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MICROALGAE PRODUCTION AND SHELLFISH FEEDING TRIALS AT THE ROSWELL TEST FACILITY

Technical Completion Report

Project No. 1423695

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By

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ABSTRACT

It has been demonstrated that several microalgae species, which are thought to be good food for filter feeders, will survive and grow in Roswell Test Facility (RTF) saline ground water at pilot scale (50 m^2 raceways). Under specific environmental and cultural conditions, several species of shellfish have demonstrated excellent survival and growth. However, the productivity and cultural stability of algae are greatly diminished during the cold months of the year in Roswell (October to March).

The potential for commercial production of bivalve molluscs in saline ground water in southern New Mexico is great if a site can be found that has a source of geothermal water for heating purposes and a source of saline water that will support the growth of marine species. A concerted effort should be made to locate such sites, and to construct and operate a large pilot-scale facility at the site. Simultaneously, algae and shellfish should continue to be grown at pilot scale in Roswell to demonstrate the technical feasibility to the private sector and to optimize methods for growing marine organisms hundreds of miles from the nearest ocean.

Key Words: aquaculture; clams; algae; oysters; saline water

INTRODUCTION

New Mexico's Saline Water Resources

New Mexico sits atop an estimated 15 billion acre-feet of saline ground water. Most of this water is saline to the degree that it cannot be used for agriculture or industry, (i.e., greater than 3000 ppm Total Dissolved Solids (TDS). One exciting possibility for economic development is to use New Mexico's abundance of saline ground water and marginal land to build a land-based marine aquaculture industry.

The Advantages of Aquaculture

Cultivating aquatic organisms on marginal lands offers a far more productive and efficient use of the land than does developing the same lands to support traditional agriculture. The cultivation of a crop that expends no energy maintaining body temperature, can be grown intensively, and has a high market volume, can result in a highly profitable industry. Aquaculture in New Mexico would be complementary to the agriculture industry and would not compete for fertile agricultural land.

Aquaculture in Southern New Mexico

The constraints on intensive land-based shellfish aquaculture in any location are: (1) the water quality, quantity, and availability; (2) availability of sufficient land area for algal production; (3) sufficient sunlight (insolation) for outdoor algal production; (4) proper temperatures for algal and shellfish growth; and (5) access to markets via relatively inexpensive trucking rather than air freight.

New Mexico's saline ground water is generally free of pollution, marine diseases, parasites, and fouling organisms. It is a clean and relatively undeveloped resource. Moreover, the saline water is often continuously available from shallow wells. Much of the saline water appears to be well suited for the purposes of aquaculture because of its temperatures (15-30°C) and salinities (fresh to ocean water salinities and greater).

Large scale aquaculture, especially when based on the production of algae which requires sunlight, needs large land areas. The cost of this land can be prohibitive in coastal areas of the U.S. and elsewhere. The abundance of relatively inexpensive land in New Mexico can be a competitive advantage for New Mexico in developing aquaculture as an industry.

Southern New Mexico has one of the highest incidents of annual insolation of any location in the country. The state's climate is generally warm enough to support many commercially valuable marine organisms. These factors create an environment that would support optimal growth of aquatic crops such as microalgae and organisms that feed on microalgae.

Possible sites for microalgae production are found in southern New Mexico. The areas surrounding Las Cruces and Roswell have been determined by the Department of Energy's Solar Energy Research Institute (SERI) (Maxwell et al 1985) to be among the most suitable in the Southwest for the production of microalgae, with respect to temperature range, availability of land, water suitability, and insolation. Inexpensive land and labor costs, as well as access to major markets via ground transportation are important assets of these areas as well. Together, these assets appear to make an almost ideal location for a viable aquaculture

industry. Of course, only extensive and careful study will determine if an industry can be established in New Mexico.

Bivalve Aquaculture in Southern New Mexico

Previous work has shown that southern New Mexico is well suited for intensive aquaculture, and that bivalves, especially oysters, are promising initial aquacultural products. The market for oysters is limited only by supply (Goldstein 1988). Bivalves are a high value crop and the accelerating degradation of natural populations and traditional fisheries in recent years has reduced supply. This will make bivalves produced in New Mexico all the more valuable. Most importantly, bivalves respond well to intensive culture.

New Mexico's Land-Based Aquaculture's Unique Position

Land-based aquaculture in New Mexico could enjoy the advantage of year-round production. This would position the state's industry as practically the only supplier of some marine products when natural populations are out of season or have been affected by disaster, disease, or pollution. The reliability of the state's aquaculture production could strengthen the industry's position in the market and the value of its products.

Summary

The establishment of an aquaculture industry in New Mexico has three distinct advantages over other forms of economic development. First, there are no competing industries for either the land or the saline water that aquaculture would require. Second, aquaculture is a clean, non-

polluting industry. Finally, the practice of aquaculture complements the existing agricultural traditions of the Southwest. Basically, aquaculture is a form of agriculture. For these reasons, aquaculture promises to gain popularity as an appropriate use of resources and as an acceptable economic activity in the Southwest. This, coupled with the economic potential of the industry, make it a prime candidate for development.

RELEVANT RESEARCH

Saline Ground Water Aquaculture in New Mexico

There is much literature on the effects of different salinities on the physiology and growth of marine organisms (Bardach et al. 1972; Friedrich 1969; Parsons et al. 1977). However, this research has focused on the effects of different dilutions of seawater. Nine ions constitute 99.5 percent of the salts in solution in seawater and these are found in remarkably constant proportions throughout the world's oceans and seas (Harvey 1955). Saline ground water in New Mexico often has a unique ionic composition as compared to seawater of the same salinity. This is true of the Roswsell Test Facility (RTF) saline water as well. For example, RTF water is higher in calcium and sulfate than seawater of the same TDS. The TDS content of this water is 10,000 - 17,000 ppm TDS. One of the objectives of this project was to explore and document the effects of Roswell water on the physiology and growth of marine organisms.

A wealth of information has been published on growing microalgae using saline ground waters; more, in fact, than can be summarized here. A publication by the Solar Energy Research Institute (SERI) provides an excellent review (Neenan et al. 1986).

Previous research on growing marine microalgae in the saline ground waters of the RTF has been promising. Several species of marine microalgae (e.g., Tahitian strain of Isochrysis galbana, Chaetoceros gracillis (SS-14), Platymonas sp.) were successfully grown indoors (in volumes of several thousand gallons) under artificial light. C. gracilis, in particular, grew very well in unmodified RTF water. Cell densities approaching 1 x $10E^6$ cells/ml were routine in indoor culture. C. gracilis also was grown

outdoors, in both batch and continuous culture modes, where cell densities of 4 x $10E^6$ cells/ml were common. Current work at the RTF has demonstrated the survival and growth in outdoor culture at pilot scale (50 m^2) of several species of marine phytoplankton on saline ground water of up to 17,000 ppm TDS.

A more immediate problem in the economic use of algae, however, is that of harvesting. Because of the small size of their cells, harvesting of microalgae mechanically has been one of the major problems in mass culture systems (Mohn 1980). Scura et al., (1979) found that oysters in intensive raceway culture systems removed 88-99 percent of the phyotoplankton from the feed water, and the conversion efficiency of algal biomass to shellfish meat was 11.4 percent. This suggests the possibility of using microalgae to collect solar energy, and bivalves to transform that energy into a useful product.

Bivalves

The edible oyster, of course, has been well studied. Galtsoff (1964) provides a thorough compendium of what is known about C. virginica. Most of this information is also true of C. gigas. The trophic dynamics of the hard clam Mercenaria have been described by Goldstein and Roels (1980).

However, there are no known, properly conducted studies of the growth of marine bivalves in saline ground waters besides those of the author. In recent research, the growth of two species of oysters fed marine microalgae grown in RTF water was demonstrated (Goldstein 1986).

Based on these results, several different species of oysters were evaluated for survival and growth in RTF water. These included populations of small C. gigas, small C. virginica, and large C. virginica. All three grew at rates which compare well with the best rates reported in the literature. Juvenile clams (Mercenaria mercenaria) also were evaluated and demonstrated similar growth rates. While promising, the preliminary nature of this work must be emphasized.

Brine Shrimp

In the last ten years, there has been tremendous interest in Artemia, the brine shrimp, and its use as food for fish and crustaceans (Sorgeloos et al. 1983; Giddings and Chenley 1981; Vanhaeche and Sorgeloos 1983; Mock et al. 1973; Bossuyt and Sorgellos 1980; and Dobbelier et al. 1980) The growth of this species in saline ground water has yet to be studied.

PROJECT GOAL

The project goal was to examine and demonstrate the technical possibility and commercial potential of intensive bivalve aquaculture in southern New Mexico. In order to achieve this goal, the project was to demonstrate that aquaculture production technology was sufficiently developed to enable laboratory aquaculture methods and results to be successfully translated and applied to consistent commercial scale production. Because bivalves must feed on live microalgae and will grow well only if they enjoy a consistently high food level, it was necessary to demonstrate that microalgae could be grown in sufficient quantity and with sufficient consistency to support the oyster crop. Consistent

microalgae production requires that algal growth levels be maintained throughout the year despite seasonal temperature fluctuations. This was to be accomplished by constructing, operating, and evaluating a small, pilot scale production facility.

The algae screening task was designed to identify a suite of algal species that would grow well in the temperature extremes an outdoor culture system would experience.

Furthermore, the project was to demonstrate that the specific techniques of microalgae cultivation would create an environment where oysters might grow rapidly and consistently to market size.

Finally, basic research was to be conducted on the survival and growth of brine shrimp in RTF water to expand the suite of commercially valuable species that feed on microalgae.

FACILITIES

The algae culturing system at the RTF consists of: (1) two greenhouse-covered, paddlewheel-driven, concrete raceways, each with a growing area of 50 square meters; (2) a complete water treatment (1 μm filtration, UV sterilization) and water recycling facility with the capability of automated fertilizer injection; (3) an indoor algal culture laboratory and inoculation system; and (4) a computerized data acquisition and control system.

Raceways

The outdoor algal production system consists of two greenhouses, each approximately 7.6 meters wide by 14.6 meters long. Each greenhouse

has two layers of polyethylene film that hold an insulating layer of air around the greenhouse enclosure. Each greenhouse covers a concrete raceway with 0.3 meter by 0.3 meter side walls. An equally sized dividing wall runs down the center of most of the raceway, which effectively creates a 2.1 meter wide channel. Algae in the raceway is kept in homogeneous suspension by a paddlewheel driven by an electric motor. There are two large exhaust fans in each greenhouse for ventilation which maintain greenhouse temperatures near outside ambient air temperatures during the warmer months of the year. There is no active heating system in the greenhouses. Rather, the massive concrete walls and floor of the raceway store much of the solar energy falling on the greenhouse. As a result, air temperatures at night inside the greenhouse are warmer than outside ambient air temperatures. In general, when the fans are not used, greenhouse air temperatures tend to be almost 10°C higher than outside ambient air temperatures. Water temperatures are more constant than air temperatures in the greenhouses.

Algal culture depth can be maintained at any level from 5 cm to 30 cm. At a depth of 15 cm, the inflow and harvest systems can be operated such that 100 percent of the raceway volume can be exchanged daily.

CO2 Injection System

A CO_2 injection system was designed to automatically provide the necessary carbon for rapid microbial growth. The system continuously monitors culture water pH with a pH meter/controller. If pH levels rise above a given set point, a solenoid valve is energized to open. The solenoid valve is in line between the pressurized CO_2 cylinder and the CO_2 dispersal tube in the culture water. Gaseous CO_2 under pressure flows into the

dispersal tube which resembles a polyvinyl chloride (PVC) pipe, with millions of micron-sized pores. CO_2 is released as tiny bubbles, which helps in the efficient dissolution of the CO_2 gas into the culture water. As more and more CO_2 goes into solution, the pH falls until it drops below the set point. The pH controller then closes the solenoid valve and CO_2 is no longer sent to the culture. This process continues automatically throughout the day and night.

Data Acquisition and Control System

The measurement and control subsystem is based upon a Zenith Z158 (IBM PC compatible) computer and Keithly System 500 data acquisition and control system. Data acquisition is managed and accomplished with the Labtech Notebook software from Laboratory Technology Corp. The system is used to monitor light and temperature conditions in and around the raceways, and to monitor comparable conditions outside the greenhouse. The light meters measure PAR (light in the photosynthetically active range--400 to 700 nanometers) at the surface and near the bottom of the culture in the raceway.

RESULTS AND DISCUSSION

Microalgae Species Screening

Species Selection. Four species of algae were selected for subsequent laboratory and pilot scale screening. Available manpower was just sufficient to do the work necessary for evaluating the growth of four species of algae at seven temperatures. The species were selected to meet two important criteria:

- Grow well in Roswell saline ground water under local environmental conditions;

- Be an adequate source of nutrition for the shellfish that were to be fed the algae.

Many species of the Chaetoceros genus have proved to be a good source of nutrition for filter feeders (Goldstein 1984). This genus has been documented to have many species/strains that grow well over a wide range of water temperatures and salinities by SERI. Discussions with the SERI staff led to the selection of three Chaetoceros strains from the SERI Culture Collection for subsequent screening: Chaet 6, Chaet 9, and Chaet 14. The species is Chaetoceros mullerii subsalsum.

The fourth species, TET1 (Tetraselmis suecica), was selected from the SERI culture collection for subsequent screening because Dr. Laws in Hawaii had previously demonstrated high productivity in outdoor culture for this species. Thus, all four algal species were selected from the SERI Culture Collection.

Algal Growth Trials--Laboratory Scale. The four algal species were screened for growth rate in RTF saline ground water at the following temperatures: 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C. These temperatures cover the range of expected water temperatures of outdoor algae raceways. All experiments were conducted at one temperature at a time in a temperature controlled plant growth chamber. Each treatment was replicated two times. An experimental run consisted of eight 500 ml flasks: 4 species x 2 replicates. In addition, there was a control of RTF water, enriched with Guillard's medium (as were all the flasks), but with no algae. Each flask initially contained 400 ml of Guillard's f/2 media

made in RTF water. All flask contents were kept in homogeneous suspension by bubbling humidified air into the flask.

The flasks were removed daily from the growth chamber, mixed well, and 10 mls were removed from each flask for optical density measurements (Bausch and Lomb Spectronic 20) at 670 and 750 nanometers. The flasks were quickly returned to the growth chamber.

This procedure was repeated daily for 10 days because previous work had shown that in most instances there was no additional net growth after 10 days, or, when the "plateau stage" of the classical growth curve had been reached. Although pigment content can increase even during the plateau stage presumably as a result of self shading, optical density readings at 750 nanometers measure turbidity (total amount of particles blocking light) and not chlorophyll content. The use of cell counts as a measure of algal biomass would have required more manpower than was available and only has an accuracy of ±20%.

The growth at laboratory scale of the four algal species at seven temperatures are graphically presented in figures 1 through 7.

An analysis of variance of the growth of all the species at all temperatures indicates that the species effect showed no statistically significant differences. As a result, a "best" species cannot be determined from these laboratory studies. There were, however, statistically significant differences due to the temperature effect.

There is no growth at 10°C for any of the species tested (figure 1). At 15°C (figure 2), TET1 and CHAET14 demonstrate little growth. CHAET6 and CHAET9, after a slow start, show good growth, with CHAET6 starting to

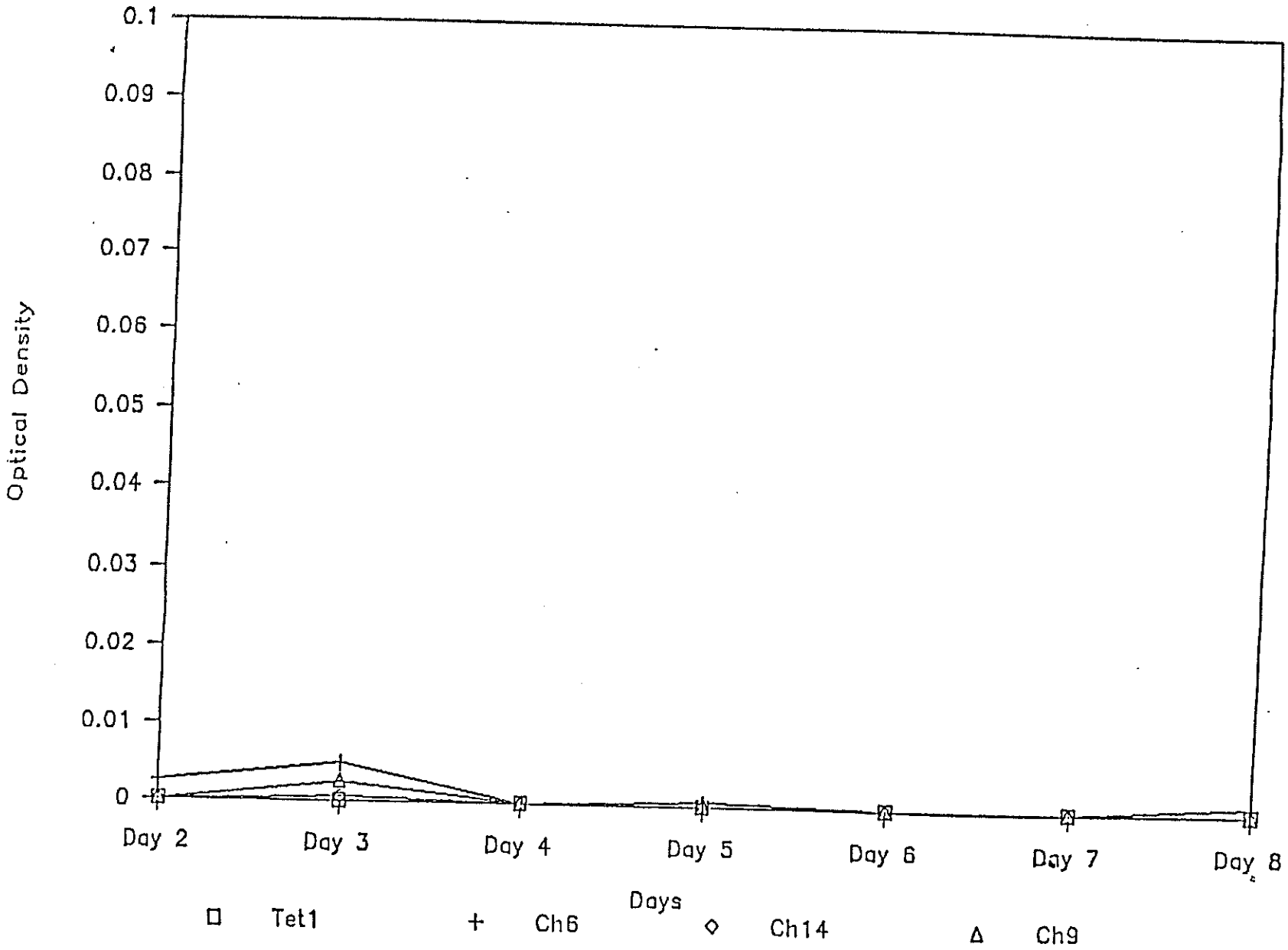


Fig. 1. Algal Growth Trials, All Species in RTF Water, at 10°C

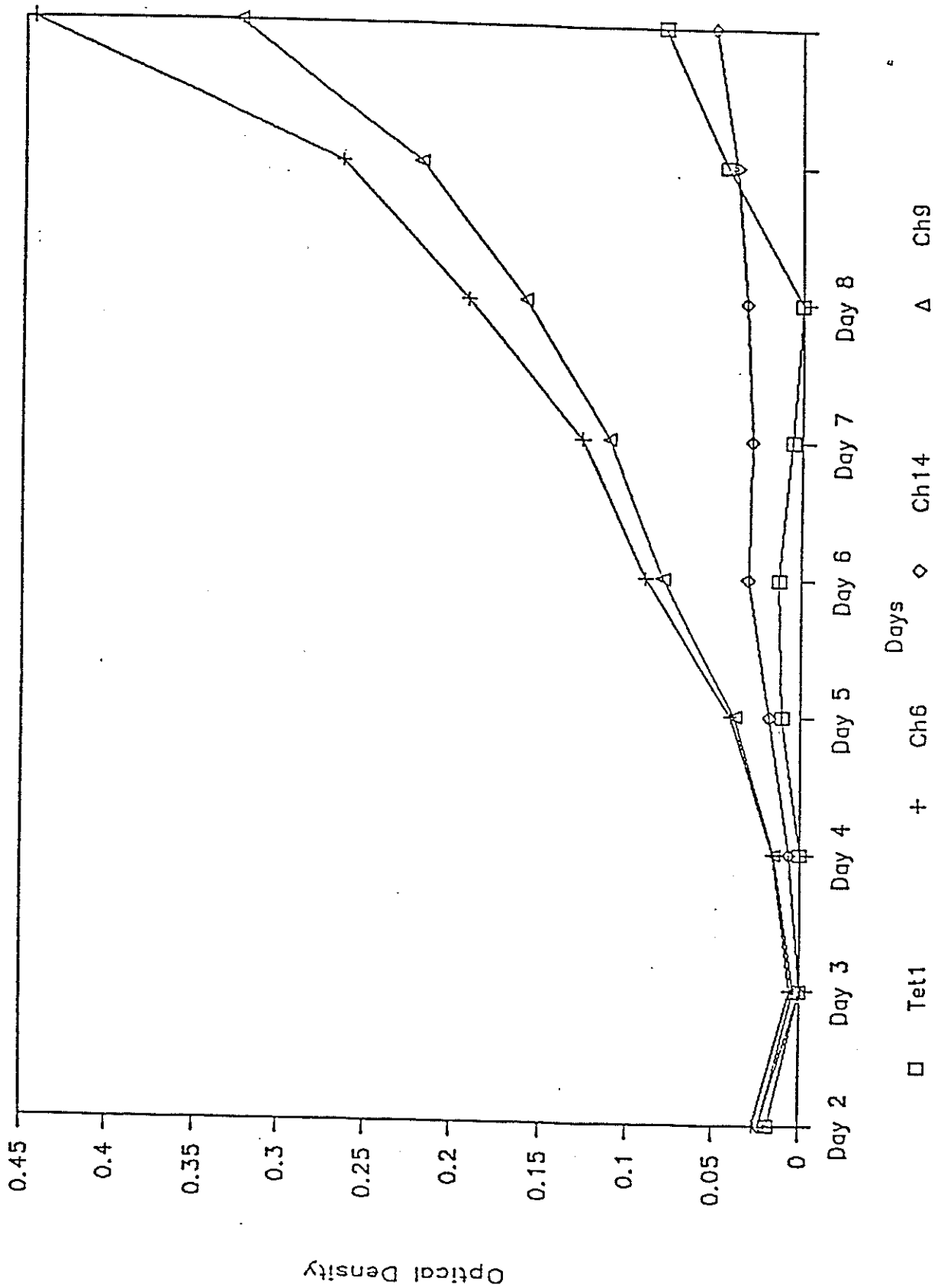


Fig. 2. Algal Growth Trials, All Species in RTF Water, at 15°C

increase its growth over that of CHAET9 toward the end of the run. This same general pattern is maintained at 20°C (figure 3).

The overall growth rate for all species is greater at 20°C than at 10°C and 15°C. The increased growth rate at 20°C for all species accentuates the superior growth of CHAET6 and CHAET9 over TET1 and CHAET14. CHAET6 is clearly the fastest growing species at 20°C.

Surprisingly, at 25°C, CHAET6's superiority is lost; indeed it is the slowest growing species at 25°C (figure 4). CHAET9 is the next slowest grower with CHAET14 doing consistently better over the trial run. TET1, after a slower start than the CHAET species, demonstrates a superior growth rate. Perhaps the CHAET 6 used in this temperature run was contaminated or diseased.

At 30°C (figure 5), the three CHAET species have similar growth rates and attain similar optical densities (OD) at the end of the trial. All are superior to TET1, although after its customary slow start, TET1 soon demonstrates a growth rate equivalent to that of the CHAET species. However, at the end of the trial run, it has achieved a markedly lower OD level.

At 35°C (figure 6), the pattern we have seen at previous temperatures (except at 25°C, which appears to be an anomalous run for CHAET 6) emerges again. Although all the CHAET species achieve essentially the same OD at Day 9, CHAET6 peaked at a higher OD (0.65) earlier at Day 6. Clearly, CHAET6 is superior to the other species in both exponential growth rate and highest OD achieved. Exponential growth was measured as the slope of the growth curve after the "lag" stage and before the plateau stage. TET1 is clearly the slowest grower.

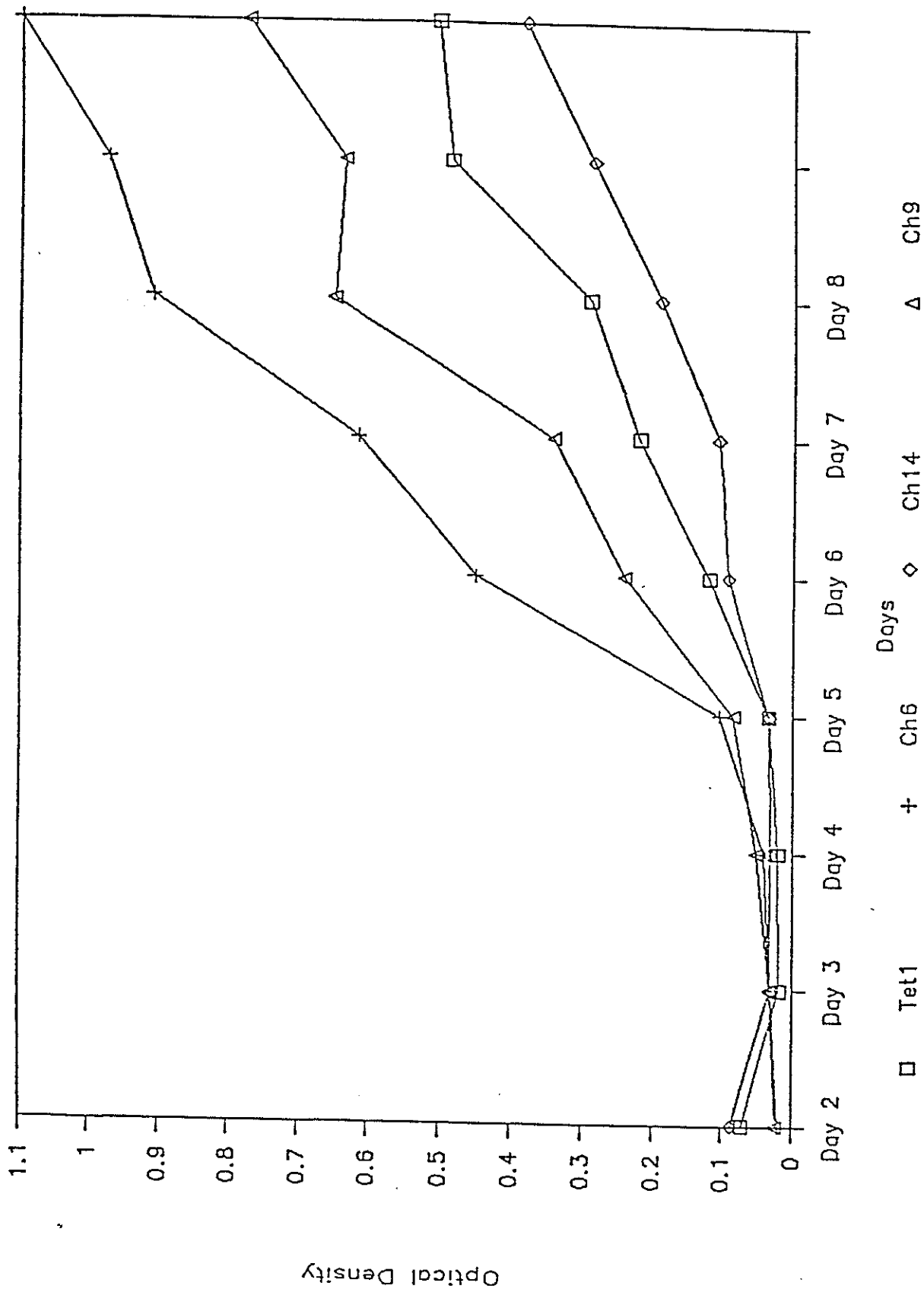


Fig. 3. Algal Growth Trials, All Species in RTF Water, at 20°C

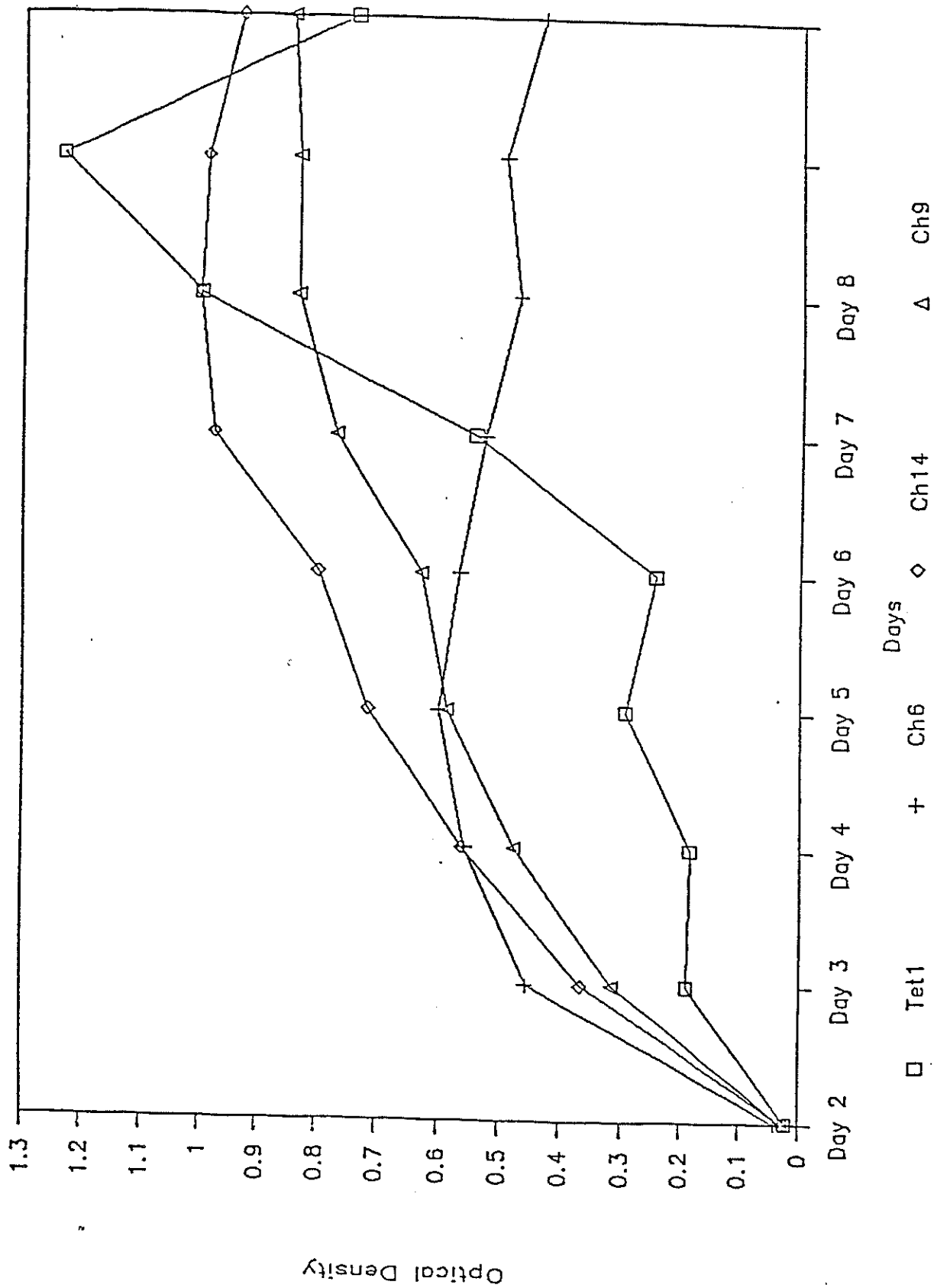


Fig. 4. Algal Growth Trials, All Species in RTF Water, at 25°C

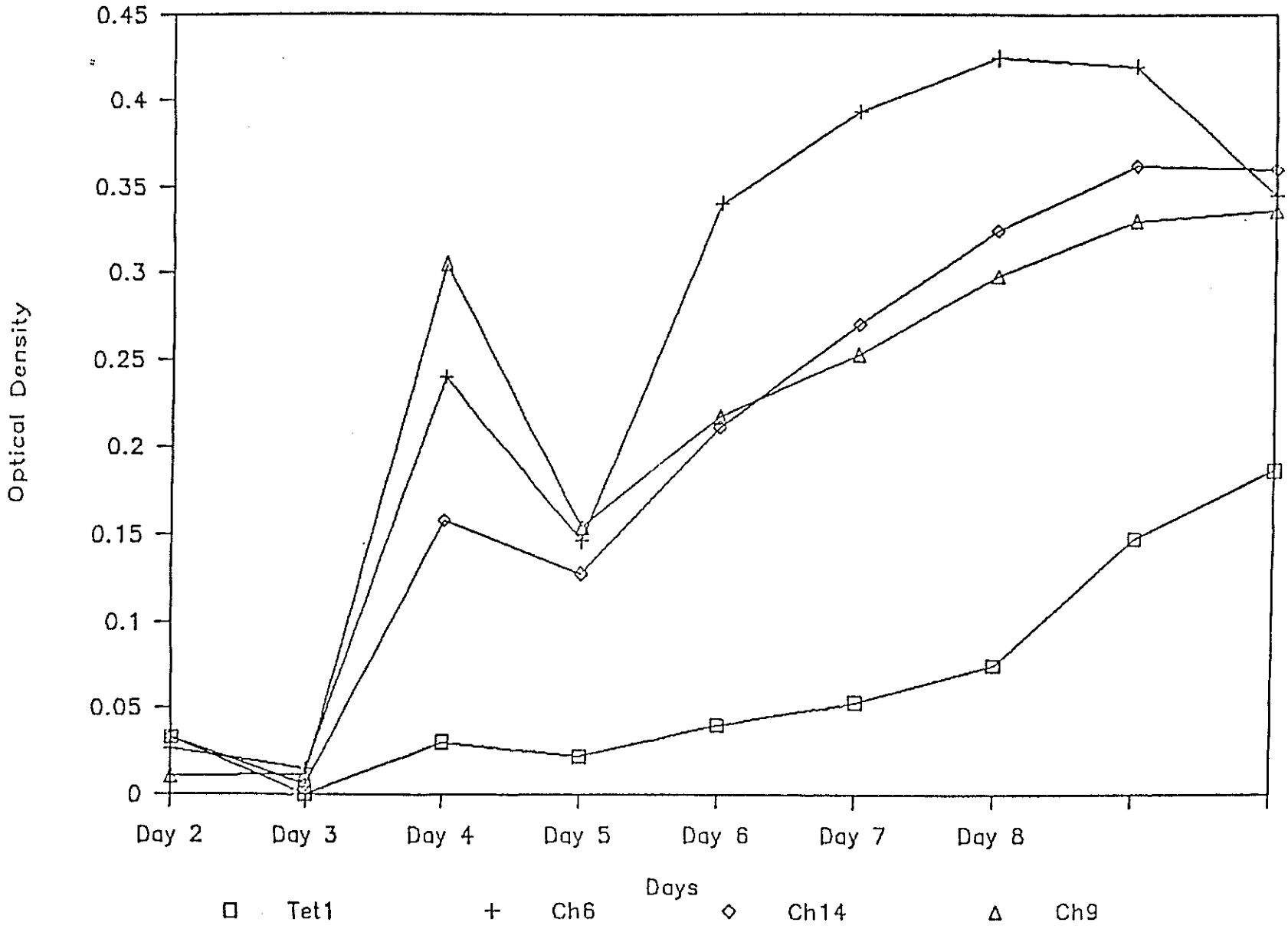


Fig. 5. Algal Growth Trials, All Species in RTF Water, at 30°C

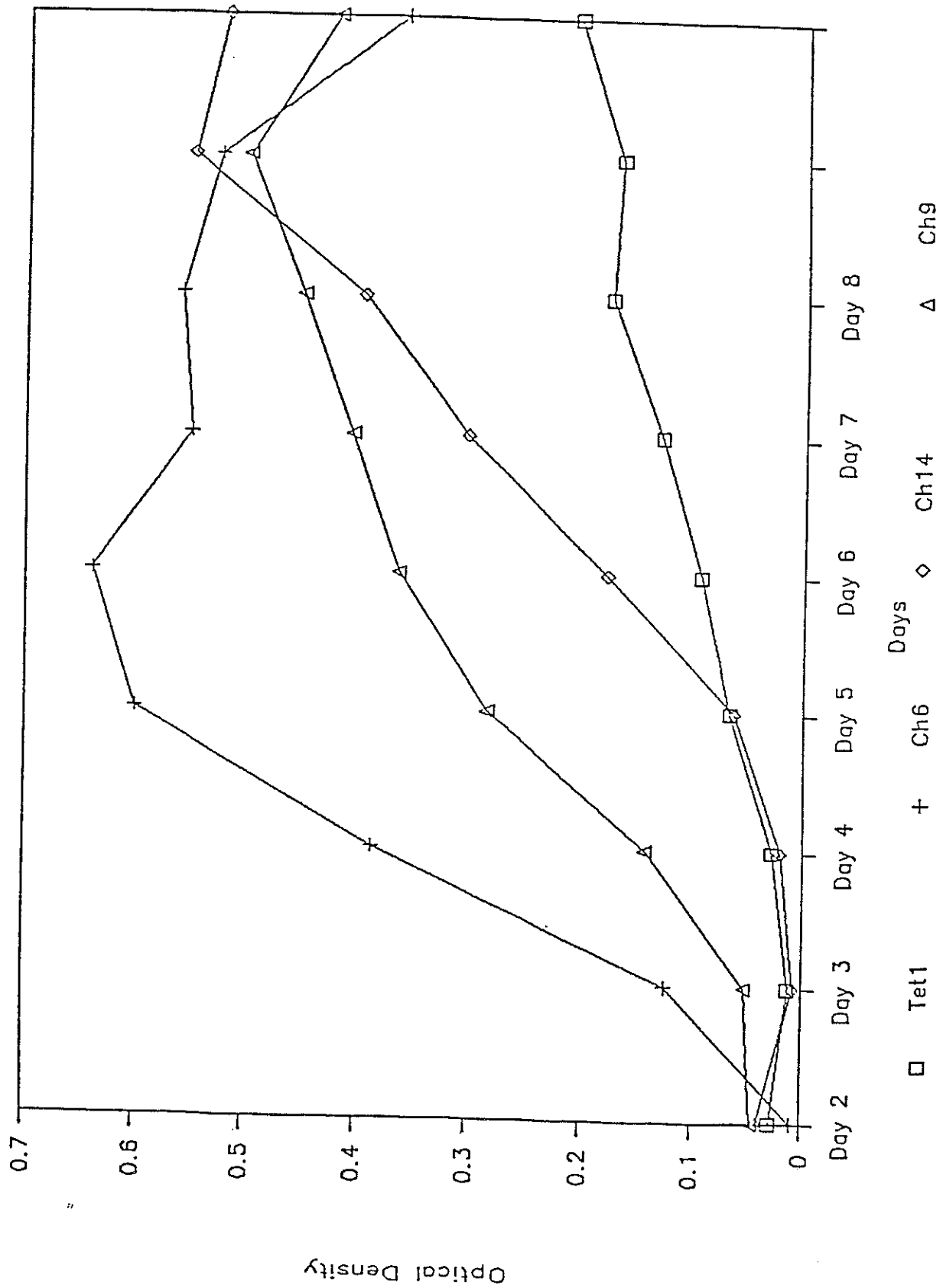


Fig. 6. Algal Growth Trials, All Species in RTF Water, at 35°C

However, at 40°C (figure 7), the general trend observed above seems to break down. Both TET1 and CHAET6 fail to grow, while CHAET9 and CHAET14 both do relatively well. It appears that TET1 and CHAET6 in this run were contaminated or diseased. The 25°C and 40°C runs should have been repeated to verify this.

At almost every temperature tested, (except the anomalous runs at 25°C and at 40°C), CHAET6 achieved both higher densities as measured by OD, and a greater rate of exponential growth. For this reason, CHAET6 seems the obvious choice for "best" species at lab scale. However, the choice of "best" species for commercial production must be determined by actual outdoor culture, pilot scale trials.

The effect of different temperatures on the growth of CHAET6 can be seen in figure 8. The greatest exponential growth rate and maximum OD is achieved at 20°C. At all other temperatures (other than 10°C), the final OD achieved was similar. The complete ranking of exponential growth rates at all temperatures is: 20°C>25°C>35°C>30°C>15°C>10°C. The ranking of maximum OD achieved is: 20°C>35°C>25°C>30°C>15°C>10°C. In both rankings, results at 25°C and 35°C are very similar, and require further statistical analysis. Clearly, however, CHAET6 growth at 20°C is maximal. This is clearly demonstrated in figure 9 where the maximum OD attained by CHAET6 in every temperature trial is compared.

If we compare the maximum OD achieved by all four species at all seven temperatures (figure 10), CHAET6 does indeed show the greatest achieved OD four times out of seven, but also shows a great deal of variability. CHAET9, on the other hand, while rarely the "star" performer, seems to produce at a fairly consistent rate across all temperatures. This is illustrated more clearly in figure 11 where the average maximum OD, for

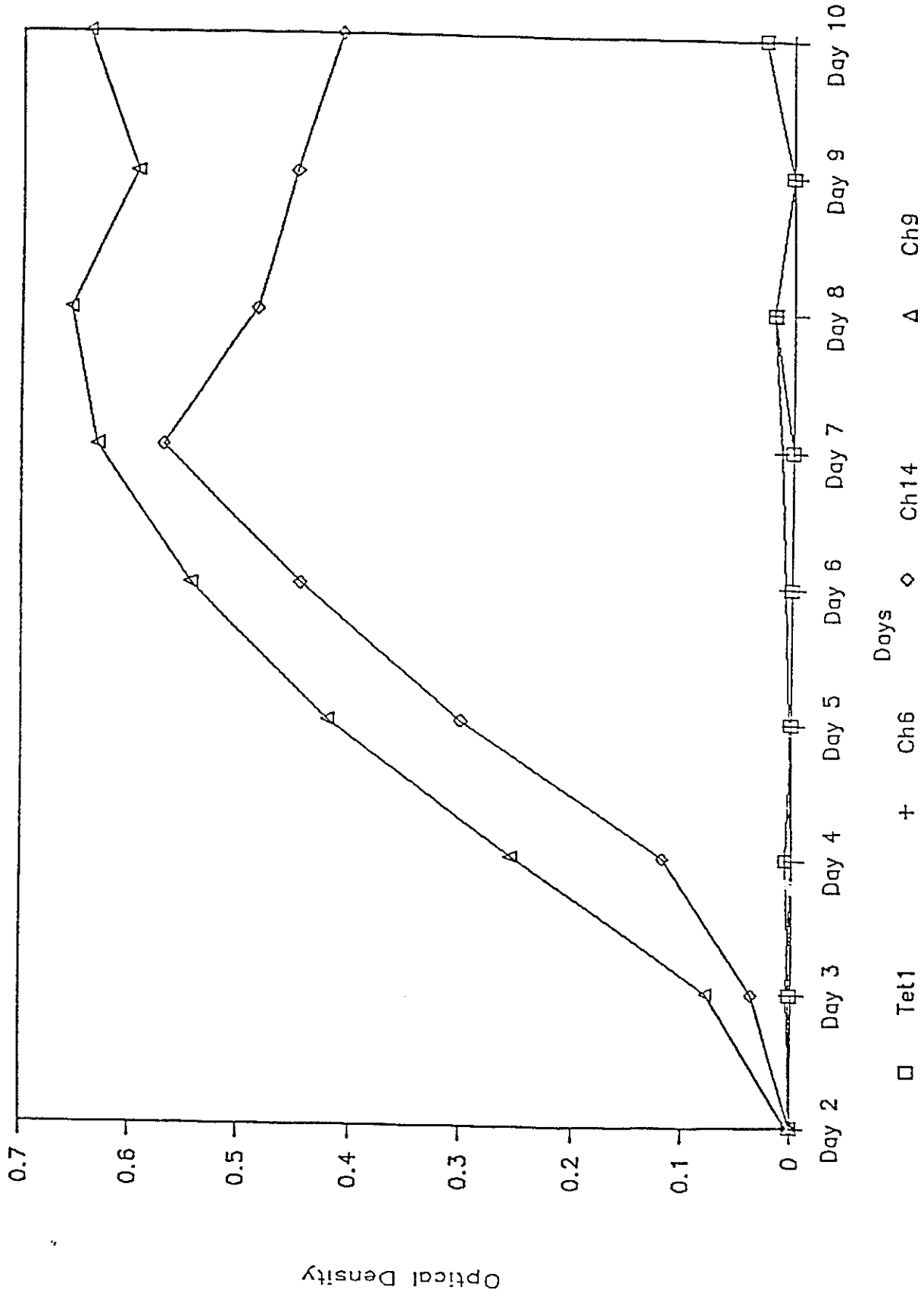


Fig. 7. Algal Growth Trials, All Species in RTF Water, at 40°C

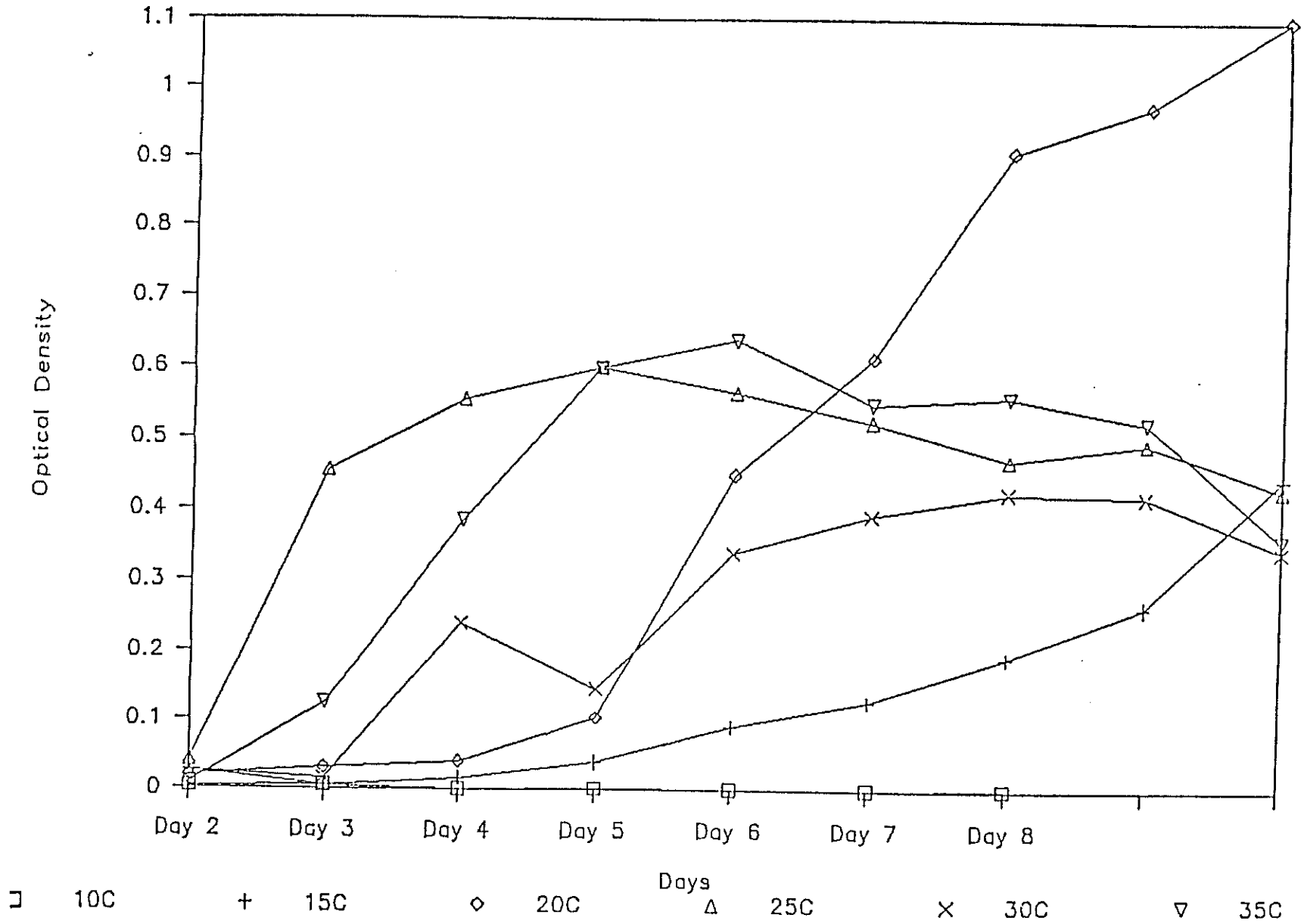


Fig. 8. Algal Growth Trials, CH6 in RTF Water, for All Temperatures

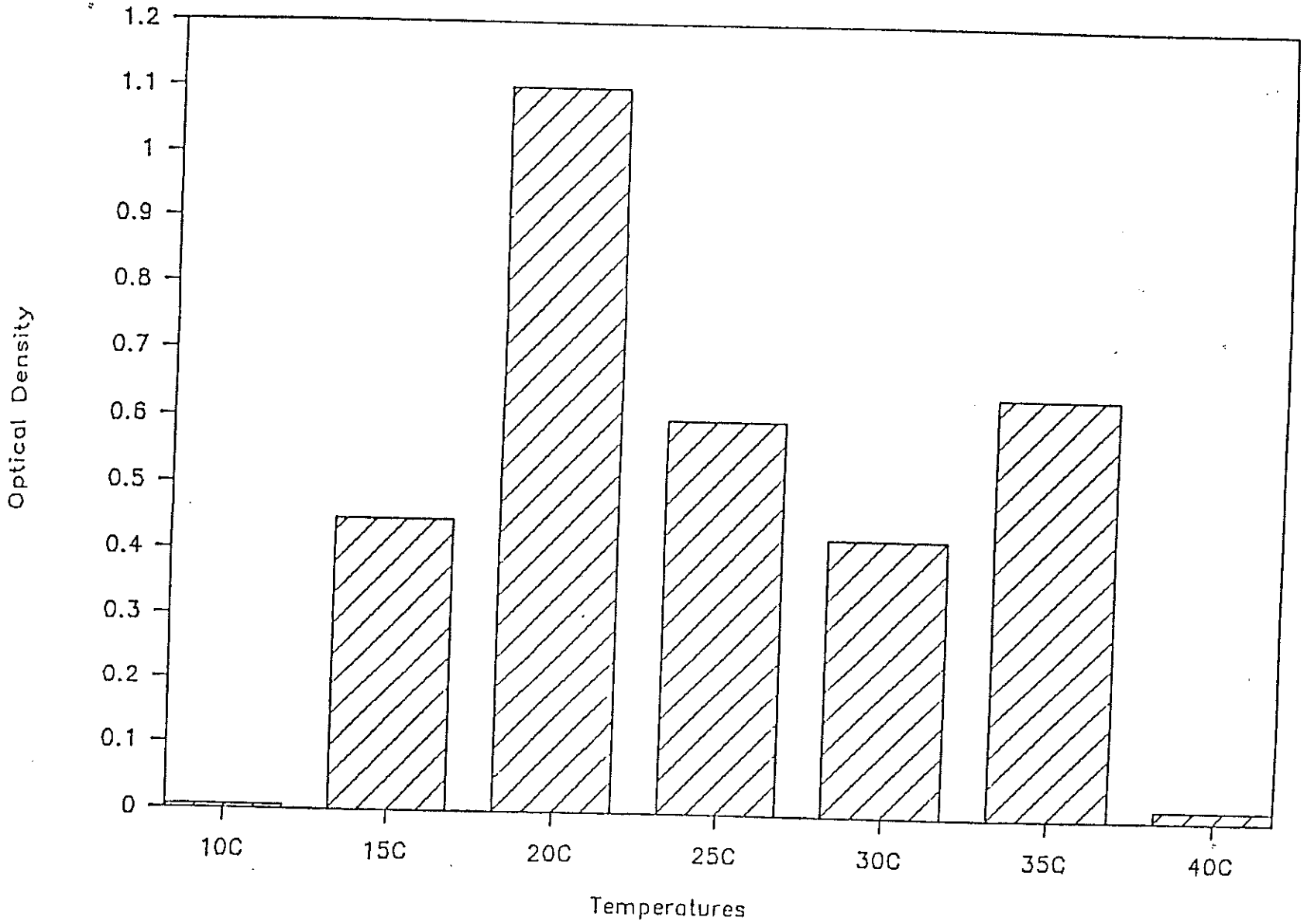


Fig. 9. Algal Growth Trials, Max OD, for Ch6 in RTF Water, at All Temperatures

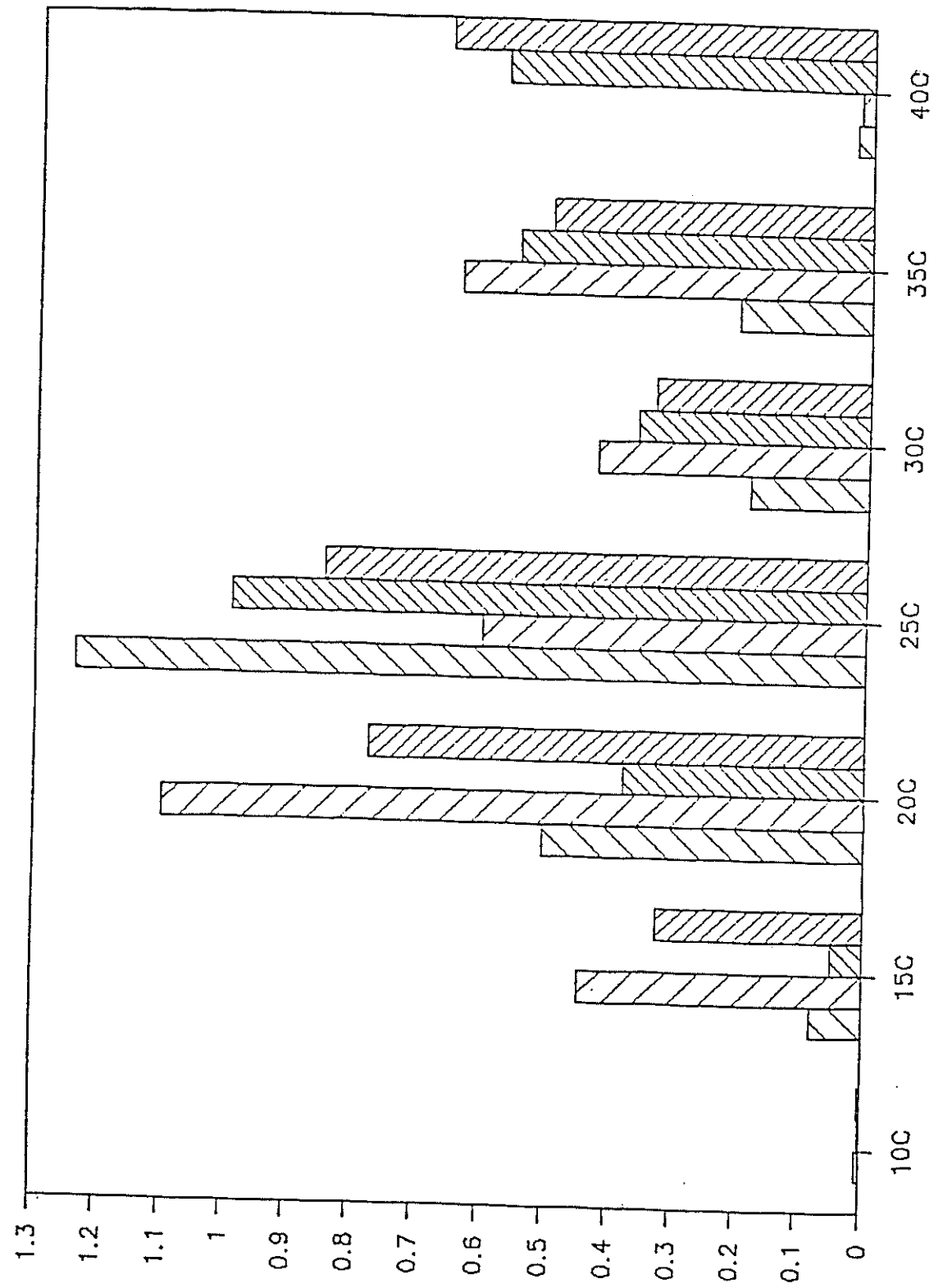


Fig. 10. Algal Growth Trials, Max OD, for All Species, at All Temperatures

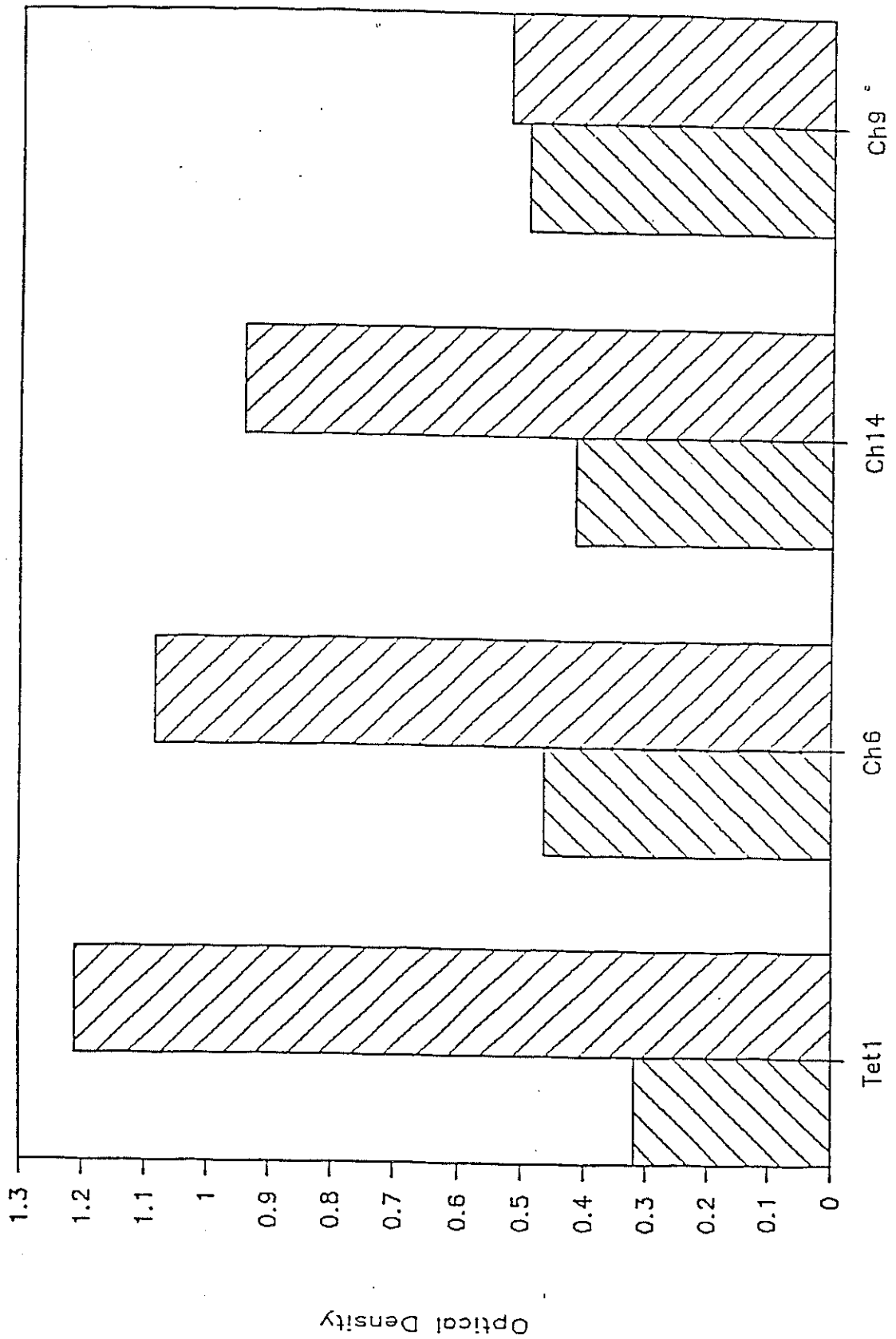


Fig. 11. Algal Growth Trials, Avg. Max OD and Variation for All Species

each species, across all seven temperatures, is compared. CHAET9 edges out CHAET6 for average maximum OD. The amount of variation in maximum OD for each species is also compared. This value was obtained by finding the difference between the greatest and the lowest maximum OD achieved by each species, not counting the run at 10°C where essentially no growth occurred. As indicated in figure 11, CHAET9 shows the smallest range of variation between greatest and least maximum OD achieved across all temperatures. In other words, CHAET9 is the most consistent species across all temperatures.

Statistical Analysis of Algal Growth Trials. Growth rates and maximum optical density data were analyzed to determine the statistical significance of the effects of temperature and species. In general there was no significant variation in growth measures among species at each temperature. However, variation due to differing temperatures was statistically significant in all species.

To avoid biasing the analysis toward runs where the algae had grown spectacularly well and then died off rapidly, we performed the analysis on four indices of growth performance. These indices were: maximum instantaneous slope ([OD for day n] - [OD for day (n-1)]), average slope (the average of all the slopes obtained by calculating the instantaneous slope for each day of the growth trial), maximum optical density achieved, and average optical density during the duration of the growth trial. The averaged measures were used to filter the non-robustness that an analysis of the simple maximums might hide.

The observed significance level (P) from the analysis of variance caused by species and temperature for each growth index is summarized in table 1.

TABLE 1
Observed Significance Level (P) for Species and Temperature Compared Among the Four Indicies of Growth

Source	Maximum Slope	Average Slope	Maximum Density	Average Density
Species	0.2318	0.2541	0.5572	0.2611
Temperature	0.0001	0.0020	0.0007	0.0003

As shown in table 1, temperature was the only cause of significant variation in growth measures.

Tables 2 and 3 record the pairwise comparisons for the different species and temperatures respectively. These tables should be read as follows: Species or temperatures with marks appearing in the same column under the "Homogeneous Groups" heading are not significantly different from one another. Those with marks in different columns are significantly different from one another.

The range of the means in table 3 should be noted. This is further indication of the tremendous effect of temperature on algal growth rates. These results also illustrate the temperature envelope for optimal growth. Specifically, the algal species tested grow well when temperatures are between 20°C and 40°C, and seems to prefer temperatures around 25°C.

TABLE 2

Least Significant Difference Pairwise Comparisons of Growth Measures by Species for Each of the Four Growth Indicies

MAXIMUM SLOPE*

Species	Mean	Homogeneous Groups
Ch6	2.0064E-01	•
Ch9	1.8171E-01	•
Ch14	1.3350E-01	•
TET1	1.2029E-01	•

AVERAGE SLOPE*

Species	Mean	Homogeneous Groups
Ch9	5.733E-02	•
Ch6	4.569E-02	•
Ch14	4.361E-02	•
TET1	2.807E-02	•

MAXIMUM OPTICAL DENSITY*

Species	Mean	Homogeneous Groups
Ch9	4.912E-01	•
Ch6	4.617E-01	•
Ch14	4.109E-01	•
TET1	3.197E-01	•

AVERAGE OPTICAL DENSITY*

Species	Mean	Homogeneous Groups
Ch9	2.4007E-01	•
Ch6	2.4979E-01	•
Ch14	2.3070E-01	•
TET1	1.2573E-01	•

*There are no significant pairwise differences among the means.

TABLE 3

Least Significant Difference Pairwise Comparisons of Growth Measures by Temperature for Each of the Four Growth Indices

MAXIMUM SLOPE*

Temp	Mean	Homogeneous Groups
25	3.747E-01	•
20	2.367E-01	•
30	1.785E-01	• •
35	1.502E-01	• •
40	9.775E-02	• •
15	7.387E-02	• •
10	1.375E-03	•

AVERAGE SLOPE*

Temp	Mean	Homogeneous Groups
25	8.832E-02	•
20	8.012E-02	• •
35	4.330E-02	• •
30	3.522E-02	• •
40	3.350E-02	• •
15	2.532E-02	• •
10	0.000	•

MAXIMUM DENSITY*

Temp	Mean	Homogeneous Groups
25	9.200E-01	•
20	6.900E-01	• •
35	4.740E-01	• •
30	3.275E-01	• •
40	3.167E-01	• •
15	2.250E-01	• •
10	2.000E-03	•

AVERAGE OPTICAL DENSITY*

Temp	Mean	Homogeneous Groups
25	5.629E-01	•
20	2.793E-01	•
35	1.945E-01	• •
40	1.866E-01	• •
30	1.862E-01	• •
15	7.092E-02	• •
10	4.200E-04	• •

*There are 4 groups in which the means are not significantly different from one another.

A final note should be added about the differences in the order of species and temperatures among the four indices. There is no one definitive index, and so ranking the variation of one species and/or temperature among the indices is largely unimportant. What is important is to note the overall consistency of the pattern across all the indices.

Outdoor Microalgae Production Technology

Carbon Dioxide Injection System. CO_2 is the algae's primary carbon source. Because microalgae grow so rapidly, atmospheric CO_2 (which constitutes only a small fraction of the composition of air) cannot diffuse into the culture water quickly enough to satisfy the algae's carbon requirements. Therefore, it is necessary to introduce pure CO_2 into the cultures to satisfy the algae's carbon requirements, especially during the highly productive afternoon hours. A good index of CO_2 availability in culture water is the pH; as dissolved CO_2 in the culture water decreases via microbial uptake, the pH increases. If it rises too high, the algae's growth is depressed not only because of insufficient carbon, but also because the cell's enzymes function suboptimally at the higher pH values.

The CO_2 system has generally worked well. A minimum pressure differential of 5 psi was found to be necessary to prevent the solenoid valve from leaking badly. Even with the correct differential, the valve often failed to close completely; this resulted in wasted CO_2 and non-optimal pH. A replacement valve (same kind) was better, but not perfect. A different solenoid valve that doesn't have any minimum differential pressure requirements has been ordered.

Initially, four sections of dispersal tubing were used for the 50 m^2 raceway. The four sections were spaced at even intervals around the

raceway. Each section was installed perpendicular to culture flow. It was found that gas dispersal through the sections was very sensitive to the depth of the sections. If each was not exactly at the same depth, or if some part of a section was uneven, gas would flow out of the sections or parts of the sections that were closer to the culture surface. It was also found that the pH was lowered to the set point of pH 8.0 very rapidly during short periods of gas flow, and often overshot the set point. As a result, the pH was undergoing rapid oscillations which is a non-optimal situation. All but one of the sections were removed and this section was carefully and evenly attached to the raceway bottom. As a result, the CO_2 is evenly dispersed from this section and the correct pH is maintained with only a few oscillations of small amplitude.

The Effect of CO2 on Algal Growth Rates. During the installation and debugging of the CO_2 injection system, it was observed that culture densities increased several fold and that cultures remained monospecific for longer periods of time when supplied with injected CO_2. As a result, CO_2 injection was used for all 50 m^2 raceway growth trials. All results reported in section 5.2.4, therefore, represent growth trails with cultures enriched with pure CO_2.

An examination of the literature (Neenan et al. 1986) indicates that CO_2 enrichment is necessary for high production rates, regardless of culture facility type. The important question is not whether to use CO_2, but rather how most efficiently to accomplish carbon enrichment in a reliable and cost-effective manner. Further research is required.

Although carbon enrichment is needed for high productivity, a growth trial was run to quantify the effect of CO_2 enrichment on productivity. The best algal species for outdoor culture at the 50 m^2

raceway level is TET1, as determined by a series of growth trials discussed below. The content of one 50 m^2 raceway was divided and half of the culture was pumped to the second raceway. When densities of 200 mg/l were reached in both raceways, the CO_2 enrichment was added in one of the raceways. Densities were measured daily, along with culture temperature, and incident photosynthetic active radiation (PAR). Daily microscopic observations were made to determine species purity. Figure 12 compares the production rate of TET1 with and without CO_2 enrichment. The dramatic effect on production of CO_2 enrichment is clearly shown.

Data Acquisition and Control System Performance. The data acquisition and control (DAC) system was initially developed for use with Spirulina cultivation, which is characterized by high densities, relatively slow (for microalgae) growth, high pH, higher than RTF average conductivity, and continuous harvest. Only one species/strain of Spirulina was evaluated at the 50 m^2 level. These conditions are quite different from the current conditions of the 50 m^2 growth trials. These differences necessitated changes in the DAC. The major change was to discontinue use of the test rack to monitor sequentially four sample streams (inflow and effluent of each raceway) with one set of sensors. Use of the test rack was deemed inadvisable because of the tremendous potential for cross contamination of the cultures in the two raceways. The danger of invalidating the experiment was particularly great because of the difficulty of distinguishing among the three strains of Chaetoceros. Continuous culture of the algae (as used with Spirulina) was discontinued in favor of a more simply executed and monitored daily batch harvest. In essence, there was no inflow or effluent that changed diurnally and needed

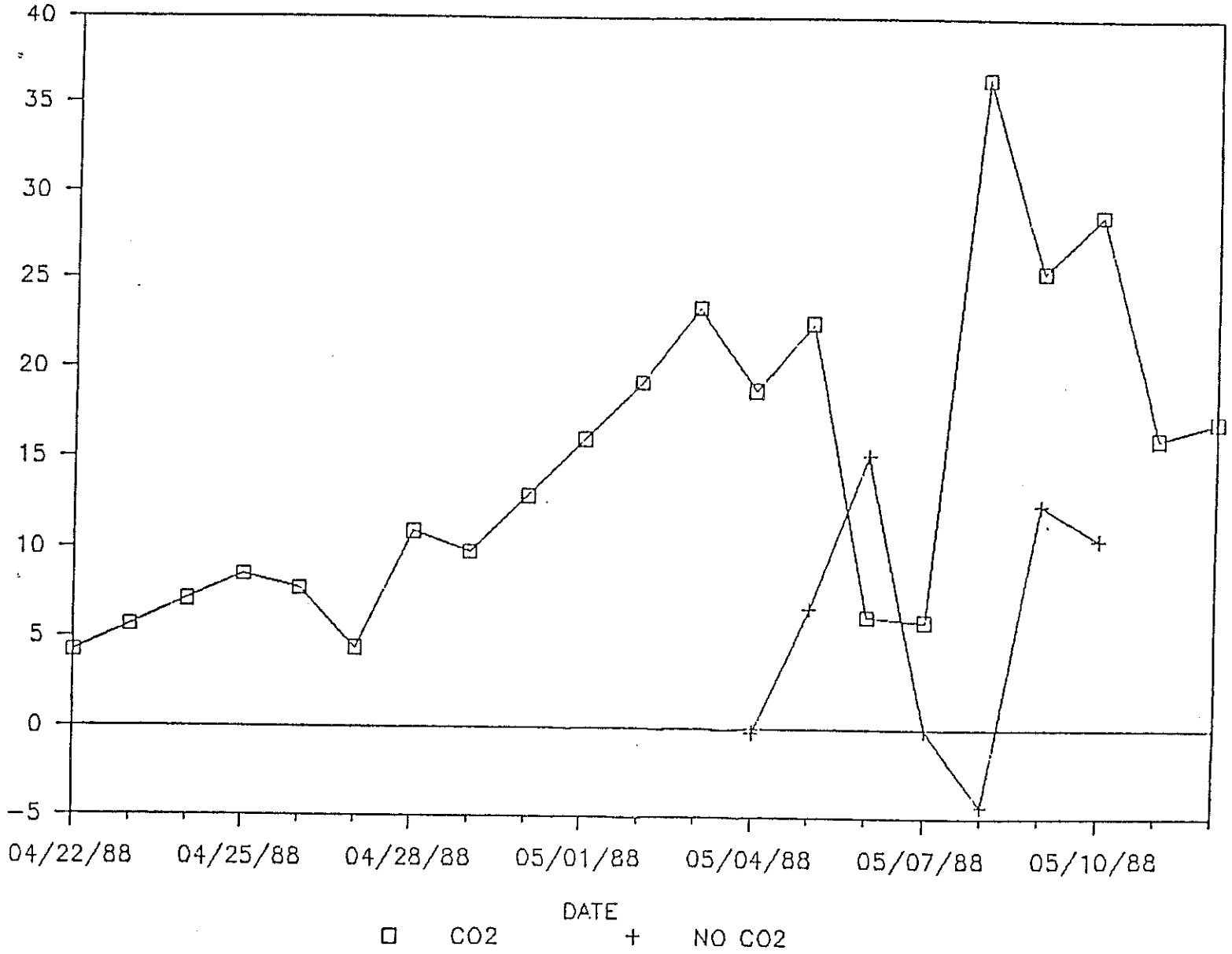


Fig. 12. Effect of CO2 Enrichment on TET 1

continuous monitoring. Rather, algal densities were determined analytically before harvest and by calculation after harvest and dilution.

The measurement and control system, in its current form, only monitors water temperature and incident PAR on a continuous basis. Once the signal is received at the analog to digital board of the Keithly unit, it is routinely and reliably conditioned and the data sent to disk files for storage. The data is also graphically displayed on the computer monitor. The whole operation is controlled and directed with the LABTECH NOTEBOOK software.

NOTEBOOK is a good systems development tool, but is somewhat ungainly and inflexible for actual applications work. Probably, efficient custom applications software will be executed in Soft500, an expanded BASIC provided by Keithly.

We are awaiting the delivery of an in situ sensor/transmitter that contains sensors for pH, salinity, temperature, and dissolved oxygen (DO). It transmits the data for these parameters directly to a computer via an RS232C port. If this equipment proves itself in field use, a future DAC system might consist of the computer, a multiplexer, and multiple transmitters for its culture monitoring functions. A Keithly DAC system, appropriate software (NOTEBOOK or custom BASIC program), and sensors (thermocouples and PAR sensors) could be used for continuous environmental monitoring. Automatic harvesting and filling of the raceway could be similarly controlled with the Keithly system and appropriate software.

Outdoor Growth Trials. The same four algae species tested at laboratory scale were used for outdoor growth trials in the two greenhouse

covered, 50 m^2 concrete raceways at the RTF. These pilot-scale trials are more predictive of algae performance at commercial scale than lab scale is of pilot scale because pilot scale and commercial culture experience the same light levels, diurnal temperature fluctuations and both are not axenic.

Figure 13 compares the best growth trials of each species. TET1 is clearly superior to the other species in long-term culture stability and resistance to competition from weed species, as well as productivity. TET1 lasted 45 days before it was drained and cleaned to allow other species to be tested. For most of the run, productivities equaled or exceeded 15 g/m^2/day, the productivity goal for this year's work. Higher productivities are expected in warmer weather.

CHAET6 and CHAET9 demonstrated productivity and longevity similar to one another. CHAET14 was much less productive, but had a longevity similar to the other Chaetoceros species. It should be noted, however, that CHAET14 was last tested during the period of 1/29/88-2/19/88, unlike the others which had runs one to two months later and were therefore influenced by warmer temperatures. CHAET14 grown in the warm months might well demonstrate higher productivity.

Figure 14 compares the productivity of TET1 at different times of the year with all other conditions identical (i.e., water type, fertilizer, harvesting regime, etc.). The 3/23/88 to 4/15/88 run lasted much longer and was much more productive than the 12/8/87 to 1/8/88 trial. Although PAR light levels are lower in the winter, there still is enough light to produce higher productivities than demonstrated. Statistical analysis of laboratory data has confirmed that temperature is a very influential variable affecting productivity.

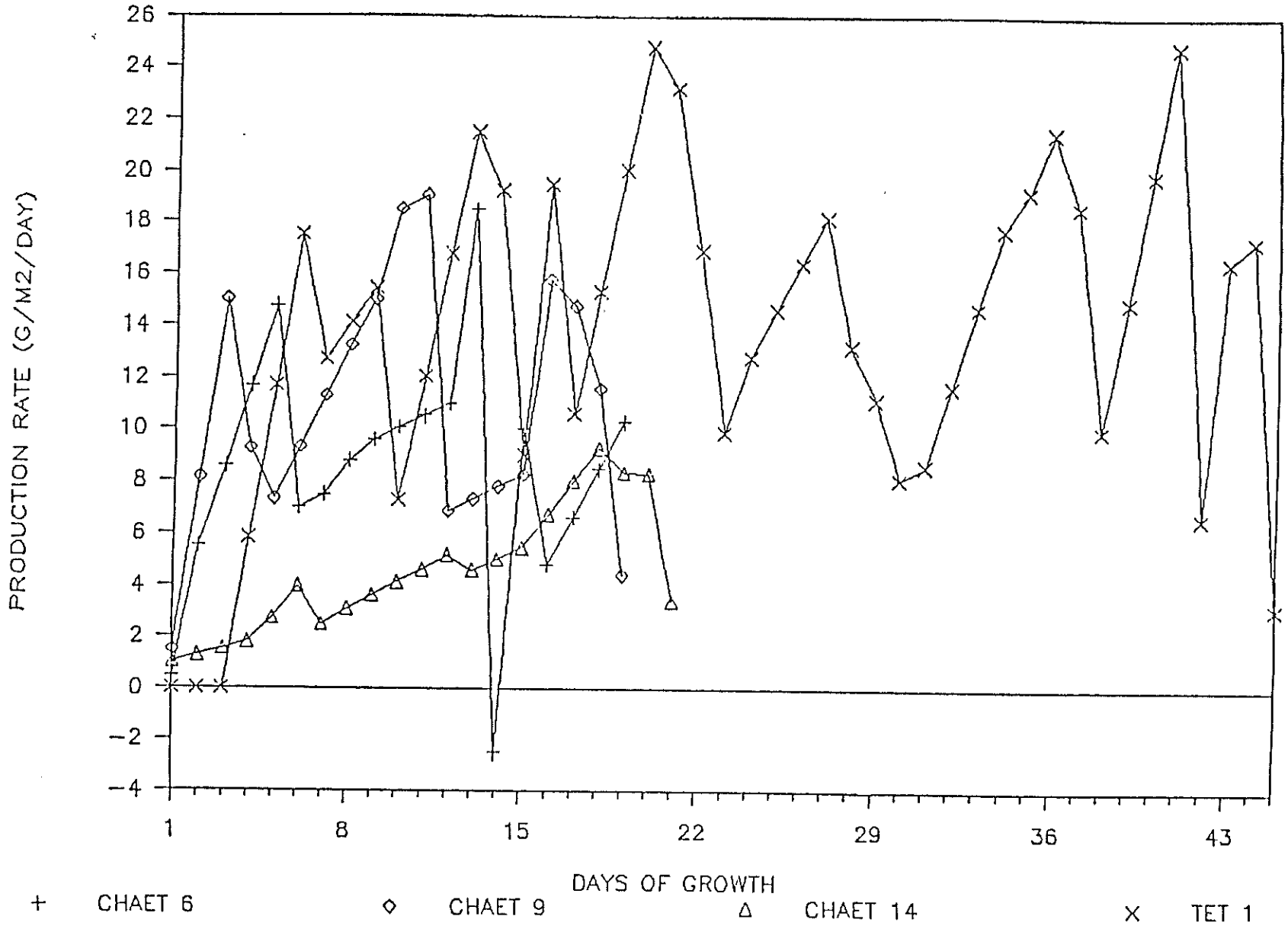


Fig. 13. Production Rate Over Time, Best Run of Each Species

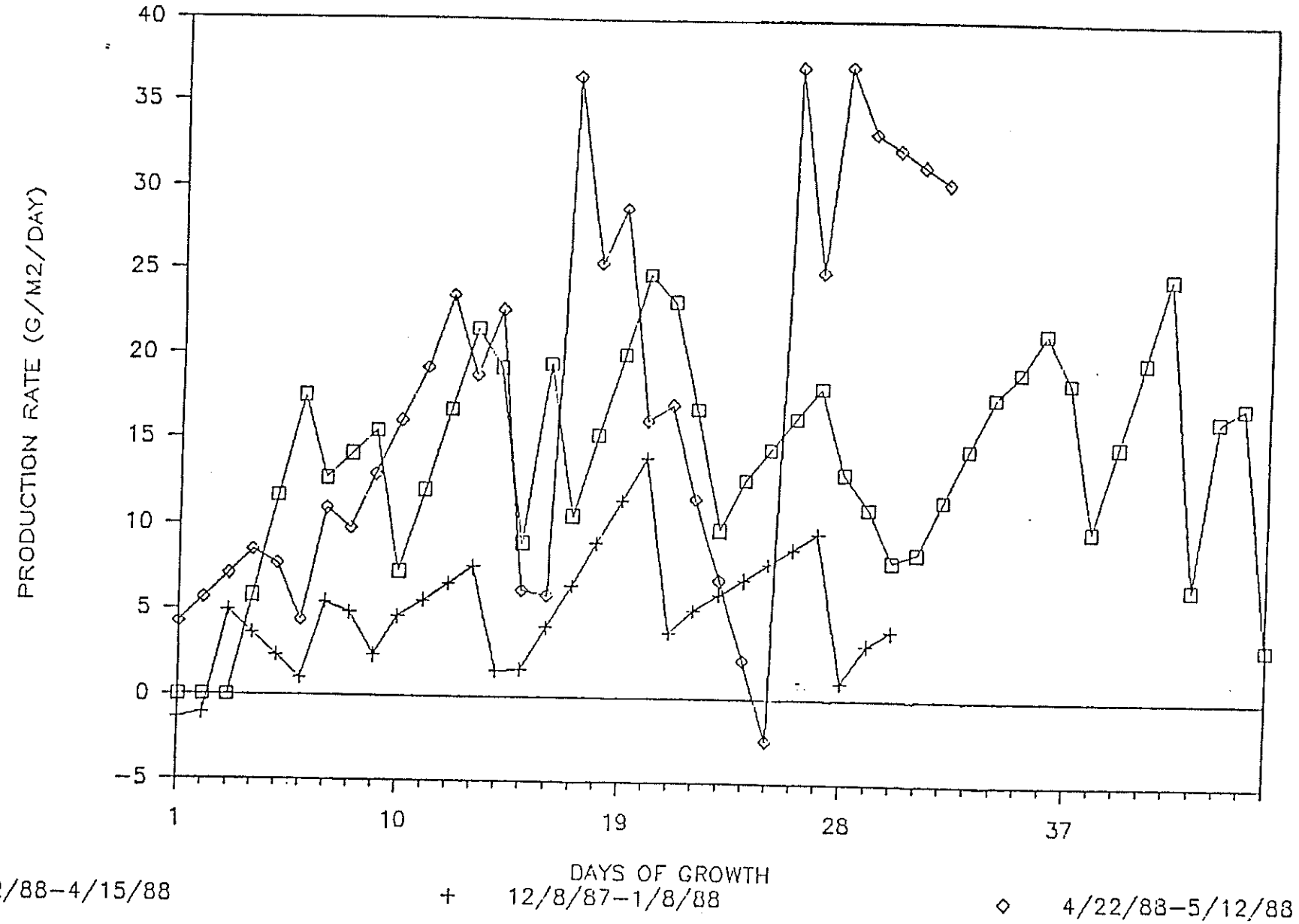


Fig. 14. Production Rate Over Time, Tetraselmis I

Comparisons of the lab and pilot-scale trials indicate that species that do well at one scale do not necessarily do equally well at the other. The laboratory work's purpose is to provide information that may be used to optimize pilot-scale production.

Apparently, the large diurnal temperature swings experienced by the algae at pilot scale (but not at lab scale) greatly effected the algae's growth. In future work, comparative growth trials at lab scale will be run with four diurnal temperature cycles representative of each of the seasons during a year to simulate more fully outdoor conditions. Higher light levels will also be used. Unfortunately, the bacterial loads and protozoan contamination often found in outdoor culture cannot be effectively simulated in lab scale work.

Shellfish Feeding Trials

Methods and Procedures. Three species of bivalve molluscs were evaluated for survival and growth in RTF water and two different blends of RTF water and additives. Previous research documented increased shellfish growth in RTF water when blended with a local potash product, KayMag. Blend 1 and blend 2 represent two different concentrations of KayMag in RTF water. Blend 1 contained 0.76 g/l, while Blend 2 contained 3.86 g/l.

The species evaluated were Crassostrea gigas, C. virginicia, and Mercenaria mercenaria. Each treatment was replicated three times. All shellfish received Tetreselimis suecici, an algal species shown to be a good food by Walne et al. Temperatures were the same for all populations and ranged from 20 to 29°C over the experimental period.

The culture vessels were rectangular fiberglass boxes with dimensions of 915 mm (long) x 203 mm (deep) x 158 mm (wide). A standpipe maintained the water depth at 152 mm. Two air-water lift (AWL) systems, placed at the opposite end of the vessel from the standpipe, were used to aerate and mix the vessel's contents. All treatments received the same type and amount of algae via drip irrigation emitters at a rate of 30 ml/min, 24 hours a day for a total input volume of 43.2 l per day. The density of the feed algae was 10 mg Ash Free Dry weight/l. Thus, each population received 0.43 g AFDW algae per day. Each treatment received the same volume of saline ground water in a similar manner. Those treatments receiving blended saline water received blends at double strength so that after dilution with algae (grown only in RTF water), the final concentration of the inflows received by the treatment was at normal strength. It was not logistically feasible to daily measure effluent algal densities for the 27 vessels.

The shellfish were placed in rectangular mesh bags made from fiberglass windowscreen and open at the top. The bags were placed on two longitudinal plastic pipe supports that suspended the shellfish 75 mm off the bottom of the growth vessel, so that the outflow of the AWL system washed over the shellfish.

C. gigas, the Pacific oyster, with an average individual live weight of 2.78 mg, was stocked at 10 g live weight per growth vessel, for a stocking density of 71.9 g live weight/m^2. C. virginica, the American oyster, could be obtained only in larger sizes; sufficient individuals, with an average individual live weight of 1076 mg, were stocked to provide an initial population live weight of 72.2 g per vessel, for a stocking density of 519.1 g live weight/m^2. The other bivalve mollusc evaluated, M. mercenaria

(the hard clam), with an initial average individual live weight of 8.24 mg, was stocked with 17.2 g live weight per vessel for an initial stocking density of 123.7 g/m^2. These stocking densities were chosen on a dry meat basis: C. gigas and M. mercenaria were stocked at the same dry meat density, while C. virginica was stocked at ten times this level. If growth of C. gigas and M. mercenaria was sufficient to bring the population weight up to the range of C. virginica (which was the preferred stocking density), then periodic culling would be initiated to maintain all populations at the higher stocking density. Whole live weight measurements were used to assess shellfish growth because it does not require the death of the animal and its removal from the experimental system as does dry meat weight measurements. When periodic culling began, dry meat weight measurements could be performed on the culled animals in addition to whole live weights of the remaining population.

The growth trials commenced January 20, 1988, and the results through mid-June 1988 are reported herein. Results are reported both as weight increase and as G30, a thirty-day "instantaneous" growth rate calculated as G30 = (30/t)*ln(wt/wo), where wo = initial live weight, wt = final live weight, and t = elapsed time in days. Great efforts were made to perform live weight measurements reliably. Of particular note, was the drying of the animals for two hours under a strong fan. Since water on the shell is the greatest contributor to non-reliable results, the methods used eliminated a major source of error.

M. mercenaria. M. mercenaria did not grow well in any of the water types tested. In straight RTF water, (figure 15) the average population weight increased only to 19.3 g, a 12.2 percent increase. It did

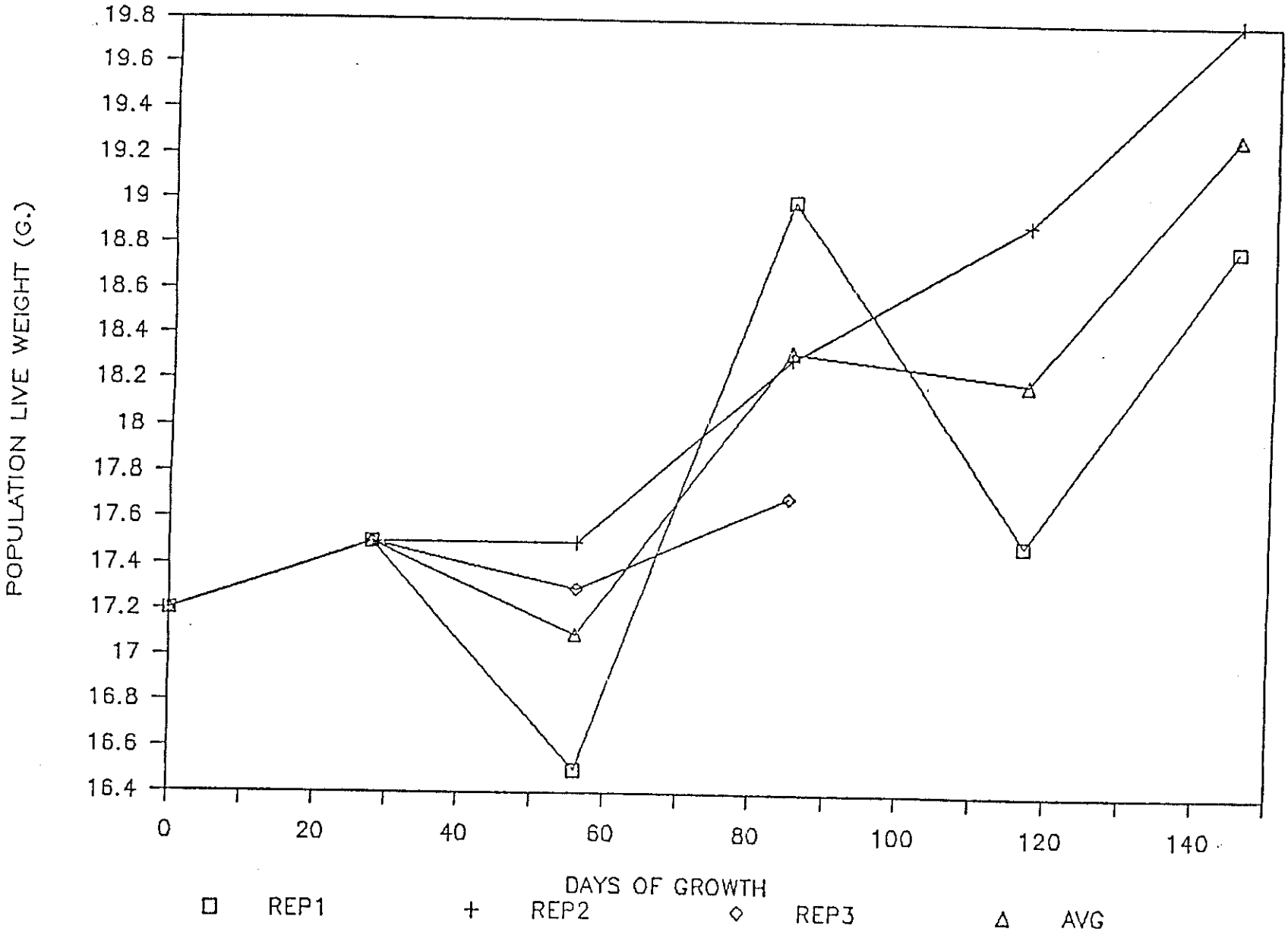


Fig. 15. Population Growth in RTF Water, M. mercenaria Juveniles

less well in Blend 1, increasing only to 18.5 g, a 7.6 percent increase (figure 16). It did least well in Blend 2 where the population weight decreased 1.6 percent to 16.9 g (figure 17). Figure 18 summarizes these results.

The principle reason for the poor growth of M. mercenaria is believed to be the suboptimal salinity of the saline water. The lowest tolerable salinity for these organisms is considered to be 20,000 ppm. Throughout the growth trials salinity ranged from 10,000 to 15,000 ppm (Goldstein and 1989 Roels). RTF water exceeds 20,000 ppm at times, but not reliably throughout the year. Thus, at this time, it is not recommended that this species be grown at the RTF unless an effort is made to increase and maintain higher water salinities. Other shellfish species, especially clam species, that are more tolerant to low salinities should be evaluated.

C. gigas. C. gigas experienced an initial rapid weight loss when placed into straight RTF water (figure 19), but soon stabilized at an average population weight of 5.5 g, a 45 percent decrease in weight. A similar pattern was observed in Blend 1 (figure 20), but the weight decrease was somewhat less drastic at only 32 percent.

In Blend 2, however, C. gigas demonstrated excellent growth (figure 21). On average, population live weight increased 450.3 percent to 55.03 g.

The leveling of growth in later weeks could be an indication of a sublethal stress related perhaps to water chemistry, but more likely due to insufficient food. This insufficiency was caused by a series of facility and equipment failures that prevented full delivery of algae to the shellfish.

Moreover, the reduced amounts of food were distributed unevenly. This may account for the anomalously low growth in replicate 1, compared

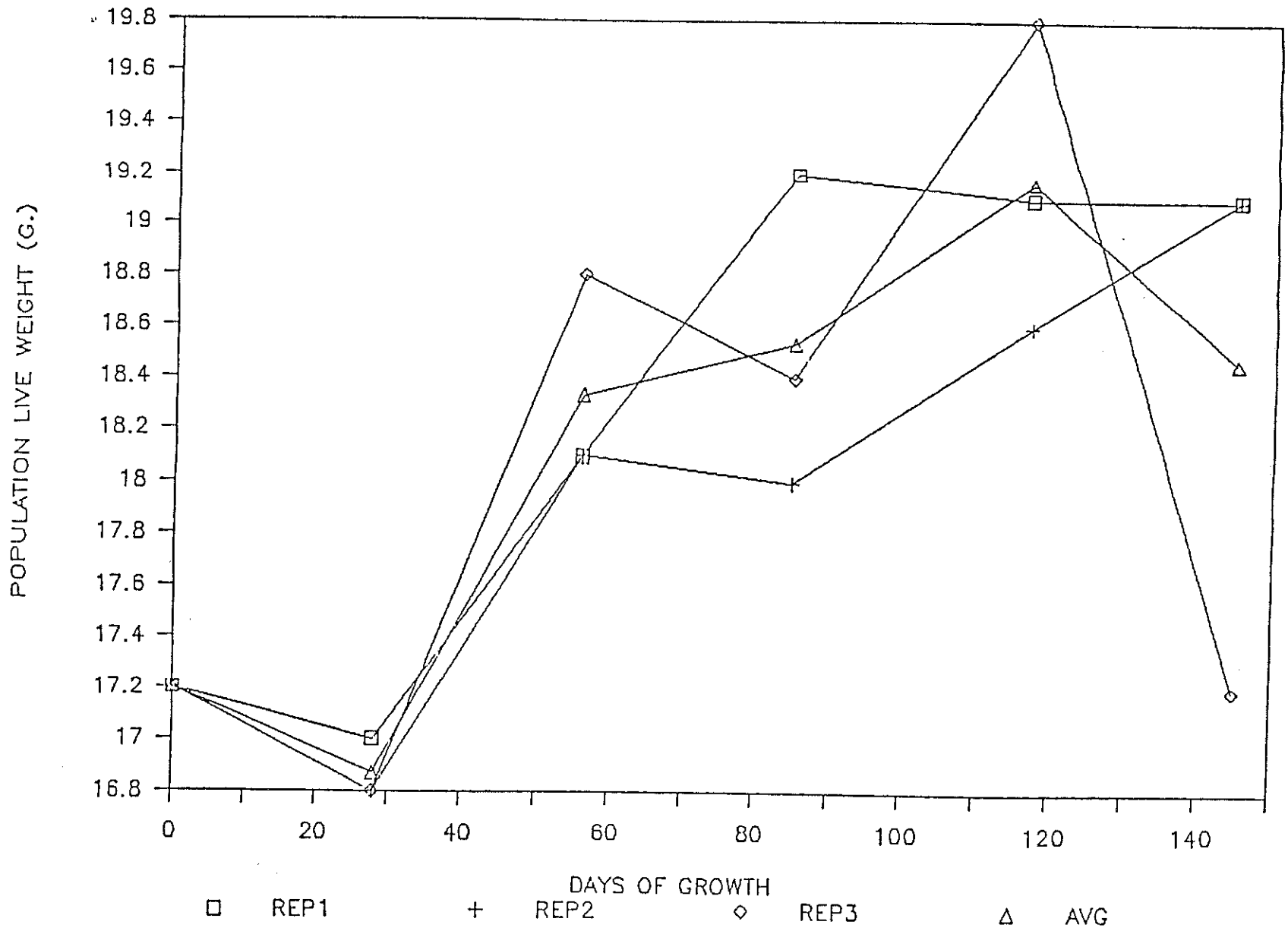


Fig. 16. Population Growth in Blend 1, M. mercenaria Juveniles

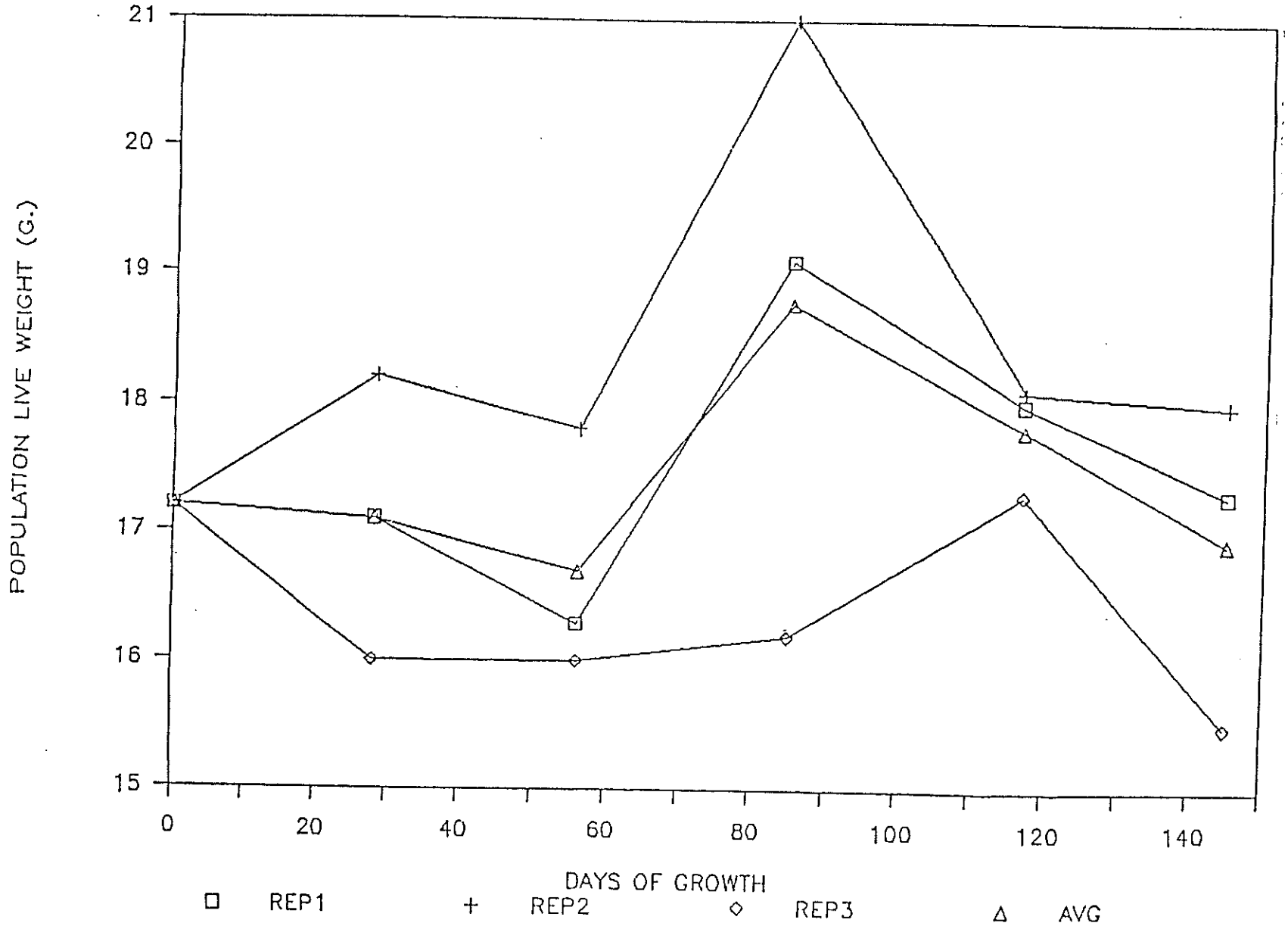


Fig. 17. Population Growth in Blend 2, M. mercenaria Juveniles

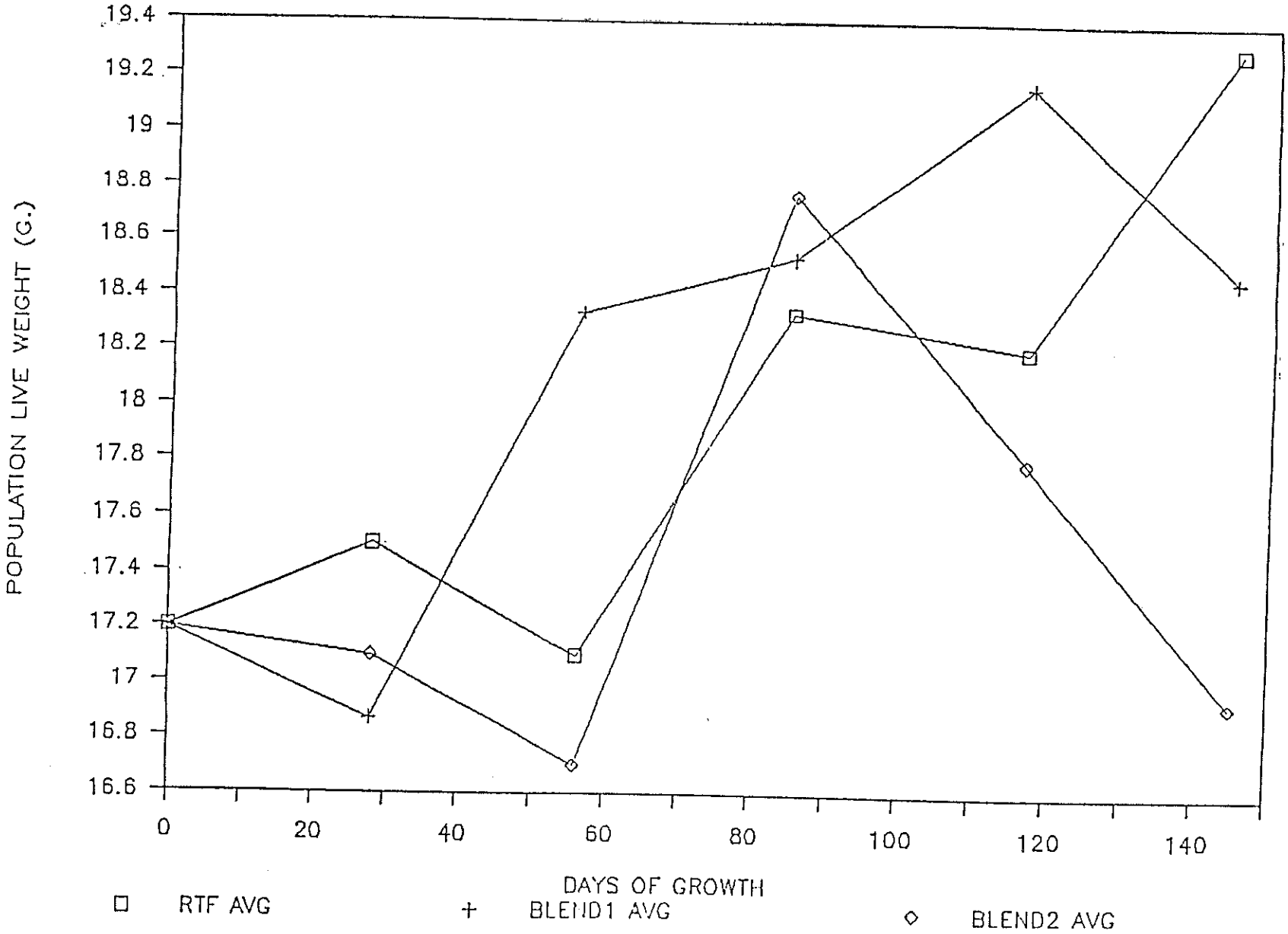


Fig. 18. Population Growth in Different Waters, M. mercenaria Juveniles

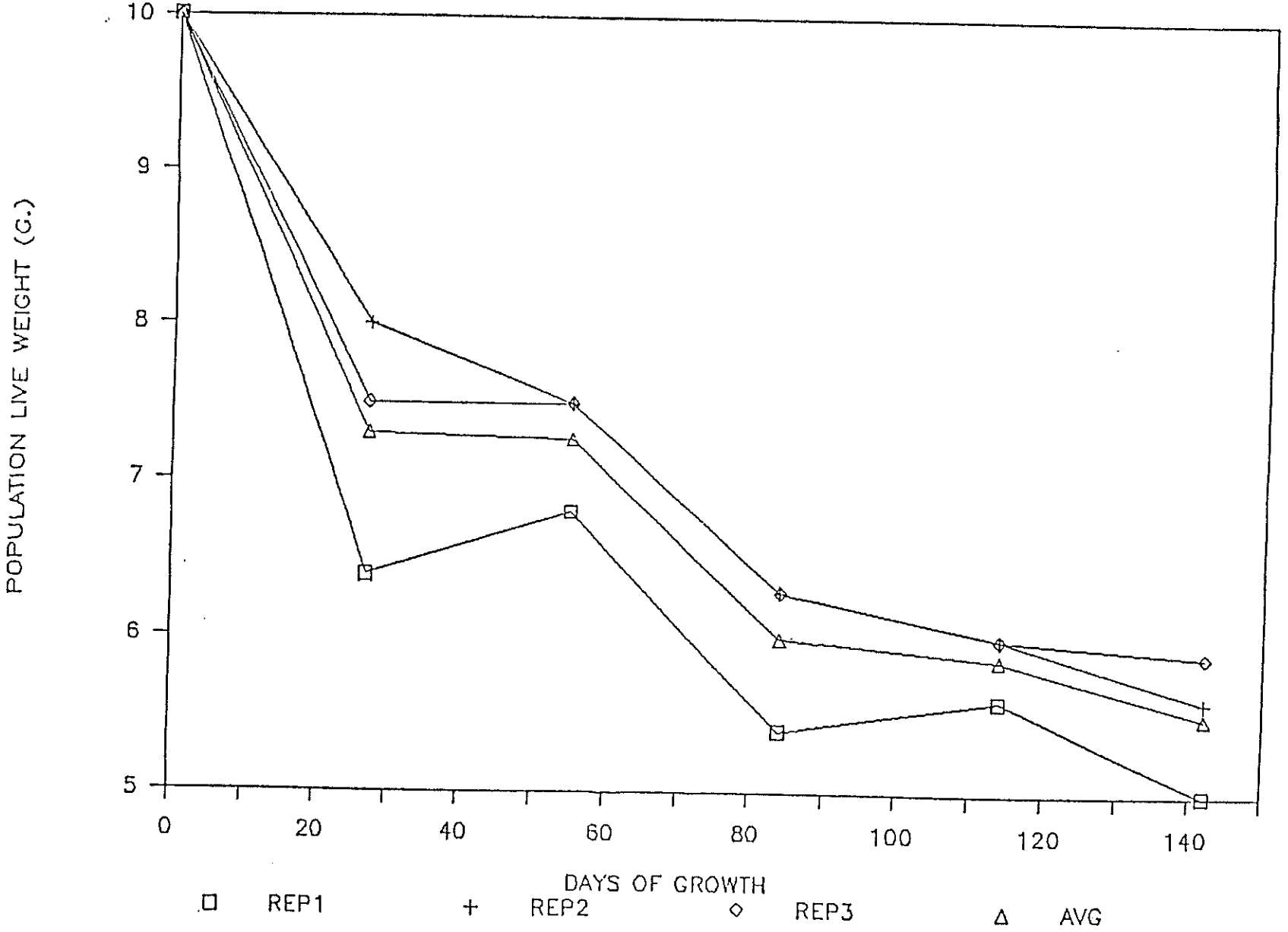


Fig. 19. Population Growth in RTF Water, C. gigas Juveniles

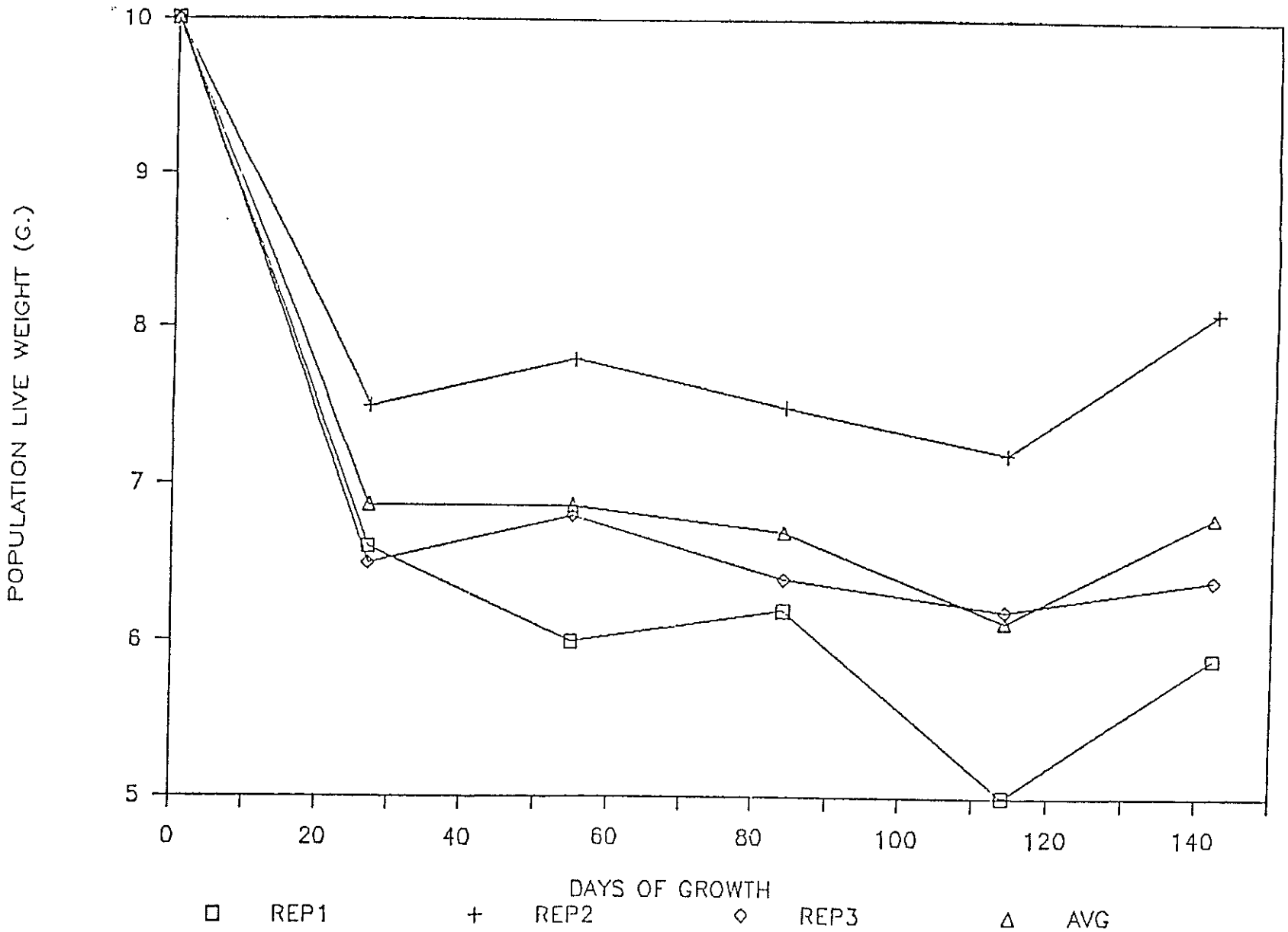


Fig. 20. Population Growth in Blend 1, C. gigas Juveniles

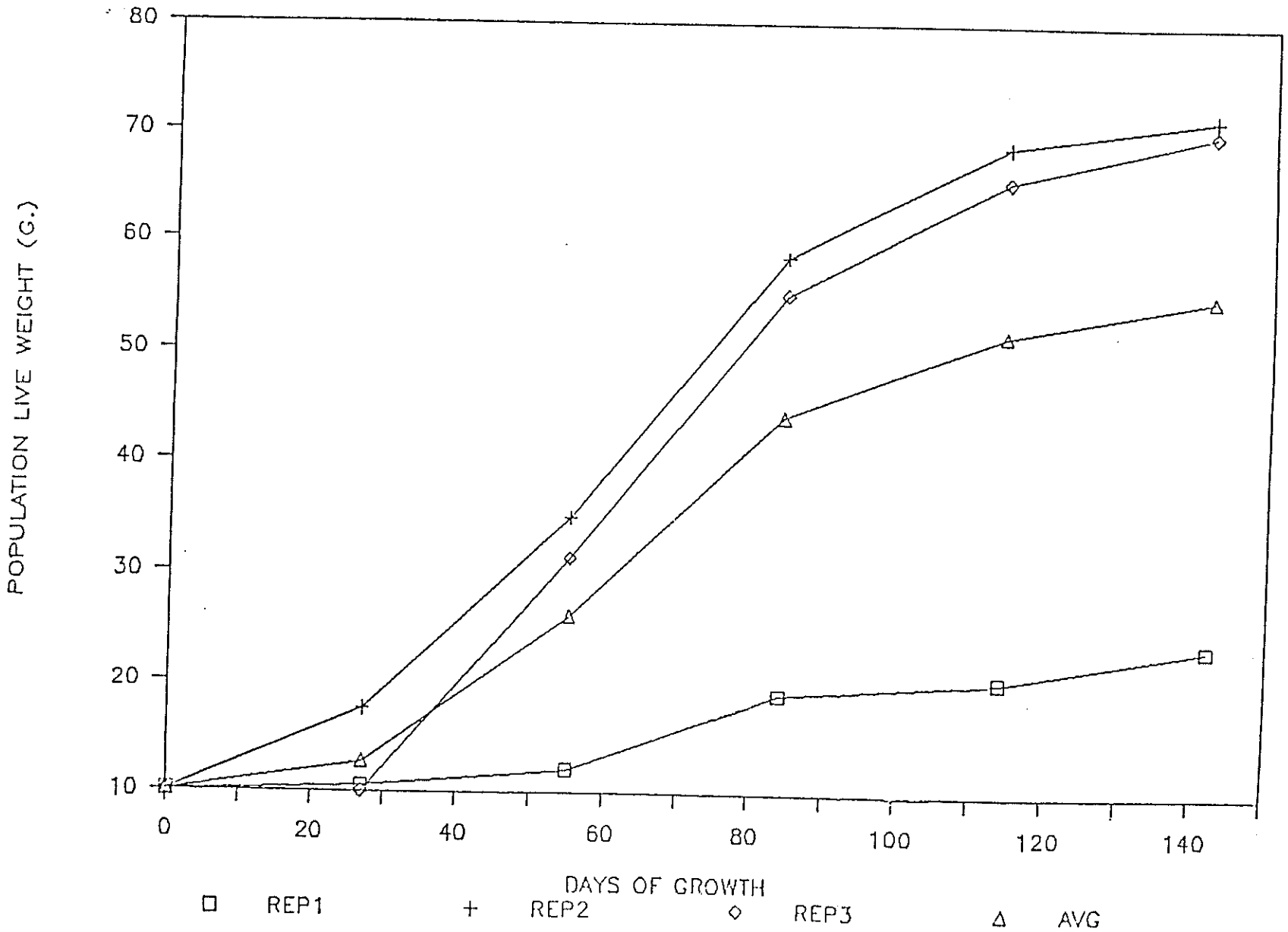


Fig. 21. Population Growth in Blend 2, C. gigas Juveniles

to the other replicates. These failures have been remedied, and, if limited food was the cause of lower growth, increased growth should be evident in following months.

The effect of water type on the growth of C. gigas is summarized in figure 22. There is excellent growth of C. gigas in Blend 2 water and no net growth in RTF and Blend 1 water.

C. gigas, G30 Comparisons. G30 is an excellent index for comparing the growth of a species in different systems. Claus (1981) has compiled G30 numbers for several different bivalve species reared in different nursery systems. There is much variability (+/- 100 percent) in the data for C. gigas, but the average G30 calculated by regression analysis for C. gigas in other systems with an individual live weight of 70mg, is 1.4. C. gigas in Blend 2 demonstrated a G30 of 0.68, or 49 percent of the average G30 of the other systems. This is quite remarkable given that shellfish in the other systems were being reared under the best conditions and in natural seawater. C. gigas in our system, as indicated, may have suffered from fluctuating food levels and the growth reported took place during winter and spring. Little or no growth would have occurred in the other systems in the winter. G30 is very sensitive to temperature (Claus 1981). The effect on individual G30 of C. gigas of different water types is summarized in figure 23.

Prior to placement in the experimental growth vessels, all shellfish had been held in Blend 2. The initial weight loss experienced by C. gigas in RTF water and Blend 1 may be explained as acclimation. However, after 140 days in RTF Water or Blend 1, any acclimation would have occurred. It is safe to state that C. gigas prefers Blend 2 over RTF water or Blend 1.

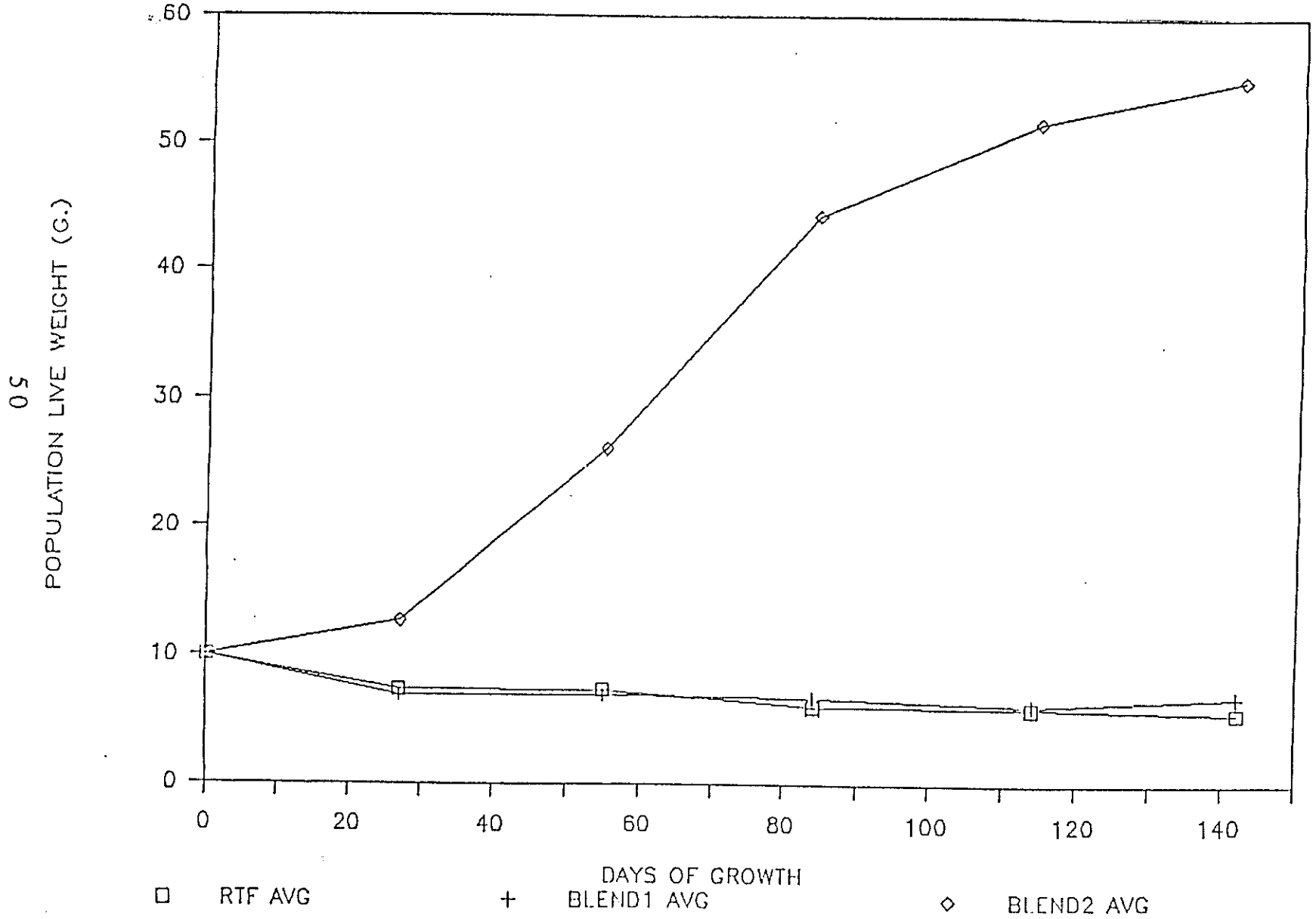
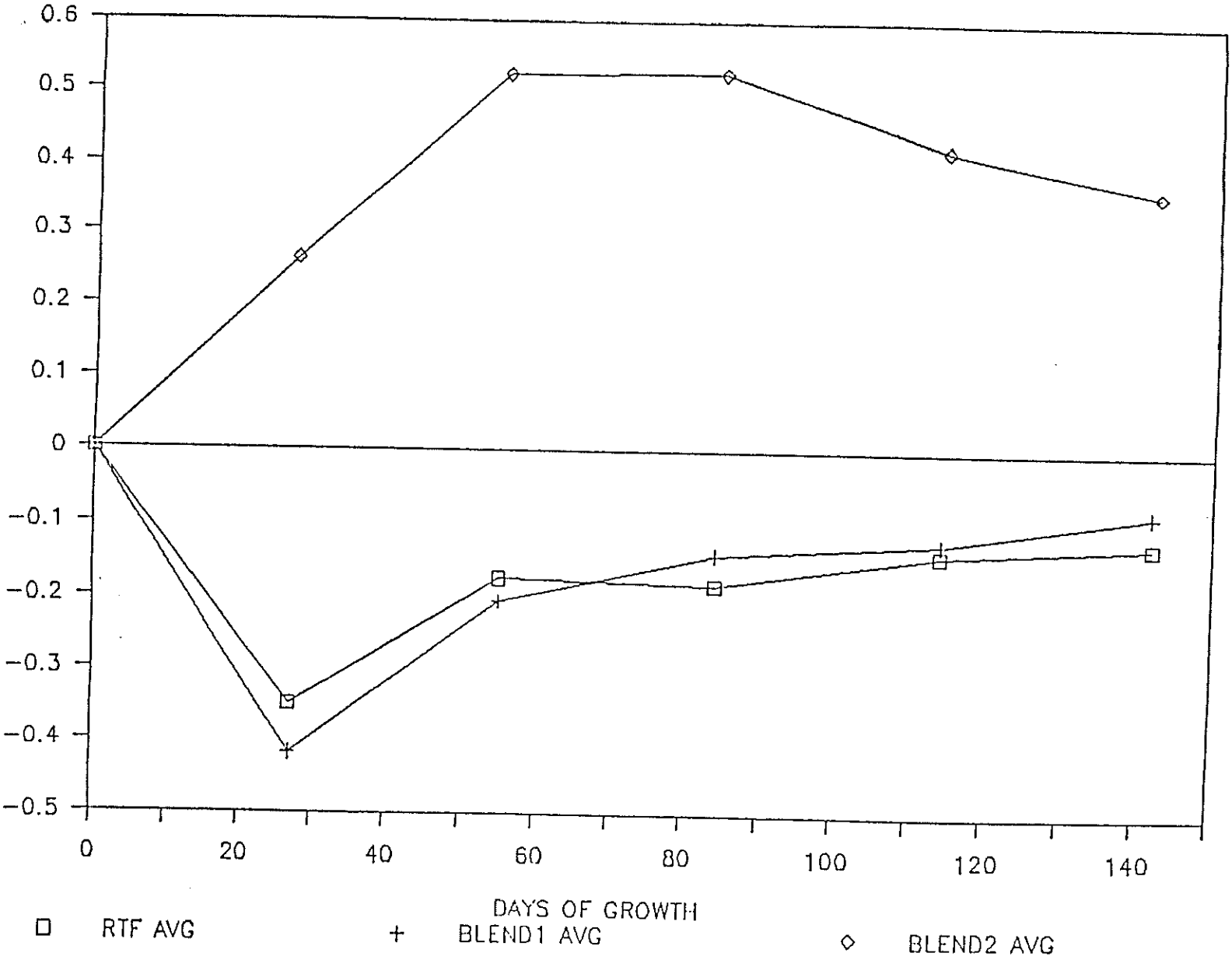


Fig. 22. Population Growth in Different Waters, C. gigas Juveniles



G30

Fig. 23. Population G30 in Different Waters, C. gigas Juveniles

It should also be noted that because individual weights and thus individual G30 are determined by measurement of a subsample of each population, there is appreciably more scatter in the data as compared to other data where the whole population is weighed. For this reason, wherever possible, most of the results of the growth trials are reported for population growth which are more constant and reliable indicators of growth then are individual growth. Unfortunately, Claus (1981) only lists individual G30. Thus, we had to use our individual G30 for comparison purposes.

C. virginica. C. virginica demonstrated the same growth responses as C. gigas to RTF water and to Blend 1, i.e., an initial rapid weight loss followed by a leveling of growth (figures 24 and 25). The weight loss is more pronounced in RTF water.

Similarly to C. gigas, C. virginica demonstrates excellent growth in Blend 2 (figure 26) with an 84 percent increase in live population weight. The effect of water type on population growth is summarized in figure 27. Claus (1981) does not list individual G30 for animals larger than 1000 mg, nor specifically for C. virginica. She states, however, that the extant data for C. virginica compares well with that of C. gigas. Accepting these limitations, we documented an individual G30 for C. virginica of 1076 mg live weight of 0.09, which closely approximates the individual G30 calculated for all systems compiled by Claus (1981). Individual G30 for C. virginica in all water types is summarized in figure 28.

If one makes the assumption that the average individual weight of the members of a population increases in direct proportion to that population's weight and should yield the same G30, then a G30 calculated on the basis of our C. virginica population weight data may be used to

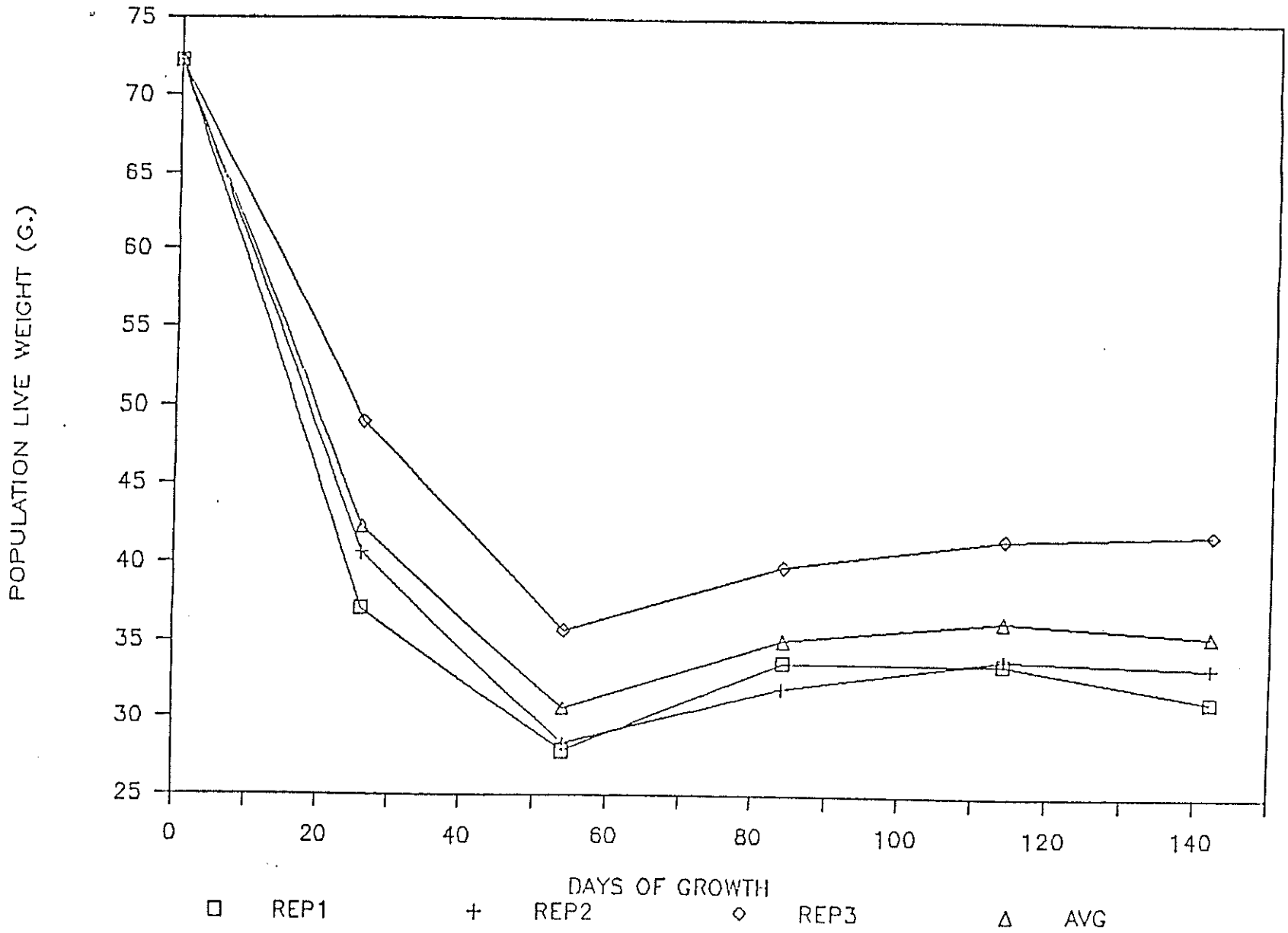


Fig. 24. Population Growth in RTF Water, C. virginica Juveniles

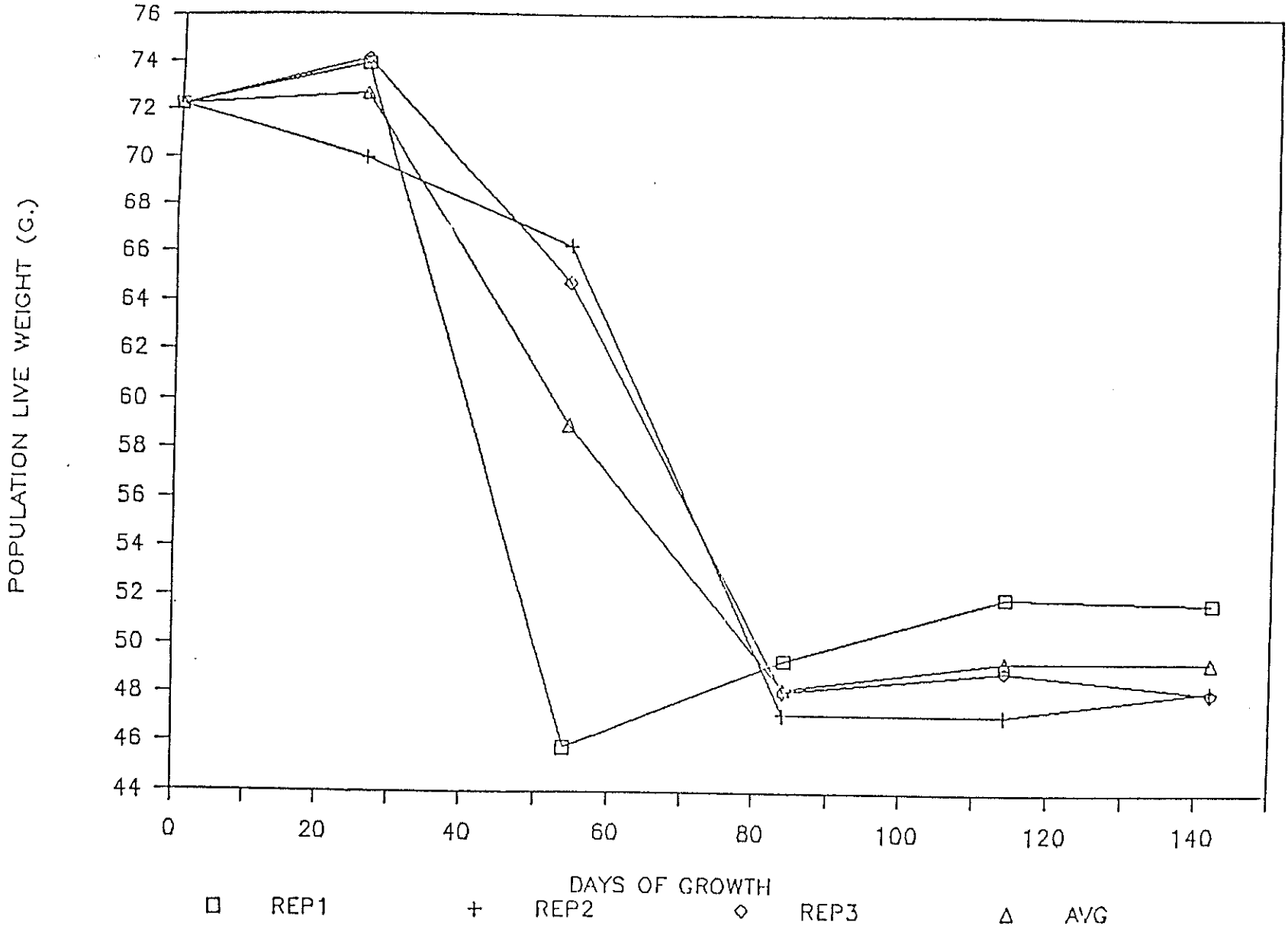


Fig. 25. Population Growth in Blend 1, C. virginica Juveniles

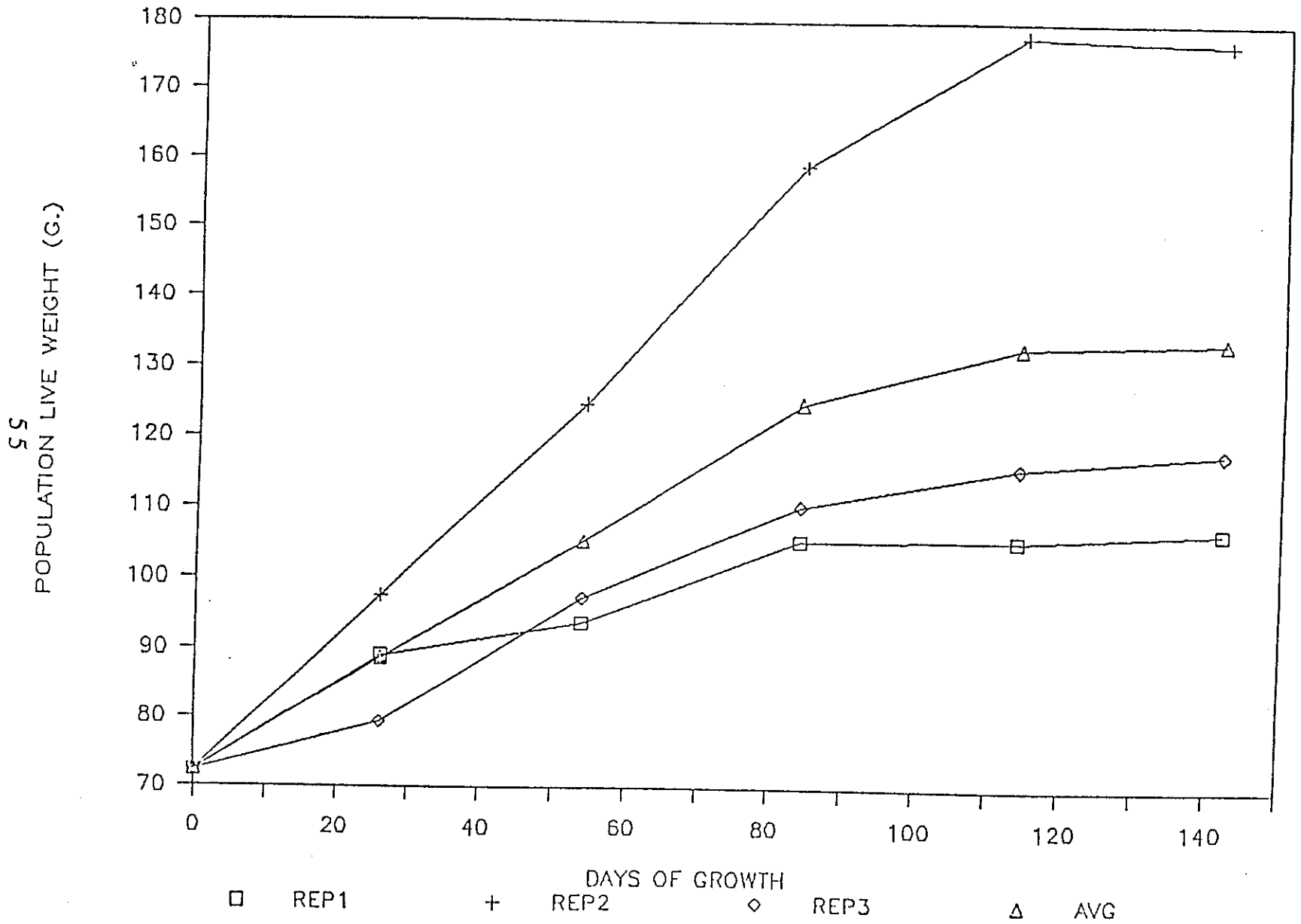


Fig. 26. Population Growth in Blend 2, C. virginica Juveniles

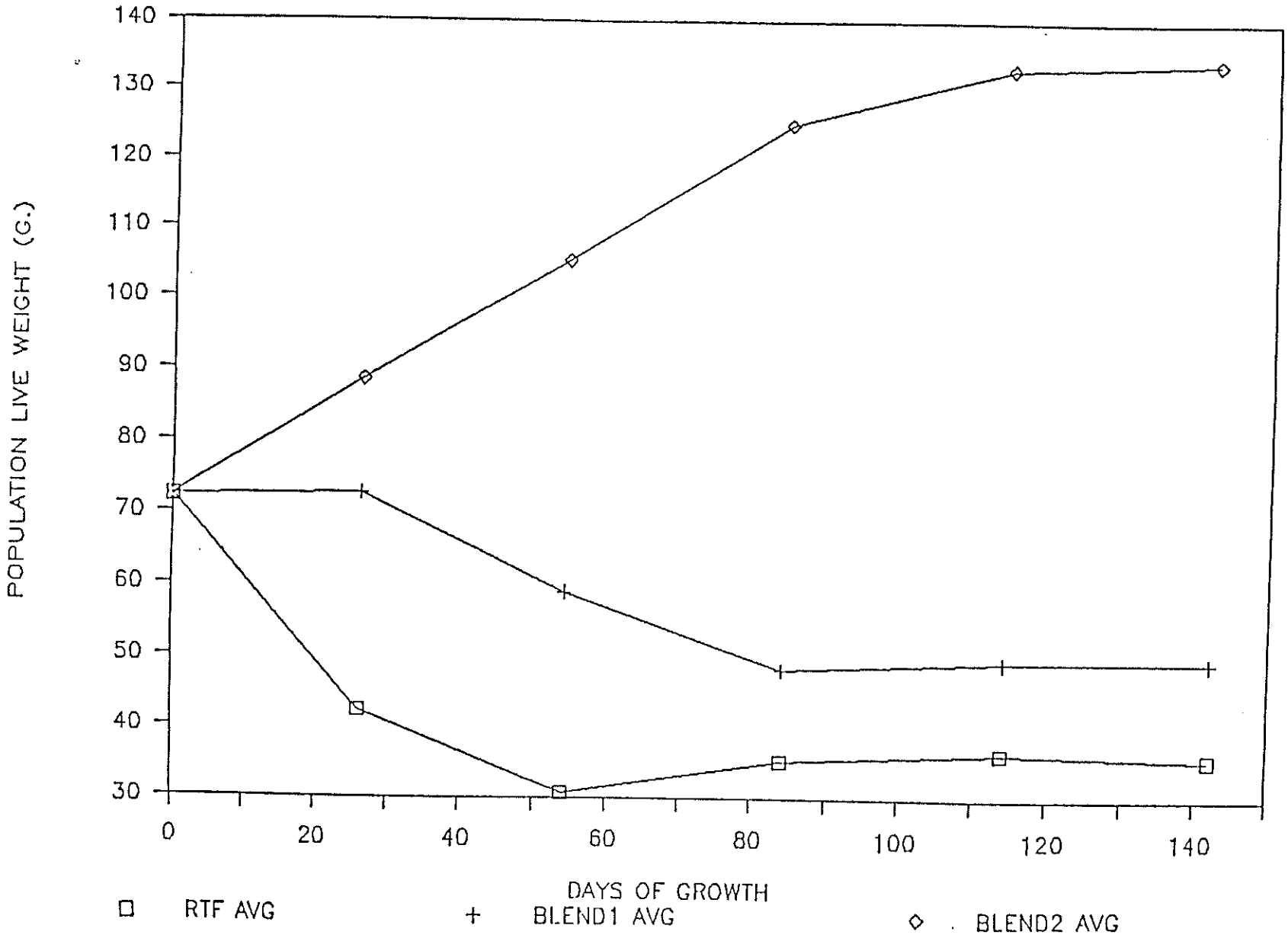


Fig. 27. Population Growth in Different Waters, C. virginica Juveniles

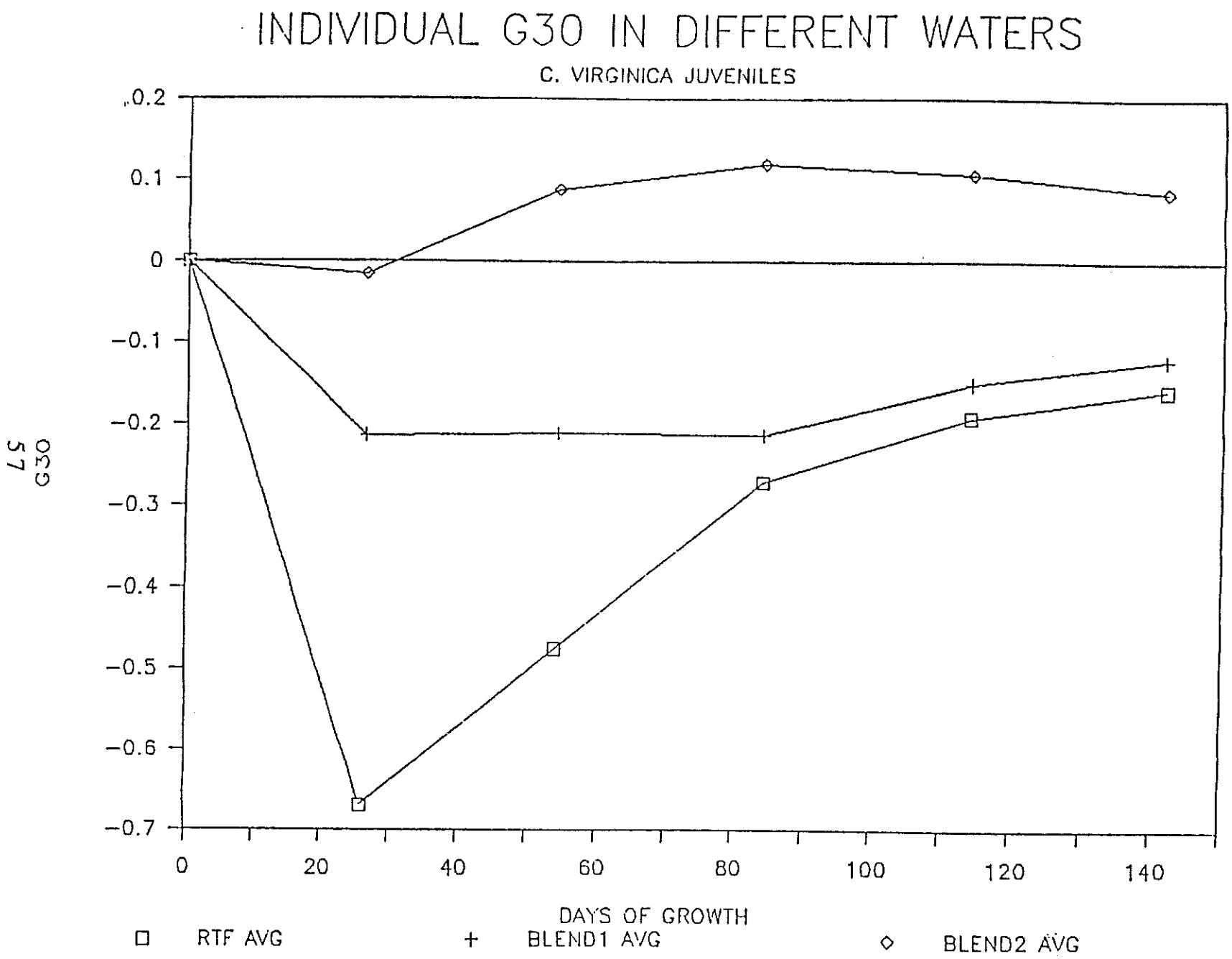


Fig. 28. Individual G30 in Different Waters, C. virginica Juveniles

compare to the individual G30 of other systems (figure 29). On this basis, C. virginica G30 exceeds that of other systems. However, given the variability of both our data and of that compiled by Claus, and because we are using the extreme range of her data, the safest statement which can be made is that C. virginica grown at the RTF appears to grow as well as C. virginica grown in other nursery systems around the world. This is a remarkable achievement given the suboptimal conditions (erratic and, at times, insufficient food) experienced by our shellfish, especially as compared to the excellent conditions of the other nursery systems.

Brine Shrimp Survival and Growth

In order to expand the suite of filter-feeding species that might be grown at the RTF, several series of experiments were conducted to choose the better strains of Artemia and the more optimal growth densities.

Hatching Efficiency. To determine the number of nauplii hatched per gram of cysts in 48 hours at 22°C, the following procedure was used: three 15 liter hatching containers were set up (one for each species) and filled with 14 liters of RTF water. The containers were illuminated from above using fluorescent lights.

The appropriate amount of cysts for each of the three species were calculated, weighed and placed in 1.5 liters of distilled water for one hour of prehydration.

The cysts were then placed in the hatching containers and aerated vigorously from the bottom of the containers for 48 hours at temperatures between 22°C and 24°C. Temperatures of the containers were recorded at 0, 12, 24, 36, and 48 hours.

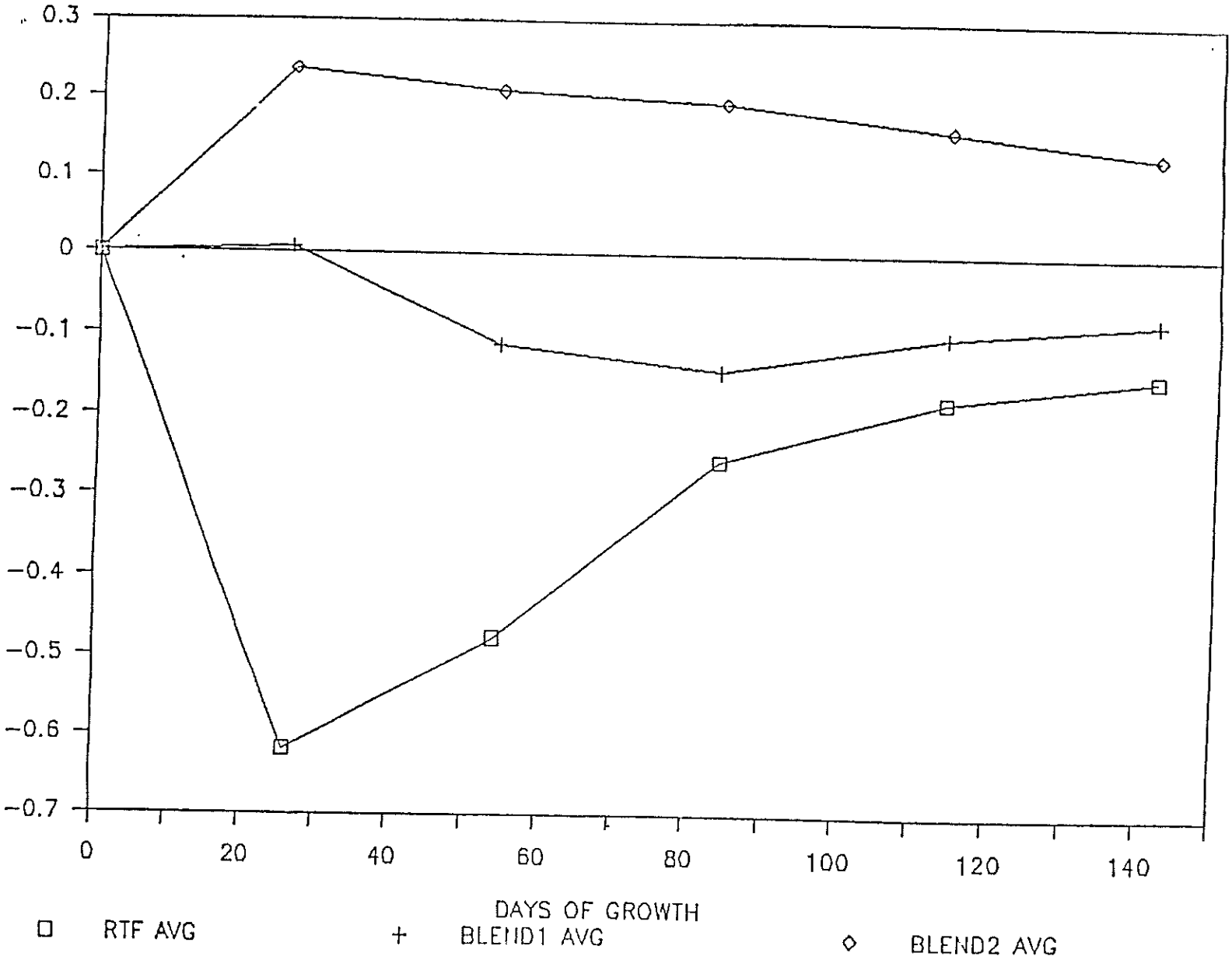


Fig. 29. Population G30 in Different Waters, C. virginica Juveniles

Aeration was stopped, the nauplii settled to the bottom of the hatching containers, and were collected in a total of one liter of RTF water. From each liter of nauplii, a 10 ml sample was taken, the nauplii collected on filters, and counted. The number of nauplii per ml was calculated for each species.

Three varieties of brine shrimp cysts (obtained from Cultured Aquatics of Northport, NY) were placed in RTF water in the following densities:

Great Salt Lake (GSL)	0.0357	mg/ml
Chinese	0.0357	mg/ml
Canadian	0.2500	mg/ml

The Canadian variety was stocked at a much higher level because of impurities which could not be separated from the cysts.

The cysts were hatched and surviving nauplii were collected and counted; the resulting yields were:

Great Salt Lake	0.857	nauplii/ml
Chinese	0.642	nauplii/ml
Canadian	5.357	nauplii/ml

Dividing nauplii/ml by the initial densities gives the following hatching efficiencies in nauplii produced/mg of cysts hatched:

Great Salt Lake	24.006	nauplii/mg/cysts
Chinese	17.983	nauplii/mg/cysts
Canadian	21.428	nauplii/mg/cysts

The number of cysts in one mg is on the order of thousands of individuals, hence these results represent very low hatching efficiencies. What is important is not that so few of the cysts became nauplii, but that they hatched at all. The experiments reported here are preliminary in nature. That some few brine shrimp did manage to survive and grow in

pure RTF water is significant enough to merit further study of the ways in which RTF water may be modified to provide a more suitable environment for brine shrimp growth. Simply establishing and maintaining a pH of 8.0 of the RTF water (by simple additions of acid or base) may improve hatching efficiencies.

Growth Rates at Different Densities. Using the nauplii hatched above, each of the three varieties of brine shrimp was observed for its growth rates at high and low densities of 1 nauplii/ml and 1 nauplii/5 ml respectively.

Twelve growth chambers were set up (3 species x 2 densities, done in duplicate) and partially filled with RTF water. An algal suspension grown in RTF water was added to the growth chambers to reach a final density of 1 x $10E^5$ cells/ml. The chambers were then filled to a total volume of 14 liters with RTF water.

An 8 quart basket was suspended in each container and fitted with the smallest size mesh. The appropriate number of nauplii were placed inside the baskets and the airlift assemblies installed.

Each day the temperatures of the growth chambers were recorded. The chambers were also swabbed with a bottle brush to dislodge settled algae, then cell densities were calculated. If necessary, algae were added from an algal suspension grown in RTF water to maintain an algal density of 1 x $10E^5$ cells/ml or higher.

Once weekly the culture water was changed and a new algal suspension was added to the chambers. The mesh size of the 8 quart baskets was also changed weekly from 125 to 250 and 500 μm.

At the conclusion of the experiment (2 weeks, 2 days at 22-24°C), the length of 20 randomly selected brine shrimp from each container were measured. The remaining brine shrimp were collected, counted, and placed on filters for wet and dry weight measurements. The average length, wet weight, and dry weight were calculated.

The variability in survival among the replicates is shown in table 4.

TABLE 4
Total Number of Shrimp in Each Growth Chamber

Density	GSL	Chinese	Canadian
One per ml	457.00	359.00	38.00
One per ml	169.00	287.00	241.00
One per five ml	213.00	194.00	458.00
One per five ml	6.00	0.00	285.00

Despite the variability, however, those individuals that survived, from each variety and at each density, all grew to about the same average length. The overall average length, not excluding the sample with no growth, for all varieties and densities, was 8.55 mm. The breakdown length for each variety and from both replicates of each initial stocking density is shown in table 5. The average length for Chinese (8.8 mm) and Canadian (8.7 mm) varieties is somewhat higher than the Great Salt Lake variety (8.2 mm), but these may not be statistically significantly different.

It must be reiterated the most significant result of this work is not the specific quantification, but rather the demonstration that brine shrimp can survive and grow in pure RTF water, on a diet of algae grown in the same water. Most other species that have been cultured in pure RTF water

TABLE 5
Average Length of Brine Shrimp in Each Growth Chamber (Millimeters)

Density	GSL	Chinese	Canadian
One per ml	8.00	8.80	7.30
One per ml	8.70	8.70	10.20
One per five ml	8.50	8.90	8.30
One per five ml	7.60	0.00	9.00

and blends of RTF water, have preferred the blends. The same may be true of the brine shrimp, and deserves further study.

SUMMARY

It has been demonstrated that several microalgae species, which are thought to be good food for filter feeders, will survive and grow in RTF saline ground water at pilot scale (50 m^2 raceways). However, the productivity and cultural stability of algae is greatly diminished during the cold months of the year in Roswell (October to March). System Culture, Inc., the operators of the world's first and biggest commercial land-based oyster farm in Hawaii, averaged microalgae productivity of 15g AFDW/m^2/day. This was the current year's project goal. Several of the algae species tested this year at the RTF met or exceeded this goal, but not during the colder months and only at the level of 50 m^2. The result of the algae growth trials at the RTF are very promising, especially since productivities as high as 38g AFDW/m^2/day have been achieved. However, it is absolutely necessary to demonstrate this productivity level at larger scale before technical feasibility can be firmly documented.

As a result of the shellfish feeding trials, it appears that the low salinity of RTF water and blends made with RTF water currently preclude the growth of M. mercenaria at the RTF. Blend 2 supports the excellent growth of both C. gigas and C. virginica. Favorable comparisons to growth in other nursery systems around the world may be made with the growth documented in the RTF system and this is particularly true of C. virginica. Considering the erratic and often insufficient food experienced by the shellfish at the RTF, growth rates might be greater than were documented.

Future research should focus on C. gigas and C. virginica, on evaluation of alternative blends of water and on improved and reliable supply of food to the shellfish. The dramatic differences in growth for all species evaluated between water types strongly indicates that greatly enhanced growth could result from an optimal blend. The technical and economic feasibility of using additives to enhance the growth of commercially valuable marine organisms in RTF water needs evaluation.

The apparent requirement of the algae for heated greenhouses, coupled with the possible requirement of the shellfish for a recycling system, are actually synergistic. It is logistically easier if the algae are grown in the same water type as the shellfish. Because this water has to be heated for algal growth (and preferably for the shellfish) during the colder months, less energy input would be needed if the same water is recycled rather than used once and discarded. Also, the recycled water would also require fewer additives than water used once. Of course, appropriate water treatment (protein skimmer) will be necessary to deal with dissolved organics generated by both the algae and the shellfish. Because of the requirements for continuous monitoring of the water

temperature and water chemistry, a computerized measurement and control system is required.

The picture that emerges is the feasibility of production of commercially valuable marine organisms 1000 km from the nearest source of natural seawater via the use of a highly controlled environment maintained by computer. The increased capitalization (i.e., greenhouses) and operating costs (i.e., fuel costs, additives) of this approach over shellfish production in a natural environment may be offset by both increased production and reliability, and by the reduced cost of mariculture in New Mexico as compared to coastal sites: i.e., lower land costs, unpolluted water, lower labor costs, greater insolation, and better access to inland markets. The resulting product may sell for more in the marketplace because it will be disease and pollution free.

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