Studies were conducted to evaluate the potential of monosex hybrid tilapia (♀️ T. mossambica x ♂ T. hornorum) in waste-heat polyculture systems. The optimum growth temperature for this hybrid was found to be 32°C in laboratory experiments. Experiments in sewage pond cage culture in the temperature range of 23-33°C at stocking densities of ~53 fish/m³ were also conducted. At fish sizes between 5 and 12 cm TL, estimated annual production is approximately 50,000 kg/ha/yr (50,000 lb/acre/yr). Fish in the sewage oxidation ponds grew significantly faster than fish fed trout chow at optimum temperature in the laboratory, even though temperatures in the sewage ponds averaged below the optimum growth temperature. Techniques to accelerate growth rates are being explored. Exposure to gamma radiation (500 rads), known to cause significant increases in channel catfish growth rate, was found to have a similar effect on tilapia. After a 20-week growth period, exposed fish weighed an average of 20% more than controls.
INTRODUCTION

Aquaculture research at Oak Ridge National Laboratory (ORNL) is part of a large effort to develop methods to utilize waste heat (the heat rejected from condenser cooling systems). The magnitude of this potential resource is great; about 70% of the fuel energy is rejected as waste heat by electric generating stations. A 1000 megawatt station would support (at a minimum) some 314 ha of aquaculture ponds (Olszewski, 1977a).

The basic precept of our research on aquaculture is that production systems should be designed to produce low-cost protein. This orientation is in contrast with the approach of most aquacultural efforts in the U.S. today, which produce expensive (luxury) protein. Our basic thinking has been that although protein supplies are adequate in the U.S. at present, a large part of the world is already protein-poor, and conditions of surplus will not last much longer here. We decided that such a system should be based on polyculture rather than monoculture, for reasons such as increased system efficiency and stability. A preliminary system design was developed (Olszewski, 1977b) and subjected to extensive economic and engineering analysis. The resulting conceptual design is shown in Figure 1. While details of the system may change as a result of future analyses, the basic concept is expected to remain.

After examining several different species arrays, we decided to concentrate our initial efforts on tilapia. Several reasons underlay this choice. First, tilapia are excellent fish, and are readily marketable (Crawford et al., 1978). Second, tilapia are cultured extensively around the world and have relatively few disease problems. Third, as a result of worldwide culture activities a good data base exists on many aspects of their biology. Fourth, literature reports on several species indicate growth-temperature optima from 25-35°C (Chimits, 1955) substantially above that recorded for temperate-latitude fish, a factor that suits the species well to waste heat systems where relatively high temperatures might be expected.

Tilapia have one major drawback as a food culture organism; they become reproductively active at a small (< 100 g) size. Thus the potential for production of fish too small to be marketed as human food is a problem which must be solved if the fish are to be practically used in a commercial operation. There are, at present, two possible solutions. The first is sex reversal by oral administration of hormone (Guerrero, 1975). This technique involves the administration of androgenic hormones to young fish to accomplish sex reversal and is about 95-99% effective. The second method is hybridization. Several tilapia species, when crossed, produce monosex hybrids. Done carefully, this technique is 100% effective (M. Sipe, personal communication).

For our system, we decided to use monosex (male) hybrids which were obtained from Natural Systems, Palmetto, Florida. The hybrid used was the cross between female Tilapia mossambica and male Tilapia hornorum.
Our experimental programs involved both field and laboratory studies and were designed to help develop the information base necessary for commercial development of a waste heat aquaculture system incorporating the tilapia hybrids.

GROWTH-TEMPERATURE EXPERIMENTS

The objective of our growth-temperature experiments was to define the optimum growth temperature for Tilapia mossambica♀ x Tilapia hornorum♂ hybrids.

Materials and Methods

Fish between 45 and 55 mm standard length were divided into 16 groups of ten fish each. Two groups of 10 fish each were placed in each of eight glass aquaria, 45 x 89 x 38 cm, and separated by wire mesh partitions. Each aquarium was maintained at a different temperature: 20, 23, 26, 28, 30, 32, 34, or 37°C, with a continuous flow-through of about 3.5 liters/minute. Purina trout chow (#2) was fed to the fish ad libitum and the tanks were vacuum-cleaned daily.

At the initiation of the experiment, tilapia were acclimated to test temperatures for one week. Laboratory lighting throughout the experimental period was maintained at a seasonal (summer) photoperiod; activated by an outdoor photocell. All fish were weighed and measured biweekly after anesthesia in tricane methanesulfonate. The length of the experiment was 39 days.

Confidence limits for \( P \), the % daily increase in weight were established based on the definition of \( P \) as follows:

\[
P = \frac{100}{D} \left( \frac{Y - X}{X} \right),
\]

where \( Y = \) mean weight at time \( D \), the end of the experiment

\( X = \) mean weight at time \( 0 \), the beginning of the experiment

then

\[
P = \frac{100}{D} \left[ \frac{Y}{X} - 1 \right]
\]
and \[ p = \frac{Y}{W} - \frac{100}{D} \]

where \[ W = \frac{DX}{100} \]

Kendall and Stewart (1973) have derived the confidence limits for the ratio of two means where \( x \) and \( y \) are distributed as a bivariate normal. This method requires an estimate of the correlation \( \rho_{xy} \) between \( x \) and \( y \). Though we cannot estimate this from the data (since the fish were not individually tagged) we can obtain conservative confidence bounds by setting the correlation equal to 0. This will be a conservative estimate as long as \( \rho_{xy} \) is positive and this is true for the fish growth rate data in the region that is being considered in this paper.

Results

Growth at all temperatures was approximately linear throughout the experiment (Figure 2). The largest increase in biomass occurred with those fish maintained at 32°C with the fish at 26, 28, 30 and 34°C also showing substantial growth. Even though the fish at 32°C produced the most biomass, the confidence intervals on % increase in weight per day (Figure 3) indicate that there is not a statistically significant difference between the growth rates at 26, 28, 30, 32 and 34°C. Statistical significance may not be the most important criterion for selecting aquaculture temperatures, however, when the trend of the results is so clear.

SEWAGE POND EXPERIMENTS

The objective of our sewage pond experiments was to assess the viability of our waste-stream nutrient concept (Figure 1).

Methods and Materials

The Oak Ridge National Laboratory (ORNL) sewage oxidation ponds were used as an experimental facility. It was felt that they were an excellent simulation of a waste-nutrient-loaded pond system, and would produce results similar to a system devoted to aquaculture.

The two ORNL sewage ponds are sequential. Raw sewage flows from a preliminary mechanical chopper into the first pond, resides there for about six days, then flows into the second pond, is retained for another six days,
then is discharged (receiving chlorination during the process) to a stream. Table 1 lists some salient characteristics of this pond system during the experimental period. The sewage ponds are rectangular, and are lined with polyvinyl chloride. To facilitate the oxidation process each pond is equipped with large bubble aerators, designed and installed to produce a 9-minute turnover time. This aeration system is very effective; in several months of observation, dissolved oxygen readings did not drop below 5.5 ppm. The thermal regime of the ponds is shown in Figure 4. During the experimental period (July and August) average daily temperatures were 26-31°C, which were found to be favorable in the laboratory growth-temperature experiments. Due to the rapid turnover caused by the aeration system, the ponds were isothermal.

The tilapia were stocked in the ponds at a density of 53/m² in floating cages made of 6 mm mesh Vexor netting and wood frames, and were weighed and measured biweekly after anesthesia in tricane methanesulfonate.

The plankton assemblages in the two ponds were strikingly different. The first pond, into which raw sewage was discharged from the preliminary treatment facility, was almost a monoculture of *Euglena* for the duration of the experiment. This condition appears to be the "natural" equilibrium situation. The second pond was a fairly stable zooplankton culture, dominated by a large strain of *Daphnia pulex*. Numbers of this organism declined during the peak temperatures in mid-July, but became re-established as temperature began to fall in August, again dominating the plankton assemblage.

**Results and Discussion**

Change in mean weight of the tilapia is shown in Figure 5. As can be seen by inspection, average weight gain was essentially linear over time; about 7.5 g/week (= 1.07 g/day). Although the differences are not statistically significant, it appears that the fish in pond 2 (the one dominated by zooplankton) grew faster than those in pond 1. Growth rate as % change in body weight per week is shown in Figure 6. Growth is an exponentially decreasing function of body weight over time.

The production potential of a waste-nutrient system was calculated from the results, and is quite large. From these experimental data, we calculate production rates of 397 g/m²/wk or 3970 kg/ha/wk or 206,440 kg/ha/yr.

This production estimate must be viewed with caution, as it is based on extrapolation from experimental results, not the operation of a full-scale system. Several assumptions underly the estimate, some of which make it excessive. The first is that the entire pond surface is assumed to be covered with cages. This is unreasonable, as it allows no room for harvesting equipment (boats). Assuming one half of the pond surface to be open allows room for boats and reduces the production estimate by a factor of 2. We also assumed that the fishes' daily growth increment remained constant up to marketable size (3–400 g). Our experimental data indicate no decrease in growth increment as fish size increases up to around 80 grams, but we feel that such a decrease is likely in larger fish, and that production estimates
should be reduced by a factor of 2 to account for this. We have assumed that the system operates near the optimum growth temperature for the fish year-round, a situation which is feasible in temperate latitudes only with the addition of heat for much of the year, making our production estimates applicable only to waste heat aquaculture systems (any other source of heat, excepting geothermal, would be too expensive).

It is probably not necessary to reduce production estimates to account for density-dependent effects if pond design and stocking densities are similar to those discussed in this paper. Density-dependent effects may alter production rates in two basic ways. First, fish crowding may lower growth rates. This is probably not the case at the stocking densities postulated here; experiments carried out by the senior author (Suffern, unpublished) indicated no difference in growth rate between fish stocked in the sewage ponds at 10/m$^3$ and those stocked at 53/m$^3$. A second density dependent effect on production is the effect of fish feeding on food organisms. If consumption by the fish exceeds food organism production rates, fish production rates will decline. Assuming Golueke et al.'s (1973) reported production rates for algae in hypereutrophic, intermittently aerated ponds, a conversion efficiency from algal biomass to fish biomass of 10%, rapid mixing, half the surface area covered with cages, and a pond depth of about 2.5 meters, fish production between 25,000 and 50,000 kg/ha/yr could be supported. This is probably a low estimate of supportable production because Golueke's estimates were based on ponds which were aerated only during the winter as versus all-year round aeration in our proposed system. Also, the support estimate does not account for auxwuchs utilization by the fish, a component of their ration in this cage culture system. Given all these considerations we feel it is reasonable to expect production on the order of 50,000 kg/ha/yr from a waste-heat waste-nutrient aquaculture system.

GROWTH ACCELERATION EXPERIMENTS

The objective of our growth acceleration experiments was to explore ways of boosting production rates in waste-heat aquaculture systems. There is evidence that changes in growth rates in some animals can be caused by exposure to moderate doses of hard radiation (Donaldson and Bonham, 1964). This effect was tested on tilapia hybrids.

Methods and Materials

Twenty-five young tilapia hybrids (mean weight 2.7 g, mean standard length 6.60 cm) were exposed to 500 rads of gamma radiation in a cobalt$^{60}$ source. From previous experiments at ORNL with channel catfish (Ictalurus punctatus) (Blaylock, unpublished) there was evidence that this dosage might enhance growth rates. The irradiated tilapia were placed in a flow-through tank kept at 30°C. A control group of 25 fish was kept in an adjacent tank with
identical flow and temperature conditions. Both groups were fed ad libidum
with Purina #2 trout chow. Both groups were weighed and measured at 2, and
later \( \frac{1}{4} \) week intervals.

**Results**

The results of this experiment for the first 20 weeks (it is continuing)
are shown in Figure 7. The mean weights of the fish for the control and
treated groups are shown for six dates. The data clearly show that up to
week 8 there was no difference in the growth patterns of the two groups.
Any observed differences can be attributed to random variations. However
on the next two sampling dates one observes a possible pattern with the
irradiated group growing faster than the control group, i.e., the growth
curve of the irradiated group is diverging from the control group. One
indication of this divergence is the difference between the means of the control
and treatment groups at the two dates. If this difference becomes significant
it is an indication of divergence. At week 14, the data are:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Irradiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \bar{x} )</td>
<td>51.80</td>
<td>59.48</td>
</tr>
<tr>
<td>( s )</td>
<td>12.935</td>
<td>8.793</td>
</tr>
<tr>
<td>( n )</td>
<td>19.00</td>
<td>13.00</td>
</tr>
</tbody>
</table>

The variances are significantly different only at the 0.085 level, so the
usual t-statistic is used to evaluate the difference between the means. The
means are not significantly different at the 0.05 level.

At week 20, the data are:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Irradiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \bar{x} )</td>
<td>58.32</td>
<td>72.57</td>
</tr>
<tr>
<td>( s )</td>
<td>15.921</td>
<td>10.175</td>
</tr>
<tr>
<td>( n )</td>
<td>16.00</td>
<td>13.00</td>
</tr>
</tbody>
</table>

The variances are significantly different at the 0.06 level. Since this is
reasonably close to the 0.05 level, we assume the variances are different and
use the Subhatme d-statistic (Finney, 1971):

\[
d = \frac{\bar{x}_T - \bar{x}_C}{\left( \frac{s_T^2}{n_T} + \frac{s_C^2}{n_C} \right)^{1/2}}
\]
This analysis indicates that the means are significantly different at the 0.05 level, hence there is an indication of real divergence. There were some mortalities in both groups; these were felt due to handling, within-group aggression and jumping out of the tank rather than to the experimental regime.

This experiment is ongoing; the fish will be grown to a mean weight of 300-400 grams. At that time, detailed autopsies will be performed in attempt to ascertain the mechanism whereby gamma radiation causes growth rate changes. Our present hypothesis is that the administered radiation induced gonadal atrophy causing growth rate changes parallel to, for example, those seen in cattle which have been made steers.

Summary and Conclusions

Tilapia mossambica ♀ x Tilapia hornorum ♂ hybrids appear to have great potential as waste-heat aquaculture organisms. With an optimum growth temperature around 30°C, and the ability to utilize waste-stream-generated plankton assemblages for food, this fish is a promising source of low-cost, high-quality protein. Based on pilot experiments in cage culture, it seems reasonable to expect production on the order of 50,000 kg/ha/yr. Exposure to moderate doses of gamma radiation appears promising as a mechanism to further boost production by these fish.

Obviously, much more research is needed on this fish. Particularly relevant is the examination of growth-temperature relationships with larger fish. It is known that the temperature of optimum growth decreases as fish grow (Coutant and Suffern, 1977). The magnitude of this shift with hybrid tilapia should be defined. The production potential of waste-stream-driven systems should be explored on a larger scale to substantiate the results shown here. Perhaps the issue of greatest practical concern is that of governmental regulation. At present, a maze of policies, regulation and laws on both the state and federal levels affect the use of exotic fish and waste nutrients. A detailed examination of the regulatory controls (and the possibility for change) is a necessary step for the development of this branch of aquaculture.

LITERATURE CITED


Table 1.  - ORNL sewage pond characteristics

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area (each)</td>
<td>0.24 ha</td>
</tr>
<tr>
<td>Depth (each)</td>
<td>2.44 m</td>
</tr>
<tr>
<td>Volume (each)</td>
<td>4644 m³</td>
</tr>
<tr>
<td>Flow (average)</td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>913453 liters/day</td>
</tr>
<tr>
<td>August</td>
<td>748317 liters/day</td>
</tr>
<tr>
<td>Retention time (each pond)</td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>5.07 days</td>
</tr>
<tr>
<td>August</td>
<td>6.19 days</td>
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<tr>
<td>BOD (influent)</td>
<td>79 mg/l</td>
</tr>
<tr>
<td>Suspended solids (influent)</td>
<td>61 mg/l</td>
</tr>
<tr>
<td>Ammonia, total (influent)</td>
<td>4.5 - 27 ppm (daily cycle; low in early morning, high in early afternoon)</td>
</tr>
</tbody>
</table>
Figure 1. Conceptual design of the ORNL waste heat aquaculture system.

Figure 2. Growth of tilapia hybrids at different temperatures in the laboratory.

Figure 3. Percent increase in weight of tilapia hybrids per day as a function of temperature, means and 95% conf. int.

Figure 4. The thermal regime of ORNL sewage ponds during the summer of 1977.

Figure 5. Growth of Tilapia hybrids in sewage pond cage culture.

Figure 6. Growth rate (% change in body weight) of tilapia grown in sewage ponds.

Figure 7. Growth of irradiated and control tilapia hybrids in the laboratory.
WATER FLOW

TEMPERATURE CONTROL VIA HEAT EXCHANGES

WATER FLOW

POND I
ALGAE, BACTERIA, ZOOPLANKTON

WASTE STREAM NUTRIENTS
(SEWAGE, CATTLEWASTES, PROCESS WASTES HIGH IN N & P, ETC.) + DILUENT

POND II
FISH (SEVERAL DIFFERENT KINDS)

POND III
ALGAE, BACTERIA, ZOOPLANKTON (AGAIN)

POND IV
CLAMS, CRAYFISH

POND V
ROOTED AQUATIC VEGETATION

PRODUCT PROTEIN
Total Biomass of *Tilapia.*
Tilapia Growth vs Temperature After 39 days.
Growth of *Tilapia* Hybrids in Sewage Pond Cage Culture
% CHANGE IN BODY wt/week

ORNL Sewage Pond Cage Culture
Tilapia

TIME (weeks)

ORNL-DWG 77-20819
Tilapia Growth x Time

- CONTROLS
- IRRADIATED (500 rads OF GAMMA)
  (ERROR BARS: ±1 STANDARD DEVIATION)