This paper not to be cited without prior reference to the authors

International Council for the Exploration of the Sea

C.M. 1977/M:20
Anadromous and Catadromous Fish Committee

Changes in Na^+K^+ATPase activity as an indication of smolt status in Atlantic salmon

THÜNEN
Digitalization sponsored by Thünen-Institut

by

Richard L. Saunders*

and

Eugene B. Henderson

Canada Department of Fisheries and Environment
Fisheries and Marine Service, Biological Station
St. Andrews, New Brunswick, Canada

ABSTRACT

Gill ATPase activity increased during winter-spring in Atlantic salmon held at 10°C and subjected to simulated natural or reciprocal (light-to-dark ratio opposite that of natural) photoperiods. ATPase activity increased earlier and more sharply in the reciprocal than in the natural photoperiod regime. Body lipid decreased and moisture content increased more rapidly in reciprocal than in natural photoperiod fish. Salinity tolerance (to 40‰) increased between March and April. Exposure to 40‰ salinity for periods up to 14 days gave marked increases in ATPase activity over levels measured in fresh water. Although ATPase activity is a sensitive indicator of the ability of Atlantic salmon to osmoregulate in sea water, it is of less value as an indicator of smolt preparedness to migrate to sea than lipid-moisture content, tolerance to high salinity and migratory behavior.

INTRODUCTION

Among the various physiological and behavioral changes taking place prior to migration of salmonid smolts to sea is an increase in Na^+K^+ activated ATPase activity in the gill microsomes (Zaugg and Wagner, 1973). The gill ATPase system is involved in excretion of monovalent ions. This and other physiological and behavioral changes comprise the parr-smolt transformation which pre-adapts certain salmonids, while still in fresh water, for life in the sea (Hoar, 1976).

* Present address: North American Salmon Research Center
St. Andrews, New Brunswick, Canada

The objective of this study was to learn whether or not Atlantic salmon gill ATPase activity changes in response to photoperiod manipulation as do many of the other physiological and behavioral aspects of the parr-smolt transformation (Saunders & Henderson, 1970 and Komourdjian et al 1976). In production of salmonids for aquaculture or for release in salmon enhancement projects it is important to plant smolts at the appropriate time when they are ready in all respects to make the transition from fresh to salt water. Through the present study we hope to assess ATPase activity in comparison with other criteria, such as lipid-moisture content, salinity tolerance and migratory behavior, used as bases for deciding when to plant or release smolts.

MATERIALS AND METHODS

Presmolt Atlantic salmon were brought to the laboratory in December and held in groups of 100 at 10^0C in 2 m diameter fiberglass tanks. One group was held under a simulated natural photoperiod; the other was held under a reciprocal photoperiod, i.e., 16 hr. light/day decreasing to 8 hr/day between December and June. The fish were fed several times daily with pelleted Ewos salmon food.

ATPase measurements were made at first monthly and then twice monthly using the method of Miller et al (1976) using five fish from each photoperiod group. Measurements were based on the entire gill system from which the filaments were trimmed and placed in an iced solution at 5 mM EDTA and 250 mM sucrose. The filaments were ground using a teflon pestle in a glass homogenizer. Three ½ ml aliquots of the homogenate were frozen in vials in a mixture of dry ice and acetone. Samples were then freeze-dried for 24 hours and stored at -20^0C. Samples were reconstituted by adding 5 ml of EDTA sucrose solution to each vial. Protein levels were determined using the method of Lowry et al (1951). ATPase levels were expressed as μ moles pi/mg protein/hr.

Moisture determinations were made on the fish minus gills by drying to a constant weight at 80^0C - about 48 hours.

Lipid determinations were made on aliquots of dry ground tissue by column extraction as described by Korn & Macedo (1973) using monofluorotrichloromethane (MF Freon, refrigerant - 11).

RESULTS

Gill ATPase activity did not increase greatly during the period January - March in the fish subjected to a simulated natural photoperiod (Fig. 1). Activity increased sharply in mid March and continued increasing through June. ATPase activity increased during late winter - early spring in response to the long day-length provided by the reciprocal photoperiod. In both the natural and reciprocal photoperiod groups there were abrupt changes in ATPase activity near the middle of the observation periods. The increase in ATPase activity was more abrupt and occurred earlier in the reciprocal than in the natural photoperiod fish. The slope of the regression equation for reciprocal photoperiod fish was greater and significantly different ($P>0.01$) from that for the natural photoperiod.

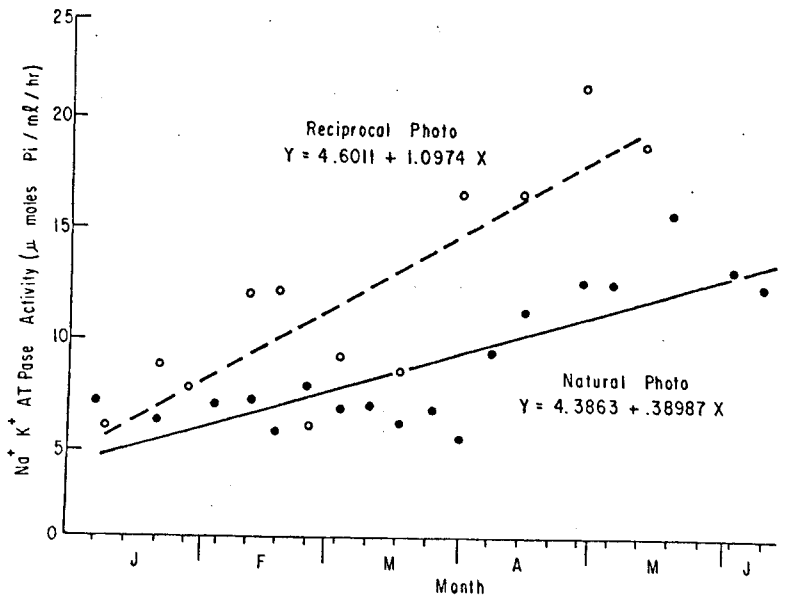

Figure 1. Gill ATPase levels in response to photoperiod. Each point represents the mean of five determinations.

Total body lipid levels decreased (Fig. 2) and moisture content increased (Fig. 3) during the period January through June. The rates of decrease of lipid ($P>0.05$) and increase of moisture ($P>0.01$) were significantly greater in the reciprocal than in the natural photoperiod fish.

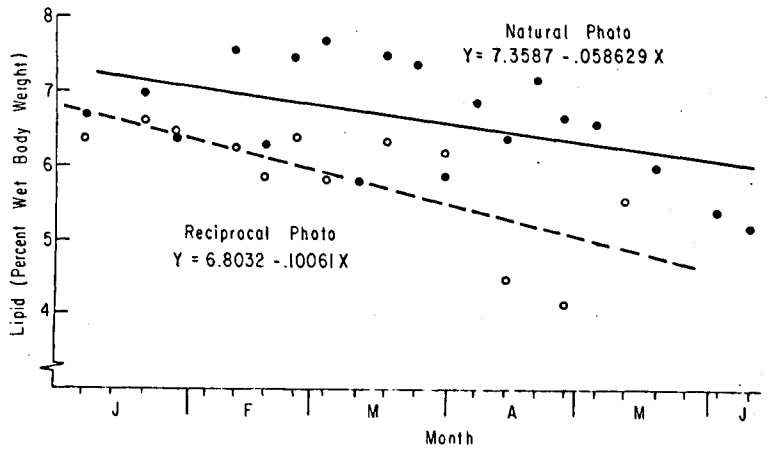

Figure 2. Changes in total body lipid level in response to photoperiod during winter-spring. Each point represents the mean of five determinations.

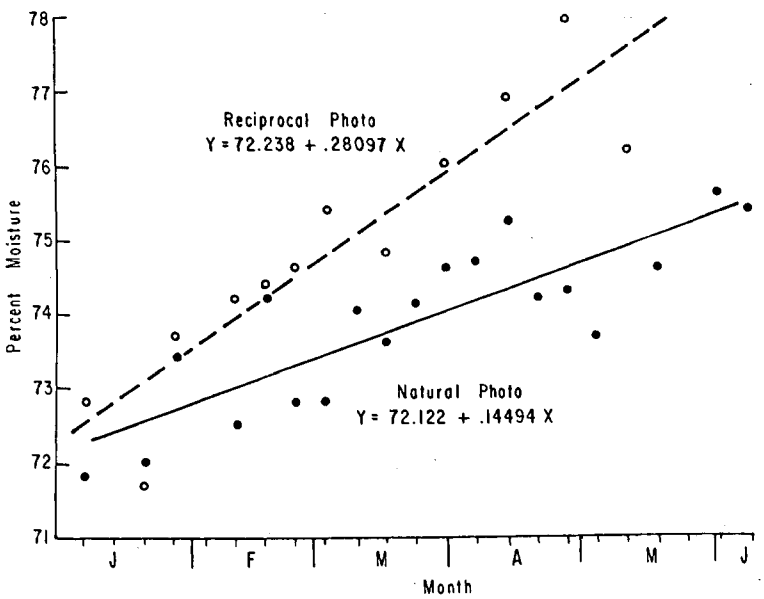

Figure 3. Changes in moisture content in response to photoperiod during winter-spring. Each point represents the mean of five determinations.

In both the natural and reciprocal photoperiod groups there were highly significant ($P>0.01$) correlations between ATPase activity and moisture content but the two regressions were not significantly different one from another (Fig. 4). There was a significant ($P>0.05$) regression between ATPase activity and lipid content in the reciprocal but a non-significant regression for the natural photoperiod group.

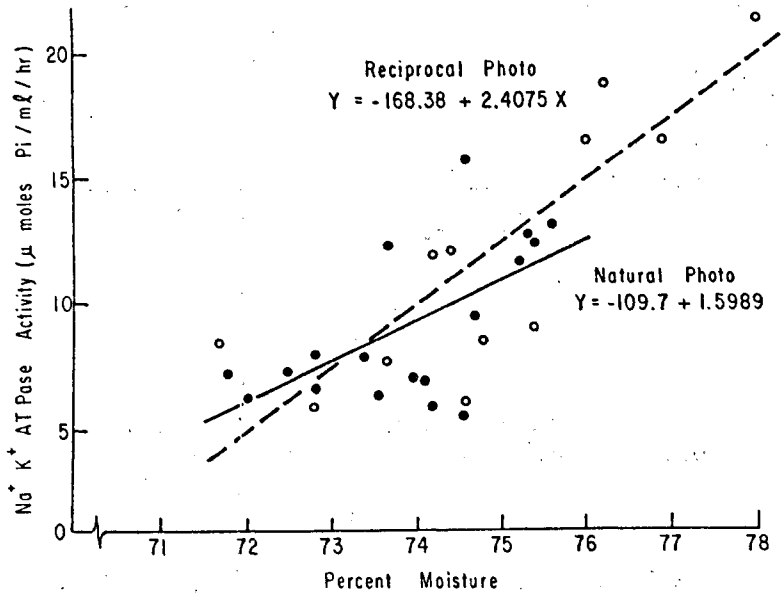

Figure 4. Relations between ATPase level and moisture content in response to photoperiod.

There were highly significant correlations between lipid and moisture content in both groups but the two regressions were not significantly different from each other (Fig. 5). Two salinity tolerance tests were conducted at 40‰ salinity with fish from the natural photoperiod group, one in late March and one in late April. Because of earlier mortalities, there were too few fish in the reciprocal photoperiod group to test for salinity tolerance. In the first test, 50% of the fish were dead after 50 hours. When the fish were challenged with high salinity in April, they displayed a marked tolerance; only one of eight fish had died after 112 hours. Following both salinity tolerance tests the surviving fish were retained in 40‰ for various periods before being sampled for plasma chloride level and ATPase activity. The result following both salinity tolerance tests was that ATPase activity increased with length of exposure to high salinity. Plasma chloride level increased and remained *ca.* 20 meq/l higher than for fish in fresh water.

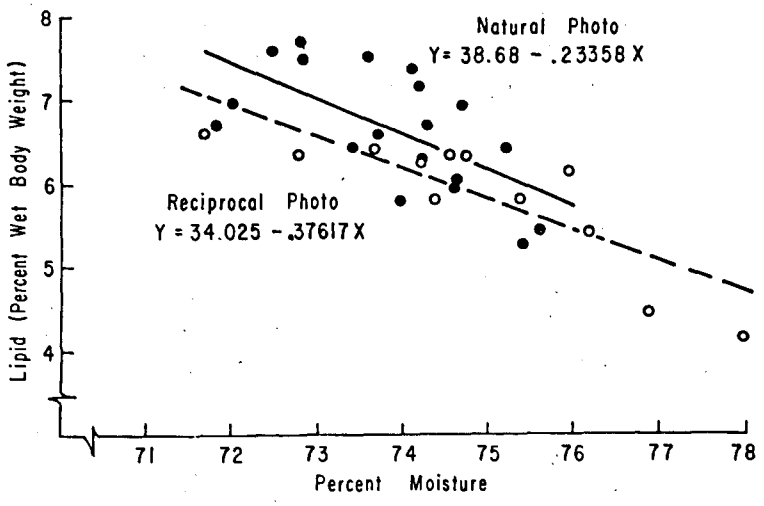

Figure 5. Relations between body lipid and moisture content in response to photoperiod.

DISCUSSION

Our findings are in agreement with those of McCartney (1976) who reported increased gill Na^+K^+ATPase activity in Atlantic salmon during spring and peak values corresponding with the usual time of migration to sea. ATPase activity, like the other smolt criteria studied by Komourdjian et al (1976), responds to photoperiod manipulation and can be advanced on a temporal basis by subjecting Atlantic salmon to an artificially accelerated spring. The temperature and photoperiod regime in our study was patterned after that used by Komourdjian et al (1976) and in the same laboratory. Hence, the results of the two experiments may be compared in respect to certain parameters in addition to ATPase activity which was not measured by Komourdjian et al. Body lipid declined and moisture content increased in the reciprocal fish much earlier than in natural photoperiod fish in both studies. The salinity tolerance data in the present experiment are in agreement with those of Komourdjian et al for natural photoperiod fish.

Our findings of a dramatic increase in ATPase activity during prolonged residence in sea water was in agreement with those of Zauggand McLain (1970) and Giles and Vanstone (1976) both working with Coho salmon. Not only were the control values for ATPase activity in fresh water higher in late April than in late May, but also the resulting increases in ATPase activity

were greater resulting from long exposure to 40‰ salinity. This high ATPase activity together with greater salinity tolerance in late April than a month earlier indicated a greater degree of preadaptation for entry to the sea and the requirement for the ability to hyperosmoregulate. Our data suggest that plasma chloride regulation improved slightly between tests in March and April.

The present study provides an indication that there is not the degree of temperature dependence in ATPase activity in Atlantic salmon as found by Adams et al (1973) in steelhead trout. Adams et al showed that the increase in ATPase activity and consequent salinity tolerance of steelhead trout are temperature dependent. At 6.5 and 10^0 there was a two-fold increase in ATPase activity during the spring; at 15^0 or higher there was no increase in enzyme activity and little salinity tolerance. Zaugg and McLain (1976) report that ATPase activity in Coho salmon is less temperature sensitive than in steelhead. Elevated temperatures resulted in increased ATPase activity in Coho and accelerated smoltification. Enzyme activity was preserved during long exposure to 6^0 whereas at 10 and 15^0 there were decreases following earlier increases. Since our Atlantic salmon developed elevated ATPase levels and salinity tolerance and maintained them while being held during winter-spring at 10^0, it appears that this temperature is not too high for effective smoltification. Indeed, Komourdjian et al (1976) found no inhibition of various physiological processes associated with smolting in Atlantic salmon held at 10^0. Possible temperature dependence of ATPase activity in Atlantic salmon at $>10^0$ like that in Pacific salmonids should be investigated because hatchery and aquaculture facilities often have such temperatures close to the time that naturally produced smolts are moving to sea.

ATPase activity appears to be a sensitive and excellent criterion of salmonids' ability to osmoregulate in an hypertonic environment. However, like pituitary activity (Komourdjian et al 1976), it is time consuming and difficult to measure on a routine basis. In comparison with changes in body lipid and moisture content, with which it is correlated, ATPase activity is less convenient and perhaps less sensitive an indicator of preparedness of salmonids to move to sea. Farmer et al (1977) recommend use of lipid-moisture contents and condition factor as indicators of the appropriate time to release hatchery smolts.

A physiological challenge to a salinity as high as 40‰ appears to be an excellent test of sea worthiness but not necessarily of any disposition to move to sea. Wagner (1974b) makes the point that salinity tolerance in salmonids develops many months before smoltification and is separate from smolting. Indeed, salinity tolerance is size dependent (Parry, 1958; 1960) and Atlantic salmon of 10-12 cm will live

for long periods in sea water of 30‰ salinity (Saunders and Henderson, 1969). By challenging juvenile salmonids to >40‰, Komourdjian et al (1976) and the present study have shown a degree of salinity tolerance that is undeveloped before smoltification and which is an excellent indicator of preparedness for going to sea.

Migratory activity as described by Zaugg and Wagner (1973) and Wagner (1974a) and observed by many salmon hatchery workers is a useful indicator of readiness of salmonids to move downstream when planted in a river.

We conclude that ATPase activity is one of the parameters by which to judge a juvenile Atlantic salmon to be a smolt. Like many of the other parameters studied, ATPase activity in Atlantic salmon responds to photoperiod manipulation. Together with growth pattern, lipid-moisture dynamics, body and fin color, pituitary histology and hormone activity, osmotic-ionic regulation and salinity tolerance and migratory behavior, ATPase activity serves as an indicator of smolt status and particularly the readiness to begin hyperosmotic regulation. From a fish culturist's or aquaculturist's point of view, ATPase activity appears to be of less value as an indicator of smolt preparedness to migrate than lipid-moisture content, tolerance to high salinity and migratory behavior.

ACKNOWLEDGEMENTS

This study was supported financially by The Marine Farming Research Branch of the Cape Breton Development Corporation.

We are grateful to Mr. Jeff Ray who did the ATPase determinations, to Mr. A. Sreedharan who assisted with statistical analysis of our data and to Messrs. P.W.G. McMullan and F. Cunningham who prepared the drawings.

REFERENCES

Adams, B. L., W. S. Zaugg & L. R. McLain. 1973. Temperature effect on parr-smolt transformation in steelhead trout (*Salmo gairdneri*) as measured by sodium-potassium stimulated adenosine triphosphatase. Comp. Biochem. Physiol. 44A: 1333-1339.

Farmer, G. J., J. A. Ritter and D. Ashfield, 1977. Seawater acclimation and parr-smolt transformation of juvenile Atlantic salmon, *Salmo salar* L. ICES. Anad. Catad. Fish Comm. C.M. 1977/

Giles, M. A. and W. E. Vanstone. 1976. Changes in ouabain sensitive adenosine triphosphatase activity in gills of Coho salmon (*Oncorhynchus kisutch*) during parr-smolt transformation. J. Fish. Res. Bd. Can. 33: 54-62.

Hoar, W. S. 1976. Smolt transformation: evolution behavior and physiology. J. Fish. Res. Bd. Canada. 33:1233-1252.

Komourdjian, M. P., R. L. Saunders and J. C. Fenwick. 1976. Evidence for the role of growth hormone as a part of a 'light-pituitary axis' in growth and smoltification of Atlantic salmon (*Salmo salar*). Can. J. Zool. 54: 544-551.

Korn, S. and D. Macedo. 1973. Determination of fat content in fish with a nontoxic, noninflammable solvent. J. Fish. Res. Board Can. 30:1880-1881.

Lowry, H., N. H. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Miller, David S., William B. Kinter, David B. Peakall, and Robert W. Risebrough. 1976. DDE feeding and plasma osmoregulation in ducks, guillemots, and puffins. Am. J. Physiol. Vol. 231, No. 2.

McCartney, T. H. 1976. Sodium-potassium dependent adenosine triphosphatase activity in gills and kidneys of Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. 53A: 351-353.

Parry, G. 1958. Size and osmoregulation in salmonid fishes. Nature, 181: 1218.

Parry, G. 1960. The development of salinity tolerance in the salmon, *Salmo salar* (L.) and some related species. J. Exp. Biol. 37:425-434.

Saunders, R. L. and E. B. Henderson. 1970. Influence of photoperiod on smolt development and growth of Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada. 27: 1295-1311.

Wagner, H. H. 1974a. Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). Can. J. Zool. 52: 219-234.

Wagner, H. H. 1974b. Seawater adaptation independent of photoperiod in steelhead trout (*Salmo gairdneri*). Can. J. Zool. 52: 805-812.

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

Zaugg, W. S. and H. H. Wagner. 1973. Gill ATPase activity related to parr-smolt transformation and migration in steelhead trout (*Salmo gairdneri*): influence of photoperiod and temperature. Comp. Biochem. Physiol. 45B: 955-965.

Zaugg, W. S. & L. R. McLain. 1976. Influence of water temperature on gill sodium, potassium-stimulated ATPase activity in juvenile Coho salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol. 54A: 419-421.