEFFICIENTS (Gills)	
	Males	Females	Males	Females	Males	Females
K1 =	10.78	8.90	10.78	8.90	10.95	8.84
K2 =	10.71	8.20	10.71	8.20	10.61	8.31
K3 =	14.58	11.27	14.58	11.27	14.30	11.19
K4 =	12.75	9.08	12.75	9.08	12.84	9.08
K5 =	16.72	12.45	16.72	12.45	16.50	12.11
K6 =	30.26	22.87	30.26	22.87	29.32	22.09

significant.

The replication differences demonstrated an increasing level of significance from 3- to 5- months of age in all observed traits. This source of variation represented the contemporaneous effect between cages.

In Table 14, variance components were re-calculated after partitioning out replicate effects derived from the replicated family data. The mean squares were then re-calculated accordingly with significant among sire and among dam within sire differences observed in both males and females at 4- months of age for weight and in males only at 5- months of age for weight, drawnweight, and total length. The estimated narrow-sense heritabilities derived from the sire component of variance for these traits are shown below:

	<u>MONTH</u>	
	<u>4</u>	<u>5</u>
<u>MALES</u>		
Weight	0.39	0.39
Drawnweight	-	0.31
Total Length	-	0.31
<u>FEMALES</u>		
Weight	0.39	-

The genetic correlations for the above male traits

TABLE 14

VARIANCE COMPONENTS FOR TRAITS AT
3- TO 5-MONTHS OF AGE

<u>VARIANCE COMPONENT</u>	<u>WEIGHT</u>	<u>TOTAL LENGTH</u>	<u>HEAD</u>	<u>HEIGHT</u>	<u>DRAWN-WEIGHT</u>	<u>INTESTINE</u>	<u>GILLS</u>
<u>MALES</u>							
3 Months:							
σ_S^2	5.37	17.06	1.00	1.37	-	-	-
σ_D^2	16.47	65.79	3.59	5.50	-	-	-
σ_W^2	38.98	145.47	8.91	14.65	-	-	-
4 Months:							
σ_W^2	18.12	18.32	1.11	1.05	-	-	-
σ_D^2	29.60	57.21	2.97	4.12	-	-	-
σ_W^2	139.05	211.92	13.74	22.56	-	-	-
5 Months:							
σ_S^2	40.67	28.25	2.56	3.56	24.92	7735.07	3389.51
σ_D^2	41.95	43.32	1.28	2.14	33.76	4510.59	104.74
σ_W^2	335.14	298.99	19.36	34.99	266.78	39979.31	11814.34
<u>FEMALES</u>							
3 Months:							
σ_S^2	1.41	0.00	0.00	0.27	-	-	-
σ_D^2	5.22	31.68	1.56	7.97	-	-	-
σ_W^2	16.19	87.17	5.55	9.46	-	-	-
4 Months:							
σ_S^2	5.78	9.26	0.46	0.58	-	-	-
σ_D^2	7.77	24.11	1.10	2.10	-	-	-
σ_W^2	45.15	116.22	6.71	12.24	-	-	-
5 Months:							
σ_S^2	16.73	22.83	1.33	1.96	11.08	4510.29	769.32
σ_D^2	4.09	11.89	0.50	1.01	3.35	103.11	716.51
σ_W^2	90.17	147.61	8.64	16.10	70.29	20736.06	4610.51

σ_S^2 = Sire Component
 σ_D^2 = Dam Component
 σ_W^2 = Within Progeny Component

are as follows:

	<u>WEIGHT-4</u>	<u>WEIGHT-5</u>	<u>DRAWN WEIGHT-5</u>	<u>TOTAL LENGTH-5</u>
WEIGHT-4	-	0.92	0.88	0.87
WEIGHT-5		-	1.01	0.95
DRAWNWEIGHT-5			-	0.94
TOTAL LENGTH-5				-

The larger variance components in males than in females is a reflection of their sexually dimorphic growth rate. The current explanations for the cause or mechanisms of dimorphism in tilapia range from "incompletely understood" to "unknown." Lowe-McConnell (1982) suggests an environmental and behavioral basis of sexually dimorphic growth rather than a genetic one. It is possible that precocious sexual maturation in females for vitellogenesis may stunt growth more than in males for spermatogenesis, thus, providing smaller variance component estimates in females than in males. The variability in the onset of sexual maturity in males and females have not been adequately studied in tilapia (Jalabert and Zohar, 1982). However, if females mature earlier than in males the switch from growth to reproduction may also account for their smaller observed variances.

For both sexes, the dam variance components in 3-

and 4- month old fish measurements (Table 14) are larger than the sire variance component. However, for females at the 5- month age, the sire variance component exceeds the dam variance component. In genetic theory the dam component should be equal to or greater than the sire component because it contains additional non-additive genetic elements such as dominance genetic variation and common environmental variation (Falconer, 1981). This discrepancy perhaps has a behavioral explanation. At the time of sacrifice, most males and females had mature gonads and even a few females were caught mouthbrooding their young. Consequently, if territoriality or sexually aggressive behavior has a genetic basis in males, then the sire variance component in females at 5- months of age will be inflated relative to the dam variance component. For the male traits of head and height size, intestinal length, and gill surface area at 5- months of age the mechanisms responsible for the sire component being larger than the dam component remains unclear.

In Table 15, the additive genetic, maternal, and environmental components of variance have been calculated into percentages. The corresponding male and female estimates are different due to the sexually dimorphic growth rates. However, as in the 1- and 2- month old data the overall trend, especially in the males, appears to favor an increase in the genetic component and a decrease

TABLE 15

PERCENTAGE OF GENETIC, MATERNAL, AND ENVIRONMENTAL
VARIANCE COMPONENTS AT 3- TO 5-MONTHS OF AGE

<u>VARIANCE COMPONENT</u>	<u>WEIGHT</u>	<u>TOTAL LENGTH</u>	<u>HEAD</u>	<u>HEIGHT</u>	<u>DRAWN-WEIGHT</u>	<u>INTESTINE</u>	<u>GILLS</u>
<u>MALES</u>							
3 Months:							
V(G)	35.3	29.9	29.6	25.5	-	-	-
V(M)	18.3	21.3	19.2	19.2	-	-	-
V(E)	46.4	48.8	51.2	55.3	-	-	-
4 Months:							
V(G)	38.8	25.5	24.9	15.1	-	-	-
V(M)	6.1	13.5	10.4	11.1	-	-	-
V(E)	55.0	61.0	64.6	73.8	-	-	-
5 Months:							
V(G)	39.2	30.5	41.8	35.5	30.6	55.8	72.9
V(M)	0.3	4.1	#	#	2.7	#	#
V(E)	60.5	65.4	58.2	64.5	66.7	44.2	27.1
<u>FEMALES</u>							
3 Months:							
V(G)	24.6	0.0	0.0	6.1	-	-	-
V(M)	16.7	26.7	21.9	43.5	-	-	-
V(E)	58.7	73.3	78.1	50.4	-	-	-
4 Months:							
V(G)	39.4	24.8	22.2	15.5	-	-	-
V(M)	3.4	9.9	7.7	10.2	-	-	-
V(E)	57.2	65.3	70.0	74.3	-	-	-
5 Months:							
V(G)	54.1	47.2	47.1	39.2	47.9	60.6	50.0
V(M)	#	#	#	#	#	#	#
V(E)	45.9	52.8	52.9	60.8	52.1	39.4	50.0

V(G) = Additive Genetic Variance

V(M) = Maternal Variance

V(E) = Environmental Variance

indicates $V(M) < 0$

in the maternal component.

V. GENERAL DISCUSSION

Five founder specimens collected in Java in 1939 were responsible for the extant O. mossambicus populations in the Asia/Pacific region. As a testimony to their successful colonizing abilities, this fish has become an established exotic able to exploit a wide spectrum of aquatic environments in the tropical developing countries of Asia and Oceania. In 1951, fourteen O. mossambicus individuals were reported introduced into Hawaii from Singapore. This study investigated the genetic variation of economic traits in the Hawaiian O. mossambicus population.

In spite of the potentially depauperate gene pool in the above founder specimens and Malecha's (1968) study indicating serum esterase and transferrin loci were monomorphic, the results from this study reveal the presence of genetic variation in O. mossambicus. Significant differences among sires and among dams within sire were found in males and females for weight at 4-months of age and in males only at 5-months of age for weight, drawnweight, and total length. The estimated heritabilities on these traits were found to lie in the intermediate range with genetic correlations ranging from 0.87 to unity.

Allendorf and Utter (1979) hypothesized that the amount of genetic variation by isozyme loci in fish is an indicator of the genetic variation throughout the genome of that population. Therefore, populations with a high or low average heterozygosity should generally demonstrate high or low additive genetic variation. However, in this instance, it appears the two Hawaiian isozyme data reported by Malecha does not reflect the amount of genetic variation observed in this study. Unfortunately, no other work in or outside of Hawaii on electrophoretic variation within O. mossambicus populations has been done to compare results. Since polygenic or quantitative traits are, by definition, assumed to be controlled by many genes it is unlikely that there is no genetic variation or that all loci are fixed in the same direction in the population. Thus estimation of genetic variation by isozyme frequencies should be interpreted with caution, and is no substitute for estimating the genetic variation or heritabilities of economic traits.

The disparity between the findings of this study and Malecha's results may be explained by studying a summary of the reported tilapia introductions to Hawaiian waters (Maciolek, 1984), as follows:

<u>SPECIES</u>	<u>SOURCE</u>	<u>NUMBER IMPORTED</u>	<u>YEAR</u>
<i>Oreochromis mossambicus</i>	Singapore	14	1951+
<i>O. macrochir</i>	Congo	52	1958+
<i>Sarotherodon melanotheron</i>	?	?	>1970
<i>Tilapia melanopleura</i>	Congo	50	1957
<i>T. zillii</i>	West Indies	19	1957+

It is possible that in addition to genetic drift for monomorphism the 1 locus, 2 allele systems in serum esterase and transferrin were already fixed in the small number of introduced O. mossambicus individuals. The polymorphisms found in O. macrochir and T. melanopleura had an increased probability of detection when noting that 52 and 50 specimens were introduced respectively. Genetic variation in O. mossambicus may also have increased since 1951 from mutations, hybridizations with other tilapia species, or through subsequent unreported introductions of O. mossambicus into Hawaii.

The amount of genetic variability reported in this study is not surprising. Although O. mossambicus in Hawaii has undergone two founder events this does not necessarily lead to greatly reduced levels of variability. For example, Nei, Maruyama, and Chakroborty (1975) and Templeton (1980) have respectively studied this topic by computer simulation and empirical investigations. They demonstrated that if a polygenic trait is controlled by a

large number of loci each having small additive effects, then there is often very little change in genetic variability after the founder effect. It appears that if the founder genome is sufficiently heterozygous it can survive and genetically respond to the forces of drift and altered selective forces provided there is an open niche to allow a population flush. A clear example of such a case is provided by Carson's (1978) comprehensive studies documenting the repeated inter-island founder events by the Hawaiian Drosophila.

METHODOLOGICAL CONSIDERATIONS

One of the major problems in the methodology of experiments in animal genetics is synchronous mating or spawning dates in families. The reason for such concern is that fishes, as opposed to mammals, are known to be more sensitive to environmental variations, e.g. water temperature, density, competition, food availability, and water quality (Gall and Gross, 1978a; El-Ibiary and Joyce, 1978). Hence, uniformity of environmental conditions is particularly important when evaluating quantitative traits.

This problem of asynchronous mating of sires and dams has been overcome through artificial fertilization techniques. These are regularly practiced in salmonids.

Stripping of gametes and artificial fertilization have already been demonstrated in tilapia (Rothbard and Prugenin, 1975). In conjunction, a successful egg incubation system has been devised (Rothbard and Hulata, 1975). However, these techniques have never been attempted on a large scale. If artificial fertilization were practiced in this study, the time effects of water temperature and photoperiod would have been eliminated. Other studies could likewise have benefited from artificial fertilization, but instead have encountered difficulties using "natural service." For example, in channel catfish, Reagan et al. (1970) produced inflated heritability estimates for weight and length traits because of assortative mating. Female breeders had to be of equal or smaller size than the males to prevent injury.

Artificial fertilization paves the way for a more precise approach to estimating genetic variation in tilapia by analysis of variance techniques. The ideal experimental design would be a factorial design whereby all sires and dams would be simultaneously mated to each other. The lack of incubation units for fertilized eggs prevented the implementation of a factorial experiment for this study.

The factorial design has distinct advantages over the hierachal design. A lower error variance in the heritability estimates using a factorial design has been

demonstrated using real data with the long head poppy (Papaver dubium) (Kearsey, 1965) as well as with simulated data (Pederson, 1972). The simulation study of Pederson's suggests the required number of sires in a factorial design would be less than that required in a hierarchical design in order to get the same amount of genetic information. This is an important consideration since the number of testable sires is almost always governed by the capabilities of the testing facility.

Refstie and Steine (1978) used a hierarchical design to estimate heritabilities for weight and length of freshwater Atlantic salmon using 62 sires and 33,000 observations of offspring. They cite Henry (1968) in estimating that the same level of accuracy in their study would be obtained with only 3000 observations of offspring if sire numbers were increased from 62 to 100. However, the testing of 100 sires is a very demanding study. The advantage in the factorial design of requiring fewer sires to get the same amount of genetic information is much appreciated by any investigator.

In addition, the factorial design is superior to the hierarchical design in partitioning components of variance (Becker, 1975). Maternal variation in tilapia can be partitioned without the confounding effects of dominance genetic variation. The interaction of genotypes or sire x dam interaction can also be partitioned, whereas

this effect is confounded in the dam variance component in the hierarchical design.

PRACTICAL APPLICATION OF RESULTS

Tilapia culture is currently considered to have significant impact in food programs for alleviating protein shortages in developing tropical countries. Its importance is undisputable. However, there appears a difference of opinion among scientists on how to best improve growth rate for increased production. For example, Lowe-McConnell (1982) believes genetic factors are unimportant relative to the manipulation of environmental and behavioral mechanisms. In contrast, Gall (1983) generally supports a genetic approach in fishes because selection experiments on other fish species have resulted in genetic responses typical of animal studies. A review by Gjedrem (1983) showed that in 18 mass selection experiments in a variety of fishes, 15 of those studies showed positive genetic gains.

This study has revealed that growth in O. mossambicus is not depauperate of genetic variation, but has the potential to be improved by means of genetic manipulation. The heritability estimates for weight and total lengths were found to range between 0.31 to 0.39. The Russian study by Chan (1971) had similar realized

heritability estimates for weight that ranged from 0.12 to 0.32.

It is of interest to note that the Asian O. mossambicus which is in Hawaii is morphologically different from the African O. mossambicus. The former is long and cylindrical in contrast to the latter being deep-bodied and generally growing to a larger size (Pullin, 1982). Therefore, if growth rates in Hawaiian O. mossambicus are to be improved through selection, the introduction of O. mossambicus from Africa should be considered to increase the gene pool for optimal genetic gains.

VI. APPENDIX 1

MEASUREMENT OF FISH LENGTHS USING A COMPUTER DIGITIZER

ABSTRACT

The use of a computer digitizer as a new technique for measuring fish lengths was statistically compared against the traditional method of measurement by a hand ruler. Three judges measured four types of body lengths in Oreochromis mossambicus. Each fish was measured by each judge, three times by the digitizer method, and three more times by the hand method. A two-way analysis of variance revealed very strong (judge x method) interaction. When the two methods of measurement were analyzed independently of each other, their respective components of judge variation were about equal. Thus the digitizer appears to be a comparable new fish measuring tool that is equally applicable to other aquatic species. This study also lists its advantages and possible conditions which may affect its measurement precision.

INTRODUCTION

Fish measurements for growth rate, age classification, or taxonomic purposes have traditionally

been done by the use of a reliable ruler. More recently, still photography has captured the imagination of scientists in improving new measurement techniques. For example, juvenile salmon were photographed against a graph paper background for estimation of their lengths (Martin, 1967) and weights of live fish have been estimated by length and width measurements of their photographic images (Hawkes, 1975; Christensen, Fiandt, and Benoit, 1977).

In the continuing improvement of length measuring devices, the potential of the computer digitizer has yet to be fully exploited. To demonstrate its usefulness, Frie (1982) has measured fish scales and back calculated body length using a digitizer coupled to an Apple II microcomputer. However, the degree of reliability and efficiency in this novel method relative to the hand measurement is unknown. This study evaluates the two methods by the comparison of their respective variance components.

METHODS AND MATERIALS

A total of 24 live, unanaesthetized Cichlid fish (Oreochromis mossambicus) were used as subjects with half of them categorized as "small (juvenile)" (total length 40-70mm) and the other half as "large (adults)" (total length 130-185mm). For each method of measurement, three

judges separately measured the total length, standard length, height, and head size of the same fish. These measurements were performed on each fish three times by each judge.

HAND MEASUREMENT

The live fish subjects were placed into separate water-filled glass containers that were labeled for identification. Each of three judges randomly chose and measured each fish by hand ruler at three different times. The fish were returned to their individual containers after each measurement.

DIGITIZER MEASUREMENT

Each fish was individually photographed in a lateral position three separate times. A ruler was included in each photograph's background to later standardize measurements. Kodak ektachrome slide film was used in a hand held single lens reflex camera equipped with a macro lens. A single tensor lamp provided the light source.

A computer program written in BASIC permitted digitizing of a fish by placing the developed slide on the lighted platen of a Hewlett-Packard 9874A digitizer coupled to a Hewlett-Packard 2649G intelligent graphics terminal. The logic for determining fish lengths was the

Pythagorean theorem in calculating the distance between two points on the digitizer's platen grid. To improve the measurement of fish details from a 35mm slide, binocular magnifying glasses ("optivisor") were worn around the forehead. To standardize body measurements in millimeters, it was necessary to scale the fish using the ruler in the photographic background. All data were stored on cassette tape and later read into a Hewlett-Packard 3000 for analysis.

STATISTICAL ANALYSIS

A two-way ANOVA with interaction was performed on the following statistical model:

$$Y_{ijk} = \text{mean} + J_i + M_j + J_iM_j + R(\text{error})_{ijk}$$

where:

Y_{ijk} = the k th observation in the i th Judge and j th Method

mean = parametric mean of the population

J_i = random contribution of the i th Judge group

M_j = random contribution of the j th Method group

J_iM_j = interaction between the i th Judge and j th Method groups

$R(\text{error})_{ijk}$ = error term of the i th Judge and j th Method

A set of one-way ANOVAs on the methods of measurement estimated the method effects of each judge independent of the other two judges. The statistical model for each judge was:

$$Y_{ij} = \text{mean} + M_i + R(\text{error})_{ij}$$

where:

Y_{ij} = the j th observation in the i th Method

mean = parametric mean of the population

M_i = random contribution of the i th Method

$R(\text{error})_{ij}$ = error term of the j th item in the i th Method

A hierarchical ANOVA to estimate the variance components of judge variation in fish groups were separately analyzed for each method of measurement. The analysis was performed on the following fish sizes: (1) small fish only, (2) large fish only, and (3) all fish pooled. The statistical model was:

$$Y_{ijk} = \text{mean} + F_i + J_{ij} + e_{ijk}$$

where:

Y_{ijk} = the k th observation in the j th Judge subgroup of the i th Fish group

mean = parametric mean of the population

F_i = random contribution of the i th Fish group

J_{ij} = random contribution of the j th Judge subgroup in the i th Fish group

e_{ijk} = error term of the k th observation in the j th Judge subgroup in the i th Fish group

RESULTS

The F-values of the two-way ANOVA with interaction indicated that not only are there highly significant differences ($p < 0.01$) in both main effects of judge and method, but also highly significant is the (judge x method) interaction. These results were consistent in all four body measurements. This indicates that the effects of the two factors were not simply additive.

To investigate the cause of the significant interaction component, a least squares approach to estimate method effects of each separate judge indicated that the hand method generally gave smaller values in the four body lengths compared to the digitizer measurements (Table 16). The magnitude rather than the direction of each judge's measurement appears to be the contributor to the observed interaction component.

A clear interpretation of the two methods tested was complicated by the presence of interaction. Therefore, each method was separately analyzed by a hierarchical ANOVA

TABLE 16

LEAST-SQUARES MEANS AND STANDARD ERRORS OF METHOD
OF MEASUREMENT FOR FIXED EFFECTS OF FOUR VARIABLES
FOR EACH JUDGE

	<u>TOTAL</u> <u>LENGTH</u>	<u>STANDARD</u> <u>LENGTH</u>	<u>HEIGHT</u>	<u>HEAD</u>
<u>JUDGE 1</u>				
Overall Mean	108.19	87.00	33.39	28.00
Hand	-0.82 _{±.13}	-1.98 _{±0.18}	0.08 _{±0.06}	-0.95 _{±0.11}
Digitizer	0.82 _{±.13}	1.98 _{±0.18}	-0.08 _{±0.06}	0.95 _{±0.11}
<u>JUDGE 2</u>				
Overall Mean	107.72	85.92	32.68	28.33
Hand	-0.93 _{±0.10}	-1.38 _{±0.15}	-0.30 _{±0.04}	-0.45 _{±0.07}
Digitizer	0.93 _{±0.10}	1.38 _{±0.15}	0.30 _{±0.04}	0.45 _{±0.07}
<u>JUDGE 3</u>				
Overall Mean	107.91	86.27	32.55	28.36
Hand	0.06 _{±0.09}	-0.63 _{±0.13}	-0.40 _{±0.06}	-0.09 _{±0.07}
Digitizer	-0.06 _{±0.09}	0.63 _{±0.13}	0.40 _{±0.06}	0.09 _{±0.07}

on a between judge within fish size basis to compare their respective components of judge variation. From Table 17, the digitizer method relative to the hand method generally has a smaller pattern of variation. However, there are several exceptions which merit explanation. An example is when a judge measures the same fish three separate times, the digitizer has a large within component of judge variation in total length. In examining the replicate photographic slides of each fish subject, it was found that the fish tail was sometimes tilted in the upward direction in a replicate slide due to dorsal muscle contraction at the region of the caudal peduncle. This contributed to the increased variation which is less noticeable in the small size fish. Increased variation due to the tilted position of the tail is corroborated by the consistently smaller variation in digitizer measurements of standard length values which do not include the tail length.

The digitizer method also has a larger within component of judge variation in head size, which measures the front of the snout to the outermost edge of the operculum. This was caused by poor photographic lighting. It was difficult to clearly define the operculum edge from a slide image. This was particularly evident in the large O. mossambicus where melanin pigments obscured visual perception by turning the body a dark coloration when the

TABLE 17

THE BETWEEN AND WITHIN COMPONENTS OF VARIATION FOR JUDGES

	<u>W I T H I N</u>		<u>B E T W E E N</u>	
	<u>HAND METHOD</u>	<u>DIGITIZER METHOD</u>	<u>HAND METHOD</u>	<u>DIGITIZER METHOD</u>
<u>ALL FISH</u>				
Total Length	0.68	1.33	1.27	0.24
Standard Length	0.78	0.73	3.30	3.25
Height Size	0.32	0.29	0.80	0.02
Head Size	0.44	0.57	0.92	0.00
<u>SMALL FISH</u>				
Total Length	0.24	0.27	1.31	0.06
Standard Length	0.21	0.17	2.86	0.15
Height Size	0.11	0.10	0.11	0.03
Head Size	0.19	0.21	0.05	0.06
<u>LARGE FISH</u>				
Total Length	1.11	2.40	1.22	0.42
Standard Length	1.36	1.29	3.74	6.36
Height Size	0.54	0.49	1.50	0.16
Head Size	0.69	0.93	1.80	0.00

fish was out of the water. In the small or juvenile sized fish subjects, the melanin was not fully developed. Hence, this problem was not as evident as shown by the almost equal within component of judge variation in the hand and digitizer methods.

In general, the digitizer method maintained a smaller between component of judge variation than the hand measurement. The difference in the between component of judge variation in head size of small fish by the digitizer (0.06) compared to the hand method (0.05) is slight and considered negligible. However, standard length in large fish has a significantly larger between component of judge variation using the digitizer. This is possibly attributable to the differing subjective judgements of each judge in determining the photographic location of the caudal peduncle. The lesser variation in the hand measurement reveals each judge is better able to repeatedly locate the caudal peduncle by feel rather than sight. In contrast, the reverse is true in small fish where the caudal peduncle is more difficult to find by feel than by sight. These results may be interpreted as: when one person always performs the measurement of standard lengths in large fish, then the digitizer is a more reliable method of measurement compared to the hand method.

DISCUSSION

This study statistically evaluated the differences between a novel method of measuring fish lengths using a computer digitizer against the traditional method of measurement using a hand ruler. Four types of body length measurements were used to test for differences between the digitizer and hand methods. Analysis of variance revealed very strong (judge x method) interaction throughout the results. Consequently, each method was analyzed on a separate basis and then had their respective components of judge variation compared.

In general, all the components of judge variation were smaller with the digitizer than by hand. Exceptions from this pattern were noted. First, the larger within component of judge variation in head size could be explained by poor lighting. This can be easily remedied by improving the lighting source during the photographic sessions. Second, the larger within component of judge variation in the total length of large fish is caused by tilting of the tail at the region of the caudal peduncle. This problem can be minimized by relaxing the fish through anaesthetization.

The standard length's large between component of judge variation found in large fish by the digitizer cannot be significantly reduced. However, as previously

noted, the digitizer's within-judge component is smaller than the hand method and is deemed the more reliable method when one person consistently measures the standard length in large fish.

In summary, these two methods of measurement appear to be about equal. Although the components of judge variation in the digitizer usually had smaller variances, this study did reveal possible conditions which can increase variation (e.g. fish body color, fish size, and fish position during photography).

An investigator must take careful consideration of the advantages a digitizer offers over the hand method of measurement. In very small or delicate fish, the process of photography for digitizing minimizes the handling stress inherent in hand measurements. More important, each fish retains a permanent record on film for calculation or re-calculation of data at any time convenient to the investigator. This property of a permanent record is an asset generally unavailable in fish growth studies. Lastly, since the digitizer directly records data to memory devices, the tedium, time, and error of manual data entry is notably reduced. The disadvantages of the digitizing method in this study can be its monetary costs (i.e. photography and digitizer/computer system) as well as time and expertise required for film development if done by the investigator.

The digitizer method is a comparable performer to hand measurements and has potential applications not yet fully exploited in the fishery sciences. This technique is not limited to fish but can also be used on other aquatic species for morphometric measurements in taxonomy as well as for growth rate/size measurements in research and management. Not only can linear size data be collected from photographic slides, negatives, video tape projections, or even x-rays, but the digitizer can also map the coordinates of non-linear items, measure surface areas, and reproduce precise drawings and outlines. The broad and flexible scope of the digitizer can be a very useful device on many subjects requiring quantification.

ACKNOWLEDGEMENTS

This study was supported by the Hawaii Institute of Marine Biology, Kaneohe, Hawaii, the Pacific Islands Development Program, East West Center, Honolulu, Hawaii, and the Data Computations Unit, Cancer Center of Hawaii, Honolulu, Hawaii.

APPENDIX 2

DESCRIPTION OF BREEDERS AND BROODSHIPS

SIRE NO.	FARM ORIGIN ¹	DAM NO.	FARM ORIGIN ¹	BIRTH DATE ²	REP. NO.	CAGE LOCATION ³	DENSITY ⁴
1	P	1	H	139	1	16	24
					-	-	-
		54	P	187	1	42	25
					-	-	-
2	L	2	P	147	1	61	24
					-	-	-
		32	L	168	1	71	7
					-	-	-
3	H	3	L	152	1	74	25
					-	-	-
		21	P	164	1	35	18
					-	-	-
4	P	4	S	152	1	75	15
					-	-	-
		60	H	196	1	6	25
					-	-	-
5	H	5	L	152	1	77	22
					-	-	-
		62	L	222	1	48	25
					2	16	23
		63	L	179	1	64	0
					-	-	-

APPENDIX 2 (Continued)

SIRE NO.	FARM ORIGIN ¹	DAM NO.	FARM ORIGIN ¹	BIRTH DATE ²	REP. NO.	CAGE LOCATION ³	DENSITY ⁴
6	L	6	H	152	1	66	25
					2	67	25
		11	P	147	1	60	24
					2	43	25
7	L	7	H	152	1	38	19
					2	28	15
		8	P	152	1	69	24
					2	28	24
8	P	9	L	152	1	37	17
					-	-	-
		58	?	181	1	64	25
					-	-	-
9	S	10	H	152	1	27	25
					-	-	-
		35	S	176	1	32	4
					-	-	-
10	H	12	L	152	1	31	21
					-	-	-
		26	S	167	1	-	0
					-	-	-
11	P	13	L	158	1	24	25
					2	55	25
		38	S	172	1	12	25
					2	76	25

APPENDIX 2 (Continued)

SIRE NO.	FARM ORIGIN ¹	DAM NO.	FARM ORIGIN ¹	BIRTH DATE ²	REP. NO.	CAGE LOCATION ³	DENSITY ⁴
12	P	14	L	158	1	2	24
					2	54	25
		27	P	164	1	17	25
					2	45	25
13	P	15	P	158	1	1	12
					2	22	13
		55	L	187	1	23	25
					2	59	25
14	P	16	L	176	1	40	15
					-	-	-
		51	L	158	1	26	20
					-	-	-
15	P	17	P	158	1	18	20
					-	-	-
		52	P	179	1	50	25
					-	-	-
		56	P	187	1	21	17
					-	-	-
16	P	18	S	167	1	39	16
					2	62	16
		39	H	172	1	14	22
					2	80	22

APPENDIX 2 (Continued)

SIRE NO.	FARM ORIGIN ¹	DAM NO.	FARM ORIGIN ¹	BIRTH DATE ²	REP. NO.	CAGE LOCATION ³	DENSITY ⁴
17	P	19	L	158	1	57	25
					-	-	-
		28	P	164	1	41	18
					-	-	-
18	P	20	L	161	1	63	25
					-	-	-
		61	L	222	1	19	25
					2	2	21
19	H	22	P	167	1	25	24
					-	-	-
20	L	23	L	161	1	5	15
					-	-	-
		49	L	174	1	56	8
					-	-	-
21	H	24	P	164	1	3	14
					2	47	14
		25	L	161	1	51	17
					2	58	17
22	L	29	L	168	1	72	22
					-	-	-
		57	H	189	1	65	25
					-	-	-

APPENDIX 2 (Continued)

SIRE NO.	FARM ORIGIN ¹	DAM NO.	FARM ORIGIN ¹	BIRTH DATE ²	REP. NO.	CAGE LOCATION ³	DENSITY ⁴
23	L	30	H	172	1	73	25
					-	-	-
24	L	31	P	168	1	70	22
					-	-	-
		43	H	176	1	79	25
					-	-	-
25	P	33	H	165	1	20	19
					2	78	19
		42	L	172	1	11	11
					2	58	14
26	L	34	L	194	1	51	12
					-	-	-
		40	S	176	1	49	25
					-	-	-
27	H	36	H	173	1	9	25
					2	16	25
		37	L	172	1	13	20
					2	46	22
28	P	41	P	174	1	10	25
					-	-	-
		45	L	174	1	4	15
					-	-	-

APPENDIX 2 (Continued)

SIRE NO.	FARM ORIGIN ¹	DAM NO.	FARM ORIGIN ¹	BIRTH DATE ²	REP. NO.	CAGE LOCATION ³	DENSITY ⁴
29	L	46	H	193	1	33	13
					2	52	13
		53	L	187	1	7	22
					2	30	9
30	H	47	P	173	1	8	17
					-	-	-
		48	P	173	1	15	25
					-	-	-
31	P	50	L	174	1	53	15
					-	-	-
		59	P	196	1	44	24
					-	-	-

¹Farms:

- H = Hanohano Enterprises
- P = Seafood Plantation
- L = Amorient
- S = Kahuku Prawn Co.

²Julian Calendar date.

³See Figure 1 for reference map of cage locations.

⁴Total number of fish per cage at 5 months of age.

APPENDIX 3a

MULTIPLE REGRESSION COEFFICIENTS AND STANDARD ERRORS
OF ENVIRONMENTAL EFFECTS AT 1 AND 2 MONTHS OF AGE

<u>W E I G H T</u>		
	<u>TEMPERATURE</u>	<u>PHOTOPERIOD</u>
<u>MONTH</u>		
1	0.15+0.08	-2.01+1.47
2	2.78+0.63	-4.92+0.56

<u>T O T A L L E N G T H</u>		
	<u>TEMPERATURE</u>	<u>PHOTOPERIOD</u>
<u>MONTH</u>		
1	1.52+0.92	-23.64+1.79
2	13.62+2.36	-18.31+2.09

<u>H E A D S I Z E</u>		
	<u>TEMPERATURE</u>	<u>PHOTOPERIOD</u>
<u>MONTH</u>		
1	0.32+0.24	-6.18+0.46
2	3.18+0.60	-4.28+0.53

<u>H E I G H T S I Z E</u>		
	<u>TEMPERATURE</u>	<u>PHOTOPERIOD</u>
<u>MONTH</u>		
1	0.40+0.31	-7.88+0.60
2	5.04+0.78	-6.35+0.69

APPENDIX 3b

MULTIPLE REGRESSION COEFFICIENTS AND STANDARD ERRORS
OF ENVIRONMENTAL EFFECTS AT 3- TO 5-MONTHS OF AGE

<u>MONTH</u>	<u>TEMPERATURE</u>	<u>W E I G H T</u>	
		<u>PHOTOPERIOD</u>	<u>DENSITY</u>
Males:			
3	0.55+1.46	-6.21+1.76	-0.48+0.07
4	10.13+1.66	-33.43+5.51	-0.72+0.13
5	8.01+1.54	-25.43+9.79	-0.66+0.20
Females:			
3	2.25+1.15	-6.98+1.33	-0.50+0.05
4	5.73+1.16	-17.00+3.55	-0.45+0.08
5	5.78+1.01	-21.18+5.79	-0.42+0.10

<u>MONTH</u>	<u>TEMPERATURE</u>	<u>T O T A L L E N G T H</u>	
		<u>PHOTOPERIOD</u>	<u>DENSITY</u>
Males:			
3	5.03+2.91	-16.12+3.50	-0.84+0.14
4	14.57+2.12	-48.00+7.04	-0.86+0.16
5	8.36+1.49	-30.11+9.48	-0.54+0.19
Females:			
3	7.65+2.62	-18.92+3.10	-0.98+0.12
4	10.97+1.89	-35.16+5.81	-0.78+0.12
5	7.86+1.32	-29.68+7.55	-0.63+0.14

APPENDIX 3b
(Continued)

<u>H E A D S I Z E</u>			
<u>MONTH</u>	<u>TEMPERATURE</u>	<u>PHOTOPERIOD</u>	<u>DENSITY</u>
Males:			
3	1.77±0.71	-4.77±0.86	-0.16±0.03
4	3.67±0.53	-12.24±1.78	-0.16±0.04
5	2.23±0.37	-6.68±2.37	-0.10±0.05
Females:			
3	2.29±0.66	-5.29±0.76	-0.19±0.03
4	2.65±0.45	-8.78±1.38	-0.16±0.03
5	2.07±0.32	-7.10±1.82	-0.14±0.03

<u>H E I G H T S I Z E</u>			
<u>MONTH</u>	<u>TEMPERATURE</u>	<u>PHOTOPERIOD</u>	<u>DENSITY</u>
Males:			
3	2.00±0.90	-5.04±1.09	-0.28±0.04
4	3.75±0.67	-11.92±2.24	-0.23±0.05
5	2.28±0.48	-6.49±3.08	-0.15±0.06
Females:			
3	2.66±0.89	-5.64±1.03	-0.33±0.04
4	3.16±0.61	-9.22±1.86	-0.20±0.04
5	2.45±0.43	-9.20±2.47	-0.18±0.04

APPENDIX 3b
(Continued)

<u>DRAWNWEIGHT AT 5 MONTHS</u>			
	<u>TEMPERATURE</u>	<u>PHOTOPERIOD</u>	<u>DENSITY</u>
Males	6.99+1.38	-24.02+8.75	-0.66+0.18
Females	4.85+0.89	-18.43+5.11	-0.39+0.09

<u>INTESTINAL LENGTH AT 5 MONTHS</u>			
	<u>TEMPERATURE</u>	<u>PHOTOPERIOD</u>	<u>DENSITY</u>
Males	53.16+16.87	107.55+107.35	1.29+2.15
Females	53.44+15.04	-40.67+ 85.82	-0.40+1.55

<u>GILL SURFACE AREA AT 5 MONTHS</u>			
	<u>TEMPERATURE</u>	<u>PHOTOPFRIOD</u>	<u>DENSITY</u>
Males	19.59+11.61	91.57+73.39	1.84+1.42
Females	58.59+9.83	-169.68+56.17	0.33+0.99

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