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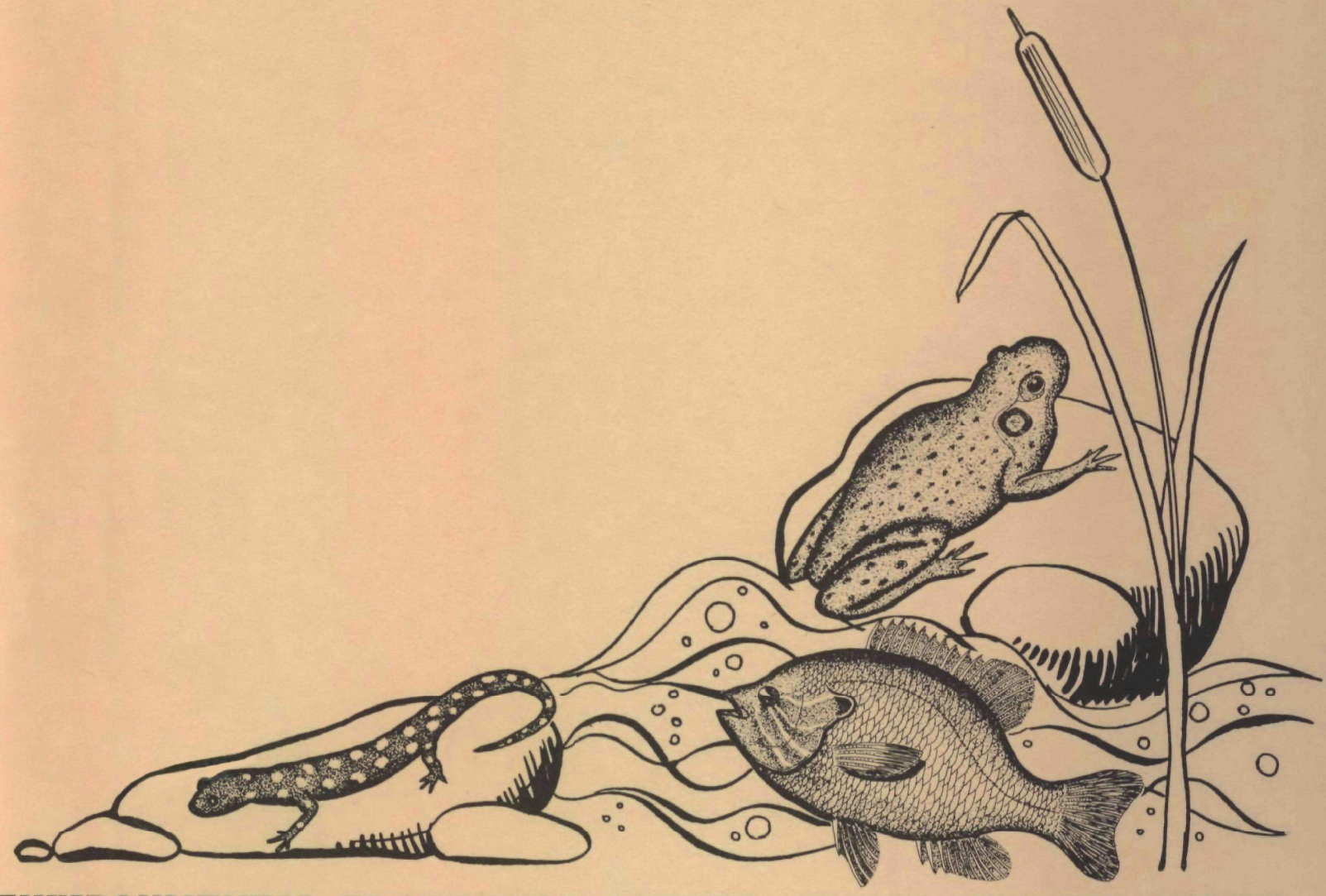
Response of Teleost Fish to Environmental Stress



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RESPONSES OF TELEOST FISH TO ENVIRONMENTAL STRESS

by

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ABSTRACT

A floating laboratory was built for conducting multiparameter physiological studies on salmon in marine, estuarine, and fresh waters. New methods were developed using a swimming chamber-respirometer for adult salmon. Normal values were measured for a variety of physiological functions, then repeated on salmon migrating through an urban estuary characterized by sewage pollution and low DO. Effects seen included decreased swimming stamina and respiratory efficiency, decreased oxygen consumption and increased lactate, decreased urine flow and ammonia excretion, especially in the presence of environmental ammonia. Longer term disruptions in hematology and lipid metabolism were seen. Most of the effects occurred at DO concentrations just below 5 mg/liter, except for synergistic effects between ammonia and low DO at somewhat higher concentrations.

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Key Words: Fish physiology, fish migration, environmental effects, Pacific salmon, oxygen sag.

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SECTION I

CONCLUSIONS

1. A floating laboratory was built as a 98.5' x 35' self-contained (but not self-propelled) barge for studying the environmental physiology of salmon migrating through the protected waters of Oregon, Washington, British Columbia, and Alaska. The idea of taking the laboratory to the fish proved advantageous in many respects, but particularly served to minimize problems of handling stress (to which salmon are highly susceptible) and also problems of simulating in a fixed laboratory the specific quality characteristics of a particular stream and estuary.

2. A number of new techniques were devised and used to make repeated physiological measurements from active salmon. Since salmon in their natural environment rarely stop swimming, a realistic assessment of the problems they face in polluted estuaries had to be made on the basis of data from swimming salmon.

3. We described for the first time several normal physiological functions of salmon in clean water for comparison with salmon in polluted water in the areas of metabolism, excretion, osmoregulation, and blood circulation. Surprising as it may seem, since salmonids are among the best studied of the commercially important fish, little is known of the basic physiology of most of their organ systems, and therefore norms had to be measured first before any interpretation of data from polluted waters could be made.

4. Normal osmoregulatory changes as young salmon migrate from freshwater into sea water and the adults return to the stream take place with a minimum of stress to the fish. Outmigrants reach osmotic equilibrium with sea water in 30 to 36 hours and appear not to need any gradual adaptation to the change in salinity. Inmigrant adults are relatively impermeable to freshwater and their kidneys readily readapt to freshwater. While tissues of spawning salmon become increasingly watery with continued residence in the stream, this is not normally the result of kidney failure or incomplete readaptation to freshwater, as has sometimes been suggested.

5. Urine output is decreased to about half by decreases in environmental oxygen or increased stressful activity. In sea water, one of the major urine constituents is magnesium ion, and therefore, continued depression of urine production could lead to problems of magnesium toxicity (which would produce muscular paralysis). We identified the potential problem, but did not determine its magnitude. There is also possible accumulation of other toxic products when kidney function is depressed, especially if the environment contains toxic products and is low in DO at the same time.

6. Ammonia is the primary excretory product resulting from the normal breakdown of proteins in fish and is excreted by the gills. Ammonia

excretion can be either depressed or elevated by decreased environmental DO, but the mechanisms governing which direction the effect will be are presently not understood. However, it would be beneficial to the well-being of the fish if the water quality standards for DO were slightly higher for waters containing ammonia than for those lacking it.

7. We do not know exactly how a salmon chooses to try to surmount the respiratory problems it faces when trying to enter a river blocked by an area of low DO. It appears that salmon stay in the bottom (salt water) layers as long as possible and swim upstream as far as the river and tidal currents and the available oxygen supply will allow. We now do know, however, most of the respiratory choices available to a salmon facing low DO and have considerable experience with salmon choosing from among the alternatives while in our swimming chamber. We are now ready to make physiological interpretations of the estuarine behavior of these fish as soon as we can get the depth-sensitive sonic tag now being developed by the Bureau of Commercial Fisheries.

8. In the past, the appearance of lactate in blood and muscle has been empirically associated with anaerobic metabolism and large amounts of lactate have been associated with delayed mortality. Our experiments demonstrated that some lactate always occurred in the blood of even rested salmon and that lactate increased proportionally to increased activity or decreased DO. The effects of increased activity and decreased DO together were additive. Thus blood lactate was an excellent indicator of immediate environmental stress, but responded to environmental changes so rapidly that great precautions were needed to prevent lactate changes due to the stress of capture and handling.

9. Production of lactate is a highly inefficient means of obtaining energy which is used when the quantities of oxygen available to the tissues are insufficient to meet the demand. Lactate accumulation also produces toxicity and pH problems, for which one solution is to excrete the lactate and throw away the chemical energy it contains. Thus production of urine containing lactate could amount to a continuous "energy leak" in salmon experiencing continuous low DO. We observed this phenomenon very carefully because adult salmon on their spawning migration do not feed and could exhaust their energy reserves before spawning. However, the loss of lactate rarely exceeded 100 µgm/kg/hr or about 75 mg/kg/month. Since an adult coho or chinook salmon may start its spawning migration with 20-30% of its body weight as lipids stored for energy reserve, this urine loss of lactate cannot be considered serious. It is probable that lactate is lost from the gills, but we have not yet completed the experiments to test this hypothesis.

10. A major problem in assessing the effects of environmental stress in migrating adult salmon is that fatal degrees of deterioration in physiological condition take place naturally. All Pacific salmon die after spawning, usually from diseases to which they become increasingly less resistant as the migration stress progresses. The effects of estuarine pollution on adult salmon is to increase the <u>rate</u> rather than the <u>kind</u> of

deterioration. We have described many of the hematological changes which occur during both the downstream and upstream migrations in both polluted and clean waters. The comparisons between all variables, species, and conditions are beginning to merge into a concept of a generalized response for most kinds of stress. The general concept of stress, in turn, is producing ideas of how to treat fish to alleviate the effects of stress, so that eventually wild fish can be treated for stress, diseases, etc., like any other of our better-managed game animals.

11. Our lipid research has had many facets. Most basic has been the description of typical fatty acid composition of structural and reserve lipids of muscle, liver, and blood in all species and ages of salmon. With five species of salmon, 28 fatty acids, and many changes in their proportional composition and distribution, this alone was a large job. Once completed, however, it became possible to demonstrate that outmigrant juvenile salmon may have numerous behavioral and physiological problems before they learn to recognize and adapt their metabolism to the new kinds of food in their new marine environment. This seemed to be a more serious problem than adapting to the salinity. Wild food organisms contain a much smaller amount of saturated fatty acids than most hatchery foods, and the conversion to eating wild food causes major reapportionment of the structural fatty acids, especially 22:6 (22 carbon atoms, 6 unsaturated bonds). Overall, the study of lipids has assisted our assessment of chronic stress in terms of the disruption of growth in juvenile fish and loss of reserve energy in the adults - i.e., the physiological cost of the stress.

SECTION II

RECOMMENDATIONS

We tested adult coho salmon under circumstances closely resembling the estuary and found that the existing standard of 5 mg O_2/liter is approximately correct. Most of the fish which had physiological difficulties did so at DO's below 4.5 mg/liter. In addition, we identified synergisms between environmental DO and ammonia, environmental DO and blood lactate, and between environmental DO and activity levels. These synergisms may later be elaborated to the point where it can be demonstrated that the required DO level for salmonid streams should be increased to 5.5 or 6.0 mg/liter when more than a few ppm of ammonia are present (as occurs in the effluent of many sewage treatment plants).

SECTION III

INTRODUCTION: STUDYING THE ENVIRONMENTAL PHYSIOLOGY OF SALMON

The time once was when the criteria for water quality consisted partly of whether fish or any other organism could live in a specified water sample for 96 hours or not. While such tests are occasionally still useful, today most people concerned with water quality recognize that there are many degrees of well-being between living and dying. Sub-lethal stresses can eventually be just as devastating to a species as a sudden death and much more subtle because they can slowly accumulate unnoticed until a point-of-no-return is eventually passed.

The projects to be described in this report represent a beginning in understanding the many interactions between salmon and their estuarine environment in the urban setting of Seattle's industrialized waterfront. Rather than choose a single indicator function of stress, we measured a combination of functions encompassing several of a salmon's major functional systems which relate to water quality - respiration, blood circulation, osmoregulation, and lipid metabolism. Thus we could observe the interactions between organ systems as well as between each organ system and the environment. The results reported here demonstrate that we pioneered in at least two areas: Descriptions of normal physiological functions in adult salmon which relate directly to water quality (kidney functions, blood lactate dynamics) which had never before been described, and developing techniques for making a variety of measurements in salmon swimming in their normal estuarine environment.

This report describes the main points investigated during the four years of the project. Most of the material here has been or is in press in scientific journals in expanded form and more will continue to appear for the next 1-2 years as additional graduate students finish theses begun under FWQA sponsorship.

The General Concept of a Floating Laboratory

Pacific salmon are a valuable resource whose decline has coincided with the degradation of our aquatic environments. The life functions of salmon are known mostly in terms of their freshwater stages. Little is known of their estuarine and marine environmental requirements for survival and reproduction. Criteria for improving these environments for salmon are not well defined. Thus the idea was proposed to the Federal Water Pollution Control Administration (F.W.P.C.A.), U.S.A. in 1966 to begin a study of several basic physiological functions of salmon which are vital to their transition between fresh and salt water in the estuaries of Puget Sound. These estuaries were considered as a crucial point in the salmon's lifelong migration where natural stressers[1] are greatest and mankind's additional stressers would most likely be overwhelming.

[1] We will use stresser to mean the agent or agents provoking stress responses in animals. Stress represents the sum of morphological, physiological, and biochemical changes resulting from the actions of the stresser.

One problem was how to study the environmental physiology of a fish whose annual migrations might exceed several thousand miles and whose estuarine problems would probably be different in every estuary. Further, a proper physiological study required instrumentation characteristic of a laboratory while the environmental aspects of the program demanded a field orientation. The eventual result was the design and construction of a self-contained laboratory aboard a 98.5-foot (30.0-meter) steel barge named the R.V. Kumtuks which could be anchored in most of the protected waterways of Puget Sound, British Columbia, and southeastern Alaska along the migration routes of the salmon. We decided to take the laboratory to the fish and study them in their chosen waters rather than bring the fish to a laboratory and attempt to simulate natural conditions (Fig. 1).

The Duwamish Estuary

An important site for studying estuarine problems faced by salmon was the Duwamish Estuary of the Green River which passes through the primary industrial section of Seattle, Washington, and empties into Elliott Bay. The river is of moderate size, the flow varying from about 200 c.f.s. in the late summer to over 4,500 c.f.s. during the winter rains.[2] The river flow is augmented by the effluent from the sewage lagoon of the suburban city of Auburn, the sewage treatment plant which serves the suburban city of Renton and southeastern Seattle, and, up until November, 1969, by the effluent of Seattle's Diagonal Street Sewage Treatment Plant and some temporarily-diverted raw sewage (Fig. 2). After that date, 95 per cent of the raw sewage and other wastes in Seattle downstream from the Renton Treatment Plant were diverted to Seattle's new METRO[3] treatment plant whose effluent goes directly into Puget Sound.

The most significant biological resources of the Duwamish are the runs of coho and chinook salmon. Juvenile fish migrate downstream in May, return from 2 to 3 years later as adults to Elliott Bay in August, and ascend the river to spawn in September during the first autumn rains. Most of the fish ascend the river to Soos Creek (Green River) Hatchery, operated by the Washington State Department of Fisheries upstream from the city of Auburn. The salmon are avidly pursued by numerous sports-fishermen who angle in Elliott Bay and the lower estuary by day and night; the city's street lights make night fishing possible. There are also other fish present in the estuary including sole, rockfish, and hake.

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[2]Data from U.S. Geological Survey, Water Resources Division, Seattle, Washington.

[3]Municipality of Metropolitan Seattle.

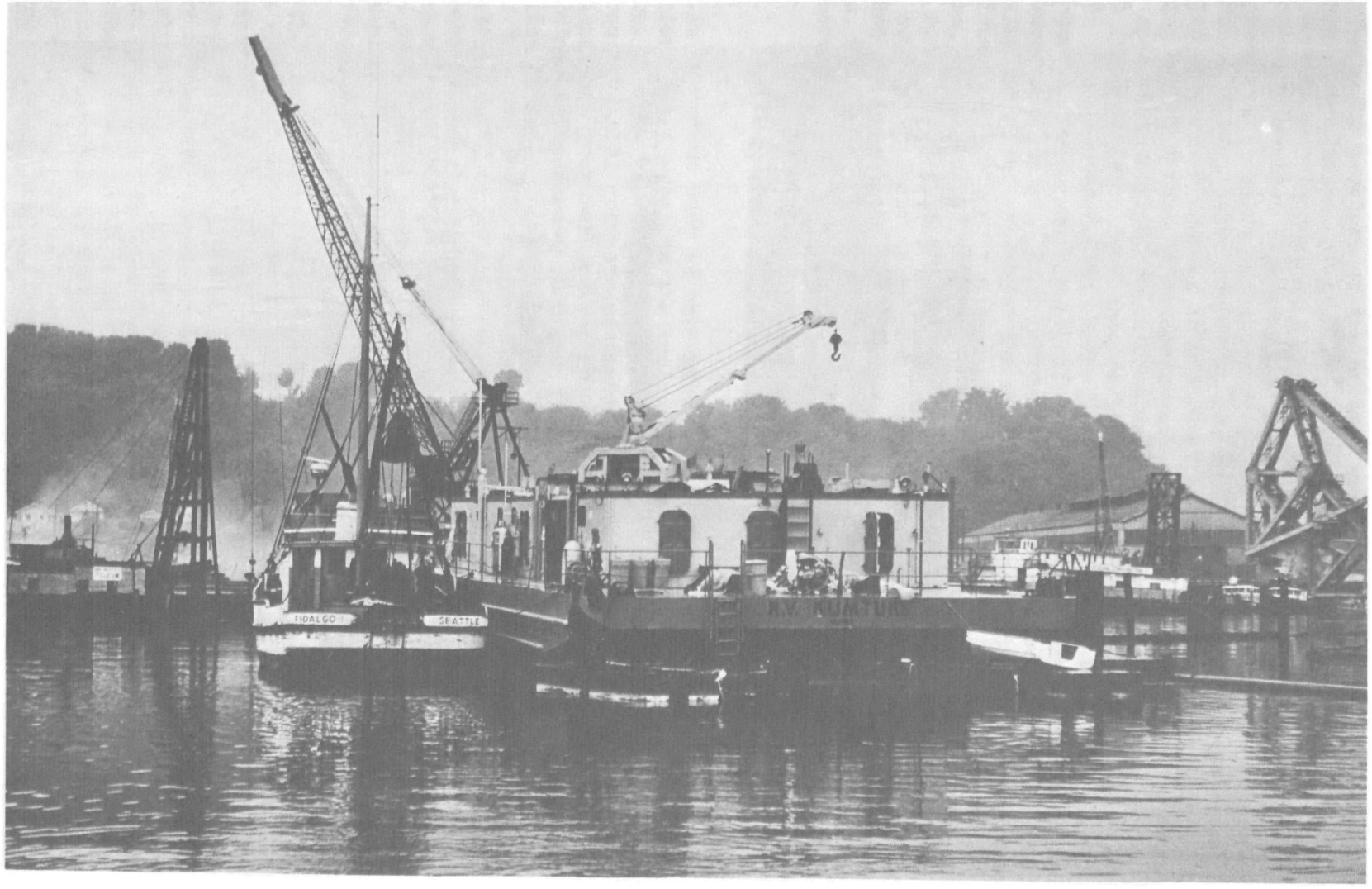


Fig. 1. R.V. Kumtuks and purse seine vessel anchored in the Duwamish Waterway (September, 1969).

Fig. 2. Site of domestic sewage outfall approximately 1,000 ft upstream from R.V. Kumtuks in the Duwamish Waterway. White arrow indicates sewage outfall; black arrows point to plume created by effluent.

The major problems of the estuary are also biological in nature. The once large amounts of organic material discharged into the river produced a heavy biochemical oxygen demand (BOD), particularly in the lower river and upper estuary. The levels of dissolved oxygen (DO) were drastically reduced in the surface waters, often nearly to zero, especially at night when there was no oxygen production by algae. In addition, the river channel is dredged for several miles upstream, creating a long tongue of rather static salt water beneath the freshwater which appears to collect organic matter and to have chronically low DO levels (Salo, 1969). From the results of our experiments on swimming stamina in salmon (described below), we believe that a major cause of the observed low DO was biological - the combination of algal blooms and raw sewage discharges. Experiments there did not detect any toxic products affecting the fish. Now that the major BOD source has been removed from the river by diversion of sewage to a new treatment plant, there still remains, we believe, a problem of the salt wedge collecting dead and dying river algae in the dredged part of the lower river and estuary. Thus, some of the continuing problems of the estuary will also be biological ones and the problems of salmon will continue to revolve around dissolved oxygen, metabolic energy, and many associated factors to be described in this report.

Training Aspects

An important part of this project which will not be published in any scientific journal is the training of new scientists in environmental science. Taking the laboratory into the field with minimum non-scientific crew required that graduate students participate in fishing, cooking, boat-handling, proposal and report writing, purchasing of supplies (including engine parts, food and chemicals), planning experiments, and arranging commuting schedules using car, boat, ferry, and floatplane. And in the case of our research in fish physiology, there were usually no readily available research tools which could be applied directly to our problems, so new methods had to be devised and tested before some of the experiments could be completed. In some cases, new equipment also had to be designed, built, and its operational characteristics measured. An example of this was our respirometer and swimming chamber for adult salmon (described in Section IV).

The most noteworthy aspect of the present program is the wide scope of the training provided for graduate students. After several years participation in one or more of the programs aboard the floating laboratory, a newly-graduated Ph.D. is capable of independent investigation in a wide variety of research lines concerning aquatic biology. The students are certainly not limited to working on problems concerning salmonid physiology. Further, they learn to work independently, as part of a team, and cooperatively with other investigators and officials from city, state, and federal agencies, and to communicate with the general public and the news media. The result of this wide scope involvement has been, internally, an excellent esprit de corps among the project staff at all levels resulting in a high work

output, and, externally, an excellent ability to compete for jobs. The first Ph.D. to graduate from the program is presently with the Biology Department at Marquette University in Milwaukee. Two Master's degrees have been completed in the program, and four additional Ph.D. degrees are expected in the next one or two years, all having been partially supported by FWPCA funds. Regardless of where their careers take them, these graduates will always be promoters of environmental conservation.

SECTION IV

MATERIALS AND METHODS

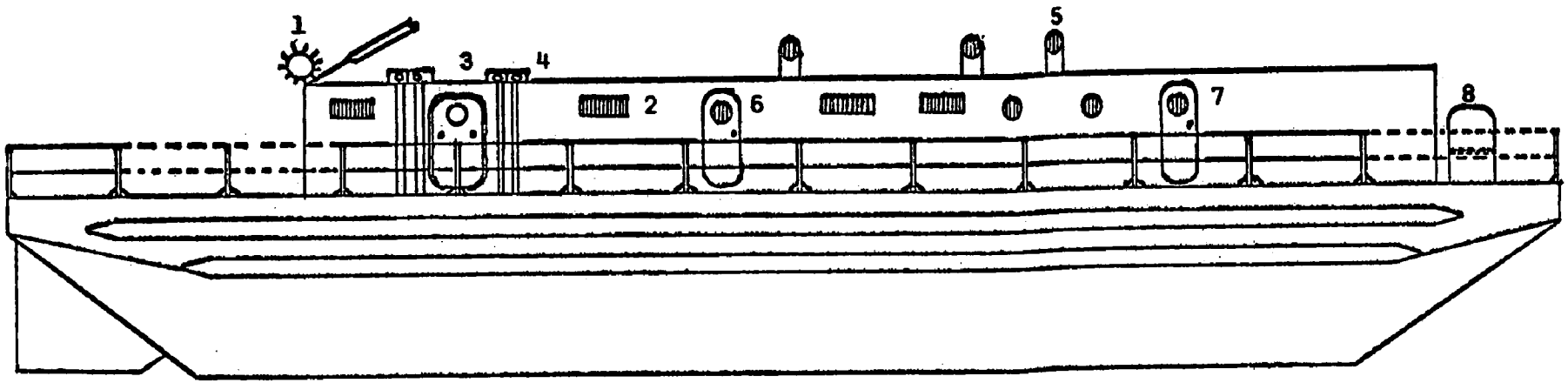
This section describes a number of general methods which are basic to most of the individual sections of the report. In addition, each section will contain further description of its own unique methods.

Designing, Building and Using a Floating Laboratory

The basic facility needed was a means of performing physiological experiments on salmon during most stages of their life cycle. The primary alternatives were shoreside laboratories with sea-water systems, a portable laboratory, and a new laboratory at the existing inland site with recirculating sea-water systems where the various environments would be simulated. Eventually the choice narrowed to a non-propelled barge because of the maximum space and stability that it would provide for a minimal cost of construction and operation. Such a vessel could anchor in bays close to the marine migration routes of salmon. Freshwater stages of several salmonids were available, along with freshwater moorage for the barge, at the University of Washington campus on the Lake Washington Ship Canal.

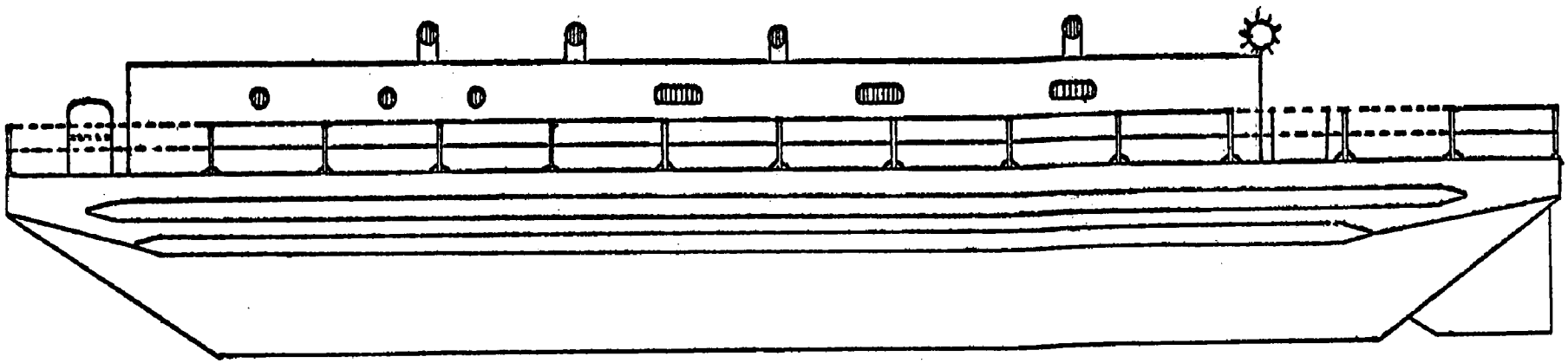
Eventually a small salvage, surplus, and marine construction company was found that had a surplus hull at the right price and would undertake the construction as well. Plans were drawn up to suit the available hull, a portion of a WW II LSM (Figs. 3, 4, 5, and 6). Living quarters for eight persons, a bunkroom for additional persons, a wet laboratory, a chemical laboratory, instrument rooms, storage and refrigeration compartments, a machine shop, and engine room with three diesel-electric generators - 2-40 kw and 1-10 kw - were provided. The barge was designed to support a crew and staff of 12 persons for eight weeks at a remote research station completely independent of shoreside services.

The basic problems that necessitated the construction of the floating laboratory were largely solved. Experiments were performed under environmental conditions that would have been very difficult to simulate ashore. Usually pitch or roll exceeded 2-3° only from vessel wakes. When experiencing vessel motion, we soon learned to weigh chemicals only during certain portions of the barge's swinging around the anchor, to limit water depth in aquaria, and to mount pressure transducers to avoid motion and engine vibration. Transporting people to the laboratory instead of the fish proved advantageous. Float plane charter flights were scheduled between one and three times weekly, making long distance commuting practical. Efficiency and productivity were high because the scientific staff enjoyed between two and seven days of endeavor away from telephones, committee meetings, and the stress of urban life. Even when the barge was anchored in the waters of metropolitan Seattle, where commuting



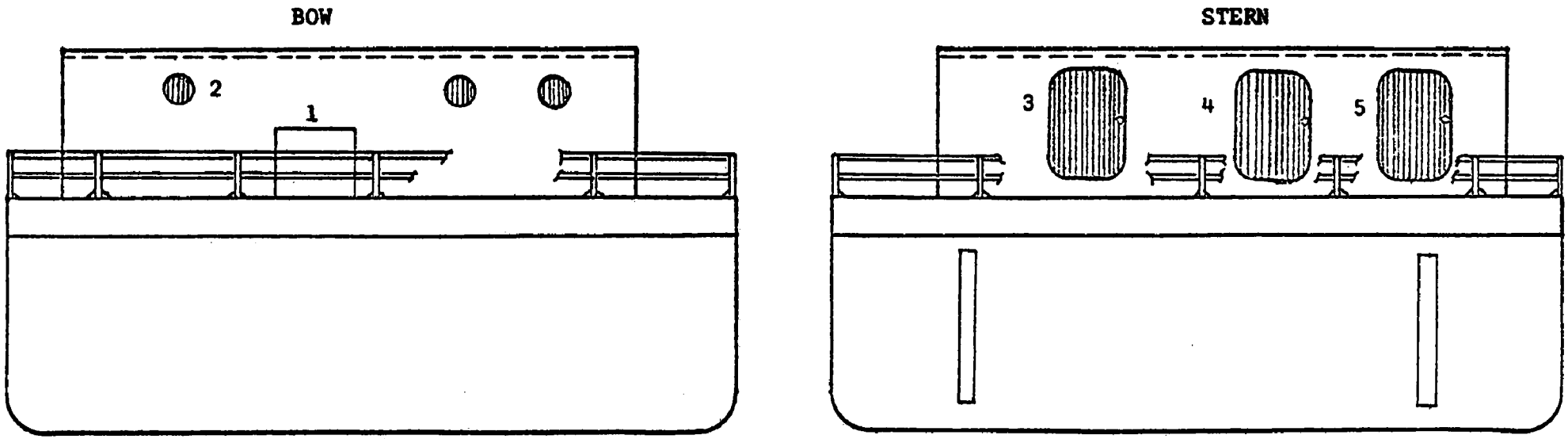
STARBOARD

1. Crane
2. Portholes
3. Entrance hatch to wet laboratory
4. Engine exhaust manifolds
5. Exhaust vent
6. Entrance hatch to engine room
7. Entrance hatch to galley
8. Anchor winch



PORT

Fig. 3. Drawings of port and starboard sections of R.V. Kumtuks.



1. Anchor winch
2. Porthole
3. Paint locker
4. SCUBA diver's locker
5. Entrance to wet laboratory

Fig. 4. Drawings of bow and stern sections of R.V. Kumtuks.

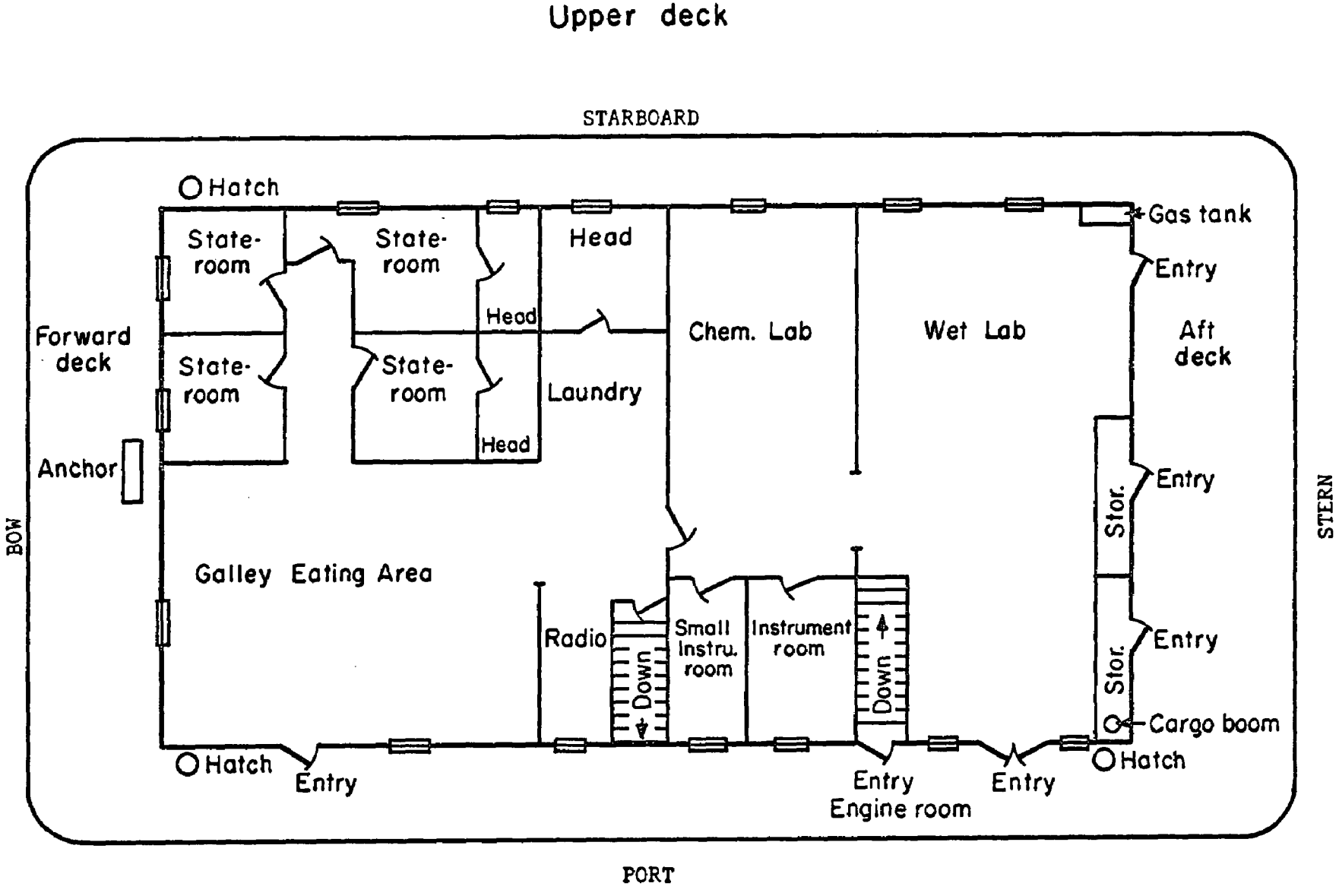


Fig. 5. Upper deck facilities of R.V. Kumtuks.

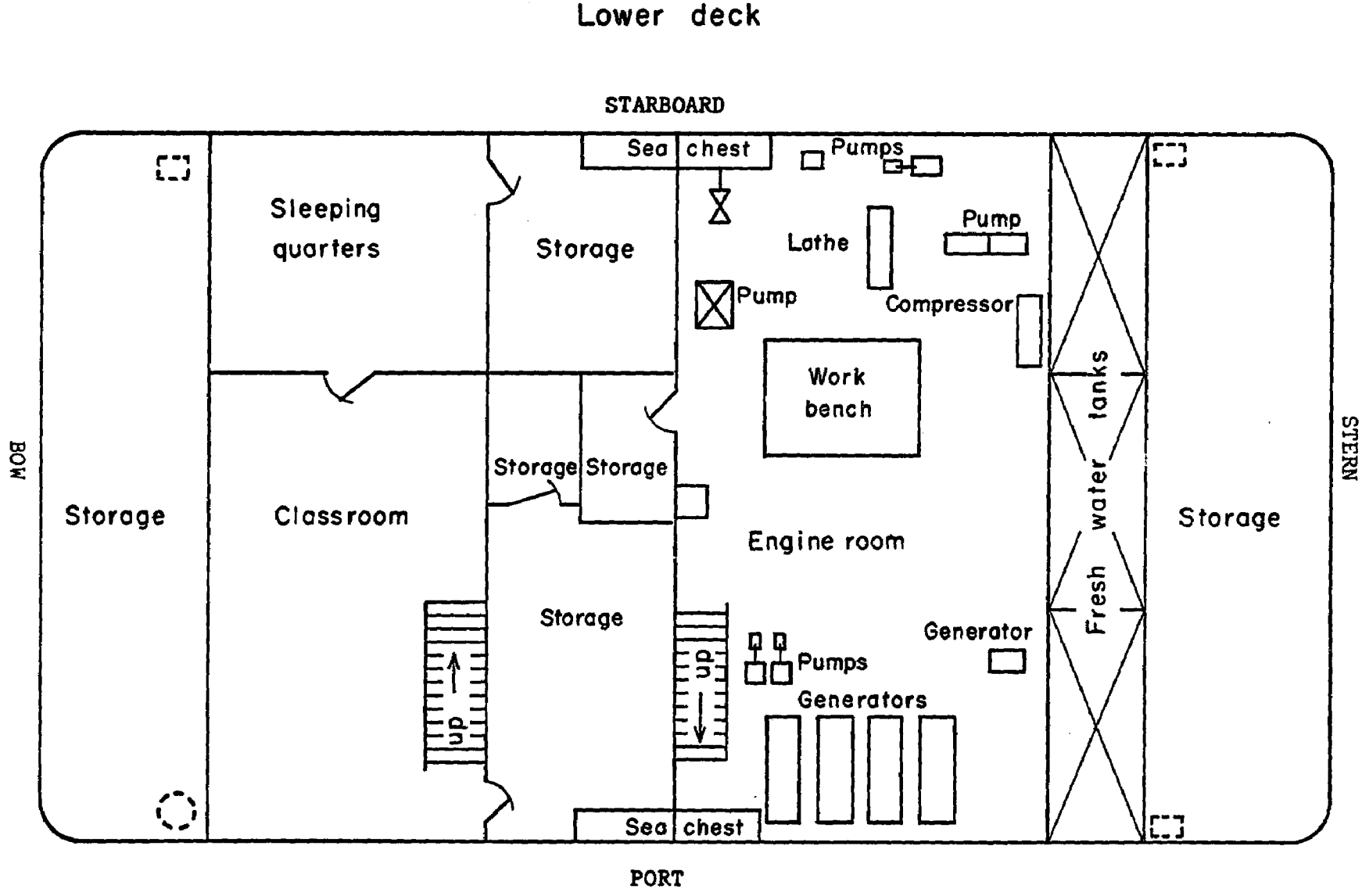


Fig. 6. Lower deck facilities of R.V. Kumtuks.

by car was practical, working for significant lengths of time - two days or longer - was most effective. Isolation from urban stress was nearly as good in the city as in rural areas, except for theft of deck equipment.

Towing the barge to the research site proved convenient and economical: it has a light displacement by barge loading standards - about 300 dead weight tons when completely loaded with fuel and water. It was towed at 5-1/2 or 6 knots by a 350-hp tug and at about 9 knots by a 1,000-hp tug and was moved at short distances under calm conditions with our 18-ft, 155 hp inboard-outboard boat. With the barge skegs under the aft rake, there was no problem of veer from side to side during towing. A 750-lb Danforth anchor proved secure in winds of over 40 knots; thus the lack of propulsion engines for maintaining position during bad weather was not a worry, at least not after we had successfully ridden out the first storm and become confident of the vessel's capabilities. We later weathered a 40-knot blow with a 90-ft seiner and a 40-ft troller moored to the barge.

Operating the Kumtuks at a research station involves a combination of shipboard and shoreside practices. Once anchored on station, the docks and small boats are lowered and assembled behind the barge. All personnel, including the scientific crew, usually help with the chores. Next the flexible suction line for the aquarium water supply is lowered, primed, and the wet laboratory activated. Masking tape is removed from cupboard doors and drawers in the chemical laboratories and instrument rooms (having been applied for the trip) and contents unpacked. Breakage during towing, even with only minimal packing, is not a problem. Twice we successfully carried out experiments while the Kumtuks was being towed from Puget Sound through the locks into freshwater. Preparations for departure from a research station are the reverse of the above.

It was originally thought that the scientific staff could contribute to engine room operations, especially when only one or two members of the scientific staff were aboard. However, the scientific staff were generally too engrossed in their work to perform regular engine checks and too valuable to be spending the time necessary for oil changes and other matters of routine engine maintenance. Conversely, once the staff had finished their activities in the late evening and most of the heavier intermittent electrical load was unnecessary, everyone could go to bed with one engine running unattended until morning. In several cases, our chief marine engineer detected minor problems that a mechanically less experienced person would not have noted and thereby probably prevented major difficulties.

The power system, while generally satisfactory, has provided some problems. Electric heat was installed, partly because its loading characteristics were supposed to minimize voltage changes when large electric motors were started. In spite of the heating ballast, voltages momentarily dropped from 120 to 100 v, and some scientific instruments were affected. After a number of possibilities were tried, a 10-kw generator was installed to supply power only to the scientific instruments.

Although 10-kw are more than would ever be needed for instruments, this large a generator was installed so that it could carry the entire shipload on warm nights and use less fuel than the larger engines. It also serves as an emergency generator.

A primary requisite of any physiology laboratory is an adequate, dependable supply of healthy animals. When studying wild animals, one must catch and maintain them in good condition. Purse seines and traps have proved to be the least injurious or stressful to salmon. Whenever possible fish were obtained in collaboration with other projects, partly for reciprocal sharing of biological and physiological information about the fish in question and partly because experience demonstrated that professional fishermen catch more fish per unit of effort than physiologists. When the staff had to conduct the fishing, an 18-ft inboard-outboard boat with a 30-ft x 600-ft hand-operated seine was used. Fish were transported in the same boat, in a flow-through live-tank. With the net aboard and live-tank filled the safe loading limit for the boat was exceeded, so it was used mostly for seining or transporting, but not for both together. The same boat with a towing post installed amidship also served as seine skiff for a chartered seiner and for towing sections of our docks lashed together as an improvised barge.

Fish were held in a variety of containers, including small-sized confinement chambers submerged in a shallow water table, 100-gal aquaria, 4-ft- and 6-ft-diameter fiberglass tanks, 8-ft-diameter plastic swimming pools, and 15-ft square pens made of nylon mesh. The latter were supported inside of the U-shaped docks and servedto hold fish safe in case of pump or power failure, although pump failure was unlikely because any of several pumps can serve in place of the laboratory pump during an emergency. We found that any confinement was stressful to salmon; the smaller the container in proportion to the size of the fish, the greater was the degree of stress. The wide range of container sizes, therefore, proved advantageous for handling many sizes of salmon.

While one reason for the inexpensive operation of the Kumtuks has been the lack of certification requirements and subsequent requirements for a three-shift licensed crew, the Coast Guard provided a courtesy inspection and made recommendations, so that the vessel and its equipment could meet all standard safety requirements. A number of short cuts were taken in the construction and operation of the vessel, but safety has never been a matter for compromise.

The costs of operation are difficult to determine exactly. Fixed annual costs are about $20,000, and 20 weeks of independent operation cost about $300/week, so that 120 days of charter at $250/day would pay crew salary, operation, and maintenance for a year. Food costs were not included in these estimates, because they vary widely, depending on numbers of people and food preferences. Also not included are the depreciation on the original capital investment of $160,000 and the cost of scientific and other non-integral equipment.

A Modified Version of the Blazka Respirometer and Exercise Chamber of Large Fish

Design

A basic problem of studying salmon, especially adults, was that they swim almost continuously. Valid physiological measurements, therefore, should be taken from swimming fish. Respirometers are devices which make such things possible and we adapted one to our needs.

Our version (Fig. 7) was made with two plexiglass (acrylic) tubes 12 inches (30.5 cm) and 8 inches (20.3 cm) in diameter with walls 1/4 inch (6.6 mm) thick. The smaller tube is loosely centered inside the larger tube at four vanes at each end. A removable set of vanes inside the smaller tube at the upstream end controls direction of flow. Flanges were machined from flat plexiglass plate and glued to the outer tube for attachment of the end blocks and for mounting of the tubes on a plywood base. The end blocks were laminated from lumberyard hardwood, turned on a wood lathe, and fiberglassed for waterproofing. The shape shown for the end blocks has proved sufficiently satisfactory so that we plan to have them duplicated in cast aluminum. The plywood base is supported on an axle and a framework that allow the whole respirometer to be tilted for insertion of fish into the respirometer while it is partly filled with water. Partly filling the respirometer in the tilted position minimizes the amount of time that the fish is out of water. A 3/4-hp motor with a variable-speed magnetic clutch drives a jet outboard impeller mounted on a stainless steel driveshaft. The driveshaft passes through the end block in a standard, graphited packing gland. The jet outboard impeller causes less spiral movement of the water than a propeller or centrifugal impeller would, and produces a flow having very low turbulence and velocities up to 131 cm/sec. When the experimental fish fills a significant portion of the cross-sectional area of the chamber, the effective velocity is considerably greater, depending on the size of the fish. Water enters the respirometer at the bottom and leaves from a large diameter, low-turbulence standpipe at the top. Tubes, wires, and other attachments to the fish also exit there.

Operation

An anesthetized fish is put into the partly-filled respirometer through the end opposite the impeller. Any tubes, wires, or other attachments are pulled through the standpipe and a hole in the inner tube with a hooked wire. The inner vanes are inserted, the end block is secured with wing nuts, and the respirometer leveled. The impeller is rotated while the respirometer completes filling so that the water movement provides a respiratory current for the fish and induces recovery from the plane III (respiratory arrest) level of anesthesia used during surgery. Between 2

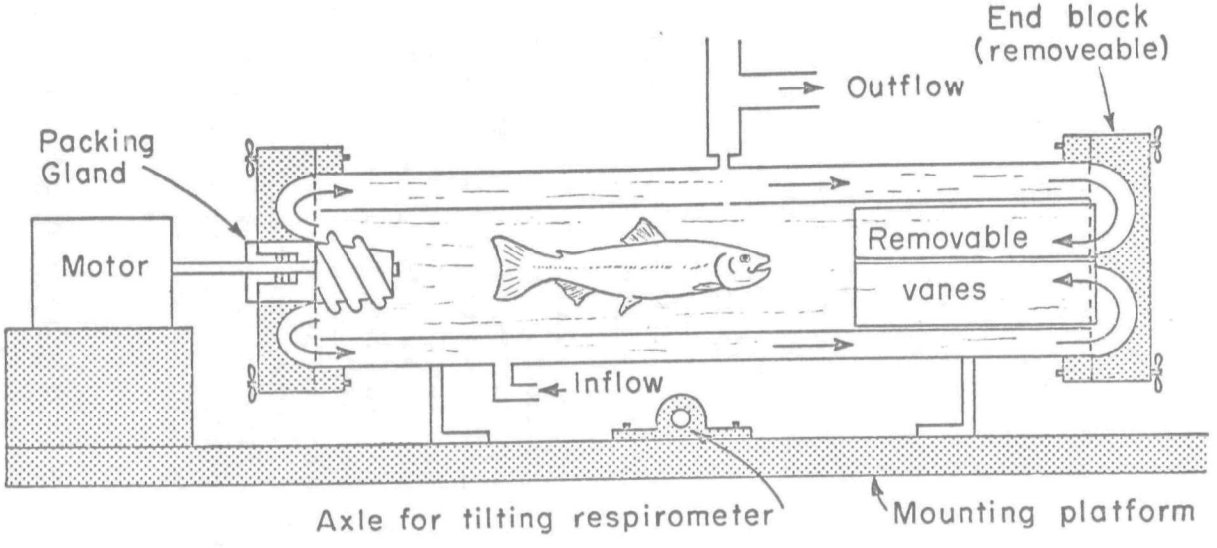


Fig. 7. Diagram of modified Blazka respirometer used for investigating physiological responses of salmon swimming in water of low dissolved oxygen.

and 24 hr of recovery in the respirometer are allowed before an experiment is begun, the length of time depending on the experimental requirements (Fig. 8).

The reverse procedure is followed for removal of the fish from the respirometer. The respirometer is tilted, the water level lowered, and the end block removed. Either the fish is pulled out by its tubes (after being disconnected from the recorder, etc.) or the respirometer is tilted down again and the remaining water with the fish poured into a large container.

Controlling the position of the fish in the respirometer without interfering with free swimming is a continuing problem. Several respirometers use screens or grids downstream from the fish to keep the fish out of the pump and provide electrification of a grid to stimulate swimming. However, we wished to minimize frictional losses and electrolytic products in sea water are toxic; therefore, screens were not included in the design. We had already found that small adult salmon gave no overt response when towed by a cannula anchored in their nasal cartilage. Preliminary trials in this respirometer showed that most fish will swim either when the water velocity increases or when their tail touches the rotating impeller. However, larger fish may break, pull out, or damage most of the tubing and routine attachments. Thus we inserted a heavy nylon cord through the muscles under the anterior edge of the fish's dorsal fin - the approximate node of its swimming oscillations and also its approximate center of gravity. We call this cord a "tether" or "towline."

In use, the towline is led out of the standpipe and tied with minimal slack while the fish recovers from surgery or previous periods of exercise. Swimming is stimulated by increasing the water velocity and giving the fish more slack in the towline. Brightly illuminating the downstream half of the respirometer and darkening the upstream half help the fish to learn to hold position in the swimming chamber. The towline system has been used successfully on salmonids ranging in weight from 0.3 to 10 kg for up to 2 weeks. The towline does not seem to cause necrosis or promote infection at the puncture site, even in the presence of the fungus Saprolegna elsewhere on the fish. Fish become accustomed to the towline usually within minutes. Because water flow produced by the impeller is sufficient to meet the fish's respiratory needs, fish have an extremely low level of ventilatory activity while they are being towed.

Anesthesia, Multiparameter Sampling, and Surgery

Problems of Sampling Live Swimming Fish

Repeated sampling of blood, urine, and inspired and expired water is necessary for evaluating immediate physiological responses of a free swimming fish to environmental changes. Surgical installation of catheters or tubes is presently the most direct method of access to blood vessels,

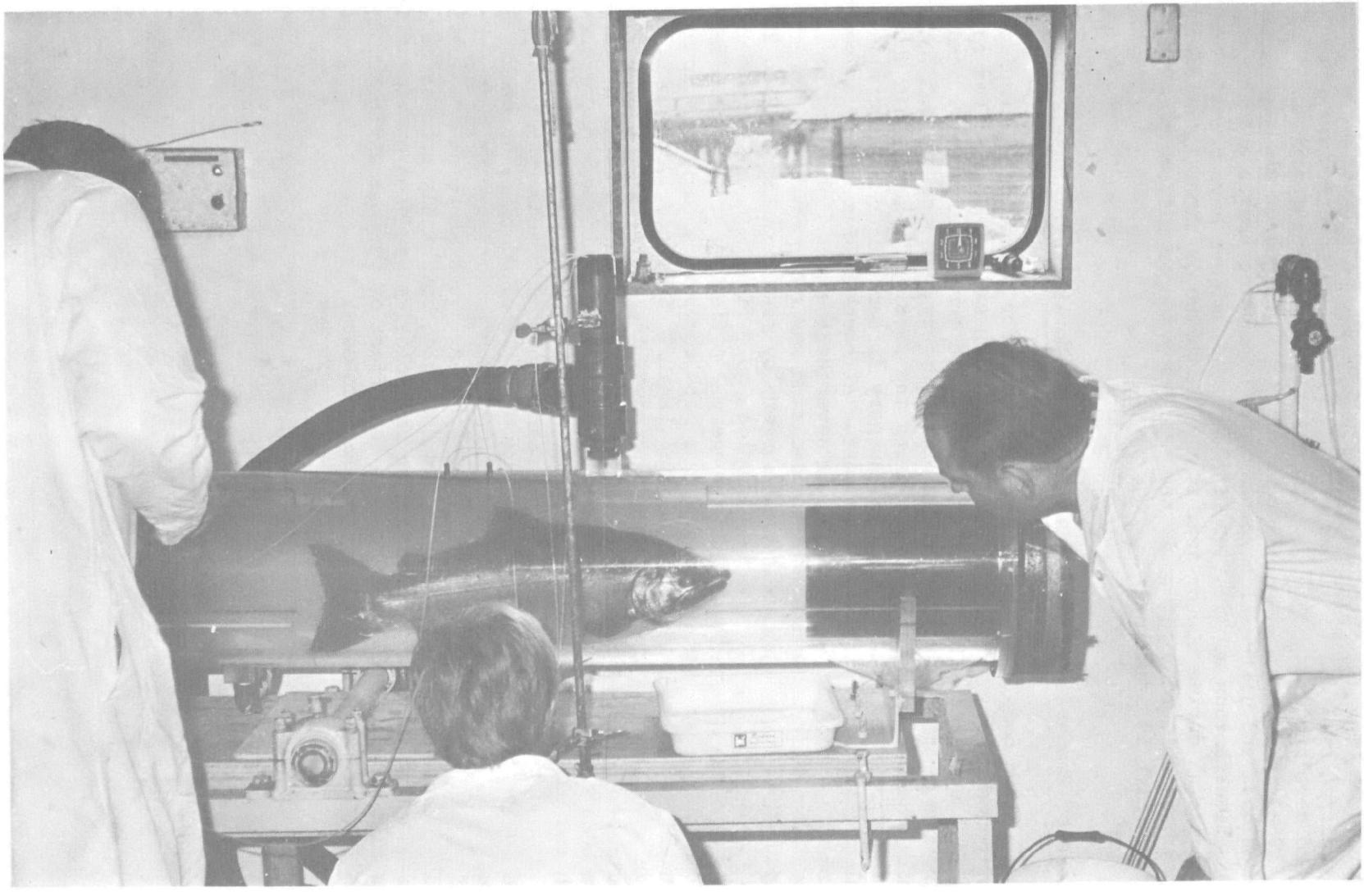


Fig. 8. Adult male coho salmon swimming in respirometer after surgical implantation of various cannulae and catheters.

urine bladder, and gill regions. A method of sampling pre-gill water was developed by Saunders (1962), and members of our project have modified and developed methods for continuous blood sampling (Smith and Bell, 1964), urine sampling (Miles, 1967, 1969; Mearns et al., in preparation), and post-gill (expired water) sampling (Davis and Watters, 1970). Simultaneous application of all the techniques have proved feasible and successful during the present study. With two or three technicians, we have been able to monitor all samples every ten or fifteen minutes while the fish is swimming.

Problems of Preparing Fish for Physiological Monitoring

A salmon completely prepared and rested for physiological monitoring of environmental responses has unavoidably been exposed to a series of prior "handling" stresses. These include initial capture, confinements in a holding pen or tank, netting, anesthesia, surgery, and acclimation to the swimming chamber. Our experience during the 1968 field season has shown that the stresses of anesthesia and surgery in particular may prove lethal to salmon in the low oxygen environment. Although indicative of a lowered stamina for these particular fish, it nevertheless posed problems for further physiological studies. Our decision was to separate the stresses of capture, netting, and anesthesia-surgery by rest periods of 12 to 24 hours for each fish. In the 1969 studies, only fish held on board the Kumtuks for two days were used for surgery. A fish was then netted from the holding tank or pen and placed in a dark chamber for 12 to 24 hours (overnight) to recover from the netting itself. Sufficient anesthetic was then infused into the dark chamber to tranquilize the rested fish in 3 to 4 minutes. The relaxed fish was removed and placed on the surgical table with adequate irrigation of aerated water and anesthetic over the gills (Smith and Bell, 1967). Catheters and tubes were installed at this time (Fig. 9) and the fish was brought to nearly complete recovery before being placed in the respirometer (stress tunnel). Finally, the fish was allowed to recover in the tunnel 12 to 20 hours (overnight) prior to the swimming performance and physiological studies. This approach (separating handling stresses) has not only proved feasible, but highly successful in insuring the validity of conducting physiological studies on wild fish. It has also given us new insight into the effect of various environments on the ability of fish to overcome handling stresses.

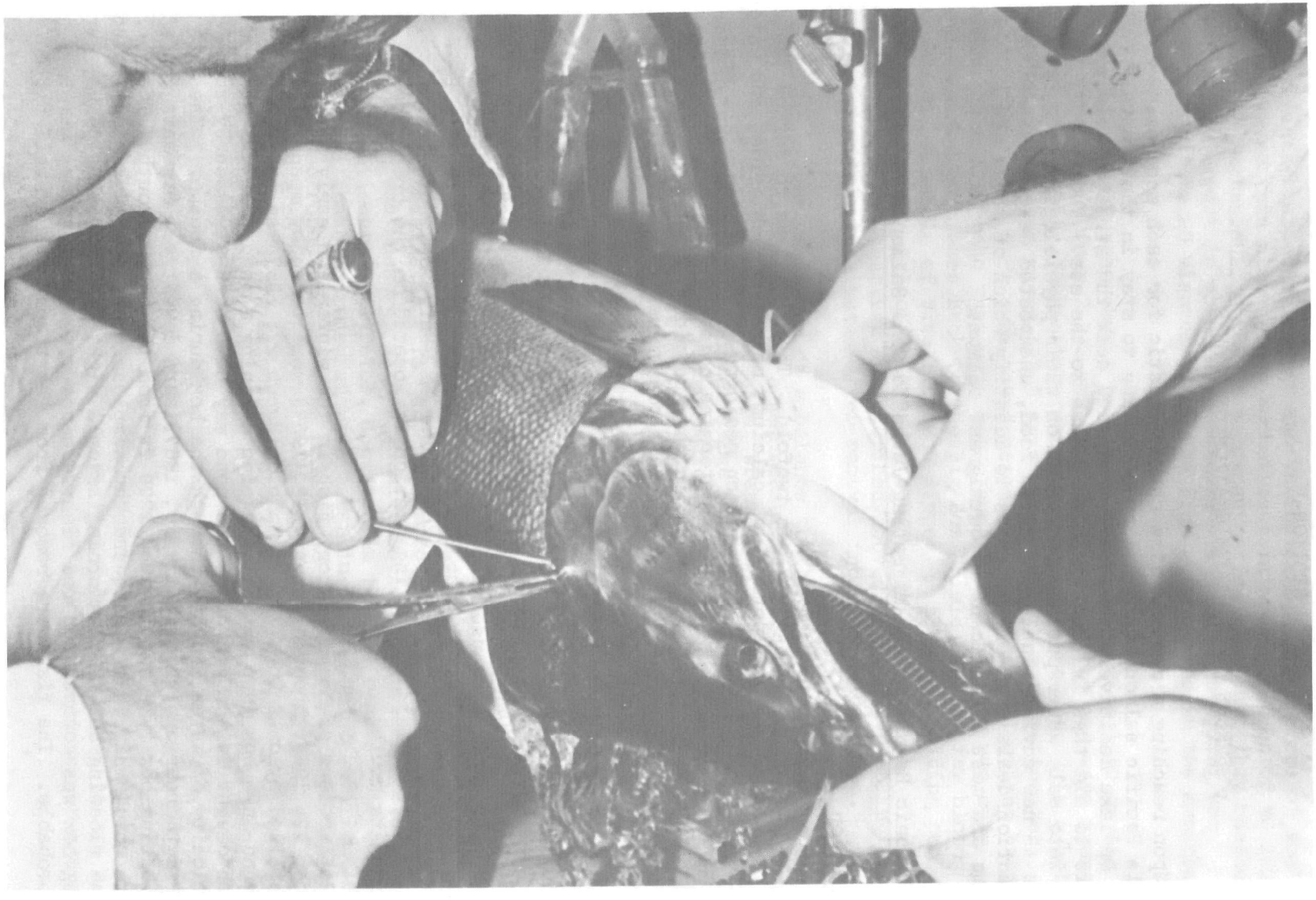


Fig. 9. Installation of catheter behind the gill in adult male coho salmon. Buccal catheter is visible arising from the snout of the fish. The tube in the salmon's mouth supplies oxygenated water and anesthetic.

SECTION V

STRESS DURING OUTMIGRATION OF SMOLTS

Upon reaching a certain size characteristic for each species, juvenile Pacific salmon which had been working to stay in their natal stream or lake decisively turn downstream and seek the strongest river currents to aid them in their pell-mell dash to the sea. Their plunge into salt water puts many strains on their physiological systems -- new environment, unfamiliar food, unexpected dangers. This section deals with our attempts to identify which of these new problems are most significant to outmigrant salmon.

Ionic Regulation in Migrating Juvenile Coho Salmon

Introduction

The purpose of this study was twofold. The first was to define the concentrations of sodium, potassium, calcium, magnesium, chloride, and water in blood plasma in juvenile coho salmon residing in freshwater. All measurements were made for each fish to determine normal values and the relationships among these parameters in the individual fish. The second purpose was to learn what changes in these constituents occur after entry into sea water.

Materials and Methods

Coho salmon, Oncorhynchus kisutch, residing in Big Beef Creek, Kitsap County, Washington, were used throughout the study. Big Beef Creek discharges into the Hood Canal near Seabeck. The facilities of the Big Beef Creek Field Station of the College of Fisheries, University of Washington, were used in the field work. The station is located on the creek about 1/4 mile from its mouth.

Collections of non-migratory fish in the winter and early spring (November 12, 1966 through February 18, 1967) were taken by means of a 15-ft braided nylon seine. The sample on March 22, 1967 was taken from fish found in a box trap set below a pipe draining a pond near the station. The fish caught in the box below the pond may have been stressed by the high velocity of water flowing through the box during a freshet. The fish collected thereafter (through May 10, 1967) were downstream migrants caught in a floating-box fyke net located 1200 ft from the stream mouth. The migrants moved mainly at night, and the samples for this study were removed from the floating box in the morning after a night of collection. The floating box was covered and the fish were not crowded; abnormal stress was improbable. The fish were removed from the traps with a brail fitted

with 1/4-inch braided nylon net and taking care to avoid loss of scales. The fish were then placed in a bucket containing a solution of MS 222 (Tricaine Methanesulfonate) in a concentration such that they reached respiratory arrest in about five minutes. The blood sampling was completed within 20 minutes after the fish were placed in the anesthetic solution.

Thirty-four coho caught in the fyke net at section 1200 on Big Beef Creek were put in four 2-1/2-gal buckets of freshwater and were transported 5 miles to Seabeck, Washington, where 29 of them were placed in a holding pen floating in sea water (25.5 ppt, measured with a refractometer). The holding pen was a 3-ft cube constructed with 1/2-inch galvanized hardware cloth. The five coho that were not placed in the holding pen were anesthetized and taken back to the Big Beef Field Station for blood sampling. A sample of five fish was dipped from the holding pen without deliberate selection every 6 hours for the succeeding 36 hours. The fish were anesthetized as described above, and blood sampling was conducted within 15 minutes after removal from the holding pen.

Wet weight was taken on a balance to the nearest tenth of a gram and total length was measured on a wet measuring board to the nearest millimeter. The dried caudal peduncle, wrapped in a tissue to prevent contamination, was severed with either large surgical scissors or a scalpel. Blood was collected into microhematocrit tubes coated with ammonium heparin as anticoagulant. The total handling time from removal of the fish from the anesthetic solution to the completion of the blood collection was usually less than 45 sec. The blood samples in the heparinized tubes were centrifuged at 13,500 x g for 5 minutes, and hematocrit was determined using a Spiracrit microhematocrit reading device. Then the microhematocrit tubes were broken immediately above the layer of leucocytes so as to separate the cells and plasma. A 20-microliter aliquot of the plasma was removed and run into a 1-ml volumetric flask containing 0.980 ml of deionized water and was thoroughly mixed by repeated inversions of the flask. When the percentage of total dissolved solids in plasma was determined, a small, hand-held refractometer ("TS" Meter, American Optical Co.) was used. Chloride ion was measured with a Buchler-Cotlove chloridimeter, an electronic potentiometric titrator, operated in the low range. The concentration of the metal ions was measured with a Perkin-Elmer, Model 290 flame absorption spectrophotometer. The blank used was deionized water and the standard was a solution containing 0.4 mEq/l Na, 0.3 mEq/l K, 0.3 mEq/l Ca, and 0.1 mEq/l Mg in deionized water. One-tenth ml of the diluted plasma was further diluted to one ml for the sodium determination.

Results

Throughout the experiment (from November 12, 1966 to May 11, 1967), length, weight, and hematocrit of the fish in samples taken in Big Beef Creek were measured. There was a total of 103 observations. Measurement

of plasma ion concentrations was begun on April 6, 1967 and measurement of total dissolved solids was begun on April 12, and both were continued for the remainder of the experiment. The means, variances, and ranges of these physical and blood parameters for freshwater and sea water observations are presented in Tables in the appendix.

Discussion

Premigratory Blood Characteristics. Hematocrit was measured in juvenile coho salmon residing in freshwater during the winter and spring months. There was considerable variation in hematocrit with time which might be explained by the variation in water temperature (Fig. 10). Snieszko (1960) reported that the hematocrit values of fish are sensitive to temperature and especially sensitive to the amount of dissolved oxygen. The changes in stream temperature may be seen to correspond with the changes in hematocrit with two exceptions. This correlation is probably due to the higher dissolved oxygen concentration and lower metabolic rate of the fish with lower stream temperatures. With a lower metabolic rate, the fish would require less oxygen delivery to the tissues while at the same time the environmental concentrations of oxygen would be higher. Both of these conditions are compatible with a hypothesis that fish have fewer circulating red cells, as indicated by lower hematocrit, at lower temperatures than at higher temperatures.

During the period between April 12 and May 10, there was no statistically detectable change in the concentration of dissolved solids (Appendix Table 3). This indicates that there was no major change in the equilibrium between excretion of water by the kidney and uptake of water across the gills. Houston (1959) reported that plasma water and chloride concentrations varied inversely with weight in premigratory development of steelhead trout. In the present study, these parameters were found to be directly correlated with weight (Table 1). There were no decreases in chloride levels as have been described from previous studies (Kubo, 1955; Houston, 1959), but compared to previously reported chloride values, those reported in this study are somewhat low. It is possible that a decrease in chloride concentration occurred before the sampling began.

A correlation matrix (Table 1) of parameters from all of the fish sampled from April 12 through May 10 was prepared without regard to sample date. Hematocrit was correlated with both length and weight, but this correlation was probably of secondary nature since growth and stream temperature both increased with time during the period of observation. The increase in length and weight was due to growth, and the increase in hematocrit was due in part to the rise in temperature. Total solids also were positively correlated with length, weight, and hematocrit. Since there was no corresponding correlation of the ion concentrations with these factors, the correlation was probably due to the changes in the protein fraction of the total dissolved solids, which may have been a function of growth. The levels of the predominantly extracellular ions sodium, calcium, and chloride were mutually related as were the intracellular ions potassium and magnesium.

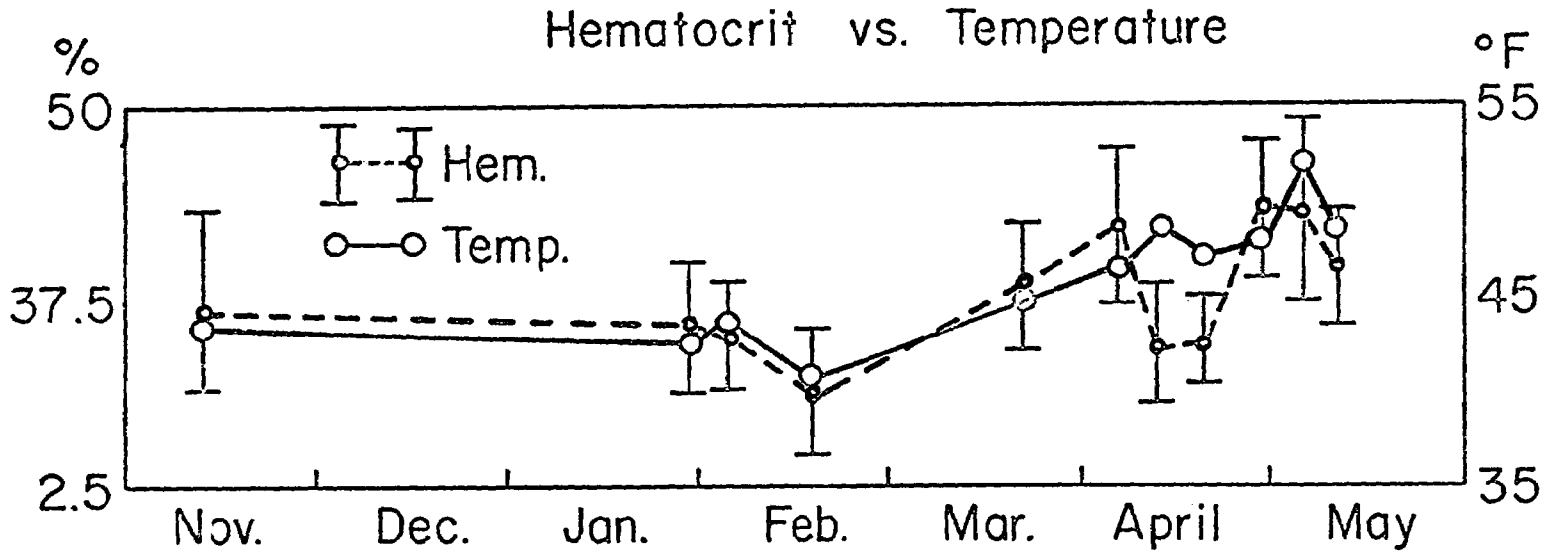


Fig. 10. Seasonal changes in hematocrit and stream temperature.

Table 1. Correlation matrix of physical and blood parameters of freshwater residents (April 12 through May 10, 1967)

	Length	Weight	Hemato-crit	Total Solids	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-
Length	1.000	0.972**	0.702**	0.466**	0.183	-0.135	0.343*	0.125	0.300*
Weight		1.000	0.675**	0.488**	0.193	-0.172	0.313*	0.150	0.333*
Hematocrit			1.000	0.557*	0.052	-0.113	0.303*	0.355*	0.083
Total solids				1.000	0.016	-0.147	0.310*	0.236	0.024
Sodium Ion					1.000	-0.352*	0.494**	-0.079	0.596**
Potassium Ion						1.000	-0.202	0.512**	-0.224
Calcium Ion							1.000	0.177	0.235
Magnesium Ion								1.000	0.075
Chloride Ion									1.000

* Correlation significant at the 95% level.

** Correlation significant at the 99% level.

The physiological and behavioral changes occurring during the parr-smolt transformation and migration are incompletely understood. In the period of these observations, there were no dramatic changes in the ionic constituents (Figs.11,12,13, and 14) of the plasma. Conte et al. (1966) found that coho throughout winter and spring were equally able to survive transfer to sea water and presumably were capable of migrating. Baggerman (1960), however, reported a salinity preference of coho for sea water only in late April and May, and a preference for freshwater in periods preceding and following this time. Conte et al. (1966) concluded that the ability to survive in sea water was independent of the parr-smolt transformation. The data gathered in the present work showed no relation between pre-migratory plasma ion concentrations and the parr-smolt transformation.

Adaptation to Sea Water. In this experiment, the juvenile coho were able to complete the adjustive phase referred to in earlier literature (Black, 1951; Fontaine and Koch, 1950; Keys, 1933) in about 36 hours. Conte et al. (1966) found this period to be about 36 to 40 hours for coho. Conte and Wagner (1965) found the period of adjustment for rainbow trout to be 60 to 100 hours, and Houston (1959) found it to be 40 to 70 hours in 50-per-cent sea water for fish of the same species. Black (1951) and Houston (1957) reported a period of 36 hours for chum fry. The adaptation to sea water in the present study was characterized by increased concentrations of plasma sodium, chloride, magnesium, and during one six-hour period, an increase in the concentration of potassium.

The percentage of packed erythrocytes rose during the first 18 hours of exposure of the fish to sea water (Fig. 15A). A rise in hematocrit can be accounted for by an increase in the size of individual erythrocytes, an increase in the number of erythrocytes, or a decrease in the plasma volume. Since the fish were osmotically losing water through the gill epithelium to the environment, the plasma must have become temporarily hyperosmotic to the erythrocytes and they must have tended to lose fluid rather than to gain it. It is also highly unlikely, although unproven, that the hemopoeitic capabilities of the fish were such that they could produce approximately a 25-per-cent increase in the number of circulating erythrocytes in a period of 18 hours. Therefore, it seems that the increase in hematocrit was due to a decrease in plasma volume. If this hypothesis is accepted, the hematocrit values obtained in this experiment can be used as measures of plasma volume.

In freshwater, the juvenile salmon is hyperosmotic to its environment, resulting in an inflow of water through the gills, which is excreted by the kidney as a relatively large volume of dilute urine. Upon entering sea water, the fish is in a hypertonic environment, and water is lost by diffusion through the gills while the kidney continues production of a smaller volume of dilute urine. Holmes (1961) reported that urine flow declined in rainbow trout (Salmo gairdneri) from 75-90 ml/kg/day in fresh-water to 0.5-1.0 ml/kg/day in sea water. A diagrammatic account is given of the changes in water flux at the three major exchange sites - gill,

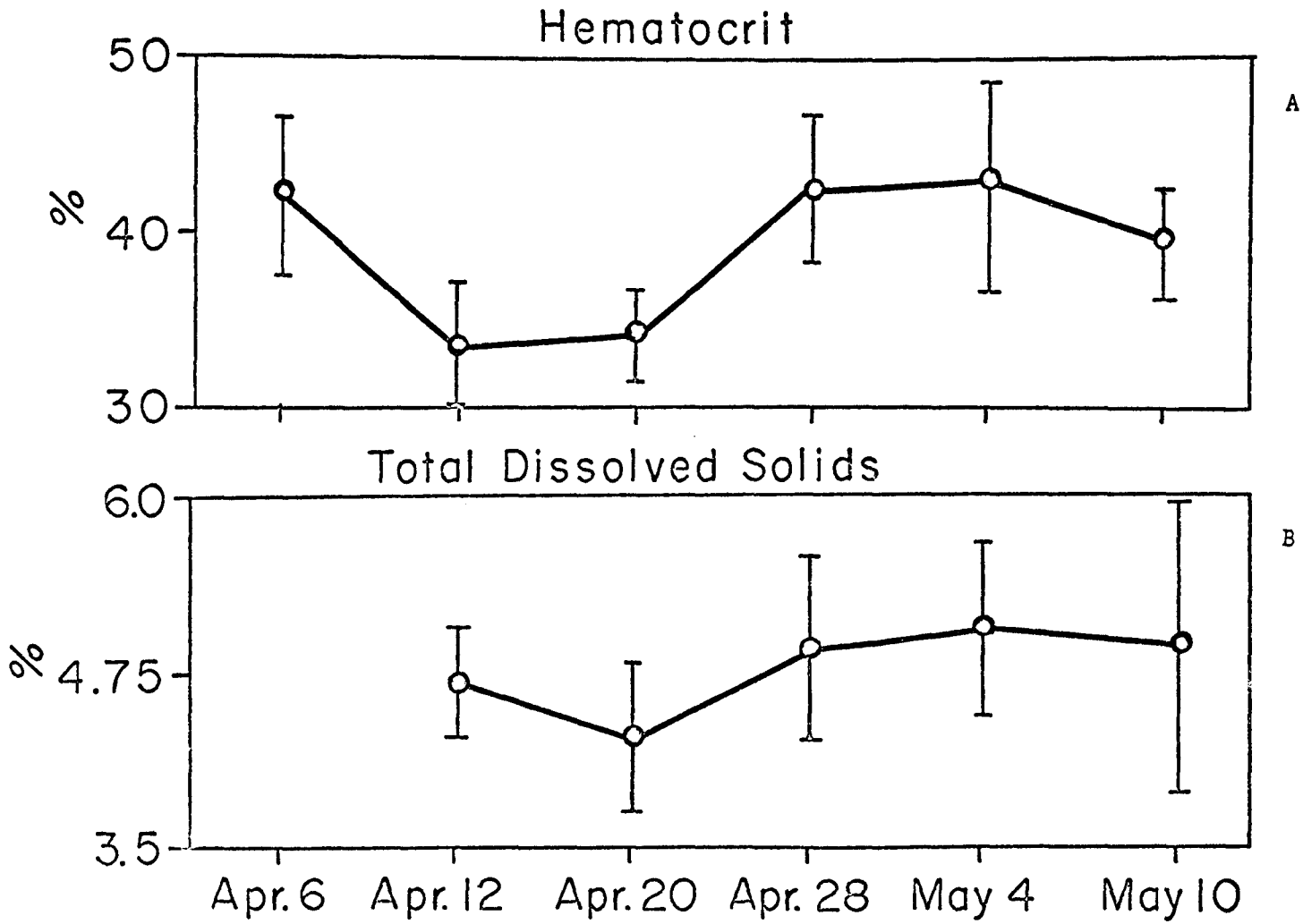


Fig. 11. Observations of hematocrit and total dissolved solids in migrating juvenile coho salmon.

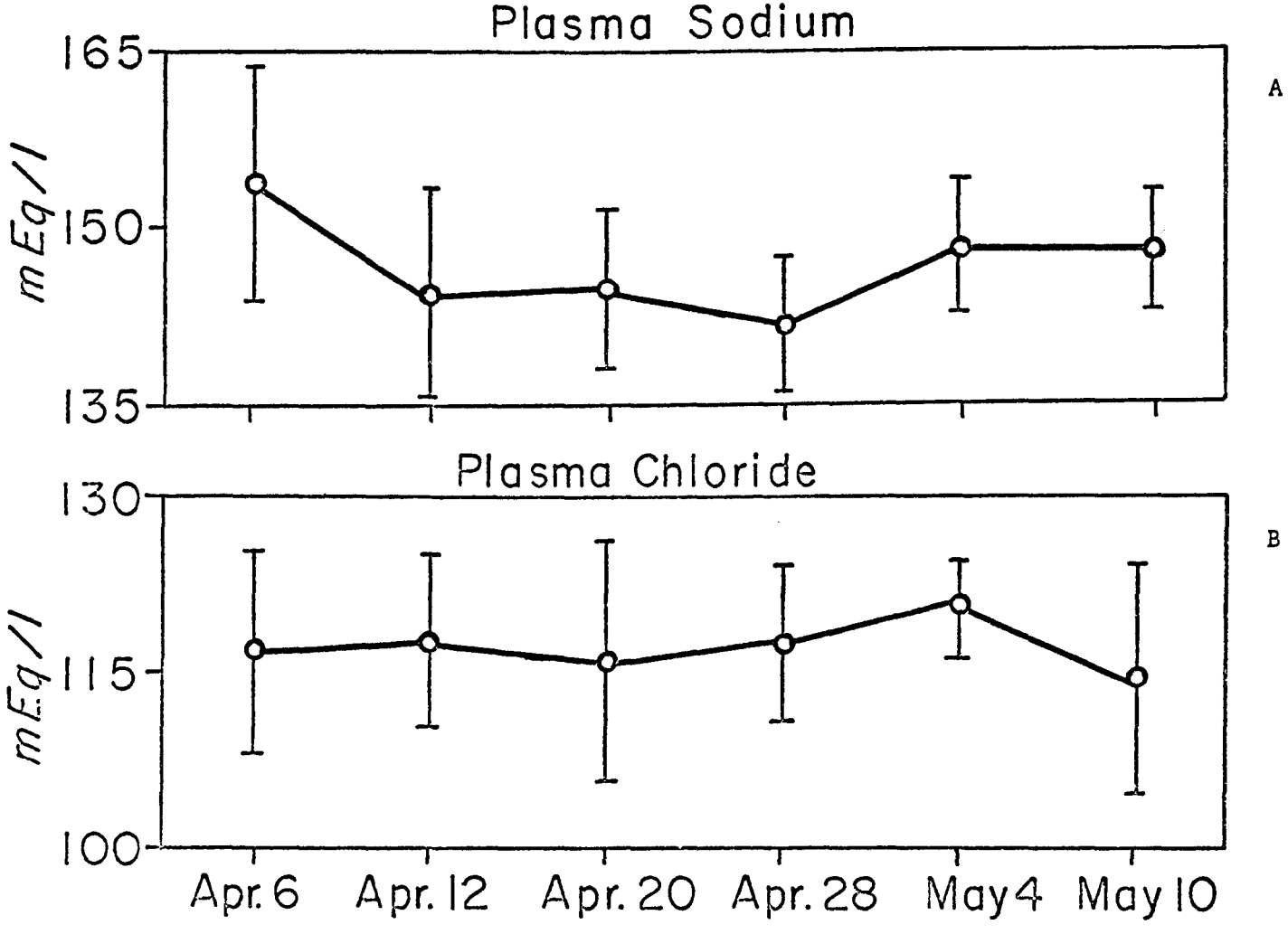


Fig. 12. Observations of sodium and chloride concentrations in the blood plasma of migrating juvenile coho salmon.

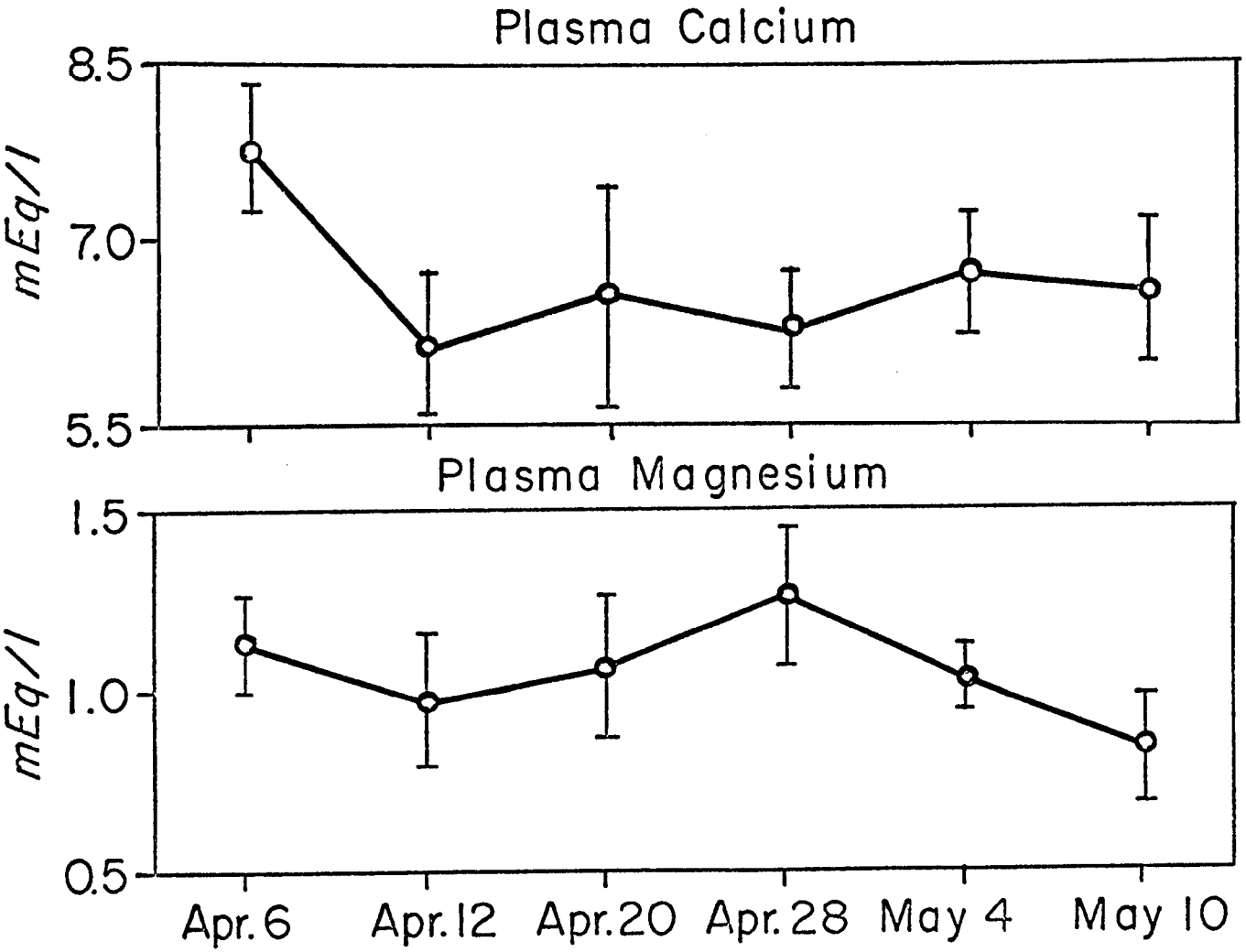


Fig. 13. Observations of calcium and magnesium concentrations in the blood plasma of migrating juvenile coho salmon.

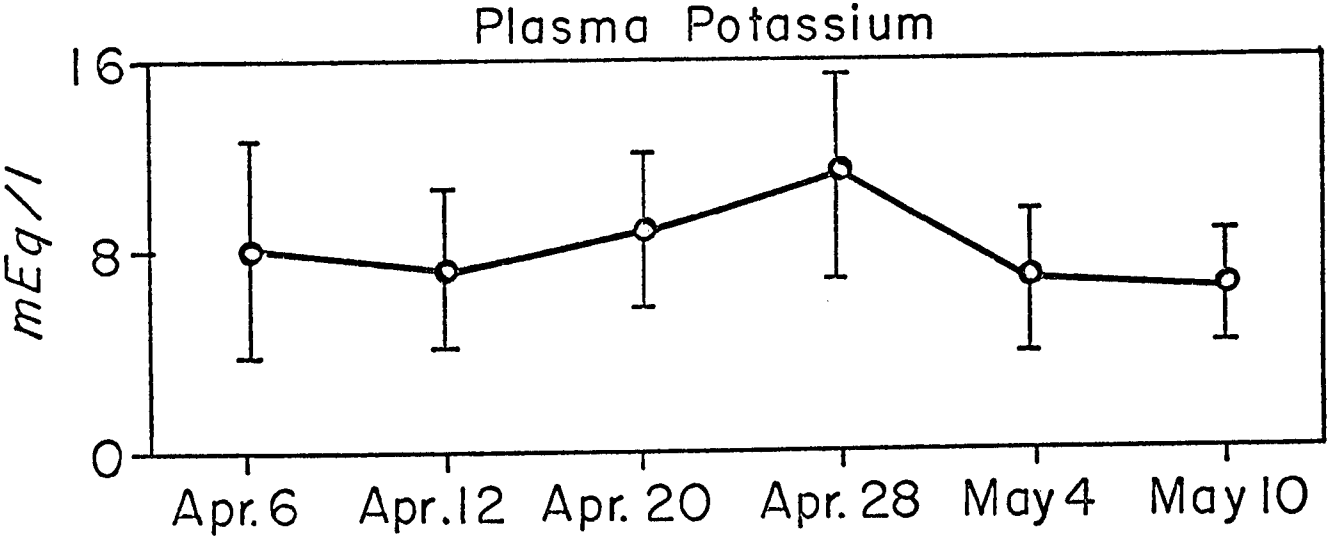


Fig. 14. Observations of potassium concentration in the blood plasma of migrating juvenile coho salmon.

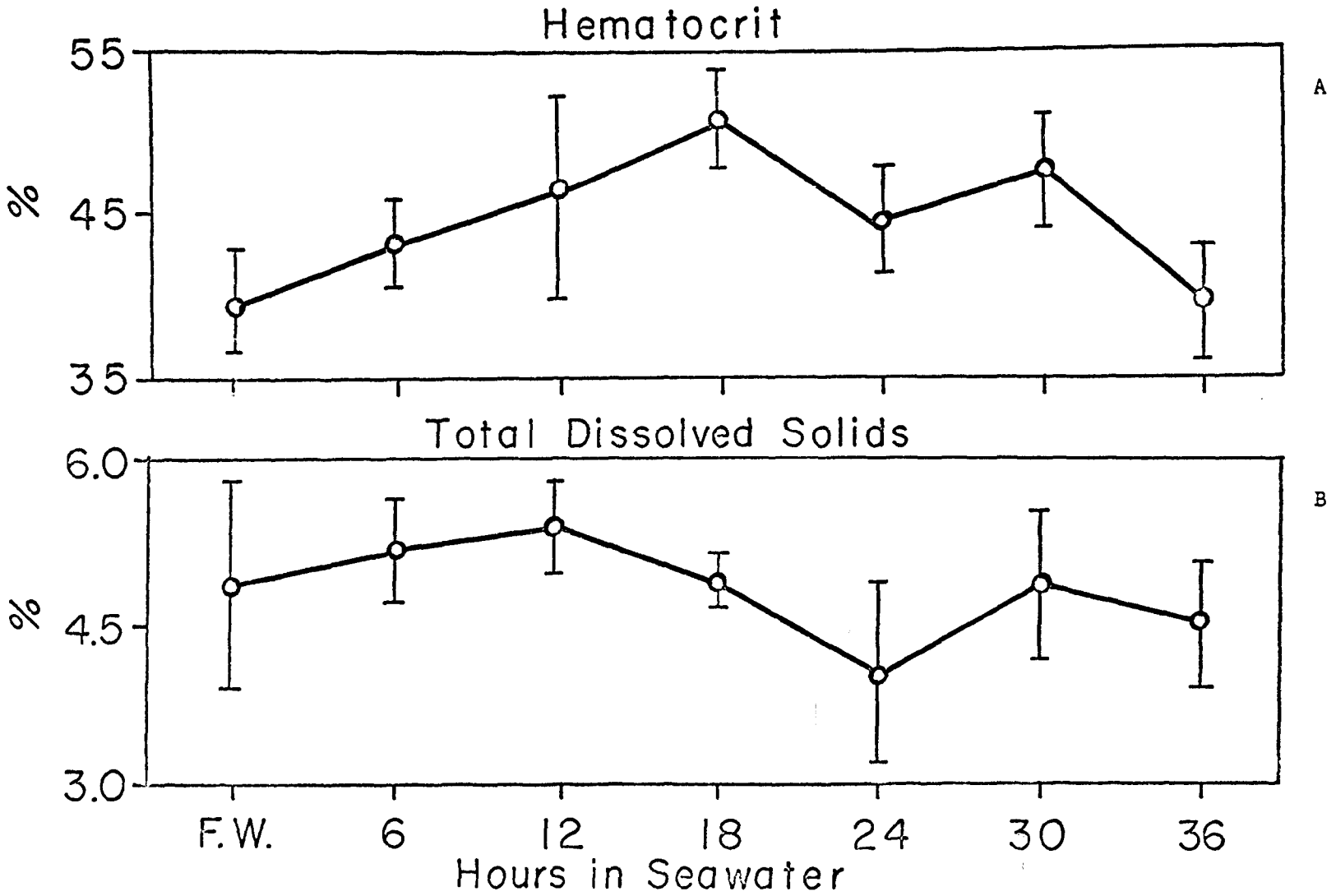


Fig. 15. Changes in the hematocrit and percentage of total dissolved solids in the blood plasma of juvenile coho salmon with adaptation to sea water.

kidney, and gut - in Fig. 16 from the data and theories of other workers and the data obtained in this study.

Upon entry of the juvenile coho salmon into sea water, there is an influx of sodium and chloride across the gills. This influx, combined with the loss of plasma water, results in increased concentrations of these ions (Fig. 17) during the first 6 hours. It appears that then the gills begin to actively transport these ions from plasma to the sea water. When continued dehydration stimulates ingestion of sea water, the fish again must cope with increased plasma levels of these ions as they are passively absorbed from the gut with the water.

Some workers have observed decreases in the sodium-chloride ratio during adaptation to sea water in several salmonid species (Gordon, 1959; Houston, 1957; Parry, 1966). Such decreases indicate a loss of some other anion, most likely bicarbonate. This is consistent with Busnel's (1943) observation of a decrease in the pH of the blood of rainbow trout after their transfer from freshwater to sea water. In the present study, a decline in the mean sodium-chloride ratio from 1.31 to 1.23 was observed during the 36-hour experimental period, but the variance of each mean was high and rendered the differences statistically insignificant. Decreases in pH have been observed to cause decrease in membrane permeability and therefore may aid passively in the adaptation to sea water (Houston, 1964).

The potassium movements observed in this study were probably passive. There was a movement of potassium from the tissue to the extracellular fluid (Fig. 18) along with the dehydration of the tissue early in the adjustive phase, but this movement reversed direction (Houston, 1959) as the potassium level reached equilibrium with the concentration in the environment. Since the concentration of potassium in sea water was 7.36 mEq/l, it is unlikely that there ever was a strong gradient of this ion between the environment and the plasma.

The concentration of calcium in the plasma remained stable (Fig. 19A) throughout the experimental period until the thirtieth hour. Houston (1959) observed that the plasma level of calcium remained rather constant along with rising tissue concentrations of this ion. Calcium ion may contribute to the decrease in surface permeability and reduce the rate of ion flux across the gills and skin. The drop in the calcium level in this experiment at the thirty-sixth hour was probably associated with initiation of divalent ion control by the hindgut.

The concentration of magnesium in the sea water used in the experiment was 39.6 mEq/l. This concentration provides a substantial gradient for passive magnesium transport across the gills. The data (Fig. 19B) show a decreasing rate of the influx of magnesium into the plasma, probably as a result of decreasing membrane permeability, and then a decrease in plasma concentration when active transport of divalent ions begins in the hindgut.

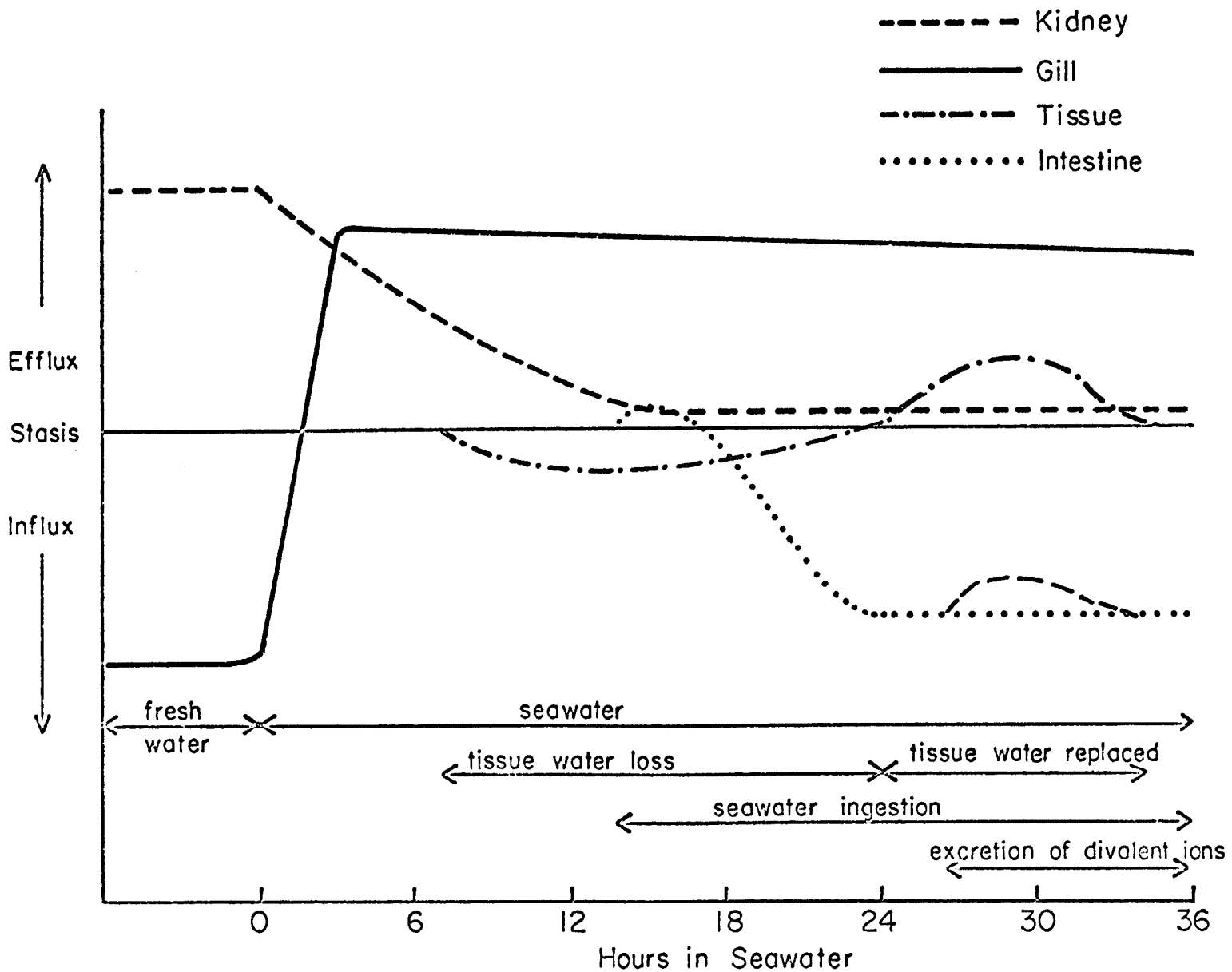


Fig. 16. Theoretical flow rates of plasma water in juvenile coho salmon during the adjustive phase.

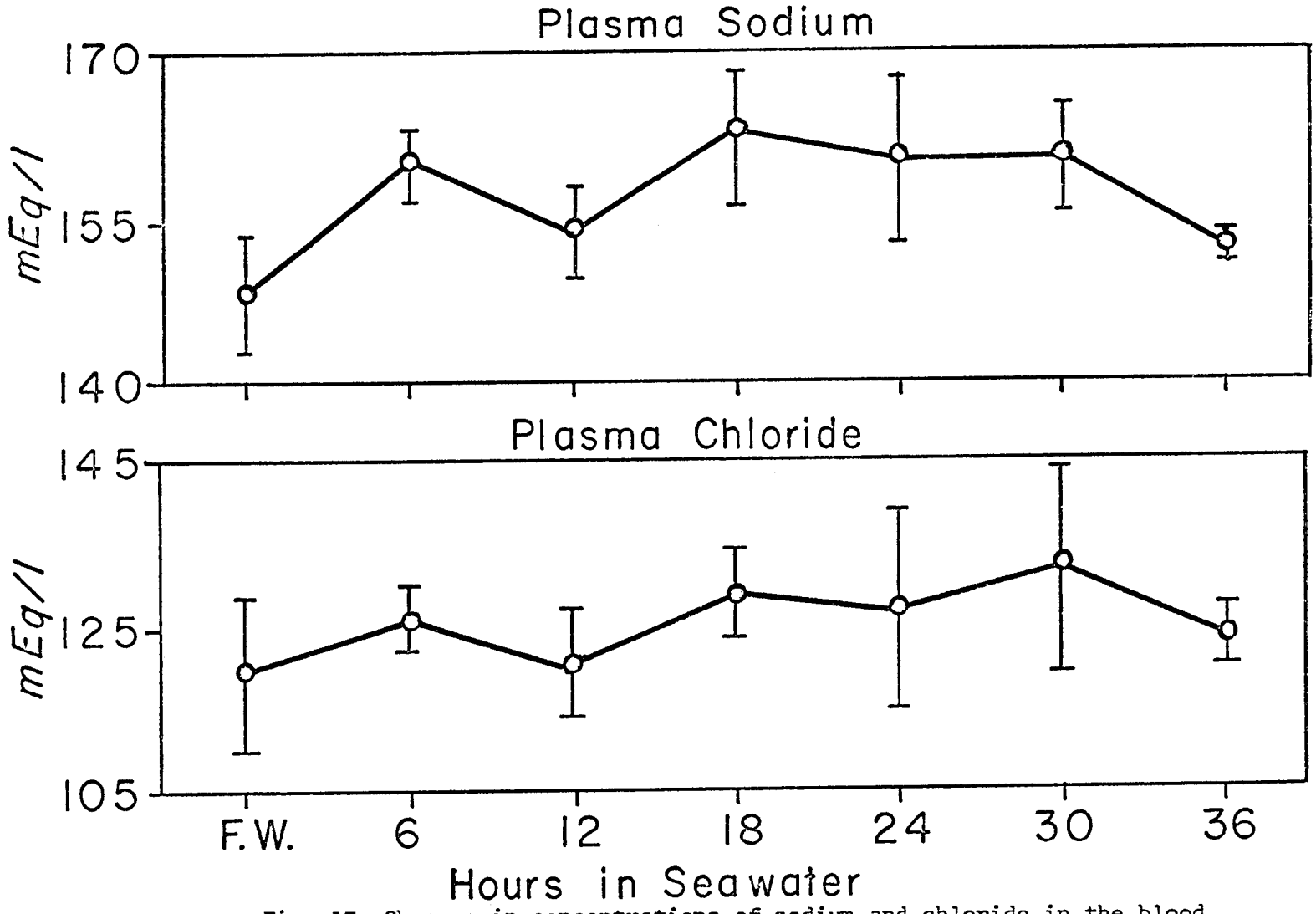


Fig. 17. Changes in concentrations of sodium and chloride in the blood plasma of juvenile coho salmon with adaptation to sea water.

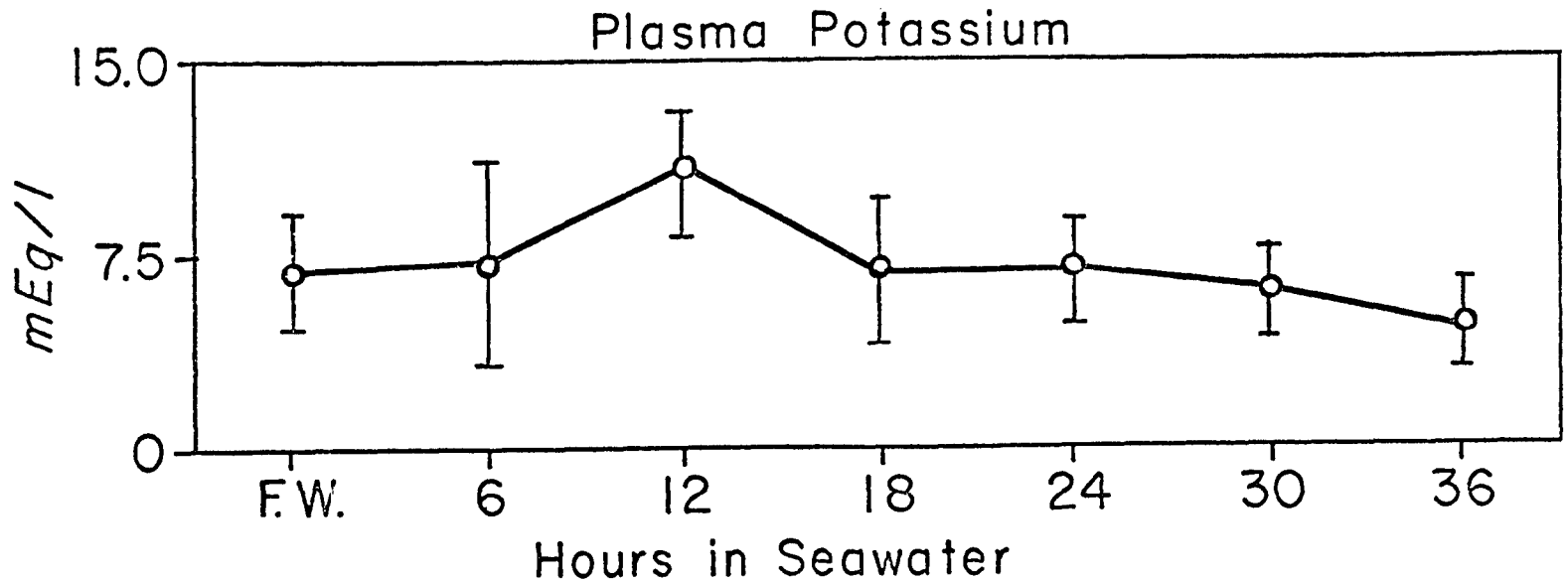


Fig. 18. Changes in concentration of potassium in the blood plasma of juvenile coho salmon with adaptation to sea water.

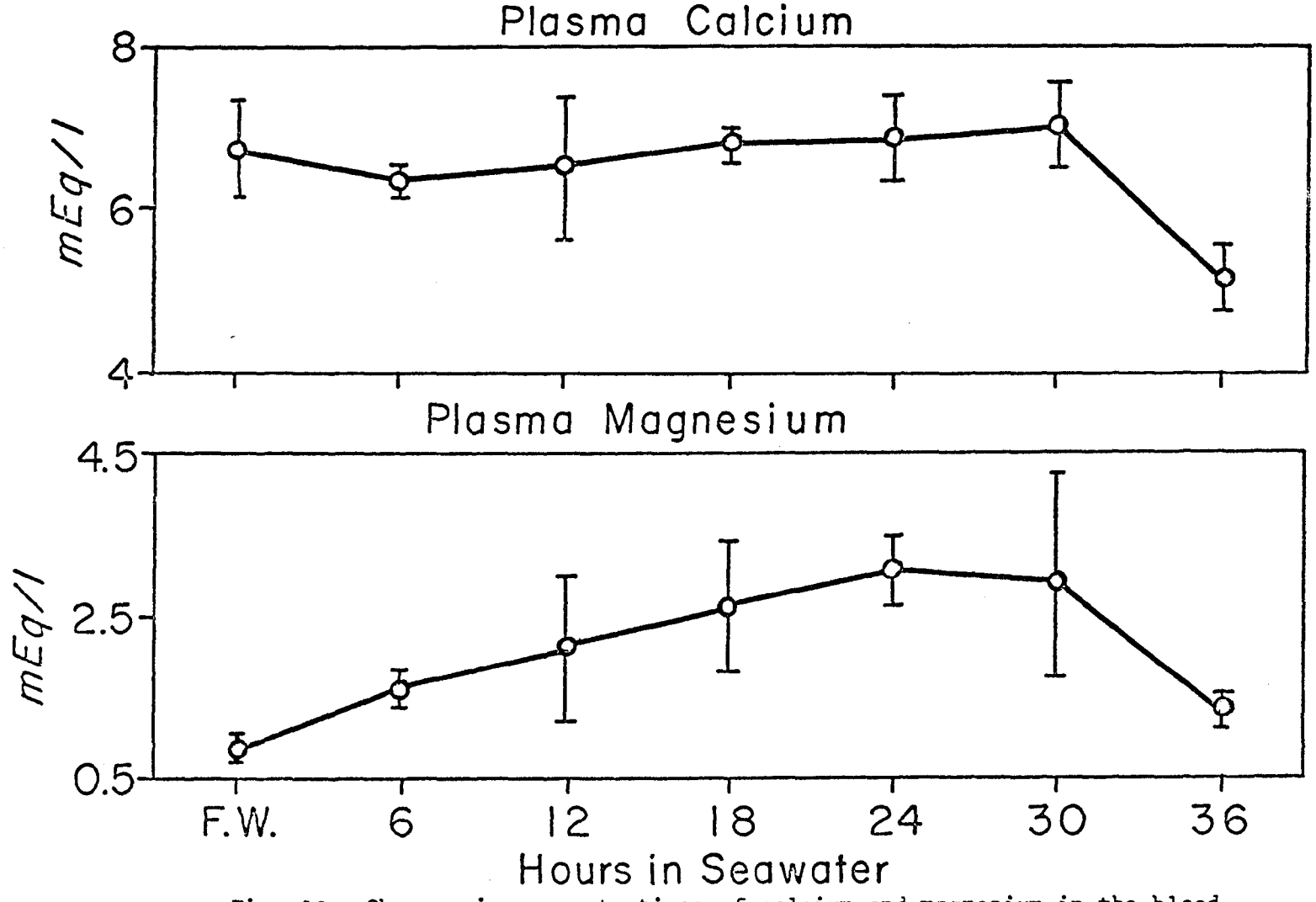


Fig. 19. Changes in concentrations of calcium and magnesium in the blood plasma of juvenile coho salmon during adaptation to sea water.

Relationship of the Sea Water Experiment to the Ecological Situation. In behavioral experiments conducted by Houston (1957), coho smolts moved into sea water (21.83 ppt) about 2 hours after they were given the opportunity to do so. Thus, the fish apparently spend little time in the estuarine environment, perhaps only the time it takes to follow the increasing salinity gradient into full strength sea water. In a stream that empties directly into sea water, such as Big Beef Creek, the fish could make the transition in a matter of minutes. Therefore, the results obtained by transferring smolts directly from freshwater to sea water very closely approximate the response to osmotic stress in wild fish migrating at will.

Lipid Composition and Hematology of Juvenile Chinook Salmon (Oncorhynchus tshawytscha) Before and After Migrating Through The Duwamish Estuary

Introduction

Chinook salmon (Oncorhynchus tshawytscha) reared at Soos Creek Hatchery near Auburn, Washington, encounter problems of feeding in their unfamiliar environment following release and downstream migration. The young salmon were reared in a hatchery environment and fed a commercial pelleted diet. They were liberated when they reached approximately 50 fish per pound (9 g each). Following release from the hatchery, the young salmon were free to migrate down the Green River into the estuary (Duwamish Waterway) and then into Elliott Bay, Washington. The young fish may remain and forage in Elliott Bay for a temporary period prior to their seaward migration through Puget Sound and the Strait of Juan de Fuca into the Pacific Ocean. While retained in the hatchery environment, the young fish were fed a pelleted diet periodically, and hence were accustomed to the feeding schedule of the hatchery attendants. In the environment of river and estuary, the young fish had to seek and catch food. The Green River, at the time of the salmon's release from the hatchery, offered few food items. The Duwamish Waterway was a polluted area near Seattle Harbor, containing reduced oxygen levels, and receiving chemical, oil, and domestic wastes. The estuary probably contained a minimal food supply for the young fish.

Young salmon were captured by tow net at specific locations along their migration route from the hatchery to Elliott Bay to study changes in their lipid composition, specific fatty acid composition, and hematology. To compare these changes quantitatively, a control sample of fish was taken from the hatchery at the time of release. Additional fish were taken from the estuarine environment and placed aboard the R.V. Kumtuks located in the Duwamish Waterway, and fed a commercial diet. Quantitative analyses of fork length, body weight, and total lipid and fatty acid concentrations were performed to assess the quality and amounts of food being ingested, and to evaluate the influence of diet upon the lipid components of the fish.

Materials and Methods

Sampling of the Fish. Downstream-migrating chinook, released from the hatchery in late May, were captured by tow net in the Duwamish Waterway (Fig. 20). The fish were taken from the tow net and immediately packed in ice for transportation to the research laboratory at the University of Washington. Some of the captured salmon were utilized for hematological experiments, and others were transported live to the R.V. Kumtuks for mortality studies and studies by other individuals in related areas of physiological work.

Lipid Analysis. Individual samples of the young salmon were homogenized in a mixture of chloroform and methanol for lipid extraction (Bligh and Dyer, 1959). Total lipid analyses were taken to determine the amount of lipid in the entire salmon, and this was related to the fish's fork length and weight. Methylation of the total fatty acids was accomplished prior to gas chromatographic analysis of the individual fatty acids present. Supportive thin-layer chromatography and gas-liquid chromatography were used to verify the identification of individual fatty acids. The fatty acids were detected by a Model 5750 research gas chromatograph manufactured by Hewlett-Packard. Detection of the fatty acids present was made at 190 C, employing a flow rate of 60 mm/min of helium gas in a column containing 15 per cent ethylene glycol succinate bound to chromasorb-p (60 - 80 mesh).

Identification of the Fatty Acids. Comparison of known fatty acid standards and related material from other salmon lipids was used to identify the fatty acids present in the lipids of the young salmon. Procedures employing retention time and supportive work with thin-layer chromatography have been described earlier by Saddler et al. (1966).

Hematology. Juvenile chinook salmon from the hatchery at the University of Washington were cultured on Abernathy dry pellet[4] in freshwater and sea water. Fish from these two environments were compared to juvenile chinook salmon cultured concurrently at Soos Creek Hatchery on Oregon moist pellet,[4] and with the same stock of salmon following their release and subsequent capture by tow netting in Elliott Bay. A portion of the fish captured in Elliott Bay were cultured for 12 days in sea water on the R.V. Kumtuks on Abernathy dry pellets.

Hematological analyses included measurements of packed cell volume (PCV or hematocrit) by the microhematocrit technique, hemoglobin concentration by the cyanmethemoglobin method, mean corpuscular hemoglobin concentration (MCHC) by standard calculations (Wintrobe, 1967), and total dissolved solids with a Goldberg refractometer. Fork length and body weight were also

[4]Obtained from R. V. Moore Company, La Conner, Washington.

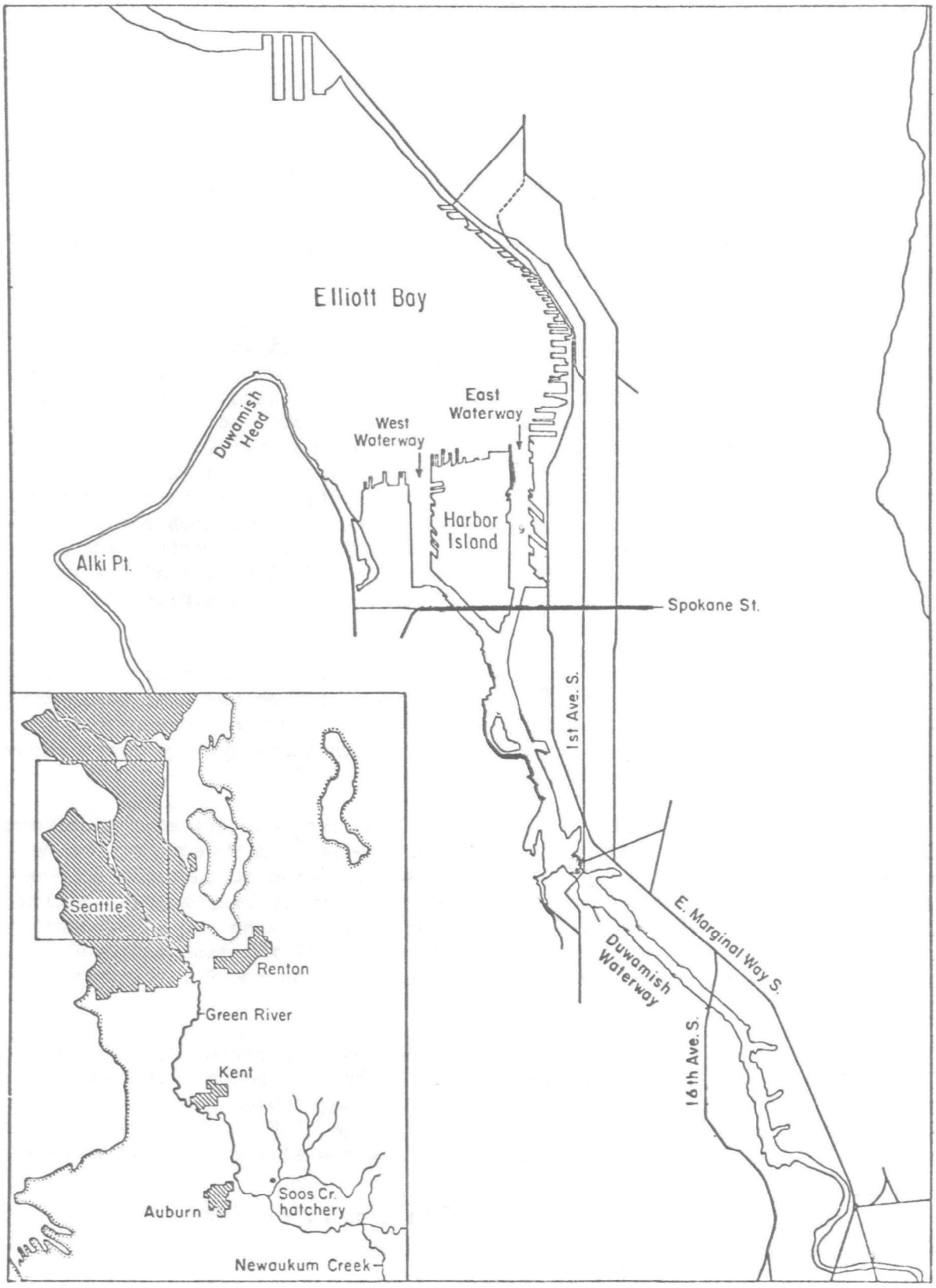


Fig. 20. The Duwamish Estuary and Green-Duwamish River.

determined for each fish. Chinook salmon sampled at Soos Creek Hatchery as well as Soos Creek chinook captured in Elliott Bay contained insufficient volumes of blood to permit measurement of all hematological variables in each fish. The values for salmon sampled at Soos Creek Hatchery represent pooled observations. Insufficient sample sizes did not permit complete evaluations of blood in chinook captured in Elliott Bay.

Results

Chinook salmon reared at Soos Creek Hatchery and those cultured in these experiments were fed either Oregon moist pellets or Abernathy dry pellets. Table 2 gives the percentage composition of the basic ingredients

Table 2. Percentage composition of basic ingredients in Oregon moist pellet and Abernathy dry pellet diets. Oil content was derived for both diets from fish, soybean, and cottonseed oils

Diet	Crude protein, %	Crude fat, %	Crude fiber, %	Carbohydrate, %	Water %
Oregon moist pellets	35.0	5.0	4.0	-	35.0
Abernathy dry pellets	43.0	10.5	-	26.0	8.0

for the two diets. The oil source is listed as crude fat and contained fish oil, soybean oil, and cottonseed oil. The plant oils contributed greater amounts of linoleic acid (18:2) than is usually found in aquatic marine food items utilized by salmon (Gruger et al., 1964). The two diets contained twenty-eight major fatty acids. These fatty acids contained from 8 to 24 carbon atoms and from 0 to 6 double bonds. Six of the twenty-eight fatty acids were selected for detailed examination because of their quantitative and physiologic importance: palmitic (16:0), oleic (18:1), linoleic (18:2), arachidonic (20:4), eicosapentaenoic (20:5), and docosahexaenoic (22:6) acids. In addition, the two diets contained the same fatty acids found in hatchery salmon and in native salmon inhabiting the Puget Sound area. Major differences in the two diets existed in the percentages of linoleic acid (18:2) and the major polyunsaturated fatty acids containing five and six double bonds. Percentage relationships for fatty acid contents sometimes are misleading and difficult to evaluate quantitatively. Basically, each fatty acid is relative to the total

number present and an increase or decrease in percentage may mislead the reader. Table 3 gives concentrations (mg) and proportions of the individual fatty acids in three-gram samples of the Oregon moist pellet and Abernathy dry pellet diets. The information in these tables indicates the amount of lipid available to the fish following consumption.

The juvenile chinook salmon released from the hatchery frequently remained in freshwater near the hatchery for seven to ten days before migrating downstream into the estuary. Tables 4 and 5 give the relative percentages of the individual fatty acids for salmon taken directly from the hatchery and for salmon that were retained in freshwater and fed Abernathy pellets. Major changes during this time included a 10-mm increase in length, and a 33 per cent increase in weight from an average of 3.8 g to 5.7 g. The total lipid percentage for the salmon fed Abernathy pellets was 3.6 per cent, while those from the hatchery was only 2.6 per cent. Major changes in fatty acids included an approximately two-fold increase in the amount of 18:2 and a major reduction in the amount of polyunsaturated fatty acids for the salmon fed Abernathy pellets. Concentrations (mg) of saturated and monounsaturated fatty acids increased in hatchery-reared fish and in hatchery fish fed Abernathy pellets in freshwater. Linoleic acid increased from 13 mg to 51 mg, while at the same time, concentrations of eicosapentaenoic and docosahexaenoic acids remained approximately the same. Tables 6 and 7 give the comparisons for the weight relationships of the major fatty acids present.

Chinook salmon migrating through the Duwamish River into the West Waterway, near the entrance of Elliott Bay, showed significant changes in the percentages of specific fatty acids. Linoleic acid accounted for 6.0 per cent of the total fatty acids present. This constituted a marked reduction relative to hatchery fish which contained 14.6 per cent linoleic acid, and hatchery fish fed Abernathy pellets which contained 29.7 per cent. Tables 8 and 9 present the percentage and weight relationships for juvenile chinook salmon captured in the West Waterway of the Duwamish River, where the proportion of docosahexaenoic acid increased 26 per cent. However, the concentration (mg) of this acid (17.7 mg) was very similar to the concentrations found in chinook salmon reared on Oregon moist pellet and residing in the environments studied. Juvenile salmon migrating into the West Waterway were captured and retained on the research vessel Kumtuks and fed Abernathy pellets. These fish showed a reversal in the original fatty acid pattern found in salmon cultured in freshwater, namely, a sharp increase in the percentage of linoleic acid and a decrease in polyunsaturated compounds. Tables 10 and 11 give the relative percentage and the weight relationships for the fatty acids obtained from these fish while on the R. V. Kumtuks.

Hematology. Soos Creek Hatchery salmon were smaller in size, and gave lower readings for packed cell volume, hemoglobin, and total dissolved solids than salmon cultured on Abernathy diet in either freshwater or sea water. Following release of the hatchery salmon into the Green River, and upon their subsequent capture and sampling in the estuary, there were further declines

Table 3. Fatty acid contents of Oregon moist and Abernathy dry pellet diets expressed in per cent and mg weight

Fatty Acid Fatty Acid	Content in Oregon moist pellet,* %	Content in Oregon moist pellet,* mg	Content in Abernathy dry pellet,* %	Content in Abernathy dry pellet,* mg
8:	0.00	0.00	0.00	0.00
10:0	0.03	0.06	0.00	0.00
12:0	0.06	0.13	0.12	0.32
14:0	3.35	7.82	1.46	3.84
14:1	0.21	0.59	0.09	0.25
15:0	0.34	0.79	0.15	0.40
15:1	0.02	0.05	0.01	0.02
16:0	16.31	38.04	12.45	32.70
16:1	4.48	10.45	2.13	5.60
16:2	0.49	1.13	0.19	0.49
17:1	0.34	0.79	0.09	0.23
18:0	3.23	7.54	3.20	8.40
18:1	18.28	42.64	19.18	50.40
18:2	20.69	48.27	40.78	107.16
18:3	0.12	0.27	6.90	18.14
18:4	2.50	5.84	3.82	10.04
20:1	7.21	15.83	0.01	0.08
20:2	0.07	0.17	0.02	0.05
20:3	0.01	0.01	0.02	0.06
20:4	0.39	0.90	0.08	0.21
20:5	13.79	32.16	6.21	16.31
22:1	0.15	0.34	0.04	0.10
22:2	0.05	0.11	0.09	0.24
22:3	0.06	0.14	0.02	0.64
22:4	0.07	0.15	0.01	0.24
22:5	0.35	0.83	0.14	0.38
22:6	7.12	16.60	2.58	6.78
24:1	0.09	0.22	0.13	0.35

*Based upon a 3-g sample of test diet.

Table 4. Proportions of the 28 fatty acids found in Soos Creek Hatchery juvenile chinook salmon fed Oregon moist pellet

Average percentage contribution of individual fatty acids							
Number of carbons	Number of double bonds						
	0	1	2	3	4	5	6
8	0						
10	0						
12	0.05						
14	2.16	0.09					
15	0.28	0.02					
16	18.20	3.53	0.64				
17		0.20					
18	5.09	18.64	14.63	0.21	1.50		
20		3.88	0.26	0.14	1.10	6.27	
22		0.09	0.10	0.12	0.38	0.57	21.16
24		0.29					

Average weight = 3.80 g
Average length = 71.6 mm
Average lipid = 2.6 %

Table 5. Percentage fatty acid composition of juvenile, hatchery-reared chinook salmon retained in freshwater and fed Abernathy pellets.

Average percentage contribution of individual fatty acids							
Number of carbons	Number of double bonds						
	0	1	2	3	4	5	6
8	0						
10	0						
12	0.07						
14	1.85	0.09					
15	0.23	0.20					
16	15.71	2.78	0.41				
17		0.18					
18	4.67	19.12	29.72	0.63	3.93		
20		3.73	0.45	0.54	0.74	3.03	
22		0.70	0.06	0.07	0.19	0.80	9.77
24		0.42					

Table 6. Concentrations (in mg) of 28 fatty acids found in juvenile chinook salmon cultured at Soos Creek Hatchery on Oregon moist pellets

Average weight (mg) of individual fatty acids							
Number of carbons	Number of double bonds						
	0	1	2	3	4	5	6
8	0						
10	0						
12	0.04						
14	1.86	0.08					
15	0.20	0.02					
16	14.80	3.09	0.52				
17		0.18					
18	4.11	15.88	12.98	0.16	1.35		
20		3.45	0.23	0.11	0.85	5.37	
22		0.08	0.09	0.12	0.34	0.52	16.78
24		0.23					

Average weight = 3.80 g
Average length = 71.6 mm
Average lipid = 2.6 %

Table 7. Concentrations (in mg) of 28 fatty acids found in juvenile chinook salmon retained in freshwater and fed Abernathy dry pellet diet

Average concentrations (in mg) of individual fatty acids							
Number of carbons	Number of double bonds						
	0	1	2	3	4	5	6
8	0						
10	0						
12	0.13						
14	3.24	0.16					
15	0.40	0.04					
16	27.35	4.90	0.72				
17		0.31					
18	8.04	33.09	51.46	1.07	6.82		
20		6.48	0.79	0.90	1.27	5.15	
22		1.20	0.10	0.12	0.34	1.28	16.47
24		0.68					

Average weight = 5.68 g
Average length = 81.8 mm
Average lipid = 3.6 %

Table 8. Percentage fatty acid composition of 28 fatty acids found in juvenile chinook salmon that had been released from Soos Creek Hatchery and captured in the West Waterway of the Green-Duwamish river estuary

Average percentage contribution of individual fatty acids							
Number of carbons	Number of double bonds						
	0	1	2	3	4	5	6
8	0						
10	0						
12	0.23						
14	1.58	0.16					
15	0.28	0.06					
16	16.24	4.13	0.82	0.50			
17		0.31					
18	5.99	21.54	5.97	0.37	0.50		
20		2.57	0.24	0.48	2.50	5.96	
22		0.10	0.12	0.23	0.38	1.32	26.37
24		0.57					

Average weight = 3.58 g
Average length = 72.2 mm
Average lipid = 2.3 %

Table 9. Fatty acid concentrations of juvenile chinook salmon that were released from Soos Creek Hatchery and captured in the West Waterway of the Green-Duwamish river estuary

Average weight (mg) of individual fatty acids							
Number of carbons	Number of double bonds						
	0	1	2	3	4	5	6
8	0						
10	0						
12	0.15						
14	1.27	0.14					
15	0.21	0.04					
16	11.29	3.32	0.59				
17		0.39					
18	4.07	15.43	4.50	0.29	0.40		
20		2.31	0.18	0.43	1.64	4.40	
22		0.09	0.09	0.17	0.21	0.97	17.72
24		0.47					

Average weight = 3.58 g
Average length = 72.2 mm
Average lipid = 2.3 %

Table 10. Percentage composition of 28 fatty acids of juvenile chinook salmon that had been released from Soos Creek Hatchery, captured in the estuary, and retained and cultured aboard the R.V. Kumtuks on Abernathy pellets

Number of carbons	Average percentage contribution of individual fatty acids						
	Number of double bonds						
	0	1	2	3	4	5	6
8	0						
10	0						
12	0.07						
14	1.56	0.06					
15	0.19	0.03					
16	16.35	2.29	0.45				
17		0.14					
18		5.57	17.70	20.42	0.50	2.21	
20		2.19	0.35	0.53	1.39	5.35	
22		0.07	0.13	0.15	0.21	0.33	20.50
24		0.34					

Average weight = 6.52 g
Average length = 76.4 mm
Average lipid = 2.2 %

Table 11. Concentrations of 28 fatty acids of juvenile chinook salmon that had been released from Soos Creek Hatchery, captured in the estuary, and retained and cultured aboard the R.V. Kumtuks on Abernathy pellets

Average weight (mg) of individual fatty acids							
Number of carbons	Number of double bonds						
	0	1	2	3	4	5	6
8	0						
10	0						
12	0.08						
14	2.25	0.10					
15	0.27	0.03					
16	20.40	3.29	0.57				
17		0.18					
18	6.55	23.48	28.48	0.69	3.13		
20		4.63	0.49	0.71	1.52	7.06	
22		0.08	0.09	0.15	0.21	0.38	19.45
24		0.31					

in blood constituents. Maintenance of these salmon, captured in the Green-Duwamish estuary, on an Abernathy dry pellet diet for 12 days restored the PCV 22 per cent (Table 12).

Discussion

The interaction between the feeding adjustments and behavioral adjustments of juvenile salmon that encounter an alien environment after having been released from a hatchery is not known. During the time spent in the hatchery the young salmon were fed periodically and showed behavioral patterns similar to the feeding schedule. Upon their release into the river, active predation was a necessity. Stomach contents taken from the migrating juvenile chinook salmon included items usually ingested by salmon, but in addition, some nondigestible items such as pieces of wood and fir needles. This could possibly suggest a lack of adequate food items or changes in the feeding behavior of the migrating fish.

Tables 13 and 14 give the average per cent contribution of major fatty acids and the weight values for six major fatty acids of the diets and feeding environments utilized in this study. The six fatty acids listed in the table often account for as much as 80 per cent of the total fatty acids present in the diet and in the young salmon.

Studies by Mead et al. (1960) on the biogenesis of polyunsaturated acids in Tilapia mossambica employed radioactive tracers. Fish were found to synthesize large amounts of saturated and monounsaturated fatty acids. However, polyunsaturated fatty acids showed the least activity and there was question as to whether or not the essential polyunsaturated fatty acids could be metabolized in sufficient concentration by the fish. Thus, eicosapentaenoic and docosahexaenoic acids, which frequently accounted for as much as 40 per cent of the total fatty acids in fish inhabiting the estuary, need to be supplied in the diet. These two compounds were present in both diets, with the Oregon moist pellets containing approximately twice the amount contained in the Abernathy diet. The weight in milligrams for these two fatty acids showed a similar relationship for the two diets. Bioenergetic studies on the swimming performance of salmon by Krueger et al. (1968) found that juvenile coho salmon swimming over twenty-four-hr periods preferentially utilized palmitic and oleic acids for energy. These results indicated preferential use of these compounds. Essential polyunsaturated fatty acids are conserved and utilized for cell structure requirements in the growing salmon (Gruger, 1964).

Different species of wild juvenile salmon taken from streams in Western Washington have been found to contain between one and two per cent linoleic acid (Saddler and Koski, in preparation). It is not known what effect the large percentages of linoleic acid retained by the juvenile chinook salmon fed artificial diets may have on their survival, growth, general nutrition, and migration following their release from the hatchery. In approximately seven to ten days, the time estimated for migration from the hatchery into the West Waterway of the Duwamish River, the percentage of linoleic acid

Table 12. Hematological characteristics of juvenile chinook salmon cultured on different diets and residing in different environments

Group	Fork length, cm	Wet weight, g	PCV, %	HB, g-%	MCHC, %	TDS, %
Soos Creek Chinook Salmon* at hatchery (May 19, 1969)*	7.40 ±0.85	4.44 ±0.12	33.27 ±0.59	6.1846 ±0.18	20.48 ---	5.11 ±0.16
University of Washington Salmon Hatchery, Chinook Salmon (June 4, 1969)**	8.04 ±0.13	5.49 ±0.32	39.13 ±1.35	6.93 ±0.30	17.74 ±0.90	6.17 ±0.15
Soos Creek Chinook Cultured R/V Kumtuks on Abernathy pellet diet (June 4, 1969)	9.63 ±0.25	9.38 ±0.90	39.7 ±1.19	8.56 ±0.31	21.60 ±0.72	4.93 ±0.10
Soos Creek Chinook captured by townetting in Elliott Bay (June 4, 1969)	7.25	3.75	25.00	---	---	4.60
Soos Creek Chinook captured by townetting on June 4 and fed Abernathy diet for 12 days (June 12, 1969)	7.56 ±0.20	3.86 ±0.48	32.00	9.03 ±0.77	28.21	4.4

*Cultured on Oregon moist pellet.

**Cultured on Abernathy dry pellets.

Table 13. Percentage composition of the six major fatty acids in juvenile chinook salmon cultured on different diets and residing in different environments

Diets and environments	16:0, %	18:1, %	18:2, %	20:4, %	20:5, %	22:6, %
Estuary-captured	16.24	21.54	5.97	2.50	2.96	26.37
Hatchery, Diet I*	18.20	18.64	14.63	1.10	6.27	21.16
Estuary-retained, Diet II**	16.35	17.70	20.42	1.39	5.35	20.50
Diet I*	16.31	18.28	20.69	0.39	13.79	7.12
Freshwater-retained, Diet II**	15.71	19.12	29.72	0.74	3.03	9.77
Diet II**	12.45	19.18	40.78	0.08	6.21	2.58

*Diet I - Oregon moist pellets.

**Diet II - Abernathy diet.

Table 14. Concentrations (in mg) of the six major fatty acids in juvenile chinook salmon cultured on different diets and residing in different environments

Diets and environments	16:0, mg	18:1, mg	18:2, mg	20:4, mg	20:5, mg	22:6, mg
Estuary-captured	11.29	15.43	4.50	1.64	4.40	17.72
Hatchery, Diet I*	14.80	15.88	12.98	0.84	5.37	16.78
Estuary-retained, Diet II**	20.40	23.48	28.48	1.52	7.06	19.45
Diet I*	28.04	42.64	48.27	0.90	32.16	16.60
Freshwater-retained, Diet II**	27.35	33.09	51.46	1.27	5.15	16.47
Diet II**	32.70	50.40	107,16	0.21	16.31	6.78

*Oregon moist pellet.

**Abernathy dry pellet.

decreased from 20.4 to 6.0 per cent of the total. In contrast, salmon fed the Abernathy diet possessed the greatest amount of linoleic acid (29.7 per cent). Metabolically, fish cannot convert linoleic acid into the longer-chained polyunsaturated fatty acids, 20:5 and 22:6. Compounds related to linoleic acid, namely 20:2, 20:3, and 20:4, do not increase or change in the same proportion as the dramatic percentage increase of 18:2 in the fish studied. It is possible that migrating salmon utilize linoleic acid for energy during migration. Saddler and Cardwell (in preparation). found that linoleic acid was the most extensively utilized fatty acid in juvenile pink salmon (Oncorhynchus gorbuscha) that had been captured in sea water near Neah Bay, Washington, tagged with the Dennison internal anchor tag (Dell, 1968), and held for 33 days. However, the fate of 18:2 in the metabolic pattern of the young salmon cannot be concluded from these studies.

Soos Creek salmon were suffering from a low-grade infection of bacterial gill disease (Myxobacteria spp.), which probably accounted for the anemia that prevailed at the time of their release. During the juvenile salmon's 25-km migration to the estuary, there was further hematological deterioration. This condition could have resulted collectively from the bacterial infection, the limited quantity of food available in the river and estuary, and reduced food consumption by the salmon as they changed to an actively foraging type of behavior. An inadequate food supply is known to produce erythropoietic depression (Zanjani et al., 1969) and plasma protein diminution (Lysak and Wojcik, 1960). Decreased numbers of red blood cells would contribute substantially to deterioration of the salmon's physiological condition by limiting the oxygen supply to the tissues. This in turn would reduce the fish's stamina for burst and sustained swimming activity, implicating that these juvenile fish would be limited in their capabilities to escape predators and capture food organisms relative to juvenile salmon possessing a healthy hemogram.

SECTION VI

NATURAL STRESS DURING SPAWNING MIGRATION OF ADULTS

The migration problems of outmigrant juveniles and inmigrant adult salmon differ in several respects. The juveniles descend into sea water with considerable speed because they combine the river velocity with that of their own swimming. Even if lost, delayed or hindered in their journey, as long as they are not killed outright enroute, they are likely to end up in the estuary, having been minimally influenced by environmental degradation along the way. Adults on the other hand may enter several estuaries, and once committed to a particular one, stay in the lower reaches of the river mouth for several weeks. Our impression was that they would venture as far into a degraded environment as their physiological capabilities would allow, being subjected to prolonged periods of exposure to low DO, toxicants, and whatever else mankind had to offer them. The waiting problem was further complicated by their cessation of feeding - any serious delay or excessive use of stored energy could cause failure to reach the spawning grounds or cause the eggs to be of such poor quality as to effectively produce the same result.

This section of the report assesses the biological significance of several estuarine problems encountered by adult salmon in the perspective of the fact that spawning Pacific salmon eventually deteriorate and die after spawning anyway.

Normal Hematological Variations During the Spawning Migration of Chinook Salmon

Concurrent with studies on the effects of low environmental dissolved oxygen on the physiology of chinook and coho salmon, an investigation was undertaken to evaluate the normal hematological variations in chinook salmon following their arrival in the Duwamish-Green River estuary and subsequent 25-km spawning migration to Soos Creek Hatchery, near Auburn, Washington. Additional observations of blood constituents were made on subadult coho and chinook salmon and on adult sockeye salmon (*O. nerka*) captured at the entrance to the Strait of Juan de Fuca. The basic problem was to distinguish between the effects of low DO or increased residence time in the estuary from the physiological change which normally accompanies the salmon's undisturbed spawning migration.

The hematological variables examined for all groups of fish were PCV (hematocrit), TDS (total dissolved solids), and TPP (total plasma protein). Five plasma protein fractions,[5] presumably fibrinogen,

[5]Fractions tentatively identified in salmon plasma using differential precipitation with sodium sulfate.

beta globulins, alpha globulins, albumin, and pre-albumin, were examined in sexually mature chinook salmon using electrophoresis and cellulose acetate support media.

Blood samples collected from estuarine adult male and female chinook salmon revealed a sexually homogeneous and above-normal hemogram. The prespawning salmon in the estuary and at the hatchery were notably polycythemic relative to subadult salmon entering the Strait of Juan de Fuca (Tables 15 and 16). Evidently there was increased erythrocyte production during the final stages of maturation. The above-average red blood cell content (polycythemia) and the elevated plasma protein content of the adult sockeye sampled in the ocean was a reflection of the advanced maturity of these fish. After spawning, however, there was a rapid decline in hematocrit, which was more acute in female than male fish. Mature female salmon, in general possess fewer erythrocytes than males during and after upstream migration. Apparently the functional deterioration so characteristic of sexually mature salmon does not include the erythropoietic mechanisms until, or after, spawning. It is unlikely that the number of erythrocytes is the physiological factor limiting the adjustments of these salmon to hypoxia. Total dissolved solids and total plasma protein related well to changes in hematocrit. Commonly these two variables are significantly correlated ($P = 0.05$). The plasma protein values in estuarine chinook and prespawning male chinook were higher than readings obtained from sub-adult and juvenile salmon, and indicate a condition of hyperproteinemia. The diminution in protein concentration in the terminal stages of the salmon's life cycle is caused directly by starvation, although an increase in the volume of the extravascular space after the fish had entered freshwater would also have initially increased the progressive reduction in protein. The declines were more marked in female fish, presumably because ovarian development required more energy and proteinaceous materials than the developing germinal epithelium of males (Tables 15 and 16). Plasma proteins and the ability of the blood to attract metabolites from the tissues (oncotic pressure) are interrelated, with reductions in TPP contributing to the tissue edema characteristic of salmon migrating upstream.

The plasma protein composition of upstream migrating salmon deviated from patterns commonly obtained from immature fish. There was a decrease in albumin and concurrently increased globulins, notably the alpha fraction and one of the least electrophoretically mobile beta globulins (Fig. 21). Relative to TPP, all plasma protein fractions were significantly reduced. Some of the beta globulins (the beta-2 fraction) possess antibodies

Table 15. **Hematological characteristics of sub-adult chinook and coho salmon, and adult sockeye salmon captured at the entrance to the Strait of Juan de Fuca. Values are given as means ± standard errors.**

Group	Location	Sex	n	Fork length, cm	Weight, g	PCV, %	TDS, %	TPP, %
Adult sockeye	Ocean[1]	Male	4	61.38 ±0.78	2,573.75 ± 283.97	54.50 ±2.11	9.96 ±0.32	6.94 ±0.15
Adult sockeye	Ocean	Female	3	59.70 ±2.14	2,657.00 ± 470.89	53.17 ±0.55	11.73 ±1.02	8.82 ±1.79
Sub-adult coho	Ocean	Male	3	51.80 ±1.91	1,571.75 ± 149.73	46.25 ±3.70	5.88 ±0.59	4.64 ±0.73
Sub-adult coho	Ocean	Female	2	59.10 ±3.11	2,201.00 ± 154.86	60.75 ±0.53	8.73 ±0.30	6.43 ±0.34
Adult chinook	Ocean	Male	2	46.70 ±4.77	1,332.50 ± 369.47	41.75 ±3.71	5.25 ±0.32	3.58 ±0.04

[1]Entrance of Strait of Juan de Fuca.

Table 16. Hematological variations in adult Green-Duwamish River chinook salmon at various stages of maturation. Values are given as means ± standard errors.

Sample group	Location	n	Fork length, cm	Wet weight, gm	PCV, %	TDS, %	TPP, gm-%
Estuarine adult[1]	Duwamish Waterway	5	73.40 ±5.12	5,830.00 ±1,107.90	- -	7.64 ±0.37	9.48 ±0.69
Unspawned adult male	Soos Creek Hatchery	5	84.00 ±4.80	7,360.00 ±1,263.10	51.26 ±4.82	6.72 ±0.60	5.24 ±0.70
Unspawned adult female	Soos Creek Hatchery	5	83.90 ±1.95	7,646.00 ± 759.20	44.76 ±2.59	5.86 ±0.40	4.20 ±0.35
Spawned-out adult male	Soos Creek Hatchery	5	80.42 ±5.42	6,358.30 ±1,020.10	38.00 ±6.86	4.14 ±0.35	3.25 ±0.39
Spawned-out adult female	Soos Creek Hatchery	4	81.25 ±1.45	6,455.00 ± 960.40	38.68 ±5.32	2.98 ±0.53	2.37 ±0.20

[1]Sample group sexually intermixed.

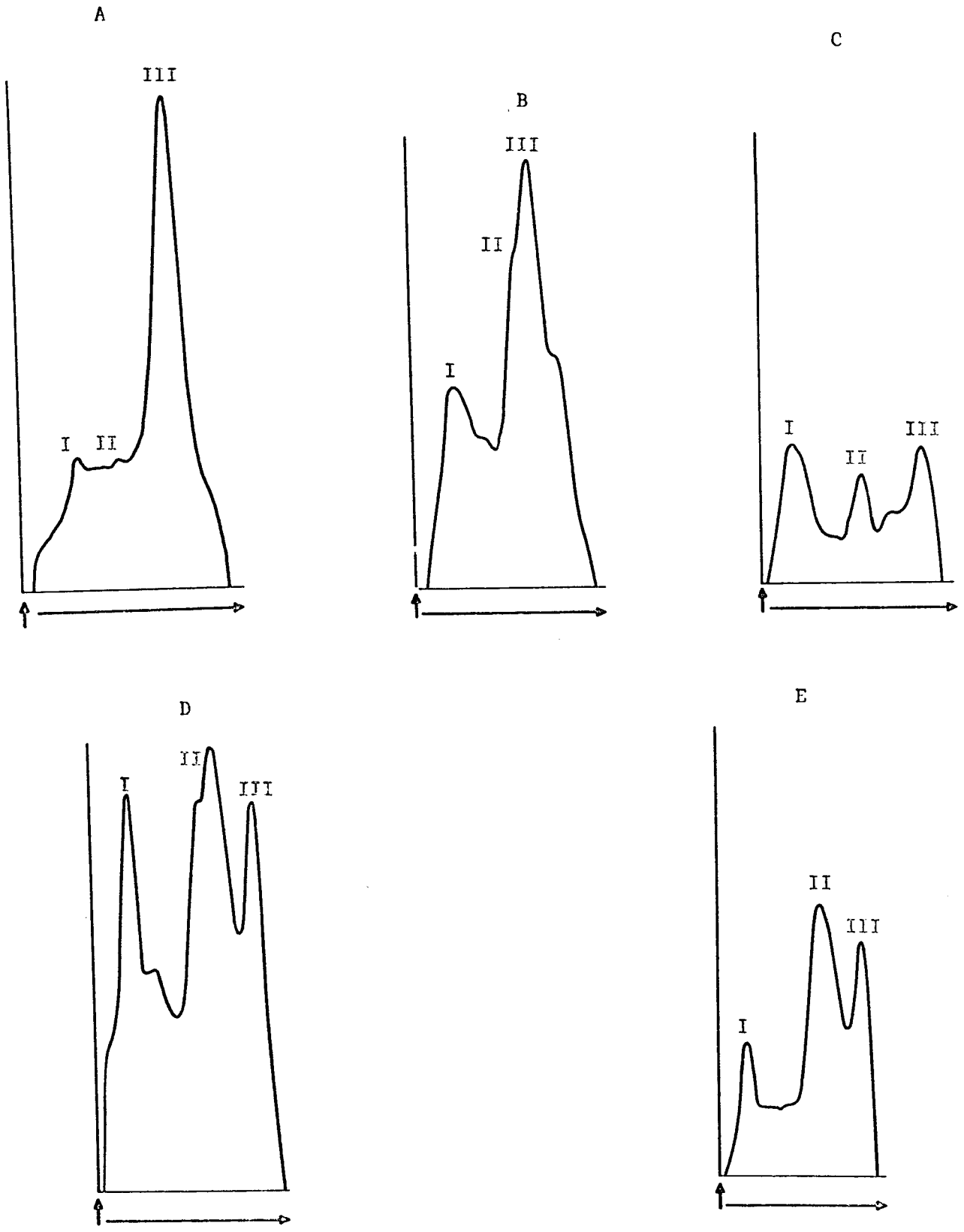


Fig. 21. Differences in the electrophoretic composition of the plasma proteins in chinook salmon at various stages of their life cycle: juvenile (A), estuarine adult male (B), adult female with severe myxobacterial infection and hypoproteinemia (C), pre-spawning adult male (D), and post-spawning adult male (E). Protein fractions corresponding to those discussed in text include beta globulins (I), alpha globulins (II), and albumin (III).

similar to mammalian gamma globulins. Although increased relative to the albumins, the depressed total globulin concentrations in spawning salmon imply a diminished resistance to stress and infection. Albumin declines are characteristic of many pathologies and protein malnutrition, and presumedly these small molecular weight proteins function indirectly in gluconeogenesis and furnish amino acid substrates for wound repair.

Renal Function in Migrating Adult Coho Salmon

Introduction

The present study was undertaken to try to define the normal changes in kidney function that occur when an adult coho salmon enters freshwater on the spawning migration. These normal changes can then be compared, in later work, with the changes observed in salmon migrating through polluted estuaries. These basic data are necessary for the future evaluation of the effects of water quality upon physiological functions of fish. A secondary purpose of the work was to evaluate the use of p-aminohippuric acid (PAH) in the measurement of renal plasma flow of the coho salmon.

Methods and Materials

Location and Collection of Animals. Coho salmon, Oncorhynchus kisutch, captured while migrating into Big Beef Creek, Kitsap County (Washington), were used in the study. An upstream trap, a facility of the Big Beef Field Station of the College of Fisheries, University of Washington, was used to capture the migrating adult fish. These fish were typically about 65-70 cm in length and 3.0-3.3 kg in weight. The fish were removed from the trap in the tidal estuary and transported by boat to the R. V. Kumtuks, anchored in Seabeck Bay. They were then placed in a 5 x 5 x 3 meter holding pen in sea water for at least one week prior to experimentation. Fish used only in freshwater were in freshwater for at least one week prior to experimentation.

Preparation of an Experimental Animal. The fish was netted from the holding pen and placed directly into sea water containing the anesthetic MS-222. When it reached plane III anesthesia ["No respiratory activity. The fish may be easily revived by removing it to untreated water." (Klontz, 1964)], it was placed on an operating table (Smith and Bell, 1967) where the gills were continuously irrigated with salt water or freshwater, as appropriate, which contained MS-222. A catheter, made from PE 160 (polyethylene, I.D. = 1.14 mm) tubing (Clay-Adams) which was shaped by gentle heating, was inserted into the bladder. This catheter was anchored with three stitches through the base of the anal fin; an additional stitch was placed on the dorsal midline posterior to the dorsal fin.

Two holes were then made in the snout of the fish using a large bore hypodermic needle. Each hole was lined with a short piece of PE 200 tubing (I.D. = 1.40 mm), that was heat flared at one end and inserted from the inside of the buccal cavity to resist being pulled out. A long (1.5 meter) piece of PE 60 (I.D. = 0.76 mm) tubing (heat flared at one end) was pulled through one liner from the inside until flush with the palate. At this time, a stitch of braided silk suture was placed through the skin of the palate on the midline just anterior to the gill arches (Smith and Bell, 1967).

A 16 gauge cannulating syringe (Aloe Co.) filled with Cortland saline (Wolf, 1963) was inserted into the dorsal aorta. The syringe and needle were withdrawn leaving the short plastic sleeve into which a PE 60 tube filled with Cortland saline was quickly inserted to minimize blood loss. The tube was then secured to the palate with the silk suture previously placed there and pushed through the other snout liner. On the outside of the snout, a tie was placed around each of the liners and pulled tight enough to secure the buccal and dorsal aorta cannulae without obstructing them. A restraining "tether" was tied through the epaxial musculature just anterior to the dorsal fin. The length of time the fish were on the operating table varied from 8 to 12 minutes.

After preparation, the animal was brought to plane II anesthesia (opercular and fin movement) and placed in an exercise chamber (Smith and Newcomb, 1970) to recover. The dorsal aorta cannula (filled with Cortland saline), and the buccal cannula were connected to differential pressure transducers (Sanborn Model 267B) which were attached to a Brush amplifier and recorder system. Heart rate, blood pressure, and breathing rate were recorded. Urine from the catheter was collected in a fraction collector (Buchler) at intervals dependent on the urine flow rate.

Insulin and PAH. ^{14}C Insulin and ^{3}H PAH (p-aminohippuric acid) were injected as a single dose (Table 1). The ^{3}H PAH was mixed with unlabeled PAH to reduce the specific activity.

Collection and Processing of Blood Samples. Blood was withdrawn from the dorsal aorta cannula at irregular intervals with a 1 ml syringe. After filling the syringe, 2 microhematocrit tubes were filled directly from the cannula. One milliliter of Cortland saline (Wolfe, 1963) was then injected through the cannula to replace the sample volume and maintain blood volume. The microhematocrit tubes were then centrifuged at 12,000 RPM (r = 9 cm) for five minutes. After centrifugation, the hematocrit (packed cell volume) was determined and the plasma supernatant was taken for measurement of per cent total plasma solids. The per cent of total solids was measured with a hand-held refractometer (Bausch and Lombe "TS Meter").

The 1 ml sample was then centrifuged at 12,000 (r = 9 cm) for five minutes and the plasma decanted. A 200 µliter aliquot was dissolved in 15 ml of "cocktail" in a standard scintillation vial. The "cocktail" used was made by dissolving 4.69 g POP (2, 5-Diphenyloxazole),0.469 g POPOP (1, 4-bis-[2-(4-Methyl -5-Phenyloxayolyl)]), and 62.5 ml "Biosolve" (Beckman BBS-3) in reagent grade toluene and making up to one liter. A 100 µliter aliquot of plasma was put dropwise while stirring on a vortex mixer into 0.900 ml of a solution containing 5% (w/v) trichloroacetic acid, 5% (w/v) HCl, and 1% (w/v) lanthanum (Willis, 1960 and 1961). This mixture was centrifuged to separate the precipitated protein. The supernatant solution was aspirated directly into an atomic absorption spectrophotometer (Perkin-Elmer Model 290) for calcium analysis, and appropriate dilutions of the supernatant solution were used for the analysis of sodium, potassium, and magnesium by use of the same spectrophotometer. A 50 µliter aliquot of plasma was used for determination

of chloride ion by use of a potentimetric titrater (Buchler-Cotlove Chloridimeter).

Plasma Ultrafiltration. Dialysis tubing was soaked in distilled water until soft and then folded to make a U-shaped tube. An aliquot of plasma was placed in the tube and the tops of the tube tied together with heavy thread (Toribara et al., 1957). This bag, containing the plasma, was then placed in a screw-top centrifuge tube; the top of this tube was screwed down over the trailing threads, thus suspending the bag in the tube. The tube was then centrifuged for 12 hours at 2,000 RPM (r = 13 cm). At the end of that time, the filtrate was removed from the bottom of the centrifuge tube and frozen for later analysis.

Collection and Processing of Urine Samples. Urine samples were collected by an automatic fraction collector at intervals determined by the urine flow rate and collecting tube size. The volume of the collection, if less than 5 ml was measured with a pipette of appropriate size. If the sample was larger than 5 ml, it was measured with a 10 ml graduated cylinder. A 2 ml aliquot was frozen for later analysis. The same set of analyses was done on the urine samples; similar methods (omitting the protein precipitation step) as used in the plasma analysis described previously were employed.

Analysis of Urinary Precipitate. After aliquots were removed for ion analysis, five urine samples containing precipitate were combined in a 15 ml centrifuge tube and centrifuged for 30 minutes at 3,600 RPM (r = 13 cm). The supernatant was decanted, the precipitate was washed and centrifuged two additional times and dried at 105° CC. A sample of the precipitate was pressed into a potassium bromide block and its infrared spectrum analyzed with a Perkin-Elmer Infrared Spectrophotometer.

Calculations. The catheter dead space was corrected by the method of Hickman (1968a). The symbols in Table 17 will be used in the equations for the calculation of the renal parameters.

Results

The results from this study are extensive and will be published in full by H. M. Miles in Comparative Biochemistry and Physiology sometime in 1971. That which follows is excerpted from his Ph.D. thesis and includes the most significant results. Individual fish are designated by letters and data from each fish is presented in tables in the appendix.

Urine Flow Rate. After the fish were transferred to freshwater, the urine rate exhibits a five- to tenfold increase over the salt water urine rate with a highly variable freshwater urine rate. This high variability persisted even after considerable time in freshwater. In fish 0 the injection of 1 mg adrenaline at 3000 minutes after the

Table 17. Symbols used in the equations for the calculation of the renal parameters (Koch, 1965)

Parameter	Symbol	Units
Urine flow rate	$\dot{V}_u$	ml/(kg x hr)
Glomerular filtration rate	$\dot{V}_g$	ml/(kg x hr)
Renal plasma flow rate	$\dot{V}_p$	ml/(kg x hr)
Excretion rate of Cl^-	$\dot{Q}_{Cl_u}$	µequiv/(kg x hr)
Plasma concentration of Na^+	$[Na]_p$	mequiv/l
Filtered load of K^+	$\dot{Q}_{K_g}$	µequiv/(kg x hr)
Proportion protein bound ion	k	dimensionless

Glomerular filtration rate.

$$\dot{V}_g = \frac{[\text{Inulin}]_u \times \dot{V}_u}{[\text{Inulin}]_p}$$

PAH clearance.

$$\dot{V}_{PAH} = \frac{[PAH]_u \times \dot{V}_u}{[PAH]_p}$$

Filtration fraction.

$$\frac{\dot{V}_g}{\dot{V}_{PAH}}$$

Excretion rate of Na^+, K^+, Ca^{++}, Mg^{++}, and Cl^- (Example Na^+ given).

$$\dot{Q}_{Na_u} = [Na]_u \times \dot{V}_u$$

Filtered load of Na^+, K^+, Ca^{++}, Mg^{++}, and Cl^- (Example K^+ given).

$$\dot{Q}_{Kg} = \dot{V}_g \times [K]_p \ (1-k)$$

Total load of Na^+, K^+, Ca^{++}, Mg^{++}, and Cl^- based on PAH clearance (Example Ca^{++} given).

$$\dot{Q}_{Ca_p} = \dot{V}_{PAH} \times [Ca]_p$$

Clearance ratio of water

$$\frac{\dot{V}_u}{\dot{V}_{PAH}}$$

Clearance ratio of Na^+, K^+, Ca^{++}, Mg^{++}, and Cl^- (Example Mg^{++} given).

$$\frac{\dot{Q}_{Mg_u}}{\dot{Q}_{Mg_p}}$$

Filtration ratio of water.

$$\frac{\dot{V}_u}{\dot{V}_g}$$

Filtration ratio of Na^+, K^+, Ca^{++}, Mg^{++}, and Cl^- (Example Cl^- given).

$$\frac{\dot{Q}_{Cl_u}}{\dot{Q}_{Cl_g}}$$

experimental period resulted in a depression of the urine rate from around 3.6 ml/(kg x hr) to about 1.8 ml/(kg x hr) that persisted for about 600 minutes when the urine rate returned to normal, if not slightly elevated levels. When all samples are pooled (Table 18) the mean urine flow in salt water is 0.406 ml/(kg x hr) while in freshwater the mean urine flow is 4.65 ml/(kg x hr).

Glomerular Filtration Rate. The glomerular filtration rate (GFR) for the pooled samples (Table 18) taken from fish held in salt water is 1.48 ml/(kg x hr) and in the samples taken while the fish were in freshwater, the mean GFR was 9.06 ml/(kg x hr). Although this increase was slightly less than the increase in urine flow, a correlation at the 1 per cent level ($r=0.769$, $df=266$) exists between the GFR and the urine rate for the pooled freshwater samples. After the injection of 1 mg adrenalin in fish 0, the GFR showed a depression from about 8 ml/(kg x hr) to about 5 ml/(kg x hr). The mean filtration ratio of water (Table 18), or the ratio of the rate of filtration of water through the glomerulus to the rate of excretion of water in the urine, changed from 0.380 in salt water to 0.525 in freshwater. This indicates that about 15 per cent more water was reabsorbed from the glomerular filtrate in sea water than in freshwater. This 15 per cent increase does not account for all of the observed increase in urine rate, yet the filtration ratio of water is correlated ($r=0.564$, $df=266$) at the 1 per cent level with urine rate in the pooled samples from fish held in freshwater. This shows that reabsorption rate does play a significant part in the rate of urine production.

PAH Clearance. The clearance of PAH, while usually being greater than the GFR, varied with and was correlated ($r=0.564$, $df=266$) at the 1 per cent level with the GFR. In fish 0 after injection of 1 mg adrenaline, the PAH clearance also varied with the GFR. For the pooled samples (Table 18), PAH clearance increased from a mean of 2.86 ml/(kg x hr) in salt water to a mean of 13.3 ml/(kg x hr) in freshwater.

Magnesium Clearance. The magnesium clearance, calculated from taking the mean values in salt water for urine flow rate, plasma magnesium concentration, and urine magnesium concentration for fish H (Appendix), is 79.6 ml/(kg x hr). The magnesium clearance value for fish L (Appendix), similarly calculated, is 4.56 ml/(kg x hr).

Urine Ion Concentrations. There was considerable variation among the ion concentrations of the individual fish. The most dramatic change common to all of them was the greatly decreased concentrations of magnesium and chloride ions in the urine. Some details for individual fish are presented in the appendix tables and are expected to be published in full by Miles in 1971.

Urine Ion Excretion Rates. The excretion rates of ions are dependent on both the urine flow and the concentration of ions in the urine. It can be seen in Appendix B and in Table 18 that the excretion rates of sodium,

Table 18. Means, standard deviations (S.D.), and sample sizes (n), pooled for renal parameters in all salt water fish and in all freshwater fish

Parameter	Salt water			Freshwater		
	Mean	S.D.	n	Mean	S.D.	n
Flow rates (ml/(kg x hr))						
Urine rate	0.406	0.217	19	4.65	3.66	309
Glomerular filtration rate	1.48	0.723	14	9.06	4.70	268
PAH clearance	2.86	1.41	14	13.3	8.04	268
Urine ion concentrations (mequiv/l)						
Sodium	55.5	50.0	19	12.3	12.1	273
Potassium	2.48	1.33	19	1.69	1.88	275
Calcium	8.21	5.34	19	2.93	1.72	275
Magnesium	160	74.6	19	10.2	35.4	275
Chloride	133	68.5	19	13.2	23.8	301
Plasma ion concentrations (mequiv/l)						
Sodium	181	2.30	16	157	8.75	282
Potassium	2.72	0.134	16	2.53	0.293	282
Calcium	2.45	1.43	16	2.38	0.904	282
Magnesium	3.35	3.33	16	2.01	0.859	282
Chloride	156	2.24	16	133	9.81	289
Excretion rates (μequiv/(kg x hr))						
Sodium	16.9	11.3	19	68.1	126	273
Potassium	0.863	0.370	19	7.05	7.08	275

Table 18. Means, standard deviations (S.D.), and sample sizes (n), pooled for renal parameters in all salt water fish and in all freshwater fish - Continued

Parameter	Salt water			Freshwater		
	Mean	S.D.	n	Mean	S.D.	n
Calcium	2.85	1.33	19	12.2	9.13	275
Magnesium	72.4	52.7	19	14.1	20.9	275
Chloride	61.0	47.0	19	60.7	115	301
Filtered ion load (μequiv/(kg x hr))						
Sodium	227	112	14	1200	631	262
Potassium	3.62	1.81	14	20.2	10.3	262
Calcium	1.41	1.37	14	6.62	4.02	262
Magnesium	5.29	6.53	14	14.3	10.7	262
Chloride	224	112	14	1180	662	268
Ion load based on PAH clearance (μequiv/(kg x hr))						
Sodium	517	261	14	2100	1380	262
Potassium	7.78	3.79	14	33.9	22.4	262
Calcium	5.90	2.02	14	30.3	20.1	262
Magnesium	7.51	4.95	14	25.9	15.7	262
Chloride	445	220	14	1740	971	268
Clearance ratios based on PAH clearance (dimensionless)						
Water	0.154	0.0191	14	0.405	0.314	268
Sodium	0.0524	0.0467	14	0.0347	0.0454	245

Table 18. Means, standard deviations (S.D.), and sample sizes (n), pooled for renal parameters in all salt water fish and in all freshwater fish - Continued

Parameter	Salt water			Freshwater		
	Mean	S.D.	n	Mean	S.D.	n
Potassium	0.145	0.0839	14	0.227	0.170	247
Calcium	0.556	0.198	14	0.504	0.310	247
Magnesium	17.1	12.6	14	1.45	4.77	247
Chloride	0.126	0.0747	14	0.0378	0.552	263
Filtration ratios (dimensionless)						
Water	0.380	0.209	14	0.525	0.343	268
Sodium	0.0876	0.0279	14	0.0501	0.0583	245
Potassium	0.297	0.0852	14	0.337	0.200	247
Calcium	4.66	2.90	14	2.23	1.24	247
Magnesium	78.5	61.6	14	4.96	22.2	247
Chloride	0.413	0.309	14	0.0538	0.0929	264
Filtration fraction (dimensionless)	0.682	0.565	14	0.765	0.262	268
Hematocrit (%)	20.5	1.02	16	14.8	6.47	299
Plasma total solids (%)	3.35	0.710	16	3.42	0.817	299
Blood pressure (mm Hg)	28.8	7.09	15	26.7	8.06	253
Dorsal aorta pulse pressure (mm Hg)	3.80	0.519	15	5.07	1.79	253
Heart rate (beats/min)	59.4	9.35	19	52.4	8.59	253

potassium, and calcium actually increase when the fish enters freshwater. The excretion rates of magnesium and chloride ions, however, decrease with entry into freshwater. While there are several significant correlations among the ion excretion rates in both salt water and freshwater (Tables 19 and 20), the closest correlation in salt water is that between magnesium and chloride and the closest correlation in freshwater is between sodium and chloride.

Protein Binding of Plasma Ions. Plasma was subjected to pressure filtration by centrifugation through dialysis membrane. Analysis of the filtrate from each sample so treated yielded the percentages of protein bound ions shown in Table 21.

Table 21. Percentages of protein bound sodium, potassium, calcium, magnesium, and chloride in blood plasma from fish M

Ion	Per cent bound
Sodium	15.0
Potassium	11.0
Calcium	69.0
Magnesium	27.0
Chloride	3.1

All of the calculations of filtered ion load utilized the percentages listed in Table 21.

Filtered Ion Load. The filtered ion load is dependent on the glomerular filtration rate and the concentration of unbound ions in the blood plasma. The means for filtered ion load are presented in Appendix Table B-6. In the pooled freshwater sample significant correlations are found between the per cent plasma total solids and the filtered load of sodium, calcium, and magnesium (Table 22).

Table 19. Correlation coefficient matrix with sample sizes of sodium, potassium, calcium, magnesium, and chloride excretion rates for the pooled freshwater samples

	Sodium	Potassium	Calcium	Magnesium	Chloride
Sodium	1.00 (273)	0.543** (273)	0.653** (273)	0.0255 (273)	0.968** (272)
Potassium		1.00 (275)	0.546** (275)	-0.143* (275)	0.500** (274)
Calcium			1.00 (275)	0.489** (275)	0.681** (274)
Mangesium				1.00 (275)	0.137* (274)
Chloride					1.00 (301)

*Correlation significant at the 5% level.

**Correlation significant at the 1% level.

Table 20. Correlation coefficient matrix (n=19) of sodium, potassium, calcium, magnesium, and chloride excretion rates for the pooled salt water samples

	Sodium	Potassium	Calcium	Magnesium	Chloride
Sodium	1.00	0.614**	0.320	-0.477*	-0.480*
Potassium		1.00	0.614**	0.226	0.242
Calcium			1.00	0.283	0.269
Magnesium				1.00	0.996**
Chloride					1.00

*Correlation significant at the 5% level.

**Correlation significant at the 1% level.

Table 22. Correlation coefficients (r) and sample size (n) for correlations between plasma total solids and the filtered load of sodium, potassium, calcium, magnesium, and chloride

Ion	r	n
Sodium	0.130*	262
Potassium	0.0775	262
Calcium	0.218**	262
Magnesium	0.226**	262
Chloride	0.199**	268

*Correlation significant at the 5% level.
**Correlation significant at the 1% level.

Ion Load and Clearance Ratios Based on PAH Clearance. The mean results from the calculation of ion load and clearance ratios from the PAH clearance are presented in Appendix Table B-7 and Table 18. The clearance ratio is the ratio of the rate of excretion of a substance to the rate at which it is presented to the kidney by the renal circulation. A priori, this ratio could not exceed a value of 1.00. However, the mean clearance ratio of magnesium (Table 18) in salt water is 17.1 and in freshwater is 1.45, both ratios being greater than 1.00.

Ion Load and Clearance Ratios Based on Magnesium Clearance. The mean magnesium clearance of 79.6 ml/(kg x hr) was used as an estimate of renal plasma flow in the calculation of the total ion load and clearance ratios presented in Table 23. The mean plasma ion concentrations and excretion rates from Table 18 were used in these calculations.

Filtration Ratios. The filtration ratio is the ratio of the rate of excretion of a substance to its rate of filtration. These values are reported in the appendix tables. If the filtration ratio is greater than 1.0, active secretion of that substance into the tubule lumen is indicated. A filtration ratio of less than 1.0 indicates active reabsorption from the glomerular filtrate into the blood. The mean filtration ratios for the pooled freshwater and salt water samples (Table 18) reveal that except for calcium and magnesium, there is a net reabsorption of water and ions by the tubule.

Filtration Fraction. The filtration fractions, the ratios of the glomerular filtration rate to the total renal plasma flow, reported in the Appendix and Table 18 are based on the PAH clearance as a measure

Table 23. Total ion load and clearance ratios based on the magnesium clearance of fish H in salt water and calculated from the mean plasma ion concentrations and excretion rates of the pooled samples in salt water and freshwater*

Ion	Salt water		Freshwater	
	Total load μequiv/(kg x hr)	No units clearance ratio	Total load μequiv/(kg x hr)	No units clearance ratio
Sodium	14,400	0.00117	12,500	0.00545
Potassium	217	0.00399	201	0.0350
Calcium	195	0.0146	189	0.0644
Magnesium	267	0.272	180	0.0881
Chloride	12,400	0.00491	10,600	0.00573

*Figures reliable to 3 significant figures only.

of total renal plasma flow. If these values are calculated using the high renal plasma flow indicated by the magnesium clearance of fish H (79.6 ml/(kg x hr)) and the mean values for GFR for the pooled salt water and freshwater samples (Table 18), filtration fractions of 0.0186 in salt water and 0.114 in freshwater are obtained.

Blood Parameters. In general, the hematocrit and per cent plasma total solids decreased with introduction to freshwater, the blood pressure remained the same or decreased slightly, and the heart rate remained relatively constant. The dorsal aortic pulse pressure increased. This indicated the occurrence of a dilation of the branchial capillary beds with introduction into freshwater.

Urinary Precipitate. The infrared spectrum for the purified material obtained from this precipitate was comparable with that for components of human urinary calculi. The precipitate in the fish urine appears to consist chiefly of the mineral brushite $CaHPO_4 \cdot 3H_2O$.

Discussion

These observations have demonstrated some of the changes in the renal function of salmon when they enter freshwater during the final portion of their spawning migration. In general, in the salt water environment, the salmon kidney has an osmoregulatory function involving excretion of magnesium and sulfate ions (Hickman, 1968c) and conservation of water. In freshwater, the kidney excretes large amounts of water while conserving salts.

Maintenance of Internal Homeostasis. When fish enter the freshwater environment from the salt-water environment, there is a decrease in the concentrations of the plasma ions. Green (1904) observed that the freezing point depression was greater in the blood from the chinook salmon (Oncorhynchus tschawytscha) taken in salt water (-0.762° C) than the mean of those taken from the salmon taken in freshwater (-0.613° C). This was probably the first published observation on the osmoregulatory physiology of the genus of Pacific salmon (Oncorhynchus). Since that time there have been no detailed observations comparing the composition of the plasma of adult salmonids in both salt water and freshwater. In the present work, there was observed a definite decrease in plasma ion concentration, except in the case of potassium and calcium. There seem to be two homeostatic levels for ion concentrations, one in salt water and one in freshwater, and both are equally functional.

The excretion of magnesium, which is one of the major roles of the kidney in salt water, is an extremely important function. It has been found (Engbaek, 1952; Del Castillo and Engbaek, 1954) that magnesium in concentrations greater than 10 mequiv/l will produce a neuromuscular

block. The kidney, the primary excretory site for magnesium, excretes most of this cation that is absorbed by the intestine (Hickman, 1968c). It thus plays a critical role.

Precipitation of magnesium and calcium salts in the tubular lumen is an efficient mode of excretion. After crystalization, these salts would therefore no longer participate in the osmotic gradient from the peritubular blood to the lumen of the tubule.

Other reports of crystalline material are by Pitts (1934), and by Grafflin and Ennis (1934) who reported $MgHPO_4 \cdot 3H_2O$ in marine fish urine. Hickman (1962b) reports $CaHPO_4$ in the urine of the southern flounder. The precipitate found in the present work on adult coho salmon was $CaHPO_4 \cdot 3H_2O$.

In his work with southern flounder in salt water, Hickman (1968b) found that this fish actively secreted magnesium and calcium into the tubule and actively reabsorbed sodium, potassium, and chloride. The same relations were found in the present work.

SECTION VII

EFFECTS OF MAN-MADE STRESSES ON ADULT SALMON

Stress During the Migration of Adult Salmon

We assume that the high seas migration of salmon is relatively unstressful. However, as these salmon approach the estuary of their natal stream, a number of major physiological changes occur in response to environmental and internal cues. The rates of swimming and general metabolism increase (Royce, Smith, and Hartt, 1968; Brett, 1965). Feeding ceases prior to entry into the river, but the exact timing of this appears to be unknown. The scales are absorbed, the skin thickens about threefold, and mucous production increases. The kidney, gills, and gut prepare to change from their marine functions of excreting both monovalent and divalent ions and conserving water to excreting excess water and conserving monovalent ions as the fish enters freshwater (Miles, 1969). The blood levels of the adrenocorticosteroid hormones increase about fivefold (Fagerlund, 1967), and in response many of the blood constituents such as blood cells and plasma proteins decline and change dramatically by the time the fish has completed spawning (Cardwell, 1968). Resistance to disease also diminishes markedly (Wedemeyer, In press). Dramatic changes take place in protein and lipid metabolism (Idler and Bitners, 1959). The fish completes spawning with its lipid reserves exhausted and much of its muscle tissues replaced by water. These changes in salmon are intriguingly similar to degenerative diseases like Cushing's syndrome in humans, and may be reversible in the salmon if food is given after spawning (McBride et al., 1963). We define all such changes as natural stress responses.

Man-made stressers which are not immediately lethal to the fish evoke many of the same responses as the natural stressers. Therefore, the problem of quantifying responses to man-made stressers is one of distinguishing between degrees of responses rather than between types of responses. Studying degrees of responses is a much more difficult kind of research than studying types of responses. It is vitally important in several ways to understand the fish's adjustments to reduced water quality. First, salmon are not feeding in the estuary or river and therefore cannot replace lost or wasted energy. If the man-made stresser requires too much energy in addition to that normally needed for migration, the fish will die prior to reaching the spawning grounds. Second, since the man-made stressers are likely to be cumulative, the two together can be fatal, although either one alone might not. Third, the fish have a limited ability to respond to stressing agents owing to chronic elevation of circulating corticosteroids (Fagerlund, 1967). Most salmon die from disease after spawning, but not because they can no longer meet their basic physiological requirements. Studies by McBride et al. (1963) on mature sockeye salmon, kept alive after spawning, showed that the primary factors causing death were vitamin deficiencies and disease, not functional insufficiencies.

To understand the full effects of stressing agents on salmon, one must understand a whole spectrum of man-made and natural stressers and the degree of response by the fish.

Effects of Low Levels of Dissolved Oxygen on Chinook and Coho Salmon in the Duwamish Estuary

General Considerations

Before beginning a description of our experiments, it is useful to point out that salmon have alternative courses of action available for coping with the problems of low DO. Each of these alternatives involves a compromise. Each action taken by the fish to remedy its difficulties in maintaining adequate oxygen consumption under conditions of decreased oxygen availability produces at least one new difficulty. Thus, the problems salmon encounter in water of low DO are difficult to predict because there are a large number of considerations and alternatives, some of which may be suitable at one DO level and not at another. The experiments described below will illustrate some of the ways in which salmon select these alternatives as best they can.

Materials and Methods

For these particular experiments, a series of four fish was put in the swimming chamber: 1) no catheterizations and no prolonged anesthesia; 2) no catheterizations but with prolonged anesthesia; 3) dorsal aorta and urethra catheters; and 4) pre- and post-gill catheterizations. The fish were rested in a chamber after capture, anesthetized, catheterized using an operating table for fish, placed in a swimming chamber, and rested overnight to recover from anesthesia and surgery. The turnover of *in situ* estuarine water (salinity 21-25 °/oo and temperature 12.0-13.5 C) in the swimming chamber was 10 l/min. For the first hour the water was aerated, giving DO's ranging from 5.5 to 6.5 ppm. The aeration was stopped during the second hour so that the DO decreased to the range of 4.0 to 5.0 ppm. The current in the chamber was set to provide a moderate swimming velocity. At the end of the two hour period, the current was reduced. The fish's recovery could then be followed with the fish resting in aerated water.

The series of four fish were repeated three times using different fish in ambient estuary water having a moderately low DO and then repeated one more time in the sea water of Elliott Bay (about two miles away from the first site), where DO was saturated and no pollution was obvious. In the latter case, water flow through the chamber was reduced during the second hour so that the fish's own oxygen consumption produced DO levels

in the chamber comparable to those we observed in the estuary. As an additional control for the coho salmon which we caught in the estuary and whose swimming might have been affected by pollution agents other than low DO, we also brought coho salmon from Hood Canal where pollution contamination was very unlikely and tested them at the Elliott Bay site on the same schedule as the other salmon.

Results

Swimming Stamina. Although it was not part of our experimental design to test swimming stamina per se, some comments on swimming stamina are warranted from our data. Swimming stamina may be some kind of integrated indicator for the status of a fish's energy transfer systems, but high speed swimming until fatigued - the usual definition of stamina - probably does not occur in nature. Just as with people, most fish will not work to complete exhaustion unless forced to do so by unusual circumstances. However, some of the fish we tested did become fatigued at our relatively moderate test velocity of 56 cm/sec within the two-hour swimming period of the experiment. Data concerning the environmental oxygen levels and swimming endurance are shown in Table 24. Fish exposed to 4.0 - 4.5 mg/liter fatigued in the test period.

Table 24. Relation between dissolved oxygen concentrations and fatigue in swimming adult coho salmon[1,2]

		First hour		Second hour	
		Fatigued	Not fatigued	Fatigued	Not fatigued
Number of fish		0	8	4	4
Dissolved oxygen	mean	-	5.8	4.3	4.8
Concentration mg/l	range	-	5.0-6.6	4.0-4.5	4.5-5.0

[1]Environmental temperature range was 12-13.5 °C.

[2]Salmon were swum at 56 cm/sec.

Ammonia concentration at low DO levels may have some effect on swimming stamina since all four of the fish which fatigued had DO levels below 4.5 mg/liter and ammonia concentrations above 0.65 ppm, while the four that did not become fatigued in the same test series had DO levels above 4.5 mg/liter and ammonia concentrations of less than 0.65 ppm. Some synergistic effects between ammonia and oxygen appear possible and will be discussed later under ammonia excretion.

Respiratory Changes. The three respiratory variables which we measured - oxygen consumption, ventilation volume, and extraction coefficient - are summarized in Fig. 22. During the first hour of swimming in high DO, the consumption remained nearly constant and dropped slightly at the end of the hour. This drop was probably real and could have been caused by a decrease in the excitement of the new swimming task being performed. Similar observations have been made by J. R. Brett (personal communication). A similar change was seen in ventilation volume, probably for the same reason. The extraction coefficient was commonly the complement of the ventilation volume. As the level of dissolved oxygen was decreased, the oxygen consumption and extraction coefficient decreased by about one-third almost immediately and then slowly recovered. From the oxygen metabolism data alone, it appeared that the fish were recovering from the initial shock of the dissolved oxygen drop.

Two observations seem especially noteworthy. First, the decreased oxygen consumption occurred during a period of constant or slightly-increased activity. The decreased consumption must therefore represent an oxygen debt and lactate accumulation. Second, the ventilation volume increased rather slowly as compared to the consumption and extraction. This indicates, we believe, that an increase in ventilation volume is more "expensive" physiologically than going into oxygen debt, and that an increase in ventilation volume occurs only when the low DO persists long enough that the lactate accumulation begins to become a problem. In our swimming chamber, the fish did not have the option of reducing its level of activity prior to fatigue as a means of reducing its lactate.

Changes in Levels of Lactate in the Blood. We have been successful in completely catheterizing relatively few individual fish. Therefore, we will present information from individual fish, since each proved to be different and each illustrates different points. In Fig. 23, fish 1C began swimming with a moderately-high blood lactate level, but improved it slightly by the end of the first hour. Upon entry into water of low DO, however, the lactate increased rapidly until the fish became fatigued just before the end of the second hour. The recovery period after fatigue was normal and uneventful, declining to the normal 5-20 mg% level of a typical rested fish.

In fish 2C (Fig. 23), the blood lactate was normal at the start of the swimming, but rose during swimming, showing a possible tendency to

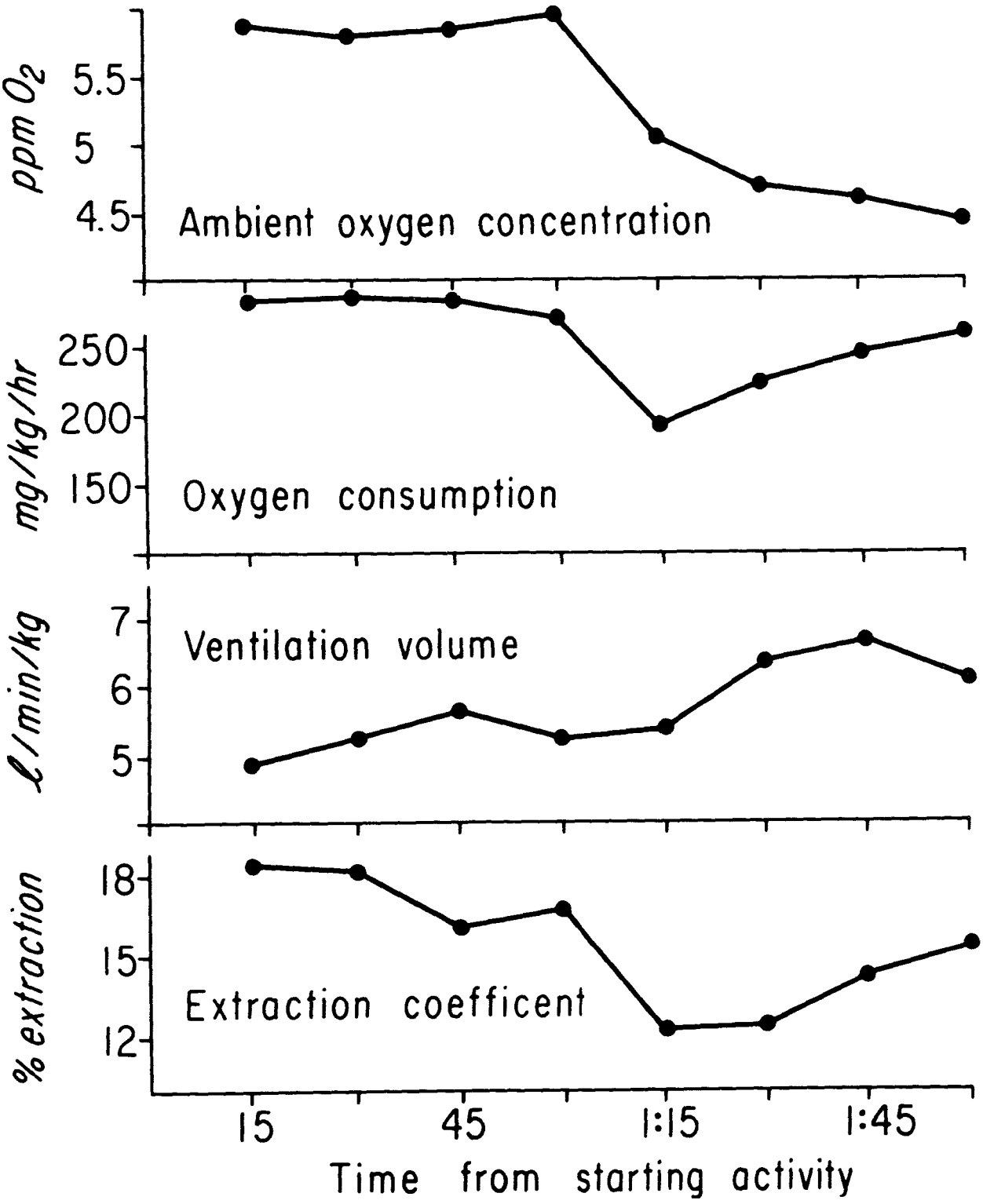


Fig. 22. Oxygen consumption, extraction coefficient, and ventilation volume with change in environmental oxygen concentration in swimming coho salmon. Temperatures ranged from 12 to 15 °C, and salinities from 20 to 28 ppt.

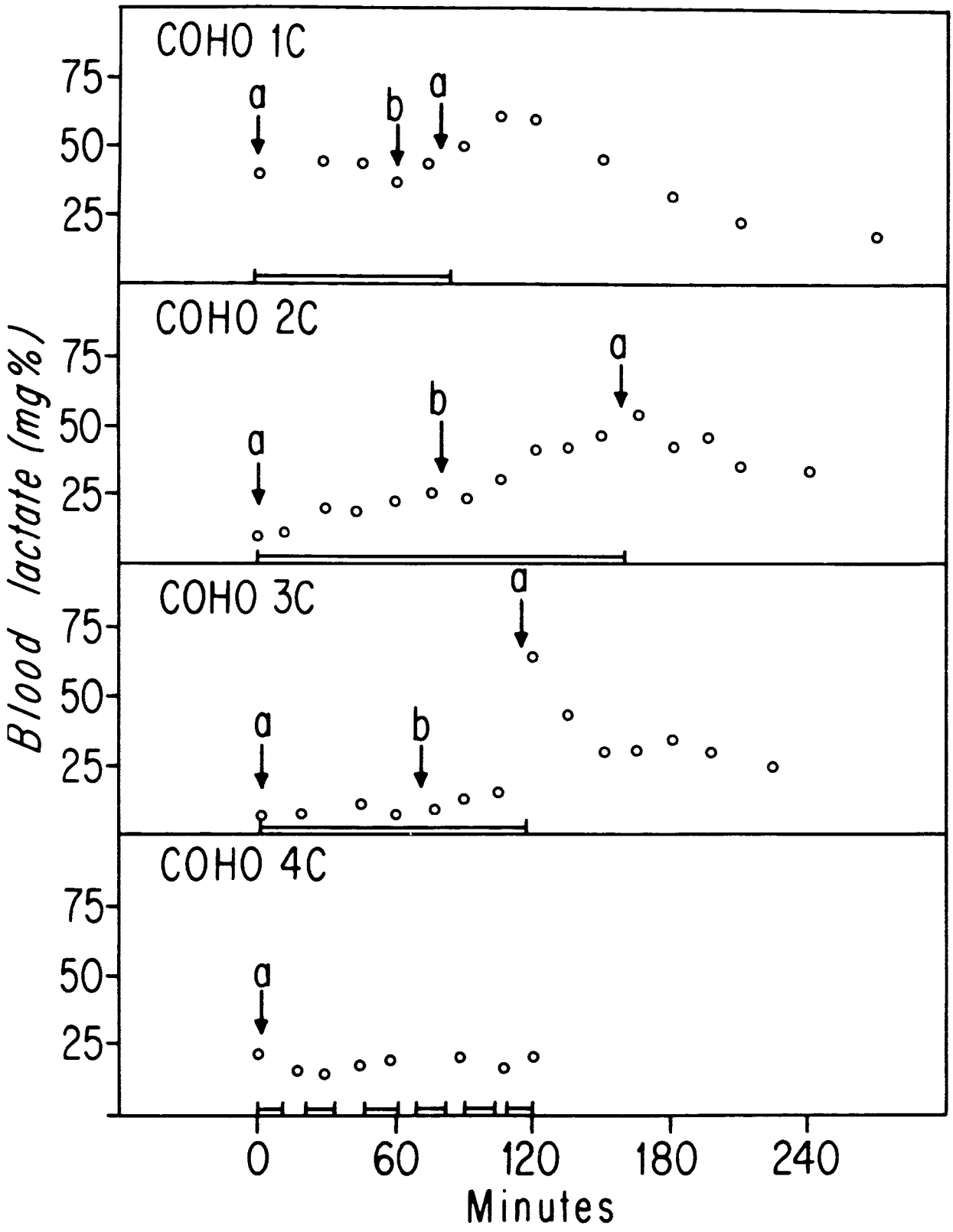


Fig. 23. Changes in blood lactate levels in four coho salmon (Oncorhynchus kisutch) swimming various periods (bar) in "a" elevated (65% saturated) and "b" low (50% saturated) dissolved oxygen concentrations. Temperature and salinity ranges were 12-14 °C and 20-28 ppt, respectively.

stabilize toward the end of the first hour. The adjustments in lactate levels in both this fish and in fish 1C probably correspond to the slight decrease in oxygen consumption as seen in Fig. 22.

Fish 3C and 4C (Fig. 23) illustrate the problems of controlling the behavior of the fish. In fish 3C the sharp peak in lactate concentration between 105 and 150 minutes resulted from violent, nonswimming activity. Thus, the fish indicated that it would have preferred to turn downstream and remove itself from that environment rather than continue to swim upstream. Low blood lactate concentrations in fish 4C resulted from the fish's refusal to swim or exert itself in any manner. These two fish also illustrate the degree to which normal values can be influenced by erratic behavior.

We believe that an average response to our swimming schedule is represented by this figure (Fig. 24). In adequate DO there was a minimal resting level of lactate which rose slightly during any increase in activity. Once some critical point was reached in either decreased DO or increased activity, the blood lactate level increased rapidly until the fish became fatigued and stopped swimming, or until it reached the end of the test period and was allowed to rest.

Figures 25 and 26 illustrate the direct application of lactate data to water quality criteria. In Fig. 25, lactate levels in the blood were determined at 15 min. intervals while the fish swam at a constant velocity in decreasing concentrations of dissolved oxygen. Although there was considerable individual variation, the general pattern seemed to be a relatively constant level of blood lactate until the DO concentration decreased below 5 mg/l. Then blood lactate increased rapidly and fatigue occurred shortly thereafter as the DO continued to decrease.

Two significant interpretations can be made from this figure. First, coho salmon experience a range of DO levels within which they can adjust their respiratory functions to meet a large fraction of their energy needs - i.e., they do not go into oxygen debt by producing much lactate. Second, they reach a critical DO level at which the respiratory adjustments are insufficient to meet their needs and they produce lactate very rapidly and soon fatigue. For the Duwamish Waterway at ambient temperatures and a swimming rate which we assumed to be typical, the critical DO level was just below 5 mg/l.

Some possible generalizations on the lactate-DO data of Fig. 25 are shown in Fig. 26. This figure suggests that the rate of lactate production is a function of DO. As DO decreased, lactate production increased so that the sum of oxygen consumption and lactate production was approximately constant. Eventually, fatigue is virtually certain if the lactate is high enough or the DO is low enough. Fish also can have high lactate levels in normal DO due to excessive activity produced by handling, chasing, or

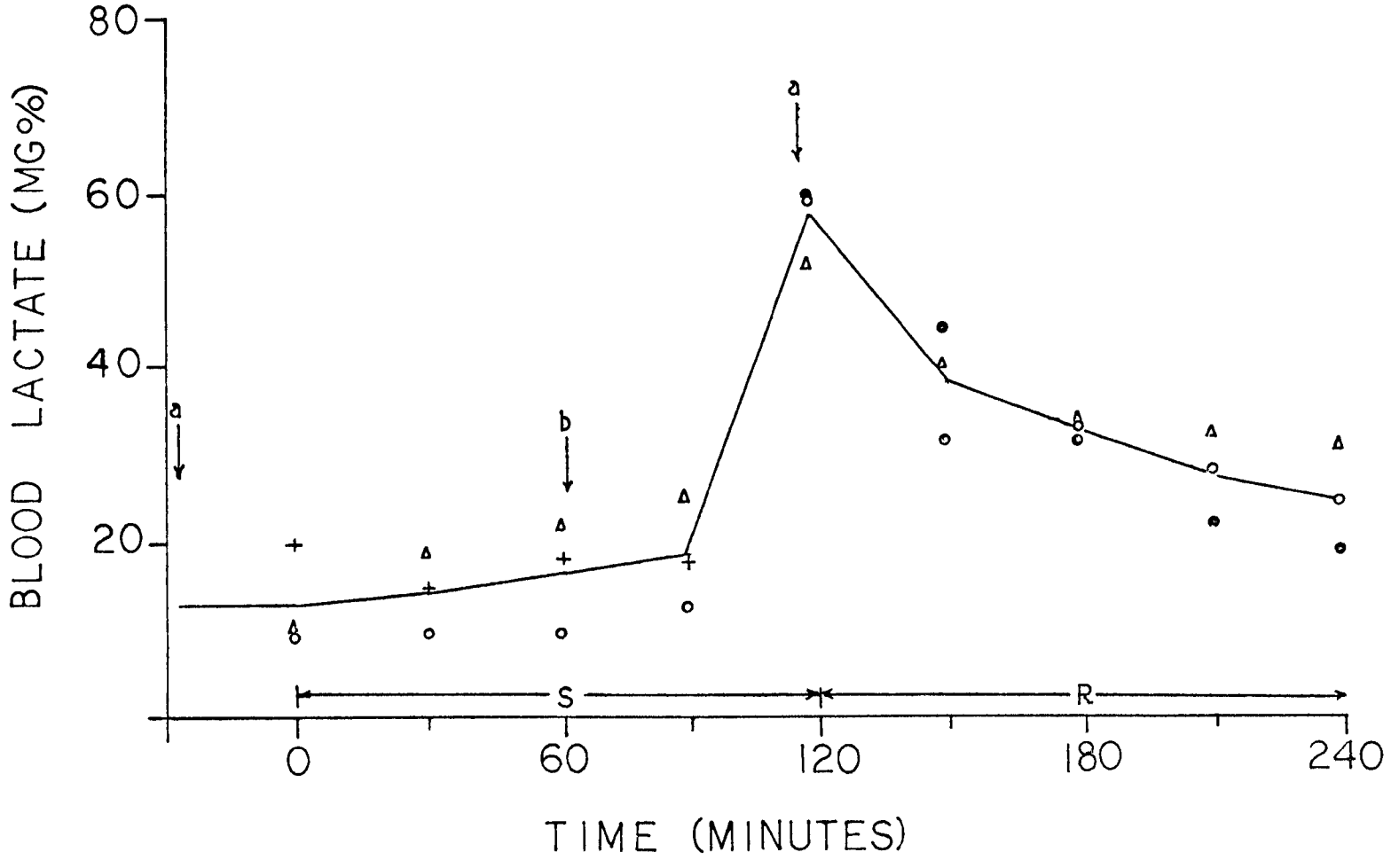


Fig. 24. Temporal variations in blood lactate concentrations in three coho salmon swimming at 56 cm/sec in aerated (100% saturation = "a") and hypoxic (50% saturation = b) waters.

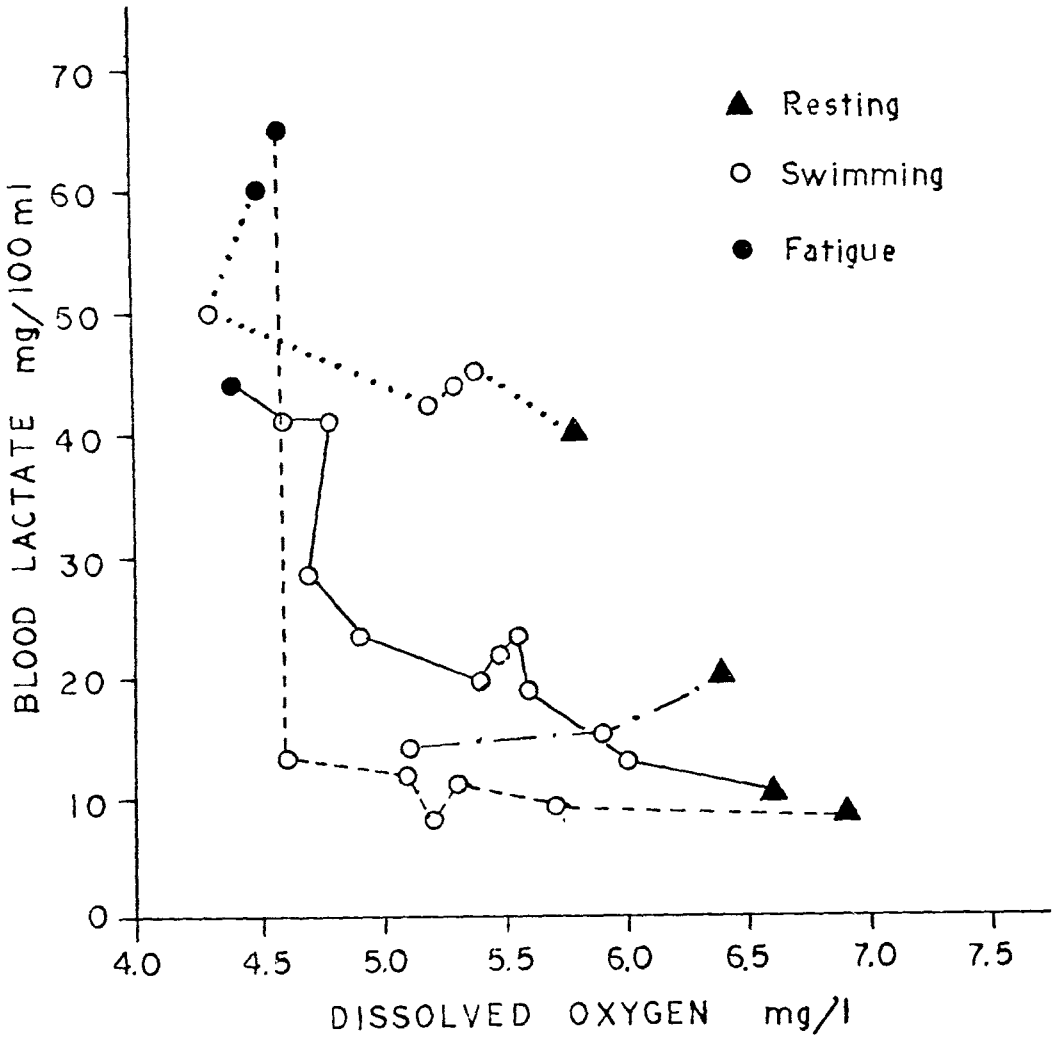


Fig. 25. Relation between blood lactate, dissolved oxygen and duration of swimming in four coho salmon from the Duwamish Waterway, 3 to 4 kg adult fish. Samples taken every 15 min proceeding from right to left. Coho 1C, dotted line; coho 2C, solid line, coho 3C, dashed line, and coho 4C, broken line.

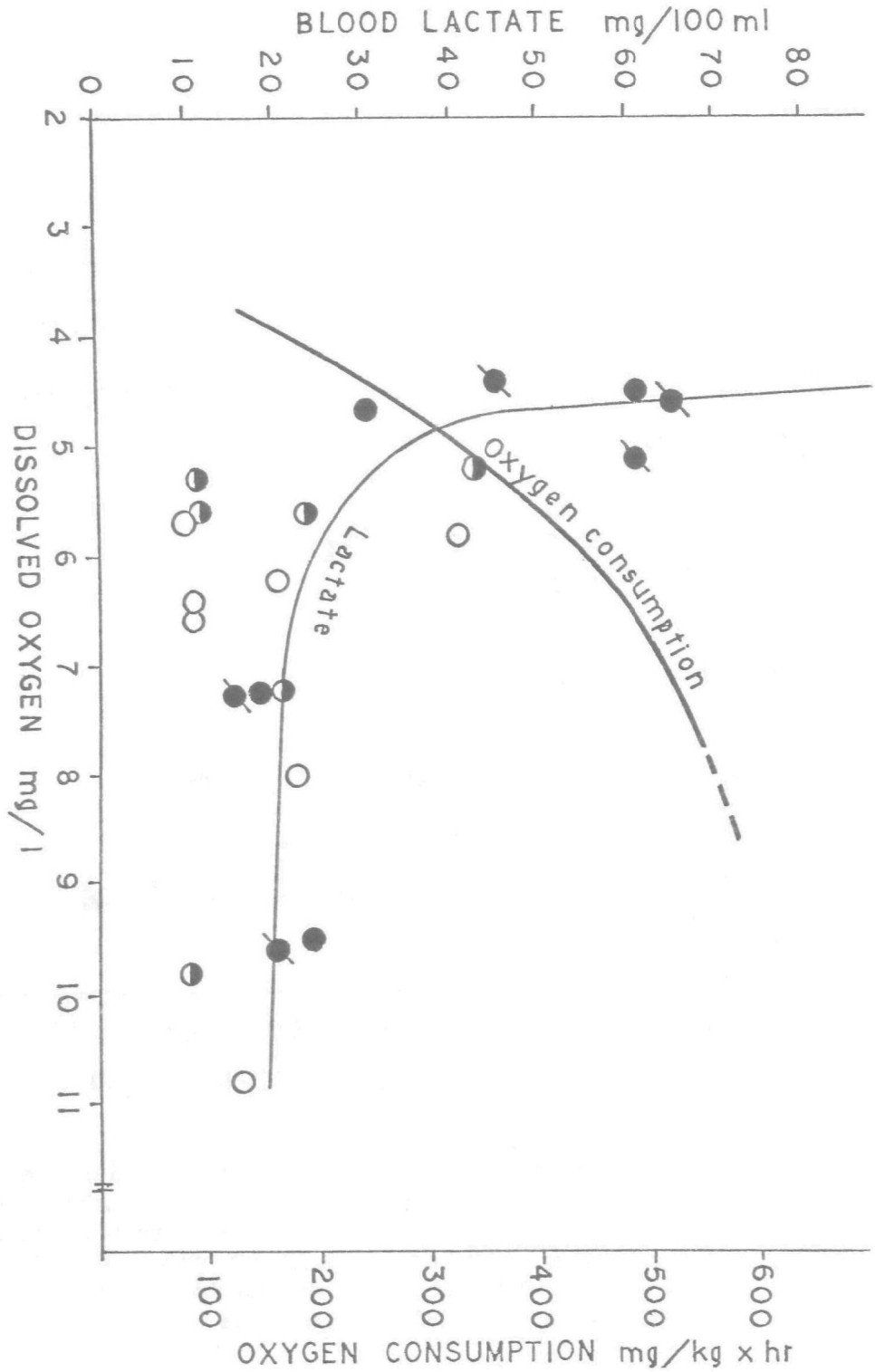


Fig. 26 Relation between blood lactate, dissolved oxygen, oxygen consumption and duration of swimming in 3 to 4 kg adult coho salmon and steelhead trout. Symbols; ○ - resting, ◓ - 45 min of swimming, ● - 90 min of swimming, ◉ - fatigue or termination of experiment.

other strenuous activity. These latter fish would not be expected to fatigue. The relation between oxygen consumption and DO is partly based on our data and partly on data from Salvelinus fontinalis (Fry, 1957).

Excretion of Lactate. The first evidence that lactate can appear in the urine of salmonids was produced by Hunn (1969). The first evidence that blood lactate levels may increase in proportion to sub-maximal levels of activity was just presented. The two facts together raise the question of how much lactate (and therefore energy) might a salmon lose if subjected to increased blood lactate while swimming in chronically low DO.

We have some experiments in progress at the time of writing which may answer that question. In a coho salmon whose blood lactate concentration ranged between 10 and 15 mg%, 30-45 gm/kg/hr were excreted in the urine. If we extrapolate to a blood level of 45 mg% and 135 gm/kg/hr urinary excretion, a one-kg fish would lose 3.2 mg/day or 97.2 mg/month, a month not being an unreasonable period of time for the fish to wait in the estuary before ascending the stream.

Whether this energy leak is significant to the fish's energy budget or not is impossible to say now, because we do not know how crucial the last few milligrams of energy stores may be to the fish when it is on the spawning grounds. Also, there probably are other routes of lactate loss, such as through the gills. This point was suggested by an experiment in which a coho salmon had a very large dose of lactate injected directly into the blood stream (concentration rose to 938 mg% - far beyond physiological levels), but only 57% of it was recovered in the urine.

Ammonia Excretion. Ammonia is the primary end product of nitrogenous metabolism in teleost fish and excretion occurs primarily through the gills. Since the gills are very difficult to isolate, we monitored ammonia excretion only as the differential in ammonia content between the water entering and leaving the swimming chamber.

Data from ten fish indicate two patterns of ammonia excretion (Fig. 27). The rate of ammonia excretion increased during activity (in water with adequate oxygen). Fish that did not fatigue during the second hour (low DO) characteristically showed a depressed rate of ammonia excretion during exposure to the low DO. Fish that did fatigue during the low DO period had rates of ammonia excretion (dotted line) which remained constant or continued to increase until fatigue occurred. It is also worthwhile to note that fish which became fatigued were exposed to low DO which was about 0.5 mg/liter lower than those which did not become fatigued, and that the nonfatigued fish elevated their rates of ammonia excretion again when adequate DO was restored. Both patterns of ammonia excretion were accompanied by the typical pattern of increased lactate levels as shown in the upper part of Fig. 27.

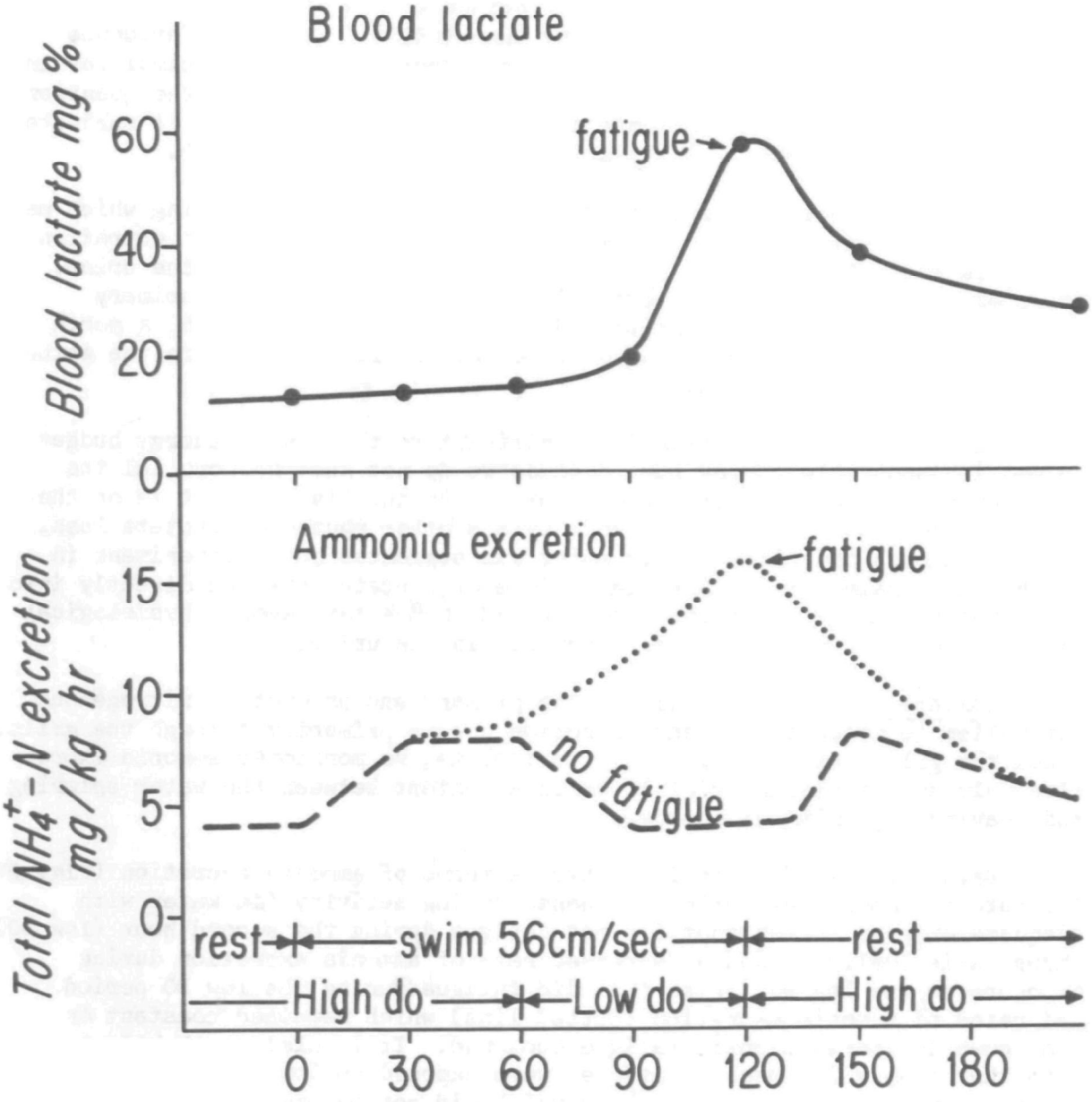


Fig. 27. Upper graph indicates average changes in blood lactate levels of adult coho salmon (Oncorhynchus kisutch) contacting a low dissolved oxygen (50% saturation) while swimming at 56 cm/sec. Lower graph indicates variations in total ammonia excretion in swimming adult coho salmon subjected to various levels of dissolved oxygen.

Rate of Urine Production. Kidney function in marine salmon is concerned primarily with the excretion of divalent ions, particularly magnesium, and organic acids including, as we have shown, lactic acid. The major excretory load of sodium, ammonium, and chloride ions is excreted by cells in the gills, so that urine volume in marine salmon is only about one-eighth of that in freshwater salmon (Hickman and Trump, 1969; Miles, 1969). However, any major decrease in the rate of kidney function could cause problems through the toxicity of the accumulated magnesium ions in marine salmon or accumulated water in freshwater salmon.

Our present data is sufficient only to say that there appeared to be a decrease in urine production when the fish was subjected to low levels of DO. We plan additional research in this area, but cannot now say how important this effect of low DO may be. However, if excretory mechanisms are impaired by low DO, then toxic levels of various blood constituents may be reached.

SECTION VIII

ACKNOWLEDGMENTS

Some of the personnel contributing to this project were partly supported by a contract, No. 14-17-0007-1114, from the Bureau of Commercial Fisheries (now National Marine Fisheries Service) for study of problems encountered while tagging juvenile Pacific salmon at sea. A number of the physiological methods devised during the 1966-1970 period proved equally valuable for studying the stress of either pollution or tagging and, therefore, the costs of developing these methods were distributed between the two agencies.

Mr. Alan Mearns was supported during part of his participation in this project by FWQA Training Grant No. 5T1-WP-175 as well as directly by this project.

SECTION IX

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SECTION X

APPENDIX A - LIST OF PUBLICATIONS FROM THE PROJECT

Published Papers

Miles, H. M., and L. S. Smith. 1968. Ionic regulation in migrating juvenile coho salmon, Oncorhynchus kisutch. Comp. Biochem. Physiol. 26:381-398.

Smith, L. S. 1970. Building and operating a floating laboratory. Lab. Practice 19(7):709-712.

Smith, L. S., and T. W. Newcomb. 1970. A modified version of the Blazka respirometer and exercise chamber for large fish. J. Fish. Res. Bd. Canada 27:1321-1324.

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Cardwell, Rick D. 1968. Hematologic responses of Pacific salmon to acute and chronic stress. M.S. Thesis, Univ. Washington, Seattle. 94 p.

Miles, H. M. 1967. Ionic regulation in migrating juvenile coho salmon, Oncorhynchus kisutch. M.S. Thesis, Univ. Washington, Seattle. 35 p.

Miles, H. M. 1969. Renal function in migrating adult coho salmon. Ph.D. Thesis, Univ. Washington, Seattle. 80 p.

Papers in Press (as of February, 1971)

Cardwell, R. D., and L. S. Smith. Hematological manifestations of a marine bacterial infection upon juvenile chinook salmon (Oncorhynchus tshawytscha). Progr. Fish-Cult.

Miles, H. M. Renal function in migrating coho salmon. Comp. Biochem. Physiol.

Smith et al. Physiological changes experienced by Pacific salmon migrating through a polluted urban estuary. "FAO Technical Conference on Marine Pollution and its Effects on Living Resources and Fishing."

Papers Submitted or in Preparation (as of February, 1971)

Mearns, A. J. Lactic acid regulation in salmonid fishes. Ph.D. Thesis, in preparation. (This thesis will eventually become 2 or 3 papers relating lactate metabolism to swimming stamina and low DO.)

Saddler, J. B., R. D. Cardwell, and L. S. Smith. Lipid composition and hematology of juvenile chinook salmon (Oncorhynchus tshawytscha) cultured on different diets and residing in different environments. (Submitted to Lipids.)

Saddler, J. B., and P. R. Dorn. Comparative fatty acid changes during rapid growth of trout in salt water ponds. (In preparation.)

Saddler, J. B., and K V. Koski. Fatty acid alterations during the early development and migration of Pacific salmon. (Submitted to Lipids.)

SECTION X

APPENDIX B - SUPPLEMENTARY TABLES

Appendix Table B-1. Means, variances, and ranges for length, weight, hematocrit, and ion concentrations in samples of coho salmon resident in freshwater

Sampling period	Parameter	Mean	Standard deviation	Standard error of mean	Maximum	Minimum	Range
Nov. 12, 1966 to May 11, 1967 (N=103)	Total length (mm)	104.21	19.22	1.89	170	72	98
	Wet weight (g)	9.87	6.22	0.61	34.24	3.14	31.1
	Hematocrit (%)	37.77	5.52	0.54	53.0	27.0	26.0
Apr. 6, 1967 to May 11, 1967 (N=55)	Total length (mm)	114.73	19.63	2.65	170	86	84
	Wet weight (g)	12.49	7.43	1.00	34.24	4.29	29.95
	Hematocrit (%)	39.33	5.75	0.77	53.0	28.5	24.5
	Sodium (mEq/l)	146.7	8.24	1.11	168	130	38
	Potassium (mEq/l)	8.58	3.69	0.50	19.01	1.80	17.21
	Calcium (mEq/l)	6.73	0.83	0.11	8.85	4.95	3.9
	Magnesium (mEq/l)	1.08	0.20	0.03	1.55	0.65	0.9
	Chloride (mEq/l)	117.33	7.66	1.03	132.57	90.85	41.72
Apr. 12, 1967 to May 11, 1967 (N=45)	Total solids (%)	4.77	0.66	0.10	6.6	3.1	3.5

Appendix Table B-2. Results of one-way analysis of variance in length, weight, hematocrit, and ion concentrations between samples of juvenile coho salmon resident in freshwater

Parameter	Source	Sum of squares	Degrees of freedom	Mean square	F ratio	Significance
Total length	Between samples	13393.0085	5	2678.6017	17.7178	**
	Within sample	7407.8991	49	151.1816		
Wet weight	Betweeen samples	1905.7651	5	381.1530	17.4304	**
	Within sample	1071.4912	49	21.8672		
Hematocrit	Between samples	898.8094	5	179.7619	9.9612	**
	Within sample	884.2639	49	18.0462		
Total solids	Between samples	3.6510	4	0.9127	2.3212	N.S.
	Within sample	15.7290	40	0.3932		
Sodium	Between samples	871.8420	5	174.3684	3.0585	*
	Within sample	2793.5737	49	57.0117		
Potassium	Between samples	120.7501	5	24.1500	1.9273	N.S.
	Within sample	614.0101	49	12.5308		
Calcium	Between samples	17.2715	5	3.4538	8.3482	**
	Within sample	20.2751	49	0.4138		
Magnesium	Between samples	0.7526	5	0.1505	5.3292	**
	Within sample	1.3840	49	0.0282		
Chloride	Between samples	195.0506	5	39.0101	0.6432	N.S.
	Within sample	2971.8135	49	60.6493		

Appendix Table B-3. Means, variances, and ranges for length, weight, hematocrit, and ion concentrations in all coho salmon samples[1] taken during sea water adaptation. (N=29)

Parameter	Mean	Standard deviation	Standard error of mean	Maximum	Minimum	Range
Total length (mm)	120.48	8.41	1.56	144	101	43
Wet weight (g)	12.69	2.78	0.52	21.06	7.55	13.51
Hematocrit (%)	45.29	5.26	0.98	55.5	33.5	22.0
Total solids (%)	4.86	0.67	0.12	5.9	3.2	2.7
Sodium (mEq/l)	158.3	5.89	1.09	170	149	21.0
Potassium (mEq/l)	7.19	2.99	0.55	13.35	3.15	10.2
Calcium (mEq/l)	6.38	0.78	0.14	7.5	4.5	3.0
Magnesium (mEq/l)	2.17	0.95	0.18	4.2	0.95	3.25
Chloride (mEq/l)	126.6	7.43	1.38	143.68	111.6	32.08

[1]A sample of five fish was taken every 6 hours over a period of 36 hours after introduction of the fish into sea water.

Appendix Table B-4. Results of one-way analysis of variance in length, weight, hematocrit, and ion concentrations between samples of juvenile coho salmon during adaptation to sea water

Parameter	Source	Sum of squares	Degrees of freedom	Mean square	F ratio	Significance
Total length (mm)	Between samples	161.4206	6	26.9034	0.3293	N.S.
	Within sample	2205.5498	27	81.6870		
Wet weight (g)	Between samples	18.7746	6	3.1291	0.3864	N.S.
	Within sample	218.6759	27	8.0991		
Hematocrit (%)	Between samples	521.6125	6	86.9354	5.6372	**
	Within sample	416.3875	27	15.4218		
Total Solids (%)	Between samples	5.2076	6	0.8679	1.9383	N.S.
	Within sample	12.0900	27	0.4478		
Sodium (mEq/l)	Between samples	859.6794	6	143.2799	5.7841	**
	Within sample	668.8299	27	24.7715		
Potassium (mEq/l)	Between samples	94.1381	6	15.6897	2.4212	N.S.
	Within sample	174.9645	27	6.4802		
Calcium (mEq/l)	Between samples	11.4946	6	1.9158	7.0804	**
	Within sample	7.3055	27	0.2706		
Magnesium (mEq/l)	Between samples	18.8704	6	3.1451	6.1663	**
	Within sample	13.7711	27	0.5100		
Chloride (mEq/l)	Between samples	1134.1743	6	189.0291	3.5485	**
	Within sample	1438.3055	27	53.2706		

Appendix Table B-5. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in salt water and freshwater for fish H

Flow rates (ml/(kg x hr))						
Urine rate	0.487	0.216	13	1.81	1.14	37
Glomerular filtration rate	1.05	0.443	9	2.57	1.05	25
PAH clearance	3.52	1.36	9	4.37	0.902	25
Urine ion concentrations (mequiv/l)						
Sodium	23.6	7.55	13	16.5	7.37	25
Potassium	1.62	0.115	13	1.47	0.517	25
Calcium	5.71	0.911	13	3.96	3.12	25
Magnesium	208	19.1	13	70.5	97.6	25
Chloride	177	11.4	13	37.8	55.5	36
Plasma ion concentrations (mequiv/l)						
Sodium	181	2.81	11	154	4.62	34
Potassium	2.70	0.155	11	2.36	0.375	34
Calcium	1.53	0.281	11	1.53	0.273	34
Magnesium	1.27	0.179	11	0.944	0.144	34
Chloride	155	1.95	11	134	5.06	34
Excretion rates (μequiv/(kg x hr))						
Sodium	11.0	4.34	13	22.1	20.3	25
Potassium	0.791	0.370	13	1.75	1.16	25
Calcium	2.72	1.07	13	3.10	0.967	25
Magnesium	100	39.4	13	35.9	50.9	25
Chloride	85.6	35.3	13	33.8	29.8	36

Appendix Table B-5. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in salt water and freshwater for fish H - Continued

Parameter	Salt water			Freshwater		
	Mean	S.D.	n	Mean	S.D.	n
Filtered ion load (μequiv/(kg x hr))						
Sodium	162	70.4	9	336	132	25
Potassium	2.54	1.09	9	5.24	1.71	25
Calcium	0.466	0.191	9	1.16	0.448	25
Magnesium	0.944	0.286	9	1.72	0.787	25
Chloride	158.	68.2	9	330	130	25
Ion load based on PAH clearance (μequiv/(kg x hr))						
Sodium	637	251	9	677	139	25
Potassium	9.53	3.92	9	10.5	2.69	25
Calcium	5.03	3.68	9	6.63	1.86	25
Magnesium	4.34	1.16	9	3.94	0.817	25
Chloride	546	215	9	584	119	25
Clearance ratios based on PAH clearance (dimensionless)						
Water	0.155	0.0159	9	0.307	0.207	25
Sodium	0.0208	0.0070	9	0.0294	0.0236	24
Potassium	0.0913	0.0136	9	0.164	0.110	24
Calcium	0.598	0.0818	9	0.466	0.104	24
Magnesium	26.0	3.22	9	9.89	12.5	24
Chloride	0.178	0.0199	9	0.0539	0.0515	25

Appendix Table B-5. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in salt water and freshwater for fish H - Continued

Parameter	Salt water			Freshwater		
	Mean	S.D.	n	Mean	S.D.	n
Filtration Ratios (dimensionless)						
Water	0.526	0.0584	9	0.478	0.166	25
Sodium	0.0828	0.0304	9	0.0557	0.0251	24
Potassium	0.348	0.0428	9	0.301	0.0978	24
Calcium	6.60	1.25	9	3.12	1.60	24
Magnesium	121	19.0	9	42.7	60.1	24
Chloride	0.628	0.090	9	0.149	0.196	25
Filtration fraction (dimensionless)	0.298	0.0543	9	0.587	0.226	25
Hematocrit (%)	21.1	0.485	11	16.9	0.850	34
Plasma total solids (%)	2.88	0.0745	11	2.54	0.0524	34
Blood pressure (mm Hg)	23.5	2.01	9	23.0	3.97	34
Dorsal aorta pulse pressure (mm Hg)	3.71	0.367	9	4.89	1.91	34
Heart rate (beats/min)	54.1	5.65	13	35.6	7.55	34

Appendix Table B-6. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in salt water and freshwater for fish L

Parameter	Salt water			Freshwater		
	Mean	S.D.	n	Mean	S.D.	n
Flow rates (ml/(kg x hr))						
Urine rate	0.232	0.0621	6	8.00	4.42	100
Glomerular filtration rate	2.24	0.404	5	12.1	5.29	96
PAH clearance	1.67	0.220	5	13.3	5.84	96
Urine ion concentrations (mequiv/l)						
Sodium	125	22.0	6	16.7	17.0	93
Potassium	4.34	0.474	6	1.09	0.586	94
Calcium	13.6	7.03	6	2.71	0.895	94
Magnesium	56.5	16.6	6	4.89	7.62	94
Chloride	35.5	8.43	6	17.1	10.3	96
Plasma ion concentrations (mequiv/l)						
Sodium	181	0.00	5	158	6.15	100
Potassium	2.78	0.0286	5	2.41	0.194	100
Calcium	4.48	0.217	5	2.47	0.262	100
Magnesium	7.91	1.88	5	2.90	0.639	100
Chloride	158	0.653	5	143	2.73	100
Excretion rates (µequiv/(kg x hr))						
Sodium	29.6	11.4	6	150	190	93
Potassium	1.02	0.348	6	9.05	6.90	94

Appendix Table B-6. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in salt water and freshwater for fish L - Continued

Parameter	Salt water			Freshwater		
	Mean	S.D.	n	Mean	S.D.	n
Calcium	3.11	1.87	6	20.1	9.63	94
Magnesium	12.6	3.43	6	19.1	15.4	94
Chloride	7.90	1.55	6	155	167	96
Filtered ion load (μequiv/(kg x hr))						
Sodium	345	62.2	5	1620	700	96
Potassium	5.55	0.990	5	25.8	11.1	96
Calcium	3.12	0.618	5	9.17	3.98	96
Magnesium	13.1	4.46	5	24.7	10.4	96
Chloride	344	62.3	5	1680	733	96
Ion load based on PAH clearance (μequiv/(kg x hr))						
Sodium	302	39.8	5	2090	898	96
Potassium	4.64	0.610	5	31.7	13.5	96
Calcium	7.47	1.07	5	32.5	14.4	96
Magnesium	13.2	3.67	5	36.6	14.6	96
Chloride	264	34.9	5	1900	832	96
Clearance ratios based on PAH clearance (dimensionless)						
Water	0.153	0.0260	5	0.645	0.390	96
Sodium	0.109	0.0263	5	0.0627	0.0616	93

Appendix Table B-6. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in salt water and freshwater for fish L - Continued

Parameter	Salt water			Freshwater		
	Mean	S.D.	n	Mean	S.D.	n
Potassium	0.242	0.0662	5	0.276	0.172	94
Calcium	0.479	0.321	5	0.655	0.272	94
Magnesium	1.13	0.506	5	0.569	0.425	94
Chloride	0.0316	0.0053	5	0.0754	0.0707	94
Filtration ratios (dimensionless)						
Water	0.116	0.0286	5	0.730	0.486	96
Sodium	0.0962	0.0231	5	0.0809	0.0823	93
Potassium	0.205	0.0597	5	0.347	0.214	94
Calcium	1.16	0.694	5	2.35	1.06	94
Magnesium	1.17	0.496	5	0.815	0.546	94
Chloride	0.0240	0.0035	5	0.0874	0.0888	94
Filtration fraction (dimensionless)	1.37	0.321	5	0.927	0.134	96
Hematocrit (%)	19.2	0.164	5	16.5	1.12	100
Plasma total solids (%)	4.36	0.0818	5	3.65	0.350	100
Blood pressure (mm Hg)	36.8	2.44	6	18.3	7.35	51
Dorsal aorta pulse pressure (mm Hg)	3.94	0.707	6	3.05	1.21	51
Heart rate (beats/min)	71.0	1.79	6	54.4	5.06	51

Appendix Table B-7. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in freshwater for fishes M and O

Parameter	Fish M			Fish O		
	Mean	S.D.	n	Mean	S.D.	n
Flow rates (ml/(kg x hr))						
Urine rate	3.38	1.57	79	3.86	1.30	44
Glomerular filtration rate	8.88	2.95	77	8.04	1.95	41
PAH clearance	13.0	5.41	77	24.4	8.67	41
Urine ion concentrations (mequiv/l)						
Sodium	9.82	10.0	69	10.6	3.83	43
Potassium	2.13	1.74	69	2.13	0.652	43
Calcium	3.92	1.94	69	2.63	0.854	43
Magnesium	6.89	11.4	69	1.13	0.539	43
Chloride	9.02	15.0	77	2.31	1.34	43
Plasma ion concentrations (mequiv/l)						
Sodium	152	2.63	72	172	4.27	44
Potassium	2.52	0.149	72	2.97	0.199	44
Calcium	1.67	0.523	72	2.78	0.922	44
Magnesium	1.80	0.555	72	1.39	0.184	44
Chloride	131	2.29	79	115	3.24	44
Excretion rates (μequiv/(kg x hr))						
Sodium	25.7	16.4	69	39.2	17.3	43
Potassium	6.66	5.76	69	7.90	3.02	43

Appendix Table B-7. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in freshwater for fishes M and O - Continued

Parameter	Fish M			Fish O		
	Mean	S.D.	n	Mean	S.D.	n
Calcium	11.5	5.14	69	9.72	3.85	43
Magnesium	12.3	12.7	69	3.85	0.910	43
Chloride	18.3	8.92	77	7.75	3.59	43
Filtered ion load (μequiv/(kg x hr))						
Sodium	1130	384	71	1180	282	41
Potassium	19.6	6.93	71	21.3	5.76	41
Calcium	4.45	1.90	71	6.65	2.87	41
Magnesium	11.0	3.82	71	8.03	2.12	41
Chloride	1130	380	77	895	221	41
Ion load based on PAH clearance (μequiv/(kg x hr))						
Sodium	1880	784	71	4210	1510	41
Potassium	31.4	13.7	71	71.0	21.3	41
Calcium	19.9	8.08	71	61.3	19.5	41
Magnesium	21.2	7.74	71	33.2	11.2	41
Chloride	1710	723	77	2790	948	41
Clearance ratios based on PAH clearance (dimensionless)						
Water	0.257	0.0849	77	0.179	0.0948	41
Sodium	0.0166	0.0150	64	0.0109	0.0080	40

Appendix Table B-7. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in freshwater for fishes M and O - Continued

Parameter	Fish M			Fish O		
	Mean	S.D.	n	Mean	S.D.	n
Potassium	0.232	0.218	64	0.124	0.0636	40
Calcium	0.638	0.289	64	0.178	0.0967	40
Magnesium	0.858	1.14	64	0.130	0.0609	40
Chloride	0.0144	0.0154	76	0.0032	0.0019	39
Filtration ratios (dimensionless)						
Water	0.365	0.111	77	0.476	0.0928	41
Sodium	0.0249	0.0187	64	0.0328	0.0117	40
Potassium	0.335	0.266	64	0.386	0.108	40
Calcium	2.73	0.972	64	1.69	0.879	40
Magnesium	1.43	1.74	64	0.516	0.179	40
Chloride	0.0215	0.0298	76	0.0088	0.0044	40
Filtration fraction (dimensionless)	0.719	0.151	77	0.369	0.162	41
Hematocrit (%)	4.35	0.800	72	16.9	0.865	44
Plasma total solids (%)	2.84	0.124	72	2.95	0.0382	44
Blood pressure (mm Hg)	29.7	3.41	77	36.6	3.83	43
Dorsal aorta pulse pressure (mm Hg)	4.89	0.819	77	7.63	0.651	43
Heart rate (beats/min)	53.8	4.92	77	56.1	5.83	43

Apendix Table B-8. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in freshwater for fish P

Parameter	Fish P		
	Mean	S.D.	n
Flow rates (ml/(kg x hr))			
Urine rate	2.74	1.59	49
Glomerular filtration rate	6.47	2.77	29
PAH clearance	6.07	2.55	29
Urine ion concentrations (mequiv/l)			
Sodium	5.83	2.76	43
Potassium	1.97	3.88	44
Calcium	1.54	0.866	44
Magnesium	1.57	1.45	44
Chloride	3.54	1.55	49
Plasma ion concentrations (mequiv/l)			
Sodium	146	2.31	32
Potassium	2.48	0.222	32
Calcium	4.10	0.159	32
Magnesium	1.65	0.0322	32
Chloride	132	1.51	32
Excretion rates (µequiv/(kg x hr))			
Sodium	15.0	8.89	43
Potassium	5.56	11.2	44

Appendix Table B-8. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in freshwater for fish P - Continued

Parameter	Fish P		
	Mean	S.D.	n
Calcium	4.39	4.62	44
Magnesium	4.05	4.06	44
Chloride	8.62	5.02	49
Filtered ion load (μequiv/(kg x hr))			
Sodium	803	342	29
Potassium	14.3	6.79	29
Calcium	8.15	3.52	29
Magnesium	7.84	3.40	29
Chloride	827	354	29
Ion load based on PAH clearance (μequiv/l)			
Sodium	883	372	29
Potassium	15.1	7.23	29
Calcium	24.7	10.4	29
Magnesium	10.1	4.29	29
Chloride	802	336	29
Clearance ratios based on PAH clearance (dimensionless)			
Water	0.408	0.111	29
Sodium	0.0195	0.0071	24

Appendix Table B-8. Means, standard deviations (S.D.), and sample sizes (n) for renal parameters in freshwater for fish P - Continued

Parameter	Fish P		
	Mean	S.D.	n
Potassium	0.252	0.0798	25
Calcium	0.157	0.111	25
Magnesium	0.336	0.279	25
Chloride	0.0098	0.0031	29
Filtration ratios (dimensionless)			
Water	0.385	0.101	29
Sodium	0.0212	0.0072	24
Potassium	0.265	0.0821	25
Calcium	0.479	0.361	25
Magnesium	0.431	0.350	25
Chloride	0.0095	0.0029	29
Filtration fraction (dimensionless)	1.06	0.0780	29
Hematocrit (%)	22.7	5.39	49
Plasma total solids (%)	4.86	0.621	49
Blood pressure (mm Hg)	24.5	7.12	48
Dorsal aorta pulse pressure (mm Hg)	5.31	1.04	48
Heart rate (beats/min)	56.4	4.29	48

BIBLIOGRAPHIC:

L. S. Smith, J. B. Saddler, R. C. Cardwell, A. J. Mearns, H. M. Miles, T. W. Newcomb, and K. C. Watters. Fisheries Research Institute, Univ. of Wash. Responses of Teleost Fish to Environmental Stress. Final Report FWQA Grant No. 18050EBK. February, 1971.

ACCESSION NO.

ABSTRACT

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KEY WORDS:

Fish Physiology
Fish Migration
Environmental Effects
Pacific Salmon
Oxygen Sag

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10 Author(s)

L. S. Smith, J. B. Saddler, R. C. Cardwell, A. J. Mearns, H. M. Miles, T. W. Newcomb, and K. C. Watters.

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21 Note

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Salmon
Environmental Effects
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Oxygen Sag
Oxygen Requirements
Animal Metabolism

25 Identifiers (Starred First)

Aquatic Environments
Fish Migration
Salmonids

27 Abstract

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