# COMPARISON OF ELECTROLYTE EXCRETION IN SMOLTING AND NONSMOLTING TROUT (SALMO GAIRDNERI)

by

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We accept this thesis as conforming to the required standard

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#### ABSTRACT

Urine was collected from the trout (Salmo gairdneri) using a cannulation technique. Total urine and electrolyte outputs decreased in smolts as compared with fresh water parr. The filtration rates, as measured using the inulin method, were concomitantly decreased in the smolts. No significant change of urine concentration, percent reabsorption or percent secretion of filtrate was recorded. There was a linear relationship between cumulative output and time indicating a steady flow rate.

An increase in sodium and chloride content in the muscle was recorded during smolt transformation. Potassium and water content remained the same as in non-smolts. In the above group, the plasma sodium concentration increased.

The decrease in renal output without dilution of body fluids is discussed with respect to a possible change in the extrarenal ion exchange mechanism.

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#### INTRODUCTION

Many salmonid fishes such as Salmo salar and Salmo gairdneri are anadromous. The fish hatch in fresh water, migrate to the sea before maturity, then return to fresh water and spawn. Immediately before and during migration to the sea, these salmonid fish undergo certain external morphological changes. Guanine is deposited on the ventral and ventro-lateral surfaces, giving the fish a silvery appearance and there is an alteration in body proportion: the weight-length relationship changing (Hoar, 1939). The structures deposited in the smolting fish have been identified as guanine both by chemical and crystallographic analysis (Hitchings and Falco, 1944; Sumner, 1944 and Neckel, 1954). The deposition of guanine was thought to be due to the increased blood thyroxine levels since it was found that silvering could be experimentally produced by the injection of thyroxine (Landgrebe, 1941; Robertson, 1949). The level of thyroxine in the blood was determined indirectly by observation of increased iodine uptake by the thyroid and by increased height of the thyroid epithelial cells during smolting (Baggerman, 1960). It has since been suggested, however, that the increased epithelial height of the thyroid may be due to endemic goitre resulting from lack of available iodine (Hickman, 1959; Eales, 1963). hypothesis is supported by the observation that the increased thyroid activity observed at smolting can be reduced by increasing the available iodine. Hickman (1959)

showed in the starry flounder, <u>Platichthys</u> stellatus, that if the environmental iodine was adjusted to similar concentrations, the demand for iodine was greater in salt water than in fresh water. The goitre shown in smolts indicates an increase in the demand for iodine may occur.

The reduced fat and increased protein content of the tissues during the smoltification of salmonids may be responsible for the change in body proportions observed at this time. The type of protein stored during smoltification contains increased amounts of cystine, tyrosine and arginine (Malikova, 1959).

Also during smolting an increased level of plasma non-protein nitrogen has been reported (Stewart, 1964). This would indicate an increased protein catabolism and the amino acids could then be built up into proteins of a different composition.

Increasing photoperiod has been found to affect the salinity preference in four species of Oncorhynchus (Baggerman, 1960). Fish which were kept on a short photoperiod retained their preference for fresh water, as do the parr, but those exposed to longer days developed a preference for salt water, as do the smolts. The fish kept at a short photoperiod did not show an increased thyroid activity but nevertheless developed a silver appearance (Eales, 1963). The role of thyroxine in inducing silvering, therefore, is questionable. Fish exposed to a short photoperiod showed no change in salinity preference during the experimental period from March to the beginning of June. This suggests that a short

photoperiod may delay or completely inhibit their salinity preference (Baggerman, 1960).

Along with the change in salinity preference at smolting, Salmo salar showed an increased ability to adjust to salt water (Houston, 1959; Parry, 1960). These smolts took only four hours to adjust their blood electrolyte concentration to the normal level of a marine fish after abrupt transfer to sea water. S. salar parr have been shown to be unable to adapt after similar transfer (Huntsman and Hoar, 1939) and always succumb during the initial phase of rising plasma electrolyte concentrations (Gordon, 1959; Koch, 1959).

A rise in adrenocortical steroid levels was found in smolting Salmo salar by Fontaine and Hatey (1954). These hormones have been found to increase the rate of salt excretion by the gills in salt loaded fresh water fish (Holmes, 1959) and to decrease plasma sodium levels in the fresh water non-smolting Salmo gairdneri (Holmes and Butler, 1963), non-smolting Salmo salar (Olivereau, 1962) and non-smolting S. trutta (Chester-Jones and Spalding, 1958). Olivereau also observed that the adrenal corticoids accelerated the adaptation of Salmo gairdneri to salt water.

Somatotrophic hormone occurs at a higher concentration in the plasma of smolts than in non-smolts. Fontaine, 1960 suggested that this increased level may be the cause of the decrease of the intra-cellular/extracellular K ratio.

The above changes in electrolyte regulatory patterns

might involve an alteration in renal and extrarenal output. During smolting S. gairdneri shows a predisposition toward adaptation to sea water. As there is an extreme change in the renal excretory pattern in fresh water fish as compared to sea water fish, it was thought a modification in renal excretory pattern may precede migration into seawater. Therefore the renal excretion was compared in smolting and non-smolting trout to determine if such a change occurred.

### MATERIALS AND METHODS

Hatchery raised <u>Salmo</u> gairdneri were maintained in running dechlorinated water at seasonal temperatures and photoperiods.

Experimental fish were starved for one week prior to anaesthesia with MS222 (tricainmethane sulfonate). fish were originally put under the anaesthetic in a 1:5000 concentration, then placed in the cooled operating tank with MS222 at a 1:1900 concentration. A polythene cannula (Band D PE60) was inserted into the urogenital papilla and secured by two ligatures. The first ligature was fastened around the papilla and a pursestring ligature passed through the body wall anterior to the papilla and tied around behind the papilla, holding the cannula in place. After cannulation the fish were placed in individual tanks supplied with running dechlorinated water and the catheter was passed through a hole in the rear of the tank and attached to a collecting cylinder (Fig. la and b). At autopsy, terminal blood samples were taken by severing the tail and immediately centrifuged at 8000 rpm at 5°C for 10 minutes.

# <u>Determination</u> of glomerular filtration rates (G. F. R.)

Glomerular filtration rates were determined in separate groups of fish using the inulin technique (Schriener, 1950). It is probable that inulin is freely filtrable in the glomerular trout, Salmo gairdneri (Shannon, 1934; McBean, 1963).

A single intra-peritoneal dose of 25 mg. inulin in .78 percent saline was administered to each fish and urine collection was started after 4 hours. At this time the blood inulin concentrations were constant and remained so during the remainder of the urine collection period. Terminal blood samples were collected into heparinized tubes and immediately centrifuged at 8000 rpm at 5°C for 10 minutes. Plasma protein was precipitated with cadmium sulphate (Fukita and Twatake, 1931, as modified by Smith et al., 1945). Plasma samples of 0.5 ml. and urine samples of 1.0 ml. were analyzed for inulin according to the Schriener (1950) modification of the direct resorcinal method of Roe, Epstein and Goldstein (1949).

# Electrolytes in the urine and plasma

The fish were left for one hour after cannulation before collection. Urine was collected from intact and .78 percent saline sham injected fish for at least 20 hours. Urine and terminal plasma sodium and potassium concentrations were determined using the Zeiss flame photometer (PF5). Chloride concentrations were measured by titration with silver ions, using a Cotlove automatic titrator (Aminco). Total osmolarity in both plasma and urine was determined using a Fiske osmometer.

All electrolyte concentrations were expressed in m.eq. or mosm./l. and the excretion of water and electrolytes were expressed in ml., mosm. or m.eq./kg. body wt./day. The percent reabsorption or secretion of water and ions was

determined for each fish (Smith, 1956).

## Muscle electrolytes

Muscle samples of approximately one gram were taken from the region below the dorsal fin and weighed accurately. Blood samples were taken from the same fish and analyzed for electrolytes as described above.

Samples for determination of sodium and potassium were digested for 48 hours in 4-5 ml.s of concentrated  $\rm H_2SO_4$  with 1 ml.  $\rm H_2O_2$ . The resultant solution was neutralized with concentrated NH<sub>4</sub>OH, using phenol red as an indicator, and diluted to 50 ml. in a volumetric flask. Concentrations were determined by flame photometry.

Samples for determination of chloride were digested in dilute NaOH at 100 C for 45 minutes and protein precipitated with ZnSO<sub>4</sub>. The method used was from Cotlove (1963) for 5-15 g chloride. Chloride concentration was determined using the Cotlove automatic titrator.

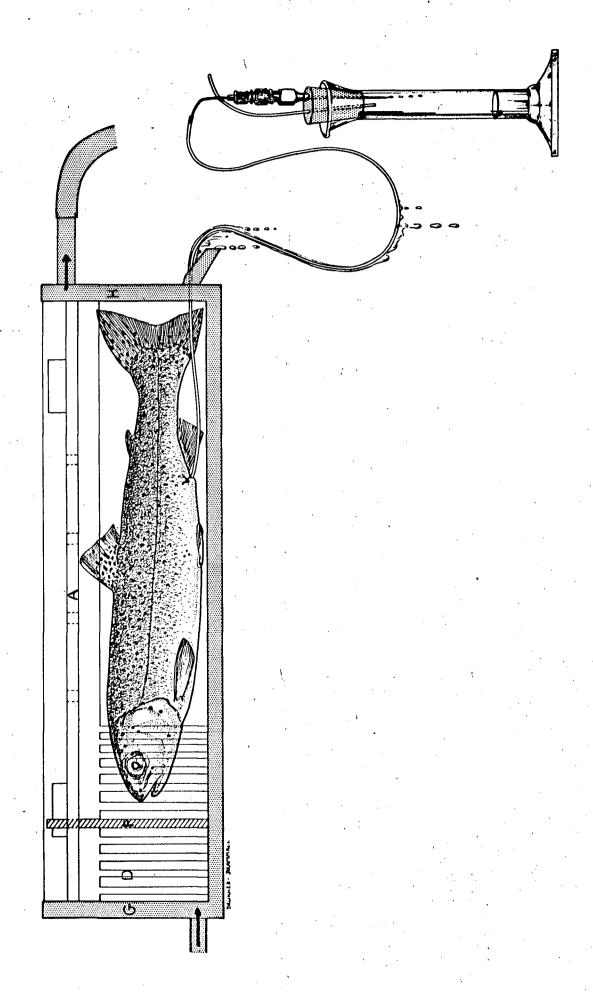
All concentrations of muscle Na, K and Cl were expressed in m.eq./kg. wet weight and m.eq./kg.  $\rm H_2O$ .

Muscle samples were weighed, then dried in an oven at approximately 108°C until constant weight was reached. The dried samples were weighed and the percent water calculated.

Statistical analysis of covariance and calculation of standard errors were according to the methods of Snedecor (1956).

# LEGEND FOR FIGURE 1a

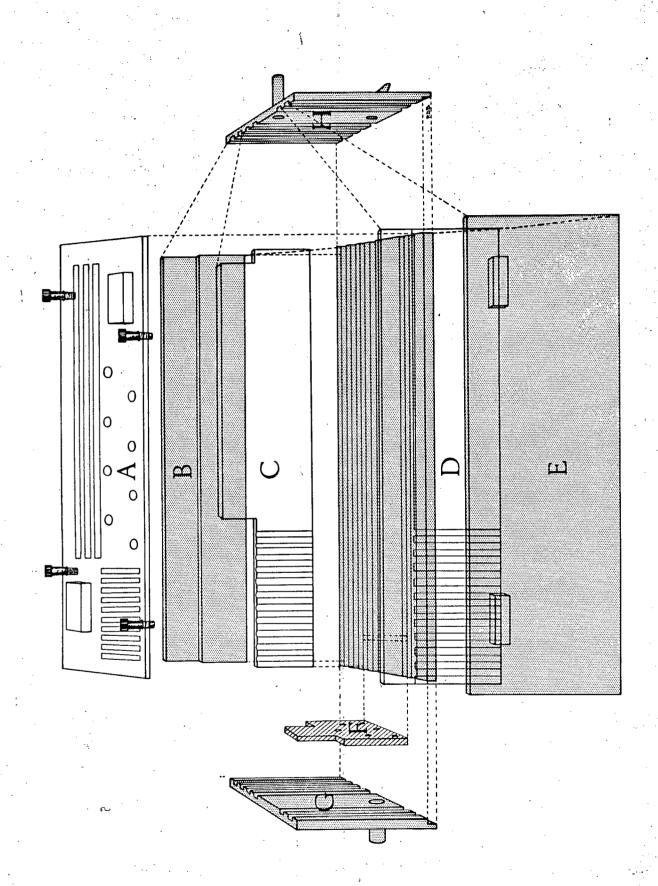
Diagram of experimental set up for urine collection



LEGEND FOR FIGURE 1b

Exploded diagram of apparatus for holding fish during urine collection

(Compare with figure 1a)



#### RESULTS

In both non-smolting and smolting trout, the rate of urine excretion was linear with respect to time over the experimental period of these studies (Fig. 2, Table I).

## Electrolytes in the urine and plasma

There were no significant differences in water or ion excretion between intact and saline sham injected fish within smolts or non-smolts. Saline sham injected fish were used as controls for the inulin injected fish.

The excretion of water in ml./kg. body wt./day by smolts was significantly lower than that of the non-smolting fish (P<.001). The potassium, sodium and total osmolar outputs were significantly lower (P<.001) in smolts than in the non-smolts (Tables II and III). Although total excretion of chloride in smolts was lower than that of non-smolts the difference was not significant. The percent reabsorption of filtrate remained the same in both smolts and non-smolts (Table IV). The potassium secretion was found to be extremely high (90%) in contrast to that of mammals (10%). Osmolarity of the urine was higher than that of non-smolts although this was not significant. Slightly increased chloride, potassium and sodium concentrations of urine were also found in smolts (Tables V and VI).

There is a significant negative correlation between osmolar concentration of urine and water output in smolts, indicating a decrease in ion concentration as the total volume

of urine excreted increases (Table VIIa). This trend can be best observed in smolts whose urine outputs vary over a wider range than those of non-smolts. The total osmolar output/kg. body wt./day, however, increases with increasing urine volume (Table VIIb). Regression lines calculated for water output against mosm. output/kg./day showed a positive correlation.

There was no change in plasma electrolyte concentrations between smolts and non-smolts in the above group.

# Glomerular filtration rates

The smolts had a significantly lower glomerular filtration rate than did the non-smolts (P<.001). As there was no change in the concentration of electrolytes in the plasma this resulted in a significantly decreased filtration rate of all ions except for potassium (Table VIII). As the concentration of plasma K varied greatly between different fish, the large standard error would probably mask any difference between smolts and non-smolts.

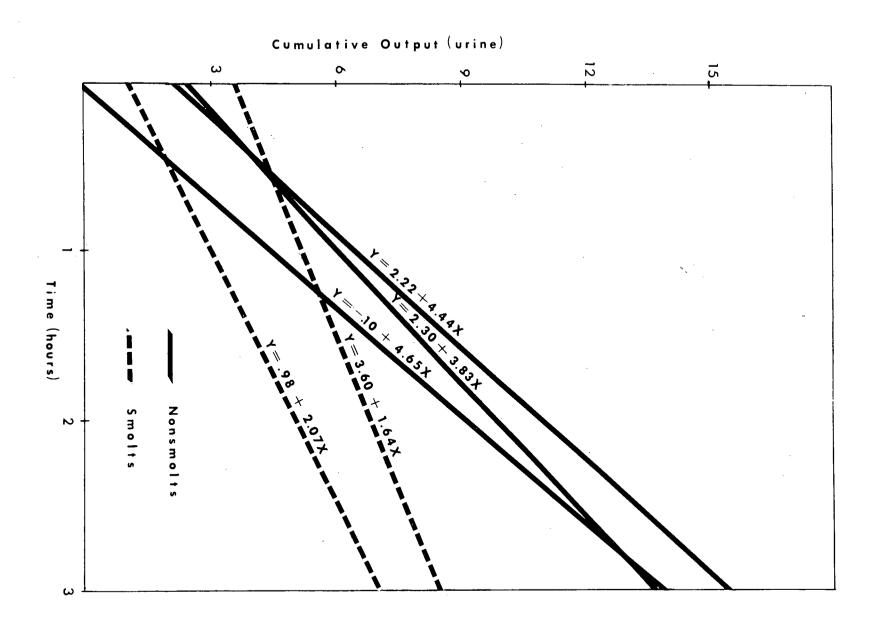
# Muscle Electrolytes

The concentration of ions in the muscle of smolts was found to increase over that of non-smolts (Table IXa). The chloride and sodium values in both m.eq./kg. wet wt. and m.eq./kg. tissue water were found to be significantly higher in smolts than in non-smolts (P < .01). The potassium concentration did not change. The plasma electrolyte concentration remained the same except for the sodium level which was higher (Table IXb).

This rise in sodium level was not found in the earlier smolts which were used for urine electrolyte determinations.

## LEGEND FOR FIGURE 2

Graph of cumulative output (ml./kg.) against time (hours) showing regression lines for each group of experimental animals as shown in Table I.



#### DISCUSSION

The smolting salmonid undergoes a number of physiological changes which increases its ability to adapt to a saltwater environment (Houston, 1959; Parry, 1960). The freshwater fish has a different osmotic problem from that of the saltwater fish. As its internal concentration is greater than that of the external environment, it is continually in danger of being flooded with water. To rid itself of the excess water it excretes copious amounts of dilute urine. In Salmo gairdneri the total urine output was found to be between 90-115 ml./kg./day. This value agrees with that found by Fromm (1963) and is slightly higher than values given by R. M. Holmes (1961), both using a cannulation technique. As some electrolytes are lost in the urine, the fish actively transports ions in through the gills to maintain a constant milieu interior (Krogh, 1939).

In the saltwater fish the active transport mechanism in the gills is reversed, the animal actively excreting ions (Smith, 1930). The saltwater animal loses water to the hypertonic external environment. To compensate for this continual loss, the fish swallows seawater, with sodium and chloride selectively taken up from the gut. In the eel, the posterior intestine is the main site of water reabsorption and is thought to be linked with the active transport of sodium (Sharratt, Bellamy, Chester-Jones, 1964). In the teleost Cottus scorpius the active transport of Sodium and water uptake was halted by addition of Potassium cyanide,

indicating that this uptake was dependent on tissue metabolism (House, 1963). Calcium and magnesium are mainly excreted through the intestine (Smith, 1930). The urine output in the saltwater fish is decreased to approximately 1 ml./kg./day with a corresponding decrease in glomerular filtration rate (G. F. R.).

In this study a decrease in urine output has been found in the smolt as compared with that of the fresh water parr. The decrease in urine output in the smolt was found to be completely due to a fall in the G. F. R. from the non-smolt value, with a resultant decrease in the renal filtration of ions, the plasma electrolyte concentrations remaining unchanged.

The apparent dependence of urine output on G. F. R. in fish varies from the mammalian mode of urine volume regulation. In man the urine output may vary over a wide range without a change in G. F. R. In this case the volume excreted is regulated by the action of antidiuretic hormone (A. D. H.) on the permeability of the distal collecting tubules of the kidney (Smith, 1955). In trout, mammalian A. D. H. does not appear to have any antidiuretic action. However, in the goldfish Carassius auratus, injection of neurohypophyseal hormone resulted in diuresis which was found to be completely due to an increase in G. F. R. (Maetz, 1963). Maetz suggested that this increase in G. F. R. was due to an increase in the number of active glomeruli. Richards (1924) first introduced the concept of active glomeruli in lower vertebrates when he observed a change in the number of active glomeruli in a

pithed frog when various substances were added, such as NaCl and adrenalin.

Variation in the number of active glomeruli have been suggested in fish. In the sculpin Myoxocephalus scorpius a recruitment of active glomeruli as determined by G. F. R. measurement followed laboratory diuresis (Forster, 1953).

Decrease in urine output was found in smolts in fresh This change would result in dilution of body fluids if water intake remained constant. It therefore appears that since neither plasma nor muscle electrolyte concentrations decreased there must be a compensatory permeability change regulating water uptake. Holmes (1959) showed that there was no significant difference between sodium loss from intact and greased fish indicating that the skin was relatively impermeable to ions. This leaves the gills or the gut as the site of water uptake. However, Smith (1930) demonstrated using phenol red that little or no water was swallowed by the eel Anguilla in fresh water. The gills, therefore, appear to be the major site of water uptake as well as of electrolyte uptake or secretion, as stated by earlier workers (Krogh, 1939; Smith, 1929), although some loss through the skin has been recorded (Krogh, 1937). Alteration of the extrarenal mechanism seems probable, especially as this mechanism is thought to change between the fresh and saltwater adapted trout (Holmes, 1959). Bellamy (1961) demonstrated in the isolated gills of a silver eel, that there is a reversal in the direction of active transport of sodium, depending on whether the animal

is in fresh or salt water. This is presumed to occur in other migrating forms, such as the trout. Houston and Threadgold (1963) observed a decrease in plasma chloride at the beginning of smolting and suggested that this was due to a premigratory activation and secondary inhibition of the extrarenal excretory mechanism. This change in plasma chloride was not found in the fish used in this study.

The change in extrarenal and renal mechanisms that occur in the smolt and the seawater adapted animal as compared to the freshwater parr, appear to be physiological rather than morphological. Motais (1961), measured the rate of 24Na exchange in the flounder Platichthys flesus. He found the animal, when transferred to seawater, took over 30 hours to adjust its extrarenal mechanism to that of a seawater adapted However, the saltwater adapted fish took under an hour to change its extrarenal mechanism back to normal after transfer to freshwater. In the smolting trout, an increase in adrenal steroids was observed (Chester-Jones, Phillips, 1962; Fontaine, 1956). These hormones have been found to decrease internal electrolyte concentrations in a saline loaded freshwater parr by increasing the net sodium output by the gills. A similar regulation may occur in the smolt. decrease in G. F. R. that occurs when a trout is transferred to seawater has been found to be a reversible process, the G. F. R. returning to the original freshwater value when the saltwater adapted fish is returned to freshwater (Holmes and McBean, 1963). In the smolting Salmo gairdneri, the G. F. R.

fell to approximately half the parr value. When these smolts remained in freshwater, however, they slowly returned to the parr condition with a concurrent return to the previously recorded urine output and filtration rate. This indicates that the excretory changes studied are reversible which would not be expected if any drastic morphological changes occurred.

In the fish used in this experiment, no significant difference was found in concentrations of electrolytes in the urine of smolts as compared to those of non-smolts. However, there was a negative correlation between the concentration of electrolytes and the total water excretion when calculating a regression line of water output against electrolyte concentration in individual fish. There was a positive correlation between water output and total electrolyte output. This indicates that although there was a decrease in electrolyte concentrations as the water output was increased this was not enough to prevent the total electrolyte output from being greater at the higher urine volumes.

This relationship was also found by Bentley, (1963) in the migrating silver eel. He suggested that the decreased concentration of ions with increased urine output indicated a change in the tubular reabsorption of these ions. No change, however, was found in the percent reabsorption of ions in the smolting Salmo gairdneri.

In nonaquatic animals such as the duck (Holmes, Phillips, and Butler, 1961), water loading results in a greatly increased urine output a short time after loading, with decreasing amounts

excreted with increase in length of time after loading. This results in a nonlinear relationship between cumulative output and time in hours of collection.

The fresh water fish is being continually water loaded so the excretion of water per hour would be expected to be relatively constant. This has been found, a significant linear relationship existing between cumulative output and hours of collection.

During smolting, an increase in sodium and chloride content of muscle was found. Concomitantly with this was found an increase in plasma sodium which did not occur in the earlier smolts used for urine collection. The percent water of the muscle remained the same.

Olivereau (1962) recorded a temporary augmentation of sodium and of chloride during one part of smolting. In the general case though, she found an increase in potassium and a decrease in sodium, chloride and percent water in Salmo salar. Fontaine (1956, 1961) found a difference in electrolyte content between sedentary smolts of Salmo gairdneri and those which were swimming actively against a current, the latter showing a decrease in chloride and sodium content of muscle which was not found in the other group. There was an increase in potassium in both groups. Fontaine (1960) suggested that the diminution of the ratio of intracellular K/extracellular K was due to an increase in somatotrophic hormone secretion. He observed that the high K/Na ratio in muscle of smolts returns to non-smolt values in seawater.

In this experiment it was the Na/K ratio of muscle which was increased on smolting. This is similar to the values given for the trout adapted for ten days to salt water (Holmes and McBean, 1963). A significant rise in plasma sodium was also recorded for the saltwater adapted trout. This suggests that during smolting the muscle electrolyte concentration may initially rise to that of the salt water animal due to a decrease in urine output occurring before the compensatory change in the extrarenal mechanism.

# LEGEND FOR TABLE I

Regression analysis of cumulative output against time in hours. Analysis of covariance between non-smolts and smolts.

•						-			
							•		
			TABLE	I			Ane	alysis of Co	varianc
	Group	No. of fish	Regression	$\frac{s}{yx}$	$\frac{s_{b}}{}$	<u>r</u>	$\underline{\mathtt{df}}$	F value	P val
	Non-smolt intact	10	Y=2.22 + 4.44X	9.18	.17	•94	_	· . —	_
	1963								
	Smolt intact	14	Y=0.98 + 2.07X	8.57	.15	.80	1,198	109.05	P .0
	1964						-	·	ŕ
	Non-smolt sham	10	Y=2.30 + 3.83X	5.74	.12	.97	_	-	_
	injected, 1963	-			•				
	Smolt sham	12	Y=3.60 + 1.64X	6.70	.12	.81	1,172	123.519	P .0
	injected, 1964	·			•	•			
	Non-smolt sham	9	Y=10 + 4.65X	9.28	.21	•94	-	_	_
	injected, 1964								
	Smolt sham	12	Y=0.98 + 2.07X	8.57	•15	.80	1,167	89.953	P .0
	injected, 1964		<i>,</i>						
								·	

# LEGEND FOR TABLE II

Total output of water and electrolytes in ml., m.eq., or mosm./kg./day in sham injected Salmo gairdneri.

TABLE II

Group	No. of fish	Body Wt. (gm.)	Water	Mosm.	Chloride	Sodium	Potassium
Non-smolts	10	185.77	94.46**	3.26	0.72	0.77	0.14**
1963		<u>+</u> 11.21	<u>+</u> 4.91	±.77	<u>+.</u> 13	<u>+.</u> 12	<u>+</u> .03
Smolts	10	174.37	50.03	2.04	0.54	0.58	0.07
1964		± 9.25	<u>+</u> 3.76	±•15	<u>+</u> .08	±.08	±.01
Non-smolts	9	176.95	115.85**	4.15**	0.88	1.23*	0.14**
1964		<u>+</u> 11.38	<u>+</u> 8.88	±.29	±.14	±.15	<u>+</u> .02

 $<sup>\</sup>ensuremath{^{\star}P}\xspace<$  .01 with respect to the corresponding smolt values.

 $<sup>\</sup>ensuremath{\mbox{**P}}\xspace<\ensuremath{\mbox{.001}}$  with respect to the corresponding smolt values.

# LEGEND FOR TABLE III

Total output of water and electrolytes in ml., m.eq., or mosm./kg./day in intact Salmo gairdneri.

TABLE III

Group	No. of fish	Body Wt. (gm.)	Water	Mosm.	Chloride	Sodium	Potassium
Non-smolts	9	171.18	115.90**	4.91*	1.05	1.03	0.23
1963		± 4.43	± 8.27	±.78	±.24	<b>±.</b> 16	<u>+</u> .02
Smolts	9	179.00	51.78	2.20	0.52	0.56	0.10
1964		±10.57	<u>+</u> 3.9	±.18	<u>+.</u> 10	±.10	<u>+.</u> 01
Non-smolts	8	163.20	112.80**	3 <b>.7</b> 9**	0.79	1.14*	0.14
1964	er i sammer er e	± 8.92	± 4.41	±.27	<u>+.</u> 12	±.14	<u>+.</u> 01

<sup>\*</sup>P < .01 with respect to the corresponding smolt values.

 $<sup>\</sup>ensuremath{\mbox{**P}}\xspace<\ensuremath{\mbox{.001}}$  with respect to the corresponding smolt values.

### LEGEND FOR TABLE IV

Percent reabsorption and percent secretion of filtrate.

TABLE IV

			•	% Secretion			
Group	No. of fish	Body Wt. (gm.)	Water	Mosm.	Chloride	Sodium	Potassium
Non-smolts	10	185.77	45.92	93.18	96.55	96.63	88.22
1963		±11.21	<u>+</u> 2.81	<u>+</u> 1.86	±0.65	<u>+</u> 0.56	<u>+</u> 28.48
Smolts	10	174.37	44.64	92.31	95.18	95.31	90.53
1964		<u>+</u> 9.25	<u>+</u> 1.31	±0.55	±0.68	±0.47	<u>+</u> 25.03
Non-smolts	9	176.95	36.31	92.11	96.08	94.08	100.71
1964		<u>+</u> 11.38	<u>+</u> 4.88	<u>+</u> 0.62	<u>+</u> 0.63	+0.62	+ 9.38

### LEGEND FOR TABLE V

Urine concentrations in mosm. or m.eq./l. in sham injected Salmo gairdneri.

TABLE V

Group	No. of fish	Body Wt. (gm.)	Mosm.	Chloride	Sodium	Potassium
Non-smolts	10	185.77	34.06	7.40	7.97	1.40
1963		<u>+</u> 11.21	± 8.07	<u>+</u> 1.13	± .97	±.82
Smolts	. 12	174.46	59.66	11.34	15.85	1.97
1964		<u>+</u> 7.90	±11.81	<u>+</u> 1.46	<u>+</u> 2.76	±.31
Non-smolts	. 9	176.95	<b>35.90</b>	8.11	10.89	1.28
1964		<u>+</u> 11.38	<u>+</u> 3.30	±1.30	<u>+</u> 1.10	<del>+</del> .11

## LEGEND FOR TABLE VI

Urine concentrations in mosm. or m.eq./1. in intact Salmo gairdneri.

TABLE VI

Group	No. of fish	Body Wt. (gm.)	Mosm.	Chloride	Sodium	Potassium
Nonsmolts	9	171.18	42.98	9.29	8.96	1.94
1963		<u>+</u> 4.43	<u>+</u> 6.61	<u>+</u> 2.02	<u>+</u> 1.29	<u>+.17</u>
Smolts	14	174.69	55.49	12.06	12.08	2.46
1964		± 9.20	<u>+</u> 6.50	<u>+</u> 2.19	<u>+</u> 1.46	<b>±.</b> 28
Non-smolts	8	163.20	35.14	7.63	11.68	1.23**
1964		± 8.92	±3.37	<u>+</u> 1.45	+2.24	±.10

<sup>\*</sup>P < .01 with respect to the corresponding smolt values.

 $<sup>\</sup>ensuremath{\mbox{**P}}$  < .001 with respect to the corresponding smolt values.

#### LEGEND FOR TABLE VIIa

Correlation of electrolyte concentration in mosm. or m.eq./l. with total urine flow in ml./kg./day ('r').

### LEGEND FOR TABLE VIIb

Correlation of electrolyte output in ml./kg./day with urine flow in ml./kg./day ('r').

### TABLE VIIa

	(Mosm.)	(Chloride)	(Sodium)	(Potassium)
Non-smolts	-0.068	-0.111	-0.001	0.043
Smolts	-0.740**	0.080	-0.268	-0.663**

# TABLE VIIb

_ v · · · · · · · · · ·	Mosm.	Chloride	Sodium	Potassium
Non-smolts	0.327*	0.248	0.478**	0.500**
Smolts	0.476*	0.632**	0.572**	0.488**

.001 < \*\*P < .01

### LEGEND FOR TABLE VIII

Filtration rates of water and electrolytes in ml., mosm. or m.eq./kg./day.

TABLE VIII

Group	No. of fish	Body Wt. (gm.)	Water	Mosm.	Chloride	Sodium	Potassium
Non-smolts	10	185.77	174.65**	49.94**	20.62**	23.39**	0.29
1963		<u>+</u> 11.21	<u>+</u> 9.64	<u>+</u> 0.90	<u>+</u> 0.96	±0.98	<u>+</u> 05
Smolts	12	174.46	90.52	26.13	10.81	11.87	0.15
1964		<u>+</u> 7.90	± 7.8	<u>+</u> 0.40	<u>+</u> 0.44	±0.44	± .03
Non-smolts	9	176.95	181.90*	52.82**	22.68**	23.83**	0.16
1964		±11.38	±22.54	<u>+</u> 0.58	<u>+</u> 0.42	<u>+</u> 0.71	±0.03

<sup>\*</sup>P < .01 with respect to the corresponding smolt values.

<sup>\*\*</sup>P < .001 with respect to the corresponding smolt values.

LEGEND FOR TABLE IXa

Muscle electrolyte concentrations in Salmo gairdneri in m.eq./kg. wet weight.

LEGEND FOR TABLE IXb

Plasma concentrations in mosm. or m.eq./l. in Salmo gairdneri used for muscle determinations.

TABLE IXa

Group	No. of fish	Body Wt. (gm.)	Chloride	Sodium	Potassium	% Water
Smolts	10	128.02	9.22	23.22	131.45	80.50
		± 5.50	±•55	<u>+</u> 1.48	± 3.97	<u>+</u> .60
Non-smolts	10	164.40	7.40*	16.47*	126.28	79.94
	·	± 7.40	<u>+.30</u>	± .96	<u>+</u> 1.48	± •39
•						
			TABLE IXb			
Group	No. of fish	Body Wt. (gm.)	Mosm.	Chloride	Sodium	Potassium
Smolts	10	128.02	309.97	134.06	155.17	1.08
		± 5.50	<u>+</u> 1.54	± 2.28	± 1.48	<u>+.</u> 08
Non-smolts	10	164.42*	289.32	126.92	135.39**	1.10
		+ 7-40	+ 6.99	+ 3.56	+ 3.77	+.10

<sup>\*</sup>P < .01 with respect to the corresponding smolt values.

 $<sup>\</sup>ensuremath{^{**}P}$  < .001 with respect to the corresponding smolt values.

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