Seasonal Changes in Osmotic Pressure of Flounder Sera

Abstract. The freezing point depression of sera collected from flounder during the winter averaged 1.15°C, whereas it averaged 0.63°C for sera collected during the summer. Elevated concentration of sodium and chloride ions were found for the winter samples of sera, but NaCl explained only a fraction of the seasonal increase in osmolarity.

During the winter of 1958–59, when temperatures of as low as −0.8°C were recorded in the bottom waters of the Mystic River estuary in eastern Connecticut, adult winter flounder, *Pseudopleuronectes americanus* (Walbaum), were observed to migrate into the estuary to spawn. Experiments during this season on minimum temperature tolerance indicated that death, which was accompanied by freezing of tissues, occurred in the fish between −1.0° and −1.5°C; these are abnormally low temperatures in view of the usual range of freezing points of teleost blood, from −0.5° to −0.8°C (*1*).

These observations led to investigation of the osmotic pressure (as determined by freezing point) of flounder sera in the winter, and to a comparison with that found in the summer. Flounder in both seasons were collected within the estuary, where the salinity was 22 to 30 per mil. Most of the fish collected in the winter were ripe adults, which do not normally feed prior to spawning; the flesh of these fish was soft and watery compared with that of fish collected in the summer. Blood was obtained by heart puncture from fish 24 to 44 cm long, during the periods June–August 1959 and January–February, 1959 and 1960. Heparinized blood samples were centrifuged at 500 rev/min for 5 min. Each serum sample was pipetted into a Pyrex vial, which was sealed and stored in a deep-freezer. The osmolarity of serum samples was measured with a Fiske osmometer in January 1960.

There was a striking seasonal difference in the osmotic pressure of flounder sera. The average freezing-point depression for 18 fish collected in the winter, including those collected in the relatively mild winter of 1959–60, was 1.15°C (S.D., 0.13), a finding in agreement with the indications of tolerance experiments. In contrast, the freezing point depression for sera from 19 fish collected in the summer was significantly lower, 0.63°C (S.D., 0.04), and within the range of osmolarity for "normal" marine teleosts.

Since chloride and sodium ions are principal electrolytes in fish blood, analyses were made to determine to what extent they contributed to the seasonal differences in serum osmolarity. Analysis for chloride was made through a microadaptation of the method of Schales and Schales as proposed by Burden (*2*), in which 5 mm^3 of serum and 50 mm^3 of indicator solution were transferred into titration tubes with the aid of constriction pipettes. Titrations were made without deproteinization by means of a microburette and magnetic stirrers. The accuracy of this method was checked through analyses in which standard sodium chloride solutions and clinical human serum of known chloride content were used. Sodium concentration was determined with a Beckman DU spectrophotometer with a flame attachment. Serum solutions and standards (0.2 to 1.6 percent NaCl) were mixed with 0.02 Sterox in the proportion of 1 to 500. A blank and a clinical serum standard were run for each fish-serum determination. The variation in replicate determinations averaged 5 percent for the sodium and 2 percent for the chloride.

Although variable, the results of the chloride and sodium analyses (Fig. 1) showed clear seasonal differences. The concentrations of both were higher in the winter. The mean difference between the chloride and the sodium contents for individual fish was not significant for fish collected in the summer (t = 1.8, 19 degrees of freedom), but the difference was significant for those collected in the winter (t = 4.4, 12 degrees of freedom); hence higher levels of sodium than chloride are indicated during the winter.

Osmolarity and concentration of sodium chloride (based on concentration of chloride) are plotted for individual fish in Fig. 2. Even though there was an elevation of sodium chloride in winter, this accounts for only a portion of the winter increase in osmotic pressure. Whereas an average of 83 percent of the total osmolarity for sera collected in the summer was accounted for by sodium chloride, only 57 percent of the winter total was attributable to sodium chloride. In other words, most of the increased osmolarity (about 0.4°C or 0.2M) was due to the presence of other substances.

These results for the winter flounder are very similar to results reported by Scholander *et al.* (*3*) for two species of fishes living near the surface in an arctic fjord; for these fish the freezing point of blood was −0.7° to −0.9°C in the summer and −1.3° to −1.6°C in the winter. Levels of sodium chloride (based on levels of chloride) accounted for about 80 percent of the total osmolarity in the summer but for only about 50 percent in the winter.

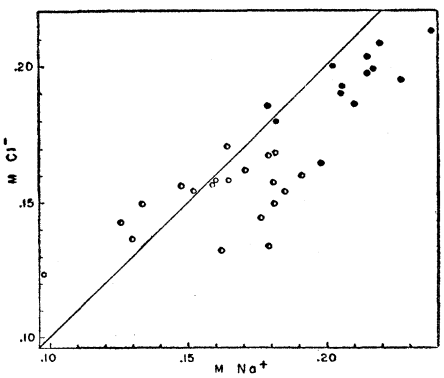

Fig. 1. Molarity of flounder sera, based on concentrations of chloride and sodium in samples collected in winter (solid circles) and summer (open circles). The diagonal line represents equivalent molarities.

Although Woodhead and Woodhead (*4*) did not report such distinct seasonal changes in the total osmotic pressure in sera from the Atlantic cod, elevations in concentrations of sodium and chloride were found in cod captured in the winter from water below 2°C. They interpreted these seasonal changes in levels of sodium and chloride as being due to a breakdown of the osmoregulatory ability at low water temperatures in the winter. (Other factors are postulated to explain the apparent lack of osmotic imbalance at low temperatures during the summer.)

If the seasonal changes in osmolarity reported here and by Scholander *et al.* were in fact due to osmoregulatory failure in the winter, as suggested by

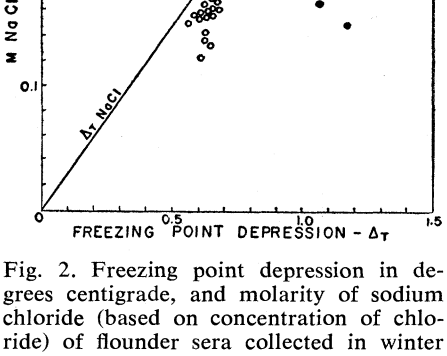

Fig. 2. Freezing point depression in degrees centigrade, and molarity of sodium chloride (based on concentration of chloride) of flounder sera collected in winter (solid circles) and summer (open circles).

Woodhead and Woodhead, then we would expect that the main increase in osmolarity could be ascribed to an increase in sodium chloride, the major sea-water salt. However, sodium chloride explained only a part of the difference in osmotic pressure, and the proportion of the total osmotic pressure due to sodium chloride decreased in the winter (see Fig. 2). Therefore the theory of osmotic imbalance does not appear to offer a complete explanation of the results of all these studies. As suggested by Scholander *et al.*, the high osmotic pressure may have some adaptive significance as a protection against freezing for fishes inhabiting cold, shallow waters (*5*).

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References and Notes

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First-Year Breakdown of Leaf Litter in Southern Appalachian Forests

Abstract. Breakdown of organic matter, an important step in the ecological circulation of chemical elements, was measured in Great Smoky Mountain and Oak Ridge forests. Greatest variation in first-year weight loss of leaves in nylon net bags was due to species (*Fagus grandifolia* 21 percent, *Acer saccharum* 32 percent, *Quercus shumardii* 34 percent, *Quercus alba* 39 percent, *Morus rubra* 64 percent). At elevations of 5200, 3400, and 850 ft, losses for all five species averaged, respectively, 29, 34, and 40 percent for leaves placed in spruce, hemlock, and pine stands, and 35, 40, and 46 percent for leaves placed in beech, cove hardwood, and whiteoak stands.

The Southern Appalachian forests have long attracted attention for their floristic diversity and complexity of vegetation pattern (*1*), but the opportunities which they offer for the study of ecological processes in contrasting natural environments deserve at least as much attention. The first-year results in a long-term experiment show remarkably systematic effects of contrasting climate, forest cover (evergreen versus deciduous), and species of leaf on the rates of breakdown of leaves on the forest floor. Such differences influence the release of nutrients to the soil and their availability to growing plants. The rate of this cycling of chemical elements from plants to litter to soil and back to plants is important in relation to the metabolism of the ecological system and to the movement through this system of radioactive isotopes originating from fallout and radioactive waste disposal operations (*2–5*).

Forests in the Great Smoky Mountains and at Oak Ridge have already been shown to exhibit a great range in accumulation of litter and humus (*6*). The differences are presumably due more to contrasting rates of breakdown than to variations in productivity (*3, 4, 7*). Differences in breakdown are influenced by both the litter species and the environment in which the litter is decomposing—factors which are confounded under natural conditions. The present study was designed to isolate the effects of these factors. Weighed samples of recently fallen leaves of five deciduous tree species were placed in bags of nylon net in paired deciduous and evergreen forest stands typical of three contrasting altitudinal climatic belts of the Southern Appalachian region (*1, 8*).

Of the field procedures previously used (*9–11*), that of Bocock and Gilbert (*10*) was most similar to ours, but we used larger samples in bags of smaller mesh size. The nylon net with 2.3-mm holes was fine enough to restrict loss of leaf fragments, yet coarse enough to admit a rich litter fauna (*12*). Rectangular net bags, 45 by 60 cm, were loaded with 50-g samples of leaves from large air-dried homogeneous supplies collected, soon after falling, at Oak Ridge (mulberry, *Morus rubra* L.; sugar maple, *Acer saccharum* Marsh.; Shumard red oak, *Quercus shumardii* Buckley), at Knoxville (white oak, *Quercus alba* L.), or at the beech stand where one set of litter bags was placed (beech, *Fagus grandifolia* Ehrh.). A safety pin closed the bag and attached it to a stainless steel nail anchored in the forest floor. The 50 g of leaves were well distributed initially over approximately 40 by 50 cm, but slow downhill creep tended to reduce this area by the end of the year.

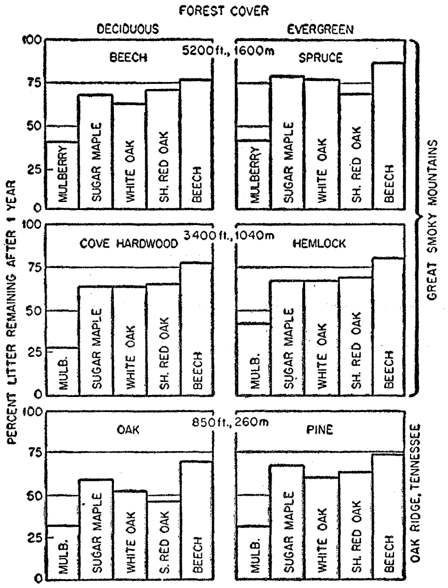

Fig. 1. Leaf litter from five tree species at end of first year, in six Southern Appalachian forests (evergreen versus deciduous stands at three elevations).

In mid-December 1958, five bags were distributed in each of four ramdomized blocks in each of the six forest stands, only one block being used in the study here reported. During early inspections at approximately monthly intervals, most herbs (*Claytonia, Oxalis, Stellaria*) growing into the bags were gently pulled out; any remaining herbs or other extraneous materials (spruce or hemlock needles) were removed before final weighing. After 5½ mo, one set of bags was dried on Tullgren funnels for extraction of the soil fauna (*12*), weighed air dry (30 percent relative humidity), and returned to the field in less than 2 wk (*4*). Extraction of the fauna, removal of extraneous materials, and final weighing were carried out at the end of 12 mo on a full set of 30 samples (Fig. 1), and the samples were retained for chemical analysis. Other blocks were left for future collection and comparison with additional bags installed in 1959.

Analysis of variance and regression (Fig. 2) shows the main effects of all three variables to be highly significant. In the split plot analysis of variance in Fig. 2, "error I" (for effects between stands) happens to be smaller than "error II" (for comparisons of leaf species within stands), but the main effects are highly significant with respect to either estimate of error. Interactions of altitude and forest cover, leaf species and cover, and species and altitude are not statistically significant ($F < 1$) and are far smaller than the main effects.