ASSESSMENT OF A DISPLACEMENT TECHNIQUE FOR THE ESTIMATION OF THE BIOMASS OF FISH IN A CLOSED TANK

A thesis
submitted to the Graduate Faculty
in Partial Fulfilment of the Requirements
for the Degree of
Master of Science
in the Department of Health Management
Faculty of Veterinary Medicine
University of Prince Edward Island

Nancy Nathalie Bruneau
Charlottetown, P.E.I.
July, 1992

ISBN 0-315-77649-8

CONDITIONS OF USE OF THESIS

The author has agreed that the Library, University of Prince Edward Island, may make this thesis freely available for inspection. Moreover, the author has agreed that permission for extensive copying of this thesis for scholarly purposes may be granted by the professor or professors who supervised the thesis work recorded herein or, in their absence, by the Chairman of the Department or the Dean of the Faculty in which the thesis work was done. It is understood that due recognition will be given to the author of this thesis and to the University of Prince Edward Island in any use of the material in this thesis. Copying or publication or any other use of the thesis for financial gain without approval by the University of Prince Edward Island and the author's written permission is prohibited.

Requests for permission to copy or to make any other use of material in this thesis in whole or in part should be addressed to:

Chairman of the department of Health Management

Faculty of Veterinary Medicine

University of Prince Edward Island

Charlottetown, Prince Edward Island

Canada C1A 4P3

SIGNATURE PAGES

iii-iv

REMOVED

ABSTRACT

The sensitivity and commercial applicability of an innovative technique that estimates the total biomass of fish in a closed tank was investigated. The technique operates on the principle of water displacement. The procedure involves adding a known quantity of inert marker to a tank of known volume and allowing it to mix completely. The amount of free water in the tank is calculated from the concentration of the marker in the water. This volume is subtracted from the total tank volume, yielding the fish biomass for that tank.

Stability under the influence of potential limiting factors (pH, reducing and oxidizing agents, chemical and physical adsorption), ease of detectability, and safety concerns for both animals and consumers were the prime criteria for the selection of an appropriate marker. Food Drugs and Cosmetics No. 1 (FD&C Blue No. 1), a triphenylmethane colour, showed better stability than three other candidates.

FD&C No. 1 was determined to be non-toxic to rainbow trout (Oncorhynchus mykiss) and arctic charr (Salvelinus alpinus). Tissue uptake of the chemical was not detected.

A comparison was made between two physical forms (granular and powder) of FD&C No. 1. The granules gave more accurate results than the powder. Mechanical water turbulence relevancy was found to significantly increase both accuracy and precision of the proposed technique. Sampling times were evaluated. At fixed concentration, tank volume did not significantly affect the time required to reach optimum dye dispersion.

A field survey on four different biomass calculation strategies -- mass weighing (standard), dip-net sampling, specific growth rate estimation, and water displacement technique -- was performed. Statistical evidence showed that 1) dip-netting yielded random samples, 2) the water displacement technique yielded positively biased estimates of the true fish biomass, and 3) biomass estimation through specific growth rate was reliable.

ACKNOWLEDGEMENTS

I would like to thank the members of my supervisory committee (Drs. A. Donald, G. Johnson, T. Ogilvie, and L. Spangler) for their help and guidance and offer special thanks to my co-supervisors -- Drs. Donald and Spangler --. I would also like to acknowledge Dr. P. Niger who gave me a bench space in his laboratory. Thanks are also due to the staff of the fish health unit and fish farm, principally Paul Lyon and Dr. J. Bayer, and the technicians of the toxicology laboratory. Finally, special thanks to Bill for his everlasting support.

This research was supported by the Cooperative Agreement for Fisheries Development, Aquaculture Diagnostic Services Program, Atlantic Veterinary College and the Department of Fisheries and Oceans.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

TABLES

FIGURES

1. GENERAL INTRODUCTION

The lack of information on the biomass of fish in a tank can create health and economic problems. The ability to accurately and easily weigh fish while they are still in the tank is useful as both a sound health management tool and as an aid to marketing. Knowing the carrying capacity (kg of fish per litre per minute) and loading capacity (kg of fish per cubic meter of water) helps to optimize the feed conversion efficiency, water quality (Poston and Williams, 1988), and administration of medication. Farmers often need to know exactly how many tanks of fish are needed to fulfil a sales contract.

Fish held at high stocking densities are generally considered to be exposed to chronic stressors (Wedemeyer, 1976; Vijayan and Leatherland, 1989) which impose severe energy demands and may increase susceptibility to disease (Wedemeyer, 1976; Refstie, 1977; Fagerlund et al, 1981; Pickering and Pottinger, 1987a). The onset of disease is also influenced by the spatial distribution of fish within the tanks (Soderberg and Krilse, 1986). Other studies have indicated that high rearing densities can affect growth rates (Sandercock and Stone, 1982; Fagerlund et al., 1983, 1984, 1987), and normal physiological development of juvenile salmonids (Schreck et al., 1985; Leatherland and Cho, 1985; Patino et al., 1986). The growth-modifying effect of stocking density has been attributed to several factors, including decreased food consumption (Refstie, 1977), increased social interaction (Fenderson and Carpenter 1971; Reftsie and Kittelsen, 1976), and water quality deterioration (Pickering and

Stewart, 1984; Pickering and Pottinger, 1987b).

To counteract the problems associated with high rearing densities, to evaluate loss and growth, and to maintain livestock at optimum rearing levels (Haskell, 1955; Burrows and Combs, 1968; Piper, 1970; Piper et al.,1982), the fish-farming industry needs an efficient means to estimate the biomass of fish in a tank.

In the following, an overview of the current measuring methods and their respective limitations is presented.

The bioenergetics models. A simple energy budget equation is designed to simulate fish growth. Parameters for the model are initial body size (weight), activity level, food consumption, diet (all-fish, all-invertebrate, or mixed), and environmental temperature (Kitchell et al., 1977; Diana et al., 1988). However, most of the coefficients for these parameters are (1) extrapolated from simulation growth models of the species of interest; (2) extrapolated from available data for other, presumably similar species; or (3) measured in the laboratory, thus more variable or poorly known under natural and commercial culture conditions.

The expected growth rate. The relationships between mean fish weight, water temperature and time on feed are used to develop equations that predict growth rates of fish (Dwyer et al.,1981; Jobling, 1983a). These rates are then used to predict total fish biomass.

More knowledge is required to increase the external validity of this method. Findings on the study of temperature-size-growth relationships refer mostly to salmonid species (Brett and Shelbourn 1975; Jobling, 1983b), and are not extended

to different fish sizes, species or various environmental conditions.

Hydroacoustic assessment. Echo intensity is proportional to the density of targets and the average scattering cross-section per target. Fish densities can be estimated in depth layers from the echo integrator outputs according to the equation (Ehrenberg and Kanemori,1987):

$$Q_i = AV_i^2$$

where

Q_i = mean density of fish for the (i)th depth interval (layer)

V_i^2= integrator output (average squared voltage) for the (i)th layer

A = integrator scaling factor.

The integrator scaling factor is made up of the object's mean backscattering cross-section and various parameters of the acoustic system.

Fish densities in rearing facilities are high (up to 100 fish/m^3), and the classical integration techniques based on a single-scattering model cannot be applied owing to high attenuation of the echo signal by the fish aggregation (0.5-3 dB/m). A multiple-scattering approximation was tested to estimate high densities of fish (>3.5 fish/m^3) in sea pens (Burczynski; 1990). In this study, density values as predicted by single-scattering with attenuation (SSA) approximation and point-scattering (PA) approximation, were much higher for uplooking than for downlooking transducers. Acker (1977) indicated that the average value of target strength within +/- 10° from a salmon's ventral aspect is roughly 5 dB higher than from its dorsal body surface. These findings suggest that fish reflect more energy from their ventral than from their

dorsal aspect. Fish densities in sea pens were underestimated acoustically with downlooking transducers by a factor ranging between 0.47 and 0.97 (average 0.75, s.d. 0.14), while with uplooking transducers they were overestimated by a factor ranging from 1.38 to 3.75 (average 2.15, s.d. 0.96). The systematic error was most likely caused by a number of simplifying assumptions used in the applied factors for the model (Burczynski et al. 1990).

Dip-net sampling. Dip-netting crowded fish appears to yield a random sample (Leitritz, 1980; Thorburn, unpublished data). Unfortunately, results from Thorburn (1989) showed that sampling of uncrowded fish does not yield random samples, even if the tank is stirred first. As has been suggested by Seeger et al. (1977), larger fish may, when stressed, be more able to evade the dip-net. Also, the distribution of fish in a water volume is dependent, among other factors, on fish size (Crittenden and Thomas, 1989).

With the exception of the acoustical remote sensing, all of these methods involve handling of the fish. It has been shown that 2 weeks is necessary for the fish to completely overcome the effects of acute handling stress associated with sampling (Pickering et al., 1982).

The rapidly expanding fish-farming industry needs a means to estimate fish biomass reliably, quickly, and with minimal stress to fish. The main purpose of this research project was to investigate the utility of a technique, valid for closed systems, that is believed to meet the above criteria.

The principle is to add a known quantity of inert marker to a tank of known

volume and allow it to mix thoroughly. The amount of free water in the tank can be calculated from the concentration of the marker obtained from a water sample. Subtracting this volume from the total tank volume gives the volume of fish in the tank.

It is immediately evident that the success of such a method depends upon the following critical control points: (1) the initial tank volume must be assessed accurately; (2) the initial weight of the marker and its final concentration must be determined accurately; (3) the marker must be allowed to mix thoroughly in the water; (4) marker selection must be made such that markers which have a tendency to cling on the sides of the tank or to the fish are avoided; (5) the chemical must be non-toxic and neither absorbed nor metabolized by the fish; (6) finally, the marker must be stable when exposed to different environmental conditions.

There were four objectives of this research:

1) to select the marker that will demonstrate the highest detectability and stability when exposed to different water chemistry conditions;
2) to assess the potential exposure effects (including possible toxicity or uptake of the marker by the fish) of the chosen chemical on the experimental fish species (Oncorhynchus mykiss);
3) To identify the optimal methodology, in terms of accuracy and precision, of the proposed technique;
4) To determine the precision and accuracy of the method in the field, and compare it to its potential competitors: fish weighing, dip-net sampling, and estimated

growth rate.

For the purpose of this study two commercial fish species were used: rainbow trout (Oncorhynchus mykiss), and arctic charr (Salvelinus alpinus). The test species were selected for their commercial or economic value, availability, and considerable geographic distribution.

2.DETECTABILITY AND STABILITY EVALUATION OF FOUR CERTIFIED FOOD DYES

2.1 INTRODUCTION

Initially, six compounds were selected as potential markers for the biomass estimation technique. They were: Pectin, polyethylene glycol, and Food Drug and Cosmetics Blue No. 1 (Brilliant Blue FCF), Yellow No. 5 (Tartrazine), Yellow No. 6 (Sunset Yellow No.6), and Red No. 40 (Allura Red). Consideration was given to the above compounds because of their desirable physical characteristics such as high hydrophilicity, low lipophilicity, high molecular weight, and apparent safety for both the target animals and consumers (Smith et al., 1949; Radomski, 1974). In preliminary testing, pectin and polyethylene glycol were rejected due to difficulty in detecting and measuring them. In contrast to the synthetic food dyes, neither compound absorbs at a single reference wavelength, and their physical characteristics (e.g. viscosity) are temperature-dependent (Windholz et al., 1983). Also, the quantitative analysis of colorless compounds that do not absorb in the ultra violet region can be altered by the presence of other solutes in the water. Because of these concerns, testing was performed only on the certified food dyes. See Table 1 for empirical formula, classification, and key characteristics of the four food colours (Noonan, 1972).

Results of several studies have indicated the carcinogenic, teratogenic, hyperkinetic, and allergenic potential of certain dyes. Perhaps the most controversial aspect of the safety of artificial colours and flavours is the one that links ingestion of

Table I. Some identification and solubility properties of FD&C colours.

Federal name (Emperical formula)	Classification	Mol.wt[1]	Water sol.(2)	
			2C	25C
FD&C Blue No. 1 ($C_{37}H_{34}N_2O_9S_3Na_2$)	Triphenylmethane	792.84	20	20
FD&C Yellow No. 5 ($C_{16}H_9N_4O_9SNa_3$)	Pyrazolone	534.36	4	20
FD&C Yellow No. 6 ($C_{16}H_{10}N_2O_7S_2Na_2$)	Monoazo	452.36	19	19
FD&C Red No. 40 ($C_{18}H_{14}N_2O_8S_2Na_2$)	Monoazo	496.42	18	22

(1) Molecular weight
(2) Water solubility (g/L)

food dyes to the functional state of the cental nervous system (CNS). Tartrazine in particular, has been linked with allergic-type reactions in humans (Lockey, 1959; Chafee and Settipane, 1967) and with the behavioral disorders in children (Feingold, 1975). Although the latter specific claim of neurobehavioral potential is based on clinical observations and disagrees with the well-controlled experimental evidence of Sobotka et al. (1977) and Kantor et al. (1984), the results of the Feingold study are enough to raise doubts about its safety. Triphenylmethane colours, including FD&C Blue No. 1, contain sulfonic acid groups and are, therefore, highly water soluble. Unsulfonated dyes would be oil soluble. In addition, lack of sulfonation seems to go along with high toxicity (Noonan, 1972). Triphenylmethanes are all poorly absorbed from the gastrointestinal tract, due to their low pKs, and are largely excreted unchanged in the faeces (Hess and Fitzhugh, 1955).

Perhaps the least toxic of the sulfonated naphthalene azo colours is FD&C Yellow No. 6. This colour has been extensively tested for carcinogenicity and chronic toxicity in mice, rats, and dogs. No pathological or toxicological effects were noted even at 2-5% of the diet (Gaunt et al., 1967). Also, no toxicological abnormalities were observed in pigs tested for 98 days at levels up to 100 mg/day (Gaunt et al., 1969).

A number of studies have been performed to test the possible teratogenic and reproductive effects of FD&C Red No. 40. In a study of Collins et al. (1989), there were no dose-related changes in maternal findings, number of fetuses, fetal viability, or external or visceral variations in treated female rats consuming up to 939.3 mg of

dye per kilogram body weight per day. This agrees with a study of Collins and Black (1980), who found that no signs of compound-related effects in the maternal or fetal animals, but disagrees with that of Vorhees et al. (1983) who found reduction in offspring survival and evidence of dose-related behavioral toxicity.

In general, the certified food colours can be said to be stable for most uses. In the dry state, no degradation has been noted, other than loss in dye strength by moisture absorption, in samples stored for up to 15 years. Factors that contribute to the instability of colorants are mostly associated with storage and processing (Noonan, 1972).

The majority of the certified food colours, however, lack stability in combination with reducing agents and retorted protein materials (e.g. canned foods). The azo and triphenylmethane dyes are rapidly reduced to colourless compounds under such conditions. Colour fading is frequently caused by ascorbic acid, a reducing agent, used as a flavour anti-oxidant and as a source of vitamin C in carbonated and still beverages. Fogg and Summan (1983) have shown that the reductive action of ascorbic acid is catalyzed by the synergistic action of light and the absence of the disodium salt of ethylenediaminetetraacetic acid (EDTA). The times for the complete degradation of food colouring material in accelerated light (500-W lamp) with and without EDTA were 23 and 10 h (Red 2G), 24 and 6 h (Sunset Yellow FCF), 12 and 4 h (Ponceau 4R), and 4 and 4 h (amaranth), respectively. Clearly in some instances the presence of EDTA not only protects the ascorbic acid from interaction with oxygen but also stabilises the food colouring with respect to

reaction with asorbic acid. With the exception of FD&C No.2 and No.3, the light stability of the dyes is good (Noonan, 1972). Trace metals including, zinc, tin, aluminium, iron, and copper are known to cause fading of some additives (Koch, 1967). Azo dyes in particular are troublesome in this regard: they often react with food cans at a rate proportional to their concentration, causing corrosion of the container and a corresponding loss in the food's dye content. Extreme heat (≥ 230-260°C) is a well known enemy of all colouring agents (Hajratwala, 1985; Lykens, 1986).

The pH conditions must also be considered, since some colorants are highly sensitive to pH variation. FD&C Red No.3, for instance, precipitates from acid solution whereas FD&C Green No.3 turns blue under alkaline conditions. Other less drastic changes with pH include loss of tinctorial strength, shifts in shade, or changes in solubility and shelf life.

Most of the literature is of limited practicality since it is mainly qualitative in nature, and provides no accurate guidance about the intensity or magnitude of exposure to potential limiting factors.

The purpose of this investigation was to 1) prepare spectral compilation and analytical standard of each dye. These preliminary steps are indispensable to achieving sensitive quantitative analysis; 2) determine the stability of the dyes in solution when they are subjected to chemical adsorption (ie. red clay), reducing agents (ie. copper and ascorbic acid), an oxidizing agent (ie. zinc), physical adsorption, as well as pH change.

2.2 MATERIALS AND METHODS

2.2.1 Preparation of Calibration Curves

The four FD&C pigments used (Blue No. 1, Yellow No. 5, Yellow No. 6, and Red No. 40) were dry powder mixtures obtained from Dyeco Limited (Kingston, Ontario). Detection was by means of a Hewlett-Packard 8452 diode array spectrophotometer (1-cm cell pathlength employed), coupled with a HP89510A UV-Vis Software and printer for data output.

The absorption spectrum for each dyestuff was determined at five different dye concentrations (Table II). After determining the absorbance (A) for each concentration, the calibration curve was prepared for each dye.

Specific amounts of each compound were accurately weighed (Table II) on an analytical balance (Sartorius model 1872) and separately dissolved with 1 litre of distilled water (pH 4.4) in a 1 litre volumetric flask. Ten millilitres was pipetted (10 mL measuring pipet) from each stock and diluted to 100 mL with distilled water to give working solutions (Table II) from which aliquots were taken for either readings or further dilutions. The concentration of a sample solution was adjusted so that its absorbance was within the range of 0.12 to 1.00 (A) to minimize instrumental errors (2% at this suggested range). The absorbance of each solution was measured at the analytical wavelength (highest peak) at room temperature (22.3°C). The reference cell, commonly called the blank, was filled with the solvent (distilled H_2O) and the sample cell with the dye solution. Such an arrangement compensates for solvent absorbtion

Table II. Method of dilution of samples for calibration curves.

Dye	Weight of dye(mg)	Sol.[1]	Dilutions[2]	Final conc.[3]
Blue No. 1	70	7	0, 1:2, 1:4, 1:8	7.0, 3.5, 1.75, 0.875
	60	6	0, 1:2, 1:4	6.0, 3,0, 1.5
	50	5	0, 1:2	5.0, 2.5
	20	2	0, 1:2	2.0, 1.0
Yellow No. 5	220	22	0, 1:2, 1:4	22.0, 11.0, 5.5
	200	20	0, 1:2, 1:10	20.0, 10.0, 2.0
	180	18	0, 1:2	18.0, 9.0
	40	4	0, 1:2	4.0, 2.0
Yellow No. 6	220	22	0, 1:2, 1:4	22.0, 11.0, 5.5
	180	18	0, 1:2	18.0, 9.0
	150	15	0, 1:2	15.0, 7.5
	100	10	0, 1:2, 1:4	10.0, 5.0, 2.5
Red No. 40	220	22	0, 1:2, 1:4	22.0, 11.0, 5.5
	180	18	0, 1:2	18.0, 9.0
	160	16	0, 1:2	16.0, 8.0
	100	10	0, 1:2, 1:4	10.0, 5.0, 2.5

[1] Concentration of dye (mg/L) in stock solution from which dilutions were made
[2] Dilutions of stock solution
[3] Concentration of solutions used in standard plot (mg/L)

and also for losses of radiation by scattering and reflection. The same cell was used for the blank and the samples. The cell containing a sample was placed in the holder in the same orientation as was used when scanning the blank. Once emptied, the cell was rinsed several times with a small amount of the sample to be scanned and the washes and sample were transferred using a Pasteur disposable pipet to avoid spilling down the outside of the cell. A graph of absorbance versus concentration for each dye was plotted. Linear relationships were expected (Beer's law). The percentage uncertainty (the ratio of the prediction interval to the concentration, in percent, for the highest absolute value of absorbance) was calculated for each plot as a measure of detectability and comparative assessment (Hewlett-Packard, 1987).

2.2.2 Testing for Potential Limiting Factors

The stability of the four FD&C colours (Blue No. 1, Yellow No. 5, Yellow No. 6, and Red No. 40) was investigated after exposure to the more common problems expected to have bearing within this study. Sample concentration was determined spectrophometrically, on a 8452 diode array (Hewlett Packard), by visible absorbance measurement at corresponding maximum wavelength. The difference between the predicted and observed concentrations was used as a measure of dye stability. Stability is a major factor in the selection of the best marker for the automated biomass technique. In all cases, the compounds were randomized prior to manipulations. All factors other than the one under investigation were kept constant, including temperature (22.3°C) and solvent (distilled water, pH ~4.42). The stock solutions, prepared as described in Table II, were exposed to the limiting agent of concern and shaken for 3 minutes before aliquots were collected (transfer pipet). The aliquots were diluted as prescribed in Table II. The resulting samples were left undisturbed for 40 to 45 minutes before reading.

The experimental design was intended to exaggerate normal conditions expected on a fish farm, thus boosting any physical changes that might take place.

Chemical adsorption. Ten grams of red clay was added to each stock solution (1 litre). Before reading, the samples were filtered by decantation with analytical filter papers (11 cm diameter).

pH change. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were added

alternately to the solvent, in order to reach a desired pH level of 8.5. Prior to measurement (Beckman 31 pH Meter) two reference solutions, pH4 and pH10, were used for standardization.

Oxidation. One millilitre was taken out of a metallic zinc solution of 1g/L and added separately to the stock dye solutions to yield final zinc concentrations of 1 mg/L.

Reduction. One millilitre was taken out of a metallic copper solution of 1g/L and added separately to the stock dye solutions to yield final copper concentrations of 1 mg/L. One hundred milligrams of ascorbic acid were added to the stock dye solutions.

Physical adsorption. For each dye, four specific amounts (Table III) were weighed (Sartorius model 1872 balance) and dissolved separately into a 100-litre fibreglass tank containing 10 litres of well water. Five minutes were allowed for complete mixing. Physical mixing was performed to ensure homogeneity. One sample was taken from every batch. The procedure was done three times.

The effect of each factor was assessed by the paired t-test comparing the true concentration with the concentration obtained using the spectrophotometer. Change in optical density following exposure was expressed in percentage (average difference in concentration X 100).

Table III. Weights of markers used for physical adsorption trial.

Dye	Weights (mg)
Blue No. 1	70, 60, 50, 40
Yellow No. 5	220, 180, 150, 100
Yellow No. 6	220, 180, 150, 100
Red No. 40	220, 180, 160, 100

2.3 RESULTS

2.3.1 Normalization and Standardization Analysis

The wavelengths of maximum absorption of samples of varying concentration were compared. The ideal is a constant result, as in FD&C Blue No. 1 and Yellow No. 6, where the maximum absorption wavelength (highest peak) was not affected by the absorbance value (Table IV).

Once the calibration curves were completed the uncertainty factor (%) for each of these was calculated as an overall measure of how well the regression model predicts new data. The uncertainty factor, a statistic developed to indicate the quality of the calibration curve, is defined as the ratio of the prediction interval to the concentration in percent for the standard with the highest absolute value of absorbance. The curve with the smallest value was judged highest on the basis of predictive ability. The following uncertainty factors were obtained; 9.623%, 7.333%, 4.337%, and 3.040% for Yellow No. 5, Red No. 40, Blue No. 1, and Yellow No. 6 respectively.

Table IV. Influence of dye concentration on maximum absorption wavelenght (nm).

Dye (FD&C)	Absorbance (A)					
	0.97	0.65	0.47	0.35	0.25	0.12
Blue No. 1	---	630	630	630	630	630
Yellow No. 5	426	426	426	428	---	428
Yellow No. 6	---	482	482	482	482	482
Red No. 40	---	502	502	504	506	506

* For each absorbance, the maximum absorption wavelenght is given in the body of the table.

2.3.2 Quantitative Effects of Potential Limiting Factors

The mean differences between the true and observed concentrations are presented in Table V.

Chemical adsorption. The mean colour difference of the four dyes were significantly different ($\alpha<0.05$) before and after clay adsorption. Average loss of 13.2%, 21.2%, 25.1%, and 32.4% of colour was observed for FD&C Blue No. 1, Yellow No. 5, Yellow No. 6, and Red No. 40 respectively.

pH change. Only FD&C Yellow No. 5 was significantly affected by pH variation. A colour reduction of 7.6% was observed.

Oxidation. Zinc caused a significant oxidation reaction in FD&C Yellow No. 5, Yellow No. 6, and Red No. 40. An increased optical density of 4.5% for FD&C Yellow No. 5 and decreases of 2.4% for FD&C Yellow No. 6 and 6.2% for FD&C Red No. 40 were obtained.

Reduction. Copper, a reducing agent, caused fading in FD&C Yellow No. 5 and Red No. 40 (3.8% and 6.4% respectively). Copper significantly intensified the color of FD&C Blue No. 1 by 4.3%.

Chemical reduction by ascorbic acid reduced significantly the colour strength of FD&C Yellow No. 6 and Red No. 40, with respective losses of 10.4% and 11.3%.

Physical adsorption. By visual assessment there was no apparent clinging of the dyes to the sides of the tanks. However, experimental data revealed significant color loss for FD&C Yellow No. 6 (5.4%). FD&C Yellow No. 6 held the slowest

Table V. Mean difference (mg/L) between the predicted and observed concentrations for the tested factors.

Dye (FD&C)	Factors					
	Chemical adsorption	pH change	Oxidation	Reduction		Physical adorption
				copper	a.acid[1]	
Blue No. 1	0.132*	-0.009	-0.013	-0.043*	0.002	0.013
Yellow No. 5	0.212*	0.008	-0.045*	0.038*	0.012	-0.024
Yellow No. 6	0.251*	0.076*	0.024*	0.009	0.104*	0.054*
Red No. 40	0.324*	0.031	0.062*	0.064*	0.113*	-0.009

* significant at $\alpha=0.05$
[1] ascorbic acid

dissolution followed by FD&C Red No. 40, Yellow No. 5, and Blue No. 1. It was also observed that with the addition of physical mixing approximatively 50 seconds was needed to complete dissolution in FD&C Yellow No. 6 and as little as 5 seconds in the case of FD&C blue No. 1.

In all cases, the observed changes were induced by color instability and not precipitation of the compounds.

2.4 DISCUSSION

This study indicated that among the four compounds only FD&C Blue No. 1 and Yellow No. 6 had the same analytical wavelength at any of the tested concentrations.

Some variation in the values of maximum absorption have been known to occur when the proportion of different absorbing species changes with concentration (Fritz and Schenk, 1973).

Also, since the tinctorial strength of a water soluble colour is proportional to the pure dye content, the presence of various combinations of trace impurities in a dye can contribute to discrimination between samples (Bell, 1976; Joyce and Humphreys, 1982).

The quality of each calibration curve based on the uncertainty factor statistic, an indicator of the fit of the data to the best-fit line, was as follows: FD&C Yellow No. 6 > Blue No. 1 > Red No. 40 > Yellow No. 5. The sources of error contributing to this uncertainty are:

. random error within measurement, primarily instrument noise;
. measurement-to-measurement error, including particulates/impurities, bubbles, schlieren effects (change in refracting index), other solution noise;
. random error in making of standards, mainly generated by the manipulator.

These all have an effect on the quality of the calibration curve. In general, the quality of the calibration curve can be improved by increasing the number of

standards measured (this applies only as long as the errors associated to the additional standards are similar to those for existing standards).

The investigation evaluating the stability of the four Food Drug and Cosmetics dyes (Table V) showed that Blue No. 1 has the best chemical integrity, followed by Yellow No. 5, Red No. 40, and Yellow No. 6.

With the exception of FD&C Blue No. 2, food colours have poor resistance to reducing agents (Noonan, 1972). This supports our findings, that all dyes were affected significantly by either copper or ascorbic acid. FD&C colours are also known for their fair to poor stability to oxidation. In this study all compounds but FD&C Blue No. 1 were significantly oxidized.

In general, these dyes show good stability to changes in pH, showing no appreciable change at pH 3 to 8 (Noonan, 1972). Joyce and Sanger (1979) demonstrated some variation in the values of wavelengths of maximum absorption of both FD&C Yellow No. 5 and No. 6 when the dyes were exposed to pH variation. This variation might explain the instability of FD&C Yellow No. 6 to changes in pH in this study, since samples were measured at the normalization wavelength obtained at pH 4.42. Because of their suitable physicochemical properties such as excellent solubility and polarity, the tested compounds have a strong solute-solvent affinity and the extent of adsorption is expected to be low because of the necessity of breaking the solute-solvent bond before adsorption can occur. Adsorption also occurs when the ligand (also known as chemical binder) and the molecule have different charges, which is why in this study chemisorption took place in all compounds.

Physical adsorption, which is not molecular site-specific and does not involve irreversible chemical bonding, was present only in FD&C Yellow No. 6. Since all these dyes are anionic and share similar chemical characteristics (structure, molecular weight, hydrophilicity, etc.), it seems likely that the physical adsorption observed with FD&C Yellow No. 6 may be artificial. FD&C Yellow No. 6 is the slowest to disperse, and may not have been fully dispersed when the samples were taken, which is suspected to have biased the outcome.

Solution concentrations suitable for both visual and spectrophotometric analysis (in 1 cm cells) are as follows for the reviewed dyes: FD&C Blue No. 1 - 4mg/L, Yellow No. 5, Yellow No. 6, and Red No. 40 - 10mg/L (Noonan, 1972). In this study, FD&C Blue No. 1 also demonstrated the strongest hue (Table II). This last information touches both economic and safety concerns (i.e., smaller amounts of chemical are needed to achieve proper quantitative analysis), and merits consideration.

Based on all of the above findings, Brilliant blue (FD&C Blue No. 1) was identified as the best of all candidates. Although there is room to deepen this study with more exhaustive data, this comparative study was sufficient to meet the objective of finding the most suitable compound for this project.

3. THE EFFECT OF EXPOSURE TO FD&C BLUE NO. 1 ON RAINBOW TROUT

3.1 INTRODUCTION

The currently certified FD&C colourings fall into five chemical categories: monoazo, indigoid, quinoline, triphenylmethane, and xanthene. FD&C Blue No. 1 (Brilliant blue FCF) is a water soluble triphenylmethane colour additive that has been in continuous use in foods, drugs, and cosmetics since the late 1800s. It was first formally approved for use in foods in 1929 by the US Department of Agriculture. An acceptable human consumption of FD&C Blue No. 1 of approximatively 12mg/kg of body weight per day was suggested by the US Food and Drug Agency (FDA, 1982).

FD&C Blue No. 1 was initially considered to have carcinogenic potential because of the development of sarcomas at the injection site (Grasso and Goldberg, 1966). Subsequent studies showed that the sarcomas developed because of an alteration in the surface activity of the subcutaneous tissue by the repeated injection of highly concentrated solutions of several colourings, including FD&C Blue No. 1, and were not attributable to the colour per se (Gangolli et al., 1967, 1972; Grasso et al., 1971).

A skin-painting study of repeated dermal applications of FD&C Blue No. 1 to laboratory mice failed to increase the incidence of neoplasms (Carson, 1984). FD&C Blue No. 1 applied to the conjuctival sac of the eye of albino rabbits in 20 mg-doses twice daily, five times a week, for four weeks was not considered an irritant

(Burnett and Opdyke, 1971). The oral administration of FD&C Blue No. 1 has been carried out by Brown et al (1980) and Phillips et al. (1980), who reported that the colouring agent is poorly absorbed from the gastro-intestinal tract, and undergoes subsequent rapid and complete biliary excretion. This partially explains the low oral toxicity of this and structurally related colourings (Randomski, 1974).

There are few published data on dyestuff toxicity to fish. Under the Japanese "Chemical Substances Control Act (1974)", new products which are not readily biodegradable, such as the synthetic organic dyestuffs, must pass a bioaccumulation test in fish before they can be introduced to the market (Kubota, 1979). Since fish accumulation testing is expensive and time consuming, considerable interest was aroused by the evidence that the partition coefficient (P) in n-octanol/water provides a useful indication of bioaccumulation tendency (Neely et al., 1974; Chio et al., 1977). The accumulation test involves measurement of chemical in the whole fish whereas the partition coefficient is more strictly a predictor of accumulation tendency in fish fat. Within the framework of the Organization for Economic Cooperation and Development (OECD) Chemicals Testing Programme (1979) it is proposed that if $P < 1000$, it may be confidently predicted that the Bioconcentration Factor (BF) in the fish will be < 100, and that the carrying out of a fish accumulation test is superfluous. Anliker et al. (1981) shows that none of the dyestuffs bearing at least one charged group, such as FD&C Blue No. 1, show a BF > 10, and such products are not exceptions to the proposed OECD scheme (Esser et al., 1980). Anliker et al. (1981) have also shown that a substantial increase of the octanol/water P of

anionic or cationic dyes is observed when sodium chloride is added to the aqueous phase. These observations suggest that a higher bioaccumulation of ionic dyes may occur in marine fish than in fresh water fish under similar conditions. This possibility merits consideration only if $\log_{10} P > 1$ (for pure water), which would be unusual for an ionic dyestuff.

The insignificant fish bioaccumulation, as reported in the literature, indicates that FD&C Blue No. 1 is not likely to cause major toxic effects. But uptake, even if negligible, will affect the dye concentration in the water and a knowledge of its extent is necessary to appraise the situation.

The present study was designed to determine the toxicity of FD&C Blue No. 1 to rainbow trout (Oncorhynchus mykiss) in laboratory toxicity tests and to measure the dye absorption and adhesion to the outside of the fish or the intestines. These data were used to determine the fate of the chemical in fish and, therefore, the utility of FD&C Blue No. 1 as the marker in the biomass estimation technique.

3.2 MATERIALS AND METHODS

Laboratory static bioassays were conducted to investigate the acute effects of exposure of FD&C Blue No. 1 to rainbow trout. Two test tanks of different concentrations, 5mg/L and 20mg/L, and one control tank were used for both bioassays. The second bioassay was a duplicate. All fish in this study originated from a population of fingerling rainbow trout (Oncorhynchus mykiss) purchased from a commercial hatchery and then kept in holding tanks at the Atlantic Veterinary College for four months.

Well water was used for the tests, and was monitored weekly for chemical characteristics. The average of the measured chemical parameters in the reservoir were: pH 7.4, total alkalinity 129.0 mg/L, total hardness 229.727 mg/L, calcium 49.77 mg/L, and chloride 85.0 mg/L. Temperature was held constant throughout the facility at 10.5°C. The bioassays were carried out in 150-Litre fibreglass tanks. Experimental fish were selected from the holding tanks by dip-net sampling and randomized among test tanks. Thirty fish per tank were used for both bioassays, which corresponded to a density of about 10.7kg of fish/m^3 of water. The fish were acclimated to the test tanks for at least a week prior to the introduction of the compound tested. The study used 180 fingerlings ranging from 11 to 91 g. Fish were fed to satiation twice per day (once per day at weekends), and were starved for one day prior to testing. Exposure lasted exactly three hours. Once the water flow was turned off, oxygenation was ensured by bubbling with atmospheric air. Dissolved gas

was determined by a saturometer (Tensinometer/300c). Before exposure, dissolved nitrogen was supersaturated in the experimental units (due to technical problems) and dissolved oxygen was maintained close to saturation. Following exposure, a drop of a factor of 1.07 for nitrogen (102% to 95% saturation) and 1.23 for oxygen (75% to 61% saturation) was measured.

Behaviour abnormalities were assessed for the whole exposure. Lastly, five fish from each tank were dip-netted and euthanized by a blow on the head for post-mortem and residue analysis.

The freshly culled fish were weighed and measured and their skin, eyes, gills, abdominal organs, heart, and swimbladder examined for any abnormalities under a compound light microscope. Treatment and preparation of fish for residues in tissues analysis followed AOAC Official Methods of Analysis (Bell, 1990; Hollingworth and Wekell, 1990). After the fish were weighed, heads, tails, and fins were discarded. The fillets were cut to obtain all flesh and skin from top of back to belly on both sides. The skin was included in the analysis, since both absorption and adsorption were of concern.

The samples were put individually into an 80-ml beaker containing 10 ml of distilled water and ground with a Polytron PT 3000 grinder (Brinkmann instruments) for several minutes. The grinder was stopped frequently in order to remove unground material from the hinge and mix it with ground material.

Each resulting mixture was transferred into a 1-Litre beaker, which was filled with distilled water, and brought to pH 10 by adding alternatively sodium hydroxide

(NaOH) and hydrochloric acid (HCL). Alkalinity ensures the extraction of the color from the tissues. After two hours, 10ml were pipetted (transfer pipet) from each stocking solution to test tubes, and centrifuged for 5 minutes on a clinical centrifuge (IEC model). Following centrifugation, the supernatant was scanned on a Hewlett-Packard 8452 diode array spectrophotometer (1-cm cell) for color detection. Concentrations of FD&C Blue No. 1 residues in the samples were expressed as milligrams of chemical per liter of solution.

Data for each tissue were initially standardized by dividing the color concentration by the eviscerated weight of the fish. The effect of dye concentration on dye residue in fish tissues was assessed using analysis of variance (ANOVA).

3.3 RESULTS

There was no concentration-dependent behavioral effects of FD&C Blue No. 1 following short exposure duration of fish, although unlike the control both avoidance reactions and schooling behaviour or absence of parallelism (typical signs of chemical stress) were observed in the test tanks but not in the control.

No mortalities were recorded for the entire course of the experiment. Post-mortem examination of the fish revealed no overt external or internal abnormalities.

In the tissue residue analysis, standardized mean concentration scanned at 630 nm (analytical wavelength of FD&C Blue No. 1) of 0.0722, 0.0573, and 0.0844 mg/L were obtained for the control, test tank 5mg/L, and test tank 20 mg/L respectively. The F-test ($F=0.45$) showed a non-significant difference in the mean residue between all fish group studied ($p=0.642$).

3.4 DISCUSSION

For the duration of the experiment, the manifestation of the two behavioral stress responses observed, avoidance reaction and lack of parallelism, represent the only negative impacts of FD&C Blue No. 1 on fish. Furthermore, the avoidance reaction or swimming outburst was uniquely encountered in the first minute of exposure and did not persist. These behaviors, although typical of chemical stress, were not severe.

The lack of toxicity and uptake of FD&C Blue No. 1 in rainbow trout (Oncorhynchus mykiss) used in this study is in agreement with the pooled results obtained by the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry (ETAD) member companies (Anliker et al., 1981).

The physicochemical properties of chemical substances that minimize the qualitative and quantitative movement of a chemical across fish biological membranes include neutral or anionic molecule, high molecular weight and volume, low lipid solubility, and small concentration in the water (Spacie and Hamelink, 1982), and low log octanol:water Partition coefficient (Schmieder and Henry, 1988). The effects of these parameters on uptake and elimination are in large part related to the binding of chemicals in the blood proteins and lipids, which affects the diffusion gradients between blood and water across the gill epithelium.

Most toxicological studies consider environmental variables that have been reported to influence the toxicity and bioconcentration of xenobiotic chemicals

(McKim and Erickson, 1991). Changes in potency of toxicants can be caused by manipulations of temperature, dissolved oxygen, pH, and dissolved organic and particulate materials (Sprague, 1985). These environmental changes may alter the physicochemical properties of the chemical or the exposed animal's normal physiology in such a way as to change the total flux of chemical that crosses the gills. Within this study, the observations were made under static environmental conditions suitable for the species under examination to provide relatively unambiguous answers to the precisely framed question of the experiment: what is the toxicity and uptake potential of FD&C Blue No. 1 to fresh water rainbow trout under farm-like conditions? The consequence of such a study design was to increase the internal validity of the experiment at the expense of the external validity, i.e., extension or generalizability to situations differing from the conditions of the experiment. Thus, extrapolating these results to other fish species and environments (e.g. sea cages) is not recommended.

In conclusion, the apparent lack of toxicity, absorption, and adhesion of FD&C Blue No. 1 to the experimental fish makes it a suitable marker, in terms of safety and sensitivity, for the automated biomass technique under study.

4. ESTABLISHMENT OF AN INNOVATIVE FISH BIOMASS CALCULATION TECHNIQUE

4.1 INTRODUCTION

To fully ensure the commercial capability of the innovative fish biomass estimation technique, a range of difficulties regarding the technique efficiency must be addressed. The ability to resolve potential technical flaws, especially early in the study design, in large part will enhance the validity and precision of the technique. The terms "accuracy" and "precision" must be distinguished, especially since each must be assessed differently. In general, these terms are concerned with two different sources of error that can occur when estimating a parameter: systematic error (an accuracy problem) and random error (a precision problem). The precision of an experiment involves the consistency of the results. If a trial always gives approximately the same value for a sample (regardless of whether or not it is the correct value), it is said to have precision. An observation is precise if multiple observations yield a small variance. The accuracy of an experiment relates to its ability to give a true measure of the factor being evaluated. To be accurate, a single observation need not always to be close to the true or expected value but the average of a large number of observations should be close to the true value.

In statistical terms, this can be explained as follows:

Let θ be the population parameter

Let $\hat{O}$ be the sample estimation

Let $\bar{O}$ be the expected value of $\hat{O}$ (i.e. its average over many observations).

The bias of $\hat{O}$ is $B = \theta - \bar{O}$. The variance of $\hat{O}$ is the average value of $(\hat{O} - \bar{O})^2$. Notice that the true value of θ does not enter into the variance. In the context of this work

θ is the expected dye concentration

$\hat{O}$ is the obtained concentration from a water sample

$\bar{O}$ is the overall mean concentration obtained.

The first hurdle pertains to fish physiology. Fish density needs to be determined, in order to derive a correction factor for the conversion of fish volume (L) to fish weight (kg). Considering the buoyancy of fat, overweight fish are suspected to have a density below one. Imprecision in the measurement of the dye's final concentration is one of the most important source of error. This can be demonstrated mathematically in the basic formula, where the dye concentration, C_d, corresponds to the denominator.

$$B_v = V_t - W_d/C_d \qquad (1)$$

where

B_v = Total fish volume

V_t = Tank volume

W_d = Dye weight

C_d = Dye concentration

In order to calculate the total fish volume, the volume of free water in the stocked tank (W_d/C_d) is subtracted from the tank volume (V_t). The tank volume may be calculated by applying the dye dilution to a tank containing only water. If the

measurements are precise (whether or not it is the correct value) and the weights of dye are the same, the error would be cancelled out since the volumes are subtracted from one another. To be more descriptive

Let B_o be the true fish biomass

Let E_{Bo} be the estimated fish biomass

Let b_w be the bias of the balance, so that a true weight of W is recorded as $W+b_w$

Let b_c be the bias of the spectrophotometer

Let W_1 be the true weight of the compound used to estimate the tank volume

Let C_1 be the the true concentration in this estimation

Let W_2 and C_2 be the true weight and concentration used to estimate fish volume.

Then, by definition

$$B_o = \frac{W_1}{C_1} - \frac{W_2}{C_2}$$

But what is observed is

$$E_{Bo} = \frac{W_1 + b_w}{C_1 + b_c} - \frac{W_2 + b_w}{C_2 + b_c}$$

So the bias of the estimation is

$$E_{Bo} - B_o = \frac{W_1 + b_w}{C_1 + b_c} - \frac{W_2 + b_w}{C_2 + b_c} - \frac{W_1}{C_1} - \frac{W_2}{C_2}$$

This does not simplify, unless it is assumed that the two concentrations are about the same, $C_1 = C_2 = C$. Then

$$E_{Bo} - B_o = \frac{- b_c(W_1 - W_2)}{C(C + b_c)}$$

Therefore, it should be standard procedure to keep the concentrations, C_1 and C_2, as alike as possible.

In terms of accuracy, overestimate C_d would overestimate B_f and vice versa. Whereas, overestimating either W_d or V_t would understimate B_f and vice versa.

To minimize this source of information bias, several questions need to be investigated.

1. Which form of colour should be used? Dyes are available in many forms, including powder, granular, plating colours, wet-dry (blends), diluted (cut blends), liquid (aqueous), liquid (non-aqueous), and paste. Table VI lists the range of pure dye percentages and the advantages and disadvantages of the various forms of colour (Noonan, 1972). The best form for this specific project is dictated by its purity, ease of handling and rate of dissolving, availability, and cost. Thus, in regard to these key features, a choice between granular and powdered form of FD&C Blue No.1 remains.
2. Does mechanical mixing significantly enhances the homogeneous dissolution of pigments throughout water? It is generally considered that pigment scattering is reinforced by shear forces (Noonan, 1972). However, water solubility of FD&C Blue No. 1 is quite high (Table I) and in most application methods, solubility is usually no problem. So two inquiries remain: should the tank content be stirred before sampling and should the samples be skaken prior to scanning.
3. Which analytical dye concentration will minimize bias and variance?
4. At fixed concentration, is tank volume a significant determinant of the time

Table VI. Purity, advantages and disadvantages of various forms of certified food additives.

Form	Pure Dye(%)	Advantage	Disadvantage
Powder (primary colour)	88-93	Ease of dissolving	Dusty
Granular (primary colour)	88-93	Dustless, free flowing	Slower dissolving
Wet dry blends	90	No flashing in dry blends when wetted	____
Aqueous liquid	1-6	Ready to use, ease of handling, accurate measurement	More costly than dry colour
Non-aqueous liquid	1-8	May be used in fatty material	" "
Cut blends	22-85	Permits larger weighings	" "
Paste	4-10	May be used in products in which water is limited	" "
Plating colours	88-93	Gives good depth to dry mixes	Not available in all primary colours

Taken from Noonan (1972)

required to attain optimum dye dispersion?

5. At a given point in time, what is the fading rate of FD&C Blue No. 1 to light? Fading of FD&C Blue No. 1 due to light exposure would underestimate the final dye concentration (C_d), which would in turn underestimate the total fish biomass (B_f).

The objective of this series of experiments was to calculate the density of rainbow trout and find answers to the preceding list of questions. Knowing the average fish density will determine the correction factor and prevent the occurrence of a systematic error associated with the mathematical model. Answering questions one to five will ensure that proper procedures are executed for the most sensitive stages of the technical process.

4.2 MATERIALS AND METHODS

4.2.1 Density of Rainbow trout

All fish in this study originated from a stock of rainbow trout (Oncorhynchus mykiss) purchased from a commercial hatchery and maintained in the Aquatic Animal Facility of the Atlantic Veterinary College for several months. The study used 10 fish with an average weight of 263g (standard deviation 214g).

The experimental fish, sampled at random, were anaesthetized with MS 222 (100 mg/L) before handling. Each fish was weighed on a Mettler J Balance (FHU SOP 311) and then immersed in 2-L plastic cylinder containing 1 litre of well water (10.5°C). The resulting net water displaced was noted.

The assumption of homogeneity of variance was not violated and a linear regression analysis was performed with fish weight and water displacement as the independent and dependent variables respectively (Glantz and Slinker, 1990).

4.2.2 Multiobjective Comparative Trial

Commercial granular and dry powder mixtures of FD&C Blue No. 1 (Dyeco Ltd, Kingston, Ontario) were used for this experiment. Samples of 74.6, 70.3, 59.9, 54.7, 49.7, 40.5 30.1, 20.4, 10.2, and 5.0 mg of both forms of FD&C Blue No.1 were weighed on a Sartorius model 1872 analytical balance. Each amount was dissolved in a 35 x 35 cm plastic container containing 10 L of distilled water. After 15 minutes, six samples (10 mL test tube) were taken from each working solution. Then, every working solution was vigorously stirred and the procedure above was repeated. Three samples were shaken and three were left undisturbed for all 40 batches. Each sample was then measured (Hewlett-Packard 8452 diode array spectrophotometer) at the optimum wavelength (630 nm). Three different readings were performed for each sample. The absorbency of each solution was noted. Lastly, the obtained absorbances were plotted against the expected concentration to yield a standard curve for each of the two forms of dye. A duplicate was performed.

The SAS statistical package (SAS Institute, 1987) was used to perform statistical analyses and report the results. The significance of the tested variables: form, expected concentration, stirring, and shaking was evaluated for both accuracy and consistency.

Several analysis were performed on this data set of 720 observations. Levene's test (a formal test of homogeneity of variance) was used to determine if the precision was the same for different sets of parameter combinations (Glantz and Slinker, 1990).

To formally test whether the equal variance assumption was violated, the Levene median test was used to compare the corrected mean squares (MS) of the absorbance of each factor level.

A nested analysis of variance, with the difference between the expected and obtained concentration as the dependent variable, was used to test the accuracy of the results. The variability between samples, the appropriate denominator for testing the factors effects, was isolated and a pseudo-F test was performed (Hicks, 1973). SAS gives the exact expected mean squares (EMS) needed to construct a pseudo-F test.

4.2.3 Dispersion Attributes of FD&C Blue No. 1

A Hewlett-Packard 8452 diode array spectrophotometer was used for determining the time required to reach optimum dissolution of granular FD&C Blue No. 1 (Dyeco Ltd, Kingston, Ont) at various water volumes.

A 2m diameter circular fibreglass tank was used throughout the experiment, with water depths of 20, 30, 40, and 50 cm. The approximate desired dye concentration of 5 mg/L was chosen. The volume of water in the tank for each depth was calculated (see Equation 1), in order to determine the amount of dye needed to achieve the desired concentration. The formula

$$V = 3.14(r^2)\, d \qquad (1)$$

where V, r, and d designate the volume, radius and depth, yielded the volumes displayed in Table VII. The different water volumes times the selected dye concentration (5 mg/L) gave the quantity of dye to be weighed (analytical balance Sartorius model 1872) for each volume (Table VII).

An air stone (9 cm^3), pumping air, was installed at the centre of the tank for the whole trial. Well water at 10.5°C was used to fill the tank. The weight of dye for each water depth, was introduced at the source of turbulence. A water sample (10-mL test tube) was taken every five minutes for 60 minutes. For the entire trial, sampling was performed at the same site. Each sample concentration was read spectrophotometrically at 630 nm, using 1-cm cells, against H_2O as reference. Three measurements were done on each sample to evaluate the overall within-sample error,

Table VII. Water volumes, depths and dye weights used for the optimum dissolution experiment.

Depth (cm)	Volume (L)	Weight of dye (mg)
20	628	3140
30	942	4710
40	1257	6285
50	1571	7885

which was attributable mainly to the instrument noise. Three assays were done at each depth. It was impossible to reproduce identical tank volume at each depth, because of the difficulty of filling the tank to exactly the same water level repeatedly. Statistical analysis was performed on 12 different tank volumes. Regression analysis was performed to test for linear relationship between time of sampling and measured tank volume. The time needed to reach optimal dispersion for each of the twelve tank volumes was calculated as follows: sampling time versus dye concentration were plotted, and the time associated with the beginning of no trend between the two variables was selected by eye. A plot of the standardized residuals on the predicted values of the dependent variable (time to reach optimal dispersion) tested the quality of the regression equation. If the regression equation represented the data well, the residuals would be distributed evenly about zero with reasonably constant variance regardless of the values plotted along the horizontal axis. The standard error of the estimate (the square root of the average squared deviation of the data about the regression line) was calculated. The error within samples was assessed by calculating the standard deviation of the mean square error (ANOVA).

4.2.4 Effect of Light on FD&C Blue No. 1

The present investigation tested the stability of granular FD&C Blue No. 1 (Dyeco Ltd, Kingston, Ont) in solution to light. Fifty four samples of FD&C Blue No. 1 of 7.013 g (+/- 0.002 g) were weighed on an analytical balance (Sartorius model 1872). Each weight was dissolved in a 2000-L fibreglass tank. The samples were collected and immediately scanned (Hewlett-Packard diode array 8452 spectrophotometer) at the dye analytical wavelength (630 nm). Each concentration was determined in mg/L. Subsequently, the solutions were assigned at random to exposure to a fluorescent light for 24, 48 or 72 hours. Following exposure, the spectrophotometric readings were repeated.

The difference in concentration before and after exposure to light was calculated. A paired t-test was used to assess if the mean difference in concentration was significantly different from zero. Regression analysis was used to investigate the relationship between time and change in concentration.

4.3 RESULTS

4.3.1 Calculation of Density of Rainbow Trout

Table VIII shows the fish weights and their respective water displacements. In order to determine the density of rainbow trout, the data in Table VIII was used to construct the following regression line (Figure 1):

$$V = 0.44\ mL + (1.02\ mL/g)\ (W) \qquad (1)$$

where V = the volume of a fish in millilitres and W is the weight of a fish in grams. The slope of the line can be used to convert fish volume to fish weight. The 95% confidence interval for the conversion factor was 0.979g to 1.061g. The density of fish, defined as the mass per unit volume under standard conditions, is the inverse of the slope, or 0.98 g/mL, assuming the experiment was conducted under standard conditions.

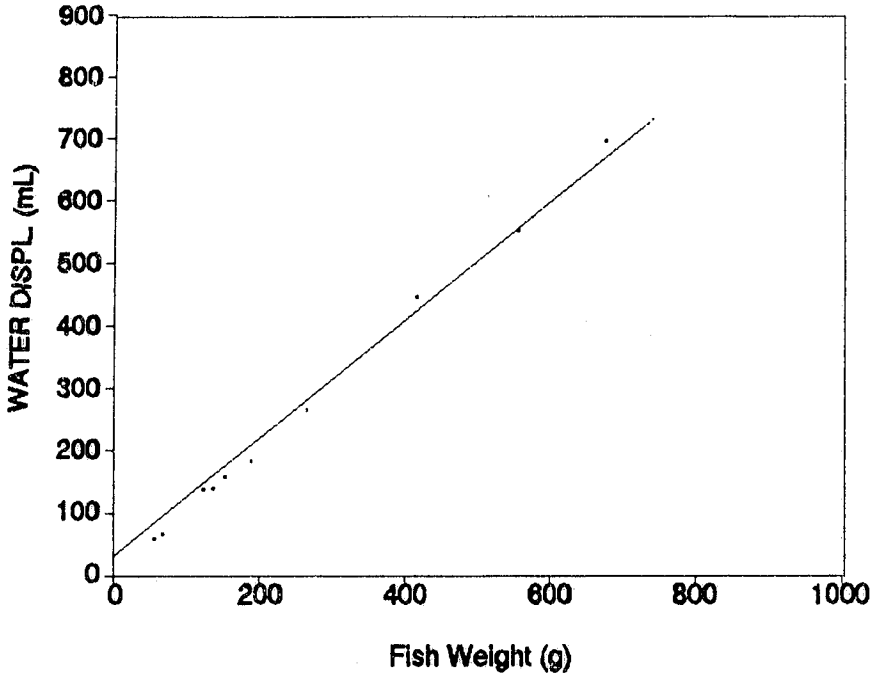

Figure 1. Regression line of water displacement on fish weight.

Table VIII. Fish weights and their respective water displacement.

Weights (g)	Water Displacement (mL)
55.9	59
67.9	66
123.4	138
137.4	140
153.8	158
189.3	183
264.9	266
416.2	447
555.0	551
675.1	696

4.3.2 Multiobjective Comparative Trial

4.3.2.1 Selection of the Physical Form of the Marker

In the consistency analysis (Levene's test), the marker's physical form was the factor of interest and bias, the difference between the expected and obtained values, was the outcome. The expected value is the concentration expected from placing a known weight of dye in a known volume of water. The obtained value is the value obtained from the spectrophotometry reading. The analysis of variance was not statistically significant ($F=3.00$, $p=0.092$). The between mean squares obtained for the powder and granular forms were respectively 0.00022 and 0.00062. Hence, the powder mixtures of FD&C Blue No.1 yielded a greater, but non significant, consistency over the granules.

The accuracy of the granular form was significantly superior to the powder dry mixture ($F=154.3$, $p<0.01$). The mean difference in dye concentration (expected - obtained) were -0.05989 mg/L and -0.01187 mg/L for the powder and granular form, respectively. The schematic geometric interpretations in Figure 2a, 2b, and 2c illustrate the significant two-way interactions between the independent variable "compound", "expected concentration" and "stirring" ($\alpha < 0.01$).

4.3.2.2 Effects of Water Turbulence

Stirring the water before sampling significantly increased both the consistency

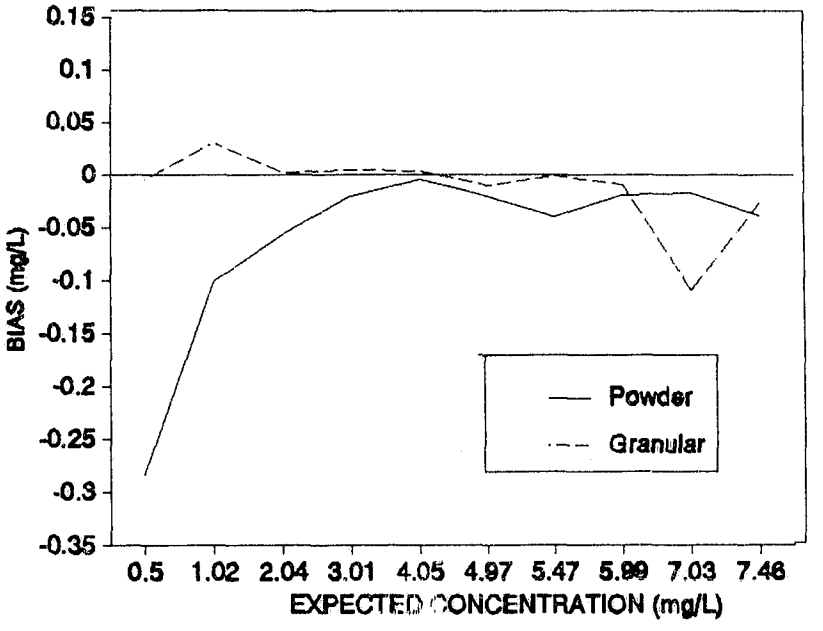

Figure 2a. Interaction between form and expected concentration versus bias.

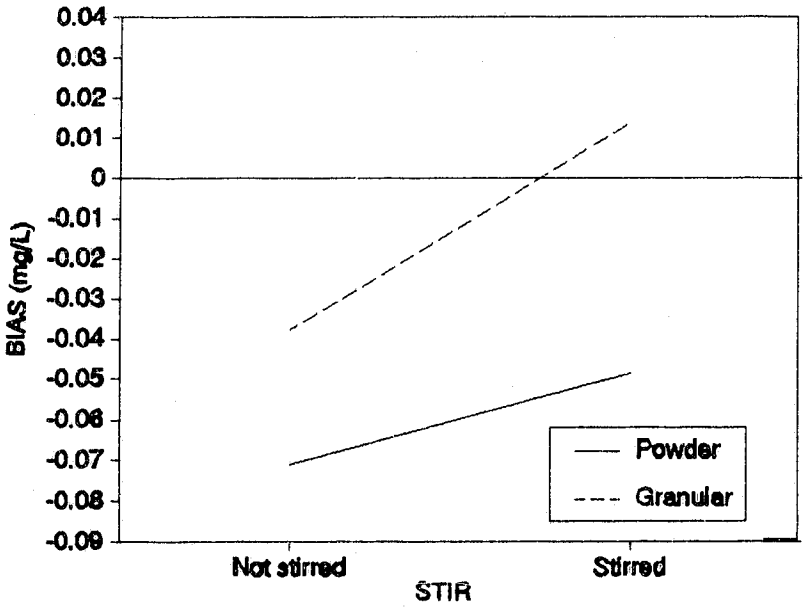

Figure 2b. Interaction between form and stirring versus bias.

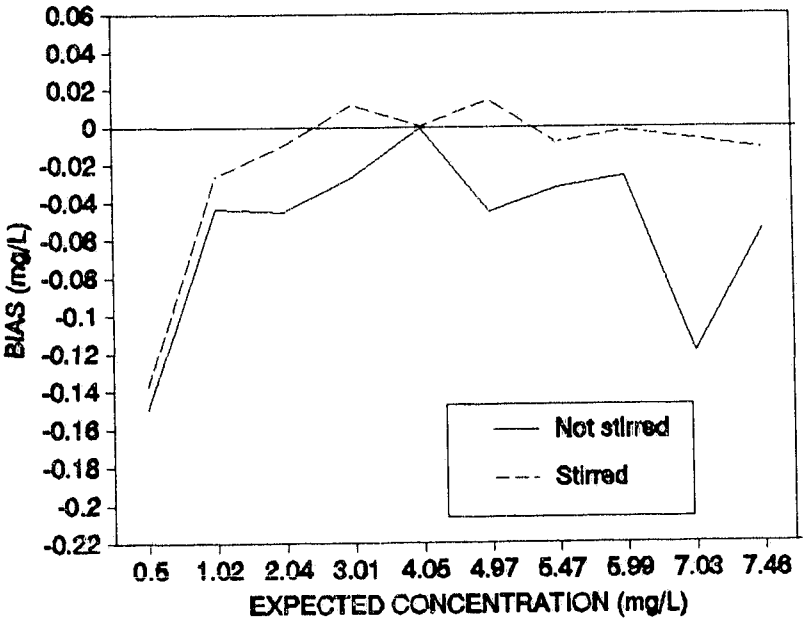

Figure 2c. Interaction between stirring and expected concentration versus bias.

and accuracy of the results. The Levene's test for corrected mean squares yielded a mean of 0.00082 for "non stirring" and 0.00002 for "stirring" ($p=0.001$).

In terms of accuracy testing, the analysis of variance for the outcome "change in dye concentration" was significant at $p<0.01$ ($F=91.29$). Means for the change in concentration of -0.05435 mg/L (non stirring) and -0.01741 mg/L (stirring) were obtained. Shaking the samples prior to scanning increased, but not significantly, the consistency ($F=1.64$, $p=0.205$) and the accuracy ($F=15.28$, $p>0.05$) of the results . For shaking and non-shaking respectively, the corrected mean squares were 0.00058 and 0.00026, and the mean change in concentration were -0.01741 mg/L and -0.05435 mg/L.

4.3.2.3 Marker Concentration Range Determination for Results Optimization

The precision of the technique was not improved by the use of a specific dye concentration ($F=1.91$, $p=0.080$). Whereas, the accuracy of the proposed technique was optimal at a working dye concentration of 4.05 mg/L ($F=46.5$, $\alpha<0.01$). The average corrected mean square and change in concentration for each experimental concentration are shown in Table IX.

Table IX. The mean variance and bias (expected - obtained) for each experimental concentration.

Concentration (mg/L)	Corr.MS	Bias (mg/L)
0.50	0.00176	-0.14346
1.02	0.00071	-0.03495
2.04	0.00019	-0.02748
3.01	0.00012	-0.00742
4.05	0.00049	-0.00016
4.97	0.00023	-0.01514
5.47	0.00025	-0.02022
5.99	0.00019	-0.01386
7.03	0.00021	-0.06303
7.46	0.00004	-0.03306

4.3.3 Dispersion Attributes of FD&C Blue No. 1

For the entire experiment, the mean time needed to reach optimal dye dispersion was 16.67 minutes (stdev 5.37). Since about 95 percent of all populations fall within 2 standard deviations of the mean, the time necessary to reach an homogeneous dye concentration is between 11.30 and 22.04 minutes.

The regression analysis demonstrates that the water volume was not a significant predictor of the time needed to reach an optimal dye dispersion (P = 0.501). The line in Fig.3 is the regression line of dispersion time on water volume. Its equation is

dispersion time = 13.0 min. + 0.00353 min./L (water volume in L).

and the standard error of the estimate ($S_{y/x}$), which estimates the variability about the regression line, was of 5.495 minutes. A correlation coefficient of 0.22 was calcutated. The closer the correlation coefficient is to zero, the weaker the relationship between the two variables.

An analysis of variance (dye concentration by water volume and dispersion time), yielded a standard deviation for dye concentration of 0.13535 mg/L (MS_{error} 0.01832). This variability is the estimation of the error within samples, corresponding mainly to the instrument error.

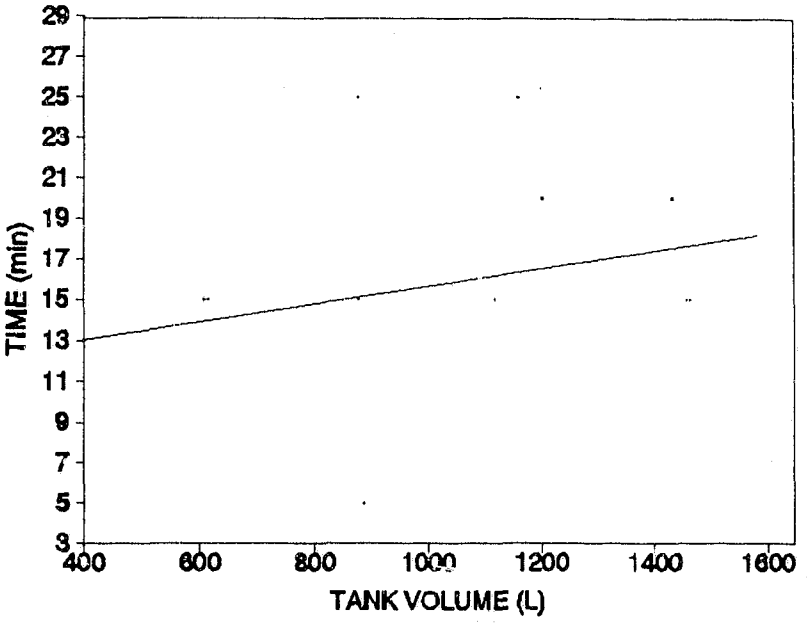

Figure 3. Regression line of the time needed to reach optimum dye dispersion versus tank volume.

4.3.4 Effect of Light on FD&C Blue No. 1

The results of the paired t-test showed that the difference in concentration, due to light exposure (24, 48, and 72 hrs), was not significant from no exposure to light ($t=-0.79$, $p=0.43$). The mean difference in concentration over time was -0.00284 mg/L.

The relationship between duration of exposure to light and change in concentration was nonlinear. It showed systematic variations, with positive values at 24 and 72 hours of exposure and negative values at intermediate time (Fig. 4).

A second-degree polynomial regression was used, which detected a significant relationship between hours of light exposure and bias or change in concentration ($F=48.33$, $p=0.000$). The regression equation is

$$\text{bias} = 0.0173 \text{ mg/L} - 0.000649 \text{ mg/L / hrs (hours)} + 0.000005 \text{ mg/L / hrs}^2 \text{ (hours)}$$

and the standard error of the estimate was 0.002219 mg/L. A partial F-test showed that the added variable increased the fit of the model significantly ($F=23.46$, $p<0.01$).

The plots of the residuals from the linear and polynomial regression appear in Figures 5 and 6. The 'U' shaped residual plot obtained from the linear model indicates the necessity for a polynomial model. The evenly distributed residuals from the polynomial model indicates that this model is adequate.

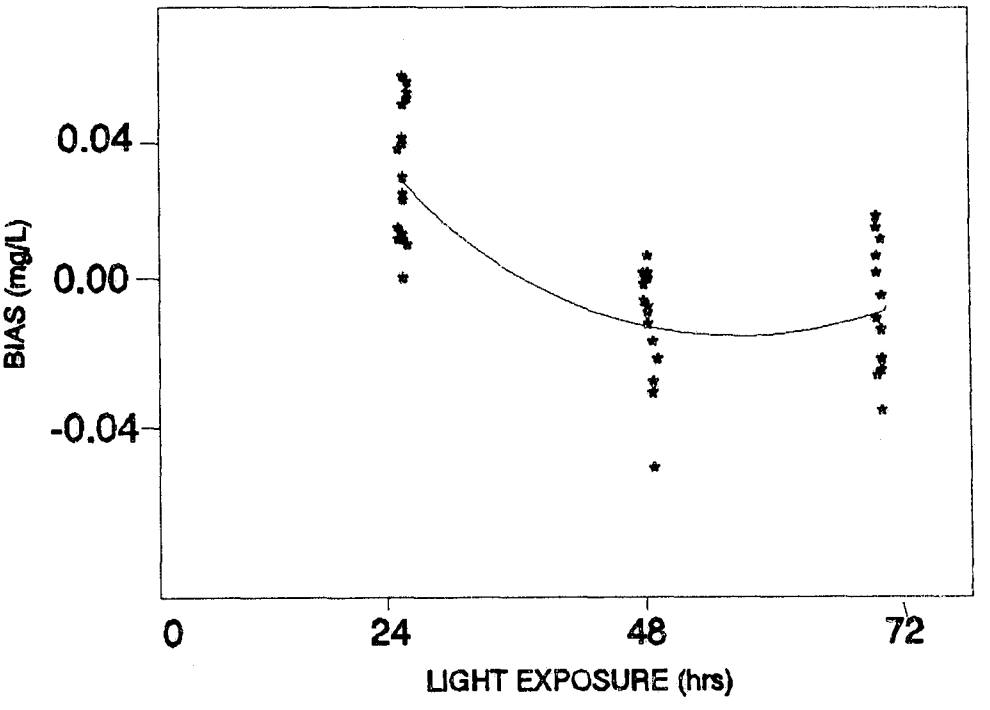

Figure 4. Non-linear relationship between change in concentration and light exposure.

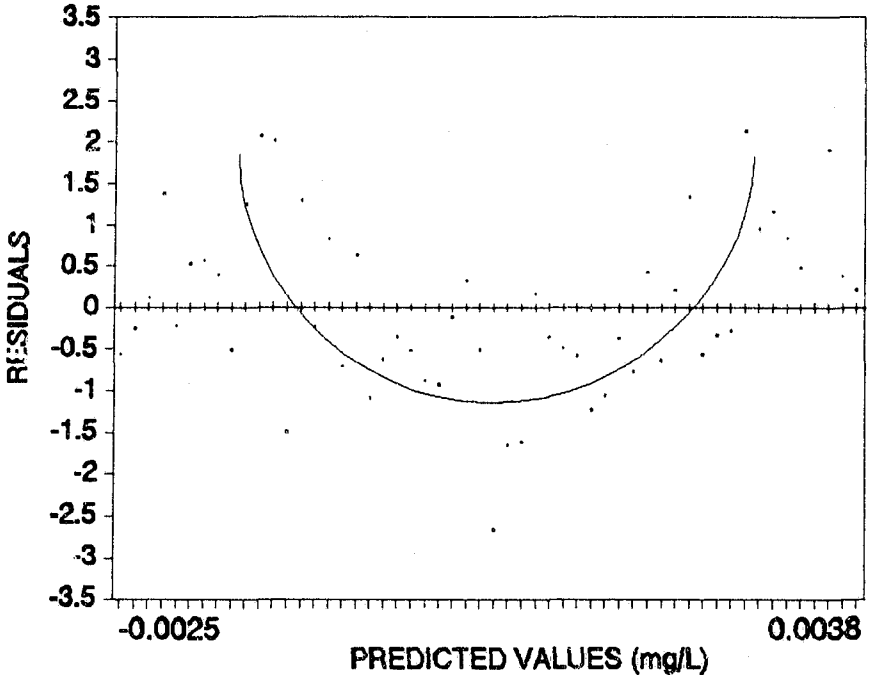

Figure 5. Plot of the residuals on the predicted values of the dependent variable.

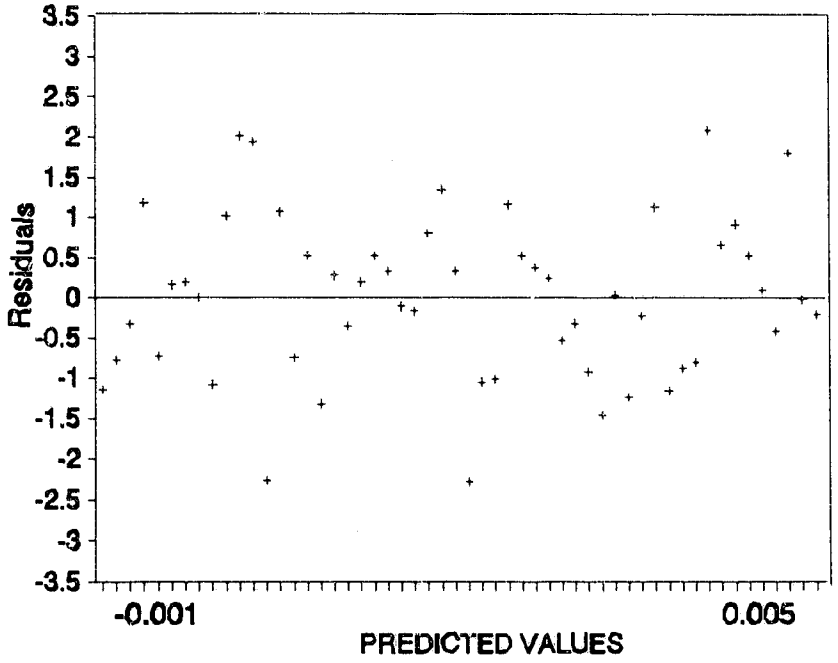

Figure 6. Residual plot of the polynomial regression.

4.4 DISCUSSION

The correlation coefficient between fish weight and water displacement was, as expected, close to one. One millilitre of water was displaced by 0.98 g of fish. All fish used in this experiment were juvenile rainbow trout (Oncorhynchus mykiss), ranging from 55.9 g to 675.1 g. Because the density of a fish is related to its fat content, the correction factor will probably depend on the fish size and species.

The accuracy analysis leads to the conclusions that the outcome -- change in concentration -- depended on the form of the marker, stirring the water and the working concentration. On the other hand, only stirring the water before sampling significantly improved the technique precision. It was shown that at lower expected concentrations the magnitude of bias (difference between observed and expected concentration) was greater for the powder form of FD&C Blue No. 1, but at higher expected concentrations the magnitude of bias decreased rapidly for powder form of FD&C Blue No. 1 and increased slightly for the granular form. It was also demonstrated that stirring had a greater effect, in terms of bias reduction, for granular than powder form. Lastly, stirring was shown to be a critical factor at greater concentration. In fact, as expected concentration increased so did the need for stirring.

This study on technique optimization also demonstrated that, at fixed concentration, there was no significant association between water volume and the time required to reach optimum dye dispersion.

The relationship between change in concentration and light exposure was clearly non-linear. The polynomial regression of the effect of light on FD&C Blue No. 1 stability suggested that the change in concentration was inversely related to light exposure. Furthermore, in contrast to those obtained with the original linear analysis, these results implied that there was some sort of saturation or rate-limiting process of the effect of light on dye stability. The certified food colours, including FD&C Blue No. 1, fade slightly when in contact with light (Hajratwala, 1974). This indicates that, most likely, there was no causal link between light exposure and change in optical density of FD&C Blue No. 1 in the present study. The two variables may be related to some third underlying variable that changed them simultaneously. A plausible explanation could be the presence of a limiting factor in the solvent that over time increased the hue or shifted the shade of FD&C Blue No. 1.

In summary, the best protocol to follow for the biomass technique under study is as follows:

1. The granular form of FD&C Blue No. 1 should be used.
2. Stirring the tank will promote the homogeneity of the dye throughout the tank.
3. An analytical concentration in the range of 4.05 mg/L (+/- 0.95 mg/L) is recommended.
4. For any water volume, assuming stirring is performed, the marker (over the range tested) should be in the tank for at least 22 minutes prior to sampling.
5. The sample concentrations should be determined as soon as possible after

sampling to reduce the likelihood of interactive actions between the compound and external factors.

6. Shaking the samples prior to reading is not mandatory.
7. FD&C blue No. 1, in solution as well as in the dry state, should be protected as much as possible from light exposure.

5. A COMPARATIVE FIELD TRIAL OF SELECTIVE CULTURED FISH BIOMASS COMPUTATION STRATEGIES

5.1 INTRODUCTION

A managed fish farm must regularly assess its stock. Farmed fish are weighed, measured or counted at least four times in their lifetime. They are weighed by the hatchery when they are sold to the farmer. They are weighed by the farmer when the fish are shipped from the hatchery. They are weighed for grading purposes. In commercial farming of salmonids, size-sorting is carried out in order to rationalize rearing practice. This assesses the survival rate and growth of the fish, optimizes the feed quantity and pellet size to feed and reduces wastage, and facilitates marketing. Finally fish are weighed when they are ready for market.

Nowadays most of the biomass assessment methods, sampling methods and yield models are performed using total or partial weighing. To determine the average size in a population from which data inferences are drawn regarding the whole population, fish can be measured either individually or in a group. Measuring a whole population is expensive, time consuming, tedious, and stressful to the fish. Fish are easily upset by such handling (Pickering et al., 1982) and can demonstrate signs of stress, such as loss of appetite. Such deleterious effects on the fish's defense systems can increase susceptibility to disease (Pickering and Duston, 1983; Pickering and Pottinger, 1985).

In many areas of application, sampling techniques are well established (Cochran, 1963, Seber, 1987). This is not proven for the sampling of fish troughs.

It is generally believed that the fish lower in the hierarchy (usually the smallest) are caught first, which underestimate the population estimate. Seeger et al. (1977), stated that the probability of catching a fish in a subsample is inversely proportional to the square root of its length. Thorburn (1989) demonstrated that the randomness of a sample depends on the method used for collecting the dip-net samples. The study revealed that crowding the fish (concentrating the fish by releasing water from the tank and then sweeping a dip-net through them) yielded simple random samples. Neither dip-netting near the water inflow without prior disturbance of the fish nor dip-netting from the centre of the tank immediately after stirring its content, however, yielded simple random samples. Without prior disturbance, larger fish were caught more frequently and, following stirring, the reverse applied.

The prediction of the specific growth rates of fish demands no unrealistic assumptions (Wandswik and Jobling, 1982; Jobling, 1983). The model examined the relationships between fish size, temperature and rates of growth and presents equations that can be used in the projection of growth rates of fish reared under a variety of conditions.

At present only one technique, the hydroacoustic assessment, is designed to estimate fish biomass in a cage and the size distribution without any handling of the fish. The classical theory of echo integration, based on a single-scattering model, can not be applied owing to the high attenuation of echo signals in dense aggregations of fish (Rottingen, 1976). A multiple-scattering approximation was tested by Burczynski et al. 1990 for estimation of high densities of fish in sea pens. The

method resulted in systematic bias of fish densities. The lack of experimental values on accoustic properties of fish is believed to be the main source of error.

A method to estimate fish biomass should have the following characteristics. It should be accurate; inexpensive; simple to perform and able to be used and handled by non-skilled labour; portable; non invasive; non-species specific; able to measure fish from all size groups; be applicable in dense or sparse aggregations of fish.

When a population is not only sampled repeatedly but also completely measured, accuracy assessment of the estimation method is possible. A method is said to be accurate when its mean estimate equals the underlying population mean. By applying a certain number of repetitions, the variation between estimates whether it is total fish biomass or mean weight of fish, can also be evaluated. One can then know the odds of getting the same estimate successively.

In this study, possible solutions to the determination of fish biomass in closed systems and the randomness of dip-net sampling are explored in the field. First, the accuracy of the specific growth rates estimation model and water displacement technique are assessed by comparing the methods with the true fish biomass (total weighing of the fish). Second, the precision of the water displacement estimates, total fish biomass, is determined as well as the precision of the dip-netting estimates and average fish weight.

5.2 MATERIALS AND METHODS

5.2.1 Fish Weighing Procedures

The Arctic charr (Salvelinus alpinus) used in these experiments were hatchery raised fish (Integrated Aquatic System, Brookvale, P.E.I.). The study involved two runs of approximately one month each. The first run used juveniles and the second yearlings of a mean weight of 138 g and 378 g respectively. The second run was executed immediately after the completion of the first run (Table X). The hatchery used well water, which was constant throughout the experimental tanks at 7.1°C. In the rearing units, oxygen was close to saturation at the source of water inflow and nitrate concentrations below toxic levels (U.S Environmental Protection Agency, 1976). During the entire course of the experiments, the fish were fed to satiation. The juveniles and the adults were fed with 3.5 mm and 5 mm pelleted dry food respectively. The amount of feed per tank per day was 0.5 to 0.9 kg and 0.3 to 0.4 kg for run 1 and 2 respectively.

The experiments were conducted in four 1.5 m^3 cylindrical fibreglass tanks. Fish were dip-netted and weighed in bulk (Mettler J Balance, FHU SOP 311) and then transferred to another tank and weighed 2 weeks later to allow time for substantial fish growth. The number of weighings per tank was 17 (standard deviation 5) for both weigh-in and weigh-out for the juvenile run and 10 (standard deviation 1) for weigh-in and 12 (standard deviation 2) for weigh-out for the adult run. There were no delays between weighings. The maximum scale capacity was 7.3

Table X. Number of replicates for each fish biomass computation method.

Run	Period	Weighing	Dip-netting	Dye technique	SGR
1	Jan 31 / Feb 4	IN	1	3	---
1	Feb 19 / 25	OUT	1	2	YES
2	Feb 28 / Mar 2	IN	1	3	---
2	Mar 18 / 21	OUT	1	2	YES

* SGR Calculated Specific Growth Rate
--- None

kg. On average, 520 and 139 fish per tank were used for the first and second runs, which corresponded to a mean stocking density of 71.7 and 58.3 kg per tank.5.2.2

5.2.2 Dip-netting Sampling Method

Fish mean weight estimation by dip-netting was based on subsamples taken in the following way. The scoop nets consisted of a circular frame carrying a net approximatively 45 cm in diameter and 58 cm in depth. The scoop net was swept from edge to edge of the tank. Subsamples were collected immediately one after another without replacement, until all fish from that tank were caught. Subsamples used for the mean weight of estimates were chosen at random. Once no water was dripping off the selected dip-net, the fish were transferred to an empty bucket for bulk weighing. Then the number of fish from that sample were counted while they were being released in another tank (initially empty). The mean weight of fish for that sample can then be calculated by dividing its overall weight by the number of fish caught. The mean fish weight for each tank was calculated from 3 to 10 dip-net subsamples. The estimations were done at the beginning and end of each run (Table X).

Levene's test (Glantz and Slinker, 1990) was used to test if the mean variations in mean weight of fish between subsamples were significantly different. Regression analysis was used to detect the absence or presence of a relationship between the mean weight of fish and the time of capture (i.e., the first , second, third,

etc. dip-net samples). The precision of the method was estimated by measuring the standard errors for all 16 groups (4 tanks x weight-in and weight-out x 2 runs]. Standard errors were computed as standard deviations of means of all subsamples (Seeger et al, 1977).

5.2.3 Specific Growth Rate Estimation

Rates of growth for each tank, for the two week interval, were calculated. Below the optimum temperature, the growth of arctic charr of a given size can be predicted using the equation (Jobling, 1983):

$$G_w = \ln 7.5\ (0.0219 + 0.0727\ T) - 0.325\ W_0$$

where G_w is specific growth rate in grams/day, T is the temperature, and W_0 is mean fish weight in grams at time 0.

The expected biomass was then calculated according to the formula (Jobling, 1983):

$$B_e = [(t_{1\text{-}0} \bullet G_w / 100) + W_0] \bullet N_f$$

where B_e is the expected biomass for a particular tank, $t_{1\text{-}0}$ is time in days (in this case, 26 days for the first run, 28 days for the second), G_w is the specific growth rate, W_0 is fish weight at time 0, and N_f is the estimated number of fish in the tank.

The accuracy of the technique was compared to bulk weighing of the fish, by a paired t-test. A two-way analysis of variance was used to assess if there was a "run" or "tank" effect.

5.3.4 Water Displacement Technique

For the entire course of the trial, the granular form of FD&C Blue No.1 was used (Dyeco Ltd, Kingston, Ont). The displacement technique was performed on three tanks, one tank was used as a control, for mortality comparison. There were three replicates at the beginning of each run and two at the end (Table X). The first application at the beginning of each run was on tanks only filled with water. Subsequent applications were on stocked tanks.

The geometrically calculated tank volumes (V_t) times the selected dye concentration (C_d), +/- 4 mg/L, gave the approximate quantity of dye to be weighed (W_d).

$$W_d = V_t \times C_d \qquad (1)$$

The volumes of tank number 1, 3 and 4 (tank number 2 was the control), calculated by the displacement technique, were 1675, 1723 and 1706 litres, respectively. The amount of dye addedd was then 7.02 g (stdev 0.01 g). The dye was weighed on an analytical balance (Sartorius model 1872), at the Atlantic Veterinary College. The dye samples were weighed and kept in opaque plastic containers to be used the same day at the hatchery. For the first ten replicates, the technique was performed as follows:

1. The water to the experimental tank was shut off and the system allowed to become completely static (no more water leaves the system). Oxygenation was kept close to optimal through the biofiltered aquacultural water reuse system.

2. The dye sample (initially weighed and identified) was discharged into a tank, at the source of the inlet.
3. The compound was allowed to disolve for a 30 minute period.
4. Three water samples, from 10 ml capped test tubes, were collected from 3 different locations. One at the inlet and two elsewhere.
5. Half of each sample was scanned on site on a Spectrophotometer 20, and the other half at the Atlantic Veterinary College on a Hewlett-Packard diode array spectrophotometer.

The overall difference between the estimated biomass and the true biomass when quantitative analysis was performed on the Hewlett-Packard spectrophotometer and on the Spectrophotometer 20 was calculated. While using the most sensitive instrument, the significance of all independent variables (tank, tank volume, fish volume, spectrophotometer, and run) were tested using a mixed model analysis of variance in which fish volume was nested in tank. Pairwise comparisons of all significant variables were tested using the Student-Newman-Keuls test.

To test for normality of the data, a normal probability plot was constructed through general linear model (GLM). A normal probability plot is a plot of the cumulative frequency of the distribution of the residuals vs. the residuals themselves, which produces a straight line if the assumption is not violated (Glantz and Slinker,1990).

5.3 RESULTS

5.3.1 Accuracy Assessment

5.3.1.1 Estimated Growth Rates

The estimated specific growth rates for each tank for both runs are shown in Table XI. The control tank (#2) had the lowest specific growth rate in the juveniles run, and was the second highest in the adult run. No mortalities were recorded in any of the four tanks.

Table XII shows the difference between the true biomass and the estimated biomass (Δ) for each tank and both runs. A paired t-test showed a nonsignificant difference between the overall means from each procedure ($t=-0.23$, $p=0.83$). The mean difference was 0.164 kg (true and estimated = 68.759 kg and 68.595 kg). This means that the total biomass was somewhat underestimated.

The two way analysis of variance showed that neither run ($F=2.28$, $p=0.228$), or tank ($F=0.29$, $p=0.835$) were significant predictors of the magnitude of the difference between the true and estimated biomass.

5.3.1.2 Water Displacement Technique

Even by using the most sensitive spectrophotometer, all independent variables were significant predictors of the outcome, bias. The overall difference between the true and calculated biomass (Δ) using the Hewlett-Packard spectrophotometer and

Table XI. Calculated specific growth rates for the whole trial.

Run	Tank	Specific Growth Rate (% day $^{-1}$)
1	1	8.3
1	2	8.2
1	3	8.5
1	4	8.7
2	1	5.8
2	2	6.0
2	3	5.8
2	4	6.1

Table XII. Difference between the true and expected biomass (Δ) using the specific growth rate method for all total fish biomass estimates.

Run	Tank	True biomass	Expected Biomass	Δ (Kg)
1	1	65.34	63.72	1.62
1	2	70.93	67.35	3.58
1	3	85.47	85.18	0.29
1	4	95.09	95.25	-0.16
2	1	53.70	53.86	-0.16
2	2	61.67	64.28	-2.61
2	3	51.87	54.29	-2.42
2	4	66.00	64.83	1.17

Spectrophotometer 20 were 5.5 kg and 17.5 kg respectively. The mean difference (Δ) for the first and second run were -0.493 kg and 11.071 kg respectively ($F=18.2, p=0.0001$). The mean Δ were 9.508 kg for the first tank volume estimate and 1.07 kg for the second estimate ($F=9.7, p=0.0023$). A strong tank effect was noted ($F=82.4, p=0.0001$). Student-Newman-Keuls test detected a significant difference for the three comparisons (tank 4 and 1, 4 and 3, and 1 and 3), the means being -12.78 kg for tank 1, -0.132 for tank 3, and 28.774 for tank 4. Table XIII shows the mean difference between the true and estimated biomass (Δ) for all replicates of the variable fish volume estimate ($F=2.3, p=0.04$).

Table XIII. Mean difference between the true and estimated fish biomass (Δ) using the water displacement technique for all replicates of fish volume estimate.

Run	Fish volume replicate	Tank volume replicate	Δ (kg)
1	1	1	5.79
1	2	1	-0.63
1	3	1	-2.74
1	4	1	12.48
1	1	2	-2.65
1	2	2	-9.07
1	3	2	-11.18
1	4	2	4.04
2	1	1	9.48
2	2	1	24.51
2	3	1	7.73
2	4	1	19.43
2	1	2	1.05
2	2	2	16.07
2	3	2	-0.71
2	4	2	10.99

5.3.2 Precision Assessment

5.3.2.1 Dip-net Method

Levene's test, a formal test of homogeneity of variance, showed no difference between the mean variation of all fish mean weights ($F=1.83$, $p=0.501$). Table XIV contains all the estimates of the mean weight of fish with their respective standard errors. A regression analysis of mean fish weight versus time of capture revealed no relationship (Fig.6) between the independent variable, time of capture, and the dependent variable, mean weight, for both the juveniles ($F=0.25$, $p=0.62$) and the yearlings ($F=1.65$, $p=21$).

5.3.2.2 Water Displacement Technique

The main source of error, variation in the amount of free water between replicates, was suspected to be generated by one of the farm staff handling the external stand pipe. Because of this, we decided to repeat the experiment. Three replicates were performed. Once the water inflow was shut off, a delay of exactly 15 minutes was allowed before the dye was added. In the second experiment, there was no handling of the external stand pipe.

For these replicates , the Levene's test was performed to assess the variability in terms of biomass estimated between replicates.

Levene's test detected a significant difference between the mean deviation for

Table XIV. Estimated mean weight of fish and inter-subsamples standards errors.

Tank	# of subsamples	Mean weight (g)	Standard errors
1	3	128.4	7.80
2	3	132.6	0.45
3	5	121.6	2.59
4	3	112.6	1.26
1	4	172.2	8.52
2	4	176.8	2.88
3	5	155.2	3.19
4	6	133.4	6.73
1	3	389.9	7.04
2	4	353.0	3.29
3	3	389.0	22.70
4	4	337.5	8.05
1	5	411.9	11.80
2	6	401.6	14.00
3	6	397.0	8.12
4	10	400.8	8.43

* The table was divided in 4 vertical quadrats. The two first quadrats represent the run on fingerlings, with the 2 week interval period. The last two quadrats belong to the run on yearlings.

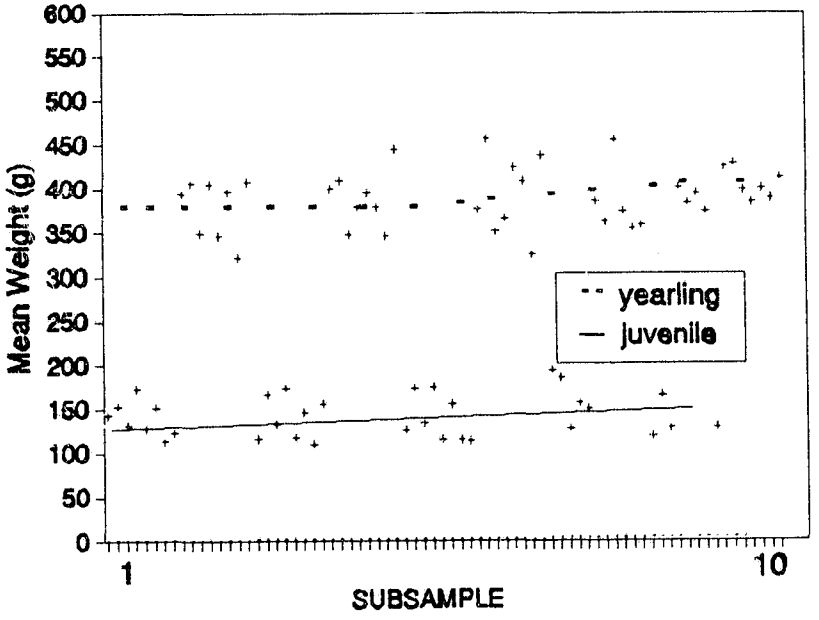

Figure 7. Regression analysis of mean weight of fish on time of capture.

both spectrophotometers ($F=6.97$, $p=0.01$). The mean deviations using the spectrophotometer 20 and the Hewlett-Packard diode array spectrophotometer were 56.6 kg and 20.92 kg respectively.

The Levene's test, applied to data from the most sensitive quantitative analytical tool (Hewlett-Packard), detected a significant difference in mean variations between replicates ($F=5.02$, $p=0.016$) and between tanks ($F=18.10$, $p=0.00$). Table XV shows the mean variations for all tanks and replicates, when quantitative analysis was performed on the Hewlett-Packard spectrophotometer.

Table XV. Mean variations in kilogram for quantitative analysis on Hewlett-Packard diode array spectrophotometer.

Replicate	Tank			
	1	3	4	ALL
1	2.10	4.34	8.41	4.95
2	5.68	78.57	2.85	29.04
3	5.40	70.68	10.22	28.77
ALL	4.40	51.20	7.16	20.92

5.4 DISCUSSION

The reported specific growth rates clearly show that the growth rate of neither fingerling nor yearling arctic charr was altered by multiple exposure to FD&C Blue No.1. Also, no mortalities were recorded in any of the four tanks. Based on growth and fish survival, FD&C Blue No. 1 was not chronically stressful to the fish. Growth rate is considered to be one of the most sensitive indicator of environmentally-based stress (Schreck, 1981; Vijayan and Leatherland, 1988). The rearing environment must be uniform, where comparisons between groups are based on weight attained after test periods. This means that the tank size, inflow rate, as well as fish density of each tank were kept nearly identical.

The growth rates of Arctic char recorded in the current study at 7.1 °C are at least as high as those reported for salmonid species reared at their optimum growth temperatures (Table XVI).

In this trial, there was no significant difference between the true biomass and the biomass estimated using the specific growth rate method. The mean difference was 0.164 kg. The short time interval (26 and 28 days for the first and second run), as well as a fairly good estimate of the population size (calculated as the true total biomass divided by the mean weight of fish) for each tank probably contributed to the accuracy of the specific growth rate model.

In this study, both juvenile and yearling arctic charr (Salvelinus alpinus), when maintained at a stocking density below usual rearing densities and captured anywhere

Table XVI. Comparison of growth rates of salmonid species reared at their optimum growth temperatures.

Species	T_{opt} (°C)	G_{opt} (% day^{-1})	Source
Arctic char (Salvelinus alpinus)	14	7.5	Jobling(1983) Brett et al. (1969)
SocKeye salmon (Oncorhynchus nerka)	15	5.4-7.7	Brett(1974) Brett and Shelbourn (1975) Kepshire (1971)
Pink salmon (Oncorhynchus gorbuscha)	15	9.8	Brett(1974)
Coho salmon (Oncorhynchus kisutch)	15.5	5.53	(cited in Brett and Shelbourn (1975))
Brown trout (Salmo trutta)	12.8	2.79	Elliot(1975) Hokansen et al.(1977)
Rainbow trout (Salmo gairdneri)	17	6.9	Papoutsoglou and Papapa.-Papoutsoglou (1978) Papoutsoglou et al.(1979) Kaushik and Lucket(1980)

Taken from Jobling (1983)

in the tank (with minimal time interval between dip-net samples), were randomly distributed. Dip-netting yielded unbiased estimates of mean weight. Contrary to the current study, the evaluation of the randomness of dip-net samples following mixing, on rainbow trout, yielded biased estimates of fish mean weight (Thorburn, 1989). Leitritz (1980) suggested that trout juveniles should be crowded in order to obtain random samples. However it is not clear how crowded the fish should be to create randomness. In this experiment, the fish were held at an average stocking density of 65 kg per tank or 33 kg per cubic meter of water. This is a low density by commercial production standard. Stirring the content of the tank is a surrogate for crowding the fish. The catch method used in this study led to a thoroughly mixed population. The method of sampling, whether it is from a crowded or sparse population or whether it is by means of larger or small dip-nets, and the size of the population, are all believed to have an effect on the randomness of the samples. At a high density of 89 kg/m^3, as opposed to a relatively low density of 37 kg/m^3, the frequency of capture per individual fish was not significantly different from that expected of a random sample (Thorburn, 1989). Seeger et al. (1977) obtained a statistically significant negative bias of averages of lengths of 2-summer old salmon when scoop nets consisting of a rectangular frame of either 45 x 25 x 23 cm and 35 x 19 x 17 cm were used. The negative bias apparently also increased with increasing heterogeneity in the populations. It was concluded that larger fish are better able to evade the scoop net. To avoid bias, weighted means (applying greater weighting to the larger fish) were used. For 1-summer-old salmon, sampled with a smaller scoop

net (25 x 16 x 7 cm), the problem of underestimation did not arise.

However, it is important not to overlook the fish species itself. Behaviours such as rates of agonistic encounters, physical displacement of individuals by social dominants, or complexity of social interactions versus population density varies between fish strains and species (Fenderson and Carpenter, 1971; Wallace et al, 1988). It is also well documented that schooling behaviour, where imitation rather than aggression is characteristic, tends to reduce size variance within population (Yamagishi, 1965, 1969). Schooling behaviour is a dominant trait in arctic char. Signs of intraspecific competition such as agonistic encounters and physical displacement of individuals by social dominants were limited during the field trial.

The real number of fish by tank was not counted but estimated by dividing the total biomass by the mean fish weight for each tank. Thus it was impossible to determine the accuracy of dip-netting in estimating the total fish biomass and true mean weight. The accuracy of the sampling method, i.e. the difference between the true and estimated biomass (Δ) and the difference between the true and estimated mean weight (D), was calculated by

$$\Delta = B_t - (W_e \bullet N_f) \qquad (1)$$

$$D = (B_t/N_f) - W_e \qquad (2)$$

where

B_t = True fish biomass

W_e = Estimated mean fish weight

N_f = Number of fish

The water displacement technique yielded biased estimates of the total fish biomass. Statistical evidence demonstrated that the main source of error was the variation in tank volume from replicate to replicate. Among all experimental units, tank number 4 had the least accuracy. The most probable source of bias was the handling of an external stand pipe by a staff member who became impatient with the slow draining of the tank. The extent of this error was not foreseen. Since a random error, unlike a systematic one, cannot be corrected, only its anticipation could have lessened its magnitude. Once the tank handling was corrected, the precision of the technique was considerably improved for tank number 1 and 4. However, there was still great variability between estimates for tank number 3. Only 3 replicates were performed, which means that such a thing as a stronger inflow in tank number 3 for one of the replicate could have caused the lack of consistency between the biomass estimates. Based on these preliminary results, the "human factor" and water system appeared to be major sources of error for estimating fish biomass through the water displacement technique, although further experimentation is necessary.

6. GENERAL DISCUSSION

A recent FAO report (British Columbia Salmon Farmer's Association, 1987) predicts that by the year 2000, world demand for foodfish will exceed supply from conventional harvests by at least 11 million tons. Increasing supplies of fish to meet expanding demand will pose the greatest challenge for the world's fish producers in this decade and beyond. As supplies of aquaculture products expand, the market will become extremely competitive, marketing will become extremely competitive, time consuming and expensive (Rackam, 1987) and cost effectiveness of production will determine the viability of individual farms (Dunn et al., 1989). The need, therefore, is for careful planning of fish stock management and well-designed market strategies.

The aspects to be considered in an effective commercial strategy for an aquaculture product are: to measure current production, to measure current demand, and to draw up production and consumption forecasts.

However, due to difficulty at estimating current production quickly and cheaply, objective information concerning fish stock assessment is not always present. On a large scale, that piece of information is regarded as the missing link in aquaculture marketing. Following is the evaluation of a low-cost approach for improving fish stocking assessment. The long-term objectives that emerge from this innovative fish biomass estimation project are: maximizing fish production and fish health (including disease prevention and control), optimizing feed conversion, and achieving effective marketing.

The innovative technique of biomass estimation, based on a water displacement principle, was subdivided in four mutually dependent objectives. The first three chapters -- detectability and stability evaluation of four certified dyes, assessment of rainbow trout exposure to FD&C Blue No. 1, and establishment of the technique methodology -- were investigated under controlled and stable conditions. The biomass estimation technique was later tested in a highly dynamic and risk adverse farm environment.

Results showed that in terms of sensitivity and safety, Food Drug and Cosmetics Blue No. 1 (Brilliant blue) was a suitable marker for the biomass estimation technique under study. The field investigation demonstrated that the conduct of good quality research on a production environment while difficult, is not only possible but also essential.

The sampling study suggested that dip-netting can yield random samples. This statement, however, only applied to the parameter estimated -- mean fish weight. Other variables, such as prevalence of disease, might have suggested otherwise. In this study, only one catch method was tested; dip-netting repeatedly nets of fish from anywhere in the tank. The method was selected on the grounds of being the most frequently used by fish farmers. The sampling investigation reported here has led to the following conclusion. With a juvenile or adult arctic charr population, an efficient sample design consisting of at least three subsamples per tank (taken immediately one after the other) can yield unbiased estimates of fish mean weight.

The purpose of the specific growth rate estimation study was to assess the

accuracy of the model under constant environmental conditions. Although the data presented in Table XII are based upon ideal conditions, in terms of temperature stability and short time interval between estimates, and therefore must be interpreted with caution, it is apparent that the specific growth rate estimation model yielded accurate total biomass estimates. It is unknown whether such results could be reproduced under different conditions, such as change in diet, season, water salinity or target species. The most obvious postulate is that the the difference between the true and expected biomass and the time interval between weighings are positively corrolated.

The major obstacle associated with the water displacement technique was neither its hazardous potential to the fish or lack of applicability in the field but rather a lack of both accuracy and precision. The estimates of fish weight generally differed from actual weight. This error was principally due to inconsistency in tank volume from replicate to replicate. A tank effect was also identified. The magnitude of this error was minimized by eliminating the "human factor" (handling of the external stand pipe and water inflow level) but it was not eradicated. Extensive experiments similar to the one conducted could probably pinpoint the exact causes of fluctuation between estimates. Furthermore, a comparative trial on the efficacy of the technique on different types of production closed systems could provide further data on the specificity or generalizability of the technique.

A second source of error was related to quantitative analysis. Quantitative analysis was performed on a computerized spectrophotometer. It was shown that a

negligible error in concentration reading can be meaningful in terms of biomass estimate. The error associated with the estimate of concentration is derived from both detection sensitivity of the instrument and the percentage uncertainty of the calibration plot of the standard (Bailey et al., 1978). Subsequent work should address the issues of other means of quantitative analysis, such as photoacoustic spectroscopy (PAS). PAS is highly sensitive for coloured pharmaceutical powders (Nakai et al., 1985). Photoacoustic spectrophotometry by total internal reflection technique has a detection limit of 0.0045 absorbance unit (Hinoue et al., 1984). Other methods such as high pressure liquid chromatography are also suggested.

If the displacement technique is to become competitive and widely used in the global industry it is imperative to study the hydrographic processes causing dispersion and flushing of the water soluble waste from fish cages in simple model systems. Concern about the release of the dye centres on aesthetic issues rather than on potential biological effects. The life-time of the chemical, flushing time, rate of input at the source, degree of mixing and shear dispersion must all be considered to correctly predict the dispersion and clearance of the compound from a farm (Turrell and Munro, 1989).

REFERENCES:

ACKER WC. Acoustic assessment of North Pacific salmon stock. -Rapp.P.-v.Reun. Cons.int.Explor.Mer 1977; 170: 189-195.

ANLIKER R, CLARKE EA, MOSER P. Use of partition coefficient as an indicator of bioaccumulation tendency of dyestuffs in fish. Chemosphere 1981; 10(3): 263-274.

BAILEY CJ, COX EA, SPRINGER JA. High pressure liquid chromatographic determination of the intermediates/side reaction products in FD&C Red No.2 and FD&C Yellow No.5: statistical analysis of instrument response. J. Assoc. Off. Anal.Chem. 1978; 61(6): 1404-1414.

BELL S, ed. Color additives. Food and Drug Administration. AOAC Official Method of Analysis 1990 (vol.2): 46; 1115-1136.

BELL S. Preparation and spectral compilation of FD&C Red No.40 intermediates and subsidary dyes. Journal of the AOAC 1976: 59(6): 1294-1311.

BRETT JR. Tank experiments on the culture of pan-size sockeye (Oncorhynchus nerka) and pink salmon (O. gorbusha) using environmental control. Aquaculture 1974; 4:341-353.

BRETT JR, SHELBOURN JE. Growth rate of young sockeye salmon, Oncorhynchus nerka, in relation to fish size and ration level. J.Fish.Res.Bd.Can. 1975; 32: 2103-2110.

BRETT JR, SHELBOURN JE. Growth rate of young sockeye salmon, Oncorhynchus nerka, in relation to fish size and ration level. J. Fish. Res. Bd Can. 1975; 32: 2103-2110.

BRITISH COLUMBIA SALMON FARMER'S ASSOCIATION. Salmon farming in Brtish Columbia. an economic impact study, November 1987.

BROWN JP, DORSKY A, ENDERLIN FE, HALE RL, WRIGHT VA, PARKINSON TM. Synthesis of C-labelled FD&C Blue No.1 (Brilliant Blue FCF) and its intestinal absorption and metabolic fate in rats. Fd Cosmet.Toxicol. 1980; 18: 1-5.

BURCZYNSKI JJ, JOHNSON RL, KREIBERG H, KIRCHNER WB. Acoustic estimation of dense aggregations of fish in sea pens. Rapp.P.-v. Reun.Cons.int.Explor.Mer 1990;189: 54-64.

BURNETT C, OPDYKE D. Chronic eye irritation and staining properties of some organic colors and lakes. CTFA Cosmet. 1971; 3: 18-20.

BURROWS RE, COMBS BD. Controlled environments for salmon propagation. Progressive Fish-Culturist 1968; 30(3): 123-136.

CARSON S. Skin painting studies in mice with 14 FD&C and D&C colors: FD&C Blue No.1, Red No.3, and Yellow No.5, D&C Red No.7, Red No.9, Red No.10, Red No.19, Red No.21, Red No.27, Red No.31, Red No.36, Orange No.5, Orange No.10, and orange No.17. J.Toxic.-Cut.Ocular Toxic. 1984; 3: 357-370.

CHAFEE FH, SETTIPANE GA. J.Allergy 1967; 40: 65-72.

CHIOU CT, FREED VH, SCHMEDDING DW, KOHNERT RL. Partition coefficient and bioaccumulation of selected organic chemicals. Environ.Sci,Technol. 1977; 11: 475-478.

COCHRAN WG. Sampling techniques. Wiley, New York, N.Y. 1963; 413 pp.

COLLINS TFX, BLACK TN, WELSH JJ, BROWN LH. Study of the teratogenic potential of FD&C Red No.40 when giving in drinking water. Toxicology and Industrial Health 1989; 5: 937-948.

COLLINS TFX, BLACK TN. Effects of FD&C Red No.40 on rat intrauterine development. Food Cosmet.Toxicol. 1980; 18: 561-568.

CRITTENDEN RN, THOMAS GL. A conditional least squares method for estimating the size of a closed population. Can.J.Fish.Aquat.Sci. 1989; 46: 818-823.

DIANA JS, KOHLER SL, OTTEY DR. A yield model for walking catfish production in aquaculture systems. Aquaculture 1988; 71: 23-35.

DUNN TP, LEITZ PA, HARRIS SL. The salmon aquaculture industry in Canada. Aquaculture: a review of recent experience 1989: 273-295.

DWYER WP, SMITH CE, PIPER RG. Lake trout growth efficiency as affected by temperature. U.S. Fish and Wildlife Service, Bozeman Information Leaflet 19, Bozeman. 1981.

EHRENBERG JE, KANEMORI RY. A microcomputer-based echo integrator system for fish population assessment. Proc. of the IEEE Conference on Eng. in Ocean Environment. 1987: 204-207

ELLIOT JM. The growth rate of brown trout, Salmo trutta L., fed on maximum ratios. J. anim. Ecol. 1975; 44: 805-821.

ESSER HO, MOSER P. Proceedings of SECOTOX Workshop "Feasibility of integrated approaches to testing of chemicals", Kyoto, 1980.

FAGERLUND UHM, MCBRIDE JR, STONE ET. Stress-related effects of hatchery rearing density on coho salmon. Trans.Am.Fish Soc. 1981; 110: 644-649.

FAGERLUND UHM, MCBRIDE JR, DOSANJH BS, STONE ET. Culture density and size effects on performance to release of juvenile chinook salmon and subsequent ocean survival. Smolt release from Capilano Hatchery in 1980 and 1981. Can.Tech.Rep.Fish.Aquat.Sci. 1987; 1572; p.24.

FDA. Final rule permanently listing FD&C Blue No.1. 47 Fed.Reg. 42563 (September 28, 1982).

FEINGOLD BF. Why your child is hyperactive? New York: Random House 1975.

FENDERSON OC, CARPENTER MR. Effects of crowding on the behaviour of juvenile hatchery and wild landlocked Atlantic salmon (Salmo salar L.). Anim.Behav. 1971; 19: 439-447.

FOGG AG, SUMMAN AM. Further differential-pulse polarographic and visible spectrophotometric studies of the degradation of permitted synthetic food colouring matters with and without the addition of ascorbic acid: degradation in the dark and in the light without the stabilizing action of EDTA. Analyst, November, 1983; 108: 1339-1344.

FRITZ JS, SCHENK H. Quantitative analytical chemestry. Allyn and Bacon, Inc., Boston, 1973.

GANGOLLI SD, GRASSO P, GOLBERG L. Physical factors determining the early local tissue reactions produced by food colourings and other compounds injected subcutaneously. Fd Cosmet. Toxicol. 1967; 5: 601-621.

GANGOLLI SD, GRASSO P, GOLBERG L, HOOSON J. Protein binding by food colourings in relation to the production of subcutaneous sarcoma. Fd Cosmet.Toxicol. 1972; 10: 449-462.

GAUNT IF, FARMER M, GRASSO P, GANGOLLI SD. Food Cosmet.Toxicol. 1967; 5: 747-754.

GAUNT IF, KISS IS, GRASSO P, GANGOLLI SD. Food Cosmet.Toxicol. 1969; 7: 9-16.

GLANTS SA, SLINKER, BK. Primer of applied regression and analysis of variance. Copyright© McGraw-Hill, Inc. 1990; 777 pp.

GRASSO P, GANGOLLI SD, GOLBERG L, HOOSON J. Physicochemical and other factors determining local sarcoma production by food additives. Fd Cosmet.Toxicol. 1971; 9: 463-478.

GRASSO P, GOLBERG L. Early changes at the site of repeated subcutaneous injection of food colourings. Fd Cosmet.Toxicol. 1966; 4: 269-282.

HAJRATWALA B. The stability of FD&C no.1. Drug Cosmet. Ind. 1985; 136(2): 50,52,86.

HAJRATWALA BR. Influence of sunscreening agents on color stability of tablets coated with certified dyes: FD&C Blue No.1. J.Pharmaceutical Sc. 1977; 66: 107-109.

HASKELL DC. Weight of fish per cubic foot of water in hatchery troughs and ponds. Progressive Fish-Culturist 1955; 17: 117-118.

HESS SM, FITZHUGH OG. J..Pharmacol.Exp.Ther. 1955; 114: 38.

HEWLETT-PACKARD. Quantitation software handbook. Copyright (c) 1987 by Hewlett-Packard Company.

HINOUE T, SHIMAHARA Y, MURATA H, YOKOYAMA Y. Photoacoustic spectrometry by total internal reflection technique. Dependence of photoacoustic signal intensity on concentration and optical path. Bunseki Kagaku 1984; 33: E459-E466.

HOKANSEN KEF, KLEINER CF, THORSLUND TW. Effects of constant temperatures and diel temperature fluctuations on specific growth and mortality rates and yield of juvenile rainbow trout, Salmo gairdneri. J. Fish. Res. Bd Can. 1977; 34: 639-648.

HOLLINGWORTH T, WEKELL MM, eds. Fish and other marine products. Food and Drug administration. AOAC Official Methods of Analysis 1990 (vol.2): 35; 867-888.

HICKS CR. Fundamental concepts in the design of experiments. HRolt, Rinehartand Winston Inc. sec. ed. 1973; 183-185.

INGRAHAM KP, PARKER JC. Guide for personnal computerr, version 6 edition. Cary, NC: SAS Institute Inc., 1987; 1028 pp.

JOBLING M. Influence of body weight and temperature on growth rates of arctic char (Salvelinus alpinus). J.Fish Biol. 1983a; 22: 471-475.

JOBLING M. Growth studies with fish overcoming the problems of size variation. J.Fish.Biol. 1983b; 22: 153-157.

JOYCE JR, SANGER DG. A procedure for the identification of soluble food dyes in illicit drug preparations. J.Forens.Sci.Soc. 1979; 19: 203-209.

JOYCE JR, HUMPHREYS IJ. The detection of trace impurities in soluble food dyes.J.Forens.Sci.Soc. 1982; 22: 253-256.

KANTOR MA, TROUT JR, LACHANCE PA. Food dyes produce minimal effects on locomotor activity and vitamin B-6 levels in postweanling rats. J. Nutr. 1984; 114: 1402-1412.

KAUSHIK SJ, LUQUET P. Influence of bacterial protein incorporation and of sulphur amino acid supplementation to such diets on growth of rainbow trout, Salmo gairdneri Richardson. Aquaculture 1980; 19: 163-175.

KEPSHIRE BM Jr. Growth of pink, chum and fall chinook salmon in heated sea-water. Proc. NW Fish Cult. Conf. 1971; 22: 25-26.

MITCHELL JF, STEWART DJ, WEININGER D. Applications of a bioenergetics model to yellow perch (Perca flavescens) and walleye (Stizostedion vitreum vitreum). J.Fish.Res.Board Can. 1977; 34: 1922-1935.

KOCH L. Some legal, chemical and physical aspects of permitted color additives. A brief review. Am. Perfumer Cosmet. 1967; 82: 35-40.

KUBOTA Y. Experience with the Chemical Substances Control Law in Japan. Ecotox.Environ.Safety 1979; 3: 256-268.

LEATHERLAND JF, CHO CY. Effect of rearing density on thyroid and interrenal gland activity and plasma and hepatic metabolite levels in rainbow trout, Salmo gairdneri Richardson. J.Fish.Biol. 1985; 27; 583-592.

LEITRITZ E. Trout and salmon culture (hatchery methods). State of California Department of Fish and Game, Fish Bulletin 1980; 164: p.123.

LOCKEY SD. Allergic reactions due to FD&C Yellow no.5 Tartrazine, an analine

dye used as a colouring and identifying agent in various steroids. Ann. Allerg. 1959; 17: 719-

LYKENS DN. Thermal stability of FD&C lake pigments. Plastics Compounding November/December 1986; 35-40.

MCKIM JM, ERICKSON RJ. Environmental impacts on the physiological mechanisms controlling xenobiotic transfer across fish gills. Physiological Zoology 1991; 64(1): 39- 67.

MINITAB INC. Minitab handbook. PWS-Kent Publishing company. Wadsworth, Inc. Sec.ed. 1985; 245-252.

NAKAI Y, YAMAMOTO K, TERADA K, SAKAI M. Some applications of photoacoustic spectroscopy to the analysis of pharmaceutical powders. Chem. Pharm. Bull. 1985; 33(7): 3068-3070.

NEELY WB, BRANSON DR, BLAU GE. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ.Sci.Technol. 1974; 8: 1113-1115.

NOONAN J. Color additives. In: Furia TE, ed. Handbook of food additives. CRC Press. Cleveland, Ohio, 1972: 587-615.

OECD CHEMICALS TESTING PROGRAMME. Sequential static procedure for measuring the bioaccumulation potential of chemicals with fishes. Report of Expert Group Degradation/Accumulation, Part I, 1979; 70-81.

P[illegible]UTSOGLOU SE, PAPAPARASKEVA-PAPOUTSOGLOU E. Effect of water temperature on growth rate and body composition of rainbow trout (Salmo gairdneri R.) fry, fed on maximum ration in closed system. Thalassographica 1978; 2: 83-97.

PAPOUTSOGLOU SE, PARAPARASKEVA-PAPOUTSOGLOU EG, DENDRINOS PK. Studies on the effect of density on body composition, growth rate and survival of rainbow trout fry reared in a semi-closed system. Thalassographica 1979; 3: 43-56.

PATINO R, SCHRECK CB, BANKS JL, ZAUGG WS. Effects of rearing conditions on the developmental physiology of smolting coho salmon. Trans.Am.Fish.Soc. 1986; 115; 828-837.

PHILLIPS JC, MENDIS D, EASON CT, GANGOLLI SD. The metabolic disposition of C-labelled Green S and Brilliant Blue FCF in the rat, mouse and

guinea-pig. Fd Cosmet.Toxicol. 1980; 18: 7-13.

PICKERING AD, POTTINGER TG. Poor water quality suppresses the cortisol response of salmonid fish to handling and confinement. J.Fish.Biol. 1987b; 30: 363-374.

PICKERING AD, POTTINGER TG. Cortisol can increase the susceptibility of brown trout, Salmo trutta L., to disease without reducing the white blood cell count. J.Fish Biol. 1985; 27: 611-619.

PICKERING AD, POTTINGER TG. Crowding causes prolonged leucopenia in salmon fish, despite interrenal acclimation. J.fish Biol. 1987a; 30: 701-712.

PICKERING AD, POTTINGER TG, CHRISTIE P. Recovering of the brown trout, Salmo trutta L., from acute handling stress: a time-course study. J.Fish Biol. 1982; 20: 229-244.

PICKERING AD, DUSTON J. Administration of cortisol to brown trout, Salmo trutta L., and its effects on the susceptibility to Saprolegnia infection and furunculosis. J.Fish Biol. 1983; 23: 163-175.

PICKERING AD, STEWART A. Acclimation of the interrenal tissue of the brown trout, Salmo trutta L., to chronic crowding stress. J.Fish.Biol. 1984; 24: 731-740.

PIPER RG, MCELWAIN IB, ORME LE, MCCRAREN JP, FOWLER LG, LEONARD JR. Fish hatchery management. U.S. Fish and Wildlife Service, Washington, D.C. 1982.

PIPER RG. Know the proper carrying capacity of your farm. Am.Fish. U.S. Trout News 1970; 15: 4-6.

POSTON HA, WILLIAMS RC. Interrelations of oxygen concentration, fish density, and performance of atlantic salmon in an ozonated water reuse system. Progressive Fish-Culturist 1988; 50: 69-76.

RACKHAM D. Scottish suppliers must leave no supply gaps. Fish farming international, November 1987.

RADOMSKI JL. Toxicology of food colors. Annu.Rev.Pharmacol. 1974; 14: 127-137.

REFSTIE T, KITTELSEN A. Effect of density on growth and survival of artificially reared Atlantic salmon. Aquaculture 1976; 8; 319-326.

REFSTIE T. Effec' of density on growth and survival of rainbow trout. Aquaculture 1977; 11: 329-334.

REFSTIE T. Effect of density on growth and survival of rainbow trout. Aquaculture 1977; 11; 329-334.

ROTTINGEN I. On the relation between echo intensity and fish density. FiskDir. Skr.Ser.HavUnders. 1976; 16: 301.

SANDERCOCK FK, STONE EJ. A progress report on the effect of rearing density on subsequent survival of Capilano coho. In Proc.North Pacific Aquacult. 1982; p.151.

SCHMIEDER PK, HENRY TR. Plasma binding of 1-butanol, phenol, nitrobenzenw and pentachlorophenol in the raibow trout and rat: a comparative study. Comp.Biochem.Physiol. 1988; 91C: 413-418.

SCHRECK CB. Stress and compensation in teleostean fishes: response to social and physiological factors. In: Pickering AD (editor), Stress and Fish. Academic Press, London, pp. 295-321.

SEBER GAF. Estimation of animal abundance. Sec. ed. London, Griffin 1987.

SEEGER P, THORRSSON U, LARSSON B, RONNINGEN K. Accuracy and precision when sampling from fish troughs. Aquaculture 1977; 10: 365-371.

SHRECK CB, PATINO R, PRING CK, WINTON JR, HOLWAY JE. Effects of rearing density on indices of smoltification and performance of coho salmon, Oncorhynchus kisutch. Aquaculture 1985; 45; 345-358.

SMITH HF, CARPENTER CP, WEIL CS. The toxicology of the polyethylene glycols. J.Am.Pharmac.Ass. 1950; 349-354.

SOBOTKA TJ, BRODIE RE, SPAID SL. Tartrazine and the developing nervous system of rats. J.Toxicol.and Env.Health 1977; 2: 1211-1220.

SODERBERG RW, KRISE WF. Effects of rearing density on growth and survival of lake trout. Progressive Fish-Culturist 1986; 48; 30-32.

SPACIE A, HAMELINK JL. Alternative models for describing the bioconcentration of organics in fish. Environ.Toxicol.Chem. 1982; 1: 309-320.

SPRAGUE JB. Factors that modify toxicity. In: RAND GM and PETROCELLI SR, eds. Aquatic toxicology. Hemisphere, New York, 1985.

THORBURN MA. The randomness of samples collected by dip-net methods from rainbow trout in tanks. Aquaculture 1992; 101: 385-390.

TURRELL WR, MUNRO ALS. Sea cage culture of Atlantic salmon: model study on the fate of soluble wastes. Aquacultue: a recent review of recent experience 1989: 92-104.

U.S. ENVIRONMENTAL PROTECTION AGENCY. Quality criteria for water. Washington, D.C.: U.S. Government Printing Office. 1976: 256 pp.

VIJAYAN MM, LEATHERLAND JF. Effect of stocking density on the growth and stress-response in brook char, Salvelinus fontinalis. Aquaculture 1988; 75: 159-170.

VOORHEES CV, BUTCHER RE, BRUNNER RL, WOOTTEN V, SOBOTKA TJ. Developmental toxicity and psychotoxicity of FD&C Red No.40 (Allura Red AC) in rats. Toxicology 1983; 28: 207-217.

WALLACE JC, KOLBEINSHAVN AG, REINSNES TG. The effects of stocking density on early growth in Arctic char, Salvelinus alpinus L.. Aquaculture 1988; 73: 101-110.

WANDSVIK A, JOBLING M. Observations on growth rates of Arctic char, Salvelinus alpinus (L)., reared at low temperature. J.Fish Biol. 1982; 20: 689-699.

WEDEMEYER GA. 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.Bd.Can. 1976; 33: 2699-2702.

WINDHOLZ M, BUDAVARI S, BLUMETTI RF, OTTERBEIN ES, eds. The Merk index.10th edition. Rahway, N.J., U.S.A. 1983

YAMAGISHI H. Comparative study on the growth of the fry of three races of Japanese crucian carp, Carassius carassius L., with special reference to behaviour and competition. Jpn. J.Ecol., 1965; 15: 100-113.

YAMAGISHI H. Postembryonal growth and its variability in three marine fishes with special reference to the mechanism of growth variation in fishes. Res. Popul. Ecol. 1969; 11 (1): 14-33.