THE SURVIVAL AND GROWTH OF SPIRULINA SPP. IN THE SALINE GROUNDWATERS OF NEW MEXICO

by

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ABSTRACT

New Mexico has some 15 billion acre-feet of saline groundwater. Much of this water has a salinity greater than 3,000 ppm Total Dissolved Solids (TDS) and thus cannot be used for potable water nor traditional agricultural activities.

The main objective of this project was to determine the technical feasibility of growing Spirulina in New Mexico using these saline groundwaters. Spirulina was chosen because it grows naturally in highly alkaline lakes in hot climates and is more readily harvested than other algae. Spirulina has many potential uses, ranging from a potential feedstock for ethanol fermentation and anaerobic digestion to a source of specialty chemicals (β-carotene, phycocyanins, tocopheral) to a highly nutritious feed ingredient for livestock and for aquatic organisms. Its highest economic value use is as a human health food.

For this project, a state-of-the-art algae cultivation facility was designed and built at the Roswell Test Facility (RTF). The cultivation facility consists of two, 50 square meter concrete raceways enclosed in greenhouses. Additionally, the facility houses a computerized measurement and control system, as well as a water treatment/recycling system. We have demonstrated that Spirulina can be successfully grown at this pilot scale plant using saline groundwaters.

Laboratory studies support empirical observations made at the pilot plant that several species of Spirulina will grow in full RTF saline water (14,000 ppm TDS), half RTF water (7,000 ppm TDS), New Mexico State University (NMSU) geothermal water (2,000 ppm TDS) and NMSU tap water (500 ppm TDS). Further, these species are capable of excellent growth at temperatures as high as 42°C. Growth appears to slow at 20°C and below.

In addition, experience has been gained in working with this saline water on a practical level. It is known how to minimize turbidity and precipitation problems that arise from the interaction of the saline water with added algal fertilizer and analytical procedures have been modified to work with the saline water and high standing densities of the *Spirulina*.

The technical feasiblity of *Spirulina* cultivation in New Mexico using saline groundwaters has been demonstrated. Market research should be performed to determine the size and location of potential markets and the price structure. If market research data is favorable, a small commercial size *Spirulina* culture operation should be established and operated for at least a year to determine actual operating and capital costs.

Key words: salinity, water management, groundwater, salt tolerance, greenhouse studies, algae

RELEVANCE OF RESEARCH

Two-thirds of the contiguous United States is underlain by brackish water aquifers containing water with total dissolved solids (TDS) of at least 1,000 ppm (Feth 1). All governmental agencies consider water of 1,500 ppm TDS and greater to be nonpotable. Water containing as much as 3,000 ppm TDS may be used for irrigation, although under most conditions it is unsatisfactory. Moderately saline water (3,000-10,000 ppm) is unsatisfactory for most uses and is seldom used for domestic supply (Hood and Kister 2). Brackish water aquifers may contain waters with a TDS greater than that of seawater (35,000 ppm).

The vast areas of semiarid lands in the world account for 36 percent of the land availability or the equivalent of a land area of 50 million square miles. The U.S. Southwest alone contains many millions of acres of semiarid land.

It is well known that the U.S. Southwest has very high levels of solar insolation. The annual average is 6.0 kilowatt hours per square meter per day. During the summer, it averages 7.8 kilowatt hours per square meter per day.

The assumed theoretical maximum photosynthetic efficiency is 12 percent. Using an efficiency of 6.6 percent, it is theoretically possible to produce 263 metric tons (dry weight basis) per hectare per year, or 116 tons dry weight per acre per year in the U.S. Southwest. A more conservative figure of 120 metric tons dry weight per hectare per year will be used, as this seems very possible from work carried out by the principal investigator (Goldstein 3).

It is evident that the U.S. Southwest has all the important criteria for high productivity algal farms: available water, land, and sunshine.

Spirulina is a particularly fine candidate for cultivation in high intensity algal farms in New Mexico because it grows naturally in many highly alkaline environments around the world. Various species of _Spirulina_ are ubiquitous in aquatic environments ranging from freshwater to hypersaline environments. However, _Spirulina_ seldom occurs as the dominant primary producer except under unusual environmental conditions such as exist in Lake Chad in Africa or Lake Texcoco in Mexico. The ability of _Spirulina_ to tolerate the extreme alkaline conditions of these lakes to the exclusion of other competing or predator species is an important biological characteristic that can be utilized to maintain unialgal conditions in culture systems. Thus, _Spirulina_ can grow and survive in New Mexico saline groundwaters and is more easily harvested than other algae.

There are many potential uses for _Spirulina_. It lends itself particularly well to many of these applications because it grows in long filaments, which makes harvesting the algae considerably easier and less energy intensive than the harvest of single-celled microorganisms such as _Chlorella_.

Spirulina can be used for energy production via anaerobic digestion to methane and/or fermentation to alcohol, as a feed for livestock and aquatic organisms (fish and shrimp), as a source of specialty chemicals for the pharmaceutical and cosmetics industry, and as a human nutritional supplementation (health food).

Methane and Ethanol Production

Many algae contain little cellulose and their biodegradable fraction consists mainly of carboxylated or sulfonated chains composed of galactose or mannose or mixed sugars. These materials are rapidly and thoroughly digested anaerobically to methane or fermented to ethanol.

It is evident that the U.S. Southwest could provide massive amounts of energy-rich fuels (methane and ethanol) from algal biomass. For example, assuming 5.1 cubic feet of methane per dry pound of algae and 55 tons of dry weight of algae per acre per year, a 1-acre algal pond could generate 561,000 cubic feet of methane yearly.

In summary, the U.S. Southwest meets all the requirements for large-scale methane and ethanol production using algal biomass as a feedstock because it has: (1) among the highest solar insolation in the U.S. and the world, (2) vast quantities of saline water, and (3) vast areas of suitable land. The Southwest (particularly New Mexico) has great geothermal reserves that could increase productivity by maintaining optimum growth temperatures and providing low-cost carbon dioxide (up to 20 percent by volume of the geothermal water).

The product, Spirulina, lends itself well to low-cost, efficient conversion to methane or fermentation to ethanol because it has a low ash content, high volatile solid contents, and requires no pretreatment (grinding or pulverizing) before bioconversion or fermentation. Furthermore, it is already in a liquid media. The by-products of the bioconversion or fermentation, carbon dioxide and effluent, can be recycled to the algal ponds for increased productivity and reduced costs.

Much of the Southwest already has the infrastructure to transport and store natural gas, and is thus well suited for such an alternative, renewable energy technology. Similarly, the growing fuel alcohol industry in New Mexico could readily (if proven effective) make use of this new alternative feedstock.

Specialty Chemicals

In addition to methane and ethanol production, *Spirulina* culture can produce organic compounds of commercial value. *Spirulina* contains photosynthetic pigments at concentrations that can exceed 20 percent on a dry weight basis. Pigments common to *Spirulina* include some with significant commercial value, such as β-carotenes, phycocyanins, chlorophylls, and xanthophylls. The pigment content and concentration of *Spirulina* varies widely as a function of species, water chemistry, and a number of other environmental parameters.

Carotenoids (3.4 - 4.0 grams/kilogram). Carotenoids are generally responsible for the red and yellow hues seen in nature. More than 300 different carotenoids have been identified, including some that do not occur naturally. Carotenoids are produced in a wide variety of algae, bacteria, and plants, but not in animals. All animal species must procure their carotenoids from dietary sources. A large number of carotenoids have been synthesized and are currently utilized in a variety of industrial applications. They are now replacing, to an increasing extent, the yellow and red azo dyes being eliminated by food regulations. Carotenoids are broadly utilized as pigments in the food, pharmaceutical, and cosmetic industries as natural coloring agents. The carotenoid pigments of commercial importance found in *Spirulina* are β-carotenes and xanthophylls.

βeta Carotenes (1.7 - 3.4 grams/kilogram). The primary significance of β-carotenes in nutrition and metabolism is as a vitamin A precursor of "provitamin A," because vitamin A is produced in animals by breaking a β-carotene molecule and attaching a water molecule in the end position. β-carotenes also are important as feed pigments. β-carotenes added to the feed for beef cattle produces a final meat product with a healthy red color.

B-carotene can also be added to the rations of dairy cattle as a means of pigmenting the butter fat, but direct addition to butter during processing is more economical. In the food industry, β-carotenes are an important coloring agent for fat-based food, including margarine, butter, fats, oils, and shortenings.

β-carotenes are often added to the artificial diets supplied to birds so that they can maintain the brilliant hues characteristic of their plumage. Because the diets of these animals in captivity differ fundamentally from their natural foods, the addition of β-carotenes must be employed to maintain the natural red coloration. For similar reasons, the diets of tropical and/or exotic fish such as the Japanese "koi" are frequently fortified with β-carotenes.

During June 1982, a new potential health food market for β-carotenes was created by the nationwide publicity afforded a National Academy of Sciences report on "Diet-Sensitive Cancers." The study concluded that β-carotenes and ascorbic acid were the only two compounds found to be inhibitory to chemically induced cancers in laboratory tests and the consumption of either compound correlates with lower cancer rates in human populations. Thus, demand for β-carotene dietary supplements (stabilized with ascorbic acid as an antioxidant) may increase in the health food market.

B-carotene was first synthesized in 1950 and is now produced commercially by Hoffman-LaRoche Corporation.

Xanthophylls (1.00 grams/kilogram). The concentration of xanthophylls in *Spirulina* depends upon the species and the environmental conditions. Xanthophylls are an important source of orange-yellow pigments. They are used in the poultry industry as a feed additive to produce highly colored egg yolk as well as meat and skin pigmentation that is pleasing to consumers.

Xanthophylls are also used to fortify the feeds of captive birds and exotic fish as discussed above to produce the strong yellow pigmentation common to these animals in the wild.

Phycocyanins (30 - 220 grams/kilogram). Some strains of Spirulina contain exceptional concentrations of phycocyanins. Phycocyanins are an important source of blue pigment for use in food colorings in Japan, although they are not apparently widely used for such purposes elsewhere.

Chlorophyll (6.8 - 11.0 grams/kilogram). Chlorophyll is an important natural green pigment used in food colorings.

Protein (600 - 700 grams/kilogram). One of the most striking characteristic of Spirulina is the unusually high concentration of protein. The protein matrix of Spirulina encompasses 18 amino acids, including the eight essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). A considerable market in the health food industry exists for protein concentrates. The wholesale value of protein concentrates depends on the mix of essential amino acids but should be favorable for Spirulina since it has an excellent ratio of amino acids. If necessary, the amino acid mix of Spirulina could be altered to more closely match the Food and Agriculture Organization/World Health Organization suggested pattern considered to be optimal for human nutrition by a combination of peptic hydrolysis and papain enzymatic treatments. This treatment results in increased concentrations of methionine, lysine, and tryptophan. Additional potential markets exist for the purified amino acids found in Spirulina as detailed below.

Tryptophan (11.3 grams/kilogram). Tryptophan is an important amino acid that often determines the nutritive value of protein because it is frequently limiting. Tryptophan has market potential as a natural sedative.

Phenylalanine (39.5 - 50.0 grams/kilogram). Phenylalanine is also an essential dietary amino acid for humans since it is not naturally produced in sufficient quantities. Phenylalanine has market potential as a natural stimulant.

Sterols (325 milligrams/kilogram). Sterols have market value either directly, as in the case of cholesterol, or as precursors for a variety of pharmaceuticals. Sterols are important biologically as hormones, components of membranes, and vitamin D. In humans, sterols are converted to cholesterol, which is then used as a precursor for other compounds. The economic value of sterols results from use either directly or as raw materials for synthetic reactions in nutrition pharmaceuticals or research.

Vitamins (variable concentrations). *Spirulina* contains a number of vitamins of potential commercial importance. These vitamins are as follows.

	milligrams/kilogram
Cyanocobalamin (B_{12})	2
d-Ca-Pantothenate	11
Inositol	350
Nicotinic Acid	118
Pyriodoxine (B_6)	3
Riboflavin (B_2)	40
Thiamine (B_1)	55
Tocopherol (E)	190
Niacin (B_3)	118

The economic viability of extracting, concentrating, and purifying these vitamins for the health food market is not clear, although the possibility of tapping this market exists.

Siderochromes (trace concentrations). Spirulina also contains a group of compounds called siderchromes that form stable complexes with metals. These metal-chelating agents have potential medicinal uses as detoxification and removal agents for heavy metals and radioactive plutonium. Presumably such compounds would be an attractive product for the health food market.

Food Additives. Unprocessed Spirulina is a potentially useful food additive for humans and/or domestic animal rations. However, the unprocessed Spirulina has a strong seaweed odor that is aesthetically unattractive to human consumers. Further, those strains with exceptionally high concentrations of phycocyanin pigments have a dark green (nearly black) coloration that is visually unaesthetic. Spirulina would be far more attractive to human consumers if the product were processed to remove the pigments before marketing. This approach is also useful because those pigments with important industrial applications can be marketed independently while increasing the marketability of the residual Spirulina product. The decolored residual would still retain its high protein and vitamin content. The depigmented product, then, could be sold through the health food industry or further processed to extract organically chelated metals, vitamins, amino acids, or protein concentrates as changing market conditions dictate.

Current Supply

Spirulina is currently harvested primarily from large aquatic ecosystems where it grows as a nearly unialgal culture as a result of extreme environmental conditions. The primary supplies of Spirulina are currently harvested from Lake Chad in Africa and Lake Texcoco in Mexico. In recent years, more intensive culture systems have become operational. Average production rates for Lake Texcoco are 10 metric tons per hectare per year. Laboratory experiments have yielded growth rates of 12 grams per square meter per day. This figure, when extrapolated, yields production rates of 43.8 metric tons per hectare per year. Highly controlled commercial production systems could be expected to produce a minimum of 25 metric tons per hectare per year when carbon is added to the culture system in a usable form such as carbon dioxide gas or sodium bicarbonate.

Because projected production costs of *Spirulina* are far below the current wholesale price, significant profit potential exists for a commercial *Spirulina* production facility.

Summary

In summary, finding uses for this underutilized resource--brackish groundwaters--could lead to expanded economic activity in the state. Those uses that make maximum benefit of the natural resources of New Mexico and yet are environmentally benign should be encouraged.

The growth of algae, such as *Spirulina*, on these brackish groundwaters makes eminently good sense in New Mexico. The state has among the highest insolation levels in the United States; abundant, inexpensive flat land; and extensive geothermal reserves. These geothermal waters can be used to maintain optimum temperature levels of algal production ponds, as well as supply substantial amounts of carbon dioxide required by the algae.

Spirulina was the chosen organism for the project because of its ability to tolerate and grow in highly alkaline waters. Indeed, *Spirulina* blooms are often found in such environments to the exclusion of other plant life because of this ability. Thus, one of the major problems of outdoor microalgae culture--contamination by nondesirable competitive species--is solved by the use of brackish groundwaters.

Additionally, *Spirulina's* larger size makes harvesting more efficient and economical. Finally, there is great present and future profit potential in the commercial cultivation of *Spirulina*.

RELATED RESEARCH

There is a relatively modest literature on the growth, biochemical composition, and potential nutritive value of *Spirulina* spp.

Leonhard and Compere (4) described the natural production of *Spirulina* in Lake Chad and its use by natives. Other investigators have described the growth and use of *Spirulina* in Mexico at Lake Texcoco (5, 6, 7). Johnston (8) and Switzer (9) discuss the history and potential use of *Spirulina* in general terms.

The interest in *Spirulina* culture stems from its high nutritive value for man and economically valuable animals. Clement et al. (5) and Clement (10) demonstrated the nutritional value of *Spirulina* for rats and chickens. Others have also discussed the great nutritional value of *Spirulina* (11, 12, 13). Arai et al. (14) have even worked on technologies to further improve the nutritional qualities of protein extracted from *Spirulina*.

Several workers have discussed analytical methods for and use of various biochemical constituents of *Spirulina* (15, 16, 17, 18, 19, 20).

Culture methods from laboratory scale to pilot scale have been discussed in the literature. Al'bitskaya et al. (21) and Aiba and Ogawa (22) have described effects of light levels and/or temperature on the growth of particular species of *Spirulina* on laboratory scale. A great deal of work on growing *Spirulina* on waste sources has been done: (23, 24, 25, 26, 27). Soong (28) describes the commercial production of *Spirulina* in Taiwan.

Berend et al. (29) has indicated that Spirulina is grown in Israel in brackish waters somewhat similar to those found in New Mexico.

These studies actively support the thesis of this project: Spirulina has a very high nutritional value and its constituents can also be economically valuable. The few lab studies on growth of Spirulina are not complete, but do indicate the general suitability of New Mexico's temperatures and sunlight levels for Spirulina growth.

None of these studies, however, has screened several species/strains of Spirulina for their ability to survive and grow on the major brackish water types found in the state. As a result, no studies have been performed on the effect of temperature, nutrient type, and concentration on the growth of those Spirulina species that grow best in New Mexico's brackish water. The research reported here is a major step in filling this gap in knowledge.

MATERIALS AND METHODS

The primary objective of this project was to determine the technical feasibility of cultivating Spirulina in the saline groundwaters of New Mexico. In order to determine the technical feasibility of pilot-scale cultivation, it was necessary to design, construct, and debug an algal culture facility.

Such a facility was built on the grounds of the Roswell Test Facility (RTF) in Roswell, New Mexico. It consists of four main systems: an outdoor, pilot-scale algal production system; an indoor water treatment/recycling system; an inoculation system; and a computerized measurement and control system. The outdoor algal production system consists of two greenhouses, each approximately 7.6 meters feet wide by 14.6 meters long. Each greenhouse has two layers of Monsanto 703 polyethylene film that are air inflated. Within each greenhouse is a concrete raceway, the walls of which are 0.3 meter thick and 0.3 meter high; the floor is 0.15 meter thick. A 0.3 meter high by 0.3 meter thick dividing wall runs down the center of most of the raceway (figure 1), which effectively creates a 2.1 meter wide channel. Algae in the raceway is kept in homogeneous suspension by the movement of a paddlewheel driven by an electric motor. There are two large exhaust fans in each greenhouse for ventilation. Based on a short operational period, these appear to be effective in maintaining greenhouse temperatures near outside ambient temperatures during the warmer months of the year. It is thought that evaporation from the raceway will cause the greenhouse to behave as a large evaporative cooler. 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, night temperatures inside the greenhouse are warmer than outside ambient temperatures. In general, when the fans are not used, the greenhouses tend to be almost 10°C higher in temperature than outside ambient air. A 3,000 watt, portable electric heater was used in the depths of winter to maintain greenhouse temperatures above 10°C; this method was mostly successful.

Algal culture depth can be anywhere from 5 centimeters to 30 centimeters. At a 15 centimeter depth, the feed and harvest systems can be operated such that a 100 percent daily turnover rate can be maintained. That is, 100 percent of the raceway volume can be exchanged daily.

The indoor inoculation system consists of an inoculation clean room where pure cultures of Spirulina are grown in ever larger volumes from test tube to 570 ℓ vats. Artificial lighting is used. The contents of two vats (1,140 ℓ total) can be pumped to either or both greenhouses as an inoculum.

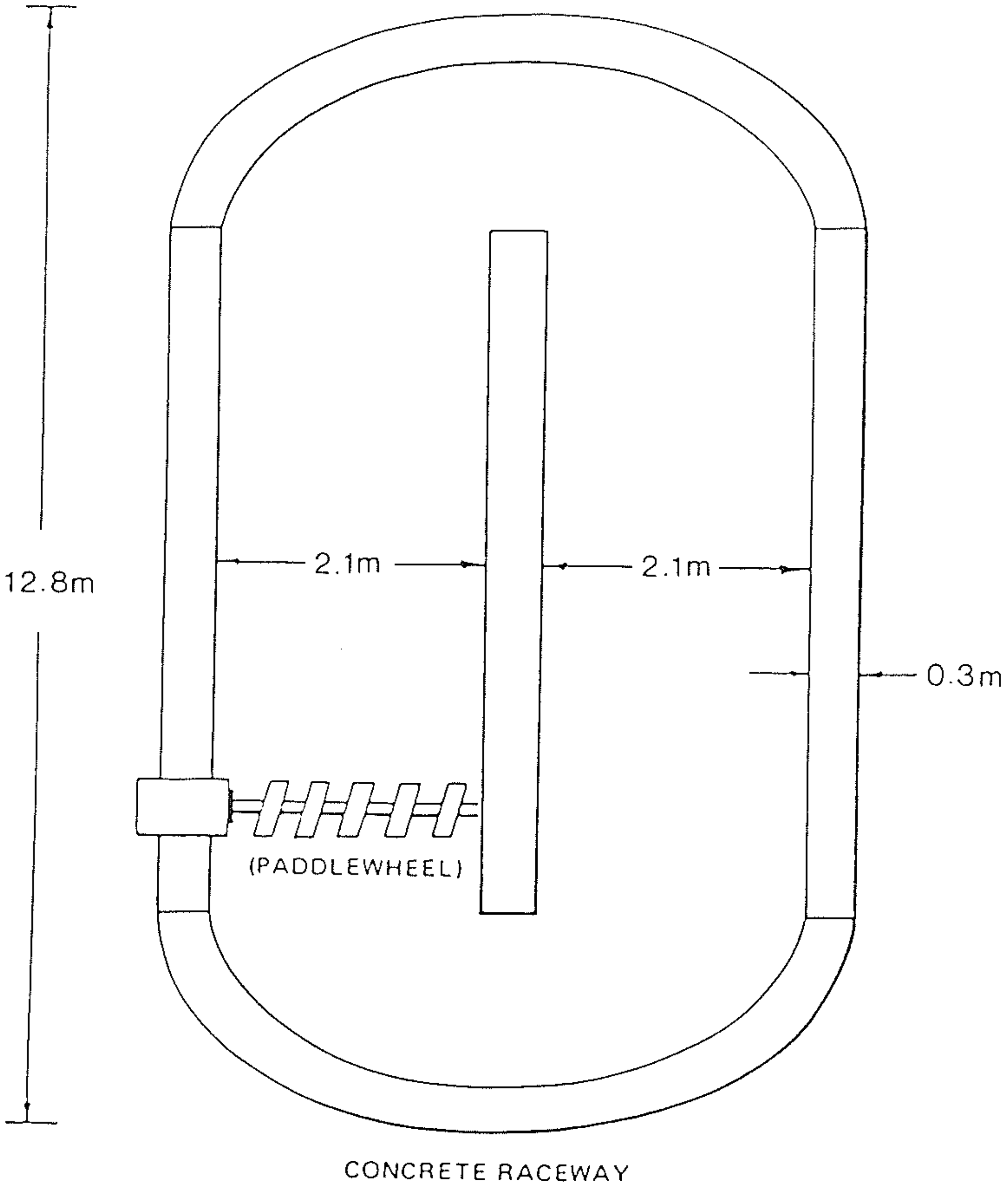

Figure 1. Concrete Raceway.

In the harvest system, algal culture is pumped from the greenhouses into the indoor pad to a harvest subsystem. This subsystem was initially 25 um cloth filter bags. It was found that a significant fraction of algae escaped. When 10 um bags were used, the insides became coated with the Spirulina and only a few hours of operation were possible before the bags needed to be recleaned. A different harvest system, now in development, consists of an inclined frame with 400 mesh screen; this system needs additional development work. The ideal system, a Sweco vibrating screen, could not be purchased because of budgetary constraints.

After most of the algae has been removed from suspension, the medium is pumped through UV sterilizers and then through 1 um depth filters. A day's worth of this treated media is stored in tanks. Daily chemical analyses on this stored media are made and the necessary chemicals added as required. This reconstituted medium can then be used as inflow water to the algal culture.

The measurement and control subsystem is based around an Apple II Plus and a Keithley DAS data acquisition and control system. Instead of installing all necessary sensors at each desired sampling point, it was decided to purchase only one set of sensors and bring the sample to the sensors. To this end, a test rack was designed and built. There are five lines running to the test rack: each inflow to the two raceways; each outflow from the two raceways; and a purge line. A series of solenoid valves controlled by the computer diverts the desired sample stream to the test rack. In-line sensors measure pH, conductivity, and turbidity (a measure of algal biomass) of the sample stream. Each of the four sample streams are sequentially diverted and monitored and then the purge line is diverted to the test rack for cleaning the lines. The duration of monitoring any one sample stream can be changed as desired; initial operation indicates that approximately 5 minutes is necessary for the sensors to reach equilibrium. Thus, the system is capable of obtaining three data points per hour for each of four sampling points for three types of sensors. Additionally, the measurement and control system monitors temperatures at five points in each raceway: (1) 0.3 meter below slab; (2) interface of slab and ground; (3) mid slab in center of channel (north side); (4) north wall; and (5) water temperature. These measurements can be used to construct heat budgets for the greenhouses and to indicate where and how much heat loss and gain is taking place. This data can be used to design a more effective passively heated/cooled greenhouse.

Finally, the measurement and control system monitors two light sensors: one that measures PAR (radiation in the photosynthetically active range--400 to 700 nm) incident to the surface of the raceway and one that measures PAR at the bottom of the algal culture. The above mentioned temperature sensors (thermocouples) and light sensors are monitored continuously to provide a very complete record of these important environmental parameters.

In order to determine the technical feasibility of pilot scale cultivation of Spirulina in saline groundwaters, it was necessary to determine which of several Spirulina species available would be best to use. This was determined by factorial laboratory studies. Four Spirulina species (UTEX #1926, #1928, #2340, and #2342) were monitored for growth rate when grown in four different water types typical of New Mexico's saline groundwaters. These water types were: (1) full RTF well water (approximately 14,000 ppm TDS); (2) a 1:1 dilution of RTF well water and distilled water (7,000 ppm TDS); (3) NMSU geothermal water (2,000 ppm TDS); and (4) Las Cruces tap water (500 ppm TDS). Each species/water type was replicated three times. An experimental run consisted of 48 flasks: 4 species x 4 water types x 3 replicates. In addition, there were controls of each water type with full Zarrouck's media. Each run was conducted at one temperature in a plant growth chamber. Growth at five temperatures were tested: 10°C, 20°C, 27°C, 35°C, and 42°C.

Each flask initially contained 400 milliliters of full Zarrouck's media made in one of the water types. All flasks' contents were kept in homogeneous suspension by bubbling humidified air into the flasks. Twenty milliliters of media were withdrawn daily from each flask. Ten milliliters were filtered onto pre-dried, preweighed Gelman glass fiber filters for dry weight determinations. The filters, held in pre-tared aluminum weighing dishes, were placed into an oven at 60°C and dried to constant weight. The same procedure was used for the controls and the control's weight subtracted from the algal medium weight to give algal dry weight.

The other 10 milliliters were used for optical density measurements (Bausch and Laumb Spectronic 20) and fluorometric determination of in-vivo chlorophyll a (Arpco Fluoro-Tec). Again, the controls' readings were subtracted from the algal medium's readings.

This procedure was repeated daily for six to nine days at which point there was no additional net growth or, in other words, when the "plateau stage" of the classical growth curve had been reached. Linear regressions were performed on the data points representing the exponential growth phase. The slope of this regression line represents the growth rate of the algae.

It should be pointed out that it is important to understand the limitations of extrapolating these laboratory results to larger scale algae production. Whereas a pilot or commercial scale algae production system would most likely be operated in a continuous culture mode, these laboratory experiments were done in a batch mode. The assumption is that exponential growth will occur in a batch culture until some "nutrient" becomes limiting and that this exponential growth represents the potential performance of the algae in continuous culture. In our laboratory experiments, all nutrients were present in excess; the limiting "nutrient" that caused a slowing of the growth rate was light. Such high biomass levels were reached ($\geq$ 1 g. dry weight/liter.) that the average light level experienced by an individual algae was very low, limiting growth. Higher light levels would probably have resulted in higher final densities.

The positive side of these limitations, however, is that the low light levels and high standing biomass densities of the laboratory experiments are probably representative of conditions experienced by the algae even in outdoor, large-scale continuous culture.

In summary, the laboratory results should be regarded as indicative of conparative growth potential of the algae, not as absolute measurements of performance.

Finally, some comments on dry weight determinations are needed. The addition of Zarrouck's media to the various water types invariably resulted in turbidity via the formation of precipitates, despite the presence of chelators. Although the precipitates were not chemically analyzed, it is likely that many were relatively insoluble phosphate salts. Additionally, the large concentrations of added sodium bicarbonate added to the turbidity.

Thus, when an aliquot was filtered for algal dry weight determinations, much of the dry weight was precipitate. Various concentrations and volumes of acidified wash waters were used, but replicable results were not possible. Dry weight determinations of the controls (Zarrouck's medium made up in various water types, but without algae) were somewhat more replicable, but could not be used to correct for true algal dry weight, because the flasks with algae were using up the nutrients (especially the bicarbonate) at an undetermined rate; this was not true of the controls. In short, the controls were only true controls for the first day of the experiment.

This problem has been faced by other workers in the field. They have resolved the problem by filtering the media free of turbidity. However, it was and is not known what the media composition is after filtration. Instead, we developed spectroscopic and fluorometric methods of algal biomass determinations that are appreciably less sensitive to the turbidity problems. These methods reduced sensitivity to turbidity is attributable to using wavelengths of light that are preferentially absorbed (spectroscopy) or preferentially reemitted at a different wavelength (fluoroscopy) by the photosynthetic pigments of the Spirulina. It is known that the pigment concentration of the algal cell (as a percentage of weight) may change with different growth conditions. However, temperature and incident light was kept constant during all experimental runs. It is possible that as the algal biomass increased that each algal cell experienced a decrease in average incident light. Photosynthetic pigments concentrations may have been increased by the cells as a response to decreased average light levels. Thus, algal biomass measurements based on pigment concentration can overestimate actual algal biomass towards the end of experimental runs. However, all species tested were taxonomically similar and probably respond to decreased light levels in a similar manner. Further, the purpose of the laboratory experimentation was to compare species. Thus, a uniform overestimation of algal biomass does not decrease the value of such experimentation.

In summary, dry weight determinations were found to be non-replicable. Spectroscopic and fluorometric determinations of algal biomass were replicable. Although no absolute measurement of algal biomass was therefore possible, these optical methods can be used to compare results. Because the purpose of the laboratory experimentation was to compare algal growth in different water types at different temperatures, the optical methods of algal biomass determination are quite appropriate and sufficient.

RESULTS AND DISCUSSION

Temperature

This study confirms literature reports of a general growth temperature optimum of 35°C for *Spirulina* spp.. This is true of almost every combination of species type and water type. (There are sixteen such combinations: four water types x four species for every temperature [figures 2-5].) It can be seen in Table 1 that the best growth rate exhibited by species Utex #1926 was at 35°C in geothermal water. Species UTEX #1928 exhibited the best growth rate at 35°C in Las Cruces water, followed very closely by growth at 35°C in geothermal water. UTEX species #2340's best growth rate was at 35°C in geothermal water, followed very closely by growth at 35°C in RTF water. Only UTEX species #2342 exhibited its best growth rate at 42°C; this was in Las Cruces water.

For any one water type, UTEX #1926's temperature optimum is 35°C (Table 2). This is also true for UTEX #1928. In every water type but Las Cruces water, the best temperature for species UTEX #2340 is 35°C; in Las Cruces water the temperature optimum for this species is 27°C, followed closely by growth at 35°C. For every water type but one, the optimum temperature for #2342 is 42°C. The exception is for RTF water, in which the temperature optimum for the UTEX #2342 is 35°C.

At the general temperature optimum of 35°C, the overall best performance was by species UTEX #1928 in Las Cruces water, followed closely by its growth

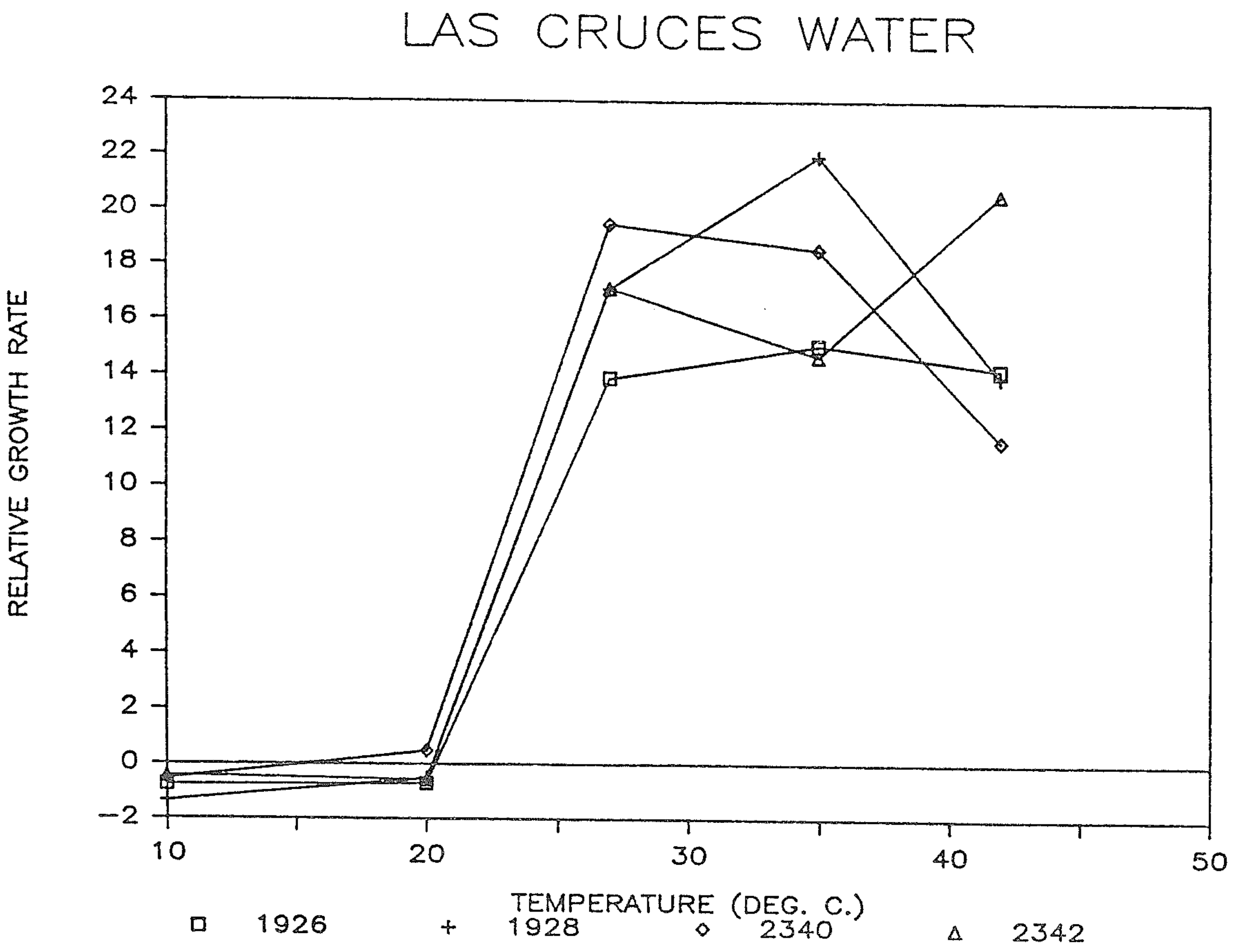

Figure 2. The effect of temperature on the growth rate of four species of <u>Spirulina</u> grown in Las Cruces Water.

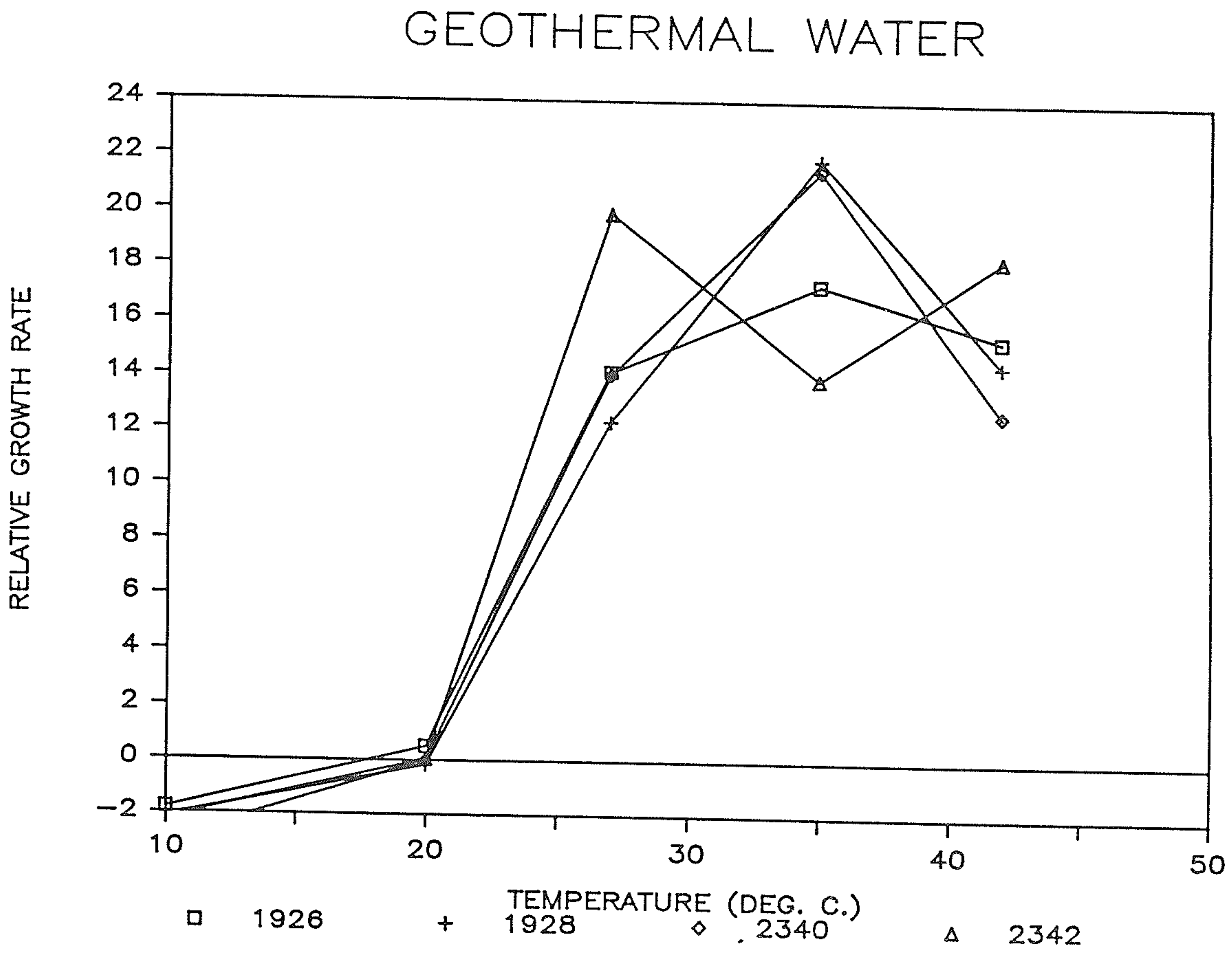

Figure 3. The effect of temperature on the growth rate of four species of _Spirulina_ grown in geothermal water.

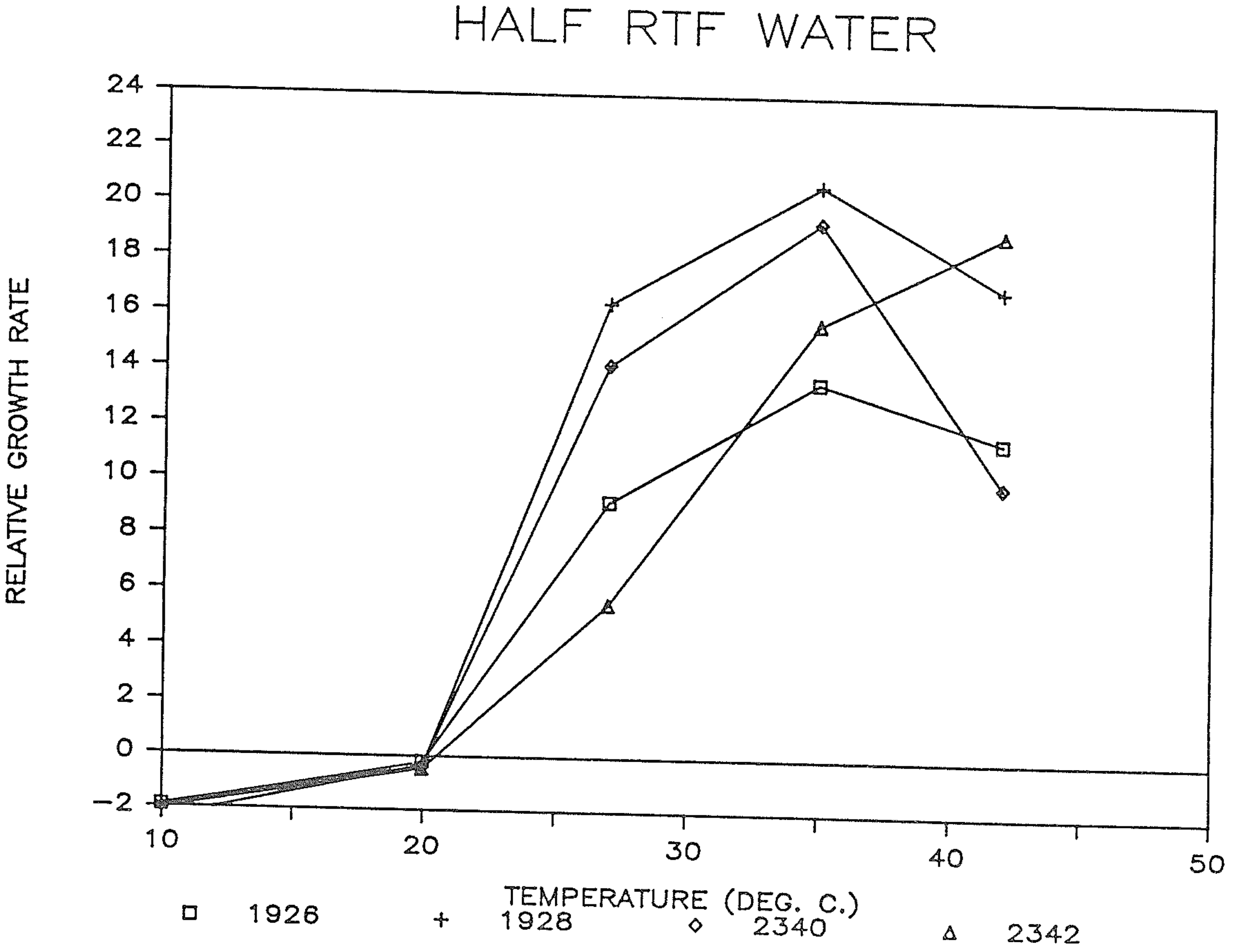

Figure 4. The effect of temperature on the growth rate of four species of _Spirulina_ grown in half RTF water.

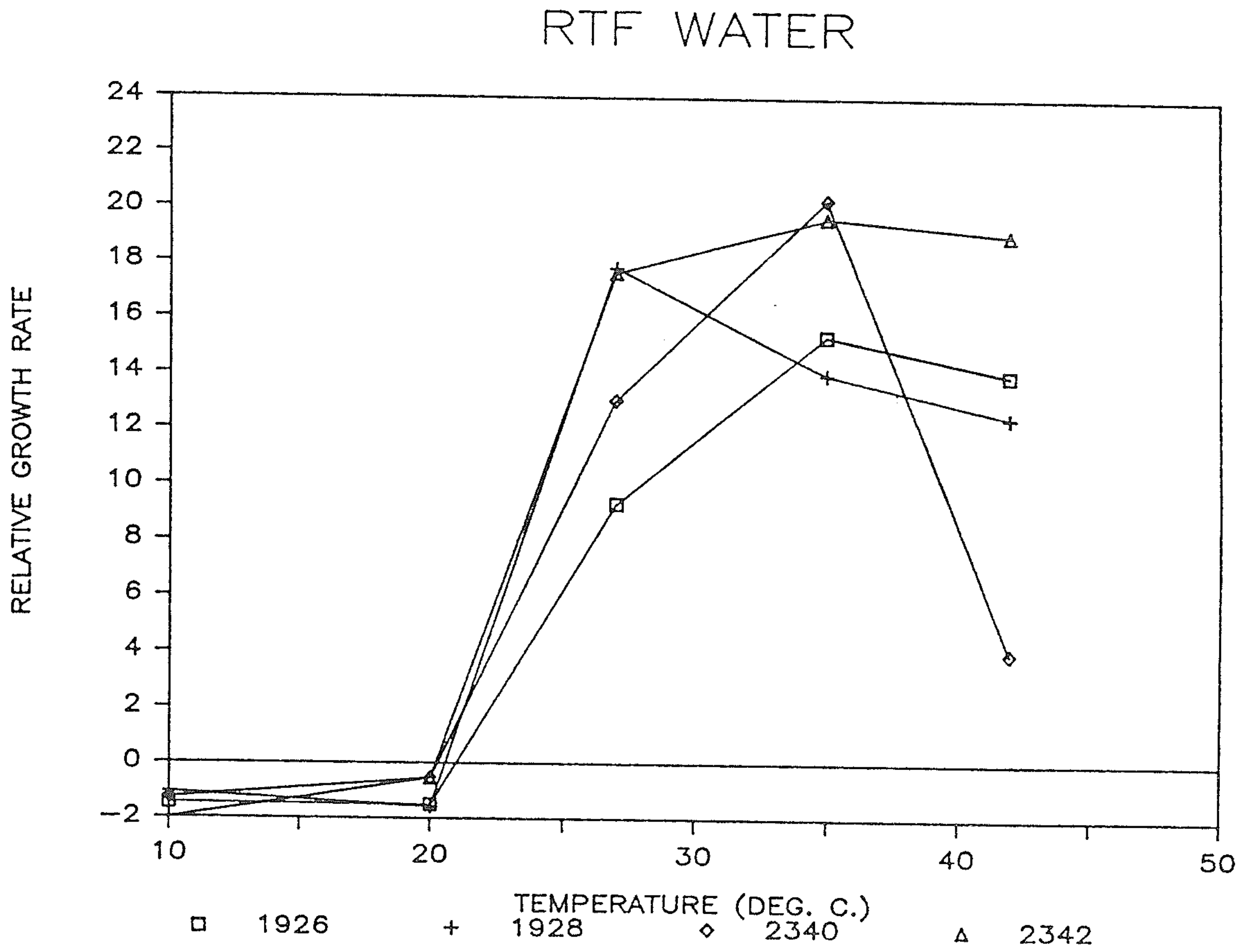

Figure 5. The effect of temperature on the growth rate of four species of Spirulina grown in RTF water.

Table 1
Relative Growth Rates Ranked by Species and Growth Rate

Species UTEX #	Relative Growthrate	Temperature Degrees (C)	Water Type
1926	-1.92	10	Half RTF
1926	-1.79	10	Geothermal
1926	-1.5	20	RTF
1926	-1.46	10	RTF
1926	-0.75	10	Las Cruces
1926	-0.7	20	Las Cruces
1926	-0.21	20	Half RTF
1926	0.52	20	Geothermal
1926	9.25	27	Half RTF
1926	9.3	27	RTF
1926	11.54	42	Half RTF
1926	13.63	35	Half RTF
1926	13.89	27	Las Cruces
1926	13.95	42	RTF
1926	14.17	27	Geothermal
1926	14.2	42	Las Cruces
1926	15.08	35	Las Cruces
1926	15.37	35	RTF
1926	15.37	42	Geothermal
1926	17.34	35	Geothermal
1928	-2.12	10	Geothermal
1928	-1.89	10	Half RTF
1928	-1.58	20	RTF
1928	-1.34	10	Las Cruces
1928	-1.06	10	RTF
1928	-0.5	20	Las Cruces
1928	-0.38	20	Half RTF
1928	-0.18	20	Geothermal
1928	12.33	27	Geothermal
1928	12.43	42	RTF
1928	13.92	42	Las Cruces
1928	13.97	35	RTF
1928	14.45	42	Geothermal
1928	16.41	27	Half RTF
1928	17.01	42	Half RTF
1928	17.12	27	Las Cruces
1928	17.8	27	RTF
1928	20.75	35	Half RTF
1928	21.88	35	Geothermal
1928	21.97	35	Las Cruces
2340	-3	10	Geothermal
2340	-2.41	10	Half RTF
2340	-1.26	10	RTF
2340	-0.53	10	Las Cruces
2340	-0.52	20	RTF

Table 1
Relative Growth Rates and Ranked by Species and Growth Rate (continued)

Species UTEX #	Relative Growthrate	Temperature Degrees (C)	Water Type
2340	-0.35	20	Half RTF
2340	0.007	20	Geothermal
2340	0.48	20	Las Cruces
2340	3.97	42	RTF
2340	9.96	42	Half RTF
2340	11.63	42	Las Cruces
2340	12.65	42	Geothermal
2340	13.03	27	RTF
2340	14.07	27	Geothermal
2340	14.18	27	Half RTF
2340	18.55	35	Las Cruces
2340	19.41	35	Half RTF
2340	19.49	27	Las Cruces
2340	20.3	35	RTF
2340	21.5	35	Geothermal
2342	-2.21	10	Geothermal
2342	-2.04	10	Half RTF
2342	-2.02	10	RTF
2342	-0.55	20	Las Cruces
2342	-0.52	20	RTF
2342	-0.47	20	Half RTF
2342	-0.44	10	Las Cruces
2342	0.04	20	Geothermal
2342	5.56	27	Half RTF
2342	13.91	35	Geothermal
2342	14.66	35	Las Cruces
2342	15.74	35	Half RTF
2342	17.13	27	Las Cruces
2342	17.63	27	RTF
2342	18.28	42	Geothermal
2342	19.04	42	RTF
2342	19.04	42	Half RTF
2342	19.65	35	RTF
2342	19.9	27	Geothermal
2342	20.57	42	Las Cruces

Table 2
Relative Growth Rates Ranked by Water Type and Species

Water Type	Temperature Degrees (C)	Species UTEX #	Relative Growthrate
Las Cruces	10	1926	-0.75
Las Cruces	20	1926	-0.7
Las Cruces	27	1926	13.89
Las Cruces	35	1926	15.08
Las Cruces	42	1926	14.2
Geothermal	10	1926	-1.79
Geothermal	20	1926	0.52
Geothermal	27	1926	14.17
Geothermal	35	1926	17.34
Geothermal	42	1926	15.37
Half RTF	10	1926	-1.92
Half RTF	20	1926	-0.21
Half RTF	27	1926	9.25
Half RTF	35	1926	13.63
Half RTF	42	1926	11.54
RTF	10	1926	-1.46
RTF	20	1926	-1.5
RTF	27	1926	9.3
RTF	35	1926	15.37
RTF	42	1926	13.95
Las Cruces	10	1928	-1.34
Las Cruces	20	1928	-0.5
Las Cruces	27	1928	17.12
Las Cruces	35	1928	21.97
Las Cruces	42	1928	13.92
Geothermal	10	1928	-2.12
Geothermal	20	1928	-0.18
Geothermal	27	1928	12.33
Geothermal	35	1928	21.88
Geothermal	42	1928	14.45
Half RTF	10	1928	-1.89
Half RTF	20	1928	-0.38
Half RTF	27	1928	16.41
Half RTF	35	1928	20.75
Half RTF	42	1928	17.01
RTF	10	1928	-1.06
RTF	20	1928	-1.58
RTF	27	1928	17.8
RTF	35	1928	13.97
RTF	42	1928	12.43
Las Cruces	10	2340	-0.53
Las Cruces	20	2340	0.48
Las Cruces	27	2340	19.49
Las Cruces	35	2340	18.55

Table 2
Relative Growthrates Ranked by Water Type and Species (continued)

Water Type	Temperature Degrees (C)	Species UTEX #	Relative Growthrate
Las Cruces	42	2340	11.63
Geothermal	10	2340	-3
Geothermal	20	2340	0.007
Geothermal	27	2340	14.07
Geothermal	35	2340	21.5
Geothermal	42	2340	12.65
Half RTF	10	2340	-2.41
Half RTF	20	2340	-0.35
Half RTF	27	2340	14.18
Half RTF	35	2340	19.41
Half RTF	42	2340	9.96
RTF	10	2340	-1.26
RTF	20	2340	-0.52
RTF	27	2340	13.03
RTF	35	2340	20.3
RTF	42	2340	3.97
Las Cruces	10	2342	-0.44
Las Cruces	20	2342	-0.55
Las Cruces	27	2342	17.13
Las Cruces	35	2342	14.66
Las Cruces	42	2342	20.57
Geothermal	10	2342	-2.21
Geothermal	20	2342	0.04
Geothermal	27	2342	19.9
Geothermal	35	2342	13.91
Geothermal	42	2342	18.28
Half RTF	10	2342	-2.04
Half RTF	20	2342	-0.47
Half RTF	27	2342	5.56
Half RTF	35	2342	15.74
Half RTF	42	2342	19.04
RTF	10	2342	-2.02
RTF	20	2342	-0.52
RTF	27	2342	17.63
RTF	35	2342	19.65
RTF	42	2342	19.04

at 35°C in geothermal water. UTEX species #2340, grown in geothermal water at 35°C, had a growth rate that is very close to that of UTEX #1928 (Table 3).

For any one water type, species UTEX #1926 exhibits equal or better growth at 42°C as at 27°C (figure 6). The situation is not as clear for UTEX #1928. For Las Cruces water and RTF water, UTEX #1928 exhibits better growth at 27°C then 42°C; the opposite is true for growth in half RTF water and in geothermal water (figure 7). UTEX #2340 consistently demonstrates better growth at 27°C then at 42°C for any water type (Table 2, figure 8). Except for geothermal water, UTEX #2342 grows better at 42°C than at 27°C (figure 9).

There is no growth for any species at 10°C (figure 10). At 20°C, there is very slight growth for UTEX #1926 in geothermal water (2000 per TDS) and for UTEX #2340 in RTF water (14000 ppm TDS) (figure 11).

In summary, the best overall growth temperature is 35°C (figure 12). Substantial growth is exhibited by most species at 27°C and 42°C (figures 13 and 14). At 20°C, it appears that only metabolic needs are met by photosynthesis and there is little, if any net growth. There is no growth for any species at 10°C (Table 3).

Water Type

Waters of lower TDS, such as geothermal water or Las Cruces water, appear to be the best water types for maximum growth of all species (Table 1, figures 2 and 3). UTEX #1926 and UTEX #2340 demonstrated maximum growth rates in geothermal water at 35°C (figures 6 and 8). UTEX #1928 and UTEX #2342 demonstrated maximum growth rate in Las Cruces water at 35°C and 42°C, respectively (figures 7, 9). However when all combinations of water type, temperature, and species are ranked by growth rate (Table 4), all four water

Table 3
Relative Growth Rates Ranked by Temperature and Growth Rate

Temperature Degrees (C)	Relative Growthrate	Water Type	Species UTEX #
10	-3.00	Geothermal	2340
10	-2.41	Half RTF	2340
10	-2.21	Geothermal	2342
10	-2.12	Geothermal	1928
10	-2.04	Half RTF	2342
10	-2.02	RTF	2342
10	-1.92	Half RTF	1926
10	-1.89	Half RTF	1928
10	-1.79	Geothermal	1926
10	-1.46	RTF	1926
10	-1.34	Las Cruces	1928
10	-1.26	RTF	2340
10	-1.06	RTF	1928
10	-0.75	Las Cruces	1926
10	-0.53	Las Cruces	2340
10	-0.44	Las Cruces	2342
20	-1.58	RTF	1928
20	-1.50	RTF	1926
20	-0.70	Las Cruces	1926
20	-0.55	Las Cruces	2342
20	-0.52	RTF	2340
20	-0.52	RTF	2342
20	-0.50	Las Cruces	1928
20	-0.47	Half RTF	2342
20	-0.38	Half RTF	1928
20	-0.35	Half RTF	2340
20	-0.21	Half RTF	1926
20	-0.18	Gothermal	1928
20	0.01	Geothermal	2340
20	0.04	Geothermal	2342
20	0.48	Las Cruces	2340
20	0.52	Geothermal	1926
27	5.56	Half RTF	2342
27	9.25	Half RTF	1926
27	9.30	RTF	1926
27	12.33	Geothermal	1928
27	13.03	RTF	2340
27	13.89	Las Cruces	1926
27	14.07	Geothermal	2340
27	14.17	Geothermal	1926
27	14.18	Half RTF	2340
27	16.41	Half RTF	1928
27	17.12	Las Cruces	1928
27	17.13	Las Cruces	2342

Table 3
Relative Growth Rates Ranked by Temperature and Growth Rate (continued)

Temperature Degrees (C)	Relative Growthrate	Water Type	Species UTEX #
27	17.63	RTF	2342
27	17.80	RTF	1928
27	19.49	Las Cruces	2340
27	19.90	Geothermal	2342
35	13.63	Half RTF	1926
35	13.91	Geothermal	2342
35	13.97	RTF	1928
35	14.66	Las Cruces	2342
35	15.08	Las Cruces	1926
35	15.37	RTF	1926
35	15.74	Half RTF	2342
35	17.34	Geothermal	1926
35	18.55	Las Cruces	2340
35	19.41	Half RTF	2340
35	19.65	RTF	2342
35	20.30	RTF	2340
35	20.75	Half RTF	1928
35	21.50	Geothermal	2340
35	21.88	Geothermal	1928
35	21.97	Las Cruces	1928
42	3.97	RTF	2340
42	9.96	Half RTF	2340
42	11.54	Half RTF	1926
42	11.63	Las Cruces	2340
42	12.43	RTF	1928
42	12.65	Geothermal	2340
42	13.92	Las Cruces	1928
42	13.95	RTF	1926
42	14.20	Las Cruces	1926
42	14.45	Geothermal	1928
42	15.37	Geothermal	1926
42	17.01	Half RTF	1928
42	18.28	Geothermal	2342
42	19.04	Half RTF	2342
42	19.04	RTF	2342
42	20.57	Las Cruces	2342

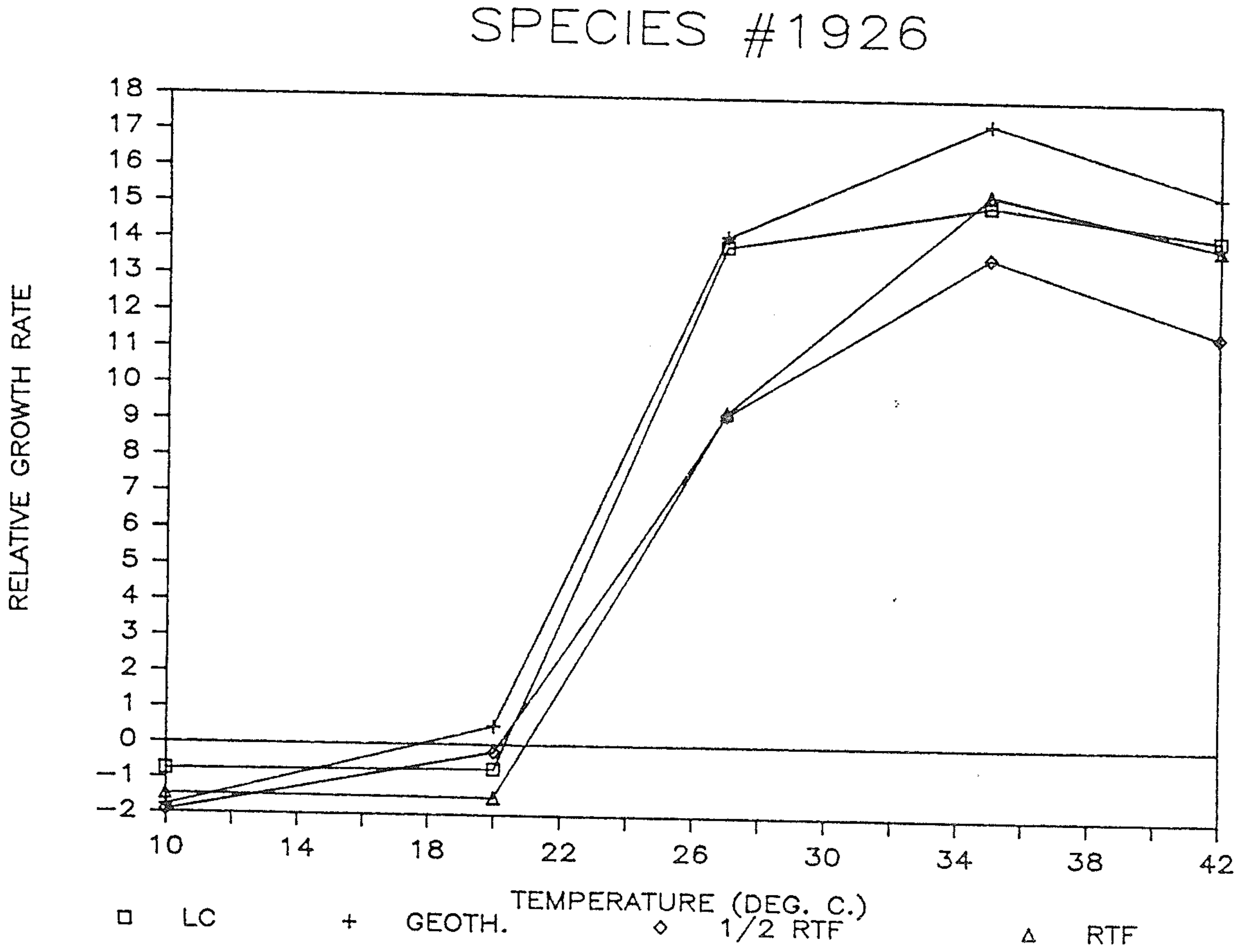

Figure 6. The effect of temperature on the growth of UTEX #1926 in four saline water types.

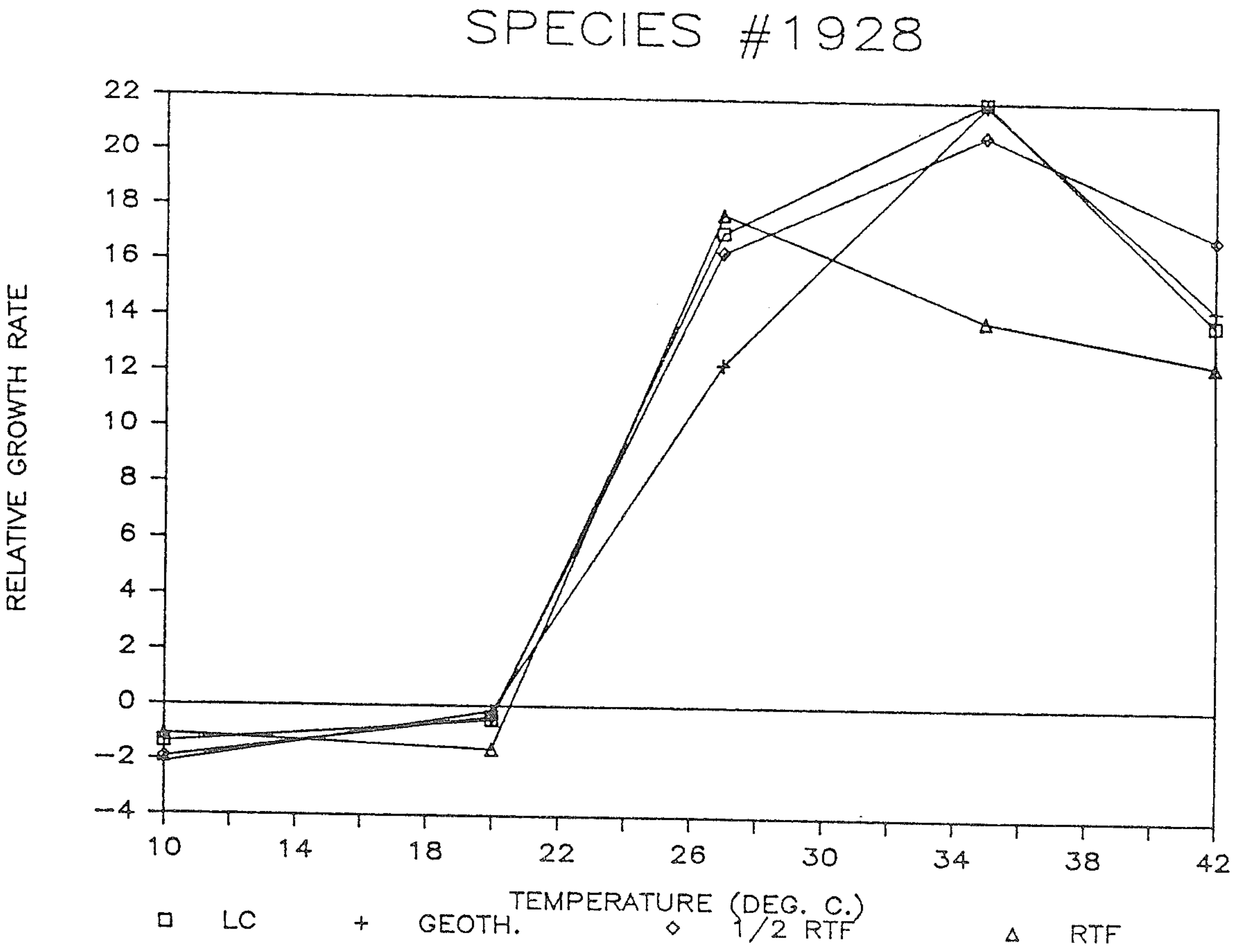

Figure 7. The effect of temperature on the growth of UTEX #1928 in four saline water types.

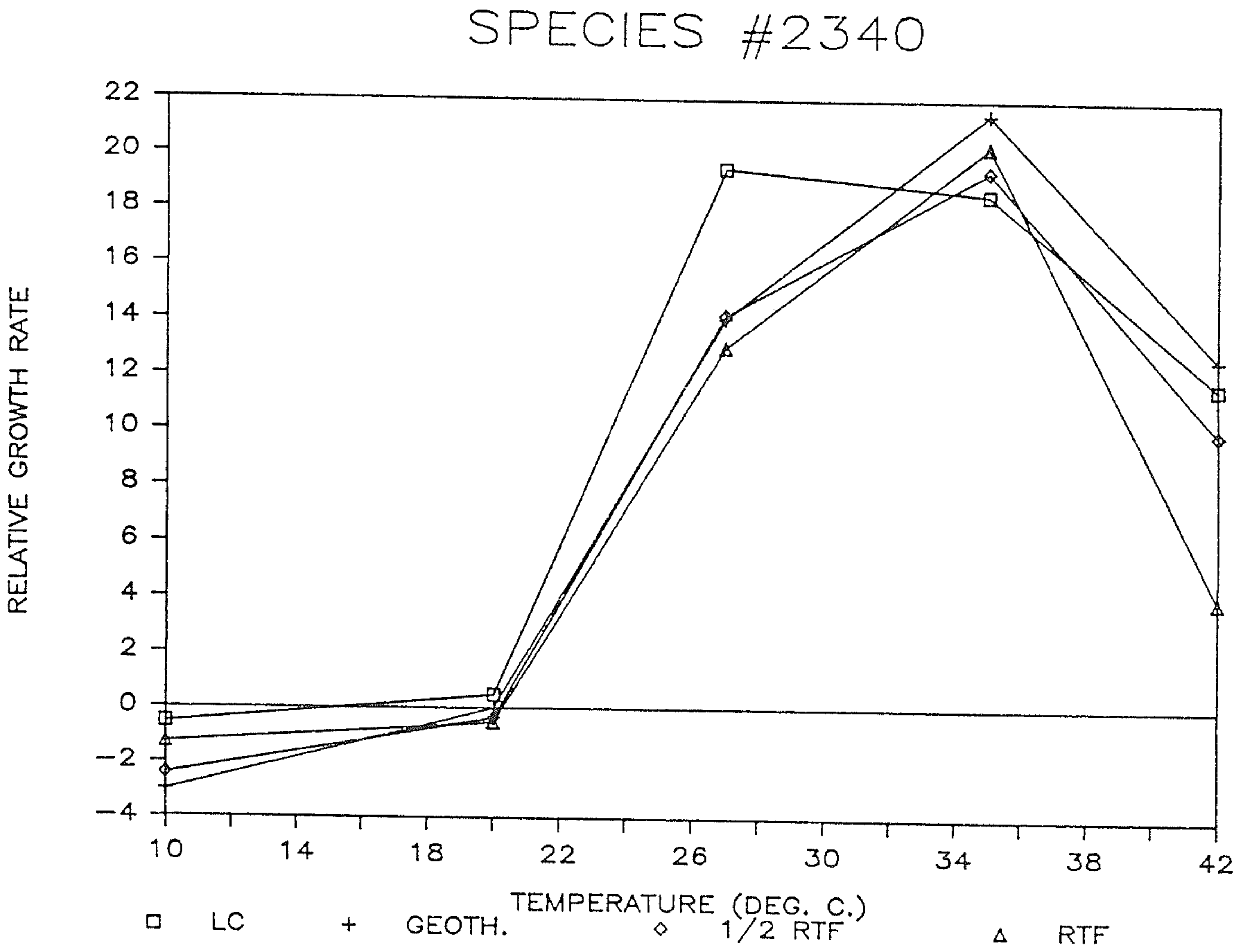

Figure 8. The effect of temperature on growth of UTEX #2340 in four saline water types.

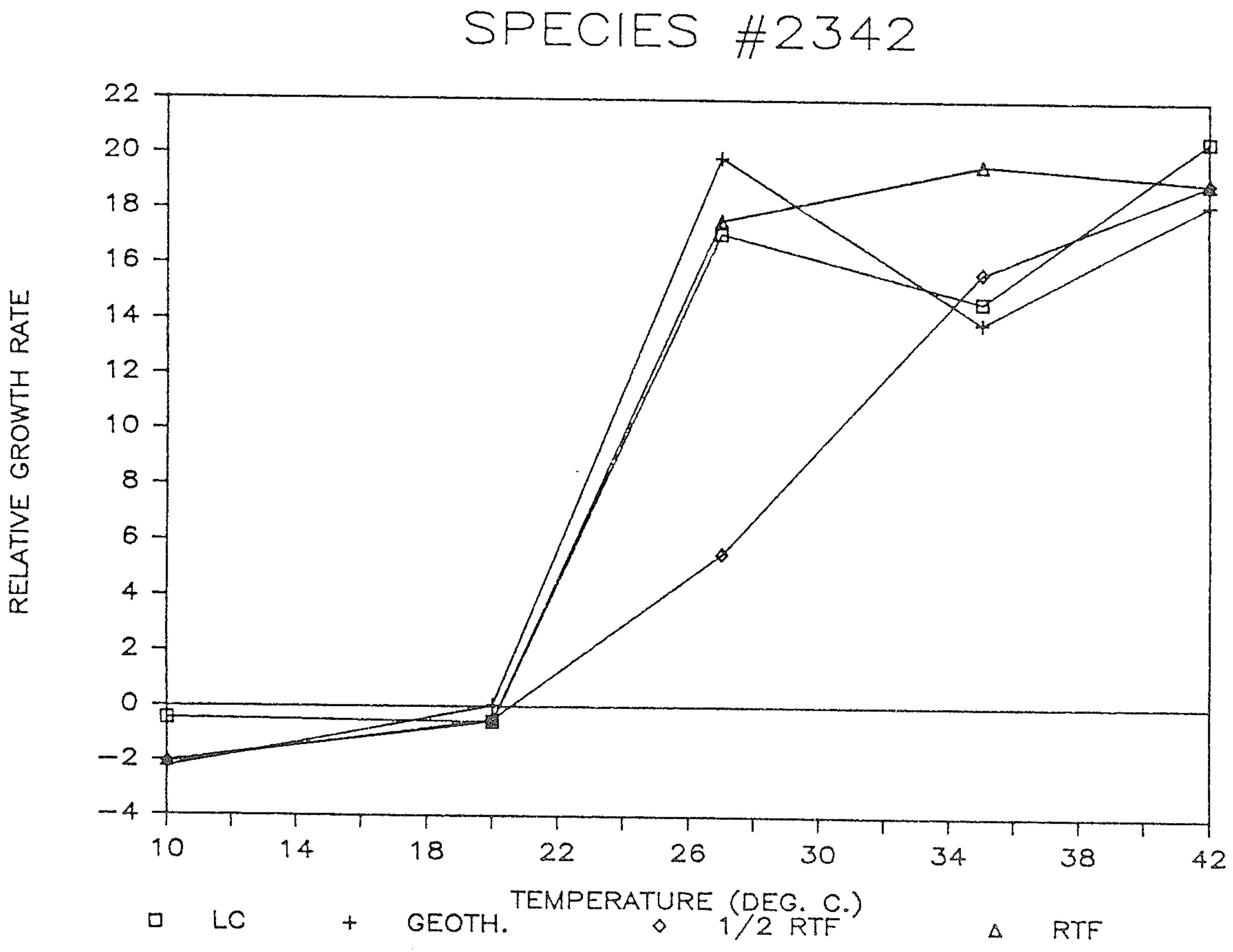

Figure 9. The effect of temperature on the growth of UTEX #2342 in four saline water types.

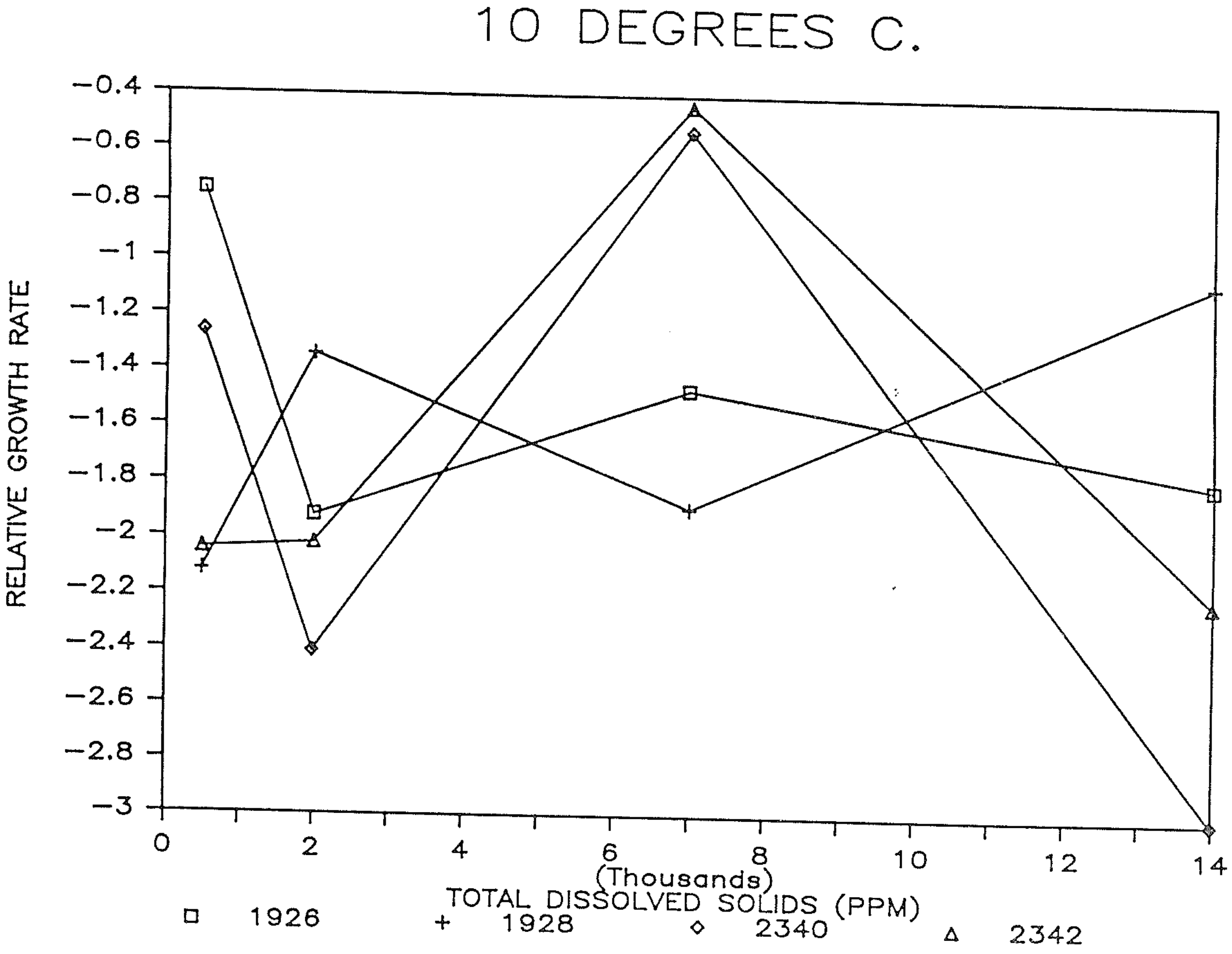

Figure 10. The growth of four <u>Spirulina</u> species in four saline water types at 10°C.

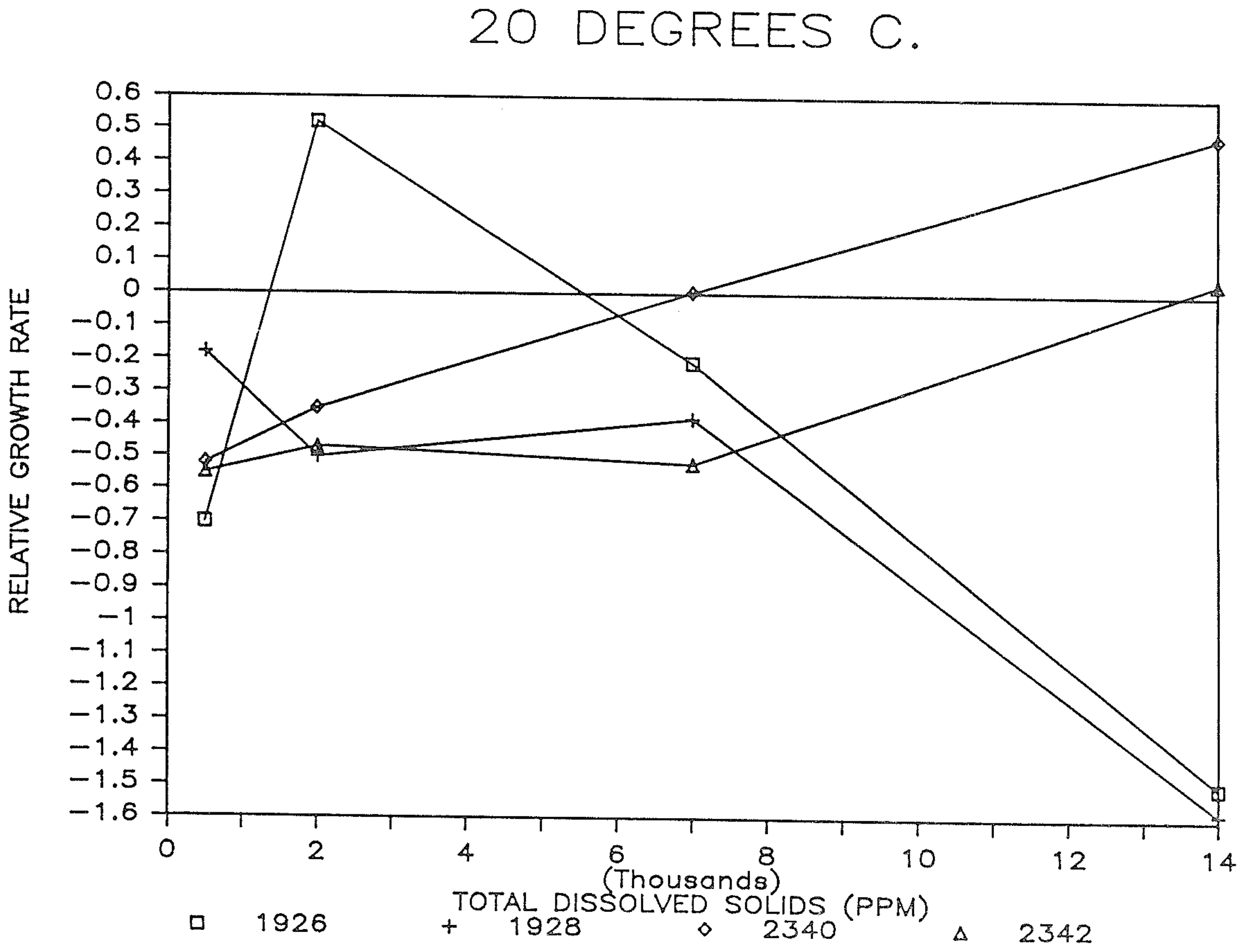

Figure 11. The growth of four Spirulina species in four saline water types at 20°C.

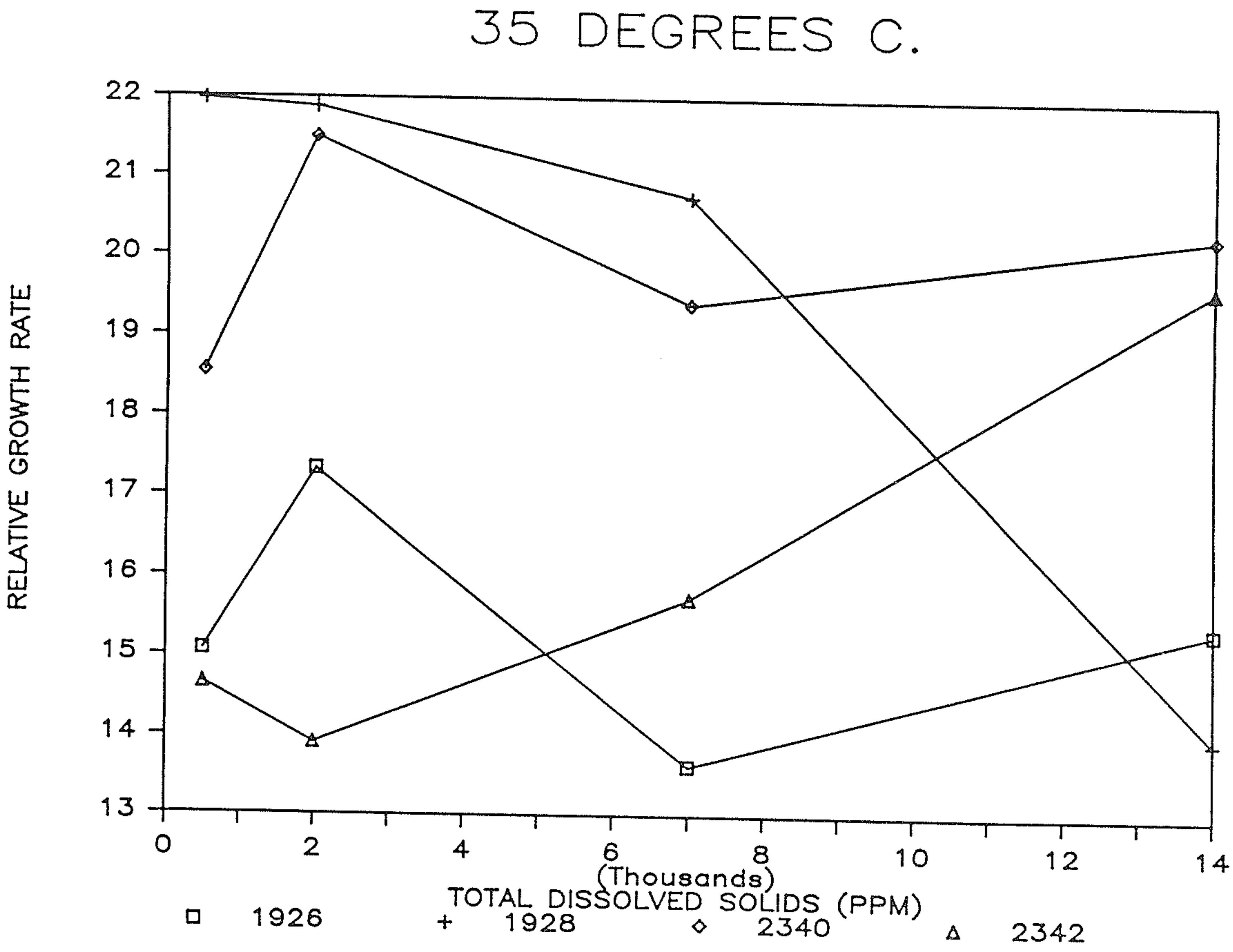

Figure 12. The growth of four _Spirulina_ species in four saline water types at 35°C.

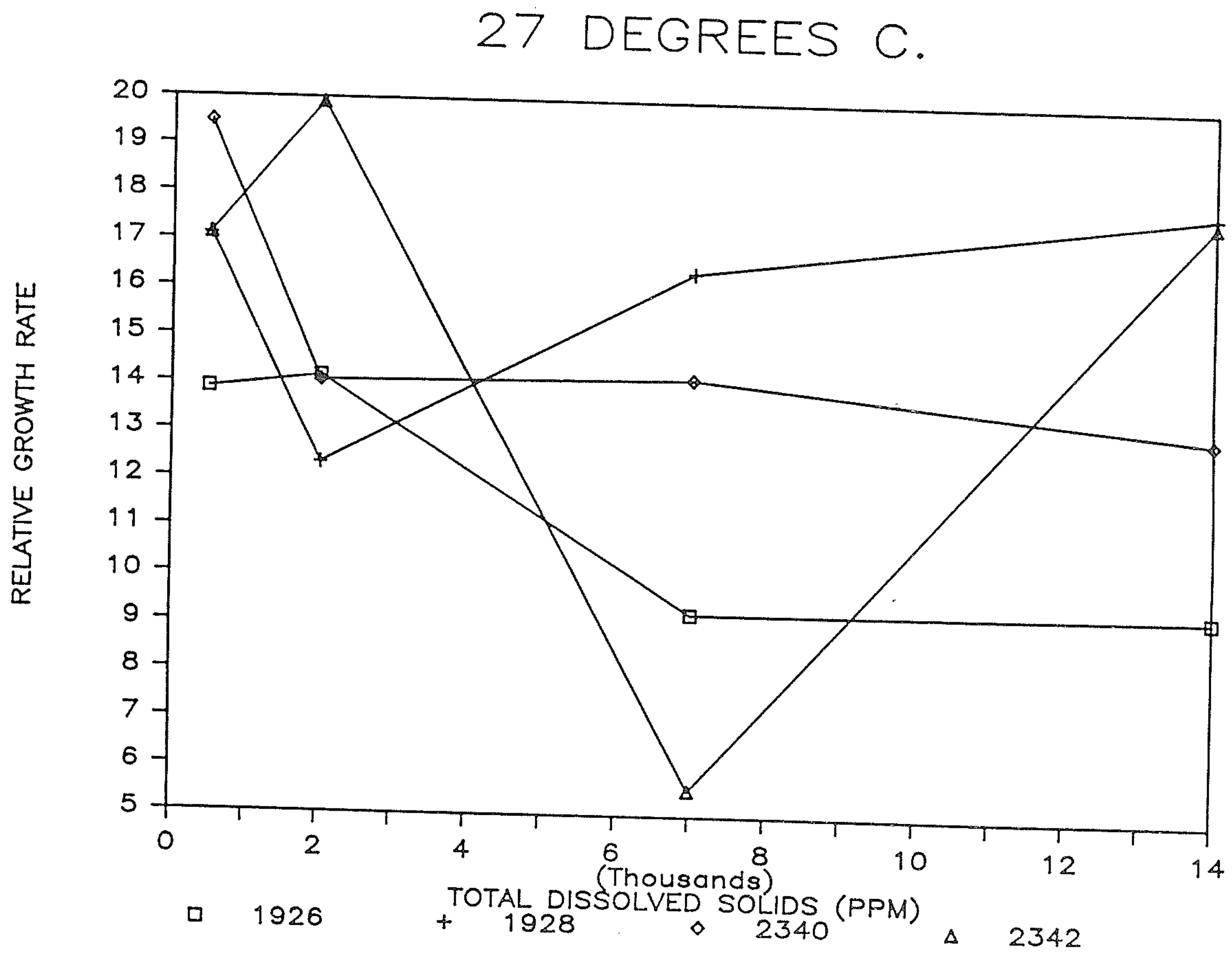

Figure 13. The growth of four Spirulina species in four saline water types at 27°C.

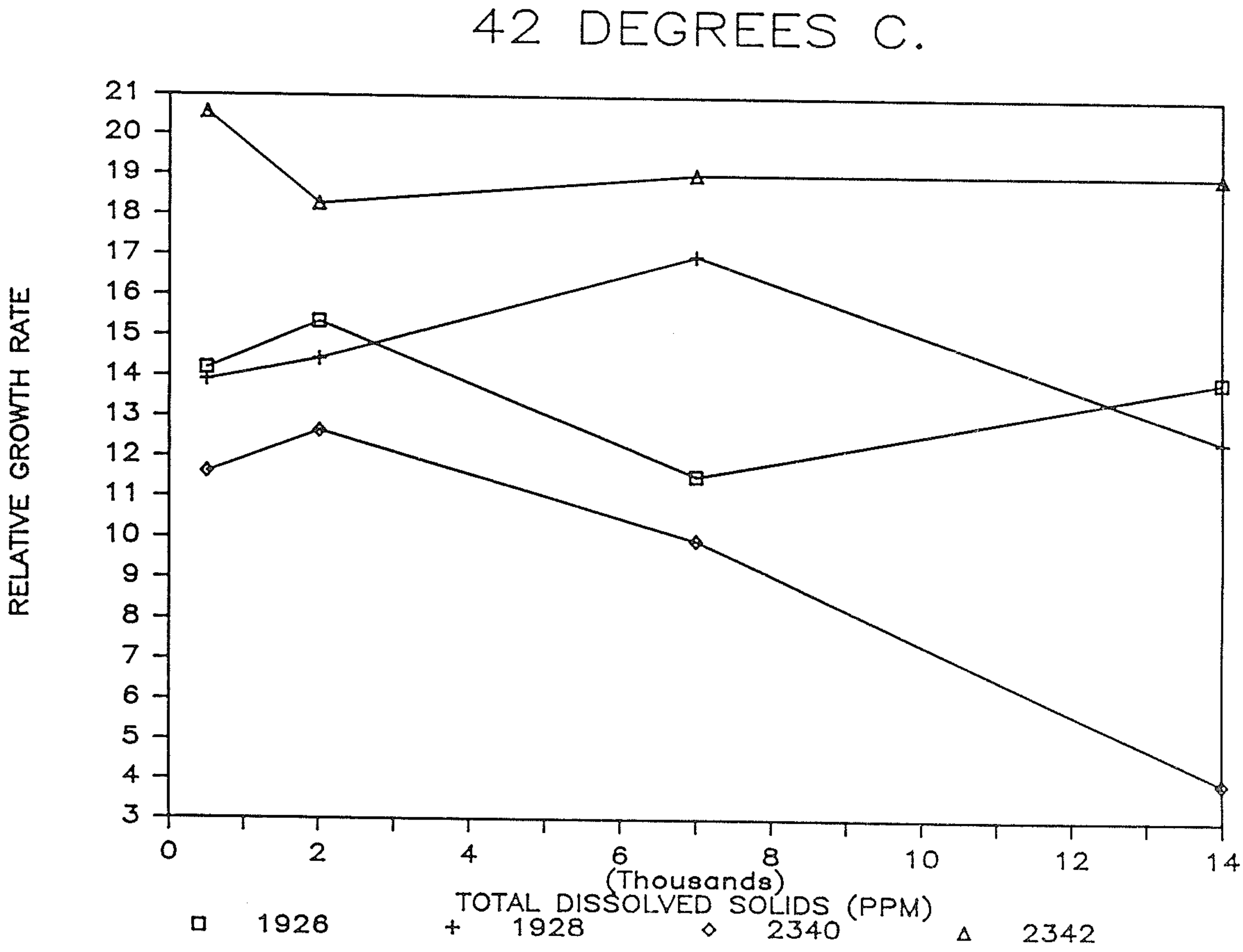

Figure 14. The growth of four Spirulina species in four saline water types at 42°C.

Table 4
Relative Growth Rates Ranked by Growth Rate

Relative Growthrate	Water Type	Species UTEX #	Temperature Degrees C
-3.00	Geothermal	2340	10
-2.41	Half RTF	2340	10
-2.21	Geothermal	2342	10
-2.12	Geothermal	1928	10
-2.04	Half RTF	2342	10
-2.02	RTF	2342	10
-1.92	Half RTF	1926	10
-1.89	Half RTF	1928	10
-1.79	Geothermal	1926	10
-1.58	RTF	1928	20
-1.50	RTF	1926	20
-1.46	RTF	1926	10
-1.34	Las Cruces	1928	10
-1.26	RTF	2340	10
-1.06	RTF	1928	10
-0.75	Las Cruces	1926	10
-0.70	Las Cruces	1926	20
-0.55	Las Cruces	2342	20
-0.53	Las Cruces	2340	10
-0.52	RTF	2342	20
-0.52	RTF	2340	20
-0.50	Las Cruces	1928	20
-0.47	Half RTF	2342	20
-0.44	Las Cruces	2342	10
-0.38	Half RTF	1928	20
-0.35	Half RTF	2340	20
-0.21	Half RTF	1926	20
-0.18	Geothermal	1928	20
0.01	Geothermal	2340	20
0.04	Geothermal	2340	20
0.48	Las Cruces	2340	20
0.52	Geothermal	1926	20
3.97	RTF	2340	42
5.56	Half RTF	2342	27
9.25	Half RTF	1926	27
9.30	RTF	1926	27
9.96	Half RTF	2340	42
11.54	Half RTF	1926	42
11.63	Las Cruces	2340	42
12.33	Geothermal	1928	27
12.43	RTF	1928	42
12.65	Geothermal	2340	42
13.03	RTF	2340	27
13.63	Half RTF	1926	35

Table 4.
Relative Growth Rates Ranked by Growth Rate (continued)

Relative Growthrate	Water Type	Species UTEX #	Temperature Degrees C
13.89	Las Cruces	1926	27
13.91	Geothermal	2342	35
13.92	Las Cruces	1928	42
13.95	RTF	1926	42
13.97	RTF	1928	35
14.07	Geothermal	2340	27
14.17	Geothermal	1926	27
14.18	Half RTF	2340	27
14.20	Las Cruces	1926	42
14.45	Geothermal	1928	42
14.66	Las Cruces	2342	35
15.08	Las Cruces	1926	35
15.37	RTF	1926	35
15.37	Geothermal	1926	42
15.74	Half RTF	2342	35
16.41	Half RTF	1928	27
17.01	Half RFT	1928	42
17.12	Las Cruces	1928	27
17.13	Las Cruces	2342	27
17.34	Geothermal	1926	35
17.63	RTF	2342	27
17.80	RTF	1928	27
18.28	Geothermal	2342	42
18.55	Las Cruces	2340	35
19.04	Half RTF	2342	42
19.04	RTF	2342	42
19.41	Half RTF	2340	35
19.49	Las Cruces	2340	27
19.65	RTF	2342	35
19.90	Geothermal	2342	27
20.30	RTF	2340	35
20.57	Las Cruces	2342	42
20.75	Half RTF	1928	35
21.50	Geothermal	2340	35
21.88	Geothermal	1928	35
21.97	Las Cruces	1928	35

types are represented in the top eight (10 percent): Las Cruces water, 2; geothermal water, 3; half RTF water, 1; and RTF water, 2.

Maximum growth rate in Las Cruces water is demonstrated by UTEX #1928 at 35°C, followed closely by #2342 at 42°C and #2340 at 27°C and 35°C (Table 5). Maximum growth rate in geothermal water is demonstrated by #1928 at 35°C, #2340 at 35°C, and #2342 at 27°C and 42°C respectively. UTEX #1928 exhibited the best growth in half RTF water at 35°C, followed closely by #2340 at 35°C and #2342 at 42°C. Maximum growth rate in RTF water is exhibited by #2340 at 35°C, followed closely by #2342 at 35°C and 42°C.

In summary, the best overall water types are Las Cruces water and geothermal water. However, good growth is exhibited in all water types (figures 2, 3, 4, 5) at most higher temperatures (27°C, 35°C, 42°C) (Table 5).

Species

UTEX species #1928 deomonstrated the best growth rate of all species tested in Las Cruces water, geothermal water and half RTF water respectively; these maximum growth rates were all attained at 35°C (Table 5). At 35°C in RTF water, #2342 and #2340 had approximately the same maximum growth rate (figures 2, 3, 4, 5, Table 1). When all combinations of water type, temperature and species are ranked by ascending growth rate (Table 4), the top eight (top 10 percent) growth rates are distributed as follows: #1926, 0; #1928, 3; #2340, 2; and #2342, 3.

In summary, UTEX species #1928 appears to be the best overall species in all water types at all temperatures except for at 42°C (figures 6, 7, 8, 9). At this higher temperature, #2342 is clearly superior in all water types (figure 14). UTEX #2342 is the best overall species to use at all temperatures in RTF water (figure 5).

Table 5
Relative Growth Rates Ranked by Water Type and Growth Rate

Water Type	Relative Growthrate	Temperature Degrees C	Species UTEX #
Las Cruces	-1.34	10	1928
Las Cruces	-0.75	10	1926
Las Cruces	-0.70	20	1926
Las Cruces	-0.55	20	2342
Las Cruces	-0.53	10	2340
Las Cruces	-0.50	20	1928
Las Cruces	-0.44	10	2342
Las Cruces	0.48	20	2340
Las Cruces	11.63	42	2340
Las Cruces	13.89	27	1926
Las Cruces	13.92	42	1928
Las Cruces	14.20	42	1926
Las Cruces	14.66	35	2342
Las Cruces	15.08	35	1926
Las Cruces	17.12	27	1928
Las Cruces	17.13	27	2342
Las Cruces	18.55	35	2340
Las Cruces	19.49	27	2340
Las Cruces	20.57	42	2342
Las Cruces	21.97	35	1928
Geothermal	-3.00	10	2340
Geothermal	-2.21	10	2342
Geothermal	-2.12	10	1928
Geothermal	-1.79	10	1926
Geothermal	-0.18	20	1928
Geothermal	0.01	20	2340
Geothermal	0.04	20	2342
Geothermal	0.52	20	1926
Geothermal	12.33	27	1928
Geothermal	12.65	42	2340
Geothermal	13.91	35	2342
Geothermal	14.07	27	2340
Geothermal	14.17	27	1926
Geothermal	14.45	42	1928
Geothermal	15.37	42	1926
Geothermal	17.34	35	1926
Geothermal	18.28	42	2342
Geothermal	19.90	27	2342
Geothermal	21.50	35	2340
Geothermal	21.88	35	1928
Half RTF	-2.41	10	2340
Half RTF	-2.04	10	2342
Half RTF	-1.92	10	1926
Half RTF	-1.89	10	1928

Table 5
Relative Growth Rates Ranked by Water Type and Growth Rate (continued)

Water Type	Relative Growthrate	Temperature Degrees C	Species UTEX #
Half RTF	-0.47	20	2342
Half RTF	-0.38	20	1928
Half RTF	-0.35	20	2340
Half RTF	-0.21	20	1926
Half RTF	5.56	27	2342
Half RTF	9.25	27	1926
Half RTF	9.96	42	2340
Half RTF	11.54	42	1926
Half RTF	13.63	35	1926
Half RTF	14.18	27	2340
Half RTF	15.74	35	2342
Half RTF	16.41	27	1928
Half RTF	17.01	42	1928
Half RTF	19.04	42	2342
Half RTF	19.41	35	2340
Half RTF	20.75	35	1928
RTF	-2.02	10	2342
RTF	-1.58	20	1928
RTF	-1.50	20	1926
RTF	-1.46	10	1926
RTF	-1.26	10	2340
RTF	-1.06	10	1928
RTF	-0.52	20	2340
RTF	-0.52	20	2342
RTF	3.97	42	2340
RTF	9.30	27	1926
RTF	12.43	42	1928
RTF	13.03	27	2340
RTF	13.95	42	1926
RTF	13.97	35	1928
RTF	15.37	35	1926
RTF	17.63	27	2342
RTF	17.80	27	1928
RTF	19.04	42	2342
RTF	19.65	35	2342
RTF	20.30	35	2340

As mentioned before, some of the most important information gathered during this project concerns the practical problems of working with RTF water, a moderately saline water with an ionic composition that is very different from seawater. When Zarrouck's medium was added to this water, appreciable turbidity resulted. Turbidity was a real problem for several reasons. First, the turbidity reduced the amount of light that can reach the algae, ultimately reducing growth. Additionally, a percentage of the concentration of one or more of the nutrients was being reduced by an unknown amount by being tied up in precipitates. This nutrient reduction could cause growth limitation. Finally, it made accurate dry weight determinations extremely difficult. Therefore, an investigation into the causes of the turbidity was launched. A very revealing experiment (figure 15) involved adding different levels of $CaCl_2 \cdot 2H_2O$ to 1 liter of RO (Reverse Osmosis) water to which had been added 0.5 g KH_2PO_4, the normal concentration of KH_2PO_4 found in Zarrouck's media. After addition of the $CaCl_2$, the contents of the flask were well mixed and filtered. Both KH_2PO_4 and $CaCl_2 \cdot 2H_2O$, at the concentrations used, should be completely soluble and thus leave no residue on the filters. However, there was a clear increase in residue weight with an increase in the amount of $CaCl_2$ added. We interpret this to mean that calcium phosphates, which are relatively insoluble, were being formed. This finding confirms that necessary nutrients, such as phosphate, are being reduced in concentration and are less available to the algae, ultimately limiting growth. Since RTF water is high in calcium, it is obvious that appreciable amounts of the phosphate added to the water are being tied up in calcium phosphates.

A similar experiment was performed to determine the effect of the added bicarbonate on turbidity. It can be seen (figure 16) that increased concentrations of bicarbonate added to RTF water (made up to full Zarrouck's

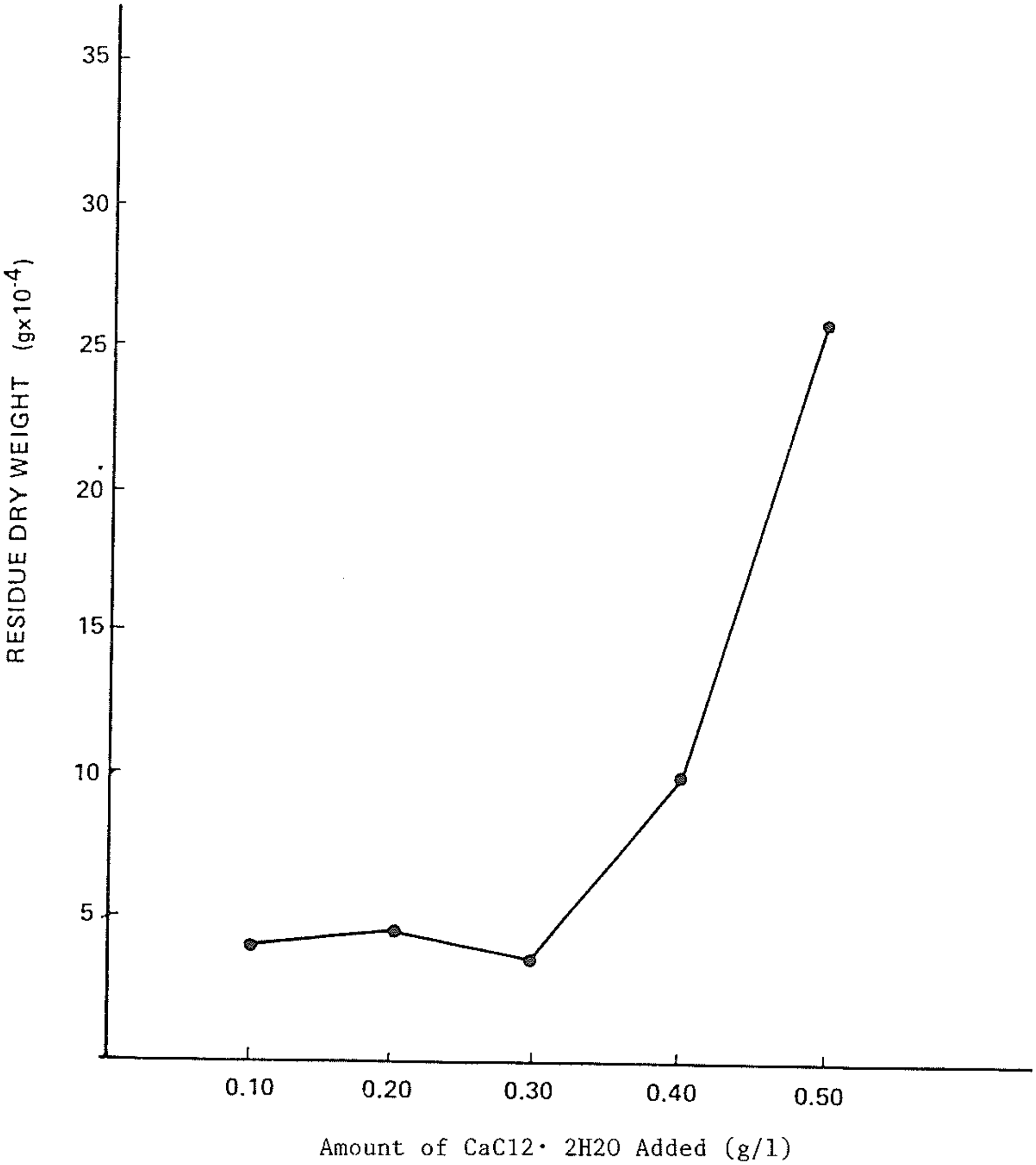

Figure 15. Interaction of CaC12 and KH2PO4.

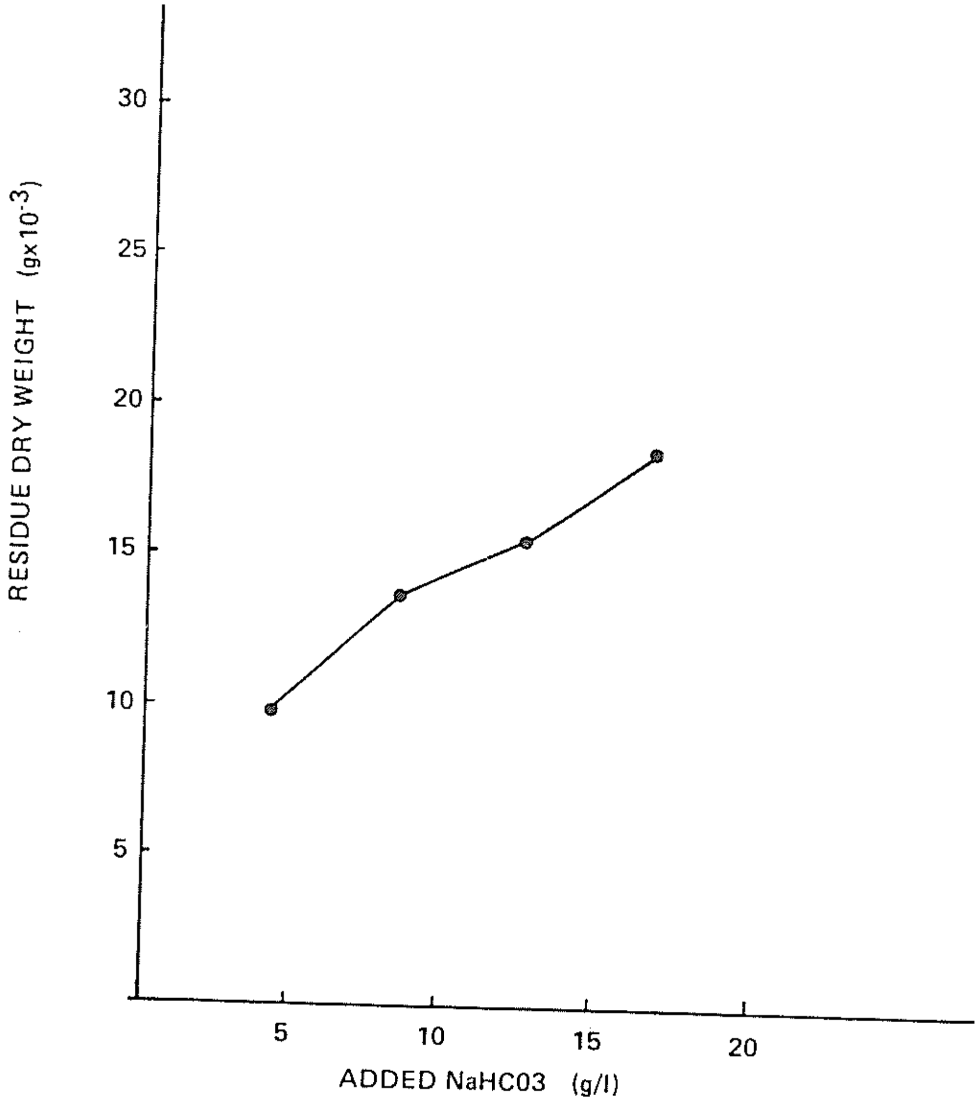

Figure 16. Effect of increased concentration of added bicarbonate on turbidity in RTF water.

media strength, but without $CaCl_2$ or bicarbonate) cause increased filter residue dry weight. Again, these concentrations of sodium bicarbonate alone should be completely soluble and thus leave no filter residue. Either the bicarbonate's solubility is reduced in the saline water (likely) or some chemical interaction resulting in precipitation, is taking place. The end result is the same-reduced light reaching the algae and ultimately reducing growth.

Potential solutions to these problems include: pretreatment of the RTF water to reduce calcium levels; using different sources of phosphate that may decrease interactions with calcium; using constant, low concentrations of bicarbonate; and, perhaps most effective of all, reusing the media. The media is essentially pretreated because the free calcium in the water already has interacted with added phosphates. Any other chemicals in the original well water that would interact with the added fertilizer ingredients already will have reacted. Reuse of the media makes good economic sense as well since less pumping for new water is required and less trace elements need be added.

SUMMARY AND CONCLUSIONS

Summary

The data presented here indicates which species should be used to achieve maximum growth rates for any particular water type used. At different temperatures for any one water type, the best species to use is also clearly indicated. For example, during summer months in New Mexico, it is possible that raceway water temperatures can approach 42°C. As indicated in figure 14, UTEX #2342 is clearly the fastest grower in any water type at this temperature. On the other hand, at 35°C (figure 12) UTEX #2342 exhibits competitive growth only in RTF water. At 27°C, #2342 is still highly competitive in RTF water. Thus, UTEX #2342 can be recommended as a good species to use in RTF water, but not necessarily in any other water type.

In summary, this data can be used to choose the best species to use for a given water type and growth temperatures. Pilot-scale studies must validate these results generated in a laboratory.

Conclusions

1. All four species of *Spirulina* tested can grow in all four water types tested when full Zarrouck's media ingredients are added to each of the water types.
2. The optimum temperature for the growth of most species of *Spirulina*, of the temperatures tested, is 35°C.
3. *Spirulina* species UTEX #2342 is the best overall species to use at temperature of 42°C, regardless of water type.
4. There is little, if any, net growth at 20°C for any species in any water type. Death occurs in all water types for all species after several days at 10°C.
5. The maximum growth rate exhibited by any species was UTEX #1928 grown in both geothermal and Las Cruces water at 35°C.
6. The interaction of the fertilizer ingredients with the saline water types tested results in turbidity of the media. The problem is most severe in full RTF well water. This turbidity can result in decreased growth via reduced light levels and reduced nutrient availability.
7. This turbidity makes accurate, replicable algal dry weight determinations extremely difficult. Optical methods of algal biomass determination are more replicable. Fluorometry, which measures in-vivo chlorophyll a, appears to be the most reliable method of algal biomass determination.

8. The pilot-scale system at the RTF is complete and operational. All subsystems have performed as expected, except for the harvest subsystem which is undergoing additional development.
9. The computerized measurement and control system currently has the capability of providing three data points for each of three parameters (conductivity, pH, and turbidity) for each of four sampling points every hour. In addition, continuous measurements of PAR (photosynthetically active radiation) at the top and bottom of the Spirulina culture water column are possible. Continuous measurements of temperature at five points in each of the greenhouses are also possible.
10. The greenhouses generally are 10°C warmer than ambient during winter. This is important in maintaining water temperatures close to the optimum 35°C during the colder months of the year.
11. Unialgal cultures of Spirulina have been grown on pilot scale at the RTF. These cultures have lasted as long as 11 weeks and reached densities exceeding 1.4 g/liter (dry weight).
12. The technical feasibility of producing Spirulina in New Mexico using a variety of saline groundwaters has been demonstrated.

RECOMMDENDATIONS

1. Continued development of the harvest system is necessary. A low-cost harvest and solar drying system for the Spirulina will enhance the economic feasibility of commercial cultivation of Spirulina in New Mexico.
2. A more thorough understanding of the chemistry of RTF water interacting with fertilizer ingredients is necessary to reduce or eliminate turbidity problems.

3. Continued development of the computerized measurement and control system will enhance the ability to acquire and analyze experimental data. This will ultimately lead to quicker progress towards project goals.
4. Since *Spirulina* growth is optimal at 35°C and its temperature tolerance range is relatively narrow, methods for maintaining optimum temperatures in large-scale cultures are necessary. The development of passive solar greenhouses is strongly recommended. Gaining practical experience with using geothermal water for its heat content as well as a growth media is strongly recommended.
5. Research on low-cost culture systems is recommended.
6. Market research should be performed to determine the size and location of potential markets for *Spirulina* grown in New Mexico.
7. A small commercial size *Spirulina* culture operation should be established and operated for at least one full year to determine actual operating and capital costs.

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