Acute and Sublethal Copper Sensitivity, Growth and Saltwater Survival in Young Babine Lake Sockeye Salmon

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ABSTRACT

Davis, J. C. and I. G. Shand. 1978. Acute and sublethal copper sensitivity, growth and saltwater survival in young Babine Lake sockeye salmon. Fish. Mar. Serv. Tech. Rep. 847: 55 p.

A series of experiments was conducted during the spring, summer and fall of 1977 to determine the potential copper toxicity hazard to Babine Lake, B.C., sockeye salmon (*Oncorhynchus nerka*) fry, lake fingerlings and smolts. Acute 96 h LC50's were determined for the three life stages utilizing static bioassays with 24 h solution replacement and $CuCl_2 \cdot 2H_2O$ as a toxicant in on-site testing using natural lake water. 96-h LC50 values complete with 95% confidence limits were obtained for fry, fingerlings and smolts and ranged from 210-220 µg Cu++/L for fry, to 240 µg Cu++/L for smolts and lake fingerlings expressed as total copper concentration as measured in test solutions. It was concluded that the present low levels of copper in the lake (4-44 µg/L total Cu++), coupled with the considerable complexing capacity of Babine Lake water (in excess of 100 µg Cu++/L), pose no acute toxicity threat to Babine sockeye at this time.

In order to study the potential hazard of low sublethal copper level exposure to fish overwintering in the lake prior to outmigration, salinity transfer studies, growth studies and mortality after seawater transfer were examined in sockeye smolts. In addition, copper sensitivity in relation to infestation with the cestode parasite *Eubothrium salvelini*, and general nutritional state of migrating smolts were examined as peripheral issues.

Osmoregulatory capability in Babine sockeye smolts was tested by examining plasma sodium levels in fish subjected to 24 or 48 h exposure to salt water in the laboratory. Two thirds of the fish tested could maintain plasma sodium levels below 170 m Eq Na+/L following salt transfer while the remaining fish exhibited elevated plasma sodium levels considered as evidence of incomplete smoltification. Laboratory exposure of Babine sockeye smolts for 144 h to 30 µg Cu++/L (total copper) in fresh water followed by 24 and 48 h salt exposure showed that fish exposed to copper in this manner were unable to regulate plasma sodium levels following salt transfer. Plasma sodium concentrations in copper-exposed individuals were approximately 200 m Eq Na+/L following salt transfer and some mortality occurred. These results confirm the findings of other workers who studied coho salmon and demonstrate that freshwater copper exposure interferes with salinity tolerance in salmonids, possibly through its effects on the Na+, K+-activated ATPase system. Calculations which included the known complexing capacity of Babine waters and the LC50 values for copper-exposed sockeye smolts in those waters, suggested that the threshold total copper concentration which might disrupt osmoregulatory capability in Babine stocks was in the range of 109-154 µg Cu++/L. This range is well above existing total copper levels in the lake at this time, hence an osmoregulatory problem in Babine sockeye due to the presence of copper is considered unlikely.

Two collection groups of smolts had low condition factors (0.69, 0.75) upon collection and exhibited a specific growth rate of approximately 1.0%/day when fed on excess ration for 60 days following salt transfer. Both groups, as well as salt-adapted stock fish,suffered a mortality of approximately 35%

during long-term exposure to salt water. Smolts tested in this manner had a 35.3% infestation with the cestode parasite *Eubothrium salvelini* and one test group had depressed lipid levels and elevated tissue water content reminiscent of starvation conditions at the time of collection. *Eubothrium* infestation did not appear related to copper sensitivity in acute toxicity tests however, it is possible that parasitism contributed to small fish size, poor nutritional state and unsuccessful seawater adaptation. It is recommended that further studies be conducted to examine smolt quality, nutritional state, success of salt water transfer and the relationship of *Eubothrium* infestation and seawater survival in Babine smolts.

Key Words: Salmon, underyearlings, smolts, copper, toxicity, osmoregulation, growth, condition, parasitism.

RÉSUMÉ

Davis, J. C. and I. G. Shand. 1978. Acute and sublethal copper sensitivity growth and saltwater survival in young Babine Lake sockeye salmon. Fish. Mar. Serv. Tech. Rep. 847: 55 p.

Du printemps à l automne de 1977, les A. ont mesuré les risques d'intoxiation pa le cuivre des alevins nouvellement éclos, de ceux de la grosseur d'un doigt et des smolts de saumon sockeye *(Oncorhynchus nerka)* du lac Babine (C.-B.) Pour chacun des trois stades, ils ont déterminé sur place la CL50 après 96 h en utilisant l'eau du lac dans des conditions statiques, avec remplacement des solutions toutes les 24 heures avec le $CuCl_2 . H_2O$ comme toxique. Avec des limites de confiance de 95%, la CL50, exprimée d'après la teneur en cuivre total des solutions expérimentales, a varié entre 210 et 220 µg de Cu^{++}/l pour les alevins nouvellement éclos, et s'est élevée jusqu'à 240 µg pour les autres. Il en ressort que, pour le moment, la teneur en cuivre du lac (4 à 44 µg de Cu^{++} total/l), associée à la trés forte capacité de complexation de ses eaux, (qui excède 100 µg de Cu++/l), ne présente pas de risques de toxicité aiguë pour le saumon sockeye.

Afin de mesurer les risques d'exposition du poisson qui hiverne dans le lac avant de migrer à une concentration sublétale de cuivre, les A. ont examiné les résultats d'études sur le passage en eau salée des smolts de sockeye, et sut leur croissance et leur mortalité en ce milieu. Chez le smolt migrateur, ils ont aussi étudié les relations entre sa sensibilité au cuivre et le parasitisme pa le cestode *(Eubothrium salvelini)*, ainsi que son état général de santé lié à la nutrition.

Ils ont mesuré la capacité osmorégulatrice des smolts d'après la teneur en sodium de leur plasma apres les avoir places en eau salee pendant 24 ou 48 heures, au laboratoire. Les deux tiers des sujets des sujets ont pu maintenir cette teneur au-dessous de 170 méde Na /l au terme de l'expérience,

tandis que chez les autres, elle a été plsu éleyée du fait de leur smoltification incomplète. En laboratoire, les smolts plongés en eau salée pendant 24 ou 48 h. après avoir séjourné 144 h dans de l'eau douce additionnée de 30 µg de Cu^{++} total/l, n'ont pu ajuster la teneur en sodium de leur plasma, laquelle s'est élevée à environ 200 mé /l, entraînant la mort de certains. Ces résultats confirment les conclusions d'autres chercheurs qui avaient montré que la présence de cuivre eneua douce diminuait la tolérance du saumon coho à la salinité en déréglant peut-être le cycle de l'ATP-ase activée par les ions Na^{+} et K^{+}. D'après la capacité de complexation des eaux du lac Babine et la C150 du cuivre pour les smolts de sockeye de ces eaux, l'osmorégulation peut se déséquilibrer lorsque la teneur en cuivre total se situe entre 109 et 154 µg de CU^{++} /l, ce qui est bien au-dessus des teneurs actuelles dans le lac; il est donc fort peu probable que le cuivre y cause un dérèglement osmotique chez le saumon sockeye.

L'état de santé de deux échantillons de smolts s'est révélé médiocre au moment du prélèvement (0,69, 0,75). La croissance a augmenté d'environ 1 % par jour durant les 60 jours après le passage en eau salée, au cours desquels les smolts ont reçu un supplément alimentaire. A la suite d'un séjour prolongé en eau salée, les deux groupes, se même que le stock adapté à ce milieu ont subi des pertes d'à peu près 35 %. Les smolts étaient infestés à 35,3 % par *Eubothrium salvelini*, et l'un des groupes expérimentaux présentait une carence lipidique et une haute teneur en eau intersticielle indices de l'état de privation dans lequel il se trouvait au moment du prélèvement. Au cours du dosage de la toxicité aiguë, le parasitisme n'a toutefois pas semblé relié à la sensibilité au cuivre. Il est possible par contre qu'il ait contribué à la réduction de la taille du poisson, à son mauvais état de santé et à son manque d'adaptation à l'eau salée. Il faudrait poursuivre les recheres en ce qui concerne la qualité des smolts, leur état de santé, le succès de leur passage en eau salée et la relation entre le parasitisme par *Eubothrium* et la survie des smolts du lac Babine en eau salée.

Mots clefs: Saumon; alevin de moins d'un an; smolt; cuivre; toxicité; régulation osmotique; croissance; état de santé; parasitisme.

INTRODUCTION

In terms of production of sockeye salmon, *Oncorhynchus nerka*, the Skeena River system located in central British Columbia, constitutes a fisheries resource area exceeded only in magnitude by the Fraser River system in terms of quantity of sockeye produced. The Babine system produces almost 90% of the sockeye reaching the Skeena (Smith, MS 1973) and has been the site of recent salmon enhancement activities to increase sockeye production based on an assessment that Babine Lake was underutilized as a nursery area for underyearling sockeye salmon (Johnson, MS 1961). Large spawning channels were constructed on Fulton River and Pinkut Creek which flow into Babine Lake (Fig. 1) and by 1973 these channels were utilized to the maximum of their design capability.

The presence of spawning channels in the Babine area has resulted in a large increase in numbers of underyearling sockeye in the lake since 1973. Smolt output has increased, however, adult returns to the system appear to be below expectations based on the number of young fish going to sea. Assessment is difficult due to the cyclic nature of fish populations, the interaction of both wild and enhanced stocks, and the relatively short time period since construction of enhancement facilities coupled with other uncertainties.

In 1977 the Salmon Enhancement Program was initiated with the objective of vastly increasing the production of Pacific salmon on a long-term basis. A considerable emphasis in the Salmon Enhancement Program is placed on increasing fish productivity in various systems by the construction of spawning channels and hatcheries in the same manner as the Babine facilities. Current uncertainty about the success of such efforts however, due to the above-mentioned post-enhancement findings in the Babine system, prompted a series of studies in Babine Lake in 1977. This report constitutes the findings of one of these studies.

Two mines producing copper concentrate utilizing an 'open pit' operation are located in the Babine Lake area. In 1976 Granisle Copper Limited, located on an island complex close to the town of Granisle, B.C. (Fig. 1), produced in excess of 50,000 tons of product with over 9,000,000 tons of waste mined in the process. Tailings were dewatered and tailings water recycled with the resultant tailings stored in a large pond separated from the lake by rock-fill dams.

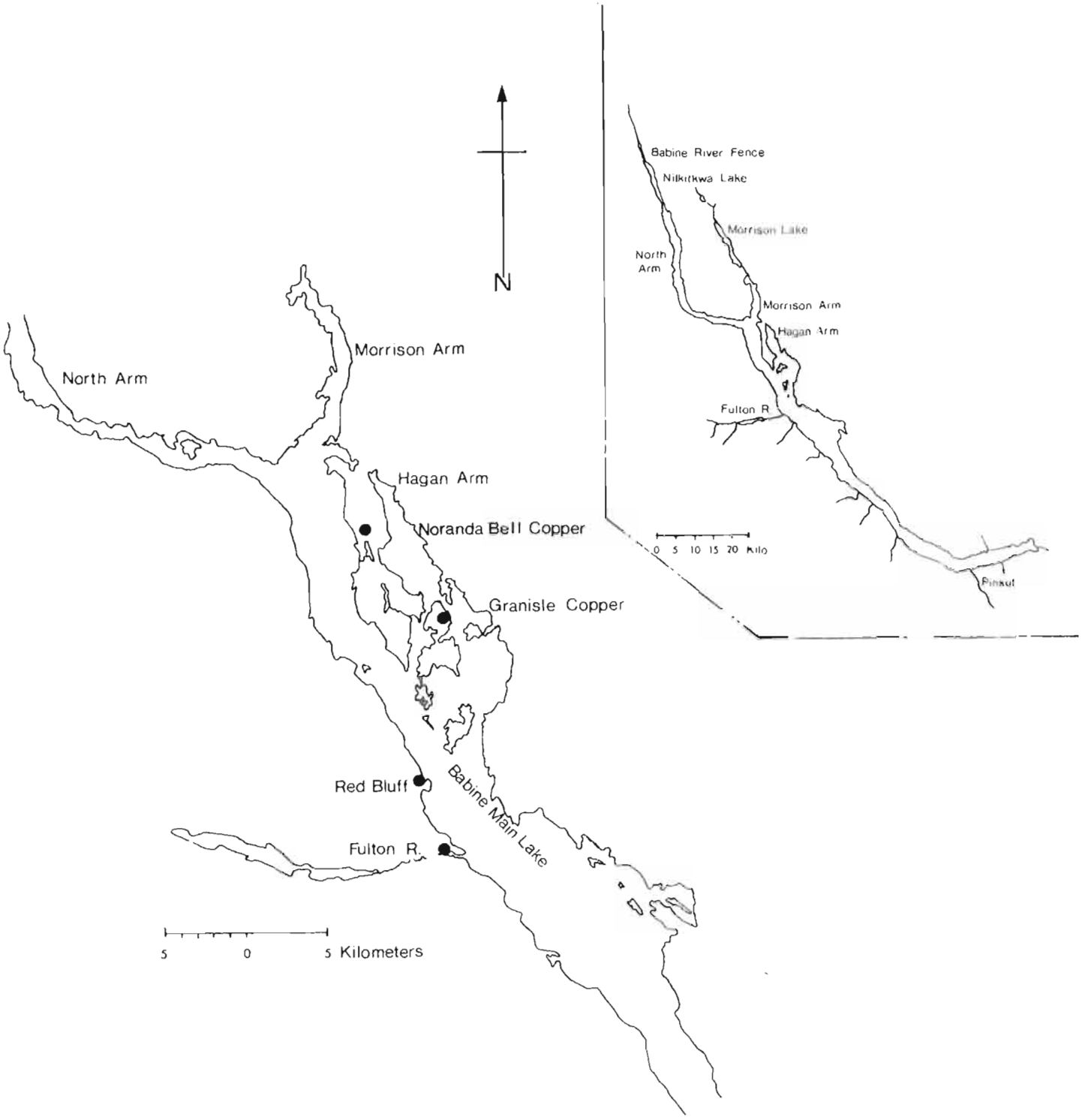


Fig. 1. Maps showing the Babine Lake area, location of copper mines and the location of the Red Bluff study site, Fulton River and Babine River counting fence.

Noranda Mines Limited, Bell Copper Division, operates the second mine, located on the Newman Penninsula NE of Granisle (Fig. 1). In 1976, in excess of 28,000 tons of copper concentrate was produced along with 1.7 million tons of waste which was used in the construction of tailings impoundment dams. In the Noranda operation, tailings impoundments are located on the Newman Penninsula where a small lake site has been used as a tailings dump.

Storage of tailings in the above manner apparently results in a generally acceptable level of water quality in the lake adjacent to the mine sites. Stockner and Shortreed (1976) reported mean total copper levels in the water column adjacent to the mines averaging 4 µg/L over five sampling dates ranging from July 1975 to October 1975. During the same period, their data indicate an average level of 4 µg/L Cu^{++} in lake water sampled from five widely spaced stations encompassing the entire lake. These data were obtained using atomic absorbtion spectrophotometry. J.A.J. Thompson (personal comm.) carried out analysis of surface lakewater samples taken in April 1977 from 3 stations in the lake including two sites in the vicinity of Fulton River. His results, using both the atomic absorbtion technique and anodic stripping yielded values in the order of 0.7-1.3 µg Cu^{++}/L. Another study (Chau and Wong 1975), using the anodic stripping technique, in October 1974 yielded levels of 16-44 µg/L total copper in surface samples collected adjacent to the mine sites. Chau and Wong determined that the copper present was in the completely bound form - i.e. it was not in the free form (ionic) or loosely bound to organic and inorganic ligands. They concluded that the metal complexing capacity of Babine water was high and that levels of Zn, Cd, Pb and Cu were not 'alarmingly high'.

Lorz and McPherson (1976) demonstrated that acute and chronic exposure of yearling coho salmon, *Oncorhynchus kisutch*, to sublethal copper concentrations (5-30 µg/L) had deleterious effects on downstream migration, gill ATPase activity and survival in seawater. Copper appeared to affect gill ATPase activity within 24-72 h of exposure and effects were often maximized within 120-144 h of exposure at 5-20 µg/L (0.09-0.35 of 96 h LC50). Lorz and McPherson concluded that such effects reduced the chances of successful migration to the ocean and adaptation to salt water.

As the concentrations of copper shown to be deleterious to coho by Lorz and McPherson (1976) approximated those described above for Babine Lake,

concern arose as to whether Babine sockeye might be similarly affected. The present study was initiated to determine the sensitivity of Babine fry, lake fingerlings and smolts to copper via acute toxicity testing in natural lake water and by salinity transfer studies utilizing smolts at the time of their seaward migration. In addition, some related aspects of growth and survival in seawater were examined.

General Methods

In situ Acute Toxicity Tests with Babine Fry, Fingerlings and Smolts

As it is well known that chemical and physical characteristics of water can have a major impact on the biological availability and toxicity of heavy metals (Lee 1973), it was necessary to conduct acute toxicity tests with young sockeye on site using natural Babine Lake water. A study site was selected at the Provincial Forest Service Red Bluff Camp located approximately 5 km N of the Fulton River facility (Fig. 1). This site was conveniently positioned for fish collection, was easily accessible by road, offered small vessel docking facilities, and had ample electrical power supplies. In addition, it allowed easy access to lake water free from any major influences of stream or river discharges that might alter lake water properties.

Details of the Red Bluff experimental site are shown in Fig. 2. The bioassay test laboratory consisted of a portable aluminum building (4.3 x 3.7 x 2:1 m) supplied with lake water via two Jabsco electrically driven, all-plastic pumps and plastic intake lines submerged in the lake. Water intake depth was 2 m early in the study and was increased to 7 m later in the season as lake water temperature rose. This system delivered a total of 10-12 GPM water flow to the facility and provided bioassay cooling water, a source of test solution diluent and a supply for holding tanks containing stock fish. Waste water from the facility was channelled to a pipe approx. 40 m long discharging to the beach south of the facility. Oil-free air was supplied to test tanks and holding tanks from an electrically-driven compressor and manifold system. Details of the laboratory set-up and test procedure are given in Part 1.

Tests conducted in the *in situ* facility consisted of static bioassays with 24-h solution replacements to determine the 96-h LC50 for Fulton River fry, lake fingerlings and smolts exposed to reagent grade cupric

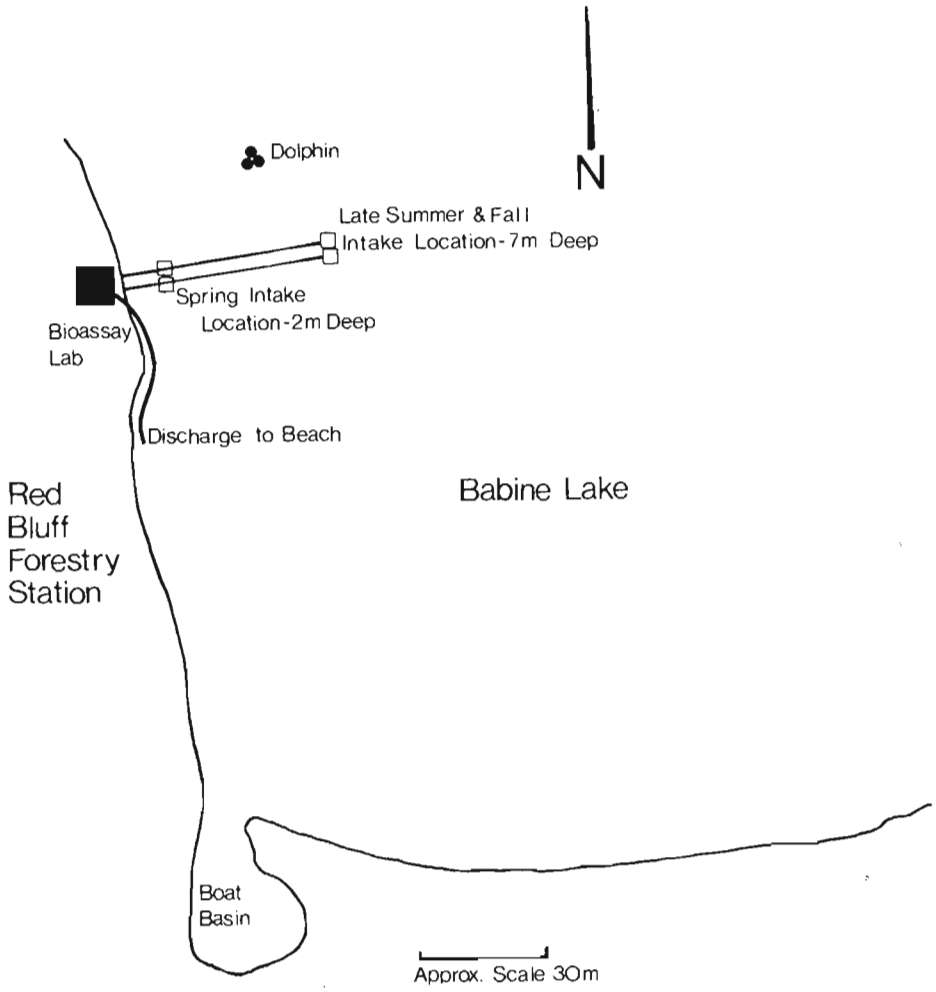


Fig. 2. Details of the Red Bluff study site on Babine Lake. Bioassays were conducted in a small building constructed on the lakeshore. Water intake locations varied with season.

chloride ($CuCl_2 \cdot 2H_2O$). The 96 h LC50 is defined as the concentration of copper lethal to 50% of the test fish within 96 hours (Sprague 1969).

Source of Test Fish and Fish Handling Procedures

Sockeye fry used for bioassays were caught in permanent fish trap structures located in the Fulton River system. All fish came from Fulton Channel No. 1 and were caught at night during their downstream migration. Fish were removed from the traps and rapidly transported in aerated plastic tubs to the test facility where they were stored in flowing aerated 100 l fibreglass holding tanks for at least 24 hours prior to bioassay. Fish were collected May 10, 14, 19, and 26th in this manner.

Sockeye fingerlings were obtained by seining in Babine Lake in the vicinity of Red Bluff by the vessel M.V. TAHLOK during August, and October 1977. These fish were collected at night and were transported to the bioassay facility in early morning hours where they were held at least 24 hours prior to testing. Collections took place August 27 and October 6 and 10, 1977. Some mortality was evident in the August stock following collection. These fish had been seined in 7 fathoms of water and it is possible that warm surface water temperature (15-16°C) during holding contributed to mortality, together with a suspected outbreak of fungus disease. For this reason, the August fingerling data is considered suspect and the October data more reliable as no holding problems were associated with the latter stocks.

Sockeye smolts that had overwintered in the lake were collected on two occasions at the Babine River counting fence located at the north end of the lake (Fig. 1). These fish were caught in smolt traps on the nights of May 17 & 31 and were air shipped in iced, oxygenated plastic bags later the same day. A portion of the May 31st collection was flown to the Red Bluff site for bioassay testing while the entire May 17 collection and the bulk of the May 31 collection was flown to Vancouver, B.C. were they were utilized in salinity transfer and growth studies as described in Part II. Figure 3 illustrates the timing of sampling of smolts at the Babine River fence in relation to the timing of the smolt migration during the month of May, 1977.

In no case, were any of the fish stocks tested at the Red Bluff site fed during holding or testing and the temperature regime was identical to

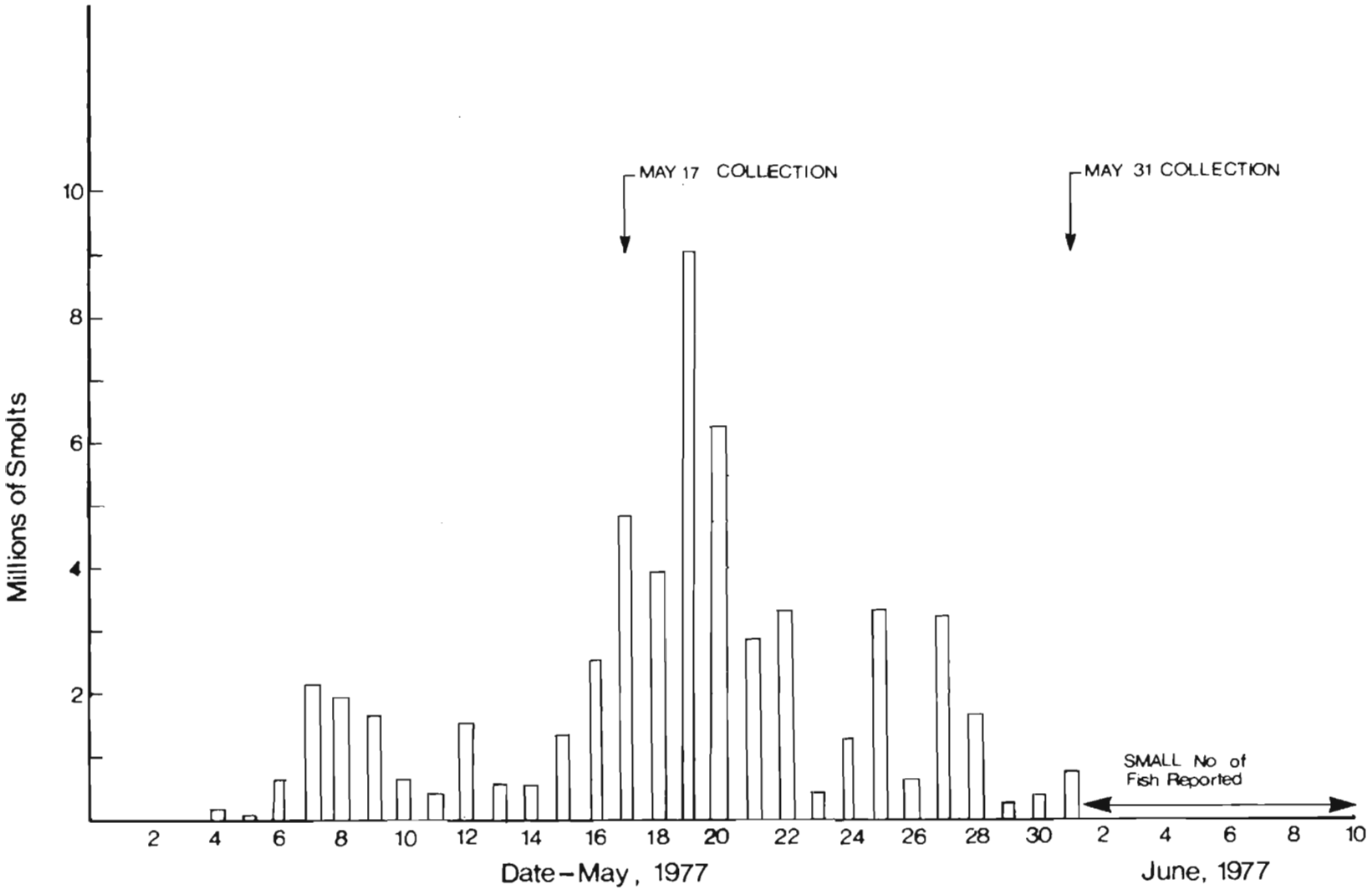


Fig. 3. Daily estimates of the magnitude of the sockeye smolt seaward migration measured at the Babine River fence in 1977. The sampling dates for the two smolt collections reported herein are indicated (data from Fisheries Service records).

that in the lake water at the intake depth at the time of the test. Natural photoperiod was maintained during holding while the bioassay test tanks received a close to normal photoperiod with overhead fluorescent lights turned off at dusk and on in the early morning.

Part I - Acute Toxicity Tests with Young Babine Sockeye Exposed to Copper Solutions

Introduction

Copper toxicity studies conducted on a variety of fish species constitute one of the better-investigated areas of fish toxicology. Detailed reviews of the subject are available [Doudoroff and Katz (1953), McKee and Wolf (1963)] and an extensive literature is evident. One of the the most important findings from all this work is the realization that copper toxicity to fish is strongly influenced by chemical properties of the test water. For this reason, it was mandatory to perform toxicity tests with Babine sockeye in the natural lake water in order to properly assess the toxicity hazard of dissolved copper. This section describes acute toxicity test procedures and results obtained with Babine sockeye fry, lake fingerlings and smolts tested in Babine Lake water.

Methods

Acute toxicity tests were conducted in acid-washed 65-l plastic tubs aerated via finely-drawn glass pipettes connected to an oil-free air source. Oxygen levels were maintained in excess of 90% saturation in all test tanks and were generally very close to the 100% saturation level. Temperature was maintained at the ambient lake water level (depending on the time of year each test was run) by immersing the test containers in the free-flowing waterbath. Test temperature in the tanks and waterbath was continuously monitored with a Taylor temperature recorder with sensor probes immersed in the waterbath.

Sockeye were tested in groups of 6 or 10 fish per test container depending upon fish size and solution volume utilized. It was desirable to keep fish loading of test solution below 0.5 g/L test solution (Sprague 1969; Davis and Mason 1973) hence bioassay tests with smolts utilized 6 fish/ 60-L of test solution while tests with fry and lake fingerlings utilized 10 fish in 20 or 60-L test volumes respectively.

Dilution water for all testing was lake water drawn from intakes located directly offshore from the testing laboratory. During the spring this water was drawn from a depth of 2 m while in late summer and the fall the intake depth was increased to 7 m in an attempt to overcome a fungus disease

outbreak in stock fish which was thought related to high surface water temperature. In all but one case, this water was not pretreated in any way and was considered representative of shallow lake water with physical and chemical properties equivalent to those in the lake at the time the test was done. The sole exception was the water used for a single bioassay determination with Fulton River fry commencing May 27, 1977. In that test, water was prefiltered with a 5 μ cartridge filter (AMF -Cuno) connected in series with an AMF-Cuno activated charcoal filter. The objective of conducting a bioassay with water filtered in this manner was to determine if filterable particulates and ligands that might act as copper complexing agents could be removed and thus influence the bioassay result. It was hoped that such a test might provide information on the nature and type of metal complexation which exists in Babine Lake water.

Stock toxicant employed in bioassays consisted of reagent grade $CuCl_2 \cdot 2H_2O$ dissolved in distilled water to make a stock solution of 0.1 or 0.2 g Cu^{++}/L. Small amounts of this stock were then accurately pipetted and diluted to the appropriate volume in each test tank to achieve a desired theoretical test concentration. Solutions were made up and gently aerated 24 hours prior to fish addition while the test tank equilibrated in the water bath. During the bioassay, fish were held in a plastic mesh basket, sized to act as a liner for the test container. Every 24 hours, test fish were transferred to a fresh test solution in an identical test container, by quickly transferring the mesh basket to the new container. In this way, fish were exposed to 24 hour replacement-type bioassay conditions in an attempt to counteract loss of toxicant from solution. Such loss might occur through uptake via the fish, complexation with fish wastes or adsorbtion on container walls or body surfaces.

As the actual nominal concentration of copper present in the water during the bioassay was of prime importance, rather than the theoretical concentration calculated by simple dilution, water samples were collected from each test tank immediately prior to starting the test or transferring fish to a new solution. To test for loss of toxicant from solution, additional water samples were also taken in some bioassays on a more frequent basis. Water samples were collected in acid-washed polyethylene bottles and were immediately adjusted to pH 1.5 by addition of HNO_3 and were then frozen. These samples were subsequently analysed for both

total and extractable copper concentration utilizing atomic absorption spectrophotometry at the Environmental Protection Service/Fisheries analytical chemistry laboratory in West Vancouver. These analyses showed that no significant difference was present between total and extractable copper concentration in a given sample hence all copper concentrations reported herein are total copper levels as measured in test solutions.

All bioassay tests were of 96 hours duration with 24-h solution replacement with the exception of range-finding tests which were sometimes of shorter duration and were used to roughly approximate the LC50 by providing a broad selection of test concentrations. Once a rough approximation of the LC50 was obtained, more rigorous 96-h LC50 tests were set up with concentrations closely spanning the LC50. Typically there were 10 test tanks including 1 control (with no copper added) with test fish roughly graded for uniform size and assigned randomly to each tank. Space limitations prevented replication of every concentration, however an attempt was made to replicate concentrations expected to give partial mortality data within 24-h. Observations of test tanks were considerably more frequent than the minimum observation regime suggested by Sprague (1969) and continued on a 24-h basis. When dead fish were observed (as determined by cessation of all movement including respiratory activity) the time was noted and the fish were removed, blotted dry, weighed and measured (fork length).

Bioassay results were treated in the following manner. For a given test concentration, cumulative percentage mortality was plotted on log-probit paper and the time to 50% mortality determined according to the graphical method of Litchfield (1949). A toxicity curve was constructed by plotting the time to 50% mortality at each concentration against concentration on log-log paper. If data were poor, with high variability between replicates, and little partial mortality at 96 h, it was sometimes only possible to estimate the LC50 from the shape of the toxicity curve or determine that it lay between certain concentrations which produced zero or 100% mortality. However, a number of bioassays yielded more complete data with several concentrations causing partial mortality at 96 h. In these cases, it was possible to prepare a log-probit plot of concentration vs partial mortality at 96 h (Sprague, 1969) and by utilizing the graphical methods of Litchfield and Wilcoxon (1949), calculate the LC50 complete with 95% confidence limits.

Water hardness was periodically determined during the study using the EDTA method (Standard Methods, 1975) and varied from 36-46 mg/L $CaCO_3$. These values are in general agreement with hardness values gathered at a variety of locations in Babine Lake at various times during 1974 and 1975 (Stockner and Shortreed 1976) and by Chau and Wong (1975).

Results

Results of all bioassay tests conducted with Babine fry, lake fingerlings and smolts tested in lake water with $CuCl_2$ added are summarized in Table 1. Over the course of the tests, water temperatures showed a warming trend from 5.0°C in early May to a maximum of 15.5°C in early September. By October, test temperature declined to 7-10°C. pH values in test tanks were generally close to neutrality with the maximum pH range existing among individual test tanks of a given series summarized in Table 1. Fish loading density in test tanks was usually below 0.5 g/L, the desired objective (Sprague, 1969), with the exception of tests done with lake fingerlings in October when loading density slightly exceeded the objective.

Although some tests only yielded a rough estimate of the 96 h LC50 for a given group of fish, LC50 values complete with 95% confidence limits were obtained for fry, lake fingerlings and smolts. Figure 4 illustrates toxicity curves obtained for copper-exposed sockeye fry in untreated Babine Lake water and in water pre-filtered through a 5 μ filter and activated charcoal filter. The shape of the toxicity curves for both water types is similar although the time to 50% mortality appeared less for non-filtered water in comparison to filtered water at the more toxic concentrations. According to the generalized rule that LC50's are not significantly different if their 95% confidence limits overlap (J.B. Sprague - personal communication) comparison of LC50's for copper exposed fry in untreated and filtered water suggests no significant difference was present.

Two LC50 values were obtained with Babine smolts exposed to copper in lake water. The first test, started on June 4, 1977, yielded a 96 h LC50 and 95% confidence limits of 240 (216,266) $\mu g Cu^{++}/L$ (total copper). These data were highly variable as suggested by the confidence limits and the second test result 103 (95, 112) $\mu g\ Cu^{++}/L$ (Fig. 5) showed considerably less variability. Stocks of smolts varied considerably in size and degree

Table 1. Babine Sockeye - Acute Toxicity Test Characteristics and Results

Test Date (Start)	# fish/ tank	Test soln. Vol.(L)	T°C (range)	pH (range)	$\bar{x}$ fish wt.(g)	$\bar{x}$ fish leng (cm)	loading density g/L	Estimated LC50 µg Cu++/L	Calculated LC50 +95% C.L. µg Cu++/L	Comments
					Fulton River Fry - May					
12/5/77	10	20	5.0	6.1-6.9	0.139	2.87	0.070	>188, >270	-	range test
16/5/77	10	20	6.5	6.4-6.9	0.138	2.96	0.069	214	-	
21/5/77	10	20	4.5-5.5	6.3-6.8	0.132	2.95	0.066	-	220(213,227)	
27/5/77	10	20	8.5-10.5	6.5-7.1	0.136	2.97	0.068	-	210(200,221)	filtered H_2O
					Babine River Smolts - June					
4/6/77	6	60	6.5-12.0	6.3-7.2	4.63	8.07	0.463	-	240(216-266)	variable data
8/6/77	6	60	8.5-11.0	-*	4.69	8.35	0.469	-	103(95,112)	
					Lake Fingerlings - Aug./Sept.					
28/8/77	10	60	16-17	6.7-6.9	2.07	6.01	0.335	-	-	control mortality fungus problems
31/8/77	10	60	15-15.3	6.9-7.4	1.94	6.05	0.323	-	-	" "
5/9/77	10	60	15.5	6.0-7.6	2.22	6.37	0.370	-	-	" "
6/9/77	10	60	14.0-15.5	6.8-7.2	2.31	6.61	0.385	<150	-	" "
9/9/77	10	60	14.0-15.5	6.9-7.1	2.13	6.67	0.355	83	-	" ""
					Lake Fingerlings - Oct.					
7/10/77	10	60	9.5-10.0	6.8-7.2	3.28	7.26	0.547	>160	-	no control mortality
12/10/77	10	60	7.0-9.5	6.8-7.3	3.90	7.17	0.650	-	240(202,285)	variable data

* pH meter malfunction

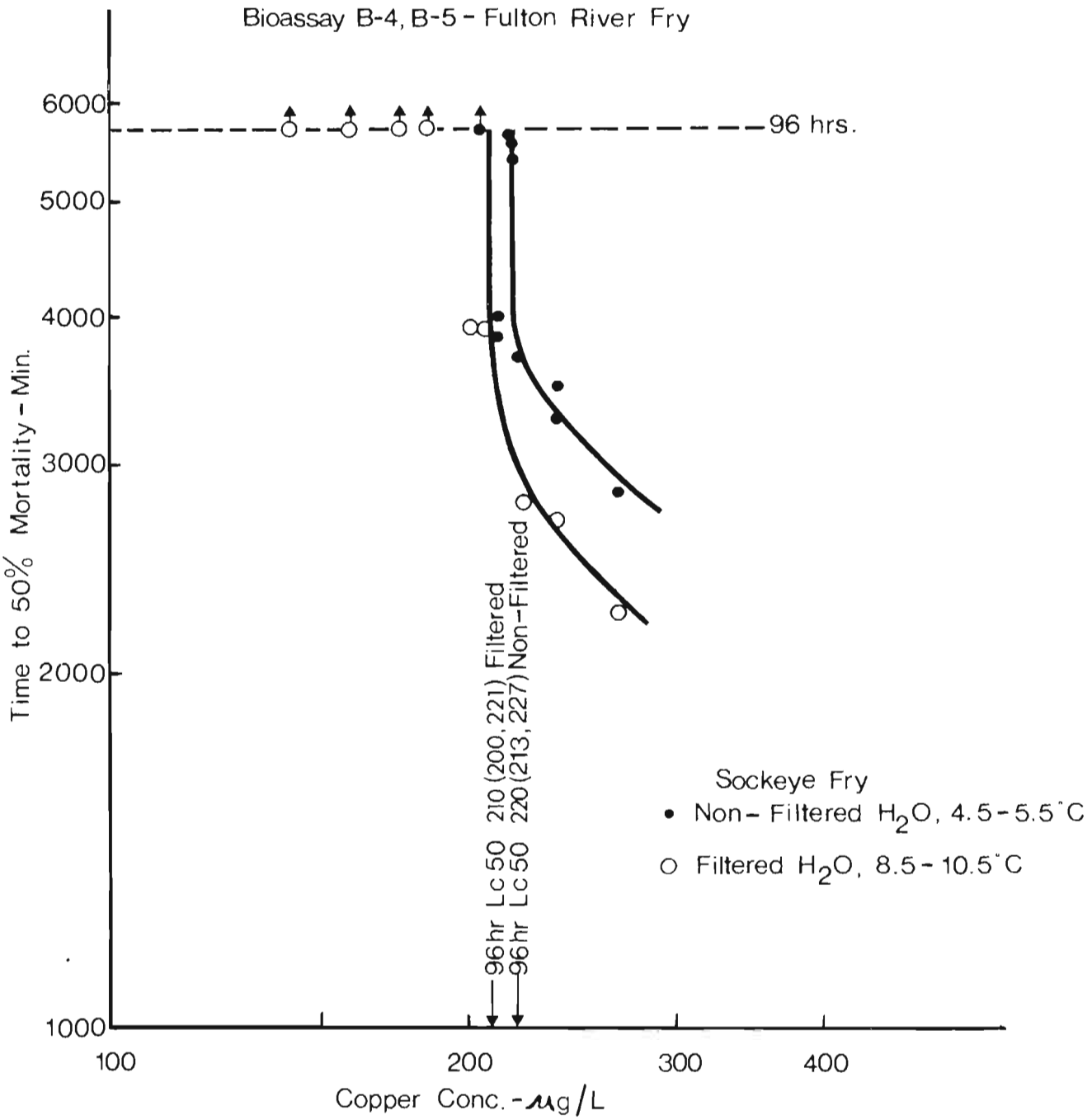


Fig. 4. Toxicity curves for Fulton River sockeye fry exposed to copper in filtered and non-filtered Babine Lake water.

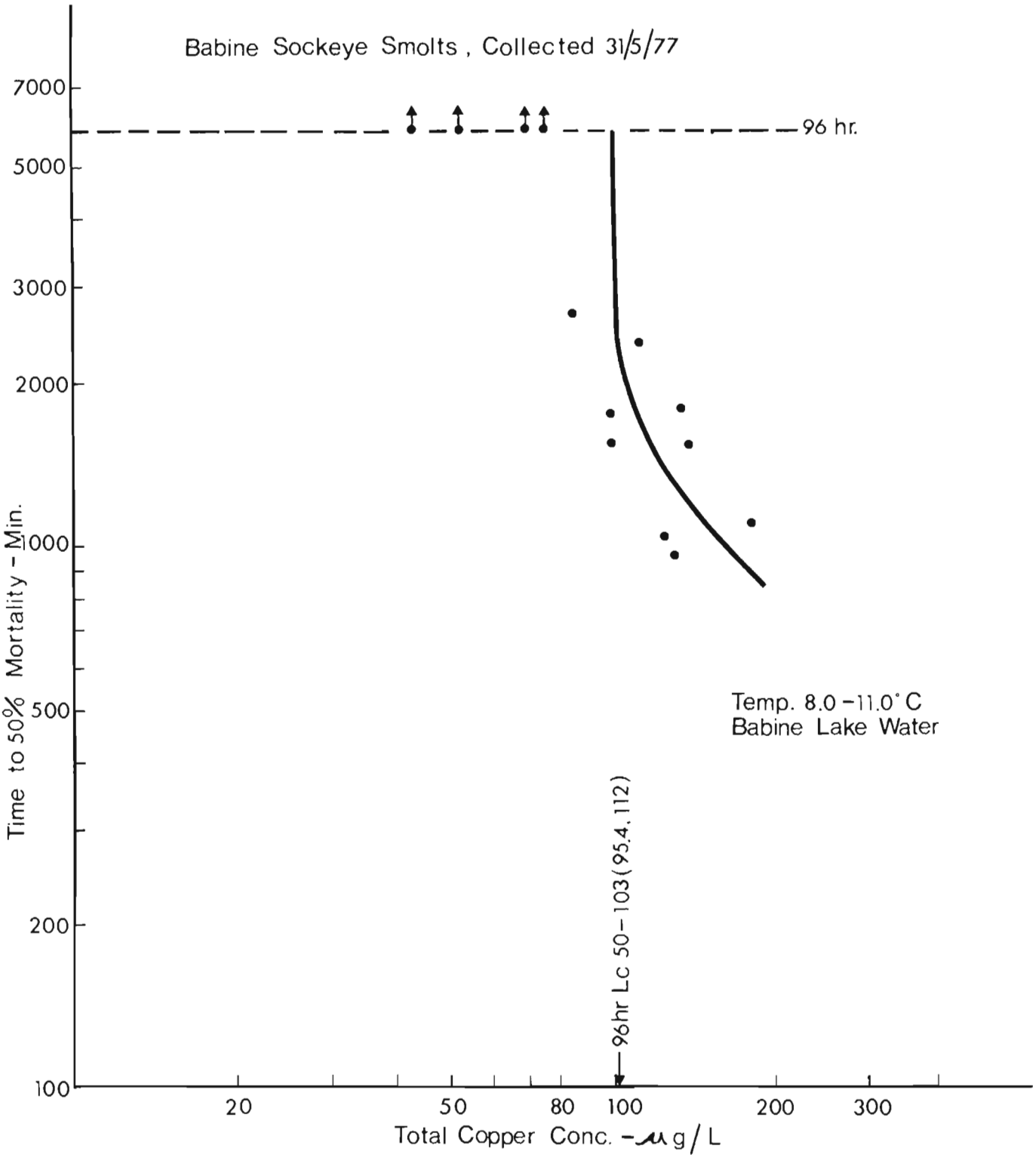


Fig. 5. Toxicity curve obtained for Babine sockeye smolts exposed to copper in lake water.

of parasitism (see Section III) and these factors may have contributed to the disparity between the above results for Babine smolts.

Bioassay tests conducted in late August and early September with lake fingerlings collected by seine were not satisfactory. No accurate determination of LC50 was obtained as experiments were complicated by control mortality and high variability among replicates. An outbreak of fungus disease, possibly related to high water temperature and handling, was suspected to be the source of these difficulties. Subsequent tests conducted with lake fingerlings in October when water temperatures were lower, revealed no problems with control mortality or outbreaks of fungus and a 96 h LC50 value of 240 (202, 285) µg Cu^{++}/L was obtained.

Discussion

Babine sockeye fry, smolts and lake fingerlings tested in natural Babine Lake water proved to be fairly resistant to copper. LC50's with confidence limits exceeded a total nominal dissolved copper concentration of 200 µg Cu^{++}/L in all but one test for all three age classes of fish. It is well known that a variety of chemical factors affect toxicant availability to fish (Lee 1973) and that metals, in particular, are susceptable to complexing with a variety of agents which render them less toxic to aquatic life compared to their free ionic forms. Lloyd and Herbert (1962) demonstrated that progressively higher copper concentrations were required to kill rainbow trout, *Salmo gairdneri*, as water hardness increased. As hardness increased from 15 to 320 mg/L as $CaCO_2$, the 48 h LC50 of rainbow trout increased from approximately 45 to 450 µg/L Cu^{++}. Stiff (1971a) and Pagenkopf et al(1974) pointed out the importance of alkalinity and bicarbonate relations in considering the toxic effects of copper at natural pH levels. Stiff demonstrated that at low pH the species of copper available are free cupric ion, Cu^{++}, and the cupric carbonate complex, $CuCO_3$. He observed that "free cupric ion represents only a fraction of total soluble copper present in a bicarbonate solution of the concentration and pH range of most natural waters (pH 6.5-8.5)".

Stiff (1971b) reviewed the physical states in which copper may be found in natural waters. Copper may exist in the particulate, colloidal or

soluble state, depending on the nature of the water body and its constituents. Particulate copper state may include copper oxides, sulphides, malachite [$Cu_2(OH)_2CO_3$], insoluble organic complexes or copper adsorbed on clay or other mineral solids. Copper in the colloidal state may be complexed with polypeptide material, some clays and metallic hydroxide precipitates. Soluble copper state, as defined by that present in water which has passed through a 0.45 µm filter, includes the free cupric ion and soluble complexes, possibly including finely divided precipitated species or clay particles small enough to be non-filterable. It is only the soluble copper state that is thought to be available to fish and thus toxic in nature.

Thus in natural waters, the soluble copper forms, which may be of a very different concentration from the total copper concentrations, are the species of biological significance. Babine Lake water is not hard (total hardness = 36-46 mg/L as $CaCO_3$), hence it is unlikely that hardness and bicarbonate relations play a dominant role in reducing the availability and toxicity of copper to fish. Indeed, Chau and Wong (1975) deduced that the lake water has ample capacity to complex ionic copper and other heavy metals. They reasoned that the high complexing capacity of Babine Lake water was related to a high organic matter content as indicated by measurements made of dissolved organic carbon and dissolved organic nitrogen. Chau and Wong found that all the copper present in their samples collected from five Babine Lake stations in the fall was in the completely bound form and cited the 'prevalence of humic materials' (Stockner and Shortreed, 1974) as being a likely source of complexing ligands. Chau and Wong, utilizing the methods of Chau et al. 1974 , determined the copper complexing capability of Babine water samples and arrived at a complexing capacity value, averaged over samples from 5 stations, of 1.64 µ moles Cu/L. This means that, according to Chau and Wong's calculations, one liter of Babine Lake water should be able to complex slightly in excess of 100 µg Cu^{++}. Assuming this value is correct, our sockeye LC50 values reported herein look reasonable in comparison to those reported in the literature. Lloyd and Hurbert (1962) obtained a 48 h LC50 for rainbow trout of approximately 100 µg/L Cu^{++} at a total hardness of 40 mg/L as $CaCO_3$. Lorz and McPherson (1976) found a 96 h LC50 of $CuCl_2$ for yearling coho salmon (*Oncorhynchus kisutch*) of 60-74 µg/L, depending on season, in well water, slightly harder than Babine water, and presumably low in organic ligands.

The above discussion also implies that the 96 h LC50 value of 103 µg Cu^{++}/L obtained for sockeye smolts in the test beginning June 8, 1977, is probably an anomaly. If Chau and Wong's calculated complexing capacity value is correct, then virtually all the copper present at a concentration equivalent to that 96 h LC50 should have been complexed. It is therefore likely that the other bioassay test which yielded a 96 h LC50 of 240 µg Cu^{++}/L for smolts is more reliable, especially as it is fairly close to the result obtained in most other tests with fry and lake fingerlings. The explanation for these two divergent test results is not apparent at this time.

Bioassays conducted on Fulton River fry commencing on May 21 and 27, 1977, were designed to test for possible filterable complexing agents in the lake water (Table 1, Fig. 3). A comparison of fry bioassays conducted with prefiltered lake water, (after passing the diluent water through a 5 µ filter and activated charcoal filter) and non-filtered water revealed no significant difference in LC50 value between filtered and non-filtered water subsequently dosed with $CuCl_2$. At the higher test concentrations, mortality did appear more rapid in non-filtered water, suggesting some effect of filtration may have been present. There results suggest that complexing ligands are not easily removed by filtration or reaction with activated charcoal.

In conclusion, it would appear that no current acute toxicity hazard due to copper exists in Babine Lake water with the present low levels of copper in the water and its considerable complexing capacity. These factors should give a considerable safety margin to fish stocks in the area , should leaching or periodic spills of copper concentrate occur from the mining operations in the area.

Some critique of methods employed in the acute toxicity test series is in order. Bioassays were conducted in plastic containers which likely adsorbed some toxicant onto their walls, reducing copper availability to the test fish over time. Comparison of theoretical and nominal total copper concentrations showed that the nominal copper levels, as measured in test solutions, averaged 12% lower than the theoretical values.

Results therefore, are expressed as nominal copper concentrations, as measured during the bioassay test from water samples taken during testing. Furthermore, tests were of the 24-h solution replacement variety with fresh toxicant added daily. These tests, which are of the semi-static type, probably yield somewhat elevated LC50 values in comparison to continuous flow techniques (Chapman and McCrady 1977). Finally, it is likely that LC50 values determined for fry, lake fingerlings and smolts over the spring, summer and fall period are not strictly comparable due to differences in fish size, age, test temperature and seasonal variation in organic content of the diluent water. Indeed, Chau and Wong (1975) speculated that both dissolved organic matter and copper complexing capacity of Babine Lake water would increase in the summer months when organic degradation rates are high.

Part II Growth, Sea Water Survival and Salinity Transfer Studies in Relation to Lake Residence and Copper Exposure at Sublethal Concentrations

Introduction

Sockeye salmon fry migrating into Babine Lake from the Fulton River, Pinkut Creek and other systems flowing into the lake, overwinter in the lake beneath the ice before migrating to the sea via the Babine and Skeena rivers as smolts. Distribution of fry and fingerlings in the lake is not uniform (McDonald, 1969) and mainlake fry from Fulton River disperse rapidly to the south. Over the summer they gradually move northward with a maximum population density off the Fulton River in the fall. These migratory pathways appear to coincide with maximum availability of phytoplankton and zooplankton in the lake, as the south basin of the lake shows considerably more production than the north (Narver, 1967; Stockner and Shortreed 1974). Three groups or demes (reproductively more-or-less isolated groups of a population) of sockeye juveniles are found in Babine Lake (Johnson & Groot, 1963): the Nilkitkwa Lake and North Arm deme, the Morrision Arm deme and the Main Lake deme. In terms of numbers of fish, the Main Lake deme predominates and contains the fry emerging from the Fulton River and Pinkut Creek enhancement facilities.

Annual seasonal migration takes place via the North Arm and Nilkitkwa Lake route with the fish moving down the Babine River to the Skeena system. A bimodal frequency of smolt out-migration frequently occurs, as determined by trapping a portion of the run at the lake outlet (Groot, 1972), when migration from all points of the lake occurs simultaneously (Johnson and Groot 1963). Smolts from the Nilkitkwa Lake and North Arm reach the outlet first, followed by fish from the Morrison Arm and Main Lake regions, producing a bimodal distribution during May and early June.

Lorz and McPherson (1976) reported that acute and chronic exposure of yearling coho salmon to concentrations of copper (5-30 ug/L) in fresh water had deleterious effects on downstream migration, gill ATPase activity and survival in seawater. As the concentrations tested by Lorz and McPherson were similar to those measured in Babine water (4 µg/L - Stockner and Shortreed 1976; 16-44 µg/L - Chau and Wong 1975), concern arose as to whether copper exposure in the lake may have similar deleterious effects on Babine smolts producing reduced saltwater survival. This portion of the study was

designed to examine saltwater survival success, growth rate following saltwater transfer, and the sublethal effects of copper exposure on osmoregulatory capability in Babine sockeye smolts.

Methods

Two groups of sockeye smolts were caught in fish traps on May 17 and 31, 1977, and were air-shipped to the Pacific Environment Institute as outlined in the General Methods. These stocks were transferred upon receipt at the institute to 750-l fibreglass holding tanks with a flowing well water supply (15-20 mg/L hardness as $CaCO_3$) and gentle aeration. Several experiments were carried out with these stock fish as follows:

1) Salinity challenge experiments to test for osmoregulatory capability in Babine smolts.
2) Salinity challenge experiments to test for osmoregulatory capability in smolts exposed for 144-h to 30 µg/L Cu^{++} in the laboratory.
3) Growth rate and survival determinations in Babine sockeye for 60 days following transfer to saltwater.

Details of the experimental design for these studies follow :

Salinity Challenge Test - Babine Smolts

The saltwater challenge test has been used as a rapid and simple method to measure smolting in juvenile salmon (Clarke and Blackburn 1977). The procedure consists of transferring fish directly from fresh water to sea water and measuring plasma sodium levels after a short time in seawater. Clarke and Blackburn have demonstrated that fully smolted salmonids have plasma sodium values of 170 m Eq/L or less within 24-h of salt transfer while non-smolted individuals have plasma sodium values in excess of 200 m Eq/L 24 hours after transfer. Non-smolted individuals also exhibit considerable mortality following abrupt transfer in addition to having elevated plasma sodium levels. In this study the salinity challenge procedure was utilized to see if Babine sockeye, having been exposed to low levels of copper in the lake, would experience osmoregulatory difficulty upon saltwater transfer.

The procedure employed was essentially that of Clarke and Blackburn (1977) with slight modifications. Three groups of 48 fish/group were tested

as follows:

1) May 17 collection, 10 days laboratory acclimation with feeding prior to testing.
2) May 31 collection, 10 days laboratory acclimation without feeding prior to testing.
3) May 31 collection, 10 days laboratory acclimation with feeding prior to testing.

The 10 day acclimation period was provided to allow recovery from transportation and holding stress as well as to simulate the length of time that these fish would take to transit the Skeena system after the time of their collection as out-migrants from the lake. Ten days is an arbitrary estimate only of migration time as no one appears to have been successful in catching Babine smolts in the mouth of the Skeena. Stocks which were fed commenced feeding 2-3 days following air-shipment and were fed twice daily to satiation on Oregon moist pellets and frozen Euphausids until 24 h prior to testing.

The salinity challenge procedure was as follows: For each test, 48 fish from the 200 L fibreglass laboratory acclimation tank were transferred in groups of 16 to three 90 L fibreglass tanks with flowing water and aeration. One of these test tanks contained flowing fresh water (T = 11°) while the remaining 2 had flowing sea water (T = 11.4-12.2°C; S = 27.2-29.6^{o}/oo). After 24 h, the fish in the freshwater control tanks and one of the seawater tanks were sampled The remaining tank was sampled the next day following 48 hr seawater exposure. Sampling consisted of anesthetizing the fish in groups of 8 from each tank in a solution of 0.5 mL/L 2-phenoxyethanol, blotting the fish dry and severing the tail with a sharp scalpel. A blood sample was collected from the dorsal aorta in a capillary tube heparinized with ammonium heparin and was sealed with capillary tube sealer at one end. Each tube was spun for 3 min at 10,000 x G in a microhematocrit centrifuge and the hematocrit (packed blood cell volume expressed in vol. %) was read using a hematocrit tube reader. The packed blood cells were then separated from the plasma by scoring the capillary tube and breaking off the segment of the tube containing the blood cells. The remaining portion of capillary tube, complete with plasma sample, was sealed at both ends, placed in a coded vial and frozen for later analysis. Care was taken to ensure that plasma within the tube did not come into contact with the sealing compound. Fish weight (g) and fork length (cm) was determined for each fish sampled.

Plasma analysis for plasma sodium determination consisted of adding 5 µL samples of plasma collected by micropipette to 200 µL $SrCl_2$ diluant (Paschen & Fuchs, 1971) and aspirating the sample into a Perkin Elmer model 403 atomic-absorbtion spectrophotometer utilizing acetylene flame. A single working sodium standard was utilized (Paschen & Fuchs 1971) with a concentration of 140 m Eq/L Na^+. Samples were processed by alternating small groups of control (freshwater) and experimental (saltwater) plasma samples and standards to check for instrument drift and stability of calibration.

Salinity Challenge Test-Smolts Exposed to a Sublethal Copper Concentration

To test the sensitivity of Babine sockeye smolts to short-term, sublethal copper exposure and confirm the findings of Lorz and McPherson (1976) with coho, a laboratory experiment was conducted with smolts from the May 31, 1977 collection group. In this test 48 smolts were acclimated without feeding for eight days in aerated, flowing freshwater at 11 ± 0.5°C in a 200 L fibreglass tank. At the start of the test, 32 fish were transferred to an identical tank with a standpipe sized to contain 140 L of well water with sufficient $CuCl_2 \cdot 2H_2O$ added to give a total copper concentration of 30 µg/L. The tank was then dosed with a fresh solution of 30 µg/l copper for 144 h utilizing a Buchler peristaltic pump at a flow rate of 900 mL/h supplemented with daily replacement of 100 L of the total solution volume. Following copper exposure, the 32 copper-exposed fish were placed in groups of 16 in two 90 L fibreglass tanks with flowing seawater (T = 11.4-11.9°C, S = 27.2-27.8°/oo) while the remaining non-copper-exposed group of 16 fish was placed in an identical tank with flowing freshwater (T = 11 ± 0.5°C). The non-copper exposed freshwater fish and one group of copper and saltwater exposed fish were sampled at 24 hours while the remaining group of copper and saltwater exposed fish were sampled at 48 hours after transfer. Anesthesia, sampling and plasma sodium analysis was identical to the procedure described previously for non-copper exposed Babine smolts.

Growth Rate and Saltwater Survival in Babine Sockeye Smolts

Growth studies were carried out on both collecton groups of Babine sockeye smolts. Upon receipt at the laboratory, the fish were held in 750 L fibreglass stock tanks with aerated, flowing freshwater. The fish typically refused to feed for the first day or two following transport but subsequently began accepting zooplankton (Euphausids) and then Oregon Moist pellets. Sixteen to twenty days after transport, a group of 60 smolts from each collection was transferred to a fibreglass tank containing 150 L of aerated, flowing freshwater. After 2-3 days recovery, gradual saltwater acclimation was started by supplying each tank with water from a header tank where salt and fresh water were mixed. Each day the flow of salt water to the header tank was gradually increased while the freshwater flow was reduced slightly. By the seventh day of this treatment, the fish were on full salt water supply (27± 1°/oo). Over a period of 60 days all fish were fed Oregon Moist pellets to satiation twice daily (early morning and late afternoon). Excess ration was offered in the amount of approximately 25% of wet wt/fish at each feeding period and uneaten food was removed at the end of each day via a siphon hose.

Growth rate in the two test groups was ascertained by periodic weighing of the fish. Every 15 days, a group of fish was removed and lightly anesthetized in 2-phenoxyethanol (0.5 ml/L). Each fish was blotted dry, weighed and measured. The usual procedure was to weigh 20 fish in this manner and remove a subsample of 5 fish at random for dry weight determination. The remaining fish were allowed to recover from anesthesia and returned to the test tank. Towards the end of the test period, small numbers of fish were sampled due to mortality and reduced availability of fish. Growth rates were calculated for the period between successive weighings according to the following formula (Brown 1957):

$$G = 100 \cdot \frac{\log_e YT - \log_e Yt}{T - t}$$

where: G = specific growth rate (% /unit time)
YT = size at end of period (wt or leng.)
Yt = size at beginning of period (wt or leng.).
T = time of final observation
t = time of initial observation

In addition to weight, length and growth rate in the experimental growth tanks, daily mortality, temperature and salinity were recorded over the 60 day study period. Utilizing weight and length data, fish condition factor was calculated from $CF = 100 \cdot wt/leng^3$ (wt = wet weight, g; leng = length in cm, CF = condition factor). This factor shows the relative fatness or condition of a fish (Lagler, 1956). A record of mortality was also maintained in the stock tanks from which the experimental fish were drawn. The stock tanks were held for 20-33 days on freshwater and were then gradually converted to salt water. Survival in salt water in the stock tanks was followed for 2 months after salt conversion.

Results

Salinity Challenge Tests - Babine Sockeye Smolts

Figure 6 illustrates the results of salinity challenge experiments with Babine sockeye smolts from the two collection groups. Plasma sodium levels are shown as mean averages ($\pm$ 2 standard errors) in fish sampled in fresh water and following 24 or 48 hours exposure to sea water. Actual data values with accompanying data on fish size, test temperature, salinity and mortality are summarized in Appendix 1. Plasma sodium levels in freshwater fish averaged 156-160 m Eq Na^+/L and tended to rise slightly upon saltwater exposure in the non copper-exposed groups. Students T-tests (Appendix 1) revealed that in some instances the elevation in plasma sodium level following salt transfer level was statistically significant.

Results of the copper-exposure experiment, conducted on fish from the May 31st collection, showed a marked effect of exposure for 144 h to 30 µg/L Cu^{++} on osmoregulatory capability. Fish exposed to copper in freshwater and transferred to salt water from 24 or 48 h exhibited mean plasma sodium levels of 192 and 203 m Eq Na+/L respectively. These levels were significantly different from the freshwater value as well as any other saltwater exposure values at the 0.005 level of significance.

Although 16 fish were utilized for each component of the tests, plasma samples were not obtained from every fish. In non-copper exposed groups, no mortality occurred in either salt water or fresh water test groups

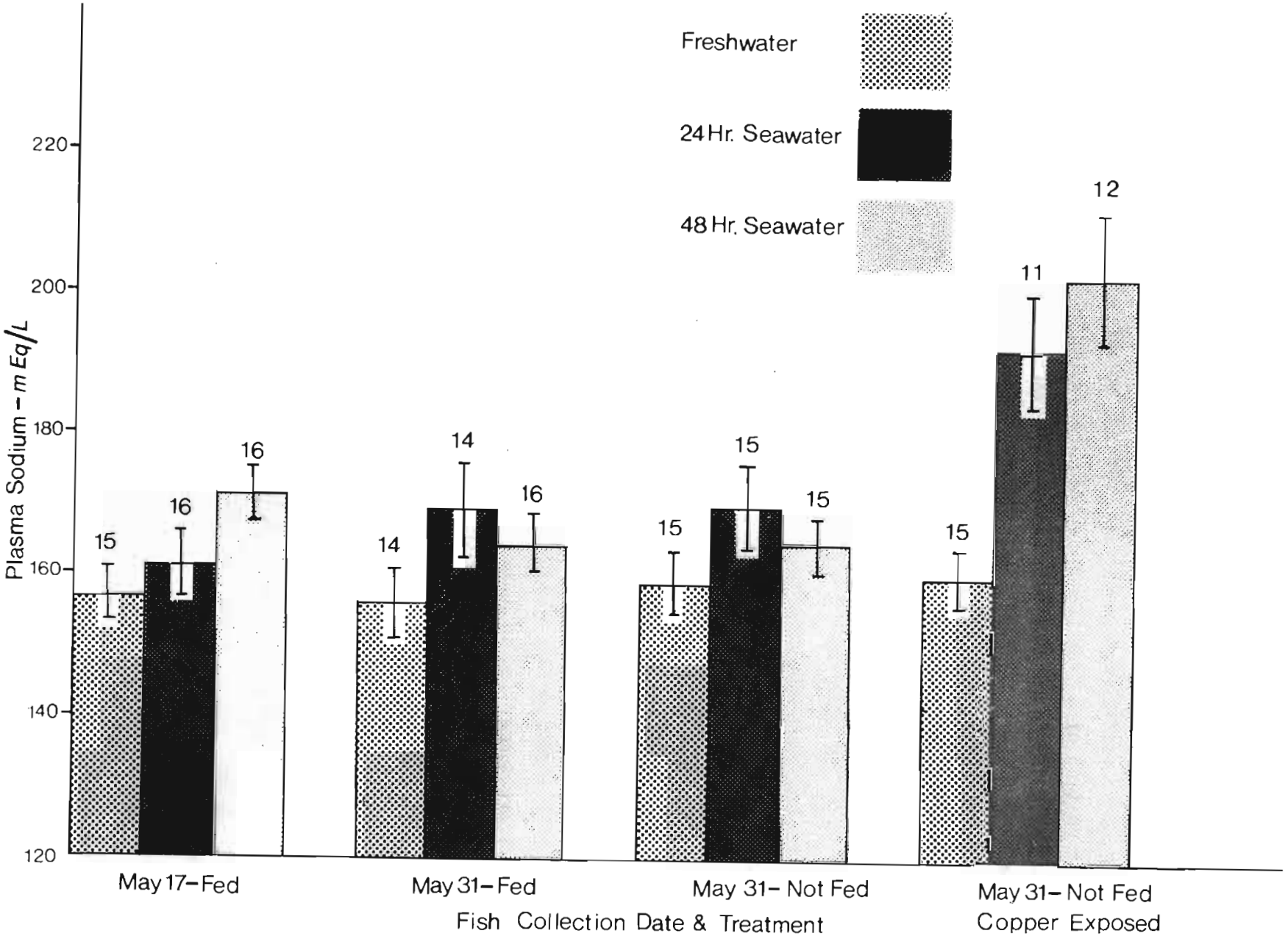


Fig. 6. Plasma sodium values in test groups exposed to 24 or 48 h of full strength sea water in comparison to freshwater control groups. Histograms are based on mean plasma sodium values shown ± 2 standard errors (vertical bars). The number of fish utilized to compute each mean is indicated.

however in some cases insufficient blood was obtained from a given fish for plasma analysis or hemolysis was present and the sample was discarded. In the case of copper-exposed fish subjected to salinity challenge however, 2 fish died prior to sampling while in sea water in both the 24 and 48 h test groups, although none had died during the freshwater copper exposure phase of the experiment. Numbers of fish utilized to compute a mean plasma sodium concentration are given above each average in Fig. 6.

Growth Rate and Salt Water Survival

Figures 7-9 illustrate the results of a sixty day salt water growth study with groups of smolts from both collections. Average weights and lengths increased during the test period and there was a trend towards increasing condition factor although that trend was more marked in the May 17 collection group. Condition factor was initially low in both groups. The May 17th collection group had a starting CF of 0.75 while the May 31 group had a CF of 0.69. Temperature increased slightly during the study period and fluctuated over a range of approximately 10-12°C due to changes in temperature of incoming seawater which were likely related to oceanographic conditions at the 20 m deep intake of the water system. Once salinity acclimation was complete, salinities in both test groups were approximately 27± 1 $^{o}/_{oo}$ with slight fluctuations over time. Both groups of test fish exhibited a steady rate of saltwater mortality that was virtually linear for 30-40 d and then appeared to decrease towards the latter portion of the test period. As 60 fish were used in each test group, this experimental mortality accounted for approximately 35% of each group by the end of the test period.

Specific growth rates, calculated on the basis of wet weight changes between successive samplings in both test groups, are illustrated in Fig. 9. Both groups showed the greatest growth in the second sampling period coincident with completion of acclimation. Subsequently growth rate declined, particularly in the May 31 group, and the growth rates in both groups in the final sampling period were approximately 1% weight/d. Over the 4 observation periods, growth averaged 1.1%/d in the May 17 collection group and 1.02% in the other test group.

Figure 7. Changes in weight and length, condition factor and cumulative mortality for the May 17 smolt collection group during the saltwater growth study. Weight and length values plotted are means $\pm$ 2 S.E. and the number of fish sampled to calculate each mean is given above the value. Temperature and salinity accompanying the test are indicated.

Saltwater Growth & Survival – May 17/77 Collection, Babine Sockeye Smolts

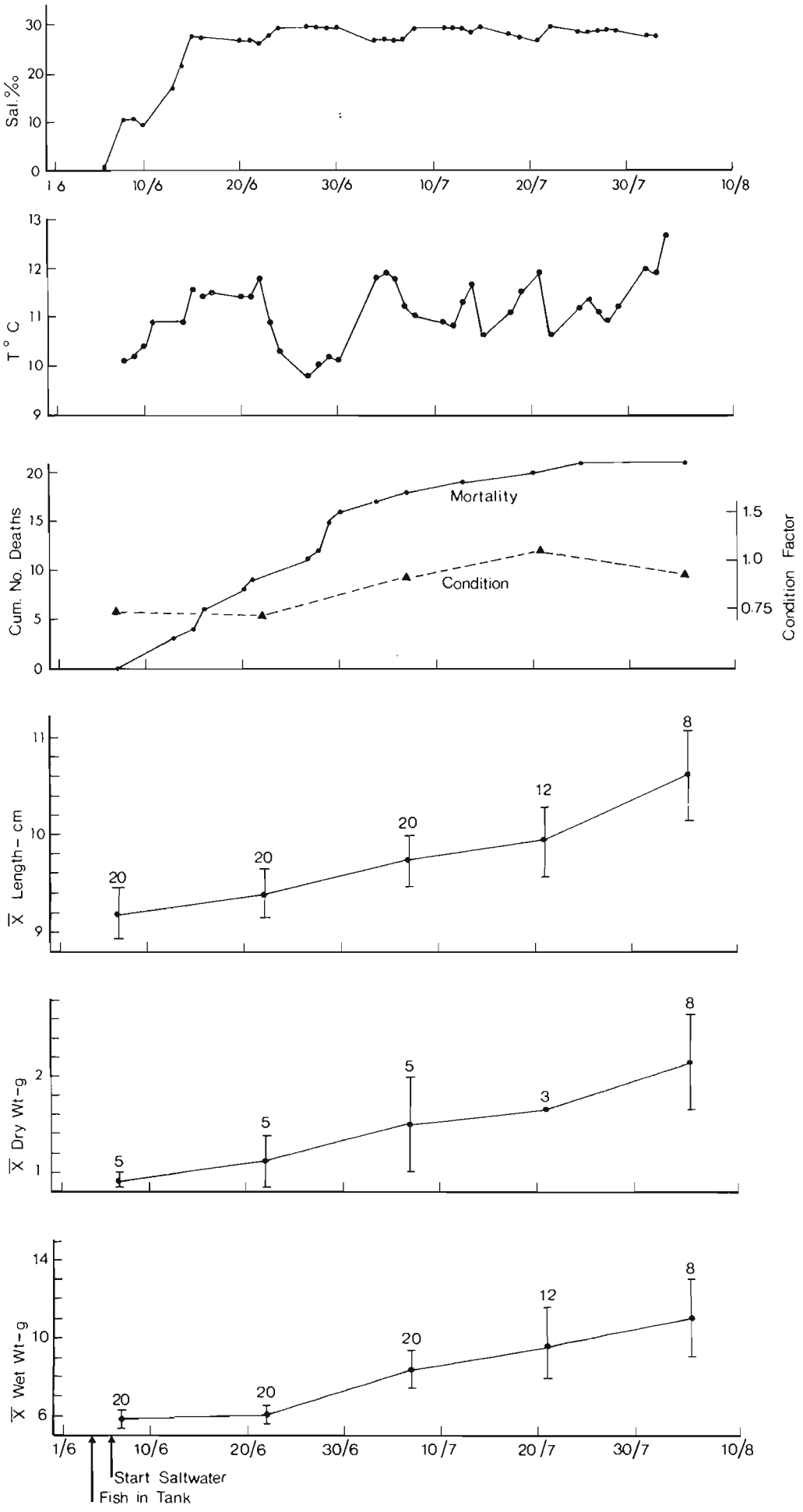


Figure 8. Changes in weight and length, condition factor and cumulative mortality for the May 31 smolt collection group during the saltwater growth study. Weight and length values plotted are means ± 2 S.E. and the number of fish sampled to calculate each mean is given above the value. Temperature and salinity accompanying the test are indicated.

Saltwater Growth & Survival - May 31/77 Collection, Babine Sockeye Smolts

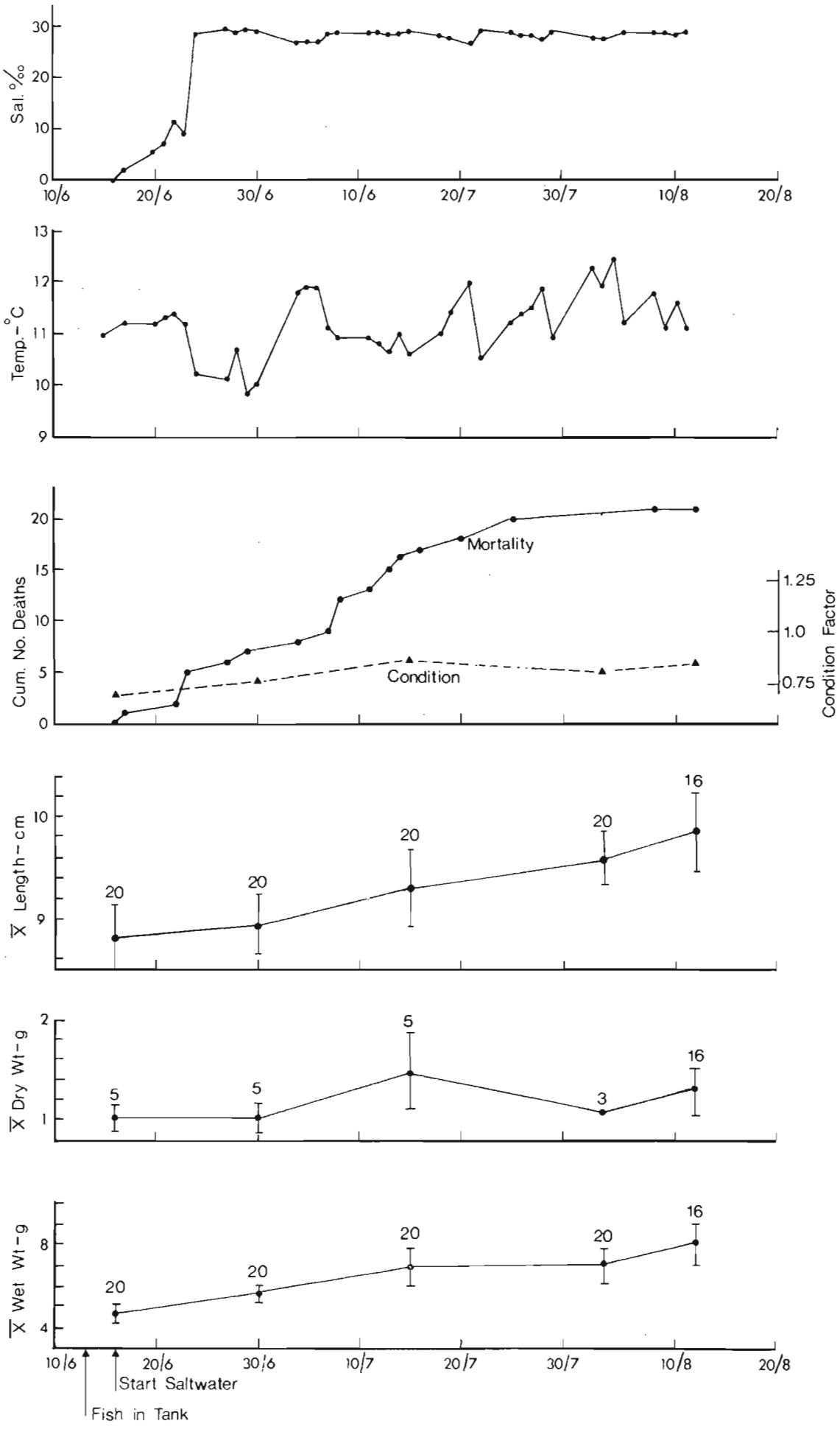
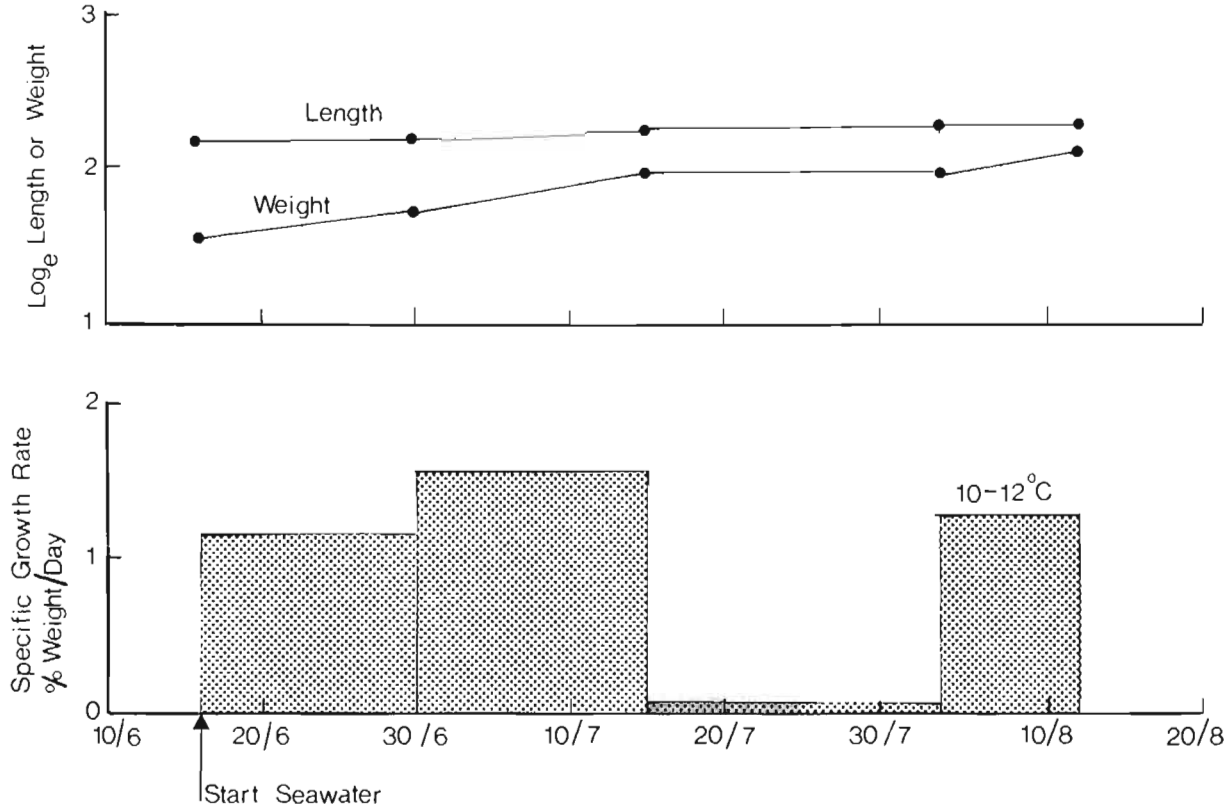
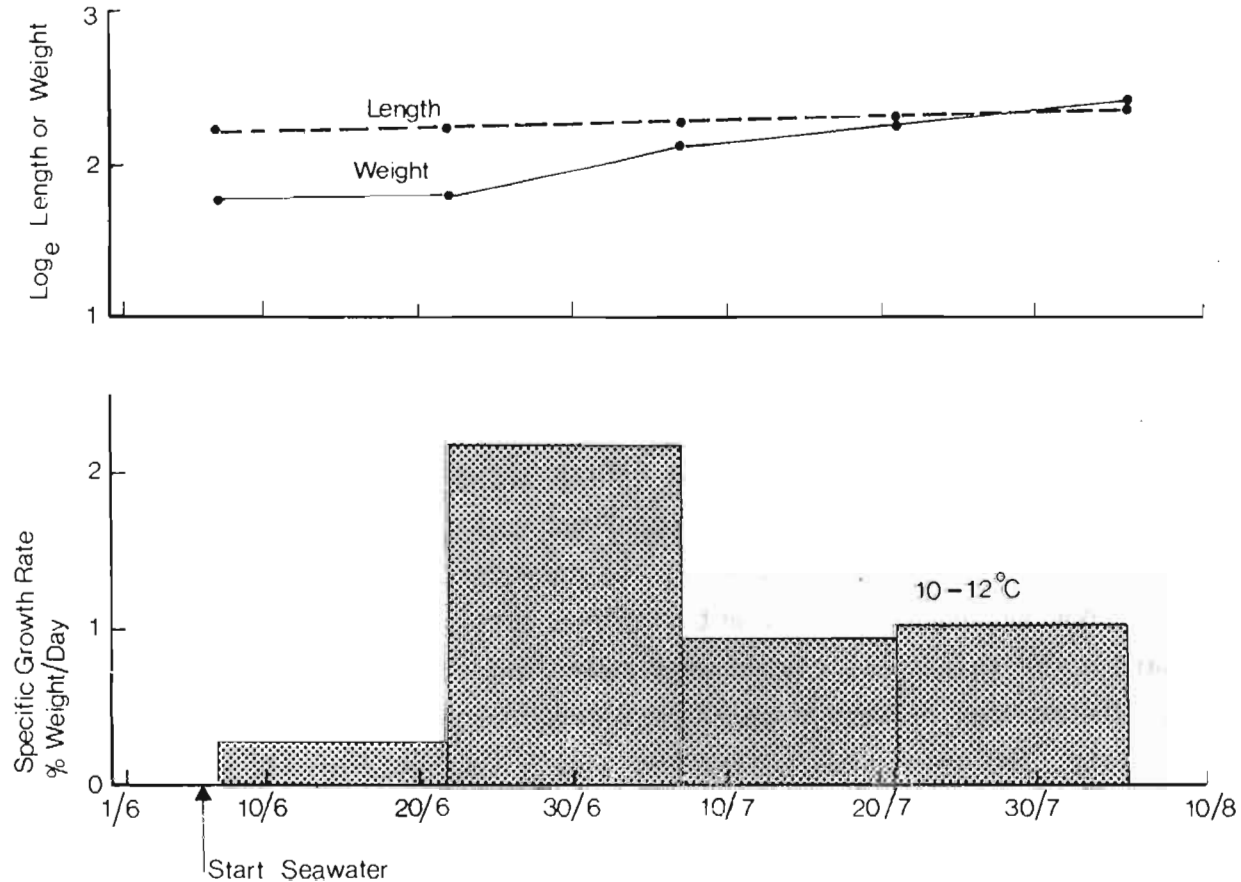


Figure 9. Logarthmic plots of wet weight, length and specific growth rates computed over the measurement intervals (see text) for both collection groups of smolts during the saltwater growth study.

Saltwater Gowth Data May 31/77 Collection of Sockeye Smolts



Saltwater Growth Data - May 17/77 Collection of Sockeye Smolts



Discussion

Salinity Challenge Test - Osmoregulatory Capability of Babine Smolts

When a teleost fish accustomed to fresh water enters the sea it is faced with a severe osmotic change which necessitates physiological adaptation. The major problem encountered is osmotic loss of water through the permeable surfaces of the fish as the osmotic concentration of the blood is lower than that of sea water. Marine teleosts conteract this water loss by drinking sea water and excreting the ingested salts via the gills, gut or kidney (Conte, 1969). Monovalent ions are mainly eliminated via secretory mechanisms in the gills while divalent ions are lost via the mucous tubes, feces, and the kidney.

In salmonids, the physiological changes which ready the fish for the sea occur in association with the parr-smolt transformation which is characterized by a number of morphological, physiological and biochemical changes (Conte 1969). Some juvenile salmonids can be transferred to full strength seawater (>30^{o}/oo) several months prior to parr-smolt transformation (Conte et al. 1966; Conte & Wagner 1965). Size, age, growth rate and species appear important factors in determining the hypo-osmoregulatory capability of salmonids (Conte 1969). It is reported that chinook and sockeye salmon must reach a minimum of 4-5 g to achieve smoltification while rainbow trout must be 50-60 g to tolerate sea water (Landless and Jackson 1976).

Clarke and Blackburn (1977) utilized the salinity challenge procedure as a rapid test for osmoregulatory capacity of juvenile salmonids. Their findings indicate that fully-smolted salmon juveniles are capable of maintaining their plasma sodium concentration below 170 m eq/L within 24 h of transfer to salt water (28-30^{o}/oo) while parr suffer a substantial elevation of plasma sodium. Clarke and Blackburn reported that parr exhibit plasma sodium levels often in excess of 200 m eq/L and usually suffer considerable mortality within 24 h of salt transfer. Our data for Babine sockeye (Fig. 6) indicate that mean plasma sodium values for non-copper exposed fish after 24 or 48 h were generally below 170 m eq/L and were, in many cases, close to the freshwater value. Some variation was evident within a test group as indicated by the standard error values associated with means and it was suspected that size or age differences between members of a group might have contributed to this variability. The grand mean weight and length averages for all non-copper

exposed fish were 4.68 g and 8.74 cm respectively, although fish as small as 1.6 g with low condition factor were occasionally included in the test groups.

Plots of fish weight vs plasma sodium concentration after 24 or 48 h salt exposure are illustrated in Fig. 10. Contrary to expectations, no clear relationship of weight and plasma sodium level is evident. It is of interest that for both the 24 and 48 h observations, that one third of the values are in excess of 170 m eg Na+/L. If indeed the 170 m Eq Na+/L criterion for successful smoltification of Clarke and Blackburn (1977) is accurate, these data might suggest that one third of the fish experienced osmoregulatory difficulty upon salt water exposure. This matter will be considered in relation to the growth and saltwater survival test result discussion which follows.

Figure 6 illustrates the large elevations of plasma sodium experienced by copper-exposed fish following saltwater transfer. Plasma sodium levels of around 200 m Eq Na+/L are generally indicative of non-smolted individuals (Clarke and Blackburn 1977). In this case, the results are likely related to osmoregulatory difficulties caused by copper exposure. Lorz and McPherson (1977) reported poor survival in sea water and reduced gill microsomal Na+, K+ - activated adenosine triphosphatase (ATPase) in coho yearlings exposed to 5-30 µg Cu++/L for 144 h prior to salt transfer. Gill ATPase appears an important enzyme for maintanance of water and ion balance in salmonids. During parr-smolt transformation, ATPase levels in the gills of coho and steelhead double (Zaugg and McLain 1970, 1972; Zaugg and Wagner 1973) and ATPase levels increase following saltwater transfer for 30 d in salmonids and other euryhaline fishes (Epstein et al. 1957; Zaugg and McLain 1970). Reduction in gill ATPase levels and resulting loss of osmoregulatory capability in our fish would explain the observed results of the salinity challenge experiment in copper-exposed fish.

A consideration of the implications of sublethal copper sensitivity and osmoregulatory difficulty following copper exposure is relevant to the Babine situation. If exposure to low levels of copper in the lake caused no acute mortality, yet through sublethal mechanisms, rendered the fish unfit for migration and salt transfer, there would be great cause for concern. Lorz and McPherson (1976) demonstrated effects of freshwater copper exposure

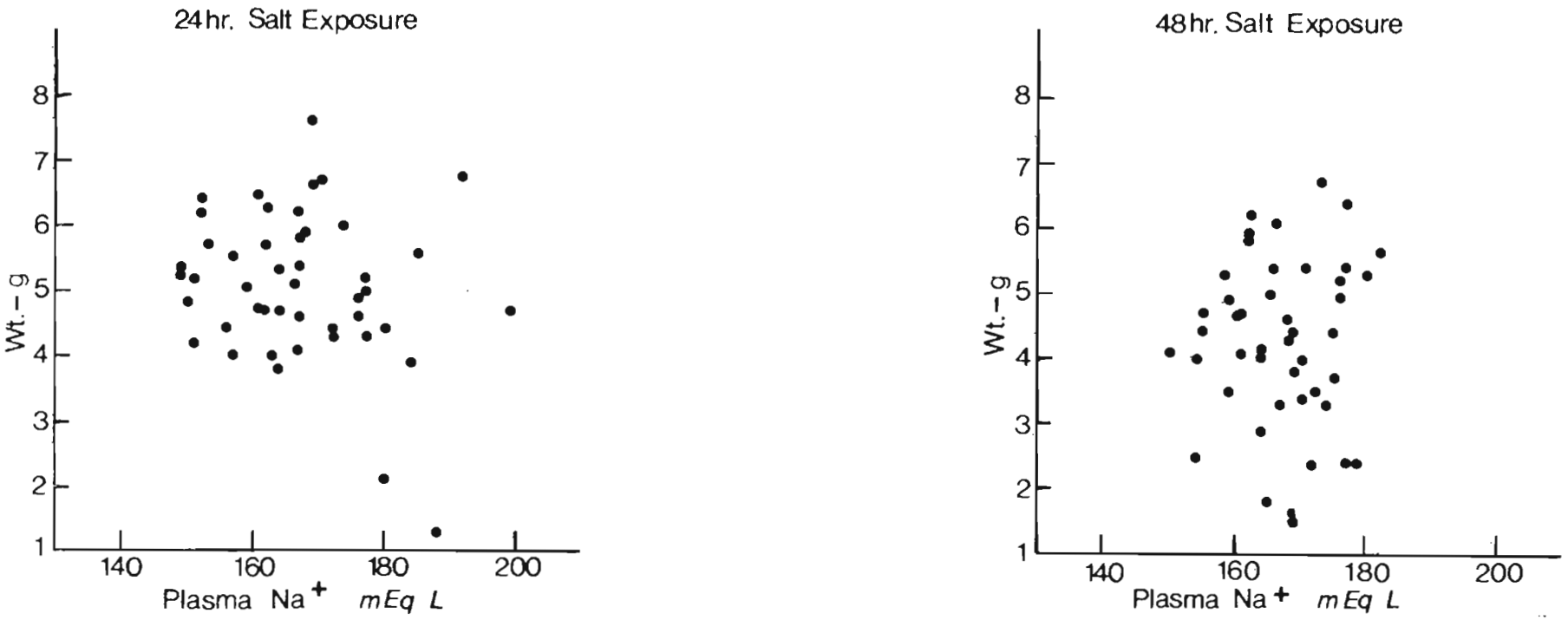


Fig. 10. Fish weight versus plasma sodium concentration in non-copper-exposed smolts transferred to salt water for 24 or 28 h. Figures represent all data for fed and non-fed groups from both collection groups.

on osmoregulatory capability of coho yearlings at a concentration of 5-30 µg Cu++/L (0.09-0.53 96 h LC50). In our laboratory tests, Babine smolts experienced osmoregulatory difficulty following 144 h exposure to 30 µg Cu++/L (0.54 96 h LC50 as estimated by acute copper toxicity tests in laboratory well water). It would therefore appear that a copper concentration ranging from 0.09 to 0.54 96 h LC50 might be considered hazardous in terms of osmoregulatory effects following exposure.

In the Babine Lake situation, we have seen that 96 h LC50 values are generally in the vicinity of 200 µg Cu++/L for sockeye juveniles. Furthermore Chau and Wong (1975) have calculated that Babine Lake water can complex slightly in excess of 100 µg Cu++/L, presumably rendering the complexed copper unavailable to fish and thus non-toxic. If we then think in terms of sublethal threshold copper concentrations expressed as fractions of the acutely toxic level or LC50 (toxic units - Sprague 1970), and assume that no complexing occurred in the experiments used to derive the 0.09-0.54 hazard range, then the range of potentially hazardous Cu++ concentrations in Babine Lake would be (assuming 96 h LC50 = 200 µg/L):

Lower limit = complexed copper + 0.09 (LC50 value - complexed copper)
= 100 + 0.09 (200 - 100)
= 109 µg Cu++/L total copper conc.

Upper limit = complexed copper + 0.54 (LC50 value - complexed copper)
= 100 + 0.54 (200-100)
= 154 µg Cu++/L total copper conc.

These levels are well above those reported in Babine Lake water at any time (4 µg/L - Stockner and Shortreed, 1976; 16-44 µg/L, Chau and Wong 1975). Hence, on the basis of these calculations, it would not appear that a hazard exists in the lake at present. As discussed earlier, at least two thirds of the fish tested via the salinity challenge test, having been previously only exposed to natural Babine water, appeared to show good osmoregulatory capability on salt transfer. The poor osmoregulatory capability of the other third of the test fish is unlikely the result of copper exposure, following the above reasoning, but may be related to other factors as discussed in the following sections.

Growth and Salt Water Survival in Babine Smolts

Specific growth rate for both test groups was variable between observation periods but in both groups was greatest in the second measurement period (Fig. 9).

Possibly this high rate of growth represents a compensation for reduced growth during or prior to saltwater acclimation or to a behavioural change associated with adaptation to seawater. Mean growth rates over the 60 day study period were 1.10% and 1.02% weight increase/d for the May 17 and 31st collection groups respectively. These rates are slightly below those measured by Brett et al (1969) in 7-12 month old sockeye at 10-15°C when fish were fed on excess ration (3 times/day). It appears likely that all fish were not feeding properly in our experiment as a steady mortality occurred throughout the test and condition factor was initially low. Indeed the low condition factors observed for the group (0.75 May 17 collection, 0.69 May 31 collection) suggest that the fish were in a poor nutritional state at the start of the experiments. Reimers (1963) reported that fish with condition factor in the range of 0.6-0.7 "may be considered marginal in vitality, tiring quickly in stamina tests". During the growth study condition factor increased, particularly in the May 17th fish, indicating that the relative fatness of the fish increased as a result of feeding.

A steady mortality occurred during the growth study period and was most apparent in the first 30 days following salt transfer. By the end of the study, mortality in both groups of test fish had reached over 33%, indicating that one third of the fish failed to survive in salt water. These findings are possibly related to the results of the salinity challenge test dicussed earlier, where one third of the fish showed plasma sodium levels in excess of 170 m Eq Na^+/L upon salt transfer. These results might suggest that one third of the fish lacked the capability of adjusting to salt water for some reason or another.

Inspection of the salinity challenge test results where fish weight was plotted against plasma sodium level (Fig. 10) showed that fish ranging from 1.3-7.0 g had plasma sodium levels in excess of 170 m eg Na^+/L - i.e. the phenomenon did not appear to be size related or confined to small fish of insufficient size for correct smoltification. Observation of mortality in the aquarium stock tanks for both groups of test fish over a four month period (with conversion of the stocks to salt early in the holding regime) also revealed high mortality (Fig. 11) which occurred simultaneously to that observed in the growth studies. In the case of the May 17 collection group, holding mortality appeared to start prior to salt transfer, suggesting factors

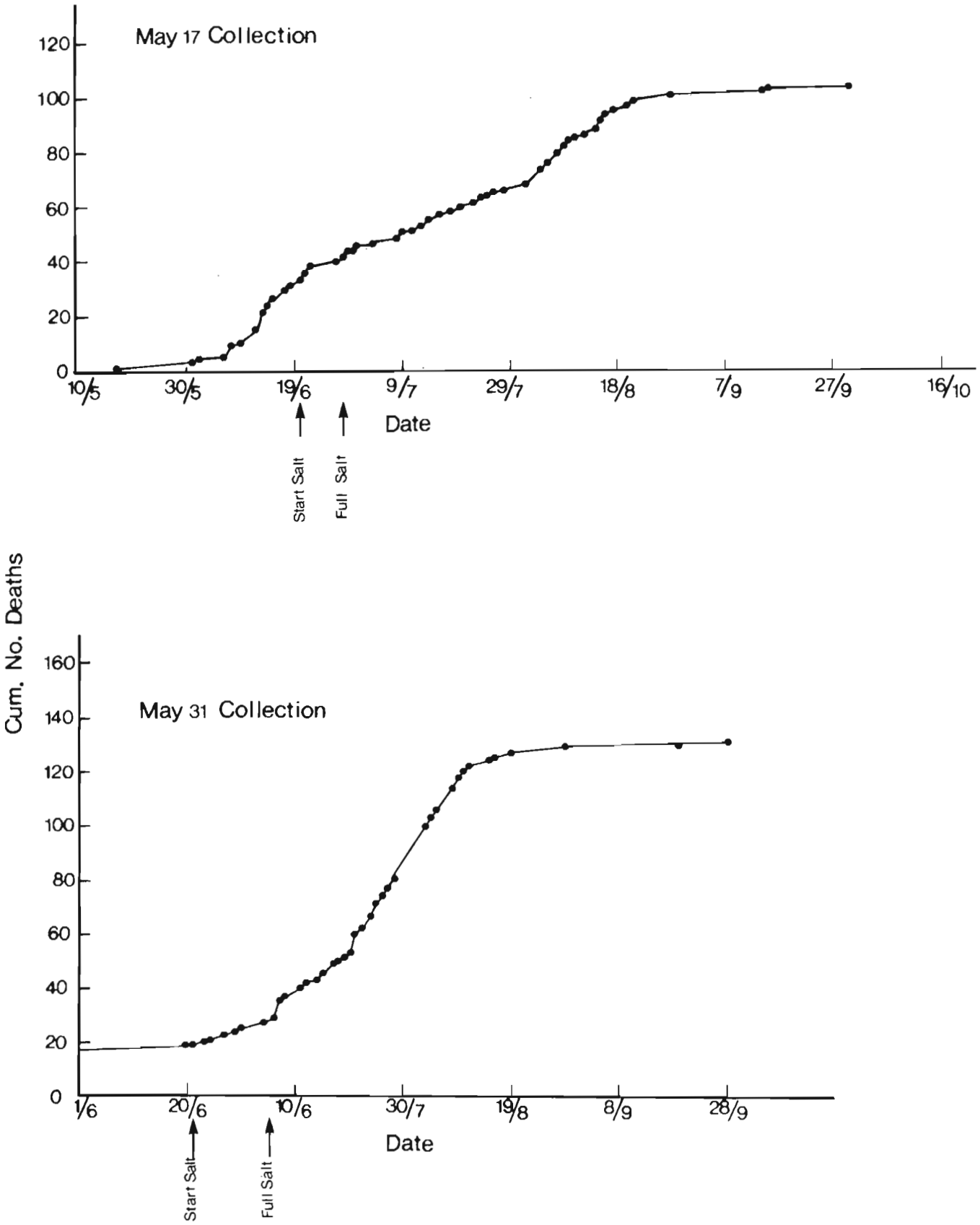


Fig. 11. Mortality in aquarium stock tanks from which fish were drawn for use in the growth studies. Like the growth study tanks, stock tanks were converted to salt water and daily mortality was recorded for both collection groups.

other than salinity stress were involved. Samples of recently dead and apparently healthy fish from the holding tanks were sent to the disease diagnostic center at the Pacific Biological Station, Nanaimo. No evidence of bacterial disease was present but all the fish were heavily infested with the intestinal cestode *Eubothrium* sp., and were in a poor nutritional state. Two fish had focal necrosis of the head kidney which may have been I.H.N. lesions (infectious hematopoietic necrosis- a virus disease). Thus heavy parasitism, nutritional state and possibly I.H.N. disease may explain the observed mortality in the study groups. The subjects of parasitism and nutritional state will be considered in Part III of this report.

These results indicate that at least a majority of the Babine smolts tested appeared to grow at a respectable rate in salt water and survive for prolonged periods. Presumably some mortality and stress was associated with capture, shipment, holding and handling of our test stocks, hence success in the wild may well be better than that shown in the laboratory. It is significant however, that a number of test fish apparently had difficulty in making the transfer from fresh to salt water and that the stock was heavily parasitized and had a low condition factor upon collection. For these reasons, further work was done with the Babine smolts as described in the following section.

Part III - Parasitism vs Copper Sensitivity and Nutritional State in Babine Sockeye Smolts

Introduction

Two factors suspected to be related to saltwater survival capability in Babine smolts, as discussed earlier, were degree of parasitism and nutritional state. Babine smolts are known to be heavily infected with the intestinal cestode parasite *Eubothrium salvelini* with the incidence of infection ranging from 12-46% during 1958-71 (Smith 1973). Infestation rate may well be higher in underyearlings inhabiting the lake with resultant mortality prior to smolt outmigration (H.D. Smith - personal comm.). Smith summarized effects of the parasite on the well-being of Babine smolts including inferior growth, poor swimming performance and aberrant behaviour and postulated that parasitism might increase susceptibility to death from other causes. Boyce and Behrens Yamada (1977) demonstrated that Babine sockeye smolts exposed to 1 mg/L zinc in laboratory studies proved significantly more susceptible to zinc when infected with *Eubothrium salvelini* than when non-infected. Low condition factor (Part II) and small size (which may be related to saltwater survival success) might also be related to parasitism or possibly food limitation due to the increased size of the lake population of underyearling fish following enhancement.

As the above factors had implications on the interpretation of the copper studies and growth and saltwater survival experiments, further analysis was carried out on copper sensitivity vs parasitism and nutritional state of Babine smolts upon collection. These studies were not designed to be definitive experiments but were peripheral items added during the study to aid in interpretation of results.

Methods

Copper Sensitivity vs *Eubothrium* Infestation in Babine Smolts

In order to ascertain if *Eubothrium* infestation contributed to copper sensitivity, all the smolts used for acute toxicity tests in two bioassays (June 4, June 8) were examined following testing to determine *Eubothrium* load. The weight and length was noted for each test fish either at the time of mortality during the test or at the cessation of the test (96 h) for individuals that did not die. All fish utilized for both bioassays were preserved in 10% formalin and were transferred to the Parisitology Group at Pacific Biological

Station where each fish was examined for presence of parasites by Mr. N.P. Boyce. Presence or absence of *Eubothrium* and their number, state of development, total weight of adults and reproductive state was determined for each fish. These results were related to size and time of death of each fish at a given copper concentration. The fish tested in this manner were obtained in the May 31, 1977 smolt collection from the Babine River.

Nutritional State - Proximate Analysis of Tissues

A random sample of 10-12 fish from each of the two smolt collections was given to the Nutrition and Applied Endocrinology group at West Vancouver for proximate analysis. Fish were collected live within 24 h of capture by sampling the holding tanks at the West Vancouver laboratory site. They were weighed, measured, frozen and ground to a fine homogenate. Weighed samples were used to determine moisture, ash, fat and protein content. Moisture was measured using the AOAC (1975) technique with a 2 g oven dried sample at 105°C for 20 h. Ash was determined via the AOAC technique with a 4 g sample ashed in a muffle furnace for 2 h at 600°C. Fat content was measured by the technique of Bligh and Dyer (1959) while protein levels were determined using a Technicon Autoanalyser II via the AOAC technique for total Kjeldahl nitrogen in food and agricultural products.

Results

Eubothrium Infestation vs Copper Sensitivity

Of the combined total of all fish from bioassays conducted on June 4th and 8th (139 fish) 35.3% were found to be infested with *Eubothrium*. Some individuals had as many as 10 parasites and the majority of parasites examined were gravid.

Appendix 2 provides a summary of size, *Eubothrium* infestation and time to death of individual fish in various copper concentrations from 2 acute toxicity bioassays. Examination of the data and various statistical analyses revealed no apparent relationship of copper toxicity to presence, absence or degree of parasite infestation. Heavily parasitized individuals do tend to be small individuals but were not necessarily more sensitive to copper than larger fish.

Nutritional State - Proximate Analysis of Tissues

Analytical results for tissues from individual fish from both collection

groups subjected to proximate analysis are summarized below:

Table 2. Proximate Analysis of Whole Body Homogenate from Two Collection Groups of Babine Smolts

	May 17, 1977 - Collection			May 31, 1977 - Collection		
	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.
Length(cm)	12	8.89	0.57	10	8.16	0.94
Weight(g)	12	6.04	1.10	10	4.37	1.59
% Moisture	12	77.43	1.99	10	78.61	1.84
% Ash	12	2.38	0.35	10	2.54	0.33
% Protein	12	16.48	0.89	10	16.28	0.86
% Fat	12	3.82	1.04	10	2.14	0.53

A comparison of means between the two groups utilizing an unpaired t-test at the 0.05 level of significance revealed that the May 31 collection groups were significantly shorter, lighter and had a lower fat content than the May 17 collection group. Condition factor, calculated from $CF = 100 \cdot wt/L^3$ (part II), using the weight and length data in Table 2 was 1.14 for the May 17 collection group and 0.80 for the May 31 group.

Discussion

Eubothrium Infestation versus Copper Sensitivity in Babine Smolts

Analysis of data in Appendix II illustrates that *Eubothrium* infestation apparently was not related to acute copper sensitivity in the smolts tested in the present experiments. These findings are in contrast with those of Boyce and Behrens Yamada (1977) who demonstrated that Babine sockeye smolts were significantly more susceptible to zinc under laboratory conditions when infected with *E. salvelini* when compared to non-infected individuals. The explanation as to why cestode infected sockeye smolts proved more susceptable to zinc and showed no significant increased sensitivity to copper is not understood at this time. Boyce and Behrens Yamada (1977) postulate that *E. salvelini* competes with its host for intestinal nutrients producing a condition akin to starvation in severe cases of infestation. Dombroski (1955) and Smith (1973) reported that infected Babine smolts were considerably smaller than parasite-free individuals and larger individuals appear to

show higher sea water survival and adult returns than smaller smolts (Foerster 1954; Ricker 1962). Indeed poor growth, reduced swimming performance and aberrant behaviour in Babine sockeye are thought related to *Eubothrium* infestation (Smith 1973) and it is possible that heavily infested fish may die in the lake (Ricker and Smith 1975). Indeed, Boyce (1978) demonstrated that experimentally induced *E. salvelini* infestation in sockeye fry had deleterious effects on growth, survival and swimming performance (tested in an active capacity tunnel). Certainly it is reasonable to postulate that parasitized fish might be more sensitive to a toxicant such as copper. Possibly such effects might be detectable if suitable sublethal tests of physiological state, stamina, etc. were devised rather than the acute toxicity tests reported here. Alternately, use of a larger sample size might have revealed an influence of copper on survival in acute toxicity tests.

The incidence of parasitism reported here (35%) is not unusual for Babine smolts (Smith 1973). Among infected individuals parasite load, expressed as a percentage, and calculated from:

$$\text{load} = \frac{w(100)}{W - w}$$ where: W = wet weight of fish, w = wet weight of worms

had a mean value of 2.41% (S.E. = ± 0.39). Such loading is not unusual and Smith (1973) reported that about 1 fish in 8 infected individuals had loading ≥ 10% in the early run of 1967.

A factor not addressed in these studies but possibly of major significance, is the effect of *Eubothrium* infestation on early sea life and estuarine survival. If smaller, less vigorous fish with reduced osmotic adaptation capability result from *Eubothrium* infestation, a considerable mortality could occur during early sea life. Indeed, this mechanism could explain, in part, the seawater mortality discussed in Part II. It is recommended that further work be done to examine the interaction of parasitism with early sea life, growth and survival in Babine sockeye smolts.

Nutritional State - Proximate Analysis of Tissues

Brett, et al (1969), in an experimental study of the effect of temperature and ration on growth and body constituents of sockeye fingerlings in freshwater, obtained the following results after 99 days:

ration	lipid levels at test temperature (%)			
	1°C	5°C	10°C	15°C
starved	3.4	1.9	1.3	0.6
1.48-1.87%/day	6.6	7.4	5.9	4.4
satiation	7.5	8.9	9.0	7.6

Parker and Vanstone (1966), reporting on the data of Kizevetter (1948), gave the following body composition data for two life stages of sockeye:

life stage	weight(g)	% moisture	% protein	% lipid
fingerling sockeye	10	79.32	13.35	6.02
migrating smolts	21.2	79.06	15.21	3.67

These data indicate the lipid, protein and moisture levels present in fish in various nutritional states as fingerlings and migrant smolts. Comparison of the literature values with the results obtained from proximate analysis of the two collection groups used in the present study (Table 2), reveals some interesting points. Levels of lipid, protein and moisture in the May 17, 1977 collection group are similar to those reported by Parker and Vanstone (1966) for migrating smolts. Lipid levels in the May 31 collection are considerably depressed however, and are accompanied by elevated body moisture content. A lipid level of around 2% of body weight is reminiscent of starvation conditions as documented by Brett et al, 1969 (see above) and the May 31 collection group was significantly shorter and lighter than the May 17 collection group.

The apparently poor nutritional state of May 31 collection group in comparison to the May 17 collection group may be related to several factors. Figure 3 shows that the May 31 group was obtained very close to the end of the smolt run while the earlier collection coincided with the peak of the run. Examination of Figure 3 together with a consideration of the historical pattern of the Babine smolt migration (Smith 1973) suggests that both

collection groups were mainly composed of late run fish which originated in Morrison Arm and the southern lake basins of Babine (main lake). It is possible that smolts migrating at the end of the of the late run (May 31 fish) were of lower quality due to parasitism (Smith 1973), reduced swimming performance (Smith and Margolis 1970) or some other factor associated with late migration. However, if the low lipid levels indicating poor nutritional state in the May 31 collection group are common to a significant portion of the smolt run, there is cause for concern. Such low levels would indicate nutritional limitations or related problems, possibly resulting from the size of the enhanced sockeye population in the system interacting with the food supply. It is recommended that further studies be initiated to examine smolt nutritional state, size and overall vigor at successive intervals during the run. Poor nutritional state and reduced vigor could produce significant mortality during migration and early sea life.

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Appendix Table 1. Results of Salinity Challange Experiments - Babine Smolts

TEST GROUP	TREATMENT		Wet Wt. (g)	Leng. (cm)	Hematocrit Vol.%	C.F. 1000 Wt/ Leng^3	PLASMA Na^+ m Eq/L
May 17 Colln. (Fed)	Fresh water	$\bar{x}$	5.69	9.13	43.5	0.74	156.6
		SD	1.08	0.57	2.51		7.23
		n	16	16	16		15
	24 h Sea water	$\bar{x}$	5.46	9.09	42.2	0.73	161.3
		SD	0.71	0.40	2.34		9.2
		n	16	16	15		16
-	48 h Sea water	$\bar{x}$	4.69	8.66	40.7	0.72	171.3
		SD	1.17	0.60	2.60		9.40
		n	16	16	15		16
May 31 Colln. (not Fed)	Fresh water	$\bar{x}$	3.95	8.44	44.8	0.66	159.1
		SD	0.96	0.46	4.88		8.08
		n	16	16	16		15
	24 h Sea water	$\bar{x}$	4.96	9.06	44.0	0.67	170.0
		SD	1.23	0.68	3.00		11.24
		n	16	16	16		15
	48 h Sea water	$\bar{x}$	4.00	8.37	41.2	0.68	164.7
		SD	1.45	0.96	2.73		7.74
		n	15	15	15		15
May 31 Colln. (Fed)	Fresh water	$\bar{x}$	5.00	8.87	47.1	0.72	156.1
		SD	1.19	0.66	4.97		8.76
		n	16	16	16		14
	24 h Sea water	$\bar{x}$	4.46	8.68	43.7	0.68	169.8
		SD	1.39	0.92	4.81		12.13
		n	16	16	15		14
	48 h Sea water	$\bar{x}$	3.93	8.32	43.9	0.68	164.7
		SD	1.22	0.70	4.51		7.44
		n	16	16	16		16
May 31 Colln. (not Fed)	Fresh water	$\bar{x}$	4.28	8.74	38.08	0.64	160.0
		SD	0.80	0.41	4.63		7.80
		n	16	16	16		15
Cu^{++} exposed	24 h Sea water	$\bar{x}$	3.26	8.11	43.4	0.61	192.5
		SD	1.15	0.91	3.29		12.28
		n	14	14	14		11
	48 h Sea water	$\bar{x}$	3.35	8.22	40.0	0.60	202.83
		SD	1.20	0.75	4.78		15.56
		n	13	13	13		12

Appendix Table 2. Sockeye Smolts - Bioassay B-6 - Mortality vs. Parasitism

Cu++ Conc. (ug/L)	Time to Death (min)	Fish Leng. (cm)	Fish Wt. (g)	Condition Factor	*Eubothrium*		
					No.	Total Wt. (g)	Condition
0	>5760	8.9	5.29	.75	1	0.02	Gravid
"	"	8.8	4.87	.71	4	0.09	Gravid
"	"	9.2	5.93	.76	0	-	-
"	"	8.8	5.47	.80	0	-	-
"	"	8.9	4.88	.69	0	-	-
190	4305	9.2	6.73	.87	0	-	-
"	4880	7.6	3.70	.84	0	-	-
"	>5760	8.6	4.94	.77	0	-	-
"	>5760	8.8	5.14	.75	0		
"	>5760	7.9	3.60	.73	4	0.09	Gravid
"	>5760	8.9	4.90	.69	0	-	-
198	2210	9.0	6.08	.83	0	-	-
"	2370	8.4	5.14	.86	0	-	-
"	3165	8.7	5.43	.82	0	-	-
"	4305	9.1	6.64	.88	1	0.03	Gravid
"	>5760	8.8	5.04	.73	0		
"	>5760	8.7	4.95	.75	0	-	-
213	1920	6.6	2.43	.84	1	0.02	Gravid
"	2370	6.3	1.87	.74	1	0.02	Gravid
"	>5760	7.8	3.53	.74	6	0.10	Gravid
"	>5760	8.5	4.11	.66	0	-	-
"	>5760	7.6	3.00	.68	2	0.02	Gravid
"	>5760	9.0	5.23	.71	0	-	-
235	1880	8.7	5.45	.82	0	-	-
"	3445	7.5	3.14	.74	1	0.01	-
"	4305	8.9	6.45	.91	0	-	-
"	4410	8.6	5.15	.80	1	0.03	Gravid
"	>5760	8.5	4.48	.72	0	-	-
"	>5760	9.0	5.07	.69	0	-	-
285	1875	7.3	3.83	.98	5	0.17	Adults, Gravid
"	2000	8.8	6.93	1.01	0	-	-
"	3540	8.5	5.29	.86	0	-	-
"	>5760	8.6	5.08	.79	0	-	-
"	>5760	7.6	2.82	.64	3	0.08	Gravid
"	>5760	9.2	5.84	.74	0	-	-
293	1180	7.9	3.83	.77	2	0.01	-
"	1780	8.3	4.51	.78	0	-	-
"	2000	8.8	6.02	.88	0	-	-
"	2880	8.5	6.55	1.06	0	-	-
"	3455	9.2	6.68	.85	0	-	-
"	4305	7.4	3.69	.91	2	0.03	Gravid

Appendix Table 2. (cont'd)

Cu++ Conc. (ug/L)	Time to Death (min)	Fish Leng. (cm)	Fish Wt. (g)	Condition Factor	*Eubothrium*		
					No.	Total Wt. (g)	Condition
303	1315	7.5	3.21	.76	10	0.15	Gravid
"	2085	7.3	2.85	.73	5	0.15	"
"	3165	9.0	6.77	.92	1	0.02	"
"	4760	9.1	6.08	.80	0	-	-
"	>5760	7.8	3.76	.79	0	-	-
"	>5760	7.4	2.70	.66	5	0.11	Gravid
333	2085	8.0	3.67	.71	4	0.11	Gravid
"	2280	7.4	4.26	1.05	0	-	-
"	3535	7.6	3.31	.75	1	0.03	
"	3620	8.1	4.94	.92	0	-	-
"	4510	8.6	5.49	.86	0	-	-
"	>5760	9.3	6.21	.77	0	-	-
355	2085	9.0	6.00	.82	0	-	-
"	2280	8.9	5.55	.78	0	-	-
"	2390	6.6	1.91	.66	7	0.10	Gravid
"	4305	6.8	2.48	.78	6	0.11	"
"	>5760	7.4	3.61	.89	4	0.19	"
"	>5760	8.8	4.74	.69	0	-	-

Appendix Table 2 (cont'd) Sockeye Smolts - Bioassay B-7 - Mortality vs. Parasitism

Cu++ Conc (ug/L)	Time to Death (min)	Fish Leng. (cm)	Fish Wt. (g)	Condition Factor	*Eubothrium*		
					No.	Total Wt. (g)	Condition
43	>5760	9.0	5.12	.70	1	0.034	Gravid
"	"	8.9	4.94	.70	0	-	-
"	"	9.1	5.29	.70	0	-	-
"	"	9.4	5.52	.66	0	-	-
"	"	9.0	5.21	.71	0	-	-
"	"	8.5	4.35	.70	0	-	-
53	>5760	8.9	4.92	.70	2	0.040	Gravid
"	"	8.9	5.03	.60	0	-	-
"	"	8.0	3.66	.71	0	-	-
"	"	9.6	6.48	.73	0	-	-
"	"	8.8	4.87	.71	0	-	-
70	1955	7.7	3.66	.80	3	0.07	Gravid
"	>5760	8.8	4.86	.71	0	-	-
"	"	9.3	5.00	.62	0	-	-
"	"	8.8	4.52	.66	0	-	-
"	"	8.7	4.49	.68	0	-	-
75	>5760	8.5	3.97	.64	0	-	-
"	"	9.2	5.88	.75	0	-	-
"	"	9.2	5.57	.71	0	-	-
"	"	8.5	4.31	.70	0	-	-
"	"	8.0	2.80	.54	6	0.126	Gravid
"	"	9.0	4.71	.64	0	-	-
85	1895	8.8	6.15	.70	0	-	-
"	1895	7.5	3.17	.75	2	0.05	Gravid
"	2850	8.2	4.57	.82	1	0.02	"
"	>5760	8.1	3.67	.69	7	0.069	"
"	"	8.8	5.08	.74	0	-	-
"	"	9.2	5.32	.68	0	-	-
100	1320	9.4	6.08	.73	0	-	-
"	1425	8.1	4.11	.77	0	-	-
"	1425	8.2	4.06	.73	1	0.019	Gravid
"	1785	8.4	4.10	.69	1	0.020	"
"	3345	8.1	4.84	.91	0	-	-
"	4022	9.2	6.29	.80	0	-	-
113	1240	9.2	6.65	.85	0	-	-
"	1355	8.6	5.28	.83	1	0.025	Gravid
"	2865	7.9	4.00	.81	1	0.029	"
"	>5760	7.2	2.87	1.07	0	-	-
"	"	8.3	4.00	.69	0	-	-
"	"	8.5	4.04	.65	2	0.030	-

Appendix Table 2. (cont'd)

Cu++ Conc. (ug/L)	Time to Death (min)	Fish Leng. (cm)	Fish Wt. (g)	Condition Factor	*Eubothrium*		
					No.	Total Wt. (g)	Condition
130	1230	9.0	5.48	.75	0	-	-
"	1230	7.9	3.59	.72	2	0.074	Gravid
"	1425	9.7	7.19	.78	0	-	-
"	2765	8.4	5.17	1.21	0	-	-
"	>5760	8.4	3.39	.65	1	0.02	Gravid
"	>5760	9.2	5.80	.74	1	0.02	Gravid
135	1230	8.8	4.87	.71	0	-	-
"	1230	8.8	5.37	.78	0	-	-
"	1510	8.6	5.18	.81	3	0.073	Gravid
"	3270	8.5	4.86	.79	0	-	-
"	>5760	7.0	2.25	.65	3	0.057	Gravid
"	>5760	9.6	6.08	.68	0	-	-