International Council for the Exploration of the Sea

C.M. 1975/M:19
Anadromous and Catadromous Fish Committee
Ref: Fisheries Improvement.

INDUCED SALT WATER TOLERANCE IN CONNECTION WITH INORGANIC SALTS IN THE FEEDING OF ATLANTIC SALMON (SALMO SALAR L.).

by Sergio Basulto x)

ABSTRACT

Changes in length, weight, condition factor, water content in tissue, Na^{+} and Cl^{-} level in the blood plasma and sea water tolerance of salmon parr were measured after different periods of feeding with control and experimental diets containing inorganic salts.

Results point out differences mainly in relation to gain in length and weight, food conversion and sea water tolerance. Salmon parr seem to be able to cope with a daily amount of inorganic salt of up to 12% of food weight consumed. Tolerance to sea water was higher in fish fed with enriched salt diets.

INTRODUCTION

The fastest possible growth in saline environments obtained with some fish reared at sea or in estuarine waters evidently plays an important role in the sea farming development. Canagaratman (1959) provides evidence from his literature survey as well as from his experimental findings on Pacific salmon (Oncorhyncus) that those species of fish, among them Atlantic salmon, which can withstand a wide range of salinity grow better in a more saline environment. This is also true for some species which normally live in fresh-water. Experiments show that trout reared in sea water grow faster than those reared in fresh water (Canaga and Ratman, 1959). Rain-bow trout, for example, has a better conversion of food in salt water than in freshwater (Sedwick, 1970).

The Atlantic salmon has a great and rapid increase in growth rate after its seaward migration, probably because, in general, the availability of food and space are better in the ocean than in fresh water. If we add to this fact that growth rate and food conversion are notably higher for young fish, it is obvious that to obtain sea water tolerance ahead of natural time will be a goal for the normal salmon farming practices. Clearly, the solution to this will come only after refining the knowledge about the factors which influence the metamorphosis from parr to smolt.

---

x) Institute of Marine Research
Directorate of Fisheries
Bergen, Norway

Through different ways investigators have been making efforts in order to get early adaptation of fishes to sea water: rearing young fish at elevated temperatures, early exposure to gradual increment in salinity, variations in the normal photoperiod, hormonal treatments, etc.

Lately, investigation in the osmoregulation field indicate that by feeding a diet enriched with inorganic salts it is possible to achieve an easier transition from fresh to sea water (Zaugg and McLain, 1969, 1970). This salt ingestion seems to produce an activation of the mechanisms involved in the osmoregulatory process. The physiological explanation is that dietary sodium chloride produces an increase in Na^{+}, K^{+} -ATPase and a decrease in Mg^{2+}-dependent ATPase activity. This is quite similar, though to a lesser extent, to one of the physiological changes reported for a number of euryhaline fishes during natural adaptation to sea water.

The results of Zaugg and McLain, who used enriched NaCl diet and afterward another inorganic salt, were for coho salmon, a Pacific salmon species (O. kisutch). Currently there is no information on similar experiments made with Atlantic salmon, and the relative importance of its incidence in the marine aquaculture economy. This encouraged us to start some trials in order to investigate the effects on this fish.

MATERIALS AND METHODS

The experiments were done in two instalments, from September to December 1974 and from March to June 1975. The second experimental period served to repeat parts of the one made in 1974, and provided opportunity to check preliminary conclusions only in relation with salt water tolerance experiments.

Fish

In both periods it was used salmon parr hatched in spring 1973 and obtained from Matredal Fish and Research Culture Station belonging to Directorate of Fisheries (Institute of Marine Research, Bergen). These fish came from Suldalslågen river for the 1974 experiment, and from the same river and Årøy river for the second one.

Fish-holding condition

Salmon were held in a dark green 250 liter fiberglass aquarium. A flow of fresh water was maintained at a rate of about 4 liters/min. The water temperature was fixed between 10-12 C. During the entire experiment a daily photoperiod of 12 hours light was used. Lights were switched on or off automatically without transition.

Feeding

The basis of fish diet was a commercial fish powder food. At our special request the factory of T. Skretting A/S prepared for us a batch of food without mineral supplement. This food contained only the natural salt of the ingredients and that was estimated to be 1%.

For the daily feeding a moist pellet described by Fowler and Burrows (1971) with carboxi-methyl-cellulose (CMC) as binder was used. For the experiments four different formulae were made with fish powder. We denoted the diets as A (control), B, C and D. Their respective conpositions were:

Diet A.

Tess food powder	98%
CMC	2%
Mineral supplement	0%

Diet B.

Tess food powder	94%
CMC	2%
Mineral supplement	4%

Diet C

Tess food powder	90%
CMC	2%
Mineral supplement	8%

Diet D

Tess food powder	86%
CMC	2%
Mineral supplement	12%

30% of water was added to the ingredients to produce a doughlike mixture.

As mineral supplement a preparation "Instant Ocean" made by Aquarium Systems, Inc., Wycliff, Ohio, USA, was used. This salt mixture is composed principally of (%) NaCl, 65.2; $MgSO_4 \cdot 7H_2O$, 16.3; $MgCl_2 \cdot 6H_2O$, 12.8; $CaCl_2$, 3.3; KCl, 1.7; $NaHCO_3$, 0.5.

In order to avoid possible action of some minerals, in particular iodide that probably has connection with natural smoltification process, the solution of trace elements enclosed with "Instant Ocean" was not used.

The pellets were supplied to salmon parr twice daily, five days a week. The food was slowly given by hand as to prevent any food loss. The food supplied was wholly consumed.

During the 1974 experiment the fish were fed a daily ration of 2% of their body wet weight. In order to asses the diet efficiency, each month the total amount of food given through the period was divided by the number of survivors in each group. The sumation of these monthly values, lot by lot, were divided by the respective mean weight increase of the fish. The resultant quantity is the food spent to get 1 gram of fish weight increase. In the 1975 experiment the fish were fed "ad libitum".

Water content in tissue

In the 1974 experiment water content of tissue was assessed. From each group a few measurements of dry weight were made according to a method described by Black (1950).

Chloride and sodium ions in blood plasma

At the same time as the test for tissue water content, some fish were sampled in order to measure the level of Cl^- and Na^+ in the plasma. The samples were analyzed at the Biochemical Clinical Department, Haukeland Hospital, Bergen. Because of the small volume of plasma available per fish, four individual samples in each group were pooled for determination of ions. For Cl^- and Na^+ a Corning EEL 920 Chloride Meter and a EEL 227 Integrating Flame Photometer, respectively, were used.

Salt water tolerance trials

The first period of salinity exposure trials was carried out by placing experimental and control fish groups in 15 liter plastic buckets containing 14 liter of water. The water was changed every 2-3 days in order to avoid accumulation of excretory products and a constant stream of air was maintained through air stones. In order to check if the casualties were caused by variations in environmental salinity or due to unsuitable oxygen content, for every salinity exposure trial carried out in static water a control fish group kept under the same conditions in fresh water was used.

In this period trials were performed at two different salinities: 75 and 100% of fully strength sea water (35 0/00). To obtain the right salinity either fresh water was added to sea water to reduce salinity or "Instant Ocean" added to increase it. In this period the salinity tests were going on for 48 hours.

In 1975 salmon groups were placed in running sea water into 250 liter fiberglass tanks. The exposure time was up to 192 hours.

In both test periods fish were starved for two days before start of each salinity test and the fish were not fed during the test. For close inspection, times of death for all fish were recorded and the corpses removed immediately after verification of death.

1974 Experiment

In September 1974, salmon parr of 9.7 cm mean fork length and 10.1 g mean weight, were divided in three batches which each received a different food mixture with 0, 8 and 12% of inorganic salts (diets: A, C and D, respectively). The experiment had a duration of three months and salinity tolerance tests were performed immediately before beginning of the experiment and after 1, 2 and 3 months on both control and experimental diet groups.

1975 Experiment

In February 1975, salmon of 12.0 cm mean fork length and 17.1 g mean weight, were divided in three groups. Each lot was submitted to different diets with 0, 4 and 12% of salts (diets: A, B and D, respectively). This experiment was quite similar to the 1974 experiment with the only differences afore mentioned.

Also, during 1975 in three occasion feeding treatments with only one month on control and experimental diets: A, B, C and D, were performed.

RESULTS

Water content in tissue

Data in Table 1 indicate that water content in tissue was higher in salmon parr groups submitted to diets with enriched inorganic salts. In any case these differences in water content do not seem to be of any noticeable harm to the fish.

TABLE 1. Average values for tissue water content after 34, 62 and 94 days on control and experimental diets.

Diet	Test 1		Test 2		Test 3	
	Number of Fish	% water content	Number of Fish	% water content	Number of Fish	% water content
A	8	69.1	4	73.7	3	68.7
C	8	71.6	4	74.1	3	72.0
D	8	72.3	4	74.5	4	74.0

Chloride and sodium ions in blood plasma

Although only a few microanalyses for ions in blood plasma were made, the data in Table 2 show that always the values for sodium and chloride were higher in fish that had a diet enriched with inorganic salts. These microanalyses were made at the same time as those made on tissue water content. Plasma samples were taken after two days or more of full starvation.

TABLE 2. Values for chloride and sodium ions in blood plasma (meq/l) after 34, 62 and 94 days on control and experimental diets.

Diet	Test 1		Test 2		Test 3	
	Na	Cl	Na	Cl	Na	Cl
A	165	115	152	124	148	102
C	168	140	150	140	154	124
D	186	148	186	136	191	144

Effect of enriched inorganic salt diets on growth

Table 3 reports mean and standard error values for weight, fork length and condition factor obtained at four different dates of the 1974 experimental period.

We denoted "probably significant" and "definitely significant" when a difference of more than two or more than three standard errors between the samples means, respectively, were found (Moroney, 1969).

Gain in weight

After three months on diet the fish belonging to the groups with 8 and 12% of salt content in the diet, had a gain weight, from the statistical stand point, that was significantly lower than that of the control group. Among the experimental groups, the fish with only 8% of salt had a higher but only probably significant than those with 12% of salt in the diet.

Increase in length

At the end of the 1974 experimental period the fish mean lengths in the experimental groups were significantly lower than those belonging to the control group, specially evident in the group with 12% of salt. It was not possible to find any significant difference between the experimental groups.

Condition factor

After two months on research, the control group showed a condition factor consistently higher than those of the experimental groups, nevertheless the condition factor for all batches decreased with time throughout experimental period.

TABLE 3 Mean length, weight, and condition factor of salmon parr tested with diets containing different amounts of inorganic salts.

Date (days elapsed on diet)	Added salts (% dry wt.)	Batch	Number of Fish	Mean fork lenght cm	SE of mean	Mean wet wt. g	SE of mean	Mean condition factor	SE of mean
12-13. 09. 74 (0)	0	A	180	9.6	.560	10.0	1.878	1.101	.0639
	8	C	180	9.8	.498	10.4	1.592	1.091	.0656
	12	D	180	9.6	.539	10.1	1.684	1.118	.0665
15-16. 10. 74 (34)	0	A	166	10.7	.786	13.3	3.138	1.072	.0603
	8	C	176	10.8	.577	13.4	2.201	1.068	.0063
	12	D	171	10.5	.636	12.8	2.363	1.096	.0494
12. 11. 74 (62)	0	A	98a	11.2	.888	15.6	3.571	1.077	.0495
	8	C	132	11.3	.573	15.5	2.432	1.064	.0423
	12	D	92b	11.1	.633	14.8	2.714	1.067	.0468
13. 12. 74 (94)	0	A	51	12.1	.843	19.5	3.727	1.087	.0417
	8	C	88	11.8	.655	17.6	.278	1.063	.0451
	12	D	50	11.6	.737	16.5	3.202	1.052	.0543

a. 29 fish died on 1.11.74, flow rate of fresh water accidentally droped under the required level
b. 22 fish died on 6.11.74, flow rate of fresh water accidentally droped under the required level

Food conversion efficiency

Table 4 list the average gain in weight in three parr groups with diets differing in the amounts of added inorganic salts.

TABLE 4. Effect on weight growth of salts added to salmon parr diets.

Added salt (% dry wt.)	Average initial weight (g)	Average final weight (g)	Average gain weight (g)	% of control
0 (A)	10.0	19.5	9.5	----
8 (C)	10.4	17.6	7.2	76
12 (D)	10.0	16.5	6.5	68

The amount of food needed to produce 1 g increase in weight was higher for the experimental groups than the control one. Table 5 illustrates these decreasing conversion efficiences. Obviously, the total amount of nutrient per unit of diet was less in diet with supplemental inorganic salts included.

TABLE 5. Effect of dietary inorganic salts on food conversion efficiency in salmon parr.

Added salt (% dry w.t.)	Average gain wt. (g)	Food eaten per average fish (g)	Weight of food per gain of wt.	Weight of food (excl.salt added) per wt. gain
0 (A)	9.5	20.7	2.2	2.2
8 (C)	7.2	19.2	2.7	2.5
12 (D)	6.5	20.0	3.1	2.8

Effect of dietary "Instant Ocean" salts on sea water tolerance of salmon parr.

Results presented in Tables 6 to 9 show the survival rates of fish submitted to abrupt changes from fresh to salt water after different periods on diets with different amounts of salt content.

1974 Experiment

Tables 6 and 7 gives the data for survival at two different salinities, 75 and 100% of fully strength sea water (35 0/00), of three lots of fish, one of them fed with control diet and the others with diets containing 8 and 12% of salt.

The results point out that in all trials, groups with enriched inorganic salts had the lower mortality rate to salinity tolerance test.

The survival rates reached for fishes after two months with control and experimental diets were lower than those registered for these same groups with only one month on diet. Trials after three months show again an increase in tolerance to the salt water exposure.

Tables 6 and 7, specially the first one, show clearly that the efficiency of the osmoregulation mechanisms was increasing gradually with time. Survival rates at 75% sea water salinity exposure rose from 0% in September to practically 100% in December. In this last month the fish of all groups were able to stand 75% sea water salinity at least for one month. For 35 0/00 salinity the survival rate rose in the same period from 0 to 40%. Table 3 allows to connect this relative higher efficiency with monthly increase in size.

TABLE 6. Survival rates of salmon parr exposed to 75% sea water salinity after receiving salt supplemented diet. 1974 experiment, up to 48 hours of exposure.

Days on diet	Number of fish per batch	Survival per group (%)			Total survival (%)
		A	C	D	
0	12				0
34	12	25.0	75.0	91.7	63.9
62	12	50.0	75.0	50.0	58.3
94	15	100.0	100.0	93.3	97.8

TABLE 7. Survival rates of salmon parr exposed to 100% sea water salinity (35 0/00) after receiving salt supplemented diet. 1974 experiment, up to 48 hours of exposure.

Days on diet	Number of fish per batch	Survival per group(%) A	C	D	Total survival (%)
0	12				0
34	12	0	0	16.7	5.6
62	12	0	0	0	0
94	15	33.3	46.7	40.0	40.0

1975 Experiment

Table 8 illustrates experiments conducted on three fish groups fed diet A (control), B and D from February to June. Salinity tests were performed immediately before the beginning of the experiment and after 1, 2 and 3 months on diet. Results obtained point out that in all the tests made groups fed with enriched salt diet had better survival rate. That was specially marked in the test performed in March (after one month on diet). In this serie the survival rate for the group with 12% of salt was significantly higher than the control group ($P < 0.02$).

TABLE 8. Survival rates of salmon parr exposed to 35 0/00 salinity after receiving salt supplemented diet. 1975 experiment, up to 192 hours of exposure.

Days on diet	Number of fish per batch	Survival per group (%) A	B	D	Total survival (%)
0	25				0
31	25	8.0	12.0	40.0	20.0
67	30	53.3	56.7	70.0	60.0
107	38	86.8	65.8	100.0	84.2

Table 9 list results of sea water exposure for four different batches of fish after approximately one month on diet. The results point out likewise that more survivors were obtained in the groups where salt was included in the diet.

TABLE 9. Survival rates of salmon parr exposed to 35 0/00 salinity after receiving salt supplement diets. 1975 experiment, up to 192 hours of exposure.

Days on diet	Number of fish per batch	Survival per group (%) A	B	C	D	Total survival (%)
0	25					0
31	25	8.0	12.0	24.0	40.0	21.0
36	34	70.6	73.5	52.9	79.4	69.0
40	38	86.8	71.1	84.2	82.0	82.0

In both series of experiments it was possible to verify clearly the natural increase with time of the sea water tolerance.

DISCUSSION

The present data indicate no detrimental effect on the enriched salt diets. Results are in that respect similar to those obtained in trout by Tunison et al.(1939), and Shterman (1960), and for perch and pike by Privol'nev (1964). These results are different from those reported by Phillips (1947), who found that excessive levels of dietary salt cause an upset in osmotic regulation of the fish´body, an upset that is characterized by an edema that eventually results in death.

Results of tissue water content analyses point out that fish fed with enriched salt diets had a higher water content in tissue, but in none of the cases there was any visual difference between experimental and control groups.

Salmon parr can cope with daily introduction of inorganic salt through feeding, at least up to 12% of food weight consumed. This figure is higher than the 10% given by Shterman (1960) and less than the 13% given by Tunison (1939) both for trout and the 15% quoted by Zaugg (1969) for salmon (one type of Oregon Moist Pellet). This capacity means that the physiological answer of the osmotic regulation is adequate, but in any case, future research should have a closer look at this aspect, because differences in water content in tissue could be an evidence of some osmotical derangement. This is clearly a very important matter, specially from the fish breeding point of view where use of salty food, requires accurate knowledge of the amounts of salt that fish of different age or species can tolerate (Privol ´nev, 1964).

Values for the levels of chloride and sodium ions in blood plasma between control and experimental groups, obtained at least after two days or more of full starvation of fish are in some way a support for Privol´nev´s (1964) assertion that the salts have a slow absorption from the intestines into the blood. Therefore, this physiological feature could be used in the fish farming practice when eventually a salty food is prescribed.

Results indicated that significant impairment on gain in weight and length were obtained after three months on diets with 8 and 12% of salt in comparison to the same parameters reached for the control group without salt supplement. Obviously, the addition of salt in the diet reduces to some extent the amount of nutrients, but it is also clear that the salt interfered with food utilization (Table 5). These results agreed with those obtained by Zaugg and McLain (1969).

The principal aim of our research was to verify if enriched salt diets had some influence upon salt water tolerance of salmon parr. As to that the results are positive. Using survival rates as an indication of greater or minor resistance to abrupt changes from fresh to salt water, it can be concluded that fish fed with enriched salt diet had, in all tests, a higher tolerance to salt water. Zaugg and McLain (1969) suggest the possibility that "ingestion of elevated quantities of salts would activate the excretory process involved in the elimination of these salts when swallowed by drinking sea water in a marine environment".

During the 1974 experiment all salt water tests performed after two months on diet show a sharp drop in survival rate in comparison to results obtained after only one month. This is possible explained by the fact that just before the start of this series of tests a serious outbreak of Costia spp. appeared in tanks from which the fish used in the test came. It is suggested that the fish of all groups died prematurely weakened by disease, not only because of osmoregulatory difficulties per se.

In spite of that the test groups had lower mean length and weight than the control group, high survival rates in all groups after sea water exposure were found in the last series of trials in 1974 as well as in 1975. This fact makes it difficult to decide whether it was a possible effect of salt feeding on the induction of salt water tolerance or not.

CONCLUSIONS

1. In all tests of salt water tolerance performed, the best results, from the survival stand point, were obtained for the groups of fish submitted to enriched inorganic salts diets. Unfortunately, the statistical scope of the experiments on acclimatization of Atlantic salmon parr to sea water is not sufficient to warrant if salt feeding could condition them to make an easier transition from fresh to sea water. Nevertheless the trends that we have noticed in our experiments rend us optimistic in this way.

2. Apparently doses of up to 12% of inorganic salts in the diet, do not adversely affect the health of the fish.

3. Taking into consideration the negative interference between inorganic salts feeding and food utilization, a shorter period of "treatment" might be better.

4. More research is needed to provide a better understanding of the effects of salt feeding in aquacultural practice.

LITERATURE CITED

Black, V.S. (1951). Changes in body chloride, density, and water content of chum (Oncorhynchus keta) and coho (O. kisutch) salmon fry transferred from fresh water to sea water. J. Fish. Res. Bd. Canada, 8. 164-176.

Canagaratman, P. (1959). Growth of fishes in different salinities. J. Fish. Res. Bd. Canada. 16. 121-130.

Canaga, and Ratman (1959). Quoted by Schäperclaus, 1961, pp. 439.

Fowler, L.G., & Burrows, R.E. (1971). The Abernathy salmon diet. Prog.Fish. Cult. 33(2), 67-75.

Moroney, M.J. Facts From Figures. 1969 ed. Penguin Books Ltd, Harmondsworth, Middlesex, England. 472 p.

Phillips, A.M., Jr. (1947). The physiological effect of sodium chloride upon brook trout. Trans. Amer. Fish. Soc. 74 (1944): 297-309.

Privol'nev, T.I. (1964). Fish Physiology in Acclimatization and Breeding. Published for the U.S. Department of the Interior and the National Science Foundation, Washington, D.C. by the Israel Program for Scientific Translation, 1970, 204 p.

Shterman, L.Ya. (1960). Dopustimye kolichestva povarennoi soli v kormovykh ratsionakh raduzhnoi foreli (Permissible amounts of salt in the diet of rainbow trout). Nauchno-Tekhnicheskii Byulleten' No. 11, GosNIORKh. Original not seen.

Tunison, A.V., Phillips, A.M., Rodgers, E.O., Mitchell, and McCay, C.M. (1939). The nutrition of trout. Cortland hatchery report N. 7 for the year 1938. N.Y. Cons. Dept., Albany, New York.

Zaugg, W.S., & McLain L.R. (1969). Inorganic salt effects on growth, salt water adaption and gill ATPase of Pacific salmon. In Fish in Research (edited by Neuhaus O.W. & Halver J.E.), 293-306. Academic Press, New York.

Zaugg, W.S., & McLain, L.R. (1970). Adenosinetriphosphatase activity in gills of salmonids: seasonal variations and salt water influence in coho salmon, Oncorhynchus kisutch. Comp. Biochem. Physiol. 35, 587-596.