THE PHARMACOKINETICS OF OXYTETRACYCLINE IN ATLANTIC COD (Gadus morhua)

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The Pharmacokinetics of Oxytetracycline in Atlantic Cod (*Gadus morhua*)

By

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Abstract

Under conditions of intense fish culture it may be necessary to use antibiotics in order to mitigate disease problems. In an effort to reduce the impact of antibiotic residuals on the environment and to eliminate the risk of exposure of these residuals to the consumer optimal treatment regimes must be developed. These regimes must account for the use of different antibiotics used on a wide range of species held under varying culture conditions. Culture of the Atlantic cod (*Gadus morhua*) shows a great deal of potential and at present little information is available on the behaviour of Oxytetracycline (OTC) in this species.

In an effort to generate baseline pharmacokinetic numbers and to determine the clearance time from organs for OTC in Atlantic cod two experiments were undertaken. Experiment 1 examined the pharmacokinetics of OTC in Atlantic cod following a single dose by oral gavage (Per Os (PO)) or intraperitoneal (IP) injection. Eighteen fish were held at 10°C and administered OTC either intraperitoneally a dose level of 132.8 mg OTC/ kg fish or orally at a dose level of 134.5 mg OTC/ kg fish. Blood samples were taken at 0.5, 3, 6, 12, 36 and 72 hrs and analyzed for OTC levels using High Pressure Liquid Chromatography (HPLC). Noncompartmental modeling identified a time to maximum serum concentration (T_{max}) for IP and PO administration of 18 hrs and 6 hrs respectively. The shorter T_{max} in the PO group as compared to the IP group is not as expected and may have been the result of precipitation in the injectable solution. Maximum serum concentrations (C_{max}) for IP and PO were 140.8 ppm and 1.04 ppm respectively. The

higher C_{max} in the IP group is as expected and both numbers are in the same order of magnitude as literature values. The relative bioavailability of OTC was calculated to be 0.8%. This low value compares well with literature values for orally administered drugs. T½ for PO administration was calculated to be 184 hours. The PO T½ value was then used to predict a clearance time for OTC of 38.3 days from the serum and 55.5 days from the muscle of cod. This number compares well with literature values and with results from experiment 2.

Experiment 2 examined the absorption, distribution and elimination of oxytetracycline from Atlantic cod held in seawater at temperatures of 10°C and 10°C decreasing to 2°C. Fish were administered OTC in the feed at a dose level of 120 mg OTC/ kg fish over an 11 day period. One group of five fish were held as controls and fed nonmedicated feed. The water temperature for half the medicated fish was maintained at 10°C and for control fish and the other half of the medicated fish was decreased from 10°C to 2°C by daily increments of 0.1°C. Samples of blood, muscle, gonad and liver were taken on days 1, 5, 10, 20, 41, 61 and 101 and analyzed for OTC levels using HPLC. OTC in the gonad was detected in one sample on day 1 only therefore no subsequent statistical analysis was conducted. Data for the remaining three tissue types was grouped by sample day and temperature regime. OTC concentration for the tissue types was plotted versus time. A two factor analysis of variance showed no significant differences in OTC concentrations between the temperature groups for serum, muscle or liver. Lack of a temperature effect on antibiotic clearance is unusual in poikilothermic animals and does not compare with results found in the literature. Insufficient differences in water temperature between the

two groups, as a result of small daily incremental decreases, are believed to account for this result.

There were significant differences in OTC concentration by sample day in the serum, muscle and liver. A Scheffes multiple comparison determined that in serum and muscle the differences were between OTC levels up to and including day 10 and OTC levels on and after day 20. In liver the differences lay between OTC levels up to and including day 41 and OTC levels on and after day 61. It was determined that clearance of OTC in both temperature groups occurred in the serum between day 20 and day 41. This compares well with the predicted value from experiment 1 of 38.3 days. Clearance from the muscle in both temperature groups occurred between day 41 and day 61. This compares well with the predicted value from experiment 1 of 55.5 days. Clearance from the liver in both temperature groups was still occurring up to day 101.

These results indicate that residuals of OTC in cod for both temperature groups fell below the legally acceptable level of 0.1 ppm by day 41 in the serum and day 61 in the muscle. These fall within the suggested withdrawal period of 80 days, at or above 10°C, for OTC in salmon. They also indicate that a withdrawal period for OTC of 80 days may be acceptable in cod held below 10°C. Clearance of OTC from the liver was not consistently below legal limits in either temperature group within the sample period. This indicates that when harvesting the liver of cultured cod extended clearance times should be considered.

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Thank God its over.

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AUC — area under the plasma drug concentration versus time curve (zero to infinity : zero moment).

AUCM — area under the concentration – time versus time curve (first moment).

β — beta. Elimination rate constant. Obtained from terminal slope of a semilogarithmic plot of plasma drug concentration versus time curve.

C_{max} — maximum observed concentration of a drug.

Cl — Volume of reference fluid (such as plasma) cleared of a drug by various elimination processes per unit time.

Cl_b — Total (or systemic) clearance. Sum of individual clearances that contribute to the overall elimination of a drug.

IP — intraperitoneal.

MRT — mean residence time.

Per Os (PO) — Oral administration.

T_{max} — time of maximum observed concentration of a drug from a tissue or organ.

$T_{1/2}$ — half – life of elimination. Time required for a given concentration of a drug in a tissue or organ to decline by 50% during the exponential terminal phase of the drug concentration – time profile.

1.0 Introduction

The Atlantic cod (*Gadus morhua*) is a bottom dwelling gadoid which inhabits the cool-temperate waters of the continental shelf (Scott and Scott, 1988). Cod has been the cornerstone of the Atlantic Canadian fishery for 500 years during which time there have been marked fluctuations in annual landings. In 1988, in an effort to alleviate marketing problems associated with these fluctuations, Sea Forest Plantation Ltd. of Newfoundland investigated the potential of cod farming. Cod were captured live during the high volume trap season in the spring and held and fed over the summer. High quality fillets then went to market during the traditionally low volume season of late fall. Although cod proved to be a suitable species to farm Vibriosis became a problem under conditions of intense culture.

Vibriosis is a disease caused by bacteria belonging to the *Vibrio spp.*. It is common in many cultured species and causes the death of infected fish if not treated. The antibiotic of choice for treating Vibriosis is oxytetracycline (OTC). OTC is a broad spectrum antibiotic that is effective against a range of Gram negative bacteria (Combes *et al*, 1972). It is used extensively in aquaculture as it is relatively effective, inexpensive, has low toxicity and is easily accessible (Alderman, 1988).

The efficacy of not only OTC, but any antibiotic regime, is dependent on the drug reaching the target organ. This, in turn, is dependent on four factors:

- absorption - transport of a drug from site of application to systemic blood supply;

- distribution - transport of drug from blood to tissues and organs. Efficacy is further dependent on the drug accumulating in tissues and organs at concentrations adequate to kill or inhibit bacterial growth. This concentration is referred to as the minimum inhibition concentration (MIC).
- metabolism - enzyme mediated anabolism or catabolism of a compound; and
- elimination - excretion of a compound from the body (Baggott, 1988).

These four factors represent the pharmacokinetics of a drug. Pharmacokinetics is affected by the properties of the particular drug, the biology of the host and the environmental conditions in which it lives. Drug properties include such factors as molecular weight, solubility, degree of ionization (pH and pKa) and chemical structure. These affect a drugs ability to be passively diffused, actively transported or metabolized (Guarino, 1987, 1991; Ingebrigtsen, 1991). Host factors include the presence of metabolic or active transport systems, plasma protein binding, fat or protein content of tissues and organs, vascularization, gastric emptying time and nutritional status (Jacobsen, 1984; *Grondel et al*, 1989; Bjorklund and Bylund, 1990, 1991; Black, 1991; Uno, 1992). Species which undergo profound changes in physiology (such as metamorphosis or smoltification) or seasonal variations in metabolism may show variations in their ability to utilize a drug (Bruno, 1989; McSwain, 1992; O'Hara *et al*, 1997). Of the many environmental factors which can potentially affect pharmacokinetics, the main one for poikilotherms is temperature. Higher temperatures increase the rate at which kinetic processes occur, lower temperatures decrease the rate (Bjorklund and Bylund, 1990; Burka *et al*, 1997).

Salinity also affects pharmacokinetics through its influence on bioavailability (*Elema et al*, 1996; Burka *et al*, 1997).

The widespread use of OTC in aquaculture implies the existence of an extensive database on its therapeutic and chemical properties. This is not the case. The main body of work conducted has been on warm/ temperate, freshwater species such as carp (Grondel *et al* 1987, 1989; Nouws *et al* 1992) or fresh water phase salmon and trout (Grondel *et al*, 1989; Bjorklund and Bylund, 1990, 1991; Black *et al*, 1991; Rogstad *et al*, 1991; Nouws *et al*, 1992; Uno, 1996; Uno *et al*, 1992, 1997). This impacts on mariculture activities in that regimes for cold/ temperate, salt water species are often based on pharmacological assumptions from warm/ temperate, fresh water species. Differences in drug disposition indicate that extrapolation of data among species may be invalid (Ingebrigtsen, 1991; Uno *et al*, 1992). Inappropriate administration of a drug may also lead to problems with environmental buildup (Bjorklund *et al*, 1991; Rogstad *et al*, 1991; Hektoen, 1995) and bacterial resistance (Lunestad *et al*, 1990; Smith *et al*, 1994).

For cod aquaculture it is intended that the liver and gonad, in addition to the fillets, will be marketed. Because there is variability in clearance times for OTC, not only among species but between organ systems as well, the industry must work to ensure that they are in compliance with the legal limit of 0.1 ppm for OTC residuals when setting withdrawal times. This study, precipitated by a lack of published data, represents a preliminary effort to establish a pharmacokinetic database for cod. This data was generated by conducting two experiments.

Experiment 1: Pharmacokinetics of oxytetracycline in Atlantic cod (*Gadus morhua*) following a single dose by oral gavage or intraperitoneal injection.

This experiment was designed to map serum concentrations of OTC over time in order to determine maximum serum concentration (C_{max}), time to maximum concentration (T_{max}), bioavailability (F) and elimination half-life ($T_{½}$). This would allow for the generation of preliminary estimates of clearance time for OTC from the blood and muscle of cod. These numbers were subsequently compared to the results of experiment 2 and with literature values for comparative purposes.

Experiment 2: The absorption, distribution and elimination of oxytetracycline from Atlantic cod (*Gadus morhua*) held in seawater at temperatures of constant 10°C and 10°C decreasing to 2°C.

This experiment was designed with three aims. To: 1) determine whether the absorption, distribution and elimination of oxytetracycline occurs differentially at two temperature regimes. One regime mimicked that of a cod farm between August (high risk of disease at 10°C) and December (harvest at 2°C) and the second served as a controlled comparison (constant seawater temperature of 10°C), 2) identify the affinity of OTC for different organs, and 3) determine the elimination time, for regulatory purposes, of OTC from the serum, muscle, liver and gonad of cod. These goals were achieved by administering OTC to cod in the feed. Blood and organ samples were analyzed and the concentration of OTC mapped over a 101 day period. These numbers were compared to results from Experiment 1 and to literature values for comparative purposes.

This project marks the first time such pharmacokinetic experiments were conducted in a 100% recirculation, saltwater system. A high degree of effort was made to establish and maintain the facility to experimental protocols. In addition, these experiments were conducted to Good Laboratory Practice (GLP) standards. GLP is a set of regulations, enforced by law in the United States, which are designed to ensure the quality and validity of non-clinical laboratory studies of materials intended for market. GLP guidelines deal with the test facility, its personnel, the Standard Operating Procedures (SOPs), the performance of the study, the reporting of study results and the archiving of records and materials. It is anticipated that GLP (or its equivalent) will be implemented in Canada. When that happens, experiments not conducted to GLP specifications will be invalid insofar as regulatory procedures are concerned. This experiment was conducted to GLP standards in order to familiarize all parties concerned with the procedures required and to commence building a database for pharmacokinetics in cold, salt water species which would have a high degree of validity from a regulatory standpoint.

2.0 Materials and Methods

A total of 189 Atlantic cod (*Gadus morhua*), targeted weight of 2 kg, were obtained from Sea Forest Plantation's growout site at Bay Bulls, Newfoundland on January 4, 1995 and land transported to the Atlantic Veterinary College facilities in Prince Edward Island where they arrived on January 5, 1995. Upon arrival the fish were randomly assigned to nine 1.9m^3 fiberglass tanks in either of two 100% recirculation units (Modules 7 and 8). Temperature during transport was 2.1°C and the arrival temperature was 3.0°C in Module 7 and 4.4°C in Module 8. The temperatures of the modules were raised over the course of the holding period until the experimental temperatures of 10°C were reached.

All fish were tagged three weeks after arrival in the facility. The fish were fed moist pellets or chopped herring *ad libitum* over the course of the acclimation period. Dissolved oxygen levels in all tanks were maintained above 8 ppm.

2.1 Pharmacokinetics

Eighteen fish were used in this experiment and had been held for 74 days prior to the start of the experiment. Spawning was in progress and fish were feeding at 1.0% body weight moist pellets/ day on commencement of experiment (Appendix 1). Food was denied for two days prior to the start of the experiment.

The fish were divided equally into two tanks on March 3 (three weeks prior to experiment start) at which time the average weights were 2755g in Tank 2 and 3443 g in Tank 3 (Appendix 2) with stocking densities of 13.1kg/ m^3 and 16.3/ m^3 respectively.

Water temperatures in both tanks were 6.9°C fourteen days prior to commencement, 7.2°C seven days prior to commencement and 10.0°C at commencement of experiment (Appendix 3).

OTC for IP administration at a dosage level of 132.8 mg/ kg fish was prepared by mixing 10.79 grams of OTC-HCl with double distilled H_2O to a total volume of 50 mL. A period of approximately 1.5 hours elapsed from time of solution preparation to injection by which time some precipitation of the solution had occurred.

For orally administered (PO) OTC, a modified Luer Monovette syringe with an outside diameter of 15.3 mm was used for delivery. The feed/ OTC mixture was made up using herring which was thawed and processed into a paste. The paste was premeasured into labeled syringes to the 3/4 mark. The weight of each fish was determined (Appendix 2) and the requisite amount of OTC injected into the syringe. The remaining volume in the syringe was packed with herring paste. Fish were orally gavaged at a dosage level of 134.5 mg OTC/ kg cod (Appendix 4).

Dose levels were to have been 120 mg OTC/ kg fish. A calculation error resulted in the administration of higher doses. As these doses were at clinically acceptable levels and of the same order of magnitude for both groups the numbers were used in subsequent pharmacokinetic calculations. They are not considered to have impacted on the integrity of the experiment.

For intraperitoneally (IP) administrated OTC, Hamilton Microlitre Syringes (calibrated to 0.1 milligram precision) were used. On administration, fish weight was determined

(Appendix 2) and the requisite amount of OTC was aspirated into the syringe. Fish were injected at a dosage level of 132.8 mg OTC/ Kg cod (Appendix 5).

To collect and hold fish for administration of the OTC and blood sampling, a 1" mesh Vexar barrier was placed in the holding tank, dividing it into three compartments of adjustable size. The fish were held in one compartment. Fish were dip-netted and anaesthetized using MS222. Anaesthetized fish were weighed and the weights used to calculate the appropriate dose for the PO and IP groups.

For PO administration, the syringe was prepared with the appropriate amount of OTC sandwiched between two layers of herring paste. The syringe was placed in the oesophagus of the fish, the plunger depressed and dose delivered. Fish were then placed in an oxygenated recovery bath and monitored for signs of regurgitation and stress for 15 minutes. After recovery, the fish were returned to compartment 2 of the holding tank. Nine fish were orally gavaged.

For injected fish, the requisite amount of OTC was aspirated into the syringe and fish injected intraperitoneally on their left, ventral side. These fish were also placed in an oxygenated recovery bath and monitored for signs of stress for 15 minutes. After recovery the fish were returned to compartment 3 of the holding tank. Nine fish were IP injected.

Blood sampling started at hr 0.5 for the IP group and at hr 3 for the PO group. Samples for both groups were taken thereafter at 6, 12, 18, 36 and 72 hrs (Appendix 6). Each fish was sampled for blood twice in order to obtain the requisite data. At the scheduled times

fish were removed from the appropriate tank compartment and anaesthetized in MS222. They were placed, ventral side up, on a damp sponge and 5 mL of blood extracted by venipuncture of the caudal vein. After the 0.5, 3, 6 and 12 hr blood collections, fish were revived in an oxygenated recovery bath and returned to the housing tank. On completion of the 18, 36 and 72 hr blood collections, fish were euthanized by submersion in the MS222 anaesthetic bath until 15 minutes after last signs of opercular beat. Necropsies were performed on all fish.

Blood was collected from the fish using 24G/ 1 ½ " needles with 5cc barrels. The blood was held in 5 ml lithium heparin vacutainer tubes until centrifuged. Lithium heparin is an anticoagulant which normally does not interfere with HPLC analysis. Blood samples were transferred from the syringe into a lithium heparin vacutainer and stored on ice. On completion of each blood collection, samples were centrifuged in a Beckman TJ-6 Centrifuge at 2,700 RPM for 25 minutes at ambient temperature. Plasma was then pipetted from the vacutainer tubes into labeled cryovial tubes and stored at -20°C until analysis.

All plasma samples were analyzed using High Pressure Liquid Chromatography (HPLC) with a method refined for cod serum to a Limit of Detection (LoD) of 0.05 ppm (Appendix 7). The instrumentation used was a Perkin Elmer ISS-100 HPLC system with a Series 410 LC pump, an LC 90 UV Spectrophotometric Detector and an LCI-100 Laboratory Computing Integrator. Spiked serum samples were run to determine the recovery levels of the HPLC procedure (Appendix 8).

Results were recorded and graphed using QuattroProPlus. The data were modeled using a least squares, nonlinear regression package, PCNONLIN version 4, SCI. This package was made available courtesy of Dr. Tor Horsberg, Norwegian School of Veterinary Medicine, Oslo. Modeling was noncompartmental with extravascular administration.

2.2 Temperature

Atlantic cod were held for 133 days in a 100% recirculated saltwater system prior to commencement of the experiment. During this time water temperatures were raised from 3.0°C in Module 7 and 4.4°C in Module 8 to 10.1°C in both modules (Appendix 9). Water quality was monitored continuously and on commencement of experiment O_2 levels were at 9.4 ppm and 11.4 ppm in Modules 7 and 8, respectively, and un-ionized ammonia levels were at 0.01 mg/l.

The fish had completed a spawning event during this period. Organic loading in the recirculation system resulted in poor water quality with a subsequent total mortality of 106 fish out of 171 over the acclimation period. Mortalities had stabilized to zero over the month preceding the experiment.

Sixty five fish were used in this experiment. They were weighed on March 29 (Appendix 10) and a sub-sample of 9 fish was again weighed on May 2 (Appendix 11). An average weight decrease of 2.6% was calculated and this number was used to determine the OTC dosage for the experiment. Ten fish each were allocated to six 1.9m^3 tanks. These were the experimental fish. Five control fish were allocated to a separate 1.9m^3 tank. The stocking densities for the fish ranged from 9.6 kg/ m^3 to 14.9 kg/ m^3.

Of the experimental fish, thirty were maintained at 10°C throughout the experiment (constant temperature group). Water temperatures for thirty experimental fish and 5 control fish were decreased from 10°C, by 0.1°C daily, to 2°C on the final sample day (decreasing temperature group). The water temperature regime of the decreasing

temperature group was calculated from the average daily temperatures at a cod farm site between August and December, 1992 (Appendix 12).

The constant temperature group, decreasing temperature group and control fish were held at 10°C for the 11 day period during which the OTC was administered (Appendix 13).

For delivery of the OTC to all experimental fish, OTC-HCL was injected into the body cavity of whole smelt in quantities calculated to deliver 120 mg OTC/ kg cod. The smelt were stored at -20°C in plastic bags in a freezer and were removed four hours prior to feeding time and thawed. The order of delivery to tanks was random. The water was disturbed by hand and smelt offered slowly to the cod. The fish were closely monitored and non-feeders were gavaged. Due to excessive noise in the tank room on Administration day 5, feeding was cancelled for the day to allow fish to reacclimate. As a result, the 10 day regime of OTC was administered over an 11 day period.

Variations in temperature control resulted in a 0.5°C difference between the two temperature groups at sample day 10 when there should have been a 1°C difference. As this was not considered significant, fish from the two temperature groups were sampled as a common temperature group (10°C) to sample day10. Thereafter they were sampled as two temperature groups: 10°C (constant temperature) and 10°C decreasing to 2°C (decreasing temperature) (Appendix 14). The fish were lethally sampled at hour 12 and on days 5, 10, 20, 41, 61 and 101. Fish to be sampled were selected randomly from within the sample groups using Minitab generated numbers. On the final sample day the

weights were recorded for the 10°C decreasing to 2°C group, 10°C group and the control (Appendix 10).

Sampled fish were placed in an anaesthetic bath of MS222 and left until moderate anesthesia was achieved. Blood was taken via caudal venipuncture using 5 mL syringes with 24G/ 1 ½" needles. The blood was held until centrifuged in 5 mL lithium heparin Vacutainer®. Centrifugation was done in a Beckman TJ-6 centrifuge at 2,700 RPM for 25 minutes at ambient temperature. Plasma was then pipetted from the vacutainer tubes into labeled cryovial tubes and stored at -20°C until analysis.

Fish were then euthanized by submersion in the anaesthetic bath until 15 minutes after the last signs of opercular beat, transferred to a laboratory and dissected using aseptic technique. Samples of liver, gonad and muscle were taken with double sets of tissue placed individually in bags marked with the date, sample type, fish number and destination. All samples were quick frozen and stored at -20°C in a controlled access freezer. One tissue set was shipped to RPC Laboratory Inc., Fredericton, New Brunswick. On shipment day, organ samples were packed in insulated, heavy duty styrofoam containers with temperatures maintained below freezing using medigrade ice packs. Shipment was via air courier with transit time maintained below four hours. The second tissue set was stored at the Atlantic Veterinary College.

HPLC analysis was conducted by RPC Laboratory on serum [Limit of Detection (LoD) 0.5 ppm], muscle (LoD 0.05 ppm), liver (LoD 3 ppm) and gonad (LoD 3 ppm). Results from the analyses were stored on QuattroProPlus. Data for the four tissue types were

grouped by sample day and temperature regime. Group means, standard errors and standard deviations were calculated. OTC concentrations were plotted versus time for the different tissue types.

The Box Cox procedure was run to examine the shape of the observed data distribution and to identify any departures from normality. A two factor analysis of variance was run with sample day and temperature as the main effects. Significance was set at $p=0.05$. If significant differences were found a Scheffes multiple comparison (post hoc, two way, pair wise $p=0.05$) was run to determine where these differences lay.

3.0 Results

3.1 Pharmacokinetics

On March 22 (initiation of experiment) the average weight of PO fish was 2445 g and IP fish was 3012 g. This represents an average weight loss of 310 g for PO fish and 431 g for IP fish from the March 3 weights of 2755 g and 3443 g respectively (Appendix 2). The PO fish received, on average, 134.5 mg OTC/ kg fish (Appendix 4) while the IP fish received an average dose of 132.8 mg OTC/ kg fish (Appendix 5).

Examination of the data revealed an outlier in the PO treatment group at hours 3 and 18 (17 ppm and 6.3 ppm respectively (Table 1). As these numbers were excessively and inexplicably high relative to the others they were not used in the statistical analysis. It should be noted that these outliers were from the same fish sampled at two time periods and this was the first fish gavaged. One fish also died in the PO group after the first sample period. The average serum concentrations for this group show an initial rise to 0.76 ppm at hour 3 with a slight rise to 1.04 ppm at hour 6. After this time the numbers level out with 0.67, 0.61, 0.49 and 0.68 ppm OTC at hours 12, 18, 36 and 72 respectively (Table 2, Figure 1).

One fish died in the IP group after the first sample period. The average serum concentrations rise initially to 67 ppm at hour 0.5 and decline to 44.8 ppm then 51.2 ppm at hours 6 and 12 respectively (Table 2, Figure 2). A maximum concentration of 140.8 ppm is reached at hour 18. This falls to 64.9 ppm at hour 36 and rises again to 88.6 ppm at hour 72.

Necropsies on all sampled fish revealed high levels of mucous in the gastrointestinal tract.

The OTC recoveries on spiked serum samples using the HPLC method were consistently high, ranging from 83 % to 108% (Appendix 8).

Modeling using PCNONLIN gave the following results: T_{max} for IP and PO administration was at 18hrs and 6 hrs respectively. C_{max} for IP and PO was 140.8 ppm and 1.04 ppm respectively. AUC for the 72 hour time period for IP and PO was 5802.5 hr g/ml and 43.8 hr g/ml. MRT (0-72 hrs) was 36.8 hr and 35.9 hr respectively (Table 3). The relative bioavailability of OTC was calculated to be 0.8%. T ½ for IP administration was calculated to be 154.2 hours. T ½ for PO administration was calculated to be 184 hours.

These numbers were compared to literature values generated from other pharmacokinetic experiments. A synopsis of pharmacokinetic numbers found in the literature is given in Table 4.

Table 1: Oxytetracycline Levels (ppm) by Sample Period (hrs) in the Serum Of Individual Fish for Intraperitoneal and Per Os Administration

Date	Sample Period (hrs)	Administration	Serum OTC Levels (ppm)
March 22, 1995	0.5	Intraperitoneal	64.3
	0.5	Intraperitoneal	71.4
	0.5	Intraperitoneal	65.4
	3	Per Os	0.67
	3	Per Os	0.85
	3	Per Os	17
	6	Per Os	0.79
	6	Per Os	1.46
	6	Per Os	0.87
	6	Intraperitoneal	46.1
	6	Intraperitoneal	18.3
	6	Intraperitoneal	70
March 23, 1995	12	Per Os	0.49
	12	Per Os	0.78
	12	Per Os	0.73
	12	Intraperitoneal	4.21
	12	Intraperitoneal	15.3
	12	Intraperitoneal	87
	18	Per Os	0.62
	18	Per Os	0.59
	18	Per Os	6.34
	18	Intraperitoneal	88.5
	18	Intraperitoneal	170
	18	Intraperitoneal	164
March 24, 1995	36	Per Os	0.61
	36	Per Os	0.37
	36	Per Os	
	36	Intraperitoneal	18.6
	36	Intraperitoneal	53.1
	36	Intraperitoneal	123
March 25, 1995	72	Per Os	0.47
	72	Per Os	0.87
	72	Per Os	0.70
	72	Intraperitoneal	105
	72	Intraperitoneal	72.1
	72	Intraperitoneal	

Table 2: Average Oxytetracycline Concentrations (ppm) in the Serum of Fish, Grouped by Sample Period (hrs), for IP and PO Administration.

Sample Interval (hrs)	Average Serum Concentrations (ppm)	
	IP	PO
0.5	67.0	
3		0.76
6	44.8	1.04
12	51.2	0.67
18	140.8	0.61
36	64.9	0.49
72	88.6	0.68

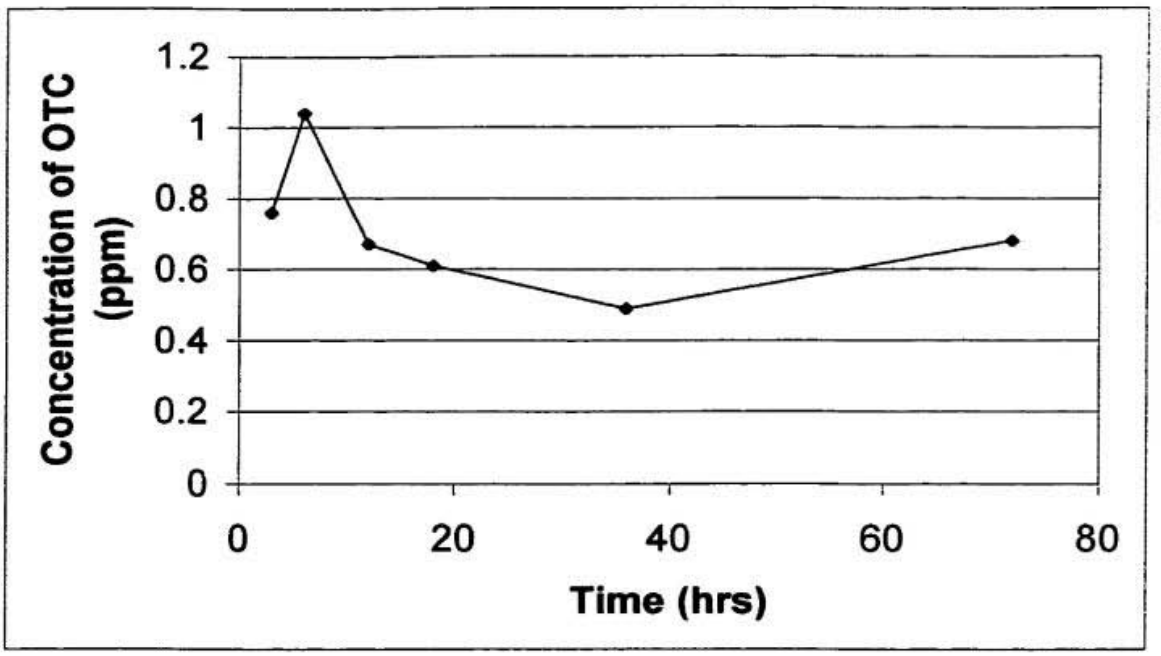

Figure 1: Average Oxytetracycline Concentrations (ppm) in the Serum of Fish (n=3), Grouped by Sample Period (hrs), for Per Os Administration over time.

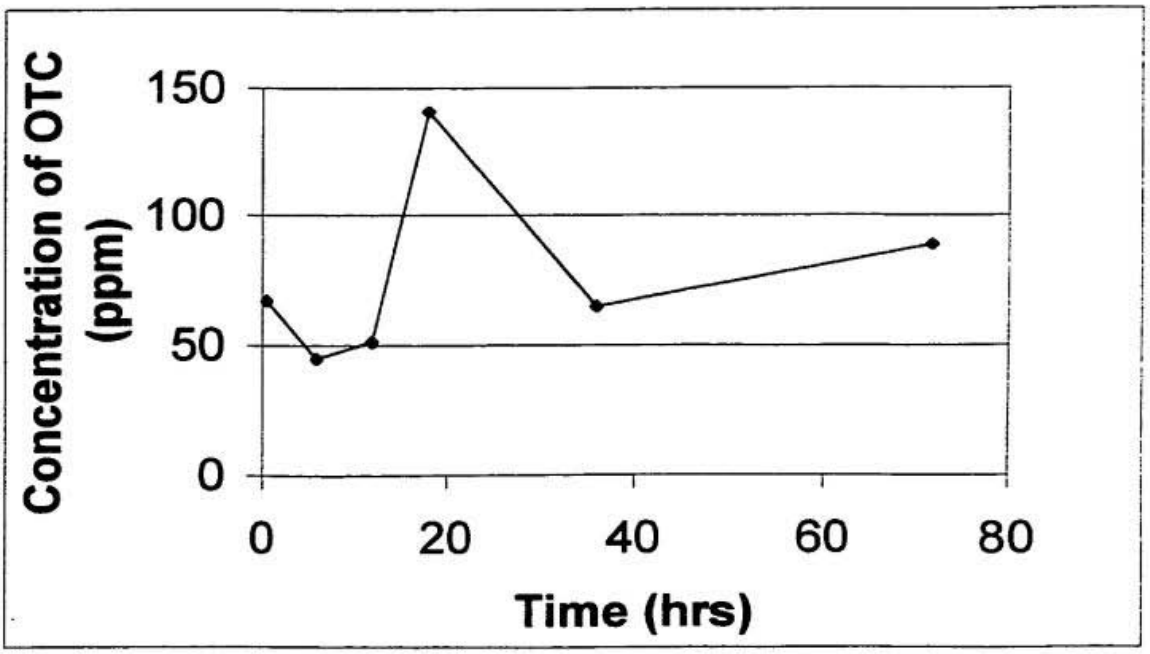

Figure 2 : Average Oxytetracycline Concentrations (ppm) in the Serum of Fish (n=3), Grouped by Sample Period (hrs), for Intraperitoneal Administration over time

Table 3: Pharmacokinetic Parameters for Per Os and Intraperitoneal Administration of OTC in Cod

	Average Values		Maximum Values		Minimum Values	
	Intra peritoneal	Per Os	Intra peritoneal	Per Os	Intra peritoneal	Per Os
*Tmax	18 hr	6 hr	18 hr	6 hr	18 hr	6 hr
*Cmax	140.8 ug/ml	1.04 ug/ml	170 ug/ml	1.46 ug/ml	88.5 ug/ml	0.79 ug/ml
*AUC (0-72 hr)	5802.5 hr•ug/ml	43.77 hr•ug/ml	8389.7 hr•ug/ml	53.37 hr•ug/ml	3251.9 hr•ug/ml	34.035 hr•ug/ml
AUMC (0-72 hr)	213653.7 hr^2•ug/ml	1572.48 hr^2•ug/ml	301142.1 hr^2•ug/ml	1957.59 hr^2•ug/ml	132466.2 hr^2•ug/ml	1158.84 hr^2•ug/ml
*MRT (0-72 hr)	36.8 hr	35.9 hr	35.9 hr	36.7 hr	40.7 hr	34.0 hr
*$T_{1/2}$ (hr)	154.2	184				
Relative Bioavailability		0.8%				

Tmax — Time to maximum concentration.
Cmax — Maximum serum concentration (ppm)
AUC — Area under the curve
AUMC — Area under the concentration-time curve (first moment)
MRT — Mean retention time
$T_{½}$ — Halflife of elimination

Table 4: Summary of Published Pharmacokinetic Parameters for Oxytetracycline for Different Fish Species Held under Varying Environmental Conditions.

Species	Researcher	Year	Salinity	Temp.	Route	Dose	Tmax	Cmax	$T_{1/2}$	Eliminaton	F
			(ppt)	(celsius)		(mg/ kg)	(hr)	(ppm)	(hr)	(days)	
Atlantic salmon	Bjorklund & Bylund	1990	Fresh water	13-17	Per Os	100/ 10 D			127	37	
Rainbow trout	Bjorklund & Bylund	1990	Fresh water	17-20	Per Os	100/ 10 D			40.8	9	
Rainbow trout	Bjorklund & Bylund	1990	Fresh water	5	Per Os	75	24	3.2	213	72	
Rainbow trout	Bjorklund & Bylund	1990	Fresh water	10	Per Os	75	12	5.3	146	41	
Rainbow trout	Bjorklund & Bylund	1990	Fresh water	16	Per Os	75	1	2.1	115.2	27	
Rainbow trout	Bjorklund & Bylund	1991	Fresh water	16	Intravenous	20	0	113	60.3		
Rainbow trout	Bjorklund & Bylund	1991	Fresh water	16	Per Os	75	12	2	74.9		5.60%
Rainbow trout	Black *et al*	1991		10	Intravenous	5	0.5	18.8	81.5		
Atlantic salmon	Elema *et al*	1996	Salt water	7	Intravenous	20			50.4		
Atlantic salmon	Elema *et al*	1996	Salt water	7	Per Os	50	12	0.42			1.94%
Carp	Grondel *et al*	1987	Fresh water	20	Intravenous	60	1	247.5	139.8		
Carp	Grondel *et al*	1987	Fresh water	20	Intra muscular	60	14	56.8	78.6		80%
Carp	Grondel *et al*	1987	Fresh water	20	Per Os	60	20	0.11			0.60%
African catfish	Grondel *et al*	1989	Fresh water	25	Intra muscular	60	7	43.4	74.4		85%

Species	Researcher	Year	Salinity	Temp.	Route	Dose	Tmax	Cmax	$T_{1/2}$	Eliminaton	F
			(ppt)	(celsius)		(mg/ kg)	(hr)	(ppm)	(hr)	(days)	
Rainbow trout	Grondel *et al*	1989	Fresh water	12	Intra muscular	60	4	56.9	94.7		80%
African catfish	Grondel *et al*	1989	Fresh water	25	Intravenous	60	0	86?	80.3		
Rainbow trout	Grondel *et al*	1989	Fresh water	12	Intravenous	60	0	753?	89.5		
Atlantic salmon	McSwain (MSc)	1992	Salt water	15	Intra arterial	100	3	31.5	58.3	85.8 ml/ hr	
Atlantic salmon	McSwain (MSc)	1992	Salt water	10	Intra arterial	100	3	39.9	62.2	80.5 ml/ hr	
Atlantic salmon	McSwain (MSc)	1992	Salt water	10	Per Os	100	3	0.22	72.5	5522 ml/ hr	1.50%
Atlantic salmon	McSwain (MSc)	1992	Salt water	15	Per Os	100	3	0.77	171.6	933.4 ml/ hr	7.40%
Atlantic salmon	McSwain *et al*	1992	Salt water	15	Intra arterial	100	3	31.5	12		
Atlantic salmon	McSwain *et al*	1992	Salt water	15	Per Os	100					
Carp	Nouws *et al*	1992	Fresh water	20	Intra muscular	60	14	57	79		
Carp	Nouws *et al*	1992	Fresh water	8	Intra muscular	60	8	48	157		
Eel	Nouws *et al*	1992	Fresh water	22	Intra muscular	60	16	100	196		
Rainbow trout	Nouws *et al*	1992	Fresh water	10	Intra muscular	60	10	35	150		
Carp	Nouws *et al*	1992	Fresh water		Intra peritoneal	60	5	65	36		
Carp	Nouws *et al*	1992	Fresh water	20	Intravenous	60		>100	52		

Species	Researcher	Year	Salinity	Temp.	Route	Dose	Tmax	Cmax	$T_{1/2}$	Eliminaton	F
			(ppt)	(celsius)		(mg/ kg)	(hr)	(ppm)	(hr)	(days)	
Carp	Nouws *et al*	1992	Fresh water	8	Intravenous	60		>100	169		
Rainbow trout	Nouws *et al*	1992	Fresh water	19	Intravenous	60		>100	76		
Rainbow trout	Nouws *et al*	1992	Fresh water	10	Intravenous	60		>100	130		
Rainbow trout	Nouws *et al*	1992	Fresh water	10	Per Os	60	48	0.35	50		1.25%
Carp	Nouws *et al*	1992	Fresh water	20	Per Os	60	24	0.46	56		0.38%
Rainbow trout	Rogstad *et al*	1991	Fresh water	7	Per Os / capsule	150	72	1.7	278.4	>14	2.60%
P. altivelis (Ayu)	Uno	1996	Fresh water	18	Per Os / capsule	100		34.2	53.1		9.80%
P. altivelis (Ayu)	Uno	1996	Fresh water	18	Per Os / capsule	100		2.17	63.2		3.80%
P. altivelis (Ayu)	Uno	1996	Fresh water	18	Intravenous	25	0	122	52.1		
Rainbow trout	Uno *et al*	1997	Fresh water	15	Intravenous	50	0	331	52		
Amago salmon	Uno *et al*	1992	Fresh water	15	Per Os	100	24	2.05	16		
Rainbow trout	Uno *et al*	1992	Fresh water	15	Per Os	100	9	1.14	23.2		0.60%
Yellowtail	Uno *et al*	1992	Salt water	22	Per Os	100	3	0.89	28		
Gadus morhua	Vatcher/ Rainnie	1995	Salt water	10	Intra peritoneal	132.8	18	140.8	154.2		0.80%
Gadus morhua	Vatcher/ Rainnie	1995	Salt water	10	Per Os	134.5	6	1.04	184	54	

Species	Researcher	Year	Salinity	Temp.	Route	Dose	Tmax	Cmax	$T_{1/2}$	Eliminaton	F
			(ppt)	(celsius)		(mg/ kg)	(hr)	(ppm)	(hr)	(days)	
Salvelinus alpinus	Haug	1994		5.7	Intravenous	10		4.02	220.6		
Salvelinus alpinus	Haug	1994		6.7	Per Os / Agar	50	29	1.07	313		3.09
Salvelinus alpinus	Haug	1994		5.5	Per Os / Agar	100	30	3.9	336		8.01
Salvelinus alpinus	Haug	1994		1.1	Per Os / Capsule	10	97	0.34	536.7		
Salvelinus alpinus	Haug	1994		6.3	Per Os / capsule	100	136	0.97	321.9		2.35

Tmax — Time to maximum concentration.
Cmax — Maximum serum concentration (ppm)
$T_{½}$ — Halflife of elimination
F — Bioavailability

3.2 Temperature

Gonadal concentrations of OTC in the experimental fish were at 1.0 ppm at Hour 12 (Table 5). There were no detectable levels of OTC at days 5, 10, 20, 41, 61 or 101.

Serum concentrations of OTC in the experimental groups rose from 0.8 ppm (standard error 0.2 ppm) at hour 12 to a maximum concentration of 1.3 ppm (standard error 0.2 ppm) at day 5 (Table 5). At day 10 OTC levels were at 1.2 ppm (standard error 0.3 ppm). After this point the groups were sampled as two temperature effects. Levels in the constant temperature group were at 0 ppm on day 20, 41, 61 and 101. The decreasing temperature group had 0.1 ppm (standard error 0.1 ppm) on day 20 and 0 ppm on day 41, 61 and 101. The average serum concentrations of OTC in the experimental groups by sample day show a T_{max} at day 5 with a C_{max} of 1.3 ppm (Table 6; Figure 3).

Muscle concentrations of OTC rose steadily from 0.43 ppm (standard error 0.16 ppm) at hour 12, 0.51 ppm (standard error 0.13 ppm) on day 5 and peaked at 0.76 ppm (standard error 0.23 ppm) on sample day 10 (Table 5). After this point the groups were sampled as two temperature effects. The constant temperature group was at 0.14 ppm (standard error 0.05 ppm) , 0.02 ppm (standard error 0.01 ppm), 0 ppm and 0 ppm on sample days 20, 41, 61 and 101 respectively. The decreasing temperature group was at 0.18 ppm (standard error 0.07 ppm), 0.08 ppm (standard error 0.05 ppm), 0 ppm and 0 ppm on sample days 20, 41, 61 and 101 respectively. The average muscle concentrations of OTC in the experimental groups by sample day show a T_{max} at day 10 with a C_{max} of 0.76 ppm (Table 6, Figure 4).

Liver OTC concentrations rose steadily from 7 ppm (standard error 2 ppm) at hour 12 to 15 ppm (standard error 0.7 ppm) at day 5 to a maximum concentration of 42 ppm (standard error 6 ppm) at day 10 (Table 5). After this point the groups were sampled as two temperature effects. The constant temperature group was at 28 ppm (standard error 5 ppm), 27 ppm (standard error 8 ppm), 2 ppm (standard error 1 ppm) and 0 ppm on sample day 20, 41, 61 and 101 respectively. The decreasing temperature group was at 17 ppm (standard error 8 ppm), 17 ppm (standard error 3 ppm), 0 ppm and 1 ppm (standard error 1 ppm) on sample days 20, 41, 61 and 101 respectively. The average liver concentrations of OTC in the experimental groups by day show a T_{max} at day 10 with a C_{max} of 42 ppm (Table 6; Figure 5).

One control fish died during the experiment. Liver samples in one control fish at day 5 and one control fish at day 10 had residuals of OTC (Table 5).

Analysis of the observed data distribution patterns from the Box Cox procedure indicated that a Gomperts Transformation (1/ SQRT(Log x+1.5) was required to normalize the data. The two factor (sample day and temperature) analysis of variance showed no significant differences in OTC concentrations between the temperature groups for serum (Table 7), muscle (Table 8) or liver (Table 9). There were significant differences in OTC concentration by sample day in the serum (Table 7), muscle (Table 8) and liver (Table 9). A Scheffes multiple comparison determined that in serum (Table 10) and muscle (Table 11) significant differences lay were between OTC levels up to and including day 10 and OTC levels on and after day 20. In liver (Table 12) significant differences were between OTC levels up to and including day 41 and OTC levels on and after day 61.

Table 5: Concentrations of Oxytetracycline (ppm), Grouped by Sample Day and Temperature Regime, in the Serum, Muscle, Liver and Gonad of Individual Fish With Mean, Standard Error and Standard Deviation.

Sample Day	Temperature Group	Fish Number	OCT Concentration (ppm)			
			Serum	Muscle	Liver	Gonad
1	Common	1	0	0.17	5	0
1	Common	2	0.5	0.21	0	0
1	Common	3	1.4	1.24	7	3
1	Common	4	1	0.18	15	0
1	Common	5	0.9	0.26	13	0
1	Common	6	1.2	0.52	4	0
Mean			0.8	0.43	7	1
St. Deviation			0.5	0.38	5	1
St. Error			0.2	0.16	2	0
5	Control	7	0	0	8	0
5	Common	8	1.1	0.52	11	0
5	Common	9	2	0	4	0
5	Common	10	1.3	0.89	8	0
5	Common	11	1.5	0.55	5	0
5	Common	12	0.7	0.61	45	0
Mean			1.3	0.51	15	
St. Deviation			0.4	0.29	15	
St. Error			0.2	0.13	7	
10	Control	13	0.5	0	18	0
10	Common	14	2.3	0.25	38	0
10	Common	15	0.5	1.28	63	0
10	Common	16	1.5	0.64	34	0
10	Common	17	1	0.21	51	0
10	Common	18	0.8	1.41	25	0
Average			1.2	0.76	42	
St. Deviation			0.6	0.5	13	
St. Error			0.3	0.23	6	
20	10°C	19	0	0.06	13	0
20	10°C	20	0	0.32	29	0
20	10°C	21	0	0.11		0
20	10°C	22	0	0.19		0
20	10°C	23	0	0	42	0
Mean				0.14	28	
St. Deviation				0.11	12	
St. Error				0.05	5	
20	2°C	24	0.5	0.49	14	0
20	2°C	25	0	0.08	3	0
20	2°C	26	0	0.14		0
20	2°C	27	0	0.12	5	0
20	2°C	28	0	0.08	46	0
Mean			0.1	0.18	17	
St. Deviation			0.2	0.16	17	
St. Error			0.1	0.07	8	
41	10°C	29	0	0	31	0

Sample Day	Temperature Group	Fish Number	OCT Concentration (ppm)			
			Serum	Muscle	Liver	Gonad
41	10°C	30	0	0.09	14	0
41	10°C	31	0	0.05	26	0
41	10°C	32	0	0	19	0
41	10°C	33	0	0	7	0
41	10°C	34	0	0	65	0
Mean				0.02	27	
St. Deviation				0.03	19	
St. Error				0.01	8	
41	2°C	35	0	0.13	23	0
41	2°C	36	0	0.33	12	0
41	2°C	37	0	0	6	0
41	2°C	38	0	0	26	0
41	2°C	39	0	0	17	0
41	2°C	40	0	0	18	0
Mean				0.08	17	
St. Deviation				0.12	7	
St. Error				0.05	3	
61	10°C	41	0	0	5	0
61	10°C	42	0	0	4	0
61	10°C	43	0	0	0	0
61	10°C	44	0	0	0	0
61	10°C	45	0	0	0	0
Mean					2	
St. Deviation					2	
St. Error					1	
61	2°C	46	0	0	0	0
61	2°C	47	0	0	0	0
61	2°C	48	0	0	0	0
61	2°C	49	0	0	0	0
61	2°C	50	0	0	0	0
61	Control	51	0	0	0	0
Mean						
St. Deviation						
St. Error						
101	10°C	52	0	0	0	0
101	10°C	53	0	0	0	0
101	10°C	54	0	0	0	0
101	10°C	55	0	0	0	0
101	10°C	56	0	0	0	0
101	10°C	57	0	0	0	0
Mean						
St. Deviation						
St. Error						
101	2°C	58	0	0	0	0
101	2°C	59	0	0	3	0
101	2°C	60	0	0	3	0
101	2°C	61	0	0	0	0
101	2°C	62	0	0	0	0

Sample Day	Temperature Group	Fish Number	OCT Concentration (ppm)			
			Serum	Muscle	Liver	Gonad
101	2°C	63	0	0	0	0
101	Control	64	0	0	0	0
Mean					1	
St. Deviation					1	
St. Error					1	

Table 6: Average Serum Concentrations of Oxytetracycline (ppm), Grouped by Temperature Regime and Sample Day, in the Serum, Muscle, Liver and Gonad of Fish

Sample Day	Temperature Group	Oxytetracycline Concentration (ppm)			
		Serum	Muscle	Liver	Gonad
1	Common	0.8	0.43	7	1
5	Common	1.3	0.51	15	0
10	Common	1.2	0.76	42	0
20	Ten Decreasing to Two	0 0.1	0.14 0.18	28 17	0 0
41	Ten Decreasing to Two	0 0	0.02 0.08	27 17	0 0
61	Ten Decreasing to Two	0 0	0 0	2 0	0 0
101	Ten Decreasing to Two	0 0	0 0	0 1	0 0
LoD		0.5	0.05	3	3

Table 7: Results of the two factor ANOVA testing the effect of Day, Temperature, and Interaction on the Concentration of Oxytetracycline (ppm) in the Serum of Fish.

Two Factor Analysis of Variance (P<0.05)				
Effect	DF*	F Value	P Value	Significance*
Temperature	1	0.161	0.6892	NS
Day	6	60.88	0.0001	S
Temperature x Day	6	0.152	0.9880	NS
Residual	62			
DF* — Degree of Freedom. Significance* — "S" denotes a statistically significant result.				

Table 8: Results of the two factor ANOVA testing the effect of Day, Temperature, and Interaction on the Concentration of Oxytetracycline (ppm) in the Muscle of Fish.

Two Factor Analysis of Variance (P<0.05)				
Effect	**DF***	**F Value**	**P Value**	**Significance***
Temperature	1	0.135	0.7150	NS
Day	6	18.735	0.0001	S
Temperature x Day	6	0.060	0.9991	NS
Residual	62			
DF* — Degree of Freedom. Significance* — "S" denotes a statistically significant result.				

Table 9: Results of the two factor ANOVA testing the effect of Day, Temperature, and Interaction on the Concentration of Oxytetracycline (ppm) in the Liver of Fish.

Two Factor Analysis of Variance (P<0.05)				
Effect	**DF***	**F Value**	**P Value**	**Significance***
Temperature	1	0.0004	0.9838	NS
Day	6	19.372	0.0001	S
Temperature x Day	6	1.012	0.4261	NS
Residual	62			
DF* — Degree of Freedom. Significance* — "S" denotes a statistically significant result.				

Table 10: Scheffe's pair-wise comparisons for Effect of Day (P<0.05) on the Concentration of Oxytetracycline (ppm) in the Serum of Fish

Day	**1**	**5**	**10**	**20**	**41**	**61**
5	NS					
10	NS	NS				
20	S	S	S			
41	S	S	S	NS		
61	S	S	S	NS	NS	
101	S	S	S	NS	NS	NS
Significance* — "S" denotes a statistically significant result.						

Table 11: Scheffe's pair-wise comparisons for Effect of Day (P<0.05) on the Concentration of Oxytetracycline (ppm) in the Muscle of Fish.

Day	1	5	10	20	41	61
5	NS					
10	NS	NS				
20	NS	NS	S			
41	S	S	S	NS		
61	S	S	S	NS	NS	
101	S	S	S	NS	NS	NS
Significance* — "S" denotes a statistically significant result.						

Table 12: Scheffe's pair-wise comparisons for Effect of Day (P<0.05) on the Concentration of Oxytetracycline (ppm) in the Liver of Fish.

Day	1	5	10	20	41	61
5	NS					
10	NS	NS				
20	NS	NS	NS			
41	NS	NS	NS	NS		
61	S	S	S	S	S	
101	S	S	S	S	S	NS
Significance* — "S" denotes a statistically significant result.						

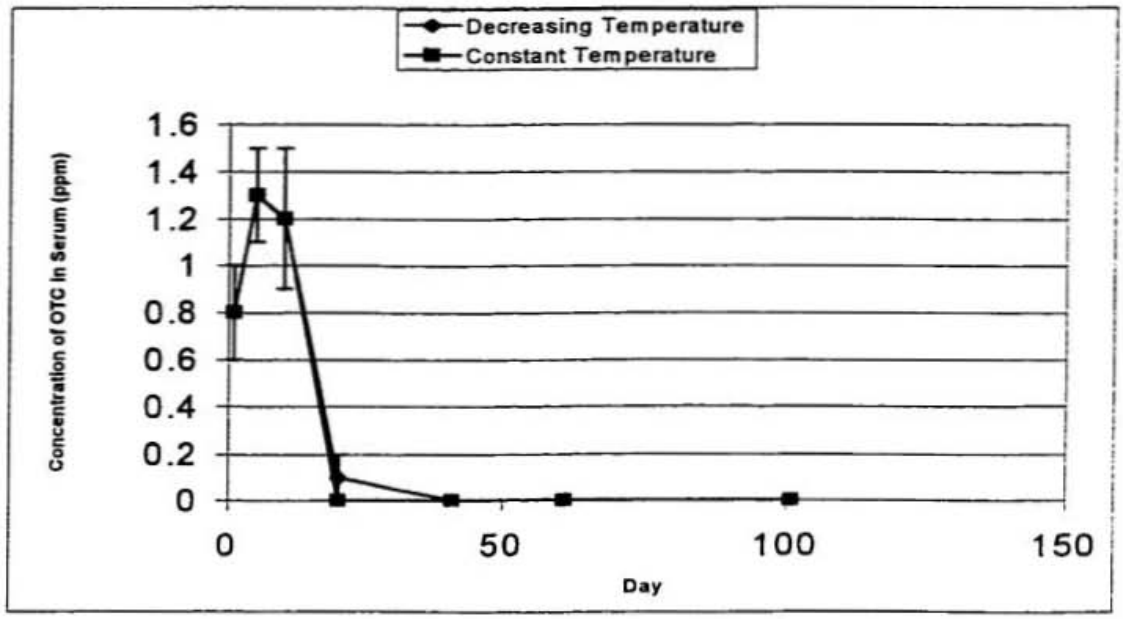

Figure 3: Average Oxytetracycline Concentration (ppm), with Standard Error Bars, in the Serum of Fish over Time (Days)

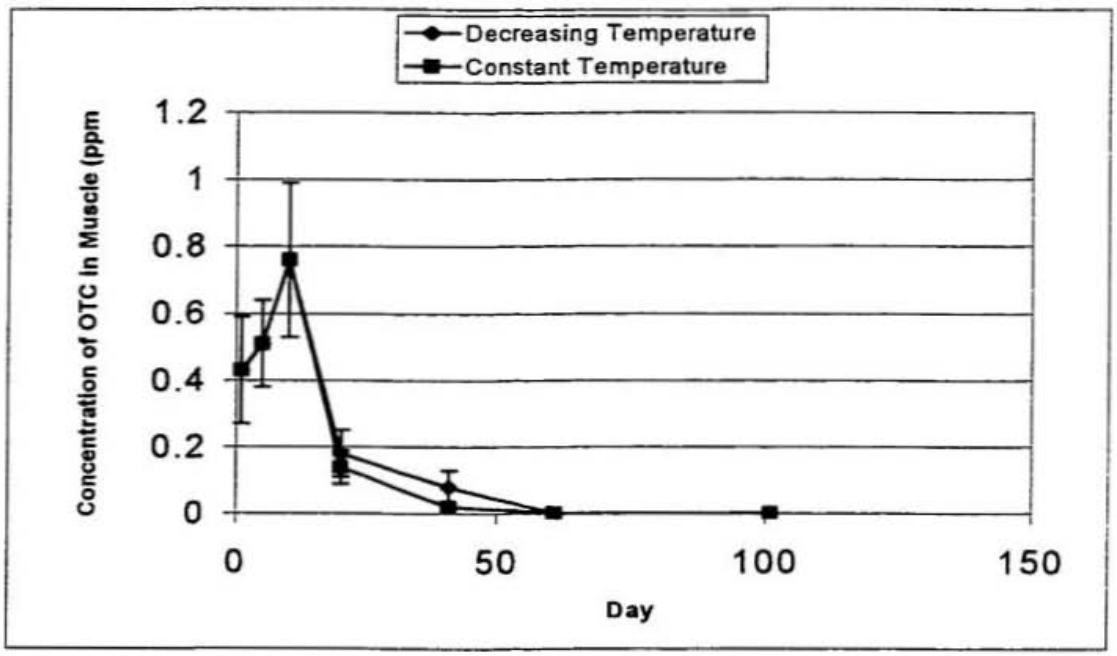

Figure 4: Average Oxytetracycline Concentrations (ppm), with Standard Error Bars, in the Muscle of Fish Over Time (Days)

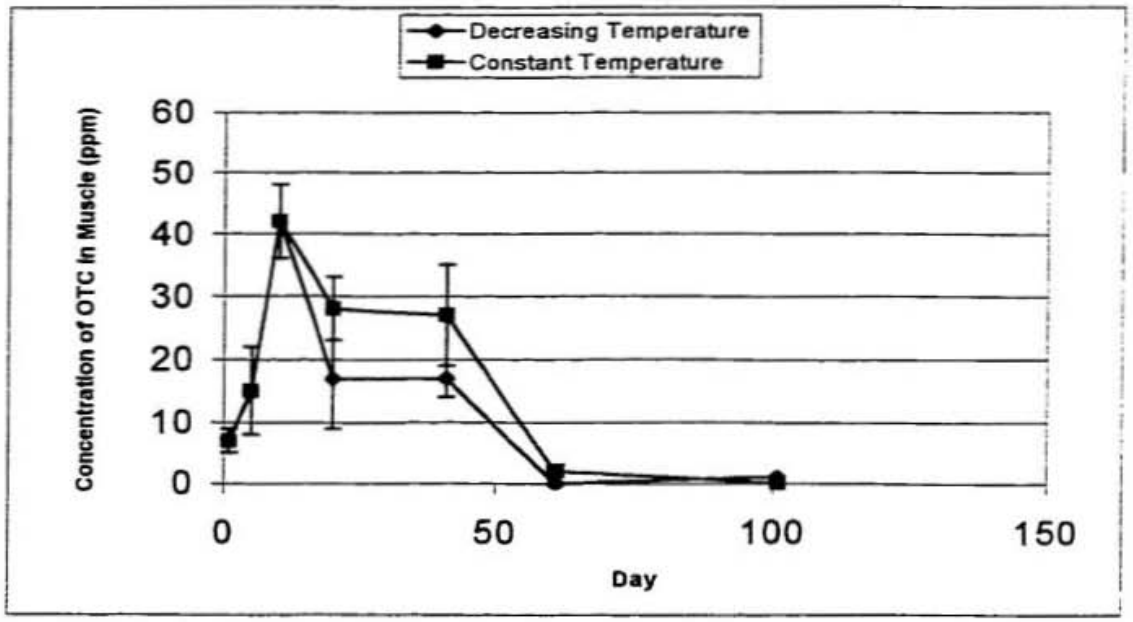

Figure 5: Average Oxytetracycline Concentrations (ppm), with Standard Error Bars, in the Liver of Fish over Time (Days).

4.0 Discussion

4.1 Pharmacokinetics

The graphed data from PO administration of OTC reveal a curve with a rise in concentration to 1.04 ppm OTC at hour 6 and a steady decline to 0.49 ppm at hour 36. The slight rise in the terminal portion of the curve occurred after the anticipated point of absorption of OTC in the serum. PO serum curves often manifest as a small peak with a slow but steady decline to negligible residues (Grondel *et al*, 1987; Nouws *et al* 1992; Uno, 1996). Dissections of the cod following the final samplings revealed that the herring paste was partially digested at the 36 hour sample period and was fully digested by the 72 hour sample period. This indicates that digestion was occurring within the normal range of 24-48 hrs at 10°C (Ellis *et al*, 1989). There were, however, large quantities of gastrointestinal (GI) mucous. GI mucous has been noted in non-feeding and newly feeding farmed cod (Brian Blanchard, Pers. Comm.). Feeding in wild and farmed cod is greatly reduced during the winter and throughout the spring spawning event. The cod used in this experiment were transported in January at 2.1°C and acclimated to 10°C over a 74 day holding period. At the start of the experiment the cod had just completed spawning and were still at low feeding levels (1.0% body weight/ day moist feed as opposed to the normal daily intake of 5% body weight/ day). The presence of GI mucous was, therefore, not considered unusual however the mucous may have interfered with the efficiency of absorption of OTC across the gut wall. Ingebrigtsen *et al* (1985) fed radiolabeled tetracycline to fish and then followed its flow through the organ systems. They found that at day 2 there had been negligible absorption of the OTC; most of it

resided in the mucosal lining of the gastrointestinal tract. This may result in a prolonged absorption phase of OTC to systemic circulation and explain why the OTC in this experiment was still being absorbed past the final sample period.

The graphed data from IP administration reveal a curve with a double peak at hours 0.5 and 18 (at levels of 67 ppm and 140.8 ppm respectively). Although the concentration of OTC declined rapidly to 64.9 ppm at hour 36, there was a slight rise in the terminal portion of the curve to the final sample point. This differs from published data in that most absorption curves (intravenous, intraperitoneal or intramuscular) are manifested as a single peak immediately after injection with a steady decline to negligible residuals (Grondel *et al*, 1987, McCracken *et al*, 1975, Uno, 1996). In this experiment, the first peak occurs at 30 minutes after injection, which is in line with published figures. The second peak at 18 hours is an anomaly and may be the result of precipitation of OTC in the injected solution. OTC which remained in solution was absorbed immediately; precipitated OTC was not absorbed until it dissolved. The presence of precipitous material in the viscera of dissected fish at 72 hours supports this theory. This may also have contributed to the rise in the curve to the final sample period.

A physiological contribution to the pattern of absorption (and therefore the shape of the curve) for both types of administration may have been enterohepatic circulation. This describes the process whereby a compound, such as OTC, is recirculated between the gastrointestinal tract and the liver. OTC is transported in blood via the hepatic portal system from the intestine (for orally administered drugs) or from systemic circulation via the coeliac artery (for IV, IM or IP administered drugs) to the liver. In the liver it is

metabolized or biotransformed, actively transported to the bile and then excreted via the biliary route into the intestinal lumen. It can then be excreted from the body via the faeces or reabsorbed back into the liver via the hepatic portal system. This creates a loop which allows for a portion of the compound to be reabsorbed and a portion to be eliminated from the body with each cycle. The rate of reduction of the compound would be dependent on hepatobiliary function (Ingebrigtsen, 1985; Bruno, 1989; Ellis, 1989; McSwain, 1992; Burka *et al*, 1997). Secondary peaks for PO data at 15°C support the presence of an enterohepatic cycle (McSwain, 1992). The cod used in this experiment had just completed spawning and were in transition from a nonfeeding phase where energy was being derived from the liver, to a feeding phase where a portion of the energy was being directed to production of the liver. Long periods of food deprivation may induce general changes in hepatobiliary function (McSwain, 1992). Strasdine and McBride (1979) found evidence of differential absorption between fasting (sexually mature) and non-fasting (immature) salmon. The effect of hepatic metabolism in species, such as cod, which undergo marked seasonal variations in feeding and use the liver as an organ of storage, might impact significantly on the pharmacokinetics of a drug and bear further investigation.

There are two methods available for modeling pharmacokinetic data: compartmental and non-compartmental. In the classic compartmental approach the body is viewed as one, two or three compartments with blood and highly perfused organs being the central compartment. Drug transport between these abstract compartments occurs with concentration differences acting as the driving force. The most frequently used model is

the two compartment model which dictates that the plasma concentration curve satisfy a bi-exponential equation. The model parameters are clearance, volume and flow. Rate constants govern transport between compartments. The majority of pharmacokinetic work in fish is modeled this way however the theoretical limitations of a model which reduces the body to two compartments must be recognized. As a result of these limitations, classical pharmacokinetic models are designed to render conservative estimates for the elimination of drugs. This provides a wide margin of safety for the consumer but results in extended holding periods for farmers with recently treated market fish. Bjorklund and Bylund (1990) predicted elimination times of 72, 41 and 27 days for OTC from Atlantic salmon held at 5°C, 10°C and 16°C respectively using the classical approach. Actual elimination times were 40, 30 and 20 days. This represents an overestimation of 27% to 45% in elimination time.

In non-compartmental pharmacokinetics the body is seen as an arrangement of organs and subsystems through which blood flows and behaves as a system controlled by positive feedback. The important parameters are the mean body transit time, cardiac output, extraction ratio, clearance, number of recirculations, mean residence time and volume of distribution. Assumptions made about the static nature of the body system are greatly reduced in non-compartmental analysis. This mathematical acknowledgement of the complex nature of the body system requires an increase in the mathematical complexity of the computer package with fewer associated assumptions. It also represents a move towards increased precision in predicting elimination times under changing conditions of administration, dose, temperature, age, disease status and species of fish.

This must be done while maintaining an acceptable margin of safety for the consumer. Brocklebank *et al* (1997) compared the predicted elimination times of classical and noncompartmental analysis for OTC in salmon. For a given dosage regime, the classic pharmacokinetic approach predicted a 180 day withdrawal period, and the non-compartmental a 108 day withdrawal period. Actual analysis of the tissues showed that there were no detectable residues at day 118. There were no samples taken on day 108. Assuming that the OTC had not cleared by day 108, the noncompartmental analysis rendered an underestimation of clearance time of 9%. The classical approach rendered an overestimation of 34%. It is important to move towards the increased precision of non-compartmental modeling however given the variations in physiology within and among species the collection of a more extensive database is required. Data in this experiment was modeled non-compartmentally.

In the following discussion it should be noted that there is little standardization between pharmacokinetic experiments. Experimental differences in design, data modeling, water temperature, salinity, therapeutic dose, route of administration, species, individual size and seasonal variations in physiology make comparisons between published numbers a benchmarking process rather than a precise comparison. Table 4 provides a summary of pharmacokinetic experiments. Only one reference to OTC in nonsalmonid, marine fish was found (Uno *et al*, 1992).

C_{max} is the maximum concentration of a drug found in the tissue and is measured in parts per million (ppm). T_{max} is the time (hr) to maximum plasma concentration and provides an estimate of absorption rate for a given dosage form (Barron *et al*, 1990). In the present

experiment, C_{max} for the IP group was calculated to be 140.8 ppm and occurred at a T_{max} of 8 hours. It is believed that the double peak in the curve may have resulted in an artificially high T_{max}. This number should be regarded as a maximum value. In the only other IP study found, Nouws *et al* (1992) reported a C_{max} of 65 ppm at a T_{max} of 5 hours for carp held at 20°C. The differences between that data and those found in this experiment are due to several of the non-standardized factors listed above. Higher water temperatures (20°C versus 10°C) may have contributed to the shorter T_{max} than our data and a combination of lower dose (60 mg OTC/ kg) and higher temperatures to the lower C_{max}.

C_{max} for the PO group was calculated to be 1.04 ppm and occurred at a T_{max} of 6 hours. These data fit well with the PO data of Uno *et al* (1992) who reported a C_{max}/ T_{max} of 1.14 ppm at hr 9, 2.05ppm at hr 24 and 0.89 ppm at hr 3 for trout, Amago salmon and yellowtail respectively. The T_{max}/ C_{max} data also fit with those found for Atlantic salmon. Elema *et al*, (1996) reported a C_{max} of 0.42 at a T_{max} of 12 hours; McSwain (1992) reported two C_{max} values for Atlantic salmon: 0.22 ppm and 0.77 ppm, both at a T_{max} of 3 hours.

Of the many factors which affect C_{max} and T_{max} the main one is route of administration. C_{max} is highest and T_{max} shortest in intravenous (IV) administered treatments, followed by intraperitoneal (IP), intramuscular (IM) and PO administered treatments. This is due to the fact that in IV administration the drug is introduced directly to the animals systemic circulation. In IP and IM administration the drug is introduced to well perfused compartments of the body and makes its way rapidly to systemic circulation. In PO

administration the drug is introduced to the body via the feed through the digestive tract where it makes its way to the circulation system. Depending on the drug and its formulation this may result in poor absorption and bioavailability. In the case of oxytetracycline, it does.

IP administration in cod rendered a C_{max} of 140.8 ppm at hour 18 versus a PO C_{max} of 1.04 ppm at hour 6. The higher IP C_{max} relative to the PO C_{max} is as expected. The delayed IP T_{max} was a result of the double absorption peak. Examples of C_{max} and T_{max} from studies done using IV, IP, IM and PO administration are provided in Table 4. In carp held at 20°C (Nouws *et al*,1992) C_{max} for IV administration was >100 ppm at hour 0, IP was 65 ppm at hr 5, IM was 57 ppm at hr 14 and PO was 0.46 ppm at hr 24. In the same paper, trout held at 10°C had an IV administration C_{max} of >100 ppm at hr 0, IM of 35 ppm at hr 10 and PO of 0.35 ppm at hr 48. The pattern of highest C_{max} and shortest T_{max} in order of IV to PO administration is well demonstrated. The same was shown in the following experiments. Grondel *et al* (1987) held carp at 20°C and achieved an IV C_{max} of 247.5 ppm at hr 1, IM C_{max} of 56.8 ppm at hr 14 and PO C_{max} of 0.11 ppm at hr 20. Bjorklund and Bylund (1991) held trout at 16°C and got an IV C_{max} of 113 ppm at hr 0 and a PO C_{max} of 2 ppm at hr 12.

For oxytetracycline administered orally in a capsule, Uno (1996) obtained a C_{max} of 34.2 ppm in healthy Ayu (time not specified). This relatively high C_{max} may be due to the fact that the OTC was administered without the interacting effect of chelation with cations in the feed. This will be discussed further in the section on bioavailability.

Dose level also affects C_{max} with high doses leading to higher maximum concentrations. This is supported by the findings in this experiment of a C_{max} of 140.8 ppm at an IP dose level of 132.8 mg OTC/ kg fish versus a C_{max} in carp (Nouws *et al*, 1992) of 65 ppm after an IP administration of 60 mg OTC/ kg fish. Rainbow trout injected intravenously with 5mg OTC/ kg (Black *et al*, 1991), 20 mg OTC/ kg (Bjorklund and Bylund, 1991) and 50 mg OTC/ kg (Uno *et al*, 1997) rendered a C_{max} of 18.8, 113 and 331 ppm respectively. PO administration in Rainbow trout of 60 mg OTC/ kg (Nouws *et al*, 1992) and 75 mg/ OTC (Bjorklund and Bylund, 1990) rendered a C_{max} of 0.35 and 5.3 ppm respectively. The PO C_{max} of 1.04 ppm at a dose level of 134.5 ppm found in this experiment falls between these numbers. Variations in experimental protocol would have influenced the C_{max} levels.

Temperature appears to affect T_{max} and C_{max} differentially. T_{max} in Rainbow trout (Bjorklund and Bylund, 1990) was achieved at hr 24, hr 12, and hr 1 at temperatures of 5°C, 10°C and 16°C respectively. T_{max} was shortest at 16°C and longest at 5°C. This may have been due to the increased metabolic rates of fish at higher temperatures. C_{max}, however, was highest at 10°C (5.3 ppm) not 16°C (2.1 ppm). It was hypothesized that the relatively low C_{max} at 16°C was the result of an accordingly increased excretion rate. A low C_{max} of 3.2 ppm at 5°C may be the result of decreased fluidity of the GI membrane at lower temperatures with a subsequent reduction in absorption (Ingebrigtsen, 1991).

Species differences exist with respect to C_{max} and T_{max} and may be due to differences in muscle perfusion, fat and protein content (Grondel, 1987; Ingebrigtsen, 1991). Grondel et al (1989) conducted experiments with African catfish (whiteflesh) and trout (redflesh).

IM injection of 60 mg OTC/ kg rendered a C_{max} of 43.4 ppm in African catfish and 56.9 ppm in trout. Experiments by Nouws *et al* (1992) with carp (whiteflesh) and eel (high fat/ protein fish) after a 60 mg OTC/ kg IM injection obtained a C_{max} of 57 ppm in carp and 100 ppm in eel. The PO C_{max} in this experiment for cod (whiteflesh) was 1.04 ppm whereas trout at a similar dose and temperature was 5.3 ppm (Bjorklund and Bylund, 1990).

The last major factor affecting C_{max} for orally administered drugs is salinity. High salinities reduce bioavailability by chelation of OTC with divalent cations in the seawater. This will be examined thoroughly in the section on bioavailability.

A factor which may influence maximum concentration of a drug achieved in the body is health status. Uno (1996) found that the C_{max} in healthy Ayu was 34.2 ppm and in Vibriosis infected Ayu was 2.17 ppm. This may be due to damage of the GI tract by the bacteria or toxin. This effect is particularly important when treating sick fish as a reduction in C_{max} could reduce the likelihood of attaining therapeutic levels (MICs) in the target organ. Little work has been conducted on pharmacokinetics in sick fish.

Two mathematical concepts are used to calculate the elimination and elimination half-life ($T_{1/2}$) of various drugs. The AUC (Area Under the Concentration vs Time Curve) and AUMC (area under the first moment curve) are mathematical representations of the graphed values. Beta is a derivation of

Elimination half-life ($T_{1/2}$), is the time required for a given plasma concentration to decline by 50% during the exponential, terminal phase of the drug concentration time profile (Baggott, 1988). PCNONLIN calculates $T_{1/2}$ by conducting a nonlinear, least squares regression analysis of the data from T_{max} to the last sampling point. Due to the unusual shape of the curve produced in this experiment (double peak with slight rise to final sample point) PCNONLIN would not calculate beta or $T_{1/2}$ for the IP group. Calculating this number by graphing the data and conducting a regression analysis from T_{max} to the final sample point rendered a $T_{1/2}$ value of 154.2 hrs (6.4 days) for IP administered OTC.

The PO $T_{1/2}$ was calculated by PCNONLIN to be 184 hrs (7.7 days). Because of the rise in the terminal portion of the curve this is considered an overestimation of half-life. Optimally, the final sample period should extend beyond any rise in the terminal portion of the graph in order to ensure that absorption is complete. These values are, however, of the same order of magnitude as the 146 hrs calculated for trout held at 10°C (Bjorklund and Bylund, 1990); the 130 hrs and 150 hrs for trout at 10°C; and the 169 hrs and 157 hrs for carp at 8°C (Nouws *et al*, 1992).

$T_{1/2}$ is a parameter derived from volume of distribution and clearance ($T_{1/2} = Vd_{area}/Cl_t \cdot \ln$) and, assuming that volume is constant, is an indicator of the rate of elimination. It is estimated that a 1°C change in temperature alters the metabolic rate of fish by 10% (Ellis *et al*, 1978). Because excretion rate is affected by metabolism, it too is temperature dependent (Ingebrigtsen, 1991). This theory is supported in an experiment by Bjorklund and Bylund (1990) in which elimination half-lives and beta values were measured for

Rainbow trout held at three different temperatures. A long half-life ($T_{1/2}$), with the associated small beta, is indicative of an extended elimination time. A short half-life, with the associated large beta, is indicative of rapid elimination. Trout held at temperatures of 5°C, 10°C and 16°C had half-lives of 213, 146 and 115.2 hours with betas of 0.078, 0.114 and 0.143 respectively. The short half-life values and large betas at high temperatures support the theory that elimination rates increase with increasing temperature. In another experiment by the same group the $T_{1/2}$ value for Atlantic salmon held at ≈15°C was 127.2 hours and for Rainbow trout held at ≈18°C was 48 hrs. Salte and Liestol (1983) calculated betas of 0.069 and 0.056 for rainbow trout held at 9.6°C and 7.5°C respectively. Nouws *et al* (1992) obtained half-life values in trout of 150 hrs at 10°C and 76 hrs at 19°C. In carp they obtained $T_{1/2}$ values at 8°C of 169 hrs and 157 hrs and values at 20°C of 52 hrs and 79 hrs.

The PO elimination half-life of 184 hrs in this study for fish held a 10°C is longer than that of 16 hours obtained by Uno *et al* (1992) for Amago salmon held at 15°C and shorter than that of 313 hrs obtained by Haug (1994) for charr held at 6.7°C. They are in the same order of magnitude found in trout at 10°C of 146 hrs (Bjorklund and Bylund, 1990) and 150 hrs (Nouws *et al*, 1992). This fits in well with the theory of temperature effect on elimination half-life.

Method of administration affects elimination in a pattern correlated to drug concentration levels. Administration resulting in high tissue concentrations (IV, IP or IM) result in rapid elimination. Conversely, methods of administration resulting in low tissue concentrations (PO) result in prolonged elimination periods (Salte and Liestol, 1983;

Jacobsen, 1989). This is well corroborated in this experiment in that the $T_{1/2}$ for OTC in cod was found to be 154 hrs for IP administration and 184 hrs for PO administration despite a higher C_{max} of 140 ppm for IP than of 1.04 ppm for PO. In trout, Bjorklund and Bylund (1991) obtained an IV $T_{1/2}$ of 60.3 hrs (C_{max} of 113 ppm) and a PO $T_{1/2}$ of 74.9 hrs (C_{max} of 2 ppm). They noted that IV and IP administration had similar $T_{1/2}$ values for OTC, however, no numbers were given. Grondel *et al* (1989) obtained $T_{1/2}$ values for IV administration of 89.5 hrs (C_{max} 753) and for IM administration of 94.7 hrs (C_{max} 56.9) for trout held at 12°C. It should be noted that at low concentrations (as found with PO administration) elimination curves reach an asymptote therefore the depletion period (and corresponding withdrawal times) can be very long (Burka *et al*, 1997).

Because $T_{1/2}$ is affected by concentration, dose levels would also affect elimination time. Rainbow trout with an OTC IV dose of 50 mg (Uno *et al*, 1997), 20 mg (Bjorklund and Bylund, 1991) and 5 mg (Black *et al*, 1991) rendered $T_{1/2}$ values of 52 hrs, 60.3 hrs and 81.5 hrs respectively. The $T_{1/2}$ values of 154.2 hrs after an IP dose of 132.8 mg OTC/ kg fish and 184 hrs after a PO dose of 134.5 mg OTC/ kg fish found in this experiment are relatively high. This is believed to be caused by an overestimation of $T_{1/2}$ resulting from the rise in the terminal portion of the IP and PO curve. This overestimation will impact on subsequent discussions on $T_{1/2}$.

Species differences were found when a general comparison of literature $T_{1/2}$ data was made. Contributing factors to this may be differences in: metabolic rates, fat and protein content and muscle perfusion. Nouwes *et al* (1992) conducted an experiment with carp (whiteflesh), trout (redflesh) and eel (high fat/ protein) held at similar temperatures. They

obtained $T_{1/2}$ values for IV administration of 76 hrs for trout and 52 hrs for carp. IM administration resulted in a $T_{1/2}$ in eel of 196 hrs, in trout of 150 hrs and in carp of 79 hrs. The $T_{1/2}$ of 154.2 hrs obtained for cod after IP administration in this experiment is high compared to an IP $T_{1/2}$ of 36 hrs in carp. Once again the high $T_{1/2}$ from this experiment may be a result of the rise in the terminal portion of the absorption curve. The role, however, of physiological differences on $T_{1/2}$ is evident overall and supports the premise that cross species pharmacokinetic assumptions should be made with caution.

In fresh water, $T_{1/2}$ can be prolonged compared to the $T_{1/2}$ in saltwater. This is because cations in saltwater chelate with OTC and so reduce the amounts of OTC absorbed and reabsorbed from the GI tract (McSwain, 1992; Burka *et al*, 1997). This trend is corroborated by $T_{1/2}$ literature values. In salt water, salmon had a $T_{1/2}$ of 50.4 hrs (Elema *et al*, 1996), in brackish water Rainbow trout a $T_{1/2}$ of 40.8 hrs and in fresh water a $T_{1/2}$ of 115.2 (Bjorklund and Bylund, 1990) to 74.9 hrs (Bjorklund and Bylund, 1991). This trend is not supported by the findings of Uno *et al* (1992). They found a $T_{1/2}$ of 16 hrs for freshwater Amago salmon, 23.2 hrs for freshwater trout and 28 hrs for saltwater Yellowtail. Species differences may have masked the effect of salinity in that experiment. It should be noted that elimination of drugs in freshwater fish can occur through the urine therefore excretion would be affected by the glomerular filtration rate (Ingebrigtsen *et al*, 1985; Guarino, 1988, 1991). The impact of this effect on elimination rate comparisons is unknown.

Uno (1996) found $T_{1/2}$ values in healthy Ayu of 53.1 hrs and in infected Ayu of 63.2 hrs. These two values were considered "similar" by the author however no statistical analysis

was conducted. Bruno (1989) found OTC residuals 8 weeks post administration of 3.7 ppm in the muscle of furunculosis infected fish and of 1.3 ppm in healthy fish. Diseases negatively affect the physiology of fish and therefore may impact on its ability to eliminate drugs. Little work could be found in the literature on the pharmacokinetics of drugs in sick fish.

Another factor which may play a role in prolonged $T_{1/2}$ values is plasma protein binding and the accumulation of drugs in pronephros, bone and scale. During elimination, free drug is removed from the reservoir and protein bound drug is then released and available for diffusion through the system. Reversible binding means that the liver and other extravascular compartments may serve as pools of active drug (Grondel *et al*, 1987, Grondel *et al*, 1989). High levels of bound drug will extend the time the drug is available within the biological system (McSwain, 1992). Values of 55% (Bjorklund and Bylund, 1991) and 51.1% (Uno *et al*, 1997) have been found for serum protein binding in trout. No evaluation of serum protein binding was made for cod in this experiment.

Due to the unusual shape of the PO curve, clearance time of OTC from the serum could not be calculated by PCNONLIN. An estimate of clearance was calculated using the $T_{1/2}$ value. This was done by multiplying the calculated half-life by a factor of five. It is estimated that after five half-lives ≈3% of the maximum concentration of OTC will remain (Baggott, 1988, Black *et al*, 1991).

5 X 184 hours = 920 hrs

= 38.3 days for OTC to clear from serum at 10°C

The clearance time from the muscle of cod was then calculated using affinity ratios. Affinity ratios are the ratio of the concentration of OTC in an organ (in this case the muscle) relative to serum concentrations over the elimination period. Affinity ratios for muscle in trout and salmon were obtained from the literature. Black (1991) calculated an affinity ratio of 2.1 for muscle at 10°C, Nouws *et al* (1992) of 0.65 at 10°C, Grondel *et al* (1987) of 1.25 at 312 hrs, and Rogstad *et al* (1991) of 1.8 at 336 hrs. The average of these muscle: serum affinity ratios is 1.45. The estimated clearance time from the serum is then multiplied by the muscle affinity ratio to give an estimate of clearance time from the muscle.

$$1.45 \times 38.3 = 55.5 \text{ days}$$

The estimated clearance time for OTC from the muscle of cod at 10°C is 55.5 days. This method of predicting clearance time is considered robust.

Another calculation of importance in pharmacokinetics is bioavailability (F). Bioavailability is a measure of the extent to which a drug, administered at a particular dosage, enters an organisms systemic circulation in active form (Baggott, 1988). Bioavailability affects the pharmacokinetics of a drug. The route of administration has a major affect on bioavailability. Drugs administered intravenously (IV) are considered to be 100% bioavailable. Intramuscular (IM) and intraperitoneally (IP) administered drugs, because they are not injected directly into the bloodstream, express less than complete systemic availability. Grondel *et al* (1989) found values for IM administration of OTC of 86% in African catfish, 85% in trout and 80% in carp. No values could be found for IP

bioavailability. The IM and IP methods of administration are expensive and impractical for large numbers of fish but can be effective when treating smaller numbers of valuable fish such as broodstock. Drugs administered orally, because they pass through the digestive tract before entering the circulation system, are considered to have the lowest bioavailability. Despite this, it is the most practical method of administering antibiotics to large numbers of fish.

There are two measures of bioavailability: absolute and relative. Absolute bioavailability refers to the absorption of a drug relative to intravenous (IV) injection; relative bioavailability refers to the absorption of a drug relative to intramuscular (IM) or intraperitoneal (IP) injection. Relative bioavailability of oral vs IP injection of OTC in this experiment was calculated using the equation AUC_{po}xDose/ AUC_{ip}xDose X 100 where AUC_{po} is the area under the concentration time curve for PO administration and AUC_{ip} the area under the concentration time curve for IP administration. The relative bioavailability was calculated to be 0.8%. Absolute bioavailability would be lower. Published literature shows that oral bioavailability of OTC is consistently low with values in Atlantic salmon ranging from 1.94% (Elema *et al*, 1996) to 1.5% and 7.4% (MacSwain, 1992). In trout, values of 8% (Cravedi *et al*, 1987), 5.6% (Bjorklund and Bylund, 1991), 2.6% (Rogstad *et al*, 1991), 1.25% (Nouws *et al*, 1992) and 0.6% (Uno *et al*, 1992) have been found. In carp bioavailabilities of 0.6% (Grondel *et al*, 1987) and 0.38% (Nouws *et al*, 1992) have been found.

Low oral bioavailability may be the result of several factors. First Pass Effect describes the phenomenon by which all compounds absorbed from the intestine enter the blood

flow of the hepatic portal system and are transported directly to the liver. Any orally administered compound is filtered through the liver first and, if there is a substantial First Pass Effect only a small fraction may reach systemic blood circulation in unchanged form. Compounds, such as tetracyclines, which are metabolized by or have an affinity for the liver will be relatively unavailable. (Grondel *et al*, 1987, McSwain, 1992). The additional impact of this effect in species, such as cod, which undergo seasonal variations in hepatic metabolism may bear further study.

An increase in salinity will also reduce the bioavailability of some orally administered drugs (Lunestad and Goksoyr, 1990; Burka *et al*, 1997). In the case of OTC, chelation with di- and tri-valent cations occurs. This results in an alteration of the molecular charge of OTC and a subsequent reduction in its ability to cross lipid rich biological membranes. The intestine of seawater-adapted fish (which drink actively for osmoregulation) contains a high proportion of cations, particularly Ca++ and Mg++. Patterns for salinity effects can be found when examining the extremes of literature bioavailability data. Values have been reported in saltwater of 0.8% in cod and 1.94% (Elema *et al*, 1996) and 2% (McSwain, 1992) in Atlantic salmon. In freshwater, values as high as 9.8% in Ayu (Uno, 1996) to 5.6% (Bjorklund and Bylund,1991) and 8% (Cravedi *et al*, 1987) in trout were found. These numbers support the theory that saltwater reduces bioavailability. There are, however, numbers which range widely in between with values as high as of 7.4% in saltwater salmon (McSwain, 1992) and as low as 1.25% (Nouws *et al*, 1992) and 2.6% (Rogstad *et al*, 1991) in freshwater phase trout. These large variations may be due to differences in experimental design and support the premise that some standardization of

methodologies used in pharmacokinetic experimentation is required. Variations in the results of pharmacokinetic experiments are discussed further in Experiment 2.

The low F of 0.8% found in this experiment is believed to have resulted from chelation of OTC with cations in the herring paste and with cations in excess saltwater in the stomach which occurred as a result of low feeding levels.

Another factor which affects the bioavailability of drugs is the pH of the environment. Typically, the pH is higher in saltwater than in freshwater and because weak acids are relatively more ionized and less lipid soluble at higher pHs their bioavailability is affected. Burka *et al* (1997) found that the bioavailability of quinilones was reduced in seawater. The same may be true for OTC (a weak acid) though the effect may be small and difficult to quantify given the high variability found in this type of data.

Dose levels were found to affect bioavailability in Oxolinic Acid with bioavailabilities being higher at lower doses. Bjorklund and Bylund (1991) did not find a dose effect for OTC in their experiment.

Finally, health status may affect bioavailability. BA in healthy Ayu was found to be 9.3% and in infected Ayu was 3.8%. This may be the result of reduced absorption due to damage to the digestive tract by the bacteria or toxin (Uno, 1996).

4.1.1 Conclusion: Pharmacokinetics

In this experiment baseline pharmacokinetic data was generated for OTC in cod and used to predict clearance time from the body. It was found that the pattern of absorption for IP administered OTC, with a double absorptive peak, varied from that found in the literature. This may have resulted from precipitation in the injectable solution. A rise in the terminal portion of the PO curve is believed to have resulted from the delayed absorption and reabsorption of OTC from the GI tract as a result of high levels of mucous. Variations in enterohepatic recirculation may have contributed to the rise in the terminal portion of both the IP and PO curves. The role of variations in hepatic metabolism and feed patterns on the pharmacokinetic behaviour of OTC in cod bears further investigation.

The maximum serum concentration (C_{max}) of OTC was higher for IP administration (140.8 ppm) than PO administration (1.04 ppm). In the literature, injected drugs are consistently found to have a higher C_{max} than orally administered drugs. The time to maximum serum concentration (T_{max}) for IP administered OTC at 18 hrs was higher than the PO C_{max} at 6 hrs. This may have resulted from the double peak in the IP absorption curve. Literature values indicate the injected drugs normally have a shorter T_{max} than orally administered drugs. These results indicate that method of delivery has a significant impact on efficiency of absorption of a drug, with injection delivery being more efficient than oral delivery. A literature search indicated that serum concentrations of a drug are also affected by temperature, species differences, salinity and health status.

The elimination half-life ($T_{1/2}$) was calculated to be 154.2 hrs for IP and 184 hrs for PO administered OTC. The rise in the terminal portion of the concentration time curves may have resulted in an overestimation of half-life however they are in order with those found in the literature. In future experiments, longer sampling periods will be used so as to ensure that absorption is complete. A literature search shows that $T_{1/2}$ is affected by temperature, method of administration, serum concentration, fat content, vascularization, salinity, health status and plasma protein binding.

Bioavailability is affected by method of administration with injected drugs having a higher bioavailability than orally delivered drugs. The low bioavailability of 0.8% for OTC administered orally to cod was consistent with values found in the literature. Given the large number of fish to be treated under farm conditions, oral delivery is still considered the most practical method for delivery of antibiotics. Oral bioavailability is also affected by salinity, food composition, drug formulation and pH. Hepatic metabolism may affect bioavailablity.

From this experiment clearance time for OTC in cod held at 10°C was estimated to be 38.3 days from the serum and 55.5 days from the muscle. These numbers will be compared with the actual clearance numbers generated in experiment 2.

4.2 Temperature

In this experiment the absorption, distribution and elimination of OTC was mapped in the blood, gonad, muscle and liver of cod over a 101 day period at two temperatures. In two of the control liver samples there were detectable levels of OTC at day 5 and day 20. These residuals are believed to have resulted from cross contamination of samples at point of analysis. They are not believed to be due to inadvertent administration of OTC as that would have resulted in residuals in all organs. Neither are they believed to be a result of prior treatment as there was a 10 month period from date of last antibiotic treatment to commencement of experiment.

As described in the pharmacokinetic experiment, T_{max} is the time at which a drug reaches its maximum concentration. For serum, T_{max} is indicative of how quickly the drug is digested out of the feed and absorbed into the blood. In the organs, T_{max} represents the distribution time from blood to the organs. The maximum concentration of a drug (C_{max}) is indicative of two distinct features: the amount of drug available for absorption by the blood and distribution of the drug to the body. The first factor is affected by bioavailability, the second by affinity. Affinity, in turn, is affected by vascularization, the presence of specific binding sites, protein content and fat content (Ingebrigtsen,1991). Overall, temperature is the major environmental variable affecting absorption, distribution and elimination in poikilotherms.

As with the pharmacokinetic experiment, scientific accommodation must be made in the upcoming discussion for environmental and species differences when comparing results among experiments.

In this experiment, a gonadal C_{max} of 1 ppm was found at a T_{max} of 12 hrs. Black *et al* (1991) found concentrations of 4.92 ppm of OTC in the gonads of trout after an IV administration of 5 mg OTC/ kg fish. Ingebrigtsen (1985), in a whole body autoradiography experiment with trout, showed relatively high levels of OTC in the gonads. For this reason, it was anticipated that gonadal OTC levels would be present at levels above the LoD of 3 ppm. They were not. One possible explanation is that the cod had just completed spawning and therefore no energy was being directed towards production of the gonads (Scott and Scott, 1988). The reduced vascularization, lipid and protein content of undeveloped gonads may have resulted in reduced concentrations of OTC in that organ. From this experiment the only conclusion that can be drawn about residual OTC in the gonads of cod is that they did not exceed 3 ppm when administered immediately post spawning. It is anticipated that any OTC present below 3 ppm on Sample day 1 would have had sufficient time to clear to the legal limit of 0.1 ppm over the 101 day elimination period. Refinement of the HPLC procedure for gonadal material to LoDs approaching 0.1 ppm would allow the confirmation of this in a subsequent experiment.

In serum, the shape of the curve shows a rise to a single peak at day 5 followed by rapid elimination to day 20 with slow, steady elimination thereafter. This pattern of absorption, distribution and elimination is similar to that found by Bjorklund and Bylund (1991), Grondel *et al* (1987) and Uno *et al* (1997). The maximum concentration of 1.3 ppm at day 5 is in order with the C_{max} of 1.7 ppm at 72 hrs in trout (Rogstad *et al*, 1991).

Serum C_{max} from the pharmacokinetic experiment was 1.04 ppm after a single, oral dose of 134 mg OTC/ kg fish. In this experiment C_{max} was 1.5 ppm after a 10 day oral dose of 120 mg OTC/ day. Given the multiple dosing of OTC it was anticipated that C_{max} would be higher. In the pharmacokinetic experiment, a distinct layer of OTC, sandwiched between a small amount of herring paste, was administered directly to the cod via syringe. This resulted in direct contact of the GI tract with the OTC. In this experiment the OTC was administered in the body cavity of smelt. Administration of the requisite amount of OTC was based on the observation that cod were consuming, on average, two smelt per feeding. Individual variations in feeding levels along with variability in digestion time, gastric emptying and the chelation of OTC with cations in both the body of the smelt and seawater in the stomach would have reduced the bioavailability of OTC in this experiment. In a disease outbreak on a farm, the compromised physiological status of sick fish and their subsequent reduced appetite would increase this variability even more.

The inefficiency of oral administration as a means of delivering OTC is emphasized in a study by Bjorklund and Bylund (1991). Trout held in the laboratory at 16°C were administered a single oral dose of 75 mg OTC/ kg fish and achieved a maximum muscle concentration of 2.9 ppm at sample day 2. In a field trial with rainbow trout held at18°C, OTC was administered for 10 days at 100 mg OTC/ kg fish/ day. Muscle concentrations of OTC were at 0.6 ppm on the last day of administration and were less than 0.05 ppm at sample day 7. These levels are significantly lower than those achieved in the laboratory

study. Despite the dubious efficacy of OTC administered in the feed, it is the only practical method presently available for treating large numbers of fish.

In muscle, the shape of the curve shows a rise to a single peak followed by rapid elimination to day 20 with slow, steady elimination thereafter. The pattern of absorption, distribution and elimination is similar to that found by Rogstad *et al* (1991) and Uno *et al* (1997). T_{max} was at day 10 with a C_{max} of 0.76 ppm. The delayed T_{max} in muscle relative to the serum (day 5) is reflective of the time required for OTC to be distributed from the blood to the organs and tissues. The same pattern was found by Rogstad *et al* (1991) with a maximum in the serum at 72 hrs and a subsequent maximum in the muscle at 96 hrs. Bjorklund and Bylund (1990) had a maximum in the serum at 12 hrs with a subsequent maximum in the muscle at day 4.

In the liver, the shape of the curve shows a rise to a single peak, rapid elimination to a plateau between day 20 and day 41, another period of rapid elimination to day 61 with slow, steady elimination thereafter. This pattern is indicative of enterohepatic recirculation and is similar to the biphasic absorption found in the liver of trout (Rogstad *et al*, 1991; Uno *et al*,1997) and Amago salmon (Uno,1992).

Bjorklund and Bylund (1990) found temperature effects on hepatobiliary function. Rainbow trout held at 5°C had a maximum of 20 ppm at day 9, a plateau in elimination to day 20 and slow steady elimination thereafter. Trout held at 16°C had a single absorptive peak of 24.1 ppm at day 2 with relatively rapid elimination thereafter. This indicates that lower temperatures magnify the effect of enterohepatic recirculation and result in

prolonged retention of OTC in the liver and body system as a whole. Variations in hepatic metabolism might affect a drugs absorption and distribution especially in species which undergo seasonal variations in feeding (Bruno, 1989; Bjorklund and Bylund, 1991; Ingebrigtsen, 1991; McSwain, 1992). Affinity of the liver for OTC may also contribute to an extended elimination period (Whittaker and Eales, 1993; Ingebrigtsen, 1985;).

These effects would significantly affect farmed cod as they go through profound seasonal changes in feeding levels and hepatic metabolism. Cod feed minimally during the winter and through the spring spawning event. Energy during this period is derived from the liver. In the fall, energy is directed towards liver production. In cod the effect of low temperature and periods of nonfeeding on enterohepatic recirculation are confounding effects as they occur simultaneously. However, acting independently or in association, they will affect the absorption, distribution and elimination of OTC. At present, the high risk period of disease is July to September. As hatchery production replaces wild supply and culture activity becomes more intensive the occurrence of disease may increase and occur over a wider period throughout the year. The possibility that OTC, administrated at different times of the year, will display different pharmacokinetic behaviour should be investigated.

Liver T_{max} was at day 10 with a C_{max} of 42 ppm. This is the same order of magnitude found in trout by Rogstad *et al* (1991) with a maximum of 44.7 ppm at 72 hrs and Black *et al* (1991) with a maximum of 50.4 ppm at 2 hrs.

In this experiment, serum T_{max} occurred at day 5 with the muscle and liver T_{max} occurring at day 10. This differs somewhat from Bjorklund and Bylund (1990) and Rogstad *et al* (1991) in that their maxima were achieved simultaneously in the serum and liver first with a subsequent maximum in the muscle. Orally administered drugs are absorbed out of the digestive tract and filtered through the liver (First Pass Effect) before they reach systemic circulation. This could result in a concurrent rise in OTC levels in the liver and blood. Distribution to the organs would be delayed relative to this.

The highest concentration of OTC was found in the liver at 42 ppm followed by serum at 1.3 ppm and muscle at 0.76 ppm. Given that all orally administered drugs are carried by the blood to the liver before they enter systemic circulation, a high accumulation of OTC in the liver was expected. There is variability in the data in the literature for whether OTC has a greater affinity for blood or muscle. In studies by Ingebrigtsen (1985) and Haug (1992) the order of maximum concentration was liver, blood and then muscle. In studies by Rogstad *et al* (1991) and Uno *et al* (1992) the order of maximum concentration was liver, muscle, and then blood. Muscle and blood appear to have an almost equal affinity for OTC. This affinity appears to be affected by temperature. In an experiment by Bjorklund and Bylund (1990) the highest concentrations of OTC at 10°C and 5°C were achieved in the liver, serum and then muscle. At 16°C the order was liver, muscle and then serum. A variety of factors such as protein binding, vascularization of tissues and organs, and differing metabolic rates may account for this.

MIC is the minimum concentration of an antibiotic required to inhibit bacterial growth. In order to achieve a therapeutic effect the antibiotic must accumulate in organs and tissues

at concentrations above the MIC of the target pathogen for a period of ≈5 days. Giles (1992) determined that the MIC_{90} for *Vibrio spp.* was 0.5 ppm at 10°C. In this experiment, therapeutic levels were achieved in the serum from day 1 to day 10, in the muscle from day 5 to day 10 and in the liver from day 1 to day 61.

Data from this experiment were subjected to statistical analysis to determine if there was an effect by temperature or sample day on the absorption, distribution and elimination of OTC in the organs and tissues of cod. The variability in the data for individual fish in this experiment resulted in relatively high standard errors (SE). SE in the serum data ranged from 0.2 ppm at a mean of 1.3 ppm to 0.2 ppm at a mean of 0.8 ppm. The SE in the muscle data ranged from 0.23 ppm at a mean of 0.76 ppm to 0.01 ppm at a mean of 0.02 ppm and those in the liver data ranged from 6 ppm at a mean of 42 ppm to 3 ppm at a mean of 17 ppm. The literature was reviewed to determine how these data compare to other results. It was found that Bjorklund *et al* (1991), Jacobsen *et al* (1989), McCracken *et al* (1976), and Uno *et al* (1992) conducted limited statistical analyses on their data. Others restricted their statistical analysis to measuring standard deviation, standard error and variance, and these tended to be high as well. Cravedi *et al* (1991) used a student t test and had standard deviations in the liver ranging from 299 ppm at a mean of 404 ppm to 14 ppm at a mean of 29 ppm with a sample size of 6. Grondel *et al* (1987) had standard deviations in the serum ranging from 10.9 ppm at a mean of 56.8 ppm to 0.1 ppm at a mean of 0.34 ppm with a sample size of 5. Strasdine and McBride (1979) had standard errors in the serum ranging from 0.11 ppm at a mean of 0.33 ppm to 0.42 ppm at a mean of 0.8 ppm with a sample size of 5. Many factors affect the variability found in the data

for this type of experiment. Bjorklund and Bylund (1990) found that variations in absorption and elimination were more pronounced at low temperatures (3.2±1.8 ppm at 5°C) than at high temperatures (2.1±0.5 ppm at 16°C). This resulted in high standard errors at low temperatures. Sample size in that experiment was five. Other contributing factors include low sample numbers, large variations in the physiological state of the experimental animal, low bioavailability, variable feed uptake, variations in gastric emptying time, metabolic rate fluctuations and variability in the method of analysis. In this experiment a source of variability was the low feed levels of the cod throughout the administration phase and its subsequent affect on delivery of the drug. Administration of OTC at the requisite dose was based on the observation that the cod would consume, on average, two smelt per feeding. There was individual variation from day to day in this pattern.

A two factor analysis of variance was run on the Gomperts transformed data and no temperature effect could be detected for the absorption and distribution of OTC in serum, muscle and liver at the temperature regime used. This is believed to be a result of the fact that C_{max} and T_{max} were achieved before there was a significant difference in temperatures between the two temperature groups. Fluctuations in temperature control resulted in a 0.5°C difference in the temperature groups at day 10 when there should have been a 1.0°C difference. As a result, the two temperature groups were sampled as a common temperature group to day 10. Absorption to the blood at day 5 and distribution to the muscle and liver at day 10 had occurred by this time and so no temperature effect

could be detected. No temperature effect was detected for elimination from the serum, muscle and liver at the temperature regimes used.

There was an effect on concentrations of OTC by sample day. In serum, the significant differences were between OTC levels up to and including day 10 (absorption and distribution phase) and levels including and after day 20 (distribution and elimination phase). In muscle, the significant differences were between OTC levels up to and including day 10 (absorption/ distribution phase) and levels including and after day 20 (distribution/ elimination phase). In liver, the significant differences were between OTC levels up to and including day 41 (absorption and distribution phase) and in levels including and after day 61 (elimination phase).

The elimination time of OTC from the serum, muscle and liver varied. Levels of OTC in the serum at day 10 were above 0.1 ppm (legal limit) in the commonly sampled temperature group. At day 20 OTC residuals had been eliminated completely from the 10°C group and were still at the legal limit of 0.1 ppm in the 2°C group. At this point there was a 1°C temperature difference between the two temperature groups. At day 41 elimination was complete in the 2°C group. As with the absorption and distribution phase, most of the OTC had been eliminated from the serum before there was a significant temperature difference between the two temperature groups. As a result no temperature effect was detected.

Levels of OTC in the muscle at day 20 were above 0.1 ppm (legal limit) in both temperature groups. At day 41, OTC residuals in both temperature groups were

detectable but below the legal limit. At this point there was a 3.9°C temperature difference between the two groups. At day 61 there were no detectable residuals in either group. There was no temperature effect detected.

Levels of OTC in the liver at day 20 were well above the legal limit of 0.1 ppm in both temperature groups. At this point there was a 1.0°C temperature difference between the two groups. There was a plateau in elimination in both groups between day 20 and day 41 with a temperature differential of 3.9°C at day 41. At day 61 the 10°C group was over the legal limit of 0.1 ppm and there were no detectable residuals in the 2°C group. Day 61 marks the beginning of the elimination phase in both groups and at this point there was a 6°C difference in temperature. At day 101 there were residuals in the 2°C group and none in 10°C group. There was a difference between the two groups from day 20 to day 101 with the constant temperature group eliminating more rapidly than the decreasing temperature group. This difference was not found to be statistically significant. It is believed that the variability within the sample groups masked the difference between the temperature groups.

There is, however, evidence in the literature of temperature effects on absorption, distribution and elimination of OTC. In the experiment on trout by Bjorklund and Bylund (1990), serum T_{max} was achieved at 1, 12 and 24 hrs for fish held at 16°C, 10°C and 5°C respectively. This indicates an obvious temperature effect with absorption occurring more rapidly at higher temperatures. T_{max} in the muscle occurred at day 2, 4 and 9 at 16°C, 10°C and 5°C. As with absorption, distribution occurred more rapidly at higher temperatures. Distribution of the drug from blood to muscle was determined to be slower

and more temperature dependent than absorption. T_{max} in the liver was achieved at day 2, 12 hrs and day 9 at 16°C, 10°C and 5°C respectively. There is a temperature effect , with OTC being distributed to the liver more efficiently at 10°C than at 16°C. An evaluation of maximum concentrations showed that they were higher at 10°C as well. C_{max} in the serum was 2.1, 5.3 and 3.2 ppm, in muscle it was 2.9, 4.0 and 2.6 ppm and in liver it was 24.1, 45.8 and 20 ppm at 16°C, 10°C and 5°C respectively. This indicates that absorption and distribution are most efficient at 10°C. Lower maxima at 5°C may be attributable to lower absorption of orally administered drugs from the intestine as a result of reduced membrane fluidity (Ingebrigtsen, 1991). Lower peaks at 16°C may be due to higher metabolic and elimination rates. The fact that C_{max} occurs at 10°C may be a reflection of the fact that this is the optimal temperature for trout.

Bjorklund and Bylund (1990) also showed a temperature effect on elimination of OTC. Elimination from the serum occurred by day 20, 30 and 40 at 16°C, 10°C and 5°C respectively. Elimination from the muscle occurred by day 32 in the 16°C and 10°C group and by day 40 at 5°C. Elimination from the liver occurred by day 32, 42 and 40 at 16°C, 10°C and 5°C respectively. Elimination of OTC from the organs and tissues was consistently most rapid at 16°C. An anomaly was that OTC eliminated from the liver more rapidly at 5°C than 10°C. No explanation could be found for that effect.

As was mentioned previously, administration of OTC in the feed under farm conditions is inefficient relative to laboratory conditions and results in lower maximum concentrations in the tissues and organs. This poor uptake would result in a subsequent reduction in clearance time as there would be significantly less OTC to eliminate from the body. In

addition to this, fish in cages are able to swim freely. Swimming causes increases in cardiac volume and a decrease in systemic resistance (Evans, 1993) and would serve to increase the rate at which OTC is eliminated. The experiment by Bjorklund and Bylund (1990) emphasizes this point. Trout held in the laboratory at 10°C and administered 75 mg OTC/ kg fish in a single oral dose had a 32 day elimination time from the muscle; trout held in cages at 18°C and fed 100 mg OTC/ kg fish for 10 days had less than 0.05 ppm OTC in the muscle at day 7.

The lack of a temperature effect in the present experiment is largely attributable to the fact that there was not enough of a temperature difference between the two groups in the initial phases of the experiment. However, Hazel *et al*, 1979 (in Ingebrigtsen,1991) found higher hepatic metabolic activity (Phase I) for cold acclimated trout than warm acclimated trout when measured at a standard incubation temperature. The possibility that acclimation plays a role in the absorption, distribution and elimination of compounds in the body of cold water fish should be considered in future experiments. The temperature regime used in this experiment mimicked that of the farm and so was appropriate for the question at hand.

The pharmacokinetic experiment (Experiment 1) resulted in a predicted clearance time for OTC from the serum of 38.3 days. The actual clearance time was between day 20 and day 41. Similarly, the pharmacokinetic experiment predicted a clearance time for OTC from the muscle of 55.5 days. The actual time was between day 41 and day 61.

4.2.1 Conclusion: Temperature

In this experiment we evaluated the effect of ambient water temperatures on the absorption, distribution and elimination of OTC from the serum, flesh, liver and gonad of cod. We also determined the affinity of tissues and organs for OTC.

The results from this experiment indicate that the liver has the highest affinity for OTC with serum and muscle showing equal but lower affinity. A lack of residuals in the gonad may be a result of the reproductive phase of the fish used in this experiment. Consideration should be given to the hypothesis that there may be seasonal variations in gonadal levels of OTC.

There was no temperature effect on the absorption, distribution and elimination of OTC in cod at a temperature regime mimicking that found on the farm. This may be a result of insufficient temperature differentials during the initial sampling period. While these results are sufficient for the original question of clearance under farmed conditions, future experiments should see larger temperature differences between the experimental groups. The role of acclimation of cold water fish to low temperatures on the clearance of OTC from tissues and organs should also be considered.

Statistical analyses showed an effect by day on concentrations of OTC. The significant differences lay between concentrations in the absorption and distribution phases and concentrations in the elimination phase for serum, muscle and liver.

Minimum Inhibition Concentrations for *Vibrio spp.* were achieved in the serum, muscle and liver.

Improvements in future methodology should see larger sample sizes and, as cod is intended as a skin-on product, skin should be included in the analysis.

From this experiment it was determined that OTC levels in the muscle and serum (both temperature groups) fell below 0.1 ppm (legal limit) by Day 61 and 41 respectively. There were no detectable levels of OTC in the gonad after Day 1. This indicates that the regulatory guidelines of an 80 day clearance time for OTC from the flesh of fish held at or above 10°C are acceptable under Newfoundland farm conditions. The 80 day limit also appears to be acceptable for cod held at or below 10°C. Predictions from the pharmacokinetic experiment for clearance from the serum and muscle were well corroborated in the elimination experiment.

Concentrations of OTC in the liver (both temperature groups) were high and are not believed to have fallen to 0.1 ppm by the end of the experimental period. Should industry decide to harvest the livers of cod, extended clearance periods should be considered.

5. General Conclusion

At present there are three antibiotics (OTC-HCL, sulphadimethoxine: ormetoprim and sulphadiazine: trimethoprim) licensed for use in aquaculture in Canada. Extended periods of use of one often results in the development of resistant strains of bacteria and necessitates a change to an alternate antibiotic. This results in a cycle of use through the three available antibiotics. Given concerns over the use of antibiotics in food animals it is unlikely that new antibiotics will be licensed and so it is anticipated that there will always be a dependence on the use of OTC in aquaculture. Physiological and environmental differences encountered by poikilotherms introduces high variability in cross species assumptions on the use of antibiotics. As a result it is critical that a standardized database be established for the pharmacokinetics of OTC in cod and other marine species so as to set acceptable treatment regimes and clearance times for the industry.

Overall research should focus on determining the effect of seasonal variations in hepatic metabolism and feeding levels on the pharmacokinetics of drugs in cod. Acclimation to optimal temperatures, health status of the fish and variable clearance times from different organ systems should also be evaluated. Ongoing generation of non-compartmentally modeled numbers could lead to increased precision and accuracy in the pharmacokinetic database for cold water, marine species. All laboratory results should be validated in the field trials to determine the efficacy of a given regime under farm conditions.

Given the practicality of administering antibiotics orally, additional work should be conducted on improving the bioavailability of these drugs. Identification of compounds

that could increase the uptake of an antibiotic, reduce the interaction with divalent cations, allow for delayed release to the body or that work optimally at pHs found in the GI tract are examples of research which would serve to improve the efficacy of a given regime. Considering the high quality of fish nutrition work being conducted for the aquaculture industry it may be advisable to work with the feed companies to develop feeds and antibiotic regimes that enhance the delivery of drugs to sick fish.

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Appendix 1: Average Weekly Feed Consumption of fish (% body weight / day) by Individual Tanks

	#of Feeds	Diet	1	2	3	4	5	6	7	8	9
February 14	1	Chopped herring	-	-	-	3.1	1.2	1.9	2.1	2.1	1.5
February 16	1	Chopped herring	-	-	-	3.4	1.9	2.2	1.7	1.6	1.6
February 21	1	Chopped herring	-	-	-	.04	.02	0	0.5	-	-
March 9	1	Herring pellets	1.0	1.2	1.1	1.4	0.8	-	0.2	0.2	0.2
March 13-19	1	Herring pellets	1.0	0.7	0.7	0.7	0.8	-	0.4	0.6	0.6
March 20-16	-	Herring pellets	-	-	-	-		-	-	-	-
27-April 2	2	Herring pellets	0.5	0.3	0.2	1.0	0.7	0.5	0.3	0.5	0.3
April 3-9	3	Herring pellets	1.0	0.8	0.5	1.0	0.8	0.7	0.3	-	0.5
April 10-16	2	Herring pellets	1.0	0.5	0.5	0.4	0.4	0.4	0.1	-	0.3
April 17-23	1	Chopped herring	1.8	1.5	-	-	-	-	-	-	-
April 24-30	2	Smelt	1.2	1.0	0.9	0.8	0.1	1.0	0.6	-	1.6
May 1-7	3	Smelt	1.9	1.2	1.0	1.6	0.6	1.3	0.6	-	1.5
May 8-14	3	Smelt	2.6	1.8	1.2	2.5	0.9	1.8	-	-	2.4
*May 15-21	4	Smelt	1.8	1.8	1.1	1.7	1.6	1.3	-	-	1.2
*May 22-28	6	Smelt	1.3	1.5	1.2	1.7	1.6	1.0	-	-	0.6
May29-June4	3	Smelt	1.7	1.5	1.2	1.6	1.6	1.5	-	-	1.1
June 5-11	3	Smelt	1.4	2.0	1.5	2.3	1.8	1.9	-	-	0.75
June 12-18	3	Smelt	1.0	1.3	1.3	2.3	1.8	2.1	-	-	0.2
June 19-25	3	Smelt	2.0	2.7	2.1	2.8	2.7	2.5	-	-	0.2
June 26-July 2	3	Smelt	2.1	3.0	2.3	3.1	2.0	2.8	-	-	0.5
July 3-9	2	Smelt	3.1	3.4	2.6	3.5	3.0	3.2	-	-	0.5
July 10-16	3	Smelt	2.9	3.0	2.4	2.6	2.6	2.7	-	-	0.5
July 17-23	3	Smelt	2.8	3.0	1.9	2.2	2.5	2.3	-	-	0.5
July 24-30	2	Smelt	1.6	2.6	1.5	2.3	2.6	1.8	-	-	0.2
July 31-Aug 6	3	Smelt	1.6	-	-	-	-	1.9	-	-	0.2
August 7-13	3	Smelt	0.9	-	-	-	-	1.5	-	-	-
August 14-20	3	Smelt	0.9	-	-	-	-	1.1	-	-	
August 21-27	3	Smelt	1.4	-	-	-	-	1.4	-	-	
August 28-September 3	3	Smelt	2.4	-	-	-	-	2.5	-	-	
Note: * Experiment started May 17, ** Experiment finished May 27											

Appendix 2: Weight of Fish Used in Pharmacokinetic Experiment.

ORAL DELIVERY: TANK 2			INJECTION: TANK 3		
TAG NUMBER	MARCH 3 WEIGHT	MARCH 22 WEIGHT	TAG NUMBER	MARCH 3 WEIGHT	MARCH 22 WEIGHT
F00978	3180	3234	F00471	3688	3613
F00984	2679	2615	F00468	3350	2783
F00979	3376	2461	F00466	4148	3970
F00976	2580	2101	F00463	3469	2395
F00952	1858	1892	F00460	2450	1635
F00985	3243	2386	F00456	4065	3140
F00980	2934	2851	F00458	2943	3065
F00981	2411	2363	F00469	3556	3335
F00983	2531	2100	F00464	3316	3176
AVERAGE	2755	2455		3443	3012

Appendix 3: Temperature Data: Acclimation and Pharmacokinetics Experiment

DATE	DAYS TO EXPERIMEN	TANK2 MIN	TANK 2 MAX	TANK 2 AVG	TANK 3 MIN	TANK 3 MAX	TANK 3 AVG
950308	14	6.737	6.902	6.86	6.654	6.845	6.78
950309	13	6.777	6.926	6.87	6.701	6.879	6.80
950310	12	6.827	7.31	7.06	6.744	7.23	6.99
950311	11	6.768	7.37	7.01	6.685	7.31	6.93
950312	10	7.11	7.44	7.31	7.02	7.38	7.25
950313	9	7.44	7.52	7.49	7.38	7.45	7.42
950314	8	6.772	7.78	7.13	6.639	7.74	7.05
950315	7	6.756	7.36	7.22	6.685	7.3	7.17
950316	6	7.3	7.84	7.73	7.24	7.8	7.68
950317	5	7.78	8.51	8.18	7.72	8.53	8.13
950318	4	8.34	8.97	8.77	8.3	8.94	8.73
950319	3	8.81	9.22	9.12	8.76	9.18	9.08
950320	2	9.17	9.69	9.53	9.12	9.65	9.48
950321	1	9.63	9.7	9.68	9.59	9.66	9.63
950322	START	9.68	10.08	9.99	9.64	10.05	9.96
950323		10.01	10.08	10.04	9.95	10.04	9.99
950.24	FINISH	9.98	10.04	10.01	9.93	10.01	9.97

Appendix 4: Dose Levels of OTC Administered Per Os for Individual Fish With Sample Calculation

Tag #	Weight (grams)	Volume OTC (ml)	Dosage mg OCT/kg fish
978	3234	2.0	136.1
984	2615	1.6	134.6
979	2461	1.5	134.1
976	2101	1.3	136.1
952	1892	1.2	133.7
958	2386	1.5	133.7
980	2851	1.7	131.2
981	2363	1.5	135.0
983	2100	1.3	136.0
		Average	134.5

Sample Calculation: Fish 984
10.79 g OTC/50 ml dd H_2O
=0.22 g OTC/ml dd H_2O

1.60 ml OTC injected × 1.22 g
=0.3453 g OTC
=345.3 mg OTC/2615 g fish
=134.6 mg OTC/kg fish

Appendix 5: Dose Levels of OTC Administered by IP Injection for Individual Fish

Tag #	Weight (grams)	Volume OTC (ml)	Dosage mg OTC/kg fish
471	3616	2.2	130.1
468	2783	1.7	134.4
466	3970	2.4	133.0
463	2395	1.5	133.0
460	1635	1.0	135.0
456	3140	1.9	133.1
458	3065	1.8	132.8
469	3335	2.0	131.9
464	3176	1.9	131.6
		Average	132.8

Appendix 6: Administration and Sample Times for Pharmacokinetic Experiment

Date	Time	Route	N	Event
Wednesday, March 22	1430	Per os	9	Fish Gavaged
	1530	Intraperitoneal	9	Fish Injected
	1600	Intraperitoneal	3	0.5 hr blood sample
	1730	Per os	3	3 hr blood sample
	2030	Per os	3	6 hr blood sample
	2130	Intraperitoneal	3	6 hr blood sample
Thursday, March 23	0230	Per os	3	12 hr blood sample
	0330	Intraperitoneal	3	12 hr blood sample
	0830	Per os	3	18 hr blood sample (Terminal)
	0930	Intraperitoneal	3	18 hr blood sample (Terminal)
Friday, March 24	0230	Per os	3	36 hr blood sample (Terminal)
	0330	Intraperitoneal	3	36 hr blood sample (Terminal)
Saturday, March 25	1430	Per os	3	72 hr blood sample (Terminal)
	1530	Intraperitoneal	3	72 hr blood sample (Terminal)

Appendix 7: HPLC Method for Detection of OTC in Cod Fish Serum

Procedure

1. Aliquot 1 ml of serum or blood into 10 ml teflon centrifuge tubes. If blood has coagulated weigh out 1 gm per tube.
2. If spiking with OTC, add directly to serum/ blood, vortex.
3. Add 1 ml 20% TCA-MeOH, vortex, refrigerate overnight at 4C.
4. Vortex again then centrifuge at 10,000 rpm for 15 minutes at room temperature. Use JA20.1 rotor.
5. Pour off supernatant into pre-marked, glass graduated tubes. Evaporate under nitrogen until final volume is 1.0 or 0.5 ml, depending on degree of concentration required.
6. Filter extract through 0.45 um syringe tip filter into HPLC vials, cap and run on HPLC.

HPLC Conditions

Column:	Ultrasphere ODS 5 um, 4.6×250 mm, C18
Solvent:	80% 50mM NaH2PO4 buffer*, pH 2.5 20% acetonitrile.
Flow Rate:	1.0 ml/min
UV detector:	353 nm
Injection volume:	25 ul
Retention Time:	4.9 to 6.7 minute range
Detection Limit:	0.05 ppm

Notes

Standards made up in MeOH; Use fresh standards each day. Run OTC standards in MeOH with each run of unknowns. Run occasional spiked sample. Use standards that compare in concentration to unknowns.

If final volume is 1.0 ml, express HPLC value as straight ppm as measured by instrument, based on appropriate OTC standards. Recovery is 100%.
If final volume is less than 1.0 ml, multiply instrument value by volume. eg. 1.6 ppm in 0.7 ml = 1.6 × 0.7 = 1.12 ppm OTC in sample.

Appendix 8: Recoveries of OTC from Spiked Serum Samples

OTC (ppm)	% Recovered
33.3	97
16.7	83
3.1	99
1.4	101
0.5	94
0.3	108

Appendix 9: Water Temperatures for Experimental Fish.

		Module 7	Module 8
January	2-8	3.0	4.4
	9-15	3.4	2.6
	16-22	3.3	2.5
	23-29	9.8	3.3
February	30-5	10.4	4.0
	6-12	10.5	4.8
	13-19	10.8	6.0
	20-26	6.9	6.0
March	27-5	6.7	6.1
	6-12	7.0	6.8
	13-19	8.6	6.8
	20-26	9.6	6.3
	27-2	8.4	7.1
April	3-9	8.8	9.3
	10-16	9.3	9.4
	17-23	8.5	8.8
	24-30	7.8	7.9
May	1-7	8.9	8.9
	8-14	10.1	10.2
	15-21	10.1	10.1
	22-28	10.1	10.1
	29-4	10.1	10.1
June	5-11	10.2	9.6
	12-18	10.2	9.0
	19-25	10.2	8.1
	26-2	10.1	7.1
July	3-9	10.1	6.3
	10-16	10.0	5.6
	17-23	10.1	4.9
	24-30	10.1	4.8
	31-6	10.1	5.0
August	7-13	10.1	4.7
	14-20	10.1	3.0
	21-27	10.1	2.3
	28-3	10.1	2.1
Note: For details on individual tank temperatures refer to specific experiment write up.			

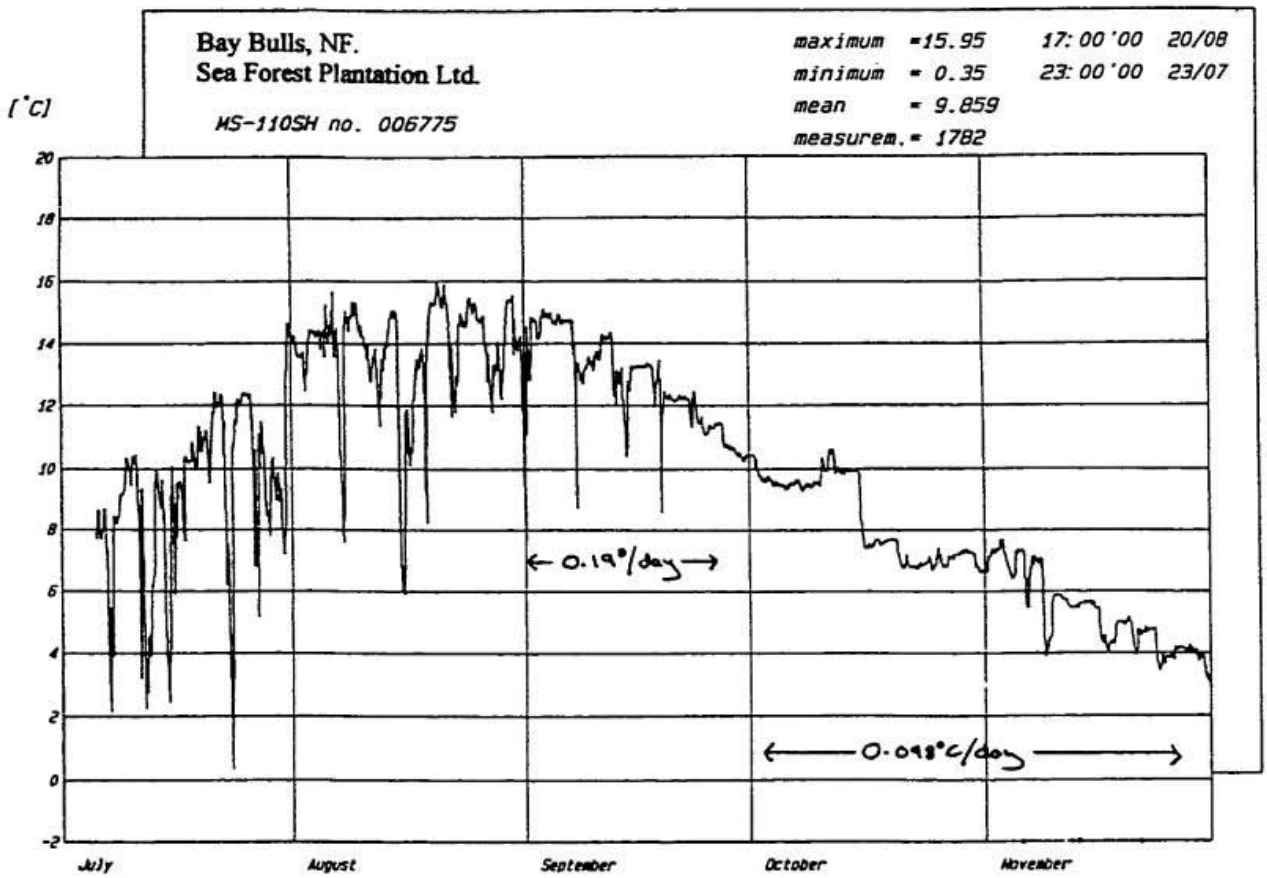
Appendix 10: Weights of Fish Used in Elimination Experiment.

Tag	Temperature	Final Samples Day	Date of Final Day	Weight March 3	Weight March 29	Weight on Final Day
F000966	10	1	950527	2756	2008	2063
W000596	10	1	950527	2876	2863	2337
W000933	5	5	950601	2531	2929	2213
W000835	10	5	950601	3731	3102	3392
W000974	10	5	950601	3304	3316	3069
W000830	10	5	950601	2733	2771	2445
W000557	10	5	950601	2832	3111	2151
W000836	5	10	950606	2680	2552	1968
W000556	10	10	950606	2724	2664	2437
F000986	10	10	950606	2983	1963	2469
F000965	10	20	950616	2493	1576	1518
W000560	10	20	950616	1553	1504	1389
W000829	10	20	950616	2812	2610	2539
W000589	10	20	950616	2848	2569	2050
W000555	2	20	950616	1916	1876	1979
F000951	2	20	950616	2502	2482	2181
W000831	2	20	950616	3793	3554	2878
F000462	2	20	950616	3004	2716	2201
F000954	10	41	950707	2753	2542	2154
F000962	10	41	950707	4053	3038	6542
W000554	10	41	950707	2586	2470	2013
W000585	2	41	950707	3627	3437	3211
W000970	2	41	950707	3099	2748	3680
W000834	2	41	950707	2744	2400	2399
F000465	2	41	950707	2518	1879	2078
F000982	2	41	950727	2695	2800	2396
F000955	10	61	950727	2471	2057	2793
F000567	10	61	950727	2556	2564	3755
W000562	10	61	950727	2015	1599	2529
W000839	2	61	950727	1623	1624	2135
W000583	2	61	950727	2868	2749	2923
W000951	5	61	950727			
W000559	2	101	950905	4449	4551	3937
W000582	2	101	950905	2147	1796	2111
W000457	2	101	950905	2958	2698	3312
W000968	2	101	950905	3035	2939	2286
W000843	2	101	950905			
F000472	2	101	950905	3519	2868	2718
W000828	10	101	950905	3618	3554	3429
W000594	10	101	950905	2806	2623	1887
F000596	10	101	950905	2967	2669	2754
W000838	10	101	950905	2904	2231	3250
Sample Day Average Weight				2853	2605	2557

Appendix 11: Sub Weights of Fish for Elimination Experiment

Tag #	Original Weight (grams)	Present Weight (grams)	Weight Difference (grams)
951	2482	2332	-150
599	4551	4461	-90
555	1876	1850	-26
459	2573	2500	-73
586	3437	3389	-48
582	1796	1736	-60
583	2749	2721	-28
591	1702	1681	-21
596	2363	2334	-29
831	3554	3501	-53
Average	2708	2650	-58

Appendix 12: Water Temperature Profile: Bay Bulls, NF Farm Site, July to November, 1994



July - November 1994

T. McKeever

Appendix 13: Water Temperatures, and Differences Between the Temperature Groups, for the 10°C, 10°C Decreasing to 2°C and Control Tanks

Date	Event	10 C	10 C to 2 C	Control	Diff. Between Temp Groups
May 17,1995	Start Admin	9.9	9.9	9.9	0.0
May 27,1995	Fnsh Admin	10.0	10.1	10.0	0.0
	Day 5	10.2	10.0	10.0	0.2
	Day 10	9.9	9.4	9.4	0.5
	Day 20	9.7	8.7	8.5	1.0
	Day 41	9.6	5.7	5.8	3.9
	Day 61	9.7	3.7		6.0
	Day 101	9.7	1.9		7.8

Appendix 14: Number of Fish Sampled Per Sample Day for the Elimination Experiment

	Common Temperature	10 C	10 C decreasing to 2 C	Control
Day 1	6			
Day 5	5			1
Day 10	5			1
Day 20		5	5	
Day 41		6	6	
Day 61		5	5	1
Day 101		6	6	1
Total	16	22	22	4