Revised Procedure for the 24 Hour Seawater Challenge Test to Measure Seawater Adaptability of Juvenile Salmonids

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January 1987

Canadian Technical Report of Fisheries and Aquatic Sciences No. 1515

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Oceans et Océans

Canada

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Cat. No. Fs 97-6/1515E ISSN 0706-6457

Correct citation for this publication:

Blackburn, J. and W. C. Clarke. 1987. Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquat. Sci. 1515: 35 p.

ABSTRACT

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An improved 24 hour seawater challenge plasma sodium test is described. Field use of static, artificial sea water is comparable to laboratory tests if density, temperature, salinity and oxygen are within limits. Reasonable use of anesthetics, netting disturbance, starvation, and sample storage will not affect test results but crowding, fish transport, and anoxia will increase sodium after seawater challenge. Circulation currents can sometimes cause cloudy eye after seawater transfer. Glass micropipets have lower variability than plastic tip micropipets. Using a modern clinical flame photometer and reference calibration sera, group means can be determined within 3 mmol/L sodium. Normal values vary with treatment, freshwater control values, and unknown factors so that group mean sodium values after seawater challenge range from 162 to 168 mmol/L for coho and chinook smolts. Plasma sodium is linearly related to seawater temperature. Increasing challenge salinity causes an exponential increase in plasma sodium. In hemolysed samples there is an inverse linear relation of potassium and sodium which may be used to adjust sodium values for the effects of lysis. Osmolality, weight loss, and mortality after hypersaline challenge are other useful measures of seawater adaptability but hematocrit, magnesium and potassium levels after challenge are not.

Key Words: plasma, blood, serum, sodium, smolt, salmon, seawater challenge

RESUME

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Les auteurs décrivent un test amélioré du sodium plasmatique après 24 h d'acclimation à l'eau de mer. L'utilisation sur le terrain d'eau salée artificielle statique donne des résultats comparables à ceux obtenus en laboratoire si la densité, la température, la salinité et la teneur en oxygène sont à l'intérieur des certaines limites. L'utilisation raisonnable d'anesthésiques, les perturbations entraînées par le mouillage des filets, le manque d'aliments et l'entreposage des échantillons n'influeront pas sur les résultats du test; par contre, l'entassement, le transport du poisson et l'anoxie entraîneront une augmentation de la concentration de sodium après l'acclimation à l'eau salée. De plus, les courants peuvent parfois causer l'obscurcissement opthalmique après le transfert en eau salée. Les micropipettes de verre ont une plus faible variabilité que celles à pointe en plastique. En utilisant un photomètre à flamme moderne et des sérums étalon on peut déterminer les moyennes des groupes à 3 mmol/L de sodium près. Les valeurs normales varient selon le traitement, les valeurs obtenues pour le groupe témoin gardé en eau douce et des facteurs inconnus; les concentrations moyennes de sodium de groupe peuvent ainsi varier de 162 à 168 mmol/L chez les saumoneaux coho et quinnat après le test d'acclimatation à l'eau de mer. La concentration de sodium plasmatique est en relation linéaire avec la température de l'eau salée. Une augmentation de la salinité des eaux d'acclimation entraîne une augmentation exponentielle de la concentration du sodium plasmatique. Dans les échantillons hémolysés, il existe une relation linéaire inverse entre le potassium et le sodium qui peut servir à l'étalonnage des valeurs du sodium afin de tenir compte de l'incidence de la lyse. L'osmolatité, la perte de poids et la mortalité après le test d'acclimatation à un milieu hypersodique sont d'autres quantifications utiles de la capacité d'adaptation à l'eau salée; par contre, les teneurs en hématocrite, en magnésium et en potassum après le test d'acclimatation ne comptent pas parmi mesures.

Mots-clés: plasma, sang, sérum, sodium, saumoneau, saumon, test d'acclimatation à l'eau salée

INTRODUCTION

We have employed the 24 hour seawater challenge test since 1974 as a measure of seawater adaptability in juvenile salmonids (Clarke and Blackburn 1977; Clarke 1982; Wedemeyer et al. 1980). It has been applied to fish from laboratory, hatchery, and field studies; the species investigated include coho, chinook, sockeye, chum and Atlantic salmon as well as steelhead and rainbow trout.

The analytical procedure has been modified since our initial description (Clarke and Blackburn 1977) and a number of experiments have been done to improve quality control and determine the effect of stress on fish performance. This report describes the revised procedure and presents the results of the various experiments.

TEST PROCEDURE

FISH SAMPLING, TRANSPORT, AND HOLDING

Fish tested should be representative of their group and be collected and handled with minimum stress. In a large pond or raceway, crowding is the best way to get a good sample, however a dip net approaching another net is often all that can be done. Our minimum sample is 12 fish for transfer (plus 8 for freshwater control if needed); for correlation with size 36 transfers are required. While at the collection site we record temperature, stock, pond, medication, history, and release date. Recent stress of transport, crowding, clipping, anesthetics, antibiotics, starvation, sickness, etc., may affect performance in the seawater challenge test. Therefore any recent information about the general condition or health of the fish being tested should be noted.

We transport fish from hatcheries to Nanaimo, a 1 to 3 hour drive, using either of two methods. Generally we use 80 L plastic garbage cans with air from a 12 volt air compressor. Ice packs are added if needed. Otherwise we put fish in plastic bags with 1/3 water and 2/3 oxygen, and pack the bags and ice into styrofoam chests. No salt or anesthetic is used in transport. At Nanaimo the fish are held for 2 or 3 days in 200 L tanks until tested; during this recovery period hatchery temperature and normal photoperiod are maintained, but fish are not fed.

TRANSFER TO SEA WATER

Fish are transferred directly into 29-30°/$_{oo}$ sea water with minimum handling (usually netted from a freshwater tank to a seawater tank). Normally we use 200 L tanks supplied with filtered sea water flowing at 300 L/h at or near the acclimation temperature. Our tanks are equipped with circulating pumps to produce a 4 cm/s current, but these pumps are used only if the fish are acclimated to the system. At hatchery or field sites sea water is brought in or made up using artificial sea salt (table salt is toxic). Artificial salts should be fresh and completely dissolved in warm water for best results. Salinity can be measured with a conductivity meter, hydrometer, or refractometer. Large (80 L or more) plastic garbage buckets with lids are good for field transfers; they can be suspended in raceways or pools for temperature control. Air or oxygen is needed, but not so much as to scare the fish or produce foam. An 80 L bucket can hold up to 240 g of fish. Normal photoperiod is maintained, no food is offered, and the fish are not disturbed during the 24 hour seawater challenge. Also we often measure plasma sodium in fish which are not transferred: these freshwater controls can indicate problems in handling or other stress.

BLOOD COLLECTION

To immobilize fish before blood collection we use 500 ppm 2-Phenoxy-ethanol (2-PE). Previously, we have used 50 ppm MS222. Anesthetic is made up using sea water for seawater challenge groups. Small groups (3-5 fish) are placed in anesthetic at one time to minimize exposure time. Each fish is measured (fork length), weighed, checked for tags, descaling, silveryness, dark fin tips, cloudy eyes etc., and is thoroughly blotted off. The tail is cut off and blood from the caudal artery is collected into a microhematocrit tube treated with ammonium heparin. Samples contaminated with urine or gut contents are rejected. The tubes are sealed with putty and spun five minutes in a hematocrit centrifuge within 15 minutes of collection. Analysis is best completed the same day; if so the tubes can be stored vertically in a refrigerator until used. If analysis is delayed the tubes should be cut and the plasma portion sealed at both ends, then stored in a refrigerator for up to 8 days or in a freezer for longer periods. Samples frozen in microhematocrit tubes may show extreme variability in sodium, so freeze only when absolutely necessary, avoid auto-defrost freezers, and expel and completely stir the thawed sample before taking the sample aliquot. Lysis in individual tubes should be noted. Lysis may indicate contamination, sickness, sample deterioration or small sample size; it lowers sodium and raises potassium values. A correction for this effect is given below.

Commercial reference calibration materials (i.e., Instrumentation Laboratory flame standard ampules, Ortho assayed control serum or Cation Cal) are used so that each analysis can be compared to those performed at other dates. These freshly opened references are synthetic or reconstituted dried human sera with assayed concentrations of various ions. Every day of sampling

we collect, spin, and store three of these preparations in the same way as the salmon blood samples so that a bias in dilution, storage, contaminated reagents, or badly calibrated analyser can be corrected for.

SAMPLE DILUTION AND SODIUM ANALYSIS

Flame photometry is the accepted way to measure sodium in blood (Ruff 1982). The sample size and dilution depend on the instrument used: from 1974 to 1980 we used a Jarrell-Ash flame emission spectrophotometer with 2 µL (µL = microliter) plasma diluted to 5 mL. We now use a Turner (Case Instrument) clinical flame photometer with 5 µL diluted to 1.0 mL. Dilutions are manual since most automatic diluters require more plasma than can be obtained from small fish. (If an outside laboratory is contracted to do the analysis, the sample size should be explained; also that samples will be outside the range of some photometers.)

We use disposable Microcap pipets to measure plasma and a piston dispenser to measure the diluent. If clotting is a problem the samples sometimes can be liquified by freezing and thawing. Frozen samples need thorough mixing to eliminate possible variations in concentration. The Microcaps must be rinsed with the diluent. Diluted samples are best analysed the same day but can be stored at room temperature for a few days. Measure sodium using instructions for the analyser used.

Every time we use the photometer we run three replicates of six sodium standards (0, 50, 100, 150, 200, 250 mmol/L [1]), three replicates of two reference sera, ten replicates of a third, main, reference serum, and three replicates of sea water. The standards correct deviation from linearity by the instrument. Then the adjustment to give the main reference material its assayed value is made to all samples. The two other reference materials ensure that the main reference is not contaminated and the sea water sample checks the challenge salinity.

ACCURACY AND PRECISION OF SODIUM ANALYSIS

Replicate analyses of our main reference (IL 160 mmol/L standard ampules freshly opened each day) are shown in Table 1. The values have been fitted to a standard curve and then adjusted to give the mean its assayed value (the same calculation applied to the blood samples). In seven of ten cases the 95% confidence interval (C.I.) is within 159 and 161, and in all

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[1]Various units and abbreviations for sodium are used by different authors. Millimoles per liter (mmol/L, mm/L, mM or mM/L), and milliequivalents per liter (mEq/L or meq/L) are synonymous for sodium. We follow Velapoldi et al. (1978) and use mmol/L.

cases between 158 and 162. The C.I. not only measures repeatability, but also the accuracy of our analysis since all values are adjusted to this reference point. Although the 95% confidence interval indicates that sodium is usually within 1 or 2 mmol/L for reference material, fish blood determinations probably are more variable than this. This is because variation in reference material is due only to dilution and instrument fluctuation during the run, but fish blood samples have additional variation due to viscosity, interferences, sample handling, and contamination. These factors have been minimized but cannot be eliminated or measured with reasonable effort (Velapoldi et al. 1978). Since our procedures are standardized we are able to make day to day, or year to year comparisons within 3 mmol/L sodium.

SMALL SAMPLE SIZE AND LYSIS CORRECTION

Fish as small as 1.5 g can provide 5 µL of blood plasma, but for analyses of fish less than 2 g it is often necessary to pool blood from two or three fish. These very small samples often show lysis, indicated by high potassium and low sodium. When potassium exceeds 10 mmol/L, sodium values should be increased by:

$$\text{sodium} = \text{observed sodium} + 1.33 * (\text{potassium} - 6).$$

This adjustment is explained below in the hemolysis experiment.

REQUIREMENTS FOR SODIUM TEST

Apart from transport of fish to the laboratory, setting up tanks, instrument preparation, and data analysis two people can collect and centrifuge blood from 100 fish in 2 or 3 hours and then one person can dilute samples and analyse for sodium in about six hours. This includes the daily requirement for 37 samples diluted and analysed for standards and calibration and 40 minutes to warm-up and clean out the photometer. A list of equipment and reagents needed to perform the seawater challenge test is given below.

balance	measuring board	anesthetic
thermometer	salinometer	sea water
hematocrit tubes	sealing putty	centrifuge
diamond pencil	calibration sera	vials and caps
photometer	micropipets	diluent dispenser
standards	diluent	

EXPRESSION OF SODIUM RESULTS

Sodium results can be expressed in (at least) three different ways: 1. the mean of the group after seawater challenge, 2. the group mean after seawater challenge minus the group mean for the fresh water controls, and 3. the percentage of fish over (or under) a threshold sodium value. This last measure is recommended by Rawson and Howe (1984); we used 170 mmol/L as the threshold. To compare these three indexes, 16 tests on three tanks of yearling coho, fitted to a cubic polynomial with 95% confidence limits of the line, are given in Figure 1. Sodium values after seawater challenge, sodium in fresh water and the percentage of challenged fish with sodium above 170 mmol/L are plotted against date. The test temperature is also included. The seawater and freshwater curves were computed using 576 individual values, but the percentage under 170 mmol/L used only 48 group values and consequently has wider confidence limits. The confidence limits of the data are not included so many group means are outside the confidence limits of the line.

As can be seen in Figure 1 the freshwater control values change with the season and/or temperature although much less than the seawater values. Other factors such as stress (or temperature) may also affect fresh water sodium values. Although it might seem desirable to express results as the difference between seawater challenge and freshwater control sodium value, we don't think the cost in fish and analytical effort is justified. Taking the difference between freshwater control and seawater challenge values also reduces the degrees of freedom and makes it more difficult to detect differences among groups.

We normally express seawater challenge results as a group mean of plasma sodium along with the number of fish tested, the standard error of the mean, and the number of mortalities if any. If available, the freshwater control values are given as well. However the mean does not reflect the performance of most fish if the population is not normally distributed. For example a population of yearling Atlantic salmon may have a bimodal size distribution with half fully developed smolts and half parr with an average value indicating incomplete seawater tolerance. Where the population is bimodal or skewed, the results can be expressed as percent of the group under or over a threshold. In Figure 1 the results are expressed as percent over 170; the coho were not a bimodal smolting population, and the percent over 170 mmol/L curve is very similar to the mean seawater sodium curve.

INTERPRETATION OF RESULTS

In our previous report (Clarke and Blackburn 1977), we stated that smolts are capable of regulating their plasma sodium levels below 170 mmol/L within 24 hours of direct transfer to 28-30°/$_{oo}$ sea water. In subsequent work, the mean sodium value after challenge for coho smolts (Fig. 1) and chinook smolts (Clarke and Shelbourn 1985) is 165 mmol/L, with individual group means between 162 and 168 mmol/L. The mean value for freshwater

controls lies within the range of 154 to 162 mmol/L. A strong, inverse correlation between plasma sodium concentration after challenge and subsequent growth rate of accelerated coho in sea water has been demonstrated (Clarke and Shelbourn 1982).

Different laboratories report different values for sodium (Hille 1982) so our normal values may not apply in all cases. Interpretation of a single sodium mean is difficult because seawater tolerance may be improving or regressing and stress, sickness, or technical problems can affect results. A series of challenge tests during the season is preferrable to a single test particularly when the purpose is to determine optimal time of entry of smolts into sea water or to study the effect of a treatment (photoperiod, temperature) upon the development of seawater tolerance. A one-time test with controls, is suitable for evaluating smolt quality after some acute treatments or fish culture procedures (diet, chemicals, crowding, etc.).

EXPERIMENTS AND RESULTS

TRANSPORT STRESS

Two experiments studied the effect of transport stress on blood sodium. In July a group of Quesnel River chinook (2) reared at the Pacific Biological Station was split, half remaining in their tank (no travel) and half were loaded into garbage buckets and driven around for two hours (travel). The oxygen remained above 80% saturation and the temperature dropped from 15 to 14°C. Fish from both groups were transferred to sea water (SW) 0, 1, 2, 3, and 7 days after travel; blood sodium was measured in freshwater controls as well as in the transfer groups. The fish in experiment 1 were poorly adapted to SW. In May, experiment 2 used Big Qualicum River chinook stock reared in the laboratory: temperature and oxygen remained at 13°C and 90% saturation, fish had 0, 1, 2, 3, and 6 days recovery, and the fish appeared to be good smolts.

The results of both travel experiments are presented in Table 2. A two-way analysis of variance tested travel, recovery time and interaction effect on four groups: exp 1 FW control, exp 1 SW challenge, exp 2 FW control, and exp 2 SW challenge. The travel effect was significant (3) in

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[2]Except for the Quesnel River chinook, all chinook in this report are fall run stocks from Vancouver Island.

[3]In this report significant difference means $p<0.05$ unless probability is specified.

both FW controls but not in either SW challenge test. Number of days recovery after travel was significant in experiment 2 for both fresh and sea water groups but not in either group in the first experiment. However, the recovery time x travel interaction was significant in all but the fresh water group in experiment 1.

FEEDING BEFORE TRANSFER

In November underyearling coho were not fed for 0, 1, 3, and 6 days before transfer to measure the effect on subsequent plasma sodium. Prior to this test these fish were held at 10°C and were fed OMP (Oregon Moist Pellet) once a day. Forty-eight fish were sampled in each treatment; most fish had parr marks. The mean sodium ± standard error for the four fasting periods were:

0 day	177.8 ± 1.2 mmol/L
1 day	175.8 ± 1.3 mmol/L
3 day	176.5 ± 1.0 mmol/L
6 day	179.4 ± 0.9 mmol/L

ANOVA showed that days of food deprivation did not significantly affect plasma sodium levels.

EFFECT OF SEAWATER TEMPERATURE

Yearling coho and rainbow trout acclimated to 15°C were transferred into sea water of 10, 12.5, 15, 17.5, and 20°C on June 22. The effect of seawater temperature at transfer was similar for coho and rainbow trout and also for size groups within the two species. The data for both species are combined in Table 3. The fish did not adapt well to sea water. ANOVA showed a significant effect of temperature, but studentized range test showed only two different pairs among the five treatments: the two low temperatures were different from the 20°C group. There was a significant, positive correlation between temperature and plasma sodium ($r = 0.31$, $p<0.01$).

TIME IN SEA WATER

We conducted a time-course experiment on May 16-23 using Rosewall Creek hatchery coho smolts at 10°C. In addition to the usual freshwater control and 24-hour challenge, blood was sampled after 8, 16, 48, 72, and 168 hours SW challenge. Sodium was highest after 24 hours challenge; the 24 h sample was significantly higher than the 48 h sample (data not shown).

NETTING DISTURBANCE BEFORE CHALLENGE

On 14 June, with laboratory reared chinook, two transfer methods were compared: a no-disturbance transfer in which the fish were not moved but the water flow was changed from fresh to salt, and a disturbed transfer where the fish were netted from fresh water and carried to a seawater tank. These fish were preadapted to 14 °/₀₀ sea water at 14°C. On 29 November a second test was done using freshwater underyearling coho parr held in a 5000 L stock tank. Three treatments were compared: 1) fish moved to 200 L laboratory tanks seven days before transfer and then transferred to sea water with no disturbance; 2) fish moved to 200 L tanks seven days before transfer, at transfer fish were netted out, tank was drained and refilled with SW, and fish returned; and 3) fish moved from fresh water stock tank to 200 L tanks full of sea water. Fresh water temperature was 5.5°C and sea water was 6.0°C, photoperiod was normal, fish were fed three times a week, and duplicate 200 L tanks were used for each treatment. The results of both tests are in Table 4. When netting and no disturbance treatments were compared there was no significant difference ('t' test) in blood sodium for the first experiment. Analysis of variance of the second netting disturbance experiment showed no treatment or replicate effects.

EFFECT OF STATIC AND ARTIFICIAL SW AND TANK VOLUME

When natural, flowing sea water is not available, transfers must be made in containers (often plastic garbage buckets) of static sea water made from artificial sea salts or transported from the coast. In six experiments we compared static to flowing SW, natural to artificial SW, and tested different container sizes. Five of these tests are in Table 5 identified by date.

Two tests compared static and flowing natural sea water. On 20 May, with similar density, volume, and salinity, there was no difference in sodium values for coho. On 2 May, transfers of yearling Rosewall coho were made in three tank sizes and two flow treatments- 300 L/h flowing and static natural sea water with air stones and temperature regulation. Total ammonia was measured at the end of 24 h challenge, pH was 8.0. Flow was not a significant factor.

Static sea water made with marine aquarium salts were compared to static natural sea water in three trials. Rila brand sea water was tested on 14 March using hatchery steelhead. There were no mortalities and oxygen was maintained with airstones. ANOVA showed that the type of SW was not significant. On 25 March we compared artificial (Hagen Biocrystals) SW, natural SW, and table salt, all at 29°/₀₀. All fish in table salt died after a few hours and chinook in Biocrystals were not different than those in natural sea water. In the third test (data not shown), 40°/₀₀ SW was made

up two ways - 100% Biocrystal, and 25% Biocrystal with 75% natural sea water. There was no significant difference in mortality of coho yearling or chum fry if the salt was dissolved in warm water and allowed to stand a few hours. However, if the salts were dissolved in cold water and fish added immediately there was high mortality in the 100% artificial seawater group.

Three experiments demonstrate the effect of container size on plasma sodium levels; density of fish (g/L) is confounded with tank volume (L) in all these experiments. On 14 March, 20 and 45 L buckets were used for sea water challenge (replicated with both natural and synthetic SW) giving two densities of hatchery steelhead. The higher densities (5.0 and 6.6 g/L) caused significant increase in plasma sodium over the lower densities (2.9 and 2.6 g/L). Second, transfers of yearling coho were made 2 May in three tank sizes (23, 53, and 200 L) and two flow conditions. Two-way ANOVA showed that the density effect was significant, but flow and interaction effects were not significant. Tukey's test (Steel and Torrie 1960, p. 114) showed the high density (10.2 g/L) significantly different from the low density (1.1 g/L). Third, on 10 June, 30 hatchery chinook smolts averaging 7.5 g were transferred into cylindrical 50 L "pot" tanks, and also into our regular 200 L "tub" tanks. Both tanks had sea water flowing at 240 L/h. The higher density (4.5 g/L) was stressful, as indicated by higher plasma sodium, compared to lower density (0.9 g/L).

SALINITY OF CHALLENGE SEA WATER

At four times, yearling coho were transferred to 60 L static sea water tanks with air stones and temperature control. To get different salinities natural sea water was mixed with fresh water or with Hagen Marine Mix Biocrystals. Plasma sodium results (24 h) are illustrated in Figure 2c,d,e,f. There is little change in sodium up to 25 °/₀₀, but sodium increases sharply at higher salinities. As the coho develop greater seawater adaptability later in the season, a higher salinity is required to produce the same elevation of plasma sodium.

BASKET vs LOOSE TRANSFERS

It would be convenient to transfer fish like french-fries in net baskets so that several groups could share a single 200 L tank. Our baskets measured 25 cm long, 12 cm wide, and 20 cm deep, so volume was only 6 L while water quality was equal that in a 200 L tank. Basket and loose SW transfers were compared using coho at two temperatures, 5 and 12°C, and using chinook at 13°C. In every test the fish in baskets had significantly higher sodium than those loose in the tank. Freshwater control sodium values in coho were lowered significantly after 24 hours in a basket indicating stress due to confinement in baskets (data not shown).

CLOUDY EYE

From February to March 1982, cloudy eyes (a milky film on the cornea in contrast to cataract deeper in the lens) were observed in some Big Qualicum coho reared in the laboratory. While freshwater controls had clear eyes certain tanks had a high frequency of either left or right eye cloudy after the 24 hour transfer but no tank had both left and right eye affected. The side affected was not determined by direction of flow in the fresh water pond but by the direction of flow (created by the circulation pump) in the seawater tank. Testing in a clockwise flow produced 89% right eye cloudy and 0% left eye cloudy; fish from the same freshwater pond in a counter-clockwise SW flow had 33% left and 0% right eyes cloudy. When returned to fresh water the cloudiness was worse after one day but virtually disappeared in four days.

The second netting disturbance experiment described above also showed cloudy eyes in one treatment and not in others, helping explain the cause of cloudy eye after sea water transfer. Coho moved from a 5000 L tank of fresh water to a 200 L tank of sea water showed a 70% incidence of cloudy eye, while fish that were acclimated to a 200 L tank showed no cloudy eye after 24 hours in sea water. In this case seawater circulation was counter-clockwise and cloudy eyes were on the left side only.

ANESTHETICS BEFORE BLEEDING

We tried to avoid chemical anesthetics by using live, killed, stunned, or chilled fish, but killed fish did not always bleed well, stunning was messy, and an ice bath either did not sedate or else froze the fish. We compared the blood sodium after short exposure to anesthetics with that of stunned fish: Tricaine methanosulfonate (MS222) was tested on Coho underyearlings on 13 August, while 2-phenoxy-ethanol (2-PE) was tested on chinook on December 20. The anesthetic treatment group had the usual immobilization before bleeding; in the other group fish were individually netted and stunned by a blow to the head with a metal rod. Coho treated with MS222 (after 24 h SW challenge, d.f.=22) were not significantly different from stunned fish. Furthermore, the second test showed no significant difference between 2PE anesthetised and stunned chinook after 24 h SW challenge (d.f.=30).

ANOXIA BEFORE BLEEDING

On 5 May, ten Rosewall Creek hatchery coho smolts were transferred to sea water in the normal manner, anesthetized and then held in air for 5 min before bleeding. Another ten fish were bled within 1 min of anesthesia as usual. The coho treated normally had average plasma sodium of 168.3 ± 1.3 mmol/L and the five minute anoxic group 174.0 ± 2.5 mmol/L. This increase in plasma sodium was found to be statistically significant (one-tailed 't' test, d.f.=18).

PLASMA STORAGE

When standards, distilled water or plasma was refrigerated for 5 days in glass collection tubes sealed at both ends there was no change in sodium or potassium concentrations (data not shown). Both ions increase if stored in unsealed tubes, as a result of sample evaporation. Plasma from adult chum salmon was stored refrigerated (5°C) or frozen (-25°C), and analysed after 0, 4, 8 and 21 days. The plasma was stored in 2 mL auto-analyser cups; three replicates from each of two fish were used. The stored values were compared to fresh (0 day) using randomized block ANOVA and Dunnett's multiple comparisons test (Steel and Torrie 1960). As shown in Table 6, 8 days refrigeration or 21 days freezing did not change the sodium values. However, all stored potassium samples were slightly higher than fresh plasma, but duration of storage did not further change potassium.

In spite of this, we have had poor results on sodium analysis of samples stored frozen in glass microhematocrit tubes. One problem is that the putty seal often falls out of the tube, but even if this does not happen the sodium results can be highly variable. Possibly the auto-defrost cycles or opening the freezer causes frequent thawing and refreezing of these very small samples, producing an uneven distribution of salt in the tube. Furthermore, thorough mixing of samples after thawing is difficult since the microhematocrit tubes are so thin that a vortex mixer cannot stir the sample. Instead, the whole sample must be blown into a vial and shaken in order to thoroughly mix the sample.

HEPARINISED OR PLAIN TUBES

We compared plain (blue tip) with heparinised (red tip) microhematocrit tubes first using a calibration serum (which does not clot) and second using pooled fresh fish blood. Twelve tubes in each group were 70% filled, centrifuged within 15 min, and sodium analysed as usual. There was no difference in sodium between the two types of tubes when the calibration serum was used. However, when fish blood was tested the measured sodium concentrations in the heparinised tubes were 157.2 ± 0.55 mmol/L while measurements from plain tubes were 155.2 ± 0.37 mmol/L, a significant difference.

HEMOLYSIS

Usually the plasma is not coloured or has a pale straw colour. Occasionally it is yellow, orange or even red due to hemoglobin released from broken blood cells. Fluid released from ruptured blood cells is high in potassium and low in sodium. Since lysis is common in very small samples a correction for the low sodium values was needed. Blood from two large (about

one kg) coho from sea water was divided into 28 hematocrit tubes and varying degrees of hemolysis was produced by freezing all or part of the tube in -50°C alcohol. After centrifuging there were 3 layers: packed cells at the bottom, a cloudy layer, and clear, coloured plasma on top. Sodium, potassium and osmotic pressure was measured in the plasma and reported in Table 7.

Lysis did not change osmotic pressure, but did increase potassium and decrease sodium concentration. In both fish there was an inverse relationship between sodium and potassium concentrations. The linear regression of sodium and potassium was significant, with the same slope for each fish, and with a common regression (from covariance) of:

$$\text{sodium} = 170.9 - 1.33 * \text{potassium}; \quad r=-.97, \; p<.01.$$

Accordingly, the dilution of sodium due to lysis can be corrected by adding 1.33 times the increase in potassium to the observed sodium. The increase in potassium is estimated by the observed potassium minus 6 mmol/L, (an average value for potassium). This relationship is not observed in samples with normal potassium so the correction should only be used when potassium exceeds 10 mmol/L.

COMPARISON OF MICROPIPETS

In addition to the glass microcaps described in the test procedure, we have tried three other types of micropipets to measure plasma. One type is a plastic or glass capillary with a wire or teflon plunger to draw up and expel the sample (Wiretrol, SMI, Labindustries). The second type has disposable conical plastic tips fitted to a piston which displaces air (Oxford, Eppendorf); they require good seals and consistent, even technique. While they are easier for a beginner to use they increase measurement error due to viscosity in fish blood and also they are so long that it is awkward to get the pipet tip into the hematocrit tube. The third type, automatic dilutors, measure both the plasma sample and the diluent. The one we tried (Cordis) was slow and the sample tip was too wide to insert into microhematocrit tubes. We compared these three types of pipets with microcaps using replicate dilutions of reference sera to find the type that is most reliable, repeatable, and fast. Since our standards and references are all diluted in the same way as the plasma samples, absolute accuracy is less important than repeatability. Table 8 gives the coefficient of variation for the different micropipets tried. Samples run on the Jarrell-Ash flame emission spectrophotometer before 1980 are not comparable to more recent trials with the Turner flame photometer. However, the benefit of experience with microcaps is shown.

INDICATORS OTHER THAN SODIUM

We have examined osmotic pressure, magnesium, potassium, hematocrit, mortality after hypersaline transfer, and weight loss 24 hours after transfer to sea water.

Osmotic Pressure

Osmotic pressure (OP) or osmolality in units of milliosmoles (mOsm) indicates the concentration of all dissolved ions and osmotically active particles and thus should be a good indicator of osmoregulatory performance. Both sodium and OP were measured in Capilano hatchery coho smolts (May, 10°C), in Rosewall Creek hatchery steelhead smolts (April, 10°C), and in four groups of laboratory reared coho parr (November, 6°C). The Capilano coho were tested in fresh water as well as after transfer, the other five groups only 24 h after transfer to SW. We used a Wescor 5100C vapour pressure osmometer which requires 8 µL of undiluted plasma collected as for the sodium test. There were no mortalities in any of these tests.

For each of the seven groups, Table 9 gives sodium and OP means, coefficient of variation, the correlation coefficient, and the probability that the correlation coefficient is not zero. The slope or intercept of each group differed when all seven groups were compared and also within the six SW groups, but the four groups of coho parr had the same regression of OP upon sodium. The average coefficient of variation for the seven groups is 3.3% (average n=20).

Magnesium

Magnesium is measured by atomic absorption spectroscopy after dilution of plasma (Foster 1974; APHA 1975; Pashen and Fuchs 1971). Magnesium measured in an air-acetylene flame using 0.2% lanthanum and 1.0% HCl diluent gives consistent results. Magnesium in sea water, urine, and gut fluids is about 50 times higher than in blood so there is much greater chance of contamination with magnesium than with sodium (sodium in sea water is about 2.5 times blood concentration). Because of this we rinse fish in fresh water and pat dry before cutting the tail. Standards and reference sera are treated the same as unknown samples. We measured magnesium, sodium, and potassium on three experimental groups (tanks A, B, and C) of yearling coho; on four dates (27 Jan, 12 Feb, 23 April, and 6 July); both in fresh water and after 24 h in sea water. (This is part of the same data set that produced the sodium curves of Fig. 1.)

Magnesium and sodium means, 't' test significance of challenge vs control means, ANOVA of date and FW/SW treatment effects, and average coefficient of variation (CV) are given in Table 10. Sodium shows low variation among FW control groups, SW means are significantly higher than FW controls in all but one case, the effect of date and SW are significant, and coefficient of variation is low. Mean magnesium values were fairly similar

among control groups but SW transfer group means were usually not different from FW controls. ANOVA showed the effect of SW/FW on magnesium was significant but the effect of date was not. The magnesium CV is much higher than for sodium. The correlation of magnesium and sodium was significant when SW challenge groups from all dates and tanks were pooled ($r=.51$, $p<.01$). To show how well each method can discriminate between two groups we calculated the sample size required to give 95% probability that the mean February and April values (all three tanks combined) are different ($p=.05$), (Sokal and Rohlf 1969, p 247). Using sodium, a sample of 7 is sufficient, but use of magnesium requires 62 fish.

Potassium

Potassium values are given at the same time as sodium on clinical flame photometers, so sample preparation is identical. Lysed samples or samples stored in collection tubes have high potassium and low sodium values. The time whole blood stands before centrifuging affects potassium values: tubes spun immediately have higher potassium than tubes which stand for 10-20 minutes before centrifuging.

Potassium was measured in the experiment described above for magnesium (Table 10). Potassium shows high variation between control groups, and in one case the controls are significantly higher than SW transfer fish. Although the treatment effects of date and water type are significant, the potassium CV is far higher than for sodium. Potassium and sodium were correlated in SW challenge groups ($r=.40$, $p<.01$). The sample size needed to find the difference between February and April potassium values (calculated as for sodium and magnesium) is only 4 fish, since the February - April difference in potassium was very high. This was due to the high potassium variation seen between groups, rather than to a change in seawater adaptability.

Hematocrit

The increase in osmolality following SW challenge may dehydrate the blood cells and reduce hematocrit. Hematocrit is easily measured on fish over 5 g by centrifuging blood and measuring the length of packed red cells relative to total blood. Hematocrit and sodium were measured in three groups of chinook in July. Fresh water temperature was 17°C, sea water was 14°C. The three group means were:

fresh water	43.6, 40.2, and 43.2 percent packed cells
24 h challenge	39.3, 36.6, and 39.4 percent packed cells.

Randomised block ANOVA showed seawater transfer to significantly affect hematocrit but when analysed for correlation of hematocrit and sodium, the slope, intercept, and correlation coefficient varied widely between groups, with no particular difference between FW and SW groups (data not shown). The correlation was significant in only one of the six groups. The hematocrit CV had an average value of 8.4% ($n=15$), higher than for sodium.

Mortality after Hypersaline Transfer

We challenged newly emerged chum (Fig. 2a) and pink (Fig. 2b) salmon fry using 50 fish per tank at different salinities for 96 hours at 5°C. Dead fish were removed twice daily for four days. Our trials with coho at various salinities (Fig. 2c,d,e,f) were done concurrently with the Salinity of Challenge Sea Water experiment described above. We first did our 24 h sodium test on 10 fish per tank, then the 96 h mortality test using 12 fish per tank.

As can be seen in Figure 2, the percent mortality changes quickly from 0 to 100% as salinity increases. The shape of the mortality curves are similar in all cases, with a shift to the right as seawater tolerance improves. Correlation of 96 h mortality upon our 24 h sodium method is not possible with this data since both methods cannot be applied to the same individual fish. Table 11 compares sodium after 24 h at 30°/$_{oo}$ with the salinity required to produce 50% mortality (from Fig. 2).

Weight Loss

Another method that we have tried is to weigh fish before and after a 24 h seawater challenge: the difference expressed as a percent of initial weight is mainly due to dehydration if the fish were not fed the day before. This requires individual holding tanks, or tags, or identifying individuals by length. We used the latter method in tests to see if weight loss and plasma sodium were related. Fish were anesthetised, measured, and weighed before and after transfer and then sodium samples were collected. On 12 September, 22 underyearling laboratory reared coho were tested and on 20 October, 12 fish each from laboratory coho, two stocks of Rosewall steelhead (2 and 3 ocean-year parents), and domesticated and wild rainbow trout stocks. The September test was at 15°C, the October tests at 13°C.

Table 12 gives correlation of percent weight loss and plasma sodium for two groups each of coho, steelhead, and rainbow trout. ANOVA of regression coefficients shows significant reduction of residuals due to grouping, so the 6 groups (and 3 species) have different regression lines. The coho are the most interesting species since they most closely approximated smolts. Seven of the October group were silvery-smolts and the remaining five looked like parr. The two groups of coho were not significantly different in slope but the sodium group means, after adjustment for weight loss, were different ($p<.005$). Whether the two coho groups were tested separately or together the correlation was highly significant. The coefficient of variation for weight loss is much higher than for sodium (Table 12).

DISCUSSION AND SUMMARY OF EXPERIMENTS

TRANSPORT STRESS

Specker and Schreck (1980) report that plasma cortisol in coho was high immediately after transport, much lower after 24 h, and had returned to resting levels in 4 days. In both our tests on chinook, the interaction of day and transport was significant, indicating that recovery time after transport is important. Our first experiment, on fish that were not SW tolerant, showed that travel stress decreased plasma sodium in FW controls, and increased sodium in SW challenge after 0 and 1 days recovery, but recovery time of 2 or 3 days eliminated the effect of travel. In contrast, the second experiment showed increased FW control values in transported fish, indicating no stress. The SW challenge results of experiment 2 also showed no sign of stress due to travel. The difference between the two experiments suggests that well-smolted fish are able to tolerate travel stress better than reverted smolts or parr.

FEEDING BEFORE TRANSFER

Davis and Shand (1978) measured blood sodium after 24 h SW challenge in sockeye at 11°C: fed groups were not different than similar groups starved for ten days. Our results show that up to six days starvation before transfer does not appear to influence results on coho parr. However it is advisable to adopt a consistent procedure. We do not feed the day before transfer, nor starve more than 3 days.

EFFECT OF TRANSFER TEMPERATURE

Our test on coho parr and rainbow trout shows plasma sodium is positively related to sea water temperature. Accordingly sea water temperatures should be close to the acclimation temperature.

TIME IN SEA WATER

We found sodium highest in coho 16 h after transfer at 15°C (Clarke and Blackburn 1977), and after 24 h at 10°C in this study. Otto (1968) tested underyearling coho (10°C, 30°/$_{oo}$) and found sodium at 32 h higher than at 16 or 64 h; 24 h was not tested by Otto. Davis and Shand (1978) examined sodium after 24 and 48 hour SW challenge of sockeye smolts (11°C, 28°/$_{oo}$); three of four groups showed no significant diffference between 24 and 48 hours

and in the fourth group 48 hour sodium was higher than at 24 hour. Iwata et al. (1982) tested chum (10°C, 33.5°/$_{oo}$) and found sodium highest after 3 hour (mainly fish smaller than 1.3 g) or after 12 h (usually 1.3 - 2.3 g fish) but always found 24 h sodium lower than 3 or 12 hours. Virtanen and Oikari (1984) reported highest sodium 48 h after transfer of Atlantic salmon at 10°C, but the 24 h value was almost as high; at 1.5°C however sodium at 48 h was higher than 24 h, and was higher still after 9 days. Hogstrand and Haux (1985) found plasma sodium in Salmo trutta was highest 24 hours after transfer to 25°/$_{oo}$ sea water. Generally the 24 h challenge period is best for detecting differences in short term sea water adaptation since sodium is then at or near the maximum, but a different period might apply in very cold or warm water, or at a considerably different salinity. The period appears to be less for chum salmon than for other species.

NETTING DISTURBANCE BEFORE CHALLENGE

Barton et al. (1980) show that rainbow trout recover from brief handling stress in 2 hours based on plasma cortisol. Our testing shows netting does not affect challenge sodium in chinook that were preadapted to 14°/$_{oo}$ sea water or in fresh water adapted coho. A small amount of netting during transfer to different tanks immediately before challenge will not strongly affect the results.

EFFECT OF STATIC AND ARTIFICIAL SW AND TANK VOLUME

Transfers into static, natural sea water can be compared to our regular 300 L/h flow conditions if temperature, oxygen, density and tank size are satisfactory. The two brands of artificial sea water that we tested were comparable to natural sea water if dissolved in warm water and allowed to stand a few hours before use.

While a maximum acceptable density is not obvious from our results, a density of 3.0 g/L seems fairly safe but tank volume should be at least 45 L with small fish. For yearling coho and steelhead an 80 L or larger bucket or tank is required. Cylindrical "pot" shaped tanks of 50 L capacity are stressful for 8 gram chinook at density of 4.5 g/L. The stress of high density may not be due to metabolite accumulation since static tanks with measurably higher ammonia do not cause higher blood sodium than tanks with flowing water. High density in a small tank probably causes a crowding stress to raise plasma sodium. The basket tests are consistent with this interpretation since water quality is equal for fish in baskets and those loose in the tank. Therefore we recommend that use of very small containers or high densities be avoided in the challenge test.

SALINITY OF SEA WATER

The salinity should be 29-30°/$_{oo}$ to be comparable with our results, but it can be adjusted to conform to the seawater adaptability of fish in particular applications.

BASKET vs LOOSE TRANSFERS

Rainbow trout (Barton et al. 1980) and coho (Redding and Schreck 1983) show severe stress during intense handling and confinement. All our tests show that 24 hours confinement in a small basket impaired sodium regulation in both sea water and fresh water. Small cages or baskets are unsuitable for this method.

CLOUDY EYE

Direction of sea water circulation determined on which side the cloudy eye occurred. Fish acclimated to our tanks and to the circulating water had no problem, while fish used to large ponds had cloudy eye. The cloudy eye is probably caused by rubbing against the tank wall while swimming against an unfamiliar current, possibly due to confusion and panic in a new, confining tank, in darkness for much of the time, while establishing a pecking order, and with stress of seawater challenge. Susceptibility to cloudy eye may change with season or development of fish because it appears to come and go in a stock of fish. Cloudy eye disappeared after a few days in fresh water. We have transferred many groups of coho from large tanks and raceways into our 200 L tanks without observing cloudy eye; just why these two stocks were predisposed to this problem is not known. Iwata et al. (1985) have observed corneal cataracts in yearling coho suffering osmoregulatory distress after transfer to 33°/$_{oo}$ sea water.

ANESTHETICS BEFORE BLEEDING

Short exposure of fish to MS222 immediately before blood collection does not significantly alter blood sodium (Soivio et al. 1977; Smit et al. 1979). The use of MS222 or 2PE anesthetic prior to bleeding in our seawater challenge test does not affect the results. Use of anesthetics before SW challenge is another matter, and like other stressors can change blood sodium (Hille 1982; Bouck and Johnson 1979).

SAMPLE STORAGE

Our results indicate that plasma for sodium analysis can be stored sealed in collection tubes for one week in a refrigerator or three weeks in a freezer. In a longer term experiment, Watanabe et al. (1978) found no change in sodium over four months at -15°C. Thawed samples need to be thoroughly stirred before use.

COMPARISON OF MICROPIPETS

For occasional use by beginners, pipets other than microcaps would be better, but if many analyses are to be done microcaps are best in terms of speed, repeatability, cost, and ability to use extremely small samples which have variable viscosity.

INDICATORS OTHER THAN SODIUM

The choice of indicators of seawater adaptability may be influenced by a number of factors including the analytical capability of the laboratory, the degree of precision required, and the number of fish available. Osmotic pressure is highly correlated with sodium and can be measured with comparable accuracy. Therefore, it can be substituted for sodium (see Grau et al. 1985), but to predict sodium the regression should be determined for the species. Plasma chloride should also be a good indicator of osmoregulatory performance but we have not tested it. On the other hand, magnesium ion is not very useful in our experience. Although magnesium levels are consistent in fresh water, there is little increase after seawater challenge. The correlation of magnesium with sodium levels in plasma is low and the variability is very much greater. Also, there is a risk of contamination since magnesium levels in sea water are about fifty times as high as those in plasma, and urine and gut contents also have very high levels in seawater fish (Shehadeh and Gordon 1969). In contrast to our findings, Hogstrand and Haux (1985) observed higher magnesium levels after seawater challenge of sea trout and suggested that it could be used as an indicator of smolting. Potassium ion is likewise not a useful indicator since potassium levels vary as widely among groups of coho in fresh water as between freshwater and seawater coho. Bath and Eddy (1979) found no changes in potassium after transfer of rainbow trout to sea water.

Hematocrit in rainbow trout decreased 8 h after exposure to sea water with return to normal after 24 h (Bath and Eddy 1979). We found a decrease in hematocrit of chinook salmon after 24 h seawater challenge. However, hematocrit is useless as a measure of smolting since it is variable in freshwater control groups and it correlates poorly with sodium. Since the change in hematocrit from FW to SW is small relative to the variance we expect this measurement will not separate groups of slightly different seawater tolerance without extensive replication.

Johnston and Saunders (1981) and Saunders and Henderson (1970, 1978) reported that challenging juvenile Atlantic salmon with 35-40°/₀₀ salinity was a useful way of distinguishing parr and smolts since only fully smolted fish are capable of surviving for 96 h. This technique is simple to perform but the resulting percentage survival value is not useful for statistical analysis. In order to determine the seasonal development of salinity tolerance and analyze the results statistically, it is necessary to perform a series of tests at different salinities (Finney 1952, page 17). Since mortality rises very steeply with increasing salinity, it is advisable to use steps of 1 or 2°/₀₀ in order to obtain at least 2 or 3 concentrations giving mortalities in the range between 2 and 98%. Unfortunately, this increases the required number of fish considerably. In our preliminary tests with juvenile coho, the salinity required to cause 50% mortality increased from 33°/₀₀ in early February to 41°/₀₀ in mid April. However, Varnavskiy and Varnavskaya (1984) reported considerable mortality of coho and sockeye salmon after challenge in 28°/₀₀ sea water. Their results are surprising, particularly since the surviving fish did not have as high a plasma sodium concentration as would be expected in groups suffering mortality.

The correlation of weight loss with sodium is generally significant, but there is no common regression for trout, steelhead, and coho. The two coho tests were similar but the intercept of the regression lines were significantly different suggesting that fish growth and development can produce changes in the percent dehydration. Evidently the regression equation should be evaluated for each application if sodium is to be predicted. Nevertheless the weight loss method shows promise since the slope of regression on sodium is similar in all cases tried over a range of fish sizes in different species, with a high correlation coefficient. The method is convenient since a balance is the only instrument required and the fish are not killed.

ACKNOWLEDGEMENTS

We are greatful for the assistance of Louise LeBrasseur, Doug Biffard, Geoff Haywood, Ian Carmicheal, Charles Lam, Bill McLean, Don Buxton, Hans Lundqvist and John Jensen.

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Table 1. Calibration for Ten Sodium Tests, mmol/L.

n	Mean	Std. dev.	S.E.	95% confidence interval
9	160.02	2.45	0.82	158.14 - 161.90
9	159.97	0.44	0.15	159.63 - 160.31
9	160.00	0.90	0.30	159.30 - 160.69
9	160.00	1.30	0.43	159.00 - 161.01
9	160.00	1.00	0.33	159.23 - 160.77
10	160.00	1.54	0.49	158.90 - 161.10
10	160.00	1.38	0.44	159.01 - 160.99
10	160.01	1.27	0.40	159.10 - 160.92
10	159.98	1.72	0.54	158.75 - 161.21
10	159.98	0.99	0.31	159.27 - 160.69

Table 2. Sodium (mmol/L) and Transport of chinook salmon.

Experiment 1, fresh water controls

Days recovery	No-travel (A)		Travel (B)		Mean	Difference (B-A)
	n	sodium	n	sodium	sodium	
0	8	156.1	8	152.1	154.1[a]	- 4.0
1	8	155.8	8	153.2	154.5[a]	- 2.6
2	8	152.6	8	150.7	151.6[a]	- 1.9
3	8	153.7	6	152.3	153.1[a]	- 1.4
7	6	153.3	6	152.3	152.8[a]	- 1.0
Mean		154.4[a]		152.1[b]		

Experiment 1, seawater challenge

Days recovery	No-travel (A)		Travel (B)		Mean	Difference (B-A)
	n	sodium	n	sodium	sodium	
0	15	190.4	12	203.4	196.2[a]	+13.0
1	15	188.4	16	192.7	190.7[a]	+ 4.3
2	15	195.1	15	195.2	195.1[a]	+ 0.1
3	16	195.4	15	193.3	194.4[a]	- 2.1
7	12	195.0	12	192.8	193.9[a]	- 2.2
Mean		192.8[b]		195.2[b]		

Table 2 (cont'd)

Experiment 2, freshwater control

Days recovery	No-travel (A)		Travel (B)		Mean	Difference (B-A)
	n	sodium	n	sodium	sodium	
0	8	153.2	8	154.0	153.6[a]	+ 0.8
1	8	152.1	8	154.8	153.4[a]	+ 2.7
2	8	154.7	8	153.1	153.9[a]	- 1.6
3	8	152.7	8	156.1	154.4[a]	+ 3.4
6	12	149.1	11	150.2	149.7[b]	+ 1.1
Mean		152.1[a]		153.4[b]		

Experiment 2, seawater challenge

Days recovery	No-travel (A)		Travel (B)		Mean	Difference (B-A)
	n	sodium	n	sodium	sodium	
0	11	171.0	12	164.7	167.7[a]	- 6.3
1	12	166.7	12	164.4	165.5[a]	- 2.3
2	12	159.2	12	162.5	160.9[b]	+ 3.3
3	12	156.2	12	160.5	158.4[b]	+ 4.3
6	13	160.8	15	159.1	159.9[b]	- 1.7
Mean		162.6[a]		162.1[a]		

(Means with different superscripts are different by Tukey's studentized range test (Steel and Torrie 1960, p. 114). Columns and rows analysed separately.)

Table 3. Effect of seawater temperature, fresh water 15°C.

SW temp	n	Sodium, mmol/L Mean	S.E.	Differences
10.0	35	186.2	2.1	(significant difference from 20.0°C)
12.5	37	190.6	2.1	(significant difference from 20.0°C)
15.0	34	191.7	2.1	
17.5	33	193.8	2.2	
20.0	30	199.4	2.5	

Table 4. Netting disturbance before SW transfer and plasma sodium, mmol/L.

Treatment	Chinook n	mean	S.E.	Coho rep 1 n	mean	S.E.	Coho rep 2 n	mean	S.E.
No disturbance	12	160.8	1.0	24	171.4	0.9	24	174.6	1.3
Net and return	12	157.1	2.0	24	173.4	1.0	24	173.8	1.5
Net and moved				24	174.8	1.1	24	175.6	1.3

(Chinook preadapted to 14°/$_{oo}$ SW, coho accclimated to FW, no significant differences.)

Table 5. Effect of static and artificial SW and tank volume.

Date	Flow (L/h)	Sea water type (°/₀₀)		Vol (L)	Density (g/L)	n	Sodium Mean (mmol/L)	S.E.	Morts (%)	Total ammonia (ppm)
14	0	Rila	30	20	6.6	7	189.3	7.2	0	
Mar	0	Rila	30	45	2.6	7	169.5	5.1	0	
	0	natural	29	20	5.0	7	194.5	7.9	0	
	0	natural	29	45	2.9	7	176.8	5.9	0	
25	0	natural	29	15	1.3	12	220.1	7.0	0	
Mar	0	NaCl	29	15	1.4	12	-	-	100	
	0	Hagen	29	15	1.4	12	209.7	2.9	0	
2	300	natural	29	200	1.1	12	170.9	1.3	0	0.00
May	300	natural	29	53	4.2	12	178.1	2.7	0	0.02
	300	natural	29	23	10.3	12	177.5	3.1	0	0.10
	0	natural	29	200	1.2	12	171.2	2.0	0	0.24
	0	natural	29	53	5.1	12	173.9	2.4	0	0.87
	0	natural	29	23	10.1	12	179.1	3.4	18	1.42
20	0	natural	27	137	3.4	16	167.9	3.2	0	
May	300	natural	29	200	2.2	12	160.9	4.7	0	
10	240	natural	29	50	4.5	10	187.1	2.2	0	
Jun	240	natural	29	200	0.9	10	165.5	1.7	0	

14 March, steelhead, 8°C, volume effect significant, SW type effect not significant.

25 March, chinook, 10°C, SW type effect not significant, salt (NaCl) toxic.

2 May, coho, 10°C, flow effect not significant, volume effect significant.

20 May, coho, 11.5°C, flow effect not significant.

10 June, chinook, 14°C, volume effect significant.

Table 6. Effect of storage of fish plasma upon sodium and potassium.

Ion	Temp.	Fresh Mean	S.E.	4 day Mean	S.E.		8 day Mean	S.E.		21 day Mean	S.E.	
Na	5 C	150.88	.33	151.20	.45	ns	151.66	.80	ns	154.53	.61	**
Na	-25 C	152.32	.86	150.83	.42	ns	151.00	.86	ns	151.83	.61	ns
K	5 C	1.80	.36	2.13	.34	**	2.00	.30	ns	2.07	.34	*
K	-25 C	1.82	.34	2.10	.33	**	2.17	.33	**	2.02	.38	**

(Both ions in mmol/L, n=6, difference from fresh by Dunnett's test; $p<.01$, $p<.05$ and $p>.05$ shown as **, * and ns respectively.)

Table 7. The effect of hemolysis on sodium, potassium and osmolality (OP).

Fish no.	Treat-ment	No. reps	Plasma colour	Hemat-ocrit (%)	Lysis (%)	Sodium (mmol/L)	Potassium (mmol/L)	OP (mOsm)
1	none	3	no colour	27	0	162	4.7	332
1	froze 1X	3	orange	8	70	139	23.0	334
1	froze 3X	3	dark red	4	85	133	26.2	335
1	froze 6X	3	dark red	3	89	132	26.3	335
1	warmed	2	no colour	27	0	160	5.3	334
2	none	3	no colour	21	0	167	5.7	346
2	froze 5 s	3	yellow	21	0	165	6.7	346
2	froze 10 s	3	orange	12	43	147	19.3	345
2	froze 20 s	3	red	8	62	145	22.1	346
2	1/3 frozen	2	orange	14	33	154	14.3	346

(Lysis = % reduction in hematocrit; warmed tubes held 1 min at 40°C.)

Table 8. Variability of different micropipets.

Volume µL	Brand	Experience on microcap	Photo-meter	Replicates n	Coefficient of variation
10	microcap	yes	Jarrell-Ash	10	3.6
10	Wiretrol		Jarrell-Ash	10	3.0
2	Microcap	yes	Jarrell-Ash	10	3.3
2	Oxford		Jarrell-Ash	10	3.9
5	microcap	no	Turner	20	2.3
5	microcap	yes	Turner	20	0.9
5	Labindustries		Turner	20	2.4
5	SMI		Turner	20	2.0
5	Eppendorf		Turner	10	1.7
10	Cordis		Turner	20	2.0

Table 9. Osmolality (mOsm) and sodium (mmol/L), sodium = A*(OP) + B.

Group	n fish	Sodium Mean	Sodium CV	osmolality Mean	osmolality CV	Slope A	Intcpt. B	Corr. coef.	P of correl.
0+ coho	22	171.6	2.4	355.5	2.4	.27	75.6	.57	**
0+ coho	24	174.8	3.1	363.8	3.0	.43	19.5	.84	**
0+ coho	24	175.6	3.7	372.3	3.7	.39	29.3	.83	**
0+ coho	23	173.9	4.2	365.4	4.8	.36	41.9	.87	**
steelhead	24	172.4	4.0	355.9	4.9	.37	39.2	.94	**
1+ coho	15	177.7	2.7	353.9	2.8	.42	29.5	.86	**
1+ coho fw	10	158.1	1.4	323.3	1.2	.48	2.4	.81	**

(All but the fw group had 24 hour transfer to sea water, ** = $p<.01$.)

Table 10. Sodium, potassium, and magnesium means, with difference ('t' test) between FW and SW, effects (ANOVA) of date (Jan, Feb, Mar, July) and treatment (FW or SW), and average coefficient of variation for three tanks.

Tank	Date/ effect	Sodium FW	Sodium SW		Potassium FW	Potassium SW		Magnesium FW	Magnesium SW	
A	Jan	158	177	**	4.5	6.3	*	1.00	1.21	ns
A	Feb	155	172	**	4.2	7.9	**	.96	1.40	**
A	April	161	164	ns	2.4	2.8	ns	1.10	1.24	ns
A	July	158	176	**	5.1	5.8	ns	1.02	1.17	ns
A	FW/SW effect			**			**			**
A	date effect			**			**			ns
A	average CV			1.9			25.8			18.0
B	Jan	158	186	**	4.4	5.8	ns	.99	1.74	**
B	Feb	158	168	**	2.0	8.6	**	1.10	1.42	ns
B	April	156	166	**	5.1	2.1	**	1.12	1.34	ns
B	July	158	180	**	5.0	4.5	ns	1.04	1.61	*
B	FW/SW effect			**			**			**
B	date effect			**			**			ns
B	average CV			2.8			31.5			26.8
C	Jan	157	185	**	4.2	7.5	**	1.14	1.50	ns
C	Feb	157	181	**	2.8	9.7	**	.99	1.43	ns
C	April	160	165	*	5.6	4.1	ns	1.28	1.08	ns
C	July	160	171	**	6.8	5.9	ns	1.00	1.13	ns
C	FW/SW effect			**			**			*
C	date effect			**			**			ns
C	average CV			2.2			23.4			23.5

(All ions are in mmol/L; n=12/cell; n=96/tank; yearling coho; coefficient of variation calculated for each cell then averaged; $p<.01$, $p<.05$ and $p>.05$ shown as **, * and ns respectively.)

Table 11. Plasma sodium 24 h after transfer to 30°/₀₀ SW and salinity producing 50 percent mortality in 96 h (from Fig. 2), with date and wet weight.

Stock	Date	Weight (g)	Sodium (mmol/L)	Salinity (°/₀₀)
Chum fry	29 Jan.	0.28	---	39
Pink fry	23 Jan.	0.20	---	41
Coho 1+	5 Feb.	16.0	186	33
Coho 1+	27 Feb.	20.6	165	37
Coho 1+	18 April	31.0	165	41
Coho 1+	16 May	39.1	168	41

Table 12. Weight loss and Sodium; Sodium = A * (wt loss) + B.

Group	n trans	n dead	Avg wt. (g)	Wt loss Mean (%)	Wt loss CV	Sodium mean (mmol/L)	Sodium CV	Slope A	Inter-cept B	Corr. coef. r	p
Coho Sep	21	0	24.9	6.8	34	182	6.5	4.51	151.7	.88	**
Coho Oct	12	0	48.4	6.4	42	171	7.6	4.19	144.7	.87	**
all coho	33	0	33.0	6.7	37	178	7.4	4.54	148.1	.84	**
3x3 sthd	14	2	9.7	14.8	25	216	8.0	3.08	170.0	.67	*
2x2 sthd	14	2	9.7	13.5	23	208	8.4	4.63	145.9	.81	**
all sthd	28	4	9.7	14.2	24	212	8.2	3.80	157.8	.74	**
dom. RBT	12	0	16.7	10.9	36	213	7.8	3.37	176.3	.79	**
wild RBT	14	7	3.8	18.8	13	231	6.3	2.96	174.6	.49	ns
all RBT	26	7	10.2	14.3	36	220	8.1	2.62	182.9	.77	**
All	87	11	18.7	11.0	47	200	12.3	4.15	154.1	.88	**

($p<.01$, $p<.05$ and $p>.05$ shown as **, * and ns respectively.)

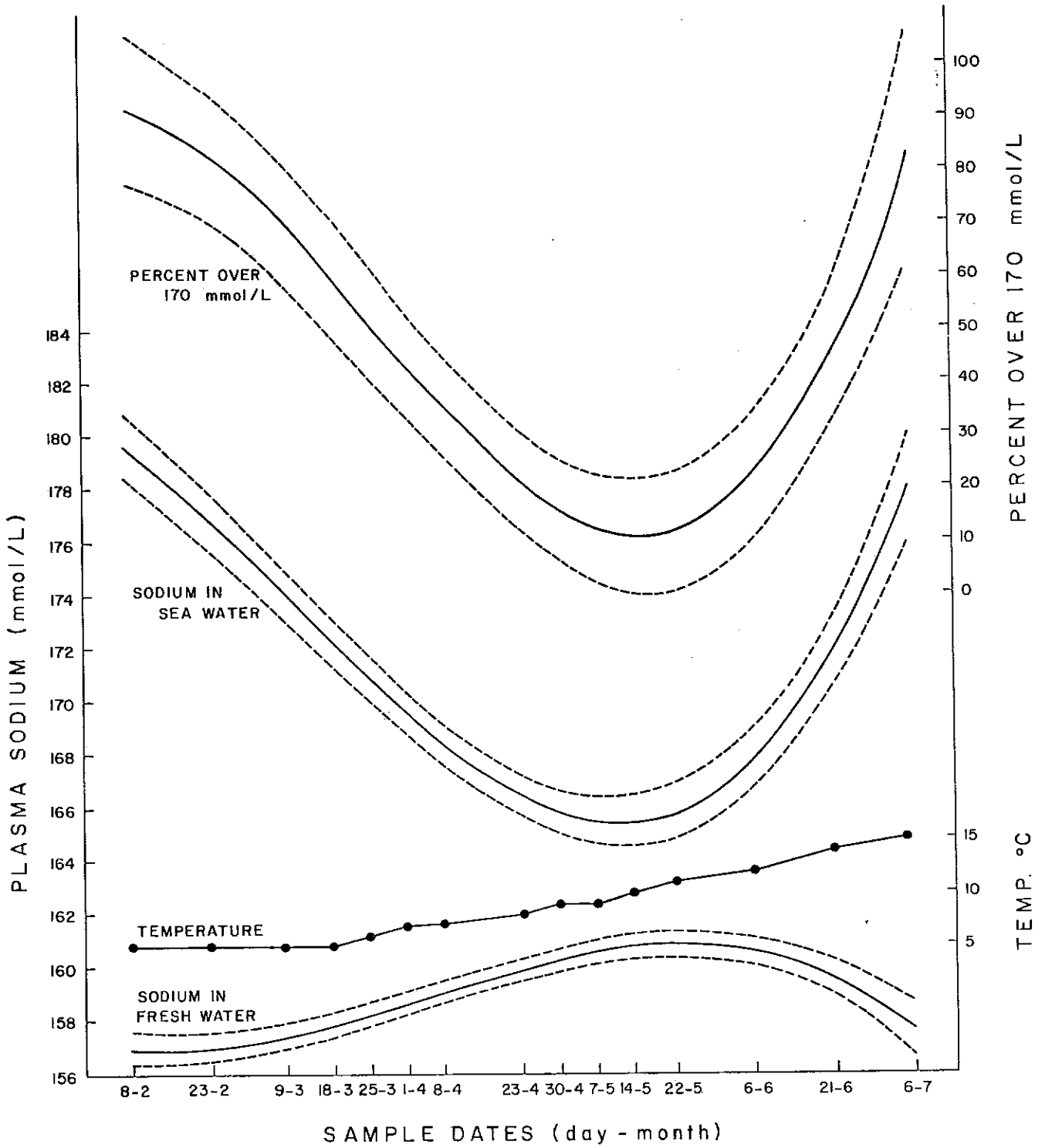

Fig. 1. Seasonal development of sodium after seawater challenge, freshwater control sodium, percent over 170 mmol/L sodium (each fitted to a cubic polynomial with 95% confidence limits of the line) and temperature in yearling coho.

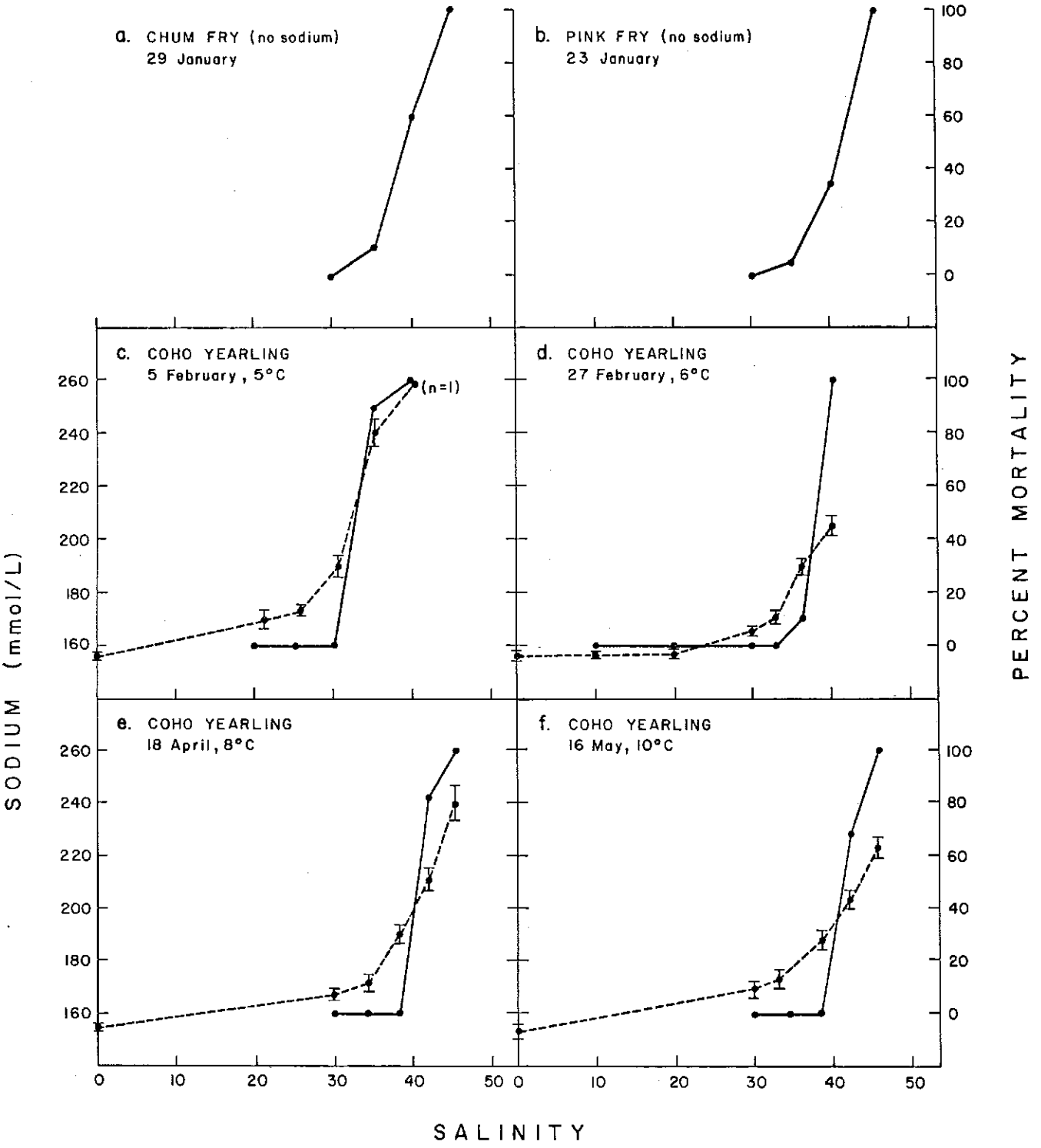

Fig. 2. Relation of 24 hour plasma sodium (--Φ--) mean ± 1 s.e., and 96 hour mortality (—●—) to transfer salinity.