FISHERIES RESEARCH BOARD OF CANADA

ANNUAL REPORT

1963 - 1964

ATLANTIC TECHNOLOGICAL

RESEARCH PROGRAM

D. R. IDLER, DIRECTOR
TECHNOLOGICAL RESEARCH LABORATORY
HALIFAX, NOVA SCOTIA

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ROGER DUHAMEL, F.R.S.C.
QUEEN'S PRINTER AND CONTROLLER OF STATIONERY
OTTAWA, 1964
82

FISHERIES RESEARCH BOARD OF CANADA

ATLANTIC TECHNOLOGICAL RESEARCH PROGRAM

INTRODUCTION

Organization

The co-ordination of technological research in the Atlantic Provinces was completed in July, 1962, when the program of the Grande Rivière Technological Station was linked with that of the Newfoundland Technological Unit and the Halifax Technological Research Laboratory to form the Atlantic Technological Research Program. Program responsibilities were assigned to the Director of the Halifax Laboratory.

A Few Program Highlights

A commonly employed diet for commercially pounded lobsters has been shown to be little better than starvation in maintaining lobster blood cell counts at the level found in a natural environment. Supplementation of the diet with liver corrected this situation.

The tolerance of lobsters to various woods and fluoridated water has been assessed. Western cedar is extremely toxic and should not be used for construction of pounds.

A diluting media for salmon sperm has been found which does not cause inactivation during the process of incorporating glycerol in the cell. This is a pre-requisite to successful freezing of the sperm.

Malpeque resistant oyster stocks have been shown by serological tests to possess an antigen which is absent in susceptible Cape Breton oysters, but it is still to be determined whether this is associated with resistance.

Dimethyl sulphide has been identified as the substance responsible for the off-odour of fillets from Labrador cod feeding on the pteropod Limicina Helicina (blackberry).

Sample thickness is indicated to be an important variable in determining texture characteristics of freeze-dried cod. A mascerated muscle residue from commercial filleting operations in Newfoundland has been shown to produce a freeze-dried product with much better texture than similar products prepared from fillets.

Very low dose radiation pasteurization of haddock and scallop meat has resulted in products with excellent storage characteristics in ice.

Fish protein concentrate (flour) has been prepared from filleting scraps and whole fish and the process has been modified for application to oily species such as herring. Nutritional and aesthetic characteristics

of all products have been very good.

Considerable progress has been made towards defining the influence of physiological and environmental factors on quality and shelf-life of fishery products, particularly in the studies on Newfoundland trap cod and non-bacterial deterioration of groundfish.

Publications

In last year's annual report it was noted that the prognosis for 1963-64 publications from the Atlantic Technological Program was not good in view of the extreme shortage of support staff, brought about in part by recruitment restrictions. It is therefore particularly gratifying to note that there was a modest increase (8%) in published and submitted scientific papers in 1963-64 as compared to 1962-63, which in turn had seen an increase of 50% over 1961-62 and 170% over 1960-61.

Laboratory and other Facilities

During the past year the Atlantic Technological Program has been expedited by additions to the running sea water facilities at Halifax and the completion of recirculating sea water facilities at Grande Rivière.

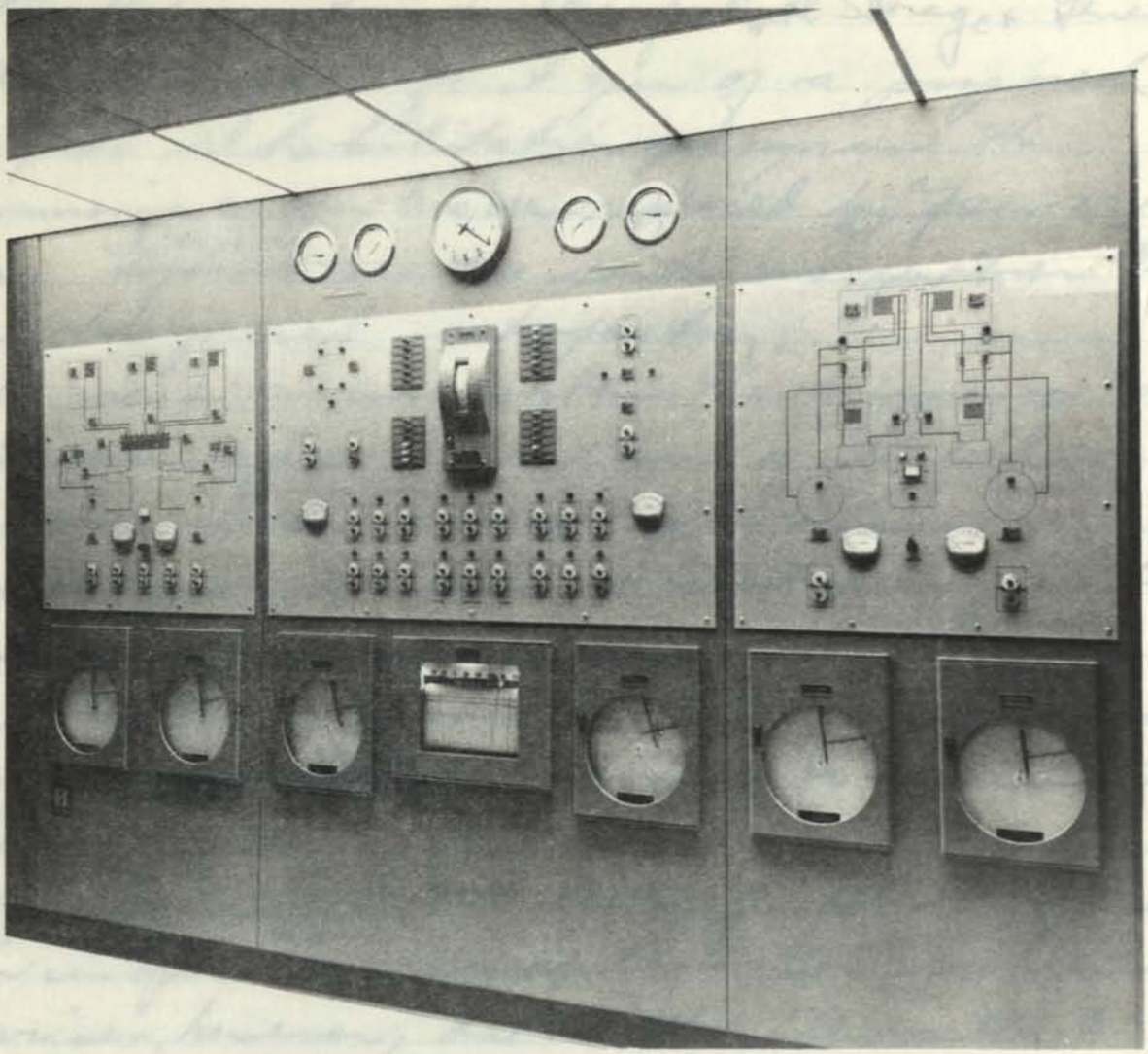
An outdoor fishtank (50' x 12' x 6') has been completed at Halifax, a seawall has been constructed, and a section of the harbour filled in an area adjacent to the Department of Fisheries wharf. Approximately half of the pilot plant area at Halifax has been converted to laboratory space and a wet work area, to expedite filleting and related operations.

There has been extensive modernization of the facilities controlled by the low temperature cold rooms at Halifax and walk-in type, near freezing workrooms have been completed at all three laboratories. An histology laboratory has been completed at Halifax.

Further details on most of the facilities will be found in Summaries 63 to 67.

D. R. Idler

D.R. Idler, Director
Technological Research Laboratory
Halifax



REFRIGERATION SUPERVISORY CONTROL PANEL

A Supervisory Control Panel, installed along with new refrigeration equipment, is now being integrated with existing equipment. Continuous records of the principal operating pressures and temperatures are provided. Graphic modules show the flow of refrigerant and brine for the two experimental storage systems, with controls indicating lights, valves and gauges in their proper location. Control of all compressors, pumps, fans and adjusting of all temperatures is provided in this control centre. All temperatures necessary for operation of the refrigeration equipment, analysis of operating efficiency changes, or experimental storage may be read at the panel.

This new equipment will permit the preventive maintenance necessary for the long term reliability required for Time-Temperature-Tolerance studies of frozen fishery products.

Summary Report Nov 13/64

~~Expt~~ Refrigeration facilities for Expt Storage + General Use. At the end of the first year of a proposed 3 - 4 year rehabilitation program the old ammonia system has been replaced by a freon-22/brine system. During the 6 months of continuous operation the system has proved satisfactory.

All power wiring within the compressor room area has been replaced, and most of the cold room wiring has been checked + brought up to minimum Can. Elect. Code standards. It has not been possible to shut down one ~~room~~ experimental storage room (-15°F). ~~no. made~~ The wiring in this room remains to be ~~rehabilitated~~ brought up to minimum safety levels.

The Supervisory Control Centre for all the refrigeration has also been in operation successfully. Some problems have arisen, however, due to troubles in the 3 phase power supply to the building. To prevent damage to the heavy equipment some units have been disconnected until such time as the power problem can be eliminated.

Work on replacing insulation and vapour seals on the 3 principal experimental storage rooms must wait until the laboratory program will permit a shut-down. Replacement of

The constant temperature cabinets within these rooms may be started next year.

I N D E X

INVESTIGATORS' SUMMARIES

H A L I F A X

SUMMARY NO. 1

QUALITY OF FRESH AND FROZEN FISH:
THE USE OF GAMMA IRRADIATION IN
THE PRESERVATION OF SCALLOP, HADDOCK AND LOBSTER.

H.E. Power, Doris I.Fraser
Wanda Neal, C.H. Castell
W.J. Dyer.

It has been known for many years that X-rays and other forms of ionizing radiation could cause the destruction of living cells and this property has been made use of in medicine for this purpose. For the past thirty years it has been known that this radiation will sterilize and aid in the preservation of food products. Only recently, however, has interest been aroused generally in this method of preserving food products. It is generally agreed that doses of ionizing radiation large enough to sterilize the product cause undesirable changes in the taste, odour and texture so as to make the product unacceptable to the consumer. Accordingly, interest has turned to lower doses where the taste and odour of the product is affected to a lesser extent. These doses, while not completely sterilizing the product, interfere sufficiently with the life cycle of contaminating organisms to cause the storage life to show a significant increase.

Pasteurization of scallop meat and of haddock fillets by low dose gamma radiation from a Co^{60} source has resulted in a 2-fold extension of the storage life at ice temperature. The results, reported last year and now prepared for publication, indicated that an optimum level of 75,000 rads extended the keeping time of iced scallop meat to 40 days and of iced haddock fillets to 23-30 days, over 18 and 12 days for controls respectively.

A study is presently underway to determine the effect of pasteurizing doses of gamma radiation on the keeping time of cooked lobster meat held in ice. Preliminary investigations have been carried out to determine a suitable range of irradiation levels, and to establish the progression of flavour and texture changes in iced lobster meat necessary for the compilation of a suitable scoring sheet for the taste panel studies.

For the principal study, cooked lobster meat sealed in polyethylene bags has been irradiated with doses of 75,000, 150,000 and 250,000 rad, and the storage life at $32^{\circ}F$ is being compared with that of unirradiated and frozen control meats by means of taste panel evaluation and chemical assessment.

SUMMARY NO. 2

QUALITY OF FRESH AND FROZEN FISH: NON-BACTERIAL DETERIORATION: CONTINUED STUDY OF METAL-INDUCED RANCIDITY IN FLESH OF LEAN FISH.

Jill MacLean
Wanda Neal
C. H. Castell

This is a continuation of the work described in Summary No. 3 in the 1962-63 Annual Report. It is primarily concerned with the use of copper and chemical tests to assess rancidity in the muscle of lean fish such as cod or haddock.

Based on previous studies an accelerated, copper-catalyzed rancidity test has been developed for determining the susceptibility of lean fish muscle to oxidative rancidity. The results of the test, in which TBA values are plotted against storage time or Cu^{++} concentration, form typical S-curves, showing an induction period, a period of active oxidation and a maximum level of rancidity for each sample tested. The accuracy, limitations and reproducibility of the results obtained by this technique have been ascertained.

Three samples taken from this last year's work will indicate how this test has been used to broaden our knowledge of the development of rancidity in cod muscle:

A. Seasonal Changes. Several hundred tests made periodically on cod muscle over a period of 15 months have shown a marked seasonal variation in the susceptibility to oxidation. It is at a maximum during the months of January, February, March and the first part of April. It decreases rapidly during late April and May and has minimum values during June, July and August. During the fall it gradually increases until it again reaches the high winter values. This means that the same treatment under a given set of conditions will develop high TBA values and strong rancid odours in cod muscle in February or March, but low values and no rancidity odours in June or July. The data so far suggests that this results from variations in the amount of antioxidants present in the muscle rather than differences in the composition of the lipids themselves.

It was also observed that fish taken from the more northern waters (Strait of Belle Isle and off Labrador) undergo this change in susceptibility to oxidation at a later period than the fish taken off the East Coast of Nova Scotia.

B. Effect of Salt on Rancidity. In the previous Annual Report it was stated that sodium chloride and certain other electrolytes accelerated the development of rancidity in fish muscle. A very large number of tests made since then indicate that the effect of salt may vary with the season and that

over most of the year salt actually retards the development of rancidity. The anti-oxidant effect is greatest with salt concentrations between 1 and 8%. In addition to laboratory tests, it has been shown that fillets dipped in very weak copper solutions made up with 3% saline, or sea water, and then stored at 0°C, will go rancid much slower than fillets dipped in similar solutions made from tap or distilled water. Under commercial conditions, fish containing various amounts of salt have been frozen and stored up to 12 months. Tests for the development of oxidative rancidity have shown that so far the salt has not increased lipid oxidation.

C. Effect of Unsaturated Fatty Acids. Deterioration of lean-fish muscle during frozen storage is usually confined to protein denaturation and the accumulation of free fatty acids. Tests made on both commercial and experimental fish held in frozen storage for periods up to several years (including samples showing considerable deterioration in quality) have shown that they have not undergone any significant amount of oxidative rancidity. Something seems to predispose this fish against oxidative rancidity. It seems probable that some of our recent studies are pointing to an explanation. It has been found that the addition of small concentrations of unsaturated fatty acids (linoleic or linolenic), or the addition of the natural fatty acids extracted from cod muscle, will inhibit the development of rancidity in the muscle. The concentrations used were at levels similar to those that occur in stored frozen fish.

In addition to this, tests were made on the susceptibility of fresh, unfrozen fish to oxidative rancidity and on similar fish held for increasing periods of time in frozen storage. It was found that the fresh, unfrozen fish was the most susceptible and that this susceptibility decreased with storage time and paralleled the increase in free fatty acids in the frozen fish muscle.

D. Low Temperature Freezing. In the range immediately below freezing it was found that decreasing the storage temperature retarded the copper catalyzed oxidative rancidity. However, when the temperature was decreased to below -50°, the picture changed. For example, fish muscle was frozen and stored at -26° and a second lot was rapidly frozen to -65° to -70°F for 30 minutes and then stored at -26°. The fish that was initially frozen at the low temperature went rancid much faster than the fish frozen and stored at -26°. This was confirmed by repeating the experiment several times.

These and other similar experiments are demonstrating the complexity in the problems involved in the oxidation of lean fish muscle, compared to the oxidation of pure fats and oils. Consideration must be given to physical and

chemical relationship between the lipids and the non-lipid portions of the muscle and to the fact that the physiological condition of the fish at the time of catching can have a profound effect on the subsequent development of rancidity.

SUMMARY NO. 3

QUALITY OF FRESH AND FROZEN FISH:
NON-BACTERIAL DETERIORATION:
PROTEOLYTIC ENZYMES OF COD

H.P. DUSSAULT
N. BISSON
P.H. ODENSE

Last year's Annual Report included a description of some preliminary work dealing with the detection of proteolytic enzymes in cod, in view of relating their activity to the various conditions of the fish either before or after death. Considerably more exploratory work has been accomplished and a better picture has been obtained with regard to the extraction and characteristics of the proteolytic enzymes present in cod tissues.

Extraction of proteolytic activity from cod muscle. After comparing various methods of enzyme extraction from cod muscle, such as salt extraction, maceration, overnight autolysis, water extraction, a procedure which gave a maximum activity in semi-purified extracts was selected. It consists of the following steps: The muscle sample is cut into approximately 1 cm cubes and distilled water is added in the proportion of 4 parts to 1 part of fish. The suspension is slowly frozen and kept overnight at 0° to 2°F. After thawing, the suspension is pressed through cheese-cloth, acidified to pH 5.0 with .1N HCl, then centrifuged at 10,000 R.p.m. for 20 minutes. The described method, which is simple and easily adaptable to the collection of a large number of samples, yields an extract which is almost water-clear and which contains approximately 1.2 mg protein per ml.

However, more recent experiments have indicated that if the same muscle sample is repeatedly extracted by the freeze and thaw procedure, activity continues to be liberated. This has been found to be true for seven successive extractions. These findings would seem to indicate that the original procedure only extracted approximately 10 per cent of the total activity and that cod muscle contains considerably more activity than was previously believed. It may be concluded that more experimentation is required before arriving at the recommendation of a better procedure for extracting proteolytic enzymes from cod muscle.

Characteristics of Cod Muscle Proteolytic Enzyme. The semi-purified cod muscle extract obtained by the procedure previously described has shown two definite activity peaks: A major one at pH 5.25 and a slightly lower one at pH 2.25. For both these activity peaks the optimum temperature was found to be 45°C. Time curves for proteolytic activity and autolysis have been drawn for both pH optima.

At pH 5.25 autolysis represented 14% of the total activity and at pH 2.25, 62%.

Enzyme stability studies have shown that both cod muscle and cod muscle extracts remain stable at 20 and 35°F for short storage periods up to 12 days. However, appreciable losses, ranging from 20 to 30% have been observed in extracts stored at 0°F for 2 to 3 months. Such losses are not observed when the muscle extracts are stored at pH 5.0.

Several determinations have shown that the dark muscle of cod is approximately 3 to 5 times more active than the white muscle and that the tail section which contains a greater proportion of dark muscle, shows approximately twice the activity of the other sections. Consequently a better sampling is obtained when one whole fillet is homogenized.

Although studies on activators and inhibitors are not completed, some results have indicated that low NaCl concentrations produce some activation whereas the high ones (10-12%) cause complete inactivation.

Heat coagulation of Cod Muscle Extract and Enzyme Inactivation. Partial purification of the muscle extract has been attempted by heating the solution for a definite time to a temperature just below that at which the enzyme is destroyed. Heating a crude cod muscle extract at 45°C for 10 minutes changed the total nitrogen content from .44 to .26 mg/ml with a 23% loss in total activity, but a net increase in specific activity from 96 to 130. Heating for 10 minutes at 50°C produced a 70% enzyme inactivation and 10 minutes at 55°C produced complete inactivation.

An experiment has been carried out to study the effect of NaCl during heat coagulation at 45°C on the proteolytic activity of the cod muscle extract. The highest specific activity has been obtained with 4% NaCl. It is not yet known if NaCl activates the enzymes or contributes to the precipitation of an interfering protein fraction. Several comparative experiments have shown that higher activity is obtained when protein is precipitated at pH 5.0 than when it is heat coagulated.

Post-mortem Changes in Proteolytic Activity of Cod Muscle. A few experiments have shown that when a fresh cod fillet is kept at room temperature for 24 hours the proteolytic activity is greatly diminished as compared to that of the fresh fillet. In another experiment where cod had been strangled to simulate capture in gill nets, and had been kept in water at 35° and 52°F for 48 hours, determinations of proteolytic activity have shown that for both temperatures, activity of the muscle is greater in the drowned cod than in the

controls, but that the activity of the blood samples remains unchanged. Haemorrhage caused by strangulation has been observed in the fish and the liberated blood may have accounted for the observed increase in activity.

Throughout the above described experiments on development of procedure and determination of enzyme characteristics, 50 analyses of cod muscle were performed. However, it is too early to be able to establish a definite correlation between proteolytic activity and some of the various conditions of the fish before or after death.

Proteolytic Activity of Cod Blood. Blood obtained from cod has been found to contain definite proteolytic activity. For determination of activity, the optimum dilution is 2.5% in water. After dilution, freezing overnight, then thawing has yielded a 35 to 37% increase in total activity. By this method maximum activity is obtained. Several determinations have shown that the proteolytic activity is located in the blood cells since the serum and plasma fractions were found to be completely inactive.

Experimental results have shown that maximal proteolytic activity is exhibited at pH 2.0. A smaller definite peak is also shown at pH 5.0 and an indefinite one at pH 9.5 to 10. At pH 2.0 the optimum temperature was 40 - 45°C and at pH 5.0, 45 to 50°C. Time curves for proteolytic activity and autolysis have shown that at pH 2.0 autolysis represents only 10% of the total activity and at pH 5.0, 42%.

So far 29 analyses of blood from different fish have been made. Since more attention was paid to procedures and determination of optimum conditions, significant differences were not observed. However, in view of the fact that blood contains appreciable proteolytic activity, which offers less variations due to sampling and which can be easily and completely extracted, more specimens will be examined. It is hoped that it will be possible to establish a correlation between proteolytic activity and some of the various conditions of the fish before or immediately following death.

Proteolytic Activity of Cod Skin. The higher proteolytic activity present in the dark muscle of cod which is located near the skin, has prompted a study of proteolytic activity in cod skin. The activity which is extracted with water by the freeze and thaw procedure shows an optimum at pH 2.25 and a smaller peak at pH 5.25. At both pH the optimum temperature is 45 to 50°C. Cod skin extracts contain very little soluble protein and therefore show very little autolysis. The activity is almost quantitatively equal to that of cod blood and approximately ten times that of cod muscle.

SUMMARY NO. 4

QUALITY OF FRESH AND FROZEN FISH:
NON-BACTERIAL DETERIORATION:
FEEDING, STARVATION AND TEMPERATURE.

M.A. BORDELEAU
P.H. ODENSE

In evaluating the roles played by lipolytic and proteolytic enzymes in the port-mortem changes in fish quality it is also essential to know the factors which influence the condition of the fish at the time of death. In the first study utilizing the live holding facilities the effect of starvation upon the condition of the fish at death has been studied.

Live cod were held in the recirculated seawater tanks. One group was held several weeks without food at a temperature of 35°F, another group was similarly starved but at a temperature of 51°F, a third group was fed on herring during this period and was kept at a temperature of 35°F.

Cod from these groups were killed by a blow on the head and were immediately transferred to the coldroom (2°C) where the weight, length, sex, liver and gonad weights, skin colour, stomach contents and flesh pH of each fish were noted. The following analyses were performed on fillets from each fish:- water content of muscle, total extractable protein nitrogen (T.E.P.N.), total non-protein nitrogen (T.N.P.N.), actomyosin nitrogen (A.M.N.), water extractable protein nitrogen (W.E.P.N.), water extractable non-protein nitrogen (W.N.P.N.), total lipids extractable (T.L.E.), free fatty acids (F.F.A.),

total volatile acids (T.V.A.) formic acid and acetic acid.

Preliminary results indicate a much lower flesh pH in the fed group compared with the starved groups. The T.N.P.N., W.E.P.N., T.L.E., F.F.A. and T.V.A. values appeared lower in the starved groups while the A.M.N. and acetic acid values were higher. These results must be confirmed in the coming season with more fish.

SUMMARY NO. 5

QUALITY OF FRESH AND FROZEN FISH:
NON-BACTERIAL DETERIORATION:
FRACTIONATION OF COD MUSCLE ALBUMIN ENZYMES WITH ACETONE.

M.A. Bordeleau
P.H. Odense

Fractionation of the enzymes present in the aqueous extracts of fresh and freeze dried cod has been attempted. Acetone has been added in stepwise quantities to the extracts to precipitate the proteins. Various conditions of pH, temperature and ionic strength have been used to try to obtain the best separation of the proteins. So far the best fractionation has been achieved at a pH of 6.5 at low ionic strength and at temperatures below 0°C. Vertical starch gel electrophoresis of the fractions revealed some overlapping of the proteins present in the different fractions.

Vertical starch gel electrophoresis has been used to follow changes in the extracts themselves during storage at various temperatures. Gradual loss of some protein constituents has been observed during storage.

SUMMARY NO. 6

QUALITY OF FRESH AND FROZEN FISH:
COLLABORATIVE EXPERIMENT WITH U.S. FISH AND WILDLIFE LABORATORY AT GLOUCESTER ON FREEZING (PLATE vs BRINE) AND THAWING (WATER vs MICRO-WAVE) METHODS.

W.A. MacCallum
W.J. Dyer
D.I. Fraser
D.R. Idler

This experiment set up last year (Summary No. 4, Annual Report for 1962-63) to examine the effect of water and microwave (Raytheon) thawing of frozen round gutted and headed cod blocks, has been concluded. The blocks were prepared at Halifax and thawed, refrozen in 1-lb waxed overwrapped cartons, and stored at -18°C at Gloucester. Quality was assessed periodically by taste panels at Gloucester and by chemical analyses on samples shipped to Halifax.

Last year it was shown that the refrozen fillets immediately after thawing and refreezing showed almost no effect due to freezing method (plate vs brine), thawing procedure (circulating water vs microwave (Raytheon)) with pre-rigor fish rating possibly slightly higher than post-rigor fish.

After 12 months' storage, no significant differences developed between the variously treated samples in taste panel assessment, free fatty acid formation

or extractable protein, with the possible exception that texture rated somewhat higher in the fish frozen pre-rigor as compared with post-rigor fish, in plate and in brine frozen samples, but only when water thawed (not in the microwave thawed samples).

Free fatty acids increased slightly on thawing and refreezing, then showed a rapid jump from about 15 to 35-40% on refrozen storage for 2 months at -18°C, followed by a slow gradual increase to almost 50% at 12 months. The rapid increase phase indicated an activation or release of enzyme activity or of substrates on thawing and refreezing agreeing with previous observations from this Laboratory on frozen cod allowed to rise in temperature to 10-15°F or above for a short period. Extractable protein decreased gradually; very little extractable actomyosin remained at 12 months. This rate of decrease is similar to that found for refrozen Newfoundland trap cod reported last year (Summary No. 10, Annual Report for 1962-63).

Taste panel assessment grades decreased to "fair" and "border-line" values after 4-6 months' storage. Discolouration and surface dehydration had become objectionable at 10 months and prior to tasting the dried surface was shaved off. Thus, some increase in acceptability occurred in the 10 and 12 month sampling. This again emphasizes the importance of proper wrapping to prevent dehydration and oxidation in frozen products. The refrozen fish therefore graded a great deal poorer than the controls even though they remained of edible quality throughout 12 months' storage at -18°C.

Control samples (overwrapped with coated cellophane) stored at -20°F showed little or no deterioration in taste panel grades or protein extractability and only a slight development of free fatty acids.

A preliminary write-up has been submitted to the Gloucester Laboratory for revision and addition of taste panel data.

SUMMARY NO. 7

QUALITY OF FRESH AND FROZEN FISH: W.J. Dyer
COLLABORATIVE TESTING OF COOKING PROCEDURES: Doris I. Fraser

It has long been evident that the taste panel appeal and also the grade assigned by a taste panel assessment of cooked fish is influenced by the method of cooking. To provide quantitative data a collaborative experiment was undertaken with the Consumer Branch of the Department of Fisheries, Ottawa. Three basic cooking procedures, baking, steaming and frying, were compared by taste panel assessment on cod fillets of three pre-freezing qualities, Grade I, II and III, each of which had been stored frozen to give products with no, medium or a great deal of frozen storage deterioration. Very little difference in

score due to initial quality (i.e. degree of initial spoilage) was shown, but differences due to frozen storage quality were large. With the latter samples much better discrimination between the quality levels was shown by baking and steaming than by frying. Texture showed greater differences than did taste. With baked samples, scores of good, medium and poor samples from the standpoint of frozen storage deterioration were significantly different at the 5% level. On steaming the poor sample was significantly lower than the good and medium, but on the fried samples only good and poor were separated at the 5% level.

Thus, baking, and possibly steaming, are the best cooking methods for detection and comparison of quality changes on storage. Frying is the method of choice for the consumer if product quality is in doubt, but any cooking method may be used for a top quality product.

This work has been submitted for publication.

SUMMARY NO. 8

QUALITY OF FRESH AND FROZEN FISH:
STORAGE LIFE OF POLYPHOSPHATE DIPPED FROZEN COD.

W.J.Dyer, H. Brockerhoff
R.J. Hoyle, Doris I. Fraser

In certain applications, frozen fish may be thawed prior to cooking, and in such a case it is desirable to minimize thaw drip as much as possible to avoid messiness and the loss of vitamins, minerals, and also protein dissolved in the fluid lost. While brine dips are commonly used for this purpose, certain phosphate compounds have recently been introduced. This experiment, to determine the effect of polyphosphate treatment on subsequent storage characteristics of frozen fillets as reported last year, Summary No. 7, has been terminated and the results published.

Dip uptake was found to be variable, although rancidity development (free fatty acid release) and decrease in protein extractability in salt solution proceeded at identical rates in control and treated paired fillets. Because of uptake of solution on dipping, thawed yields were near 100% in the treated fillets but lower in the control samples, there being no consistent effect on thaw drip. The controls were not water-dipped since this would result in considerable moisture absorption, most of which would be lost as thaw drip on thawing, and in practice, there will be some draining prior to packing. Theoretical considerations led to the conclusion that polyphosphate should be most effective at high ionic strength, under which condition the actomyosin could be dissociated into its constituent proteins which have a greater water-binding capacity. A further experiment is in progress to investigate the effect of high ionic strength.

SUMMARY NO. 9

QUALITY OF FRESH AND FROZEN FISH:
STORAGE LIFE OF POLYPHOSPHATE TREATED FROZEN COD AT HIGH IONIC STRENGTH IN THE PRESENCE OF SALT.

W.J. Dyer,
Doris I.Fraser
J.R. Dingle

Since disassociation of actomyosin with accompanying modification of the protein water-holding capacity should occur at high ionic strength (previous abstract), the effect of polyphosphate in the presence of about 1% sodium chloride was investigated on thaw drip and frozen storage characteristics in cod fillets.

Discussion on the need and effectiveness of polyphosphate for reducing drip losses in frozen fish at a conference called by the Food and Drug Laboratory showed that no satisfactory commercial method for controlling variability of phosphate pickup had yet been found, and also that there was no good evidence supporting its effectiveness. It is not yet permitted on fish in Canada, although it may be used on certain other products.

Because of the limited solubility of sodium tripolyphosphate in the presence of brine, considerable preliminary work was required to develop satisfactory methods for attaining the desired phosphate and salt concentrations in the fillet.

Paired fillets from trawler fish in ice 2 days, treated to contain about 1% brine in the control fillet and about 0.5% sodium tripolyphosphate + 1% brine in the fillet from the other side of the fish, were frozen and stored at -12°C and at -26°C (+ 10 and -15°F). Weight uptakes showed considerable scatter and were higher in the phosphate-treated fillets. Initially, thaw drip was below 2% in all samples.

Taste panel and analytical work were conducted at the Newfoundland Unit and the results follow in the next summary.

SUMMARY NO. 10

QUALITY OF FRESH AND FROZEN FISH:
STORAGE LIFE OF POLYPHOSPHATE DIPPED FROZEN COD.

Dorothy A. Chalker
J.T. Lauder
W.A. MacCallum

See preceding summary for experimental detail.

At zero time all fillets were soft to mushy to slightly fibrous in texture, grading 74 - 78%. At three months those stored at -15°F were again soft to mushy, rating 76%, while those held at +10°F were chewy and rated 34% for untreated, 41% for treated samples, not a significant difference. The polyphosphate dipped fish were similar to the untreated fish in texture and taste

in all cases. The taste of these samples was in most cases objectionably salty. In addition, those at +10°F for 3 months were stale. At the 3 months analyses tail pieces were tasted as well. These were considered so salty as to be inedible, also those held at -15°F were stale. The texture of all tail pieces held both at +10 and -15°F was soft to mushy, grading 60 - 71%.

The added phosphorus in the fillet averaged 0.1% wet weight, rising to 0.24% in the tail pieces. The sodium chloride varied from 1% wet weight in the fillet to 2.37% in the trimmings. Thaw drip was very low in all samples, polyphosphate dipped samples averaging slightly less than brine dipped fillets. The polyphosphate dip had no effect on total lipid, FFA, EPN and moisture.

SUMMARY NO. 11

QUALITY OF FRESH AND FROZEN FISH:
THE DENSITY OF UNTREATED AND
POLYPHOSPHATE DIPPED COD FILLETS.

W.A. MacCallum
Dorothy A.Chalker
J.T. Lauder
D.R. Idler

If the density of fillets were to increase or decrease significantly following dipping, the producer could conceivably overpack or underpack a carton of given volume. Actual densities of large, fresh, trawler-caught cod which were filleted and skinned, was determined by measuring displacement in water. Prior to this, respective samples were given a one-minute dip in (a) a 5% sodium tripolyphosphate solution, and in (b) a 9% sodium tripolyphosphate solution, and (c) a 20-seconds dip in NaCl brine plus a 1-minute dip in a 9% sodium tripolyphosphate solution. Other fillets were untreated. Results were consistent and densities were affected little by the polyphosphate solution added. Fillets of treatment (b) were increased in density over untreated individuals by a maximum of about one-half of one per cent and on the average of about one-quarter of one per cent only.

SUMMARY NO. 12

TRAP COD:
LIVE CARRYING FOR
IMMEDIATE PROCESSING.

W.A. MacCallum
Dorothy A.Chalker
J.T. Lauder
D.R. Idler
P.H. Odense

The possibility of benefits from handling trap cod alive for a few hours to ensure controlled conditions of killing, processing and freezing has been suggested. Data obtained in 1963 in continuing studies indicate that no benefits arise from such handling.

On July 11, 1963, fish were obtained and held alive in floating crates and later on board the research vessel INVESTIGATOR, which was anchored near several traps off St. John's. The fish in Series 100 to 400 were all frozen pre-rigor. Treatments were as follows:

Series 100 - The fish were sacrificed and gutted after 2 1/2 hours live holding. Processing and freezing (aboard ship) started immediately.

Series 200 - These samples were sacrificed, gutted and iced, after having been held 2 1/2 to 3 hours alive. They were processed and frozen after 7 hours on ice.

Series 300 - The live fish were sacrificed and gutted 2 1/2 to 3 1/2 hours after capture. They were held uniced at an ambient temperature of 65°F for about one hour, then iced and held for about 4 hours. The fish were then processed and frozen.

Series 400 - These fish were held alive about 5 1/2 hours, then sacrificed, but not bled or gutted for about 1 hour at 65°F ambient. They were then eviscerated, iced and stored for about 3 hours. Processing and freezing followed immediately.

Taste panel grades on samples held at -12°F for up to 6 months were much alike, and at no time was the grade high for fish of any treatment. Nothing in the results indicates that live carrying for a few hours contributed to improved storage quality which was little better than acceptable.

In regard to mortality, great difficulty was experienced in landing a substantial proportion of St. John's trap cod alive. Some thought should be given to the establishment of ratio of dead to live fish in commercial handling.

SUMMARY NO. 13

TRAP COD:
HOLDING COD ALIVE FOR SUBSEQUENT PROCESSING.

W.A. MacCallum
Dorothy A. Chalker
J.T. Lauder
D.R. Idler
P.H. Odense

It was stated in Summary No. 46 of last year's report that trap cod held alive for 25 and 58 days in 35 and 38°F water respectively, had very good cold

storage qualities following sacrificing, processing and freezing. Grades of the freshly frozen fish were maintained after 4 months storage at -10°F, at which time the experiment was terminated. Fillets of fish which were sacrificed at the traps, then frozen and stored were somewhat but not significantly lower in quality than those prepared from live-held fish of the same lots. Because of the suggested benefits of live holding, further comparative studies were conducted in 1963. In these tests, frozen, stored, fillets of fish handled according to treatments A, B, C and D below, were examined. (Treatment A - fish held alive in sea-water for 15 days of which 6 were devoted to lowering water temperature to 32°F; treatment B - same as A, except 5 days were required to reach a final water temperature of 42°F; treatment C - fish held alive in sea-water for 13 days of which 6 days were devoted to raising the water to a final holding temperature of 52°F; treatment D - fish sacrificed, gutted and iced at sea). Fish of treatments A, B and C were sacrificed, gutted and iced for 24 hours, then processed and frozen in-rigor, following the method used with samples of treatment D.

Order of preference given samples held at -12°F for up to 6 months was: (1) - Treatment B; (2) - Treatment A; (3) - Treatment D; (4) - Treatment C. Live holding at 42°F without feed contributed significantly to improved texture and shelf life for at least 6 months. Live holding at 32°F appeared to improve freshly frozen quality mainly. Three- and six-month stored samples were not particularly good. None of the samples prepared from 52°F live-held cod were good. They were inferior to those prepared from fish sacrificed at the traps.

The investigations were extended to include fall-caught Newfoundland cod which, after capture on October 3, 1963, were put in equal numbers in two sea-water tanks at 42°F. The temperature of one tank was changed to 35°F over a period of 5 days, that of the second to 52°F. The fish were then sacrificed, gutted, iced for 24 hours, filleted and frozen in-rigor. Taste tests have been completed on samples stored for zero time and for 3 months. At the start, samples were not significantly different in texture but those prepared from 35°F held fish were significantly better in taste. After 3 months storage, there was no difference in texture, taste or in overall grade in fillets of fish held alive at the two temperatures. Grades at 3 months (73 to 74%) were sufficiently high to suggest that the fish have quite good storage potential; probably somewhat higher than that for the average run of trap fish. When available, results of 6 months storage tests will be examined with interest. Meanwhile, present data would suggest that trap fish have lower potential for good storage quality than the fall fish, and this potential can be altered to a

greater degree by live holding at a low temperature (e.g. 35 to 42°F) than is the case with the fall catch.

Technical assistance was given by Jean Gibson, Bevin LeDrew, Gerald Curnew and Winston Barzell.

SUMMARY NO. 14

TRAP COD:
REFRIGERATION AND HANDLING IN RELATION TO CONTROL OF STATE OF RIGOR FOR FREEZING.

W.A. MacCallum
Dorothy A.Chalker
J.T. Lauder
D.R. Idler
P.H. Odense

Previously gathered data showed that eviscerated cod, iced at the traps and held in ice 24 hours prior to in-rigor freezing, could produce fillets with equal or better texture qualities than those from some iced samples frozen pre-rigor and from others frozen post-rigor. However, results of recently completed (1963) experiments with iced and uniced fish from the traps and with fish sacrificed from live holding tanks do not show that in-rigor frozen samples of trap cod were preferred. However, downgrading was for different reasons. Pre-rigor frozen samples were mealy and in-rigor and post-rigor frozen samples chewy. On the other hand, in-rigor frozen fillets of fall-caught cod, handled as described in Summary No. 13, were greatly preferred to pre-rigor frozen fillets of the same catch (when tasted soon after freezing). These investigations will be the subject of a published report.

Technical assistance was given by Jean Gibson.

SUMMARY NO. 15

TRAP COD:
TEXTURE OF PRE-RIGOR FROZEN COD.

W.A. MacCallum
Dorothy A.Chalker

A continuation of studies in 1963 on the texture of Newfoundland frozen cod showed that mealy characteristics as well as toughness can contribute to poor grades. This undesirable feature of texture was associated with pre-rigor handling, particularly with samples tasted within a few weeks of freezing. The effect, not previously reported in Newfoundland production, but recognized in the fresh fish trade, was observed in fish sacrificed from both the traps and the tanks. Special panels, which included representation from Halifax, were conducted to assess the scope and significance of mealiness. Samples with this characteristic crumble in the mouth and are dry and lack flavour.

SUMMARY NO. 16

TRAP COD:
RATES OF THAWING ON COOKING.

W.A. MacCallum
Dorothy A. Chalker

Since all taste panel results reported to date on Newfoundland cod have been obtained from rapidly-thawed samples (on cooking), it is important to learn something of the effect of speed of thawing on texture. Therefore, exploratory studies were conducted in 1963. Only two tastings, one at zero time, the other at 6 weeks, on slow and fast (on cooking) thawed samples have been completed. Results are, of course, not conclusive and the work is to be continued.

SUMMARY NO. 17

TRAP COD:
GLYCOGEN CONTENT IN TRAP FISH.

J.T. Lauder

Where experimental conditions were comparable, results on control samples and on samples from fish held alive confirmed those reported in Summary No. 23 of the Annual Report for 1962-63 on the Atlantic Technological Research Program. Initial values of 163 mg% (pH 6.48) were found in fillets frozen pre-rigor and low values of 32 mg% (pH 6.44) in fillets frozen after the fish had been iced for over 2 days. A value for uniced trap cod, not previously reported, was 35 mg% (pH 6.52). In frozen samples from fish held alive at 52°F, then sacrificed and processed, low values of 30 mg% (pH 6.40) were obtained (fish stowed 24 hours in ice subsequent to killing). Samples from fish handled similarly after being sacrificed from 32 and 42°F live holding tanks had glycogen contents of 160 (pH 6.49) and 147 (pH 6.37) mg%, respectively.

Technical assistance was given by Gerald Curnew and Bevin LeDrew.

SUMMARY NO. 18

TRAP COD:
THE REDUCTION OF GLYCOGEN IN
COD PRIOR TO KILLING.

W.A. MacCallum
G. Curnew
Winston Barzell
J.T. Lauder
D.R. Idler
P.H. Odense

Port-mortem glycolysis in fish fillets produces acid which lowers the pH of the fillet and may accompany a lowering in quality of the frozen product. In an endeavour to control post-mortem pH the experiments described below were carried out in 1963. Two studies are described; one involving the icing of live fish, the other the exhaustion of live fish held in sea-water.

Fish Iced Alive

Six fish were gutted and iced at the trap and six others were buried alive in ice. The live fish put up almost no struggle. Six hours later pH readings were taken on all fish and the iced-alive fish were gutted. The stomachs of half of the fish, including those gutted at sea, were partly filled with capelin, the others were empty. All samples were re-iced and examined for pH at 13 1/2, 39 1/2 and 54 1/2 hours post-mortem age.

The pH of all iced-alive fish dropped significantly, 5 of the 6 fish having an average pH of 6.1 at 40 hours. One fish had a pH of 6.35. Five of the 6 gutted-at-sea fish experienced the expected pH drop to about 6.1 - 6.2; one fish showed little change. Glycogen content of three of the iced-alive fish, including the individual with the pH reading of 6.35, was determined. Results were 146, 260 and 171 mg%. The struggle put up by trap fish iced alive was insufficient to deplete the glycogen content in the muscle; hence little benefit to frozen storage quality of fillets would be expected.

SUMMARY NO. 19

TRAP COD:
EXHAUSTION OF FISH IN SEA-WATER.

W.A.MacCallum, G. Curnew
Winston Barzell, J.T.Lauder
D.R. Idler, P.H. Odense

Trap cod, held alive for about two weeks at 38°F without added feed, were used. Electric stimuli were used on individual fish; the latter served as one terminal for the flow of pulsating D.C. current (6 volts). The fish were shocked twice every 10 seconds for a 10 minute interval, then once every 10 seconds for an additional 5 minute period. There was some evidence that the cod became conditioned to the shocks after 15 minutes, so manual stimulus for an additional 10 minutes was practiced on some individuals. Following exercising the cod were killed, gutted, pH taken and the samples iced. Thereafter glycogen and pH readings were taken periodically. Fish shocked electrically showed very small drop in pH following icing while the drop in control samples was substantial. Combined electric and manual treatments causing exhaustion had the desired effect as well, since the pH of samples thus treated did not drop. The pH of fish kept alive for 1 1/2 hours following exercising dropped very little and that of the unexercised control dropped significantly. Four hours after icing, the glycogen content of exercised and exercised-rested samples was 70 and 72 mg% respectively, and that of the unexercised fish 2 1/2 times as great. It would appear that stimuli of this nature are adequate to provide exercised fish for further experimental studies of the effect of muscle pH on frozen quality and shelf life.

SUMMARY NO. 20

TRAP COD:
POLYPHOSPHATE TREATMENT OF FROZEN COD. TASTE PANEL EVALUATION, CHEMICAL ASSESSMENT AND THAW-DRIP IN ONCE-FROZEN FISH.

W.A. MacCallum
Dorothy A. Chalker
Jean Gibson
Bevin LeDrew
J.T. Lauder
P.H. Odense
D.R. Idler

Summary No. 50 of the 1962-63 report described the results of chemical and physical assessment of Newfoundland cod fillets treated with sodium tripolyphosphate used to "lock in" flavours, to control drip and to increase yield in fillets. An account prepared for publication is now in Press. However, it was important in the case of polyphosphate treatment to obtain judgment in terms of taste and texture of the fillets and loss of nutrients to the drip, since in the last analyses these factors are of paramount importance to consumer and producer alike. Accordingly, in 1963 paired fillets of selected male trap cod of 57 to 59 cm average length were dipped in 10.7% sodium tripolyphosphate and in plain water. The single fillets were packed one to a waxed paper carton and the latter overwrapped in Saran, sealed, frozen and stored for up to 6 months.

Evaluation of all phases, except amino acid determinations has been completed and the work is being prepared for publication. Treated fillets were significantly less chewy than untreated controls and developed significantly less drip. Results indicate that treated fillets could have greater market acceptance than the untreated pack.

SUMMARY NO. 21

TRAP COD:
DRIP-THAWING TIME RELATION IN FILLETS OF POLYPHOSPHATE-TREATED AND UNTREATED FILLETS.

Dorothy A.Chalker
W.A. MacCallum
Jean Gibson
D.R. Idler

A number of technical questions were answered in connection with polyphosphate treatment of trap cod fillets prior to freezing. The first, reported here, deals with the form of the drip-time curve when portions of frozen fillets are allowed to thaw in air at 2-3°C. In effect, it was desirable to know if the pattern of the amount of drip collected from treated fillets over a 48-hour period was comparable to that for untreated samples.

Fillets from one side of a number of fish of uniform size and sex were dipped according to procedures laid down by Gorton's of Gloucester while those from the other side of the same fish were untreated. Tail and nape portions were excluded from the 12 oz. fillet packs which were plate frozen and stored

at -15°F. Samples were examined for drip within a few days of freezing. Duplicate determinations were made.

The rate at which the treated and untreated samples dripped was found to be the same: it depended only on the dimensions of the piece of fish thawed. All drip was yielded in 48 hours but the volume of drip collected between any two shorter periods of thawing was significant. In summary, the drip-time curves for treated and untreated fillets assumed the same form, the drip collected rising rapidly in initial stages of storage at 2-3°C and then tapering off. Thus, the technique for collecting drip over a 48-hour period at 2-3°C may be used in comparing drip from treated and untreated samples without in any way prejudicing the case of the treated as against the untreated pack.

Technical assistance was given by Gerald Curnew and Winston Barzell.

SUMMARY NO. 22

TRAP COD:
A COMPARISON OF TWO
METHODS OF ASSESSING DRIP.

Dorothy A. Chalker
W.A. MacCallum
Jean Gibson
D.R. Idler

U.S. Fish and Wildlife inspectors use one method to measure thaw-drip while the Board laboratories (on the East Coast) use another. The former thaw a given weight of fish (50 - 100 gm was used to compare directly with our method) in a closed plastic bag in 70°F water. In this method the fish comes into contact with the drip, and can therefore reabsorb it. The Board uses a method whereby 50 - 100 gms of fish are left in a closed system at 2-3°C for 48 hours and the drip as formed is collected apart from the fish (Summary No. 21). Hence, no reabsorption can occur.

Little difference was found in the amount of drip experienced using the two methods. It appears that the reabsorption that occurs at the higher temperature could be equated with the amount of drip retained at 3°C since in both methods the fish is completely thawed.

SUMMARY NO. 23

DISEASES, NUTRITION AND PARASITISM:
LOBSTERS.

James E. Stewart
John W. Cornick

Epidemics of Gaffkaemia (a fatal bacterial infection of lobsters) occur periodically. This investigation is concerned with the prevention, prediction and/or control of this disease. One phase of the study concerns the natural and inducible defences against disease possessed by the lobster.

An animal's defences against diseases can be separated fairly logically into three general categories: (1) Physical barriers, (i.e. skin or integument), (2) Non-specific responses (i.e. bactericidal or bacteriostatic properties of normal blood plasma or serum and phagocytosis of the infective agent by certain of the blood cells), (3) Induced specific responses (i.e. production of a specific antibody after infection or injection with a particular infective agent or vaccine).

(a) Non-Specific Defences. Lobster haemocytes (blood cells) are thought to be phagocytic (engulf and/or destroy foreign particles such as bacteria) and they are also involved in blood clotting. Thus it is important to know the lobster blood cell counts and the effect of captivity, feeding, starvation, infection, immunization and other factors on the numbers of cells.

Technical problems, such as blood clotting, agglutination of cells and precipitation in the suspending medium, have been overcome and suitable conditions have been found to allow the use of an electronic counter for obtaining rapid and accurate lobster blood cell counts. A correlation coefficient of 0.98 compared with haemocytometer counts has been established. This method has been applied to a study of lobsters held captive and the results compared with lobsters allowed to remain in the natural environment for 30 days longer. In addition, the blood protein values of the different sets of lobsters were compared.

Lobsters left in the natural environment had significantly higher blood protein and blood cell values than those held in captivity and fed. Those held in captivity and starved had much lower cell counts and blood protein values than the captive fed group at the end of a thirty day period. The reduction in cell counts and blood protein values at the end of a 60 day period bordered on the spectacular for the captive starved group. Electrophoretic studies show that the reduction in blood protein affects all protein components rather than any single component. Sizing of the blood cells also indicates an overall reduction rather than a reduction in any special group of cells.

Previous work would indicate that the ability to prevent serious bleeding would be poor to non-existent in the starved captive group, mediocre in the fed captive group, and good in the group left at sea. Furthermore, the physiological condition of the lobsters, including their resistance to disease should follow a similar pattern.

The food used in these studies was whole sardine herring, a material commonly used commercially for feeding captive lobsters. The results show

conclusively that this is a less than complete diet, although it is better than nothing. The incompleteness of the diet was further demonstrated by the addition of raw beef liver to the diet. A complete reversal of the declines in blood cell numbers and protein levels was noted. Injections of the vitamin thiamine, a suspected dietary deficiency, did not show the same effect. This study to date points up the need for a thorough nutritional study of a lobster's food requirements. It is possible that many of the problems connected with holding lobsters in captivity, including diseases, may stem directly from nutritional deficiencies.

(b) Specific Defences. Work on the resistance to Gaffkya infection by lobsters and the properties of normal and "immune" lobster sera has been initiated.

SUMMARY NO. 24

DISEASES, NUTRITION AND PARASITISM: James E. Stewart
John W. Cornick

LOBSTER MEAT YIELD.

The meat yield (claw plus tail meat) from lobsters held in captivity and starved for five months was approximately 10 per cent of the live whole lobster weight. The group fed on herring for the same period had approximately a 25 per cent meat yield. The females in the starved group had a slightly higher meat yield than the males. This may have been due to their absorption of the unlaid eggs which constitutes an additional reserve food supply.

SUMMARY NO. 25

DISEASES, NUTRITION AND PARASITISM: James E. Stewart
John W. Cornick

TOXICITY OF WESTERN CEDAR EXTRACTS TO LOBSTERS.

An inquiry arising from excessive losses of lobsters held in a tank constructed of cedar prompted this investigation.

Salt water extracts of wood sawdusts, including black spruce, Eastern fir, Douglas fir, Western cedar, redwood and Tennessee cedar, were added to tanks containing healthy vigorous lobsters. Seventy-five per cent of the lobsters in the Western cedar extracts died within six days and the remainder were extremely weak. No deaths occurred, nor was any weakness apparent among the lobsters held in the other extracts or among the controls. It was concluded that Western cedar contains a sea water extractable material toxic to lobsters and that it is inadvisable to build lobster tanks using this wood. The redwood extract heavily stains the lobsters but seems to have no other ill effect.

SUMMARY NO. 26

DISEASES, NUTRITION AND PARASITISM:

James E.Stewart
John W. Cornick

COLLABORATIVE STUDY WITH ST. ANDREWS STATION ON MOULTING.

Blood cell counts, Gaffkya homari survey, and blood protein levels were checked February 10-15, 1964, on a group of approximately 224 lobsters held at St. Andrews. These lobsters are being used in moulting temperature studies by the Lobster Investigation at St. Andrews. The purpose of the collaborative work is to attempt to find some correlation between diet, blood cell and protein levels, and moulting rates. A second similar check on this same group of lobsters will be made in April 1964, the results analysed and conclusions drawn at that time.

SUMMARY NO. 27

DISEASES, NUTRITION AND PARASITISM:

M.F. Li
James E. Stewart

PRELIMINARY RESULTS IN THE DEVELOPMENT OF A TISSUE CULTURE METHOD FOR THE STUDY OF DISEASES OF OYSTER (CRASSOSTREA VIRGINICA).

The possibility of farming oysters in the southern Gulf of St. Lawrence is being enhanced by the establishment of an oyster hatchery at Ellerslie, P.E.I. This hatchery will supply seed oysters to potential oyster farms. However, before this program can be soundly based or truly successful, the problems of prevention, prediction and/or control of oyster diseases must be mastered. The outstanding disease commonly called "Malpeque Disease" has had disastrous effects on oyster stocks, with mortalities reliably reported to be 95% or greater in most oyster beds. At present there is a lack of information, not only on this disease but on all shellfish diseases. A program on oyster diseases was initiated in March, 1963 to supply much needed information regarding oyster diseases in general and Malpeque disease in particular.

The available information indicates that it is possible that a virus could be involved in Malpeque Disease of oysters. If this is the case oyster tissues should be among the best tissues for propagating this particular infective agent. Little information concerning the in-vitro cultivation of marine invertebrate tissue is available even though the techniques of cell and tissue cultures for vertebrate tissue, especially that of mammals, have advanced rapidly in the past few years.

Explants from oyster heart have been cultured in a medium composed of Medium 199* minus sodium carbonate, plus 50% Hank's balanced salt solution, 10% human serum, 10% amniotic fluid, 5% fetal calf serum, 5% bovine embryo

extract, and 1% glutamine. Vigorous and rhythmical contractions persist for several months in the explants held in this medium. Amebocyte-like cells migrate from the heart explant and proliferate into spindle forms in the "low" pH medium after incubation for 24 to 48 hours at 18°C. In addition, outgrowths from the explant have been noted.

When the necessary preliminary studies on oyster tissue culture are completed the tissue will be used in studies of the infective agent and in diagnostic procedures.

* A concentrated Medium 199 (Medium 199 10x) was used. The addition of other supplements are listed in terms of their final concentration in the medium.

SUMMARY NO. 28

DISEASES, NUTRITION AND PARASITISM:
SEROLOGICAL STUDIES ON THE DISEASE SUSCEPTIBLE AND THE DISEASE RESISTANT OYSTERS.

M.F. Li
James E. Stewart

It has been known for a considerable period that the oysters from certain areas, such as Cape Breton, are highly susceptible to Malpeque Disease, and that the oysters from Malpeque Bay apparently are resistant. If there is any difference in antigenic properties between the disease susceptible and disease resistant stocks, and if this difference could be demonstrated by serological methods it would be of great value in the oyster disease studies and the hatchery program. This technique would permit an early segregation of the two stocks and allow the selection of disease resistant progeny for farming and aid in the development of a totally disease resistant stock.

Sera from disease susceptible oysters from Cape Breton and the disease resistant oysters from Malpeque Bay were prepared by centrifuging blood which was obtained from the oyster's heart. The antisera against the serum of each oyster stock were produced in healthy, adult rabbits. The "ring" or interfacial tests were employed for the serological studies. The results of cross typing reactions, shown in the following table, indicate that the resistant stock from Malpeque Bay possesses at least one antigen different from those demonstrated by the susceptible stock from Cape Breton. The significance of this finding is still to be determined by further investigation.

Antigen and Antiserum Titers of Both Homologous and Heterologus Reactions in the Disease Susceptible and the Disease Resistant Stocks of Oyster.

ANTIGENS	RING TEST BY ANTISERUM	
	C	M
C	2560	5120
M	5120	1,210,720

C = Cape Breton Stock (The susceptible stock)
M = Malpeque Bay Stock (The resistant stock)

SUMMARY NO. 29

DISEASES, NUTRITION AND PARASITISM:
A QUANTITATIVE STUDY OF THE EFFECTS OF NATURALLY OCCURRING SUPPLEMENTS ON THE GROWTH OF RAINBOW TROUT (SALMO GAIRDNERI) GONADAL CELLS.

M.F. Li
James E.Stewart
S.J. Hazelden

Rainbow trout gonadal tissue has been used to a considerable extent in viral diagnostic and descriptive studies. Attempts are now being made to use this tissue in a study of the infective agent of Malpeque Disease of oysters. A prior study qualitatively and quantitatively of the effect of various naturally occurring supplements on cell growth and appearance was essential.

The relative growth-promoting properties of animal sera, embryo extract and body fluid, and their effects on cellular morphology were tested on gonadal cells of rainbow trout. The cells were cultured in Leighton tubes at 18°C. The initial cell density was approximately 3.3×10^4 cells/ml, and duplicate tubes were employed for each test medium. At the end of the growth period the cells were counted using an electronic cell counter.

There was no significant difference among human serum, bovine serum, and ascitic fluid on the overall multiplication of the cell in the 12-day experimental period when these supplements were added at a 10% level to Medium 199* which also contained 5% fetal calf serum, 5% bovine embryo extract, and 1% glutamine. However, in the presence of ascitic fluid in the medium an early growth response of the cell and certain changes in cell appearance were noted. A deletion of any of the naturally occurring supplements used in the test medium would result in a decrease in the proliferation of the cell. Little or

no growth was observed in the medium in which the naturally occurring supplements were deleted. The results would suggest that Medium 199 supplemented with 5% fetal calf serum, 5% bovine embryo extract, 1% glutamine, and either 10% human serum or bovine serum would be suitable for the proliferation of normal appearing gonadal cells of rainbow trout.

* A concentrated Medium 199 (Medium 199 10x) was used, so that the addition of these supplements was at the expense of the diluting solution (water) and would not affect the final concentration of Medium 199 in the test medium.

SUMMARY NO. 30

DISEASES, NUTRITION AND PARASITISM:

EXOPHTHALMUS (POPEYE) IN COD

K. Ronald
B. Beaton
Heather Macnab

Preliminary experimentation has already shown that extrusion of the eye, associated with the "pop-eyed" condition of captive cod, was correlated with an increase in both gas and fluids in the intraocular spaces.

Surgical procedures, such as complete removal of the pseudobranch, prevented the appearance of the exophthalmic condition. The fish, however, did not survive a post-operative period of more than 41 days. Consequently an attempt was made to control the activity of the pseudobranch by ligaturing themandibularis afferens and efferens, the main blood vessels leading to and from the pseudobranch. Both "pop-eyed" and normal fish were treated by tying off the vessels, dorsal, ventral and dorso-ventral to the pseudobranch. Intercorneal distances and eye diameters were measured regularly over a period of one month. No change was noted in any fish. The nylon ligature did, however, cut through the blood vessels and surrounding tissues causing haemorrhage and necrosis.

To prevent subsidiary tissue damage the pseudobranch was exposed for varying periods (5-100 secs.) to a minus 70°C source. This low temperature surgery produced first a superficial blister followed by scar tissue formation, but no change in intercorneal distance or the associated exophthalmus.

Fish were obtained by various capture methods, otter trawl, hook (jig and long-line) and trap. Over a period of 6 months only the trap caught fish did not exhibit pop-eye. Hypothetically, the trap caught fish were not exposed to a sudden change in environmental conditions, a condition that must trigger the mechanism responsible for exophthalmus in fish captured by the other high stress methods.

SUMMARY NO. 31

DISEASES, NUTRITION AND PARASITISM:
PARASITISM AND THE "NORMAL" COD.

K. Ronald
Heather Macnab
Barbara Beaton.

The presence of a parasite is often taken as an indication of pathology or disease. As we cannot justify this belief, a study of the effect of parasitism on a host has some importance. A study has been started to evaluate the effects of parasitism on the normal physiology of the Atlantic cod.

One of the most stable and easily handled of the tissues is blood. Blood was therefore used to measure changes that might occur in parasitized cod.

The normal patterns have been established over a period of one year, in the greater part by sampling an inshore cod population monthly.

THE STANDARD COD

It was first necessary to establish the osmotic fragility of cellular components of the blood as "normal" physiological saline (0.85% sodium chloride) rapidly causes haemocytolysis of the cod's erythrocytes. The fragility of the cells was evaluated in various saline concentrations of from 0.6 to 1.6% by weight of sodium chloride. The cells were allowed to stand in the saline for up to one hour. Every 10 minutes the cell number was counted, and the cell size and distribution plotted on a Coulter Counter.

The cells haemolyzed rapidly at the lower concentrations (1.5 million down to 0.5 million/cubic mm. in one hour) while at 1.6% the counts dropped 10%. The final isotonic concentration was found to be 1.15%, where a reduction of only 3% occurred over a four hour period.

Red Blood Cells

The mean number of erythrocytes per fish was established over a one year period from over 300 fish at 1,618,000/cubic mm. There was an increase in cell number before and during the spawning period (April and May) which gradually declined to a low point during June, July and August. In September the counts started to increase once again. This is somewhat the opposite condition to that found in man. It is not surprising however, man being a placental mammal which has to show a large increase in volume of its circulatory system so as to include the closed foetal circulation, whereas the fish only dilates the gonadal blood vessels, so that any cellular increase would be more marked in the latter. There was no significant difference in the blood counts of the two sexes.

Packed Cell Volume

The PCV or haematocrit is a measure of volume of cellular material present in the whole blood. It is an indicator of physiological condition, and

the fish's ability to utilize respiratory oxygen. The mean figure was 30.5/100 ml of blood, or 30.5%.

This figure is low compared to that of mackerel (50+%), an indication of the different potential activity rates of the two species. The PCV of the male did not differ greatly from that of the female.

Mean Corpuscular Volume

The volume was measured using Winthrobe's formula

$$MCV = \frac{PCV \times 10}{\text{Erythrocytes (million mm}^{-3})}$$

The figure for the normal cod was established as 196.0 cubic micra. This is much lower than in elasmobranchs, which is as would be expected because in general the higher the evolutionary position of the animal the smaller the erythrocyte. The MCV of the female cod's blood was 2% higher than that of the male.

Haemoglobin

The cyanmethemoglobin method was used to measure the quantity of respiratory pigment present in the erythrocytes. In general the higher the haemoglobin concentration the greater the activity potential of the animal. The cod with a level of 5.5 g/100 ml. of blood falls between the pleuronectids and the hakes. The mackerel with a 15.0 g/100 ml. level is one of the richest haemoglobin carriers among the vertebrates.

There was no significant difference between the level of haemoglobin in the male and female cod.

Mean Corpuscular Haemoglobin

MCH is the expression in absolute units of the average weight of haemoglobin contained in an erythrocyte. It is found by dividing the number of grams of haemoglobin present in 100 ml of blood by the number of erythrocytes in millions/cubic mm. This factor is increased by a power of ten to give the average erythrocyte haemoglobin content in picograms. A low MCH is therefore an indicator of an anaemic condition.

The cod has a MCH of 34.5 pg. As it also has a greater blood cell volume (MCV) than most mammals the erythrocyte must contain more haemoglobin (man, 29 pg).

Mean Corpuscular Haemoglobin Concentration

As the MCHC is calculated from the formula

$$MCHC = \frac{\text{Haemoglobin (g/100 ml)} \times 100}{PCV}$$

it gives the average concentration of haemoglobin in each erythrocyte. Any large variation from the normal concentration (18.2%) reported here would indicate that a hypochromic condition exists if the erythrocytes were still of normal size.

As the normal value of MCHC is also the maximum value of haemoglobin molecules that can be carried by the normal erythrocyte, any increase is an indication of a macrocytic condition.

It is interesting to note that the mackerel's MCHC is 50% higher than the cod's, again an indication of the activity permissible in some of the pelagic forms.

Hydrogen Ion

Perhaps one of the most stable of all indicators in the blood system of the normal animal is the acid-base value.

The normal figure is hard to obtain, as it soon becomes apparent that fish held in confined quarters (shipping tank, etc.) show abnormally low figures(6.95), whereas in these fish that are sampled immediately the normal value is 7.235.

There is no difference in the pH of the two sexes.

Total Blood Volume

Three methods were used to measure the total amount of blood present in the cod. As there are variations in the size of the fish the final figure was calculated as a percentage of body weight.

Exsanguination could not be completed even in those fish previously injected with massive doses of heparin.

Radioactive chromium and iodine was injected using standard human medicine techniques; however, the available blood volume was too low to allow accurate readings.

The most accurate method was found to be by introducing tagged serum proteins into the circulatory system of the cod. Plasma obtained by bleeding after the injection was read against pre-injected plasma and then the dilution factor so calculated.

Using the PCV the total blood volume could then be calculated. Twenty-three fish, ranging from 0.8 to 17.0 kg in weight were used to obtain a figure of 2.4 per cent of the body weight.

Parasitism and Blood Loss

Some of the larger parasitic Copepoda (L. branchialis) feed almost exclusively on blood. As these offer an ideal experimental infection for estimating parasite effect, cod with and without parasites were separated into fed and starved groups. Two different bleeding rates were used to see if there was any effect produced by the parasites. Over a period of 14 days 5 ml and 10 ml of blood was removed from each group. In the unfed fish a reduction of 80% of the erythrocytes and PCV occurred before death inter-

vened. This indicated a decrease in cell number without change in size. This was further substantiated by recording erythrocyte size and distribution on a Coulter counter plotter.

The fed fish showed a less marked decrease in cell number, dropping by only 35%.

Those fish that were parasitized did not die sooner than the non-parasitized, in fact, the last fish to survive a 10 ml daily extraction of blood was a parasitized specimen whose blood count had been reduced by 83.5%.

The white cell volume increased under extended bleeding from 0.5% to over 1% of total blood volume.

In order that the function of the spleen could be evaluated in haemapoiesis, splenectomized and non-splenectomized fish were exposed to 3-day interval bleedings of 0.5 and 0.1 per cent of their body weight. Over a period of thirty days, the intact fish survived a 120 per cent removal of their total blood volume. The splenectomized fish usually died before the 50% level had been reached. The spleen was functional in the cod's haemapoietic regulation.

In those fish bled at the 0.1% level there was an initial increase in the erythrocyte number, and these fish outlived those at the 0.5% level.

The blood counts dropped in moribund, non-splenectomized fish to less than 20% of the original number. In the splenectomized fish they approached the 35% level at time of death. Splenectomized fish also showed the greatest weight loss (up to 20%).

The pH remained constant throughout all the experiments, indicating that although the number of cells was being reduced the buffering action of the plasma had not changed.

SUMMARY NO. 32

BIOCHEMISTRY OF COD:
POST-MORTEM BIOCHEMICAL AND PHYSICAL
CHANGES IN COD MUSCLE.

Doris I. Fraser
H.Weinstein
W.J. Dyer
J.R. Dingle
J.A. Hines
J.T. Lauder

The post-mortem biochemical and related physical changes during rigor mortis are being studied to assess the effect of these important processes on the condition of the flesh prior to and through freezing on the subsequent storage life of the frozen product. Initial levels of metabolites of the glycolytic cycle, glycogen, lactate, and high-energy phosphate compounds, are being determined under various conditions of rest, exercise and diet prior to death, and their postmortem changes followed under various handling

conditions. Both commercially trawled and aquarium-held cod were used.

Trawled, offshore cod, taken in 20 min. sets during the March 19 - 22, 1963 cruise of the A.T. Cameron, contained negligible amounts of glycogen and high-energy phosphate compounds, and high levels of ammonia, indicating glycolytic activity accompanied by nucleotide dephosphorylation and deamination during the struggle involved in catching. No significant differences in the levels of metabolites were observed whether samples, frozen on board in brine at -12°C, were taken from fish immediately on emptying the trawl, from fish allowed to struggle to death in air, or held live for one day. Low lactate levels of 110 mg/100g may indicate depleted glycogen reserves in these fish caught during the spawning season. The findings indicate that in trawled fish the energy reserves of the muscle have become exhausted or nearly so during the struggling and handling involved in dragging and emptying of the trawl net. A paper concerning this work and that of glycolytic changes in Newfoundland trap-caught cod reported last year has been prepared for publication.

Feeding, aquarium-held cod were used to determine initial levels of metabolites under various conditions of rest and exercise prior to death. The muscular activity associated with vigorous exercise of these fish depleted the glycogen reserves to very low levels and caused dephosphorylation and deamination of the high-energy phosphate compounds, with accumulation of ammonia. The production of significant amounts of lactate, associated with loss of muscle glycogen, was, however, not apparent immediately after death, but was delayed into the early part of the post-mortem period. The same effect was observed previously in the brailed trap cod samples (Summary No. 23, Annual Report for 1962-63). In so-called "rested" cod, netted quickly with negligible struggling and killed immediately by stunning, variable initial levels of lactate in the order of 100-200 mg/100 g were found, as well as appreciable deamination as indicated by ultraviolet absorption maxima characteristic of inosine compounds and by increased ammonia levels. The content of energy-rich phosphate compounds was also lower than expected from previous work. On the other hand, it was later found that, in feeding cod unalarmed and completely at rest at death, initial lactate levels were practically zero; high initial levels of creatine phosphate and of other labile phosphate compounds and a low ammonia content substantiated the lack of glycolytic activity in these truly rested fish. Resting glycogen levels of up to 700 mg/100 g, higher than any so far recorded in literature, were found in some of these feeding cod immediately post-mortem.

In following post-mortem glycolytic changes, wide variations in metabolite concentrations were encountered which could be explained only by variation with anatomical position within the fillet. Investigations showed these differences to be rather large; in general, the highest glycogen levels were found in muscle from the central part of the fillet. Contrary to reports for some other species, the red muscle of cod was not richer in glycogen than the white, or ordinary, muscle.

The development of rigor mortis has been followed by observations on stiffness and by a penetrometer technique. While the various parts of the fillet develop varying degrees of maximum stiffness, the penetrometer curves appear to parallel each other, but the tail part remained much softer. Time to maximum rigor development at ice temperature is strongly dependent on the degree of struggling in the feeding aquarium-held cod used, although some variation occurs. In strongly exercised fish maximum rigor may occur in less than 12 hours, in comparison with about 24 hours in fish killed by stunning, and still longer, almost 2 days, in the fish where no glycolytic activity occurred prior to death.

SUMMARY NO. 33

BIOCHEMISTRY OF COD: SEASONAL VARIATION IN COMPOSITION OF TERENCE BAY COD.

P. Odense, W.Shinners, K. Ronald
H. Brockerhoff, R.J. Hoyle
H.C. Freeman, N.Dambergs, R.G.Ackman
W.J. Dyer.

Since November, 1962, the cod population at Terence Bay has been sampled at monthly intervals. The purpose of this sampling was stated in the last Annual Report: "..to test for physiological homogeneity and determine seasonal variation among the body constituents". The sampling period has been extended beyond the original one year period in order to obtain sexually mature specimens and has now been completed. The data collected included the length, weight, sex, state of maturity, colour, gonad and liver weights, otoliths, estimate of the number of flesh parasites and stomach contents of each fish. Data sheets have been given to each group participating in this study and this information will be correlated with the results of each group's own particular analyses.

Hematocrit, hemoglobin and blood coagulation time has been determined on each fish as well as the starch gel electrophoresis pattern of the muscle albumins and blood plasma proteins. A quantitative determination of the water soluble muscle proteins has been made.

Lipid analysis of the liver and muscle, non protein nitrogen and TMA levels of the muscle and blood steroids have been determined. For this group of analyses, pooled samples of four male fish of the same approximate weight, length and state of sexual maturity have been used as well as a similar pooled sample of four female fish.

The nutritional condition of the fish, as measured by the ratio; - liver weight/total weight, and by the percentage of fat in the liver, reaches a peak in the month of November. It then drops off sharply in December, levels off or drops slightly until April-May, and then starts to climb again. These observations may be correlated with stomach contents. The ratio; - weight liver/total weight varies from a low of 0.6×10^{-2} to a high of 7.6×10^{-2}. A fish 60 cm long and in good condition would normally have a value of $2.5\text{-}3.5 \times 10^{-2}$ The liver oil, as extracted with petroleum ether, varied from 10 to 75% of the liver. In general, the larger the fish the more fatty the liver.

The ratio of gonad weight to weight of fish is a good measure of ripeness. Immature and spent female fish have this ratio in the range $2\text{-}4 \times 10^{-3}$. Any value higher than 6×10^{-3} indicates ripening, and in large fish with ripe gonads, the range is $60\text{-}90 \times 10^{-3}$.

The lipid content of the flesh shows little variation, although a maximum occurs in November and in December and June. The unsaponifiable material in flesh lipid is also nearly constant at 6-10%. The lipid contents of the gonads examined showed considerable variation, ranging from 0.6 to 2.3%. The unsaponifiable matter in this lipid varied from 9 to 21%. Eventually, detailed analyses of fatty acid composition and phosphorous content may show correlations not at present obvious. To ensure this an extension of the time alloted to this study to permit an overlapping period has been arranged.

SUMMARY NO. 34

BIOCHEMISTRY OF COD:
SPECIES IDENTIFICATION:
VERTICAL STARCH GEL ELECTROPHORESIS
OF FISH PROTEINS.

P.H. Odense
C.W. Shinners

The vertical starch gel electrophoresis runs of the muscle albumin extracts of various fish species have been extended to include some forty species of fish. In all cases so far examined the patterns have been characteristic of the species.

Experiments with various other gel media and buffer systems have been carried out. None have given better results than the conventional vertical

starch gel electrophoresis procedure of Smithies. One of several commercial starch products produced a resolution comparable with that obtained by Smithies procedure, but the rest were not as good. The addition of 2-mercaptoethanol or cysteine to the gel or extract has been advocated by some for improving the resolution of protein mixtures, but it had no discernable effect upon fish muscle extracts.

Resolutions obtained by the use of the disc electrophoresis system were not as good as the starch gel procedure. It is necessary to carry out the electrophoresis of fish extracts in the cold room and perhaps optimum conditions have not been established for fish tissue extracts.

European workers have reported two hemoglobin types among North Sea cod. Preliminary studies of cod blood samples from Terence Bay have revealed only one hemoglobin type.

Drip samples have been studied and as in the case of extracts from freeze dried cod, the patterns have clearly resembled the normal muscle albumin patterns of cod, thus indicating that the major protein components of drip are the muscle albumins.

SUMMARY NO. 35

BIOCHEMISTRY OF COD:
SPECIES IDENTIFICATION:
IMMUNOELECTROPHORESIS OF FISH PROTEINS.

P.H. Odense
C.W. Shinners
S. Lee

Starch gel electrophoresis of the muscle and blood proteins from different populations of both herring and alewives revealed more pattern differences between individuals of the same population than between the different populations. For this reason a different attempt has been made in a study of cod populations.

Cod blood sera and muscle albumin extracts were obtained from cod caught at Terence Bay. One set of rabbits received a course of injections of the cod blood sera, another a set of injections of the muscle albumin extracts. The sera from these rabbits was used in immunoelectrophoresis runs on cod blood sera and cod muscle albumins. Ten precipitin bands were obtained in the immunoelectrophoresis run of the cod sera. Fewer and more diffuse precipitin bands appeared in the albumin runs. These "standard" cod antisera will be used in immunoelectrophoresis runs on cod blood samples kindly provided by scientists at the St. Andrews Biological Station. These samples have come from cod from different populations and data such as the sex, maturity, size, etc. are known for each fish. It is hoped that this knowledge will make it possible to distinguish between precipitin bands characteristic of the different populations

and those bands which might be characteristic of such factors as sex, or state of maturation.

SUMMARY NO. 36

BIOCHEMISTRY OF COD:
SEPARATION OF NUCLEOSIDES AND NUCLEOTIDES.

R. Guilbault
P.H. Odense

Certain nucleotides play an important role in post-mortem deterioration of fish and other nucleotides and nucleosides are sensitive indicators of post-mortem change. It is therefore desirable to have a rapid method of analyzing for these substances.

A very good separation of the nucleosides and nucleotides in fresh cod has been obtained by the combined use both of thin layer chromatography and thin layer electrophoresis. The separation was made in a relatively short time compared to previous work made with column chromatography.

Seven compounds, adenosine, inosine, adenosine monophosphate, adenosine diphosphate, adenosine triphosphate, inosine monophosphate and coenzyme I (diphosphopyridine nucleotide) have been completely separated with two dimensional runs on thin-layer plates. After several trials, it was found that the combination of isobutyric acid, ammonium hydroxide and water (66 : 1: 33) as solvent system and MN-cellulose powder (300 diethylaminoethyl) as adsorbent, give the best results.

Following chromatography the thin layer electrophoresis separation has been performed with citrate buffer (0.05 M) at pH 4.8 and at 400 volts. These separations were carried out with standard compounds. Initial trials indicate that these compounds can be separated in the fish muscle extracts.

SUMMARY NO. 37

BIOCHEMISTRY OF COD:
EFFECTS OF TRIPOLYPHOSPHATE AND ADENOSINETRIPHOSPHATE ON MUSCLE PROTEIN EXTRACTS.

J.R. Dingle
J.A. Hines

By observing the effects of tripolyphosphate on the properties of muscle protein extracts some light might be shed on the mechanism of its action on intact muscle. A comparison with the action of the natural muscle triphosphate, ATP, could also be instructive.

Like ATP, sodium tripolyphosphate reduced the viscosity of myosin B solutions at high ionic strength (0.6), suggesting a possible dissociation into the component proteins myosin and actin. Unlike ATP, the tripolyphosphate was not dephosphorylated by myosin B, but did not appear to compete with ATP for the enzyme sites since it did not affect the dephosphorylation of ATP.

by myosin B ATPase. The effects of the two polyphosphates on the solubility of myosin B at various ionic strengths also differed. Sodium tripolyphosphate had no significant effect on the solubility whereas ATP lowered the "salting-in" concentration of the protein. The dependence of both the solubility of myosin B and of the physical appearance of the precipitated fraction on ATP concentration as well as ionic strength indicated a considerable complexity of interaction between ATP and the protein.

Evidence so far suggests that tripolyphosphate acts on muscle by preventing or disrupting a combination of the contractile proteins, actin and myosin.

SUMMARY NO. 38

MARINE HORMONES:
LOW-TEMPERATURE STORAGE OF SALMON SPERM.

Beryl Truscott
D.R. Idler
H.C. Freeman

Preliminary studies on the storage of sperm indicated that individual variations occurred in the storage life of sperm samples, and that a good sample mixed with a bad sample produced a bad sample. Sperm freezing methods used routinely in the dairy industry proved to be unsuccessful when applied to samples of salmon sperm.

This year the experimental work was designed to confirm or deny the conclusions indicated by the results of 1962; to investigate methods, other than fertility, of checking the viability of a sperm sample and to find, if possible, the conditions required to freeze salmon sperm cells for long term storage.

1. Individual Variation of Sperm Samples

Sperm was collected from 12 mature fish, 3 of them large, 6 small, and 3 smolt-size. Precautions were taken to exclude all water from the sperm samples. The fish were tagged during the first collection of samples (Series A) at the beginning of the spawning season. Sperm samples were taken from the same 12 fish after 1 week (Series B) and after 2 weeks (Series C), the end of the 1963 spawning season. All samples were stored in screw cap vials at 2°C, and protected from direct light.

The eggs required for fertility tests were collected from at least 2 females each time, were mixed thoroughly, transported, and stored at 2°C in screw cap bottles.

At zero storage time, there was no significant difference in fertility rates of individual fish, large or small. Series A, however, were 20% lower than Series B or C. This difference may or may not have been real; fertility tests at zero time were done at the hatchery and the fertilized eggs were

immediately transported to the laboratory hatchery, a practice avoided by fish culturists. Precautions against mechanical injury were taken but it is conceivable that the 2nd and 3rd trips were smoother than the first.

After storage in a closed system, all sperm samples of Series A, with one exception, maintained their fertility in varying degrees (20 - 80%), and for varying lengths of time (2 - 5 days). Samples of Series B and C, similarly stored, were not fertile after 24 hours. However, 4 samples of Series C stored in open tubes for 5 days gave a fertility rating of 80%. In Series B and C, all samples of small volume (in effect, though the vial was closed, oxygen was available), again with one exception, maintained a high fertility rating for at least 3 days. In Series B, 1 ml of 2 samples which showed 0% fertility after 24 hours storage, was then transferred to other vials, and 24 hours later gave 60 and 80% fertility ratings. The difference in storage behaviour of Series A and Series B and C is not understood but may be related to the maturity of the sperm cells. It should be noted that in the literature there are conflicting reports on the conditions required to store trout sperm. Some workers stored the sperm samples in containers sealed with paraffin, while others used cotton plugs only, allowing the free transfer of air. The success of the 2 methods possibly depended upon the original state of maturity of the trout sperm cells. Certainly the present work would indicate that samples of salmon sperm should be stored in a manner such that oxygen is freely available to them.

2. Mixing of Sperm Samples

Contrary to the results of 1962, mixing sperm samples of 0% fertility with good samples did not give a total sample of 0% fertility. In 1962, all sperm cells were collected near the end of the spawning season and therefore, when stored in capped containers, would not maintain their viability more than 24 hours whether mixed or not.

3. Tests for Viability of Sperm Cells

With the exception of fertility, the only reliable test for viability was motility of the cells upon dilution with 20% sea water. No immotile sperm sample gave a positive fertility test.

The pH of the sperm sample was indicative of the viability of the cells. In general, samples of pH lower than 7.2 rated 0% fertility and samples of pH 7.4 or over gave a positive fertility test. Samples with available oxygen during storage maintained a higher pH than those with a limited oxygen supply.

A vital stain (Fast Green dye) which distinguishes live from dead mammalian spermatoza did not stain any of the salmon sperm cells.

4. Freezing Experiments for Long Term Storage

Again this year, the freezing of salmon sperm was unsuccessful. The freezing of other sperm cells has been achieved by the use of a diluent and a protective agent, usually glycerol. Fish sperm metabolism seems to depend upon an endogenous substrate, so that once activated by dilution, the cells, unlike mammalian sperm cells, cannot maintain their activity by using metabolites in the diluent. Freezing of the sperm with no diluent but with a protective agent caused denaturation of the cell protein. A diluent which will maintain the salmon sperm alive but inactive was developed by modifying the salt composition of Cortland saline; ethylene glycol was added as a protective agent. Salmon sperm diluted 1:4 with this solution became very motile upon further dilution with water and after 4 days storage in an open vial gave a 20% fertility test. Frozen samples were immotile upon dilution and did not fertilize eggs. Further studies involving dilution ratios, freezing rates, and reactivation of quiescent sperm cells will be continued when sperm samples become available.

SUMMARY NO. 39

MARINE HORMONES:
ATTEMPT AT ARTIFICIAL INDUCTION OF SEXUAL MATURATION OF ATLANTIC SALMON.

Beryl Truscott
D.R. Idler
H.C. Freeman

Adult spawned female salmon were obtained from the River Philip, N.S. hatchery in November, 1962, and held in circular tanks supplied with running sea water. During the winter months most of the fish refused food, but as the water temperature increased, the salmon resumed feeding and through the summer months accepted liver and sardine herring. In September, 1963, the survivors, 46 in number, were weighed and measured. Increases in weight from March through September of individual fish varied from ÷20% to 40%. Post-mortem examination of 6 fish showed that gonad development had begun, the gonad weight (approximately 12 g) being the same for all 6 fish regardless of their size or condition.

For the experimental work, the fish were divided into 2 comparable groups of 20, each group containing the same number of fish in good and in poor condition. Since shortening daylight hours is known to promote maturation of trout, the lighting of the tanks was controlled and of 12 hours duration per day. Throughout the experimental period the fish were fed regularly. The sea water temperature varied from 12 to 18°C.

Beginning in mid- September, the experimental fish were given a weekly injection of 5 mg B-estradiol and 5 mg cortisol in 0.2 ml peanut oil. The

control group were given 0.2 ml injections of peanut oil only. After 4 weeks, the method of hormone treatment was changed for three reasons: first, analysis of blood samples indicated that the increased hormone levels were not maintained more than 48 hours; second, the gonads removed from fish which died during the experiment showed no change; and third, weekly handling of the fish even under mild sedation seemed to be detrimental to their physical condition. All of the experimental group of fish were then injected with 17 B-estradiol, 17-cyclopentanepropionate (0.45 mg/lb), a compound that reportedly maintains its estrogenic activity for 2 - 3 months after injection in animals. At the same time cortisol pellets were implanted subcutaneously in 13 of the experimental salmon.

The experimental fish stopped feeding immediately after this treatment and within ten days, the weaker individuals had died. Post-mortem examination of the fish showed a weight loss of 20%, a loss that could be explained only by a loss of tissue fluid. Until this time, the fish had been held in salt water. The water inflow was changed first to 50% fresh-50% salt water and then to 75% fresh-25% salt water. However, the cure either did not match the disease or was too little and too late; within a month all of the experimental fish, except two, had died. The change in water salinity did not adversely affect the control group of salmon.

The fatal result of this experiment would seem to be attributable to the estradiol ester rather than the cortisol pellets since all 6 fish which did not receive a pellet also died. The two survivors have resumed feeding and are now being held in salt water with the control group.

SUMMARY NO. 40

MARINE HORMONES:
STEROID HORMONES IN THE PLASMA OF SPAWNED ATLANTIC SALMON AND THEIR DETERMINATION BY CHEMICAL AND BIOLOGICAL ASSAY METHODS.

H.C. Freeman
D.R. Idler

Preliminary results obtained at the Vancouver Technological Research Laboratory indicated that cortisol, cortisone and 11-ketotestosterone were present in the blood of Atlantic salmon. The presence of these hormones has now been established by rigorous chemical methods. Other investigators have previously described a discrepancy in the amounts of adrenocortical hormones in Atlantic salmon, as measured by chemical and biological methods. These authors suggested the possible presence of large quantities of hormones other than those found in the present investigation. This question has now been resolved and excellent agreement has been obtained employing chemical and

biological methods for the major hormones referred to above. There is no evidence for the presence of significant quantities of hormones of the type suggested by previous investigators. A report describing this investigation in detail has been published.

SUMMARY NO. 41

MARINE HORMONES:
STEROIDS IN SEAL (HALICHOERUS GRYPUS) BLOOD.

H.C. Freeman
D. R.Idler

Total dihydroxyacetone steroids (Porter-Silber) hormones were determined in the plasma of four seals: a male of 2 years, a male of 4 years, a male of 12 years, a female of 19 years, and 4.40, 4.99, 3.74 and 15.7 µg respectively of cortisol equivalents of hormones per 100 ml.of plasma were found.

SUMMARY NO. 42

MARINE HORMONES:
STEROIDS IN COD BLOOD.

H.C. Freeman
D.R. Idler

Cortisone and cortisol have been isolated and identified as the principal plasma steroid hormones in mature, unripe male and female Atlantic cod with cortisol predominating in both sexes. This investigation is being carried out in conjunction with hormone studies on Terence Bay cod to determine the association of the hormones with the sexual condition of the fish.

When obtaining fish from Terence Bay during the past year, it was observed that very few mature ripe fish were caught making it necessary to find another source in order to obtain ripe fish. After consultation with Board biologists it was concluded that spawning cod should be caught in deeper water (about 40 fathoms) in spawning season. Bearing this in mind, the M.V. Harengus was engaged for several fishing excursions in deeper water off Terence Bay and was able to catch a limited number of mature ripe cod during the month of March (1964). A small quantity of ripe cod plasma was collected and presently experiments are being carried out to quantitatively determine the hormones present.

SUMMARY NO. 43

MARINE LIPIDS:
DETERMINATION OF FATTY ACID COMPOSITION.

R.G. Ackman
P.M. Jangaard
J.C. Sipos
R.D. Burgher

With the development of methods for the identification of gas-liquid chromatographic peaks in marine lipids (1-4,7,9,10,12,36,37 - see also Annual Reports 1961-62, 1962-63), the failure of the particular argon ionization detector employed in this laboratory to correlate indicated quantitative analyses with other

properties such as iodine value and chain length composition (9) became a major problem. Although an empirical approach (9) to correcting the observed peak areas from this detector was reasonably satisfactory, the alternative use of a flame ionization detector has proven much more successful from the quantitative point of view. Minor corrections (35,43,44) based on weight per cent carbon content of the fatty acid chain are still desirable, although not of extreme significance. With the flame ionization detector the iodine values computed from gas-liquid chromatograms seldom differ from experimental values by more than ± 5 units, and chain length correlations with hydrogenated samples are within probable experimental error. In the course of checking the validity of the results from this detector with known concentrations of materials, certain types of responses were correlated with structures of oxygenated materials on a fundamental basis not hitherto reported (35,44). The identification of, and quantitative analysis for, marine type fatty acids has now been placed on a routine basis in this laboratory unless novel techniques or apparatus can be introduced to advantage.

SUMMARY NO. 44

MARINE LIPIDS:
HOMOGENEITY OF FATTY ACID TYPE COMPOSITION.

R.G. Ackman
P.M. Jangaard
J.C. Sipos

The view that marine fatty acids conform to regular animal fatty acid types on a structural basis has been maturing for some time as evidence from different laboratories accumulated. It can thus be stated (36) that the C_{20} and C_{22} polyunsaturated fatty acids in higher marine species are chiefly of the linolenic type, the C_{18} polyunsaturated acids of both the linoleic and linolenic types, and the C_{16} polyunsaturated fatty acids atypical in having the ultimate double bonds two carbon atoms closer to the end of the fatty acid chain than in the other chain lengths. Much of the effort of earlier workers as quoted in reference books must now be disregarded. In supporting this view it was necessary to investigate in particular saury (13) and pilchard (45) oils to check on anomalous compositions reported by other workers. In each case the fatty acid distribution patterns were normal for depot fats of teleost fish. Further investigations on other marine species of importance either as commercial species (e.g., lobster, elasmobranch fish) or as important stages in the food chain to commercial species (e.g., zooplankton, shrimp, crabs, squid, etc.) will be carried out to delineate the limits of this viewpoint.

SUMMARY NO. 45

MARINE LIPIDS:
LIPIDS OF COD.

R.G. Ackman
R.D. Burgher

To provide a basis for the seasonal variation study in Terence Bay cod Summary No. 33), the lipid fatty acids found in the flesh, liver and roe of mature individual fish were examined (38 -40). The flesh lipids are chiefly phospholipids, and the fatty acid composition therefore did not appear similar to teleost depot fats (Summary No. 2). Palmitic and polyunsaturated fatty acids predominated, with a considerable exclusion of minor fatty acids of no metabolic significance. A curious similarity in proportions of certain polyunsaturated fatty acids in the flesh and liver was noted, possibly related to the normal occurrence of these acids almost exclusively in the B-position of phospholipids and triglycerides. The developing roe require highly unsaturated fatty acids, yet the liver of this particular fish was low primarily in respect to C_{20} and C_{22} monounsaturated fatty acids. The role of the latter acids in marine species has never been satisfactorily explained and tentatively these observations suggest that they form a readily accessible reserve of fatty acids for metabolic needs, specifically either for energy requirements or as source material for the synthesis of polyunsaturated fatty acids.

SUMMARY NO. 46

MARINE LIPIDS:
ORIGIN OF MARINE FATTY ACIDS.

R.G. Ackman
P.M. Jangaard
R.J. Hoyle
H. Brockerhoff

An investigation into the origin of the fatty acids characteristic of marine species has been started through examination of the lipid product of a culture of Skeletonema costatum. This phytoplanktonic diatom is of particular importance in the spring plankton bloom in temperate North American waters. The results indicate that some 20% of the fatty acids are the atypical C_{16} acids (Summary No. 44). Since these are known to be biologically inactive in mammals it is presumed that their occurrence in the higher marine species is due to their retention in depot fats from the original planktonic source common to the aquatic food chain. A further important observation was the occurrence of 5,8,11,14,17-eicosapentaenoic acid to the extent of 15-20%. Thus phytoplankton can serve as source of this acid for fish without the necessity of an intermediate zooplankton stage. Of the remaining acids the lack of octadecenoic acid and accumulation in mature cultures of tetradecanoic acid are also signifi-

cant. Caution must be used in comparing laboratory culture results with the possible compositions in pelagic plankton, but there is ample evidence from this one experiment to indicate that further work is required before even a modest understanding of the possible relationships between fatty acid composition, environment and level in the evolutionary scale can be reached.

SUMMARY NO. 47

MARINE MAMMALS:
COMPOSITION OF SEAL BLUBBER FAT AND MILK.

P.M. Jangaard
R.G. Ackman
R.D. Burgher

The use of urea complex fractions from seal oil (10) was of considerable assistance in verifying the linear log plot and separation factor procedures developed in this laboratory (Annual Reports 1961-62, 1962-63, Summary No. 1). At that time a quantitative analysis was not possible owing to the limitations of the apparatus employed. Subsequently a complete analysis of the blubber fat of the common or harbour seal was carried out. The blubber fat does not differ significantly except in minor details from that associated with marine lipids in general (Summary No. 44). The chief significant feature common to various marine mammal oils studied in this laboratory has been the high proportion of hexadecenoic acid, in this case three times the amount of hexadecanoic acid, and also a high level of tetradecenoic acid.

When a sample of the milk of the grey (Atlantic) seal became available this was also analysed. The above mentioned peculiarities were not evident in this milk sample, which conformed more to a teleost fish depot fat, although both seal lipids are somewhat different in having higher levels of 7,10,13,16,19-docosapentaenoic acid, in relation to 4,7,10,13,16,19-docosahexaenoic acid, than do the fish oils.

SUMMARY NO. 48

MARINE LIPIDS:
LIPOLYTIC ENZYMES OF FISH.

H. Brockerhoff
R.J. Hoyle
M. Yurkowski

In accordance with the hypothesis of the "retention of the β-monoglyceride structure" in the marine food chains, the pancreatic lipase of a skate was found to be specific for α-glycerides.

The phospholipase of cod muscle, which is responsible for the appearance of free fatty acids in cold storage, attacks lecithin without accumulation of lysolecithin. Lysolecithin was therefore treated with cod muscle extract; it was attacked ten times faster than lecithin. Further characterization and purification of the enzyme is now being attempted.

SUMMARY NO. 49

MARINE LIPIDS:
THE ASYMMETRIC STRUCTURE OF TRIGLYCERIDES.

H. Brockerhoff

The known biosynthetic pathway to triglycerides proceeds via asymmetric precursors; it can therefore be assumed that natural triglycerides themselves are asymmetric in that they contain different fatty acids in either of the α positions of the glycerol. According to previous results one would expect marine fats to be more symmetrical than mammalian fats. As a preliminary to further studies of this subject, a method was developed that allows the stereospecific analysis of triglycerides, that is, the differentiation of the two α-positions.

SUMMARY NO. 50

MARINE LIPIDS:
RETENTION OF THE STRUCTURE OF MARINE FATS
THROUGH THE FOOD CHAIN.

H.Brockerhoff
R.J. Hoyle
K. Ronald

In the typical fats of marine fish and invertebrates, the polyunsaturated essential fatty acids are accumulated in the β-position of glycerol. The hypothesis has been advanced (Annual Report 1962-63, Summary No. 26; Arch. Biochem. Biophys., 100, 9 (1963)) that this typical pattern originates in the phytoplankton and is retained through the marine food chains by virtue of a "retention of the β monoglyceride structure". In order to verify this hypothesis, a β-labelled triglyceride was fed to lobster, cod, trout, and rat, and then the distribution of the label was determined.

In the invertebrate and in the fish 50 to 80% of the original structure had been retained after absorption and deposition of the fat. There was still retention of structure after 4 weeks. On the other hand, no retention was observed in the triglycerides of rat.

It appears, therefore, that fish and invertebrates, though not mammals, can deposit dietary fat without disruption of the β-monoglyceride bonds as required in the original hypothesis.

SUMMARY NO. 51

MARINE LIPIDS:
STRUCTURE OF TRIGLYCERIDES OF MARINE PLANKTON.

H.Brockerhoff
M.Yurkowski
R.J. Hoyle
R.G. Ackman

The triglycerides of a sample of zooplankton and those of a common diatom, Skeletonema costatum, were found to have a typical structure: they contain the polyunsaturated "essential" fatty acid accumulated in the β-position of glycerol. This finding is a further confirmation of our hypothesis of the

"retention of the β monoglyceride structure" through the marine food chains.

SUMMARY NO. 52

THE "BLACKBERRY" PROBLEM IN LABRADOR COD.

R.G.Ackman
J.C.Sipos

"Blackberry" refers to the appearance of the stomach contents of cod from Labrador waters at a specific time when the fillet quality must be downgraded as a result of an offensive and persistant odour. The actual odour has been identified by gas-liquid chromatography as dimethyl sulphide.

The precursor of this material, known to occur in marine invertebrates, is dimethyl-β-propiothetin. In the Pacific the problem is apparently peculiar to chum salmon, where the precursor accumulates and is primarily broken down on cooking. In Labrador cod only the dimethyl sulphide has been as yet identified, but it is possible that different enzyme systems in the cod are responsible for the accumulation of dimethyl sulphide in vivo. Any modification of processing to render these fish commercially useful will depend on the outcome of a complete examination of the food species and the fish for dimethyl-β-propiothetin as well as dimethyl sulphide.

SUMMARY NO. 53

FISH PROTEIN CONCENTRATES:
NUTRITIONAL QUALITY OF PROTEIN.

H.E. Power

While the use of cod fillets as a source of raw material for the manufacture of fish protein concentrate results in a very high quality, white, odourless fish protein concentrate, many people, both potential producers and consumers, are of the opinion that a lower cost product made from cheaper raw material would better suit a larger part of the market for protein concentrate. For many markets it is not necessary, or in some cases even desirable, that the fish protein concentrate be white, of soft texture, or even odourless. However, it is necessary that in all cases the protein be of high nutritional value.

With the above in mind, it was decided to evaluate the protein concentrate which could be produced from lower cost starting material as well as to explore the suitability of the improved process for use with these materials.

The starting materials studied were: whole cod, headed, eviscerated cod, cod trimmings, cod presscake, and herring. Fish protein concentrate produced from these materials has been evaluated in terms of its protein efficiency ratio (P.E.R.), microbiological lysine and available lysine.

The percentage of protein (N x 6.25, dry basis) varied between 93% for the sample of fish protein concentrate produced from cod fillets, and 70.6% for fish protein concentrate produced from presscake. In the latter case, the presscake is made by cooking cutting table trimmings with live steam and then pressing to lower the moisture content of the fish to 60%. In this process a considerable quantity of nitrogenous material is lost.

The quality of the protein in these types of FPC, as measured by the Protein Efficiency Ratio, shows some variation, although all samples were superior to casein, with the exception of those produced from presscake. The P.E.R. for casein was 2.5, while for the samples of FPC this value varied from 2.97 for protein concentrate made from cod fillets to 2.12 for the protein concentrate from cod presscake. It appears that a larger percentage of the more valuable amino acids were lost during the pressing. The percentage of lysine in the presscake samples was slightly lower than that for the samples produced from other raw materials.

The amount of fat deposited in the liver of the test animals is another measure of quality of the protein being fed to these animals in the experimental diets. Where the quantity or quality of the protein is low, increased quantities of fat are deposited in the liver. The animals used to evaluate the quality of the FPC protein showed some variation in the percentage of lipids contained in the livers. The liver of the rats being fed casein showed a higher liver fat than did the livers of the rats which received their protein from FPC. The values for liver fat ranged from 7.33% for headed, eviscerated cod to 10.52% for herring. The value of liver lipids for casein was 12.03%.

Morrison and Campbell have found that over the range of lysine content from 2.0 to 6.4 g/16 g N there is a highly significant correlation between the per cent lysine in the protein and the resulting P.E.R. when fed to experimental animals, the P.E.R. being a direct function of the percentage lysine in the protein.

When the percentage of lysine in the concentrate which covered a range of 10.7 to 14.5 was plotted against the P.E.R. obtained by feeding tests, no such relationship was noted, either for microbiological or available lysine. However, the number of samples used were too few to permit a definite conclusion to be drawn.

SUMMARY NO. 54

FREEZE-DRYING OF FISHERY PRODUCTS:

A.L. Wood
H.E. Power

The objects of this investigation are:

1. To determine what changes are introduced by this process when applied to fishery products and seek an explanation for such changes.

2. To define the characteristic quality of freeze-dried fishery products.

3. To study the effect of pressure and temperature cycles and the pre-treatment of the product with regard to improved quality.

A review of the literature on freeze-drying of foods has revealed that a moderate number of products have been successfully freeze-dried commercially. With the exceptions of shrimp and crab, fishery products have not enjoyed the same success as coffee, soup mixes, eggs, certain vegetables and meats.

Preliminary experiments indicate that the lack of acceptability of freeze-dried fish is because of its poor texture and possibly lack of flavour.

The equipment used initially in this investigation has now been abandoned for reasons outlined in the 1962-63 Annual Report. A Stokes Model 21, Weigh-Scale Freeze-Dryer has been in operation since August, 1963, and is giving reproducible results. It is equipped for programmed temperature control and weight-loss indication during drying.

To limit variations in the raw material, live fish are held in sea water tanks at 2° tc 3°C for at least 3 days before being killed, headed and gutted. These are then iced for 24 hours, then frozen, sealed in polyethylene and stored at -25°C until required. Only male fish are used.

The effect of temperature is being studied using radiant heat. The rate of sublimation varies with the heater plate temperature between $37.5^{\circ}$and 148°C.

An upper limit for the sample temperature has been determined, above which discolouration of the sample occurs, accompanied by definite "off" flavours.

A more efficient heat input cycle is in the process of development by programming the plate temperatures during the drying period.

To date, the variation between freeze-dried cod steaks and good quality frozen fish is so wide that comparisons are difficult. An attempt is being made to produce a freeze-dried sample at maximum sublimation rate which can be reproduced as required to provide a control for future quality evaluations.

Shredded cod, frozen in blocks, both salted and unsalted, and prepared commercially, have been freeze-dried. The unsalted made a reasonably acceptable

product. However, the salted was judged to be unacceptable not only because of flavour but because the structure of the sample was most unsatisfactory.

Variations in chamber pressure, heater plates and sample temperature, as well as drying rate, are being recorded for all samples processed.

SUMMARY NO. 55

FREEZE-DRYING OF FISHERY PRODUCTS

R. Legendre
H.E. Power

The lyophilization, or freeze-drying, generally calls for rapid and deep freezing, followed by sublimation of the ice without thawing. The objective is to preserve the food for an indefinite period under normal temperature conditions. To be successful, the freezing operation should be conducted with the minimum of change to the physico-chemical structure of the food and the sublimation of the ice in the food should be conducted at conditions which will not cause thawing nor permit ice-free tissue at any stage to be subjected to any damage by heat. If all these requirements are observed, the resulting product can be so little altered that the mere addition of water amost immediately restores it to its original condition of size, shape, flavour and texture.

In previous experiments, texture was found to be the main problem with freeze-dried codfish and a study of reconstitution was undertaken during the year. Since the method used for reconstitution influences moisture uptake, it has also an influence on texture. The moisture content of the material after freeze-drying was found to be about 2.5% wet basis. After reconstitution, (soaking for 5 minutes in cold water, followed by draining for 1 minute) the final moisture content was 75%. With perfect reconstitution, the final moisture content should be around 80%. It was found that longer soaking times (up to 15 minutes) did not improve reconstitution and using weak brine (3%) instead of water did not produce better results. The temperature of the reconstituting water was also investigated but no appreciable difference was found between temperatures of 5°C, 10°C, 25°C and 40°C. Slices of codfish steaks of 1/4", 1/2" and 3/4" thickness were then prepared and it was found that reconstitution was much better with thin slices. In fact, it was possible to obtain a final moisture content of about 78% with slices of 1/4" thickness as compared to about 74% with other thicknesses. It was thought possible that the improvement in moisture uptake could be a consequence of the increase in sublimation rate which varies with thickness. In fact, the overall drying time was about 6 1/2 hours with 1/4" slices, 13 hours with 1/2" slices and 18 1/2 hours with 3/4" slices.

A co-operative program was then initiated during the summer between the Halifax and Grande-Rivière Laboratories to study the application of the process to fishery products. To limit variations in the raw material, live fish are being held at Halifax in tanks maintained at 2° to 3°C for at least 3 days before being killed, headed and gutted. Then they are iced for 24 hours, after which they are frozen in a Dole Plate Freezer, sealed in polyethelene and stored at -25°C until required. A taste panel has been organized for organoleptic evaluation and other data are being obtained from moisture regain curves and some physical and chemical determinations.

Up to now, sample thickness has been investigated at Grande-Rivière and some modifications have been made to the freeze-drying equipment in order to enable the samples to be weighed during the drying process. Using samples supplied from Halifax, slices of various thicknesses have been prepared and sublimation rate curves obtained with slices of codfish steaks of 1/4", 1/2" and 3/4" thickness. For these experiments, the method used to supply heat to the material was not very efficient and the rate of sublimation was comparatively low. In spite of this, however, organoleptic evaluations of odour, flavour and texture have shown a good product with slices of 1/4" thickness as compared to not edible with slices of 3/4" thickness. The slices of 1/4" thickness have been noted as being the best texture in freeze-dried cod steaks examined to date. The good results obtained with slices of 1/4" thickness could probably be explained by the fact that the temperature of the material was relatively low during the sublimation process and the difference in results between slices of 1/4" and 3/4" is apparently a consequence of the difference in sublimation rate. Better results should therefore be possible by increasing the sublimation rate without unduly increasing the temperature of the fish.

Further experiments are needed to confirm these results and the freeze-drying equipment available at Grande Rivière is undergoing modification to enable the effect of pressure to be studied and to improve the method of applying heat.

Vacuum System

The rate of sublimation depends on the heat input and therefore methods of increasing heat transfer are very important. For a given method of supplying heat, the heat transfer is proportional to the difference in temperature between the ice and the surrounding.. Since there are some limitations in temperature (with codfish) to obtain a good quality product, it seems that the best way to increase the heat transfer and improve sublimation rate is to decrease the temperature of the ice in the material by using lower pressures. For example, the ice

temperature is equal to -17°C at 1 torr and -74°C at 1 millitorr. The system is therefore being changed in order to improve pumping capacity and efficiency and permit the use of very low pressure. An Alphatron gauge has been installed for the measurement and control of pressure. Lower pressures will also permit a lower moisture content of the fish at the end of the operation.

Heating System

It is well known that dehydrated foodstuffs are very poor conductors of heat and the gradually increasing layer of insulating material which is formed retards the transfer of heat to the ice phase boundary. The rate of sublimation becomes then progressively slower and the removal of the last amount of moisture is comparatively very long. The method used for applying heat is therefore very important. Because of the penetrating properties of microwaves, dielectric heating should be very efficient in this application. In previous experiments (Annual Report 1960-61) it was found that infra-red heating is also efficient. For that reason, infra-red heaters have been installed in the oven together with means of varying the intensity of the heating systems and controlling the temperature. With infra-red heaters it is hoped that it will be possible to supply the required amount of heat for the sublimation of ice with the minimum increase in the temperature of the already dry portion of the fish.

SUMMARY NO. 56

DETERMINATION OF MEAT CONTENT OF LIVE OYSTERS. H.E. Power

For the purpose of studying nutrition, growth, etc. of certain shellfish it is often desirable to follow the changes in the weight of the meat of individual shellfish over a period of time. An exploratory experiment is under way to determine the feasibility of measuring the percent of meat in live oysters using mechanical vibrations. It is too early, as yet, to predict with certainty the possibility of success with this method.

SUMMARY NO. 57

MARINE BACTERIA:
A PATHWAY OF CHOLINE METABOLISM. H.S. Shieh

Choline, a basic lipid, is recognized as an essential dietary component and its aerobic metabolism in mammalian tissues has been intensively studied. Generally, the enzymic oxidation of choline proceeds in two steps; in the first, choline is oxidized by choline oxidase in the presence of FAD to form betaine aldehyde, which is then oxidized to betaine by a DPN-dependent betaine aldehyde dehydrogenase. In the presence of the enzyme "betaine-homocystaine

transmethylase", betaine donates one of its methyl groups to homocysteine with the formation of methionine and dimethylglycine. Dimethylglycine is progressively demethylated through sarcosine to glycine with the formation of "active 1-carbon fragments". The anaerobic degradation of choline in bacteria has been demonstrated, with the formation of ethanol, acetate, and trimethylamine by some investigators. This summary reports a new pathway of choline metabolism by the marine bacteria.

Marine bacteria, isolated by an enrichment technique, are able to utilize choline as the sole carbon and nitrogen source in a medium containing various salts. The rate of growth of these bacteria is fast and the concentration of choline falls steadily and ammonia is accumulated. Trials indicated that choline metabolism is more rapid in a well aerated medium. The rate of metabolism of choline was enhanced by the addition of iron in trace amounts to the usual salts medium. In the absence of iron, the oxidation of choline is accompanied by a substantial accumulation of pyruvic acid.

With resting cells in a phosphate buffer containing choline, betaine, dimethylglycine, sarcosine or serine, production of pyruvic acid and ammonia is fast. Experiments with cell free extracts indicated that the conversion of serine to pyruvic acid and ammonia is almost quantitative. Pyruvic acid was isolated as 2,4-dinitrophenyl-hydra-zone from various culture liquors and was identified by its melting point and by its absorption spectrum.

Paper chromatography of the water soluble materials from the resting cells or the cell free extracts in a tris-buffer containing choline, betaine, or dimethylglycine, revealed the identical Rf value and colour reaction of sarcosine and serine.

The aerobic metabolism of choline in marine bacteria indicates that choline is metabolized via betaine, dimethylglycine, sarcosine, serine and pyruvic acid.

SUMMARY NO. 58

ASSESSMENT OF AN ELECTRONIC FISH TESTER. C.H. Castell

It has long been known that deterioration of fish muscle is accompanied by increased conductivity of the tissues. Based on this principle, a German firm has developed an instrument for measuring the freshness of fish. It has many advantages over most other methods for detecting spoilage. The instrument is portable and the readings can be made almost instantaneously. The producers claim the instrument will not only measure freshness, but by use of an attached scale, it will predict the keeping time of the fish in ice.

In order to determine the value of this instrument under commercial conditions, more than a thousand tests have been made on fish as they were being discharged from the vessels at Lunenburg. The previous storage period and storage conditions for each fish had been recorded; each fish was graded by two of our best fresh fish inspectors and each fish was subsequently re-examined in detail for the presence of bruises, muscle breaks, softness and other physical blemishes.

The results show that the instrument does in general indicate degrees of spoilage resulting from bacterial and enzymatic activity. But it is also extremely sensitive to minor physical blemishes in the tissue adjacent to where the readings are made. The results do not always give an accurate picture of the degrees of freshness as claimed. It could not be used in place of the organoleptic judgments of an experienced grader but it might be very useful as an additional tool for measuring changes occurring in fish under defined experimental conditions.

SUMMARY NO. 59

ASSESSMENT OF AN ELECTRONIC FISH TESTER.

W.A. MacCallum
Dorothy A.Chalker
J.T. Lauder

Interest has been shown by the Newfoundland Industry and by the Fish Inspection Branch of the Department of Fisheries in the potential of the above instrument (which operates on the electrical conduction principle) for grading landed and iced fish. One series of storage runs was conducted at the Unit after which the apparatus was loaned to the Fish Inspection Branch on a temporary basis.

Fall-caught cod, exhausted on trawl-lines, were used. Organoleptic grading and TMA assessment of gutted, iced fish were conducted and pH and fish tester readings (giving additional days iced storage life) were taken periodically over a 14-day period. Glycogen determinations were made as a check on pH readings.

Results confirmed data obtained in previous years relating to the exhausted condition at time of killing of some trawl-caught fish. Glycogen content was very low and pH readings practically constant throughout the whole of the storage time. The latter effect is particularly interesting in the case of fish iced longer than 5 or 6 days when bacterial decomposition normally causes a rise in pH. Likewise TMA values remained lower than those customarily found in trawler (bank) -caught cod.

Notwithstanding, organoleptic grades worsened with storage and this worsening was reflected in reduced storage life registered by the tester. The latter gave quite consistent appraisal of total storage life of the samples independent of days storage at time of reading.

SUMMARY NO. 60

TASTE PANELS IN INDUSTRY.

Dorothy A. Chalker
W.A. MacCallum

Two large producers of frozen blocks and fillets in Newfoundland requested assistance and information for setting up taste panels.

One company was given written instructions on tasting cod. Later representatives were sent from two plants to the Unit to receive personal instruction and training. The candidates seemed most interested. A taste panel is in the process of being set up at one plant by company staff and it is hoped that a panel will be started in the other plant in the Spring.

Earlier, the second company had two members of its technical staff report to the Unit for similar written and personal instructions. Subsequently, a panel was set up by the company. A check of the Company panel and the Fisheries Research Board panel (on similar samples) showed that the scores were not at all comparable. It is evident that the industry panel had not been given sufficiently thorough training required to make it an efficient instrument for assessment.

It appears that, if staff were available for this purpose, industry could be helped but satisfactory results can be expected only if industry itself have people on staff capable of setting up, operating and checking the panels periodically.

SUMMARY NO. 61

INFORMATION PROVIDED TO FISHERY INDUSTRY, BY REQUEST, DURING 1963-64 ON PRODUCT AND PROCESS RESEARCH.

A.L. Wood
H.E. Power

1. Data re drying of salt fish - Brantford, Ontario.
2. Freezing of pre-cooked whole lobster - Clark's Harbour, N.S.
3. Filleting, skinning, candling equipment - Dept. of Fisheries.
4. Refrigeration - Cape Sable Island, N.S.
5. Fish meal production - Cape Sable Island, N.S.
6. Smokehouse drawings and specs. - Shediac, N.B.
7. Smokehouse construction - Canso, N.S.
8. Smokehouse drawings and specs. - Quebec.
9. Meal production - Montreal.
10. Meal production - N.S. Dept. of Trade and Industry.
11. Economic development of industry - N.S. Dept. of Trade and Industry.
12. Smoking eels -
13. Salt fish dryer drawings and specs. - Peru.
14. Fish processing - Uganda.

15. Conference - Voluntary Economic Planning - Truro, N.S.
16. Salt fish dryer drawings and specs.- Blandford, N.S.
17. Smokehouse drawings and specs. - Yarmouth, N.S.
18. Packaging and shipment of smoked fish - Halifax, N.S.
19. Information re fish plant at Sambro - Halifax, N.S.
20. Information re possible new plant site - Toronto.
21. Freeze drying - N.S. Dept. Trade and Industry.
22. Freeze drying information - Toronto.
23. Salt fish dryer information - Halifax.
24. Freeze-drying discussion - Visitor from Torry (Dr. Jason).
25. Refrigeration - freezing - Dept. Trade and Industry (N.S.).
26. Cold storage design information - Sambro.
27. Information re local manufacturer of meal plant equipment - Toronto.
28. Filleting equipment - Dept. of Trade and Industry (N.S.).
29. Information re heavy salted cod - Newfoundland.
30. Information re dryer specs. and costs - N.S. Dept. Trade and Industry.
31. Salt fish dryer plans and specs. - Dept. of Fisheries.
32. Discussion re freeze-drying - Legendre.
33. Freeze drying information - CBC.
34. Information re plant construction - Cape Sable Island, N.S.
35. Ice manufacture and storage - Clark's Harbour, N.S.
36. Cold storage design information - Ingonish Beach, N.S.
37. Information re operation of tunnel type smokehouse - Shippegan, N.B.
38. Information re rates for filleting, skinning, packaging - Halifax,N.S.
39. Freeze drying information - N.S. Research Foundation.
40. Bait freezer and storage design information - Truro, N.S.
41. Salt fish dryer drawings and specs. - Sambro, N.S.
42. Bait freezer and storage design discussion - Dept. of Fisheries.
43. Producing smoke - Newfoundland.
44. Smokehouse plans and specs. - Newfoundland.
45. Salt fish plant design information - Petit de Grat, C.B.
46. Freeze drying equipment - India.
47. Information re smokehouse temperature, R.H. and velocity - Halifax.
48. Information re storage of salt fish - Brazil.
49. Smokehouse drawings and specs. - Halifax.
50. Cold storage design - Digby.
51. Dryer costs and specs. - Halifax.

52. Dogfish meal - Mass.
53. Dryer design - Victoria Beach, N.S.
54. Meal plant design - Yugoslavia.
55. Storage and display of fresh fish - N.S. Research Foundation.
56. Stickwater disposal - Dartmouth, N.S.
57. Salt fish dryer design information for Chile - Lunenburg.
58. Smokehouse drawings and specs. - Newfoundland.
59. Smokehouse drawings and specs. - Labrador.
60. Information re fillet skinning machine - North Sydney.

SUMMARY NO. 62

NEWFOUNDLAND UNIT: MISCELLANEOUS — W.A. MacCallum.

During the year a demanding schedule for taste testing was maintained. Particular thanks are extended to the following personnel of the Biological Station who served as panel members: Catherine Allan, L.N. Cluett, E.J. Elms, H. Lear, M.H. Manuel, A. Elizabeth Moore, Jean E. Percy, Catherine Philpott, Mary D. Rowe, W.E. Squires, M. Lorene Tuck, G.E. Tucker, R.J. Tucker, and to Mr. E.E. Coldwell, Unit Technician, who assisted Miss Chalker in obtaining and in cutting the frozen samples. Panel members from the Unit were Dorothy A. Chalker, Mary A. O'Brien, W.A. MacCallum and H.S. Shieh. As well, Mr. Coldwell assisted materially in preparatory work required for several other studies. Captains William Barbour and Fraser A. Winsor and members of the crews of the research vessels INVESTIGATOR and MARINUS co-operated wholeheartedly and effectively in the prosecution of studies on trap and fall fish obtained alive, then landed or frozen at sea. The co-operation of Dr. Templeman and staff in arranging Biological Station schedules to meet our needs when possible permitted Unit activities to proceed successfully. The help is noteworthy since handling the cod alive in small numbers and quick transfer of fish to shore made possible by the research vessels' crews alone has proved to be a reasonably efficient and successful means to land fish in suitable condition for live holding.

SUMMARY NO. 63

FACILITIES:
LIVE FISH AND SHELLFISH HOLDING FACILITIES AT HALIFAX. — James E. Stewart, P.H. Odense

In the 1962-63 Annual Report, Summary No. 41, it was stated that "increasing emphasis is being placed on the need for a better definition of the raw materials with which technologists work". It was further stated

that it was essential that the facilities be provided to permit the scientist to control and study the effect of a variety of variables on the live animal whose condition decides, to a marked degree, the quality of the product offered to the consumer.

Implementation of this policy was continued during the past year. The following modifications and additions were made to the live holding facilities.

(1) Three additional 3 inch diameter, polyethylene pipelines, identical to the one laid in 1962, were installed in January, 1964. Thus it will be possible to draw, using all 4 pipelines at once, a total of 400 U.S. gallons of seawater per minute.

On March 24, 1964, a ship dragging its anchor in the area of the submerged pipelines severely damaged one pipeline. The pipeline had to be completely removed following this accident since in its condition it constituted a navigational hazard. The installation of the additional lines prevented the loss of any fish during this emergency.

(2) The continuous flow sea water system has been improved by the addition of 3 large sand and gravel filters. These filters are operating well and permit all of the sea water pumped continuously to tanks in the Mitchell Building to be filtered, removing the troublesome suspensions previously experienced.

(3) The sea water piping system has been extended so that running sea water can be supplied to tanks in the main building.

(4) Two new, corrosion resistant, high capacity centrifugal pumps have been purchased for installation as the main sea water pumping unit. When these are installed and connected to the 4 pipelines it will be possible to pump the full 400 U.S. gallons of sea water per minute previously mentioned.

(5) A waterproofed concrete tank 50 ft x 12 ft x 6 ft deep, surrounded by a security fence, has been built outdoors at the eastern end of the Mitchell Building. Its outside wall forms a part of the sea wall for the newly filled area. This tank will be supplied with running sea water and will be used for holding and working with a variety of marine species.

(6) Equipment consisting of filters, valves and pipes has been acquired and is being installed for the purpose of supplying a large number of tanks with a continuous stream of clean, chlorine free, fresh water. These facilities will be used for working with fresh water species and for maintaining brackish water conditions for work with all species.

Filters for the large recirculating sea water tanks have been modified. Each filter unit consists of three fiberglass baskets which are stacked in an insulated fiberglass cylinder or drum. For ease in removal and cleaning

the baskets vary in size, the smallest one being placed first in the bottom of the cylinder and the largest one last at the top. These baskets contain the glass wool, gravel and activated carbon filtering material.

New tanks have been constructed and will be used in a system which will permit their operation in either a continuous flow or a recirculated water system. Each unit of the new system consists of a rectangular 30 gallon insulated fiberglass tank connected to a pump, heat exchanger coil, filter and a temperature control unit. An interchangeable standpipe system and control valves will permit a tank to be maintained at a constant temperature in a recirculation system or it can be switched over quickly to the continuous flow system. Overhead tanks provide a constant head of either fresh water or sea water so that the flow to any tank may be adjusted without altering the flow in the other tanks of the system.

SUMMARY NO. 64

FACILITIES:
DESIGN OF REFRIGERATION FOR EXPERIMENTAL USE AT HALIFAX.

H.E. Power
D.G. Ellis

In order to provide the scientific staff with cold storage facilities which may be depended upon to operate continuously for prolonged periods with the maximum reliability possible, a new refrigeration system has been designed and installed to replace the former ammonia refrigeration equipment. The new system consists of two complete Freon 22 compressor systems cooling a large tank of calcium chloride brine. Both condensers and compressors are air cooled, making the system independent of city water supplies, as well as effecting a saving in total water consumption of the laboratory.

One system shall operate at a time with the other being an automatically operated standby. Should the operating system fail, the standby would maintain the brine at the required temperature. If both systems should fail, sufficient cold brine is available to maintain the storage rooms at the required temperatures for approximately five hours. Since this equipment is still dependent on electric power, it is proposed to install a standby power system to operate the pumps circulating brine to the storage rooms. The five hours' refrigeration provided by the cold brine tank is sufficient to take care of any power failures in recent years.

A supervisory control panel has been installed for operation and maintenance of these systems, and an existing duplicated direct expansion Freon 22 system for one experimental storage room.

Graphic layouts have been prepared of the equipment used for experimental storage. Operating components are shown, with connecting pipes and control lines colour coded for the function performed. Indicator lights show the equipment

operating or available for operation. Centralized control of all mechanical equipment is provided. All operating temperatures and pressures are indicated on the supervisory panel. Weekly records of the main operating pressures and temperatures are provided. Special records of equipment under maintenance or adjustment are possible.

Basic instrumentation only has been provided for general cold storage equipment, as distinguished from experimental storage facilities. Centralized control is also provided for this equipment.

Electronic controls are replacing the present mechanical controls for the new refrigeration equipment and will be provided also for the individual Experimental Storage Cabinets now in use. All power wiring in the refrigeration system is being brought up to minimum Canadian Electrical Code standards.

SUMMARY NO. 65

FACILITIES:
LIVE HOLDING FACILITIES AT GRANDE RIVIÈRE.

M.A. Bordeleau
R. Guilbault
P.H. Odense

The installation of live holding facilities at Grande Rivière has been completed during the year. The system includes four separate units of the recirculating type with filtration and refrigeration. Each unit has a 700 gallon tank and cod have been kept in these tanks at regulated temperatures of 35, 45, and 55°F. A fourth tank has been used as a supply tank in which fish have been kept at 40°F when initially caught. Nylon mesh partitions make it possible to hold fed and starving fish in the same tank for experimental purposes.

During the winter time, to avoid various technical difficulties, it was decided to bring the temperature of all the tanks to a temperature range of 35°F to 40°F. It was then easier to transfer fish from one tank to another when necessary. Tris buffer was used to maintain a normal seawater pH. Without the use of buffer the pH varied and gradually dropped. All the problems in maintaining live fish are not solved. On one occasion 25 cod were transferred from a tank at 36°F and pH 7.0 to a second tank at 36°F and pH 6.8. Eleven of these fish died within 48 hours for no apparent reason. These cod had been well fed and had been kept for three months

months previously. On the other hand some cod remain which have been held in the tanks for nine months, since the first tanks were installed.

SUMMARY NO. 66

FACILITIES:
WALK-IN COLD ROOM AND LIVE-HOLDING FACILITIES AT GRANDE RIVIÈRE.

P.H. Odense
R. Legendre

One of the rooms at the Grande Rivière station has been converted into a cold-room. This fulfills a long standing need for an adequate low temperature laboratory in which to work with the heat labile proteins of fish. The room is fully equipped with electrical outlets, water supply and sinks, laboratory benches and shelves for equipment. Extractions, starch gel electrophoresis, column chromatography, etc., will be done here. The room will be kept at a temperature of $2^{o}C$.

SUMMARY NO. 67

FACILITIES:
NEWFOUNDLAND UNIT.

P.H. Odense
W.A.MacCallum

Two units of a portable acetone - dry ice refrigerated plate freezer, designed in collaboration with Dr. Odense at the Halifax Research Laboratory, were built under contract and used successfully at sea and ashore. Faster freezing rates than obtained in commercial plate freezers can be realized if desired. A request for data on freezing rates for fillets frozen from one side only was received from an Industry member. The data were obtained using the above freezer.

A walk-in refrigerator, required as a cold laboratory for current analytical tests, was installed in the basement of the Main Building. Existing live-holding facilities were improved. Non-corrosive heat exchangers were designed and are now being installed. Designs for a volatile solvents storage room and for renovations to the stairs, library and storage facilities in the Main Building and for the final stage of renovations to the Refrigeration Building were completed. Work on separate contracts for the above work was

completed and sole occupancy of the Refrigeration Building was obtained. Cold storage boxes were designed for use in this space. Installation is scheduled for April, 1964. Preliminary work concerned with the design and selection of appropriate refrigeration and monitoring equipment for the rooms is continuing and increasingly demands the attention of the Unit Chief.

PUBLICATIONS 1963-1964

H A L I F A X

The following papers were published during the period April 1, 1963 to March 31, 1964:

1. Ackman,R.G. Structural correlation of unsaturated fatty acid esters through graphical comparison of gas-liquid chromatographic retention times on a polyester substrate. Journal of the American Oil Chemists' Society, Vol. 40, pp. 558-564, 1963.

   The correlation of structures of esters of certain unsaturated fatty acids is possible through a linear relation when logarithms of retention time on polyester substrates are plotted against the number of carbon atoms in the fatty acid chain.

   This correlation is independent of any relation to the retention times of the esters of the saturated fatty acids, and depends solely on the fatty acid chain length, the number of double bonds, and the length of the end carbon chain. It appears to depend on the influence of the latter on vapor pressure.

   A sound basis is thus provided for advocating a modified equivalent chain length system, virtually independent of temperature effects, and based on certain commonly occurring monounsaturated acids rather than on the saturated acids.

2. Ackman,R.G. An analysis of separation factors applicable in the gas-liquid chromatography of unsaturated fatty acid methyl esters on a polyester substrate. Journal of the American Oil Chemists' Society, Vol. 40, pp. 564-567, 1963.

   The employment of gas-liquid chromatography (GLC) separation factors between methyl esters of unsaturated fatty acids is feasible as a means of tentative identification, either between acids of one chain length and differing numbers of double bonds, or between acids of one chain length and the same number of double bonds in differing positions, provided the acid structures are appropriately grouped by end carbon chain. The modification of separation factors by temperature, chain length, number of double bonds, or position of double bonds is apparent from examination of a larger number of examples than was hitherto available. Examples of the usefulness of separation factors in identifying unknowns or predicting retention times are given.

3. Ackman,R.G. Observations on the gas-liquid chromatography of n-monoalkenes with reference to the systematic identification of esters of unsaturated fatty acids through separation factors and log retention time plots. Journal of Chromatography, Vol. 12, pp. 271-276, 1963.

On limited evidence it appears that as operating temperatures increase, inductive effects in n-alkenes governing the polarity of the double bonds decrease, suggesting that other influences must govern the gas-liquid chromatographic separation of isomerically unsaturated materials on polar substrates at elevated temperatures.

The examination of hydrocarbon retention times on various substrates from the point of view of separation factors and in one case of the linear log plot system supports the viewpoint that these systematic relationships in the gas-liquid chromatography of unsaturated fatty acid esters on polyester substrates may be largely based on similarities in volatility due to contributions from similar structural elements.

The application of systematic separation factors to the analysis of hydrocarbon mixtures on strictly inert substrates may be useful if such mixtures are essentially straight-chain alkenes and if the relationships proposed can be shown to hold for longer chain materials where reference compounds of known structure may not be readily available.

4. Ackman, R.G. Influence of column temperature in the gas-liquid chromatographic separation of methyl esters of fatty acids on polyester substrates. Journal of Gas Chromatography, Vol. 1, pp. 11-16, 1963.

Operation of any polyester column at a given temperature often will not be completely satisfactory for the detection of some of the components present in complex lipid systems. Use of the same column at different temperatures to resolve components known or suspected to be present may in many cases be simpler than the use of two different columns.

5. Ackman, R.G. Quantitative gas-liquid chromatography of fatty acids. Chemistry in Canada, Vol. 15, No. 4, p.16, April, 1963.

(Abstract of a paper given at the meeting of the Chemical Institute of Canada, Toronto, June 6-8, 1963.)

The qualitative separation, and to some extent the quantitative estimation, of fatty acids of either short or long chain lengths has been possible for some time. Quantitative results may now be achieved for the lower volatile fatty acids in aqueous solutions as dilute as 0.01% (W/W). Very satisfactory separations of the longer-chain polyunsaturated acids on a polyester substrate are also feasible on a quantitative basis. These analyses are carried out with a flame ionization detector, employing formic acid vapor, which gives no response in this detector, in the carrier gas to suppress the undesirable reversible adsorption of the weaker acids.

6. Ackman, R.G., and R.D. Burgher. Quantitative gas liquid chromatographic estimation of volatile fatty acids in aqueous media. Analytical Chemistry, Vol. 35, pp. 647-652, 1963.

The quantitative analysis by gas liquid chromatography of volatile fatty acids in aqueous solutions has been rendered difficult by adsorption and the appearance of ghost peaks on subsequent injection of samples. The addition of formic acid

vapor to the carrier gas eliminates these problems and gives reproducible quantitative results in conjunction with a flame ionization detector. Excessive amounts of water under certain conditions may cause double peak formation, but the method is applicable to analyses with Silicone, polyester, and Tween substrates on Chromosorb W support.

7. Ackman, R.G., and R.D. Burgher. A proposed basis for the systematic identification of unsaturated fatty acid esters through gas-liquid chromatography on polyester substrates. Journal of Chromatography, Vol. 11, pp. 185-194, 1963.

Systematic separation factors may be established among various unsaturated fatty acid methyl esters analysed by gas-liquid chromatography on polyester substrates. These are apparently dependent on differences in the magnitude of the carboxyl end chain and end carbon chain parts of the fatty acid chain as a survey of all relevant literature data indicates that the ratio of separation factors in the C_{18} acids is independent of operating variables.

8. Ackman, R.G., and R.D. Burgher. Employment of ethanol as a solvent in small scale catalytic hydrogenations of methyl esters. Journal of Lipid Research, Vol. 5, pp. 130-132, 1964.

The method of analytical hydrogenation of ester mixtures for gas chromatography employed in this laboratory has been investigated for the possible formation of ethyl esters from methyl esters through interesterification in the ethanol employed as a solvent. This does not take place. The recovery procedures following hydrogenation were also investigated and small possible losses of certain lower fatty acid esters were observed.

9. Ackman, R.G., and R.D. Burgher. Component fatty acids of the milk of the grey (Atlantic) seal. Canadian Journal of Biochemistry and Physiology, Vol. 41, pp. 2501-2505, 1963.

A gas-liquid chromatographic examination of the component fatty acids of the milk of the grey (Atlantic) seal confirms previous findings that in general composition the milk fats of marine mammals resemble the depot fat. An empirical correction system is suggested for quantitation in certain argon ionization detectors.

10. Ackman, R.G., R.D. Burgher and P.M. Jangaard. Systematic identification of fatty acids in the gas-liquid chromatography of fatty acid methyl esters: a preliminary survey of seal oil. Canadian Journal of Biochemistry and Physiology, Vol. 41, pp. 1627-1641, 1963.

The fatty acids of a commercial seal blubber oil have been surveyed by application of the linear log plot and separation factor procedures to the gas-liquid chromatograms of methyl esters obtained from urea complexing. These techniques, coupled with the use of two polyesters, have permitted the tentative identification of virtually all of the components. The overall composition of the seal blubber oil appears to cover the same range of fatty acids as the body oils of menhaden and herring.

11. Ackman,R.G., R.D. Burgher and J.C. Sipos. Quantitative gas-liquid chromatography of the higher fatty acids. Nature, Vol. 200, No. 4908, pp. 777-778, 1963.

The addition of formic acid vapour to the carrier gas effectively suppresses adsorption of weaker acids on the support and possibly in the substrate as well. This procedure can only be carried out with a flame ionization detector, since in this detector formic acid gives no response, but the presence of the formic acid does not affect the response characteristics of other carbon compounds.

The extension of this quantitative technique to the higher fatty acids, using an efficient polyester substrate to give separations of the unsaturated acids equivalent to those obtained with their methyl esters, has been found to be practical.

12. Ackman,R.G., and P.M. Jangaard. Identification of the major polyunsaturated C_{16} acids of marine oils by GLC separation factors on normal and organosilicone polyesters. Journal of the American Oil Chemists' Society, Vol. 40, pp. 744-745, 1963.

The tentative identification of the unsaturated C_{16} acids of marine oils is facilitated through analysis on both normal and organosilicone polyester substrates. Two different separation factors can then be approximated from the more accessible separation factors appropriate to unsaturated acids of longer chain lengths.

13. Ackman,R.G., and P.M. Jangaard. On the occurrence of 4,7,10,13,16-docosapentaenoic acid in saury (Cololabis saira) oil. J.Fish.Res. Bd., Vol. 20, pp. 1551-1552, 1963.

A sample of saury oil has been analyzed by gas-liquid chromatography on a number of polyester substrates with provisional peak identifications by the linear log plot and separation factor procedures. The results indicate that the proportions of 4,7,10,13,16- and 7,10,13,16,19-docosapentaenoates, contrary to a previous suggestion for this oil, conform to those normally observed in marine oils (about 1:10).

14. Ackman,R.G., R.D. Burgher, M.L. Hughes and W.A. MacCallum. Observations on the oil and component fatty acids of the oil from Newfoundland capelin. J. Fish. Res. Bd., Vol. 20, pp. 591-596, 1963.

The oil recovery from beach-spawning Newfoundland capelin has confirmed an earlier report that the fat content is of the order of 3-4%. The oils recovered from both sexes were examined, that from the female having a significantly higher iodine value. Owing to the sex ratio on the beach a commercial oil might be essentially that from the male capelin and is similar in properties and distribution of major fatty acid components to herring oil. The content of unsaponifiable matter was somewhat higher than in commercial herring oil.

15. Ackman,R.G., and J.C. Sipos. Ketoacid polymers as gas-liquid chromatography substrates. Journal of Chromatography, Vol. 13, pp. 337-343, 1964.

A purely organic polymer with sufficient carboxyl group content to permit direct analysis of reasonably high levels of volatile fatty acids has been prepared. Stability and the use of appropriate supports are discussed.

16. Brockerhoff,H., and R.J. Hoyle. On the structure of the depot fats of marine fish and mammals. Archives of Biochemistry and Biophysics, Vol. 102, pp. 452-455, 1963.

Fish oils and fish liver oils were found to contain poly-unsaturated fatty acids accumulated in the β position of the glycerol. Blubber oils from pilot whale and two species of seal had a different triglyceride structure; the shorter fatty acids prevailed in the β, the longer acids in the α position, independent of unsaturation. This pattern resembles that of pig depot fat.

17. Brockerhoff,H., R.J. Hoyle and K. Ronald. Retention of the fatty acid distribution pattern of a dietary triglyceride in animals. Journal of Biological Chemistry, Vol. 239, pp, 735-738, 1964.

A β-labelled triglyceride was fed to lobster, cod, trout and rat, and the distribution of label in the fats of these animals was then determined. In the invertebrate and in the fish, 50 to 80% of the structure of the dietary triglyceride had been retained. No retention was observed in the triglycerides of rat. It appears, therefore, that fish and invertebrates, though not mammals, can deposit dietary fats without disruption of the β-monoglyceride bonds.

18. Castell,C.H. A report to the fishing industry on the significance of botulism in the Canadian fisheries. Fisheries Research Board of Canada, Technological Research Laboratory, n.s. Circular, No. 14, Feb., 1964. 11 pp.

This circular was written specifically to give the industry an accurate but non-technical account of botulism in relation to fishery products. It tells of the nature of the disease, the characteristics of the casual organisms, and their distribution in nature. Although it stresses the fact that the botulinum toxin is the most deadly substance found in nature, it also points out that outbreaks of botulism in Canada from Canadian fish, caught and processed by commercial firms in Canada, are non-existant at the present time. No such cases have occurred in the last 40 years. There is need for constant care, but nothing to substantiate the recent unnecessary alarm.

19. Castell,C.H., and Jacqueline Dale. Antibiotic dips for preserving fish fillets. Fisheries Research Board of Canada, Bulletin No. 138, 1963. 70 p.

This bulletin deals with some of the problems encountered in the use of chlortetracycline as a preservative dip for fresh fillets. It points out that CTC acts chiefly as a chelating agent, binding trace amounts of metals that are necessary for

microbial growth and for enzyme activity. An excess of these metals will therefore inactivate the CTC as a preservative. The antibiotic is also destroyed by oxidizing agents and decomposes under alkaline conditions. CTC has a selective action on fish-spoiling bacteria, and if care is not taken, it is possible to build up a microflora in the plant that is insensitive to the antibiotic. A study has been made of some of the factors that affect the amount of CTC taken up by commercial fillets and of the factors that affect the keeping time of CTC-treated fillets. Suggestions are given as to how the operators might obtain the most efficient use of the antibiotic under commercial conditions, as they exist in some of our large east coast processing plants.

20. Dambergs,N. Extractives of fish muscle. 3. Amounts, sectional distribution, and variations of fat, water-solubles, protein and moisture in cod (Gadus morhua L.) fillets. J. Fish. Res. Bd., Vol. 20, pp. 909-918, 1963.

The amounts, sectional distribution and some variations of fat, water-solubles, protein and water in cod fillets have been determined on defined, pre-rigor samples. It was found that the head-end of the fillet is richest in protein. The middle section is the richest in water-solubles while the tail-end contains more fat and water than any other part of the fillet. Only slight differences in the major components were detected between the male and female specimens.

21. Dingle,J.R., D.G. Ellis, J.A. Hines and J.T. Lauder. Proteins in fish muscle. 18. Sedimentation patterns of myosin-B extracts of pre-rigor cod muscle. Canadian Journal of Biochemistry and Physiology, Vol. 41, pp. 1915-1926, 1963.

Ultracentrifuge patterns (schlieren optics) of extracts of pre-rigor cod muscle, obtained by blending with 0.6 M salt solutions, showed only one major component together with a few very minor ones provided the concentration of adenosinetriphosphate (ATP) derived from the muscle was greater than 1.5×10^{-4} M in the extract. At lower ATP concentrations, caused by its enzymic decay in aged extracts or by exhaustion of the muscle either by exercise or electric shock, peaks of heavier components appeared at the expense of the original lighter one. Ultracentrifugation of extracts at the higher ATP level prevented to some extent this change upon aging. Amino acid analyses showed that the sediment from this operation and also the residue from the original extraction had the composition of actin.

22. Dyer, W.J. Basic quality changes in frozen seafood. ASHRAE Journal, Vol. 6, No. 1. pp 77-78, 1964.

A review of the basic quality changes occurring in frozen fish and their effect on final quality from the consumers' viewpoint. The importance of quality loss during frozen storage under poor conditions was contrasted with the lesser effect of bacterial and antolytic changes occurring prior to freezing. Frying gave increased acceptability over baked or steamed products when quality was less than perfect.

23. Dyer, W.J.,H.Brockerhoff, R.J. Hoyle and D.I. Fraser. Polyphosphate treatment of frozen cod. I. Protein extractability and lipid hydrolysis. J. Fish. Res. Bd., Vol. 21, pp. 101-106, 1964.

Tripolyphosphate dipped and undipped control paired cod fillets stored at -12°C showed no differences occurring on storage in extractable protein, lipid hydrolysis, or thaw drip. A higher yield of frozen and of thawed fillets was obtained from the dipped samples due to water uptake.

No specific effect of tripolyphosphate on thaw drip was obtained at the ionic concentrations used, but it is postulated that such effects might occur in the presence of about 1% salt.

24. Dyer,W.J., D.I. Fraser and S.N. Tibbo. Composition and palability of porbeagle flesh. J. Fish. Res. Bd., Vol. 20, pp. 1153-1158, 1963.

Frozen porbeagle (Lamna nasus) steaks from samples obtained under conditions typical of goodand of poor handling were examined and their chemical composition determined. Lipid content was low, about 1% in the light muscle and 2% in the red. Phospholipase activity was absent, but considerable hydrolysis of triglyceride occurred. There was no ammonia production from the high urea content, 1.4-1.6%. Taste testing of cooked steaks showed only fair acceptability, an objectionable sour-to-bitter flavour being noted. Although its flavour may be relished by some, it is concluded that frozen porbeagle would not be generally acceptable to North Americans as a top-quality fisheries product.

25. Idler, D.R., H.C. Freeman and B. Truscott.. Steroid hormones in the plasma of spawned Atlantic salmon (Salmo salar) and a comparison of their determination by chemical and biological assay methods. Canadian Journal of Biochemistry, Vol. 42, pp 211-218, 1964.

11-Ketotestosterone, cortisone and cortisol were isolated and identified as the principal steroid hormones in spawned male Atlantic salmon plasma and the dihydroxy-acetone steroids in spawned male and female Atlantic salmon plasma were determined.

A large plasma sample from spawned female Atlantic salmon was analyzed for adrenocorticosteroids using both the mouse liver glycogen deposition bio-assay and the Porter-Silber chemical method. The results were in agreement and it was thus shown, contrary to a previous suggestion, that the major steroids have the dihydroxyacetone structure in the side chain and there is no indication of large quantities of steroid lacking the hydroxyl group at C-17.

26. Idler, D.R., and B. Truscott, with the collaboration of H.C.Freeman, V. Chang, P.J. Schmidt and A.P. Ronald. In vivo metabolism of steroid hormones by sockeye salmon (A) Impaired hormone clearance in mature and spawned Pacific salmon (O. nerka)

(B) Precursors of 11-ketotestosterone. Canadian Journal of Biochemistry and Physiology, Vol. 41, 875-887, 1963.

Intra-arterially injected cortisone-4-C^{14} and cortisol-4-C^{14} were cleared from the plasma of sexually mature and spawned sockeye salmon (O. nerka) at a much slower rate than from the plasma of immature sockeye and spawned Atlantic salmon (Salmo salar). The results explain the elevated hormone levels found in the blood of mature and spawned sockeye salmon. The normal clearance rate found with Atlantic salmon, which frequently survive spawning, would indicate that the impaired hormone metabolism was associated with the imminent death of of the Pacific salmon rather than with the act of spawning.

Testosterone and 17α-hydroxyprogesterone were found to be precursors of 11-ketotestosterone, a sex hormone found in high concentrations in the blood of mature sockeye salmon. Testosterone was also formed in vivo from 17α-hydroxyprogesterone. The results suggest more than one pathway for the synthesis of 11-ketotestosterone in salmon. Cortisol was converted to cortisone but no conversion of the former to 11-ketotestosterone could be demonstrated.

27. Jangaard, P.M., R.G. Ackman and R.D. Burgher. Component fatty acids of the blubber fat from the common or harbour seal Phoca vitulina concolor de Kay. Canadian Journal of Biochemistry and Physiology, Vol. 41, pp. 2543-2546, 1963.

The component fatty acids of the harbour seal, as determined by gas-liquid chromatography, are qualitatively the same as those demonstrated in the harp seal. In contrast to teleost fish depot fats the ratio of palmitoleic acid to palmitic acid is very high. (ca 3:1).

28. Stewart, James E. An arrangement for automatically and reproducibly controlling and varying illumination in biological experiments. J. Fish. Res. Bd., Vol. 20, pp. 1103-1107, 1963.

A control unit allowing the amount of light in biological experiments to be varied continuously and reproducibly is described. Repetition of a 24-hour program is automatic. Control is achieved through voltage changes in the power fed to the operating unit. Thus the controls can be adapted readily to any instrument whose performance can be varied by voltage changes.

29. Stewart, James E., P.L. Hoogland, H.C. Freeman and A.E.J. Waddell. Amino acid composition of representatives of eight bacterial genera with reference to aquatic productivity. J. Fish. Res.Bd. Vol. 20, pp. 729-734, 1963.

Representatives of eight bacterial genera were grown under identical conditions and the harvested, washed cells. were analyzed quantitatively for eighteen amino acids using chemical methods. All of the bacteria contained the eighteen amino acids and, although quantitative differences were noted, variation in composition was not great. Cell nitrogen content averaged 11.91% of the dry weight. Some of the nutritional implications of the data are discussed.

30. Stewart, James E. and H.E. Power. A sea water aquarium for marine animal experiments. J.Fish.Res. Bd., Vol. 20,pp. 1081-1084, 1963.

An aquarium with a continuous flow sea water supply is described. The system consists of 42 tanks suitable for holding and experimenting with fish and shellfish and an extensive pipeline and filter arrangement.

31. Stewart, James E. and H.E. Power. A continuous flow, seawater aquarium suitable for experimental work with live marine animals. Fisheries Research Board of Canada, Technological Research Laboratory,Halifax n.s. Circular,No. 13, 1963, 7 pp.

The construction of an aquarium with a continuous flow sea water supply suitable for holding and experimenting with fish and shellfish is described and discussed in detail. The system consists of 42 animal holding tanks, each tank having a capacity of 84 Imperial gallons. Sea water is pumped continuously from Halifax harbour, through filters, into the holding tanks and the displaced water is drained back to the harbour through an overflow system.

32. Townsley, P.M., H.G. Wight and M.A. Scott. Marine fish tissue culture. J. Fish. Res. Bd., Vol. 20, pp. 679-684, 1963.

Cell proliferation of tissue explants of different organs from marine fish has been achieved in a nutrient solution composed of Medium 199 plus 10% human serum. Fin, spleen, heart, kidney, liver, gonad, brain, uterus, and thymus tissues have been cultured. The tissues were obtained from sexually mature Atlantic cod (Gadus morhua), white perch (Roccus americanus) winter flounder (Pseudopleuronectes americanus) thorny skate (Raja radiata), American goosefish (Lophius americanus), pollock, (Pollachius virens), and shorthorn sculpin (Myoxocephalus scorpius). An actively dividing cell culture of flounder kidney cells prepared by mechanical disruption of the kidney tissue was maintained through serial transfers over several months. Heart explants from the cod vigorously pulsated in tissue culture.

33. Townsley, P.M., H.G.Wight, M.A. Scott and M.L. Hughes. The in-vitro maturation of the parastitic namatode, Terranova decipiens,from cod muscle. J. Fish. Res. Bd., Vol. 20, pp. 743-747, 1963.

The larvae of the parasitic nematode, Terranova decipiens, from fish muscle will mature to the adult in in-vitro culture. This stage in the life cycle of this nematode normally occurs in the digestive tract of the seal. A rather complex medium was used. This medium was composed of a commercially available tissue culture preparation, Medium 199, to which had been added glucose, beef embryo extract, beef liver extract and antibiotics. The incubation time required for the appearance of the adult nematode in culture was approximately the same as in the seal digestive tract.

34. Wood, A.L. Accelerated cooling of wet, heavily salted fish. J.Fish.Res. Bd., Vol. 20, pp. 997-1000, 1963.

"Green" heavily salted split fish may be pre-cooled within a cold storage area with ice and salt, without imposing an excessive load upon storage refrigeration facilities and with little additional labour. Twelve pounds of ice plus 4 lb of salt per 100 lb of fish, uniformly applied between 3- to 4-inch thick layers of fish, will reduce the bulk fish temperature by 25°F (14° C) in less than 8 hours.

No mechanical equipment is required if flake ice be deemed a commodity. The direct cost may be estimated at less than 15 cents per 100 lb of finished (dried) cod.

Pre-cooling is not essential under normal conditions but is recommended for heavily salted fish in which the degree of red bacterial contamination is unknown or suspected to be critical, and the storage refrigeration is not adequate to cool the product in a sufficiently short time.

The following papers have been submitted, or are in press:

35. Ackman,R.G. Fundamental groups in the response of flame ionization detectors to oxygenated aliphatic hydrocarbons. (Journal of Gas Chromatography)

A survey of the relative molar responses for a wide variety of oxygenated aliphatic hydrocarbons indicates that additive responses may be based on a number of fundamental "groups". The particular responses of the polyatomic "groups" are probably associated with the weight percent carbon content of each group.

36. Ackman,R.G. Structural homogeneity in unsaturated fatty acids of marine lipids: a review (J. Fish. Res. Bd.)

A survey of the literature data indicates that marine lipids, at least those from teleost fish, contain only a limited number of fatty acids in readily detectable amounts. Minor variations in the proportions of the major components, especially 5,8,11,14,17-eicosapenthaenoic and 4,7,10,13,16 19-docosahexaenoic acids, result in the wide range of iodine values found in different species, but more interesting and significant variations of these and other acids may be found in different tissues of one species.

37. Ackman,R.G., and R.D.Burgher. Cod liver oil fatty acids as secondary reference standards in the GLC of polyunsaturated fatty acids of animal origin: analysis of a dermal oil of the Atlantic leatherback turtle. (Journal of the American Oil Chemists' Society)

It is shown that cod liver oil fatty acids, although primarily of the linolenic type, may be used as secondary reference standards in the gas-liquid chromatography of other animal lipid fatty acids where the linoleic type acids

predominate. The leatherback turtle is the only marine species examined to date in which it has been shown that high proportions of the polyunsaturated fatty acids belong to the linoleic family.

38. Ackman,R.G., and R.D. Burgher. Cod liver oil: component fatty acids as determined by gas-liquid chromatography.(J. Fish. Res. Bd.)

A thorough analysis of cod liver oil fatty acids has been compared with other reports on this lipid. The general agreement on a qualitative basis is good, but the present study includes a number of minor fatty acids not previously reported.

39. Ackman,R.G., and R.D. Burgher. Cod-flesh: component fatty acids as determined by gas-liquid chromatography. (J. Fish. Res.Bd.)

The component fatty acids of cod flesh are shown to contain high proportions of polyunsaturated fatty acids. These occur chiefly in the phospholipids (>80% of the lipid). Comparison with a similar analysis of North Sea cod flesh suggests that the lipid fatty acid composition of the flesh of this species does not vary particularly.

40. Ackman,R.G., and R.D. Burgher. Cod roe: component fatty acids as determined by gas-liquid chromatography. (J.Fish. Res. Bd.)

The fatty acid composition of the roe is compared with the flesh and liver analyses of cod and suggestions are made as to the metabolic role of certain fatty acids.

41. Ackman,R.G., P.M. Jangaard, R.J. Hoyle and H. Brockerhoff. Origin of marine fatty acids. I. Analyses of the fatty acids produced by the diatom Skeletonema costatum. (J.Fish.Res.Bd.)

The culture of this diatom indicates that the introduction of longer-chain more highly unsaturated fatty acids, common in fish, to the aquatic food chain may occur in phytoplankton as well as in the intermediary zooplankton. Shifts in the biosynthetic pathways to linoleic or linolenic type acids as the culture matures indicate that mature laboratory cultures may give misleading results when compared with natural plankton blooms.

42. Ackman,R.G., and J.C. Sipos. Increasing the sensitivity of the Aerograph A-90-S with W-2 detector cell filaments. (Research Notes, published by Wilkens Instrument and Research Inc.).

The use of a new type of catherometer filaments provides a ten-fold increase in sensitivity for this apparatus. Simple bridge circuit alterations employing a maximum of parts from the original circuit are described.

43. Ackman,R.G., and J.C. Sipos. Application of specific response factors in the gas chromatographic analysis of methyl esters of fatty acids with flame ionization detectors. (Journal of the American Oil Chemists' Society).

It is shown that the weight response of longer chain fatty acid methyl esters in the flame ionization detector is proportional to the weight per cent carbon content of the fatty acid chain. The significance of correction factors in individual analyses is discussed.

44. Ackman,R.G., and J.C. Sipos. Flame ionization detector response for the carbonyl carbon atom in the carboxyl group of fatty acids and esters. (Journal of Chromatography.)

A tabulation of the relative molar responses of acids and esters is presented. This shows that the relative molar responses of esters of relatively short chain acids and alcohols have carbon atom deficiencies ranging from 1.5 to 1.0. The significance of this in relation to other literature reports is discussed.

45. Ackman,R.G., and J.C. Sipos. Pilchard oil: an analysis for component fatty acids with particular reference to the C_{24} chain length. (J. Fish. Res. Bd.)

The gas-liquid chromatographic analysis of the oil from the Pacific sardine or pilchard (Sardinops sagax (Jenyns)) indicates, contrary to a previous report, that only the proportions of C_{24} acids commonly found (ca. 1%) in marine oils are present. The relative proportions of the acids of this chain length (24.1> 24.5 > 24.6) are also in accord with those observed in other cases.

46. Ackman,R.G., and J.C. Sipos. Carboxyl group response in flame ionization detectors. (Submitted to the Chemical Institute of Canada. Paper to be read at meeting in June, 1964 and to appear as an abstract in "Chemistry in Canada".)

The response of the carboxyl group in flame ionization detectors has been explored for both free acids and for certain aliphatic esters. In the case of the acids the carboxyl group carbon atoms makes an increasing contribution to molecular response as the fatty acid chain length increases, until with acids of six or more carbon atoms, the response is nearly the same as for the methylene carbon atoms. The response pattern for esters is more complex, but methyl esters of the longer chain fatty acids have a total molecular response equivalent to the number of carbon atoms in the fatty acid chain. Experiments with formate esters, which have a relatively higher response in the case of the shorter chain lengths suggest that different modes of scission of the ester group linkages may be involved.

47. Brockerhoff,H. Stereospecific analysis of triglycerides. (Journal of Lipid Research.)

A method was developed to determine the fatty acid distribution in the three positions of an asymmetric triglyceride. The method was tested on two synthetic triglyceride mixtures.

48. Brockerhoff, H., M. Yurkowski, R.J. Hoyle and R.G. Ackman. Fatty acid distribution in lipids of marine plankton. (J.Fish.Res. Bd.)

In the diatom Skeletonema costatum, as well as in a zooplankton sample, the polyunsaturated fatty acids of the triglycerides were found accumulated in the β position. This fatty acid distribution pattern is typical for animal fats, in particular for fish oils. Together with the recent demonstration that fish and invertebrates retain in part the structure of ingested triglycerides, the findings of the present study show that the typical structure for marine triglycerides originates in phytoplankton and is to a large degree retained through the marine food chains.

49. Castell,C.H., and Jill MacLean. Rancidity in lean fish muscle. 2. Variations in the susceptibility to rancidity of muscle from different parts of the fish and in fish caught at different seasons of the year as measured by a copper-catalytic method. (J.Fish.Res.Bd.)

In a study of copper-catalyzed rancidity of cod fillets it has been found that: (1) muscle from the tail section goes rancid faster than muscle from the head or centre sections; (2) there is no apparent difference in the development of rancidity in the inner and outer halves of fillets that have been sliced lengthwise; (3) sensitivity to rancidity changes with the season and is greater in the winter and early spring than in the summer and fall.

50. Dambergs, N. Extractives of fish muscle. 4. Seasonal variations of fat, water-solubles, protein and water in cod (Gadus morhua L.) fillets.(J. Fish. Res. Bd.)

The concentrations of fat, water-solubles, protein and water in cod muscle are subject to seasonal variations. During the period of spawning there is a decrease in fat (-20%) and protein (-5%) paralleled by an increase of water-solubles (+10%) and water (+5%). The return of these major components to their average annual values occurs at various rates. The seasonal emaciation of the cod is closely linked to the physiological processes taking place in the animal's body during the reproductive activities.

51. Dyer,W.J. Basic quality changes in frozen seafood. (Complete paper submitted to ASHRAE Journal)

A review of the basic quality changes occurring in frozen fish and their effect on final quality from the consumers' viewpoint. The importance of quality loss during frozen storage under poor conditions was contrasted with the lesser effect of bacterial and autolytic changes occurring prior to freezing. Frying gave increased acceptability over baked or steamed products when quality was less than perfect.

52. Dyer, W.J., Doris I. Fraser, Ruth G. MacIntosh and Margaret Myers. Cooking method and palatability in frozen cod fillets of various qualities. (J. Fish. Res. Bd.)

Cooking method modified the taste and texture scores of frozen cod fillets of various qualities. All methods gave high scores with good quality fish, but on samples with frozen storage deterioration, much better discrimination between quality levels was shown by baking and steaming than by frying; texture showed greater differences than taste. Initial quality levels of the material prior to freezing, Grade I, II or III, had little effect on scores or on the subsequent deterioration of the stored frozen product. Thus baking or possibly steaming is the best cooking method for detection and comparison of quality changes on storage. Frying is the method of choice for the consumer if product quality is in doubt, but any method may be used for a top quality product.

53. Fraser, Doris I., H.M. Weinstein and W.J. Dyer. Postmortem glycolytic and associated changes in the muscle of trap- and trawl-caught cod. (J. Fish. Res. Bd.)

Glycolytic activity in the muscle of heavily-feeding, trap-caught cod during the struggle involved in catching, and the subsequent postmortem changes during holding in ice and at ambient temperatures were investigated. A "rested" cod from the trap had a high initial glycogen content of 550 mg/100 g; a rapid drop ensued during boating to levels of about 200 mg/100 g, while lactate production was delayed into the early post-rigor period. Further decreases in glycogen and high-energy phosphate compounds took place during storage in air or ice, although still incomplete at 5 hr. Little change occurred on freezing and storage at -26°C for 4-6 weeks. In contrast, trawled off-shore cod and haddock, regardless of antemortem treatment, contained little or no glycogen; almost complete dephosphorylation and deamination of the nucleotide compounds had occurred during the struggle involved in catching. Therefore, at the time of boating, the energy reserves in the trawled fish are exhausted or nearly so, whereas in trap cod they have been only partially depleted.

54. Li, M.G., and J.E.Stewart. Preliminary results in the development of a tissue culture method for the study of diseases of the oyster (Crassostrea virginica). (Abstract of paper submitted for presentation at a meeting of the Canadian Society of Microbiologists, 14th Annual Meeting, University of New Brunswick, Fredericton, June 4-6, 1964.)

Explants from the oyster heart have been cultured. Vigorous and rhythmical contractions persist for several months in the explant. Amebocyte-like cells migrate from the heart explant and proliferate in spindle form in the "low" pH medium after incubation for 24 to 48 hours at 18°C. In addition outgrowths from the explant have been noted. The medium, tissue growth and some aspects of the disease are discussed.

55. MacLean, Jill and C.H.Castell. Rancidity in lean fish muscle. 1. A proposed accelerated copper-catalyzed method for evaluating the tendency of fish muscle to become rancid. (J. Fish. Res. Bd.)

A method that can be carried out within 24 to 72 hours has been suggested for determining the tendency of fish muscle to become rancid. It consists of adding measured, trace amounts of copper ion to muscle that has been blended 1:2 with water followed by storage at 0°C.

The rancidity is observed subjectively by noting the odours that develop and objectively by means of the Thiobarbituric acid reaction.

56. Power, H.E., D.I. Fraser, W. Neal, W.J. Dyer and C.H. Castell. Gamma irradiation as a means of extending the storage life of haddock fillets. (J. Fish. Res. Bd.)

Studies of the storage life of iced haddock (Melanogrammus aeglefinus) fillets after treatment with doses of gamma radiation of 75,000, 150,000, and 250,000 rads, have shown that a useful extension of the storage life is made possible by irradiation. The fillets irradiated with the 75,000 rad dose and assessed after cooking became unacceptable after about 25 days at 0°C. The unirradiated control became unacceptable after 12 days. Fillets irradiated at the two higher levels were of lower quality initially than the fillet irradiated at the 75,000 rad level but showed no significant decrease in acceptability up to 27 days storage in ice. Raw fillets became unacceptable just prior to becoming unacceptable to the taste panel.

57. Power, H.E., D.I. Fraser, W. Neal, W.J.Dyer, C.H. Castell, H.C.Freeman and D.R. Idler. The use of gamma radiation for the preservation of scallop meat. (J. Fish. Res. Bd.)

Storage studies of iced irradiated scallops (Placopecten magellenicus Gmelin) have shown that treatment with a dose of 75,000 rads of gamma radiation increases the useful storage life at 0°C with negligible changes in odour and texture. The raw scallops remained acceptable for 28 days, while the scallops assessed after cooking remained unchanged for 35 days and did not become unacceptable until 43 days storage. Doses of gamma radiation greater than 75,000 rads caused undesirable changes in texture and odour with a consequent loss of quality. Neither the irradiated scallops nor the fresh iced control scallops were rated as high by the organoleptic assessment panel as the frozen control scallops which had been rapidly frozen in a pre-rigor condition and immediately stored at -26°C.

58. Sipos, J.C., and R.G. Ackman. Association of dimethyl sulphide with the "blackberry" problem in cod from the Labrador area. (J.Fish. Res. Bd.)

An offensive and normally persistant odour in Labrador cod (termed blackberry owing to the appearance of the stomach contents of the cod) has been investigated with gas-liquid chromatography. Only one major component was found in chromatograms. Through retention times and chemical tests this peak was associated with the odour and identified as dimethyl sulphide.

59. Tamura, T., T. Wainai, B. Truscott and D.R. Idler. Isolation of 22-dehydrocholesterol from scallop. (Canadian Journal of Biochemistry.)

22-Dehydrocholesterol has been isolated from the sterols of the scallop Placopecten magellanicus Gmelin. The azoyl esters of the unsaponifiable fats were separated into four zones by chromatography. The Δ^{22}-sterol accounted for 31.3% of the chromatographic δ-zone and 14.1% of the total sterol fraction. The homogeneity and structure of the isolated sterol was established by gas chromatography. Further confirmation of its identity was afforded by comparison of its physical properties with synthetic 22-dehydrocholesterol and with 22-dehydrocholesterol isolated from a species of red algae.

60. Townsley, P.M., and M.L. Hughes. Early stages in the recovery to injury in the dorsal fin of the Atlantic cod (Gadus morhua). (J. Fish. Res.Bd.)

The early stages in the recovery of the dorsal fin of the Atlantic cod (Gadus morhua) to a "clean cut" injury are described. It is concluded that the observed rapid epidermal migration, wound closure and cell mitosis is essentially the same in in-vivo as in in-vitro experiments. An accumulation of carbohydrate material occurs in the outermost layer of epidermal cells. There is a change in the carbohydrate composition or structure in the dermal layers at the site of injury. The basal epidermal cells rapidly divide in the in-vitro culture whereas only those basal epidermal cells in an in-vivo injury in close proximity to the injury divide. The surrounding nutrient medium in in-vitro cultures does not appear to be involved in the initial cell migration. However, ascorbic acid does stimulate epidermal migration, mucus secretion, and basal epidermal cell mitosis.

N E W F O U N D L A N D

Published (See Halifax for Collaborative Publications)

61. MacCallum, W.A., and D.G. Ellis. Water-thawing of frozen cod blocks. J. Fish. Res. Bd., Vol. 21, pp. 115-131, 1964.

Thawing time for frozen blocks of dressed cod (Gadus morhua) was investigated. Observations were made on the effect of size of individual fish, water recirculation

rates, water temperature, block height, thickness and orientation, separation of individuals from the block at some intermediate time, and the use of sodium alginate to promote quicker thawing. Over the range investigated, the first two variables did not affect thawing rate; the remainder were of importance. Blocks 4 and 4 1/2 inches (102 and 115 mm) thick, weighing 75-80 lb (34-37 kg), were separated and thawed in 60°F (15.5°C) water in about 3 and 4 1/2 hr respectively. These times were equal to or better than the times for freezing between plates under best local commercial conditions. In comparison, similar blocks can be thawed in about 1 1/2 hr in a commercially available dielectric thawer. Thawing blocks of dressed fish in recirculated water thus merits consideration with other methods.

62. MacCallum,W.A., and D. G. Ellis. Water thawing of frozen blocks of eviscerated, headed cod. Canadian Fisherman, Vol. 50, No. 12, pp. 61-66, Dec., 1963.

Block, frozen, gutted cod may be thawed in water at a rate comparable to the rate of freezing. With mechanical breakup, or hand separation, of the partially thawed blocks a commercial operation would be practical.

63. MacCallum, W.A., and D.G. Ellis. Water thawing of block-frozen, gutted cod. Fishing News International, Vol. 3, No. 1, pp. 56-57, 1964.

64. MacCallum,W.A., and D.G. Ellis. Water thawing blocks of eviscerated, headed cod. Fish Merchant and Processor, Jan./Feb., 1964.

65. MacCallum,W.A., and D.G. Ellis. Thawing cod in water. World Fishing, Vol. 13, No. 2, 56-59, Feb. 1964.

66. MacCallum,W.A., M.W. Mulland and Isabel N. Plaunt. Factors influencing the effectiveness of fresh fish washing operations. J. Fish. Res. Bd., Vol. 20, pp. 1231-1244, 1963.

The effects of ratio of the amount of fish to that of wash water, kinetic energy of impinging water, method of water application and concentration of available chlorine in the wash water on the washing of eviscerated fresh cod of various caught-ages were studied. The studies were designed to provide information of practical value to engineers interested in designing machines to wash slime and bacteria from fish received at filleting plants. A quantitative relation between kinetic energy of impinging wash water and fish to water ratio was established for one practical type of spray washing equipment. Effective spraying was found to be more efficient than fluming or dipping. However, fish dipped several times in water deposited less bacterial load on a clean surface than fish washed effectively on the skin side only by a jet of water. Thus, washing out the body cavity was found to be important. Caught-age was found to influence the results only for washing

methods of relatively low effectiveness, namely in fluming and in washing inefficiently with a jet or spray of water. Ineffective spray washing was shown to be associated with waste of equipment and high costs while serving no useful production purpose. Highly effective methods of spray washing fish of all caught-ages were shown to be suitable for efficient application in fish filleting and skinning lines. Free chlorine concentrations as high as 100 ppm in the wash water did not increase washing effectiveness as measured by a bacteriological method..

67. Slavin, J.W., and W.A. MacCallum. North American experience in chilling and freezing on board vessels. Proceedings XIth International Congress of Refrigeration. Munich, 1963.

The following papers have been submitted, or are in press:

68. MacCallum, W.A. Temperatures and thaw-drip associated with electronic thawing of Newfoundland cod. (J. Fish. Res.Bd.)

Uniformity of temperature in the region of the initial freezing point (30.5°F or 0.08°C) was observed in blocks of dressed fish and skins-on shore cod fillets thawed in a 38 mc/s commercially installed electronic thawer. In general, highest surface temperatures were about 40°F 4.5°C) only.

Drip losses on thawing were 2.4% for plant-prepared skins-on fillets of trap cod dipped in a 9% solution of sodium tripolyphosphate prior to freezing and about 1% for fall-caught shore fish similarly handled. This was much less than the estimated gain (approximately 7%) resulting from the application of dip. Two lots of untreated skins-on fillets of fall-caught shore fish developed drip losses of 2.2 and 2.5% respectively.

69. MacCallum, W.A., Dorothy A. Chalker, J.T. Lauder, P.H. Odense and D.R. Idler. Polyphosphate treatment of frozen cod. 3. Taste panel evaluation, chemical assessment and thaw-drip in once-frozen Newfoundland trap-caught cod. (J.Fish. Res. Bd.)

Sodium tripolyphosphate treatment before packaging and freezing of fillets of trap-caught cod (Gadus morhua, L.) was compared with treatment in plain water. The once-frozen treated fillets, evaluated by an analytical taste panel after frozen storage at -12°F (=24.5°C), were significantly less chewy than untreated control fillets. Also, percentage thaw-drip was significantly smaller in treated samples. Lipid hydrolysis proceeded at the same rate in both treated and untreated fillets.

Vertical starch gel electrophoresis demonstrated that the drip samples contained a high concentration of the normal sarcoplasmic proteins of muscle. There was no significant difference between the gel patterns of treated and untreated samples.

Results indicate that treated fillets could have greater market acceptance than the untreated pack. The producer would benefit from improved quality of product and from increased yield.

70. MacCallum, W.A., E.J. Laishley, Dorothy A. Chalker, W.J.Dyer and D.R. Idler. Thawing temperature and the subsequent storage quality of refrozen cod. (J. Fish. Res. Bd.)

The thawing operation on block-frozen, headed, eviscerated cod was conducted in water at 60°F and at 45°F and was followed by processing, refreezing and storing the frozen fillets. Taste panel evaluation of trawler-caught cod showed that after long-term storage the 45°F thawed samples were preferred in some but not in all instances. When trap-caught cod were investigated, no preference was shown for either thawing temperature.

Similar comparative studies on trap-caught cod thawed in water at 60°F and in a dielectric device showed no effect attributable to the method or temperature of thawing.

Trawler-caught cod of high initial quality yielded refrozen fillets having good storage characteristics. Refrozen fillets prepared from frozen trap-caught cod were rated highly at first, but depreciated in frozen storage at a faster rate than trawler-caught fish.

71. MacCallum,W.A., H.S. Shieh, Dorothy A. Chalker, W.J. Dyer and D.R. Idler. Polyphosphate treatment of frozen cod. 2. Effect on drip, yield, lipid hydrolysis and protein extractability in twice-frozen Newfoundland summer trap and fall cod. (J.Fish. Res.Bd.)

Two freezings combined with sodium tripolyphosphate treatment between freezings and subsequent storage at -23°C were carried out with drip losses from fall-caught Newfoundland cod (Gadus morhua, L.) equal to or less than those from once-frozen, untreated controls. With summer trap fish drip values were not affected by treatment. Estimated yields from twice-frozen fall and trap fish were greatly improved by dipping prior to the second freezing, and in the case of fall fish, by dipping twice.

Treatment of twice-frozen cod from the two sources did not appreciably affect lipid hydrolysis or protein denaturation.

72. Slavin,J.W., and W.A.MacCallum. North American experience in chilling and freezing on board vessels. (Fishing Gazette)

ATLANTIC TECHNOLOGICAL PROGRAM

S T A F F

April 1, 1963 to March 31, 1964

H A L I F A X

ADMINISTRATIVE

Director	D.R. Idler, D.F.C., Ph.D. (Wisconsin)
Assistant Director	C.H. Castell, M.S.A. (Toronto)
Executive Assistant (Administrative Officer 2)	I. Jean Rattray
Administrative Assistant	Gwendolyn A. Gordon
Secretary (Stenographer 3)	Patricia E. Murphy
Clerical Assistant (Clerk 3)	Mary C. Dalrymple
Receptionist (Typist 1) (to June 19, 1963)	Nancy C. Horne
Receptionist (Typist 1) (from June 20, 1963)	Marilyn M. Latter
Stenographer 2 (from November 4, 1963)	Gloria E. Marleau

LIBRARY

Librarian (Clerk 4)	Marjorie E. McPhail

SCIENTIFIC

Quality of Fresh and Frozen Fish and Postmortem Biochemistry of Fish.

Principal Scientist	W.J. Dyer, Ph.D. (McGill) F.C.I.C.
Senior Scientist	J.R. Dingle, Ph.D. (Toronto) F.C.I.C.
Associate Scientist	D.G. Ellis, B.Sc. (Queens)
Associate Scientist	Doris I. Fraser, B.Sc., B.Ed. (Acadia)
Associate Scientist	J.A. Hines, B.Sc. (St.Francis Xavier)
Associate Scientist (to June 15, 1963)	J.T. Lauder, B.Sc. (Dalhousie)

Marine Lipid Research

Senior Scientist	R.G. Ackman, Ph.D. (London)
Senior Scientist	H. Brockerhoff, Ph.D. (Cologne)
Associate Scientist	P.M. Jangaard, B.Sc. (California)
Associate Scientist	J.C. Sipos, M.A. (Toronto)
Associate Scientist (from October 1, 1963)	M. Yurkowski, M.Sc. (Saskatchewan)
Assistant Scientist	R.J. Hoyle, M.S. (Cambridge)
Assistant Scientist (to June 15, 1963)	R.D. Burgher, B.Sc. (Acadia)

Marine Bacteria

Associate Scientist (from July 1, 1963)	H.S. Shieh, Ph.D. (McGill)

Non-Bacterial Deterioration of Fish and Shellfish

Principal Scientist	C.H. Castell, M.S.A. (Toronto)
Associate Scientist	N. Dambergs, Ing.Chim. (Nancy)
Assistant Scientist (to March 17, 1964)	Jill E. MacLean, B.Sc. (Dalhousie)
Assistant Technician 3	Wanda E. Neal
Laboratory Helper	Mabel M. Shiers

Diseases and Nutrition of Shellfish

Senior Scientist	J.E. Stewart, Ph.D. (Iowa)
Associate Scientist	M.F. Li, Ph.D. (Alberta)
Associate Scientist (from January 15, 1964)	J.W. Cornick, M.S.A. (Ontario Agricultural College)

Endocrinology and Physiology of Fish

Director	D.R. Idler, Ph.D. (Wisconsin)
Senior Scientist (from March 20, 1964)	M.S. Mounib, Ph.D.(Aberdeen)
Associate Scientist	Beryl Truscott, M.S. (Queens)
Associate Scientist	H.C. Freeman, M.Sc. (Acadia)

Biochemical Indicators of Fish Species and Populations

Senior Scientist	P.H. Odense, Ph.D. (Oklahoma)
Assistant Scientist	C.W. Shinners, B.Sc. (St.Dunstan's)

Pathology of Fish

Senior Scientist	K. Ronald, Ph.D. (McGill)
Assistant Scientist (From August 12, 1963)	Heather C. Macnab, B.S.A. (Ontario Agricultural College)

Process and Product Research

Senior Scientist (to August 31, 1963)	P.M. Townsley, Ph.D. (California)
Senior Scientist	A.L. Wood, B.Sc. (Dalhousie) B.E. (Nova Scotia Technical College)
Associate Scientist	H.E. Power, B.E. (Nova Scotia Technical College)
Assistant Technician 3 (to June 5, 1963)	Mary Lou Hughes, B.Sc.(New Brunswick)
Technician 1	C.A. Campbell

MAINTENANCE

Maintenance Supervisor 5	R. Greening
Caretaker 5	J.D. Groat
Cleaning Service (Part time)	Mary Greenwood
Cleaning Service (Part time)	Lillian O'Keefe
Cleaning Service (Part time) (to November 25, 1963)	Ola F.Townsend
Cleaning Service (from December 2, 1963)	Muriel E. Miller

TECHNICAL SERVICES

Supervisor - Buildings and Grounds, Technical Services (Technician 4)	R.C. Palmer
Technical Assistant (Technician 2)	D.J. Casavechia
Machinist (Technician 2)	R.C. Edmonds
Electrical Technician (Technician 1)	J.W. Gates
Technical Assistant (Asst. Technician 3)	D.E. Turner

G R A N D E R I V I È R E

ADMINISTRATIVE

Director (Senior Scientist)	R. Legendre, , M.Eng. (McGill)
Executive Assistant (Administrative Officer 1)	R.Dubé
Stenographer 3	Jacqueline Sutton

SCIENTIFIC

Senior Scientist	R. Legendre, M.Eng. (McGill)
Senior Scientist	H.P. Dussault, M.Sc. (McGill)
Associate Scientist (to October 1, 1963)	M. Yurkowski, M.Sc. (Saskatchewan)
Assistant Scientist	F. Maltais, B.Sc. (Montreal)

TECHNICAL

Technician 3	M.A. Bordeleau
Technician 2 (to December 1, 1963)	G. Vaillancourt
Technician 2	R. Guilbault
Technician 1	N. Bisson
Assistant Technician 2	S. LeMoignan

MAINTENANCE

Maintenance Supervisor 5	C. Chartré
Maintenance Craftsman 4	E. Roussie
Caretaker 5	Y. Lambert
Watchman	G. Duguay

N E W F O U N D L A N D

ADMINISTRATIVE

Unit Chief (Principal Scientist)	W.A. MacCallum, B.E. (Nova Scotia Technical College), M.Sc. (Dalhousie)
Stenographer 2 (to August 30, 1963)	Mary A. O'Brien
Stenographer 2 (from November 18, 1963)	Bertha L. Hodder

SCIENTIFIC

Principal Scientist	W.A. MacCallum, B.E. (Nova Scotia Technical College), M.Sc.(Dalhousie)
Associate Scientist (to July 1, 1963)	H.S. Shieh, Ph.D. (McGill)
Associate Scientist (from June 15, 1963)	J.T. Lauder, B.Sc. (Dalhousie)
Assistant Scientist	Dorothy A.Chalker, B.Sc. (Memorial)

TECHNICAL

Technician 1	E.E. Coldwell

TEMPORARY STAFF

Barzell, W.E. Student Assistant. Term May 8 to July 19, 1963. (Technological Unit)

Beaton, B.H. Student Assistant. Term May 6 to September 10, 1963. (Products and Process Research)

Buyco, Lydia. B.Sc. (University of San Agustin). Under a Research Grant from the U.S. Department of Health,Education and Welfare. Full time to October 31, 1963. (Marine Natural Products)

Cornick, J.W. M.S.A. (Ontario Agricultural College). Under a Grant from the Nova Scotia Research Foundation. Full time to January 15, 1964. (Marine Microbiology)

Curnew, G.P. Student Assistant. Term May 1 to September 30, 1963. (Technological Unit)

Gibson, E.J. Student Assistant. Term May 6 to September 30, 1963. (Technological Unit)

Hazeldon, S.J. Assistant Technician 3. Term December 2, 1963 to February 28, 1964. (Marine Microbiology)

LeDrew, B.R. Student Assistant. Term July 22 to September 23, 1963. (Technological Unit)

Lee, Yew Shum. Student Assistant. Term May 3 to September 11, 1963. (Marine Natural Products)

Nagayama, Fumio. Dr. Agric. (Kyushu University, Japan). On leave from the Tokyo University of Fisheries on a National Research Council Postdoctorate Fellowship from August 27, 1963. (Marine Natural Products)

Purcell, E.M. Stenographer 3. Casual. July 2 to August 31, 1963. (Administration)

Tamura, Toshitake. M.Sc. (Nihon University). On leave of absence as lecturer from Nihon University, Tokyo, Japan. Under a Research Grant from U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health. Full time to October 31, 1963.

Weinstein, H.M. Student Assistant. Term May 21 to September 3, 1963. (Proteins: Fresh and Frozen Fish)

Wight, H.G. Student Assistant. Term May 28 to September 14, 1963. (Products and Process Research)