AN ECOLOGICAL ASSESSMENT OF GENETIC ENGINEERING

EFFECT OF GROWTH HORMONE ON RAINBOW TROUT:

AN ECOLOGICAL ASSESSMENT OF THE POTENTIAL IMPACT OF GENETIC ENGINEERING ON ORGANISM DESIGN.

BY

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ABSTRACT

Trout genetically engineered to possess extra functioning growth homone genes were simulated, using injections of fish and bovine growth hormone. This was done to test potential and ecological impact of such genetic engineering on trout. Through analysis of growth and dry-mass budgets, it was determined that elevated levels of growth hormone resulted in increased growth and consumption. Potential constraints pertaining to respiration, density and evolutionary history were considered. Surprisingly, bovine growth hormone appeared to act as a super-normal stimuli, being more potent than the natural trout hormone. In fact the natural hormone inhibited growth at high dosages. Juvenile growth rates were less sensitive to elevated levels of growth hormone than those in more mature fish. Thus, improved growth was achieved by altering its normal ontogeny. This suggests that duplicating copies of growth hormone gene in an organism is not equivalent to a speciation event. Evidently, other manipulations would be required to increase the intrinsic metabolic power. Growth rate apparently is used as a cue for determining a particular ontogenetic trajectory (Stearns and Crandall, 1984). The fact that various species conform to one of at least four plastic developmental trajectories that are shaped by natural constraints and mortality patterns, means that growth hormone may elicit different responses in different species. The findings and implications of this study underscore the importance of using the holistic scope of ecology to achieve effective and efficient genetic engineering of target species.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Increasing demands for food, diminishing space for traditional forms of food production and increasing concern for environmental and wildlife preservation have encouraged the development of aquaculture. Aquaculture is an efficient, environmentally sound method of producing palatable, high grade protein. Attempts to improve production and minimize costs have focused scrutiny on the bioenergetics and mass budgeting of cultured organisms, particularly salmonids. Major avenues of investigation include the use of exogenous hormones (Pickford, 1959; Higgs et al., 1975), altered ploidy of fish (Thorgaard, 1986; Chourrout and Quillet, 1982) and development of bioengineered organisms (Chen personal communication). Application of such techniques also provides general insights into the design of organisms.

The present study is intended to direct further research into the various aspects of organism design (energetics, behaviour, development and life history). Such research can be of particular benefit to the field of genetic engineering.

To date, genetic engineering has been in the realm of the molecular biologist, and there has been a conspicuous lack of ecological involvement. This project was initiated to provide complimentary ecological assessment of molecular biological initiatives by Dr. T. Chen at McMaster University. As such, the work represents the first "organism impact assessment" of genetic engineering on an animal. Specifically the study addresses the implications of altering the growth rate of rainbow trout (Salmo gairdneri) using recombinant fish growth hormone or purified mammalian growth hormone.

Dr. Chen, now at the Center of Marine Biotechnology, Baltimore has successfully produced trans-genic carp, highlighting the immediate need for ecological involvement in this new field.

To increase cost effectiveness in fin-fish aquaculture, two basic strategies have been adopted. One tactic is to increase the growth rate of the fish thereby decreasing the time required to reach market size. The other strategy is to decrease the cost of food. Food accounts for up to 50% of the operating cost of fin-fish aquaculture (Gill et al., 1985).

The traditional way to improve growth rate and efficiency of fish is through selective breeding. However, simply selecting fish which grow the fastest and continually crossing them does not ensure the development of superior fish. You may not be selecting for a trait that is heritable or perhaps the fastest growing fish out-compete their neighbors and do not possess inherently superior growth rates or conversion efficiencies (Weatherley, 1976). For this reason it is necessary to study the resource allocation of the organism to identify promising characteristics for selection and to determine their heritability (Kleiber, 1961). It is best to study fish individually, thereby controlling social interaction, but one must examine the species carefully for normally social fish may function abnormally when solitary (Weatherley, 1976).

The energy consumption of fish can be partitioned into several distinct categories. Resources are allocated to somatic growth, reproduction, standard metabolic rate (measured when animals are fasting), activity, specific dynamic action (SDA) and waste products (faeces and nitrogen excretion) (Ricker, 1968). Growth is the target factor to be maximized (whereas natural selection has presumably maximized reproduction). In

particular, it is most desirable to increase muscle mass with its high protein content. Specific dynamic action (SDA) is an attribute thought to possess a sizable amount of reallocatable resources (Weatherley, 1976). It has often been linked with the deamination of proteins (Warren and Davis, 1967 ; Beamish, 1974). This is still a subject of controversy, therefore for our purposes, it is best thought of as a price paid for converting food into more usable components. The magnitude of the SDA is linearly related to the size of the ration consumed (Kerr, 1971; Beamish, 1974).

Activity in fish is very difficult to measure. It is known that the scope of activity for rainbow trout (Salmo gairdneri) increases with increasing temperature and peaks at 15°C for hatchery trout and 20°C for wild trout (Dickson and Kramer, 1971). It has also been determined that movements of the brown trout (Salmo trutta) in Scottish lochs is very comparable to laboratory trout (Holliday et al., 1974). However, generalizations in this particular area are dangerous for there is considerable variation among species and environments.

Assimilation efficiency and waste production of fish depends greatly on the material being consumed and its digestibility (Lien, 1978). Many energetic studies have examined the assimilation efficiency and waste production of fish. Rainbow trout fed 17 diets had assimilation efficiencies ranging from 52-95% (Halver, 1972). However, most commercial feeds have absorption efficiencies of 80-90% (Beamish et al., 1975). When fed Gammarus pulex L., brown trout had an assimilation efficiency of 75-85%. They lost 15-20% of their consumed energy as faeces and 4-12% as excretory products, which makes for a 20-30% total energy loss (Elliot, 1976a). Energy loss in the yellow perch (Perca fluviatilis L.) was found to be 26.5%

(Solomon and Barfield, 1972). Assimilation efficiency increases with increasing temperature up to 20°C in rainbow trout fed earthworms (Brocksen and Bugge, 1974). Some controversy exists regarding the effect of ration size on growth efficiency. Paloheimo and Dickie (1966), using published data claim, to have discovered the "K-line" which showed that the proportion of consumed energy allocated to growth decreased with increasing ration size (feeding to satiety is considered a full ration). Studies conducted on sockeye salmon, (Oncorhynchus nerka), found that conversion efficiency in fish fed at a fixed percentage of their body weight remained constant and then declined (Brett et al., 1969; Brett and Shelbourn, 1975). Furthermore, salmon fed on full ration also exhibited a drop in conversion efficiency due to an allometric change in growth (Brett and Shelbourn, 1975). Kerr (1971) reconciled the two findings by proposing a model with an additional phase with a slope that was positive or zero at low ration. Given that in most cases fish will be fed to satiety the importance of the "K-line" to aquaculture is diminished.

Within the Salmonidae there is a wide variety of reproductive strategies. However, in comparison to most animals salmonids are considered to be iteroparous. Salmonids that are relatively semelparous still produce large numbers of eggs (e.g. Salmo malma produces 1412 eggs) (Hutchings and Morris, 1985). Therefore, much material is expended in reproduction but little or no energy is spent on parental care. Depending on the maturity of the fish and environmental conditions (e.g. season) a large amount of somatic resources may be devoted to reproduction. The resources allocated to reproduction have evolved as part of the trout's survival strategy in nature. In hatcheries, lower rates of reproduction may be sufficient for

replacement of stocks. This would free resources to be reallocated to growth. Thus, the life cycles of natural fish may be poorly designed for hatchery environments and human goals.

If any of the above requirements can be reduced or performed more efficiently, more material can be rerouted to growth, thus making the fish more cost effective to culture.

One very successful method of enhancing growth in salmonids has been through inducing polyploidy (Thorgaard, 1986). Induced polyploidy refers to the production of individuals with extra sets of chromosomes. This can be achieved by the use of temperature shock (Chourrout and Quillet, 1982), hydrostatic pressure (Chourrout, 1984) or chemical treatment (Refstie, et al., 1977; Refstie, 1981) of eggs. Much of these data are still being collected but it appears that when mature, polyploids sustain better growth than diploids (Thorgaard, 1986). The growth improvement may be due to the polyploids possessing a higher degree of heterozygosity (Allendorf and Leary, 1984). However, it may also be due to a reallocation of resources away from gonadal development and reproduction to somatic growth. In support of this, female rainbow trout that are triploid show inhibition of ovarial development (Lincon and Scott, 1983). This suggests that the best hatchery product might be polyploids of fish genetically engineered with extra growth hormone genes (i.e. enhanced growth rates, suppressed reproduction).

A very promising method for enhancing growth is through elevation of levels of growth hormone in cultured animals. Ultimately extra copies of growth hormone genes may be engineered into the genome of the fish, as has been accomplished by Dr. T. Chen (personal communication). Presently

elevated levels are achieved by injection or implantation of pellets containing the hormone. Administration of growth hormone orally has been avoided because the protein is digestible. However, some evidence suggests that the hormone is only partially digested before absorption into the blood stream (Cantilo and Regalado, 1942; Pickford and Atz, 1957).

Growth hormone is produced in the pituitary gland and secretion is under very complex control (Fig. 1). Its secretion is controlled from the pituitary itself; from the hypothalamus via releasing hormones such as thyrotrophin releasing hormone (TRH), growth hormone releasing factors (GRFs) and inhibitors such as somatostatin (SRIF); from the gastro-intestinal tract by gastric peptides such as vasoactive intestinal polypeptide (VIP) and cholecystokinin octapeptide (CCK-8); from the nervous system by neurotransmitters such as gamma-aminobutyric acid (GABA) and acetylcholine; by metabolic factor such as phosphorus and calcium; by other hormones such as oestrogen and finally, by external factors such as stress and exercise (reviewed by Hall et al., 1986).

The control of growth hormone secretion has not been extensively studied in poikilotherms (Hall et al., 1986). Most studies have been carried out on humans and economically important mammals. Among its many roles in mammals, growth hormone stimulates fat mobilization. It activates lipase which catalyses triglyceride hydrolysis. The energy from the oxidized lipid is believed to be used to spare dietary protein which is reallocated to muscle growth (Frye, 1971). In support of the above contention it has been found that protein meals stimulate the release of growth hormone (Pallotta and Kennedy, 1968; Shreiber, 1974). The effects of mammalian growth hormone on a variety of animals are summarized in Table 1.

Recent advances have made it possible to obtain a large supply of piscine growth hormone. Recombinant techniques have been used to produce rainbow trout and chum salmon (Oncorhynchus keta) growth hormone (Agellon and Chen, 1986; Sekine et al., 1985). Hopefully, extra functional copies of the growth hormone gene will eventually be inserted into the genome of many cultured fish, as has been done with carp. This is no simple task, for even if the gene is inserted, it must have the correct accompanying promoter sequences for the gene to be functional. There may also be a problem with the body strictly regulating the production of the hormone. If this is the case the fish may "shut down" the extra copies or it may use all copies to a lesser extent. If a fish could be produced that did have elevated growth hormone levels and it exhibited more rapid and efficient growth it would be invaluable to the aquaculture industry.

The complexity of fish resource allocation and the dilemmas associated with genetic engineering show the importance of selecting the correct gene or attribute to alter. The cost and time involved with specifically altering the genome of an organism is considerable. To make decisions that are both effective and economical it would be invaluable to have a framework of the general principles governing the organism's design.

A relative homogeneity exists in the basic biochemistry of eukaryotes (Frankel, 1959; Bonner, 1974). From this similarity the field of allometry has emerged. Allometry attempts to relate differences in organism physiology, development, morphology and behaviour to body mass according to the equation $Y = aM^b$ (where Y = variable, M = body mass, a = a coefficient

that is characteristic of a particular species or phylogenetic group and b = an exponent relating body mass to the variable)(Peters, 1983; Calder, 1984; Schmidt-Neilsen, 1984).

Metabolism has long been a favorite topic of allometrists. In 1838 Sarrus and Rameaux theorized that metabolic rate should vary with surface area rather than body mass. In support of this theory a study conducted on starving dogs yielded a mass exponent of 0.66 (Rubner, 1883). This theory came to be known as the "surface rule" which states that metabolism is geared to counteract the energy loss to the environment at the surface of the organism. Kleiber measured the metabolism of a variety of mammals covering a vast range of shapes and sizes. He arrived at the "Kleiber exponent" which was 0.75 and not the expected 0.66 (Kleiber, 1932). This led to a great deal of controversy and many explanations of the disparity were put forward. Huesner (1982) stated that the difference was due to a statistical artifact in Kleiber's data. Blumm (1977) explains the disparity as due to the existence of a fourth dimension. Wieser (1984) put forward a widely accepted view that there was no disparity between the two findings. He claimed that 0.66 was an intraspecific trend while 0.75 was an interspecific trend. The additional 0.09 was an increase due to an increase in the coefficient of basal metabolism. This shift in coefficient of basal metabolism was said to occur as species became larger through evolution (Cope's Law) (Cope, 1885; Newell, 1949). Wieser asserts that the selective advantage that size is supposed to confer (Rensch, 1960; Bonner, 1965) is not sufficient, an increase in metabolic intensity was also required. There are many studies which show the competitive superiority of species with such higher intensity (Grant, 1972; McNab, 1980).

Since allometry is a key tool for examining aspects of design, growth is not presented as weight versus time, as is conventional, but rather it is converted to a growth versus weight axis so that it can be treated the same way as other allometric attributes. This analysis was adopted to standardize time and allow comparison among fish varying in size.

Injection of growth hormone simulates the effect of having extra working copies of the growth hormone gene. Moreover, this method has the advantage of knowing exactly the amounts of extra hormone involved among treatments. Engineered fish may have unknown and variable numbers of introduced genes. Even then the number actually functioning may not be known or may vary among tissues. This pilot study may give the engineer a basis for determining the optimal number of genes to insert for maximal growth. By changing the genome, speciation may potentially be achieved. Therefore, the use of growth hormone presents a unique opportunity to gain insight into the allometry of salmonids and the principles that govern organism design.

Rules that constrain the design of organisms would serve as very useful heuristics for genetic engineers.

MATERIALS

A. Fish Used

The fish used in the study were rainbow trout (Salmo gairdneri) obtained from local hatcheries. All of the fish used in the dosage response experiment were obtained from Goossen's trout farm and were selected to a weight of 50 g.

Due to some disease problems in the fish from Goossen's the fish used in the density study were obtained from the Spring Valley Trout Farm. They were also selected to a weight of 50 g.

The allometry experiment required a wide range of sizes of fish. Therefore two hatcheries (Spring Valley Trout Farm and Rainbow Springs Hatchery) were required to span the sizes needed in the experiment.

B. General Rearing Techniques

Trout were reared in a "flow through" system which utilized conditioned tap water (Fig. 2). The temperature of the tap water varied seasonally from 22°C in August to 4°C in January. For the second and third experiments a temperature regulation system was used. This system held water in a conditioning tank before it was delivered to the rearing tanks. The conditioning tank's temperature was controlled by a solinoid valve which injected hot water when its sensors detected that the temperature had dropped below the desired level. From the conditioning tank the water was pumped through two dechlorinators (Anderson Co.) connected in series. From the dechlorinators the water went through a manifold distributing it to 24 rearing tanks (Fig. 2).

The plastic rearing tanks were 54 by 55 by 30 cm deep. They were fitted with perforated mylar inserts (37cm diameter) which made the square tanks circular. The rigid square walls of the tanks caused abrasions on the trout which sometimes became infected. The inserts decreased such injury and subsequent morbidity in the subjects by providing a continuous and flexible contour which the trout could follow. Holes at the bottom of the insert allowed the faeces produced (which sank) to flow to the outside walls of the tank (out side of the insert) (Fig. 3). This effect was useful in that it prevented possible copraphagy and simplified the collection of faeces. The tanks were covered with plastic grate lids. A 12L:12D photoperiod was maintained during all of the experiments but a cover of dark plastic diminished the light which appeared to stress the fish. Each tank received a flow rate of 150 ml/min (9l/hr). Tanks were vacuumed with a siphon to remove faeces when it was necessary (usually once every two days).

The fish were fed to satiety twice daily with trout chow (Martin Feed Mills: Protein, 40%; Fat, 8%; Fiber, 3%). Particle size of the food was adjusted to the size of the fish. Floating pellets were used and uneaten material was removed after the fish stopped feeding (20-30 min) and before the pellets began to sink. Thus, the food and faeces were effectively partitioned.

All fish were acclimated for several weeks when received from the hatchery. Preceding each experiment the fish were further allowed to acclimate in the treatment tanks for at least two weeks. Fish were selected from the holding tanks such that each treatment received fish of approximately equal size. Otherwise, individuals were randomly assigned to treatments.

C. Factors Measured

Fish were weighed each week using a "fish holder" to prevent movement during weighing. The holder was a block of silicon-covered styrofoam with a fish-shaped depression in its center (Fig. 4). Several different sizes were constructed to accommodate the growing fish. Water was placed in the holder and the water and holder were tared on a Sartorius 1265mp balance. The fish was netted, excess water was removed with a damp cloth and the fish was weighed in the holder. If wrapped in material or hand held the trout struggle violently, making estimates of weight difficult or highly inaccurate. The fish ceased struggling once in the holder, thus eliminating the need for the commonly used anesthetic (Higgs et al., 1976). This reduced the possible stress associated with anesthetic or possible drug interactions with the hormone. Higgs et al.,(1976) site the use of anesthetic to weight fish as a cause of mortality. There was no mortality due to experimental procedures in any of the experiments in the present study. Fish lost during the first experiment was due to the accidental disconnection of the air supply to one tank.

In studies involving many fish per tank the fish were distinguished using brands. The brands were placed on the backs of the fish using a copper tipped rod cooled in liquid nitrogen. Dry-mass budgets were conducted using the principle

$$C = G + F + R + U$$

(Ricker, 1968) (Fig. 4). Where C= consumption; F= faecal production; G= growth and reproduction; R= respiration; U= the energy value of excretory products. However, in this study the release of excretory products and special products (mucus production etc.) are included in the measurement of

respiration. This results in high estimates of respiration. The presence of shed scales and mucus mixed with the collected faeces must have inflated estimates of faecal production, but visual inspection suggested that this was very minimal.

The mass budgets were conducted over a one week period. During this period the subjects were fed to satiety with pre-weighed food (Cb) twice daily. The remainder of the food was removed from the tank by net (the use of floating pellets made this task considerably easier), and then was dried to constant weight (Ca) at 60°C. The use of the mylar insert allowed the faeces to settle to the bottom near the sides of the tank without the fish disturbing or eating them. Since water exited the tanks at the top, there was no loss of faeces from the tanks. The faeces were removed daily or every other day using a siphon which consisted of a glass tube with a brush at one end and Tygon tubing at the other. The faeces were collected in buckets. The contents of each bucket was vacuum filtered through two pre-weighed coffee filters (P). The filters and faeces were then placed in the drying oven and dried to constant weight (Pf) at 60°C. The dry weight to wet weight ratio of both the fish chow (Dc) and coffee filters (Dp) was determined by placing unused controls in the drying oven. The dry weight of food consumed and faeces produced was then calculated using the following equations:

$$C = (Cb*Dc)-Ca$$

$$F = Pf-(P*Dp)$$

Weight of the fish was measured at the beginning and end of the budget to determine growth (G). Dry weight of the fish was determined by drying whole trout to constant weight (60°C) and used to calculate a dry weight to

wet weight ratio. Due to the low numbers available, the actual ratio used in the calculations was an average of the value found in this study and those found in two other studies (Milligan and Wood, 1982; Denton and Yousef, 1976). Growth was calculated as the actual wet weight increase in fish body mass multiplied by the fish wet weight to dry weight ratio.

Respiration was determined by subtracting the growth and faecal production from the consumption:

$$R = C-(G+F)$$

Assimilation efficiency (AE) is a measure of how efficiently the consumed food is absorbed and was calculated as:

$$AE = (C-F)*100$$

Gross production efficiency (GPE) measures the efficiency with which the food consumed is channeled into growth. The equation for GPE was:

$$GPE = (G/C)*100$$

Net production efficiency (NPE) is a measure of how efficiently absorbed energy is channeled into growth and was calculated using the equation:

$$NPE = [G/(C-F)]*100$$

D. Preparation and Origin of the Hormone

The dosage-response experiment was conducted using rainbow trout growth hormone obtained through recombinant techniques (Agellon and Chen, 1986). This hormone was received as a crude extract and required 12 hours dialysis against distilled water to remove the tris buffer that the hormone was suspended in. The activity of the hormone was not known.

Due to problems with supply of the recombinant growth hormone, bovine growth hormone obtained from I.C.N. (cat.# S301003) was used in the density

experiment. Bovine growth hormone has been reported to stimulate growth in rainbow trout (Chartier-Baraduc, 1959) and a number of other fish species ranging from other salmonids such as chinook salmon Oncorhynchus tshawytscha (Higgs et al., 1978) to fish such carp Cyprinus carpio (Adelman, 1977) (refer to Table 1 for further examples).

The bovine growth hormone used in the allometry study was received as a grant from the U.S.D.A. Animal Hormone Program in a purified lyophilized state.

All forms of growth hormone were suspended in sterile isotonic saline and diluted to dosage specification. Injections were administered interperitoneally using disposable 1cc syringes with 23G1 tips.

E. Experiments conducted

Experiment 1: Dosage response using recombinant rainbow trout growth hormone.

Individual trout were injected with 0, 0.01, 0.05, 0.1, 1, and 5μg of recombinant growth hormone/10 g body weight/week for seven weeks. In each treatment density was maintained at six fish per tank. Each treatment was replicated so that twelve tanks were required. All 72 fish were branded making it possible to monitor growth of individuals. This study lacked temperature control and there was a gradual decline in water temperature from 13°C in October down to 5°C in December, 1986. Immediately following the series of injections a dry-mass budget was conducted.

Experiment 2: Density and Enhanced Growth

Three densities (1,2 and 4 fish/tank) of fish were injected with 0, 0.001, 0.01 and 0.1 μg of Bovine growth hormone/ 10 g body weight/ week at each density. All 56 fish were branded so that individual growth could be monitored. Although the temperature control system guarded against temperature decreases, there was still a slight increase in temperature. The temperature climbed from 11.5°C in May to 17°C in July,1987. Two dry-mass budgets were conducted: one at the beginning of the study, prior to any hormone injections (at 11.5°C), and the other immediately following termination of injections (at 17°C).

Experiments 3: Allometry and Growth Enhancement

Fish ranging in size from 10 to 30 g were individually reared in each of 24 tanks. Eight groups of fish were selected to span this range. Within each group of 3, the fish were selected to be as similar in size as possible. Fish in these groups received doses of 0, 0.1, or 1μg/g wet fish/week for four weeks. Immediately following the injections a dry-mass budget was conducted.

F. Analysis of Data

The growth data were analyzed to remove the effects of the size of the fish. Growth is usually plotted as weight versus time (Fig. 6a). To convert the data to standard allometric form ($Y=am^b$) the data were first plotted as log weight (g) versus time (Days) and fitted to a first order linear regression (Fig. 6b). If the growth conforms to a constant exponential rate this transformation will fit these data to a straight line. The slope of this regression line then describes the exponential rate of growth per day. The slope and the intercept of this least squares line were

growth per day. The slope and the intercept of this least squares line were then used to generate an estimate of each days growth as well as a cumulative record of the organism's increasing weight. It was then possible to plot the daily growth rate versus the log of the weight of the organism (Fig. 6c). This plot describes how growth rate changes with the weight of the animal. Linear regression of these data yields a Y intercept that is the log of the basal coefficient of growth. This number is the extrapolated value of the log growth of the organism being observed if it had a weight of 1g. This log specific growth rate is thus a very useful way to compare animals of differing sizes. The value of the coefficient is known to decrease with increasing body weight intraspecifically (Wieser, 1984) (Fig. 6d).

RESULTS

Experiment 1: Dosage response with recombinant rainbow trout growth hormone.

Analysis of variance determined that the extract used had a significant effect on fish growth ($P<0.05$). Doses of 0.01 and 0.1 (μg/10g body weight/week) elevated the growth rate of the fish (Table 2). These are exponential rates that look deceptively similar to control rates (i.e. differences are exponential rather than incremental). A standard 100g fish growing at these rates for 100 days would attain weights of 252$\pm$119 g, 311$\pm$131 g and 287$\pm$60 g in the control, 0.01 and 0.1 treatments. The best enhancement achieved was 19% larger than controls. Figure 7 shows that doses of 0.5 has little effect and doses higher than 0.5 actually depressed growth. A Student-Newman-Keuls test only distinguished the high (1 and 5) dosages from the controls and the lower more effective dosages (Table 2). This is probably due to the sample size being too small for statistical resolution.

Because the dry-mass budgets were conducted on tank populations rather than individual trout, there were only two replicates per treatment. It is uncommon for such studies to obtain sufficient replicates for statistical analysis due to the logistic infeasibility of the labour required and expense of the hormone. Consequently, it is not surprising that the analysis of variance did not reveal significant differences between any of the measured variables.

The same basic trends found in the growth data, however, are also seen in the measurements of consumption, faecal production and respiration (Table 3). However, the 5 μg dosage group exhibited unusually high rates during

the budget period. This dry-mass budget was conducted at low temperature (5°C), which probably accounts for the relatively low growth rates and production efficiencies.

Experiment 2: The effects of density and bovine growth hormone on growth and energetics of the rainbow trout.

Prior to the injection of hormone, a dry-mass budget was conducted. Although none of the data collected were statistically significant, very clear trends emerged upon analysis. All of the measured rates and efficiencies decreased with increasing density (Table 4).

After injection with bovine growth hormone a 3 by 4 way ANOVA was conducted that did not detect significant effects of density or dosage on the growth rate of the fish. Again, this is probably due to the low sample size since very obvious trends were apparent. Tanks with a density of four possessed the highest growth rates followed by densities of one and two fish (Table 5). The hormone had different effects at different densities. At a density of one fish per tank doses of 0.1 and 0.01 had virtually equal effectiveness in elevating growth rate while doses of 0.001 depressed growth rate relative to controls (Table 6). Rates from the dry-mass budget closely mirrored growth rates. Efficiencies, however, were relatively constant (Table 7).

In tanks containing two fish the hormone apparently had an adverse effect, with only the lowest dosage showing any enhancement of growth (Table 6). These findings correspond to those found in the dry-mass budget. The only finding of note, concerning efficiencies at this density was that at a dosage of 0.001 the fish exhibited improved gross and net production efficiencies (Table 7).

The growth data collected from tanks with a density of four fish showed enhanced growth at all dosage levels, however, the effectiveness of the dose was inversely proportional to its concentration (Table 6). Although the dry-mass budget showed enhanced growth for the lowest dosage, the two higher dosages inhibited growth. The mass budgets are calculated over much shorter periods of time and so growth estimates are not as reliable as for the overall study. The group given the lowest dosage possessed improved gross and net production efficiencies (Table 7).

Experiment 3: The effect of bovine growth hormone on allometry

One replicate (3 fish) was dropped from the allometry data due to their visibly poor health and consequent abnormally slow growth. The probability of this removal biasing the data is very low for the fish in each replicate were selected to be as similar as possible before the treatments were randomly assigned.

Both the 0.1 and 1 g/g/week treatments resulted in elevated growth rates (Table 8). A standard 100g fish growing at these rates would have attained a weights of 419±29 g, 593±28 g and 744±40 g after 100 days in the control, 0.1 and 1 g/g/week treatments. This means that the .1 and 1 treatments resulted in sizes 29% and 44% larger than controls respectively. Fish given the highest dosage exhibited enhanced growth that was significantly greater than that of controls ($P<0.05$) (Table 8).

When the log daily growth was plotted against the log weight of the trout (Figs. 8-10) their growth rate in the high dosage group did not decline with increasing weight as expected from allometric theory. This same effect is seen in the low-dosage treatment. However, the controls

showed the expected decrease in growth rate with increasing size (Brody, 1945; Medawar, 1945; Cooper, 1953; Winberg, 1956; Bertalanffy, 1957; Calder, 1984). It was also evident that the hormone had a greater effect on the larger trout than it did the small (Figs. 8-10).

When the log of the basal coefficient of growth was plotted against log weight, the controls had a mass exponent of 0.85, the low dosage has an exponent of 0.89, and the high dosage had a mass exponent of 1.01 (Table 9).

Although administration of the growth hormone had a positive effect on the growth of all fishes, effects appeared to differ with the size of the fish. To substantiate this, the subjects in each treatment were divided according to weight into two groups of equal numbers, one containing the largest individuals the other the smallest. The small fish exhibited a marked drop in growth rate after cessation of high dosage injections. This drop took the form of a scallop in the growth curve (Fig. 11). The low-dosage group exhibited the drop, but the scallop was much less pronounced than that of the high dosage group. No such inhibition of growth was seen in the controls or in any of the large trout (> 100g at time of cessation) (Fig. 11 and 12).

It was not known at the time when the dry-mass budget was conducted that the small fish were experiencing depressed growth associated with the absence of the hormone. To correct for this, data for large and small fish were analyzed separately. As was found in the growth data, the budget data for large trout also showed that the high dosage group displayed the highest rates (Table 10). However, in the budget the control group exhibited higher rates than did the low dosage group. The hormone did not affect the efficiencies of the large trout (Table 10).

The dry-mass budget of the small trout did not yield any statistically significant findings. However, it was evident that the various rates of the treated fish were suppressed (Table 10). In particular, the consumption and growth of the high dosage group were low. In addition to decreased rates the treated fish also experienced decreased efficiencies (Table 10). Therefore both a suppression of feeding and a reduction in processing efficiency accompanied cessation of BGH injection in small trout.

Dry-mass budgets were unavoidably conducted over a wide range of temperatures (5-17°C). It was also noticed that water temperature was the only parameter that had any influence on assimilation efficiency. Using an unbalanced anova it was found that controls from the different experiments run at temperatures of 5, 11.5 and 17°C had significantly different assimilation efficiencies ($P<0.05$)(Table 11). Other parameters were not compared due to the density differences between studies. Density had no effect on the assimilation efficiency therefore fish maintained at different densities were compared.

DISCUSSION

The present study simulated insertion of extra copies of functioning growth hormone genes into rainbow trout. By examining each component of the mass-budget (consumption, faeces, assimilation efficiency, respiration, gross and net production efficiencies and growth) it was intended that the impact of genetic engineering on whole-organism functioning would become apparent. Consequently, the discussion is organized around the key components of physiological resource allocation. The results of experiments that pertain to each component are synthesized from across all experiments. In addition, interactions are considered and an integrated picture of holistic function is assessed.

Consumption

Many studies reporting enhanced growth with augmented levels of growth hormone observed increased consumption over controls (Cantilo and Regalado, 1942; Komourdian *et al.*, 1976; Gill *et al.*, 1985). In the present study fish with hormonally enhanced growth also exhibited increased consumption during the dry-mass budget (Tables 3, 7 & 10). Small fish in the allometry study showed a drop in consumption following cessation of hormone injections but this was associated with a sharp decline in growth rate (Table 10). Many factors are known to control feeding such as blood glucose level and gut stretch receptors, however, it may ultimately be under hormonal control. The above result suggests some hormonal control of feeding. Wade (1974) proposed that growth hormone acted directly on centers in the hypothalamus to control food intake in weanling rats. In support of this, electrical

stimulation of the hypothalamic region of bluegills and cichlid fish elicited feeding responses (Demski, 1973; Demski and Knigge, 1971). The hypothalamus releases growth hormone releasing hormone (GHRH) suggesting that there may be a feedback system involved in the process (Hall et al., 1986). It appears that the hormonal system of the trout responds in an integrated manner so that feeding rate increases proportionally to growth potential.

Fairly rigorous studies might be required to confirm that the hormonal system does not set a feeding rate that actually constrains growth. This could occur if the system regulating feeding is not entirely integrated with that governing growth. The data suggest, however, that the system "scales up" in an entirely integrated manner.

Even greater growth may have been possible if a continuous feeding system had been available (i.e. the two daily feedings to satiety may have been insufficient to fulfil full growth potential (Kerr, 1971). Because gut size is limited, increased consumption might be achieved by more frequent feeding rather than larger amounts during one meal.

If food quality or quantity is limiting growth, then increasing consumption is an effective manner in which to obtain improvements. This reduces the time required to reach market size, thus enabling the aquaculturalist to increase the volume of production. If, however, there is an eventual decline in the efficiency of utilization of food, the increase in production may not be economically viable. There is generally an inverse relationship between processing rates and efficiencies (Waldbauer, 1968; Calow, 1977; Scriber and Slansky, 1981).

Evidently there is enough plasticity in the design of the digestive system to accommodate and process the required greater volumes of food. However, such treated fish might have a reduced ability for compensatory feeding under dietary stress (Rollo 1986; Rollo and Hawryluk, 1988).

Faeces & Assimilation Efficiency

Estimates of faecal production were slightly inflated due to contamination with mucus and shed scales. Dry-mass of faeces accounted for close to 20% of the consumption in all the experiments (Tables 3, 7 & 10). Faecal production and assimilation efficiency are heavily dependent on the nature of the food consumed (Lien, 1978). Commercial feed that has been developed ensures physical consistency and high assimilation efficiency (Beamish et al., 1975).

Enhanced levels of growth require greater flow of food through the digestive tract. Dietary requirements may change when animals grow more rapidly (Gordon, 1972). Amino acids normally produced in sufficient quantities metabolically may become limiting during rapid growth, thus creating the need for dietary supplements. Consequently, reformulation of commercial feeds may be necessary for faster-growing, genetically altered fish (e.g. to restore optimal amino acid balance). Chemical analysis of food and faeces could suggest whether such imbalances are potentially important. Strictly on the basis of mass, there is no evidence of reduced digestive efficiency with enhanced growth. Since production efficiencies were similarly unresponsive to augmented growth (see below), this study suggests that at least moderate growth enhancement can be achieved without altering diet quality.

The assimilation efficiency exhibited by the fish is relatively high compared to other organisms, therefore, it is not a likely target for enhanced productivity.

Respiration

Measurements of respiration were slightly inflated because they included mucus and soluble excretion. As expected, the absolute rate of respiration decreased with decreasing temperature since decreased metabolic rate accompanies decreasing temperature in poikilotherms. Variation in consumption makes measurements of respiration that are relative to the amount of food consumed more informative. Respiration accounted for 75-80% of the consumed food in fish treated with recombinant trout growth hormone (Table 3). With bovine growth hormone respiration accounted for 50-60% of consumption (Tables 7 & 10). The budgets with recombinant hormone were conducted at 5°C, those with bovine growth hormone were conducted at 11.5-17°C. The fact that both the controls and treated fish in the study using recombinant hormone exhibited similar respiration rates suggests that the relatively elevated rates were due to the cold temperature and not the hormone (Table 3). Perhaps the fish were more active in the cold water thus incurring higher behavioural costs.

Faster growing fish have higher absolute metabolic rates. If this was not the case they would possess higher gross production efficiencies than controls. To realize the potential growth associated with hormone enhancement, sufficient oxygen must be available. Under low oxygen regimes, fish with elevated metabolisms may become stressed. This could be an

environmental factor selecting for the exhibited growth rate of natural fish.

The density study (Table 4) found that the proportion of dry-mass respired also increased with density in the controls, probably due to the aggressive interactions among fish. Administration of bovine growth hormone apparently offsets the density effect, which if true, holds promise for the high-density production of engineered trout.

Cold water slows growth and lowers the capacity for high volumes of production. It also increased relative rates of respiration which wastes expensive commercial fish food. The above findings inform us that warm (>10°C) water is essential to cost effective aquaculture. Since oxygen concentration decreases with increasing temperature, and since fish may become more mobile to improve oxygen flow across the gill, good aeration is also critical for efficient production.

Gross and Net Production Efficiency

Productivity is dependent on the rate and efficiency of processing materials. The results suggest that efficiencies were not decreased by increases in growth or feeding rates, but were constrained by other factors. Gross production efficiency is influenced by both assimilation and respiration. Net production efficiency is mainly affected by respiration. Since assimilation efficiency was relatively constant, there is little need to differentiate these efficiencies.

The low production efficiencies (0.0-2.5%) found in the study using recombinant trout hormone may have been due the low (5°C) temperature and higher relative rates of respiration at which the budget was conducted.

Brocksen, et al. (1968) similarly found that the production efficiency of sculpins (Cottus perplexus) declined sharply with cold winter temperatures. Controls maintained at the same temperature had comparable efficiencies, suggesting that the low efficiencies were not due to the hormone injections (Table 3). The low temperature and super-abundance of food may have led to the fish expending energy through exercise to maintain a core body temperature slightly higher than the surrounding water. This expenditure of energy may have lowered the production efficiency of the trout.

The administration of bovine growth hormone did not affect the production efficiencies of the trout relative to controls but efficiencies were higher than those found at lower temperatures (17-59%). However, the absence of the hormone caused a decline in the production efficiencies in the small trout in the allometry study (Table.10).

Clearly, any adaptations that reduce respiration associated with a unit of production will increase production efficiency. The results suggest that increased respiration associated with behavioural activities can constrain production efficiency. Studies of behaviour may reveal potential attributes that could be modified to achieve this end.

Growth

Considering all the experiments, the results show that growth hormone injections produce fish 19-44% larger than controls over a 100 day period. The trend found in growth using the recombinant rainbow trout growth hormone (Fig. 7) has several possible explanations. The difficulty lies in explaining why the hormone had a deleterious effect on growth at high dosages. One possibility is that at high levels the natural growth hormone was recognized by, and elicited compensatory reactions from, system(s) that regulate growth hormone secretion and removal. Perhaps an inhibitory substance such as somatostatin (SRIF) was released in a manner which over-compensated for the injection of growth hormone. Alternatively, the extract of fish recombinant growth hormone was contaminated with a number of bacterial amino acids. It is possible that the immune system of the fish may have responded to these with an adverse impact on growth rate (Chen, personal communication). Chen et al. (unpublished) reported that fish at high dosage were morphologically modified and more firmly muscled than controls. Fish in all treatments appeared completely normal in the present study.

Density also affected growth. The effect of density alone showed that growth was inversely related to population density (Table 4). With the addition of bovine growth hormone this effect was obscured (Table 6). The effect of the hormone appeared to be to counteract the effect of increasing density. However, increasing dosages at each density, except for fish maintained individually, led to a decrease in growth rate relative to lower dosages (Table 6). In fact, in the two fish tanks the two highest dosage levels had deleterious effects on growth even when compared with controls.

There was an obvious increase in aggressiveness associated with increased hormone that may account for the deleterious effects on growth. Weatherley and Gill (1982) also noticed increased biting in rainbow trout treated with bovine growth hormone. The aggressiveness was so severe that they were forced to sacrifice the tank of fish. The confusion caused by high density may make it difficult for aggressors to focus on any one target. The data from the present study support this idea, for in the two fish tanks where aggression was most easily focused, the fish were more adversely affected by the hormone. In tanks containing four fish the deleterious effects were less severe. The large numbers of fish per tank found in aquaculture would diminish the effect of the aggressiveness. It is interesting that most studies do not site behavioural changes as part of their results. One study that did include behavioural changes (Weatherley and Gill, 1982) used low dosages of growth hormone (0.13 μg/g/2 weeks). Possibly the effect on behaviour is dosage dependent and can only be detected at low dosages. Perhaps the behaviour is affected to a greater extent than physiology at low concentrations. The resulting aggressive behaviour would have a deleterious effect on growth. Another possibility is that at high concentrations the growth hormone triggers one of the many complex controlling mechanisms which then block the behavioural effects.

The allometry study yielded some interesting results for the small trout. The growth of the high-dosage small fish exhibited a pronounced scallop when the injections ceased (Fig. 11). This same scallop was seen to a much lesser extent in the low dosage group (Fig. 11). These findings are obscured when the data from various size classes are lumped together (Fig. 13). This may be the reason that this phenomenon has not been reported

before. The drop in growth rate may have been due to the artificially high levels of growth hormone causing a suppression of endogenously produced growth hormone. Once the exogenous source was removed a deficit of hormone followed. The fact that the fish did not recover for four weeks (Fig. 11) suggests that the high levels of bovine growth hormone not only inhibited the release of the endogenously produced hormone but also suppressed its synthesis. If only the release of hormone were affected a more rapid recovery would be expected. Higgs et al. (1976) found that bovine growth hormone had a negative feedback effect on the pituitary somatotropins of coho salmon. They found that the treated fish had decreased mitotic activity in the pituitary indicating reduction in the synthesis of endogenously produced growth hormone. Somatostatin (SRIF) may play a role in this phenomenon. Peptides identical to the tetra decapeptide SRIF14 have been isolated from angler fish and similar peptides have been found in teleosts (Reviewed by Gomez-Pan and Rodrigiez-Arnao, 1983). Synthetic SRIF has been found to reduce the plasma GH levels in gold fish (Carassius auratus) when measured by a homologous carp radio immunoassay (Cook et al., 1983; Cook and Peter, 1984). Synthetic SRIF was also found to inhibit synthesis and release of growth hormone from Poecilia latippina pituitary in vitro (Wigham and Batten, 1984; Batten and Wigham, 1984).

The reaction to the absence of bovine growth hormone in the small trout may be tied to some maturational event. Data supporting this view are that all fish which displayed the reaction were under 100 g when the injections were discontinued and all those above this weight were not adversely affected. Perhaps the SRIF systems of the adults are more developed and capable of adapting rapidly to varying amounts of growth hormone, thus

eliminating the lag period (Hall et al., 1986). In ducklings, for example, infusion of SRIF caused the plasma levels of growth hormone to fall and then there was a rebound after the infusion was discontinued. No such reaction is seen in adult ducks (Harvey, 1983; Strosser et al., 1984, 1985).

Another possible explanation of the above results is that there may exist a set point (concentration) of growth hormone that triggers the inhibitory response. Faster-growing children commonly have higher plasma levels of growth hormone than adults (Donovan, 1970). It is possible that although the injections were adjusted to a per g basis, the concentration of hormone when added to the endogenous levels was sufficient to trigger the inhibitory response in the small, but not the large trout.

In large trout the effect of discontinuing injections was unremarkable. Both the high and low treatments sustained high growth rates that eventually returned to control levels. However the size advantage gained due to the growth hormone was maintained (Fig. 12).

As the trout mature and become larger their growth rate becomes proportionally smaller (Medawar, 1945; Cooper, 1953; Needham, 1964). The controls exhibited a drop in their log basal coefficient of growth with increasing weight (Fig. 8). However this trend was less pronounced in the low dosage group and there was no decrease in the high dosage group (Figs. 9 and 10). What appears to be happening is that the growth rate is being pushed against a "ceiling". The value of this maximum growth rate converges onto that of the infantile growth rate. Interestingly, a mathematical model of the growth process also predicted that the juvenile growth rate would be the species' maximum (i.e. it would be refractory to growth hormone). Later stages were suggested to grow sub-maximally and could thus be adjusted by

hormone (Sibly et al., 1985). These findings suggest that interspecific comparisons of growth would be best be accomplished by comparing juvenile rates. Under the influence of the growth hormone the growth of the fish scales in direct proportion to their body weight (Table 9). The regression lines were not statistically significantly different, however, an obvious trend emerged. The addition of the hormone straightened the intraspecific line.

IMPLICATIONS, INTEGRATION AND CONCLUSIONS

This study provides considerable insight into the possible effects of inserting extra copies of growth hormone genes into the genome of rainbow trout. Hopefully it will stand as an example to direct further ecological/physiological research in this new field. The molecular biologist and ecologist must work in concert because the variability introduced at the genetic level has emergent properties that are only obvious at the ecological level. The field of genetic engineering requires that the problems be solved from both a reductionist and holistic viewpoint.

The study clearly demonstrated that the addition of working copies of growth hormone genes to the genome of rainbow trout would lead to an increased growth rate. The study found increases of up to 19% with the recombinant hormone and 44% with the bovine growth hormone over 100 days. This requires increased food intake. There was no accompanying change in relative respiration, assimilation efficiency or gross and net production efficiency. This decreases the time required for fry to reach market size, which allows the aquaculturist to increase productivity.

The effect of cold (5°C) was found to be detrimental, not only to growth rate, but also to assimilation and conversion efficiency. These effects were due to a drop in consumption and production efficiency with a simultaneous increase in the relative respiration rate in cold water. These results underscore the importance of water temperature to cost effective aquaculture.

Surprisingly, bovine growth hormone was more effective than natural growth hormone in promoting faster growth (Tables 3 & 10). In support of this, a study conducted on coho salmon (O. kisutch) found that bovine growth hormone was more potent and economical than a pituitary extract from chinook salmon (O. tsawytscha) in promoting growth (Higgs et al., 1978). Possibly the different conformation of bovine growth hormone relative to that of the rainbow trout is the cause of its greater potency. The different shape of the hormone might inhibit its release from the binding site and/or its degradation. This same argument could explain why bovine growth hormone exhibits no inhibitory effects at high dosages compared to recombinant hormone. The different conformation may not be recognized by the systems that control growth hormone secretion and therefore the compensatory reaction is not triggered. Therefore, it may be more effective to insert copies of the bovine growth hormone gene into the genome of trout to increase growth. Such alien proteins may circumvent normal controls and act as a molecular super-normal stimuli. This result is contrary to most current work which stresses to use the species' own genes for insertion.

The use of growth hormone altered the manner in which growth scales intraspecifically. The normal slope of 0.66 was nowhere to be seen as larger fish grew like juveniles. The fact that it is possible to manipulate

the scaling of growth with such ease suggests that there exists some variability in this attribute. That means that the way in which growth scales intraspecifically is submaximal. It is likely a selected attribute that is not simply maximized within the constraints of physical and/or chemical factors. However, the omnipresence of the scaling factor (eg. 0.66) suggests that the forces selecting it are quite strong and pervasive. The effect of the hormone was to "straighten" the normally descending curve. However, the entire line itself was not shifted upwards as occurs in interspecific comparisons (Fig. 14). Perhaps to achieve a phylogenetic change the juvenile growth rate must be altered. To achieve such a jump in the metabolic power of the system evidently requires more than a change in growth hormone secretion. Such a shift may be a requirement for speciation and appears to be analogous to the interspecific "jump" discussed by Wieser (1984) that entails that animals not only grow larger but also operate at a proportionally higher metabolic rate.

Weatherley and Gill (1987) noticed that the effectiveness of growth hormone in promoting growth of fishes was directly related to the fish's ability to recruit new muscle fibers. Perhaps the fish on which the hormone has limited effect on are at or near the earlier described ceiling. For such an animal, improved growth would require a more fundamental phylogenetic change. The ability to recruit new muscle fibers could be a useful indicator of growth potential to genetic engineers interested in food technology.

The ecological study of organism design and genetic engineering are very complimentary. Genetic engineering allows one to examine organisms operating at physiological extremes not naturally available. Pushing these

limits should enable researchers to gain new insights into the principals that govern organism design and natural evolution. Such rules in turn should help identify problems or potential avenues for genetic engineering. Natural selection has acted to adapt organisms to their natural environment. Environmental constraints often require large resource expenditures to overcome. The controlled and stable conditions used in aquaculture makes many of these adaptations useless or even counter productive. Perhaps genetic engineers can make cultured organisms better adapted to their new, artificial environment. With the use of new technology organisms can be changed in a directed manner to reallocate resources into the desired attribute (i.e. away from reproduction and into growth).

An understanding of organism design, including life history, could be invaluable to the genetic engineer. Since the genome is a holistically integrated entity, changing any one component can have a profound effect on the entire organism. Some evidence in support of this comes from Stearns and Crandall (1984) who assert that growth rate acts as a cue that fish use to target a whole range of ages and sizes for maturation that will maximize reproductive success. There is good evidence of just such effects. In trout treated with high levels of growth hormone, extremely high levels of testosterone were induced, leading to very early maturation of male fish (Chen, personal communication). The above theory suggests that the population of fish have encountered different environmental constraints on growth rate in their evolutionary history and these impose varying ages and/or sizes at maturity that would be advantageous. The organism consequently is pre-programmed to follow a particular plastic developmental trajectory based on its growth rate.

This selection at the level of multi-generational genetic templates, rather than at an individual level, has profound implications for the field of genetic engineering. Changing one attribute could very well induce the organism to express a previously inactive portion of its genome. An example of such "sleeping" programs is seen in Daphnia which only develops protective spikes in response to chemicals produced by a predator. This adaptive response can be elicited even after remaining dormant for several generations (Hebert and Grewe, 1985). Gould (1977) cites many examples of organisms that express unusual maturational phenotypes under hormonal inducement. Some salamanders that do not normally transform, for example, still contain the code for expressing ancient adult features and do so following hormonal inducement. In the present study, changing the growth rate with growth hormone could alter both the age and size at which the fish reach maturity. Changes such as these are aquaculturally very important since there are at least four basic types of template predicted (Stearns and Crandall, 1984).

Despite the new technology, time-tested methods should not necessarily be abandoned. Traditional artificial selection may be usefully applied to "tune" altered fish to ensure that the genome is holistically integrated and functional. This might be one way to ensure that feeding is not constraining potential growth, for example.

Genetic engineering dramatically changes the fundamental process of evolution. It marks the beginning of the use of a non-random mutational device, which means that evolution has essentially "become self-aware". However, without the ecological knowledge of the rules constraining holistic organism design, no advantage is gained over natural or artificial

selection. Therefore, the process remains one that is blind. To take maximum advantage of the potential of this new technology requires linking molecular techniques to ecological vision.

Table 1

SPECIES	GROWTH	DOSAGE (ug/g/week)	PROTIEN SYNTH.	CONV. EFF.	INCREASED CONSUMP.	REFERENCE
Salvelinus fontinalis	Y	c.p.e.			Y	Cantilo & Regalado, 1942
Salmo salar	Y	3.5			Y	Komourdjian et al., 1976
Salmo gairdneri	Y	15				Chartier-Baraduc, 1959
Salmo gairdneri	Y		Y			Enomoto, 1964
Salmo gairdneri	Y	0.07				Weatherley & Gill, 1982
Salmo trutta	Y	10				Swift, 1954
Oncorhynchus kisutch	Y	[10 & 30]	Y			Higgs et al., 1976
Oncorhynchus kisutch	Y	[10 & 100]	Y			Higgs et al., 1975
Oncorhynchus kisutch	Y	10	I	Y	Y	Markert et al, 1977
Oncorhynchus kisutch	Y	[1 & 5]*		Y		Gill et al., 1985
Oncorhynchus nerka	Y					Clark, 1976
Ictalurus melas	Y	3.5	Y			Kayes, 1977a
Fundulus	Y	30				Pickford, 1959
Esox americanus verniculatus	Y					Weatherley & Gill, 1987b
Lepomis macrochirus	N					Weatherley & Gill, unpublished
Pimephales notatus Rafinesque	N					Weatherley & Gill, 1987a
Ryinchthus cataractae	N					Weatherley & Gill, 1987a
Cyprinus carpio	Y					Adelman, 1977
Poecilia formosa	Y					Ball, 1969
Poecilia latipinna	Y					Ball, 1969
Chelydra Serpentina	Y	[17.5-175]	Y	Y	Y	Brown et al., 1974
Pseudomys script elegans	Y	[17.5-175]	Y	Y	Y	Brown et al., 1974
Bufo boreas	Y	[0.1-17.5]			Y	Zipser et al., 1969
Bufo marinus	Y	[0.1-17.5]			Y	Zipser et al., 1969
Rana pipens(post metamorphic)	Y	[35-700]				Snyder & Frye, 1971
Rana pipens(larval)	Y	[700]**				Snyder & Frye, 1971
Anolis carolinisis	Y	7			Y	Licht & Hoyer, 1967
chickens	Y			Y		Myers & Peterson, 1974
weanling rats	Y				Y	Wade, 1974
pig	Y					Machlin, 1972
quinea pig	N					Knobil et al., 1969

Y = an observed increase

I = an observed decrease

c.p.e. = crude pituitary extract

* = used natural and recombinant hormone

** = only prolactin would work

Table 2

Effect of Recombinant Rainbow Trout Growth Hormone
On Growth Of Rainbow Trout

Dosage [ug/10g body weight/week]	Growth Rate [log. g/day]		N** [fish/ treatment]
	Mean	S.D.	
0.00	.0093 AB*	.0044	12
.01	.0107 AB	.0045	12
.10	.0115 A	.0024	12
.50	.0094 AB	.0026	12
1.00	.0073 CB	.0026	12
5.00	.0055 C	.0042	12

* values with the same letter were not distinquished according to a Student-Newman-Keuls test

** a density of six fish /tank was maintained

TEMPERATURE = 5 C

Table 3

Dry-Mass Budget on Rainbow Trout Following a Series of Injections With Recombinant Rainbow Trout Growth Hormone

	DOSAGE [ug/10g body wt./week]					
	0		.01		.1	
YIELD VARIABLE	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
CONSUMPTION**	1.038	.547	1.322	*-	1.309	.023
FAECES	.231	.106	.287	-	.274	.021
GROWTH	.002	.039	.025	-	.021	.009
RESPIRATION	.805	.402	1.010	-	1.014	.195
AV. DRY. WT.	31.590	5.120	38.200	-	37.460	.269
A.E. (%)	77.300	1.711	78.290	-	78.890	1.980
G.P.E. (%)	0.000	0.000	1.920	-	1.540	.396
N.P.E. (%)	0.000	0.000	2.460	-	1.950	.453

	DOSAGE [ug/10g body wt./week]					
	.5		1		5	
YIELD VARIABLE	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
CONSUMPTION**	.880	.345	.785	.629	1.034	.371
FAECES	.225	.067	.182	.135	.215	.055
GROWTH	-.001	.022	.005	.014	.023	.026
RESPIRATION	.656	.256	.598	.479	.796	.289
AV. DRY. WT.	28.100	2.687	43.915	23.002	25.675	6.838
A.E. (%)	73.985	2.581	76.085	2.029	78.790	2.235
G.P.E. (%)	0.000	0.000	0.000	0.000	1.850	1.895
N.P.E. (%)	0.000	0.000	0.000	0.000	2.310	2.334

* one tank of fish was lost making calculation of standard deviation impossible
** all rates were measured as dry weight/g dry fish/week
TEMPERATURE = 5 C
DENSITY = 6 FISH/TANK

all efficiencies are averages of several tanks

Table 4

The Effect of Density On the Dry-Mass Budget of Rainbow Trout Prior to Treatment with Bovine Growth Hormone

	DENSITY (fish/tank)					
	1		2		4	
YIELD VARIABLE	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
CONSUMPTION*	.498	.046	.469	.068	.454	.088
FAECES	.107	.014	.100	.015	.094	.018
GROWTH	.112	.021	.089	.030	.082	.035
RESPIRATION	.279	.032	.280	.042	.278	.056
AV. DRY WT.	44.245	6.443	36.826	5.750	48.560	16.576
A.E. (%)	78.539	1.650	78.765	1.278	79.326	.897
G.P.E. (%)	22.536	3.564	18.830	5.397	17.976	5.753
N.P.E. (%)	28.728	4.727	23.789	6.706	22.630	7.116

* all rates were measured as dry weight/g dry fish/week

Temperature = 11.5 C, all treatments contained 8 replicates

Table 5.

Overall Effects of Density and Dosage of Bovine Growth Hormone On the Growth of Rainbow Trout

Overall Density Effects

DENSITY [fish/ tank]	GROWTH RATE [log. g/day for a 1g fish]		N [fish/ treatment]
	MEAN	S.D.	
1	-4.281	.205	8
2	-4.385	.158	16
4	-4.262	.078	32

Overall Dosage Effects

DOSAGE [ug/10g body wt./week]	GROWTH RATE [log. g/day for a 1g fish]		N [fish/ treatment]
	MEAN	S.D.	
0	-4.317	.113	14
.001	-4.309	.193	14
.01	-4.275	.157	14
.1	-4.335	.198	14

TEMPERATURE = 17 C

Table 6.

Effects of Density and Bovine Growth Hormone
On the Growth of Rainbow Trout

DENSITY [fish/ tank]	DOSAGE [ug/10g body weight/week]	GROWTH RATE [log. g/day for a 1g fish]		N [fish/ treatment]
		MEAN	S.D.	
1	0.000	-4.305	.168	2
1	.001	-4.499	.262	2
1	.010	-4.160	.163	2
1	.100	-4.161	.125	2
2	0.000	-4.297	.178	4
2	.001	-4.259	.049	4
2	.010	-4.420	.158	4
2	.100	-4.565	.026	4
4	0.000	-4.349	.015	8
4	.001	-4.171	.023	8
4	.010	-4.247	.049	8
4	.100	-4.281	.083	8

TEMPERATURE = 17 C

Table 7.

The Effects of Density and Bovine Growth Hormone On the Dry-Mass Budget of Rainbow Trout

DENSITY = 1 (fish/tank)

DOSAGE [uq/10q body wt./week]

YIELD VARIABLE	0 MEAN	0 S.D.	.001 MEAN	.001 S.D.	.01 MEAN	.01 S.D.	.1 MEAN	.1 S.D.	OVERALL MEAN
CONSUMPTION**	.516	.064	.449	.016	.601	.011	.511	.042	.519
FAECES	.102	.011	.086	.007	.107	.004	.095	.001	.098
GROWTH	.137	.047	.118	.002	.187	.069	.118	.075	.140
RESPIRATION	.277	.100	.245	.007	.307	.052	.298	.033	.282
AV. DRY WT.	95.795	20.329	86.818	13.120	96.337	12.492	95.798	7.003	93.687
A.E. (%)	80.145	.276	80.885	.884	82.190	1.061	81.335	1.351	81.139
G.P.E. (%)	27.275	12.594	26.210	.509	31.005	10.826	22.610	12.756	26.775
N.P.E. (%)	34.065	15.832	32.400	.269	37.645	12.693	27.675	15.224	32.946

DENSITY = 2 (fish/tank)

DOSAGE [uq/10q body wt./week]

YIELD VARIABLE	0 MEAN	0 S.D.	.001 MEAN	.001 S.D.	.01 MEAN	.01 S.D.	.1 MEAN	.1 S.D.	OVERALL MEAN
CONSUMPTION**	.570	.079	.485	.060	.536	.004	.519	.069	.527
FAECES	.104	.001	.088	.005	.095	.001	.092	.006	.095
GROWTH	.136	.011	.159	.078	.137	.050	.132	.090	.141
RESPIRATION	.330	.069	.238	.023	.304	.054	.295	.025	.292
AV. DRY WT.	68.726	7.120	88.073	9.938	60.742	3.778	64.108	4.882	70.412
A.E. (%)	81.515	2.850	81.890	1.188	82.320	.057	82.155	1.237	81.970
G.P.E. (%)	24.020	1.344	31.915	12.141	25.565	9.539	24.320	13.888	26.455
N.P.E. (%)	29.515	2.680	38.875	14.262	31.050	11.611	29.480	16.461	32.230

density = 4 (FISH/TANK)

DOSAGE [uq/10q body wt./week]

YIELD VARIABLE	0 MEAN	0 S.D.	.001 MEAN	.001 S.D.	.01 MEAN	.01 S.D.	.1 MEAN	.1 S.D.	OVERALL MEAN
CONSUMPTION**	.511	.064	.390	.083	.513	.012	.465	.060	.469
FAECES	.091	.001	.074	.011	.082	.004	.094	.004	.085
GROWTH	.164	.109	.185	.008	.126	.025	.107	.079	.145
RESPIRATION	.256	.047	.131	.064	.305	.017	.264	.043	.239
AV. DRY WT.	67.210	8.074	82.632	12.951	87.554	3.117	94.260	10.790	82.914
A.E. (%)	82.150	2.022	80.975	1.223	84.030	.481	79.540	3.507	81.674
G.P.E. (%)	31.090	17.409	48.385	8.139	24.510	4.271	24.295	20.146	32.070
N.P.E. (%)	37.590	20.266	59.835	10.953	29.185	5.254	31.000	26.700	39.403

** all rates were measured as dry weight/q dry fish/week

TEMPERATURE = 17.5 C

Table 8.

Effect of Bovine Growth Hormone On the Allometry of Growth in Rainbow Trout

DOSAGE [ug/g body weight/week]	GROWTH RATE [log. g/day for a 1g fish] MEAN	S.D.	N** [fish/ treatment]
0	-4.232 A*	.292	7
.1	-4.015 AB	.189	7
1	-3.896 B	.209	7

* values with the same letter were not significantly different according to the Student-Newman-Keuls test

** tanks were maintained at a density of one fish/tank

TEMPERATURE = 11.5 C

Table 9.

Effect of Bovine Growth Hormone On the Scaling of Growth

DOSAGE [ug/g body wt./week]	SLOPE [b]	PROBABILITY	r
0.0	0.86	}0.05	0.35
0.1	0.89	}0.05	0.51
1.0	1.07	}0.05	0.03

TEMPERATURE = 11.5 C

DENSITY = 1 fish/tank

Table 10.

Effects of Bovine Growth Hormone On the Dry-Mass Budget of Rainbow Trout

FISH SMALLER THAN 100 g	DOSAGE [ug/g body wt./week]					
	0		.1		1	
YEILD VARIABLE	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
CONSUMPTION*	.434	.165	.444	.223	.282	.172
FAECES	.090	.034	.106	.070	.054	.052
GROWTH	.109	.031	.077	.087	.060	.107
RESPIRATION	.235	.113	.261	.080	.168	.037
AV. DRY WT.	17.947	9.103	16.696	9.041	14.214	7.199
A.E. (%)	79.230	.747	77.155	3.789	81.220	12.811
G.P.E. (%)	25.670	3.809	13.368	14.869	11.143	23.446
N.P.E. (%)	32.385	4.682	17.543	19.274	13.080	29.948

FISH LARGER THAN 100 g	DOSAGE [ug/g body wt./week]					
	0		.1		1	
YEILD VARIABLE	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
CONSUMPTION*	.451	.035	.397	.087	.589	.125
FAECES	.100	.012	.080	.022	.116	.031
GROWTH	.098	.041	.092	.034	.128	.040
RESPIRATION	.253	.020	.225	.034	.345	.060
AV. DRY WT.	45.750	22.222	59.803	16.851	66.055	6.755
A.E. (%)	77.880	1.578	80.050	1.494	80.403	1.780
G.P.E. (%)	21.540	8.137	22.663	3.516	21.480	2.685
N.P.E. (%)	27.580	10.005	28.327	4.548	26.713	3.280

* all rates were measured as dry weight/fish/g dry body wt./week

DENSITY = 1 fish/tank

N = 4 replicates in the small fish and
3 replicates in the large fish

TEMPERATURE = 11.5 C

Table 11.

The Effect of Temperature On Assimilation Efficiency

TEMPERATURE [deg. C]	ASSIMILATION EFFICIENCY MEAN [%]	S.D.	N [fish/ treatment
5	76.27	5.81	2
11.5	78.84	1.29	26
17	81.27	1.82	6

FIGURE 1.

SECRETION OF GROWTH HORMONE

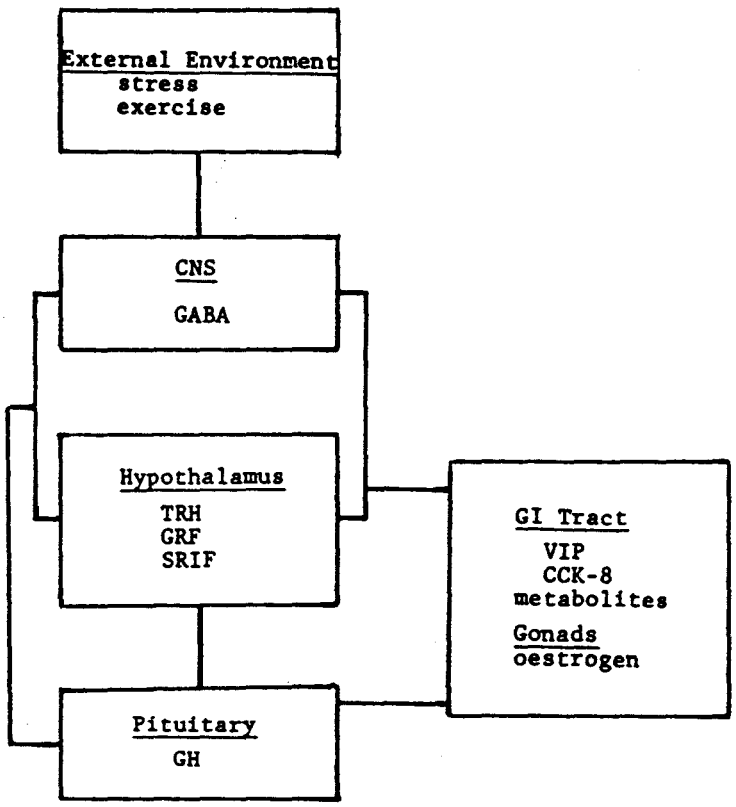

External Environment
stress
exercise
CNS
GABA
Hypothalamus
TRH
GRF
SRIF
Pituitary
GH
GI Tract
VIP
CCK-8
metabolites
Gonads
oestrogen

FIGURE 2.

WATER TREATMENT SYSTEM AND REARING TANKS

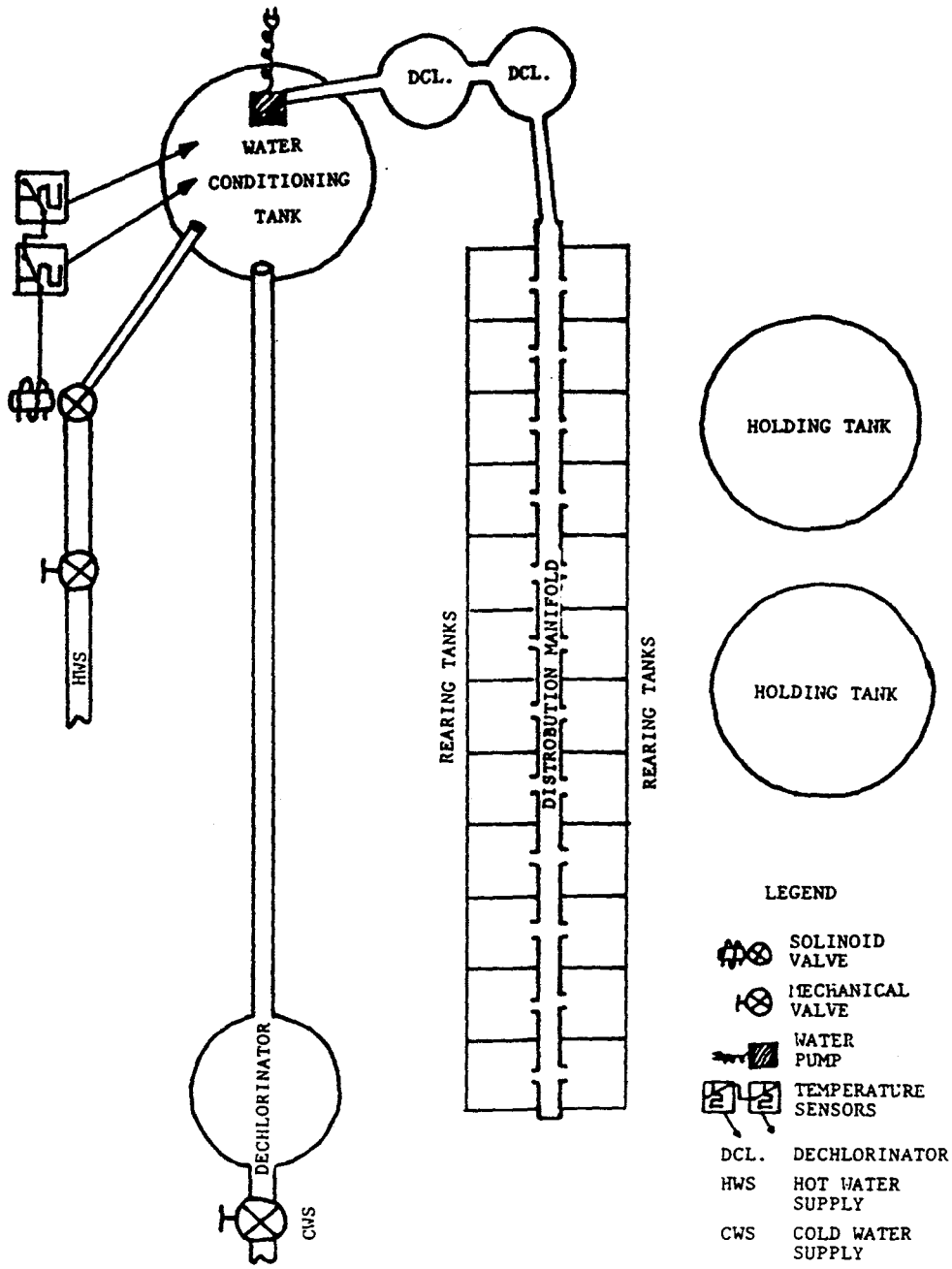

DCL.
DCL.
WATER
CONDITIONING
TANK
HWS
REARING TANKS
DISTROBUTION MANIFOLD
REARING TANKS
HOLDING TANK
HOLDING TANK
DECHLORINATOR
CWS
LEGEND
SOLINOID VALVE
MECHANICAL VALVE
WATER PUMP
TEMPERATURE SENSORS
DCL. DECHLORINATOR
HWS HOT WATER SUPPLY
CWS COLD WATER SUPPLY

FIGURE 3.

REARING TANK

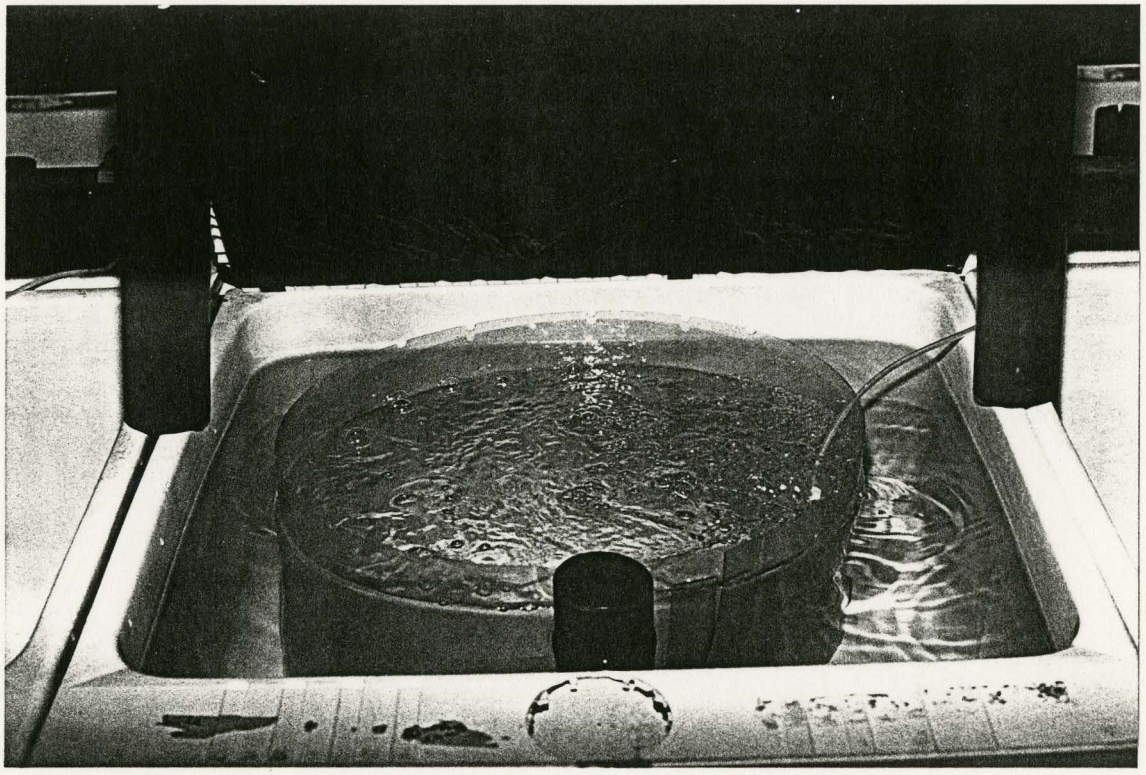


FIGURE 4.

FISH HOLDER

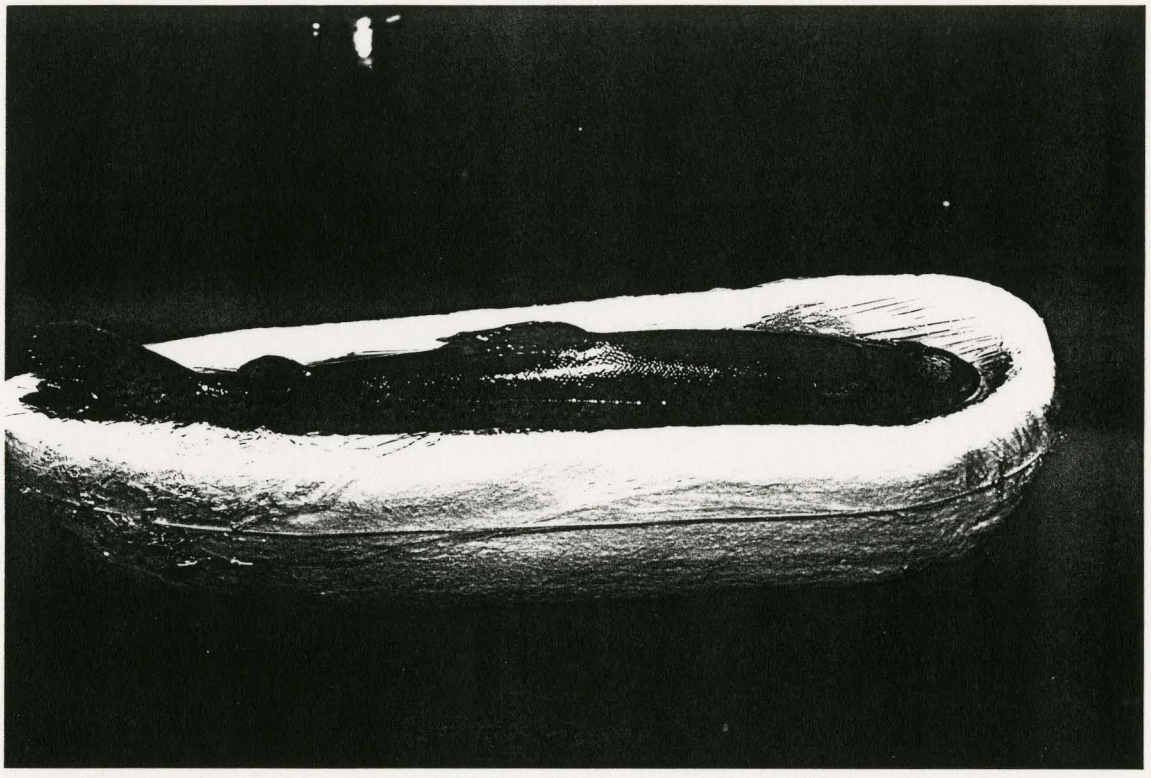


FIGURE 5.

RESOURCE ALLOCATION AND DRY-MASS BUDGETING

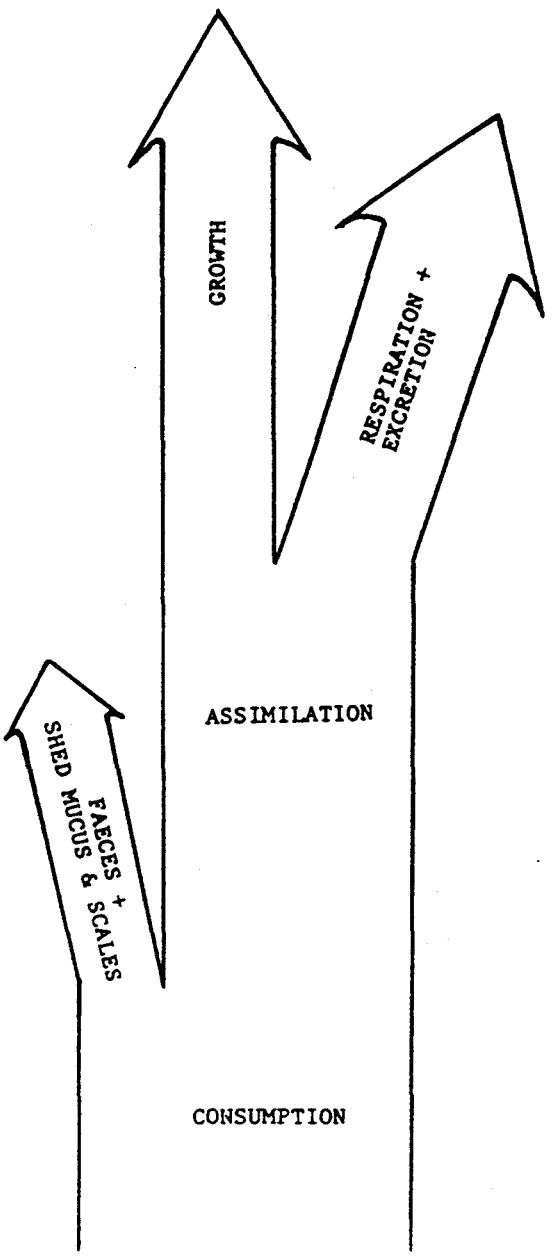

GROWTH
RESPIRATION + EXCRETION
ASSIMILATION
FAECES + SHED MUCUS & SCALES
CONSUMPTION

FIGURE 6.

ANALYSIS OF DATA

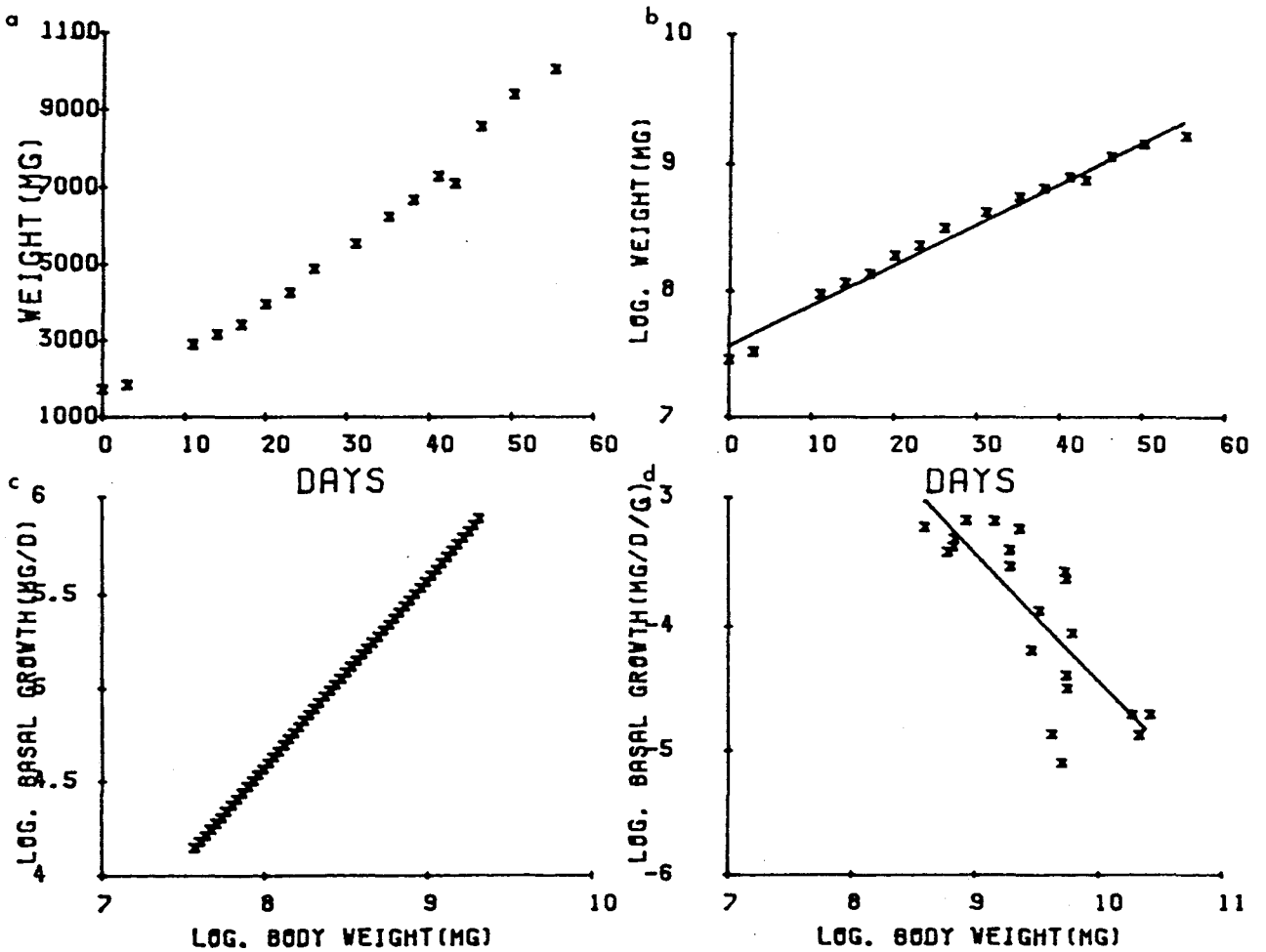

a
WEIGHT (MG)
1100
9000
7000
5000
3000
1000
0
10
20
30
40
50
60
DAYS
b
LOG. WEIGHT(MG)
10
9
8
7
0
10
20
30
40
50
60
DAYS
c
LOG. BASAL GROWTH(MG/D)
6
.5
.5
4
7
8
9
10
LOG. BODY WEIGHT(MG)
d
LOG. BASAL GROWTH(MG/D/G)
3
4
5
6
7
8
9
10
11
LOG. BODY WEIGHT(MG)

FIGURE 7.

EFFECT OF RECOMBINANT RAINBOW TROUT GROWTH HORMONE

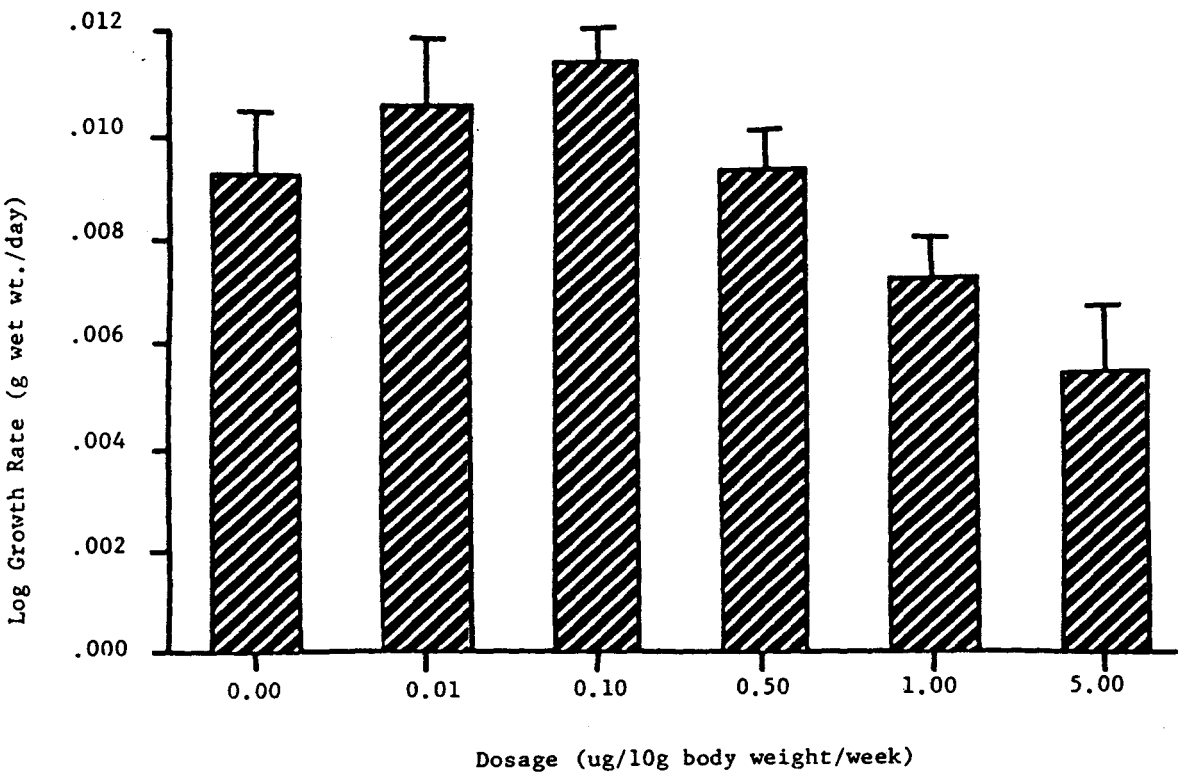

.012
.010
.008
.006
.004
.002
.000
Log Growth Rate (g wet wt./day)
0.00
0.01
0.10
0.50
1.00
5.00
Dosage (ug/10g body weight/week)

FIGURE 8.

ALLOMETRY IN GROWTH RATES OF CONTROL TROUT

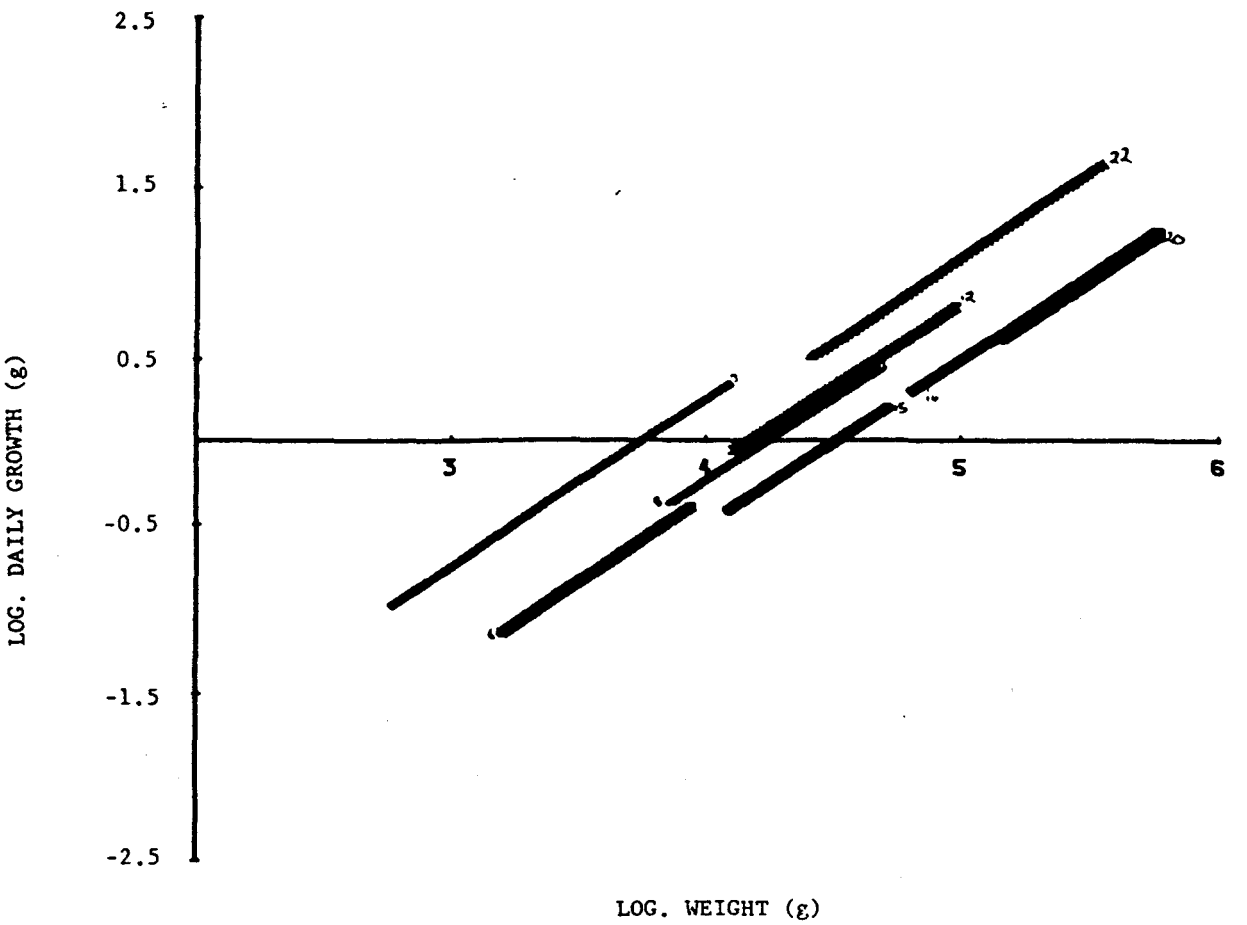

2.5
1.5
0.5
-0.5
-1.5
-2.5
3
4
5
6
LOG. DAILY GROWTH (g)
LOG. WEIGHT (g)

FIGURE 9.

ALLOMETRY IN GROWTH RATES IN TROUT RECEIVING LOW DOSAGE OF GROWTH HORMONE

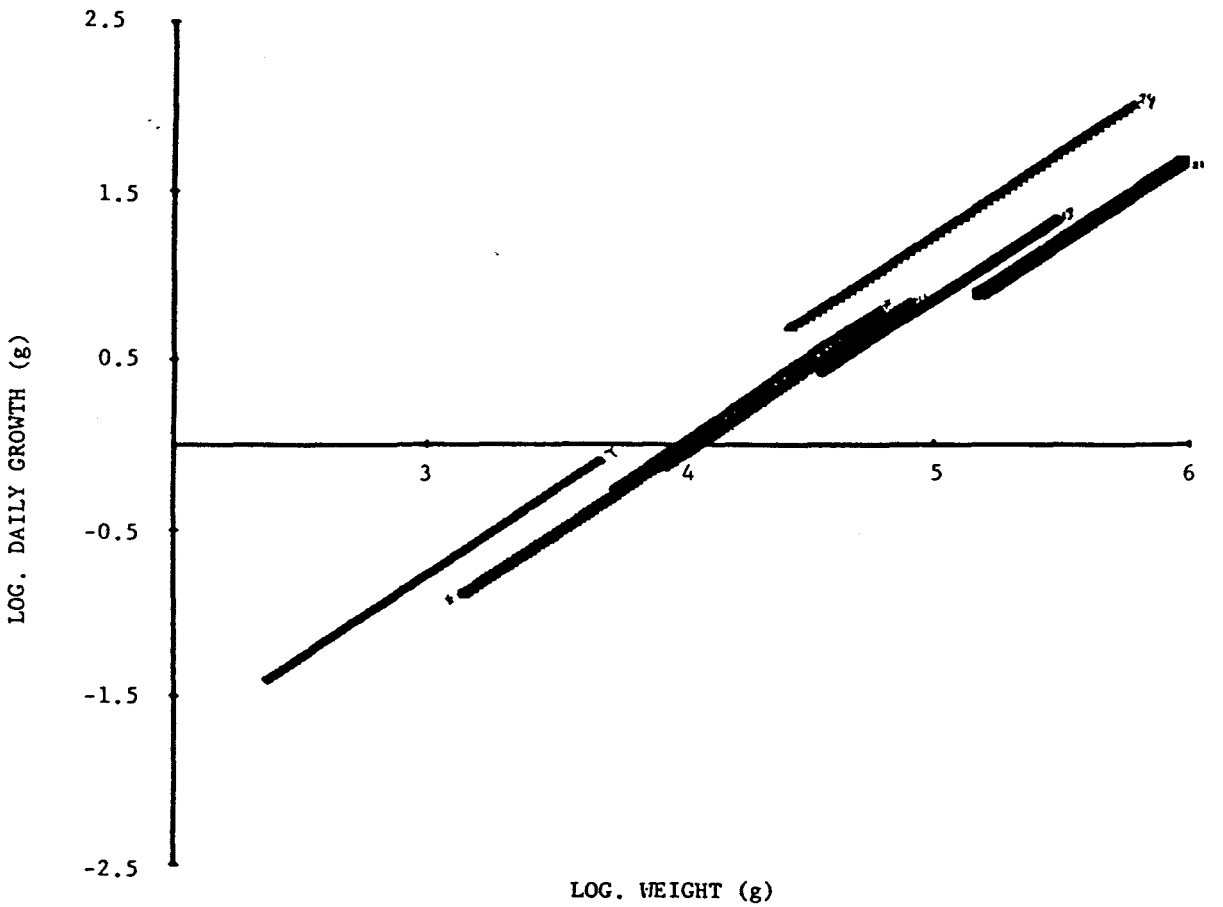

2.5
1.5
0.5
-0.5
-1.5
-2.5
3
4
5
6
LOG. DAILY GROWTH (g)
LOG. WEIGHT (g)

FIGURE 10.

ALLOMETRY IN GROWTH RATES IN TROUT RECEIVING HIGH DOSAGE OF GROWTH HORMONE

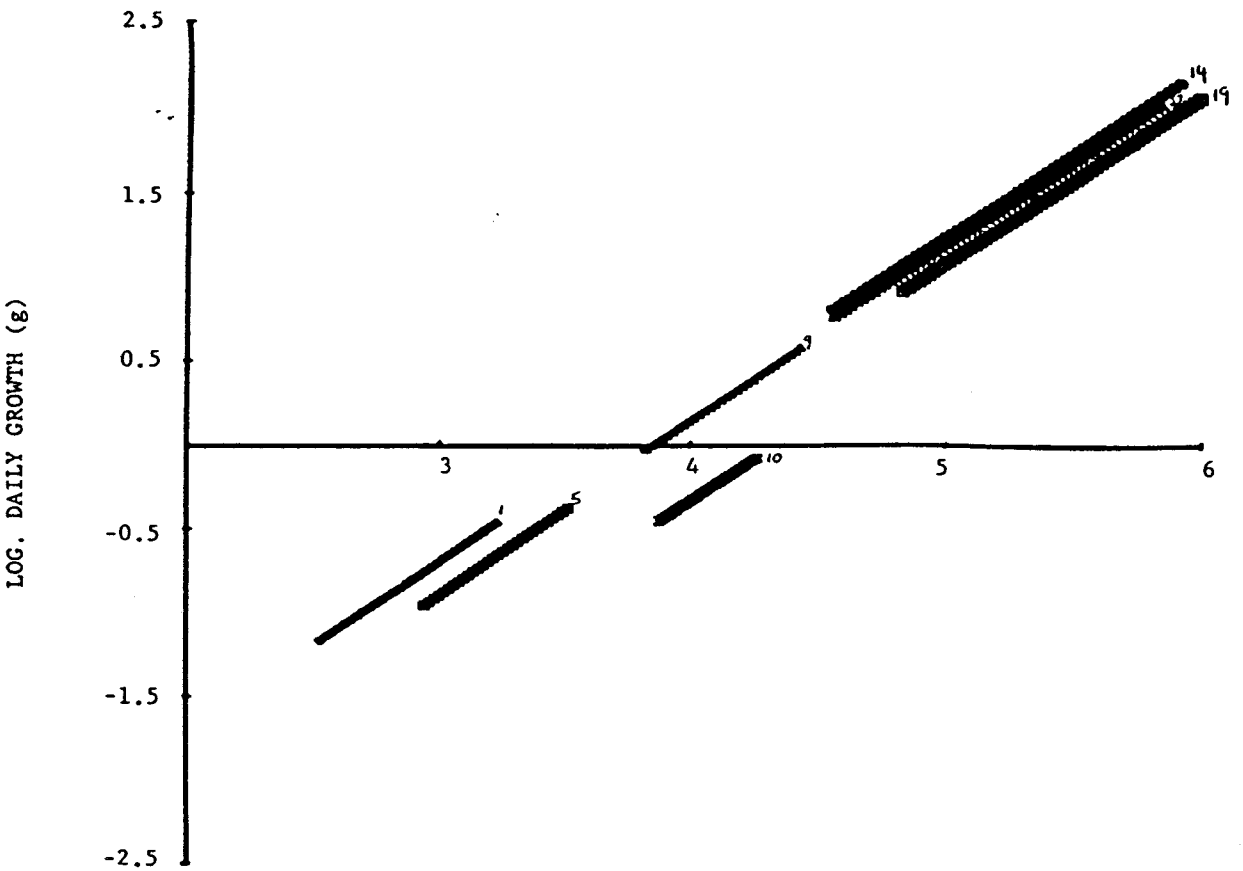
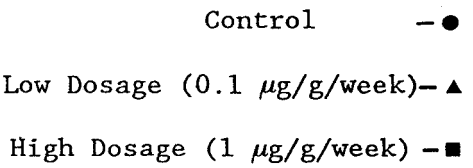

2.5
1.5
0.5
-0.5
-1.5
-2.5
3
4
5
6
LOG. DAILY GROWTH (g)
LOG. WEIGHT (g)

FIGURE 11.

EFFECT OF BOVINE GROWTH HORMONE ON "SMALL" RAINBOW TROUT



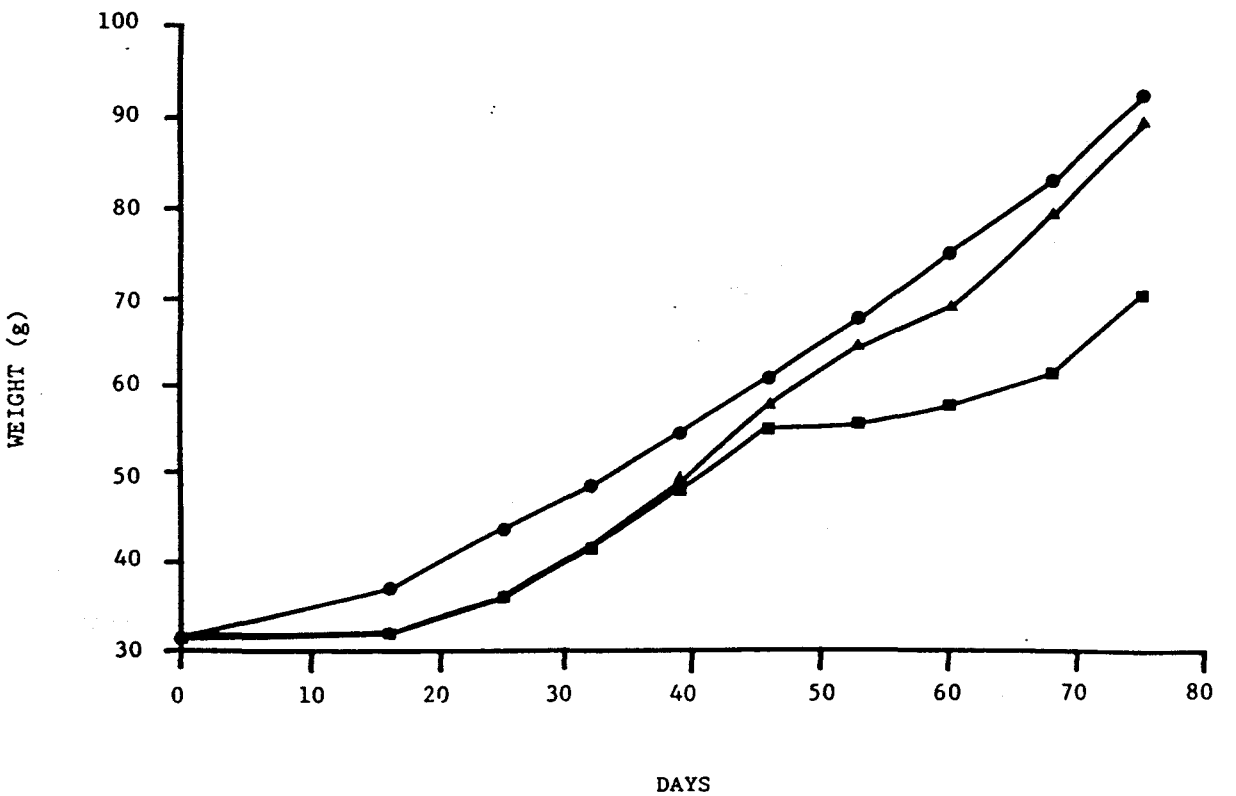
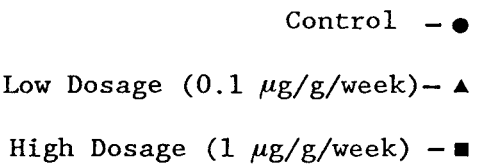

100
90
80
70
60
50
40
30
WEIGHT (g)
0
10
20
30
40
50
60
70
80
DAYS

FIGURE 12.

EFFECT OF BOVINE GROWTH HORMONE ON "LARGE" RAINBOW TROUT



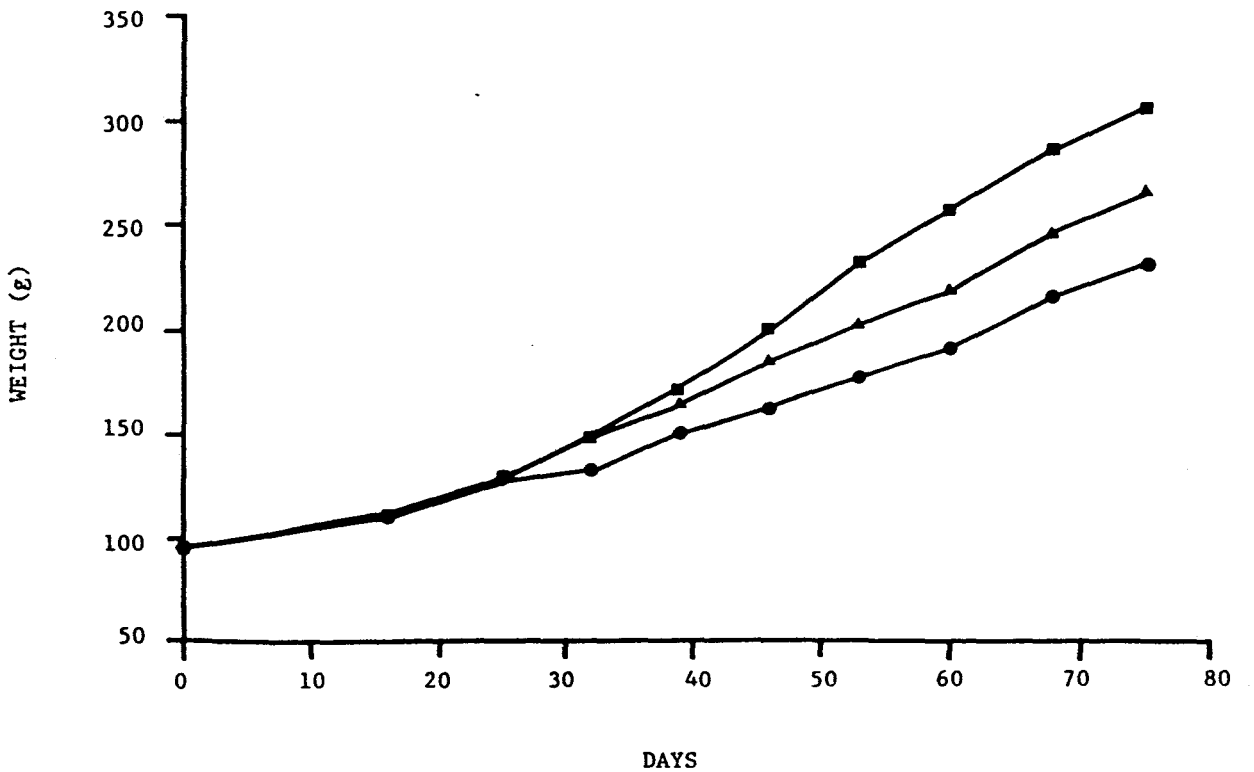

350
300
250
200
150
100
50
WEIGHT (g)
0
10
20
30
40
50
60
70
80
DAYS

FIGURE 13.

EFFECT OF BOVINE GROWTH HORMONE ON RAINBOW TROUT

Control – ●

Low Dosage (0.1 μg/g/week)– ▲

High Dosage (1 μg/g/week) – ■

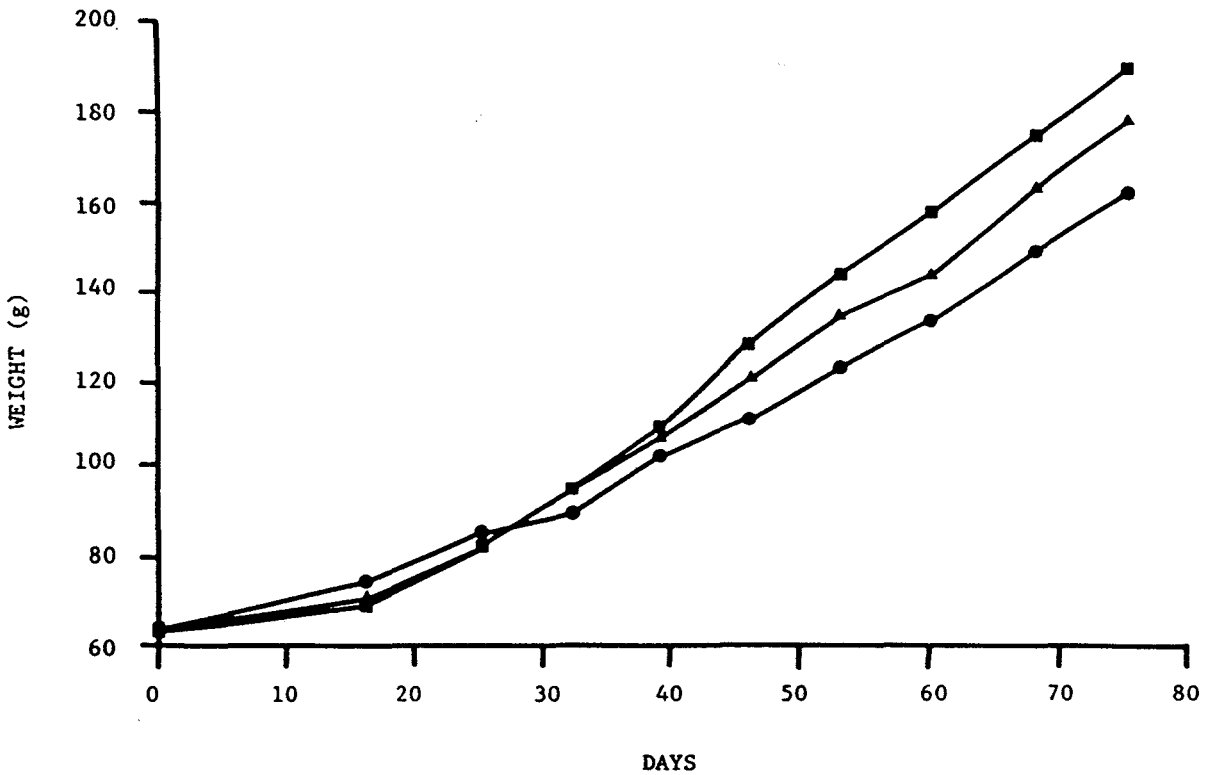
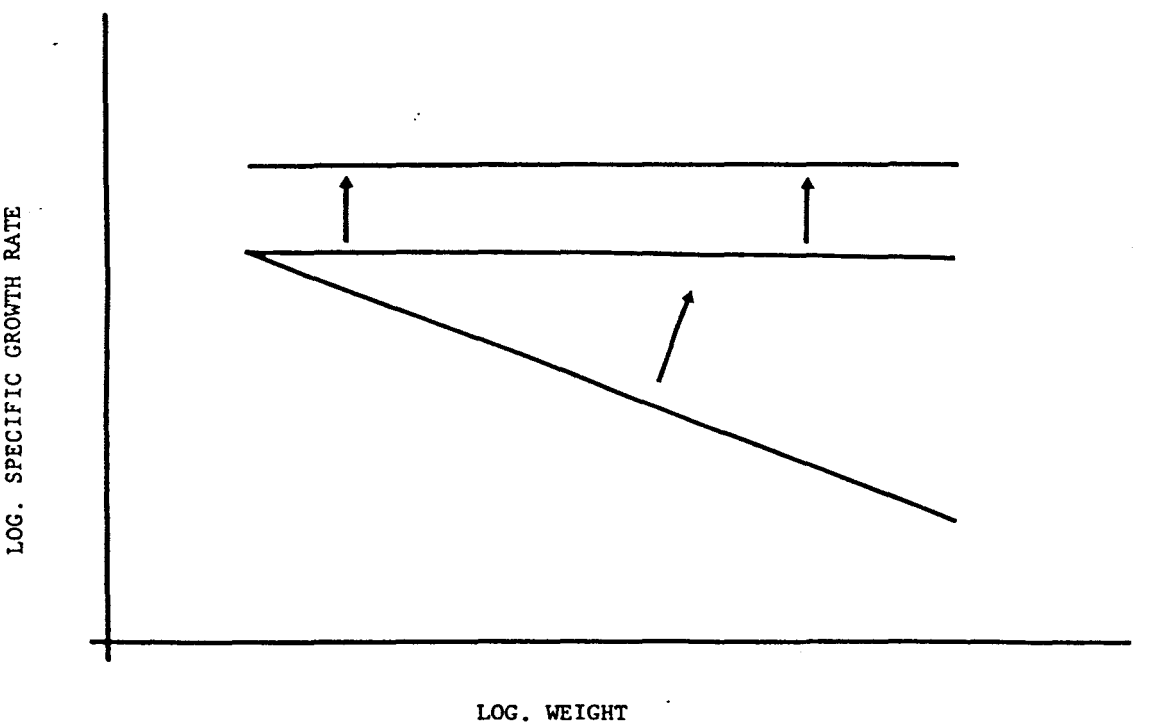

200
180
160
140
120
100
80
60
WEIGHT (g)
0
10
20
30
40
50
60
70
80
DAYS

FIGURE 14.

ALLOMETRIC CHANGES IN GROWTH RATES


LOG. SPECIFIC GROWTH RATE
LOG. WEIGHT

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