EFFECTS OF REARING DENSITY ON STRESS RESPONSE IN TANK-REARED JUVENILE STEELHEAD TROUT, (*SALMO GAIRDNERI*)

by

KEVIN KENNETH LOFTUS

B.Sc., Guelph Univ., Guelph, 1978

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
in
THE FACULTY OF GRADUATE STUDIES
(Department of Zoology)

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
January 1983

© Kevin Kenneth Loftus, 1983

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of ZOOLOGY

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date March 25, 1983

ABSTRACT

The effects of rearing density on stress response in juvenile steelhead trout (2-60 grams) were examined in a series of experiments. Fish reared at high densities showed evidence of stress, however, both the magnitude and the nature of the response varied with size.

In the first study, fry (initial weight 2.35 gm) were reared at 2 temperatures (12.5 and 15.0 °C) and 3 densities (0.6 to 3.0 times conventional densities). Fish reared at the high temperature showed a greater response to density than those reared at the low temperature. At 15 °C, growth rates were depressed by high densities, however the effect was of short duration and evident only during the first 2 of 8 weeks. Whole- body proximate composition was affected. At high densities, moisture and protein levels were elevated, and lipid levels were lowered. Condition factors were also low in fish reared at high densities. Both condition factors and growth rates followed a curvilinear pattern with time. At 12.5 °C, growth rates, proximate composition and condition factors followed trends close to those at 15 °C, however, tests were rarely significant. Plasma cortisol concentrations were unaffected by either density or temperature. Activity was affected by density, but not by temperature.

Fingerling trout (initial weight 15 gm), were differently affected over an 8-fold range in density (0.6 to 4.8 times conventional levels). At high densities, growth rates were suppressed, and the effect was of longer duration than observed in the first experiment. Whole body composition followed the same pattern with density as in the first experiment, but condition factors responded in the opposite direction, increasing at high densities. Plasma cortisol concentrations were unaffected by density. Rapid increases in density induced a response in growth rates over and above that due to density alone, which suggested that fish become conditioned to rearing densities. Rapid reductions in density did not affect growth.

Growth rates and plasma cortisol concentrations of pre-smolts and smolts were unaffected by an 8-fold density range (0.4 to 3.2 times conventional levels). However, after an acclimation period, sudden increases in density caused significant reductions in growth (greater than that expected on the basis of density alone) and elevations in plasma cortisol concentrations. Whole body composition followed a similar pattern with density as observed with smaller fish suggesting that the lack of growth responses to density, does not necessitate a lack of stress response. Condition factor data were inconclusive. Activity levels were unaffected by density, but did vary with time. After exsposure to a salt water challenge test, fish reared at high densities regulated plasma sodium levels less efficiently than fish reared at low densities. Flow rates and container volumes did not

significantly affect stress response.

These results indicate that high density rearing induces physiological, and possibly behavioral changes, in steelhead trout. This suggests the fish are showing a stress response. There is evidence that, in some cases, fish may adjust to densities, and that changes in density, not density per se, may influence the stress response. Growth rates, when used alone, are inadequate indicators of stress.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to express gratitude to my supervisor, Dr. T.G. Northcote for his encouragement, and financial support. Dr. J.D. McPhail deserves considerable thanks for assuming supervisory duties during Dr. Northcote's sabbatical. His patience and thorough efforts in reviewing the drafts were appreciated.

Special thanks to Dr. A.F. Tautz of the Fish and Wildlife Branch for his encouragement, guidance and support, without which, this study would not have been completed. He and I spent many hours planning and discussing this research. Dr. Tautz arranged access to the Fraser Valley Trout Hatchery and provided financial assistance. He also reviewed several drafts.

Dr. P.A. Larkin provided encouragement and advice at several stages during the study. His financial contribution, which covered a major portion of the material costs, was both indispensible, and appreciated. His comments on the final draft were most useful.

Mr. U.H.M. Fagerlund and Dr. D.A. Higgs of the Department of Fisheries and Oceans provided advice whenever requested, and were instrumental in defining the problem. Mr. Fagerlund also reviewed a number of drafts. Their support and encouragement was greatly appreciated.

Drs. G. Greer, J.R. McBride, and D.J. Randall provided advice on several occassions. Dr. Greer kindly arranged for the use of the video equipment.

Special thanks to Bakhshish Dosanjh, Helen Dye and Glen van der Kraak for providing technical advice with sample analyses.

The following people deserve credit and thanks for providing technical assistance with sample collection: Paul Bentzen, Linda Berg, Gary Birch, Michael Fattori, Ron Griffiths, Mike Henderson, Michele Skwarok, Al Stockwell, Leonardo Tiro, and John Werring.

Sincerest thanks are extended to the research staff at the hatchery; Bob Land, Larry Mitchell, and Morley Rempel, who cared for my fish when I was not at the hatchery. These gentlemen provided technical assistance, encouragement and advice whenever requested. Their skillful efforts ensured the health of my fish, and success of the project. Their abilities to suppress what must have been near-hysterical laughter during some of my more inventive moments, will be remembered.

Dr. Dave Zittin, of the Bioscience Data Centre provided essential help with programming and data analysis. His rather remarkable patience and good humour was appreciated.

Finally, a special thanks to my family for moral and material support throughout this effort.

Personal support during this study was provided by a Graduate Research and Engineering Technology Award from the B.C. Science Council, and from Dr. Northcote's NSERC grant.

GENERAL INTRODUCTION

Fish culturists in North America have been raising salmonids in hatcheries since the late 1850's (MacCrimmon et al. 1974) but with little scientific data guiding their selection of rearing densities. In this sense, fish culture has remained as much an art as a science, and establishing adequate criteria for selecting rearing densities remains an important problem in fish culture.

Over the past 50 years, commercial catches of some Pacific salmon, (Oncorhynchus spp.), have declined (MacLeod 1977; Larkin 1975). Similarly, catches of the most important sports fish in the province, steelhead trout, (Salmo gairdneri), have declined (Ford 1982; Hart 1973). Concomitant with these declines, has been an increase in artificial propagation as a method to replenish and supplement natural stocks. Associated with this has been an effort to increase the efficiency of hatcheries through improvements in design, operation, and management.

In nature, stream dwelling salmonids regulate densities primarily through behavioral mechanisms (Dill 1981; Allen 1969; Fraser 1969; Chapman 1966, 1962). In hatcheries, most of the natural determinants of density no longer operate, and it is the fish culturist who determines optimal density. Usually this is a balance between economic efficiency and fish survival (Birks et al. 1981). Under hatchery conditions it is probable that fish experience some degree of stress. If such stress reduces their ability to survive in the hatchery and when released, then understanding this stress response is essential to fish

culturists (Donaldson 1981; Schreck 1981a).

Salmonid culture presently involves artificial spawning, and the subsequent rearing to a development stage where the fish can be successfully released into the environment. Economic viability depends on adequate survival of released fish, and their contributions to commercial and sports fisheries, as well as to the reduction of pressures on natural stocks. To achieve success, released fish must be physiologically and behaviorally capable of surviving and growing in the natural environment. Larkin (1981) suggested that the high densities used in hatcheries may impose behavioral and physiological changes on the reared fish, and these changes may affect survival when released. He also noted that large-scale genetic interference with wild salmonid stocks may be an inevitable consequence of hatchery stocking programs, even with careful management.

Several authors have discussed the possible consequences of hatchery rearing experience on subsequent behavior and survival in the wild. Chapman (1966) suggested that artificial conditions in hatcheries influence behavior upon release. Fenderson and Carpenter (1971) noted differences in the behavior of natural and hatchery-reared Atlantic salmon (Salmo salar) smolts. Kalleberg (1958) observed a suppression of "normal" behavior patterns (aggression, territoriality) in fish reared in hatcheries. In addition, behavior on release was different from that of wild fish, and the over-crowding influence was found to be long lasting. These studies suggest that hatchery densities, often several orders of magnitude above densities in the wild,

may cause changes in behavior, and in turn these may affect survival.

High density rearing of salmonids is known to influence a number of physiological processes. Since most species are released as either smolts or pre-smolts, environmental conditions affecting the parr-smolt transformation are potentially important. Extensive research has been directed at identifying and understanding the physiological and behavioral changes associated with smoltification. Many authors (Wedemeyer et al. 1981; Schreck 1981b; Folmar and Dickhoff 1980) note that the requirements of smolts are exacting, and it is during the process of smoltification that fish are most sensitive to hatchery rearing practices.

A fundamental problem in fish culture is the establishment of adequate criteria for selecting rearing densities. Increasing rearing densities does not necessarily increase production. In fact Sandercock and Stone (cited in Fagerlund et al. 1981) observed reductions in returns of fish reared under high densities. In most hatcheries density criteria are established primarily by trial and error (McLean 1979) and the physiological basis underlying density effects is not understood (McLean 1979; Schreck 1981a).

A number of studies examine the effects of density on growth, survival, and competition in natural or simulated streams. Kalleberg (1958) noted that territories disintegrated when densities of S. salar and brown trout, Salmo trutta, were high and that under such conditions subordinates were displaced

to areas where growth rates were reduced. Chapman (1966) suggested that the minimal space requirements of territorial salmonids are fixed but that they can be moderated by physiological and psychological factors associated with food. Allen (1969) observed that territory sizes in some species would not shrink below a certain minimum size, even under conditions of high densities. Mason and Chapman (1965) observed that increased food abundance allowed increased densities of juvenile coho salmon, Oncorhynchus kisutch, without affecting growth. Slaney and Northcote (1974) made similar observations on rainbow trout, Salmo gairdneri, in artificial streams. They noted increased levels of aggression when prey levels were decreased. Kawanabe (1969) described an unusual behavioral response to density in the ayu, Plecoglossus altivelis. At high densities and under surplus food conditions, territorial behavior in this fish was inhibited and all individuals grew well. At middle densities, some fish were territorial and grew well, while others were displaced from preferred areas and grew poorly. At low densities, all fish occupied territories and grew well.

These studies made under natural and semi-natural conditions, suggest several generalizations. In many salmonids as densities increase under conditions of limited food, aggressive behavior increases. Territory sizes also may decrease (but rarely disappear), and growth rates of some individuals will be suppressed. In some species under surplus food conditions, as densities become high, territories disintegrate. In others, however, when minimum territory sizes are reached

surplus fish are displaced and regardless of food supply, grow poorly.

Several laboratory studies have examined the consequences of density on growth and aggression. In the medaka, (Oryzias latipes, Magnuson (1962) found that regardless of food availability, at high densities the advantages of territories diminished and eventually they were abandoned. Minchen (1972) made similar findings on a cichlid Aquidens pulcher. Brown (1946a,b) grew S. trutta at different densities and detected an optimum degree of crowding in fry and 2 year olds.

The high densities found in hatcheries make the extrapolation to fish production facilities of the results from ecological and some laboratory studies of limited value. More meaningful are studies conducted in hatcheries with higher (but economically viable) densities. Under hatchery conditions reduced growth rates associated with high density rearing have been observed in catfish, Ictalurus punctatus (Andrews et al. 1971), and various salmonids (Fagerlund et al. 1981; Trzebiatowski et al. 1981; Refstie 1977; Refstie and Kittleson 1976; Brauhn et al. 1976). Other studies also have described density-related growth depression; however these growth differences could be attributed to either water quality or the rearing methods were too poorly described to allow critical evaluation. In most studies however, density effects were evaluated on the basis of final size differences alone, and no effort was made to detect changes in growth rates over time within each experiment. Such analyses may be essential to

understanding density-related growth phenomena.

Growth alone is probably not an adequate indicator for assessing density effects (Birks et al. 1981). Fagerlund et al. (1981) examined the consequences of density-induced stress on a number of variables and found them useful in assessing fish health. As mentioned earlier, Sandercock and Stone (cited in Fagerlund et al. 1981) reported reduced returns of coho reared at high densities. Although density/return trends are useful, they must be interpreted cautiously. Since both size and time of release can affect salt water tolerance and rate of return (Conte and Wagner 1965; Wedemeyer et al. 1980), the results of studies describing reduced returns by fish reared under high densities may be confounded either by size or time-related effects. Therefore such studies may not be measuring density-related survival effects. Bilton (1978) and Bilton et al. (1980) demonstrated that both time and size at release, when varied independantly, affected adult returns. They found that optimum values exist for each.

Because it is unclear if size differences have affected returns from juveniles reared under different densities, the effects of rearing densities on ocean survival are not well established.

To summarize, the effects of rearing density on the growth and survival of hatchery-reared salmonids are unclear. There is evidence that high rearing density may reduce growth rates in some species. However, in some studies, inappropriate feeding regimes, water quality variations, and difficulty in comparing

growth rates among groups of different weights have distorted findings. Because of this, suitable density guidelines have not been established. Furthermore, the significance to survival of growth rates per se , not absolute size, is far from clear.

One approach to gaining an understanding of the consequences of different rearing densities on fish health, and ultimately, survival in the natural environment, may lie in developing an understanding of the levels of stress perceived by the fish, their response to stress, and culture strategies for controlling stress. If growth rates of fish reared at high densities are suppressed, then the fish must have experienced some stress. Evidence of this may be demonstrable through examination of physiological and behavioral parameters.

Before proceeding further, a definition of "stress" is required. The concept of stress as applied to biological systems has received considerable attention. Selye (1950) proposed one of the first definitions:

> "the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force."

Since then, stress has been re-defined many times. One of the most useful definitions is that of Brett (1958):

> "stress is a state produced by any environmental or other factor which extends the adaptive responses of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced. "

Seyle's (1950) account of stress introduced the concept of

the general adaptation syndrome (G.A.S.). In simple terms this is a common response to a wide variety of stress stimuli (including disease, fright, pain, etc.). The totality of responses to a stressor is composed of some reactions specific to that stimulus, and others common to all stimuli regardless of their nature. The G.A.S. can be conveniently divided into 3 stages: an alarm reaction, a stage of resistance, and a stage of exhaustion (Wedemeyer 1980; Selye 1973, 1950). The initial or alarm reaction, is usually accompanied by the release of various stress hormones. If the stress is not lethal, adaptation occurs during the resistance stage, in which biochemical, physiological, morphological and behavioral changes take place. Even if adaptation occurs, an animals' capacity to absorb other stresses may be reduced. If the stress is severe enough, adaptation will not occur, or if it is of long enough duration, the stage of exhaustion may be reached. This is usually lethal.

Three levels of response to stress have been defined in fish (Schreck 1981a; Wedemeyer and McLeay 1981; Wedemeyer 1980; Mazeaud et al. 1977). These are primary, secondary and tertiary effects (Table 1).

Over the past 15 years, a considerable interest in the effects of stress on fish has developed, and many stress-inducing stimuli have been examined (for a review, see Pickering 1981). Stimuli investigated include transportation (Barton et al. 1980), anaesthesia (Mazeaud et al. 1977; Strange and Schreck 1978; Wedemeyer and Yasutake 1977), loading and density (Fagerlund et al. 1981; Wedemeyer 1976), social heirarchies

Table 1. Summary of primary, secondary and tertiary level responses to stress (from Wedemeyer and McLeay, 1981)

Primary Effects

(i) release of adrenocorticotrophic hormone (ACTH) from the adenohypophysis;
(ii) release of "stress hormones" (catecholamines and corticosteroids) from the interrenal.

Secondary Effects

(i) blood chemistry and hematological changes (hyperglycemia, hyperlacticemia, hypochloremia, leucopenia, reduced blood clotting time);
(ii) tissue changes (depletion of liver glycogen, interrenal vitamin C depletion);
(iii) metabolic changes such as negative nitrogen balance and oxygen debt;
(iv) diuresis with resultant osmoregulatory dysfunction due to electrolyte loss.

Tertiary Effects

(i) impaired growth rate and food conversion;
(ii) delayed mortality;
(iii) impaired parr-smolt transformation;
(iv) impaired normal migratory behavior;
(v) reduced spawning success;
(vi) altered body composition;
(vii) increased susceptibility to disease.

(Ejike and Schreck 1981; Noakes and Leatherland 1977; Erickson 1967), high density confinement (Specker and Schreck 1980; Barton et al. 1980), fright induced by chase, capture or handling (Barton et al. 1980; Strange and Schreck 1980; Bouck et al. 1978; Wedemeyer 1976; Wedemeyer 1972), disease (Mazeaud et al. 1977; Wedemeyer 1976; Wedemeyer 1970), contaminants (Schreck and Lorz 1978; McLeay and Gordon 1977; Donaldson and Dye 1975; Hill and Fromm 1968), temperature (Strange et al. 1977), smolt migration and behavior (Schreck 1981b; Specker and Schreck 1980) and exercise (Zalnik and Goldspink 1980). Most of these studies demonstrated measurable stress responses, and all have

contributed to an improved understanding of the requirements of cultured fish. However, only Fagerlund et al. (1981) have attempted to measure the levels of stress experienced by fish reared for extended periods under different densities, and its consequences on physiological variables.

This study attempted to examine the effects of density, and changes in density, on growth and on a number of other physiological and behavioral variables that might be useful in assessing stress in juvenile steelhead trout. A primary consideration was that the results be of immediate use to steelhead production facilities. To attain a thorough understanding of the effects of stress, one primary indicator of stress (plasma cortisol), and several tertiary indicators (growth rate, proximate compostion, body condition, activity, and salt-water tolerance) were monitored. Cortisol was selected as a primary indicator because it responds quickly to stress (Donaldson 1981) and it has important effects on metabolic and osmotic processes. Effects of excess cortisol levels as observed in mammals are described in Lee and Laycock (1978). The other measures were selected either because of their sensitivity, or because changes in them would signify metabolic or behavioral changes.

In all experiments the null hypothesis was that density had no effect on any of the measured variables.

STUDY SITE AND REARING FACILITIES

All experiments were conducted at the Fraser Valley Trout Hatchery in Abbotsford, British Columbia. This provincial facility is operated by the Ministry of the Environment. Hatchery output includes production of parr and smolts of anadromous stocks of coastal cutthroat trout, Salmo clarki, and steelhead trout.

Water supply and quality

Ground water, obtained from a local aquifer, is available in sufficient quantities for all phases of production. Before use, water is passed through an aeration tower to remove excess nitrogen, and to bring oxygen levels to saturation. Temperature is constant throughout the year at 9.5° C.(± .2), and pH is nearly constant at 7.5 - 7.8 units. Specific conductance ranges from 200 to 230 umho/cm. Alkalinity and total hardness are 71.0 and 84.0 mg/l respectively, and for a coastal source the water is moderately buffered. Overall, water quality is ideal for fish culture.

Environmental control facilities

Most experiments were conducted in the quarantine room in the research section of the hatchery. Twelve oval, 500 litre, fibreglass tanks (137 cm by 77 cm) were available. These were fitted with central bottom drains, and were largely self-cleaning. Header boxes, each with a single overflow and two valve-regulated outflows enabling accurate flow control, were constructed for each pair of tanks. Each unit received water from three inflows: one heated, one chilled, and one at ambient groundwater temperature. By mixing temperature could be controlled accurately.

Each tank was equipped with an automatic Ewos feeder, either model 504 or 708, both of which operate silently and vibration free. Frequency of feeding, duration of each feeding, and quantity of food fed at each feeding were controllable. Automatic timers provided photoperiod control. Illumination was provided by fluorescent lighting.

GENERAL METHODS

Culture practices

Broodstock collection and initial rearing

The collection of broodstock, artificial spawning, and initial rearing of the fish used in these experiments were carried out by the research staff at the hatchery. Adult early run, Chilliwack-Vedder system steelhead were captured by angling in the headwaters of the system. The collection period extended from late December, 1979, until mid-April, 1980.

During egg take, fish were anaesthetized with 2-phenoxyethanol. Ripe females were bled by cardiac puncture, wiped clean, and then slit anteriorly from the vent allowing the eggs to fall into a clean, dry bucket. Sperm from at least two males was mixed in and then the eggs were rinsed several times. During water hardening, erythromycin phosphate (40 ppm) was used as an antibiotic.

Fertilized eggs from individual females were placed in separate Heath trays. To synchronize time to swim-up, different egg batches were reared at temperatures ranging from 7 to 12 °C.

When approximately 10% of the alevins reached the swim-up stage, they were transferred to 170 litre (2.4 m) troughs at densities up to 8000 fish per trough. They were fed Silver Cup starter until they reached a mean weight of 1.2 gm at which time they were transferred to 1.8 m circular tanks. Rearing continued in these tanks until their weights averaged 2.3 gm. At this

weight the experiment was started.

Food and feeding

Feeding schedules in density experiments should be designed to ensure that all fish are exposed to excess rations, thereby minimizing the likelihood of aggressive interactions and their consequences. Furthermore, frequent feedings should on average reduce appetite and decrease hunger-induced aggressive interactions. Slaney and Northcote (1974), and Magnuson (1962) provided evidence of the importance of prey supply and appetite on both frequency of aggression interactions and the outcome of those interactions in territorial fish.

Grove et al. (1978) demonstrated that appetites of rainbow trout correlated closely with the rate of gastric evacuation and that evacuation rate increased with temperature. Brett and Higgs (1970) found that 30-40 gram sockeye, Oncorhynchus nerka, reared at 15°C or above, could fully digest their maximum daily ration in less than 24 hours. Shelbourne et al. (1973) observed that sockeye fry fed continuously at 20°C exhibited greater growth than fish fed to satiation 3 times daily.

Given this information, it was decided that fish should be fed frequently, approximately every 20 minutes for small, 2-20 gram fish, and every 50 minutes for larger fish. Feeders were set to turn on 15 minutes after the lights were turned on, and off 15 minutes before lights off. The duration of the feeding varied between 30 and 70 seconds. At any time, however, all treatments were on the same frequency and duration schedule. The

quantity of food fed during each feeding could be controlled by adjusting the feeder plates.

To estimate food requirements, projected growth rates were calculated using a model developed by Iwama and Tautz (1981). Their model, $Wt^{.333} = Wo^{.333} + (T/1000)*t$: where Wt = final weight in grams, Wo = initial weight in gm, T = temperature in degrees Celcius, and t = time in days) was modified by incorporating a seasonal adjustment factor calculated from hatchery data. This model enabled reasonably accurate predictions of increase in weight of individual fish. Calculating the minimum amount to feed was a simple matter of multiplying projected weight increases by a conversion factor and the number of fish per tank. Since some food is always wasted, and to ensure that competition for food would be minimal, I multiplied this amount by a factor of 3. Fish were therefore provided with sufficient food to allow conversion efficiencies as poor as 9:1 on a dry weight food to dry weight fish basis (assuming an average fish moisture content of 70% and an average feed moisture content of 10%). Given that conversion efficiencies of 3:1 (dry weight basis) are not uncommon in hatcheries, it is clear that, even allowing for waste, excess food was available.

Ideally, fish should have been fed to satiation early each morning, and then frequently thereafter. Logistically, this was not possible. To confirm that rations were adequate, periodic visual inspections of food waste were made. Surplus feed was always found on the tank bottoms immediately after first feeding

in the mornings, and the amount wasted at successive feedings increased throughout the day. This suggests a voluntary reduction of food intake.

The food used in these experiments was the Abernathy diet obtained from the Moore-Clarke Company in LaConner, Washington. This brand was used since its formulation specifications are published and it is known to be a suitable feed. Feeds were stored at -20°C until required in order to reduce oxidation.

Environmental control

Temperature

The available temperature range extended from 7 to 18°C When adjusted, it could be maintained within ± 0.5°C of the desired level and fluctuations from those levels are summarized by experiment in Table 2.

Photoperiod

As described earlier, the quarantine room was equipped with fluorescent lights. Dimmers were not available.

Brett (1979) noted that changing photoperiods can affect growth rates in salmonids. To eliminate this influence, simple averages of the number of hours of daylight at the start and finish of the experiments were used to establish photoperiods.

Table 2. Summary of temperature fluctuations between tanks within experiments. Fluctuations were not calculated for tanks held at 9.5 C., since most were receiving well water, and well water temperatures fluctuated only slightly. In column 4, the greatest difference between mean tank temperatures over the duration of each experiment is given.

EXPERIMENT	DESIRED TEMPERATURE celcius	MAX DIFFERENCE BETWEEN TANKS AT ANY TIME celcius	MAX DIFFERENCE BETWEEN TIME AVERAGES celcius
1a	15.0	0.17	0.13
1b	12.5	0.21	0.15
2	12.5	0.66	0.21
3	9.5	0	0

Daily maintenance

Each morning feeders were manually switched on and individually examined to ensure proper functioning. This routine also allowed feeding activity to be observed. At the same time, header box outflows were checked. Temperatures were checked frequently.

To minimize stress, all tanks were scrubbed thoroughly only once per week. The time spent on each tank rarely exceeded 30 seconds (excluding partial draining time). Fish were not removed during cleaning. More frequently, tanks were partially drained without scrubbing and excess food and wastes were removed. This procedure was quick and apparently not stressful since fish usually fed vigorously at the next feeding.

Water quality

Generally, two approaches can be taken to ensure adequate water quality in density experiments. In one, flows can be proportionally increased in high density tanks to ensure that all treatments receive the same amount of water per unit of time and per unit of fish weight. This approach, while ideal from a water quality viewpoint, can have serious consequences. Increased flows in the high density tanks, if high enough, would necessitate higher energy expenditures for position maintenance. Fry (1957) showed that the metabolic cost of swimming varies approximately as the square of the sustained swimming speed. Brett (1964) recorded that in sockeye (30-50 gm), oxygen consumption increases logarithmically with velocity. These findings suggest that scope for growth, as defined by Warren and Davis (1967), could be reduced at high flows.

An alternate method, and the one used in these experiments, was to estimate the maximum water requirements (next section) and provide all tanks with the same water flow. Differences in water quality between treatments are expected.

Several other variables were monitored. These were dissolved oxygen and nitrogenous compounds. Dissolved oxygen was usually measured by the azide modification, iodometric (Winkler) method (Standard Methods 1976). Samples were analyzed for nitrite, nitrate, total nitrite and nitrate, and ammonia nitrogen at the Environmental Laboratory of the Ministry of the Environment. In addition pH, turbidity and specific conductance were determined. Samples were always collected near the central

bottom drains. Collection time varied, but was always in the afternoon.

Selection of rearing densities

The selection of densities was based upon: (i) the maximum amount of water available, and (ii) a knowledge of conventional steelhead rearing densities.

In the research section of the hatchery, rearing densities in circular tanks rarely exceed 20 gm/l for fish 2 to 60 gm in weight. At Capilano hatchery, steelhead rearing densities are only half that high. McLean (1979) examined rearing densities of chinook, Oncorhynchus tshawytscha and coho, O. kisutch at Quinsam, Robertson Creek, and Big Qualicum hatcheries, and found that for fish weights greater than 2 gm, 20 gm/l was a common upper density. Therefore, 20 gm/l was used as an estimate of conventional maximum rearing densities for steelhead.

To determine the number of fish that could be reared in a given flow of water, minimum acceptable oxygen levels were used instead of maximum safe metabolite levels. The minimum permissible oxygen level was set at 6.0 mg/l. This closely approximated Davis' (1975) "B" safety level. This level is conservative for three reasons: (i) oxygen levels should not approach this level until the final stages of the experiments, (ii) the frequent but short duration feeding schedule should minimize feeding-induced oxygen level reductions, and (iii) Brett and Blackburn (1981) found that in young coho and sockeye reared at 15°C, as long as oxygen levels were maintained above a

critical minimum of 4.0 - 4.5 mg/l, neither growth nor conversion efficiency were limited during 6 to 8 week experiments.

Although metabolite production rates were not calculated, rough estimates were made. It became clear that ammonia concentrations would remain low even at high densities. Un-ionized ammonia levels (the toxic form) were estimated by referring to a chart (Liao et al. 1974) which expresses the quantity of the toxic form at different pH and temperature levels. The EPA (1973) handbook recommends that un-ionized levels should remain below 0.010 mg/l. Burrows (1964) recommended 0.003 mg/l as a safe maximum level, however Westers and Pratt (1977) found errors in Burrows method and recommended 0.0125 mg/l.

The procedure for calculating maximum densities involved five steps. First, the amount of available oxygen (above 6 ppm) based on the quantity of water available per tank, and the rearing temperature, was calculated. Second, probable final fish sizes were estimated using the growth model described earlier. Third, knowing the probable final size, approximate oxygen consumption rates were calculated using the following formula (Tautz, pers. comm.):

$$\text{mg oxygen/fish/hour} = wt^{e} * \text{temp } (^{\circ}C) * 0.048$$

Fourth, to determine the maximum number of fish per tank, the total available oxygen was divided by the expected maximum

consumption rate. Finally, to achieve the desired weight/volume density, tank volumes could be adjusted once the number of fish per tank were known.

Sample collection

Anaesthetic

When required, the anaesthetic 2-phenoxyethanol was used. The concentration was varied according to the speed required by the sampling proceedure. Mortalities were rare, and recovery was usually rapid.

Lengths and weights

These measurements were usually taken every two weeks. Fish were quickly netted, anaesthetized, and 50 were randomly selected for measurement. Fork lengths were taken to the nearest millimeter, and weights to the nearest 0.1 gm, although during the first weeks of the first experient, they were weighed to the nearest 0.01 gm.

Total weights

To obtain an exact measure of mean weight by tank, total weights were determined approximately every two weeks by anaesthetizing and sampling all fish in a tank. The entire procedure rarely lasted more than 5 to 7 minutes. The experience did not appear traumatic since fish usually fed actively at the

next opportunity.

Proximate analysis

To determine the effects of rearing conditions on whole body composition, fish were killed and subsequently analysed for moisture, protein, lipid and ash content.

In the first two experiments, fish were killed at the end of the growth studies following 24 hours of starvation. Samples for the third experiment were collected mid-way through the experiment and fish were not starved, since this would have affected growth.

When killed, fish were weighed and measured, and then frozen until required. During processing, fish from each tank were divided into two groups of 2-5 fish on the basis of size. This enabled testing for sized-related differences in proximate composition within tanks. Following Higgs et al. (1975) fish were homogenized before analysis. The automated Kjeldahl procedure was used for protein determinations on a Technicon AutoAnalyser II.

Blood collection

Blood samples were collected for cortisol determination. Considerable care was required to minimize the effects of stress during sampling. Fagerlund (pers. comm.; 1967) suggested that 5 minutes between netting and taking of the last sample was an acceptable maximum time. Wedemeyer and Yasutake (1977)

recommended similar guidelines.

For 24 hours prior to sampling, fish were not disturbed. When fish were removed from a tank, care was taken to avoid disturbing fish in adjacent tanks. Fish were quickly netted and immediately placed in a bath containing strong anaesthetic. The concentration was such that most fish "rolled over" within 20-30 seconds. Several authors have demonstrated that anaesthetization prior to sampling reduced elevations in stress hormones associated with sampling (Mazeaud et al. 1977; Strange and Schreck 1978; Wedemeyer and Yasutake 1977). As soon as fish began to roll, they were removed individually, measured and weighed, and handed to one of two assistants. They severed the caudal peduncle and collected blood in heparinized 500 lamda micropipettes. Blood was quickly emptied into 2ml centrifuge vials and iced until all fish were processed. If insufficient blood volume was collected a second fish of similar size was selected, and its blood added to the same vial. Vials were centrifuged for three minutes at high speed and then the serum was removed with individual Pasteur pipettes and placed in labelled vials. All samples were frozen on dry ice, and remained frozen until analysed.

Cortisol samples were analysed by radio-immunoassay, using a kit available from Clinical Assay of Canada. Proceedures are outlined in the manual (see Ref.).

Behavioral Observations

The initial objective of the behavioral observations was to enable a quantitative assessment of both the levels of aggression and activity within different treatments. Because rearing densities were often high, it was difficult to follow and observe individual fish. Thus a video recorder, which enabled repetitive viewing, was used. This system could be used with little disturbance to the fish. Additionally, because all observations could be made within a 2-3 hour period, the possible effects of diurnal changes in behavior were reduced.

Prior to filming, fish were left undisturbed for at least 12 hours. With the television monitor operating, the camera could be positioned over the tank edge with little disturbance to fish. Activity was then followed on the monitor for at least five minutes to ensure that fish were behaving "normally". In most cases, activity appeared "normal" within two to three minutes. Fish were then filmed for five minutes.

Analysis of the films showed that both qualitative and quantitative estimates of aggression were difficult to obtain and subject to observational bias. In the low density tanks, individual fish could be followed and agonistic encounters, though uncommon, could be measured. However, at high densities, individual fish could be followed for only short times. Additionally, distribution in these tanks tended to be non-uniform, and the ease of making observations varied considerably within tanks.

Therefore only gross measures of activity were recorded.

The two variables measured were total activity (the total distance that a given fish moved in 15 seconds) and net activity (the shortest distance between a fish's position at the start and end of a 15 second interval).

A total of 100 observations was recorded for each tank (50 total and 50 net) on each day that activity was filmed. To reduce observational bias, and to decrease the influence of periods of erratic behavior, five separate time intervals, each 15 seconds in duration, evenly spaced throughout the recording period, were analyzed. At the start of each interval, ten fish were selected, five each on the right and left half of the field of view, and both measures of activity were made on each fish. Selection criteria required only that each fish be distinguishable in terms of position from its neighbours. Immediately adjacent fish were not measured, since the behavior of one could affect the behavior of the other.

During observation active fish would occassionally swim out of the field of view. To avoid selecting less active fish, the total distance moved by those fish, was corrected by a factor proportional to the fraction of the required viewing time completed. Measures of net activity were not possible for these fish. Another problem associated with high densities, was that some fish could not be followed for the required length of time. In these cases, alternates were selected.

EXPERIMENT 1: STRESS RESPONSES IN FRY

Introduction

It is known that relative growth rates of fish tends to decrease as body size increases. Therefore, when animals are small, their potential for growth is greatest. When growth is rapid, small variations in rates, regardless of cause, are more easily detected than in slower growing individuals. One might expect then, that the consequences of density-induced stress should, if present, be readily demonstrable in small fish.

In this experiment, steelhead fry were reared for eight weeks at three densities and two temperatures. Maximum densities attained were several orders of magnitude above natural stream levels, and three times conventional hatchery densities. Since in all groups, mean weights were expected to increase 400-600%, the physiological/endocrinological consequences of density-induced stress, if present, should be readily detectable. Under natural conditions, juvenile steelhead of this size are territorial (Cole and Noakes 1980), and under these highly artifical conditions, considerable differences in activity levels between densities might result if the fish attempt to establish territories.

Materials and Methods

Replicate groups of steelhead fry were reared at either 12.5 or 15°C, at one of three densities. In total, there were 6 treatments and 12 tanks. Initial fry weight averaged 2.35 gm. The experiment began on August 26, 1980 and was terminated in late October, 1980.

Maximum permissible densities were calculated as described earlier (General Methods). Water flows were set at 25 l/min. The three densities were 200, 600, and 1000 fish per tank. On a weight per volume basis, predicted final densities extended from 0.6 to 3.0 times conventional densities at the hatchery. The design is summarized in Table 3.

Photoperiod was fixed on a 12L:12D cycle. Lights were automatically switched on at 0700 hours and off at 1900 hours. Initially, fish were fed every 18-19 minutes for 60 seconds each time. On day 39, feeders were adjusted to feed every 23-24 minutes for 68 seconds. Feeder plates were adjusted frequently to further control food quantity released. Also on day 39, feed size was switched from 3/64ths to 4/64ths inches.

Total weights, and individual lengths and weights, were taken on days 1, 14, 32, 46, and 61. Plasma samples for cortisol analysis were collected on day 58, and on day 60 samples were collected for proximate analysis.

On day 36, water samples were collected from the four high density tanks, and from the inflow. On day 58, after cortisol sampling was complete, water samples were again collected, this time from all tanks. All samples were refrigerated and kept in

Table 3. Design of experiment 1. Initial densities (gm/l) based on initial mean weight of 2.35 grams. Final densities are projected values based on growth slope model (Iwama and Tautz 1981) using the seasonal adjustment factor of T/1000 (see text, page 15).

TANK	TEMP Celcius	NUMBER	VOLUME litres	FLOW l/m	DENSITY INITIAL f/l	DENSITY INITIAL gm/l	DENSITY FINAL gm/l	LOADING gm/l/min inflow
1	15.0	1000	250	25	4.0	9.4	60	600
2	15.0	600	250	25	2.4	5.6	36	360
3	15.0	200	250	25	0.8	1.9	12	120
4	15.0	1000	250	25	4.0	9.4	60	600
5	15.0	600	250	25	2.4	5.6	36	360
6	15.0	200	250	25	0.8	1.9	12	120
7	12.5	1000	250	25	4.0	9.4	48	480
8	12.5	600	250	25	2.4	5.6	29	288
9	12.5	200	250	25	0.8	1.9	10	96
10	12.5	1000	250	25	4.0	9.4	48	480
11	12.5	600	250	25	2.4	5.6	29	288
12	12.5	200	250	25	0.8	1.9	10	96

darkness until delivered to the environmental laboratory within 24 hours.

Dissolved oxygen concentrations were determined on all tanks on days 46 and 61. A YSI model 54 oxygen meter was used.

Video recordings of fish behavior were made on days 25, 39, and 53.

Mortalities were recorded daily, but were rare, and were usually caused by either physical damage while being netted, or by gilling in the perforations of the inflow pipe.

Results

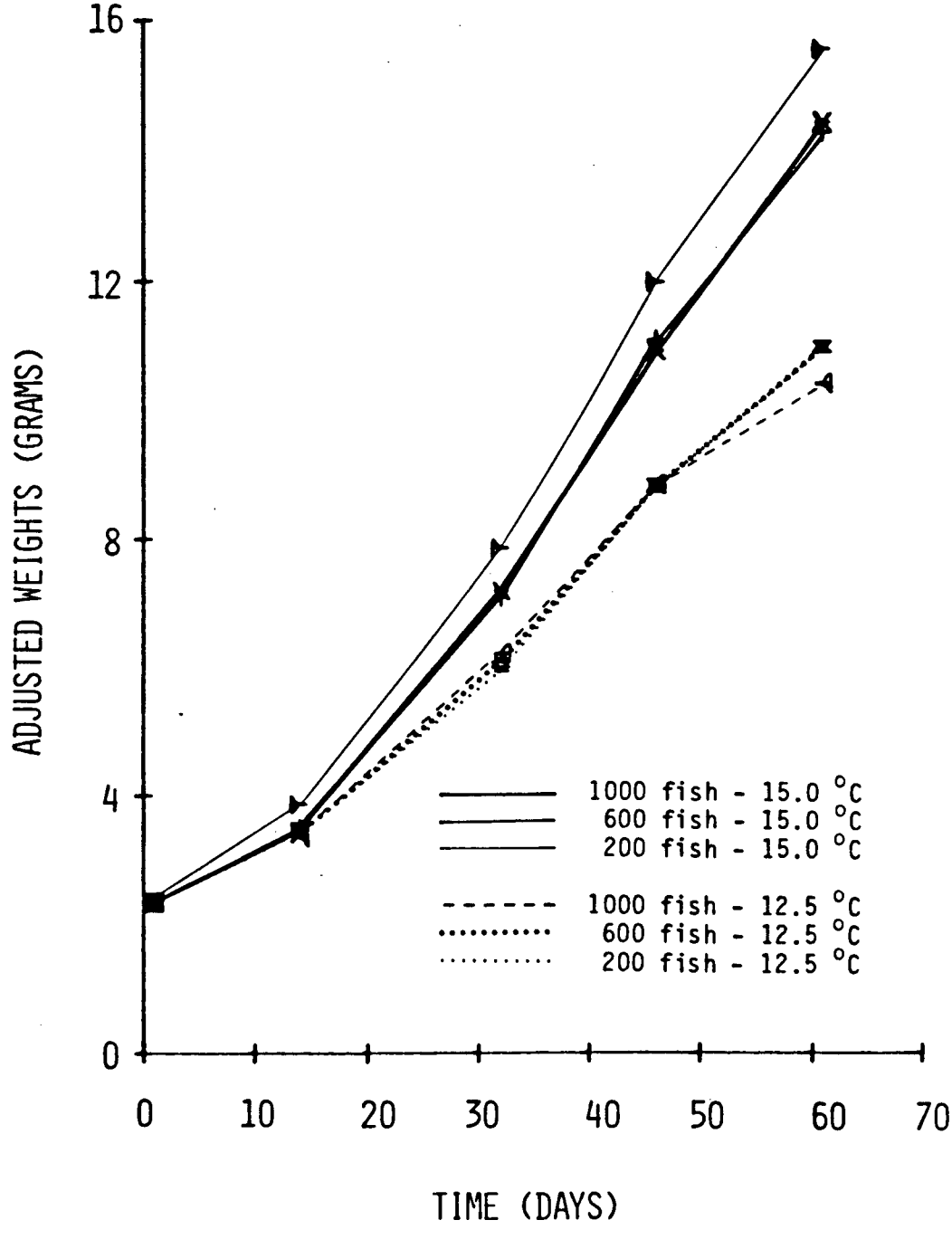
Growth

During the course of the experiments, technical difficulties resulted in the loss of some data. In tank 4, feeder problems lasting four days, between days 14 and 32, resulted in the loss of growth slope data for that interval. Similarly, growth slope data was lost between days 32 and 46 for tank 12.

A plot of adjusted fish weights against time is given (Figure 1) in which weights were corrected to a common initial size. This was accomplished using the grand mean starting weight as a common initial weight and recalculating sizes at specified times using the growth slopes from successive intervals. This figure shows the relative changes in weight over time. As expected, at 15°C, fish grew faster than at 12.5°C. At 15°C, as density increased, mean final weight decreased. At 12.5°C, the trend was similar, but less clear.

Linear regressions of mean fish weights to the one-third power against time were plotted for each treatment. The regression equations fit the data closely (all correlation coefficients were greater than 0.99). However, close inspection revealed a consistent pattern in the residuals between all treatments. It is unlikely that the slight curvilinearity in the data fit was of sufficient magnitude to either invalidate the use of the growth model (Iwama and Tautz 1981), or to alter the conclusions of any of the analyses.

Figure 1. Change in weight over time by temperature and density for experiment 1. All weights have been adjusted to a common initial weight using the grand mean starting weight (2.35 gm) and the individual growth slopes by time interval.


16
12
8
4
0
ADJUSTED WEIGHTS (GRAMS)
0
10
20
30
40
50
60
70
TIME (DAYS)
1000 fish - 15.0 °C
600 fish - 15.0 °C
200 fish - 15.0 °C
1000 fish - 12.5 °C
600 fish - 12.5 °C
200 fish - 12.5 °C

Growth slopes (both temperatures)

Growth data were first analyzed in a 3-way ANOVA (temperature, density, time) using growth slopes calculated according to Iwama and Tautz (1981) (Table 4). The temperature effect was, as expected, significant. At both temperatures the calculated growth slopes pooled by density were higher than model predictions. That is at 15°C growth slopes averaged 0.0182 (model prediction is 15/1000, or 0.0150). Similarily, at 12.5°C, the observed value was 0.0143 (model prediction is 0.0125). Density effects were significant. The two extreme densities were different from each other, but not from the mid-density (Duncan's multiple range test (5% level)). On a percentage basis, the low density tanks were growing about 10% faster than the high density tanks. Time effects were significant. Examination of the data by time interval revealed a clear pattern (Figure 2); low slopes initially, rising to a peak by the second interval, and then declining thereafter. Further discussion of the pattern will follow. The density by temperature interaction was not significant indicating that differences with temperature were similar at each density. The other interactions, temperature by time, and density by time were significant. These suggest that the influence on growth of density and temperature changed over time. The 3-way interaction was also significant.

Of all interactions, the most interesting was density by time. Since both factors taken alone were significant, this suggested that one or more intervals within the growth period

may be contributing to most of the observed density effects.

Table 4. Analysis of variance tables of growth slope against treatment for experiment 1. Part A includes both density and temperature effects. In parts B and C, analyses of the 15 and 12.5 C data are given. The degress of freedom values for both the residual and total terms in each analysis are lower than expected since at each temperature, data from one time was lost for one tank.

	SOURCE	DF	MEAN SQUARE	PROBABILITY
A BOTH TEMPERATURES	Temperature	1	0.00017606	0.0
	Density	2	0.00000468	0.0152
	Time	3	0.00018975	0.0
	Temp*Density	2	0.00000227	0.1072
	Temp*Time	3	0.00000700	0.0011
	Density*Time	6	0.00000383	0.0060
	Temp*Dens*Time	6	0.00000337	0.0112
	Residual	22	0.00000092	-
	Total	45	-	-
B 15.0 C	Density	2	0.00000444	0.0292
	Time	3	0.00011205	0.0
	Density*Time	6	0.00000393	0.0167
	Residual	11	0.00000090	-
	Total	22	-	-
C 12.5 C	Density	2	0.00000225	0.1367
	Time	3	0.00008380	0.0
	Density*Time	6	0.00000327	0.0352
	Residual	11	0.00000094	-
	Total	22	-	-

To investigate this, it was decided to analyze the data over selected time intervals. A cursory inspection indicated that the first interval may have been the most crucial. Consequently, two more ANOVAs were performed, one for the first interval, and another for the remaining three.

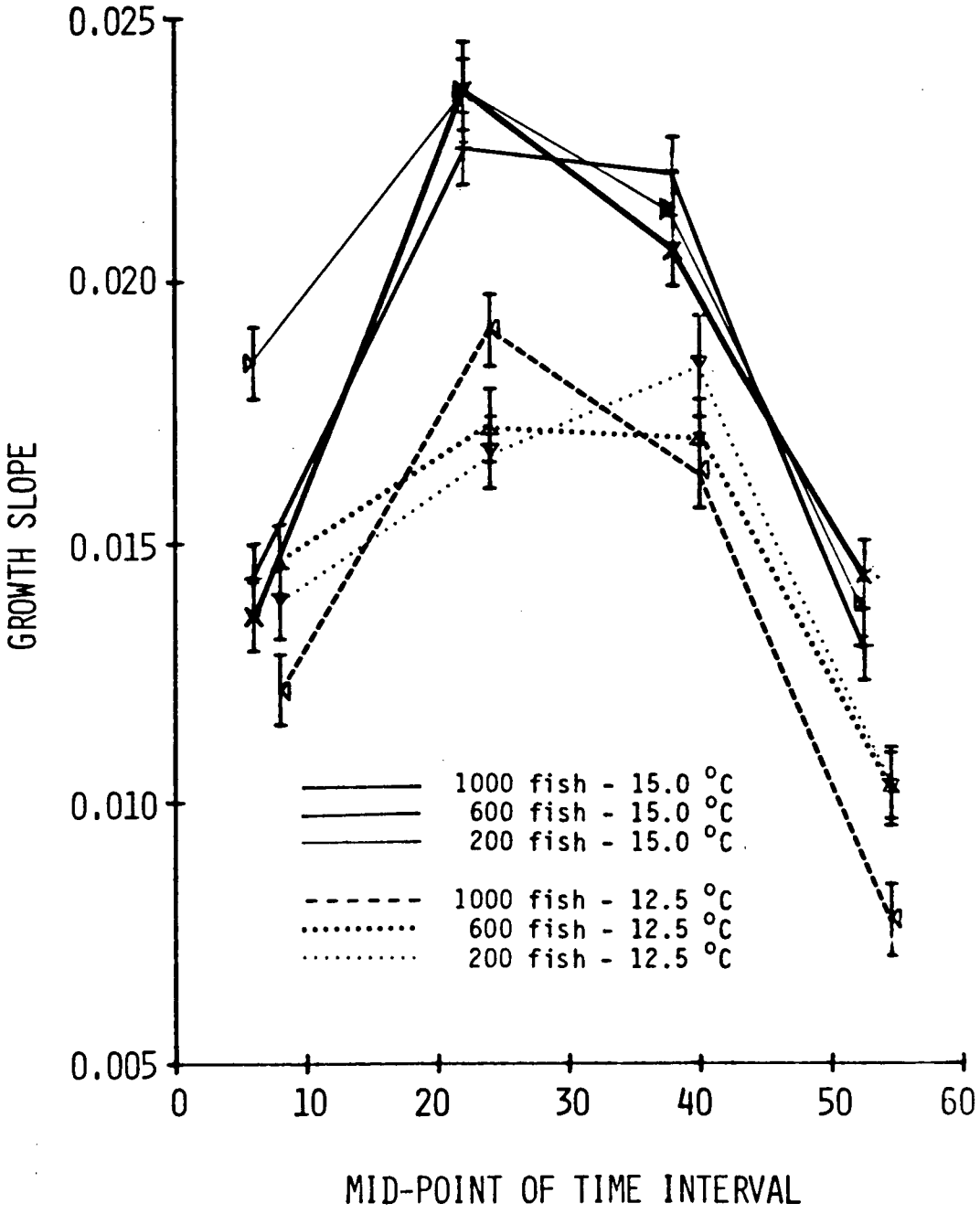
Data for the first time interval were analyzed in a 2-way

ANOVA (temperature, density). As expected, temperature produced significant effects ($df=1,11$ $p<.01$). Density also proved significant ($df=2,11$ $p<.01$). This clearly demonstrated that density affected growth during the first time period. Duncan's test showed that all three densities were significantly different from each other. Mean growth slopes increased as density decreased. Unexpectedly, the interaction of temperature and density was found to be significant ($df=2,11$ $p<.05$), indicating that the effect of density on growth varied with temperature.

The remaining 3 intervals were analysed in a 3-way ANOVA (temperature, density, time). As before, the effect of temperature ($df=1,33$ $p<.001$) and time ($df=2,33$ $p<.001$) were significant. Growth slopes declined steadily with time. Surprisingly, density was not significant ($df=2,33$ $p=.74$) and therefore, did not affect growth after the first time interval. This means that most of the observed differences in growth rate, and density effects on final sizes, occurred during the first 14 days. Neither interaction of density by time, or density by temperature, were significant. However, both were nearly significant ($df=4,33$ $.05<p<.1$ and $df=2,33$ $.05<p<.1$, respectively) suggesting that the effects of temperature and time may have been different at different densities.

To further clarify the data, it was desirable to eliminate temperature effects. This was justified since the interaction terms suggest differences.

Figure 2. Growth slopes against time (mid-point of sampling interval) for experiment 1. Mean values are given. Growth slopes were calculated according to Iwama and Tautz (1981). Verticle bars are standard errors.


0.025
0.020
0.015
0.010
0.005
GROWTH SLOPE
0
10
20
30
40
50
60
MID-POINT OF TIME INTERVAL
1000 fish - 15.0 °C
600 fish - 15.0 °C
200 fish - 15.0 °C
1000 fish - 12.5 °C
600 fish - 12.5 °C
200 fish - 12.5 °C

Growth slopes (15°C)

The high temperature data were first analysed in a 2-way ANOVA (density, time) (Table 4). The results, computed over all four time periods, indicated that both density and time had significant effects on growth. Clearly, at 15°C, density affected growth. However, as before, the density by time interaction was significant indicating that the effect of density changed over time.

To determine which times were most affected by density, the data were subdivided by time. Density effects on growth were significant ($df=2,5$ $p<.01$) during the first time interval, confirming the findings of the previous interaction effect. When analysed over the last three times, density had no effect ($df=2,16$ $p=.87$), confirming that the effect of density was of short duration. Time effects were significant ($df=2,16$ $p<.001$), but the interaction was not.

Growth slopes (12.5°C)

When growth data from all times were analysed for the low temperature treatments, density had no effect (Table 4). However both time and the interaction term were significant. The lack of a density effect was unexpected in view of the results at 15°C and was initially thought to be a function of small sample sizes. However, replicate tanks were close, suggesting that the lack of effect was real. Furthermore, the trend with density was similar, though less clear than at 15°C, and the probability level attained was close. In addition, the significant

interaction term suggested that the same process which occurred at the 15°C was occurring here.

These data (12.5°C) were then reanalyzed by time interval. Again, density effects were not significant during the first time interval (df=2,5 p=.18), although a similar, but less clear trend to that observed at 15°C, was found. Over the remaining 3 intervals, density had no effect (df=2,16 p=.59).

Proximate analysis

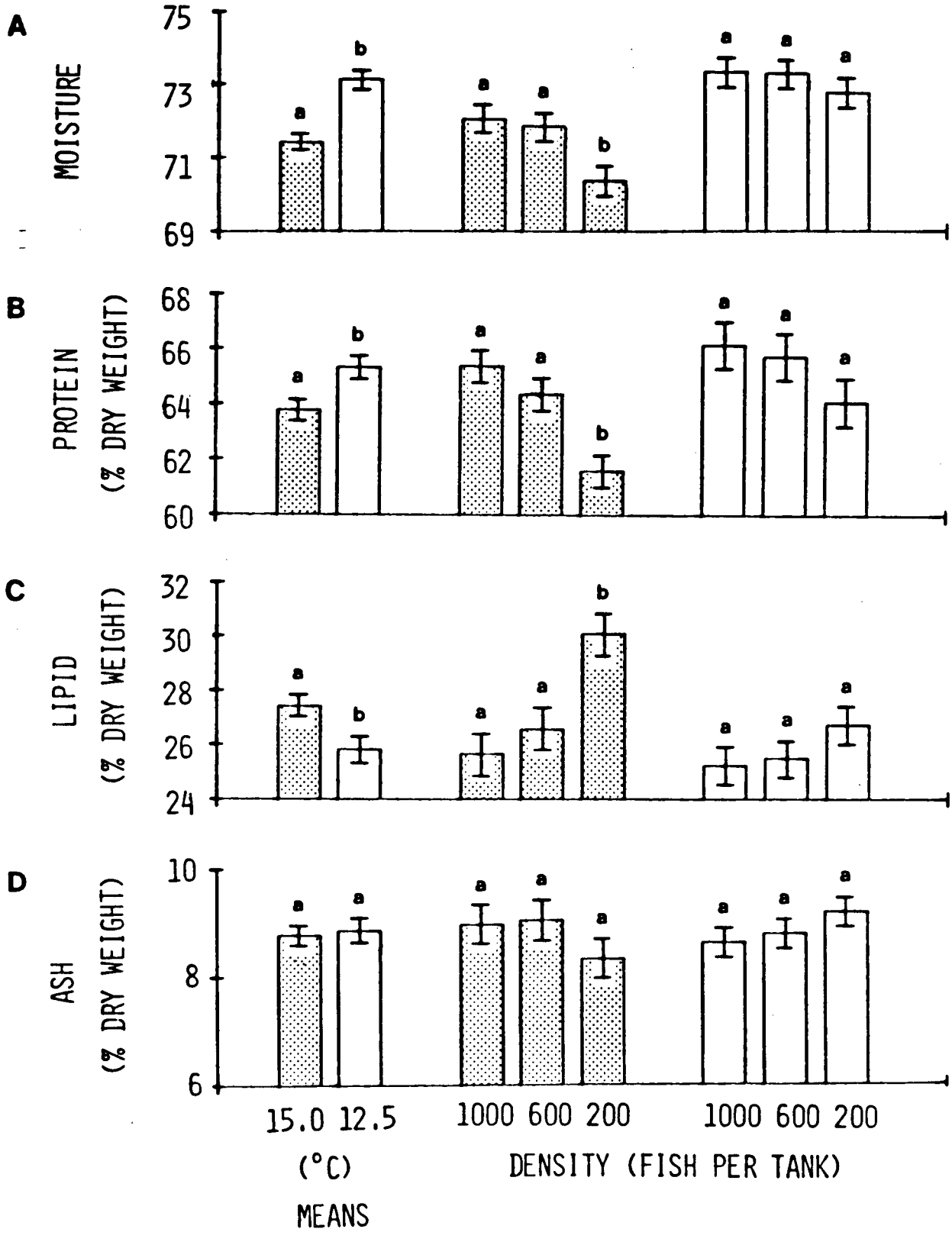
Moisture

A 2-way, nested ANOVA (temperature, density, replicates) was performed for the moisture data. Replicates were not different. A subsequent analysis on pooled data revealed that both temperature (df=1,29 $p<.001$) and density (df=2,29 $p<.01$) had significant effects. Moisture content decreased as temperature increased. As density increased, so did moisture. The interaction was not significant.

In view of the unusual temperature effect on growth slopes it was decided to analyse the data for each temperature separately. At 15°C the effect of density was significant (df=2,17 $p<.05$). At 12.5°C it was not, however a similar trend was apparent. These results are summarized in Figure 3a.

To determine whether weight affected moisture, linear regressions of mean fish weight by sample against percentage moisture content were calculated for different treatments. In all cases, slopes were not significantly different from zero.

Figure 3. Histograms of proximate composition data for experiment 1. A-moisture, B-protein, C-lipid, D-ash. Stippled bars are 15 °C data, and open bars are 12.5 °C data. Each figure is divided into 3 sections. The first 2 bars are mean levels at each temperature (densities pooled). Bars 3-5 are 15 °C data at each of the three density treatments. Bars 6-8 are 12.5 °C data at each of the three density treatments. Vertical bars are standard errors. Within each section, common letters refer to treatments not statistically different (Duncan's or Scheffe's multiple range tests - 5% level).


A
MOISTURE
75
73
71
69
B
PROTEIN
(% DRY WEIGHT)
68
66
64
62
60
C
LIPID
(% DRY WEIGHT)
32
30
28
26
24
D
ASH
(% DRY WEIGHT)
10
8
6
15.0 12.5
(°C)
MEANS
1000 600 200
1000 600 200
DENSITY (FISH PER TANK)

Protein

Protein values were expressed as percentage dry weight (Figure 3b). With replicates pooled, a 2-way ANOVA was calculated. Density ($df=2,29$ $p<.001$) and temperature ($df=1,29$ $p<.05$) effects were significant. As with the moisture data, protein levels increased with a decrease in temperature, and decreased with decreasing densities. The interaction term was not significant.

The data for each temperature were analysed separately to determine if density effects were influenced by temperature. At 15°C, the effect of density was significant ($df=2,17$ $p<.001$). Protein levels increased with density. At 12.5°C, the effect, as with the moisture data, was not signficant ($df=2,11$ $p=.24$). Again however, a decreasing trend in protein levels with decreasing density was observed.

Linear regressions of sub-sample mean fish weights against percentage protein were calculated to test for size-dependant effects. Only at the middle density at 15°C was a slope significantly different from zero obtained. Consequently, it appears unlikely that size affected protein content.

Lipids

Lipid values were expressed as percent of dry weights as were the protein data. Without pooling of replicates, density effects were significant, and temperature effects were nearly so.

When replicates were pooled, the effects of density ($df=2,29$ $p<.001$) and temperature ($df=1,29$ $p<.05$) were significant. The interaction was not. Lipid content decreased as temperature decreased and increased as density decreased. Both patterns were opposite in direction to those observed for moisture and protein.

To investigate temperature-related differences in density response, temperatures were analysed separately. At 15°C, density effects were significant ($df=2,17$ $p<.005$), lipid levels decreasing as density increased. At 12.5°C, the effect was not significant ($df=1,11$ $p=.29$). However, as with moisture and protein levels, the trend at this temperature was in the same direction as at 15°C. The results of these analyses are presented in Figure 3c.

Linear regressions were calculated to investigate size-related effects. Most regressions produced slopes not different from zero. As before, there was one exception - the middle density at the high temperature. This occurrence was considered unimportant for the reasons described earlier.

Ash

There were no density effects on ash content, either when temperatures were analysed together, or separately. There were no trends in the data. It therefore appeared that whole-body ash content was relatively insensitive to the effects of density and temperature. Linear regressions were not calculated.

Cortisol

To increase assay precision, each plasma sample was analysed in duplicate. However, there were cases in which one of the duplicates produced questionable results, or plasma volumes were insufficient to enable duplication. To avoid inappropriate weighting of the results, duplicates were pooled so that only one cortisol value per sample was entered in the analysis.

There were no effects of either density or temperature when data were analysed in a nested ANOVA. The replicates term was significant. When reanalysed by temperature, again no density effects were observed, and importantly no trends were distinguishable. There was a trend however, to higher values at the low temperature. As mentioned, this trend was not significant. These data are summarized in Table 5.

Because the variances were heterogeneous for temperature, and this was not corrected by any of the transformations tested, a number of non-parametric Mann-Whitney U-test comparisons were made. None of the tests produced significant differences with the 2-tailed test. Some were significant with 1-tailed tests, however there was little *a priori* justification for its use.

Weight length relations

Weight-length relations were examined in a two step procedure. First, a functional, geometric mean (G.M.) regression was calculated for the natural log (ln) length, natural log (ln) weight relationship. Secondly, using this regression, a

Table 5. Summary of cortisol data results. There were no significant effects due treatment. Values are given in ng/ml.

fish/tank replicate		1000 1	1000 2	600 1	600 2	200 1	200 2
15.0 C.	n	12	13	12	14	16	15
	mean	10.8	19.8	14.4	16.0	12.9	22.9
	S.E.	2.88	2.77	2.88	2.67	2.50	2.58
12.5 C.	n	15	16	15	15	15	16
	mean	19.6	27.6	22.7	12.3	16.1	24.7
	S.E.	2.58	2.50	2.58	2.58	2.58	2.50

condition factor equation "customized" to the data, with a value of one, was created. This technique is preferred by Ricker (1973, 1975). It was important to have a customized equation since different populations of fish have variable weight length relations. Small errors in certain parts of a condition equation, especially the length exponent, b, can produce artificial curvilinearities in condition trends with size. Such trends can lead to errors in interpretation.

As described earlier, weight and length data (50 pairs per tank), were collected at 5 times throughout the course of the experiment. A preliminary overview of the data indicated that in those tanks where feeder problems had existed, weight length relations were not strongly affected. However, to maintain consistency with other analyses, the affected tanks, 4 and 12, were excluded.

The G.M. functional regression of ln length against ln weight produced the following equation:

$$\ln Wt. = -5.017 + 3.269 \ln L.$$

When antilogged, the equation becomes:

condition factor = weight/(.00662437 * length$^{3.269}$),

which is the condition equation.

Using this equation, 50 condition values were calculated from the length weight data from each tank. The statistical analyses presented were performed on data with replicates pooled although in some of the comparisons they were significantly different.

Condition factors (both temperatures)

Condition factor data were first analysed in a 3-way, nested ANOVA (temperature, density, time, replicates). Replicates were significantly different. However, on close inspection, it appeared that only one or two cases caused the significance. To identify overall trends, replicates were pooled. The patterns observed in main effects and interactions were similar to those observed in growth slope data. Temperature effects were not significant. However, both density ($df=2,2499$ $p<.005$) and time effects ($df=4,2499$ $p<.001$) were significant. Condition tended to increase with decreasing densities, although the pattern was not always consistent. Scheffe's test produced two homogeneous groups which were the highest and lowest densities, and the low and middle densities. Duncan's test indicated that condition factors at each time were different than at all other times. The pattern in condition over time was identical to that for growth slope. The interaction of temperature by density was not significant, but was close

(p=.09). However, all others, temperature by time, density by time, and temperature by density by time were significant (df=4/2/2,2499 p<.001). Most importantly, the effects of density on condition changed over time. Plots of condition factors over time, by density, are presented in Figure 4.

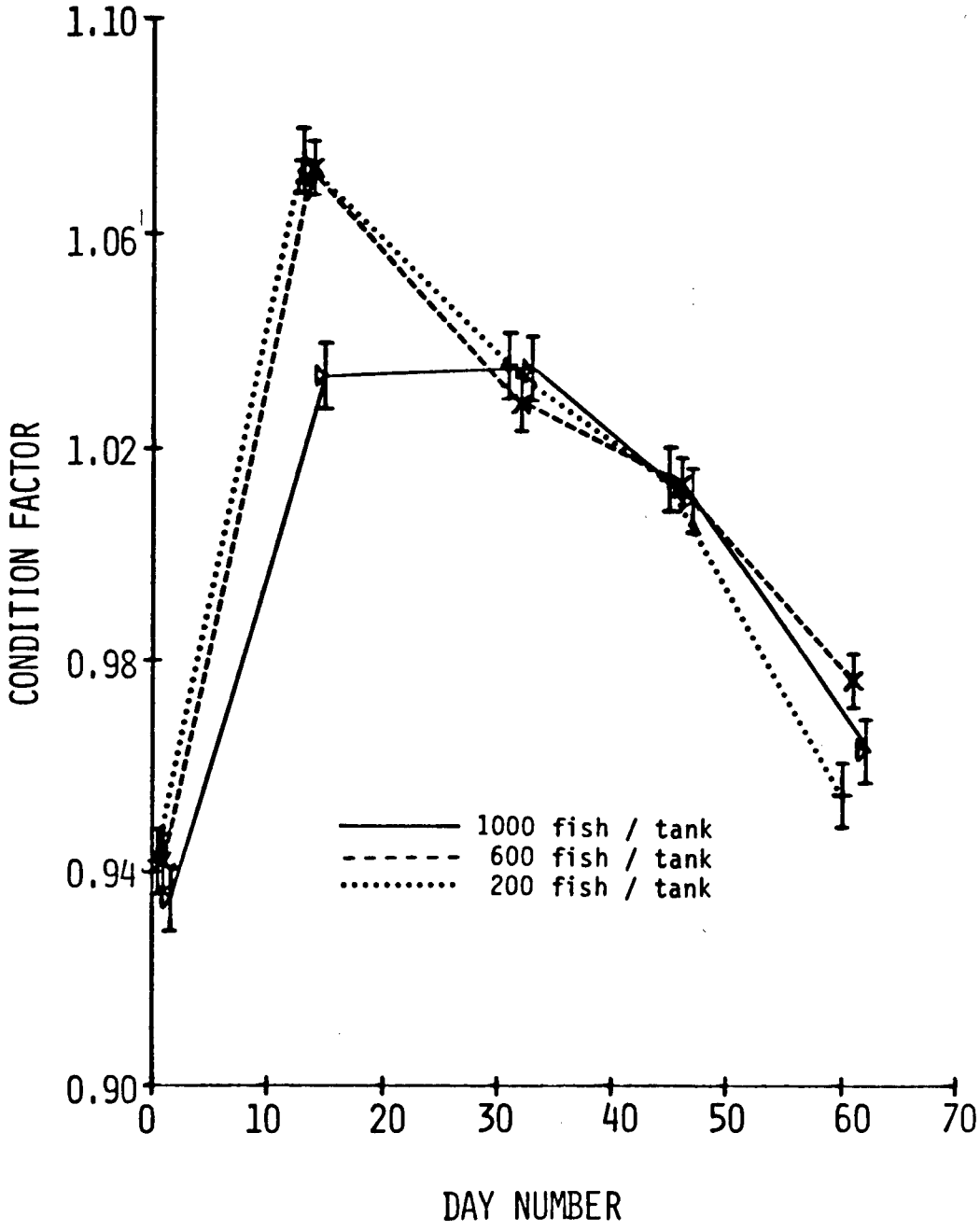
Table 6. Condition factor data summary by temperature and time for experiment 1. Conditions were calculated from the GM functional regression of natural log of length against natural log of weight from samples of 50 lengths and weights taken from each tank. In all cells, n=50 and S.E. of the mean = 0.00923.

		DAY				
TEMP	DENSITY	1	14	32	46	61
	1000	0.919	1.009	1.030	1.009	0.985
		-	-	-	-	-
15.0 C.	600	0.938	1.036	1.061	1.025	0.981
		0.915	1.071	1.007	1.030	1.005
	200	0.930	1.057	1.026	1.014	0.938
		0.948	1.100	1.071	1.014	0.974
	1000	0.941	1.046	1.039	1.005	0.949
		0.945	1.045	1.036	1.017	0.955
12.5 C.	600	0.954	1.055	1.023	0.986	0.961
		0.959	1.127	1.023	1.013	0.959
	200	0.949	1.064	1.010	1.015	0.952
		-	-	-	-	-

Condition factors (15°C)

To simplify interpretation, temperatures were analyzed separately (Table 6). At 15°C, with replicates pooled, density effects were significant (df=2,1249 p<.005), condition

Figure 4. Condition factors against time for experiment 1. Vertical bars are standard errors. Since temperature effects were not significant, temperature treatments were pooled. Each point represents 200 length weight observations.


1.10
1.06
1.02
0.98
0.94
0.90
CONDITION FACTOR
0
10
20
30
40
50
60
70
DAY NUMBER
1000 fish / tank
600 fish / tank
200 fish / tank

increasing as density decreased. Scheffe's test indicated that the high density treatments had conditions significantly lower than the others. The effect of time was significant ($df=4,1249$ $p<.001$), with the previously described pattern occurring. The interaction of density by time was significant ($df=8,1249$ $p<.001$). As density increased, the time required to peak increased, and the magnitude of the peak decreased.

Condition factors (12.5°C)

At 12.5°C, the density effect was not significant, and the trend followed exactly that for growth slope. The effect of time was significant ($df=4,1249$ $p<.001$), and followed the expected pattern. The interaction term was also significant ($df=8,1249$ $p<.001$), but the pattern was less clear than at 15°C as had been the case for growth slope data.

Behavior

Behavior data is often nominal or ordinal, or if on a measurement scale may frequently not meet the homogeneity of variance assumption required for statistical analysis. However, with large sample sizes, F-values are relatively insensitive to heterogeneity problems (Dr. T. Kozak, Faculty of Forestry, UBC, pers. comm.). Therefore, it was decided to use parametric methods in these analyses since they are more powerful. As a check, some comparisons which were markedly heterogeneous were reanalysed using non-parametric tests. All observations were transformed as follows:

natural log (activity + 1.1).

It should be kept in mind that the units of measurement although arbitrary, were consistent.

Unfortunately, technical problems with the recorder resulted in poor film quality and only one of three films could be analysed. In addition, data from tank 4 was deleted since at the time of filming feeder problems had caused the fish to become hyperactive.

Net activity

When the pooled data were analysed in a 2-way ANOVA (temperature, density) density effects were significant ($df=2,514$ $p<.01$). Scheffe's multiple range test indicated two homogeneous groups as follows: (i) the lowest and highest densities, and (ii) the highest and middle densities. The middle density was significantly different from only the lowest density. Station maintenance was least precise at the middle density, and most precise at the low density. Neither temperature nor the interaction terms were significant. Bartlett's test indicated that the variances were homogeneous when grouped by density, but not when grouped by temperature. This lack of homogeneity was not considered important because of the robustness of the test procedure.

Next, it was decided to analyse the data for each temperature separately in view of the different effects of the two temperatures on the other variables measured. At neither temperature were significant density effects found. However,

both were close (15°C df=2,233 p=.055; 12.5°C df=2,280 p=.08). It was interesting to note that at both temperatures, the pattern in net activity with density was similar (Figure 5a).

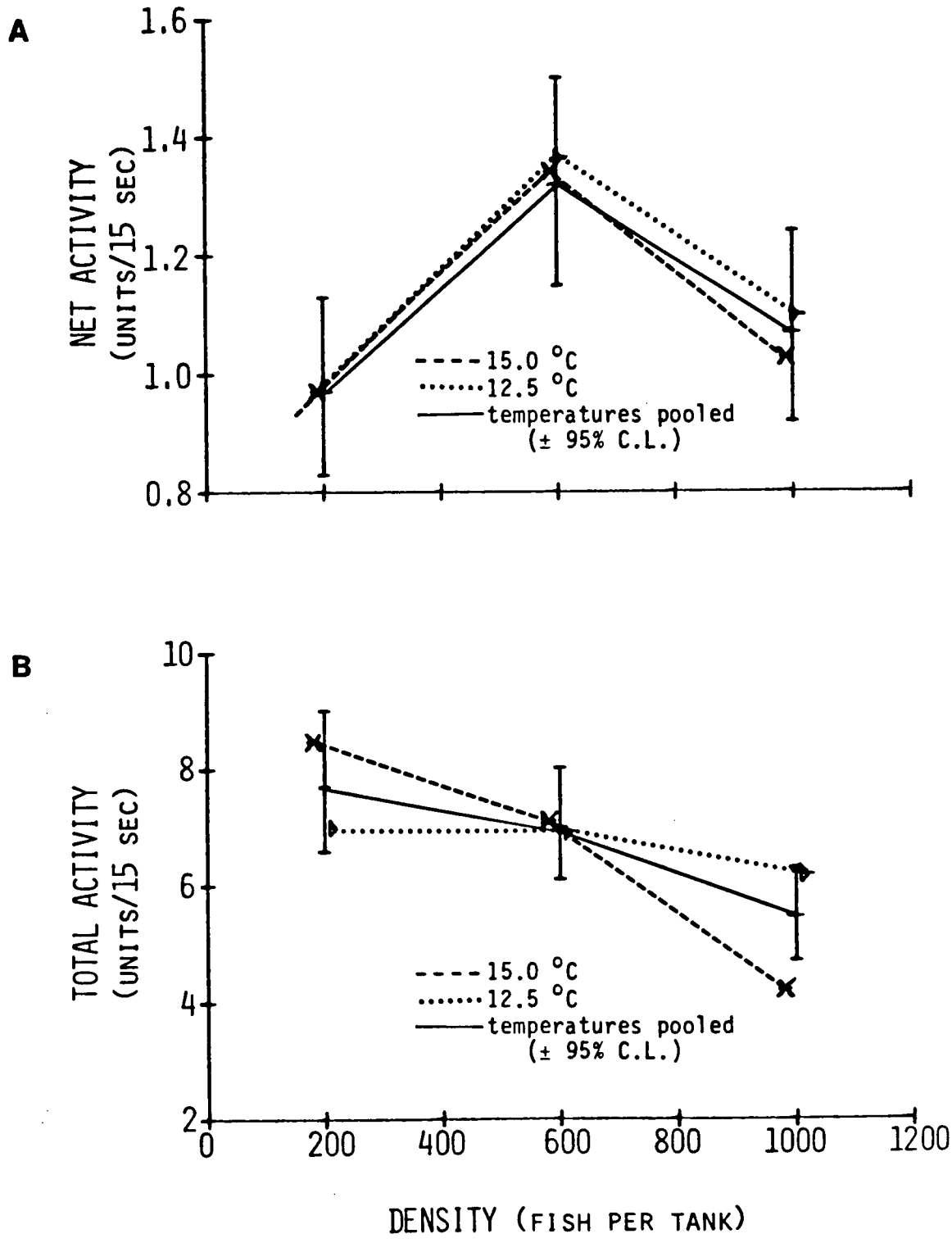
Total activity

Analysis of total activity data revealed a different pattern (Figure 5b) from that observed for net activity. Without pooling, a 2-way, nested ANOVA (temperature, density, replicates) did not produce significant effects. Consequently, replicates were pooled and the data were reanalyzed. As with the net activity data, the effects of temperature were not significant. However, both density (df=2,549 p<.005) and the interaction (df=2,549 p<.05) were significant. Total activity increased as density decreased.

To investigate interaction effects, data for each temperature was subsequently analyzed separately. At 15°C, the effect of density was significant (df=2,249 p<.005). When the data were untransformed, total activity doubled from 4.2 units per 15 second interval at the high density to 8.5 at the low density. Scheffe's test indicated that the high density tank had significantly lower activity levels than the other two which were not different from each other. At 12.5°C, the effect of density was not significant (df=2,299 p=.59), although a similar trend was apparent.

To examine variance heterogeneity effects, and to confirm that parametric tests were acceptable, 2-tailed, Mann-Whitney U-test comparisons were calculated for all density combinations at

Figure 5. Effect of fish density on activity levels. Part A is net activity, the tendancy to maintain a fixed position. High values indicate less precise station maintenance. Part B, total activity, is the total distance moved in a given time. All values are given in arbitrary units per 15 second interval.


A
1.6
1.4
1.2
1.0
0.8
NET ACTIVITY
(UNITS/15 SEC)
15.0 °C
12.5 °C
temperatures pooled
(± 95% C.L.)
B
10
8
6
4
2
0
200
400
600
800
1000
1200
TOTAL ACTIVITY
(UNITS/15 SEC)
15.0 °C
12.5 °C
temperatures pooled
(± 95% C.L.)
DENSITY (FISH PER TANK)

each temperature. The results confirmed those listed above.

Water quality

Oxygen levels were determined on days 46 and 61 (Table 7). The pattern in dissolved oxygen concentrations with density was consistent with that expected. The observed levels were close to expected levels when temperature, fish size, and fish number were accounted for. Therefore the models used in determining flows and densities were considered adequate.

On day 46 the lowest oxygen observed was 8.0 mg/l. By day 61 this had declined to 7.1 mg/l. In most of the calculations, the actual oxygen levels were slightly higher than expected. Apparently, the model predicted oxygen consumption rates on the high (conservative) side. Errors in this direction were acceptable.

As expected, oxygen levels were lowest in the high density tanks. While this may be suspected of contributing to reduced growth rates in these tanks, there were no differences in growth rates during the last 70% of the experiment when oxygen demand was greatest. In addition, there was a non-significant tendency for fish from high density tanks at 15°C to grow faster during the final weeks.

Water samples were collected on days 36 and 58 for analysis. The results are presented in Table 7 along with calculated un-ionized ammonia levels. It is clear that metabolite levels were well within safe levels.

Table 7. Water quality data for experiment 1. Un-ionized ammonia values were determined from Liao (1974) using pH and temperature data. Nitrite levels were below detectable levels (<0.005 mg/l).

TANK	TOTAL AMMONIA mg/l	UN-IONIZED AMMONIA mg/l	TOTAL NITRATE mg/l	pH
	DAY 36			
1	0.105	0.0018	5.60	7.8
4	0.069	0.0012	5.80	7.8
7	0.125	0.0022	5.80	7.8
10	0.161	0.0028	5.90	7.8
INFLOW	0.030	0.0005	5.90	7.8

TANK	TOTAL AMMONIA mg/l	UN-IONIZED AMMONIA mg/l	TOTAL NITRATE mg/l	pH	SPECIFIC CONDUCTANCE umho/cm	DISSOLVED OXYGEN mg/l DAY No. 46	61
	DAY 58						
1	0.201	0.0024	4.90	7.6	211	8.8	7.1
2	0.132	0.0017	4.80	7.7	210	9.3	8.5
3	0.045	0.0006	4.90	7.7	209	10.0	9.8
4	0.153	0.0020	5.00	7.7	209	8.8	7.8
5	0.139	0.0017	5.00	7.6	209	9.1	8.2
6	0.044	0.0006	5.00	7.7	208	10.0	9.4
7	0.110	0.0010	5.00	7.6	210	8.5	8.6
8	0.154	0.0014	5.10	7.6	209	8.7	8.0
9	0.064	0.0009	5.10	7.8	209	9.0	8.9
10	0.194	0.0023	5.00	7.6	211	8.0	7.6
11	0.183	0.0022	5.00	7.7	210	9.0	9.0
12	0.041	0.0006	5.20	7.8	210	9.7	10.0

Conclusions

Results of this experiment demonstrated clear treatment-induced differences in growth, proximate composition, and condition factors (tertiary responses to stress). Additionally, density related differences in activity were detected. Plasma cortisol levels (a primary response to stress) showed no evidence of treatment effects. The effect of density varied

between temperatures. At the high temperature, 15°C, effects were usually significant. At the low temperature, the trends observed were usually in the same direction as at the high temperature. Evaluation of the most important water quality data, dissolved oxygen and un-ionized ammonia levels, demonstrated that water quality did not at any time prove either limiting, or alter growth responses.

As expected, temperature effects were significant when all treatments were compared with respect to growth slopes. Additionally, density and time were significant. Several interactions were significant, the two most important being density by time and temperature by time. Together these suggested that the effects of density on growth were different at different times and that the effects of temperature varied over time.

Consequently, growth was analysed separately for each temperature. In these analyses, further significant density by time interaction terms suggested that density effects varied over time. Therefore, data were analysed by time interval grouping.

At the high temperature, density effects on growth were significant only during the first of four time intervals. The short duration of the density effect was indicative of conditioning. About 90% of the differences in final weight could be ascribed to the first time interval.

At the lower temperature, trends similar to those at the higher temperature appeared during the first interval, but were

not significant during that or the remaining intervals. Overall however, growth rates were suppressed at the high density. A non-significant, but persistent trend towards greater growth slopes in the middle density over the low density was observed for most time intervals.

In all growth analyses, there was an unusual pattern with time. Growth rates in all treatments were initially low, increased to a peak by the second interval, and then declined steadily thereafter. The decline with time was not a fish size effect, since growth slope analysis compensates for size. This pattern, as with the short duration density effect, was suggestive of a conditioning effect.

Whole body composition data revealed both density and temperature effects. With temperature entered as a factor in the analysis, significant density effects were observed in moisture, protein and lipids, but not in ash content. In several analyses, the density by temperature interactions were significant, necessitating further analysis by temperature.

At the high temperature, moisture and protein levels were significantly lower, and lipid levels significantly greater, than at the low temperature. Ash was unaffected.

At 15°C, moisture and protein levels increased significantly, and lipid levels decreased significantly, as density increased. As before, ash content was not affected.

At 12.5°C, significant density effects on proximate composition were not found. Although few of the comparisons were significant, some were close, and trends in moisture, protein

and lipid data suggested similar relationships to those at the higher temperature.

With temperatures pooled, there were significant differences in net activity (the tendency to maintain positions) attributable to density effects. However, at each temperature, probabilities while not significant, were close, and the patterns observed were identical. Levels were greatest at the middle density, and lowest at the low density. The consistency of this pattern and the near significance, suggest that the pattern was a real consequence of density, and not a chance occurrence.

Total activity decreased as density increased (significant at 15°C, not so at 12.5°C). The possible significance of these findings in conjunction with the net activity results will be discussed later.

No trends in plasma cortisol concentrations were present. This would indicate that fish were not differentially stressed by different densities, or that if initially stressed, a compensatory adjustment of interrenal activity as described by Schreck (1981) may have occurred. The first hypothesis, that of no stress appears unlikely in view of all other results presented.

Condition factor data showed similar trends to those of growth slope data. At both temperatures, conditions varied significantly with time; the pattern being identical to that for growth slopes. Only at 15°C were density effects significant, condition factors decreasing as density increased. At 12.5°C, no

trend was apparent. The interaction term was significant at both temperatures, and was reflected by a tendency towards delayed peaking at higher densities. From these data it is clear that at any time, a strong correlation between growth slope and condition existed.

In summary, the response to rearing density is different at the two temperatures. At 15°C, there are clear and consistent indications of stress-induced metabolic and behavioral differences associated with density. At 12.5°C, the differences noted, although not statistically significant, followed trends similar to those at 15°C, suggesting that either the magnitude of the response is reduced at the lower temperature, or that its manifestation is delayed.

EXPERIMENT 2: STRESS RESPONSES IN FINGERLINGS

Introduction

This experiment examined the effects of density on growth and stress in juvenile steelhead trout (15 gm initial weight). Weights were expected to double during the experiment and thus enable detection of growth differences and other physiological consequences of density-induced stress.

Since the results of the first experiment suggested that the first few days (or weeks) at a new density (the period immediately after transfer to a test density from a common density) may be most sensitive, the effects of sudden changes in density were examined. If it is is the relative change in density first experienced upon transfer, and not the actual density, that determines the magnitude of the stress response this could have important implications in both hatchery management and in the interpretation of results of density experiments.

Lastly, this experiment examines changes in growth over time to determine if the pattern observed in the first experiment is repeated with larger fish. Since the observed pattern suggests a conditioning effect, sudden density changes should either accelerate or decelerate changes in growth over time.

Materials and Methods

The experiment was started on December 6, 1980. Replicated groups of steelhead juveniles were reared at one of two numerical densities (200 or 800 fish per tank). Initial mean weight over all tanks was 15.1 ± 0.61 gm, (range 13.9-16.1). Tank volumes were adjusted to either 250 or 500 litres. This resulted in four density groups and represented an eight-fold range. On a weight/volume basis this was 6 to 48 gm/l (1/3 - 3 times standard densities). The experimental design is presented in Table 8. All fish used in this experiment had similar rearing histories. On November 13, 1980, about 4 weeks before the start of the study, the fish were transferred from 2 m circular tanks to the oval tanks and held at 12.5 °C. There were approximately 650 fish per tank, corresponding to a density of about 40 gm/l immediately prior to the start of the study. Fish were fed reduced rations until one week before the experiment began, when excess rations were given.

To examine the effects of sudden changes in density on a weight/volume or number/volume basis (not a numerical density basis) three volume treatments were used. In one treatment, tanks initially held at 250 litres were doubled in volume to 500 litres. In a second treatment, the opposite was done. In the third treatment, the control, volumes were unchanged. It was decided that tank volumes would not be adjusted until the pattern in growth slopes with time showed evidence of a decline, similar to that observed in the first experiment. Unfortunately, the number of tanks was limited, and the experimental design

could not be balanced. Changing the available space per fish (density) was expected to alter the rate of change in growth slopes. These changes were expected over density ranges not showing evidence of growth differences prior to volume changes.

Table 8. Design of experiment 2. Tank volumes were changed on day 32 (denoted by the separation in the table). Expected maximum and final densities were calculated according to Iwama and Tautz (1981) using T/1000.

		FIRST				SECOND				
		FIRST	---DENSITY---			SECOND	------DENSITY-------			
TNK	NUMBER FISH	VOLUME litres	INITIAL f/l	gm/l	MAX gm/l	VOLUME litres	-INITIAL- f/l	gm/l	FINAL gm/l	AVE f/l
1	200	250	0.8	12	18.8	500	0.4	9.4	12	0.6
2	800	250	3.2	48	75.2	500	1.6	37.6	49	2.4
3	200	250	0.8	12	18.8	500	0.4	9.4	12	0.6
4	800	250	3.2	48	75.2	500	1.6	37.6	49	2.4
5	200	500	0.4	6	9.4	500	0.4	9.4	12	0.4
6	800	500	1.6	24	37.6	500	1.6	37.6	49	1.6
7	200	500	0.4	6	9.4	500	0.4	9.4	12	0.4
8	800	500	1.6	24	37.6	500	1.6	37.6	49	1.6
9	200	500	0.4	6	9.4	250	0.8	18.8	24	0.6
10	800	500	1.6	24	37.6	250	3.2	75.2	98	2.4
11	200	500	0.4	6	9.4	250	0.8	18.8	24	0.6
12	800	500	1.6	24	37.6	250	3.2	75.2	98	2.4

All fish were reared at 12.5°C Photoperiod was set at 10L:14D. Lights were automatically switched on at 0730 hours and off at 1730 hours. All tanks received flows of 25 l/min. Since an optimistic projected growth rate was used, maximum density estimates were conservative. Feeding rates were calculated as for the previous experiment.

The design of the experiment necessitated that several different analyses be run on the same data, but on different

groupings. Part of the experiment was analyzed in the same manner as the first experiment. Details will be given where appropriate.

Total weights were measured on days 1, 10, 17, 31, 46, and 53. Length and weight data were taken on days 3, 10, 31, 46, and 53. During the course of the experiment, growth slopes were plotted against time. At the first indications of the anticipated changes in growth pattern tank volumes were altered. The trend was detected on day 31, and changes were made on day 32.

Plasma samples for cortisol analysis were collected on day 56. After two days of starvation, on day 58, samples were collected for proximate composition. Dissolved oxygen levels were monitored on two days, 41 and 49, using Winkler reagents. Water samples were collected for analysis of pH, metabolites, etc. on the same days. Behavioral observations were not recorded. Mortalities were recorded daily but were rare. Temperatures and flows were checked every 2-3 days.

Results

Growth

During the course of this experiment some data were lost due to technical difficulties. During the first time interval, feeder problems in tank 2 resulted in the loss of growth slope data for that period. More persistent feeder problems in tank 5 resulted in the loss of growth slope information for three

intervals. Changes in adjusted weights over time are given in Figure 6.

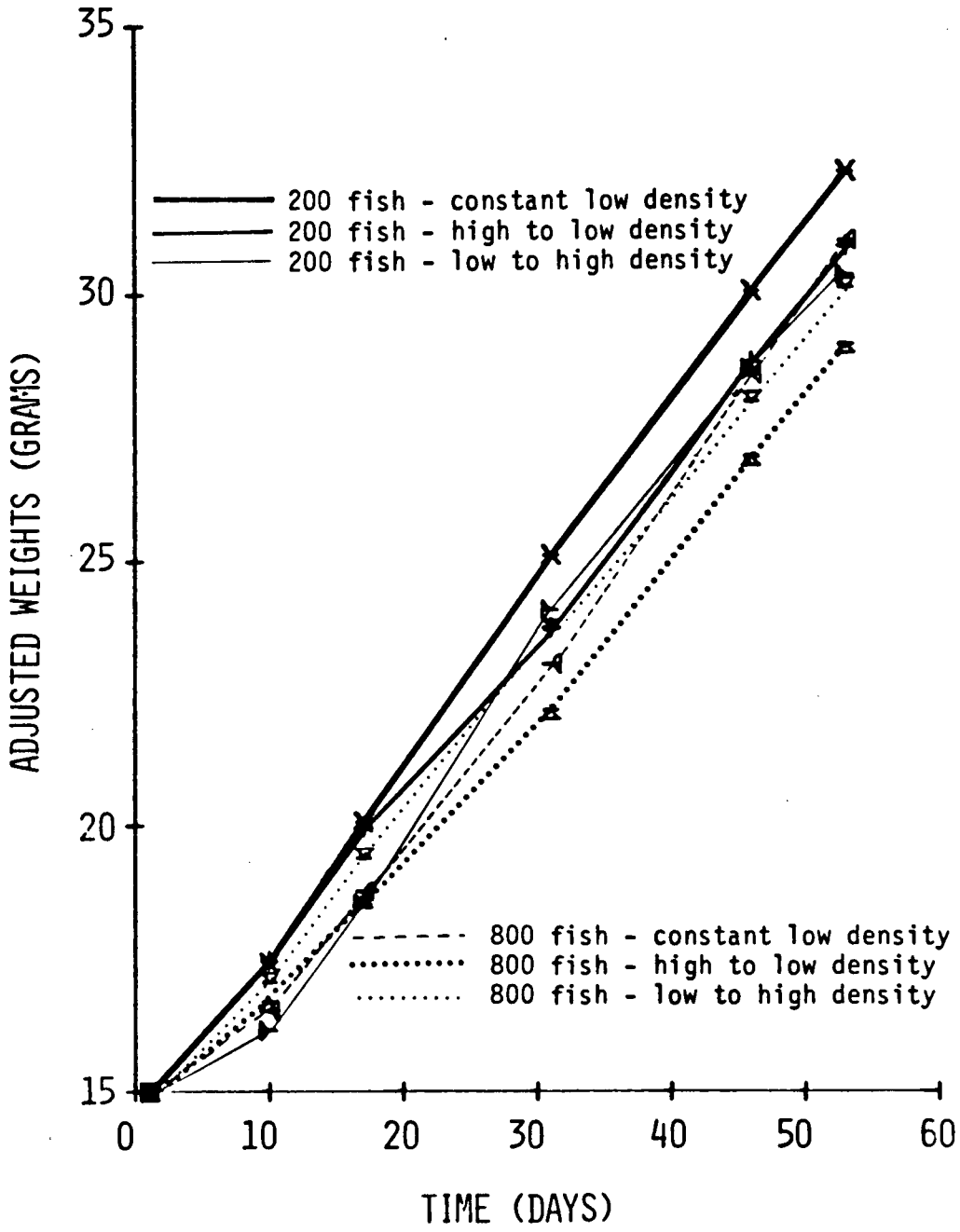
Growth slopes in constant density treatments

To examine the effects of constant density on growth slopes, the control tanks (5,6,7,8) were analyzed separately. On both a weight/volume and number/volume basis, these treatments spanned most of the range tested in the first experiment. On a fish/litre basis, density ranged from 0.4 to 1.6 (about 0.33 to 1.3 times conventional maximum hatchery densities). The data were analyzed in a 2-way ANOVA. Density effects were not significant ($df=1,16$ $p=.11$). However, fish in the high density treatment appeared to grow at 90% of the rate of the low density treatment. Although the low density treatment reached a higher peak growth slope, there was a trend for a more rapid decline in growth slope with time than at the high density. For such a trend to be detectable statistically, the interaction term would have to be significant. It was not ($df=4,16$ $p=.11$). The effect of time, however, was ($df=4,16$ $p<.05$) and the pattern closely followed that expected.

Growth slopes (average densities)

Growth rates were analysed based on average density groups (Table 8, last col.). Accordingly there were six treatments, each defined by one of two numerical densities and one of three volume treatments. It was not possible to enter both fish number and volume into an ANOVA since they covaried almost perfectly.

Figure 6. Change in weight with time for experiment 2. All weights have been adjusted to a common initial size using an initial weight of 15 grams and the growth slopes by time interval.


35
30
25
20
15
0
10
20
30
40
50
60
ADJUSTED WEIGHTS (GRAMS)
TIME (DAYS)
200 fish - constant low density
200 fish - high to low density
200 fish - low to high density
800 fish - constant low density
800 fish - high to low density
800 fish - low to high density

Following the work of Li and Brocksen (1977) it was assumed that volume effects would be minimal (General Discussion). Therefore, when volumes were changed, it was assumed that any effects were due to density changes. The data were entered in a 2-way ANOVA (average density, time) (Table 9). Average density, time and the interaction terms were all significant. As average density increased overall growth slope decreased, although the pattern was not consistent. Scheffe's test indicated that only the tanks containing 200 fish (constant density) and those containing 800 fish in which densities were doubled, were different. The interaction suggested that the effect of density changed over time, however this could have been a function of sudden density changes. The overall pattern with time was as expected -- low initially, rising to a peak, and then declining. Differences between times were of sufficient magnitude that Scheffe's test produced 3 homogeneous groups. When individual treatments were examined, patterns over time did not always follow this trend (Figure 7). This probably reflected the sudden changes in density.

Growth before density changes

To examine growth diffences before tank volumes, and therefore weight densities were changed, the first three intervals were analyzed separately. There were four groups covering an 8-fold density range (Table 8, col. 4), either 200 of 800 fish, and either high or low volumes. The data were entered in a 2-way ANOVA. Density effects were significant

Figure 7. Growth slopes against time (mid-point of sampling interval) for experiment 2. Growth slopes were calculated according to Iwama and Tautz (1981). Vertical bars are standard errors.

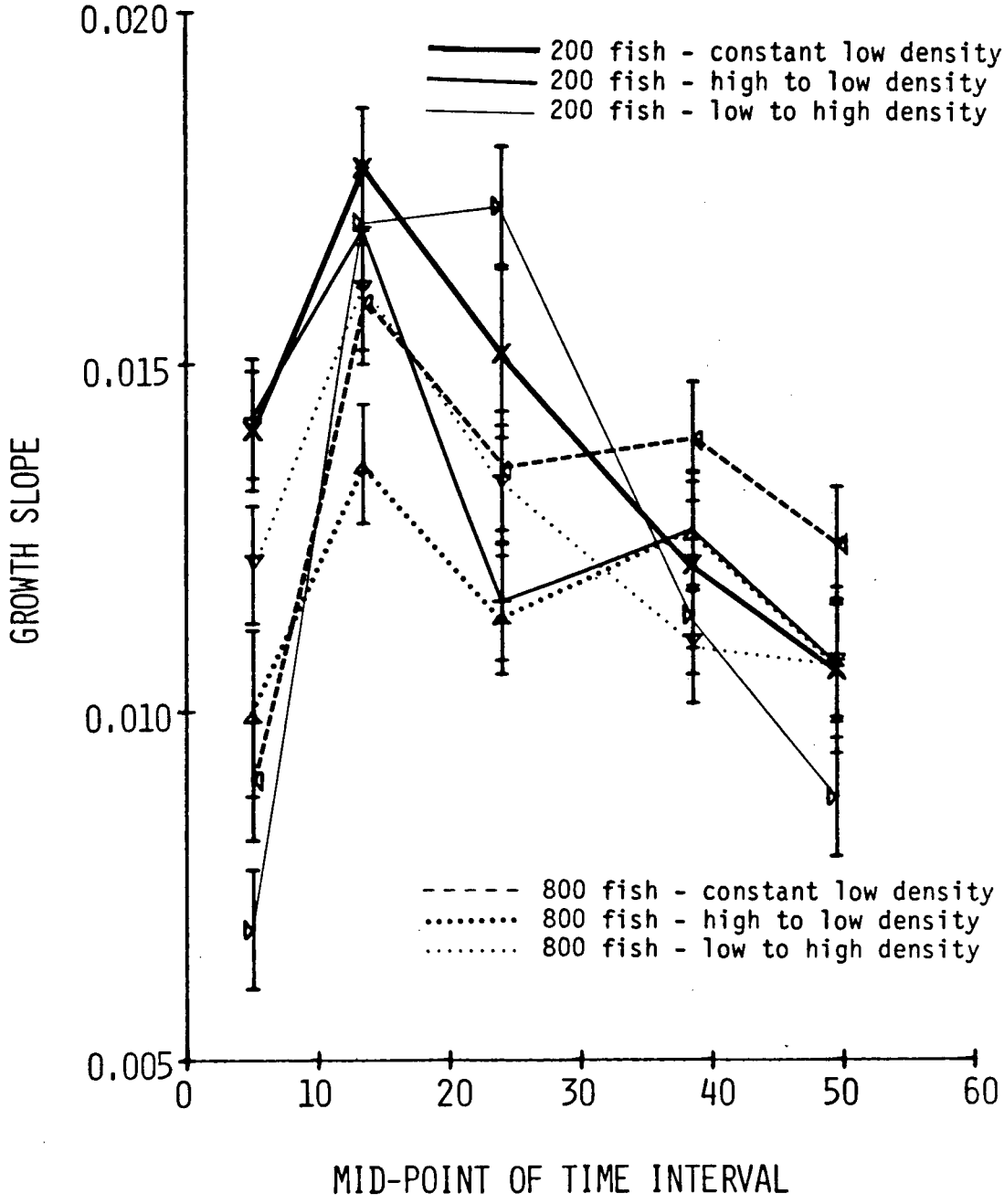

0.020
0.015
0.010
0.005
GROWTH SLOPE
200 fish - constant low density
200 fish - high to low density
200 fish - low to high density
800 fish - constant low density
800 fish - high to low density
800 fish - low to high density
0
10
20
30
40
50
60
MID-POINT OF TIME INTERVAL

Table 9. Analysis of variance of growth slope data for experiment 2. Average density treatments were entered into the analysis (Table 8, last column). Tests are considered significant if $p < 0.05$.

SOURCE	DF	MEAN SQUARE	PROBABILITY
Density	5	0.00000637	0.00483
Time	4	0.00005694	0.0
Density*Time	20	0.00000731	0.00080
Residual	26	0.00000144	-
Total	55	-	-

(df=3,33 p<.05). With each increase in density, within the lowest three groups, there was a slight reduction in mean growth slope. However, between third highest (1.6 f/l) and the highest (3.2 f/l), the decline was marked. This suggests a threshold effect. The effects of time were significant (df=2,33 p<.001), and the means of each of the three times entered were different. The interaction term, though not significant was close (df=6,33 p=.10), suggesting that it may have been the change in density inherent in the previous analysis on all 6 treatments that caused the significant interaction effect.

Growth after density changes

To examine growth changes after sudden density changes, the last two times were examined separately. Four new density groups (Table 8, col. 8) were compared. Density effects were significant (df=3,21 p<.05), as were the effects of time (df=1,21 p<.005). As before, the pattern showed a reduction in growth slope with increases in density. The more rapid decline in growth rates at the lower numerical densities was especially

apparent. The interaction term was not significant (p=.54).

Did the changes in density alter growth slopes to a greater extent than expected on the basis of density alone? Recalling that the interaction effects were significant when average treatments were compared over all times, but not when densities were analyzed before and after volume changes, it is apparent that the density changes had altered growth. To test whether growth changes were less than or greater than that expected from density effects alone, those tanks in which volumes were not changed were used as controls. Changes in growth slope over time could therefore be compared with expected changes. Linear regressions for the last three intervals were calculated for various treatment combinations, and compared using equality of slope tests.

First, the controls were compared, and as expected from the earlier analysis, their slopes were not different (p=.09). However, it appeared that the rate of decline in growth slopes was accelerated at the lower density. Unfortunately, data from one of the controls were missing, reducing the sensitivity of subsequent comparisons.

When the low density controls (200 fish, constant volume) were compared with the low density treatment in which volumes were increased (densities decreased, i.e. both had equal densities at time of comparison), slopes were not significantly different. However, there was a trend towards a less steep decline in growth slope in the group which had undergone the sudden density reduction, and in fact, in the first interval

following density changes, growth rates actually increased.

When the same controls were compared with the low density treatment in which volumes were halved, slope differences were significant ($p<.05$). The pattern was consistent with the prediction, i.e. an increase (2-fold) in the rate of decline of growth slopes with time. Although this comparison was between two different densities, it is unlikely that the absolute density difference was important, since in none of the earlier analyses were there significant difference in growth over this density range. Decreasing the space per fish (increasing the density) after the fish had been accustomed to one density, appeared to affect growth more than one would expect on the basis of density alone.

In neither comparison at the high numerical density, were slopes significantly different. In both, however, trends were in the expected direction.

If conditioning had no effect, it would seem improbable that when densities were decreased (i.e. volumes increased), there would be a consistent (although non-significant) trend for faster growth than the controls that had continuously experienced the same density.

Proximate analysis

Statistical analyses were performed on average density groupings. In all analyses, weight was used as a covariate.

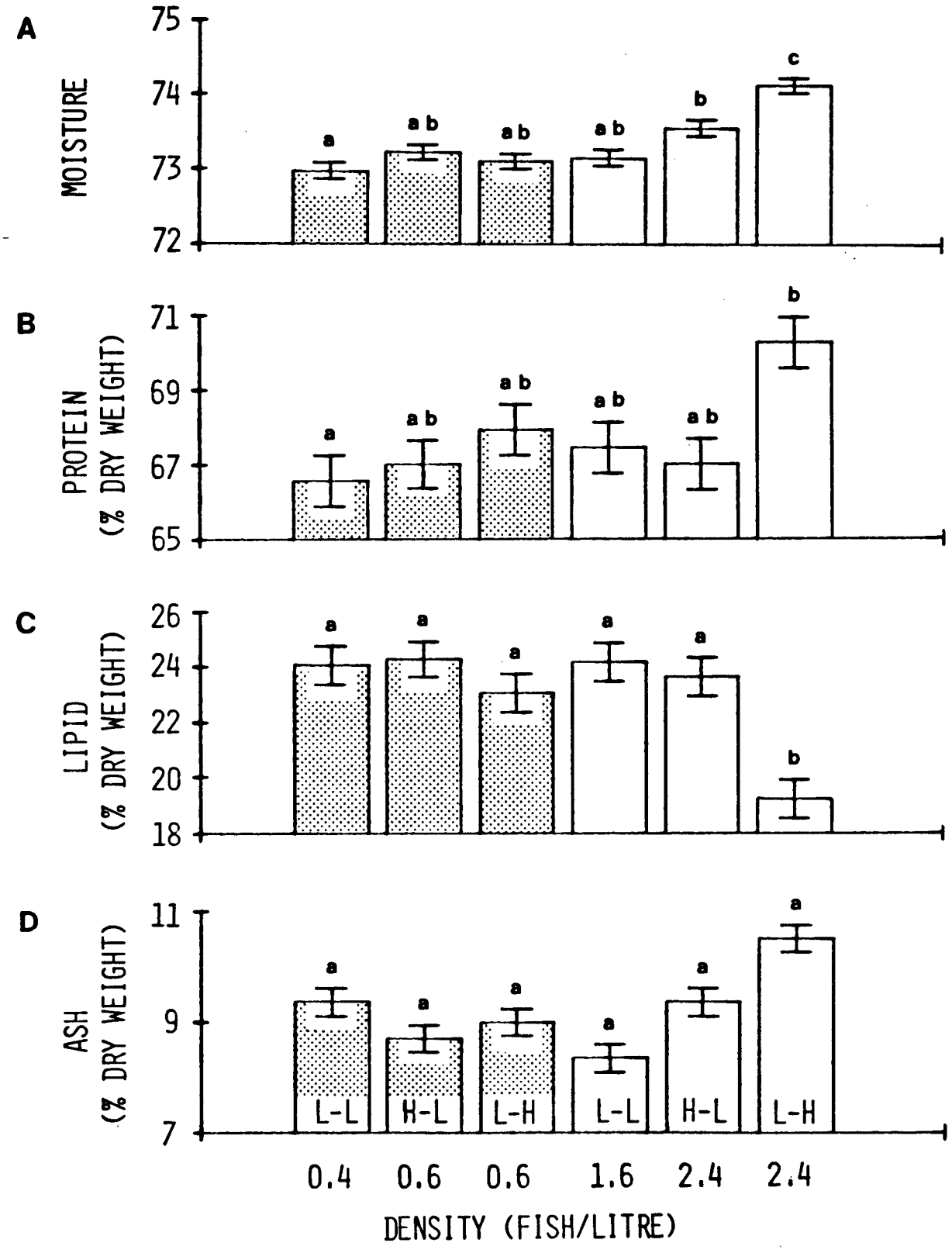
Moisture

The results of a 1-way nested ANOVA with weight as a covariate for the 6 average density treatments are presented in Figure 8a. The effects of density were significant ($df=5,49$ $p<.001$). As average density increased, so did moisture. The effect of the covariate, weight, was significant ($df=1,49$ $p<.001$), indicating that moisture levels were affected by fish weight. The equality of slope term, defined by the interaction of the covariate with density, was also significant ($df=5,49$ $p<.005$). This suggested that the effect of weight was not the same for all treatments. Consequently, it is likely that significant effects between treatments would disappear as their covariate - density regression lines approached and crossed each other. However, since the mean sample weights were close, the observed density effect is likely real.

Protein

The results of the 1-way, nested ANOVA with weight as a covariate, produced significant density effects ($df=5,49$ $p<.05$). Protein levels tended to increase with average density, although the pattern was irregular (Figure 8b). At the time that samples were collected, one of the high density treatments had a density of 3.2 f/l, even though its average density was 2.4 f/l. The other high density treatment, which had a density of 1.6 f/l at the time of sampling, had lower protein levels. Since both had similar average densities, this suggests that the metabolic

Figure 8. Histograms of proximate composition data for experiment 2. A-moisture, B-protein, C-lipid, D-ash. Stippled bars are treatments containing 200 fish. Error bars are included. Bars bearing common small-case letters are not statistically different (Duncan's or Scheffe's multiple range tests - 5% level). Upper case letters at the base of the bottom graph indicate density treatments (e.g. H-L means initially high density, then decreased).


A
MOISTURE
75
74
73
72
a
a b
a b
a b
b
c
B
PROTEIN
(% DRY WEIGHT)
71
69
67
65
a
a b
a b
a b
a b
b
C
LIPID
(% DRY WEIGHT)
26
24
22
20
18
a
a
a
a
a
b
D
ASH
(% DRY WEIGHT)
11
9
7
a
a
a
a
a
a
L-L
H-L
L-H
L-L
H-L
L-H
0.4
0.6
0.6
1.6
2.4
2.4
DENSITY (FISH/LITRE)

consequences of density on body composition appear quickly when densities increase, and also may disappear quickly when densities are reduced. As with moisture, both the covariate and the equality of slopes test terms were significant ($df=1,49$ $p<.05$ and $df=5,49$ $p<.005$). The pattern in covariate slopes suggested that at high densities, within tank size variations had a greater effect on protein levels than in the low density tanks. This may be indicative of greater differences in levels of stress within high density tanks than within low density tanks.

Lipids

The results of the lipid analysis (Figure 8c) were less clear. This was due to a higher degree of variability between replicates.

In the 1-way nested ANOVA with weight as a covariate, only the replicates term was significant ($df=6,49$ $p<.01$), although the effects of both density and the covariate were nearly so ($df=5,49$ $p=.09$; $df=1,49$ $p<.09$). Upon visual inspection it was clear, however, that differences did exist especially between the high density treatment, in which volumes were halved, and all others. There was a trend towards a reduction in lipids at high densities.

Ash

As with the previous analysis for lipids, differences between replicates were greater than between treatments. Unlike the lipid analysis, the probability value for the density effect was not significant. There were no trends in the data (Figure 8d).

Cortisol

Eight plasma samples from each tank were assayed in duplicate. This resulted in 16 cortisol determinations per tank. In all cases, duplicates were successfully analysed and there was, therefore, no need to pool duplicates.

Before the data were analyzed statisticaly, plots were made of cortisol values against both sample weight and sampling time. There was a trend towards increased cortisol levels as sample time progressed. However, within tank variation was large making it difficult to apply a correction factor. Since sample processing times were close (within 1 min), the effect was probably constant between tanks. No trends in cortisol with fish weight were apparent.

Data were analysed in a 1-way, nested ANOVA (6 average density treatments, 2 replicates). The treatment groups were those described previously analyses (Table 8, last col.). There were no effects of average density on plasma cortisol concentrations. The replicates term was significant. Bartlett's test indicated a marked heterogeneity of variance between

Table 10. Summary of cortisol data. There was no evidence of significant differences between treatments. Values are given in ng/ml. Samples sizes in all cells were 16. Standard errors (of means) were 0.71.

fish/tank	200		800	
replicate	1	2	1	2
high to low density	15.1	11.3	11.4	12.4
constant density	12.3	12.6	14.9	12.1
low to high density	12.0	12.5	12.3	15.4

treatments. Several transformations were tested to eliminate this problem, but none were successful. Consequently, non-parametric comparisons were performed on the data with replicates pooled. Only 4 of the 15 Mann-Whitney U-test comparisons indicated significant differences. However, there were no patterns in the data (Table 10) suggesting the differences were possibly chance occurences and were therefore considered unimportant.

Weight length relations

Statistical treatment of the length-weight data was the same as that outlined in the first experiment. In calculating the linear regression relationship between ln weight and ln length, data from time intervals where feeder problems occurred were excluded. Consequently, 2650 of 3000 data pairs were accounted for in the equation.

A functional GM regression of natural log of length against natural log of weight was calculated. The data fit was good (r^2 = .93). The equation of the line was:

$$\ln Wt. = -4.317 + 2.956 * \ln L.$$

Rearranged and antilogged, the equation becomes:

$$\text{condition factor} = \text{weight}/(0.0133398 * \text{length}^{2.956}),$$

which is the working equation.

Table 11. Condition factor data summary. Values are given for treatment groups on the basis of average density (see Table 8). Densities were changed on day 32 as described in the methods section. Replicates were not significantly different and were therefore pooled. Letters in column 2 refer to relative density changes. For example, H-L, means initial density was high, and then on day 32 it was halved.

AVE f/l	DENSITY TREAT.	DAY 3	10	31	46	53	n	S.E.
0.4	L-L	0.989	0.985	1.017	0.981	1.016	50	0.0096
0.6	H-L	1.003	0.995	1.017	0.996	1.030	100	0.0068
0.6	L-H	0.984	1.010	1.014	0.997	0.983	100	0.0068
1.6	L-L	0.988	0.980	1.015	1.009	1.026	100	0.0068
2.4	H-L	1.009	1.014	1.024	1.010	1.041	50	0.0096
2.4	L-H	0.988	0.991	1.025	0.996	1.024	100	0.0068

Condition factor data

The condition data (Table 11) were analysed in a 2-way ANOVA (average density, time), with replicates pooled. Density (df=5,2499), time (df=4,2499) and the interaction (df=20,2499) terms were significant ($p<.001$). The range in mean values was small, and surprisingly, the lowest mean value was found in the lowest density treatment (200 fish, constant volume), while the highest was found in one of the treatments containing 800 (low to high volume). Only these treatments were significantly different. Notably, this pattern was in the opposite direction

to the one observed in the first experiment. There was no clear pattern with density within the other treatments tested.

The pattern with time did not follow that in growth slopes as had been the case in the first experiment. However, during the first part of the experiment, before volumes were adjusted, the two variables followed each other closely. The significant interaction was attributable to the sudden changes in density.

Water quality

Oxygen levels in the high density tanks were measured three times on day 41, between 1000 and 1500 hours. Differences between times were small (Table 12). Since this time interval corresponds to the time of day when oxygen levels should be maximally depressed due to feeding, activity, and specific dynamic action, it is unlikely that growth was constrained by oxygen levels. Furthermore, this supports the view that single-point-in-time measures of oxygen levels were adequate for the purpose intended. Additional measures were collected near the end of the experiment when oxygen demand should have been greatest. In all tanks, levels were above the minimum acceptable levels.

The results of the laboratory analysis of water samples collected on day 41 (Table 12) also indicate that water quality was acceptable and unlikely to have affected either growth or stress response. Un-ionized ammonia levels were well below the guideline maximum discussed in the General Methods. It was unlikely that, in the remaining days of the experiment,

Table 12. Water quality data summary for experiment 2. To investigate variations during the time of day when oxygen demand is greatest, oxygen levels were determined at three times on day 41. Water samples for laboratory analysis were collected the same day. Oxygen data were again collected on day 49. Nitrite levels were below detectable levels (<0.005 mg/l). Un-ionized ammonia levels were determined from Liao (1974).

	DAY 41					
TANK	TIME hrs.	OXYGEN mg/l	TIME hrs.	OXYGEN mg/l	TIME hrs.	OXYGEN mg/l
2	10:14	8.35	13:00	8.50	15:22	8.60
4	10:44	8.48	13:00	8.20	15:25	8.50
6	10:44	7.75	13:00	7.80	15:29	7.40
8	11:16	7.10	13:00	7.10	15:20	7.00
10	11:16	7.00	13:00	7.30	15:15	7.60
12	11:16	7.60	13:00	8.30	15:17	7.80

	DAY 41						DAY 49
TANK	TOTAL AMMONIA mg/l	UN-IONIZED AMMONIA mg/l	TOTAL NITRATE mg/l	pH	SPECIFIC CONDUCT umho/cm	TURBIDITY J.T.U.	DISSOLVED OXYGEN mg/l
1	-	-	-	-	-	-	9.75
2	0.260	0.0018	5.65	7.5	219	1.5	-
3	-	-	-	-	-	-	9.90
4	0.306	0.0021	5.70	7.5	220	1.5	-
5	-	-	-	-	-	-	-
6	0.464	0.0032	5.65	7.5	225	4.1	-
8	0.470	0.0033	5.70	7.5	224	2.7	-
10	0.414	0.0024	5.70	7.4	221	3.5	-
12	0.265	0.0019	5.75	7.5	219	1.4	-

metabolite levels increased sufficiently to alter this conclusion.

Conclusions

The results of this experiment were less clear than those of the previous experiment. However, there was evidence of a stress-related response to density. As in the first experiment, plasma corticosteroid levels, a primary level stress indicator, showed no evidence of density effects. However, both growth slopes and proximate composition, tertiary level indicators, produced results that suggest increased stress at high densities. Condition factor results were inconclusive, and if decreased body condition is truly associated with stress, these results were somewhat contradictory. The curvilinear growth pattern observed in the first experiment was repeated in these data. This supports the idea that fish become conditioned to their rearing environment. It also removes doubt that the trend with time was an artifact of the model. Additionally, after a period of acclimation to rearing densities, sudden changes in density tended to alter the growth response more than expected on the basis of density alone. This further supports the idea of a conditioning influence on growth response. Inspection of the water quality data indicate that oxygen and metabolite levels did not influence the results.

Density effects on growth were not observed when treatments containing 0.4 and 1.6 fish/litre were compared; however, the expected pattern over time occurred. When all 6 treatments were compared, only the lowest and one of the highest densities were different. In treatments with similar average densities, increasing densities mid-way through the experiment produced

lower overall growth rates than those in which densities were decreased. This suggests that decreasing the space per fish after a period of acclimation has a greater effect on growth than increasing the space after a similar acclimation period. In all comparisons growth rates responded in the expected direction to changes in density (although 3 of 4 of the tests were not significant).

Changes in whole body composition with average density followed the same pattern as in the previous experiments. However, the data were less clear. As average density increased, moisture and protein levels tended to increase. Although no significant effects of density on lipid content were found, there was evidence of reduced levels at high densities. No effects on ash content were detected.

There were no trends in plasma corticosteroids associated with average density.

Significant differences in condition data were detected with both density and time. As discussed earlier in this section, the results were contradictory. It appears that for fish 15-30 gm in weight, over the density range tested, body condition may not be a useful indicator of density-induced stress.

EXPERIMENT 3: STRESS RESPONSES IN SMOLTS AND PRE-SMOLTS

Introduction

This experiment was divided into 3 sections in which the responses to rearing density, tank volume, flow rate and sudden density changes were examined. Initial weights averaged 30 grams. The period over which these studies were conducted corresponded to the last few months of rearing before release. This is probably the most critical period in the rearing process. From February until April, wild stream dwelling populations of juvenile steelhead of appropriate size undergo the process of smoltification. This process involves biochemical, physiological, morphological and behavioral changes. Hoar (1976), Folmar and Dickhoff (1981), and Wedemeyer (1981) have described these changes (Table 13). These, and other authors have emphasized the sensitivity of the smoltification process to hatchery rearing practices. The experiments described here examined stress responses during this period.

The first of these (3a) compared stress-related responses at two widely different and constant densities. The second, (3b), was a partial repetition of the second major experiment. The third, (3c), examined the effects of tank volume at equal densities and loading rates, on growth and stress response. Although Li and Brockson (1977) found no volume effect on growth of rainbow trout, other authors have detected such an effect (General Discussion). Most of these studies were not conducted under hatchery conditions. Aulstad and Refstie (1975) noted

Table 13. Physiological changes associated with the parr-smolt transformation in salmonids (from Wedemeyer et al. 1980).

PHYSIOLOGICAL CHARACTERISTICS	LEVEL IN SMOLTS COMPARED WITH PARR
- body silvering, fin margin blackening	increases
- hypoosmotic regulatory capability	increases
- salinity tolerance and preference	increases
- weight per unit length (condition factor)	decreases
- growth rate	increases
- body total lipid content	decreases
- oxygen consumption	increases
- ammonia production	increases
- liver glycogen	decreases
- blood glucose	increases
- endocrine activity	increases
thyroid (T4)	
interrenal	
pituitary growth hormone	
- gill microsome Na+ K+ -ATPase enzyme activity.	increases
- ability to grow in full strength sea water	increases
- buoyancy	increases
- migratory behavior	increases

significant differences in growth rates in coho reared at different tank depths, but they did not detect this effect in rainbow trout.

The null hypotheses for each of the experiments are that there will be no differences, attributable to treatment effects, in any of the variables measured.

Materials and methods

These experiments began on 13 February 1981, and were designed s a continuations of the first two experiments. Maximum permissible densities were calculated as before. All fish used in these experiments were held in circular tanks at 9.5 to 10 °C for at least two weeks prior to the start of this study. All fish were fed full rations during the final week. Rearing densities during this period were 20-30 gm/l.

In the first experiment, (3a), a simple density study was conducted in which the effects of widely varying densities on growth and stress in presmolts were examined. Initial fish weight averaged 30-32 grams. The rearing temperature was 9.5°C Tank volumes were adjusted to 350 l, and the flows were set at 24 l/min. These conditions allowed a maximum density of 400 fish per tank. This corresponded to a maximum expected density about 3.8 times the maximum density commonly used at the hatchery. The low density (50 fish) was one-eighth the high density and had a projected final density half of traditional levels. While an intermediate density was desirable, it was more important to ascertain the effects of flow rates on growth since, due to water supply constraints, the other small-scale experiments would have different flows. Therefore, two more tanks, each containing 50 fish, received a flow of 10 l/min. The design of this experiment is summarized in Table 14, tanks 1-6. Photoperiod was set at 10L:14D.

In the second study, (3b), as in the second major experiment, the effects of changes in density were examined. All

Table 14. Design of experiment 3. Tanks 1-6 (upper table), comprised study 3a. Tanks 13-16 (upper table), comprised study 3c. Tanks 7-12 (lower table) comprised study 3b. All tanks were held at 9.5 C. Flow rate in study 3b was 18 l/min.

TANK	NUMBER FISH	VOLUME litres	FLOW l/min	DENSITY -- INITIAL -- fish/l	DENSITY -- INITIAL -- gm/l	DENSITY FINAL gm/l	PHOTOPERIOD hours light
1	400	350	24.0	1.14	34.2	62.7	10
2	50	350	24.0	0.14	4.2	7.8	10
3	50	350	10.0	0.14	4.2	7.8	10
4	400	350	24.0	1.14	34.2	62.7	10
5	50	350	24.0	0.14	4.2	7.8	10
6	50	350	10.0	0.14	4.2	7.8	10
13	250	250	12.5	1.0	30.0	55.0	14
14	250	250	12.5	1.0	30.0	55.0	14
15	500	500	25.0	1.0	30.0	55.0	14
16	500	500	25.0	1.0	30.0	55.0	14

TNK	FISH	FIRST VOLUME litres	--DENSITY-- INITIAL f/l	INITIAL gm/l	FINAL gm/l	SECOND VOLUME litres	----DENSITY---- INITIAL f/l	FINAL ----	AVE gm/l----	PHOTO hrs light
7	300	500	0.6	18	27	500	0.6	27	33	0.6	10
8	300	500	0.6	18	27	500	0.6	27	33	0.6	10
9	300	500	0.6	18	27	250	1.2	54	66	0.9	10
10	300	500	0.6	18	27	250	1.2	54	66	0.9	10
11	300	250	1.2	36	54	500	0.6	27	33	0.9	10
12	300	250	1.2	36	54	500	0.6	27	33	0.9	10

tanks contained 300 fish, and received flows of 18 l/min at 9.5°C. Again, three replicated treatments of 2 tanks each, were used. As before, two tanks were held at a constant volume of 500 l throughout the study. Two tanks had their volumes halved (i.e. densities doubled) midway through the study, and two others were given the reverse treatment. This experiment is summarized in Table 14, tanks 7-12. Based on projected final weights, densities would approach 66 gm/l in the high density (low volume) tanks, and half that in the low density tanks.

Photoperiod was set at 10L:14D.

In the third small-scale study, (3c), the effects of different tank volumes on growth and stress were investigated. Both tanks had identical densities (on a gm/l basis), and were equally loaded (on a gm/l/min of inflow basis). To achieve similar loading rates flows differed by a factor of 2. Tanks containing 500 litres had 500 fish and tanks containing 250 litres had 250 fish. Expected final density in all tanks was about 55 gm/l. Pertinent details of the design of the experiment are given in Table 14, tanks 13-16. This study, unlike all the others, was conducted in the wet laboratory, not in the quarantine room. Photoperiod here was set on a 14L:10D cycle. This study was important since interpretation of the results of experiment 3b assumed no volume effects. Unfortunately, insufficient numbers of progeny of 3-ocean adults were available for this study. Therefore, it was necessary to include 2-ocean fish. This effect is considered in the analyses.

Since most tanks received well water directly, temperatures were measured infrequently. Well water temperature decreased throughout the experiment from 9.5 to 9.2°C. Because of water shortages some tanks received water from several sources. These tanks were monitored more frequently, and temperatures rarely fluctuated by more than 0.3°C from the well source.

The quantity of food and frequency of feeding were determined as before. For the first 8 days of the experiment, all fish received Abernathy 6/64th inch crumbles. After this date 3/32 pellets were used which, because of their different

shape, were larger.

Total weights were collected on days 1, 11, 18, 32, 39, 48, 54, 61 and 72. Not all tanks were measured on each of the specified days. Length-weight data were collected on the following days: 1, 8, 18, 32, 61 and 72. No measurements were collected between days 32 and 61 since fish were frequently being sampled for cortisol and it was desirable to minimize handling stress. In experiment 3b densities were adjusted on day 48.

To assess cortisol concentrations mid-way through the experiments, and to obtain pre-density adjustment data for study 3b, blood samples were collected on day 47. To assess the effects of sudden density changes, samples were again collected from study 3b tanks on day 54. All tanks were sampled again on day 74.

Samples were collected on days 47 and 74 for proximate analysis. Since the growth experiments were still in progress on day 47, it was not appropriate to starve fish before sampling. However, the effect of food in the stomach should have been both small and constant between treatments. Fortunately, these samples enabled inspection of stomach content fullness. All fish sampled had full stomachs. For logistic reasons, only samples collected on day 47 were processed.

Behavioral observations were recorded on video on days 17, 31, 45, 52, 59, and 73. Technical problems on day 17 resulted in a poor film and these data were excluded from the analysis.

Water quality was monitored less frequently in these

experiments than in the previous ones. This was acceptable for two reasons. First, results of the first two experiments suggested that the method of calculating maximum densities was conservative. Secondly, the maximum densities actually used in these last experiments were only 70-80% as high as predictions allowed. Consequently, samples were collected at the end of the experiment from the high density treatments only. In addition however, oxygen levels were periodically checked. At all times, oxygen levels were satisfactory. Oxygen data for day 73 are summarized in Table 15.

Table 15. Summary of dissolved oxygen data for experiment 3. Measurements are presented for day 73.

TANK	TIME hrs.	OXYGEN mg/l	TANK	TIME hrs.	OXYGEN mg/l
1	12:45	8.80	9	11:05	8.35
2	13:15	10.95	10	11:30	9.40
3	13:15	10.75	11	11:30	8.50
4	13:55	9.15	12	12:35	8.90
5	13:55	10.90	13	15:00	8.30
6	14:22	10.90	14	15:00	8.50
7	10:40	9.20	15	15:00	8.60
8	10:52	9.00	16	-	-

The growth phase of the experiments ended on day 74 (27 April 1981); however, feeding continued and on May 6 (day 82) groups of fish were given a salt water challenge test.

Salt water was not available at the hatchery and transporting test fish to Vancouver may have produced stress. Transport stress has been shown to reduce the ability of smolts to regulate salts in a hyperosmotic environment (Iwama 1979).

Consequently, the test was conducted at the hatchery using artificial sea water.

Ideally, a flow-through salt water system should have been used, or a closed system equipped with bio-filters. Neither were available, and it was therefore necessary to use a static bath. To minimize the liklihood of metabolites reaching stressful levels, ammonia production rates were approximated using information presented in Brett and Zala (1975) and the oxygen model (Tautz, pers. comm.). The effects of fish size, temperature, pH, carbon dioxide production, and the additional stress due to salt water were considered. With this information the minimum required volume of water for the test was approximated.

A vexar mesh holding cage, that allowed free circulation of water within compartments, was constructed. Adjacent compartments were separated with black polyethylene sheets. This enabled fish to be removed from one section without disturbing neighbours. Minimizing stress during sampling was important since catecholamine release associated with stress can cause rapid shifts in gill permeability and osmoregulatory performance (Eddy 1981). Individual compartments were approximately 30 cm wide by 27 cm across by 50 cm deep. Temperature was set at 9.5°C, and to prevent it from increasing to ambient air temperature, a heat-exchanging coil was improvised to circulate chilled water.

The artificial salt used was "Forty Fathoms Marinemix" brand. The chemical composition is provided in Appendix I. A

hydrometer (Fisher Scientific), accurate to ± 0.1 °C, was used to check salinities. Table 2.1 in Riley and Chester (1977) was used to correct for temperature effects on specific volume anomaly. The salt solution was aerated vigorously for 24 hours prior to introducing the test fish and less vigorously after.

The test procedures corresponded closely to those described by Iwama (1979) and Clarke and Blackburn (1977). Fish were not fed for 24 hours prior to testing. Fish to be used in the challenge were not anaesthetized, but were individually weighed in a pre-tared container of water. All fish from one treatment were held and introduced into the salt bath as a group. Twenty fish from each treatment were used in the test. Time of introduction was recorded.

As near to 24 hours as possible, fish were removed from the appropriate compartment, and placed in a bucket containing salt water and a strong solution of anaesthetic. Before sampling, the caudal peduncle was dried to prevent dilution of the plasma. Plasma was collected with either syringes or microcapillary tubes. Both had been treated with cation-free heparin. Samples were centrifuged and iced.

Plasma sodium levels were determined on samples diluted with a lithium standard, and measured in an Instumental Laboratories Inc., model 443 flame photometer. Zero and 140 meq Na^+ /l solutions were used as standards. Samples were assayed in duplicate. If variation between duplicates was greater than 10 units, they were re-run, and if differences were still large, they were discarded.

Results

Growth

Experiment 3a

In this experiment, the effects of density and flow on stress response were examined (Table 14). Feeder problems during four of the first eleven days necessitated the deletion of growth slope information from tanks 5 and 6 for that interval. Changes in adjusted weights over time are given in Figure 9.

A 2-way ANOVA (Table 16) was calculated for the growth slope data. Treatment effects were not significant. However, Bartlett's test showed that variances were heterogeneous, and it was therefore necessary to analyse by non-parametric means. Mann-Whitney U-test comparisons (2-tailed, 5% level) were calculated. No significant effect of either flow or density was detected.

Over the range tested, density had no effect on growth. Although there was a non-significant trend towards reduced growth at reduced flows, the effect was not considered important.

Referring back to the ANOVA presented in Table 16, Bartlett's test indicated that growth rates could be analyzed by time without heterogeneity problems. The effects of time were significant ($df=6,39$ $p<.05$). In contrast to the results of the previous experiments, growth slopes did not follow the expected pattern (i.e. rise to a peak after a low initial period, and

Figure 9. Change in weight over time for experiments 3a, 3b, and 3c. All weights have been adjusted to a common initial weight of either 30 or 33 grams (33 grams if first interval growth data had to be deleted). Changes over time were calculated using growth slope information.

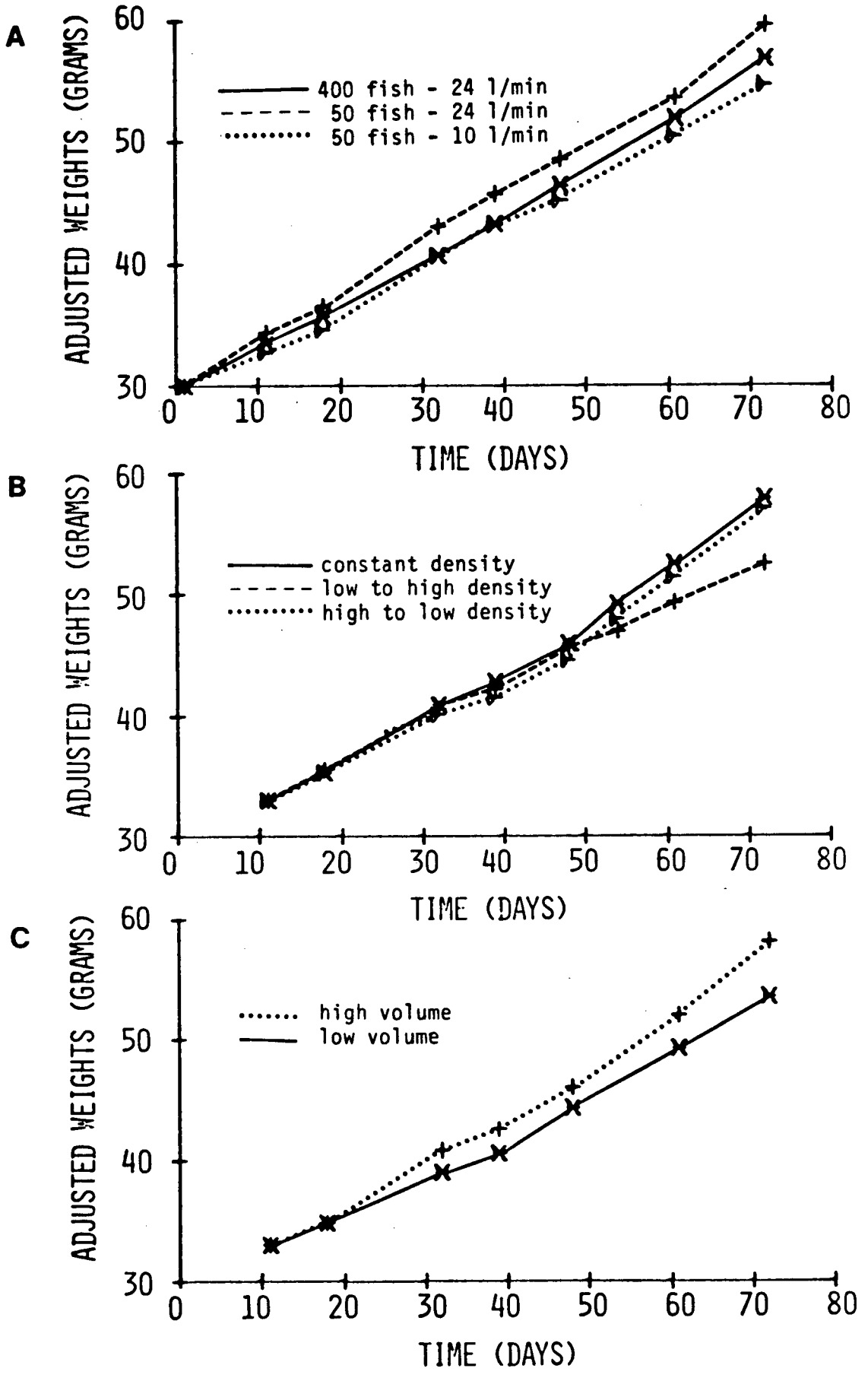

A
ADJUSTED WEIGHTS (GRAMS)
60
50
40
30
400 fish - 24 l/min
50 fish - 24 l/min
50 fish - 10 l/min
0
10
20
30
40
50
60
70
80
TIME (DAYS)
B
ADJUSTED WEIGHTS (GRAMS)
60
50
40
30
constant density
low to high density
high to low density
0
10
20
30
40
50
60
70
80
TIME (DAYS)
C
ADJUSTED WEIGHTS (GRAMS)
60
50
40
30
high volume
low volume
0
10
20
30
40
50
60
70
80
TIME (DAYS)

Table 16. Analysis of variance of growth slope data from experiment 3, part A (see table 14, part A). Tests are considered significant if $p < 0.05$. Data from all time intervals were analysed.

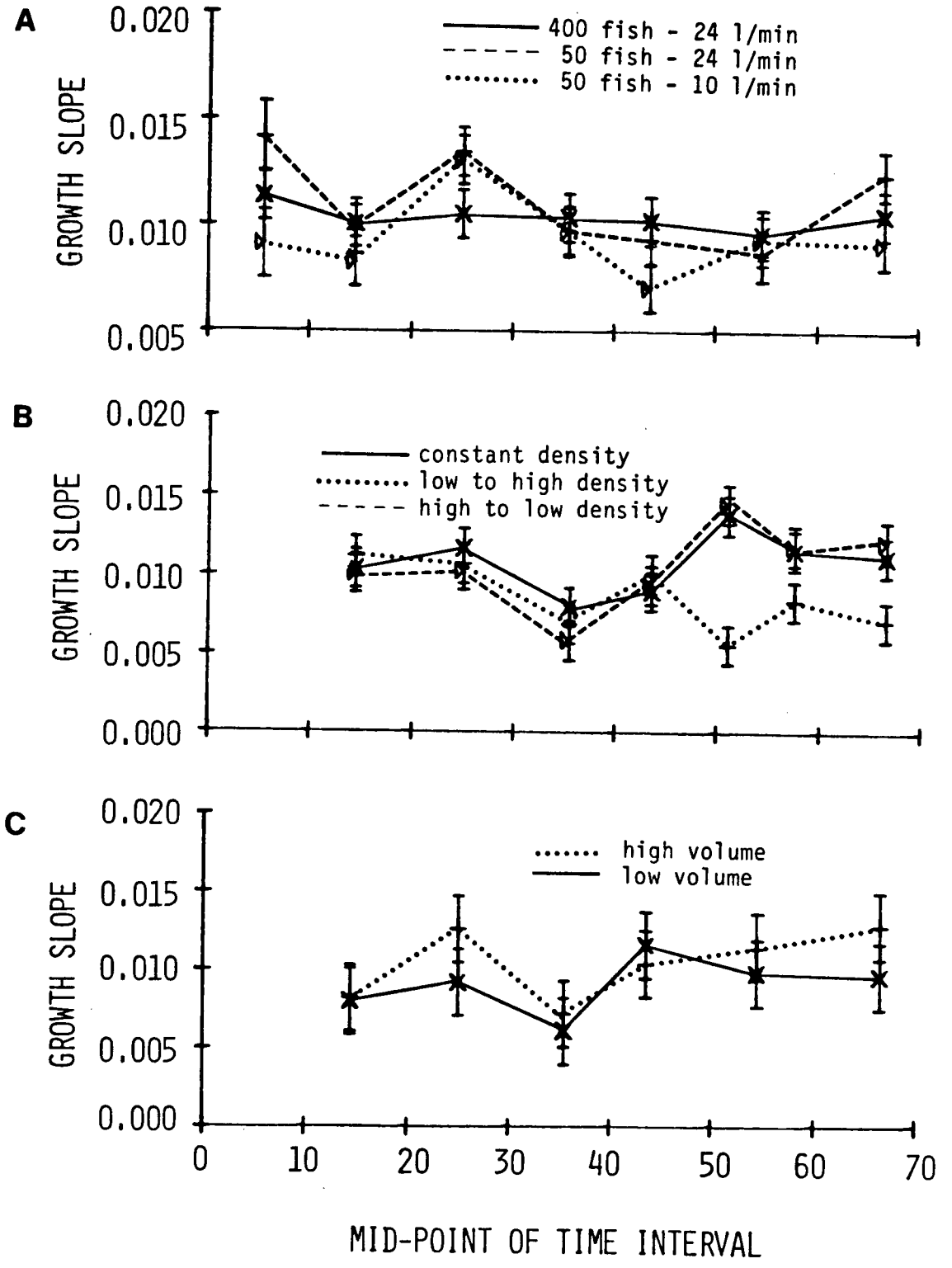
SOURCE	DF	MEAN SQUARE	PROBABILITY
Treatment	2	0.00000666	0.09846
Time	6	0.00000944	0.01284
Treatment*Time	12	0.00000298	0.36346
Residual	19	0.00000253	-
Total	39	-	-

then decline thereafter). During the first 4 times, growth was erratic (although close to model predictions). Over the last three intervals, there was a trend for growth to increase. Although the differences between the last three times were not significant (Duncan's test), all 6 tanks showed this pattern. This increase if real, may be part of the growth acceleration process associated with smoltification. Growth slopes against time are plotted in Figure 10a.

Experiment 3b

During the first days of this experiment, feeder problems in 4 of 6 tanks, resulted in unreliable data. Consequently, growth slope information for all 6 tanks was deleted for the first interval. As a result of the feeder problems, fish became hyperactive, and large numbers jumped out of some tanks. This necessitated recounts at the time of next weighing. All feeder problems were corrected within a few days of the start of the experiment, and fish appeared to behave "normally" by day 7. Because the first time interval used in the analysis extended

Figure 10. Growth slopes against time (mid-point of sampling interval) for experiments 3a, 3b, and 3c. Growth slopes were calculated according to Iwama ad Tautz (1981). Vertical bars are standard errors.


A
B
C
GROWTH SLOPE
0.020
0.015
0.010
0.005
0.000
400 fish - 24 l/min
50 fish - 24 l/min
50 fish - 10 l/min
constant density
low to high density
high to low density
high volume
low volume
0
10
20
30
40
50
60
70
MID-POINT OF TIME INTERVAL

from day 11 to 18, it is unlikely that the feeder problems affected subsequent results.

The data were analysed in a 2-way ANOVA (Table 17). Both density and time effects were significant. Duncan's test produced two homogeneous groups. Growth in the treatment in which volumes were reduced (density doubled) was significantly lower than in the other groups which were not different. The two groups with the same average density, but different volume treatments (low to high, or high to low), were not expected to have significantly different mean growth slopes. Growth slope patterns over time are given in Figure 10b.

Table 17. Analysis of variance of growth slope data from experiment 3, part B (see table 14, part B). Test terms are considered significant if $p < 0.05$. Due to technical difficulties, data from the first time interval were deleted. The significant interaction term was the basis for reanalysis by selected time intervals.

SOURCE	DF	MEAN SQUARE	PROBABILITY
Treatment	2	0.00002167	0.00270
Time	6	0.00001294	0.00335
Treatment*Time	12	0.00000908	0.00773
Residual	21	0.00000273	-
Total	41	-	-

Duncan's test produced several groups on the basis of time, however, none of these groups offered insight into growth processes since no patterns were detected. The curvilinear pattern observed in the previous major experiments was absent.

The interaction term was significant, and it was therefore

decided to reanalyze the data by time groupings before and after volumes were adjusted.

When the first four times were analyzed in a 2-way ANOVA density effects were not significant ($df=2,23$ $p=.42$). Therefore growth was unaffected over a 2-fold range in density during the first 47 days of the experiment. On a weight/volume basis, by day 47, the high density tanks were slightly more than twice conventional densities at the hatchery. Time effects were significant ($df=3,23$ $p<.005$). Growth slopes were constant over time with the exception of the third interval which was significantly lower in all groups. The interaction term was not significant.

The results of a 2-way ANOVA for the last 3 times produced treatment-related differences. Density effects were sgnificant ($df=2,17$ $p<.001$). Those groups at high density (low volume) grew about 60% slower than the low density groups. Both low density treatments had statistically equivalent growth slopes, despite the fact that they had different density histories (i.e. two of the tanks had been reared at the high density until day 47). The period immediately following density changes elicited the greatest response.

Interestingly, mean growth slopes over the last 3 intervals were considerably higher than for the first four intervals (0.0125 versus 0.0097) when tanks 7, 9, 10, and 12 were compared. This supports the trend observed in experiment 3a.

Experiment 3c

Technical difficulties during the first few days of the experiment, necessitated deletion of growth data from the first time interval. Consequently, only the remaining 6 time intervals were analysed.

The data were analyzed in a 2-way ANOVA (Table 18). Volume effects were not significant. However, fish in the low volume treatments tended towards lower growth, slightly below model predictions (0.0091), than those fish at high volume which grew slightly above predictions (0.0109). Growth slopes varied significantly with time ($df=5,23$ $p<.05$), and the overall pattern (Figure 10c) approximated those in experiments 3a and 3b. Especially important was the trend towards increased growth over the last three intervals, since it reduced the possibility that the pattern was an artifact of the room in which the research was conducted.

Since the treatment means were different by almost 15%, it was surprising that significance was not obtained. Obviously variances were large and the most likely source of variation was the two different stocks used. To examine this possibility, similar stocks were pooled and analysed in a 1-way ANOVA. The stock effect was not significant, possibly due to the variance associated with volume effects. However there was a trend for faster growth in the fish which the were progeny of 3-ocean parents. Therefore some of the variance in the ANOVA conducted on volume data may be due to stock effects. A volume effect may have been present but, if so, it was small.

Table 18. Analysis of variance of growth slope data from experiment 3, part C (see table 14, part C). Tests are considered significant if $p < 0.05$. Due to technical difficulties, data from the first time interval were deleted.

SOURCE	DF	MEAN SQUARE	PROBABILITY
Volume	1	0.00001175	0.06749
Time	5	0.00001449	0.01063
Volume*Time	5	0.00000311	0.42484
Residual	12	0.00000291	-
Total	23	-	-

Proximate Analysis

Experiment 3a

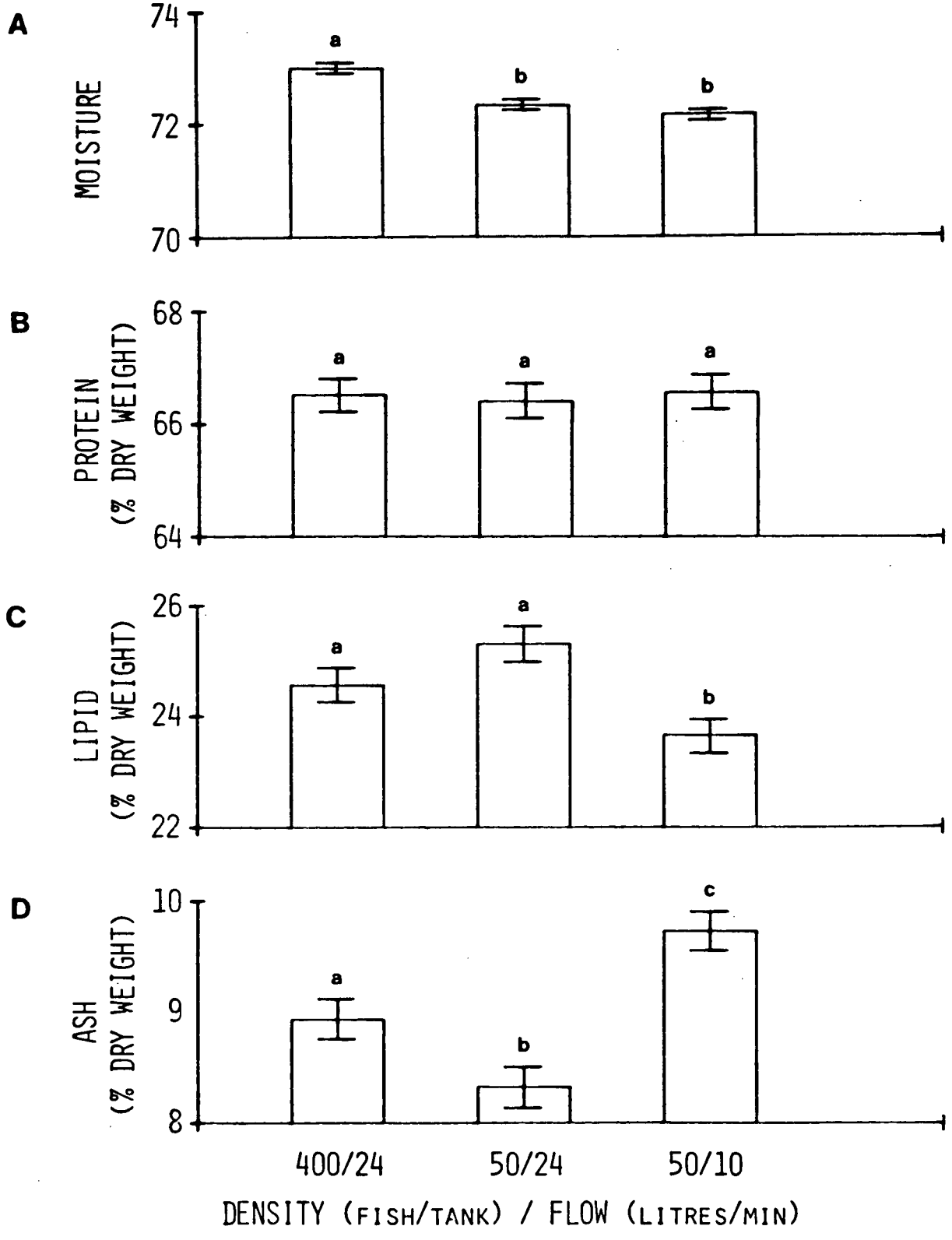
Results of these analyses are summarized in Figure 11.

Moisture: The first ANOVA calculated demonstrated that non-parametric comparisons were necessary. Mann-Whitney U-test tests were calculated for treatment replicates. In one case only were replicates statistically different. Subsequent comparisons were done on data with replicates pooled. The high density treatment had significantly higher moisture levels than the low density tanks (Us = 57, Uc = 49). This trend agreed with that observed in the first two experiments. No differences were found between treatments containing 50 fish with different flows.

Protein: Parametric tests were adequate for these data which were entered in a 1-way ANOVA with weight as a covariate. Treatments had no effect on protein content. Neither the covariate nor the equality of slope test were significant.

Lipid: With replicates pooled, density effects were examined in a 1-way ANOVA. The effect, although not significant,

Figure 11. Histogram of proximate composition data for experiment 3a. A-moisture, B-protein, C-lipid, D-ash. Means are given with standard errors. Bars bearing common small-case letters are not statistically different (Duncan's or Scheffe's multiple range test - 5% level). Statistical comparisons were made between the first 2 columns (different density, equal flows), and the last 2 columns (equal density, different flows).


A
MOISTURE
74
72
70
a
b
b
B
PROTEIN
(% DRY WEIGHT)
68
66
64
a
a
a
C
LIPID
(% DRY WEIGHT)
26
24
22
a
a
b
D
ASH
(% DRY WEIGHT)
10
9
8
a
b
c
400/24
50/24
50/10
DENSITY (FISH/TANK) / FLOW (LITRES/MIN)

was close (df=1,15 p=.12), and the trend followed the expected direction, i.e. decreased lipid content at the higher density. Furthermore there were no significant effects of the covariate or the interaction. The effects of varying flow rates were then examined in tanks containing identical densities. At the decreased flow, lipid levels were significantly reduced (df=1,15 p<.005).

Ash: With replicates pooled, the density data were analyzed in a 1-way ANOVA. There was a significant effect of density (df=1,15 p<.05), ash content decreasing as density was reduced. Flow effects were also significant (df=1,15 p<.05). At low flows ash content was higher. Effects of the covariate were not significant.

Experiment 3b

As mentioned in the methods, samples for proximate analysis were collected just prior to volume adjustment. Until that time, there were four tanks at the low density and two at the high density. There were, therefore, two treatments. Results of these analyses are summarized in Table 19.

Moisture: Using weight as a covariate, a 1-way ANOVA was calculated. The effect of density was not significant, although replicates were different (df=4,23 p<.001). Neither the covariate term nor the equality of slope test term were significant.

Protein: A similar analysis performed on these data indicated that replicates could be pooled. When pooled the

Table 19. Whole body proximate composition of juvenile steelhead trout from samples collected from experiments 3b and 3c after 47 days of rearing. Five fish were collected from each tank, and divided into two size groups. Fish from each group were homogenized and analysed in duplicate. The two studies were analysed separately with analysis of covariance and Duncan's test. Common letters represent no significant differences.

		----DENSITY----		-----VOLUME-----	
		0.6 fish/l	1.2 fish/l	250 litres	500 litres
	n	16	8	8	8
MOISTURE	mean	72.81a	72.71a	73.78a	73.61a
	S.E.	0.058	0.082	0.102	0.102
	n	16	8	8	8
PROTEIN	mean	66.11a	66.53a	67.96a	68.26a
	S.E.	0.313	0.446	0.549	0.549
	n	16	8	8	8
LIPID	mean	25.16a	25.31a	22.40a	23.48a
	S.E.	0.284	0.404	0.219	0.219
	n	16	8	8	8
ASH	mean	8.74a	8.16a	9.64a	8.27a
	S.E.	0.270	0.384	0.562	0.562

effects of density were not significant (df=1,23 p=.45). The covariate term was significantly less than zero (df=1,23 p<.001), indicating that as fish weight increased, protein levels decreased. The interaction term was not significant.

Lipid: As with the protein analysis, only the replicates term was significant (df=4,23 p<.05). No effect of density was detectable. None of the other terms were sigificant.

Ash: With replicates pooled, no density effects of density on ash content was detected. All other terms were non-significant.

Experiment 3c

As with the growth data analysis, stock differences between replicates probably increased variances. Accordingly all data were analyzed both for volume and stock effects. Only the results of the analyses on volume effects will be presented (Table 19); however, the stock analyses did suggest that stock effects existed.

Moisture: A 1-way nested ANOVA, using weight as a covariate, indicated no volume effects ($df=1,15$ $p=.92$). All other terms were non-significant with the exception of the replicates term ($df=1,15$ $p<.001$).

Protein: Similar analyses were performed on protein data. As with moisture, volume treatments had no effect on protein content. Variations between replicates were large ($df=1,15$ $p=.051$) but not quite significant. There was a trend towards increased protein levels at the lower volume.

Lipid: Volume did not significantly affect lipid content. Replicates were different ($df=1,15$ $p<.001$).

Ash: Although, not significant, there was a trend ($df=1,15$ $p=.15$) towards lower ash content at high volumes.

Cortisol

As described earlier, all tanks were sampled at least twice and those from experimient 3b were sampled three times. In some analyses variances were heterogeneous. Several transformations were tried but none were sufficient to correct the problem. In

these cases non-parametric tests were required.

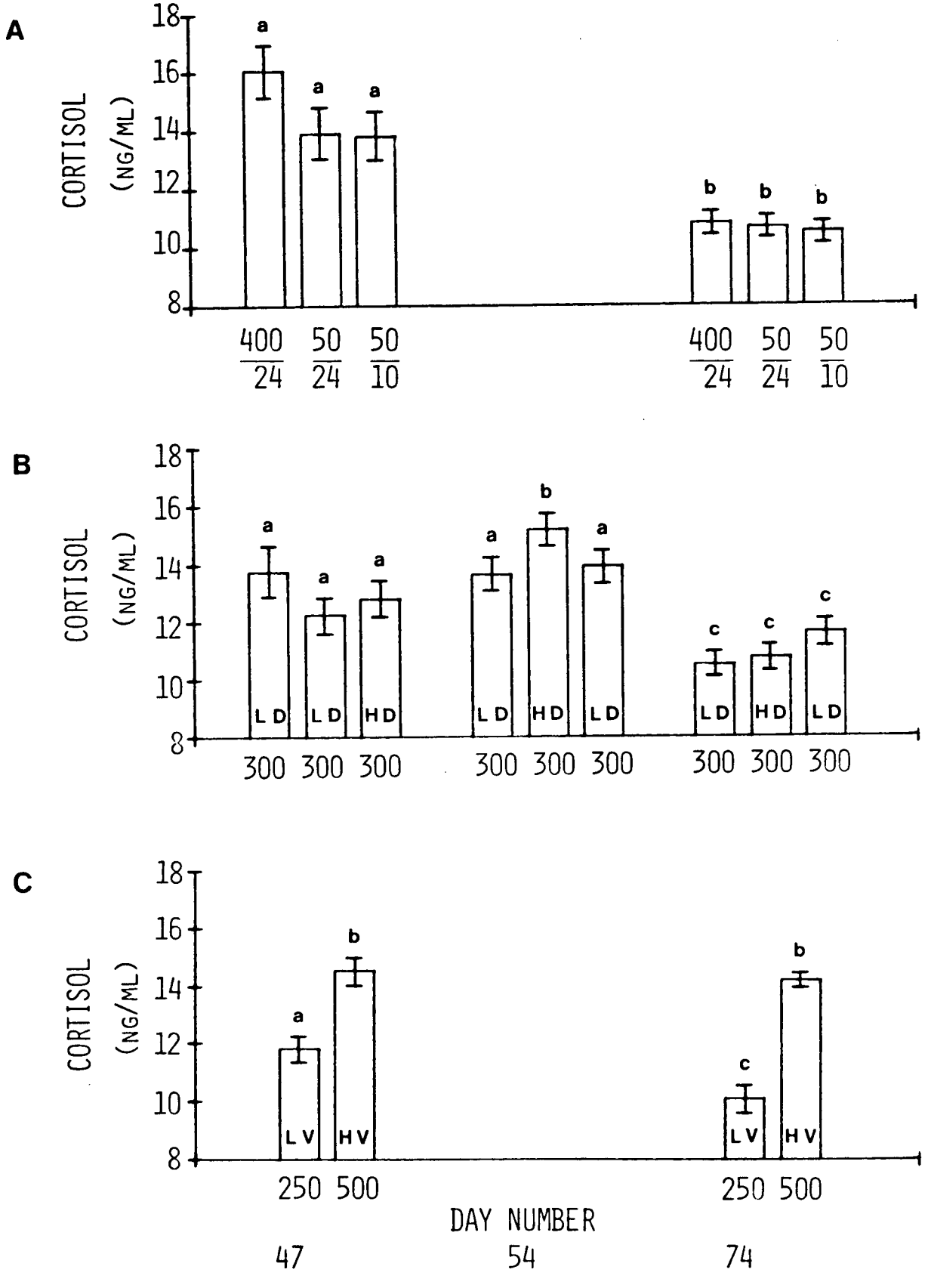
Experiment 3a

Cortisol data (Figure 12a) from the first sampling date were initially analyzed by ANOVA. Bartlett's test indicated the need for non-parametric tests. Mann-Whitney U-test comparisons between replicates indicated they could be pooled with the exception of those from the high density treatment. When the effects of flow were examined no differences were detected. Comparisons between the two density treatments (400 versus 50 fish), were not significant due to high variance between the high density tanks. It appeared unlikely that density effects were present.

Data from the second sampling date were analysed in a 1-way nested ANOVA. There were no significant differences due to either treatment (df=2,91 p=.93) or within replicates.

When the two times were compared, non-parametric tests were again required. With replicates pooled, comparisons were made between times for each treatment separately. In all cases cortisol levels were significantly reduced by the second date (400 fish, 24 l/min - calc t=2.8685; 50 fish, 24 l/min - calc t = 3.064; 50 fish 10 l/min - calc t=4.405)(critical t=1.96). On the second date, tests indicated that there were no differences between replicates. Due to the high variance, it is unlikely, without pooling, that a decline with time would have been detected at the high density.

Figure 12. Histogram of plasma cortisol concentrations for each of experiments 3a, 3b, and 3c, from samples collected on days 47, 54, and 74. Mean values are given with standard errors. Experiments 3a and 3c were sampled only on days 47 and 74. These data demonstrate the reduction in plasma cortisol concentration with time. Data from part B, experiment 3b, collected on days 47 and 54 demonstrate the cortisol response to changing densities. Bars bearing common letters are not statistically different (Duncan's or Scheffe's multiple range tests - 5% level).


A
CORTISOL (NG/ML)
18
16
14
12
10
8
a
a
a
b
b
b
400/24
50/24
50/10
400/24
50/24
50/10
B
CORTISOL (NG/ML)
LD
LD
HD
LD
HD
LD
LD
HD
LD
300 300 300 300 300 300 300 300 300
C
CORTISOL (NG/ML)
LV
HV
LV
HV
250 500 250 500
DAY NUMBER
47 54 74

Experiment 3b

Plasma samples were collected on three dates, the first and last were the same as in the other two studies. The first date was one day prior to the density (volume) adjustments. The second date was one week later and was intended to detect stress responses associated with the changes in density.

When data from the first time were analysed in a 1-way ANOVA (replicates pooled), the effects of different rearing densities were not significant ($df=2,79$ $p=.36$).

Data from the second sampling date were analysed in a 1-way ANOVA (replicates pooled). Although density treatment effects were not significant ($df=2,95$ $p=.13$), there were interesting trends in the data (see below).

The results of the last sampling period showed no differences between density treatments on pooled data ($df=2,87$ $p=.19$). Interestingly, mean plasma cortisol levels had decreased to levels similar to those of the last sampling date in experiment 3a.

To test for changes with time, the data were analysed in two 2-way, nested ANOVAs. Each analysis included two times. When data from the first two times were compared, replicates were not significantly different ($df=5,170$ $p=.17$) and were pooled. When pooled the effects of time (or more correctly, the effects of sudden changes in density) were significant ($df=1,170$ $p<.005$). Treatment effects were not significant ($df=2,170$ $p=.95$), however, the interaction term was nearly so ($df=2,170$ $p=.08$). This suggests that the effect of 'time' may not have been the

same in each treatment. This was expected since each treatment underwent different density changes. Inspection of the data revealed that cortisol levels from those tanks in which densities were unchanged did not change with time. In tanks in which volumes were halved (i.e. density doubled) cortisol levels increased significantly, indicating a stress response. Surprisingly, in tanks where volumes were doubled (i.e. density halved), there was a slight, although not significant ($df=1,61$ $p=.19$) increase in plasma cortisol levels.

In the 2-way ANOVA comparing the second and last sampling dates replicates were pooled. The effect of time was significant ($df=1,178$ $p<.001$), confirming the pattern observed in experiment 3a. Although not specifically tested, it is likely that the decrease with time was significant in all treatments. These data are presented in Figure 12b.

Experiment 3c

As with the growth and proximate analysis data, interpretation of these data was confounded by stock differences. Non-parametric testing procedures were required.

For simplicity, replicates were pooled for the pairwise comparisons. This procedure is not strictly correct; however, much of the variance was likely attributable to stock differences and so should be of similar magnitude between volume treatments.

When the effects of different volumes were compared at both sampling dates (Figure 12c), fish reared in low volume

containers had significantly lower plasma levels (time 1, $t=3.417$ $p<.001$; time 2, $t=4.231$ $p<.001$). The magnitude of the differences was similar in both cases. It was surprising that cortisol levels were higher for fish in the high volume tanks, since they tended to be growing somewhat faster.

To investigate time effects, further comparisons were computed. Cortisol levels were significantly reduced by the second sampling date in the low volume tanks ($t=2.591$ $p<.01$) and were in fact almost identical to those levels obtained in the quarantine room with the other two experiments. Cortisol levels were therefore unaffected by photoperiod. No differences were found between times when the high volume tanks were compared. This result was unexpected in view of all other cortisol trends. Consequently replicates were analysed separately. Fish in one tank did show a significant reduction while the other, surprisingly, showed an increase. The reason for this anomalous result is unclear. However, this tank was most susceptible to disturbance, as it was near a walkway, and near other frequently visited tanks.

Weight Length Relations

Experiment 3a

The GM functional regression of ln length against ln weight produced the following equation ($r^2 = .9731$ $n = 1383$ data pairs):

$$\ln Wt. = (-4.438) + 2.993 * \ln L.$$

Table 20. Condition factor data summary. Values were calculated from a GM funtional regression of natural log length against natural log weight. Details of the design are in Table 14. In part 2, densities were changed on day 48. The 8 in brackets refers only to part 2. In both other groups the first sampling date was on day 1.

	TANKS	DAY 1 (8)*	18	32	61	72	n	S.E.
	1	0.998	1.011	0.998	0.962	0.991	50	0.00645
	4	1.001	1.026	1.019	0.988	1.035	50	0.00645
part 1								
	2	0.984	1.016	1.017	0.942	1.001	50	0.00645
	5	0.995	1.024	1.032	1.008	1.020	50	0.00645
	3	0.993	0.999	1.010	0.942	0.984	50	0.00645
	6	1.000	1.010	1.018	0.991	0.992	50	0.00645
	7	1.005	1.002	1.014	0.976	1.001	50	0.00647
*	10	1.003	1.006	0.990	0.986	1.014	50	0.00647
part 2								
	8	0.996	1.023	1.014	0.975	1.012	50	0.00647
	11	1.004	1.014	1.003	0.968	1.019	50	0.00647
	9	0.996	1.007	0.976	0.994	1.017	50	0.00647
	12	0.993	1.010	0.997	0.992	1.024	50	0.00647
	13	0.997	1.004	1.015	0.968	0.995	50	0.00702
	14	0.968	1.002	1.000	0.975	0.997	50	0.00702
part 3								
	15	0.988	1.015	1.016	1.008	1.043	50	0.00702
	16	0.997	1.002	1.026	1.002	1.033	50	0.00702

When reorganized and antilogged, the working condition equation was:

$$\text{condition factor} = \text{weight}/(0.01181955 * \text{length}^{2.993}).$$

A 2-way nested ANOVA produced no significant treatment effects ($df=2,1382$ $p=.47$). Replicates were significantly different ($df=15,1382$ $p<.001$). Condition varied significantly over time ($df=4,1382$ $p<.01$). The pattern with time was similar to that for growth slope with time and was consistent in all tanks. These data are presented in Table 20.

Experiment 3b

The GM functional regression of ln length against ln weight produced the following equation (r^2 = .9779, n= 1500):

ln Wt. = (-4.549) + 3.035 * ln L.,

which when converted to the working formula was:

condition factor = weight/(0.01057778 * length$^{3.035}$).

The condition data were subsequently analysed in a 2-way ANOVA (replicates pooled, df=15,1499 p=.14). Treatment effects were not significant (df=2,1499 p=.55), however, the effects of time were (df=4,1499 p<.001). The pattern observed followed that of growth slopes over time. The same trend occurred in experiment 3a. The interaction term was significant (df=8,1499 p<.001). Inspection of the data revealed only minor pattern differences between treatments and the source of the interaction was not readily apparent. There was no evidence of a clear relation between the effects of changing density and condition factor response. Mean values are presented in Table 20.

Experiment 3c

The GM functional regression of ln weight on ln length (r^2 = .9707, n = 1000) is presented below:

ln Wt. = (-4.849) + 3.138 * ln L.,

which rearranged becomes:

condition factor = weight/(0.00783621 * length$^{3.138}$),

and is the working formula.

When condition data were analysed in a 2-way ANOVA

(replicates pooled - df=10,999 p=.55), both volume and time effects were significant (df=1,999 p<.001; df=4,999 p<.001, respectively). Fish from the low volume treatments had lower condition factors than those in the high volume tanks. This is in general agreement with the trend for increased growth at higher volumes. The pattern with time closely followed that in experiments 3a and 3b, and mirrored its own growth slope pattern. These data are summarized in Table 20.

Behavior

Behavioral analyses will be presented separately for experiments 3a and 3b. Data were first analysed by individual times and then analysed over time. Data from tank 5 (50 fish, 24 l/min) were deleted from the analysis. A faint shadow, only visible on the tapes, affected activity and fish distribution. In addition, data from the last three dates were deleted for tank 12 (300 fish, low to high volume), since surface glare obscured activity.

Experiment 3a

Net Activity: No differences between treatments were found during the first three dates when data were analysed in 1-way nested ANOVAs. On the last day activity in the high density treatment was greater than in the other treatments (df=2,209 p<.05).

To identify patterns with time, the data were reanalysed in

a 2-way nested ANOVA. No significant treatment effects were found. There was a slight trend for increased activity in higher densities. Replicates were significantly different ($df=8,819$ $p<.005$). Net activity did not vary significantly with time ($df=3,819$ $p=.07$), however, there was a pattern, repeated in each tank, for an increase in activity with time over the first 3 periods, and then a marked decline on the last day (Figure 13a).

Total Activity: When times were analysed separately in 1-way nested ANOVAs, only during the first date (day 31) were differences found. On this day, total activity in the low flow treatment was reduced ($df=2,230$ $p<.001$).

As with net activity, the data were analysed to detect trends with time. Treatment effects were not significant. As in the net activity data, there was a trend towards higher activity in the high density treatment. Replicates were different ($df=8,980$ $p<.001$). Time effects were significant ($df=3,980$ $p<.05$) and the observed pattern matched that for net activity (Figure 13b). The interaction term was not significant ($df=6,980$ $p=.84$).

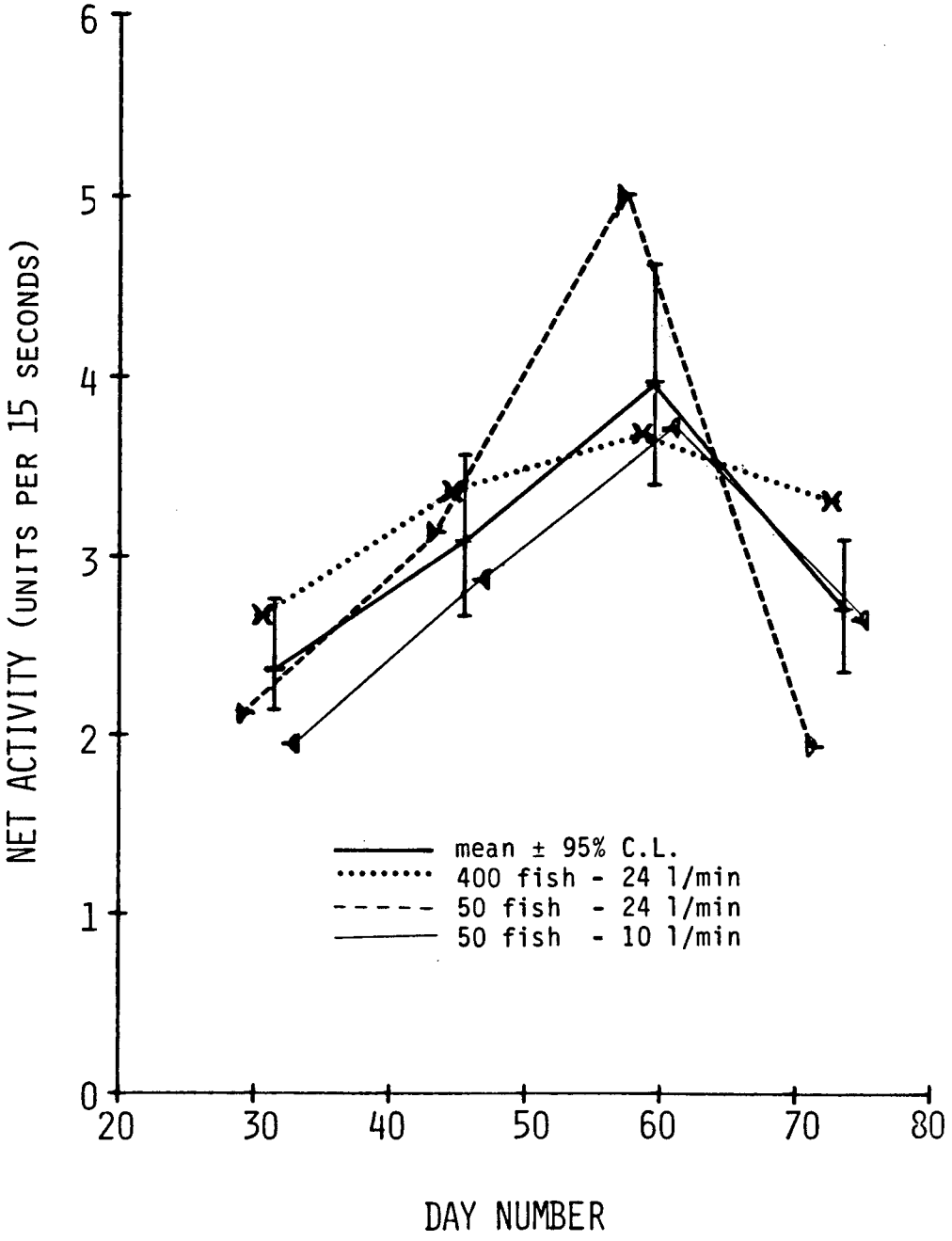
Experiment 3b

Net Activity: Significant differences were found between treatments in all but one sampling date. Only on day 59 were differences not significant. Treatments in which the space per fish was large (high volume, low density) at the time of sampling, exhibited greater activity levels than those in which the space per fish was low. These data are summarized in

118

Figure 13. Activity against time for experiment 3a. Part A- net activity, part B-total activity. Mean values are indicated by heavy solid lines with 95% C.L. Lines are offset by one day either side of the true sampling day for clarity.

A


NET ACTIVITY (UNITS PER 15 SECONDS)
0
1
2
3
4
5
6
20
30
40
50
60
70
80
DAY NUMBER
mean ± 95% C.L.
400 fish - 24 l/min
50 fish - 24 l/min
50 fish - 10 l/min

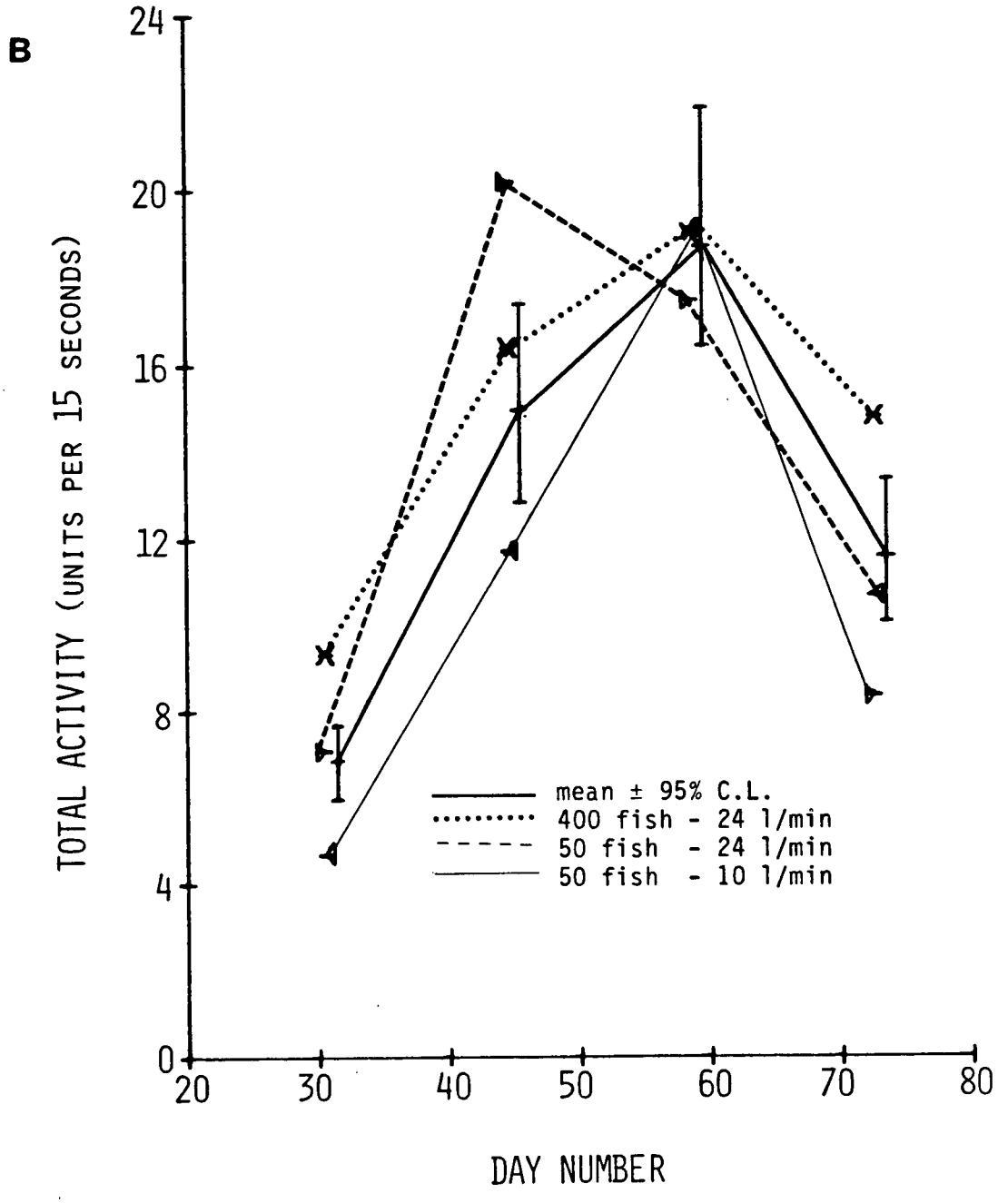

B
24
20
16
12
8
4
0
TOTAL ACTIVITY (UNITS PER 15 SECONDS)
20
30
40
50
60
70
80
DAY NUMBER
mean ± 95% C.L.
400 fish - 24 l/min
50 fish - 24 l/min
50 fish - 10 l/min

Table 21.

Table 21. Summary of net activity data by time and treatment for the second study, 3b, (sudden density changes) of the third experiment. Values presented are transformed (natural log + 1.1), and are expressed in arbitrary units per 15 seconds. To determine the effect of sudden changes in density, follow treatments across time intervals. The mean values over all times (last column) shows the effect most clearly. As mentioned in the text, the observed effects may be due to volume differences.

		FIRST --DENSITY--		SECOND ------DENSITY-----			
	DAY	31	45	52	59	73	ALL
L-L	n	38	54	32	78	49	267
	mean	1.819	1.736	1.877	1.656	1.742	1.731
	S.E.	0.1174	0.1003	0.1203	0.0817	0.0974	0.0441
L-H	n	70	83	90	73	83	412
	mean	1.765	1.410	1.262	1.480	1.279	1.364
	S.E.	0.0865	0.0809	0.0717	0.0844	0.0748	0.0354
H-L	n	86	56	29	30	39	210
	mean	1.246	1.347	1.708	1.659	1.449	1.461
	S.E.	0.0781	0.0985	0.1264	0.1310	0.1092	0.0496

L-L - constant low density (high volume)
L-H - low density to high (high volume to low)
H-L - high density to low (low volume to high)

When analysed in a 2-way ANOVA over all 5 times the results were consistent with the above analyses. Activity in treatments in which volumes were unchanged was greater ($df=2,888$ $p<.001$) than in treatments in which it was not. At high volumes (low densities) fish spent less time holding positions than at low volumes. As expected, the interaction term was close to significance ($df=8,888$ $p=.06$) suggesting that changing volumes had influenced activity.

Table 22. Summary of total activity data by time and treatment for study 2 (sudden density changes) of experiment 3. Values presented are transformed variables (natural log + 1.1), and are given in arbitrary units per 15 seconds. To determine the effects of changes in density, follow mean values across time intervals. The mean values over all time intervals (last column) depicts the effect most clearly. As mentioned in the text, the observed effects may be due to volume.

		FIRST DENSITY		SECOND DENSITY			
	DAY	31	45	52	59	73	ALL
L-L	n	100	100	100	100	99	499
	mean	3.861	3.450	3.688	2.788	3.651	3.487
	S.E.	0.1041	0.1041	0.1041	0.1041	0.1046	0.0467
L-H	n	100	100	100	100	100	500
	mean	3.079	2.543	2.470	2.818	2.630	2.708
	S.E.	0.1041	0.1041	0.1041	0.1041	0.1041	0.0466
H-L	n	100	100	60	50	50	360
	mean	2.376	3.018	3.104	3.428	2.611	2.859
	S.E.	0.1041	0.1041	0.1344	0.1472	0.1472	0.0553

L-L - constant low density (high volume)
L-H - low density to high (high volume to low)
H-L - high density to low (low volume to high)

Total Activity: When analysed in 1-way nested ANOVAs, significant differences were found at most times. However, the differences were less consistent than with net activity. This suggests that total activity is less affected by density (and/or volume) than net activity. The general pattern was the same (Table 20).

When analyzed in a 2-way nested ANOVA over all 5 times, the pattern was identical to that above. Treatment effects were significant ($df=2,1358$ $p<.001$) and replicates were significantly different ($df=13,1358$ $p<.001$). In addition the interaction term

was significant (df=8,1358 p<.005) and this indicates an effect of volume and/or density changes.

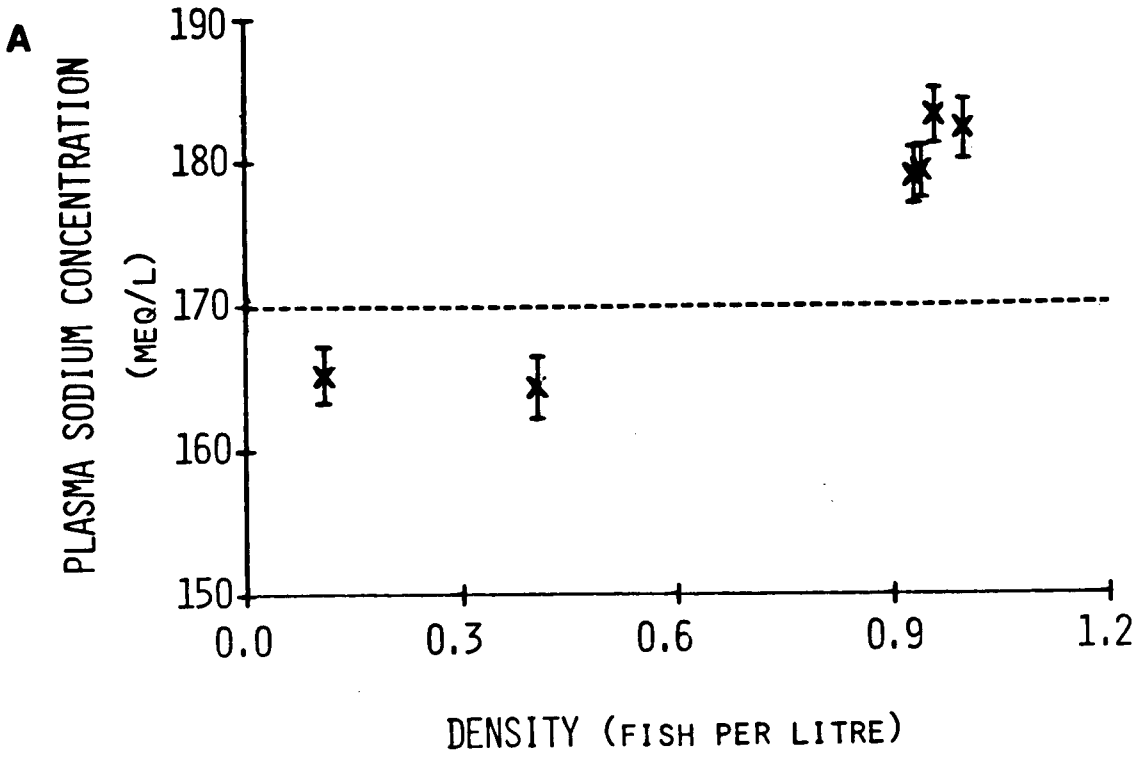
Salt Water Challenge Test

Technical difficulties, including inadvertent mixing of fish from different treatments necessitated an early termination of the first test. The second attempt was successful. For logistical reasons fish from replicate tanks of each treatment were pooled. Additionally, the two treatments containing 50 fish with different flows were pooled. The treatment containing 300 fish (low to high volume) was not tested. A total of 20 fish from each treatment was randomly selected (equal numbers from replicate tanks). Design details are presented in Table 23.

Data were first analyzed parametrically, however, heterogeneity of variance problems necessitated non-parametric tests. Evidently sample sizes were large enough that F-values were unaffected by the heterogeneous variances, or differences were large, since the results of both methods were identical. The parametric analyses are presented here.

Because fish from study 3c were reared under a different photoperiod than those from 3a and 3b, these data were initially analysed separately. When the four treatments from 3a and 3b were tested a clear density effect was apparent (df=3,72 p<.001) (Figure 14). Scheffe's test produced two groups. Fish from treatments containing high densities, approximately 1 fish/l (50-60 gm/l), regulated plasma sodium levels poorly. Fish from treatments containing low densities, either 0.14 f/l (9 gm/l) or

Figure 14. Plasma sodium concentrations following salt water challenge test. Part A- sodium concentrations against rearing density, part B- sodium concentrations against tank volume. Vertical bars are standard errors. The dashed horizontal line is an arbitrary cut-off value. Plasma sodium values above this level after 24 hours, indicate that test fish were incompletely smolted, and not fully capable of surviving in salt water.


A
PLASMA SODIUM CONCENTRATION (MEQ/L)
190
180
170
160
150
0.0
0.3
0.6
0.9
1.2
DENSITY (FISH PER LITRE)

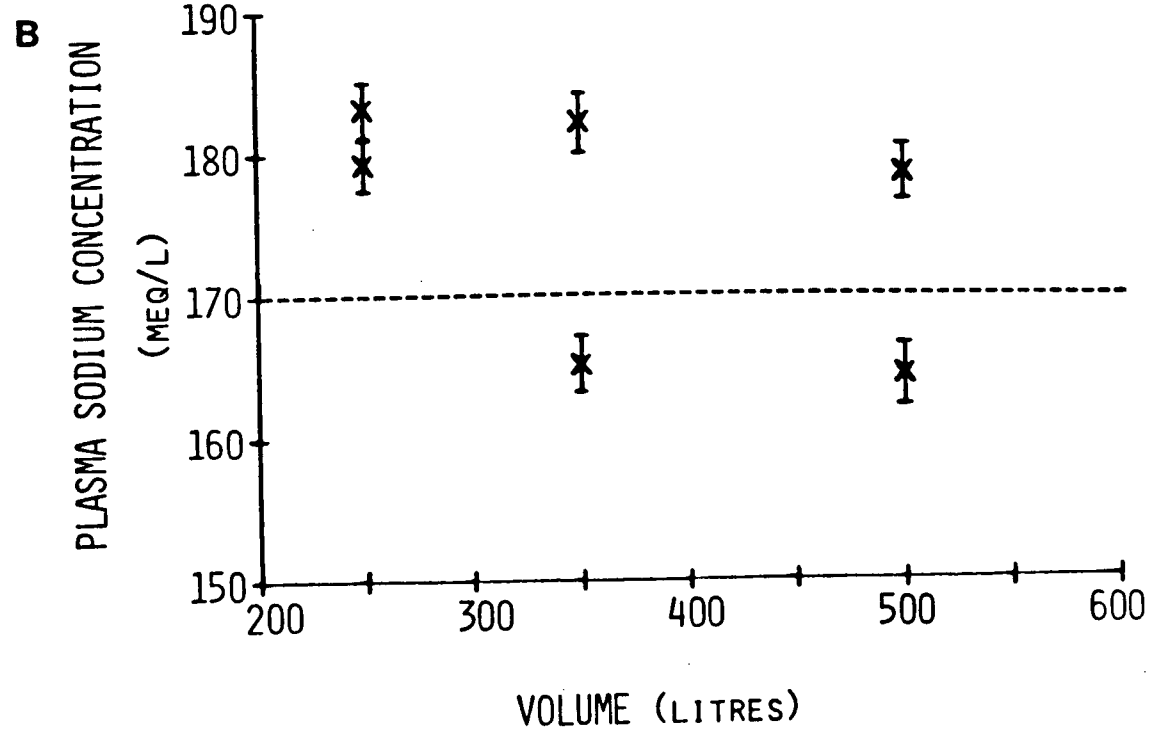

B
PLASMA SODIUM CONCENTRATION (MEQ/L)
190
180
170
160
150
200
300
400
500
600
VOLUME (LITRES)

Table 23. Design of salt water challenge test. Fish from replicate tanks were pooled. The densities given are the actual rearing densities experienced prior to testing (i.e. mortalities and sampled fish are accounted for).

GROUP	TANKS	NUMBER SAMPLED per tank	REARING DENSITY fish/l	REARING DENSITY gm/l	PHOTOPERIOD hours light	MEAN WEIGHT gm	STD. DEV. gm
1	1,4	10	1.01	59.6	10	58.3	15.1
2	2,3,5,6	5	0.10	5.6	10	56.5	11.5
3	7,10	10	0.41	21.0	10	66.4	12.7
4	8,11	10	0.96	53.0	10	51.6	9.2
5	13,14	10	0.93	46.5	14	56.9	14.7
6	15,16	10	0.96	52.8	14	52.9	10.3

0.45 f/l (30 gm/l) were similar in their capacity and regulated sodium well.

The effect of tank volume on salt regulation was examined by comparing the two treatments from experiment 3c. No differences were found (df=1,59 p=.21).

To determine if the different, but constant, photoperiods had affected the development of hyperosmoregulatory capability, treatments containing similar densities from the two rooms were compared. No differences due to photoperiod were detected (df=3,77 p=.12).

Since the mean size of the test fish used in each treatment varied, it was desirable to determine if individual weights were correlated with plasma sodium levels. This was not unlikely since smoltification is modified by fish size. Linear regressions of mean fish weight against plasma sodium were calculated for each treatment. In most cases, slopes were not significantly different from zero. In one case, however, a

significant negative slope (b = -0.39) was obtained. Since this case was isolated, and because the mean fish weight in the treatment was about the same as in other groups of comparable mean sodium levels, its effect was ignored.

There was some concern that tank volumes may have affected plasma sodium levels. This was considered unlikely in view of the results of comparisons of treatments from study 3c. Plots of plasma sodium levels against volume (Figure 14b) reinforce this conclusion.

Conclusions

Experiment 3a

Growth slope data showed no evidence of density effects over an 8-fold range from 0.5 to 3.8 times hatchery levels. Flow rate did not affect growth. Growth slopes, initially erratic, showed evidence of a non-significant increase during the last 3 periods.

Proximate composition data were less clear than in previous experiments. Fish from the high density treatment had elevated moisture and ash levels. Protein content was unaffected. Lipid content was not affected by treatments although the probability was near the significant level and content was reduced at high density. These findings are similar to those from the first two studies, and to others reported in the literature.

There were no treatment-related differences in plasma cortisol concentrations, however, there was a reduction with

time.

Condition factors showed no evidence of treatment effects. As with the growth data, differences with time were significant and a pattern mirroring growth slopes was observed.

Neither net nor total activity were significantly affected by either density or flow (except for some isolated cases). There was a pattern with time, however, that was significant for total activity and nearly so for net activity. In all tanks activity increased steadily from day 31 until it peaked at day 59. On the last date levels declined.

Experiment 3b

During the first four sampling intervals there were no density effects on any of the variables tested, with the exception of the activity data. The density range extended from 1.8 to 3.6 times standard hatchery densities. Density effects were apparent during the last 3 intervals. Using growth slope information from the treatment in which volumes remained unchanged from this study and the high density treatment from study 3a as controls, the possibility of conditioning was explored. When tanks in which volumes were doubled (i.e. densities halved - 0.6 f/l) were compared with tanks held at the high volume (0.6 f/l), no differences in growth slope were found. However, when tanks in which volumes were halved (i.e. densities doubled - 1.0 f/l) were compared with tanks of similar density from study 3a, there was evidence of significant growth depression. This clearly suggests the possibility of a

conditioning effect. Since the tanks used as standards for this second comparison contained different volumes and flows, it was necessary to establish that these did not affect results. Consequently treatments of similar densities but different volumes were compared for the period when volumes were changed. No volume effect was found. When the effect of flow was examined in study 3a, it was found to be non-significant. Since the range tested was twice that of the groups being compared between studies 3a and 3b, the effect of flow was unimportant.

Density did not affect proximate composition prior to the time of volume changes.

When cortisol data were analysed by separate sampling dates no differences between treatments were detected. However, when the first two periods were compared, differences were detected. This was expected since the sampling dates were those immediately before, and one week after, volumes were changed. Tanks in which densities were doubled had significantly higher cortisol levels than before densities were doubled, indicative of a stress response. Tanks in which densities were reduced showed evidence of a slight (non-significant) increase. When the first and last sampling dates were compared, all evidence of the stress effect present in the second period was gone. In all treatments cortisol levels decreased significantly by the last sampling time.

Condition data were unaffected by treatment. Overall patterns with time tended to follow those for growth. A significant interaction term ($df=8,1499$ $p<.001$) demonstrated

that sudden changes in density caused measureable changes in body form.

Although behavioral data, particularly total activity measures, were not always clear at individual times, when all times were pooled, a consistent pattern emerged. Under high density (low volume) conditions both activity measures were significantly below levels obtained under low density (high volume) conditions. When densities were changed activity levels adjusted very quickly. This suggests that prior experience did not have a lasting effect. The responses observed may have been artifacts of tank volumes.

Experiment 3c

The results of this study were mixed. A nearly significant trend for reduced growth at low volumes was detected. There were no volume related effects on proximate composition. Fish from low volume tanks had reduced conditions. Condition factors followed a pattern with time which approximated the pattern in growth slopes.

In apparent contradiction with growth and condition data, fish reared at high volumes had consistently higher plasma cortisol levels. As in experiments 3a and 3b cortisol levels decreased significantly with time in all but one tank.

Periodic qualitative observations indicated that activity was consistently higher in the high volume tanks. Since densities were similar, it appears likely that the response was a function of volume.

Salt Water Challenge: The salt water challenge test detected stress-related differences in smolt fitness attributable to rearing conditions. The results could not be attributed to fish size, flow rate, volume, or photoperiod effects. Fish reared at densities as high as 30 gm/l regulated salt balance well (as well as fish reared at much lower densities). Fish reared at high densities (60 gm/l.) showed evidence of a marked reduction in osmoregulatory performance.

GENERAL DISCUSSION

The stress-related physiological consequences of extended periods of high density rearing have not been thoroughly investigated in salmonids. In coho salmon Fagerlund et al. (1981) examined the effects of densities on growth, body composition, condition factor, and interrenal nuclear diameter. They found that growth and body condition was suppressed by high densities. In addition, fish reared in high densities had reduced whole-body lipid levels and elevated moisture levels. Furthermore, small fish selected from the high density treatments had significantly increased interrenal nuclear diameters. This is one of the few thorough investigations in this area. However, for part of their study, fish were fed reduced rations. Although this followed normal hatchery practices, a desired objective, it may also have contributed to the observed density-induced effects. Wedemeyer (1976) examined the short-term stress responses to crowding in both coho and steelhead and found that when densities were increased from 8.5 gm/l to 17, 34, 68, 102, or 204 gm/l., all groups exhibited hyperglycemia. This rise in blood glucose was transitory and in most groups levels returned to normal within a few weeks. This suggests adaptation. Growth responses were not examined during that stress period. Furthermore the higher densities tested were more typical of transport than rearing densities.

I examined the consequences of rearing juvenile steelhead trout of three size ranges under various density regimes. The responses to density were not the same in each of the three

experiments. Therefore generalizations regarding response are not easily made, and for clarity, each experiment is discussed separately. Where appropriate, generalizations are attempted.

Stress responses in fry

Results of the first experiment demonstrated that small steelhead trout (2-16 grams) are physiologically affected by rearing density. As described earlier, density effects at the high temperature (15°C) were statistically clear. At 12.5°C, similar effects, although not statistically significant, were observed. However the consistency in the observed patterns strongly suggest that density did have effects similar to those at 15°C. Perhaps at lower temperatures density tolerances may be higher, or if responses to density are related to metabolic rate, then more time may be required for certain physiological consequences to be manifested. The different density responses could not be ascribed to differences in swimming activity at the two temperatures.

Growth rates were affected by density during an initial short period (less than 2 weeks). After the first few weeks there were no longer statistical differences between treatments. Such short term density effects are not mentioned in the literature prior to 1981. Recently however, Birks et al. (1981) described similar short-duration density effects on growth in experiments with chinook salmon. Perhaps the short duration of density effects should not be surprising, since Wedemeyer (1976) found that both coho and steelhead adapt (as measured by 2 blood

variables) to high densities within 2-3 weeks. Furthermore, Schreck (1981) pointed out that complete adaptation (in terms of cortisol levels) to some types of chronic stress (not including crowding) can occur within a few weeks (1-3). If reduced growth resulting from high density rearing is indicative of stress, then my results demonstrate a clear stress response (Table 4, Figures 1,2). Many authors have demonstrated density-induced growth suppression in hatcheries (Fagerlund et al. 1981; Trzebiatowski et al. 1981; Refstie and Kittleson 1976; Refstie 1977; Brauhn et al. 1976; Andrews et al. 1971). None have examined temporal changes in growth.

The absence of significant differences in plasma cortisol levels between treatments appears to indicate that density effects were of short duration. However, as Schreck (1981) points out, some indicators including plasma cortisol show adaptation under conditions of chronic stress. Furthermore Hill and Fromm (1968) observed that for some forms of stress elevations in plasma corticosteroids are transitory. Unfortunately few studies have followed the cortisol response to crowding stress under hatchery conditions. Also, adaptation to chronic stress could involve behavioral as well as physiological mechanisms. Ejike and Schreck (1980) noted that conditioning alters stress response to handling in salmonids. Possibly salmonids can become conditioned to other types of stress, including high density rearing.

If, as the cortisol data suggests, complete recovery from the effects of density-induced stress had occurred by the end of

the experiment, and by the end of the first week or so according to growth slope data, then various other indicators of metabolic state (and stress) also should show no differences. This was not the case. Both proximate composition and condition factor data reflect persistent density effects. Furthermore rearing density also induced changes in behavior (activity). The exact consequences of differences in the physiological indicators of stress (proximate composition, condition, etc.), in terms of survival and ultimate fitness, are not well understood. However it is clear that different rearing densities produced measurable physiological changes in the fish, and thus altered tissue growth and energy metabolism.

Associated with increases in density were elevated moisture and protein levels and reductions in lipid content. Fagerlund et al. (1981) also observed significant elevations in moisture and reductions in lipid and a non-significant trend towards reductions in protein content at high densities. They suggest that reduced lipid reserves could signify reduced capabilities for sustained activity and predator avoidance. Lee and Laycock (1978) observed that impaired lipid metabolism is associated with elevated corticosteroids in mammals. Burrows (1969) suggested that lipid reserves of 8% (32% of dry body weight assuming 75% moisture) are optimal for survival of released fingerlings. Li and Brocksen (1977) and Li (1973) observed reductions in lipid levels in subordinate rainbow trout reared at high densities. Noakes and Leatherland (1977) demonstrated clear stress responses in rainbow trout associated with

heirarchies and found this reflected in lipid reserves. These studies suggest that the tissue changes which accompany high density rearing may reduce chances of survival.

The significance of increased protein levels at high densities is obscure. In severe cases of stress in mammals a negative nitrogen balance develops and this can lead to muscle wasting (Lee and Laycock 1978). Such a response may be adaptive in starving animals but it is difficult to comprehend in this case. In fish, under crowded conditions but with excess food, it appears that the efficiency of lipid energy storage is reduced. This may have contributed to the observed relative increase in body protein. Even though food may be provided in excess at high densities, food conversion and growth efficiencies may be reduced (Fagerlund et al. 1981; Li and Brocksen 1977; Andrews et al. 1971).

High condition factors are sometimes associated with increased physical fitness and are routinely calculated in most hatcheries as a means of assessing the general health of the fish. The assumption is that fish of a given length with a high condition factor have greater energy reserves than fish with a low condition factor. In rainbow trout fed to excess Refstie (1977) noted decreased condition factors at high densities. He attributed this to reduced food intake by small fish (subordinates). After six months of rearing over a 2-fold density range Fagerlund et al. (1981) found no evidence of condition differences in coho salmon. However, four months later, significant differences were found. Condition factors

were lower at the high densities. Since fish were receiving reduced rations for much of this last period, competition for food may have contributed to the development of heirarchies at the higher densities, and these in turn resulted in unfavourable growth conditions for much of the population. My first experiment demonstrated that condition factors were reduced at high densities, even when fish were fed excess rations. Growth rate and body condition were closely correlated. Under conditions of rapid growth condition factors were high. This suggests that during periods of good growth fish increase energy reserves. Density related differences in condition were marked during the first time period.

Interpretation of the behavior data was difficult. Since the observed patterns were consistent and clear at both temperatures, it appears likely that they were real consequences of density. At both temperatures, net activity levels were greatest at the middle density and lowest at the lowest density. This indicates that at the lowest density fish maintained positions most rigidly. Total activity (total distance moved in a given time) decreased as densities increased. Available space per fish may have limited total movement. There are no studies reported in the literature of activity levels of salmonids reared at high densities under hatchery conditions.

Quantitative measures of aggression were not collected, and there are no reported studies on aggression in fish at hatchery densities. For this reason the following discussion is entirely speculative. High total activity levels, combined with precise

station maintenance at low densities, may indicate territorial behavior. High activity levels may be the result of periodic, short-duration chase sequences. Some acts of aggression were visible at the low density; however, these were not quantified since aggressive acts could not be reliably estimated at the higher densities. At the middle density, position maintenance was least precise, and total activity levels were decreased. This could reflect continued attempts to defend territories under conditions where nearest neighbour distance is small and, therefore, perceived intruder encounter rate may be elevated. This may result in an increase in defence time, but in a localized area. At the highest densities, intruder pressure may have been so high that territorial behavior was inhibited, and fish passively maintained position.

If the relatively low net and total activity levels at the highest density indicate the absence or reduction of agonistic behavior then these results agree with those of Magnuson (1962) and Kawanabe (1969).

Another use of these measures, was to obtain a qualitative estimate of the energy expended in swimming activity, since such expenditures, if high enough, could limit growth. Minchen (1972) found that in A. pulcher, as absolute space increased activity increased. Energy expended on swimming was given as a cause of reduced growth in large containers. Li and Brocksen (1977) found no such effect in rainbow trout. In my experiments growth differences can not be attributed to activity differences, since the most active fish grew fastest.

Although the significance of the observed patterns in behavior are unclear, they do demonstrate that rearing conditions affect behavior. As noted earlier, several authors have described differences in the behavior of wild and hatchery-reared salmonids in streams. Perhaps altered hatchery rearing strategies could be used to beneficially influence the behavior of released fish.

Stress responses in fingerlings

This experiment was designed to answer two questions: first do larger trout (than tested in the first experiment) respond similarly to density stress; secondly, since density effects on growth are of short duration and the growth pattern is curvilinear (both indicative of a conditioning effect) what is the effect of sudden changes in density? McDonald et al. (1968) suggested that prior experience may be important in determining behavioral reactions in fish. Perhaps prior experience also influences physiological reactions.

The results demonstrated that juvenile steelhead trout, 15-30 grams in weight, were physiologically affected by rearing density. Growth rate, proximate composition and condition factor data supported this view.

In those treatments where volumes were not adjusted densities were not high enough to elicit a growth response. When all treatments were compared there was evidence of growth suppression at high densities, and the degree of growth suppression suggests a threshhold effect. In the literature,

evidence for threshhold density effects is rare although Brown (1946a) found that intermediate densities produced optimal growth in tank-reared brown trout fry and fingerlings. Growth rates in the second study followed a curvilinear pattern with time, similar to that in the first experiment, and again suggest a conditioning effect. As in the response to density in the first experiment, density effects were greatest in the first time interval. However, the magnitude of the response during this interval was smaller. Unlike the first experiment, the effect of density on growth persisted over time. No other studies have demonstrated a moderating influence of fish size on density response in salmonids.

To detect density-conditioning or acclimation effects, observed growth rates were compared with expected rates after volumes (i.e. densities) had been changed. There was some concern that tank volume may have influenced the results, however, Li and Brocksen (1977) and Brown (1946b) found no effects of volume in their studies. Only one of the four comparisons proved significant. That is, only in one treatment was the response to changing densities greater than that which would be expected on the basis of density alone. In this comparison, a doubling of densities in tanks containing 200 fish, resulted in a large reduction in growth rate. In fact, fish in tanks in which densities were doubled, began growing at a rate less than that of some other tanks with higher fish densities.

As in the first experiment there was no evidence of stress

from the cortisol data. For reasons described earlier this was not surprising although in this study, at the time of sampling, there was evidence of density-induced, stress-related differences in growth rates. This finding provides further evidence that the use of plasma cortisol as an indicator of chronic stress is of limited value.

Although plasma cortisol, a primary level stress indicator, did not indicate a stress response, there were changes in proximate composition, a tertiary level indicator. The effects, while less pronounced than those of the first experiment, did show similar trends. The lack of clear results was probably due to the effects of sudden density changes, and also the smaller relative change in weight than that which occurred in the first experiment. With respect to energy metabolism, it appears that juvenile steelhead trout (2-30 grams), respond similarly to density stress. Under high densities lipid reserves, the most important energy source for sustained exercise, are compromised.

Differences in the response of body condition to density were observed between the second and the first experiment. Although significant differences between densities were found in this experiment, the trend was in the opposite direction to the first experiment. In addition, there were no clear patterns with time in either body condition or growth slopes as had been observed in the first study. These findings suggest that condition factor may not always provide an accurate assessment of the well-being of a group of fish.

Stress responses in smolts and presmolts

Interpretation of the results of the final group of experiments was complicated by the fact that normal growth processes and responses to density may have been moderated by smoltification. Table 13 provides a list of some changes that occur during smoltification. Some of the variables used to assess stress also change during the transformation from parr to smolt. In particular, condition factor and whole body lipid content tend to decrease, whereas growth rates increase (Folmar and Dickhoff 1980; Hoar 1976). In addition, the capacity to osmoregulate in a hypertonic medium increases (Komourjdian et al. 1976a; Wagner 1974a).

Results of the first small-scale experiment (3a) were complex. Growth rates, condition factors, and plasma cortisol levels suggested no differences in stress response between treatments. However, body composition and hyper-osmoregulatory capability suggested otherwise.

Trout (30-60 grams) reared over an eight-fold density range showed no differences in growth rates. This suggests that larger trout (near smolt size) are unaffected by rearing densities up to three times conventional hatchery levels. There are no similar findings in the fish culture literature; however, in principle, these findings agree with those of Kawanabe (1969) and Magnuson (1962).

Cortisol levels were unaffected by rearing density. However, there was a decline with time to common levels in all treatments. If fish were stressed, adaptation may have occurred.

If this was the case then, during the period of smoltification, a time sensitive to rearing stress (Schreck 1981; Fólmar and Dickhoff 1980), cortisol should not be used to assess chronic stress.

As mentioned earlier, proximate composition patterns were not clear. However, both ash and moisture content were higher at high densities. Protein levels were unaffected, and lipids showed a tendency to decline as density increased. These findings compare favourably with those of Fagerlund et al. (1981), but are different from those of my first two experiments in that protein levels did not increase with density. If increased moisture levels indicate reduced ability to survive, then high density rearing caused stress.

Interpretation of lipid data is difficult. Reductions in lipid content are associated with smoltification (Hoar 1976; Komourdjian et al. 1976b; Vanstone and Markert 1968). Therefore, reduced levels at high densities could be attributed to either a stress-related reduction, or a more advanced state of smoltification. Burrows (1969) found that smolt survival increased as lipid levels increased. Clearly, trends in lipids must be used with caution as an indicator of either smolt readiness or stress response in density experiments, especially if the data are taken near the time of smoltification. Although the significance of the altered body composition is unclear, it does imply changes in metabolism associated with rearing density. This suggests that under certain conditions growth rates are inadequate indicators of stress. Birks et al. (1981),

studying growth of chinook salmon, reached similar conclusions. In density experiments, they found growth suppression to be short-lived. After several months of apparently stress free growth (i.e. equal growth) fish which had been reared at high densities had reduced osmoregulatory capability.

Condition factors showed no evidence of density effects. However, they did follow a pattern similar to growth rates. As with the proximate analysis data, interpretation of the changes in condition during this period is difficult. Many authors describe reductions in condition (increased streamlining) during smoltification (Folmar and Dickhoff 1980; Komourdjian et al. 1976a; Wagner 1974b; Hoar 1976). Indeed, Wagner considered reduction in condition factors to be a satisfactory means of assessing smolt status. Clearly the effects of stress were not manifested in changes in condition factor in my data, and therefore appear to be of limited value as a means of assessing stress under these conditions. Furthermore, their value in assessing smolt status appears limited since they did not indicate differences in streamlining, yet differences in salt water readiness were detected.

There were no treatment-related differences in either net or total activity levels. Energy expenditure differences could not be attributed to activity. However, the pattern with time was interesting. As described earlier, both measures of activity increased evenly from day 31 to day 59 (3 intervals), and then sharply declined. It seems probable that the increase may have been associated with the process of smoltification. Several

reviews (Wedemeyer et al. 1981; Schreck 1981b; Folmar and Dickhoff 1980), note increases in activity of smolts and an associated tendancy to school. Migratory urge also increases. If the increased activity observed in my data is a part of the smoltification process, then all groups were equally prepared. The decline in activity on the last date may indicate that the period of optimum smolt readiness had passed. Conte and Wagner (1965) and Wagner (1974b) noted that the smolt transformation in steelhead is transient.

The results of the salt water challenge test suggested differences, attributable to treatment effects, in the ability of smolts to survive. At low densities, plasma sodium levels were regulated to 165 meq/l after 24 hours, slightly above the optimum of 150-160 (Clarke and Blackburn 1978) but still below the arbitrary cut-off value of 170 meq/l. Above this level fish are considered less capable of surviving in sea water. Fish from high density treatments regulated plasma sodium levels less efficiently (average 180 meq/l), indicating that high density rearing imposed some degree of stress.

Results of the second small-scale experiment (3b) confirmed, in part, some of the results from study 3a. Until volumes were changed, density had not affected either growth rate, condition factor, proximate analysis, or plasma cortisol. This suggests that large fish are relatively insensitive to rearing densities. However, when volumes were changed, fish in tanks where densities were doubled responded with a marked decrease in growth rate and a slight reduction in body

condition. The decrease in growth rate was greater than expected on the basis of density alone. Fish in tanks where densities were halved did not show changes in any of the variables tested. These results suggest that fish become "conditioned" to their environment, including rearing density, and that sudden reductions in space per fish (increases in density) are more stressful than increases in space per fish. That sudden reductions in space per fish caused stress was evident from growth and condition data and from plasma cortisol levels. Changes in cortisol levels were small although statistically significant. Since plasma samples were collected one week after volume changes, perfect adaptation as described by Schreck (1981) had not occurred. Therefore plasma cortisol levels appear to be useful indicators of chronic stress for at least one week after the imposition of the stress. This interpretation is supported by the findings of Wedemeyer (1976).

Cortisol measurements collected on day 74 confirmed the temporal decline observed in experiment 3a providing evidence of a seasonal trend. The lack of treatment effects at the last sampling date, despite the significant differences in growth which persisted after the volume adjustment, indicate that cortisol levels do adapt and after a few weeks are not sensitive to chronic stress.

In view of the absence of significant differences in activity over the eight-fold density range in study 3a, it is likely that the activity differences observed in study 3b were artifacts of volume differences. At low densities (high volumes)

both net and total activity levels were consistently higher than at the high density (low volume). However, the density range in experiment 3a bracketed the range tested here. Furthermore Minchen (1972) found that activity increased as absolute rearing volume increased. In addition, qualitative observations on activity levels in study 3c (different volumes, equal densities), suggests that increased volumes induced increased activity.

Only two treatments were tested in the salt water challenge: those in which volumes were unchanged, and those in which volumes were halved (densities doubled). Therefore, at the time of the test, fish had recently been exposed to densities of either 0.5-0.6 or 1.0-1.1 fish/l. Those fish reared at the lower density had significantly better osmoregulatory performance. These finding agree with those of experiment 3a and the results of Birks et al. (1981).

Results of the third small-scale experiment (3c) indicated that rearing volumes had a slight, though not significant, effect on growth rate. This is in contrast to the findings of Li and Brocksen (1977) and Brown (1946a). Because the trend was not significant, it is unlikely that volume effects influenced the results of other studies. Rearing volume had no effect on salt water tolerance. When fish from experiment 3c (14L:10D photoperiod cycle) were compared with fish reared under similar densities from experiments 3a and 3b (10L:14D photoperiod cycle), no differences in salt tolerance were detected. This indicates that photoperiod effects were unimportant. Fish reared

at low volumes had reduced condition factors. Although they tended to grow slower, they had lower plasma cortisol levels than fish reared under high volumes.

In summary, the stress-related consequences of rearing density on steelhead trout vary with fish size. In each of the studies described here, there was evidence from some or most of the variables tested, of stress in fish reared at high densities. Both the nature and the apparent duration of the stress varied between experiments. Different variables often produced different conclusions regarding either the intensity or the duration of the response. It is clear that none of the variables tested, when used alone, provide an accurate assessment of the degree of stress experienced by steelhead trout as a consequence of high density rearing.

Practically, these results suggest that the rearing densities used in hatcheries for steelhead trout culture may be more conservative than necessary. On a carefully managed basis, it would appear reasonable to experimentally increase rearing densities in some hatcheries by 50 to 100%. However, to fully understand the mechanisms by which density induced stress affects hatchery production and survival of the released fish, additional, fundamental research at both behavioral and physiological levels is required. The significance to fish of density induced changes in body composition, growth rate, and behavior, is far from clear. Furthermore, the nature of the conditioning effect is unclear. Lastly, the rate at which the "adverse" effects of high densities appear as densities

increase, and disappear as they decrease is unknown, yet this knowledge could have considerable management value.

REFERENCES CITED

Allen, K.R. 1969. Limitations on production in salmonid populations in streams. pp. 3-18 In, T.G. Northcote [ed.] Symposium on salmon and trout in streams. H.R. MacMillan Lectures in Fisheries. U.B.C.

Andrews, J.W., L.H. Knight, T.W. Page, Y. Matsuda, and E.E. Brown. 1971. Interactions of stocking density and water turnover on growth and food conversion of channel catfish reared in intensively stocked tanks. Prog. Fish Cult. 33: 197-203.

Aulstad, D. and T. Refstie. 1975. The effect of water depth in rearing tanks on growth and mortality of salmon and rainbow trout fingerlings. Prog. Fish Cult. 37:113-114.

Barton, B.A., R.E. Peter, and C.R. Paulencu. 1980. Plasma cortisol levels of fingerling rainbow trout (Salmo gairdneri) at rest, and subjected to handling, confinement, transport and stocking. Can. J. Fish. Aquat. Sci. 37:805-811.

Bilton, H.T. 1978. Returns of adult coho salmon in relation to mean size and time at release of juveniles. Fish. Mar. Serv. Tech. Rep. 832. Dept. of the Environment. 73pp.

Bilton, H.T., D.F. Alderdice and J. Schnute. 1980. Increasing adult returns of hatchery-produced coho salmon through optimization of time and size at juvenile release. Proc. North Pacific Aquaculture Symp. August 1980. Anchorage, Alaska. pp 171-181.

Birks, E.K., A.R. Hemmingsen, J.L. Dentler and R.D. Ewing. 1981. Hatchery practices. Annual Progress Report. Fish Research Project. Oregon Dept. of Fish and Wildlife. Project F-104-R-2.

Bouck, G.R., M.A. Cairns and R.R. Christian. 1978. Effect of capture stress on plasma enzyme activities in rainbow trout (Salmo gairdneri) J. Fish. Res. Board Can. 35:1485-1488.

Brauhn, J.L., R.C. Simon and W.R. Bridges. 1976. Rainbow trout growth in circular tanks: consequences of different loading densities. Tech. Rep. 86. U.S. Dept. Interior. Fish and Wildlife Service, Washington, D.C. 16 pp.

Brett, J.R. 1958. Implications and assessments of environmental stress. pp. 66-97. In, The investigations of fish power problems. H.R. MacMillan Lectures in Fisheries. University of British Columbia.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21:1183-1226.

Brett, J.R. 1971. Satiation time, appetite, and maximum food intake of sockeye salmon (Oncorhynchus nerka). J. Fish. Res. Board Can. 28:409-415.

Brett, J.R. 1979. Environmental factors and growth, pp. 619-624. In, W.S. Hoar and D.J. Randall [ed.] Fish Physiology. Vol. VIII. Academic Press Inc., New York, N.Y.

Brett, J.R. and D.A. Higgs. 1970. Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, Oncorhynchus nerka. J. Fish. Res. Board Can. 27:1767-1779.

Brett, J.R. and J.M. Blackburn. 1981. Oxygen requirements for growth of young coho (Oncorhynchus kisutch) and sockeye (O. nerka) salmon at 15°C. Can. J. Fish. Aquat. Sci. 38: 399-404.

Brett, J.R. and C.A. Zala. 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (Oncorhynchus nerka) under controlled conditions. J. Fish. Res. Board Can. 32:2479-2486.

Brown, M.E. 1946a. The growth of brown trout (Salmo trutta Linn.) I. Factors affecting the growth of trout fry. J. Exptl. Biol. 22:118-129.

Brown, M.E. 1946b. The growth of brown trout (Salmo trutta Linn.) II. The growth of two-year-old trout at a constant temperature of 11.5°C J. Exptl. Biol. 22:130-144.

Burrows, R.E. 1964. Effects of accumulated excretory products on hatchery-reared salmonids. U.S. Fish Wildl. Serv. Res. Rep. 66. 12pp.

Burrows, R.E. 1969. The influence of fingerling quality on adult salmon survivals. Trans. Am. Fish Soc. 98:777-784.

Chapman, D.W. 1962. Aggressive behavior in juvenile coho salmon as a cause of emigration. J. Fish. Res. Board Can. 19: 1047-1080.

Chapman, D.W. 1966. Food and space as regulators of salmonid populations in streams. Am. Naturalist 100:345-357.

Clarke, W.C. and J. Blackburn. 1977. A seawater challenge test to measure smolting in juvenile salmon. Dep. Environ., Fish. Mar. Serv. Res. Div. Dir., Tech. Rep. 705. 11pp.

Clarke, W.C. and J. Blackburn. 1978. Seawater challenge tests performed on hatchery stocks of chinook and coho salmon in 1977. Can. Fish. Mar. Serv. Tech. Rep. 761, 19pp.

Clinical Assays. Division of Travenol Laboratories Inc., Cambridge, Massachusetts. 02139.

Cole, K.S. and D.L.G. Noakes. 1980. Development of early social behavior of rainbow trout, Salmo gairdneri (Pisces, Salmonidae). Behavioral Processes. 5:97-112.

Conte, F.P. and H.H. Wagner. 1965. Development of osmotic and ionic regulation in juvenile steelhead trout, Salmo gairdneri. Comp. Biochem. Physiol., 14:603-620.

Davis, J.C. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. J. Fish. Res. Board Can. 32:2295-2332.

Dill, L.M. 1981. Food abundance and territory size in juvenile coho salmon (Oncorhynchus kisutch). Can. J. Zool. 59:1801-1809.

Donaldson, E.M. and H.M. Dye. 1975. Corticosteroid concentrations in sockeye salmon (Oncorhynchus nerka) exposed to low concentrations of copper. J. Fish. Res. Board Can. 32:533-539.

Donaldson, E.M. 1981. The pituitary-interrenal axis as an indicator of stress in fish. pp. 11-47. In, A.D. Pickering [ed.]. Stress and fish. Academic Press. New York, N.Y.

Eddy, F.B. 1981. Effects of stress on osmotic and ionic regulation in fish. pp. 77-102. In, A.D. Pickering [ed.] Stress and Fish. Academic Press. Toronto.

Ejike, C. and C.B. Schreck. 1980. Stress and social heirarchy rank in coho salmon. Trans. Am. Fish. Soc. 109:423-426.

Environmental Protection Agency. 1973. Water quality criteria: A report of the committee on water quality criteria. National Academy of Sciences, National Academy of Engineering. Washington, D.C.

Erickson, J.G. 1967. Social heirarchy, territoriality, and stress reactions in sunfish. Physiol. Zool. 40:40-48.

Fagerlund, U.H.M., J.R. McBride and E.T. Stone. 1981. Stress-related effects of hatchery rearing density on coho salmon. Trans. Am. Fish. Soc. 110:644-649.

Fenderson, O.C. and M.R. Carpenter. 1971. Effects of crowding on the behavior of juvenile hatchery and wild landlocked Atlantic Salmon (Salmo salar L.). Animal Behavior 19:439-447.

Folmar, L.C. and W.W. Dickhoff. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids: A review of selected literature. Aquaculture 21:1-37.

Ford, B.S. 1982. Steelhead harvest analysis 1980-81. Fisheries Technical Circular 52. B.C. Ministry of the Environment. 87pp.

Fraser, F.J. 1969. Population density effects on survival and growth of juvenile coho salmon and steelhead trout in experimental stream-channels. pp. 253-266. In, T.G. Northcote [ed.]. Symposium on salmon and trout in streams. H.R. MacMillan Lectures in Fisheries. U.B.C.

Fry, F.E.J. 1957. The aquatic respiration of fish, pp. 1-63. In, M.E. Brown [ed.] The Physiology of fishes. Vol. I. Metabolism. Academic Press Inc., New york, N.Y.

Grove, D.J., L.G. Loizides, and J. Nott. 1978. Satiation amount, frequency of feeding and gastric emptying rate in Salmo gairdneri. J. Fish Biol. 12:507-516.

Hart, J.L. 1973. Pacific fishes of Canada. Fish. Res. Board Can. Bull. 180. pp. 128-131.

Higgs, D.A., E.M. Donaldson, H.M. Dye, and J.R. McBride. 1975. A preliminary investigation of the effect of bovine growth hormone on growth and muscle composition of coho salmon (Oncorhynchus kisutch). Gen. Comp. Endocr. 27:240-253.

Hill, C.W. and P.O. Fromm. 1968. Response of the interrenal gland or rainbow trout (Salmo gairdneri) to stress. Gen. Comp. Endocr. 11:69-77.

Hoar, W.S. 1976. Smolt transformation: evolution, behavior, and physiology. J. Fish. Res. Board Can. 33:1234-1252.

Iwama, G.K. 1979. Saltwater challenge tests of steelhead trout in 1979. Ministry of the Environment. B.C. Fish and Wildlife Branch. Fish Culture Section.

Iwama, G.K. and A.F. Tautz. 1981. A simple growth model for salmonids in hatcheries. Can. J. Fish. Aquat. Sci. 38:649-656.

Kalleberg, H. 1958. Observations in a stream tank of territoriality and competition in juvenile salmon and trout (Salmo salar L. S. trutta L.). Rep. Inst. Freshwater Res. Drottingholm. 39:55-98.

Kawanabe, H. 1969. The significance of social structure in production of the "Ayu", Plecoglossus altivelis. pp. 243-251. In, T.G. Northcote [ed.] Symposium on salmon and trout in streams. H.R. MacMillan Lectures in Fisheries. The University of British Columbia.

Komourdjian, M.P., R.L. Saunders and J.C. Fenwick. 1976a. The effect of porcine somatotropin on growth and survival in Atlantic salmon (Salmo salar). Can. J. Zool. 54:531-535.

Komourdjian, M.P., R.L. Saunders and J.C. Fenwick. 1976b. Evidence for the role of growth hormone as a part of a "light-pituitary axis" in growth and smoltification of Atlantic salmon (Salmo salar). Can. J. Zool. 54:544-551.

Kozak, T. personal communication. Faculty of Forestry. University of British Columbia.

Larkin, P.A. 1974. Play it again Sam - an essay on salmon enhancement. J. Fish. Res. Board Can. 31:1433-1459.

Larkin, P.A. 1981. A perspective on population genetics and salmon management. Can. J. Fish. Aquat. Sci. 38:1469-1475.

Lee, J. and J. Laycock. 1978. Essential endocrinology. Oxford University Press. Toronto. 227pp.

Li, H.W. 1973. A bioenergetic analysis of intraspecific and interspecific competition in two teleost fishes. Doctoral dissertation. U. of California, Davis, California, U.S.A.

Li, H.W. and R.W. Brocksen. 1977. Approaches to the energetic costs of intraspecific competition for space by rainbow trout (Salmo gairdneri). J. Fish Biol. 11:329-341.

Liao, P.B. 1974. Ammonia production rate and its application to fish culture system planning and design. Kramer, Chin and Mayo, Inc. Consultants. Wa. Technical Report no. 35.

MacCrimmon, H.R., J.E. Stewart, and J.R. Brett. 1974. Aquaculture in Canada: The practice and the promise. Bulletin 188. Fisheries Research Board of Canada.

MacLeod, J.R. 1977. Enhancement technology: a positive statement, pp. 137-147. In, D.V. Ellis [ed.] Pacific salmon management for people. University of Victoria press. Victoria, B.C.

Magnuson, J.J. 1962. An analysis of aggressive behavior, growth and competition for food and space in medaka (Aquidens pulcher (Pisces, Cyprinodontidae). Can. J. Zool. 40:313-363.

Mason, J.C. and D.W. Chapman. 1965. Significance of early emergence, environmental rearing capacity, and behavioral ecology of juvenile coho salmon in stream channels. J. Fish. Res. Board Can. 22:173-190.

Mazeaud, M.M., F. Mazeaud and E.M. Donaldson. 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans. Am. Fish. Soc. 106:201-212.

McDonald, A.L., N.W. Heimstra, and D.K. Damkot. 1968. Social modification of agonistic behavior in fish. Animal Behavior. 16:437-441.

McLean, W. E. 1979. A rearing model for salmonids. MSc. Interdisciplinary studies. University of British Columbia.

McLeay, D.J. and M.R. Gordon. 1977. Leucocrit: a simple hematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulpmill effluent. J. Fish. Res. Board Can. 34:2164-2175.

Minchen, J.D. deC. E. 1972. The effect of space at constant densities on growth in a cichlid Aquidens pulcher Gill. MSc thesis. Dept. of Zoology, University of British Columbia.

Noakes, D.L.G. and J. Leatherland. 1977. Social dominance and interrenal cell activity in rainbow trout, Salmo gairdneri (Pisces, Salmonidae). Env. Biol. Fish 2:131-136.

Pickering, A.D. 1981. Introduction: The concept of biological stress, pp. 1-9. In, A.D. Pickering [ed.] Stress and fish. Academic Press. Toronto.

Refstie, T. 1977. Effects of density on growth and survival of rainbow trout. Aquaculture 11:329-334.

Refstie, T. and A. Kittleson. 1976. Effect of density on growth and survival of artificially reared Atlantic salmon. Aquaculture 8:3319-326.

Ricker, W.E. 1973. Linear regressions in fishery research. J. Fish. Res. Board Can. 30:409-434.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Fish. Res. Board Can. Bull. 191. 382 pp.

Riley, J.R. and R. Chester. 1971. Introduction to marine chemistry. Academic Press. New York, N.Y.

Sandercock, F.K. and E.T. Stone. cited in Fagerlund et al. 1981.

Schreck, C.B. 1981. Stress and compensation in teleostean fishes: response to social and physical factors. pp. 295-321. In, A.D. Pickering. Stress and fish. Academic Press. New York, N.Y.

Schreck, C.B. 1981. Parr-smolt transformation and behavior. Proc. salmon and trout migratory and behavior symposium. Seattle, Washington.

Schreck, C.B. and H.W. Lorz. 1978. Stress response of coho salmon (Oncorhynchus kisutch) elicited by cadmium and copper and potential use of cortisol as an indicator of stress. J. Fish. Res. Board Can. 35:1124-1129.

Selye, H. 1950. Stress and the general adaptation syndrome. British Medical Journal 1:1383-1392.

Selye, H. 1973. The evolution of the stress concept. Am. Sci. 61:692-699.

Shelbourne, J.E., J.R. Brett and S. Shirahata. 1973. Effect of temperature and feeding regime on the specific growth rate of sockeye salmon fry (Oncorhynchus nerka), with a consideration of size effect. J. Fish. Res. Board Can. 30: 1191-1194.

Slaney, P.A. and T.G. Northcote. 1974. Effects of prey abundance on density and territorial behavior of young rainbow trout (Salmo gairdneri) in laboratory stream channels. J. Fish. Res. Board Can. 31:1201-1209.

Specker J.L. and C.B. Schreck. 1980. Stress response to transportation and fitness for marine survival in coho salmon (Oncorhynchus kisutch) smolts. Can. J. Fish. Aquat. Sci. 37:765-769.

Standard methods for the examination of water and wastewater. 14th ed. pp. 441-449. Prepared and published jointly by: American Public Health Association, American Water Works Association, Water Pollution Control Federation. Washington, D.C.

Strange, R.J. and C.B. Schreck. 1978. Anaesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (Oncorhynchus tshawytscha). J. Fish. Res. Board Can. 35:345-349.

Strange, R.J. and C.B. Schreck. 1980. Seawater and confinement alters survival and cortisol concentration in yearling chinook salmon. Copeia. 1980:351-353.

Strange, R.J., C.B. Schreck and J.T. Golden. 1977. Corticoid stress response to handling and temperature in salmonids. Trans. Am. Fish. Soc. 106:213-218.

Tautz, A.F. personal communication. British Columbia Ministry of the Environment, Fish and Wildlife Branch. University of British Columbia.

Trzebiatowski, R., J. Filipiak and R. Jakubowski. 1981. Effect of stock density on growth and survival of rainbow trout (Salmo gairdneri Rich.). Aquaculture 22:289-295.

Vanstone, W.E. and J.R. Markert. 1968. Some morphological and biochemical changes in coho salmon, Oncorhynchus kisutch, during parr-smolt transformation. J. Fish. Res. Board Can. 25:2403-2418.

Wagner, H.H. 1974a. Seawater adaptation independant of photoperiod in steelhead trout (Salmo gairdneri). Can. J. Zool. 52:805-812.

Wagner, H.H. 1974b. Photoperiod and temperature regulation of smolting in steelhead trout (Salmo gairdneri). Can. J. Zool. 52:219-234.

Warren, C.E. and G.E. Davis. 1967. Laboratory studies on the feeding, bioenergetics and growth of fishes, pp. 175-214. In, S.D. Gerking [ed.] The biological basis of freshwater fish production. Blackwell Scientific Publications. Oxford.

Wedemeyer, G.A. 1970. The role of stress in disease resistance of fishes. pp. 30-35. In, S.F. Snieszko [ed.] A symposium on diseases of fishes and shellfishes. Special Publ. 5. Am. Fish. Soc. Washington.

Wedemeyer, G.A. 1972. Some physiological consequences of handling stress in juvenile coho salmon (Oncorhynchus kisutch) and steelhead trout (Salmo gairdneri). J. Fish. Res. Board Can. 29:1780-1783.

Wedemeyer, G.A. 1976. Physiological response of juvenile coho salmon (Oncorhynchus kisutch) and rainbow trout (Salmo gairdneri) to handling and crowding stress in intensive fish culture. J. Fish. Res. Board Can. 33:2699-2702.

Wedemeyer, G.A. 1980. The physiological response of fishes to the stress of intensive aquaculture in recirculation systems. pp. 3-18. In, Proc. world symp. on aquaculture in heated effluents and recirculation systems. Stavanger 28-30. 1980. Vol. II. Berlin.

Wedemeyer, G.A. and D.J. McLeay. 1981. Methods for determining the tolerances of fishes to environmental stressors. pp. 247-275. In, A.D. Pickering [ed.] Stress and fish. Academic Press. Toronto.

Wedemeyer, G.A. and W.T. Yasutake. 1977. Clinical methods for the assessment of the affects of environmental stress on fish health. Tech. Rep. 89. U.S. Fish and Wildlife Service. U.S. Dept. of the Interior. Washington, D.C. 18 pp.

Wedemeyer, G.A., R.L. Saunders, and W.C. Clarke. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. National Marine Fisheries Review. 42:1-14.

Wedemeyer, G.A., R.L. Saunders, and W.C. Clarke. 1981. The hatchery environment required to optimize smoltification in the artificial production of anadromous salmonids, pp. 6-20. In, C.J. Allen and E.C. Kinney [ed.] Proceedings of the bio-engineering symposium on fish culture. Fish Culture Section, American Fisheries Society.

Westers, H. and K.M. Pratt. 1977. Rational design of hatcheries for intensive salmonid culture, based on metabolic characteristics. Prog. Fish Cult. 39:157-165.

Windell, J.T., D.O. Norris, J.K. Kitchell, and J.S. Norris. 1969. Digestive response of rainbow trout, Salmo gairdneri, to pellet diets. J. Fish. Res. Board Can. 26: 1801-1812.

Zelnik, P.R. and G. Goldspink. 1980. Effects of exercise at different swimming speeds on blood cortisol levels in the rainbow trout, Salmo gairdneri Richardson. Abstract In, A.D. Pickering [ed.] Stress and fish. Academic Press. Toronto.

APPENDIX I. ANALYTICAL COMPOSITION OF ARTIFICAL SEA SALT.

Average solution of Forty Fathoms Marinemix hydrated to a density of 1.025 using distilled water. Figures cited from actual independant laboratory analysis. Concentratioins in parts per million (ppm). Upon hydration, pH is 8.3.

aluminum	0.06	manganese	0.008
antimony	0.0005	mercury	0.0007
argon	trace	molybdenum	0.005
arsenum	0.01	neodymium	trace
barium	0.12	neon	trace
bicarbonate	174	nickel	0.009
beryllium	0.0002	niobium	trace
bismuth	trace	nitrogen	0.85
boron	2.1	palladium	trace
bromide	62	phosphorus	0.04
cadmium	0.009	potassium	380
calcium	410	praeseodymium	trace
carbonate	10	protactinium	trace
cerium	0.0007	radium	trace
cesium	trace	radon	trace
chromium	0.02	rubidium	0.06
chloride	18600	ruthenium	trace
copper	0.007	samarium	trace
cobalt	0.0025	scandium	trace
dysprosium	trace	selenium	trace
erbium	trace	silicon	4.5
europium	trace	sodium	10400
fluoride	1.9	strontium	12.4
gadolinium	trace	sulfur (sulfate)	2600
gallium	0.0004	tantalium	trace
germanium	0.00005	tellurium	trace
gold	trace	terbium	trace
hafnium	trace	thalium	0.00007
helium	trace	thulium	trace
holmium	trace	tin	0.006
indium	trace	titanium	0.004
iodine	0.03	tungsten	0.004
iron	0.03	uranium	0.00005
krypton	trace	vanadium	0.0009
lanthanum	trace	xenon	trace
lead	trace	ytterbium	trace
lithium	0.24	yttrium	trace
lutetium	trace	zinc	0.24
magnesium	1290	zirconium	trace