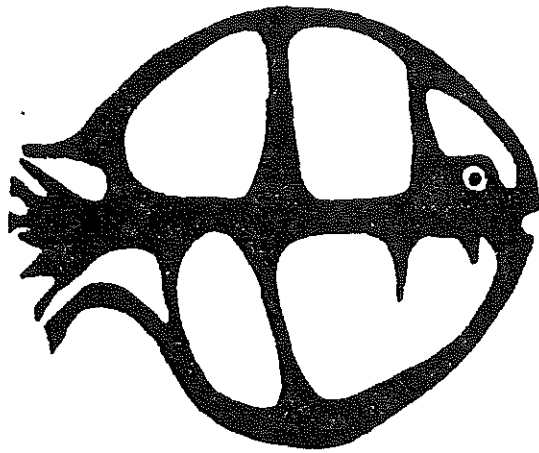


Abstracts

Fisheries Society of the British Isles



International Symposium

Feeding and Nutrition in Fish

JULY 10 - 13 1984

University of Aberdeen

Abstracts of Papers

FISHERIES SOCIETY OF THE BRITISH ISLES
SYMPOSIUM ON FEEDING AND NUTRITION

ABERDEEN, JULY 10—13, 1984

TUESDAY, JULY 10th

PROTEINS

Chairman

J.R. SARGENT

09.00 - 09.10

Introduction

09.10 - 09.55

R.P. Wilson (Dept. of Biochemistry, Mississippi State University, Mississippi, U.S.A.)

Amino acid and protein requirements of fish

09.55 - 10.30

✓*

B. Fauconneau (I.N.R.A., Laboratoire de Nutrition des Poissons, St. Pée-sur-Nivelle, France).

Protein synthesis and protein turnover in fish

10.30 - 11.00

COFFEE

11.00 - 11.35

✓

M.J. Walton (N.E.R.C., Institute of Marine Biochemistry, Aberdeen).

Aspects of amino acid metabolism in fish

11.35 - 12.10

R. Ash (Dept. of Applied Biology, University of Bradford).

Digestion and absorption of proteins and amino acid transport.

12.10 - 13.10

LUNCH

Chairman

A. KANAZAWA

13.10 - 14.05

S.J. Kaushik & C.Y. Cho (I.N.R.A., Laboratoire de Nutrition des Poissons, St. Pée-sur-Nivelle, France & Department of Nutrition, University of Guelph, Guelph, Canada).

Effects of nitrogen intake on ~~metabolizable energy~~ ^{digestible utilisable dietary} value of diets for fish. _{energies}

14.05 - 14.40

A.G.J. Tacon & A.J. Jackson (Institute of Aquaculture, University of Stirling, Stirling and S.M.B.A., Oban).

Utilisation of conventional and unconventional protein sources in practical fish feeds.

14.40 - 15.15

A.J. Matty & K.P. Lone (Dept. of Biological Sciences, University of Aston, Birmingham).
Hormonal stimulation of protein deposition

15.15 - 15.45

TEA

15.45 - 16.45

POSTER SESSION I. POSTER NUMBERS 1 - 18

16.45 - 18.00

DISCUSSION OF POSTERS

Room
B21

WEDNESDAY, JULY 11th

FEEDING BEHAVIOUR

Chairman

A.D. HAWKINS

09.00 - 09.35

A.M. Mackie & A.I. Mitchell (N.E.R.C, Institute of Marine Biochemistry, Aberdeen).

Identification of gustatory feeding stimulants for fish -applications in aquaculture.

09.35 - 10.10

P.J. Bromley (M.A.F.F., Fisheries Laboratory, Lowestoft).

Weaning diets for marine fish larvae

10.10 - 10.45

B. Knights (Biology Dept., Polytechnic of Central London, London.)

Feeding behaviour and fish culture

10.45 - 11.05

COFFEE

11.05 - 11.40

P. Higgins & C. Talbot (D.A.F.S., Pitlochry, Perthshire).

Feeding and growth in juvenile Atlantic salmon

11.40 - 12.15

K.T. O'Grady & P.B. Spillett (Dept. of Zoology, Royal Holloway College, Surrey and Thames Water Authority, Reading).

Gross nutrition and conversion efficiency of intensively and extensively reared carp (*Cyprinus carpio* L.)

12.15 - 13.30

LUNCH

14.00

Assemble for visits to:

CASTLE AND BIRD SANCTUARY **GLENFIDDICH AND BALVENIE DISTILLERIES**

19.30

Buses to Amatola Hotel for Conference Dinner

THURSDAY JULY, 12th

NUTRITION AND METABOLISM OF LIPID AND CARBOHYDRATE

Chairman

09.00 - 09.45

T. WATANABE

A. Kanazawa (Faculty of Fisheries, University of Kagoshima, Kagoshima, Japan).

Essential fatty acid and lipid requirement of fish

09.45 - 10.25

C. Leger (I.N.R.A., Jouy-en-Josas, France)

Digestion, absorption and transport of lipids

10.25 - 10.55

COFFEE

10.55 - 11.30

C.B. Cowey & J.G. Bell (N.E.R.C., Institute of Marine Biochemistry, Aberdeen).

Roles of vitamin E and selenium in the prevention of pathologies related to fatty acid oxidation in salmonids

11.30 - 12.05

R.J. Henderson & J.R. Sargent (N.E.R.C., Institute of Marine Biochemistry, Aberdeen.)

Fatty acid metabolism

12.05 - 12.45

E. Pfeffer (Dept. of Animal Nutrition, University of Bonn, West Germany).

Digestion, absorption and utilization of carbohydrates

12.45 - 13.45

LUNCH

Chairman

J.E. HALVER

13.45 - 14.20

J.R.C. Springate, N.R. Bromage & P.R.T. Cumararatunga, (Dept. of Biological Sciences, University of Aston, Birmingham).

The effects on fecundity and egg quality of feeding broodstock rainbow trout (*Salmo gairdneri* R.) at two different ration levels.

14.20 - 15.05

T. Watanabe (Fish Nutrition Laboratory, Tokyo University of Fisheries, Tokyo, Japan).

Effect of nutritional quality of broodstock diets on egg development

15.05 - 15.40

M. Keith (Unilever Limited, Aberdeen).

Salmon flesh pigmentation

15.40 - 16.00

TEA

16.00 - 17.00

POSTER SESSION II. POSTER NUMBERS 19-35

17.00 - 18.15

DISCUSSION OF POSTERS

FRIDAY, JULY 13th

MINOR DIETARY COMPONENTS:
PRACTICAL ASPECTS

Chairman

R.P. WILSON

09.00 - 09.45

J.E. Halver (School of Fisheries, University of Washington, Seattle, U.S.A).

Recent advances in vitamin nutrition and metabolism in fish

09.45 - 10.30

H.G. Ketola (Tunison Laboratory of Fish Nutrition, Cortland, New York, U.S.A.).

Mineral nutrition : Effects of phosphorus in trout and salmon feeds on water pollution

10.30 - 11.00

COFFEE

11.00 - 11.45

D.A. Bengtson, A.D. Beck & K.L. Simpson (University of Rhode Island and U.S. Environmental Protection Agency, U.S.A.).

Standardization of the nutrition of fish in aquatic toxicological testing.

11.45 - 12.30

V.O. Crampton (EWOS Baker Ltd., Bathgate, Scotland).

Application of nutritional findings to the formulation of practical diets

12.30 - 13.30

LUNCH

14.00

Visit to I.M.B.

Amino Acid and Protein Requirements of Fish

Robert P. Wilson

Department of Biochemistry, Mississippi State University, Mississippi, U.S.A.

Dietary protein requirement values have been estimated for a wide variety of fishes. These estimated values range from about 30 to 55% of the diet and are much higher than those for other animals. In general, these values have been based on the dietary protein level that produced maximum growth. Various factors have been shown to influence the growth response of fish fed different protein levels. Some of these include fish size, water temperature, amount of nonprotein energy in the diet, and dietary protein quality. A detailed summary of the estimated requirement values will be presented along with some discussion as to how some of these factors may affect the requirement value.

Several species of fish have been shown to require the same ten essential amino acids as other animals. However, the establishment of the quantitative amino acid requirements has been limited to the chinook salmon, common carp, Japanese eel, and channel catfish. Limited information is also available on selected amino acid requirements for coho salmon, rainbow trout, lake trout, gilthead bream and tilapia. The various requirement values will be summarized and discussed with respect to apparent species differences. Differences in the methodology used to quantitate the amino acid values will be reviewed. The potential use of serum amino acid levels to confirm the requirement values will be discussed.

The essential amino acid composition of the whole body protein of a fish appears to be a good index of the proper dietary amino acid balance required by that species. The estimated amino acid requirement values for channel catfish and common carp were found to be highly correlated with the essential amino acid composition of the whole body protein of the respective fish. The essential amino acid composition of the whole body protein of the channel catfish remained relatively constant for fish weighing from about 30 grams to 860 grams; thus, indicating that the amino acid requirement, when expressed as a percentage of dietary protein, should not change with fish size. Because of this direct relationship between amino acid requirement and essential amino acid composition of whole body protein, the whole body essential amino acid composition should serve as an excellent index in formulating test diets for fish.

Protein Synthesis and Protein Turnover in Fish

B. Fauconneau

Laboratoire de Nutrition des Poissons

Institute National de la Recherche Agronomique, St Pée-sur-Nivelle, France

Composition of the body is maintained and modified by dynamic renewal of its major constituents like proteins. Measurement of protein synthesis and protein breakdown provide prospective tools for studying protein metabolism.

Mechanisms of protein synthesis and protein breakdown in fish are similar to those observed in mammals. Some specific factors in regulation of protein synthesis are briefly mentioned. Reliable methods of measurement of protein synthesis and protein breakdown in fish are now available. For protein synthesis these include isotopic methods. For measurement of protein breakdown an isotopic method can be used but generally it is arrived at as the difference between protein synthesis and protein deposition. These methods are analysed from different viewpoints: conceptual (model of protein metabolism), metabolic (choice of precursor pool for protein synthesis) and technical.

Based on results accumulated up to now, the relative magnitudes of protein synthesis and protein breakdown in different tissues are reviewed. Liver, gills, kidney and digestive tract have high protein synthesis rates: 10 to 20% of protein synthesized per day and about 90% of protein synthesis represents protein turnover. Muscular tissues have a very low protein synthesis rate: from 0.15 to 2%/d, in which more than 50% is integrated in protein deposition. Interspecific variation of protein synthesis in these different tissues is quite important. The integration of the different tissues in whole body protein synthesis is also analyzed.

Intraspecific changes in protein synthesis and protein breakdown could be related to changes in some biotic factors such as physiological (age) or nutritional state, and also to changes in abiotic factors: temperature, oxygen.

Protein turnover in 'regulatory' tissues (liver, gills, digestive tract) show small changes during fasting but very high changes are noted due to differences in environmental temperature. Protein turnover in these tissues account for the most part of energy expenditure linked to protein metabolism in the overall basal metabolism.

In muscle, protein synthesis is correlated to growth rate. It is difficult to analyze protein turnover of resident protein because firstly it is masked by synthesis of new proteins and secondly it is a very low process involving relatively high errors of measurement. However the decrease in protein deposition rate throughout development can be associated to a decrease in protein turnover rate.

In the light of these results, validity of the concept of protein turnover in fish is discussed. Possible means of manipulating protein turnover in order to increase whole body protein deposition implies either a direct stimulation of protein deposition rate in the muscle or an increase in muscle mass.

Aspects of Amino Acid Metabolism in Fish

M. J. Walton,
N.E.R.C., Institute of Marine Biochemistry,
St. Fittick's Road, Aberdeen, U.K.

The metabolism of amino acids is probably basically similar in all vertebrates. This review will outline the main metabolic pathways as they apply to fish concentrating on catabolism of amino acids and relating to nutritional studies when appropriate.

Fish require the same essential amino acids as growing mammals and are able to incorporate radioactivity from [U-¹⁴C]-glucose into non-essential amino acids. However, in contrast to many mammals, carbohydrates are relatively poorly utilised by fish and the principle sources of energy are amino acids and lipids, the relative contributions depending on the species. Amino acids are derived both from dietary protein and from catabolism of body protein which is in a state of constant turnover. As there are no known body stores of amino acids, most of those not required for protein synthesis are deaminated producing a keto acid and releasing ammonia; the remaining amino acids are required for synthesis of purines, hormones, etc.

There is no functional urea cycle in teleosts and the liver has been identified as the main source of blood ammonia. Most amino acids have specific deaminating enzymes (not all of which have yet been investigated in fish) which tend to have K_m values many times higher than the enzymes initiating protein synthesis, hence offering one means of metabolic control. It is not yet clear which are the most significant ammonia forming reactions: many people favour the transdeamination pathway, whereas some evidence has been presented for the roles of glutaminase and for the purine nucleotide cycle.

After deamination the keto acid is mainly oxidised to CO₂ via the tricarboxylic acid cycle for energy, or in some cases converted to glucose or lipids. The rate of oxidation depends on several factors including nutrition. In turbot there was very little effect of dietary protein level (16 or 50%) on production of ¹⁴CO₂ from labelled leu, phe, glu or ala. When trout were given graded levels of the essential amino acids trp, lys or arg in otherwise adequate diets, oxidation of these amino acids was low in fish fed sub-requirement levels of the amino acid, but increased once the requirement level was surpassed. However, the activities of several amino acid catabolising enzymes were unaffected by either dietary levels of protein or levels of the relevant amino acid. In contrast activities of some gluconeogenic and glycolytic enzymes were affected by dietary protein level.

Hence in many fish amino acid metabolism appears to be permanently adapted to deal with high dietary levels of protein.

Digestion & Absorption of Proteins: Amino Acid Transport

R. Ash

University of Bradford, Bradford, West Yorkshire

Many factors, both biotic and abiotic, affect protein requirements of fish (Cowey & Luquet, 1983). However, if it can be assumed (see later) that intact protein absorption is nutritionally unimportant with respect to fish, then it must be accepted that dietary protein is only useful to the animal if it can be digested and the degradation products (peptides and amino acids), made available via absorptive processes. Information regarding these physiological processes and those factors which may affect their overall efficiency are therefore of considerable relevance to all concerned with the (intensive) culture of fish.

The minimum dietary protein level necessary to achieve optimal weight gain in most fish species is high and, in theory, could simply arise due to inherent inefficiencies at the level of those processes responsible for protein digestion and terminal end-product (amino-acid and peptide) absorption. Evidence will be reviewed which clearly demonstrates that such a supposition is untenable and that, far from being inefficient, the processes of protein digestion and end-product absorption in fish are, under most circumstances, extremely efficient.

The individual components (enzymes) which collectively provide for efficient hydrolysis, their organisation, and those factors relating to intensive fish feeding and husbandry practice which might compromise their overall efficiency will be discussed.

Metabolic availability of dietary derived amino acids requires the initial passage of these molecules (either as free amino acids or low-molecular weight peptides) into the enterocyte and the subsequent release of a proportion of this material into the portal vasculature. A consideration of those mechanisms available for amino acid and peptide absorption will be presented. Reference will be made to the use of diets containing either supplemented free (essential) amino acids or partially hydrolysed proteins, and the effects of such practices on amino acid transport and subsequent amino acid availability will be discussed.

Attention will be focused upon the phenomenon of intact protein absorption in fish. Evidence for the existence of such an absorptive mechanism in various fish species will be reviewed and the question of its physiological and nutritional significance considered.

Reference:

- Cowey, C. B. & Luquet, P. (1983) Physiological Basis of Protein Requirements of Fishes. Critical Analysis of Allowances. In: Protein Metabolism and Nutrition. Ed., INRA Publ. I (les Colloques de l'INRA, No 16) p 365-384. INRA Publ., Versailles.

Effects of Nitrogen Intake on Metabolizable Energy Value of Diets for Fish

C. Y. Cho and S. J. Kaushik

Fish Nutrition Laboratory, Ministry of Natural Resources
and University of Guelph, Guelph, Ontario, Canada
and

Laboratoire de Nutrition des Poissons, Institute National,
de la Recherche Agronomique, St.-Pée-Sur-Nivelle, France

Current knowledge on the interrelationships between dietary energy and protein in fish is reviewed with special consideration of the different intermediate steps involved in nutrient flow. As an index of nutrient intake, the level of digestible (available) energy (DE) in the feed is deemed to be a valid estimator. However, the proportion of metabolizable (utilizable) energy (ME) from the digestible energy is greatly dependent upon the balance between ingested energy and protein. This is especially true in the light of recent work demonstrating the protein energy-sparing action of other energy-yielding nutrients, particularly lipid. The utilization of ingested nutrients is determined by the balance of essential nutrients per unit intake of DE.

The amount of metabolizable energy has frequently been estimated using physiological fuel values (PFV) of the major nutrients in the diet. Being based on data obtained in mammals and birds, the extension of such values to different varieties of fish fed diets of varying complexity has often led to misinterpretations of the significance of ME values. The indissociable nature of energy-yielding and protein-yielding nutrients in a diet calls for a reconsideration of ME rather more in relation to complete diets than with respect to individual dietary constituents.

Heat increment associated with protein intake is estimated to be lower in ammoniotelic teleosts compared to ureotelic or uricotelic animals. This heat increment due to feeding is mainly biochemical in nature after absorption and the extent of variability depends on the quantitative nature of DE. The net energy yield and the efficiencies of protein and lipid deposition are likewise affected by the relative proportions of protein and non-protein digestible energy.

Despite their higher levels of energy and protein in fish diets, fish convert more efficiently dietary energy to body energy merely due to lower requirements of energy for maintenance and heat increment of feeding. The optimal protein:energy ratio (g protein per MJ of DE) is, on the other hand, higher for poikilotherms than for homeotherms, reflecting the importance of nitrogen intake to overall energy balance and especially on metabolizable energy. This close interrelationship should be borne in mind in the formulation of diets. Optimization characteristics of protein and lipid retentions will have to be defined recognizing that there are species differences and with the purpose of the fish production: direct food for man or to enhance natural resources for recreation.

Utilisation of Conventional and Unconventional Protein
Sources in Practical Fish Feeds

Albert G. J. Tacon* and Andrew J. Jackson**

*Institute of Aquaculture, University of Stirling,
Stirling FK9 4LA

** Dunstaffnage Marine Research Laboratory, P O Box 3, Oban,
Argyll, Scotland

The use of conventional and unconventional protein sources as a partial or complete replacement for fish meal within practical fish feeds is discussed. Conventional protein sources are viewed on the basis of methods currently available to the animal feed compounder to maximise their nutritional value to fish, including heat processing techniques (grinding, micronisation, extrusion, expansion), enzyme stabilisation techniques (Silages, hydrolysates), ingredient supplementation with limiting amino acids/minerals, or a combination of all three. Feed stuffs considered under this category include plant oil-seed meals, animal silages, meat and bone meal, and blood meal. Unconventional or novel protein sources which are assessed include single cell proteins (bacteria, yeast and Algae), milk replacers (potato protein), whole food organisms (aquatic and terrestrial lumbricid worms), aquatic macrophytes, and animal and food processing wastes (animal byproduct meals, waste sludges).

The nutritive value of individual protein sources is compared on the basis of their biochemical composition and performance in feeding trials with a variety of cold water and warm water fish species.

* Loss of TRP - follows entilage & storage.

Hormonal Stimulation of Protein Deposition

A. J. Matty and K. P. Lone

Department of Biology, University of Aston in Birmingham,
Gosta Green, Birmingham

Hormones play an important part in the physiology and biochemistry of protein deposition in all fish but with the development of fish farming and pelleted feeds there has been interest during the past decade in their potential role as growth promoters, i.e. as stimulants of protein deposition. A number of hormones present naturally in fish produce protein anabolic effects although protein catabolism is also influenced by hormones in certain physiological and pathological conditions. A balance between these anabolic and catabolic hormones will determine whether growth (protein deposition) or net loss from the protein reservoirs will take place.

It is proposed in this presentation to review briefly the normal role played by hormones in protein metabolism of fish and then to assess the effects of exogenous hormonal supplementation on protein anabolism and deposition. Finally, hormones will be evaluated as possible growth promoters in aquaculture and compared with the possible use of non-hormonal agents such as antibiotics and non-protein nitrogen supplements as growth promoters.

In addition to pituitary growth hormone the hormones most concerned with nitrogen metabolism and the increase in tissue proteins of fish are certain steroid hormones, thyroid hormones, and endopancratic hormones. These hormones together with some of their synthetic derivatives will be evaluated in terms of their effects on protein turnover with the main emphasis on protein deposition in different fish species. It is possible that some or all of these hormones play a permissive role in protein deposition, potentiating and synergising the action of growth hormone which itself may potentiate the formation of somatomedin. Other hormones such as prolactin, gastrin and cholecystokinin promote cell multiplication. Hormones may also influence growth (protein deposition) by a modulation of food intake, appetite and food conversion, this will be discussed as will be the effects of temperature and age upon hormonally induced protein deposition. As in higher vertebrates nutritional levels appear to alter blood levels of endogenous hormones and hence the rate of formation and amount of tissue protein; therefore, in conclusion this phenomenon will be examined.

Androgen on muscle.

↑ WT & Protein mass
A. N. Ret.

11 July 79

Identification of Gustatory Feeding Stimulants for Fish - Applications in Aquaculture

A. M. Mackie and A. I. Mitchell,
N.E.R.C. Institute of Marine Biochemistry,
St. Fittick's Road, Aberdeen AB1 3RA, U.K.

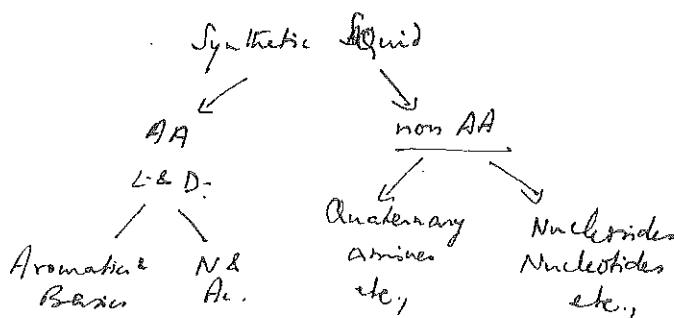
All fish utilize to some extent their chemical senses, olfaction and gustation, during feeding. Rainbow trout and turbot are sight feeders, attacking any object resembling food, and taste receptors within the buccal cavity then determine whether that object is swallowed or rejected. In contrast, dogfish, eels and Dover sole use olfaction or gustation in the long-range detection of food.

The aspect of feeding that has been investigated in greatest detail at the Institute of Marine Biochemistry is the nature of gustatory feeding stimulants - those chemicals inducing the swallowing of food. Several assay systems have been employed, including measuring the number of pellets of test diet eaten during a short feeding period, long-term feeding trials where the food consumption and growth rate were both measured, and utilization of a demand-feeder system to measure food preferences in the rainbow trout.

Mixtures of L-amino acids have been identified as the feeding stimulants for rainbow trout, European eel, sea bass and plaice. Considerable species specificity was observed and substitution of D-amino acids for the natural L-forms abolished activity in all cases, indicating absolute stereospecificity at the receptor surface. Glycine betaine was the feeding stimulant for adult Dover sole, while juveniles required the presence of amino acids as well as betaine. In the turbot and brill, inosine and inosine 5'-monophosphate were the specific feeding stimulants.

The attractiveness and palatability of the diets are factors which, therefore, must be taken into consideration in feed formulation for aquaculture, particularly when soyameal or other plant proteins are substituted for fish meal. Furthermore, fish meal prepared from teleost waste is generally low in glycine betaine and this material must be added to diets for sole, either as the pure chemical or in the form of Nephrops or scallop waste.

What is
Edicol sunset yellow?



Weaning Diets for Marine Fish Larvae

P. J. Bromley,
MAFF Fisheries Laboratory, Pakefield Road,
Lowestoft, Suffolk, NR33 0HT

Weaning, in the marine fish cultivation context, is the process of changing from live to artificial foods. This is achieved either during the larval stage or early in the juvenile stage, both types of which are considered here. Success usually depends on the implementation of specialised feeding techniques and the provision of high quality feeds.

The necessity of weaning stems from two pressures. First, difficulties in rearing the early stages of marine fish larvae on artificial foods has led to an initial dependence on live foods. Second, such foods are normally too costly for prolonged use and economic pressures dictate an early transfer to more practicable artificial feeds suitable for growing fish to market size.

Original studies on cod, sole and turbot are presented, along with a selective review of the techniques used for other species. Special attention is given to the way in which the techniques can be adapted to the particular behavioural characteristics of individual species.

The object is to maximise the number of encounters with food. In addition, the food should emit sufficient stimuli to elicit the full sequence of feeding behaviour which consists of food recognition and a feeding strike followed by ingestion and swallowing of the food particle. Beyond this, the food must be digestible and nutritionally adequate. Evidence is also presented which shows that the quality of the live food used prior to weaning can have a profound effect on the acceptability of artificial foods.

Progress in identifying the stimuli which elicit feeding has been hindered by the small and delicate nature of early larvae. This has precluded the use of many of the classical techniques for studying behaviour. In addition, information on the development of sensory systems is fragmentary. The number of stimuli to which larvae respond can be expected to increase markedly during the larval phase. The results of preliminary studies aimed at identifying these stimuli are reported. This will, it is hoped, lead to improvements in the specifications of weaning diets, possibly enabling the live food phase to be shortened or even eliminated.

Trout weaning diet

Wfm	30.4
Trout starter	30.4
Vit	1
Min	1
Cod live oil	
Anenicolan	16.4
Mussels	16.4

*some
PVFA
plan?*

Rotifers + Isotrichia in 1 culture tanks

- San Francisco nauplii poorer than Brazilian nauplii of *Artemia* for turbot larvae!
- Best step for weaning of Turbot:
Rotifers, then *Artemia* (San Francisco) -

- ~~cod~~

- first feeding unsuccessful.

* Feeding Behaviour and Fish Culture

B. Knights,
Polytechnic of Central London, London

An understanding of feeding behaviour of fish in culture is important in order that feeds and feeding regimes can be designed to encourage consumption and hence growth whilst minimising metabolic energy expenditure in feeding. Furthermore, wastage needs to be reduced because of high feed costs and the potentially deleterious effects of waste food on water quality. Feeding behaviour is discussed with special reference to dry particulate diets and to studies on warmwater culture of eels, Anguilla anguilla L.

Hunger motivation and appetite are controlled by metabolic, neurophysiological and hormonal mechanisms. These relate to innate biological rhythms but are strongly affected by commercial feeding practices (i.e. ration sizes and feeding frequencies and timings). They are also affected by culture stresses such as low oxygen, temperature shocks and handling. Learning is of great importance in arousal, selectivity and feeding success in intensive culture using standard feeds.

Initial location of food particles is by sight and/or chemoreception. Reactive distance in visual feeders is dependent on particle size, shape, movement and contrast. The latter factors are related in turn to water velocities, whether particles float or sink, light and turbidity. Chemoreception is mediated by distance and/or contact receptors and attractiveness may be enhanced by addition or supplementation of appropriate chemicals.

Capture of food particles is usually achieved by orobranchial suction processes. Subsequent manipulation and then ingestion or rejection is affected by flavour and by physical characteristics such as size, shape, hardness and texture. Interspecific variations in feeding behaviour often occur with age and body size. Social interactions and size-hierarchy effects may also be important, social facilitation encouraging consumption but agonistic behaviours possibly causing differential consumption and hence growth.

The implications of the above aspects of feeding behaviour of fish in culture are discussed in relation to feed formulation and manufacture and to general management, with particular reference to design of feeding regimes (ration sizes and feeding frequencies and timing) and to automatic and self-demand feeding systems.

Arousal & Search : Rhythmicity (learnt & innate), Conditioning, Social facilitation.

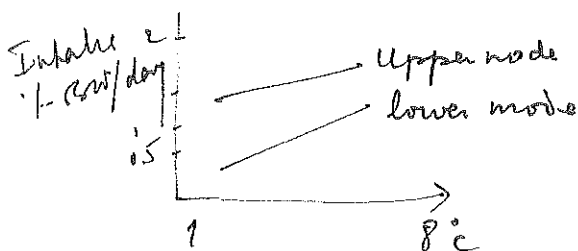
Location & Search: Rhythmicity (learned & innate),
Location & identification: Rate / encounter, location efficiency & relative distance,
 Localise supply in time & space $\downarrow$ match feed properties
 to perceptual abilities & ^{coulture} conditions.
Appetite & satiation: Gut fullness, metabolic factors, physiological factors
 $\downarrow$ appetite - satiation.

Feeding and Growth in Juvenile Atlantic Salmon

P. Higgins and C. Talbot,
Department of Agriculture and Fisheries for Scotland,
Pitlochry, Perthshire

The complex nature of the biology of juvenile Atlantic salmon (Salmo salar L.) must be taken into account in any study of the energetics of the species. Differential growth rates of individuals within a sibling population result in a bimodal length frequency distribution and in some individuals emphasis may change between somatic and gonadal growth at certain times of the year. One consequence of this is the production of smolts of different ages from a sibling population. As growth rate is dependent on a number of interacting factors it is clearly advantageous to follow processes such as growth and food intake in individual fish rather than the conventional procedure of using group mean estimates. In order to carry out such a study fish within the two modes of a hatchery reared bimodal population were marked with individually coded x-ray readable microtags. Their individual 24 hour food intake was estimated one day a week by feeding with a pelletised diet containing a small amount of iron powder as a radiopaque marker. This has allowed comparison of growth and food intake and thereby conversion efficiency in individuals within the two modes. Use of these techniques has also facilitated investigations into variations in food intake and evacuation rate with temperature, photoperiod, time of day and season.

- bimodal ? - g/s. Muscle fibre Numbers ↑ in upper modal sp & Area plateaus off in "
- Photoperiod plays a greater role than Temp during presmolt growth.
- Iron particles as markers of food intake & evacuation.



Proportion of daily food intake
at night - $\approx \underline{30\%}$
Which period is year?

evacuation 35h. what temp?
- is influenced by photoperiod rather than by temperature!!

Gross Nutrition and Conversion Efficiency of Intensively and Extensively
Reared Carp (Cyprinus carpio L.)

K. T. O'Grady¹ and P. B. Spillet²

- 1) Department of Zoology, Royal Holloway College, Englefield Green, Surrey
TW20 9TY
- 2) Thames Water Authority, Nugent House, Vastern Road, Reading, Berks RG1 8DB

A review of the literature shows that while detailed laboratory work on carp nutrition has been carried out in Britain, this aspect of carp in outdoor ponds has been little studied. Pond culture of carp is of increasing importance in Britain because of the need to grow-on for restocking as well as for the developing table market.

Experiments were undertaken to assess the conversion ratios and costs of a variety of artificial diets in small scale tank trials, and the results extrapolated to outdoor pond conditions and compared with actual performance. Indoor trials were of 10 - 12 weeks duration in aerated recirculated systems at 23°C using 30 individuals per treatment.

The food types investigated included; pelleted fish diets, herbivore pellets, grain and activated sludge. This gave ranges in composition by weight of 9 - 47% for protein, 1.5 - 17% for lipids and gross energy contents of 14 - 30 kJ. The most efficient diet, taking into account growth rate and cost, was trout pellets with conversion ratios of 1.8 - 2.0. For this diet a range of ration sizes indicated that the optimum ration was 5% body weight per day for carp of 4 - 7 g. No attempt at grading was made and as the carp grew changes in population size structure occurred which were related to the composition of the diet, possibly the relative proportions of protein and lipid. Limited experiments on co-culture with grass carp gave an increased overall conversion rate without detriment to carp growth rate.

Extensive and intensive outdoor trials in 0.5 ha, 1.7 m deep ponds exposed carp to ambient growing season (May - September) temperatures of 10 - 25°C. The pond water was naturally productive and the net extensive carp production was relatively high (300 - 400 kg ha⁻¹) and related to abundant pond zooplankton (mean 511 and peak 1600 individuals l⁻¹). The conversion ratio of this food source was estimated and considered in relation to its nutrient composition. Intensive feeding trials with trout pellets and barley gave carp production of 1400 - 2200 kg ha⁻¹. Overall conversion ratios for trout pellets were about 2.0 and allowance for consumption of natural pond foods increased the value by about 20%. Because natural pond foods were abundant and of different composition to the artificial foods given, the composition of the actual carp diet differed from the apparent, based solely on the artificial diet. Overall, naturally occurring pond foods were important both qualitatively and quantitatively, and their estimated economic equivalent appeared to be significant.

Lea Bridge carp farm 0.5 ha.
- Gravel substrates.
- Concrete banks
- eutrophic pond.

	Extensive	
	'80	'82
Stock	100	108 kg.ha ⁻¹
Net Prody	303	351 "
SGR	1.04	1.43

Essential Fatty Acid and Lipid Requirement of Fish

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Fish are incapable of synthesizing *de novo* 18:2 ω 6, 18:3 ω 3, 20:5 ω 3, and 22:6 ω 3, and require dietary sources of these fatty acids as essential fatty acids (EFA). Fish generally require ω 3-fatty acids rather than ω 6-fatty acids in contrast to mammals. Exceptionally, Tilapia nilotica requires ω 6-fatty acids such as 18:2 ω 6 and 20:4 ω 6 rather than ω 3-fatty acids. The efficacy of 18:3 ω 3 and highly unsaturated fatty acids (HUFA) such as 20:5 ω 3 and 22:6 ω 3 for fish varies from species to species. For the freshwater fish such as the rainbow trout Salmo gairdnerii, Ayu Plecoglossus altivelis, and eel Anguilla japonica, the efficacy of 18:3 ω 3 was found to be almost equal or similar to that of 20:5 ω 3 or 22:6 ω 3. On the other hand, 18:3 ω 3 was ineffective for the marine fish such as globefish Fugu rubripes, flatfish Paralichthys olivaceus, and yellow-tail Seriola quinqueradiata, and HUFA such as 20:5 ω 3 exert an EFA efficacy for these marine fish. The variation in the efficacy of 18:3 ω 3 among the fish was presumed to be related to the discrepancy in the capacities for elongation and desaturation of dietary 18:3 ω 3 to ω 3-HUFA.

Recently, we have succeeded in rearing the larval and juvenile fish with the artificial microparticulate diets and obtained information on lipid nutrition in larval stages of fish. Larval fish of species such as the red sea bream Chrysophrys major, knife jaw Oplegnathus fasciatus, flatfish, P. olivaceus, and Ayu, P. altivelis, were shown to require not only EFA but also dietary sources of phospholipids for their normal growth and survival. In the case of the Ayu larvae, the incidence of malformation was notably reduced by the addition of phospholipids to the diet. The effectiveness of dietary phospholipids was found to vary with the phospholipid classes and their constituent fatty acid moieties.

Digestion, Absorption and Transport of Lipids

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True lipase (glycerol ester hydrolase) and pancreatic colipase seem to be present in all fish. The recent discovery in primitive fish of colipase homologous to mammalian colipase appears to definitively prove that the lipase-colipase system exists in all vertebrates. Specificity of lipase is comparable to that in mammals. Triglycerides are split mainly or uniquely in glycerol positions 1 and 3. The presence of non-specific and of bile-salt dependent lipases has also been reported in either the pancreas or the intestinal contents. These enzymes hydrolize wax esters more slowly than triglycerides.

Apparent digestibility of dietary lipids depends on their nature. It is particularly high for unsaturated fats and seems to augment with the water temperature. Absorption occurs at the pyloric caeca and the proximal intestine. It is much slower than in mammals but progresses in a comparable way. After crossing the brush border, the fatty acids are esterified into triglycerides and exported out of the enterocyte as chylomicrons or VLDL-like particles. The presence of fat droplets in the enterocyte is also mentioned. These droplets, localized outside the reticulum, may be a form of temporary storage of the triglycerides synthesized in the enterocyte.

As in man, circulating plasma lipids are esterified in association with specific proteins (apoproteins) to constitute three main classes of lipoproteins (LP), VLDL, LDL and HDL. A special LP appears in the female during vitellogenesis. In rainbow trout, HDL is usually the largest LP class and its plasma level is often very high (3 to 5-fold that of man). The LP have a high docosahexaenoic acid (n-3 series) content. Lipoprotein lipase is present in trout plasma and probably contribute to the chylomicron and VLDL catabolism needed for tissue uptake of exogenous fatty acids.

Roles of Vitamin E and Selenium in the Prevention of Pathologies Related to Fatty Acid Oxidation in Salmonids

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Highly unsaturated fatty acids are essential components of fish diets. They are also readily susceptible to peroxidative attack leading to the formation of organic peroxides that break down to secondary and often even more toxic products. The initiating agent in fatty acid autoxidation is usually one of the free radical intermediates of O_2 metabolism. Such free radicals arise in the normal course of metabolism and the multi-tier defence system evolved in vertebrates to prevent their uncontrolled formation will be briefly described.

The elements in this system that have received most attention from fish nutritionists are vitamin E, an anti-oxidant terminator of free radical initiated chain reactions and selenium, or one of the metalloenzymes within which it is known to function, glutathione peroxidase.

Rainbow trout given purified diets lacking vitamin E have shown neither pathologies, nor reductions in growth rate in controlled laboratory experiments. Vitamin E requirement levels have usually been arrived at by indirect means such as erythrocyte fragility tests. Even the inclusion of moderately oxidized fish oil in practical type diets did not apparently lead to changes in growth rate nor in the appearance of gross pathologies except when the diet was totally deficient in vitamin E.

By contrast rainbow trout grown in outdoor tanks at variable temperatures (down to 6°C) developed severe muscle damage when the diets lacked vitamin E - irrespective of whether fat in the diet was oxidized or normal. This indicates that temperature is an important factor in the development of pathologies associated with vitamin E deficiency.

The size of the fish may also be an important factor. Deficiency diseases have been described in Atlantic salmon of less than 1 g initial weight given diets with a normal lipid component but lacking vitamin E.

Rainbow trout fed diets depleted of selenium did not show any gross differences (growth rate, feed conversion, pathologies) from control fish although tissue selenium levels and hepatic GSH peroxidase activity decreased. The possibility that the lack of effect of selenium deficiency may have been due to the induction of a non-selenium dependent GSH peroxidase, as has been described in mammals, was examined both in controlled feeding experiments and by examining characteristics of the purified enzyme. No selenium independent GSH peroxidase activity could be found.

Fatty Acid Metabolism

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The biosynthesis, oxidation and modification of fatty acids are all metabolic processes performed by fish to varying degrees.

Several fish species, most notably salmonids, have been shown to be capable of biosynthesizing fatty acids de novo from amino acids and glucose. The liver is the most active tissue site of de novo fatty acid biosynthesis whilst adipose tissue is very active in the uptake and esterification of preformed fatty acids into triacylglycerols, the main reserve lipid in fish. Such preformed fatty acids can arise directly from the diet and to a lesser extent from synthesis de novo in the liver.

Lipid is normally a major component of fish diets and the oxidation of dietary fatty acids is quantitatively important for the production of energy. In the marine food chain, many fish consume large amounts of lipid-rich zooplankton and thereby ingest large amounts of the long-chain monoene 22:1 (n-11) which appears to be specifically utilised by the fish for energy production. In mammals, the oxidation of long-chain monoenoic fatty acids such as 22:1 (n-11) proceeds not so much by the conventional beta-oxidation system in mitochondria but more by the cyanide-insensitive oxidation system present in the peroxisomes which proliferate in the livers of rodents fed diets rich in these fatty acids. In fish, however, peroxisomal beta-oxidation activity is low compared to that of the mitochondria and the latter organelles can oxidise 22:1 (n-11) at a rate similar to that of shorter fatty acids. The oxidation of fatty acids released from reserve lipid is critical for the provision of energy for the biosynthesis of ovarian components during the non-feeding period of sexual maturation in wild stocks of the lipid-rich capelin.

The ability of fish to modify dietary fatty acids, especially polyunsaturated fatty acids, by desaturation and/or elongation is dependent on species. In comparison with freshwater species, especially salmonids, carnivorous marine species such as turbot generally have low elongation and/or desaturation activities. In addition to their role as components of structural phospholipids in biomembranes, polyunsaturated fatty acids in fish are also converted in small amounts to prostaglandins which profoundly influence fish physiology. The relative importance of the (n-6) and (n-3) series of polyunsaturated fatty acids as precursors of prostaglandins in fish is currently under study.

The Effects on Fecundity and Egg Quality of Feeding Broodstock
Rainbow Trout (Salmo Gairdneri R) at Two Ration Levels

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Broodstock ration level is a much neglected area of fisheries research. At present commercial rainbow trout farmers practise a bewildering range of feeding regimes, varying from constant satiation to starvation for long periods. Very little is known about the consequences that these treatments may have on fecundity and egg quality. This study investigates the effects on fecundity and egg quality of feeding rainbow trout broodstock with two different ration levels.

A group of 128 two year old post-ovulated rainbow trout of the caribou strain were evenly distributed amongst 4 fibreglass square tanks (2m x 2m x 1m) supplied with 5 L⁻¹ min⁻¹ of constant 10±1°C borehole water. During the next twelve months the feed manufacturer's (Ewos-Baker) recommended ration of 0.7% of body weight per day was fed to two groups whilst the other two groups were fed half this ration. From each group 3 fish were sacrificed pre- and post-spawning for histological examination of levels of atresia and oocyte development. At spawning the fish weight, length, fecundity and ova diameter of the remaining fish were measured. Some batches of eggs from fish on both ration levels were monitored for survival levels to the swim-up fry stage. These eggs were also analysed for biochemical composition.

The fish on the full ration were significantly larger at the end of the experiment and produced significantly more and larger eggs. However, evidence is presented which suggests that the fish fed at the recommended level have a lower relative fecundity than the fish fed at the lower level. The effects of these two ration levels on egg quality and fecundity, their relationship to oocyte development and the implications of these findings to fish culture are discussed.

Effect of Nutritional Quality of Broodstock Diets on Egg Development

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Economically productive aquaculture is heavily dependent upon an adequate supply of seed, of fertile eggs and juvenile fish, with which to stock the ponds, enclosures, and other cultivation systems. One of the most important and fundamental approaches to the artificial seed production to satisfy the ever-growing demand of fish breeders is to ensure a year-round (rather than seasonal) supply of fertile eggs of high quality which give as high survival and growth rates as those occurring naturally. The former requirement - all year round production - has been achieved with some species by adjustment of the endocrine balance through variation in photoperiod regime.

Nutrition is known to have a considerable effect upon gonadal growth and fecundity although precise information on the nutritional requirements for gonadal maturation in broodstock is lacking.

Thus nutritional experiments on broodstock of both rainbow trout and red sea bream have been carried out to examine the relationship between quality of broodstock diets and that of eggs produced.

Adult or fingerling groups of rainbow trout were fed on one or other of a low protein-high energy diet, an EFA-deficient diet or a diet without trace metal supplement. The diets were given for either 3 months or 3 years. The results have demonstrated that a diet containing a lower protein content than that normally employed for juvenile trout but with a high energy level is as effective for both fingerlings and broodstock of rainbow trout as the more conventional diets of high protein content. By contrast trace metal and EFA supplements were shown to be indispensable for reproduction of rainbow trout.

A close relationship between nutritional quality of broodstock diets and reproduction was also observed in red sea bream. The quality of eggs produced by the broodstock receiving a low protein diet and diets deficient in available phosphorus or EFA was markedly lower than that of the control group. In addition cuttlefish meal was found to be superior to white fish meal as a protein source in the diet of red sea bream broodstock. The nutritional quality of diets given to broodstock shortly before spawning was also found to affect reproduction of red sea bream greatly, and in fish like red sea bream which can accept diets actively even during spawning, the quality of diets fed to broodstock during spawning becomes very important for their reproduction and egg quality. Frozen raw krill was found to be suitable as a food for red sea bream broodstock: one of the principal factors in the food value of raw krill may be its content of astaxanthin.

Salmon Flesh Pigmentation

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Although the occurrence of carotenoids in fish is widespread, it is only among the salmonids that carotenoids are found in the flesh. Most fish have pigmented skin, but only the species Salmo, Onchorynchus and Salvelinus have pigmented flesh.

In spite of an apparent biological need for carotenoids, the flesh pigmentation of Atlantic salmon under farming conditions is not without problems.

Of a wide range of carotenoids tested, only canthaxanthin, astaxanthin and astaxanthin esters are efficiently utilised from the diet. The efficiency of utilisation is dependent on season and level in the diet. Even so, there is considerable variation between fish. Methods of measuring colour will be outlined.

Astaxanthin and canthaxanthin are transported in the blood, mainly in the high density lipoprotein fraction, and are stored in the muscle in the sacroplasmic membranes.

Astaxanthin and its esters have been extracted from natural sources and fed to salmon. Free astaxanthin is utilised more efficiently than its esters, but no more efficiently than canthaxanthin. Salmon flesh pigmented this way with astaxanthin is very close in colour to that with canthaxanthin.

Astaxanthin can exist in any of three isomers. The levels of the three isomers found in wild fish is dependent on diet.

A biological role for flesh carotenoids is discussed.

Recent Advances in Vitamin Nutrition and Metabolism in Fish

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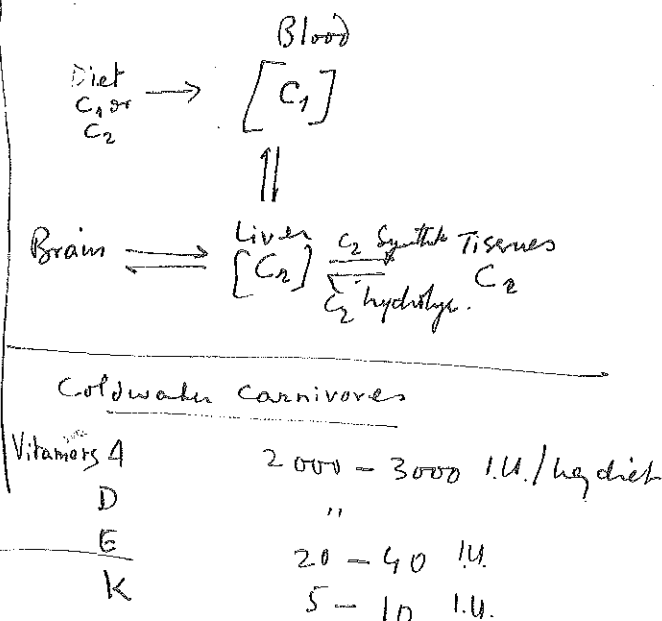
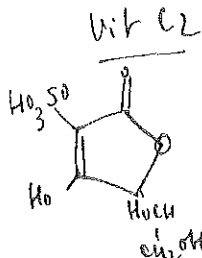
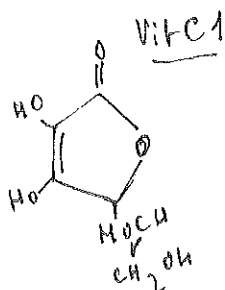
New knowledge has accumulated on the specific quantitative water-soluble and fat-soluble vitamin requirements of fish with respect to different fish species, fish size, and environment in which these are reared. The role of water-soluble vitamin intake and tissue titre on fish health and resistance to disease or other stressors has been investigated yielding interesting dividends for practical applications in fish husbandry. Dietary additions of various vitamins are dependant upon formulae and fish feed technology used, and upon species reared in various husbandry systems. Results of these advancements in knowledge have been summarized in two NAS/NRC Bulletins on Nutrient Requirements of Coldwater Fishes and Nutrient Requirements of Warmwater Fishes and Shellfishes.

Distribution, half-life, and turnover rates of ascorbate-2-sulfate and L-ascorbic acid in trout have disclosed three active body pools for vitamins C in fish. One pool is a rapidly exchanging pool equilibrated with dietary intake through digestion and absorption of ascorbate, one pool is a slowly exchanging pool mediated by the enzyme L-ascorbic acid-2-sulfohydrolase with feedback control over enzyme activity by L-ascorbic acid in circulation. The body pool total size is equivalent to 100 mg/kg and approximates that of man. Details of ascorbate intermediates and enzyme systems involved have been reported. Interrelationships between C intake and resistance to stressors and survival from some pathogens have been reported for catfish and trout. Recent evidence suggests C levels in tissues also influences maturation and larval survival. New information has accumulated on interrelationships between vitamins E, polyunsaturated fatty acids in the diet and Selenium role in maintenance of critical phospholipid membrane vitality in gill, liver, and gonadal tissues. Extension of recent research evidence into practical dietary formulations will be discussed.

Bill Woodward:

D. Amino acid oxidase - Riboflavin status.

J. Nutr., 84 - June issue. - Scurvy



* [Mineral Nutrition: Effects of Phosphorus in Trout and Salmon
* Feeds on Water Pollution

H. G. Ketola

Presented by D. KNOX, IMB

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An ecological study indicated that phosphorus (P) discharged in the effluents from a salmon hatchery in Michigan significantly hastened the eutrophication process in a small lake. The source of this P appeared to be the hatchery diet. Therefore an experiment (Salmonid, magazine 6(2):12, 1982) was conducted with rainbow trout fed the hatchery diet and various other diets. A new experimental diet (B) was formulated by computer to contain minimal excess or unavailable forms of P and was supplemented with defluorinated rock phosphate (DRP). DRP is only slightly soluble in water and may reduce water pollution. Diets were fed for 12 weeks.

Chemical analyses of P in feeds, carcasses, and sludge (faeces and solid wastes) showed significant differences between diets concerning the fate of dietary P. About 57 and 45 percent of the P in the hatchery and experimental diets, respectively, polluted the effluent waters. Calculations indicated that 1,000 kg of the hatchery diet generated 12.4 kg of effluent P, whereas the diet with DRP generated only 4.5 kg. Overall, diet B (containing DRP) supported growth at 93% and reduced pollution by 62-64% relative to that for the hatchery diet as previously used in Michigan. //

Another study was conducted to test a more economical diet (diet C) to which DRP from two commercial sources and other potential forms of P were added in an attempt to reduce effluent P pollution. Results showed that trout fed diet C with added DRP grew 94% as well as those fed the more costly experimental diet B and 84% as well as those fed the Oregon Moist Pellet (OMP), the diet then being used in Michigan. DRP in diet C reduced P pollution in effluents by about 33 to 49% relative to that for OMP. Both sources of DRP gave similar results. DRP reduced blood hematocrit readings by about 10% without any apparent effect on overall performance or health of the trout. Other forms of P tested, but not showing antipollutional properties, included feed grade dicalcium phosphate, phosphoric acid and sodium phosphate. Feeding diet C to trout in another study showed that steamed bone meal could replace DRP with comparable growth and antipollutional properties.

A large scale experiment was conducted on 300,000 coho salmon in the Platte River Hatchery in Michigan. The salmon fed the DRP diet grew 80% as well as those fed the standard OMP diet. Effluent phosphorus was reduced by about 40 to 50% by the DRP diet relative to the OMP diet. Further, it was shown that the level of dietary DRP could be reduced 10% or replaced by cattle bone meal with little effect on growth or pollution. Thus, the new diet C containing DRP or bone meal supported fair growth and markedly reduced P pollution of effluent waters in the fish hatchery.

The P level in diet is not given.

Annual discharge: 1500 kg P/yr.
Off. recommendation: 640 "

Standardization of the Nutrition of Fish in Aquatic Toxicological Testing

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E.L.S.
The nutrition of organisms used in aquatic toxicology has historically not been recognized as a problem, because the tests were short-term, in static systems, and the feeding of the animals was prohibited. Recent changes in test methodology (e.g. flow-through testing systems, long-term tests and use of larval organisms) have mandated that the animals be fed during the tests. Nutrition is especially important in the fish "early-life-stage" tests, in which both survival and growth of the larval test fish are measured. No real standards have been established for the nutrition of those organisms, however, in the way that some standards exist in mammalian toxicology. Aspects of the diet that require standardization are both nutritional composition and maximum level of contaminants. The literature indicates that commercially available diets for fish (as well as mammals) vary substantially in levels of both specific nutrients and specific contaminants. Other literature and our own studies have shown that different diets fed to fish can lead to significantly different results in toxicity testing.

Our attempts at standardization thus far have included a detailed study of nutritional variation in geographical strains of brine shrimp nauplii as part of the International Study on Artemia (ISA) and the designation of one large, homogeneous, well-characterized collection of Artemia cysts as Reference Artemia Cysts II for use as standard food by the U.S. EPA. However, natural sources of brine shrimp are not a good long-term solution to the problem because of inherent variability, decreasing availability and increasing cost. Our ISA colleagues in Belgium are presently studying production of cysts by brine shrimp in the laboratory under controlled conditions, and we are testing the nutritional quality of those cysts, in an effort to obtain standard brine shrimp nauplii.

Good-quality prepared diets for freshwater fish are available from several sources. Freshwater fish larval diets, while increasingly successful, are not universally acceptable, however. There has been little success in the development of prepared diets for marine fish larvae. Our present efforts center on the development of such diets and our recent research results will be discussed.

The Role of animal nutrition as an experimental variable has been overlooked for too long.

- * 1) test of the material-toxicity of a given compound*
- 2) Susceptibility of the organism under test.*

difference between San Francisco Bay & San Pablo Bay Artemia

Application of Nutritional Findings to the Formulation of Practical Diets

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Least cost linear programming (LP) provides a valuable aid in formulating practical diets and application of nutritional findings is most easily facilitated through this aid. The subject is discussed with regard to:

1. Requirements of LP systems: information is required on the cost and nutrient levels of competing raw materials and on constraints to be imposed on the levels of new materials offered for inclusion.
2. Outputs from LP systems: the raw materials included in the optimal diet are given as well as the cost of the diet and the analysis of nutrient levels. Also shown are those raw materials not included and the price they would need to be offered to merit inclusion; the effect of price changes upon the inclusion level of raw materials which were accepted and finally the effect of changing the specification of the limiting nutrients and raw materials upon the cost of the ration. The sophistication of such models means that a large data base on the nutrient requirements of fish can be effectively used.

Non-nutritional considerations are also discussed. These include ease of manufacture, availability of raw materials, pellet durability, marketing considerations and the palatability of the feed.

Producing a diet which gives the fish farmer the highest return on capital is the ultimate criterion upon which to judge the fitness of a diet. The economically optimum diet will vary according to the market value of fish and the non-food costs of production. Thus the relative importance of growth rate and feed conversion are discussed and the implication of these to nutritional studies are reviewed.

→ fish farmers can reconcile money-making and
Quality producing.

£ 34.

Poster No 1

The Effect of Digestible Energy on Nitrogen Balance in Rainbow Trout

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An experiment was conducted to study the influence of dietary energy sources on different parameters of nitrogen and energy balance in growing rainbow trout. Two experimental diets composed of fish meal (550g/kg), fish oil (100g/kg), vitamins and minerals (20g/kg each), binder (10g/kg) and starch (300g/kg) were used. The two diets differed from each other in the quality of dietary starch that was incorporated : crude (diet AC) or gelatinized (diet AG). A commercial diet was used as a control (C).

The experiment consisted of three phases : growth trials in a hatchery, study of postprandial plasma levels of ammonia, free amino acids and glucose, and postprandial patterns of ammonia nitrogen excretion. A marker (chromic oxide) was incorporated in the diets to estimate digestibility coefficients.

Trout fed the diet (AG) containing gelatinized starch fared better than the other groups, both in terms of growth and in terms of efficiency of feed utilization (FCR, PER and Energy retention). Both postprandial patterns and rates of ammonia excretion differed between the three groups of fish fed the different diets. The metabolic losses amounted to 44% of the nitrogen intake in the control group (C); these losses were lower in trout fed AG (29%) than in those fed crude starch diet (40%). Retention of nitrogen in the whole body was likewise affected by the dietary treatments, being better for AG-fed trout (38%) than for those fed either AC (34%) or the control diet (29%). Trout fed crude starch diet (AG) had a greater deposition of fat in the carcass than the other two groups of fish.

It is concluded that inclusion of highly digestible energy-yielding nutrients would improve overall nitrogen balance by its effect on the metabolizable energy available to fish; this is also accomplished without any severe adverse effect on the body composition of the trout.

Bob : high glu in blood $\rightarrow$ Insulin? $\rightarrow$ FAA?

Colin : high retention of fat - does it come from glucose - lipogenesis from glucose

Mu : I'd guess so, yes.

John Halver : It's always a case, when for ex - you have extruded diets - you get lipogenesis.

Evaluation of Supplemental Dietary Lipid Sources for Juvenile Chinook Salmon

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West Vancouver, B.C. V7V 1N6, Canada

Canola oil, pork lard and herring oil singly and in combination were assessed as supplemental sources of dietary lipid for juvenile chinook salmon held in running, aerated 10 - 12°C well water on a natural photoperiod for 62 days. Each of the six different types of supplemental lipid comprised about half of the lipid content (16% of dry matter) in an experimental dry diet (West Van 32). Some groups received Oregon moist pellets (OMP) which is presently the standard hatchery diet in British Columbia federal hatchery facilities. All groups were fed two to three times daily to satiation.

The type of supplemental dietary lipid did not significantly influence juvenile chinook salmon growth. All groups fed the test diets had significantly better food and protein utilization than those receiving OMP. Best food and protein utilization occurred in fish receiving West Van 32 supplemented with canola oil or an equal mixture of canola oil and herring oil. Mortality was negligible in all groups. Dietary treatment in freshwater did not influence the ability of chinook to grow and survive in seawater when all groups received OMP. Data will be presented on the composition of dietary and whole body lipids.

It is concluded that canola oil, pork lard and a blend of these lipid sources are excellent alternative types of supplemental lipid for juvenile chinook salmon. They are more available, often less expensive and less prone to oxidation than marine oil. An identical conclusion was reached when these supplemental dietary lipid sources were assessed in a previous study on juvenile coho salmon.

Poster No 3

The Effect of Handling Stress on the Nitrogen Budget of the Goldfish, *Carassius auratus* *

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Fishes are unavoidably subjected to a variety of stressors in both the farm and laboratory environments. Handling, in particular, is known to exert an acute, physical stress. The aim of the study was to determine the effects of a standardised netting stress on the major components of the nitrogen (N) budget of goldfish: ingested-N, N-retention, excreted faecal-N, and ammonia-N. Initially pelleted food was offered once daily until the first sign of feeding hesitation. Handling stress caused a significant decrease in ingested-N. In subsequent experiments the direct effects of stress on the other major components of the budget were investigated in fish which were fed 2% of wet body weight over 2 to 4 feeding sessions. Handling stress caused no significant change in N-retention. The digestibility of nitrogen, however, is significantly reduced ($p < 0.05$) from approximately 59% in unstressed fish to 47% in the stressed group. This is reflected in a significant ($p < 0.05$) increase in faecal-N output in the stressed fish. By contrast handling stress suppressed the excretion of ammonia-N ($p < 0.001$), but did not alter the daily profile of excretion. The observed deficit in the N budget is unlikely to be due entirely to the excretion of urea and other minor end products of N-metabolism. It is suggested that much of the deficit may be accounted for by the loss of mucus.

Nutritional Requirement of Dover Sole (*Solea vulgaris*)
1 - Effect of Varying Dietary Protein : Carbohydrate
Ratios at two Lipid Levels on Growth and Protein Utilization

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The dietary protein requirement of juvenile Dover sole was investigated using thirteen casein-gelatin semi-purified diets, containing different levels of protein at two lipid levels, over a 56 day feeding period at $18 \pm 1^{\circ}\text{C}$. Crude protein ranging from 24 to 77% dry diet were balanced with dextrin, and lipid (8 and 16%) were balanced with cellulose.

The best growth rate, protein efficiency ratio (P.E.R.) and protein utilization coefficient (P.U.C.) were obtained with 61:19 protein:carbohydrate diet containing 8% lipid. Better results were consistently obtained with 8% lipid as opposed to 16%. The optimum protein levels for growth at 8 and 16% lipid content were defined by the following equations:

$$y_8 = 28.34 + 2.29 x - 0.0199 x^2 \text{ and } y_{16} = - 60.38 + 4.86 x - 0.04717 x^2$$

they were equal to 57.4% and 58.3% respectively and their confidence limits are reported. The effects of protein:carbohydrate levels on feed conversion, P.E.R., P.U.C., liver somatic index and carcass and liver composition for the two lipid levels were determined and discussed in relation to other fish species.

Young Dover sole adapted well to high dietary protein levels (P.U.C. values reaching 53% for 61.1% Protein). However, this experiment did not allow separation of the effects of protein and carbohydrate. In fact, this species appeared to utilize carbohydrate efficiently as an energy source while their ability to utilize lipid seemed to be limited.

* CONACYT MEXICO

Poster No 5

Effect of Diet Composition and Feeding Procedures on in vitro Protein *
Synthesis in White Trunk Muscle of Atlantic Cod (Gadus morhua)

Einar Lied

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Four feeding experiments were conducted. In experiment I cod were fed diets containing protein energy to total energy levels (PE/TE) of 30, 45 and 60% for 25 days. In experiment II cod were fed five different diets, in which lysine accounted for 3, 4, 5, 7 and 10%, respectively, of the protein, for 8 days. In experiment III cod were fed ad libitum and ration sizes of 25, 50 and 75% of the ad lib.-fed fish for 10 weeks. Finally, in experiment IV cod were fed ad libitum at intervals of twice and once a day, and every second and fourth day for 10 weeks.

Ribosomes were isolated from the white muscle tissue, the capacity for protein synthesis in vitro determined and related to muscle tissue wet weight, ribosomal RNA and DNA. The ratio RNA/DNA was calculated. The ribosomal capacity for protein synthesis per gram wet weight and per mg DNA, and the tissue contents of ribosomal RNA and ratio RNA/DNA were reduced in muscle tissue from fish fed 30% PE/TE as compared to the fish fed 45 and 60% PE/TE diets. Likewise, the protein synthesising capacity was reduced in muscle tissue from fish fed lysine levels lower than 5% of dietary protein. Maximal protein synthesising activity was found in cod at libitum. A reduction of ration size led to a reduction of the protein synthesising activity both per g wet weight and per mg DNA, and a reduction of the muscle content of ribosomal RNA.

The capacity for protein synthesis was found to be reduced in muscle tissue from fish fed twice a day as compared with fish fed once a day, every second and every fourth day.

Measurements of the capacity for protein synthesis in vitro in white skeletal trunk muscle tissue is a sensitive and significant indicator of nutritional condition in cod, measuring the total effectiveness of a feed or feeding condition to support growth-related intracellular functions.

Protein Digestion in Rainbow Trout in Saline and Fresh Water

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Rainbow trout of an individual weight of 300-600 g maintained at 13 - 14°C were fed casein based dry diet (43.9% protein).

After feeding, fish were kept in fresh or artificial sea water (440 m Eq. 1^{-1} Na^{+} , 520 m Eq. 1^{-1} , Cl^{-}) and sacrificed after 10 or 20 hours. Digestive tract was separated into five parts : stomach, pyloric caeca region, middle intestine and two equal lengths of posterior intestine. The contents of these parts were analysed for free, peptide and total amino acids, protein and ions : Na^{+} , K^{+} and Cl^{-} . Apparent absorbability of protein and amino acids was calculated on the basis of Cr_2O_3 content in the diet and digestive tract content.

Although water content in the stomach of fish kept after a meal in fresh or saline water was not different, ion concentration was several times higher in fish kept in saline (Na^{+} 336.5; K^{+} 35.4; Cl^{-} 577) (all as expressed as m Eq. 1^{-1}) in comparison to those in fresh water (Na^{+} 12.7, K^{+} 14.0; Cl^{-} 251.6). In the intestine, ion concentration was more or less equal in both lots of fish. Free amino acids level was enhanced in sea-water fish (middle intestine, 20 hr after a meal 27.8 mM) in comparison to fish kept in fresh water (21.8 mM). The principal free amino acids were glycine (up to 4.14 mM) and glutamate (up to 3.95 mM). Protein apparent absorbability was enhanced in stomach of fish kept in sea water, 9.2 and 23.7%, 10 and 20 hr after a meal in comparison to fresh-water fish. Apparent absorbability measured at the level of posterior intestine did not differ in two lots of fish.

• Pepsin activity ?
was it measured

Utilization of Energy and Protein by Carp (Cyprinus carpio L.)

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In two experiments (I and II) with two growth periods each, carp (Cyprinus carpio L.) were given a total of 12 (2 x 6) different feed rations at a water temperature of 24°C and over a mean weight range of 170 g to 1150 g (I) or to 890 g (II). Experimental schedule:

	Digestible Energy (MJ/kg DM)	Crude Protein (g/kg DM)		
Exp. I	18.3	413	464	514
	20.1	412	465	513
Exp. II	16.9	200	299	411
	18.3	198	302	415

The daily amount of feed offered was about 2% of the live weight. The protein and fat gains as well as the energy retention were established by comparative carcass analysis (2 x 6 carps per treatment).

The main daily protein gain varied between 0.7 and 1.9 g per carp depending on the treatment. A significant increase of the protein gain was obtained by increasing the crude protein in the feed from 199 to 413 g, but a further increase to 514 g had no effect. The increase in the energy supply resulted in a slightly improved protein retention but only in the lower range (16.9 - 18.3). The protein utilization (PPV and utilization of the digestible protein) decreased with increasing crude protein supply, whereas the energy supply did not have such a clear influence on protein utilization.

In experiment I, increasing energy concentration of the feed from 18.3 to 20.1 MJ DE resulted in an increase in mean daily energy retention of 78 to 98 kJ/carp. Likewise, in experiment II, increasing energy concentration from 16.9 to 18.3 MJ DE resulted in mean daily energy retention of 46 and 69 kJ/carp. Protein concentration in the feed had no effect on energy retention. This proportionate increase of the energy content consists of differing fat and protein gains. The total utilization of the gross energy varied between 30 and 50%, that of the digestible energy between 36 and 54%. Significant differences ($P < 0.05$) were seen only at the lower range of energy and protein concentration. Taking into consideration an energetic maintenance requirement of 42 kJ of metabolizable energy/kg^{0.75}, a partial utilization of the metabolizable energy of 0.46 - 0.69 for growth (k_g) was calculated depending on the treatment. On an average of all groups there was a k_g -value of 0.60. The energy expenditure per g protein gain was 53.9 kJ, and per g fat gain it was 41.7 kJ ME. Thus the partial utilization of the metabolisable energy for protein (k_p) and for fat (k_f), respectively, was 0.46 and 0.89.

J.H: Do you infer from these that 1 carp require 40% Prot?
Reply: Depends on the P/E ratio. When it's high, Prot used is high & when P/E ratio ↓, need ↓.

The Energy-Protein Requirement of Chanos chanos Fingerlings

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Chanos chanos fingerlings weighing 0.5 to 8.0 g were fed semi-purified dry diets consisting of casein and gelatin (4:1) corn oil and cod liver oil (1:1), dextrin, vitamin and mineral mixes, celufil and carboxymethyl cellulose. A 3x3 factorial in an incomplete block design was followed. The treatments consisted of 27 combinations for three levels of protein (15, 30 and 45%), fat (0, 6 and 12%) and carbohydrates (10, 20 and 30%) with two replicates each in a different room.

Fish were acclimatized to laboratory conditions for a week and anaesthetized with 2-phenoxy ethanol prior to stocking. Energy levels were calculated by using energy values of 4 kcal/g protein 9 kcal/g fat and 4 kcal/g carbohydrate.

Twenty milkfish or a total of 40 per treatment were reared for 8 weeks in 40 liters of seawater in each rectangular fiberglass tank, 61 cm long, 35 cm wide, and 24 cm deep, in a flow through system. Seawater was pumped through a 2 micron filter. Temperature and salinity ranged from 26 to 31°C and 30 to 32 ppt., respectively. Feeding rate of dry pellets was 10% of total biomass.

Results suggest that protein level required by the fingerlings is around 30 - 40% depending on the size of the fingerlings, fat 10% and carbohydrate 25%. Weight gain did not improve when energy levels were beyond 3500 kcal/kg diet. One hundred and sixty kcal from protein, 100 from CHO and 90 from fat, a ratio of 190 kcal from fat and CHO to 40 g protein or 211 mg protein/kcal of fat and CHO is recommended. Energy values are tentative until metabolizable and digestible energies have been worked out.

Apparently not one of the diets could be singled out as the diet that would provide for "good" survival over time or one that would stabilize mortality. However, of the 27 treatments, 5 diet combinations gave mortality rates of less than 50%. The diet combinations are: 15-0-30-1800, 30-12-10-2680, 45-0-10-2220, 45-0-30-2800 and 45-12-20-3680 as protein-fat-carbohydrate-kcal/kg levels, respectively. Surface response analyses also showed that an optimal diet without fat might be possible.

On the basis of response surface analyses a summary of possible optimal combinations of diets are:

Response variable	% Protein	% Fat	% CHO	kcal/kg diet
Survival rate	30	12	10	2540
Weight gain	30	6	20	2680
Protein deposition	40	6-10	20-30	2960-3740
Fat deposition	40	6	10-30	2560-3360
Ash deposition	40	6	20-30	2460-3740

Poster No 9

Properties of an Elastase-like Enzyme in Dover Sole, Solea solea L.

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During studies on juvenile Dover Sole proteases, using casein as substrate, three areas of optimal activity were noted. An acidic (pepsin-like protease) at pH 1.7, a neutral region at about pH 7 - 8 (corresponding to trypsin-like and chymotrypsin-like activities) and a third and very strong protease activity at pH 9.5 - 10.5. Initial experiments using elastin-orcein as substrate indicated that this latter activity may be the result of an elastase-like enzyme. In an attempt to study this observation further, a number of different substrates were examined. In addition to using casein and elastin-orcein as substrates, elastin, elastin Congo red, N-succinyl-L-alanyl-L-alanyl L-alanine p-nitroanilide and N-benzoxycarbonyl L-alanine p-nitrophenyl ester were examined. There are difficulties with all of these substrates. The use of elastin itself is a fairly insensitive method when used in conjunction with absorbance readings at 280 nm or even with the Lowry protein assay and the use of the ninhydrin method may in fact be measuring the action of exoproteases acting on the elastin. With dyed elastins, problems arise because different batches of the substrates may have different degrees of dye substitution. Other substrates (N-succinyl-L-alanyl-L-alanine p-nitroanilide and N-benzoxycarbonyl L-alanine p-nitrophenyl ester have relatively low solubility in water and in addition this latter substrate has the disadvantage of being unstable at alkaline pH values. A further complication arises from the fact that, with crude enzyme preparations, each of these substrates give a different value for the optimum pH.

In experiments to examine the alkaline protease activity further, a series of inhibitor studies have been carried out. A number of these (iodoacetate, N-ethylmaleimide N-bromosuccinimide, p-chloromercuribenzoate, EDTA, TPCK and TLCK) all gave a fairly uniform degree of inhibition of the neutral and alkaline proteases. However, using phenylmethylsulphonyl fluoride (PMSF) at a concentration of 0.55 mM and casein as substrate, the neutral proteases were readily inhibited (only 6 - 8% of the original activity remained) whereas the alkaline protease still showed 65% of its original activity. Further studies are now in progress to separate and purify the alkaline endoprotease activity and to examine its specificity.

Sargent: Use of Elastins?
— for highly resistant proteins.

The Development of Digestive Proteases in Dover Sole Solea solea L.

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Extracts of the digestive tract of Dover Sole have been examined for endo- and exo-proteases. In initial studies using adult fish and casein as substrate, three pH regions of activity were noted. In each of these regions extracts of digestive tract homogenates caused a rapid reduction in the viscosity of gelatin indicating the presence of random or endo-acting enzymes. These major activities were at pH 1.7 (pepsin-like), pH 7 - 8 (trypsin and chymotrypsin-like) and at pH 9.5 - 10.5 (probably mainly elastase-like). The strongest activity was present in the alkaline region and the weakest at the acidic pH as measured by both the casein-hydrolysis and the gelatin-viscosity assays. Using artificial substrates, homogenates of adult Dover sole intestine were also shown to contain exoprotease activities corresponding to leucine amino peptidase, carboxypeptidase A and carboxypeptidase B. Further experiments indicated that these activities were located mainly in the lower regions of the intestine.

The object of the present work was to examine the level of these enzymes at different stages in the development of the fish and thus to gain information on the digestive capabilities and nutritional requirements. Since it becomes more difficult to assay digestive enzymes in younger fish and in fish larvae, comparative experiments have been carried out on fish of different ages. In initial studies, intestinal enzymes have been assayed in 50 day old, 80 day old, 200 day old and in adult fish. Pepsin-like activity was absent in the 50 and 80 day old fish but at 200 days a small amount of pepsin-like enzyme could be detected. It would therefore appear that, in common with certain other species, pepsin only appears in the gut as the fish matures. In contrast to this, the alkaline and neutral protease levels are very high in 50 day old fish, and relative to gut weights and extractable protein content, the alkaline and neutral proteases diminish gradually as the fish ages. At no time however is the pepsin-like activity greater than the alkaline protease.

Poster No 11

Digestibility Measurement in Feedstuffs for Atlantic Salmon in
Freshwater and Seawater

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Experiments were conducted with Atlantic salmon, Salmo salar, in freshwater and seawater to determine the apparent digestibility coefficients and digestible energy values of feedstuffs (herring meal, soybean meal, canola meal, corn gluten meal, wheat gluten, brewers yeast, poultry feather meal, poultry byproduct meal, blood meal, dehydrated whey and wheat middlings) widely available in Canada. The digestibility tank system developed at University of Guelph by C. Y. Cho, which uses a settling column to separate feces from effluent water, was employed with some modifications. All measurements were made with post yearling Atlantic salmon (50 g) in freshwater and on the same group of fish after seawater adaptation at 15°C. Chromic sesquioxide (1% of diet) was used as an inert digestion indicator.

Digestible energy (D.E.) values of most feed ingredients were significantly higher in seawater than freshwater. D.E. approached the gross energy values for wheat gluten meal, herring meal and dehydrated whey indicating a high degree of digestion and absorption of nutrients. However, the feedstuffs which contained substantial levels of carbohydrate such as wheat middlings, canola meal and corn gluten meal were approximately half their gross energy values, confirming that starch is not effectively utilized by Atlantic salmon. D.E. content of most ingredients, with the exception of corn gluten meal and canola meal, showed close agreement with the digestible energy values reported for rainbow trout by the Guelph system. Canola meal was more efficiently utilized by salmon than trout whereas the reverse was true for corn gluten meal.

Crude protein and crude fat in most ingredients were well digested. However, the crude protein in blood meal was poorly digested, and hydrolyzed feather meal protein was moderately well digested. In general, the apparent protein digestibility coefficients of all feedstuffs was approximately 3 - 5% higher in seawater than freshwater. Herring meal containing a higher level of ash showed lower digestibility of protein in seawater. Significance of mineral excretion in the feces of Atlantic salmon in seawater and freshwater environment is also discussed.

Digestibility in Rainbow Trout: Influence of Low Temperature,
Ration Size, Type of Food and Type of Feeding

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Using the automatic feces collector (Choubert, 1979), we measured the influence of temperature, ration size, type of food and type of feeding on the apparent digestibility coefficient (ADC).

Rainbow trout (Salmo gairdneri) of 90 g (mean weight) were used. 15 trout were kept in each lot. The study has been conducted in a government hatchery during autumn 1983. During this period, the temperature varied from 10°C to 5.5°C. In each trial (lasting 9 days), the temperature was relatively stable. During that period, duplicate lots received the following treatments:

<u>Lot #</u>	<u>Treatment</u>	
	<u>Type of Feeding</u>	<u>Ration Size</u>
1-2	manual	1.35% of live weight
3-4	manual	0.74% of live weight
5-6	demand feeder	-

From one trial to another, we used, alternatively, two types of food: a low protein high lipid diet (Lip-12 diet; 40.5% protein, 12.5% lipid) and a high protein low lipid diet (reference diet; 67% protein, 5% lipid).

At the end of the experiment, we measured the ADC of dry matter, protein (N x 6.25), lipid and energy by the indirect method (chromium oxide marker).

In our study, the temperature did not significantly influence the ADC of nutrients. However, the ADC were lower than those obtained by other workers at higher temperatures.

We also found that the ADC of dry matter was significantly higher with the Lip-12 diet. On the other hand, the ADC of lipid and energy were significantly higher with the reference diet.

With the Lip-12 diet, the ADC of dry matter, protein (N x 6.25) and energy were significantly lower when fish received 1.35% of their live weight as compared to 0.74% of the live weight.

With the reference diet, fishes did not ingest as much food as those receiving the Lip-12 diet. This might explain why we obtained less significant differences. For dry matter, lipid and energy, we had significant differences when the low ration (0.6% live weight) at 5.2°C was compared to the high ration (0.9% live weight) at 8.5°C and 5.2°C.

At no time did we observe interactions between the different conditions used in our treatment on the ADC of nutrients.

Poster No 12 (cont'd)

It was found that the slightly higher ADC obtained when fish received food from the demand feeder was not due to the type of feeding. It was better related to the amount of daily ingested food. Thus, fish fed with the demand feeder ingested approximately 0.70% of their live weight per day. Their ADC was comparable to those obtained with the low ration in other lots.

With the reference diet, there was no significant difference in the ADC of nutrients, no matter the type of feeding used or the temperature.

The results are further discussed in the poster.

Effects of Artificial Diets on the Digestive Processes of Fish Larvae

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The short and less differentiated intestine of fish larvae is primarily adapted to accept animal diets which are easily digestible and of high nutritive value. In several species like the roach (Rutilus rutilus) this may lead to a low growth rate, even to total mortality when the larvae are fed exclusively on artificial diets. In roach larvae artificial diets cause some striking changes in digestive processes. In general specific proteolytic activities in the gut content of naturally fed larvae are considerably lower than those of adult fish. Moreover, in roach larvae up to 25%, in white fish larvae up to 70%, of the proteolytic activity measured in the intestinal contents may originate from the animal diet (zooplankton). When roach larvae are fed artificial diets the production of proteolytic enzymes increases rapidly up to the level of adult fish. Although proteolytic enzymes of fish are very stable against autolysis, in adults they are not voided with the feces but reabsorbed in the second half of the intestine. Thus in adult roach only 2% of the secreted enzymes are lost by the feces. As demonstrated earlier, efficiency of reabsorption correlates positively with the length of the intestine. Thus the larval intestine is too short to allow efficient enzyme recovery. Tryptic activity in the contents of the second half of the intestine comprises 46% of total gut activity in larvae but only 12% in adults. Consequently, high enzyme production combined with inefficient enzyme recovery results in increased losses of body proteins.

Moreover, since intestinal length and gut passage time correlates positively, the short larval intestine suggests reduced digestibility, particularly of dehydrated, artificial diets. "Proteolytic action" (proteolytic activity of intestinal contents per g fish multiplied by the gut passage time) is three times higher in the adults than in the larvae of roach. In consequence, increased losses of body proteins and inefficient protein utilization may lead to a negative protein balance in several artificially fed fish larvae.

An in vitro Method for Measuring Protein Digestibility of Fish Feed Components

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The decreasing production of fishmeal in recent years has led to a search for alternative protein sources as a replacement for fishmeal in compounded fish feed. A long term feeding trial is the most dependable method of measuring the value of a fish feed but is often too slow and expensive. In vitro methods of evaluating protein digestibility are important not only because they are less expensive and rapid, but also because they allow close observation of the dynamics of the breakdown of proteins.

In the in vitro digestion test to be presented, the following conditions of the digestive tract of the fish were simulated: physiological temperature optima, pH-value, dry contents of digestives in stomach and gut, enzymatic activities and passage time through stomach and gut.

Small amounts of raw materials are sufficient to follow the dynamics of protein digestion, three approaches of increasing resolution are being used.

Firstly the intensity of protein-splitting at each moment of the digestive process was studied by an automatic titration method. Only when investigating pure protein extracts is the registration exact (no interference by fatty acids). Secondly, the break-down products were separated by HPLC (high-performance size-exclusion liquid chromatography). The planimetric interpretation of the plotted results obtained above allows a better resolution of the time sequence of proteolyses.

Thirdly, soluble fractions of different molecular weight as well as undigested solids are subjected to amino acid analysis.

In order to describe the inherent potential of this method, the in vitro digestion of "PRUTEEN", a single cell protein (SCP) of a methanophilic bacterium, is presented under the conditions of the alimentary tract of rainbow trout.

CBC: very interesting.

Att: for to go further - see 1 effect / diff. proteins
& 1 kinds / peptides

Influence of Varying Dietary Protein: Lipid Ratios and Water Temperature on Growth and Body Composition of Juvenile Coho Salmon (Oncorhynchus kisutch)

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This study was conducted employing a three-factor composite design to determine the optimal balance of dietary protein and lipid for growth and food (protein) utilization of underyearling coho salmon (Oncorhynchus kisutch) at temperatures of 7 to 19°C. Nine experimental dry diets each with one of five levels of protein (28.5, 32, 42, 52 or 55.5% of dry matter) and lipid (6.25, 8, 13, 18 or 19.75% of dry matter) were each fed to groups of 100 coho dry reared in 197-L tanks for 9 weeks. The estimated dietary metabolizable energy contents varied between 3.7 and 4.1 kcal/g. All fish were fed their respective test diets three times daily to satiation during the study. Response surface analysis was employed to estimate optima for growth, food and protein utilization and cost per kg gain.

Personal
Growth rate was maximal at 17% dietary lipid content and 16.5°C and rose in direct relation to dietary protein level. Maximum food utilization occurred when dietary protein and lipid contents were respectively 52 and 16.5% and water temperature was 15°C. Protein utilization as measured by protein efficiency ratio and apparent net protein utilization was inversely related to dietary protein content and directly related to lipid content and generally to water temperature. Maximal protein gain per kcal of metabolizable energy intake occurred when dietary levels of protein and lipid were 54 and 18.6%, respectively and water temperature was 14.7°C. Cost per kg dry body weight gain was influenced primarily by dietary lipid level and rearing temperature and was least when lipid level was 16.6% and temperature was 16.4°C. Predictable patterns for body protein and lipid content were found in relation to the three factors investigated.

It is concluded that practical diets for juvenile coho salmon should contain 52 to 54% protein and 16 to 18% lipid and rearing temperatures should range between 15 and 16°C to maximize growth, food utilization and protein gain per kcal of metabolizable energy consumed at least cost.

*See end of poster #18.
for References*

Virginomycin as a Growth Promotor in Mirror Carp (Cyprinus carpio L.)

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Antibiotics as growth promotors in fish have not yet been demonstrated. A trial was conducted to ascertain the use of virginomycin, which is a composite antibiotic produced by Streptomyces virginiae, in mirror carp (Cyprinus carpio L.). Twenty eight fish weighing an average of 17.4 g were reared in a continuous circulating system of four fiberglass tanks (15 gallon capacity) at $22 \pm 2^{\circ}\text{C}$ for ten weeks. Virginomycin was incorporated at concentrations of 0, 40 ppm, 80 ppm and 100 ppm in a diet that contained 38% protein. The diet was offered three times a day at 3% of body weight of fish for eight weeks and 2.5% for the last two weeks.

One way analysis of variance showed a significant gain in weight ($P < 0.05$) of the treated fish 52% (80 ppm), 49.5% (40 ppm) over that of the control. Food conversion ratios were 1.22 (100 ppm) 1.22 (80 ppm), 1.16 (40 ppm) and 1.61 for the control. At the termination of the experiment some fish were killed, stripped of faeces for digestibility study, then dried at 105°C for 48 hours for proximate analysis (Moisture, protein and fat). Liver, kidney and intestine were removed, weighed and somatic indices determined. Before weighing the intestine it was emptied of contents and parts were fixed in Bouin's fluid for histological investigation of changes in the wall of the intestine after antibiotic treatment. The remaining fish were used for a bacteriological study to determine whether or not virginomycin affected the microbial flora of the intestinal tract of fish.

The details of these investigations will be discussed in the poster.

Poster No 17

The Effect of Feeding Level and Feeding Frequency on Growth
and Feed Conversion Efficiency in Fingerlings of Cyprinus
carpio, Labeo rohita and Cirrhinus mrigala

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Feeding experiment conducted with finely sieved rice bran by weights equivalent to 2 to 10% of the initial body weight with Cyprinus carpio, Labeo rohita and Cirrhinus mrigala fingerlings showed that in Cyprinus, the growth, feed conversion rate and conversion efficiency were best with 6% body weight ration. In Labeo and Cirrhinus the best growth, feed conversion and conversion efficiency were observed at 5% body weight ration. In all the cases, both higher and lower levels showed minimum growth response in terms of all growth parameters. The feeding levels from 2 to 6% showed their suitability in terms of improved water quality than 7 to 10% body weight ration. Feed consumption rate was found to be negatively correlated with the increase in ration level. It is concluded that higher rates of feeding above the levels of 6% body weight in C. carpio, and 5% in L. rohita and C. mrigala are not useful.

Effect of Stabilized Krillmeal in the Diet for Pigmentation and Growth of
Atlantic Salmon (Salmo salar)

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and

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INTRODUCTION

As a qualification for Norwegian farmed rainbow trout and Atlantic salmon a fresh red-coloured fillet is a necessity to meet the market demand for the normal sized fish of about two kilos and above. In wet and moist salmon feed shrimp waste or synthetic canthaxanthin used as pigmentation sources. Canthaxanthin dominates in dry feed. In this experiment krill meal has been tested both as pigment and protein source in dry pelleted feed for Atlantic salmon.

METHODS

Krill meal was produced from raw frozen krill with addition of 400 ppm antioxidant during the drying process. The content of astaxanthin in the krill meal was 92 mg/kg dried product, and the crude protein content was 600 g/kg krill meal. A normal dietary level of pigment in dry feed for salmon is about 50 mg/kg. To reach this level, 270 g krill meal per kg diet was added. With this high inclusion, the krill meal supplied about 1/3 of the total dietary protein, which was analysed to be 482 g/kg dry feed. One diet without pigment and one diet with 50 mg canthaxanthin was used as references and fish meal was used as the sole dietary protein. All the diets were isonitrogenous, and with inclusion of krill meal in the third group the fish meal portion was similarly reduced.

The experiment was conducted on Atlantic salmon with an average body weight of 560 g at start. Ninety salmon were allocated to three tanks per diet. The duration of the feeding experiment was 16 weeks, with weight and length measurements at 4-weekly intervals. For each registration period, six fish per diet were randomized, taken out for proximate analyses and determination of fillet pigmentation.

RESULTS

Both astaxanthin and canthaxanthin were analysed in feed and fish fillet. During the 16-week period both pigmented diets lost 25% of the original pigment content (at start canthaxanthin 47 mg/kg, and astaxanthin 38 mg/kg). The fish fillet produced on the krill diet contained 0.94 mg astaxanthin/kg, which corresponds to F.3 Pink minimum according to the colour scale by the Norwegian Fish Farmers Association. Use of canthaxanthin resulted in higher fillet pigmentation, 2.1 mg pigment/kg fillet. This corresponds to F.2 minimum after the above mentioned scale. The pigment accumulation in the fillet constituted 3.2% of the pigment intake in the krill group, and 5.2% for the canthaxanthin group.

The average weight gains in the two reference groups based on fish meal were equal (410 g). The krill meal group gained 343 g on average, which was significantly lower than the reference groups.

CONCLUSIONS

- * Higher fillet pigmentation was obtained on pelletized dry feed for Atlantic salmon using synthetic canthaxanthin compared with natural astaxanthin from stabilized krill meal.
- * The pigment accumulation in the fish fillet was 3.2% and 5.2% of the intake from the astaxanthin in krill and synthetic canthaxanthin respectively.
- * When krill meal replaced 1/3 of the protein from fish meal in the diet, the growth was significantly depressed and lower feed efficiency ratios were registered.

Schulte & McKinnell 1984 = Resp. surface analysis.
Can J Fish Sci.

Alderdice & Thomson, 74.

Fish Res Bd Tech. Rept 493: 70 p.

Commercial Production and Application of a Naturally-Occurring
Crustacean Astaxanthin Pigment in Fish Diets

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Louisiana crawfish (*Procambarus clarkii*) heat-processed waste has been identified as a unique and abundant source of natural, biologically-active, astaxanthin based on > 30 million lbs waste/year, with noteworthy pigment concentration of 153 µg/g. An efficient commercial carotenoid extraction process, using a vegetable or fish oil for recovery of the oil-soluble pigment, has been developed. An annual production of 250 metric tons of astaxanthin fortified oil is anticipated with minimal concentration of 650 ppm. Improvement of pigment extraction efficiency by 40% has involved enzymatic and acid ensilage treatment as well as comparison of different vegetable and fish oils. Application of the crustacean pigment has been demonstrated in performance trials with a variety of aquatic species including rainbow trout, coho salmon, American lobster, sea bream and tropical fish. In feeding trials with rainbow trout, as high as 9 µg astaxanthin/g tissue was found in fish raised on a diet containing 45 mg astaxanthin/kg feed supplemented with the pigmented oil. The significant salmon-red coloration of the fish flesh was shown to be stable under normal cooking conditions. Sensory studies have confirmed the intrinsic value of the pigment application.

Apart from its overall value on sensory qualities, such as color, astaxanthin has been postulated to have a beneficial effect on the endocrinology of cultivated fish, including gonadal development, egg maturation and viability, and ultimate larval survival and growth. Other physiological functions, similar to those of α-tocopherol and provitamin A in protecting sensitive tissues, also have been suggested for astaxanthin during fish cultivation and embryonic development. Other ancillary components, including sterols and crustacean lipid fractions containing essential fatty acids for fish nutrition, have been shown in analysis of the pigmented oil. Aspects of the pigment extraction process and features of the crawfish astaxanthin, including stability studies are demonstrated.

9000 µg/kg.

Oxidation of lipids - enzymes hydrolyse & oxidise
Carotenoids

Pigment Uptake and Deposition in 2 Size Classes of
Arctic Charr, Salvelinus alpinus L. (Salmonidae)

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Two successive year classes of Arctic charr, originating from a single, wild, anadromous population in northern Norway, were reared from eggs in the laboratory. When the 2+ fish had achieved an average weight of 125 g the 1+ fish weighed 17 g. These 2 size classes, which hitherto had been fed unpigmented food, were then weaned on to a diet containing the pigment canthaxanthin at a concentration of 40 mg/kg food. During the feeding experiment the fish were held in freshwater at a temperature of 5.5°C. They were fed at appetite level and sampled initially, after 3 weeks and after 6 weeks on the pigmented diet, by which time the mean individual weights in the 2 groups were 25 g and 200 g. The muscle flesh of the fish in both groups had become pigmented. In the larger (2+) fish the pigmentation extended throughout the entire muscle, while in the small (1+) fish only the muscle posterior to the adipose fin had taken up pigment. The distribution of carotenoid pigment in the skin and in the muscle, as well as the fatty acid composition in the tail muscle and anteriorly in the 1+ fish were analysed. The results of these analyses are discussed in relation to pigmentation and lipid metabolism in charr.

Smith

Pantothenic Acid Supplementation of Practical Diets
for Young Rainbow Trout (Salmo gairdneri) ✓

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Two experiments were conducted at 15°C with diets (40% crude protein, 15% ether extract) formulated from fish meal (35%), soybean meal (20%) and wheat middlings (32%). The diets contained a basal level of 16 mg pantothenic acid (PA) per kg determined by microbiological assay with L. plantarum. In both experiments the control diet contained 40 mg/kg of supplemental PA supplied as D-calcium pantothenate. In experiment #1 fry initially weighing 1.9 g each exhibited, over 12 weeks, reduced live weight gain (10.6 vs. 27.6 g/fish) and inefficient utilization of diet for growth (1.5 vs. 1.1 g feed/g gain) when fed a PA-unsupplemented diet. Similar results were obtained in experiment #2 in a 5-week period with fish initially weighing 1.6 g each (1.7 vs. 4.9 g gain/fish; 3.0 vs. 1.3 g feed/g gain). Gross deficiency signs among the fry fed PA-unsupplemented diets included (both experiments) lethargy, reduced feeding response, emaciation and mortality. Lamellar hyperplasia affecting about 50% of the gill surface was evident histologically in these fish. Comparison with other fry fed (at the same time) semipurified diets containing graded levels of PA suggests that the naturally-occurring PA in our practical diets was about 50% biologically available. Finally, in experiment #2, the fish initially fed the PA-supplemented diet were fed the unsupplemented diet from the 5-week point (6.5 g/fish) until 20 weeks (63.2 g/fish). During the last 4 weeks of the experiment these fish exhibited reduced feed intake, lethargy, loss of equilibrium and grossly distended swim bladders. Gill lamellar hyperplasia did not occur. During the same period (5 - 20 weeks) the fish initially fed the PA-unsupplemented diet were given the supplemented diet and exhibited recovery from deficiency signs (final liveweight 74.2 g/fish). The results indicate that PA supplements are important for practical trout diets and demonstrate that PA deficiency signs are age-dependent in rainbow trout. (Supported by OMNR and OMAF research grants.)

✓ Vitamin E Uptake, Transport and Distribution in Rainbow Trout (Salmo gairdneri)

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Vitamin E uptake, transport and distribution in rainbow trout were studied using two radiolabeled Vitamin E analogues. The appearance of radioactivity after oral administration of 3 μ Ci D- α -[5-methyl- 3 H]tocopherol and 10 μ Ci DL- α -[3',4'- 14 C]tocopheryl acetate in plasma, liver, kidney, spleen and heart showed an exponential increase up to 32 hours, followed by plateau or slight decline from 32 to 64 hours. Radioactivity in the skeletal muscle, however, showed an exponential increase up to 8 hours but followed by a slower linear increase up to 64 hours. Results of this study showed that uptake of D- α -tocopherol was 6 to 18 times greater than DL- α -tocopheryl acetate in the first 4 hours and 2 to 3 times greater between 8 and 64 hours after the oral administration. The absorbed Vitamin E was found to be transported as free α -tocopherol by the plasma low density lipoprotein. Distribution of the absorbed Vitamin E in decreasing order are: liver > kidney > plasma > spleen > heart > skeletal muscle.

Tissue Distribution of Ascorbate in Milkfish

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The tissue distribution of L-ascorbic acid (C_1) and L-ascorbic acid-2-sulfate (C_2) in pond-grown milkfish was investigated. The ascorbate concentration was determined spectrophotometrically by ozazone formation using a modified 2,4-dinitrophenylhydrazine method. The tissues analyzed included the liver, spleen, kidney, brain, heart, muscle, eyes and skin. All milkfish tissues tested contained both C_1 and C_2 but levels of C_2 were generally higher than C_1 . The highest C_1 concentration was detected in the brain while highest C_2 level was found in the liver. Active metabolic organs such as liver, spleen, brain and kidney had relatively higher total ascorbate concentration than muscle, skin and eyes. Both C_1 and C_2 were also detected in the 21-day old wild caught milkfish fry and in the gonads of broodstock fish maintained on commercial fish feed. The ubiquitous distribution of C_2 in various organs and tissues of milkfish suggests an important physiologic role of C_2 in ascorbate metabolism in the fish. Consequently, it would be of interest to determine if dietary ascorbate requirement for milkfish could be provided through supplementation with C_2 instead of C_1 . Since C_2 is far more stable than C_1 , the keeping quality of formulated feeds for milkfish aquaculture stored under tropical conditions of high temperature and high humidity would be greatly enhanced.

Nutritional Requirement of Dover Sole (Solea vulgaris)
II - Phosphorus Requirement

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Dover sole juveniles were fed four semi-purified diets containing P at different levels (218, 462, 706 and 1195 mg per 100 g dry diet provided by $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) over a 42 day period.

Significant differences were observed in growth rate and feed utilization. The best performances in growth, feed conversion, protein efficiency ratio and protein utilization coefficient were obtained with the 706 mg P diet. The theoretical optimum P level calculated with polynomial regression analysis was 840 mg. The carcass protein and lipid content showed no difference between groups. However the P, Ca and phospholipid contents presented a maximum at 706 mg P.

From these findings, it appears that the optimum P level for growth and protein utilization was around 0.8% dry diet for Dover sole juveniles.

Effect of Varying Dietary Calcium, Zinc and Phytic Acid Content on Cataract Incidence, Growth and Histopathology in Juvenile Chinook Salmon

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During the summer of 1981, several hatcheries on the Pacific coast of Canada and the United States reported a high incidence of bilateral lens cataracts in their chinook (Oncorhynchus tshawytscha) and coho (O. kisutch) salmon stocks. A thorough investigation of the food, environmental factors and fish health revealed that the most probable cause was an induced zinc deficiency stemming from inclusion of a high ash content herring meal in the diet and consequently an excessive level of calcium in relation to zinc. However, this tentative conclusion was based upon experimental work on trout because little is known about the nutritional basis for cataract formation in Pacific salmon.

Therefore, this study was undertaken to determine the effects of wide variations in dietary levels of calcium, zinc and phytic acid (mineral binding agent present in plant protein sources) on performance and cataract incidence in juvenile chinook salmon. Nine semi-purified diets each containing one of three levels (g/kg) of calcium (4.4, 17.7, 48), zinc (0.05, 0.15, 0.40) and phytic acid (1.62, 6.46, 25.8) were each fed to chinook salmon held at 10 to 11°C for 105 days. All fish were fed their prescribed diet treatments three times daily to satiation.

High dietary phytic acid concentration (25.8 g/kg) was found to depress chinook growth, food and protein utilization and thyroid function, increase mortality, promote cataract formation (when zinc 50 ppm) and induce anomalies in pyloric caecal structure. Calcium at 51 g/kg increased the effects of high dietary phytate and low zinc content on growth and cataract formation. Moreover, calcium at 48 to 51 g/kg significantly impaired the growth and appetite of low phytate (1.62 g/kg) groups and led to nephrocalcinosis in low and high phytate groups. Plasma zinc levels were directly related to dietary zinc concentration and inversely related to dietary phytic acid level. Calcium (51 g/kg) reduced zinc bioavailability when the diet concurrently contained 0.05 g zinc and 25.8 g of phytic acid per kg.

It is concluded that zinc is essential for normal eye development in juvenile chinook salmon. Further, zinc deficiency could not be induced in chinook fed diets with high ratios of calcium to zinc alone. This required the simultaneous presence of a strong mineral (zinc) binding agent.

• 400 mg Zn - required if high phytate levels are

Progress towards Standardising the Experimental Design of
Fish Nutrition Studies

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The profusion of experimental designs used in fish nutrition studies creates difficulties in comparing the results between experiments. Variation in design can be due to the differing aims and objectives of particular investigations, but is frequently due to different approaches to the same problem. The latter situation, which is the principal concern here, stems from the lack of any obviously ideal experimental format which is suitable for solving most classes of nutritional problems, apart perhaps from investigations into the effects of starvation.

Several of the more persistent problems which beset the design of experiments are illustrated. Suggestions are made as to how these difficulties might be ameliorated. The topics include:

1. Variation in fish quality

Problems here include the general lack of genetically defined reference strains for most species of fish. In addition there is no agreed standard for the conditions under which fish are kept prior to experimental use. It is becoming clear that the way in which fish utilise food is largely dependent on the plane of nutrition to which they have been acclimated. This can generate problems, particularly in short term experiments.

2. Feeding strategy

There is no concensus view on the optimum feeding frequency for fish or whether it is preferable to feed to satiation or to a proportion of body weight, to name but a few of the methods currently in use.

3. Feed formulation

Some of the main problems here include the uncertainty over the precise requirements for many of the essential growth factors. In addition, foods based on purified diets are not always readily accepted and the quality of the constituents of semi-purified and practical feeds can vary considerably. Independent tests of the nutrient value of foods would greatly facilitate the interpretation of comparative studies. Inert filler such as cellulose is often incorporated in foods to create a range of nutrient concentrations. Analysis of published data shows that there are frequently strong negative correlations between filler level and factors such as growth rate and protein efficiency ratio. Such relationships have previously been ignored but they do serve to confound the experimental results and must cast a shadow on the validity of the conclusions which are drawn in such studies.

A number of suggestions are made regarding the practicality of introducing agreed standards for nutritional work. One option considered is the use of a series of diagnostic type tests enabling performance to be scored over a range of nutritional situations. In this way, comparison between species would be greatly facilitated.

Effects of Inhibitors in Wheat Meal on the Amylase Activity in Fish

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Although carbohydrates represent an important energy source in several artificial diets particularly for cyprinids, only little effort has been expended in optimizing their digestibility. Proteolytic inhibitors are destroyed by extruding the natural material, but amylase inhibitors remain untreated and may reduce the utilization of starch.

The effects of inhibitors on amylase activity in trout and carp were checked in in vitro experiments at a temperature of 25°C. Digestion was simulated by mixing substrate and intestinal fluid of trout (pH 7.7, activity: 21 mU/ml), and carp (pH 7.0, 616 mU/ml) in natural proportions (19% gut solids).

With extruded wheat meal amylase activity is reduced within 4 hours to 80% of initial activity due to proteolysis. With untreated wheat meal the animal amylase is immediately inhibited to 16% in trout and 30% in carp due to the action of an inhibitor. However, with time the inhibitor appears to be destroyed by proteolytic enzymes and the activity of the animal amylase increases again, reaching the values of the uninhibited enzyme within 4 hours in trout, more slowly in carp. Wheat also contains the less efficient β -amylase which, because of its instability against proteolysis, disappears completely within two hours.

The in vitro experiments suggest a reduced utilization of carbohydrates when untreated wheat is used in artificial diets.

Does the Spawning Fast of Captive Turbot (Scophthalmus maximus L.)
Females affect the Essential Fatty Acid Content of their Late Season Eggs?

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Early-, mid- and late-season eggs from 3 captive turbot (Scophthalmus maximus L.) were assayed to investigate if the 8 - 10 week 'spawning fast' of mature females caused a reduction in the essential ω 3 long-chained, polyunsaturated fatty acid (PUFA) content of their late-season eggs. Total lipid, extracted by Folch's method, was separated into neutral and phospholipid fractions on a silicic acid column and quantified spectrophotometrically. The neutral and phospholipids were converted to fatty acid methyl esters and analysed by gas-liquid chromatography. The water content of eggs was determined by freeze-drying.

No obvious variations were found in the water content or in the percentage concentrations of total, neutral or phospholipids in early or late-season eggs. The percentage concentrations of 22:6 ω 3 and, to a lesser extent, 22:5 ω 3 decreased in the phospholipid fraction of late season eggs. However, it was not possible to assess whether 20:5 ω 3 levels fluctuated because the chromatograph did not resolve the 20:5 ω 3 and 22:1 ω 11, 9 & 7 peaks. Phospholipid 16:0 levels showed a marked increase later in the season. The percentage concentrations of 22:5 ω 3 and 22:6 ω 3 in egg neutral lipid did not show any marked trend throughout the spawning period. Neutral lipid 18:0, 18:3 ω 3 and 22:4 ω 6 levels increased slightly as the spawning season progressed whilst 16:1 ω 9, 7 and 18:1 ω 9, 7 levels fell.

The results of experiments comparing growth and survival of larvae hatched from these early- and late-season egg hatches suggest that late season larvae performed just as well in the laboratory as did early larvae. It therefore appears that the reduced levels of ω 3 long-chained PUFA present in the phospholipid fraction of late-season eggs were not sufficiently low to have a detrimental effect on larval viability.

Effects of Three Commercial Fish Foods on the Quality and Degree of Smoltification in Release Salmon (Salmo salar)

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Three dry salmon diets (A, B, C), all commonly used in Finland, were tested for their possible influence on the physiological condition and smoltification of young hatchery-reared salmon. The experiment started with swim-up fry and continued until the fishes were released as 2-year-old smolts. Blood and tissue samples were taken from the fish at the beginning and at the end of the second growth period and during the last spring before the fishes were released as smolts.

The fishes were analysed for the following parameters: Condition factor, the degree of silvering, the degree of fin injuries, and total lipid content (whole fish); haematocrit value, haemoglobin concentration, MCHC (= mean cellular haemoglobin concentration)(whole blood); glucose, lactate, Cl^- , Mg^{2+} , total protein concentration and osmolality(plasma); the total lipid, glycogen and water content (muscle); and the glycogen content (liver). Before the fishes were released as smolts their osmoregulatory capacity in sea water (48 h; 28‰ salinity) was tested.

The results throughout the growth period reveal significant differences in the ability of blood to carry oxygen and in the energy metabolism of the fish fed the different diets. The average mortalities and cannibalism with the diets were A = 8.5%/5.0%, B = 10%/7.4%, C = 6%/3.8% during the second growth period.

Also the degree of fin injuries differed significantly. The time and the degree of smoltification seemed to differ between the groups. In the autumn the fishes in group C were most silvered and their glucose concentration was increased, while in the spring their osmoregulatory capacity in sea water was somewhat lower than that of the other groups.

The results indicate that the diets have an influence on the physiological condition of hatchery-reared smolts. They also show that the conventional "farming parameters" (e.g. growth) do not necessarily give an indication of how suitable the diet is for salmon which are reared for stocking.

One thousand parr were tagged with Carlin tags from each experimental group. The recapture results will show the relation between the physiological condition of the released fish and their 'release value'.

Food Chain Structure in Seagrass Beds and the Role of Seagrass
in the Nutrition of Fish

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Trophic relationships within a seagrass community on the southern Australian coast were studied to determine the dependence of major commercial fish species on seagrass as a food source. The study also examines aspects of nutrition in herbivorous species, such as the southern sea garfish Hyporhamphus melanochir, with particular emphasis on digestion of seagrass and subsequent assimilation of its nutrient components. Results show that seagrass is a major contributor to the carbon source of the food web, and that crustaceans, especially crabs, are an important link in the transfer of energy from plant production to the higher trophic levels. The detrital food chain is probably the main pathway, but some invertebrates and a few fish are able to utilise seagrass directly. The diet of the garfish underwent shifts from green seagrass (Zosteraceae) tissue during the day to mainly amphipods and other invertebrates at night. The shifts occurred sharply at dawn and dusk and coincided with the nocturnal vertical migration of the crustacean prey. The feeding strategy of the garfish appears to be an adaptation to diel changes in food availability in addition to the fishes' metabolic requirements. The garfish fed on seagrass for 13 h during daylight, ingesting an estimated 3 gut volumes equivalent to $500 \text{ kJ d}^{-1} \text{ kg}^{-1}$ dry wt. and assimilated this at an efficiency of 38% (organic matter), 28% (energy), 50% (protein) and 76% (lipids). This is equivalent to the total nutrient content in the "juice" of seagrass which the garfish extracts with the aid of its pharyngeal plates. Although crustaceans consumed were only a third of the quantity of seagrass, it was reasoned to be an essential source of protein, and at least as important as seagrass in satisfying energy demands of the garfish. Seagrass tissue was judged to be an inadequate diet for fish in nature which seem to rely on an omnivorous diet to provide essential fatty acids and amino acids.

The Nutrition of Stomachless Phytoplanktivorous Fish in
Comparison with Tilapia

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The contents of alimentary tracts of stomachless phytoplanktivorous fish, including silvercarp (Hypophthalmichthys molitrix), Esomus danrica thermoicos and Amblypharyngodon meletinus were investigated microscopically and compared with those of Tilapia (Oreochromis mossambicus, a fish with stomach) to illuminate the problem of energy supply of these so-called herbivorous fish. In addition, the digestive enzyme pattern (trypsin, amylase, cellulase and lysozyme) of silvercarp was examined to ascertain the possibility of diverse components in the diet of stomachless filterfeeder.

Most of the ingested algae could not be utilized by stomachless fish whereas fish with stomach (Tilapia) efficiently digested phytoplankton. A general comparison of stomachless fish (Esomus and Amblypharyngodon) and Tilapia shows a clear distinction between the two physiological groups, Diatomophyceae are the most predominant group of algae in the fore-gut of Tilapia, Esomus and Amblypharyngodon but, due to a less efficient digestibility by Amblypharyngodon (52.5%) and Esomus (26.1%), compared with Tilapia (93.5%), the nutritional benefit from these algae is much higher for Tilapia. In silvercarp most of the algae that were found in the fore-gut appeared intact in the hind-gut. This result is corroborated by the observations on fresh gut contents of silvercarp. For example, Euglena was even more mobile in the hind-gut than in the fore-gut.

The predominance of detritus in the food, the low efficiency with which phytoplankton is utilized, prove that stomachless fish are not primarily herbivorous. The absence of cellulolytic activity in the intestine of silvercarp corroborate this assumption. Detritus may constitute an important energy resource for stomachless fish. However, not all components of detritus (microbes, morphous and amorphous particles) are equally digestible by these fish. Silvercarp, for instance is lacking lysozymatic activity and therefore cannot utilize microbes. !!

To what extent zooplankton is important in the food of stomachless herbivorous fish remains unclear. Freshly examined samples of fore-gut and hind-gut of silvercarp as well as formalin fixed subsamples of alimentary tracts of Tilapia, Esomus and Amblypharyngodon did not contain intact zooplankton. However, incubation of gut contents of silvercarp with fresh plankton from the pond showed that zooplankton is digested immediately and hence cannot be found in the alimentary tracts.

Nutritional and Physiological Effects of Dietary Sodium Chloride on
Rainbow Trout (Salmo gairdneri Richardson)

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Normal trout pellets supplemented with NaCl at levels of 4.5%, 9.2% and 11.6% were fed to groups of rainbow trout (S. gairdneri) (average weight 15 g) reared in glass aquaria.

Fishes fed on "salty food" showed lower growth rate than those fed on normal trout pellets. The presence of dietary salt up to 9.2% did not affect the food intake, but the food conversion efficiency decreased as the amount of dietary NaCl increased. Increased dietary salt influenced food conversion to a much greater extent than appetite because it interfered with food utilization since the nutrient per unit of food was less for salty diets. Decreased growth rate may also be a reflection of increased energy demands for ionic regulation.

The fate of ingested NaCl showed that there was a significant increase in the Na^+ and Cl^- concentrations of gut contents after 1/2 hr of feeding and these ions decreased to normal values after 24 hrs with plasma Na^+ and Cl^- showing the greatest increase after 24 hrs. This finding was noted in all three salt supplemented diets and most noticeable in the 11.6% salty diet. The concentrations of K^+ , Ca^{++} and Mg^{++} in the gut contents and plasma showed a little change.

Sodium and chloride contents of the gills, gut and kidney were higher in fishes fed on high levels of dietary NaCl than those fed on normal diets. This was expected since these tissues are the main sites of salt regulation in the body. Because of the efficiency of these tissues in removing the extra salt load, retention of Na^+ and Cl^- could hardly be detected in the other tissues such as liver, muscle, bone, brain and skin. No remarkable changes in the tissue content of K^+ , Ca^{++} and Mg^{++} could be observed due to high salt feeding.

Further experiments dealing with other aspects of physiological effects of dietary NaCl are in progress.

The Mineral Composition of Some Tilapia Tissues

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A number of investigations have been made of the mineral requirements for fish diets but more information is needed. Few studies of the tissue mineral content of farmed fish have been made and in order to extend our knowledge of fish mineral utilisation the mineral content of some tissues of Tilapia nilotica of different ages and sex have been determined.

Fish of circa 4.0, 8.0 and 17.0 cm in standard length were used for the estimation of Ca, P, Mg, K, Na, Zn, Cu and Fe in skin, muscle and bone. Tissues were dried, digested and all elements, excluding P were measured by atomic absorption. Significant differences in tissue minerals were observed between small and large fish. Some of these differences were as expected, for example increase in Ca and P in bone with age. However, other significant changes were found, for example a considerable decrease in the Zn content of the skin from circa 18.8 mg% dry weight in small fish to 11.6 mg% dry weight in larger fish and of the muscle Zn from 0.7 mg% dry weight to 0.2 mg% dry weight. Other elements such as Fe showed little variation with age. No sex differences were observed. The poster to be presented will give in tabular and graphical form details of the tissue mineral variations in Tilapia nilotica and will be compared with data obtained from other farmed fish species.

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✓ Biochemical Alterations in the Tissues of a Fresh Water Carnivorous Teleost
Anabas scandens during Nutritional Stress through Starvation and
High Carbohydrate Loading

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The metabolic pattern as manifested in different physiological parameters in response to starvational stress and carbohydrate loading was investigated in a fresh water carnivorous teleost Anabas scandens. Three groups of fish were subjected to the experiments for a period of eight weeks. One group was fed with protein free-high carbohydrate diet while the second group was starved. A third group served as control and this group was fed with Commercial fish meal. After the experimental period the protein and carbohydrate profiles in different tissues were observed and the activity levels of the enzymes in tissues were estimated.

The fish were able to adapt to high carbohydrate diet without deleterious effect. However there was no significant weight gain at the end of the experimental period. The activity level of metabolites and enzymes in different tissues showed a varied response to protein free-high carbohydrate diet. The total protein content of all the tissues except liver decreased. In the liver tissue there was no significant change. There was an increase in the glycogen content in all the tissues.

There was a general decline in the level of alanine aminotransferase (ALAT), aspartate aminotransferase (AAT) and glutamate dehydrogenase involved in protein catabolism. The enzyme lactate dehydrogenase decreased in all the tissues except in liver.

Under starvational stress there was no mortality. However the body weight decreased, showing that the catabolism predominated during prolonged starvation. There was a decline in the tissue protein and glycogen. The enzymes ALAT and AAT linking carbohydrate and protein metabolism responded positively under starvation. The glutamate dehydrogenase increased in all the tissue except in muscle where there is not significant change. The lactate dehydrogenase decreased in the liver but elevated in muscle, gill and kidney tissue. The enzyme level is not affected in brain.

The adaptive response towards the maintenance of the regulatory mechanisms of tissue functions while the fish is under nutritional stress is discussed.

Gustatory Stimulants for Flatfish N

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Studies of the nature of feeding stimulants have been undertaken in two species of flatfish, the turbot (Scophthalmus maximus) and the Dover Sole (Solea solea).

The effect of stimulants and their analogues has been determined by assays at three levels, the whole animal behavioural response, the electrophysiological response and the binding activity of the stimulant to the receptor. Correlation between the responses has revealed structure-activity relationship for both Sole and Turbot.

Glycine Betaine has previously been identified as a feeding stimulant for Dover Sole (Mackie et al., 1980) and the employment of a range of betaine analogues has allowed some conclusions to be drawn about the structural requirement of the gustatory binding site of sole.

Turbot have been shown to be dependent on the presence of inosine and a limited range of close analogues for feeding activity (Mackie & Adron, 1978). The nature of these active analogues suggest a structural model for the binding site of turbot.

MACKIE A. M. & ADRON J. W. (1978) Identification of inosine and inosine 5'-monophosphate as the gustatory feeding stimulant for the turbot, Scophthalmus maximus. Comp. Biochem. Physiol. 60A 79-83.

MACKIE A. M., ADRON J. W. & GRANT P. T. (1980) Chemical Nature of feeding stimulants for the juvenile Dover Sole, Solea solea (L). J. Fish Biol. 16 701-708.

Growth and Gill Structure in the Turbot (Scophthalmus maximus) in
Relation to Dietary Polyunsaturated Fatty Acids

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SUMMARY

Three groups of turbot were maintained on different diets containing: 1, 10% of the dry wt of the diet as natural fish oil, equivalent to 2.5% (n-3) PUFA and 0.23% (n-6) PUFA; 2, 10% of the dry wt of the diet as palmitic acid, i.e. no PUFA; 3, 8.7% palmitic acid and 1.3% of the dry wt as (n-3) PUFA and negligible (n-6) PUFA. Only the fish on the diet containing natural fish oil showed significant growth over a 15 week period. In addition there were high mortalities on the two experimental diets (2 and 3). Changing the ratio of 20:5(n-3)/22:6(n-3) from 13.8 to 2.2 in the diet containing 1.3% (n-3) PUFA and negligible (n-6) PUFA markedly decreased the mortalities. Fish fed the two experimental diets (2 and 3) developed gross changes in gill structure, and the tissue ultimately disintegrated to leave a skeleton of connective tissue and a mass of cellular material in the interlamellar spaces. The results are discussed in relation to essential fatty acid requirements of the turbot. It is concluded that 22:6(n-3) is an essential fatty acid for turbot and that the gill epithelium is a sensitive indicator of essential fatty acid deficiency in this species.

Takeshi: The EPA need / turbot varies from one paper to another.
It is known ^{that} when fed rotifers, w3 needs of turbot remains low!

Investigations of Food Acceptance by the Cod, Gadus morhua L.

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To determine which chemicals are important in eliciting the swallowing response during feeding in cod (Gadus morhua), two experimental diets have been used in feeding trials at the Marine Laboratory. The diets were casein based with the addition of an ethanol extract of squid (Loligo forbesi) in one and a synthetic squid mixture in the other. Various concentrations of each diet were fed to juvenile cod and the amount consumed monitored. The results obtained indicate the optimum chemical concentrations in the diets necessary for successful ingestion of food by cod. The results also demonstrate that the lipid fraction present in the squid ethanol extract is not important in determining food acceptability.

Effect of dietary protein level on glucose utilization in rainbow trout

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The effect of different levels of dietary protein on the utilization of glucose was investigated in rainbow trout. Increased levels of dietary protein did not appear to affect either the hepatic glycolytic activity or the glucose tolerance in the trout. Therefore, the levels of dietary protein used in this study (40-50%) do not appear to increase glucose utilization in the trout. Furthermore, the significant decrease ($P < .05$) in the growth of trout reared on the highest level of dietary protein (50%) may indicate that the utilization of dietary glucose is reduced in rainbow trout reared on high protein diets. The actual cause of the growth depression in trout on the 50% protein/25% glucose diet may be due to an inability of the trout to effectively regulate gluconeogenesis. Increasing the dietary protein and glucose levels had no effect on liver alanine-aminotransferase and aspartate-aminotransferase enzyme activities. The significant reduction ($P < .05$) in the final body weight of the trout reared on the high glucose diets indicates that glucose is not effectively utilized as an energy source and that the metabolizable energy value suggested for glucose (16.74 kJ/g) in salmonids is too high. The significant reduction in final carcass lipid levels may indicate that neither glucose nor amino acids are effective precursors for lipogenesis in rainbow trout.

The effect on rainbow trout performance of the replacement of fish meal with meat meal in the diet

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Seventy five rainbow trout (Salmo gairdneri) initial mean live wieght 213 g, were kept in 5 meter long raceways with running water over-saturated with O₂ and at a mean temperature of 17.3°C, over 59 days. They were randomly allocated to 3 groups of 25 fish each, each group being offered a different diet. Three compound diets were offered, three times a day, at the rate of about 25 per cent of fish live weight, depending on water temperature. The diets were designed as approximately iso-proteic (control - 47.6 per cent crude protein) and iso-energetic. The control (C) diet contained 46.8 per cent fish meal of 59 per cent crude protein, whereas, in diets M₁ and M₂ 50 and 100 per cent of the fish meal in the control diet was replaced by a² solvent extracted meat meal, on an approximately equal protein level basis. The remainder of the protein in these diets was mostly of vegetable origin. An amino acid analysis performed on the control ration showed that it had levels of essential amino acids at least as high as published amino acid requirements for chinook salmon. With a view to estimating chemical composition of fish weight gain over the trial period, a comparative carcass analysis was performed. Comparison of weight gains at the end of the experiment showed similar values obtained with each of the three treatments (about 185 g mean individual gain). This was also the case with relative growth (about 87 per cent for each treatment). Total dry matter intake was equally similar between the three treatments (about 300 g), which, combined with similar live weight gains, originated also similar ratios for conversion of D.M. in the feed into live weight (an average 1.63); as well as for the ratio: live weight gain/feed protein consumed, P.E.R. (an average 1.24). Estimated chemical composition of the fish live weight gain was also similar between the three treatments, which, when combined with live weight gains, also very similar, gave rise to estimated total amounts for the various chemical components deposited in the carcass over the experimental period, non-significantly different between the 3 treatments, One can therefore conclude that, during the growth period under consideration, meat meal satisfactorily replaces fish meal in rations for rainbow trout, providing that suitable supplementations of lysine and essential fatty acids are made.

Utne: Control fish meal contains 55% Prot.!
& ash 25%!

Poor fish meal

PL: But this is 1 first time - meat meal is shown to be
a food growth promoter.

Satiation Feeding Versus The Use of Limited Rations
In The Evaluation of Post-larval Diets

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It is a common practice to test the quality of post-larval diets for fish based on ad-libitum feeding of rations. The danger in this practice arises with diets which differ in their attractability. Growth rate is considered a simple and common criteria for the evaluation of diets. Quite often improved growth rates result from the higher attractability of a diet rather than its improved nutritional quality. In our experiments with the gilthead sea bream (Sparus aurata), this experimental approach has produced misleading results recommending live food intake. It is our feeling that nutritional research is aimed at finding a more efficient diet in terms of its nutritional composition with attractability being a secondary requirement.

We propose in this poster to deviate from the common practice of evaluating diets based on the growth rate produced on ad-libitum feeding, to an evaluation based on growth rate produced as a function of ration size. In other words, evaluating the quality of new diets based on the slope of the relationship between ration size and growth rate of these diets. These slopes represent the nutritional quality of diets independent of their attractability to the fish.

In our comparison of the two experimental methods, it was clearly shown that when the post larvae of Sparus aurata (250 ± 50 mg wet weight) were fed adlibitum Artemia salina and a number of post-larval diets, artemia gave significantly ($P < 0.05$) better growth results. When artemia and one of the dry diets were again compared in terms of their effect on growth rate, but at different ration sizes (0, 3, 6, 9, & 12% of post-larval body weight/day on a dry/wet food fish basis) the results were reversed. The slope of the relationship between growth and ration size was greater for the post-larval diet in comparison to artemia, thus, indicating greater nutritional benefit was obtained by the fish from the dry diet. Only at the 9% feeding ration did artemia provide a similar growth rate to the post-larval diet's rate at the 3% feeding level. This suggested a much poorer food conversion of the artemia on the part of the fish.

Maximum consumption of the post-larval diet was reached at the 9% ration as food was left uneaten. This was not the case with artemia where even at the 12% ration level no food was ever left uneaten. This suggested a greater attractability of the artemia to the fish.

The conclusion reached from the two separate trials was that although the post-larval diet was superior nutritionally, the greater attractability of the artemia resulted in greater consumption on the ad-libitum regime with the resulting better growth rate. It follows that our dry diet is a nutritionally better diet than artemia. To fully, exploit its growth promoting potential feeding attractants have to be supplemented to the diet.

✓ Effects of dietary 17- methyltestosterone and bovine growth hormone on developing elvers

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Effects of dietary addition 17- -methyltestosterone (MT) and bovine growth hormone (bGH) on growth, food conversion and percent total body protein and energy were investigated. Both MT (1 ppm) and bGH (2, 10 ppm) increased significantly the mean weight of elvers as compared to controls. Although higher concentrations of MT (10 ppm) increased mean body weight, the increases were not significant. The effects of MT were more pronounced in normally developing elvers as compared to slowly-developing elvers. Reduction of water temperature had a much stronger influence on weight gain on slow-developing elvers receiving MT as compared to normally developing eels receiving MT.

Food conversion was improved by both MT and bGH. In general, food conversion by slower developing elvers was lower than food conversion by more normally developing elvers.

Total body protein of elvers was related to body weight. Total body protein was significantly lower ($p < 0.05$) in elvers receiving MT as compared to control elvers. However, there was no significant difference in total body protein in elvers receiving bGH as compared to controls.

Evaluation of mixed microbial biomass in rainbow trout diets

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The nutritional quality and safety of a predominantly yeast mixed SCP biomass, consisting of the organisms Hansenula anomola, Candida kruzei, Saccharomyces cerevisiae and Geotrichum candidum, grown on malt whiskey spent wash was evaluated using feeding trials in rainbow trout.

The composition of the SCP was as follows in g 100 g⁻¹ dry weight: crude protein (total N x 6.25), 49-55 g; Crude lipid, 4.27 g; Ash, 6.11 g, Moisture 4.58; Crude Fibre, 1.8 g and Total NPN, 1.76 g. The total energy content was 22kJg⁻¹ dry biomass.

In semi-purified diets of 40% crude protein content, up to 50% of the dietary casein N could be isonitrogenously replaced by SCP without any loss in growth rate (SGR) or dietary N utilization (nitrogen efficiency ratio (NER), nitrogen productive value (NPV) in fingerling trout. With total replacement of casein N by SCP however, NER and NPV were reduced to 50-65% that of trout fed casein alone.

Digestibility of the SCP total N content was low (64%) and accounted to a large extent for the poor performance of the SCP alone in trout diets. Processing the wet SCP slurry by five different regimes did not give any marked improvement in SCP N digestibility.

The SCP biomass was also deficient in the sulphur amino acids (1.97 g SAA 16 g⁻¹ N), particularly in L-methionine and supplementation of diets containing SCP with L-methionine (0.5-0.75 100 g⁻¹ diet) improved the dietary N utilization slightly in fingerling trout, but did not improve the N digestibility. With L-methionine supplementation (0.5 g 100 g⁻¹ diet) the SCP could partially replace white fish meal on an amino acid-N basis at up to 27% SCP w/w diet in commercial type trout diets without any loss in growth rate, dietary N utilization or without adversely affecting the gross trout carcass composition. The quality of the biomass was generally similar to brewer's yeast and has potential as a supplemental protein feedstuff at low to intermediate inclusion levels in commercial trout diets.

Despite the high copper content of the SCP biomass, (generally 120µgCu g⁻¹ but can be as high as 569µgCu g⁻¹). The copper was not accumulated in trout fed low to high dietary SCP and consequent copper levels (8µg - 142µgCu g⁻¹). The high copper content does not therefore pose a problem in the commercial use of SCP for trout.

Effects of a High Carbohydrate Diet on Glycolytic Metabolites
in Rainbow Trout Liver

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The effects of high carbohydrate (CHO) feeding on hepatic metabolism in rainbow trout were investigated by use of the freeze-clamp technique to measure prevailing liver concentrations of glycolytic metabolites in fish fed a practical control diet (0% CHO) or a diet containing supplemental d-glucose (30% CHO), both after feeding and following an overnight fast. High CHO feeding significantly depressed growth rate and feed efficiency while increasing the liver glycogen concentration and hepatosomatic index. Serum glucose level was elevated in fed high CHO fish compared to controls but this effect was abolished by fasting. In contrast, liver glucose concentration was unaffected by diet or sampling time. Hepatic glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, triose phosphates, pyruvate and lactate concentrations were measured. Other than a significant fasting decline in lactate, control fish showed similar concentrations at both sampling times. The only change induced by high CHO feeding were increased glucose-6-phosphate and fructose-6-phosphate levels which were significantly elevated after fasting. It is suggested that the accumulation of liver glycogen in rainbow trout fed high carbohydrate diets may be explained by the mass action effect of persistently elevated hexose phosphate concentrations such diets induce.

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