AN ABSTRACT OF THE THESIS OF

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Title: An Analysis of Depot Lipid of Ocean-caught Juvenile Coho Salmon and Comparisons to a Laboratory Fasting Study.

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In recent years, increased production of hatchery-reared coho salmon has been used to supplement natural stocks and reverse the trend in declining ocean catch and returns to hatcheries. These efforts have been met with limited success. Several investigators have suggested that the observed low survival rate is due to a limited carrying capacity of the ocean and that food limitation may cause high juvenile salmon mortality. This investigation is relevant to the food limitation hypothesis.

Though condition ($K = wt \times 10^5/length^3$) decreased continuously in the laboratory study, ocean-caught, hatchery-reared fish showed a significant increase in condition with time after release. Liver lipid content did not change in the fasting study but an exponential decline was observed in ocean-caught fish. The viscera and muscle exhibited a shallow linear decline in lipid content in fasting fish, whereas ocean-caught fish showed an exponential decline in visceral lipid while muscle tissue remained constant. Declining liver lipid in ocean-caught

fish is interpreted to be the result of decreased lipogenic enzyme activity as compared to fasting fish. Rapid decreases in visceral lipid content in ocean-caught fish may be the result of decreased dietary lipid intake and increased physical activity compared to hatchery-reared fish. Muscle lipid declined in fasting fish due to utilization of lipid components for energy. But, reduced lipid intake does not affect muscle lipid content.

Fatty acid composition was observed to change in fasting fish. The contribution of 20:5ω3 in the triglyceride declined significantly in all three tissues of fasting fish. Levels of this acid increased in ocean-caught fish from values near those of the hatchery diet to values similar to those in zooplankton. No change in 20:4ω6 levels were observed in fasting fish, but exponential declines were observed in viscera and muscle tissue of ocean-caught fish. The proportion of total ω3 essential fatty acids remained approximately constant in fasting fish, but increased in all three tissues in ocean-caught fish. Proportions of total ω6 essential fatty acids declined in all three tissues in ocean-caught fish while no changes were observed in fasting fish.

Results from the fasting study are interpreted to be responses to energy deficiency. Lipid weights decline as lipid is oxidized for energy. The composition of lipid changes for similar reasons; certain fatty acids undergo β-oxidation more readily than others. In contrast to

these results, observations on ocean-caught fish suggest that feeding is occurring and is sufficient to maintain and improve growth and condition. Decreasing lipid content in ocean-caught fish is in response to reduced lipid intake as compared to the hatchery diet. Higher physical activity in ocean-going fish is also probably a factor in reducing lipid content.

The evidence from the analysis of depot lipid does not support the food limitation hypothesis. However, the evidence does suggest a 30-40 day period during which juvenile salmon adapt to the marine conditions of nutrition. During this time, the composition of tissue lipids changes from reflecting a hatchery diet to reflecting a marine diet.

An Analysis of Depot Lipid of Ocean-caught Juvenile Coho Salmon and Comparisons With a Laboratory Fasting Study

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Sincere appreciation is expressed to Deborah Lumpkins who put up with the many changes and additions that were required to finish this paper.

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INTRODUCTION

Natural runs of salmon (Oncorhynchus spp.) have declined over the last 40 years in streams and estuaries in British Columbia, Washington and Oregon (Dawley et al, 1981; Peterman, 1978; Larkin, 1975; ODFW, Coho Plan, 1982). The decline in natural populations has been attributed to overfishing (Folmar and Dickhoff, 1980), hydroelectric development and forestry management techniques (Dawley et al, 1981). In an effort to compensate for the loss of natural stocks, major salmon enhancement programs have been initiated. More than 300 million fish are released annually from 150 public supported hatcheries in the Northwest alone. Many million more are released from commercial ranches and net-pen culture systems in Oregon and Washington (Folmar and Dickhoff, 1980). Even with this tremendous production effort, increased ocean catch and hatchery returns have not been realized.

Most hypotheses proposed to explain continued declines in salmon abundance in the face of increasing production, assume some kind of density-dependent effect on survival (Peterman, 1982; Peterman, 1978; Walters et al, 1978). Several authors have suggested that density-

dependent food limitation may, in some cases, prevent increased salmon populations (Peterman, 1978; Gardiner and Geddes, 1980). In the past, it has been assumed that the ocean is a large, inexhaustible reservoir of food, unaffected by increased smolt production. Such a view fails to consider that salmon are restricted by their behavioral and physiological characteristics, to a small fraction of the marine environment. Enroute to the ocean from fresh water, salmon are often forced to pass through estuaries where large numbers of fish may seriously deplete the food resources (Meyers, 1980; Judkins and Johnson, 1981).

Analyses of the food limitation hypothesis have placed emphasis on obtaining estimates of prey abundance, salmon abundance, salmon feeding and digestion rates and prey population growth rates (Peterson et al, 1980; Walters et al, 1978). These estimates are difficult to make with great precision, owing to the technical problems associated with sampling in the marine environment. At best such estimates are an indirect answer to the question of food limitation. A more direct approach is the analysis of physiological parameters known to be affected by limited food and dietary changes. Comparison of these parameters in ocean-collected fish and fish that have undergone nutritional insufficiency (fasting) may provide evidence

for or against the food limitation hypothesis. This approach is used in this paper.

The diet of marine fish is rich in lipids but relatively deficient in carbohydrate (Love, 1970; Warman, 1978). Singh and Nose (1967, cited by Cowey and Sargent (1977)) demonstrated that rainbow trout do not efficiently utilize complex carbohydrates such as starches and dextrin. Instead, lipid stores are preferentially used. Chang and Idler (1960) observed increased concentrations of liver glycogen in Oncorhynchus keta during the latter part of the spawning run. They attributed the increasing carbohydrate concentrations to an excess of gluconeogenesis over glucose demand. However, fat reserves declined continuously throughout the run.

Many authors have shown that the quantity and fatty acid composition of fish tissue lipids are affected by the quantity and fatty acid composition of dietary lipids (Kelly et al, 1958; Brenner et al, 1961; Brenner et al, 1963; Kayama et al, 1963a,b; Lovern, 1964; Gruger, 1967; Mead and Kayama, 1967; Kreps et al, 1969; Castell et al, 1972a,b,c; Lee and Sinnhuber, 1972; Cowey and Sargent, 1977; Kinsella et al, 1977; Yu et al, 1977; Ratnayake and Ackmann, 1979; Leger et al, 1981; and many others). The reflection of dietary fatty acids in tissue lipid depends on the ability of the fish to modify the dietary fats. Dietary similarity will be greatest where modification is

the least. Deviation from the dietary pattern is expected when modification of dietary fat is extensive. Normal synthesis in the fatty-acid-synthetase enzyme complex produces a 16-carbon saturated fatty acid as a final product. Further chain elongation and desaturation occurs in specialized enzyme systems. Because fish and mammals are unable to insert double bonds beyond carbon 9 in the chain, two major families of fatty acids must be supplied in the diet. These are referred to as essential fatty acids and include the $\omega 3$[1] and $\omega 6$ homologous series (Stryer, 1975). In fish, starvation induces a change in the ratio of total weight to length because as needed energy stores are utilized but not replaced, weight declines while length remains essentially unaffected.

To compensate for a period of acclimation to the new environment, hatcheries have provided the young salmon with substantial reserves of energy stores in the form of lipids (mostly triglycerides). These lipids from a hatchery diet rich in fats, presumably serve as the major source of metabolic energy from the time of release to the time when

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[1] Fatty acids are usually named using the accepted notation, X:Y, where X is the number of carbons in the chain and Y is the number of double bonds. The location of the first double bond, counting from the terminal methyl end, is denoted by the Greek letter ω. Subsequent double bonds are separated by single methyl groups. Thus, 20:5ω3 is a 20 carbon fatty acid with five double bonds. The first double bond is between the third and fourth carbons from the methyl end.

normal feeding commences (Cowey and Sargent, 1977), a time when fish must adapt to a feeding regime that differs substantially from that in the hatchery. Lipid reserves may permit a degree of flexibility in this acclimation period that does not constrain the animals to begin feeding immediately. Analysis of the stored lipids and how they change in juveniles soon after release may be a test of the usefulness of hatchery feeding practices. Similar analyses made in fish undergoing starvation may provide a basis for determining if fish are feeding in the ocean.

In this study, changes in the condition factor and the quantity and quality of the depot lipids of juvenile coho salmon is documented during the first four months of ocean life and compared with data taken from a laboratory fasting study.

MATERIALS AND METHODS

Collection Area

Juvenile coho salmon were collected with a purse seine during four cruises conducted during the summer of 1981. These cruises covered the time periods from May 16-25, June 9-18, July 9-19 and August 8-19, in the region from 20 nautical miles north of the Columbia River, to 10 nautical miles south of Coos Bay, Oregon. Stations were located at 4, 7, 10, 15 and 20 nautical miles along transects in the region. When high abundances of juvenile salmonids were present at any station, multiple seine sets were made to maximize the recovery of marked fish (Wakefield et al, 1981).

Ocean Collections

Two different commercial fishing vessels and purse seines were used. In May and June, the F/V KRISTEN GAIL, a 32m crab fishing vessel with a seine skiff was used to fish a 457m long purse seine. The main section of the seine was constructed of 32 mm stretch-measure mesh and was 800 meshes deep. A 73m, 600 mesh deep section was attached to the end towed by the boat. An additional 30 mesh deep panel of 127 mm stretch-measure mesh was sewn along the bottom. The bunt was constructed of 19 mm stretch-measure mesh. This seine was estimated to fish to 9m depth,

and sample a volume of about 150,000 m^3 (Wakefield et al, 1981).

In July and August, the F/V SOUPFIN, a 21 m drum seiner was used. The seine used was also 457 m in length. The main section was constructed of 32 mm stretch-measure mesh and was 1800 meshes deep. Additional 3 and 30 mesh deep panels of 101 mm stretch-measure mesh were sewn along the top and bottom of the seine respectively. The bunt was constructed of 32 mm stretch-measure mesh and was 37 m in length and 1200 meshes deep. This net had a measured fishing depth of 15 m and was estimated to sample a volume of 250,000 m^3.

In addition to purse seine sets, zooplankton hauls using 505 um and 333 um mesh in 70 cm bongo nets were routinely made in order to obtain samples of typical dietary items of the juvenile salmon.

Processsing the Catch at Sea

Juvenile coho salmon caught in the purse seine were immediately placed in MS 222 anesthetic to prevent physical damage and reduce stress. Each fish was then measured for fork length, placed in a plastic bag and frozen. An effort was made to maintain the temperature of the freezers at or near -20°C in order to prevent auto-oxidation of lipids (Lee et al, 1967). Zooplankton samples were placed in glass jars and frozen.

Fluorescent Pigment Marking

In order to obtain a larger number of fish of known origin than that expected from typical releases of coded-wire-tagged fish, a fluorescent pigment marking program was initiated. In cooperation with Oregon Aqua Foods, Inc. (OAF), approximately 1.5 million coho smolts were marked at the OAF hatchery at Springfield, Oregon. Marked fish were transported to release sites at Yaquina Bay and Coos Bay, Oregon.

Three colors of pigment (red, yellow and green) were obtained from Scientific Marking, Inc. in dry powdered form. These colors were used on various groups of fish in such a way that all of the fish of a particular group were released over the same short time period. The fish to be marked were herded from freshwater holding ponds into a long, central raceway. Small groups of fish were then herded over a grading device and onto a perforated metal plate that separated the water from the fish. Using a sandblaster with approximately 100 psi of air pressure, the dry fluorescent pigment was applied to the fish's skin. The fish moved fast enough through the system so that no fish received more than a few particles of pigment. Pigment applied in this manner was usually invisible to the naked eye. Recognition and identification of marked fish was accomplished under ultraviolet light.

Subsamples of fish marked by fluorescent pigment were maintained in holding tanks at the Yaquina Bay release facility of OAF for observation of dye retention and mortality due to the procedure. After nearly three months, an average of 83% of fish marked with red pigment were still marked. For yellow pigment, 68% of the fish were still marked. The data for green pigment were not sufficient to determine retention. Mortality of marked fish was insignificant.

Fasting Study

To identify the effects of fasting in ocean-caught juvenile coho salmon, a fasting study was undertaken at the Oregon State University Marine Science Center at Newport, Oregon. Four, approximately one meter diameter, one meter deep, fiberglass tanks were used. Filtered, cooled sea water from Yaquina Bay was continuously pumped into the tanks. Temperature was monitored daily.

The experiment was begun using two groups of juvenile coho salmon smolts from OAF's salt water ponds at South Beach, Oregon. Accelerated[2], zero-age[3], fish were used in

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[2] Juvenile salmon raised in warm water and fed so that they obtain larger size in the same period of time than under normal production conditions.

[3] Zero-age fish are less than one year old. Yearling-age fish are older than one year but less than two years.

one group, and yearling-age[3] fish were used in the other. For each group, one tank served as a "control" in which the fish were fed at the rate of 1% of the estimated total weight of the fish in the tank per day. This ration ("maintenance ration") was chosen as one to maintain growth and condition but was less than the 2-4% per day ration typically used in hatchery production (Suzamoto, OAF, personal communication). The second group received no food during the experiment.

Random samples were taken at intervals from each of the four tanks. Ten fish were taken from each tank at the beginning of the experiment. Subsequent samples consisted of approximately 20 fish per tank. Because of the starvation-induced mortality in the fasted fish, the last sample of these fish consisted of only 16 individuals.

Each fish was measured for fork length, damp dried, and weighed on a triple-beam balance to the nearest 0.1 g. The fish were labeled with this information and placed in separate plastic bags for freezing. The samples were stored at approximately -20°C until processed (6 to 9 months).

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[3] Zero-age fish are less than one year old. Yearling-age fish are older than one year but less than two years.

The zero-age-group portion of the experiment was continued until all of the fish in the fasting tank had died or were removed as samples. The yearling-age-group experiment was terminated after 43 days due to excessive mortality caused by the disease furunculosis. Because the disease was apparently present from the beginning of the experiment in this group, these data were not analyzed.

Laboratory Processing

All samples were processed as rapidly as possible to avoid complete thawing of the internal organs and subsequent loss of lipid materials. Ocean-caught fish were examined for coded-wire tags and for the presence of fluorescent dye. The presence of other marks such as metacercarial scars, eroded fins, etc. was noted. The weight of each fish was determined to 0.1 g on a Mettler model PL 200 electronic balance.

Dissection was begun after thawing the outer portion of the fish but before the internal organs had thawed appreciably. The liver was removed and weighed to the nearest 0.01 g and then placed in a 2 dram glass vial fitted with a snap-cap lid, and placed in the freezer. The pyloric caeca together with the spleen and fatty deposits were stripped off of the stomach and intestine (posterior to the last pyloric caecum), and were weighed to the nearest 0.01 g. This "visceral mass" or "viscera" was then

placed in a 2 dram vial and frozen. The remaining carcass was refrozen for later use for a dorsal, epaxial muscle subsample. These samples were maintained at approximately -20°C until extraction of lipids.

Lipid Extraction

The lipid extraction followed the method of Bligh and Dyer (1959), as modified by R. R. Lowry (OSU Agri. Chem., personal communication).

The sample to be extracted (not exceeding 2 g) was weighed to the nearest 0.001 g on a Fisher model 20 electronic balance. The sample was then homogenized at approximately 16000 RPM in 15 ml of 2:1 chloroform:methanol (v/v) in a test tube using a modified Servall Omni-Mixer. The homogenized material was then filtered through analytical filter pulp and the filtrate collected in a centrifuge tube fitted with a screw cap. The residue was washed with an additional 15 ml of 2:1 chloroform:methanol and the washings added to the filtrate. A volume of distilled water was added sufficient to make the total volume of water in the sample equal to 7.7 ml. For fish materials, the volume of water originally in the tissues was assumed to be equal to approximately 80% of the wet weight (Bligh and Dyer, 1959; Gardiner and Geddes, 1980; John Oh, OAF, personal communication). This ratio is within the range described by Bligh and Dyer (1959) to

yield a maximum extraction of lipid (greater than 94% of total lipid present in original sample).

The filtrate with the added water was shaken vigorously and centrifuged at high speed for approximately four minutes. The upper layer (methanol, water and non-lipids) was removed by careful aspiration. The sample in chloroform was dried at 50°C in a pure nitrogen atmosphere (by blowing nitrogen at low pressure over the surface of the solution) to prevent oxidation of the highly polyunsaturated fatty acids. The sample was then picked up in pure chloroform by rinsing the centrifuge tube with 3 ml chloroform and transferring to a screw-cap culture tube. Additional rinses of 2 ml and 1 ml chloroform were used to assure complete transfer of the lipid. The sample was stored in pure chloroform, tightly capped and refrigerated at approximately 4°C until determination of the lipid weight.

Lipid Weight Determination

The sample was again taken to dryness at 50°C in a pure nitrogen atmosphere. The lipid was then dissolved in exactly 5 ml of pure chloroform. A 100 ul subsample was placed in a tared, aluminum, microbalance pan and the solvent removed by heating at 100°C for approximately three minutes. The pan was cooled and reweighed on a Perkin-Elmer AD-2 Autobalance to the nearest 0.001 mg. The weight

of lipid in the subsample was calculated from the difference in weights and the weight of lipid in the original sample was determined through extrapolation. These data were then expressed as a percent of the total tissue wet weight.

The precision of the overall extraction method was tested by extracting eight successive samples of Oregon Moist Pellet. The coefficient of variation ($s/\bar{x}$) for the lipid weights was 4.53%. The precision of the weighing technique alone was estimated by weighing 10 aliquots from the same visceral sample. The coefficient of variation was found to be 1.39%.

Methylation Procedure

The methylation technique employed was developed by R. R. Lowry (OSU Agri. Chem., personal communication). It utilizes a mild base catalyzed saponification reaction so that efficient methylation of only the mono-, di- and triglyceride fractions of the total lipid will occur. Because phospholipid methylates only very slowly under these conditions of concentration and temperature, interference from this lipid fraction should be minimal.

Except where insufficient lipid was present in the sample, between 10 and 20 mg of the total lipid was subjected to methylation. An appropriate volume of lipid/solvent was placed in a clean, dry, screw-cap culture

tube and the solvent was removed by heating at 50°C in a pure nitrogren atmosphere. A volume of 3 ml of anhydrous diethyl ether was added along with 3 ml of 0.1 M KOH in methanol (0.56 g KOH in 100 ml anhydrous methanol). The tube was capped, mixed vigorously and then heated for 5 min. at 50°C. Following heating, the sample was allowed to cool to room temperature. The solution was then acidified by the addition of 3 ml of 0.15 N HCl. The methyl esters of fatty acids were then extracted from the solution by the addition of 3 ml of hexane. The tube was capped and mixed well and then centrifuged for approximately 2 min. The hexane-ether/methyl ester layer was transferred to a clean, dry, screw-cap culture tube by means of a transfer pipet. A second extraction using 3 ml of 50% hexane in diethyl ether was performed in the same way. The two extracts were combined in the same tube.

Gas-Liquid Chromatography

Methylated samples were injected into a F & M Scientific Corporation Model 700 Dual Column Gas Chromatograph. The 60.96 meter long capillary column (inside diameter = 0.08 cm) was coated with ethylene glycol succinate (EGS). The injection port was maintained at 200°C while the detector was kept at 230°C. All injections were made isothermally with an oven temperature of approximately 140°C. The helium carrier gas flow rate

was 0.02 l per minute. These conditions provided a total sample retention time of between 60 and 90 min. The output from the flame ionization detector was amplified and recorded on a Sargent model SRG strip chart recorder. The area under the peaks was calculated and the relative percent composition by weight of each peak of interest was determined by a LDC model 308 integrating computer. The relative percent by molecular weight[4] was then calculated by the equation:

$$\text{Relative \% by Molecular Weight} = \frac{A_i/MW_i}{\Sigma(A_i/MW_i)} \times \Sigma A_i$$

Where A_i = % by carbon weight of peak i; MW_i = molecular weight of methyl ester i.

Identification of peaks was aided by injection of standards and comparison of retention times relative to that of 18:0 (relative retention time = retention time of methyl ester/retention time of 18:0). In addition, the $\log_{10}$ of relative retention times of standards

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[4] Fatty acid composition is expressed in terms of percentage of the total weight of fatty acids identified in the chromatograph. The flame ionization detector responds to the amount of carbon in the sample. Percent by carbon weights (from the integrator) are converted to percent by molecular weight to avoid giving too much emphasis to large molecules and not enough to small molecules.

were plotted versus the number of double bonds in the fatty acid carbon chain. Comparison of relative retention times of unknowns to this plot provided an additional means of identification. By plotting $\log_{10}$ relative retention time of standards versus the number of carbons in the chain, it was possible to assign probable membership in a homologous series (saturated; $\omega 7$, $\omega 9$ series; $\omega 6$ dienoic; $\omega 3$ trienoic; $\omega 3$ pentaenoic; $\omega 3$ tetraenoic; $\omega 3$ hexaenoic) to unknown fatty acids (Kinsella et al, 1977). Family identity was further supported by comparison with similar GLC analyses from the literature (Farquhar et al, 1959; Saddler et al, 1966).

Precision of the GLC technique was measured by injection of a mixed standard with percent by areas of 3%, 6%, 45%, and 50% per peak (6 peaks). The coefficient of variation ($s/\bar{x}$, N=20) for all peaks ranged between 0.38% and 5.55%. In general, the best precision was obtained for the largest peaks. The percent accuracy [(actual value - measured value/actual value) x 100] of the technique measured in this same series of injections was to within approximately 2.1% for small peaks (i.e. 3% by area), 0.9% for moderate-size peaks (i.e. 6% by area), and 0.5% for large-size peaks (i.e. 45 to 50% by area).

Statistical analysis was accomplished on a Cyber 70/73 computer using the Statistical Interactive Programming System (SIPS) (Rowe and Brenne, 1982). An "F-test" was

applied to test regression significance and "p" values were determined for the null hypothesis, $\beta_1 = 0$. In some cases, "lack of fit" tests were not significant for both linear and power function relationships. In these cases, choice of model was made on the basis of the larger R^2.

RESULTS

Condition Factor

The fasting study was maintained for 91 days during which time, condition factors were determined for a total of 222 zero-age fish (Figure 1A). These were divided evenly between control and fasting groups. The condition factor for the control group remained high (near 1.10) and relatively stable during the study. The highest mean condition was observed at the 91 day sample where K = 1.18. Condition factor in the fasting group decreased throughout the experiment. The highest mean value occurred for the initial sample, K = 1.14, declined approximately 27 percent during the first 40 days of fasting and reached the lowest mean value after 91 days of fasting (K = 0.51). Mortality in the control group was low, amounting to only about 10% cumulative mortality at 91 days. In the fasting group, mortality increased exponentially reaching 50% cumulative mortality by approximately 74 days of fasting. Virtually no mortality was observed in either group during the first 30 days.

Condition factor was calculated for 62 coded-wire tagged and spray-dyed fish caught in the ocean (Figure 1B). These fish had been released from 2 to 114 days before recovery. Linear regression of K versus time since release, revealed a significant trend ($P < 0.01$) for

condition to increase with time after release. Substantial variability was observed during the first 30 days. During this time, the lowest condition factors were observed (K = 0.91). After 30 days, no condition factor was less than 1.03 and the highest value was observed for an individual at 87 days since release (K = 1.37).

The regressions for OAF zero-age fish and yearling-age fish were not significantly different ($p > 0.10$). However, the condition factor of zero-age, ocean-caught fish was on average lower than zero-age fish from OAF salt-water holding ponds before release. Similarly, lower average condition factor was observed in yearling-age, ocean-caught fish than in ODFW yearling-age fish before release into the Columbia River. These lower condition factors in ocean-caught, zero-age and yearling-age fish are responsible for the initial lower average condition factor in figure 1B as compared to the initial sample of the fasting study (Figure 1A).

Lipid Weight

Results of lipid weight measurements in the fasting study are shown in figure 2. Variability was high in lipid weights from livers and as a result, no significant ($p > 0.05$) trend with time was observed. Lipid weights of viscera samples showed significant changes with time for both groups (Figure 2B). In the control, lipid content

declined 27.8% ($p < 0.01$) in 91 days while fed the "maintenance" diet. The fasting group was observed to decrease ($p < 0.01$) more than three times faster, 86.5% in 91 days. The fasting group lost 50% of its initial visceral lipid after about 53 days of fasting. A significant decrease ($p = 0.02$) in lipid weight was observed in the dorsal, epaxial musculature of the fasting group (Figure 2C). The lipid content of the muscle fell 38.3% in 91 days of fasting. No changes were evident in the muscle lipid content of the control during the study.

In contrast to observations on lipid weight for liver in the fasting study, liver lipid weights in ocean-caught fish declined rapidly as a power function with time since release (Figure 3A). Liver lipid content fell from about 6.5 to 8.5% to stabilize around 3 to 3.5%. The most rapid change occurred before 30-40 days.

A significant decline ($p < 0.01$) was also observed in the visceral lipid of ocean-caught fish (Figure 3B). Again, the most rapid changes occurred during the first 30-40 days with the lipid content falling from 25-30% to stabilize around 2-5%. The trend of declining visceral lipid content with time since release was observed in both zero-age and yearling-age fish. However, yearling-age fish tended to have lower visceral lipid content initially, averaging only about half that of zero-age fish. No significant change in lipid content was observed in the

muscle tissue of ocean-caught fish (Figure 3C). Lipid content was about 1% by weight.

Essential Fatty Acids

No significant changes ($p > 0.05$) with time were observed for liver triglyceride total $\omega 3$ (Figure 4A), or viscera total $\omega 3$ in the fasting study (Figure 4B). A significant decrease in the proportion of total $\omega 3$ fatty acids by weight was observed in muscle tissue triglyceride ($p = 0.01$) of fasting but not "control" fish.

Significant increases ($p < 0.01$) in the proportion of $\omega 3$ fatty acids in all three tissues of ocean-caught fish were observed (Figure 5). Though both linear and power function curves were significant ($p < 0.05$) in describing the relationship, the power function curve had the largest value of r^2 and was chosen to represent the increase in total $\omega 3$ fatty acid in the liver and viscera of ocean-caught fish with time since release. For all three tissues, the most rapid changes occurred during the first 30 to 40 days after release. In this time, the liver triglyceride total $\omega 3$ content increased from about 38% by weight to about 44% by weight. Visceral total $\omega 3$ content increased from about 8.9% by weight to about 40% over 114 days. The rate of change of the curve is more rapid than for liver, and the greatest change also occurred during the first 30 to 40 days after release.

No difference was observed between zero-age fish and yearling-age fish in initial visceral ω3 content. After release, total ω3 content increased at the same rate in both groups.

The proportion of muscle triglyceride total ω3 increased ($p < 0.01$) from about 33% to about 51% of the total identified fatty acids by weight (Figure 5C). Again, the most rapid change was observed during the first 30 days, after which the increase in ω3 was more gradual.

The major ω3 component of the triglyceride (22:6ω3) showed no significant trends with time in any of the three tissues in either the fasting study or ocean-caught fish ($p > 0.05$). However, the next most important contributor to the ω3 family in the triglyceride (20:5ω3) was observed to decrease in all three tissues of the fasting group (Figure 6). The contribution of 20:5ω3 to the total identified fatty acids in the triglyceride decreased in the liver of fasting fish from about 5.7% to 3.9% by weight in 91 days (Figure 6A). The control group exhibited an increase in this fatty acid from about 5.7% to 6.7% by weight in 91 days. In the viscera, 20:5ω3 decreased in both groups (Figure 6B). However, the decrease in fasted fish was about 2.7 times steeper from 5.6% of the total identified fatty acids by weight, to about 2% in 91 days. The viscera of the control group experienced a 23.6% decline in triglyceride 20:5ω3 during the 91 day

experiment. The decline in this fatty acid in muscle tissue (Figure 6C) was very gradual in fasting fish. The 20:5ω3 decreased ($p < 0.01$) by about 42.7% in 91 days. In the control, the decrease was only about one-third as rapid as in the fasting fish. Muscle tissue in the control group experienced a 12.5% decline during the experiment.

Results for 20:5ω3 analysis on ocean-caught fish show that this fatty acid increased ($p < 0.01$) in the liver though variability remained high over the range of the observations (Figure 7A). The lowest values were observed during the first 30 days when the rate of change of 20:5ω3 content was greatest. The relative proportion of 20:5ω3 increased from approximately 4.4% by weight in the triglyceride to about 12%. Visceral triglyceride 20:5ω3 (Figure 7B) increased ($p < 0.01$) from about 2.1% by weight to about 14% by weight. The highest variability and most rapid change took place during the initial 30-40 days after release. After this time, the proportion of 20:5ω3 remained more or less constant in the viscera. The 20:5ω3 content of muscle tissue triglyceride in ocean-caught fish increased ($p < 0.01$) from about 4.1% to about 12.6% of the total identified fatty acid by weight (Figure 7C). The 20:5ω3 content of the viscera triglyceride followed the same trend in both zero-age and yearling-age fish.

In the fasting study, no significant differences were observed in the ω6 content in any of the three tissues between the control and fasting groups. In contrast, the total ω6 fatty acid contribution in the triglyceride was observed to change in ocean-caught fish (Figure 8). The ω6 fatty acids declined ($P < 0.01$) as a group in the liver tissue. Variability was high but the ω6 decreased from about 9.3% to about 5% by weight (Figure 8A). The highest variability and most rapid changes again occurred during the first 30 to 40 days after release. Greater stability was observed after this time. Visceral ω6 declined ($P < 0.01$) from about 27% to about 3-4% by weight (Figure 8B). Again, the most rapid change and highest variability were observed during the first 30 to 40 days after release. The total ω6 content of visceral triglyceride declined similarly in zero-age and yearling-age fish. Significant decreases ($P < 0.01$) in total ω6 were also observed in dorsal epaxial muscle tissue triglyceride (Figure 8C). The total ω6 in muscle triglyceride declined from about 10.6% by weight to about 2-3% in 114 days in the ocean. As in the other tissues, the most rapid decline was observed during the first 30 to 40 days after release.

The major component of the ω6 family (20:4ω6) did not change proportionally in any of the three tissues during the fasting study. In ocean-caught fish however,

significant trends were observed in viscera and muscle tissue triglyceride but not in liver (Figure 9). Visceral triglyceride 20:4ω6 content decreased ($P < 0.01$) from about 14.7% by weight to about 2% by weight (Figure 9B). Most of this change occurred during the first 30 days after release. After the period of rapid change, approximate stability near 2% by weight was observed. The decline of 20:4ω6 content of visceral triglyceride was about the same in both zero-age and yearling-age fish. Muscle tissue in ocean-caught fish experienced an approximate 74% decrease in 20:4ω6. Muscle tissue triglyceride 20:4ω6 decreased from about 7.7% by weight to about 2% by weight. This decline was most abrupt during the first 30 days following release and was more stable after this time.

Differences between tissues were observed at all levels in both the fasting study and in ocean-caught fish. Visceral tissue was always highest in lipid content followed by liver tissue. Muscle tissue exhibited the lowest lipid content of the three tissues. Liver tissue of laboratory-maintained fish contained the most triglyceride total ω3, but muscle tissue contained more of this family of fatty acids than liver in ocean-caught fish. Visceral triglyceride was deficient in ω3 fatty acids compared to the other tissues. Total ω6 content was similar in liver and muscle in both ocean-caught fish and in the fasting study. Visceral triglyceride contained more ω6 family

fatty acids than the other tissues in the fasting study fish. In the ocean, visceral ω6 content was the highest of the three tissues initially but declined to less than the other tissues after about 30 to 40 days.

DISCUSSION AND CONCLUSIONS

Under normal growth conditions, the weight of salmonids increases as the cube of the length (Weatherley, 1972). Comparison of these two parameters of the allometric growth relationship was suggested as a measure of condition by Fessler and Wagner, 1969.

The "condition factor", calculated as the ratio of weight to the cube of the length, (see Fessler and Wagner, 1969) decreased throughout the 90-day period for the fasting fish. Though the control group was fed at only about half of the normal hatchery feeding rate, condition factor was essentially unaffected and even increased in the later part of the experiment.

In contrast, the condition factors for ocean-caught juveniles increased over the 114-day period after release. Such an increase suggests that the fish are feeding and growing. The lowest value of condition factor observed in ocean-caught fish corresponds to a maximum of only about 27 days fasting. The cumulative mortality after 27 days of fasting was only about 1.9% in the fasted fish and 0.72% in the "control" fish. Such mortality may not be significant and is well within the range experienced in normal hatchery production (Oregon Aqua Foods, confidential report, 1981).

These results provide little insight into the causes of mortality in the ocean. For example, nothing is known about the fate of fish with condition factors lower than the mean condition factor of the control group in the fasting study. The condition factor regression for ocean-caught fish is heavily influenced by relatively few fish caught after 40 days. The possibility remains that fish with low condition factor during the first 30 to 40 days after release do not survive. After 40 days, mortality among the survivors may be very low.

The lower average condition factor observed in ocean-caught fish just after release as compared to condition just before release may represent a period of acclimation to the new environment. In addition, yearling-age fish typically have lower condition factors than zero-age fish just before release. This may explain the lower mean condition factor of ocean-caught fish than zero-age fish from the fasting study. After 30-40 days, the condition factor of all ocean-caught fish exceeded that of the control group in the fasting study.

A decreasing condition factor during fasting is suggestive of utilization of body components for metabolic energy requirements. Important among these are lipids, primarily triglycerides (Love, 1970). Rapid utilization of lipid reserves is expected in fish undergoing fasting

(Weatherley and Gill, 1981). Visceral fat was clearly an important source of reserve energy in juvenile coho salmon since lipid content declined in the fasting study. The decrease observed in the control group, though less drastic than for the fasted fish, is not unexpected given the reduced ration fed these fish as compared to their former daily ration in the hatchery. This decreased lipid content is analogous to fat loss in mammals on reduced rations (e.g. dieting).

Leger et al. (1981), concluded that the lipogenic enzyme activity of muscle tissue in rainbow trout (Salmo gairdneri) under conditions of dietary lipid shortage was greater than that of visceral tissue, but much less than liver. They found that muscle tissue serves an important role in lipid storage but that viscera is the primary locus for storage of lipids. Data from the fasting study show that lipid loss in visceral tissue was nearly 42 times faster than in muscle tissue of fasting fish. Depletion of lipid reserves progresses more rapidly in viscera where lipid is maintained for energy storage purposes. The decrease in muscle tissue occurs less rapidly probably because maintenance of cell membrane phospholipid content is more important than in viscera and lipogenic enzyme activity is higher. Because the liver is the site of substantial lipid metabolism (Norris, 1980) and mobilization of lipid reserves progresses rapidly during

starvation, no decrease in lipid content is expected in liver tissue.

Results of lipid weight measurements in ocean-caught fish suggest that the dietary intake of lipids is substantially less than in the hatchery. Analysis of the Oregon Moist Pellet diet used by Oregon Aqua Foods, Inc., reveals a lipid content of about 15.2% by weight. In comparison, the mean lipid content of two samples of frozen Euphausia pacifica, a common prey for juvenile salmon off Oregon (Peterson et al, 1981), was 1.31% by weight. In addition, three specimens of juvenile Ophiodon elongatus had an average lipid content of 2.60% by weight. The combination of reduced dietary lipid intake and probable increased physical activity in ocean-going salmon compared to hatchery salmon, produces a not unexpected reduction in visceral lipid weights.

The lower visceral lipid content observed after release in yearling-age fish than zero-age fish, may be due to the hatchery feeding program. The accelerated rearing program used for production of zero-age smolts at OAF includes higher-than-normal feeding rates. Such feeding programs may produce fish with higher fat content before release.

The decline in liver lipid content in ocean-caught fish is in sharp contrast to the lack of significant change observed in the fasting study. Lin et al. (1977a,b) noted

increased lipogenic enzyme activity in hepatic tissue when dietary lipid declined. However, Leger et al. (1981) found that increased amounts of ω3 fatty acids in the diet had an inhibitory effect on lipogenic enzyme activity in the liver. Gas chromatographic analysis of the fatty acids in the triglyceride of E. pacifica and O. elongatus demonstrated that salmon feeding on these organisms consume from 2.4 to 3.0 times as much ω3 fatty acids respectively than while feeding on Oregon Moist Pellet in the hatchery. If these levels of ω3 fatty acids are typical of the marine diet of juvenile coho salmon, lipogenic enzyme activity may reduce liver lipid content. In addition, the change in dietary lipid intake experienced by these fish may not be sufficient to induce the increased lipogenic enzyme activity observed by Lin et al. (1977a,b).

The lipid content of muscle tissue in juvenile coho salmon is not affected by the lipid content of the diet (Lin et al, 1977a). As a result, though the lipid content of the marine diet appears to be much lower than the hatchery diet, no changes in muscle lipid content occurred.

A large number of analyses of the fatty acids of marine organisms have found more ω3 family fatty acids and fewer ω6 family fatty acids than in freshwater organisms (Brenner, et al, 1961; Lovern, 1964; Gruger, 1967; Kreps et al, 1969; Lee et al, 1971; Ratnayake and Ackman, 1979). Oregon Moist Pellet is also relatively high in ω6 fatty

acids but deficient in ω3 fatty acids. Thus hatchery-reared salmon fed OMP and fish feeding in rivers before ocean-entry would be expected to have a fatty acid composition altered in the direction of the "marine pattern" after feeding in the ocean. The increasing amounts of ω3 fatty acids and decreasing amounts of ω6 fatty acids with time since release (Figures 5 and 8) demonstrate that this is true in coho salmon. In visceral tissue, the ω3 fatty acid content at release (10-15%) resembled very closely the ω3 content of OMP (16.2%). After 30 to 40 days after release, visceral ω3 content had increased to 30-35% resembling much more closely the ω3 content of E. pacifica (38.3%). Similarly, the ω6 content at release resembled that of OMP (10-15% v.s. 11.1%), but after 30-40 days, ω6 content decreased to 4-5%, similar to the 4.5% in E. pacifica.

Agreement with dietary values is closest in visceral tissue but is much less so in liver and muscle tissue. Increases in these two tissues are most likely in response to increases in dietary ω3 intake, but the specific metabolic requirements for ω3 fatty acids in these tissues may be more important than dietary composition (Lin et al, 1977a,b).

The decreasing 20:5 ω3 observed in liver, viscera and muscle tissue in the fasted group suggests selective utilization and a role in energy metabolism. A primary

energy storage role for visceral tissue is again suggested since the decline in 20:5ω3 was more than twice as rapid as liver, and more than 1.5 times faster than muscle. Changes in fatty acid composition in fasting fish are associated with the need for metabolic energy. Murata (1979a,b) investigated the role of polyunsaturated fatty acids in β-oxidation in muscle and liver tissues of rainbow trout. He found that 22:6ω3 does not contribute to the energy metabolism in these tissues, whereas 18:1ω9 was readily metabolized. Murata and Higashi (1980) concluded that the fatty acid composition of muscle triglyceride in Cyprinus carpio was altered during fasting due to selective utilization of fatty acids in energy metabolism. In this species, 18:1ω9 and 18:2ω6 were metabolized for energy, but 22:6ω3 was not.

No change was found in muscle 20:4ω6 in fasting carp (Murata and Higashi, 1980), and a similar observation was made in coho salmon muscle where the 20:4ω6 content remained similar to that of OMP throughout the fasting study. In viscera, the fasting group demonstrated an increased triglyceride 20:4ω6 content. This increase is not real, however. Barring endogenous sources of 20:4ω6 precursors, increased 20:4ω6 must be the result of decreases in other fatty acids.

The changes in fatty acid composition observed in the fasting study, were not apparent in ocean-caught fish. In

all three tissues analyzed, 20:5ω3 was observed to increase with time after release, rather than decrease as was found for fasting fish. In visceral tissues at release, the 20:5ω3 content was low (4-6%) and reflected the composition of OMP (8.7%). After release, the 20:5ω3 content increased steadily (to 12-14%) until it resembled more closely the composition of O. elongatus (13.2%) and E. pacifica (18.6%). A similar pattern was observed in liver tissue, though the resemblance to either diet was less than for viscera. Muscle tissue composition reflected that of OMP initially and O. elongatus and E. pacifica after a gradual linear increase.

The contention of Sykora and Valenta (1980) that fatty acid patterns do not exhibit tissue specificity is refuted by these data. In their analysis of the fatty acids of Salmo trutta, Salmo gairdneri, Salvelinus fontinalis, Coregonus lavaretus and Coregonus peled no differences were observed between muscle, liver and brain tissue. In the present study, differences were apparent between liver, viscera and muscle tissues. However, liver and muscle tissues exhibited the least differences and this may account for the observations of Sykora and Valenta (1980). Had they analyzed visceral lipid, tissue specificity may have been apparent.

The observed rapid declines in lipid reserves toward stable tissue levels suggest that large lipid reserves may

be an unnatural feature in juvenile coho salmon. Meyers (1980) found that hatchery but not wild smolts had visible lipid reserves investing the visceral tissue prior to ocean entry. In the present study, juvenile coho salmon caught in the ocean from 30 to 40 days after release did not have large lipid reserves. In addition, the composition of tissue lipids of hatchery and ocean smolts is quite different. Use of a more "marine-like" diet in the hatchery may produce fish with higher condition factors and with lower levels of tissue lipids.

These data on lipid analyses of juvenile coho salmon caught up to 114 days after release do not support the thesis that food limitation exists. The observed changes in fatty acid composition are very different in ocean-caught fish than fasting fish and suggest that feeding on a marine diet is occurring for many individuals and is sufficient to maintain and improve condition of the fish relative to the moment of release. These data do indicate, however, that a period of acclimation to the marine environment occurs approximately 30 to 40 days after release. This adjustment period is illustrated by the differences in lipid characteristics of the fish at release and after the acclimation period. If mortality is high during this period and fish with low condition factor, high levels of tissue lipids, low levels of $\omega 3$ fatty acids and high levels of $\omega 6$ fatty acids are eliminated from the

population, then these conclusions become valid only for survivors. However, until data on differential survival based on these parameters becomes available, the search for an answer to the observed low survival rates in juvenile coho salmon should turn elsewhere.

Figure 1. (A) Condition factor in the fasting study. Lines pass through the mean and vertical bars are one standard deviation. Upper line is the control, lower line is the fasting group. (B) Condition factor in ocean-caught fish. Dots are zero-age and open triangles are yearling-age.

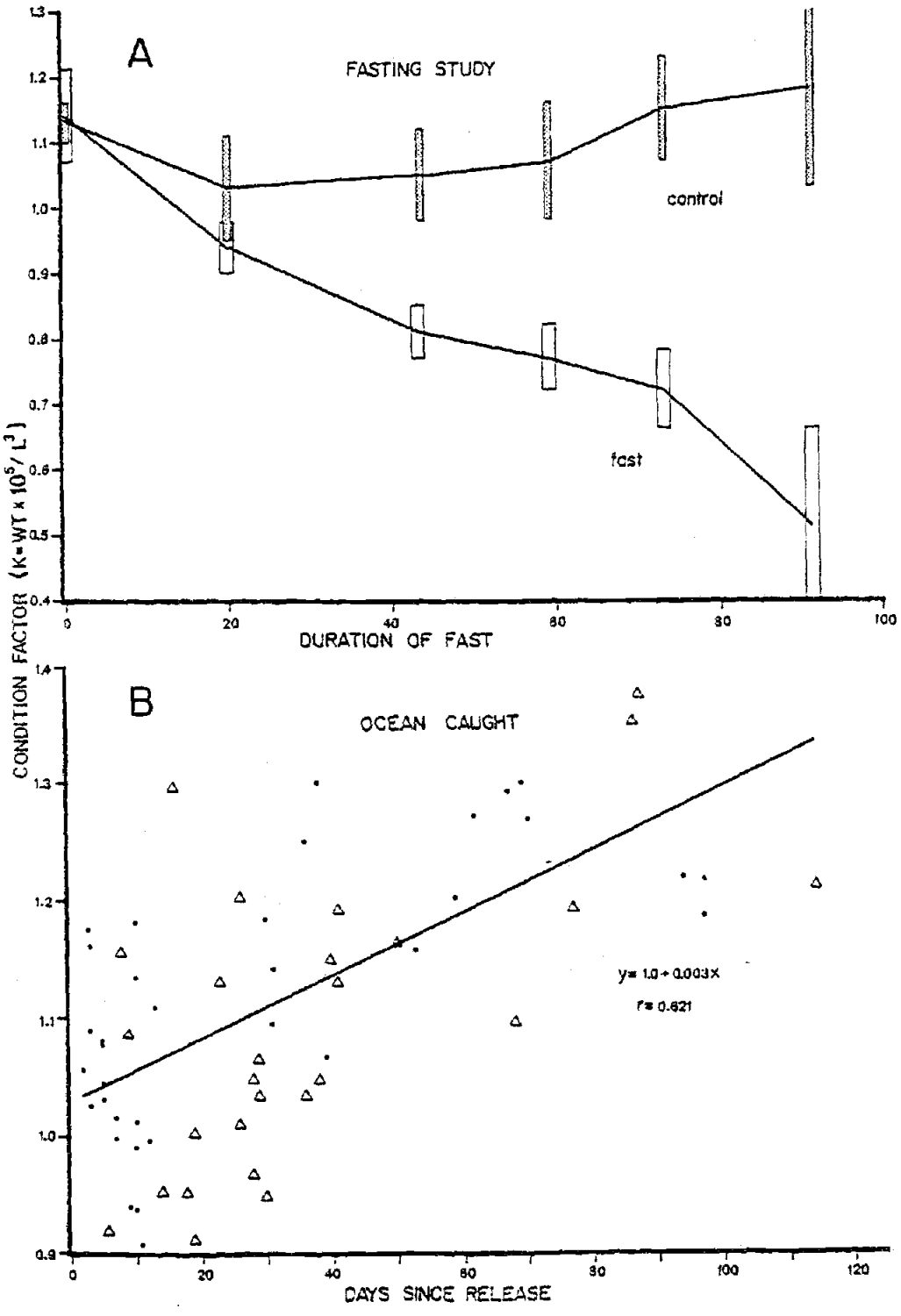
A
FASTING STUDY
control
fast
CONDITION FACTOR (K=WT x 10^5/L^3)
DURATION OF FAST
B
OCEAN CAUGHT
y= 1.0+0.003x
r= 0.621
DAYS SINCE RELEASE

Figure 1

Figure 2. Percent lipid by weight during fasting study. (A) Liver, (B) Viscera, (C) Muscle. In all cases, large dots are the fasting group and open squares are the control. C equations are for control group regression lines. F equations are for fasting group regression lines.

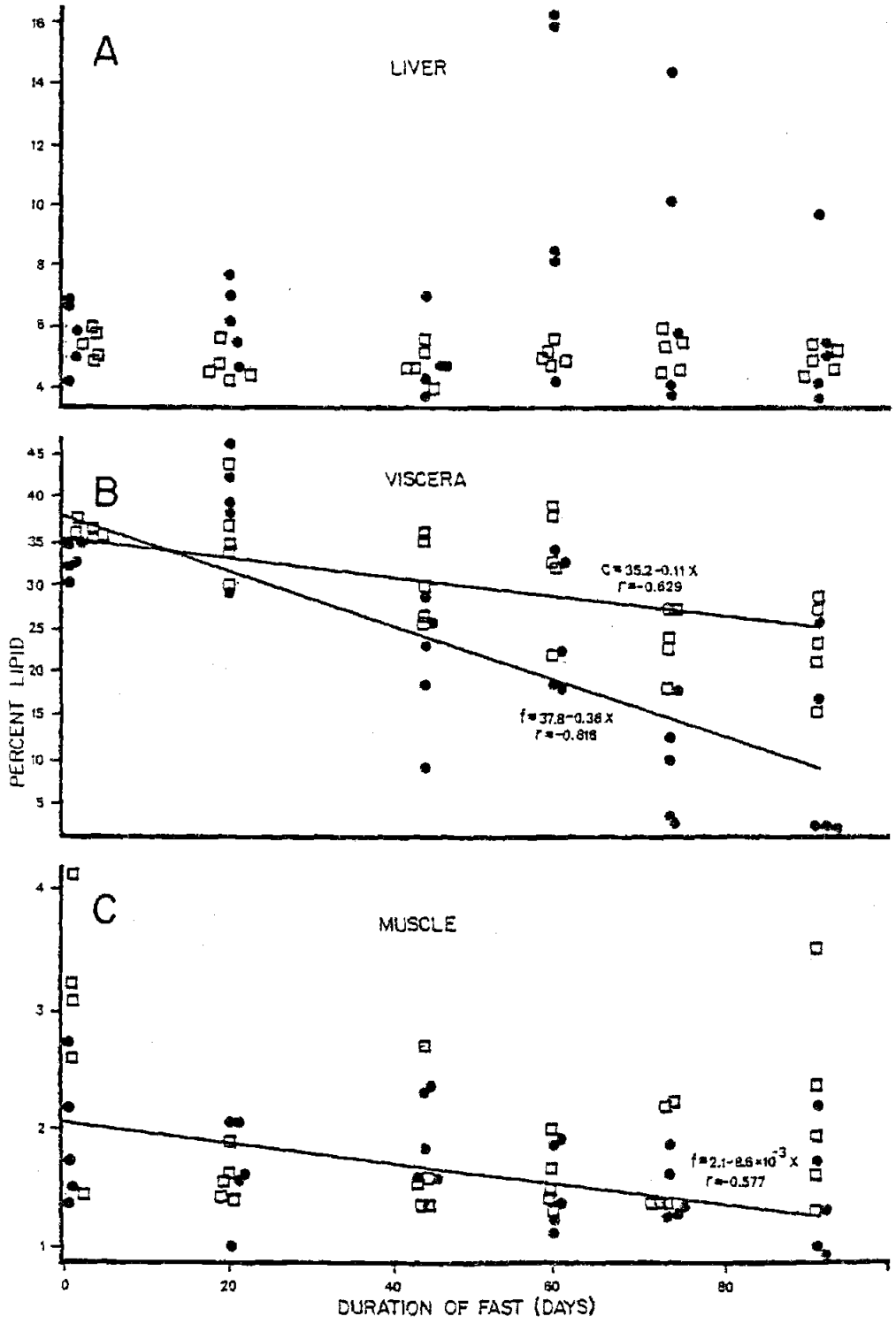
A
LIVER
B
VISCERA
C = 35.2 - 0.11 X
r = -0.629
f = 37.8 - 0.36 X
r = -0.816
C
MUSCLE
f = 2.1 - 8.6 × 10^-3 X
r = -0.577
PERCENT LIPID
DURATION OF FAST (DAYS)

Figure 2

Figure 3. Percent lipid by weight in ocean-caught fish. (A) Liver, (B) Viscera, (C) Muscle. In all cases, dots are zero-age and open triangles are yearling-age.

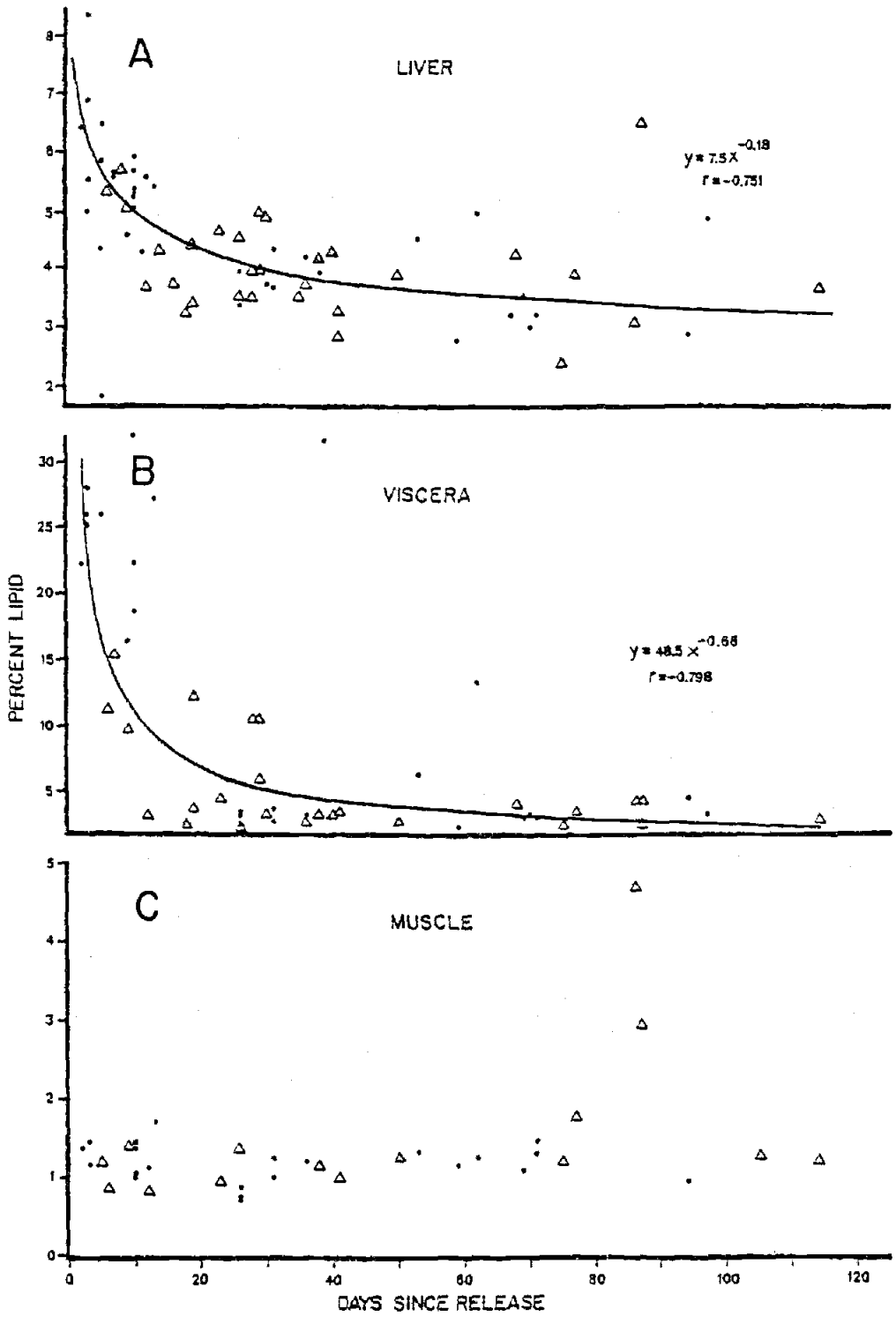
A
LIVER
y = 7.5 X^-0.18
r = -0.751
B
VISCERA
y = 48.5 X^-0.66
r = -0.798
C
MUSCLE
PERCENT LIPID
DAYS SINCE RELEASE
0
20
40
60
80
100
120

Figure 3

Figure 4. Sum of Omega 3 fatty acids (percent by molecular weight) in the fasting study. (A) Liver, (B) Viscera, (C) Muscle with F equation for fasting group regression line. Large dots in each case are for the fasting group and open squares are the control group.

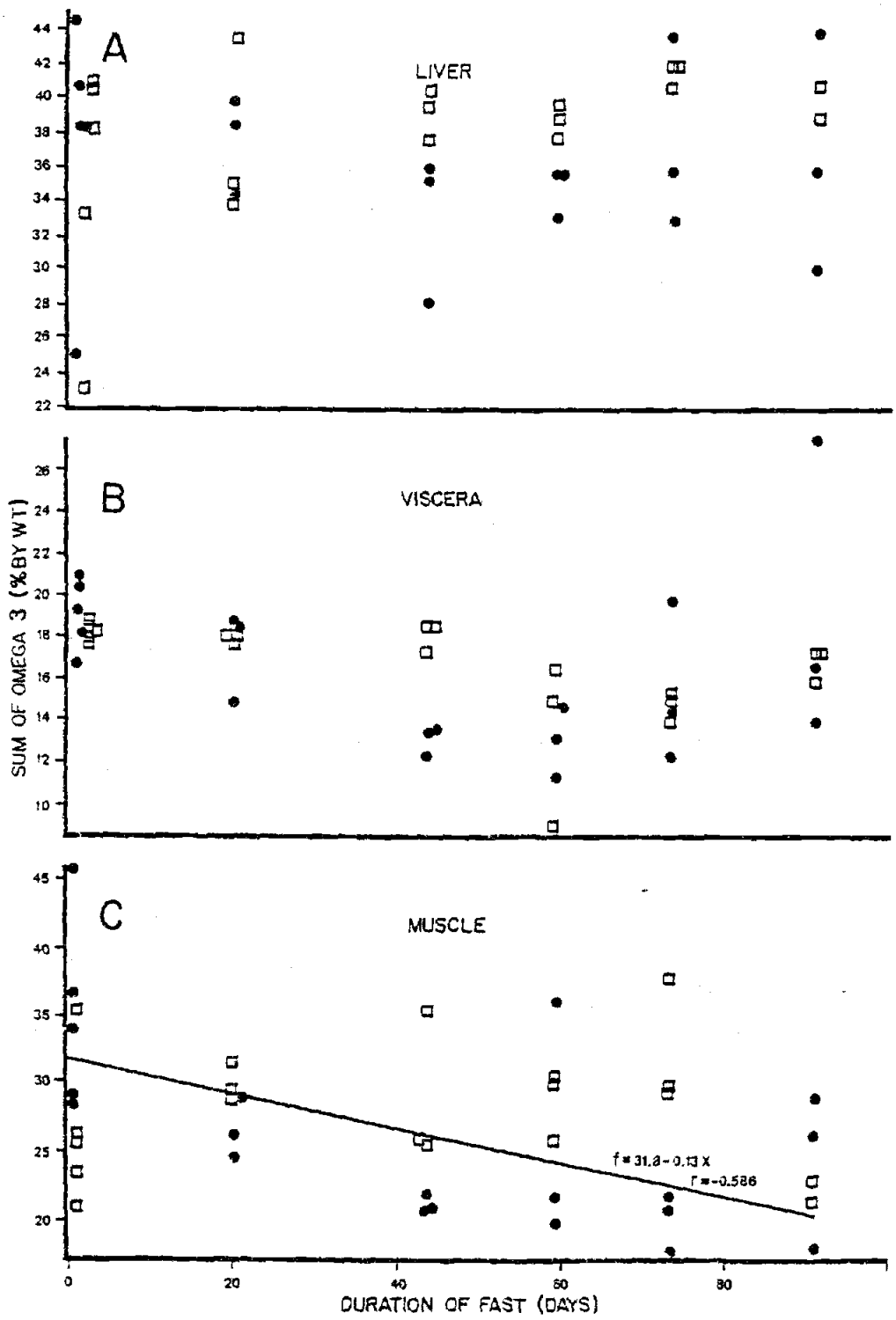
A
LIVER
B
VISCERA
C
MUSCLE
SUM OF OMEGA 3 (%BY WT)
DURATION OF FAST (DAYS)
f = 31.8 - 0.13 X
r = -0.586

Figure 4

Figure 5. Sum of Omega 3 fatty acids (percent by molecular weight) in ocean-caught fish. (A) Liver, (B) Viscera, (C) Muscle. In all cases, dots are zero-age and open triangles are yearling-age.

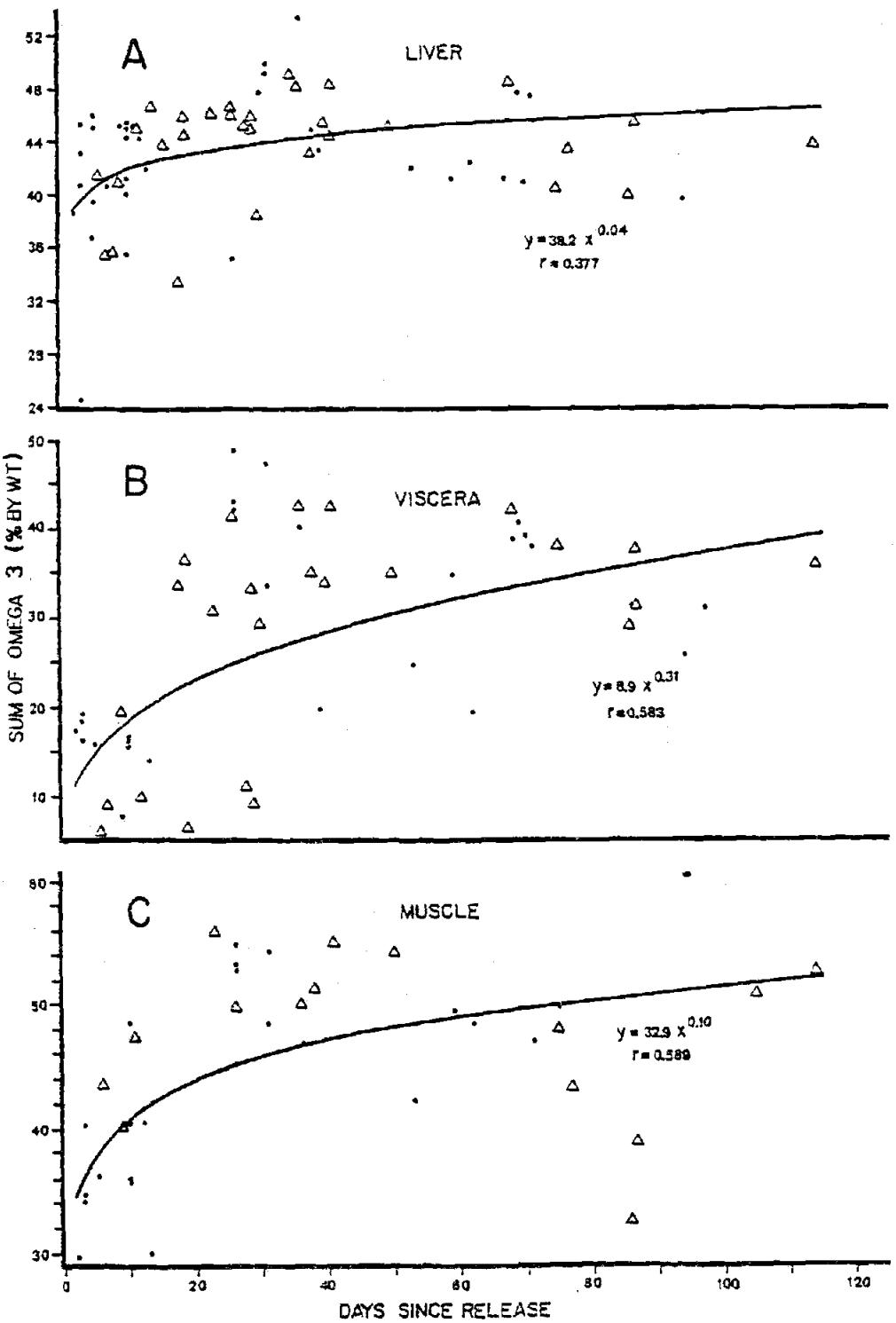
A
LIVER
y = 38.2 x^0.04
r = 0.377
B
VISCERA
y = 8.9 x^0.31
r = 0.583
C
MUSCLE
y = 32.9 x^0.10
r = 0.589
SUM OF OMEGA 3 (% BY WT)
DAYS SINCE RELEASE

Figure 5

Figure 6. 20:5 Omega 3 fatty acid by weight in the fasting study. (A) Liver, (B) Viscera, (C) Muscle. Large dots are fasting group and open squares are the control. C equations are for control group regression lines. F equations are for fasting group regression lines.

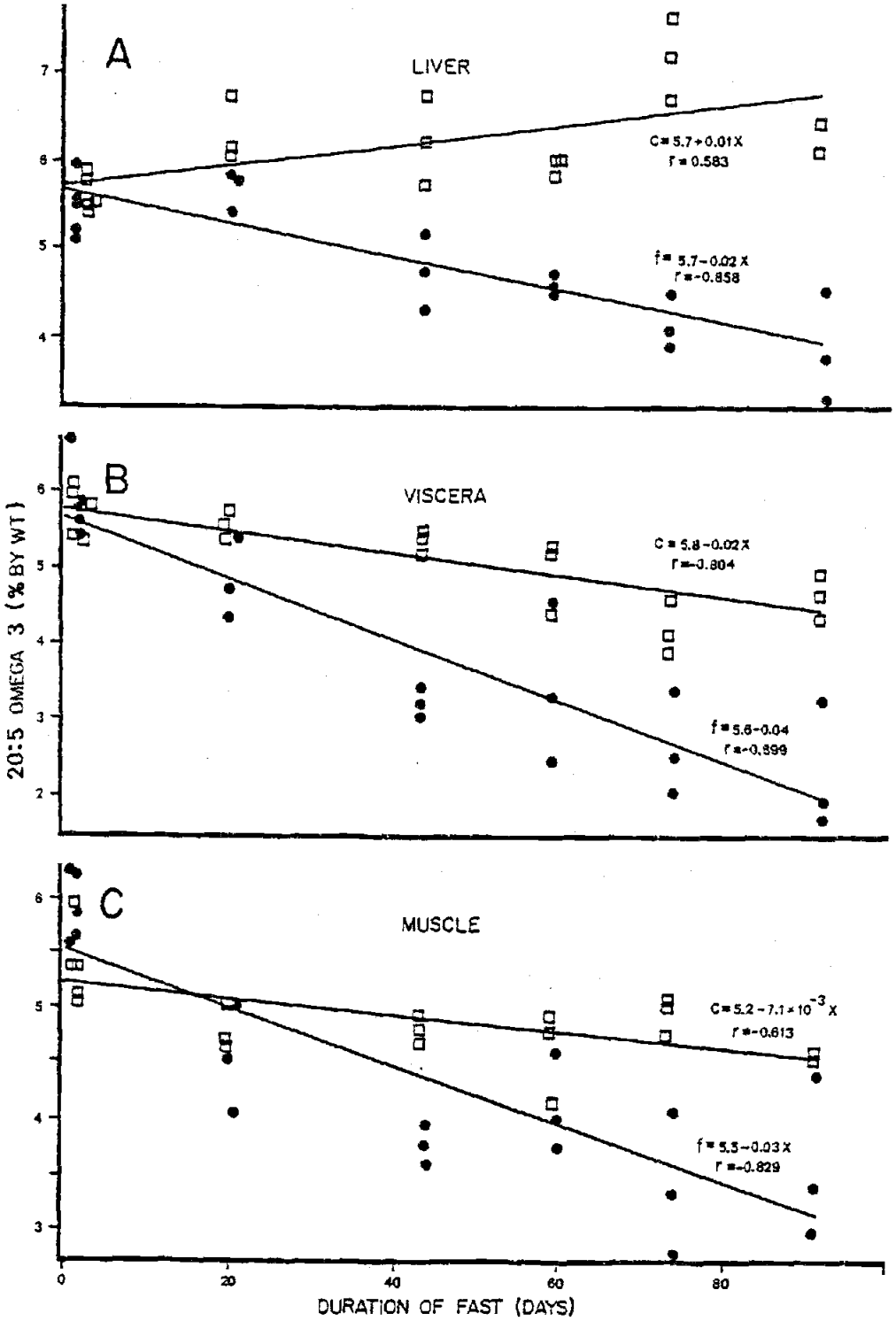
A
LIVER
C= 5.7+0.01X
r = 0.583
f= 5.7-0.02 X
r=-0.858
B
VISCERA
C = 5.8-0.02X
r=-0.804
f=5.6-0.04
r=-0.899
C
MUSCLE
C=5.2-7.1×10⁻³X
r=-0.613
f=5.5-0.03X
r=-0.829
20:5 OMEGA 3 (% BY WT)
DURATION OF FAST (DAYS)

Figure 6

Figure 7. 20:5 Omega 3 fatty acids by weight in ocean-caught fish. (A) Liver, (B) Viscera, (C) Muscle. In all cases, dots are zero-age and open triangles are yearling-age.

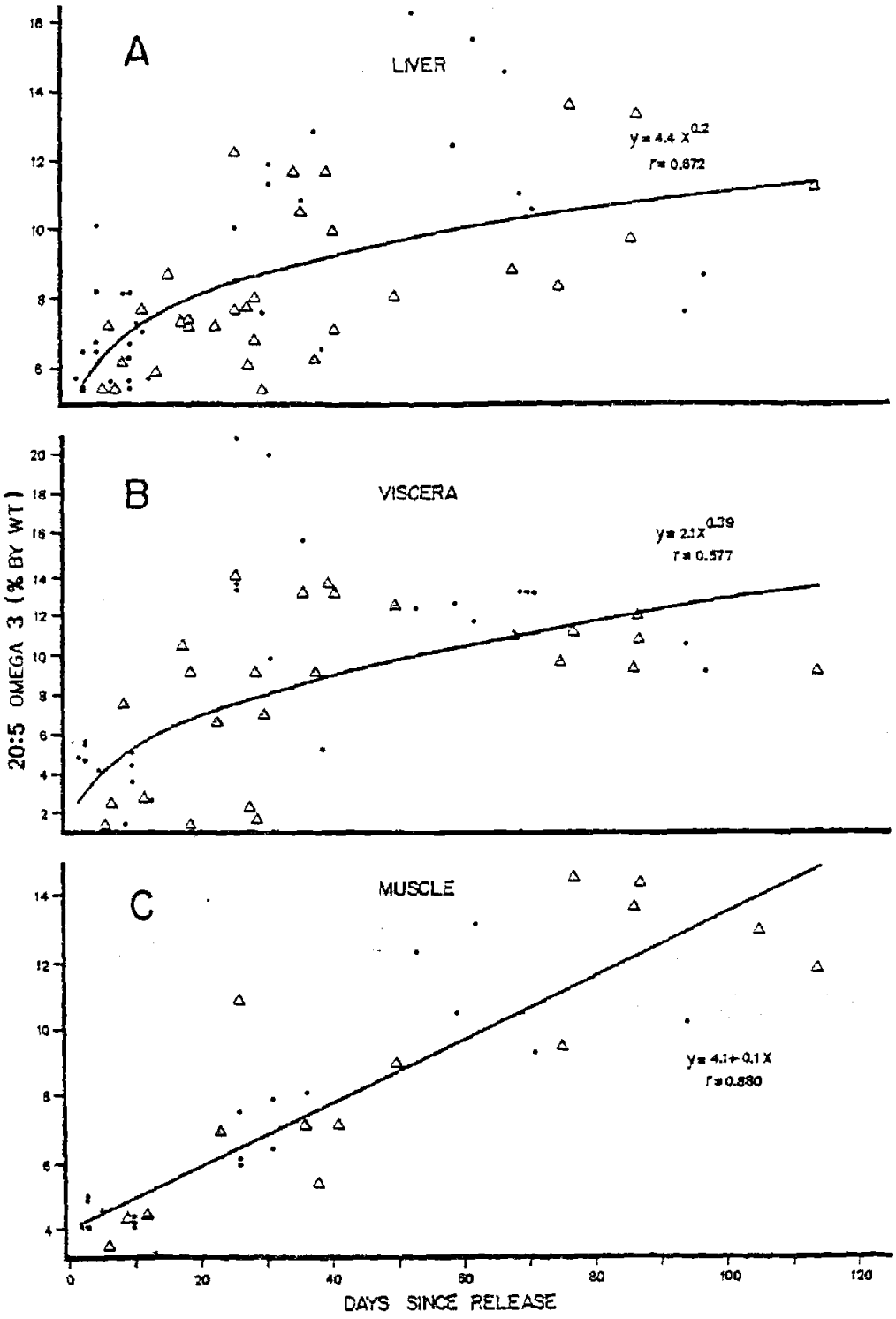
A
LIVER
y = 4.4 X^0.2
r = 0.672
B
VISCERA
y = 2.1 X^0.39
r = 0.577
C
MUSCLE
y = 4.1 + 0.1 X
r = 0.880
20:5 OMEGA 3 (% BY WT)
DAYS SINCE RELEASE

Figure 7

Figure 8. Sum of Omega 6 fatty acids by weight in ocean-caught fish. (A) Liver, (B) Viscera, (C) Muscle. In all cases, dots are zero-age and open triangles are yearling-age.

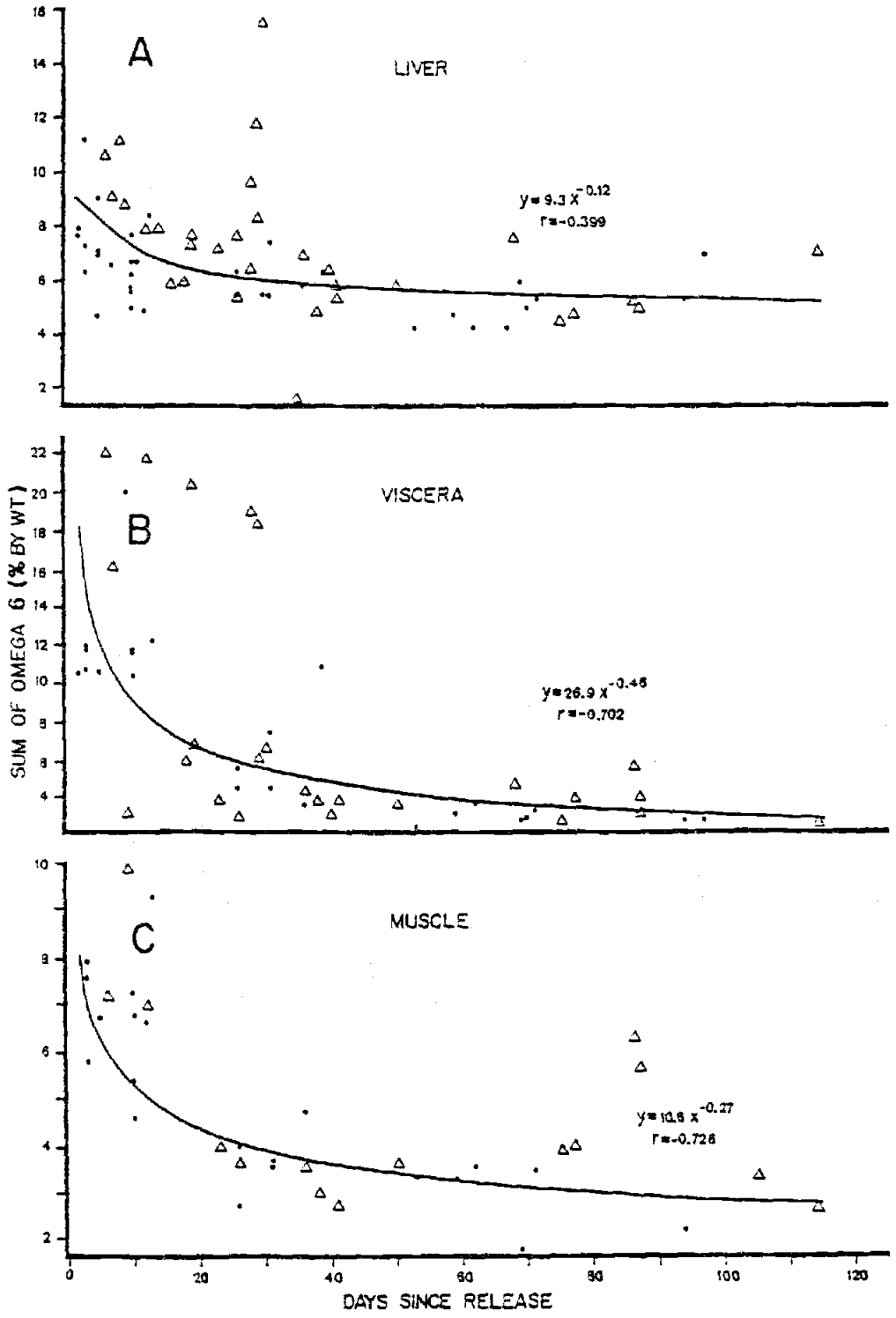
A
LIVER
y=9.3 x^-0.12
r=-0.399
B
VISCERA
y=26.9 x^-0.46
r=-0.702
C
MUSCLE
y=10.6 x^-0.27
r=-0.726
SUM OF OMEGA 6 (% BY WT)
DAYS SINCE RELEASE

Figure 8

Figure 9. 20:4 Omega 6 fatty acid by weight in ocean-caught fish. (A) Liver, (B) Viscera, (C) Muscle. In all cases, dots are zero-age and open triangles are yearling-age.

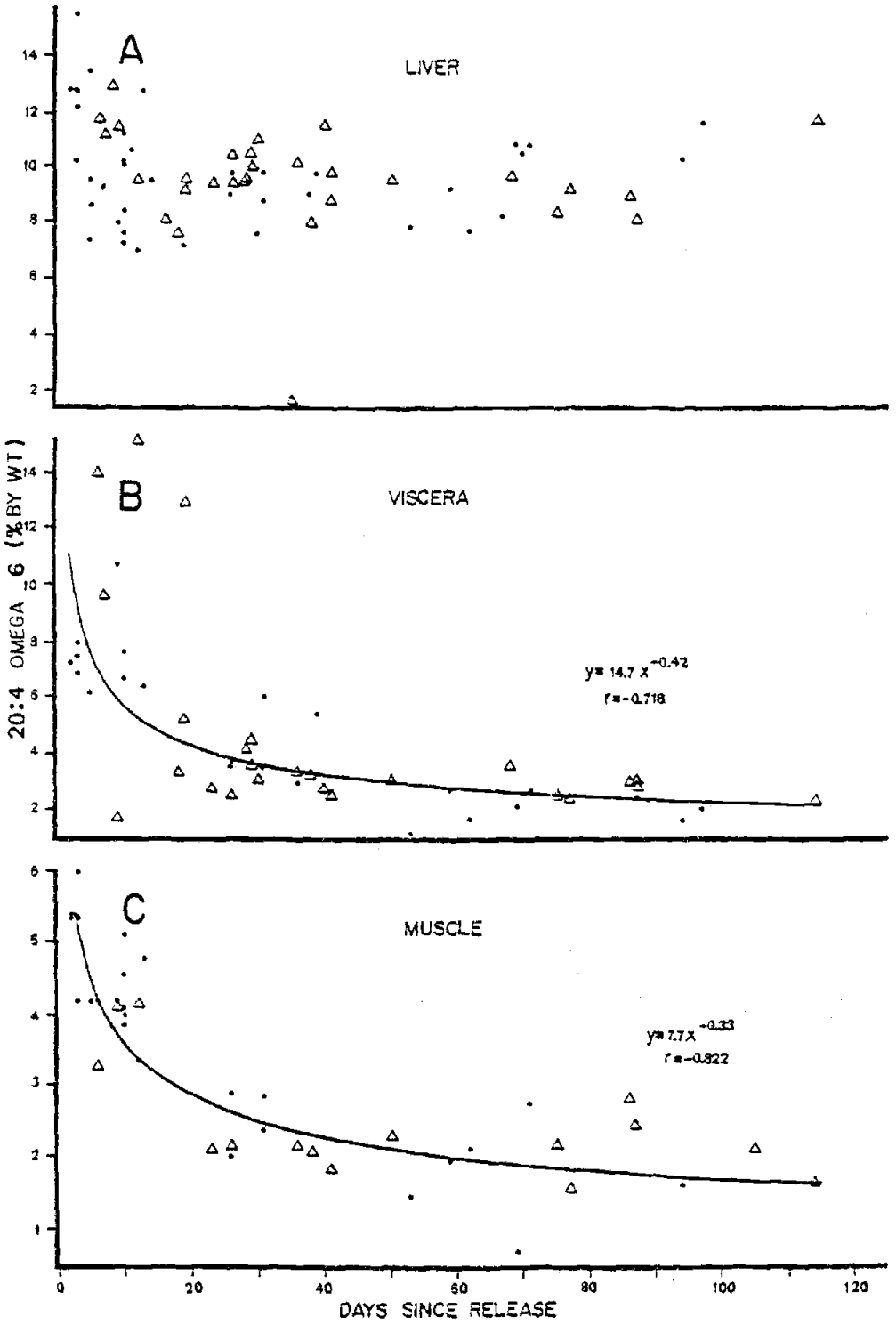
A
LIVER
B
VISCERA
y= 14.7 x^-0.42
r=-0.718
C
MUSCLE
y= 7.7 x^-0.33
r=-0.822
20:4 OMEGA_6 (%BY WT)
DAYS SINCE RELEASE

Figure 9

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APPENDIX

KEY TO TABLE HEADINGS

ID#,TS	Fish ID number and tissue type
LNG	Length (mm)
WT	Weight (g)
SEX	Sex
OR	Agency of origin
DYS	Days since release
DIS	North/South distance from ocean entry point (Naut. miles)
TWT	Tissue wet weight (g)
%LIP	Percent lipid content of tissue (lipid wt/TWT x 100)

KEY TO TABLE CODES

Tissue types (TS)	1 = liver
	2 = viscera
	3 = muscle
Sex (SEX)	1 = male
	2 = female
Agency of origin (OR)	1 = OAF
	2 = ODFW
	3 = WDF
	4 = FWS
	5 = Anadromous

Table 1. Liver samples. Fish characteristics.

ID#,TS	LNG	WT	SEX	GR	DYS	DIS	TWT	%LIP
60061	146.0	31.4	1.0	2.0	26.0	97.0	.47	4.54
60131	154.0	39.5	1.0	1.0	5.0	2.0	.45	5.89
70041	140.0	29.6	1.0	1.0	5.0	2.0	.88	1.79
80071	147.0	32.7	1.0	1.0	5.0	2.0	.48	4.35
100031	159.0	38.2	2.0	3.0	18.0	97.0	.61	3.22
150161	140.0	27.8	2.0	1.0	7.0	11.0	.36	5.59
170171	142.0	27.7	1.0	2.0	28.0	110.0	.52	3.96
300031	150.0	34.9	1.0	4.0	29.0	20.0	.52	4.96
300061	143.0	32.1	2.0	4.0	68.0	110.0	.63	4.25
300091	146.0	31.1	2.0	2.0	19.0	20.0	.51	4.44
300111	140.0	24.9	1.0	2.0	19.0	20.0	.40	3.38
300141	139.0	25.2	2.0	1.0	9.0	77.0	.44	4.61
300151	150.0	35.9	1.0	4.0	29.0	20.0	.53	3.95
300561	153.0	37.5	1.0	4.0	28.0	20.0	.72	3.53
350141	144.0	28.3	1.0	4.0	30.0	5.0	.22	4.89
770021	190.0	77.5	2.0	2.0	41.0	5.0	1.56	3.29
790111	139.0	25.5	2.0	3.0	14.0	5.0	.28	4.31
800561	199.0	102.6	2.0	5.0	16.0	170.0	2.00	3.76
820121	179.0	67.9	2.0	1.0	30.0	93.0	1.56	3.76
830271	183.0	63.4	1.0	2.0	36.0	5.0	1.33	3.73
830521	203.0	91.5	2.0	1.0	31.0	93.0	1.86	3.70
900061	173.0	60.2	2.0	4.0	50.0	5.0	1.12	3.89
910041	200.0	83.8	1.0	2.0	38.0	145.0	1.14	4.17
1040011	124.0	20.1	1.0	1.0	2.0	2.0	.31	6.48
1040081	126.0	21.8	2.0	1.0	3.0	2.0	.33	8.41
1070061	123.0	21.6	2.0	1.0	3.0	2.0	.20	6.89
1090071	136.0	25.1	1.0	1.0	7.0	77.0	.41	5.65
1100021	122.0	18.6	1.0	1.0	3.0	2.0	.35	4.98
1100031	126.0	23.5	2.0	1.0	3.0	2.0	.36	5.56
1240051	156.0	43.9	2.0	5.0	8.0	0.0	.42	5.73
1270011	173.0	58.6	2.0	5.0	23.0	10.0	.79	4.67
1270081	126.0	19.8	1.0	1.0	10.0	10.0	.29	5.96
1270111	123.0	17.4	1.0	1.0	10.0	10.0	.25	5.73
1270171	127.0	24.2	1.0	1.0	10.0	10.0	.20	5.02
1270201	169.0	52.4	2.0	5.0	9.0	10.0	.65	5.04
1270211	124.0	19.9	1.0	1.0	5.0	65.0	.32	6.50
1270411	118.0	18.6	2.0	1.0	10.0	10.0	.28	5.25
1270421	121.0	17.6	2.0	1.0	12.0	10.0	.28	5.63
1270531	130.0	24.9	2.0	1.0	10.0	10.0	.33	5.37
1290021	123.0	18.8	2.0	1.0	10.0	10.0	.34	5.31
1640021	138.0	24.1	1.0	2.0	6.0	5.0	.33	5.35
1770011	155.0	46.5	1.0	1.0	36.0	154.0	.91	4.24
1770031	223.0	0.0	1.0	5.0	35.0	154.0	2.29	3.53
1800061	161.0	54.2	2.0	1.0	38.0	154.0	.84	3.96
1800071	195.0	89.2	1.0	5.0	26.0	154.0	1.71	3.50
1830021	240.0	0.0	2.0	3.0	75.0	20.0	3.13	2.39
1900031	153.0	40.9	2.0	1.0	31.0	77.0	.70	4.38
1900071	186.0	76.6	2.0	2.0	41.0	97.0	1.93	2.84
1910101	170.0	0.0	1.0	1.0	26.0	2.0	.81	3.96
1910291	181.0	0.0	2.0	1.0	26.0	2.0	1.25	3.38
1930181	139.0	0.0	2.0	1.0	11.0	2.0	.48	4.33
1980061	156.0	0.0	1.0	2.0	12.0	80.0	.71	3.71
2140061	226.0	138.7	2.0	1.0	59.0	103.0	2.63	2.80
2240181	262.0	247.1	1.0	3.0	87.0	7.0	5.38	6.49
2300011	286.0	283.7	1.0	4.0	114.0	20.0	3.31	3.70
2310131	203.0	96.8	2.0	1.0	53.0	77.0	1.67	4.54
2320031	212.0	123.4	1.0	1.0	67.0	154.0	2.22	3.22
2320061	194.0	92.8	2.0	1.0	62.0	77.0	1.43	5.01
2320091	213.0	117.9	1.0	1.0	94.0	77.0	3.78	2.87
2330051	256.0	200.3	2.0	3.0	77.0	20.0	3.34	3.93
2430101	213.0	125.5	2.0	1.0	69.0	77.0	1.86	3.58
2430121	144.0	33.1	2.0	1.0	13.0	2.0	.49	5.45
2430131	138.0	28.0	2.0	1.0	39.0	2.0	.38	7.20
2470011	202.0	94.8	2.0	2.0	40.0	96.0	1.84	4.32
2540021	197.0	97.1	1.0	1.0	70.0	91.0	2.09	2.99
2540041	144.0	35.4	1.0	1.0	97.0	14.0	.44	4.88
2620051	245.0	179.0	1.0	1.0	71.0	63.0	3.84	3.24
2750021	337.0	517.6	1.0	5.0	86.0	40.0	6.37	3.07

Table 2. Liver samples. Fatty acid composition (% by mol. wt)

ID#,TS	14:0	15:0	16:0	16:1	18:0	18:1	18:2	20:1	18:4	20:0	20:2	20:3	20:4	20:5	22:4	22:5	22:6
60061	2.111	.506	22.401	2.748	4.131	13.430	2.455	2.768	.380	0.000	.341	0.000	4.916	7.479	0.000	2.721	35.949
60131	2.220	.590	21.749	2.847	7.648	14.535	2.266	2.989	.214	0.000	.197	0.000	6.529	6.468	0.000	2.029	27.925
70041	2.354	.531	20.724	1.288	6.116	11.900	2.115	3.453	.432	.270	.542	.621	4.439	10.054	0.000	0.000	34.439
80071	1.885	.485	21.306	2.551	5.216	11.393	2.729	2.187	.282	.139	.345	.117	3.919	6.748	0.000	2.870	36.989
100031	2.024	.729	27.721	1.633	9.833	14.339	2.542	2.710	1.066	0.000	0.000	0.000	3.453	7.288	0.000	0.000	25.159
150161	3.022	.369	20.908	4.225	4.349	17.273	2.886	3.824	.275	0.000	.870	0.000	5.300	7.203	0.000	2.931	25.054
170171	1.556	.500	20.767	2.077	7.657	11.720	1.665	2.279	.305	0.000	.367	.127	4.446	7.760	0.000	2.300	34.896
300031	1.952	.566	22.043	1.369	5.535	10.276	4.325	1.156	0.000	0.000	.601	0.000	4.947	6.865	1.859	2.497	35.717
300061	1.212	.491	19.853	.727	8.662	10.020	1.727	2.048	.262	.354	.399	0.000	4.491	8.803	.915	.920	38.452
300091	1.890	.484	21.517	3.126	5.351	13.604	2.819	2.778	0.000	0.000	.380	0.000	4.429	7.349	0.000	.897	36.373
300111	1.722	.414	20.070	1.171	4.490	14.396	1.860	3.846	0.000	0.000	.398	0.000	4.258	7.222	.900	3.203	35.599
300141	1.969	.433	20.790	3.714	4.867	12.661	2.941	2.438	.267	0.000	.395	0.000	3.647	8.202	.585	2.529	34.346
300151	1.402	.522	20.591	1.922	6.379	10.652	2.477	1.769	.247	.253	.580	0.000	4.491	8.043	.569	1.145	36.460
300561	1.695	.500	22.511	1.610	5.967	10.590	4.542	1.241	.214	.212	.538	.307	4.530	6.038	0.000	1.863	37.059
350141	1.232	.544	18.151	3.895	6.367	12.694	3.989	1.884	0.000	.251	.737	.348	5.188	5.422	5.604	2.743	30.272
770021	.939	.884	17.790	1.990	11.511	12.316	.709	1.540	0.000	0.000	.511	0.000	4.101	7.125	0.000	2.141	35.351
790111	1.234	.327	18.122	2.112	6.755	13.675	2.635	2.521	0.000	0.000	.444	0.000	4.455	5.910	.335	2.955	37.997
800561	1.447	.570	18.965	1.971	10.124	13.086	1.344	2.177	.264	.178	.844	0.000	3.689	8.758	0.000	1.974	32.841
810121	1.113	.587	14.852	2.909	8.186	13.445	1.153	2.313	.230	0.000	.488	0.000	3.430	7.600	.448	3.143	36.687
830271	1.417	0.000	14.947	2.643	7.633	13.634	.838	2.687	0.000	0.000	.508	0.000	4.766	10.588	.780	2.637	35.107
830521	.984	.557	17.182	3.085	6.847	13.064	.961	1.829	0.000	0.000	.451	0.000	4.048	11.317	0.000	3.644	34.201
900061	1.028	.409	19.188	2.176	7.821	13.928	.776	1.880	0.000	0.000	.557	0.000	4.457	8.093	0.000	2.364	34.745
910041	1.145	.458	21.779	2.501	9.934	10.967	.831	1.732	.243	.501	.445	0.000	3.663	6.285	0.000	2.084	34.720
1040011	2.124	.642	17.801	5.783	5.759	15.072	1.234	4.195	.360	.151	.309	0.000	6.161	5.678	.172	2.480	30.319
1040081	6.485	.421	16.035	7.993	3.672	20.991	2.685	7.119	1.007	.367	.326	.159	7.617	5.263	.489	2.530	15.814
1070061	2.272	.412	20.769	5.338	6.702	14.064	1.247	3.454	2.753	.188	.265	0.000	6.166	5.307	0.000	2.501	30.214
1090071	2.570	.453	20.559	5.797	4.748	14.202	1.813	2.869	.326	0.000	.310	.201	4.313	5.604	.146	2.453	32.443
1100021	1.995	.390	20.025	5.119	4.369	12.209	.998	2.792	.298	0.000	.263	0.000	4.814	5.357	.269	2.075	37.432
1100031	1.869	.408	20.645	2.969	7.077	13.224	1.143	2.665	.185	0.000	.272	0.000	5.842	6.416	0.000	1.854	34.835
1240051	2.297	.282	16.728	3.848	10.922	12.958	4.364	3.561	.552	.352	.612	.449	6.251	5.352	0.000	2.128	27.782
1270011	.936	.415	19.075	2.516	9.072	10.329	1.717	2.622	0.000	0.000	.526	0.000	4.399	7.219	.502	2.681	36.360
1270081	3.769	.447	19.225	6.552	4.478	17.008	1.919	4.418	.584	.222	.320	0.000	5.368	5.616	0.000	2.316	27.026
1270111	2.299	.411	21.626	5.814	6.235	14.020	1.170	2.634	.248	0.000	.276	0.000	4.769	6.270	0.000	2.592	30.789
1270171	1.915	.503	19.801	4.658	6.747	13.370	1.591	2.327	.206	0.000	.331	0.000	4.834	5.365	0.000	2.409	33.310
1270201	1.107	.341	20.642	2.433	10.000	11.880	2.797	2.434	0.000	0.000	.475	.299	5.488	6.166	0.000	1.796	33.105
1270211	2.749	.932	24.401	6.037	4.864	12.371	1.122	1.868	.284	.142	.265	0.000	3.336	8.165	0.000	1.926	29.117
1270411	1.796	.454	18.961	5.615	4.690	13.318	1.771	2.263	.242	0.000	.381	0.000	3.452	6.203	0.000	3.132	35.949
1270421	2.311	.429	20.340	6.020	4.669	13.379	1.516	2.237	.253	0.000	.282	0.000	3.145	7.059	0.000	3.101	33.860
1270531	2.052	.781	20.655	5.685	5.290	13.233	1.398	1.722	.195	0.000	.347	0.000	3.274	6.591	0.000	3.269	34.138
1290021	2.073	.394	9.353	5.751	4.973	12.754	1.406	2.221	.238	0.000	.279	0.000	3.908	8.162	.191	2.672	34.041
1640021	2.102	.293	17.250	4.234	4.694	15.077	3.857	4.038	.353	0.000	.431	0.000	5.576	5.385	.712	3.038	32.669

Table 2. Liver samples. Fatty acid composition (cont.).

ID#,TS	14:0	15:0	16:0	16:1	18:0	18:1	18:2	20:1	18:4	20:0	20:2	20:3	20:4	20:5	22:4	22:5	22:6
1770011	1.186	.338	11.542	2.153	8.226	13.742	.392	2.600	.234	0.000	.672	0.000	4.760	10.851	0.000	4.883	37.456
1770031	1.013	.279	11.166	1.837	10.618	15.306	.741	2.393	.223	0.000	.716	.252	.176	11.703	0.000	4.344	33.045
1800061	1.212	.343	17.740	2.804	10.894	14.878	.335	1.478	0.000	0.000	.315	0.000	4.234	12.821	0.000	5.006	27.116
1800071	.987	.353	15.991	2.881	9.468	12.851	.479	1.743	.199	0.000	.518	0.000	4.373	12.297	0.000	4.493	31.365
1830021	.981	.371	18.042	2.904	9.919	18.496	.296	2.061	0.000	0.000	.438	0.000	3.820	8.347	0.000	4.345	27.825
1900031	2.005	0.000	14.913	3.772	5.971	11.061	.546	4.408	0.000	0.000	.745	0.000	4.595	11.893	1.569	4.600	33.530
1900071	.794	.343	15.925	3.406	10.301	11.151	.417	2.194	0.000	0.000	.414	0.000	4.593	9.998	.418	2.817	35.669
1910101	1.194	.384	15.149	2.978	10.072	12.653	1.041	3.000	.230	.221	.733	0.000	4.566	8.535	0.000	4.662	32.647
1910291	1.231	.422	15.137	2.441	10.466	13.341	.565	5.568	0.000	0.000	.668	0.000	4.195	10.042	0.000	3.361	33.415
1930181	1.821	.814	18.922	3.892	6.351	11.273	1.145	3.731	0.000	0.000	.464	0.000	5.029	7.279	0.000	2.076	35.856
1980061	1.418	.475	17.501	2.949	5.861	15.168	2.807	3.480	.298	0.000	.620	.297	4.470	7.662	0.000	4.224	32.771
2140061	1.358	.329	22.357	2.786	10.615	14.125	.287	1.581	0.000	0.000	.272	0.000	4.252	12.448	0.000	4.291	24.497
2240181	2.924	.560	13.463	5.558	8.629	14.945	.579	2.300	.322	0.000	.261	0.000	3.668	13.359	.477	5.154	26.667
2300011	1.183	.406	14.948	2.464	14.777	12.707	.257	1.832	0.000	0.000	.274	0.000	5.572	11.254	.946	3.525	28.879
2310131	1.233	.210	23.209	3.532	11.296	12.141	.228	1.609	0.000	.503	.222	0.000	3.571	16.322	.286	3.878	21.759
2320031	1.320	.233	24.124	2.658	12.526	11.410	.238	1.394	.265	.251	.312	0.000	3.731	14.614	0.000	3.860	22.426
2320061	1.786	.205	23.295	3.892	8.496	13.099	.469	1.681	.190	.230	.215	0.000	3.509	15.551	.124	3.460	23.421
2320091	.726	.315	25.347	1.450	11.345	14.284	.257	1.027	0.000	0.000	.155	0.000	4.858	7.545	0.000	3.146	29.139
2330051	1.640	.230	18.380	2.544	12.688	12.966	.225	2.548	0.000	.228	.249	0.000	4.282	13.593	0.000	3.467	26.474
2430101	.675	.410	13.208	1.887	11.948	14.532	.327	1.896	0.000	.205	.389	.108	5.167	11.041	.080	4.630	32.186
2430121	1.700	.518	16.426	3.768	8.238	14.135	1.905	2.816	.175	.152	.339	.181	6.140	5.693	0.000	2.359	33.651
2430131	2.274	1.940	17.124	4.406	4.276	14.764	1.189	2.625	.267	.211	0.000	.244	4.410	6.494	.583	1.899	34.820
2470011	1.046	0.000	16.212	3.706	9.984	15.202	.596	1.898	0.000	0.000	.339	0.000	5.470	11.689	0.000	4.691	29.166
2540021	1.233	0.000	18.913	2.044	11.693	16.637	0.000	3.457	.355	0.000	0.000	0.000	4.956	10.391	0.000	3.956	26.345
2540041	1.242	.692	18.947	1.983	9.586	12.828	1.411	2.230	.407	0.000	0.000	0.000	5.550	8.656	0.000	2.997	34.011
2620051	1.195	.260	15.490	2.596	10.932	11.486	0.000	3.669	0.000	0.000	.322	0.000	5.100	10.596	0.000	3.942	32.880
2750021	2.114	.290	20.441	4.019	12.029	13.912	.790	1.179	.417	0.000	.332	0.000	4.133	9.673	0.000	4.067	25.761