

CONTRIBUTIONS FROM THE BIOLOGICAL LABORATORY OF THE BUREAU OF FISHERIES AT WOODS HOLE, MASS.

**THE PHYSIOLOGICAL EFFECTS UPON FISHES OF CHANGES IN THE
DENSITY AND SALINITY OF WATER**

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INTRODUCTION.

It is a commonplace to both fisherman and zoologist that certain fishes dwell exclusively in salt water and others exclusively in fresh water, while others yet may live indifferently in either medium. It is well known, also, that death is frequently the result of an abrupt and considerable change of density.

Numerous problems of great physiological importance are here involved. Why is an extreme change of density so fatal in some cases and so harmless in others? And is it the change of density which is responsible for the harmful effects, after all? May not salt water be toxic, in a narrower sense, to fresh-water fishes, and vice versa? In any case, what is the immediate cause of death? Are the limiting membranes of a fish permeable to both water and salts, or are they only semipermeable? Or, are they, perhaps, impermeable to both? And are all of the limiting membranes alike in this regard? Likewise, is their condition the same for all species and under all circumstances? These are closely related questions. They have received many and quite contradictory answers. It is hoped that the experiments discussed in the ensuing pages have contributed something toward their solution.

The first of these experiments were chiefly concerned in determining whether a given change in water density was harmful to a given species of fish, records being kept of the rate of death. In a second series weight determinations were made with a view to ascertaining whether such changes in the density of the surrounding medium were accompanied by appreciable osmotic effects upon the fishes. Third, it was sought to discover whether the membranes were permeable to water only or to salts as well. The passage of salts from the fish into the surrounding water was tested chemically, and likewise the salt content of the tissues of fishes of several species under different conditions was determined. Finally, a series of experiments was performed with a view to discovering whether such osmotic exchanges were confined to the gills or whether the general body integument was likewise concerned.

The experiments here described were carried on during the summers of 1904 and 1905 at the biological laboratory of the Bureau of Fisheries at Woods Hole, Mass., and during the spring of 1905 at the New York Aquarium. In the earlier portion of this work I was aided by Mr. D. W. Davis, assistant in this laboratory. The work at the New York Aquarium was rendered possible through the kindness of the director, Mr. C. H. Townsend, who placed at my disposal a room equipped for research, and provided me with abundant material throughout the course of the experiments. I must acknowledge, also, the ever-ready help of several members of his staff. My thanks are likewise due to Prof. W. C. Sabine, of the department of physics of Harvard University, for valuable criticism.

THE DEATH OR SURVIVAL OF VARIOUS SPECIES AFTER CHANGES IN THE SALINITY OF THE WATER.

THE FATAL EFFECT OF FRESH WATER UPON VARIOUS SALT-WATER FISHES.

My experiments were originally undertaken from a biometric rather than a physiological point of view. It was my object to determine measurable indices of fitness and unfitness among the individuals of a species, somewhat as Bumpus had done for sparrows and Weldon for crabs. Different modes of elimination were chosen, chiefly asphyxiation and change from salt to fresh water. The individuals of the more and the less resistant halves of each lot of fish so treated were subjected to statistical study and differences in type and in variability were noted. These biometric studies are still far from complete and no statement of the results is here attempted. My attention has for a time been diverted to the physiological questions which form the basis of the present paper.

The first experiments recorded illustrate the fatal effects of transfer to fresh water upon our three local species of killifish (*Fundulus*). These are all small fishes, restricted to shallower waters. *F. majalis* and *F. heteroclitus* are marine littoral forms, occurring particularly on weedy shores, though both species, and especially the latter, pass into the brackish waters. Bean (1903) says of *heteroclitus* that it sometimes ascends streams beyond tidewater, and regarding *majalis* he sees "no reason to doubt its occurrence, even in fresh water," while Eugene Smith (1897) states of *heteroclitus* that it "is often found landlocked in ice or quarry ponds." Dr. H. M. Smith informs me that the latter species "is found permanently in the vicinity of Washington, in the Potomac and its tributaries, and also in ponds." Mr. Vinal Edwards likewise reports a case where this fish was found in fresh water above a milldam. Fresh water is not, however, the more usual habitat of *F. heteroclitus*. *F. diaphanus*, on the contrary, is predominantly a brackish and fresh water fish, extending from the coast far into the interior, and occurring in lakes and streams as far west as the Mississippi River, and northward to Minnesota.^a These three species are readily distinguishable from one another in form and color. They are very different in their habits and in various physiological characters. Accordingly, the species should always be specified in any work dealing with a member of this genus.^b

^aThe western representatives of the species have been given the rank of a distinct variety, *menona*.

^bFor descriptions and illustrations of these fishes the reader is referred to Jordan and Evermann's "Fishes of North and Middle America," and to Garman's "The Cyprinodonts" (1895).

The first three experiments deal with *F. majalis*.

Experiment 1.^a

August, 1902. Three hundred and forty-four fishes put into tank of salt water and latter displaced by fresh. Fifty per cent of the fish died during first 24 hours; only 1 fish living at end of 3 days.

Experiment 2.

August, 1904. Twenty-five picked specimens placed abruptly in tank of fresh water. All but 1 dead in 18 hours; all dead within 24 hours.

Experiment 3.

September, 1904. Twenty-five picked specimens placed abruptly in fresh water. Fish fed throughout experiment. Thirty-six per cent dead at end of first day; last fish dead in 6 days.

The fishes here used had been taken in sea water of full strength, and kept, prior to the experiments, in tanks of running salt water of a mean density of about 1.023 (reduced to 4° C.) and a mean temperature ranging from 20° to 22° C. The change of temperature in the transfer from salt to fresh water was very slight (probably never exceeding 3° C.) and can have played no part, here or in later experiments, in causing the death of the fishes.

The next two experiments illustrate the relative mortality of *F. majalis* and *F. heteroclitus*, and, in the second case, of *F. diaphanus*.

Experiment 4.

July, 1904. Thirty-three *F. majalis* and 71 *F. heteroclitus* put into tank of salt water, this being displaced by running fresh water as in experiment 1.

F. majalis. No deaths during first day; 42 per cent dead within 2 days; 88 per cent dead within 3 days; the last in 4½ days.

F. heteroclitus. First death in 3½ days; 75 per cent dead within 6½ days; all dead within 7½ days.

Experiment 5.

July, 1904. Twenty-eight *majalis*, 32 *heteroclitus*, and 22 *diaphanus* were put directly into a tank of running fresh water, at 20° C. The *majalis* and *heteroclitus* came directly from sea water of full strength, the *diaphanus* from a tank of brackish water of specific gravity 1.006^b (originally from a brackish pond having water of specific gravity 1.005).

F. majalis died in from less than 12 to 36 hours; *F. heteroclitus* died in from 12 hours to 10 days; *F. diaphanus* died in from 5 to 14 days. All of *majalis* had died at a time when only 7 *heteroclitus* (22 per cent) and when no *diaphanus* had died; and all of *heteroclitus* had died at a time when only 59 per cent of the *diaphanus* had died.

The order of death of these three species when subjected to asphyxiation in stale water was found to be (1) *diaphanus*, (2) *majalis*, (3) *heteroclitus*.

Experiment 6.

Eighty-seven per cent of the *diaphanus* had died when only 35 per cent of the *majalis* had died and when all of the *heteroclitus* were still living.

Experiment 7.

Results similar to experiment 6.

^a These experiments are not numbered in chronological order, but are throughout arranged solely with reference to subject-matter. The first twelve are among those conducted at Woods Hole.

^b See p. 60.

Throughout all of these experiments, healthy fishes were selected unless otherwise specified. Fishes having the same history as those used for experiment were commonly kept in the reserve tanks and served for purposes of control. Special control experiments were likewise carried on from time to time, as will appear.

In a number of other experiments the harmful effects of transfer to fresh water upon *F. heteroclitus* were manifest.

Experiment 8.

August, 1904. Of 26 fishes, none died until the lapse of 6 days, when they began to succumb. Fifty-eight per cent had died in 10 days, when the experiment was discontinued.

Experiment 9.

August and September, 1904. The fishes began to die in 3 days, and 50 per cent died during the first 10 days. From the thirteenth to the nineteenth day, however, no deaths occurred and the experiment was discontinued.

Experiment 10.

September, 1904. Of 202 fishes used, 3 died during the first day, while 95 per cent died within 10 days.

Garrey (1905), speaking of some experiments of his own upon this species of fish, states: "It was found that if care was taken to select individuals which were not injured in catching, about 80 per cent lived in fresh water for 6 weeks, when the experiment was discontinued. This is as high a percentage as can be kept alive in the sea-water aquaria of the laboratory." Garrey's statement is surprising in view of my own results already cited, and of the confirmatory experiments recorded below. It is to be remarked that many of my experiments were carried on at about the same time as those of Garrey, and that the fresh water employed came from the same water system as that used at the Marine Biological Laboratory, where Garrey's work was done. The fishes that I used were generally treated with the utmost care and in many specified cases were fed throughout the experiment. Fishes from various localities were used. The conditions were likewise varied from time to time so that irrelevant factors might be eliminated.

Experiment 11.

July, 1905. Thirty specimens of *F. heteroclitus* put into a hatching box filled with running fresh water. Another 30 specimens taken from the same lot of fish were placed in an adjacent hatching box filled with running salt water. Both sets of fishes were fed, although those in the fresh water showed little desire for food. The temperature of the salt water was about 3° C. higher than that of the fresh.

In the fresh-water tank 1 fish died within 2 days, and the last died within 14 days. In the salt-water tank not a single fish died during this period.

Experiment 12.

Summer of 1905. The first fish died after 7 days in fresh water, and a single fish remained living at the end of 20 days.

It is to be added that I have given the results for all of the experiments of this nature performed by me, no contradictory figures being suppressed, here or elsewhere.

That the fresh water here used was not contaminated with any actively poisonous substance is shown by the fact that the addition of even a very small proportion

of salt water entirely prevented the harmful effects. No trace of fungus was to be observed and no surface lesions of any sort were discoverable. Lack of some or all of the saline ingredients of sea water seems to have been responsible for the death of the fishes. Perhaps the most curious feature in these results is the prolonged period of resistance shown by some of the fishes in each experiment. This suggests certain of the results of Bert (see p. 67).

Some observations made in New York City during the past winter and spring are interesting by way of comparison with the preceding results. In these later experiments the fishes came from marshes of brackish water, and were kept for some days or weeks prior to the experiments in water of a salinity slightly over half that of water from the open sea. The temperature at the time ranged from 2° to 16° C., that at Woods Hole having ranged from 20° to 22° C. These experiments were complicated by the appearance of fungus (*Saprolegnia*), which invariably devastated the tanks within a week or two after *F. heteroclitus* was placed in fresh water. Even here, however, it was noted that a certain proportion of those which died showed no traces of *Saprolegnia*.

Experiment 13.

New York, November and December, 1904. Two hundred and eleven *F. heteroclitus* transferred to fresh water. Thirteen died within the first 15 days before the disease appeared; 82 per cent of the remainder died during the next 16 days, after which the experiment was discontinued.

A certain proportion of these fishes died without being affected by the fungus, but it is of course impossible to determine what the death rate would have been if the *Saprolegnia* had not appeared. It must be remembered that fishes which are already weakened by other causes are more ready victims to the fungus. It was found in experiment 41, *c*, and others that the addition of even a small percentage of sea water served as a decided check to the appearance of the parasite. It is my belief that this preventive action of the sea salt was due not so much to a toxic effect upon the fungus as to a beneficial physiological effect upon the fishes, resulting in a greater power to resist the parasite.

Experiment 14.

It was here observed that the fungus, when once it had gained a foothold upon the fishes, continued to thrive after the latter were transferred to water of density 1.005, while few traces appeared upon fishes which were transferred in a healthy condition to water of this density.

In some cases (experiment 41, *c*) water of a much lower degree of salinity acted as an effective check.

It is evident from experiment 13 (which was repeated with similar results) that, under the conditions existing at the time, fresh water proved much less rapidly fatal to *Fundulus heteroclitus* than in the Woods Hole experiments, and this in spite of the fungus. The factor responsible for this difference may have been either (1) the lower temperature, or (2) the previous history of the fish (life in diluted sea water), or perhaps the two combined. It nevertheless seems quite probable, although no definite control experiments were made, that even here the fresh water had a deleterious effect.

It will be recalled that in experiment 5 the *F. diaphanus* finally succumbed to the effects of the fresh water, though outliving, on the average, both of the other species. The death of this species in fresh water was very surprising, since the specimens here used came from a brackish water pond (Tashmoo Pond, Marthas Vineyard) having a specific gravity of only 1.005 (corrected), and had been kept in the laboratory prior to the experiment in water of about the same specific gravity. It is to be remembered, too, that this species occurs in nature in fresh-water lakes and streams. The above results, however, were sustained by further experiment.

Experiment 15.

Woods Hole, September, 1904. Twenty-five healthy specimens of *F. diaphanus*, from a lot which had been kept in the laboratory (water 1.004 to 1.006) for 11 days, put directly into running fresh water. Fishes fed throughout. Two fishes died within 24 hours; 16 (64 per cent) died within 8 days, when the experiment was discontinued. Meanwhile, in a control tank of brackish water containing large numbers of this species, very few died.

Cyprinodon variegatus, a brackish and salt-water fish belonging to the same family as *Fundulus*, was chosen as a subject for similar experiment.

Experiment 16.

Woods Hole, August, 1904. Two well-fed specimens of *C. variegatus* from salt water placed directly in fresh water. Both were dead on the following morning.

The "white perch" (*Morone americana*), as is well known, may occur either in fresh, salt, or brackish water. It is not certain, however, that in nature the change is abruptly made.

Experiment 17.

Woods Hole, September, 1904. Of 10 specimens of *Morone* from brackish water (1.004 to 1.006), which were transferred to fresh, 4 had died and 1 sickened within 8 days, when the experiment was discontinued. Experiment 36 (p. 67) serves as a good control.

Experiments with *F. diaphanus* and the white perch during the summer of 1905 show that the capacity of different individuals to endure transfer either to fresh or to salt water varies greatly, depending upon the condition of the fishes. It seems likely that a white perch from brackish water may in some cases survive indefinitely after transfer to fresh water, though this is certainly not true of all specimens. Those which survive the initial period of mortality seem qualified to resist the harmful effects.

Of the typically marine fishes few were tested as to their ability to withstand abrupt transfer to fresh water, since this is well known to result fatally in the case of most species. Scup (*Stenotomus chrysops*) were found to die in from 1 to 3 hours, the fishes being taken from water of a specific gravity 1.023 and temperature about 20° C. Two puffers (*Spheroides maculatus*) died within 3 hours under the same conditions. Some cunners (*Tautoglabrus adspersus*) were dead at end of 12 hours (perhaps much less), and of 2 tautog (*Tautoga onitis*) 1 died within 12 and a second within 24 hours. Sculpin (*Myoxocephalus octodecimspinosus*), sea raven (*Hemitripterus americanus*), and sea bass (*Centropristes striatus*) all died within a day (probably much less).^a The

^a Experiments with these and the next two species mentioned were performed at the New York Aquarium, where the salt water had a much lower density. (See p. 68.)

winter flounder (*Pseudopleuronectes americanus*) survived considerably longer, as appears from experiment 48, while the tomcod (*Microgadus tomcod*), or at least some individuals, continued to live indefinitely after the change. It is to be remembered, however, that the tomcod is an anadromous fish.

EFFECT OF DISTILLED WATER.

Loeb (1900, p. 334) has stated that "*Fundulus* can be thrown from sea water into distilled water without any considerable swelling, or without any visible injurious effects." It is possible that Loeb would not regard death as a "visible injurious effect." In four experiments with *F. heteroclitus* (the species studied by Loeb) I found that death resulted after an interval of from less than a day to 3 days. Ten fishes at a time were put into several liters of distilled water. The latter was aerated in all cases but one, though this precaution was quite needless, since even a greater number of fishes may be kept in an equal quantity of sea water which is neither changed nor aerated. The fishes in my experiments came not from full-strength sea water but from the supply system of the New York Aquarium (at this time density=1.015). Whether or not "swelling" was evident before death I did not determine, but the dead fishes were found in one case to have gained more than 10 per cent in weight.

SYMPTOMS PRECEDING THE DEATH OF SALT-WATER FISHES IN FRESH WATER.

Fundulus heteroclitus, as stated above, took little or no food when in fresh water, sometimes tasting and then rejecting it, while those in the control tank crowded eagerly to get it. They were ordinarily far more sluggish in their movements than the normal fishes, but at times, especially when disturbed, they were subject to peculiar paroxysms. A fish so affected would start suddenly upon an erratic course around the tank, swimming in a zigzag or circular path and ending in a state of tetanus, the body rigid and twitching slightly. For a period the fish would remain nearly or quite motionless, commonly somewhat flexed, the mouth open and the gill covers widely extended. After a few minutes respiration was resumed and the fish gradually assumed a normal aspect. Sometimes my approach to the tank was sufficient to provoke these paroxysms in one or more fishes.

These same phenomena I have likewise observed very plainly in *F. diaphanus*, and in one instance in *F. majalis*. Most marine fishes, however, die much more rapidly than either of these, and quite different symptoms are manifested. Scup (*Stenotomus chrysops*), when placed in fresh water, settle at once to the bottom, and sooner or later "keel over," though this is generally deferred for some time. Respiration is at first nearly or quite normal, both as to rate^a and manner. Later it becomes irregular, the normal rhythm being interrupted at intervals by spasmodic gulps accompanied by general agitation. The fish from time to time darts violently about the tank, as if in an effort to escape, sometimes even leaping from the water. These frantic movements of the body recur at intervals, even after respiration has ceased. The rate of respiration does not decline gradually, but the rhythmic movements give place rather abruptly to convulsive ones, which soon cease altogether. As already stated, death occurs in from 1 to 3 hours after transfer to fresh water.

^aSix specimens were observed, their rate of respiration being determined by means of a stop watch. These individuals differed considerably in their reactions.

Puffers (*Spheroides maculatus*) upon the approach of death were found to inflate and deflate their bodies and to gnash their teeth quite audibly. The other symptoms were much the same as those described for the scup.

These phenomena (in those fishes which die speedily) undoubtedly suggest death from asphyxiation, and indeed this explanation has been offered to account for the fatal effects of fresh water upon salt-water fishes and vice versa (see p. 101.). A careful comparison of the preceding phenomena with those manifested by scup which were allowed to die from asphyxiation (the water supply to the tank being stopped) undoubtedly showed points of resemblance. There were in both cases disturbances of respiration, frantic endeavors to escape, and convulsive movements ending in death. In both cases, fishes were observed to eject water from the mouth while at the surface. There were, however, some characteristic differences in the symptoms, indicating differences in the physiological effects.

Bert (1871) states of the goldfish that when placed in salt water its respiration is at first accelerated and then retarded. No such rule was found to obtain in the case of the scup when allowed to die in fresh water, and indeed no constant change of rate occurred until shortly before respiration ceased.

EFFECT OF SURFACE ABRASIONS.

Bert (1883) notes that the removal of the mucus from a portion of the skin of an eel (fresh-water specimen) renders it vulnerable to the effects of salt water, which may otherwise work no harm.

Garrey (1905), like Bert, holds that the integrity of the skin is an important factor in determining whether or not certain changes of medium shall prove fatal. Furthermore, he argues that at least one salt-water fish (*F. heteroclitus*) will die in its normal medium if the skin be injured extensively:

A large number of healthy specimens were selected and about one-half the body surface denuded of scales by gentle scraping with the edge of a scalpel, or the skin was removed over an area of one square centimeter on each side; then they were divided into three lots and placed respectively into fresh, sea water diluted with an equal volume of distilled water, and normal sea water. Of those kept in fresh water in every experiment from eighty to ninety per cent died within twenty-four hours, while all died in less than thirty-six hours. In normal sea water the fish suffered a similar fate, although death did not intervene so soon. But of those kept in sea water of one-half its normal concentration only three per cent were dead at a time when all those in the other two media had died, and seventy per cent were kept alive for four weeks, when the wounds were all healed and the experiments discontinued. In these experiments, therefore, no deleterious effects obtain when the internal and external media are approximately isotonic.

In the case of the hypertonic or hypotonic solutions, however, Garrey believed that the damaged integument rendered possible a fatal osmotic action.

A priori it does not seem probable that every considerable surface abrasion should prove fatal either to a marine or a fresh-water fish in its normal medium. Indeed, we have abundant proof that the more hardy species can survive serious mutilations. Experiments of my own, moreover, suggested by those of Garrey, give results entirely contradictory to his.

In experiment 5, five of the 28 specimens of *F. majalis* were deprived of their scales throughout an area of about 1 square centimeter on the shoulder region. Of the 18 fish (64 per cent) which died during the first 12 hours, none were found to be the scraped ones.

Again, in experiment 45 (p. 74), specimens of *F. heteroclitus*, taken from salt water of density 1.007+^a, were thoroughly denuded of their scales throughout a strip about 1 centimeter in width and extending from the head to the dorsal fin. The fish were divided into lots of 16 each and put into water of five different densities.

In the fresh-water lot 2 died within the first 2 days and 6 within the first 7 days, when the experiment was given up owing to fungus, which appeared early and thus complicated the result. (Compare with experiment 41, *b*, in which normal fishes were used.) All of Garrey's fresh-water lot died within less than 36 hours.

The next lot were placed in water having a density of about 1.001 (sea water = 1.020 to 1.028). Only one fish (apparently one otherwise injured) died during 5 days, after which the experiment had to be given up owing to fungus.

The third lot, placed in the salt water of the aquarium supply (1.007), were all well at the end of 5 days, when the experiment was discontinued.

The fourth lot were put into a stronger salt water brought from Sandy Hook (density 1.014, i. e., about one-half the salinity of normal sea water). They were kept in a tub without aeration. One fish died in 5 days.

The fifth lot were placed in water of a density 1.025 (Sandy Hook water, strengthened by the addition of a commercial sea salt, thus raising the density somewhat above that of the sea water at Woods Hole). Three died within the first 4 days, after which 6 days more elapsed without a single death. It was found that the injured surfaces had healed and that the scales had begun to regenerate. All of Garrey's salt-water lot died, though he does not tell us how soon.

The changes in weight undergone by these scraped fishes are very instructive and will be dealt with later.

The temperature of the fresh water at this time was 8° to 9° C., that of the salt water being 4° to 6°. The fishes had not been fed for more than 16 days prior to the experiment.

During the summer of 1905 a few similar experiments were performed. The conditions were, however, necessarily different. The salinity of the water from which the fishes came was here more than three times that of the water at the New York Aquarium, while the temperature was of course very much higher.

Experiment 18.

Woods Hole, August, 1905. Thirty *F. heteroclitus* denuded of scales over one entire side of the body. Divided into lots of 10 each which were put into salt water, fresh water, and water of density 1.001, respectively. During the period covered by the experiment (14 days) the results were as follows: (*a*) In salt water, 3 dead, 7 alive and apparently well; (*b*) in fresh water, all dead in from 2 to 13 days; (*c*) in 1.001 water, none dead. In the last case (*c*) the water contained 1 part of fresh to about 25 parts of sea water.

Experiment 19.

Woods Hole, summer of 1905. Seven fishes in fresh water all died within 24 hours.

^a This being the density of New York Bay water at this time of the year. See p. 68.

Experiment 20.

This lot of fishes, in salt water, likewise showed a higher mortality than those in experiment 18, though here other causes probably complicated the results.

ACCLIMATIZATION.

Since certain of the fishes used in the above experiments are, in nature, often found in fresh water, it was thought that they might be accustomed to it in the laboratory by a comparatively rapid process of acclimatization.

Experiment 21.

Woods Hole, August and September, 1904. Twenty-five selected specimens of *F. heteroclitus* transferred from salt to fresh water through hourly steps of about 0.001 in specific gravity.^a First 2 fishes died 6 days after reaching fresh water; 40 per cent (10 fishes) died within first 11 days; only 2 more died within the following 19 days, when the experiment was discontinued.

It is evident that the harmful effects of the change to fresh water have not been prevented, though comparison with preceding experiments suggests that they have here been diminished.

Experiment 22.

Woods Hole, August and September, 1904. Twenty-six *F. heteroclitus* transferred from salt to fresh water through steps of 0.001 per day.^b First fish died 6 days after reaching fresh water; 1 more dead and another sick on the following day, when the experiment had to be discontinued.

It is to be noted that no deaths occurred during the 20 days occupied by the transfer and that all of the fish were healthy at the time of entering fresh water. Taken by itself this experiment would not seem convincing, but in connection with others it is significant.

If complete acclimatization to fresh water within a short period failed in the case of *F. heteroclitus*, it is not surprising that this proved true of *F. majalis* as well.

Experiment 23.

Woods Hole, August, 1904. Twenty-four large selected *F. majalis* transferred to fresh water through a series of hourly steps of 0.001 in specific gravity. (See footnote under experiment 22.) First 2 fishes died within 2 days after reaching fresh water; 50 per cent died within 3 days; last one died in 8 days.

Comparison with experiments 1 to 5 shows that the period of resistance was somewhat prolonged by even this brief period of acclimatization. The same phenomenon is even better illustrated by the next experiment.

Experiment 24.

Woods Hole, August and September, 1904. Same as last, except that daily steps of 0.001 (approximately) were substituted for hourly ones. Fishes fed throughout. First 2 died 4 days after reaching fresh water; last fish lived 9 days (then disappeared).

^a These fishes remained through one night, however, about midway in the scale.

^b The actual reading of the salinometer at 20° C. was here used, no correction being made for temperature. Hence the last step was much greater than the preceding ones, being in reality from specific gravity 1.003 to 1, instead of from 1.001.

Eugene Smith (1902) says of this species that it "can be accustomed to the [fresh water] aquarium by gradual steps," but he does not state how gradual these steps must be.

Experiment 25.

Woods Hole, August and September, 1904. Three *Cyprinodon variegatus* treated as in preceding case. Two died 3 days and one 4 days after reaching fresh water. (Compare experiment 16.)

Experiment 26.

Woods Hole, August and September, 1904. Thirteen cunners (*Tautoglabrus adspersus*) treated as were the fishes in the two preceding experiments. All died within 12 hours after reaching fresh water though apparently healthy till this occurred.

Experiment 27.

Woods Hole, August and September, 1904. Nine black-fish (*Tautoga onitis*) treated as were fishes in experiments 24, 25, and 26, except that an additional step was interposed just before the change to fresh water, which was thus reached somewhat less abruptly. Nearly all of these fish died within 12 hours and all died within 24 hours after reaching fresh water. Up to this time they appeared to be in perfect health.

The foregoing experiments prove, if anything, that fresh water itself is fatal to the fishes under consideration, and that the degree of abruptness with which the change is made is of secondary importance. There is evidence, nevertheless (experiments 21 to 25), that a certain modification may result whereby the fatal effect of the final change is deferred. A partial acclimatization of a somewhat different character is reported by Bert (1871), who succeeded in accustoming various fresh-water fishes to water of half the density of that of the sea, though abrupt change to this proved fatal. De Varigny (1892), likewise accustomed young eels to water containing five-tenths per cent sodium chloride, though the additions of salt had to be very gradual.

ENDURANCE IN WATER OF LOW SALINITY.

It is plain from certain of the preceding experiments that some fishes which die quickly in pure fresh water will endure, for short periods at least, water of a very low degree of salinity. Special experiments were made with a view to testing this point.

Experiment 28.

Woods Hole, September, 1904. Seventy-five specimens of *F. heteroclitus*, from same lot of fishes as those used in experiment 10, were changed from salt water (density 1.023)^a abruptly to water of density 1.004. No deaths in 11 days, after which experiment was discontinued. Compare with experiment 10, for which this serves as a good control.

Experiment 29.

Woods Hole, September, 1904. Twenty-five *F. majalis* subjected to same treatment and with same results.

^a Unless otherwise specified the density here given has been corrected for temperature—i. e., the specific gravity at 4° C. is given.

Experiment 30.

Woods Hole, September, 1904. Agrees with the preceding except that the water density was 1.003. The results were the same.

Experiment 31.

Water of density 1.002 was employed, and only 1 fish died, on the 10th day, after which the experiment was discontinued.

During the season of 1905 some individuals of this species were found to survive 15 days (i. e., until the experiment was discontinued) in water having only about 3 per cent of the salinity of the local sea water (experiment 31 bis). In this case, however, a large majority of the fishes had died in the meantime, and it would therefore seem that the physiological limit of dilution had been passed. In a few cases the same symptoms were observed as in the case of *F. heteroclitus* in pure fresh water. (See p. 61.)

The same result is less strikingly shown in the case of sculpins (experiment 46). Two of these fishes put into fresh water died within 24 hours (perhaps much less), while of 3 from the same lot which were put into water having a specific gravity of about 1.001^a 1 survived 6 days, when death occurred, the remaining 2 for 3 days, when they were put back (living) into salt water.

The water used in the last two experiments had only 3 to 4 per cent of the salinity of pure sea water. If the mere change in the osmotic pressure of the surrounding medium were responsible for the harmful effects, it would not be expected that transfer to water of a very low degree of salinity would result in little or no harm to the fish, when transfer to pure fresh water was so quickly fatal. This salutary influence of a very small proportion of salt in the water is later shown to be related to an important difference in its effect upon the salt content of the body (pp. 90 et seq). The latter is shown to undergo a serious diminution in fresh water, while little or no diminution occurs if the water be even slightly saline.

EFFECT OF TRANSFER OF BRACKISH AND FRESH WATER FISHES TO SALT WATER.

Experiments 32-35.

Woods Hole, July, August, September, 1904. *Fundulus diaphanus* from a brackish pond (density 1.005 to 1.006) transferred directly to sea water. The record of deaths in the four cases reads:

Experiments.	1 day.	2 days.	5 days.	13 days.
32 (30 fish).....	1	1	2	13
33 (25 fish).....	12(?)	^a 20
34 (50 fish).....	30	34	88	(?)
35 (25 fish).....	0	1	5

^a Discontinued.

These differences in the death rate are doubtless due to the condition of the fishes at the time of experiment. Those used in experiment 33, for example, had remained unfed for about 3 weeks. During the summer of 1905 the experiments were repeated with similar results, confirming (1) the harmful effects of this abrupt change of water density, and (2) the variability of different lots of fishes in their capacity to endure

^a On some occasions the salinity fell to about one-half of this.

the change (in some lots a large majority survived). These experiments show furthermore that those fishes which survive the first few days after the transfer to salt water may continue to live in the latter for an indefinite period (one set observed as much as 23 days). It is curious to relate that the survivors in experiment 32 (9 fishes at the end of 19 days), upon being transferred abruptly to fresh water, continued alive for the next 10 days, after which their history was not noted.

Several experiments with the white perch (*Morone americana*) indicate that an abrupt change from slightly brackish water to full-strength sea water is generally fatal, under laboratory conditions at least. Here again a great deal depends upon the condition of the fishes. During the present season, in several lots transferred to salt water, the majority of individuals were dead within 24 hours. One experiment during the preceding summer, however, gave different results.

Experiment 36.

Ten fishes transferred to salt water survived 8 days, after which they were not observed.

It must be noted, however, that the brackish water was more saline in the latter experiment, and consequently the change in density was not so great.

Young of the chinook salmon (*Oncorhynchus tshawytscha*), weighing from 8 to 30 grams, which had been reared in fresh water, were placed abruptly in water of density 1.013^a without harm, but this is not surprising in view of the life history of this species.

A few species of exclusively fresh-water fishes were likewise experimented upon, but it was not thought necessary to do so with many, since the commonly fatal effects of salt water upon these fishes are already well known. Three yellow perch (*Perca flavescens*) and three sunfish (*Eupomotis gibbosus*) were dead after 14 hours (probably much less) in the diluted salt-water supply of the New York Aquarium (density 1.015 at the time). One catfish (*Ameiurus nebulosus*) was found dead after 18 hours in water of density 1.014. Several of this species in another experiment were nearly dead after two days, but in this latter case the density of the water was 1.010. Hence the harmful effects were naturally diminished. Four rudd (*Leuciscus erythrophthalmus*), put directly into water of density 1.010, died some time between 24 and 48 hours after the change. In another case (experiment 50) 8 of these fishes died within 24 hours in water of density 1.011.

Bert (1871, 1873, 1883) has discussed at considerable length the fatal effects of transferring fresh-water fishes to salt water. He gives a list of species experimented upon, with the average time which elapsed before death in each case, ranging from eighteen minutes for the "ablette" (a cyprinoid) to a period of from one day to a month or more in the case of the eel. Bert points out the effect of higher or lower temperature in hastening or retarding the death of fishes under these conditions. He likewise describes the symptoms attending the death of a goldfish when it was thrown into sea water: violent agitation, followed by quiescence; rise of the fish to the surface, due to lower specific gravity; disturbances of respiration (at first accelerated, then retarded); changes in the color of the gills; opacity of the crystalline

^aThis was the density of the salt-water supply of the New York Aquarium at the time. It is likely that they would have withstood the transfer to full-strength sea water equally well.

lens; increased secretion of mucus; loss of weight. The muscles responded to stimuli, and the heart continued beating after all external signs of life had disappeared. Bert found that his fishes would endure abrupt transfer to mixtures of fresh and salt water, provided that not more than one part of sea water to two parts of distilled water were used. He states that the fatal effects commence at the point where the water is capable of exercising an exosmotic action on the fish, though he gives no evidence for this view. By a gradual process of acclimatization, however, he accustomed fresh-water fishes to live in water having one-half the salinity of that of the sea. The specific gravity of such individuals was thereby raised, for, if returned to fresh water, they were found to sink to the bottom.

Conversely, Bert states that in the case of certain unspecified sea animals "acclimatization takes place easily up to a diminution of about a third in the salinity of the sea water, and that beyond that death comes very readily." That this latter conclusion is unjustified in the case of the great majority of marine fishes is shown in the next section.

Bert's explanation of the "mechanism of death," in the case of fresh-water fishes transferred to salt water, will be discussed later.

CHANGES IN WATER DENSITY AT THE NEW YORK AQUARIUM.^a

The present salt-water supply of this aquarium is derived from New York Bay, which, as is well known, receives the waters of the Hudson River and a number of lesser streams, and contains in consequence a much-diluted sea water. Its specific gravity rarely attains and perhaps never exceeds 1.016, while during the spring months it falls at times to 1.006, and occasionally lower. Considering the density of water in the open sea as 1.027 (the mean for the North Atlantic), the water supply of the New York Aquarium never reaches two-thirds, and occasionally falls to one-fifth of this. Nevertheless, marine fishes of 173 species^b, belonging to 72 different families, have been kept in this water, some of them surviving for a considerable number of years. Except in the case of fishes from the immediate vicinity, where the water is also of low density, the new arrivals undergo an abrupt change from full-strength sea water to this much-diluted water of the aquarium. This is true, for instance, of more than 50 species of fishes from Bermuda, many of which have been kept very successfully under these unnatural conditions, some individuals having survived as much as 7 years. It must be added, however, that these semitropical fishes are received only during the summer months and that the water containing them is warmed during the winter.

EFFECT OF ALTERNATION OF FRESH AND SALT WATER.

Experiment 37.

Woods Hole, August and September, 1904. Twenty-five *F. heteroclitus*, from sea water, put into fresh and salt water alternately, one change being made daily. During the 13 days of alternating density only 1 fish died. Compare with experiment 9, in which more than half of the fish (taken from the same stock but kept in fresh water) had died in this period.

^a These facts are furnished through the courtesy of the director, Mr. C. H. Townsend.

^b Based upon an incomplete list.

Experiment 38.

Woods Hole, September, 1904. Twenty-five *F. majalis* changed daily from salt to fresh water, and vice versa. No harmful results during the 8 days through which the experiment continued. (Compare with experiments 1 to 5.)

Experiments 39 and 40.

Woods Hole, August and September, 1904. *F. diaphanus* was used. In each case the effect of this treatment was the death of a larger or smaller proportion of the fishes, though perhaps the daily handling had something to do with the result.

In experiments 37 and 38 it was seen that no harm resulted from the alternation of fresh and salt water within the period of observation. Previous experiments had shown, however, that fishes of the same species when left uninterrupted in fresh water sooner or later succumb. Giard (1900) narrates the case of a stickleback which lived in this way for many weeks. Rutter (1904) states that young Pacific salmon when transferred from fresh water to diluted sea water fared better "when the density alternated from low to high and back again." There is an obvious parallel between such fluctuations of salinity and those which result from the ebb and flow of the tide at a river's mouth.

AGE IN RELATION TO ENDURANCE OF CHANGE OF MEDIUM.

According to Rutter (1904), the young of the quinnat salmon could endure progressively higher degrees of salinity as they grew older, ranging from 25 per cent sea water at 6 days to practically pure sea water at 2 months.

Loeb (1894) records a similar correlation between the age of the embryo of *Fundulus (heteroclitus)* and the proportion of NaCl which could be added to the sea water without arresting development.

Brown (1903) states that "there is a gradual increase in susceptibility to osmotic changes and to the electric current as the embryo develops." He regards the latter susceptibility as a function of the former.

A considerable series of measurements of *F. majalis* failed to reveal any selective mortality in relation to size among fishes dying from the effects of fresh water. Such a selective mortality was very obvious, on the other hand, in the case of death from asphyxiation, the "eliminated" set having an average length about 7 per cent greater than the "surviving" set (i. e., those last to die).^a

ADAPTATION TO CHANGES IN THE MEDIUM IN NATURE.

That many species in nature may be found in either medium is well known. All of the anadromous fishes of course belong to this class, together with the eel (catadromous). Many other salt-water fishes venture into streams and may even become landlocked. Again, fresh-water fishes may be carried down to the sea by freshets and survive in waters of considerable salinity. In most cases it is impossible to determine how abruptly the changes are made. The ascent of a large river may occupy weeks, and during the earlier stages of the journey a zone of gradually decreasing (generally of fluctuating) density is passed through. I am informed by

^aI hope before long to present these results in a form which will meet the demands of the biometrical critic.

Dr. Evermann, however, that in the case of the Pacific salmon the passage into the fresh water may be made quite abruptly at the mouths of the smaller streams.

Mather (1881) publishes a list, based mainly upon the authority of J. W. Milner and G. B. Goode, of "fishes which can live in both fresh and salt water." This list aggregates 33 species, and it would probably be possible to enlarge it very greatly.^a Nothing is said, however, regarding the duration or circumstances of the change of medium, and physiologically these are of course highly important. By sufficiently slow acclimatization almost any change of habitat seems possible, and indeed such a process must have occurred on an enormous scale in the evolution of fishes.

CHANGES OF WEIGHT RESULTING FROM CHANGES IN WATER DENSITY.

CHANGES OF WEIGHT IN LIVING FISHES.

Thus far the discussion has been confined mainly to the death or survival of the various fishes under the conditions of experimentation, but little analysis being attempted of the phenomena involved. It is evident that such changes of density as we have dealt with involve enormous changes in the osmotic pressure of the medium with which the fish is bathed. In relation to the latter, the limiting membranes of a given fish might be (1) impermeable both to water and to salts therein dissolved; (2) semipermeable, permitting the passage of water, but barring the salts; (3) permeable to both in greater or lesser degree. If the first condition were realized, no change in weight ought to occur following the transfer of the fish from one medium to another; if the second condition obtained, we should expect an increase of weight in hypotonic solutions and a decrease in hypertonic ones; while in the third case we should expect the same results in a somewhat lesser degree, unless, indeed, the membranes were equally or almost equally permeable to water and salts, in which case the conditions necessary for osmotic action would be wanting.

It must be remembered, however, that we are dealing with living matter, bounded by living membranes, and that the conditions may in consequence be much more complicated than outlined in the foregoing scheme. Various so-called "vital" (i. e., as yet unexplained) factors may intervene. Thus the degree of "permeability" of a given membrane may not be a constant quantity; it may be found to be in some way under the regulative control of the organism, and to vary greatly at different moments and under different conditions. Again, it may be that chemical factors intervene, and that an imbibition of water may at times occur which is not accounted for by the laws of osmotic pressure alone; and indeed we have already noted a vast difference between the physiological effects of fresh water and of slightly saline water, a phenomenon not to be accounted for by their difference of osmotic pressure.

If the weight of a given fish remained constant in a given medium^b, it might, then, be concluded either (1) that the medium was nearly or quite isotonic with the body fluids of the fish, or, if not, (2) that the membranes of the fish were for the time being impermeable to water, or (3) that they were more or less permeable to both salts and water, but to one in nearly the same degree as to the other.

^a Dr. Gill has informed me of several examples not included in Mather's list.

^b Always allowing for loss due to waste.

If the fish lost in weight, it would be necessary to conclude that the medium was hypertonic.^a But the limiting membranes, in order to produce this effect, might be either semipermeable (entirely preventing the passage of salts), or permeable to both water and salts, though to the latter in a lesser degree than to the former.

If a gain of weight occurred, it might be concluded either (1) that the medium was hypotonic, in which case the membranes might belong to either of the categories mentioned in the preceding paragraph; or (2) that an imbibition of water had taken place, due to a chemical union of some sort. It will be shown that in the case of dead fishes such an imbibition takes place regardless of the osmotic pressure of the medium.

It must be repeated that the behavior of the membranes of a given fish varies greatly with the conditions, and that seemingly contradictory results^d may be obtained with fishes of the same species. An extensive series of experiments which was carried on during the spring of 1905 at the New York Aquarium gave results that were almost wholly in harmony with one another, and certain conclusions were framed which seemed to be supported by nearly all of the facts then known to me. A repetition of some of these experiments during the summer of 1905 gave, in certain cases, quite different results, and led to a modification of some of my conclusions. The only differences in the conditions, so far as I am aware, were the temperature of the water and the previous history of the fishes used. The New York experiments, since they form a harmonious series, will be described first, after which the Woods Hole experiments will be discussed and an attempt made to harmonize some apparent contradictions.

The method employed throughout this portion of the work was to weigh the fishes carefully before and after the change in the density of the medium. This procedure involves numerous difficulties. In the first place, fishes under such treatment should not be fed, since the gain or loss of weight through feeding or defecation would confuse the results. Fortunately, most fishes may be kept unfed for days or weeks without any appreciable detriment to their health. In the second place, it is necessary to determine the normal loss of weight through waste which would occur throughout the period of the experiment. This rate of decrease for the fishes used was in many cases determined before the commencement of the experiment; in other cases a control set was kept under observation. In the third place, and this is all-important, the same conditions must be maintained each time the fishes are weighed. The difference in the amount of moisture adhering to the body surface may make a serious difference in the weight recorded, even 1 or more per cent in the case of smaller fishes.^b

In most of my experiments the aggregate weight of a number of fishes subjected to the same treatment was taken. They were commonly laid upon a dry towel for one-half minute, then laid upon a soft cloth for one-half minute more, the cloth meanwhile being moved in such a way that the fishes should be gently rolled over the surface. Where the procedure was varied, care was taken that the same condi-

^aThe question of gain or loss of weight through the taking in or passing out of matter from the alimentary canal is here left out of account. It will be dealt with later.

^bIt was at first thought necessary in each case to dip the fishes into water of the same density before weighing, in order that the water adhering should be of the same specific gravity, but this was found not to be worth while.

tions should obtain throughout the entire experiment. To meet the criticism that the removal of mucus from the surface introduced an abnormal condition into the experiment, fishes were in some cases weighed after being simply drained for a period upon a towel. Of course the weight determinations in these cases were not so accurate.

Experiment 41.

New York, April, 1905. *F. heteroclitus*, from salt water of the New York Aquarium (density 1.008, temperature 5° C., at commencement of experiment), unfed for 4 days, divided into lots of 25 each, and treated as follows:

(a) Kept in salt water (1.008 to 1.010, during period of experiment).

	Grams.
April ^a 8.....	249.5
9.....	247.6
10.....	247.0
11.....	245.8
12.....	245.0
13.....	244.0
15.....	243.5

Loss of weight 1 per cent during first 2 days, 2.4 per cent during first 7 days.

This lot of fish serves as a good control for the others of the series.

(b) Put into running fresh water (temperature 8° C.).

	Grams.
April 9, 3 p. m.....	194.2
9, 3.05 p. m.....	194.9
9, 4.05 p. m.....	196.7
10.....	198.5 (all well)
11.....	197.3 (all well)
12.....	195.6 (a few beginning to sicken)
13.....	194.6 (fungus appearing)

During the next 4 days a number of fishes were removed, some of these having fallen prey to fungus. April 17, 5.30 p. m., the remaining 14 fishes weighed 109.6 grams. They were then transferred to salt water of density 1.018. At 6.20 p. m. they weighed 108.6 grams; April 18 (p. m.), 100.6 grams.

Results: An appreciable gain in 5 minutes after transfer to fresh water; a gain of 1.3 per cent in 1 hour; a gain of 2.2 per cent in 1 day; followed by loss, as in *a* above; after change to salt water, a loss of 8.2 per cent in 1 day.

It is to be noted that the loss of weight after the second change was much greater than the gain after the first.

(b') Boiled (fresh) water used, instead of running water, resulting in a gain of 5 per cent in 1 day, 14 per cent in 2 days.

In this experiment 4 fishes were sick on the second day, while all but 4 were dead on the third. Here and elsewhere the death of the fish was accompanied by much more rapid imbibition of water.

(c) Fishes put into water averaging about 1.001 (ranging from a half point below to a half point above this figure).

	Grams.
April 8.....	248.6
9.....	250.9
10.....	246.9
11.....	245.5
12.....	244.7
13.....	243.2
15.....	242.9
17, 5.15 p. m.....	241.5

Fishes then put into 1.025 water.

	Grams.
April 17, 6.15 p. m.....	239.6
18, p. m.....	228.5
19.....	225.6

Fish in good condition up to this time.

Results: In water of density 1.001, a gain of nearly 1 per cent in first day; a loss of 2.2 per cent in 2 days, of 3.7 per cent in 8 days. After change to salt water, a loss of 6.6 per cent in 2 days.

(d) Water of density 1.015 (sea water from Sandy Hook, slightly diluted). Loss of 1.3 per cent in 2 days, of 3.1 per cent in 11 days. The fishes remained in perfect health throughout. It is not at all probable that the decrease during the first 2 days is significant.

(e) Water of density 1.025 (Sandy Hook water, strengthened by addition of commercial sea salt). This particular lot of water had, for some reason, a harmful effect upon the fishes, 16 of them being removed dead or in a dying condition during the first 9 days. The results are none the less worth recording: Loss of 1.3 per cent during first hour; of 4.1 per cent during first day; of 8.3 per cent during first 2 days. After this the decrease was far less rapid.

At the end of 9 days the 9 remaining fishes were put into the salt water of the aquarium supply (now 1.010). The weight remained practically stationary (loss of less than 1 per cent) during the next 3 days.

Comparison of *c* with *b* figures is interesting, the gain following transfer to this slightly saline water being much less than that in fresh water. It is also to be recorded that no deaths occurred in the 9 days during which the fish were in the slightly saline water, while 11 fishes were in this time removed, either dead or sick, from the fresh water. The absence of fungus from these fishes seems to be due not so much to the fatal effect of the salts upon the fungus (for the latter will thrive in water of much greater salinity, see p. 59) as to a salutary effect upon the fishes themselves. It is well known that fishes which are in good health are most resistant to fungus, while those which are in poor condition most readily succumb to it.

The loss of weight in *F. heteroclitus* when changed from diluted sea water (1.013) to nearly full strength sea water (1.023) is shown once more in the case of the fishes used in experiment 86; but in this case no harmful effects resulted to the fishes, and the experiment is therefore of greater significance. The loss of weight here was 2 per cent during the first day.

In another experiment (experiment 81) in which fishes from (diluted) salt water (1.013) were transferred to fresh, there was a gain of 2 per cent during the first day, followed by a loss of more than 1 per cent during the second.

Experiment 42.

New York, April, 1905. Nine *F. heteroclitus* which had been for six days in fresh water were put back into the salt water of the aquarium (1.009). A loss of nearly 3 per cent occurred during the first day, after which the weight remained stationary for a day.^a It would thus appear that water of this density was hypertonic to fishes which had lived in fresh.

Experiment 43.

In this experiment *F. heteroclitus* was transferred to fresh water. The fishes were throughout weighed individually. The results are in agreement with the foregoing, but the figures are not given, since the method of procedure was not so careful, this being the first of my experiments in weighing.

It seems to me that all of the results thus far tabulated are in harmony with the following provisional hypothesis: The body fluids of the fishes used had, at the commencement of the experiments, an osmotic pressure lying somewhere between that of water of density 1.005 and water of density 1.015. Transfer to a medium which was decidedly hypertonic resulted in loss of weight; transfer to a medium which was decidedly hypotonic resulted in a gain in weight. In the former case, the osmotic pressure of the fluids of the fishes was raised, in the latter case it was lowered. In neither case, however, was an osmotic equilibrium between the "internal and external medium" established. The osmotic pressure of the body fluids of the fishes fluctuated within a much narrower range than did that of the surrounding water. Furthermore, the change in weight bore no constant ratio to the change in the osmotic pressure of the water, as a comparison between the *b* and the *c* figures shows. Again, it is evident here and elsewhere that greater changes in weight occurred in those cases in which the fishes were affected harmfully by the new medium.

It is only fair, however, that I should mention one experiment with *F. heteroclitus*, in which the results are hard to reconcile with the others.

Experiment 44.

New York, April, 1905. Twenty-five fishes changed from water of density 1.010 to water of density 1.018. Not only was there no loss, but apparently a slight gain during the first day, followed by a decrease of about $1\frac{1}{2}$ per cent on the second. The accidental dropping of a bit of food into the tank might have been responsible for the result, though this can not be assumed as an explanation.

The experiment in which the scales were removed prior to the change of medium has already been discussed (pp. 62-64) in reference to the survival of the fishes. An analysis of the changes in weight is here worth while.

Experiment 45.

New York, April, 1905. For an account of conditions see page 63.

^a It must be recorded here that the 9 fishes were all that survived of a lot of 50, the remainder of which had died from fungus. These survivors showed no traces of fungus, however, and seemed to be in perfect health.

(a) Fishes in (dilute) salt water (1.007 to 1.009). Loss of 1 per cent in 5 days. In experiment 41, *a*, where normal fishes were used, there was a loss of 1 per cent in 2 days, but the fishes had been much more recently fed than in the present experiment (here unfed 16 days), which may account for the difference.

(b) In fresh water (running). Gain of 2.8 per cent in 2 days, 4 per cent in 4 days, after which the increase ceased. Here the gain for the first 2 days is only slightly in excess of the gain in 1 day in the case of the normal fish (experiment 41, *b*).

(c) In 1.001 water (running). Gain of 1.4 per cent in 2 days. (Unfortunately the gain in 1 day was not determined). In experiment 41, *c*, the fishes gained 1 per cent during the first day, then lost.

(d) In 1.014 water. Loss of 1.2 per cent in 2 days. This loss is not more than is accounted for by waste.

(e) In 1.025 water. Loss of 4.3 per cent in 1 day; 6.1 per cent in 2 days. In experiment 41, *e*, these losses were 4.1 and 8.3 per cent, respectively, but this is hardly a fair comparison, since in the latter case the water was for some reason harmful to the fishes.

It will thus be seen, by comparing the experiments upon sound fishes with those in which the fishes were scraped, that the removal of scales throughout a large area did not result in increasing the influx or efflux of water resulting from changes in the density of the medium. Garrey's contention (p. 62) thus appears to be disproved. Indeed, later experiments seem to indicate that it is through the membranes of the gills and not through the general body integument that these osmotic changes mainly occur.

The following experiment shows strikingly the difference between the effect of pure fresh water and water which is slightly saline. It also illustrates the alternate gain and loss of weight which a fish may undergo in consequence of repeated changes of density.

Experiment 46.

New York, April, 1905. Five specimens of the 18-spined sculpin (*Myoxocephalus octodecimspinosus*) taken from salt water of density 1.009.

(a) Two specimens put directly into fresh water. After 24 hours both were dead. Average increase in weight, 4.3 per cent.

(b) Three put into 1.001 tank (density ranging from a half point above to a half point below that figure). Average increase of 2.4 per cent during first day; 8.8 per cent during 3 days.

At this time two of the fishes (Nos. 1 and 3) were sick, the other (No. 2) appearing quite well. It is to be noted that the gain in weight was considerably less in the case of this latter fish. The two former fishes were accordingly put back into the salt-water tank (now of density 1.011), No. 2 being left in the 1.001 tank. No. 2 gained an additional 2 per cent during the following day and died after 2 days more (6 days after beginning of experiment). Nos. 1 and 3 underwent loss of 6.5 per cent during next 4 days (in salt water).

These last were then put for a second time into 1.001 tank, when there resulted a gain of 4.8 per cent in 1 day.

The experiment was discontinued after 4 days of 1.001 water. At this time 1 fish was dead, the other feeble.

Experiments 47 and 48.

New York, April, 1905. Six tomcod (*Microgadus tomcod*) and 10 winter flounders (*Pseudopleuronectes americanus*) were used, respectively. With each species a comparison was made between the effects of fresh water and of the slightly saline water. With the former species the gain was slight (about 1 per cent in each case, though here slightly less in the fresh water), and even the transfer to fresh water seemed to have little or no harmful effect. Most of the fishes lived until killed by fungus, from 1 to 4 weeks later.

In the case of the flounders there was a gain of about 5 per cent in 1 day in each sort of water. In fresh water, however, the fishes died in 2 days, in the slightly saline water in from 4 to 5 days.

Weight determinations were likewise made in the case of certain fresh-water fishes transferred to salt water.

Experiment 49.

New York, April, 1905. Three catfishes (*Ameiurus nebulosus*) put into salt water (1.010+). Average decrease for all, 8.3 per cent in 17 hours; average decrease for two, 15.8 per cent in 48 hours.

One of the fishes, being put back into the 1.001 tank at the end of 17 hours, regained in 2 days nearly all that it had lost. Those in salt water were very feeble at the end of the second day.

Experiment 50.

New York, April, 1905. Eight rudd (*Leuciscus erythrophthalmus*) had lost over 4 per cent at end of first day in water of density 1.011. All dead.

It seemed particularly worth while to test the changes of weight in the case of certain fishes which may survive abrupt change from fresh to salt water, and which in nature inhabit both. Hence the next six experiments.

Experiment 51.

New York, May, 1905. Five white perch (*Morone americana*), taken from a fresh-water pond, lost on the average 4.7 per cent during the first day after the change to salt water (1.012). During the preceding 2 days the loss from waste had been only 2.2 per cent or slightly over 1 per cent per day. The fish all appeared perfectly well after the change.

Experiment 52.

Five other specimens of the white perch (taken from salt water) were transferred to fresh. The average weight of these remained almost exactly stationary during the following day, after which they were not weighed. It must be remembered, however, that stationary weight implies a gain sufficient to cover the loss through waste. Nevertheless, such a result was not what I had anticipated, and this experiment is one of the two among the entire New York series whose results are not entirely in harmony with the others.

The case of the chinook salmon (*Oncorhynchus tshawytscha*) is yet more instructive.

Experiment 53.

New York, March and April, 1905. Six specimens which had been reared in fresh water at the New York Aquarium were changed abruptly to salt

water (density during experiment 1.007 to 1.009). Result, a decrease of 2.6 per cent during first day; 3.8 per cent during 2 days; 4.7 per cent during 4 days; 6.1 per cent during 6 days.

Experiment 54.

New York, March and April, 1905. Performed as control of above. Six fishes of same lot left in fresh water and weighed to determine loss due to waste. It was found that there was a decrease of 1.6 per cent during first day; 2.6 per cent during 2 days; 4.7 per cent during 4 days; 6 per cent during 6 days.

The initial loss is thus seen to be considerably greater in the fishes transferred to even this dilute sea water. But the total loss during the first 6 days, and even during the first 4 days, is practically the same in each case. This is precisely what we should expect if the salt water were hypertonic and the membranes permeable both to water and (in lesser degree) to salts. There would result, first, a loss of weight, followed by a relative gain in weight as equilibrium was reestablished. This experiment I regard as an extremely important one from the present point of view. Accordingly I repeated it, though the density of the salt water was considerably greater at the time of the second experiment.

Experiment 55.

New York, May, 1905. (a) Six *Oncorhynchus* put into salt water (density 1.013). Loss 3.1 per cent during first day, 4.5 per cent during 4 days.

(b) Six fishes left in fresh water. Loss of 1.9 per cent during first day, 5 per cent during 4 days.

(c) Six fishes put into salt water (density 1.023) in a large can, without aeration. The fishes, as might have been expected, were all dead (probably asphyxiated). The loss in 1 day was nearly 12 per cent.

That water of the density 1.013 should prove hypertonic to these fishes is not perhaps surprising. But that water of density 1.007 to 1.009 should have done so (experiment 53) was, I confess, a source of astonishment, since such water has between one-fourth and one-third the salinity of ocean water.

Experiment 56.

In this experiment the water was of a density 1.007 to 1.008, and 4 fishes showed an average decrease of 3.6 per cent in 24 hours. These fishes, it is true, had had a patch of scales removed on one side, but this does not affect the result as determining whether or not the water was hypertonic. All of the fishes in this and in the three preceding experiments survived this treatment and continued to live in health as long as they remained under observation.

After making due allowance for gain or loss of weight through various accidental circumstances, in no way related to osmosis, and for the imperfections of the method employed, it seems to me to be proved by the foregoing figures that water may enter or leave the body of a fish after immersion in solutions having a different osmotic pressure from the latter. It may be questioned, however, to what extent this process is a normal one, and to what extent it is pathological. In certain cases, without doubt, it is pathological, the death of the fish resulting from, or at least being accompanied by, a considerable gain or loss of water. In earlier experiments it was found that *Fundulus heteroclitus* sooner or later died from the effects of fresh

water. It may be objected, therefore, that any osmotic change occurring in this fish when placed in fresh water must be classed as pathological. I do not think that this conclusion is a necessary one, for the following reason: Both the gain and subsequent loss in weight occur long before any ill effects are manifested, and if the fishes are removed in time to their normal medium no harm will have resulted. In any case such objections do not apply to most of the other experiments with this species, nor do they apply to any of the experiments with the salmon, nor probably to either of those with the white perch.

A few words are likewise necessary in reply to another possible criticism of my interpretations. It may be asked, may not the increase or decrease in weight be due to water being taken in or passed out of the alimentary canal?^a Have we necessarily to do with osmotic phenomena at all? While the former possibility has not been absolutely excluded, and could not be excluded, save by ligaturing both ends of the alimentary canal, I have very strong reasons for rejecting it. In the first place, there is strong a priori improbability that the effects of osmotic action should be so closely paralleled in any other way. Why should a fish from salt water swallow fresh or brackish water, and why should a fish from fresh or brackish water pass out a part of its intestinal contents when placed in salt water? And why should this latter initial loss be followed by a relative gain, as shown by experiments 53 and 55?

Yet more definite evidence on this question is furnished by certain experiments described in the next section. It is there shown that salts pass from the body of a salt-water fish when placed in fresh water. It is likewise shown that the entire contents of the alimentary canal (indeed the entire viscera of the fish) are not adequate to furnish as much salt as leaves the body in the course of a few hours. It seems probable that the water enters or leaves the body along the same path as do the salts, that path being through one or more of the bounding membranes. Other experiments show that in some fishes, at least, the gills are the organs chiefly concerned.

As before stated, the results of certain experiments at Woods Hole failed to conform to those previously obtained. This is not true, however, of all these later experiments, although as a whole the results are certainly not as clear-cut and unvarying as in the New York series. The only really serious difficulties to be encountered relate to the behavior of *Fundulus majalis*. Description of these experiments will accordingly be deferred until after the others have been considered.

Eight experiments with *F. heteroclitus* are included in this later series.

Experiments 57-64.

Woods Hole, July and August, 1905. Out of 4 experiments in which fishes were transferred to fresh water, an average gain of about 1 per cent (in $\frac{1}{2}$ to 1 day) was shown in 3 cases; in the fourth case, however, a loss of 1.5 per cent is recorded.

In 2 experiments in which water of density 1.001 was used, a gain of about 1 per cent in each case was likewise recorded.

Of 7 lots which were put into water of density 1.009, 3 showed a gain (in 1 case as high as 2 per cent), and 3 a loss, while 1 remained stationary. These

^aThis criticism has been made by Bottazzi and Enriques (1901) of the weight determinations of *Aplysia* by Quinton (1900).

figures may indicate a relative average gain, since 4 lots of control fishes, kept in sea water (1.023), together with 6 lots kept in water of density 1.013, and 1 in water of density 1.018, all, without exception, showed a loss. It is to be noted that the loss in the 1.013 water was about the same as that in the full strength sea water.

Experiments 65-68.

Four sets of experiments with *F. diaphanus* may be briefly summarized. The fishes came from Tashmoo Pond, which had, at the time, a density of 1.002, and were kept at the laboratory in running water of the same specific gravity.

In 1 case, in which these fishes were transferred to fresh water, the normal loss through waste is the only change to be recorded, but here the difference in density was of course slight.

In 3 cases the fishes were transferred to sea water, a decided loss following in each case, averaging 9.3 per cent in from 12 to 20 hours. In one of these experiments the fishes, after losing 10 per cent of their weight in 20 hours, were found to gain again. Four of the fishes here used were removed in a dying condition, but the remainder gained 6 per cent during the following day, at the end of which they appeared to be in a perfectly healthy state, and might have lived indefinitely. This is the only case in which the changes during the second day were followed with this species, but the percentage of increase is too great to be accidental. It is, moreover, what would result if two fluids of different osmotic pressure were separated by an elastic membrane, permeable to water and (though in less degree) to the salts in solution.

In one of the foregoing experiments the fishes, after 12 hours in salt water, were transferred to fresh. There followed a gain of 9 per cent, this being greater than was the initial loss in this case. This experiment is interesting in comparison with another one (66, *d*), in which fishes that had lived for 8 days in salt water were transferred to fresh. No gain whatever resulted. The fishes had reached a new equilibrium, and the normal tension of the membranes had been restored. It is none the less surprising, in view of other experiments, that no endosmotic flow of water here occurred, for, as will be shown later, fishes of this species have a considerably greater salt content after a sojourn of some days in salt water. It might therefore have been expected that they would conduct themselves, in relation to fresh water, just as do the other salt-water species employed in the preceding experiments.^a

In 2 cases in which the fishes were transferred from the 1.002 water to water of density 1.013 there was no significant loss, in a third case there was a loss of about 3 per cent in one-half day.

Transfer from water of the above density to fresh (experiment 66, *e*) led to no increase. On the contrary, a decrease of over 3 per cent is recorded.

In most of these experiments with *F. diaphanus*, the changes did not appear to result in any harm to the fishes, and can not therefore be regarded as pathological. Even when the change was made from the faintly brackish water in which they lived to full strength sea water no deaths had occurred at the time of the second

^aIn a repetition of this experiment the fishes showed a gain of 2.5 per cent in one day, but in this case 2 had died.

weighing, in 2 cases out of 3. Indeed, the fishes in these 2 cases all remained in perfect health for another 24 hours, after which they were returned to their normal medium. Thereafter no deaths occurred (with one possible exception) for 4 days more, after which the fishes were no longer observed. The fishes used had been "seasoned" for a long period in the laboratory, and a large majority would survive indefinite transfer to sea water (see p. 67).

Experiments 69-71.

Six scup, weighed separately, were found to have gained to a scarcely appreciable extent if weighed immediately after death. If allowed to remain long in the water, however, the gain was much more pronounced.

In interpreting the foregoing figures it must be borne in mind that these experiments were made during the summer months when metabolism in general was more active, and when the loss of weight through waste, in these unfed animals, was consequently more rapid. Owing to this fact, and probably, also, to the discharge of matters from the reproductive organs, such accurate determinations of gain or loss in weight as were previously made were here found to be impossible. It will be seen by reference to the above notes that in no case has a gain been recorded where it would not have been expected according to hypothesis. On the other hand, loss or stationary weight is recorded in some cases where a gain might reasonably have been expected. The discharge, at any time, of eggs or of ovarian fluid from one or more of the fishes in a given lot might well have produced some of these aberrant results. This, of course, was not likely to occur during the winter and early spring.

Even such allowances do not seem sufficient, however, in the case of *Fundulus majalis*, as the following experiments show:

Experiments 72-76.

Of five lots transferred to fresh water two showed a considerable gain, but in both of these cases the fishes had begun to die, and dead fishes rapidly imbibe water, as has already been pointed out. In a third case a slight (scarcely significant) gain is to be noted. In a fourth lot there was an initial loss of about 1 per cent during the first 12 hours, followed by a gain of more than 2 per cent during the next 12 hours. But it is to be noted that in this case the control fishes in salt water likewise showed a loss during the first half day, followed likewise by a slight gain. In the fifth fresh-water lot a loss was recorded at the end of the first day, as well as at the end of the first half day, the total loss amounting to about 3 per cent, while that of the control lot amounted to only a trifle over 1 per cent.

Of the five lots put into 1.001 water, three were found to have lost during the first half day, and likewise throughout the entire day, the total loss amounting in each lot to more than that shown by the control fishes in salt water. The other two lots gained during the first half day, but lost during the second.

Of the control fishes, three lots lost, one remained stationary, while one first lost and then gained slightly.

Had I begun my experiments in weighing during the summer and used *Fundulus majalis* at the outset, it is likely that I should have despaired of discovering any significant changes in weight, and, indeed, it may appear to the reader that such fig-

ures as have just been recorded may well have been due to accident or error. I will promptly add that I can not myself satisfactorily explain these differences in my results.

It must be urged at the outset, however, that these aberrant cases in no way invalidate the results of my earlier experiments. From the earlier series we are forced to conclude that certain changes of weight may occur as the result of osmotic action. Some of the later experiments show that under different conditions such changes may not occur. The only differences in the conditions, so far as I am aware, relate (1) to temperature (20° to 22° C. at Woods Hole, 3° to 15° C. at New York) and (2) to the previous history of the fishes used. Those employed at the New York Aquarium had been living in water of density 1.007 to 1.012, those at Woods Hole (i. e., the salt-water ones) in sea water of nearly or quite full strength. I can not believe, however, that this was a significant factor in the case.

It is well known that osmotic pressure varies with temperature. In the present case, however, it seems to be a difference in the permeability of the membranes that demands explanation. During the summer months metabolism in general is of course more active, and various chemical and physical changes are in progress which are dormant during the colder season. It is not unlikely, therefore, that the osmotic permeability of a fish, always dependent upon the physiological condition of the animal, should present considerable differences during the summer and winter months. This being granted, it remains to be asked just what changes in permeability would account for some of these aberrant results. In certain of the foregoing experiments with the three species of *Fundulus*, where a decided gain was to have been expected in view of earlier results, a decrease was found to have occurred instead. Assuming that this decrease is attributable to normal waste as in the case of fishes left in their customary medium (or to some other irrelevant circumstance, see p. 80), how are we to account for a stationary weight in a strongly hypotonic fluid? As has already been stated (p. 70), a stationary weight in a medium which is not approximately isotonic may mean either (1) that the membranes are impermeable to water; or (2) that water and salts may pass through with equal freedom, in which case the conditions for osmosis are wanting. The latter alternative does not necessarily imply a freely porous state of the membranes, such as obtains in the case of a filter. It is quite conceivable that this indiscriminate permeability of the body surface should be subject to the controlling influence of the organism, just as we know that the differential permeability, which renders osmosis possible, may be so controlled. I have thus emphasized this second possibility, inasmuch as I regard the first one as being thrown out of consideration in certain cases. Although no unquestionable change in weight resulted in either *F. majalis* or *F. diaphanus* when transferred from salt to fresh water, I shall later show that a decided change occurred in both cases in the salt content of the body. It will likewise be shown to be highly improbable that this salt passed from the body by way of the alimentary canal or the reproductive organs, leaving as the only alternative a diffusion through one or more of the limiting membranes of the body.

In some other cases, on the contrary, the facts are best explained by assuming a condition of complete impermeability, as in the case of *F. majalis* when transferred from salt to slightly saline water. Here no certain increase in weight occurs, and

there is apparently no decrease in the salt content of the body. (See experiment 103).

Bert (1871) has noted that a goldfish dying in salt water lost about one-fifteenth of its weight, if the specimen were a small one. The loss was insignificant, on the other hand, if the fish weighed several hundred grams. These fishes died, on the average, in an hour's time, a period not long enough to permit of any extensive loss, just as no considerable gain was found to occur in the case of the scup dying in fresh water (experiments 69-71), above. Loaches and young eels, according to Bert, lost one-tenth or even one-sixth of their weight; but both of these fishes have a naked skin. In all of Bert's experiments the results of the changes were fatal, and the phenomena were hence pathological.

Fredericq (1904) records that the blood plasma of elasmobranchs increases in volume in more dilute sea water, decreasing in less dilute. Of course a gain or loss of weight would be entailed thereby. According to Fredericq, however, the conditions which obtain in sharks relative to osmosis are radically different from those which hold for the teleosts.

Various authors have described weight changes, due to osmosis, in the case of invertebrates, but these need not be considered here.

CHANGES OF WEIGHT IN DEAD FISHES.

It has been noted that many of the fishes used in the foregoing set of experiments died from the effect of the change of medium, and it likewise appears from the records that in those cases in which death occurred the gain or loss was usually much greater in a given time than in those cases where no harmful effects were apparent. Compare, for example, experiment 81 or 41 *b* with 41 *b'*; also experiment 41 *c* with experiment 86.^a The catfish in experiment 49 lost over 8 per cent of their weight in 17 hours (probably 10 to 12 per cent in 1 day) and ultimately died, while the white perch (experiment 51) lost 4.7 per cent and the salmon (experiment 55 *a*) only 3.1 per cent in a day in water of somewhat greater density, these two species not being harmfully affected by the change. It is only fair to add, however, that in the case of the rudd (experiment 50), which died after transfer to salt water, a loss of only 4 per cent is recorded. This may be due, it is true, to a subsequent gain through imbibition of water after death (see below).

I had at first supposed that the determination of a solution which was isotonic with the body fluids of a given species of fish could be made simply enough by immersing dead specimens in water of various degrees of salinity and noting the effects upon their weight. A solution in which the weight remained stationary would be isotonic. As a matter of fact, however, some species were found to increase in weight in water of any specific gravity which was employed.

Experiment 77.

New York, April, 1905. The chinook salmon (*Oncorhynchus tshawytscha*),^b the sunfish (*Eupomotis gibbosus*), and the carp (*Cyprinus carpio*) among the

^a See pp. 73-74 for weight changes undergone by fishes used in experiments 81 and 86. They are not recorded in notes for those experiments.

^b The salmon is here counted among the fresh-water species because the earlier months of its life are passed in fresh water and those specimens here used were taken from fresh water.

fresh-water species, and *Fundulus heteroclitus*, the tomcod (*Microgadus tomcod*), and the sea raven (*Hemitripterus americanus*) among the salt-water ones, were used. The more sensitive species were allowed to die out of water, while the more resistant ones were killed by vapor of chloroform. The fishes were weighed first before immersion, again after 3 to 5 hours of soaking in the solutions, and once more after another interval of about 12 hours. Seven sorts of water were employed—fresh water, and salt solutions having the densities 1.002½, 1.005, 1.010, 1.015, 1.020, and 1.025. These solutions were prepared with a commercial "sea salt." It was found that the salmon, carp, and *Fundulus* all increased^a from the first in water of all grades. This was practically true of the sunfish and tomcod likewise, except that during the first interval there was no appreciable increase (in one case a slight decrease) in the three stronger solutions, an increase following, however, during the next 12 hours. The sea raven alone gave results which conformed in some measure with what one would expect from osmotic action. Here a loss occurred in the three stronger solutions (1.015, 1.020, and 1.025) during the first 3½ hours, this amounting to 1 per cent in the first case and 1.8 per cent in the last. Even with this species, however, there were irregularities which confused the results.

Experiment 78.

In this additional experiment with *Oncorhynchus*, in which fresh water and two different strengths of salt water were used (1.008 and 1.014), a gain resulted in each case, though in the third case this was not certain during the first day.

This increase in weight of dead fishes in the foregoing experiments is in striking contrast to those cases (particularly 41, *e*, and 55, *c*) in which fishes of the same species dying in strong salt water lost weight in considerable measure. The following two experiments likewise illustrate this contrast:

Experiment 79.

Specimens of the tautog, sea raven, and tomcod, which after death were put into water of density 1.014, were all found to have increased in weight at the end of 18 hours.

Experiment 80.

A living tautog put into a tub of water of about the same density lost 1.8 per cent during the first 20 hours, although death from asphyxiation occurred some time during this period. It is true that the dead fish underwent an additional decrease of about one-half per cent during the next 24 hours, but the loss of mucus through wiping the fish perhaps more than accounts for this.

The increase in weight of dead fishes in solutions which are undoubtedly hypertonic as compared with the body fluids may be explained in a number of ways. It is, of course, possible that water enters the alimentary canal at either end; or it is possible that after death the limiting membranes of the body become so completely permeable that no osmotic action is possible, and that a process of imbibition occurs, the water combining in some way, perhaps, with the dead proteids of the tissues. The latter hypothesis seems to me to be far more probable, since the gain in weight

^a Percentages of increase or decrease are omitted here as being of little importance for the present discussion.

is progressive, lasting, it may be, for days, at the end of which time the fish has a decidedly water-soaked appearance.

Loeb (1900) in experiments with pieces of muscle, which he immersed in various solutions, found that such pieces might either gain or lose in weight in solutions which were equimolecular, but differed in respect to the salts employed. Chemical action, rather than simple osmotic action, he believed to be responsible for the influx or efflux of water.

If this explanation be applicable to the dead fishes used in my experiments, it will be seen that the attempt by Fredericq (1904) to ascertain the osmotic pressure of the fluids of the tissues of various fishes by similar weight determinations was not likely to yield reliable results, and, indeed, he admits that the figures thus obtained differed widely from those obtained by the cryoscopic method.

The case of those fishes which died in hypertonic solutions and likewise lost weight in those solutions is perhaps to be explained on the ground that the loss of weight occurred before and shortly after death, though this explanation does not seem to cover all cases (experiment 80, second day).

PERMEABILITY OF THE MEMBRANES TO SALTS IN SOLUTION.^a

Some of the preceding experiments seem to prove conclusively that considerable amounts of water may enter or leave the body of a living fish if the latter be transferred to water of a density much lower or much higher than that to which it has previously been accustomed. That in all probability this water does not enter or leave the body by way of the alimentary canal has already been noted, in anticipation of results to be discussed later. There is thus left, as the most likely alternative, an osmotic passage through one or more of the membranes bounding the body. It has been shown, likewise, that these changes occur, in many cases, without apparent harm to the fish.

The question next to be discussed is whether or not salts in solution may likewise diffuse through these membranes. If the gain or loss of weight shown in the preceding experiments be due to osmotic action, it is of course impossible that the passage of salts should occur as readily as that of the water. Does it occur at all?

This problem I have attacked from both sides. In the first place, the passage of salts (strictly speaking, of chlorides) into fresh water from fishes taken from salt or brackish water was tested chemically. In the second place, the salt content of the tissues of various fishes which had lived in water of various degrees of salinity was likewise determined. It will be found that the results from these two methods present some striking points of agreement. The former method will be discussed first, although the results are as a whole less satisfactory than in the second case.

DETERMINATIONS OF THE CHLORINE PASSED INTO THE WATER BY THE FISHES.

Fishes of known weight taken from salt or brackish water were transferred to known volumes of fresh (in a few cases of distilled) water. The fishes were, in all cases, given a preliminary rinsing (10 to 30 minutes) in fresh water before being placed in the water to be tested. This was obviously necessary in order that all salt

^a I have spoken throughout of *salts* rather than *ions*, because I can not see the advantage, for present purposes, of borrowing the language of the electrolytic dissociation hypothesis.

water adhering to the surface should be removed. They were weighed without any preliminary drying, such as was necessary when very accurate weight determinations were required. Except in a few cases, the fishes used had been kept unfed for 3 or more days prior to the experiment.

The chief difficulty which beset this method of procedure was the fact that the fishes had to be kept in comparatively small volumes of water. Aeration was in many cases effected by the use of tanks of compressed air, but, even thus, many of the fishes were overcome by asphyxiation before the experiment had proceeded far. In such cases we may feel sure that the osmotic phenomena were not wholly normal. These complications, however, are indicated in the records cited below, where due allowance is made for them.

My reasons for taking the amount of chlorine as an index of the quantity of salts passing from the fish are obvious: (1) The test is an extremely simple one; (2) the chlorides (sodium chloride and magnesium chloride) together form more than 88 per cent of the entire saline ingredients of sea water.^a The bromine, which reacts like chlorine to the silver test, may be omitted as too insignificant in amount. The test employed by me was Mohr's silver nitrate titration method. According to this, a given volume of the solution to be tested is first tinted yellow by the addition of a few drops of a solution of neutral potassium chromate. Silver nitrate solution of a given strength is added from a burette until, after all of the chlorine has been combined as silver chloride, any excess of silver is free to combine with the chromic acid. At this point an abrupt change of color results, due to the appearance of the red silver chromate. The amount of chlorine present is of course readily computed from the amount of silver employed.

For the determinations here recorded I do not claim any very high degree of accuracy. Nor was this either necessary or possible under the circumstances. In general, the larger the proportions of chlorine the more exact are the figures. Where the amount was slight, however, the determination was more difficult, and it is probable that my figures in such cases are commonly too high. Again, in those cases in which death occurred and decomposition commenced, the presence of various organic matters in the water obscured the reaction. In such event the solution was sometimes evaporated and the residue charred.

Allowance was made in all cases for the quantity of chlorine already present in the fresh water used, this being about 0.003 gram per liter.

Experiment 81.

New York, May, 1905.^b Ten *F. heteroclitus*, of aggregate weight 110.3 grams, taken from water of density 1.013, were put into fresh water. Percentages of chlorine passed out (i. e., amounts per 100 grams of body weight) were 0.008 during the first hour, 0.030 during first day, 0.041 during 2 days.

At the end of this period the fishes were all in seemingly normal condition.

In this experiment several facts of interest are to be noted: (1) A quite appreciable amount of chlorine passed from the bodies of the fishes during the first hour. (2) The amount passing out during the entire first day is less than four times the

^a Dittmar (1884), in his hypothetical formula for the "proximate composition" of sea salts, gives the percentage of NaCl as 77.758, that of MgCl₂ as 10.878. In whatever combinations it may actually occur, however, the percentage of chlorine is given by him as 55.292.

^b This entire series was carried on during May and June of 1905 at the New York Aquarium.

amount during the first hour, and similarly only 37 per cent more passed out during two days than during the first day alone. Thus the loss of chlorides from the body occurs at a steadily diminishing rate. This conclusion is confirmed by all the succeeding experiments.

Experiment 82.

Fishes with same history as last. About twice as much chlorine was recorded for the first hour. An accident prevented further determinations.

Experiment 83.

F. heteroclitus, which had been in water of density 1.005 (1.004 to 1.006) for 5 days, and were taken originally from a salt-water tank (1.013): 0.036 gram per 100 in 1 day, 0.051 gram per 100 in 2 days.

Experiment 84.

Same species, with same history: 0.022 gram per 100 in 1 day, 0.025 gram per 100 in 2 days.

Experiment 85.

Same species from same tank originally, but in 1.005 water 10 days: 0.034 gram per 100 in 1 day, 0.044 gram per 100 in 2 days.

In each case the fishes were well at the end of the second day of the experiment.

Experiments 83 and 85 agree fairly closely, but experiment 84, for some unknown reason, shows a much smaller loss of chlorine. The mean results of these three experiments are 0.031 gram per 100 in 1 day; 0.040 gram per 100 in 2 days. In experiment 81 these figures were 0.030 and 0.041, respectively—i. e., there is a practical identity between the two sets of figures. Where such great variations occur as in the above results the mean of a large number of determinations should of course be taken. Provisionally it may be concluded, however, that fishes from water of density 1.005 will yield about as much chlorine in fresh water as fishes from water of density 1.013.

Experiment 86.

In this case the fishes had been kept 3 days in water of density 1.023 before the commencement of the experiment (originally from 1.013). Results: 0.015 gram per 100 in 1 hour, 0.042 gram per 100 in 1 day.

The figures are larger than any of those previously given.

In order to test the possibility that the chlorides thus found had left the body by way of the alimentary canal or reproductive organs, the following control experiment was performed.

Experiment 87.

Ten fishes having the same history as those used in the preceding experiment were killed, and from them were taken the entire alimentary canals and the gall bladders, testes, and ovaries. These were cut into fine bits; then macerated 15 minutes or more in fresh water. This latter was found to yield 0.005 gram chlorine. Thus this lot of fishes, weighing 106 grams, could have furnished from the contents of their alimentary canals and reproductive organs not more than five thousandths of a gram of chlorine, and probably much less than this.

It will be seen by reference to experiment 86 that three times this amount of chlorine (per 100 grams of body weight) was yielded by the living fishes in a single hour. A number of confirmatory experiments are recorded later.

Experiments 88 and 89.

Fishes (*F. heteroclitus*) were used which had been kept in fresh water for 4 and 5 days, respectively. The mean yield of chlorine during the first day in these two cases was 0.016 per 100 grams weight of the fish.

This figure was surprisingly large, being somewhat over one-half the quantity yielded during the first day by the fishes from the 1.013 water, and considerably greater than that yielded during the second day by the latter. The present fishes, it will be remembered, had already spent 4 or 5 days in fresh water. It is to be noted, however, that in each of the present experiments one or more of the fishes had died before the chlorine test was made, and that dead fishes yield up their salts more readily than living ones.

Several experiments were made with the tomcod, a fish which will, under favorable conditions, survive the abrupt transfer to fresh water, and live for considerable periods in the latter medium. Great difficulty was found, however, in maintaining sufficient aeration.

Experiment 90.

Here 3 tomcods, weighing 116 grams, were placed, after the preliminary rinsing, in 40 times their weight of fresh water. Results: 0.027 gram chlorine per 100 in 6 hours, 0.049 gram chlorine per 100 in 1 day.

The fishes were all well at the end of the first 6 hours. At the end of a day 1 fish was found dead (death probably quite recent).

Experiment 91.

This was a control experiment similar to experiment 87. In this case all of the viscera were removed, chopped up and macerated, and the body cavities, containing more or less blood, were rinsed out, the water so used being added to the rest. Even in this case the quantity of chlorine thus yielded was only 0.010 gram for each 100 grams weight of the fish.^a

This is seen to be only a little more than one-third the amount passing from the living fishes in the course of 6 hours. It can not be seriously maintained, then, that any large part of the salts which were found in the water had left the bodies of the fishes by way of the alimentary canal. That it passed out through the organs of excretion might be argued with somewhat greater plausibility.

In two other experiments in which this species was employed the aeration was quite insufficient and the fishes soon began to die in consequence.

Experiment 92.

Two of the fishes had died during the first hour, and the proportion of chlorine passed out during this period was 0.021 per 100 grams. Here the phenomena were of course pathological.

Experiment 93.

Death did not occur so soon, and the figure for the first hour was very much lower.

^aThe original weight of the fish used is of course intended here, not the weight of the viscera from which the salts were extracted.

Experiments 94, 95, and 96.

These experiments, dealing with sea raven (2 cases) and sea bass, will be briefly mentioned, though I attribute far less importance to them, since fresh water is soon fatal to both of these species and we are hence not dealing with normal phenomena. In the case of one sea raven transferred to fresh water, the loss of chlorine was 0.018 per 100 grams during the first hour and 0.028 during first 2 hours. Death probably occurred not long after this.

Another sea raven was placed in distilled water and death occurred in about 2 hours. Up to this time 0.039 gram chlorine per 100 had passed out. Here and in the preceding case almost the entire epidermis was sloughed off, even before the death of the fish.

A sea bass yielded 0.012 gram per 100 during the 40 minutes which elapsed before death.

The most instructive cases should be those of fishes which in nature live in either medium, and may be transferred with impunity from one to the other. The chinook salmon is of course such a fish, and experiments were attempted with this species, specimens being used which had previously lived 4 days in salt water. It was found impossible, however, to keep up sufficient aeration in the limited volumes of water employed, and the fishes died quickly. The white perch (*Morone americana*) is a much more favorable subject for such experiments. It has already been noted (p. 60) that perfectly healthy specimens will, in many cases at least, survive transfer to fresh water. For example, two of the same lot as used below were put into running fresh water and remained in good health for four days, when they were removed for other purposes. This alone would not of course be fully conclusive.

Experiment 97.

Two white perch from salt water (density 1.015), unfed for 2 days, after preliminary rinsing put into separate jars, each containing fresh water 40 times the weight of the fish. Mean results for 2 fishes, 0.036 gram chlorine per 100 in 1 day, 0.058 gram chlorine per 100 in 2 days. Both of these figures are considerably higher than the ones for *F. heteroclitus*.

The fishes at the end of the first day were in perfect health. By the end of the second day aeration had stopped and both fishes were dead.

Experiment 98.

The control experiment of extracting the salts from the viscera was performed once more with fishes having the same history as the preceding. Not only were all of the viscera removed and the body cavities rinsed, but the former were boiled for 15 to 20 minutes. It was found that the chlorine derived from all of these sources was (in proportion to the weight of the fishes used) considerably less than two-thirds as much as passed from the body in a single day and only about one-third as much as passed from the body in 2 days.

This does not of course entirely dispose of the objection that the salts may leave the body in the excretions of the kidneys.

It remains to be considered whether chlorine in appreciable quantities is given off by fresh-water fishes while in their normal medium. It will be remembered that

in experiments 88 and 89 some *F. heteroclitus* which had been living in fresh water for some days were found to pass out about half as much chlorine in a day as did the fishes recently taken from salt water. In qualification of these figures it will be recalled (1) that small amounts of chlorine were apt to be overestimated, and (2) that in each of these two experiments one or more of the fishes had died.

Experiment 99.

A catfish (*Ameiurus nebulosus*) was found to pass out during 19 hours 0.004 gram chlorine per 100.^a This is thus between one-eighth and one-seventh the first day's output of *F. heteroclitus* from salt water.

Experiments 100 and 101.

These experiments, in which carp were used, are decidedly puzzling. The output of chlorine per 100 grams of body weight was in one case 0.009 gram in 5 hours, in another it was 0.015 gram in 16 hours.

Since these fishes had not been fed for 10 and 13 days, respectively, the salts here indicated can not have been passed out in feces. I can only mention, by way of possible explanation, that the fishes here used had been living in fresh water which received a slight, though undetermined, admixture of salt water. It will be shown below that even very small proportions of sea water have a pronounced effect upon the salt content of the body.

It will be better to defer any general discussion of the foregoing results until the analyses of the bodies of the fishes themselves have been considered. Certain doubts which the reader may have formed regarding the significance of the preceding figures will then perhaps be dispelled.

Experiments in which the fishes died, or in which they were killed and returned to fresh water, seem to show (1) that a dead fish will yield a larger proportion of its salts than a living one, but that (2) there is no sudden increase in the diffusion of these after death. These conclusions were not tested very thoroughly by experiment, however, and they are hence only offered provisionally.

DETERMINATIONS OF THE CHLORINE IN THE TISSUES OF FISHES.

Each lot of fishes here used was first rinsed in fresh water for 5 to 10 minutes in order to remove any salt water which might adhere. They were then weighed, after which the entire fishes were cut up into fine pieces and placed in a porcelain evaporating dish or crucible. Considerable quantities of a mixture of equal parts of sodium (or potassium) nitrate and sodium carbonate were now added in order to facilitate combustion, and the whole mass was heated to the point required for ignition. It was, of course, necessary that all chemicals used should be chlorine-free, and it was likewise necessary to avoid any contamination of materials with salt water.

The soluble salts were extracted from the residue with boiling water. The resulting solution was first treated with nitric acid to transform any phosphates present, and then rendered neutral with calcium carbonate. After filtration the solution was titrated with silver nitrate according to the method already described. If care had been taken to insure the complete combustion of all organic matters, the determination was simple and fairly exact.

^a This figure is probably too high (see p. 85).

One source of error in this method is the loss of a certain portion of the chlorides through volatilization. This loss is probably slight, however, and in any case the error was likely to be a nearly constant one throughout, leaving the relative values unaffected.

Since it was thought possible that differences in the bodily salt content of the fishes used might depend upon differences in the water contained in their alimentary canals, certain tests were made with fishes from which the latter had been removed. The results of such experiments were such that this procedure was not thought to be worth while.

Let me add that I have presented here the figures for all of the analyses made by me, no discordant results being suppressed.

Experiment 102.^a

(a) Five *F. heteroclitus* from a lot which had been kept unfed for some weeks in water of density 1.023 were tested together. The alimentary canals, livers, and gall bladders were removed, and the fishes were weighed after the removal of these parts; thus the percentage of chlorine given represents the proportion found in the eviscerated bodies. The percentage was here 0.167.

(b) Five fishes from same lot were kept in fresh water for 1 day. The subsequent treatment was the same. Percentage of chlorine 0.129.

(c) Five fishes (from another lot) were kept in 1.001 water^b for 1 day. Alimentary canals, etc., removed as above. Percentage of chlorine 0.123.

(d) Eight fishes (same lot as last) kept in 1.002 water (see table on p. 96) for 1 day. Percentage of chlorine 0.159.

It would thus appear that in fresh water about 23 per cent of the total chlorides of the body passed out during a single day. Let it be recalled that in experiment 86, in which the fishes used had been kept in water of the same density as those here employed, it was found that the loss of chlorine was 0.042 gram per 100 grams of body weight. Assuming the original percentage of chlorine to have been the same as in the present experiment (0.167), the fishes in experiment 86 lost about 25 per cent of their chlorine. Thus the results reached by these two methods are found to be in striking agreement. It would be rash, however, to expect such a close correspondence in every case.

The effect of the 1.001 water was practically the same as that of fresh water, the difference of 0.006 per cent in the analyses probably having no significance. On the other hand it is a fact of the highest interest that fishes in the 1.002 water lost little if any of their chlorides. It would thus appear that the degree of dilution which may be endured with impunity by this fish lies somewhere between these two limits.

Experiment 103.

Fundulus majalis was here used, two fishes being taken for each analysis. The digestive tracts, etc., were not removed.

(a) Fishes from salt water (1.023). Percentage of chlorine 0.179.

(b) This lot, originally from salt water, had spent 2 days in water of density 1.001 (approximately). Percentage of chlorine 0.178.

^a All of this series were done at Woods Hole during August, 1905.

^b The water here used gave a salinometer reading (corrected) of about 1.001. A chemical analysis of water giving this reading, however, showed that it contained only 3 per cent as much chlorine as the local sea water.

(c) Had spent 1 day in fresh water. Percentage of chlorine 0.126.

(d) Fresh water 2 days. Percentage of chlorine 0.129.

It will be noted, first, that the lot from salt water showed a percentage of chlorine not very different from that of *F. heterochlitus* (preceding experiment). Second, the percentage was practically the same in the fishes from water of density 1.001 as in those from pure sea water; while third, fishes which had spent one day in fresh water showed a loss of 30 per cent of their chlorine. Fourth, there was practically no difference in this regard between those which had been in fresh water 1 day and those which had been there for 2 days.

The second of the results just enumerated is rather surprising, in view of the fact that *F. heterochlitus* was found to yield up a considerable fraction of its chlorine in water of approximately the same density ($1.001 \pm$), though retaining it in water of density 1.002. Whether this difference of results is due to any real physiological difference between these two species may be doubted. Where the water is so near to what may be called the critical degree of salinity, a slight difference in one direction or the other might suffice to produce a very considerable difference of results. It was previously found (experiments 31, 31 bis) that water of density 1.001 was slowly fatal to *F. majalis*, while water of density 1.002 effected little or no harm.

Despite minor discrepancies, the last two experiments agree in showing a great difference between the effects upon the chlorine content of the body of pure fresh water and water having a certain small percentage of salt. This difference is extremely significant in view of the difference, already discussed, in their effects upon the life of the fishes.

In another series of experiments (104-107) *F. diaphanus* was used. These fishes had been taken in Tashmoo Pond, in water of density 1.002, and kept in the laboratory unfed for a number of days prior to the experiment in water of the same density. Analysis of this water showed that it contained 7 per cent as much chlorine as the local sea water. The entire fishes were used for analysis, the alimentary canals, etc., not being removed. The specimens were first rinsed thoroughly, here as always.

Experiment 104.

Five different analyses were made with fishes taken directly from the brackish water (1.002). In each case 4 fishes were treated together. These analyses gave the following percentages of chlorine: *a*, 0.175; *b*, 0.136; *c*, 0.128; *d*, 0.139; *e*, 0.132. The mean of these figures is 0.142.

It will be seen that with the exception of *a* these figures do not present a wide variation. I regard the first result as probably due to an error, both on account of the exceptionally large amount of chlorine indicated and because the test was not a very satisfactory one. Strict fairness compels its insertion here, however. The mean of the other four figures is 0.134, and this figure, being in my mind the more probable one, will be used in subsequent comparisons. The reader is at any time free to substitute the other figure.

Experiment 105.

Here the fishes were kept in fresh water for a varying period prior to the analysis.

(a) 1 day (4 fishes)	0.112
(b) 3 days (4 fishes)	0.108
(c) 11 days (8 fishes)	0.085

In 1 day the fishes lost over 16 per cent of their chlorides; in 11 days nearly 37 per cent. As I have previously pointed out in discussing the analyses of chlorine in the water, the loss of salts from the body takes place at a diminishing rate. In the present case nearly as much passed out during the first day as during the next 10 days.

Experiment 106.

Fishes kept in salt water.

(a) 1 day (4 fishes 0.181) }	mean..	0.188
(a') 1 day (4 fishes 0.196) }		
(b) 5 days (2 fishes)		0.143
(c) 10 days (3 fishes)		0.151

For the rather anomalous fact that in two different tests the 1-day fishes gave much higher percentages of chlorine than the 5 or the 10 day ones, I will offer the following explanation: (1) As shown above (experiments 65-68), the immediate result of the immersion of these fishes in salt water is a loss of water amounting in 1 day to about 10 per cent of their weight. This would of course result in a higher concentration of the solutions in the body. It was found, however, that part at least of this loss of water is made up later. (2) A certain (often a large) proportion of these fishes succumb within a few days after transfer to salt water. It is quite possible, therefore, that the lots analyzed included individuals which would soon have died and thus had undergone greater changes than normal fishes would have done. Those, on the contrary, which had survived 5 or more days in salt water were probably individuals that had not been harmed by the change and would probably have lived indefinitely.^a

Omitting the figures for the fishes which had passed 1 day in salt water, it will be seen that those for the other tests fall into a very suggestive series.

Fresh water	{ 11 days	0.085
	{ 3 days	0.108
	{ 1 day	0.112
Brackish water (1.002)		0.134 [or 0.142]
Salt water	{ 5 days	0.143
	{ 10 days	0.151

It will be seen that the last of these figures is about 78 per cent greater than the first. It will be seen likewise that whichever figure be regarded as the more correct one for the brackish-water fishes, the latter agree much more closely with the salt-water than with the fresh-water individuals (the comparison being of course with the extreme members of the series).

Experiment 107.

Eight fishes kept in water of density 1.013 for 7 days. The percentage of chlorine for this lot was 0.134, i. e., the same as for those kept in brackish water.

It was thought desirable to test some species which in nature inhabits fresh, brackish, and salt water equally well, using individuals from each of these sources. The white perch (*Morone americana*) seemed likely to be a favorable object for such

^a Another explanation of this apparent anomaly was suggested to me by Prof. W. C. Sabine. It is quite possible that after the first shock of change, resulting in considerable osmotic disturbances in the body of the fish, an effort would be made by the latter to reduce its salt content to the original (normal) level, the excess or part of it being eliminated by way of the organs of excretion.

studies. The following experiments, however, show a high range of variability in the individuals used—much higher than in *F. diaphanus*.^a Hence the results are somewhat less decisive. Owing to a scarcity of material, it was found necessary to use fishes of very different sizes; but this fact certainly does not account for the variations in the proportion of chlorine.

Experiment 108.

Morone from Tashmoo Pond (1.002) kept for some days (unfed) in water of same density in laboratory before being used for experiment. One small specimen used for each test. (a) Percentage of chlorine, 0.116; (b) percentage of chlorine, 0.135; (c) percentage of chlorine, 0.151; mean, 0.134.

This mean figure, it will be recalled, is identical with that for the *F. diaphanus* from this same water. In view of the great range of variation here, however, such precise agreement is doubtless due to chance.

Experiment 109.

Morone from Lagoon Pond (head of Vineyard Haven). A water sample taken near shore gave a reading of 1.016, but it is likely that the water in which the fishes lived was considerably more saline. The fishes were kept in the salt water of the laboratory (1.023) 1 and 2 days, respectively, before the analysis. One fish was used in each experiment. (a) Percentage of chlorine, 0.136; (b) percentage of chlorine, 0.142; mean, 0.139.

The difference of this figure from that of the brackish-water specimens is perhaps not great enough to be significant. Strict candor compels me to record the analysis of another fish from this source, which appeared to contain only 0.101 per cent of chlorine. This figure falls so far below all others obtained from this species, however, that I can not but regard it as due to an error. If this analysis were included with the foregoing, the average would become 0.126.

Experiment 110.

Fishes from a fresh-water pond (landlocked). The water of this pond, though fresh to the taste, was found to contain 0.051 gram chlorine per liter, or about 18 times the proportion in the fresh-water supply of New York City. One fish was used in each test. (a) Percentage of chlorine, 0.112; (b) percentage of chlorine, 0.130; mean, 0.121.

Were there no other facts upon which to base conclusions, it might be objected that the differences in salt content of these fishes from various sources might be due to differences in their food and not to any osmotic relations between the animals and the surrounding water. Such an objection is of course inapplicable to the foregoing experiments with the various species of *Fundulus*. It is likewise out of question as regards the next two experiments with *Morone*. Here fishes coming originally from brackish water were analyzed after a stay of some days (unfed) in salt or fresh water in the laboratory.

Experiment 111.

Fishes from Tashmoo Pond were kept 6 days in water of density 1.023 before the analysis. In the second of the two specimens used the alimentary canal was removed. (a) Percentage of chlorine, 0.136; (b) percentage of chlorine, 0.162; mean, 0.149.

^aOwing, perhaps, to the fact that in the case of the latter species a number of specimens were used at a time, thus tending to level down individual differences.

This figure is somewhat greater than that for the fishes taken from a somewhat diluted sea water (experiment 109). It will be noted that the second fish gave a very much higher percentage of chlorine, in spite of the removal of the alimentary canal. The contents of the latter, therefore, played no part in determining the amount of chlorine present.

Experiment 112.

Fishes from Tashmoo Pond, kept 5 days in the fresh water of the laboratory prior to analysis. In the first case, 1 fish was used, in the second 2. In *b*, the alimentary canals were removed. (*a*) Percentage of chlorine, 0.120; (*b*) percentage of chlorine, 0.109; mean, 0.114.

This figure will be seen to be somewhat lower than that for the fishes from the fresh-water pond (experiment 110), but in view of such large variations it would be absurd to draw any conclusions from this fact.

If, on the one hand, the mean figure for the *Morone* from the fresh-water pond is averaged with that for those kept in the laboratory fresh-water for 5 days; and if, on the other hand, the figure for the Vineyard Haven fishes is averaged with that for those kept in the laboratory salt water, the figures thus obtained, together with that for the brackish-water fishes, may be arranged in the following series:

(<i>a</i>) Fresh-water specimens	0.117
(<i>b</i>) Brackish-water specimens	0.134
(<i>c</i>) Salt-water specimens	0.144 [or 0.137]

The figure for the "fresh water" fishes is thus 19 per cent [or 15 per cent] less than that for the "salt water" ones. It was found in experiment 97 (also with *Morone*) that 0.036 gram chlorine per 100 grams body weight was given out in the course of a single day. Assuming the percentage of chlorine originally present to have been 0.144 it would appear that 25 per cent of the chlorine of the body was lost in a single day. Here, then, the figures obtained by the two methods are not in full accord.

It may be regarded as abundantly proved by the preceding experiments that some species of fishes, at least, undergo considerable changes in the salt content of their bodies in consequence of changes in the salinity of the surrounding water. These changes are fairly rapid, resulting, at times, in differences of 25 per cent or more in a single day. Moreover, they do not necessarily result in any harm to the animal. It is plain, however, that these changes in the bodily salt content are in no way proportional to the changes in the salinity of the medium. Indeed, very great alterations in the latter may sometimes be made without any apparent effect upon the former. (Experiments 103, *b*, and 107.) The exact extent of the correlation between the two might be determined by a sufficient number of analyses, but it is quite unlikely that it could be expressed by any single mathematical formula. This is because fishes do not conduct themselves as simple dialyzers.

Whether or not all of the tissues are equally affected by these changes in the bodily salt content I can not say. The body as a whole loses or gains in its proportion of salts (chlorides), and these leave or enter it by some path other than the alimentary canal. The occurrence of the weight changes described in the preceding section seems to show the existence of membranes which are permeable in some measure to water, at least. In the absence of any other plausible hypothesis it may be assumed that the salts enter or leave the body through these same membranes.

Which of the limiting membranes of the body are thus concerned will be considered later.

Fredericq (1885) states that the blood of salt-water fishes tastes scarcely more salt than that of fresh-water ones. Surely this is hardly an exact quantitative test. He repeats (1891) that it is "not much more salt." Fredericq likewise states (1885) that it has long been known that the muscles and glands of a salt-water fish are not more salt than those of a fresh-water one. I can not find upon whose authority this statement is based. Griffiths (1892) makes the equally unsupported assertion that "the blood of a sole, a haddock, and a weever does not contain more soluble salts than the blood of fresh-water fishes" (p. 140).

Atwater (1891) gives the percentage of chlorine in the flesh of two salt-water species analyzed by him as follows: Black-fish (*Tautoga onitis*) 0.23, mackerel (*Scomber scombrus*) 0.24; mean, 0.235.

Three fresh-water fishes^a gave the following percentages: Salmon (Penobscot River—spent) 0.18; salmon (landlocked—spent) 0.20; shad (Connecticut River) 0.22; mean, 0.20.

It thus appears from the figures of Atwater that the flesh of the fresh-water fishes analyzed by him had about 15 per cent less chlorine than that of the salt-water ones. In comparing his percentages just quoted with mine it must be remembered that Atwater used the flesh alone while I used the entire fish, including the skeleton. The proportion of chlorides in the latter we should expect to be smaller than in the flesh.

The figures given by Almén (cited by Atwater) show such enormous differences in the amounts of chlorine in different fishes (his maximum figure being nearly 15 times as great as his minimum) that they are certainly to be regarded with suspicion. It may be noted, however, that the average figure for salt-water species is much higher than that for the fresh-water ones (0.122 and 0.076, respectively, provided that we count the salmon and eel among the fresh-water ones).

Katz (1896) gives the mean percentage of chlorine in the dorsal muscles of 2 large eels (apparently from fresh water) analyzed by him as 0.03448; the mean figure for 2 pike was 0.03191; that for 2 haddock ("Schellfish") was 0.24093. Such results certainly demand confirmation. It may be noted that Almén's figures for the eel and pike were 0.013 and 0.186, respectively.

Church (1903) states that he found 0.2 per cent of "common salt" in a "mackerel in good condition." This would place the percentage of chlorine at about 0.121. Atwater's figure was almost exactly twice as great.

As regards the salinity of the blood, the determinations of Quinton (1904, pp. 439-440) give the mean percentage of chlorine^b in the blood of 8 species of marine teleosts as 0.651, that for 3 fresh-water species as 0.411. From these figures it would appear (1) that the salinity (at least the proportion of chlorine) of the blood is several times that of the body as a whole, and (2) that the salinity is over 50 per cent greater for salt-water than for fresh-water species. Mosso (1890) likewise states that sea fish have more salt in their blood than fresh-water ones, though he offers no figures in support.

^aThe shad and the Atlantic salmon are of course anadromous, but the present specimens appear to have been taken in fresh water.

^bQuinton's original figures are given in terms of NaCl, but I have reduced them to corresponding values in chlorine.

Numerous authors have determined the percentages of "ash" in the flesh of fishes, though none, so far as I know, have had in view a comparison between the fresh and the salt water species.

The average percentage of ash in 7 fresh-water^a species analyzed by Atwater was 1.30; for 11 salt-water species, 1.41.

From the determinations of Payen and Kostytscheff (cited by Atwater) it may likewise be gathered that the averages for the salt-water fishes are substantially greater than those for the fresh-water ones.

The figures of Balland (1898) and of Milone (cited by Lichtenfeld, 1904) also give average percentages of ash which are considerably greater for salt-water than for fresh-water species. According to Balland, the figure for eels from fresh water is 0.76, that for salt-water specimens being 0.87.

An exhaustive search of the literature of this subject would perhaps reveal figures which would not harmonize with the general trend indicated above. But, so far as I have been able to learn, one important conclusion may be drawn from the records as a whole, despite great inconsistencies—namely, that both the proportion of ash in general, and the proportion of chlorides in particular, are, on the average, greater in salt-water fishes than in fresh-water ones. The ratio between the two can not, of course, be stated with any exactness. For such a ratio to have any value it would be necessary that each of the mean percentages should be based upon a very large number of exact determinations. Leaving out of account the figures of Katz, however, the mean percentage (either of chlorine or of ash) for the salt-water fishes is in every case less than twice that for the fresh-water ones. In most cases it is much less. On the other hand, the ratio between the amount of chlorine found in sea water and that in ordinary fresh water is as several thousand to one. The following table gives the percentages of chlorine found in several of the grades of water used by me in the experiments:

	•Parts of chlorine per 100.
New York City (Croton) water.....	0.0003
Woods Hole (Falmouth) water.....	.0011+
Daggetts Pond (Marthas Vineyard) ^b0051
Water giving salinometer reading about 1.001.....	.0584
Water giving salinometer reading about 1.002.....	.1252
Local sea water (1.023).....	1.8180

It is thus seen that the water of specific gravity 1.002 (equivalent to that of Tashmoo Pond during the present summer) contains a percentage of chlorine not very far different from that of the fishes (*F. diaphanus* and the white perch, experiments 104 and 108) living in it and, indeed, of the same order of magnitude as that of any of the teleosts analyzed by me. That such water can not even be approximately isotonic with the body fluids of these fishes seems evident from the cryoscopic determinations of other investigators.^c Nevertheless the approximate equality between the percentage of chlorides in this slightly brackish water and in the tissues

^a I have counted the shad and salmon among the fresh-water fishes, the smelt among the salt-water ones.

^b From which came the white perch used in experiment 110.

^c The figures obtained by me do not of course indicate the proportions of chlorine in the fluids, but rather those for the body as a whole. It is likewise to be remembered that the osmotic pressure of the body fluids is in part determined by various organic matters in solution and not entirely by salts.

of teleost fishes is profoundly interesting in view of the great physiological importance to certain species of even such a small proportion of salt in the water. It is interesting to recall in this connection that the "1.001" water did not appear to be quite saline enough to support the life of *F. majalis* indefinitely. (Experiment 31 bis.)

The records of other investigators (to be discussed later) show that the osmotic pressure of the blood of salt-water teleosts is somewhat higher than that of fresh-water ones, though this fact has been almost lost sight of in the zeal to prove that the internal medium is not isotonic with the external and that its osmotic pressure is relatively constant.

It may be objected once more at this point that all this discussion of the relative salt content of fishes inhabiting fresh and salt water, together with the resulting differences in osmotic pressure, is quite irrelevant, since the differences found may depend upon the degree of salinity of the food eaten, and not directly upon the salinity of the water which bathes the body. This objection can only be met by reference to the changes which certain species were found to undergo in the course of a single day, no food being taken. It is theoretically possible, even in these cases, on the one hand that salt water was swallowed and the salts absorbed, on the other that salts should have left the body by way of excretion. The former possibility I regard as sufficiently met by the analyses of the contents of the alimentary canals (indeed, of the entire viscera) described above. The second possibility is not entirely excluded, though it seems unlikely a priori that the decrease in salinity which occurs in fresh water should be due to a cause quite distinct from that responsible for the increase in salt water.

THE PART PLAYED BY THE GILLS IN OSMOTIC EXCHANGES.

The thin membranes covering the gill filaments are especially adapted to facilitating exchange between the gases contained in the water and those contained in the blood. It would therefore be natural to look here for one path of diffusion for water and salts as well. The greater part of the body surface, on the contrary, is in most teleosts covered with a layer of scales, which would seem to present a barrier to any great amount of osmotic exchange between the tissues and the water which bathes the body. The lining of the alimentary canal is of course readily permeable to fluids and to various substances in solution, but this, it is needless to say, is not freely exposed to the surrounding medium. Water or salts, in order to be thus absorbed, must either be swallowed or force their way in through the anus. Such an entry of fluids would not, however, be consistent with the decrease in the weight of a fish which frequently follows its transfer to a stronger salt solution. Yet it seems to be proved by some of the foregoing experiments that salts are in some way taken into the body after such a transfer.

The part played by the gills in this process I have demonstrated by a series of comparatively simple experiments. A piece of apparatus was devised, by the aid of which it was possible to pass salt water through the gills, while the remainder of the body was bathed in fresh water, or vice versa. A wooden frame was made (fig. 1), consisting of a rectangular piece of planed board, near one end of which a shelf was

fastened, containing an aperture large enough to admit the head of a fish. The body of the animal was loosely bandaged, the cloth being tacked to the vertical piece of wood in order to restrain movement (fig. 2). A piece of sheet rubber was then perforated and drawn over the head in such a way that it fitted snugly around the body, just behind the gill covers. The margin of the rubber being tacked to the wooden frame, a nearly water-tight partition resulted, which served to bound off the head from the trunk region. The frame, bearing the fish, was now immersed in a rectangular jar of water, the head being uppermost, except in two specified cases. Fresh (or salt) water was supplied to the jar through a rubber tube, while another tube carried salt (or fresh) water into the mouth, allowing it to pass out through the gills. A second bandage held the head in position, while the pectoral fins were securely bound to staples.

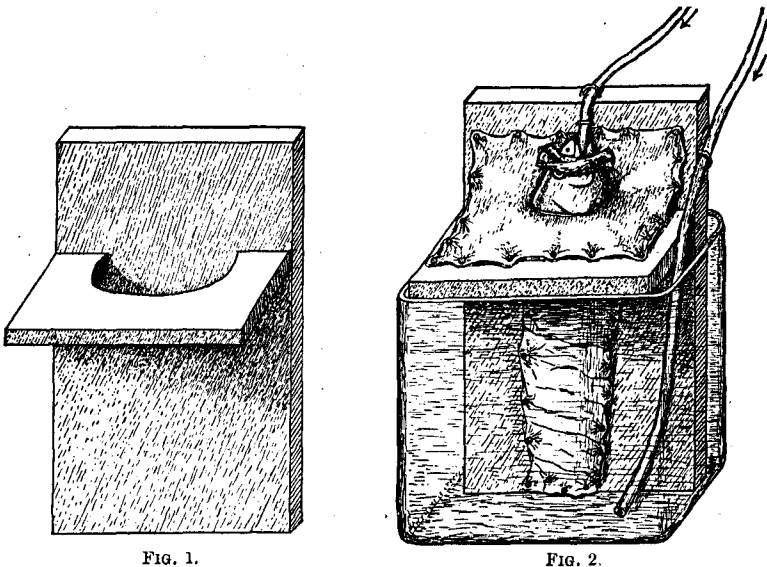


FIG. 1.

FIG. 2.

Device used in experiment to determine the part played by the gills in osmotic exchanges.

The following six experiments were made with the carp. The fishes were weighed before and after the treatment, the same precautions being taken as have already been described for earlier experiments.

Experiment 113.

New York, May, 1905.^a One carp (fed 24 hours previously), weight 86.1 grams. Salt water (1.014) through gills, fresh on body. After 5 hours alive, though feeble; weight 80.9 grams. The fish thus lost 6 per cent.

Experiment 114.

One carp (fed 24 hours previously), weight 63.6 grams. Fresh water through gills, salt on body. After 5½ hours fish well; weight 63.8 grams. Weight thus practically unchanged.

^a Date and place the same for all of this series.

Experiment 115.

One carp (unfed for 22 days), weight 353.5 grams. Salt water through gills, fresh on body. After 15 hours fish dead; weight 338.7 grams. Loss of about 4 per cent.

Experiment 116.

One carp (unfed for 22 days), weight 34.2 grams. Fresh water through gills, salt on body. After 14½ hours fish in good health; weight 34.5 grams. Weight practically unchanged.

Experiment 117.

One carp (same specimen as used in 116), weight 33.6 grams. Salt water through gills, fresh on body. After 16½ hours fish dead; weight 31.1 grams. Loss of 7.4 per cent.

Experiment 118.

One carp (fed preceding day), weight 476.7 grams. Fresh water through gills, salt on body. After 7 hours weight 476.8 grams. Weight practically unchanged.

It will be noted that a considerable loss of weight occurred in all of those cases in which salt water passed through the gills and fresh water over the body, while the weight remained practically stationary in those cases in which the conditions were reversed. Under such harsh treatment the death of any of these fishes would not have occasioned surprise. As a matter of fact, however, the three whose gills were bathed by fresh water all remained well till the end of the experiments, while of those whose gills were bathed by salt water, two died and one sickened.

The part played by the gills in the death of fresh-water fishes in salt water has been discussed by Bert (1871, see p. 101). Bert states that "a tench suspended in a vessel full of sea water, the head remaining outside, lives for a long time, if care be taken to bathe the gills with fresh water." No weight determinations are recorded. In the case of (practically) naked-skinned fishes, such as the eel and loach, Bert held that the general integument made possible the extraction of water from the tissues when the fishes were transferred to salt water.

Two scaleless species were tested by me according to the method described above. The results were here far less satisfactory than those for the carp.

Experiment 119.

Sea raven (unfed for 8 days), weight 334.8 grams. Salt water through gills, fresh on body. After 6 hours fish dead (for some time); weight 332 grams. Loss of about 0.8 per cent.

Experiment 120.

Sea raven (unfed for 7 days), weight 456.3 grams. Fresh water through gills, salt on body. After 19½ hours fish dead (how long?); weight 462.5 grams. Gain of 1.4 per cent.

The loss in the former experiment is scarcely significant, being perhaps fully accounted for by the removal of mucus in drying the fish for weighing. The gain in the second experiment is what would be expected on hypothesis, though the fact that the fish had been dead for an indefinite period lessens greatly the force of this evidence.

Experiment 121.

Catfish (*Ameiurus nebulosus*) (unfed 17 days), weight 121.6 grams. Salt water through gills, fresh on body. After 22 hours fish dead; weight 115.1 grams. Loss of 5.3 per cent.

Experiment 122.

Catfish (unfed 17 days), weight 159.8 grams: Fresh water through gills, salt on body. After 23½ hours fish dead; weight 167.3 grams. Gain of 4.7 per cent.

This gain is probably due to the absorption of water after death, as discussed earlier. It may be due, however, to the animal's having swallowed water.

So far none of these experiments have given results actually contradictory to my view regarding the part played by the gills. The following pair of experiments, however, are not in harmony with the others:

Experiment 123.

Catfish (fed 24 hours previously), weight 156.9 grams. Salt water through gills, fresh on body (fish inverted). After 14 hours fish still stirring; after 22 hours fish dead; weight 157.1 grams. Weight practically unchanged.

Experiment 124.

Catfish (fed 24 hours previously), weight 227 grams. Fresh water through gills, salt on body (fish inverted). After 14 hours fish dead; weight 220.5 grams. Loss of nearly 3 per cent.

Occasional inconsistencies in the results such as these might naturally be expected under the conditions of experiment. The swallowing of water at any time would result in an increase in weight, the discharge of feces in a decrease. In this connection it will be noted that the fishes used in the last two experiments had been fed 24 hours before.

It is possible, however, that naked-skinned fishes react somewhat differently from scaly ones, as was supposed by Bert. Experiments with such fishes should of course be repeated.

If water or salts be taken in or passed out of the body through the membranes of the gills, it seems to follow that the blood must be the medium of such exchanges. Such differences in salinity as were indicated for the fishes used in the analyses above could not, however, have depended entirely upon differences in the salinity of the blood, since the volume of the latter is not sufficient to account for them. Indirectly, then, some or all of the other tissues of the body must have been affected. Where, in the preceding pages, I have referred to the "body fluids," I have meant to include, not merely blood and lymph, but the liquid content of the tissues in general.

It has been assumed by Fredericq (1885) that in certain invertebrates the osmotic equilibrium which is maintained between the body fluids and the surrounding water is due to the permeability of the gills. Concerning fishes, however, he declares "the gills, so permeable to the gaseous exchanges of respiration, seem on the contrary to constitute an almost impassable barrier to the salts dissolved in sea water."

^a Of course living fishes only are here intended.

HISTORICAL REVIEW.

The work of Bert (1871, 1873, 1883) has been more than once referred to in the preceding pages. Bert's explanation of the "mechanism of death" in the case of the fishes studied by him deserves some attention. This writer explained the fatal effects of salt water upon fresh-water fishes by assuming an osmotic action upon the gills. The capillaries of the latter became contracted, and the blood cells, distorted by the action of the salt water, soon plugged them up and thus arrested the branchial circulation. The immediate cause of death was thus believed to be asphyxiation. In the case of scaly fishes, this occurred, he believed, before any considerable amount of water was abstracted from the body osmotically and before the blood in the larger vessels was altered to any appreciable extent. With naked-skinned fishes, on the other hand, osmotic action occurred throughout the entire surface of the body, and the consequent loss of water from the tissues was one factor in causing death. Bert is not entirely consistent in his views, however, for he likewise tells us that "death is definitely due to the chlorides," and "inversely [referring to the death of salt-water fishes in fresh water] it is the suppression of the sodium chloride which causes death." This salt he found could not be replaced by any other substance which he tried. Thus, the importance of chemical factors is recognized by him, though he denies that the salts in sea water act as poisons to fresh-water fishes. The differences in the reactions of different species of fishes are "due to differences in the chemical composition of the branchial epithelium and in the exosmotic properties of this epithelium."

Mosso (1890) also held (for sharks, at least) that the death of the fishes, when placed in fresh water, was the result of asphyxiation due to the blocking of the gill capillaries by disintegrated blood cells. In such a fish he found it impossible to force salt solution through the branchial capillaries, though this could be done readily in the normal animal. Mosso likewise records differences in the resistance of the blood cells of various fishes to the hæmolytic action of dilute salt solutions, implying that such differences may be accountable for the relative power of these animals to withstand changes in the density of the medium. In the case of certain migratory fishes, which inhabit either medium (*Acipenser*, *Salmo*, *Anguilla*, *Petromyzon*) he found the corpuscles to be particularly resistant, being able to remain many hours in a salt solution as dilute as 0.3 to 0.4 per cent without giving up their hæmoglobin. Sea fishes as a rule he found to have less resistant corpuscles than fresh-water ones. Other investigators (Hamburger, 1887; Bottazzi and Ducceschi, 1896, and Rodier, 1899) have performed similar experiments upon the erythrocytes of various fishes, likewise finding great differences in their resisting powers, though not in all respects agreeing with the determinations of Mosso.

Of course any such effect of the surrounding medium upon the cells of the blood presupposes some way by which it may reach them. Bert believed that in fishes (at least the scaly ones) death occurred before any considerable portion of the blood was affected, the osmotic effects being restricted to the gills.

In any discussion of the effects of changes in water density upon aquatic organisms, the osmotic factor must play a leading part. Much light has in recent years been thrown upon the osmotic relations maintained between the "internal and

external media." The now rather celebrated utterance of Claude Bernard (1865, p. 110) is perhaps worth repeating here; I do not recall its having been quoted in an English work:

In all living beings the internal medium, which is a true *product of the organism*, preserves the necessary relations of exchange and of equilibrium with the external cosmic medium, but in proportion as the organism becomes more perfect, the organic medium specializes and isolates itself, in a certain manner, more and more from the surrounding medium.

The Belgian physiologist, Fredericq, was one of the first to investigate the osmotic relations between the body fluids of marine organisms and the medium in which they live. By the method of dialysis, and by determinations of the salinity of the body fluids, he found that in various marine invertebrates these fluids were nearly or quite isotonic with the sea water. He likewise showed that changes in the salinity of the water resulted in corresponding changes in the body fluids. As regards fishes (both elasmobranchs and teleosts) Fredericq maintained that the osmotic pressure was considerably less (about one-half) than that of the external medium.

Bottazzi and his colleagues, working at Naples, employed the cryoscopic method, using Beckmann's apparatus. By this method the freezing point of a solution is determined with precision, and from this the osmotic pressure, according to well-known physical principles. Bottazzi arrived at the same conclusion as Fredericq, relative to the osmotic pressures of the body fluids of marine invertebrates. The reduction in the freezing point (Δ) due to osmotically active substances in solution was found to be nearly constant, ranging from -2.195° C. to -2.36° C. The mean of all his determinations was -2.29° C., which is the same figure as his mean determination for the local sea water.

Practically identical results were obtained for elasmobranch fishes, the mean osmotic pressure for the blood of three species being expressed by: $\Delta = -2.356$ C. The difference between these results and those of Fredericq is explained by the fact that the latter inferred a lower osmotic pressure for the blood of elasmobranchs from its lower salt content. The blood is rendered isotonic with sea water, however, by the presence of an unusually large proportion of urea. This correction has been accepted by Fredericq himself.

For the blood of two species of marine teleosts four determinations by Bottazzi yield the mean figure: $\Delta = -1.036$. The blood of these fishes thus appears to have an osmotic pressure about half that of the water which they inhabit.

Rodier, Quinton, and Garrey have also determined that the vascular fluids of various marine invertebrates are practically isotonic with the water which they inhabit. Rodier and Garrey, employing the cryoscopic method, have likewise confirmed the conclusions of Bottazzi regarding the elasmobranchs, while Rodier, Fredericq, and Garrey have by this method obtained confirmatory (though not identical) results for teleosts.

The mean value of Δ for two elasmobranchs tested by Garrey at Woods Hole is -1.92° . The mean value for five teleosts is -0.872° , the figures ranging from -0.80° (minimum for conger eel) to -0.96° (maximum for swordfish). The value of Δ for the sea water of the neighborhood was about -1.82° .

The testimony of a number of investigators seems, then, to be in full accord upon certain main points, which may be provisionally accepted as proved. Fredericq (1904) has classified the three sorts of aquatic organisms, relative to osmotic conditions, as follows: (1) Molecular concentration and salt content both approximately the same for the [vascular fluids of] animal as for the surrounding water (marine invertebrates); (2) molecular concentration the same, but proportion of salts less—the deficiency being compensated for by organic matters in solution—(elasmobranchs); (3) both molecular concentration and salt content very different from those of external water (teleosts, both marine and fresh water; fresh-water invertebrates).

It is with the third class that we are especially concerned in the present paper. The molecular concentration (hence the osmotic pressure) and the salt content are both very different from those of the surrounding medium. It seems never to have been fully appreciated that there is even here a certain correlation between the inner and the outer fluids, both as regards osmotic pressure and salt content. But reference to the various cryoscopic determinations shows that not all teleosts have blood of the same osmotic pressure. Rodier found the latter to range between $\Delta = -0.62^\circ$ and $\Delta = -0.80^\circ$ in *Lophius* alone. What is more significant is that the blood of fresh-water fishes has been found to possess, on the average, a considerably lower osmotic pressure than that of marine fishes. The mean figure given by Fredericq (1904) for two marine teleosts is $\Delta = -0.80^\circ$, that for three fresh-water species is about -0.53° . Of course in the case of these fresh-water fishes, the osmotic pressure of the blood, though lower than that of marine fishes, is nevertheless very much higher than that of the fresh water in which they live. Satisfactory determinations of both fresh and salt water individuals in the case of species inhabiting both have not, so far as I know, been made. As noted above (p. 96), Balland found that the flesh of eels from salt water contained a considerably greater percentage of ash than those from fresh water. Similar differences in chlorine content were obtained by myself in the case of several species, and in general it has been shown that the percentage of salts is greater in salt-water fishes than in fresh-water ones. It must be borne in mind, however, that these determinations of salts were made for the flesh of the fishes, while those of osmotic pressure were made upon the blood. So far as I know the only recorded comparison of the salt content of the blood of fresh and salt water fishes was made by Quinton (p. 95). A considerably higher percentage was indicated for the marine forms. If the figures of Quinton are correct, it is likewise to be noted that the percentage of chlorides in the blood is several times as great as in the flesh. Of course the osmotic pressure of neither is entirely dependent upon the percentage of salts, but may depend upon organic matters in solution, as was found in the case of elasmobranch blood. Fredericq (1904) has attempted to determine the osmotic pressure of the solutions contained in the various tissues of fishes and invertebrates, partly by extracting the soluble ingredients by boiling, partly by noting the changes of weight in strips of tissue suspended in solutions of varying concentration. Reasons for doubting the value of the latter method have already been given (p. 84).

Where a correlation is found to exist between the osmotic pressure of the body fluids of an animal and that of the surrounding water, the question arises, How is this correlation maintained? For various invertebrates, it seems to have been gen-

erally held (Fredericq, Atwater^a, Quinton, Garrey) that the bounding membranes, or some of them, are permeable both to water and salts. Botazzi and Enriques, on the other hand, from experiments upon the excised gut of *Aplysia*, conclude that, in a normal condition, this and presumably the other limiting surfaces of the body are only semipermeable. This condition, of course, would be sufficient to insure an osmotic equilibrium between the organism and its environment. Equivalence in the proportions of the various saline ingredients is maintained, according to these writers, on the one hand through the process of (nutritive) absorption, occurring chiefly in the ducts of the digestive gland; on the other through the organs of excretion.

For the elasmobranchs, a permeability to water seems to follow from the facts above stated. An unlimited permeability to salts must, on the other hand, be excluded, if, as seems proved, the salt content of the blood is so far below that of sea water. Whether the (gill?) membranes are in any degree permeable to salts has not been determined experimentally.

In the case of teleosts, it does not seem to have been generally appreciated that there is a certain correlation between the inner and outer fluids, both as regards osmotic pressure and salt content; and certain authors have been free to state that the membranes of teleost fishes form an effective barrier against osmotic changes. Fredericq (as quoted above, p. 100) makes this assertion broadly; while Garrey says of *Fundulus heteroclitus*: "The integument and gills are therefore impermeable." Garrey is cautious enough, however, not to postulate an absolute impermeability either for *Fundulus* or for teleosts in general.

SUMMARY AND CONCLUSIONS.

The more important results of the foregoing experiments may be very briefly summarized as follows:

(1) Certain brackish and salt-water fishes were unable to survive even a gradual transfer to pure fresh water, though enduring an abrupt transfer to water of a very low degree of salinity. Thus fresh water, as such, proved fatal to these fishes, the degree of abruptness of the change being of secondary importance.

(2) Considerable changes of weight were found to result, in many cases, from changes in the salinity (hence the osmotic pressure) of the surrounding medium.

(3) Considerable changes in the salt (chlorine) content of the body were likewise found to result, in many cases, from changes in the salinity of the water.

(4) Careful control experiments excluded the possibility that the water or salts entered or passed from the body through the alimentary canal, leaving as the only probable alternative an osmotic exchange through one or more of the limiting membranes.

(5) In certain fishes, at least, it was found that the membranes chiefly concerned in such exchanges were those of the gills.

Accordingly, we can not conclude from the absence of osmotic equilibrium between the fish and its environment that no osmotic interchanges normally occur. On the contrary, abundant experiments seem to prove that both water and salts may under certain conditions be transmitted in either direction without any harm result-

^a For oysters, *op. cit.*, p. 814.

ing to the fish. These conditions seem impossible to state in advance for a given case. In general we may say that:

(1) Measurable changes in weight result only from considerable changes in the density of the surrounding water, but—

(2) Not all such changes of density suffice to produce changes of weight, even when the fish is transferred to a medium which is known to be strongly hypertonic or hypotonic to its own body fluids.

(3) Changes in the salinity of the water may or may not result in changes in the salt content of the body.

(4) Changes in the body salt content may or may not be accompanied by changes in weight.

(5) Neither the changes in weight nor in salt content are at all proportional to the changes in the density of the external medium.

It would appear that there is normally a tendency on the part of the fish to resist osmotic changes and to maintain the fluids of the body at a definite degree of concentration. Under various conditions, however, this resistance is overcome and a certain degree of permeability is established. This is generally a differential permeability, resulting in osmosis and consequent changes of weight. In such cases, however, the membranes are not strictly semipermeable, but transmit salts in some measure. Indeed, it would seem that at times the permeability is indiscriminate, in which case the salts may diffuse freely, but no changes in weight occur. These various changes continue until a new level of stability is established, after which the normal resisting power of the fish reasserts itself and no further alteration occurs so long as the medium is constant. Complete osmotic equilibrium between the fish and the water is probably never attained except in waters having roughly a medium degree of salinity. The osmotic pressure of the "internal medium" fluctuates within a much narrower range than that of the "external medium."

The foregoing conclusions are intended to apply only to normal fishes. It seems certain that the enfeeblement of the fish may result in an increased permeability of the membranes, which in turn would doubtless result in a further enfeeblement of the fish. The death of those fishes which can not withstand transfer to a medium very different from that to which they are accustomed is thus probably in part a cause and in part an effect of these changes. Death is accompanied (perhaps in some cases caused) by a giving way in the power to resist an abnormal degree of osmotic exchange. The body becomes water-soaked (if in fresh water), or dehydrated (if in salt). The difference between the more hardy and the more delicate species in this regard seems to lie partly in the resisting power of the limiting membranes (chiefly those of the gills); partly, also, in internal differences, such as composition of blood, etc., which determine whether a given influx or efflux of water or salts shall prove fatal.

The actual cause of death following a change in the salinity of the water seems to differ in different cases. With those fishes which succumb rapidly with but a slight change of weight (e. g., scup, experiments 69-71), it is unlikely that any appreciable alteration occurs in the tissues at large. Such changes are probably confined to the blood, perhaps, as Bert held, to that in the gill capillaries. In those cases, on the contrary, where the fatal effects are not manifested for some days, it

seems likely that the manner of death is different. In the case of *F. heteroclitus* it was found in most instances that the endosmotic flow of water had ceased, and that a decrease in weight had ensued within one or two days after transfer to fresh water. On the other hand, it will be remembered that fishes of this species commonly did not die for a considerable number of days, while many survived for a week and some even for several weeks. Again, it will be recalled that the fatal effects of fresh water upon this and some other species were nullified by the admixture of a very small percentage of salt water. Analyses showed that in this latter case there was little or no decrease in the salt content of the body. A rough approximation was pointed out (p. 96) between the percentage of salts in this faintly saline water and that in the fishes themselves. All of these facts point to the conclusion that one factor in the death of salt-water fishes in fresh water is the extraction from their tissues of an amount of salts sufficient to reduce the percentage below a certain necessary minimum.

If the question be asked, Why are not fresh-water fishes thus affected in their own medium? it is replied that their membranes have been adapted to resisting such an extraction of salts. It is perhaps also true that the irreducible minimum of salts in these species is lower than in the case of salt-water ones. In any case the percentage actually present is, on the average, less.

Whether or not salt water ever has a toxic effect, in the narrower sense, upon fresh-water fishes can not be stated definitely. Bert denied that such was the case, but, as already stated, he is not entirely consistent in his position. In view of the fatal effects upon salt-water fishes of some of the individual components of sea salt, when taken separately (Loeb, 1900; Siedlecki, 1903), it seems quite possible that sea water itself may act as a poison to fresh-water organisms, independently of any osmotic effects. Indeed, both of these writers have shown that it is the chemical nature of the solutions used rather than their osmotic pressures which determines, in many cases, whether they shall prove fatal.

SUPPLEMENTARY NOTE.

It gives me great pleasure to find in a paper by Prof. C. W. Greene on the physiology of the chinook salmon, just published, that certain of the results obtained by him lend strong support to the views upheld in the preceding pages. Greene finds a very considerable difference between the osmotic pressure of blood from salmon taken in sea water and of that from fishes taken at the spawning grounds far upstream. The mean values for Δ given by Greene are -0.762° and -0.628° , respectively, showing a decrease of 17.6 per cent in the fresh-water individuals. Greene is not convinced, however, that osmotic changes have been responsible for this decrease, but is of the opinion that "the absence of food and the important metabolisms occurring during the eight to twelve weeks' sojourn in fresh water are to be considered in this connection, and possibly are sufficient to account for the change" (p. 455).

Another of Greene's results is of great interest to me, namely, that he found but a slight reduction (3.3 per cent) in the osmotic pressure of the blood of salmon

taken in faintly saline water near the (Sacramento) river's mouth. Greene's conclusion appears to be that the slight extent of this reduction was owing to the short time which had elapsed since the fishes had left the sea. Unfortunately the length of this interval is not stated. According to Rutter's account, the first steps of the passage into the Sacramento River are rather gradual, the fish falling back somewhat with each ebb tide. My own experiments show that with certain fishes, at least, a single day's sojourn in fresh water is sufficient to effect a considerable reduction in the salt content of the body. On the other hand, even a very slight degree of salinity was sufficient to prevent this change. In the absence of further data I suggest the same explanation for the results obtained by Greené with the brackish-water salmon.

LIST OF WORKS REFERRED TO.

1891. ATWATER, W. O. The chemical composition and nutritive values of food fishes and aquatic invertebrates. Report of U. S. Commission of Fish and Fisheries for 1888, pp. 679-868.
1898. BALLAND. Sur la composition des poissons, des crustacés et des mollusques. Comptes rendus de l'Académie des Sciences, t. 126, pp. 1728-1731.
1903. BEAN, T. H. Catalogue of the fishes of New York. Bulletin no. 60, New York State Museum.
1865. BERNARD, C. Introduction à l'étude de la médecine expérimentale. Paris, 1865.
1871. BERT, P. Sur les phénomènes et les causes de la mort des animaux d'eau douce que l'on plonge dans l'eau de mer. Comptes rendus de l'Académie des Sciences, t. 73, pp. 382-385, 464-467.
1873. ————. La mort des animaux d'eau douce que l'on immerge dans l'eau de mer. Comptes rendus de la Société de Biologie, 5^e série, t. 3, pp. 59-61.
1883. ————. Sur la cause et la mort des animaux d'eau douce qu'on plonge dans l'eau de mer et réciproquement. Comptes rendus de l'Académie des Sciences, t. 97, pp. 133-136.
1896. BOTTAZZI, F., and DUCCESCHI. Résistance des érythrocytes, etc. Archives italiennes de Biologie, t. 26, pp. 161-172.
1897. BOTTAZZI, F. La pression osmotique du sang des animaux marins. Archives italiennes de Biologie, t. 28, pp. 61-72.
1901. BOTTAZZI, F., and ENRIQUES, P. Ueber die Bedingungen des osmotischen Gleichgewichts und des Gleichgewichtsmangels zwischen den organischen Flüssigkeiten und dem äusseren Medium bei den Wasserthieren. Archiv für Anatomie und Physiologie. Supplement-Band, p. 109-170.
1903. BROWN, O. H. The immunity of *Fundulus* eggs and embryos to electrical stimulation. American Journal of Physiology, vol. 9, pp. 111-115.
1903. CHURCH, A. H. Food. London, 1903.
1897. DAVENPORT, C. B. Experimental morphology. New York, 1897.
1884. DITTMAR, W. Report on researches into the composition of ocean water. Challenger Reports: Physics and Chemistry, vol. I.
1885. FREDERICQ, L. Influence du milieu ambiant sur la composition du sang des animaux aquatiques. Archives de Zoologie Expérimentale, 2^e série, t. III, Notes et Revue, pp. xxxiv-xxxviii.
1891. ————. Sur la physiologie de la branchie. Archives de Zoologie Expérimentale, 2^e série, t. IX, pp. 117-123.
1904. ————. Sur la concentration moléculaire du sang et des tissus chez les animaux aquatiques. Archives de Biologie, t. 20, pp. 709-730.
1895. GARMAN, S. The cyprinodonts. Memoirs of the Museum of Comparative Zoology, vol. XIX, 1895.
1905. GARREY, W. E. The osmotic pressure of sea water and of the blood of marine animals. Biological Bulletin, vol. VIII, no. 4, pp. 257-270.
1900. GIARD, A. Sur l'adaptation brusque de l'épinoche (*Gasterosteus trachurus* Cuv. et Val.) aux eaux alternativement douces et marines. Comptes rendus de la Société de Biologie, t. 52, pp. 46-48.

1905. GREENE, C. W. Physiological studies of the chinook salmon. Bulletin U. S. Bureau of Fisheries 1904, vol. xxiv, pp. 429-456.
1892. GRIFFITHS. Physiology of the Invertebrata. London, 1892.
1887. HAMBURGER, H. J. Ueber die Salz- und Rohrzucker-Lösungen bewirkten Veränderungen der Blutkörperchen. Archiv für Anatomie und Physiologie, 1887, p. 31-50.
- 1896-1900. JORDAN, D. S., and EVERMANN, B. W. The fishes of North and Middle America. Bulletin United States National Museum No. 47.
1896. KATZ, J. Die mineralischen Bestandtheile des Muskelfleisches. Archiv für die gesammte Physiologie, Bd. 63, p. 1-85.
1904. LICHTENFELD, H. Ueber die chemische Zusammensetzung einiger Fischarten. Archiv für die gesammte Physiologie, Bd. 103, p. 353-402.
1894. LOEB, J. Ueber die relative Empfindlichkeit von Fischembryonen gegen Sauerstoffmangel und Wasserentziehung in verschiedenen Entwicklungsstadien. Archiv für die gesammte Physiologie, Bd. 55, p. 530-541.
1900. ————. On ion-proteid compounds and their rôle in the mechanics of life phenomena. I. The poisonous character of a pure NaCl solution. American Journal of Physiology, vol. 3, no. vii, pp. 327-338.
1900. ————. On the different effect of ions upon myogenic and neurogenic rhythmical contractions and upon embryonic and muscular tissue. American Journal of Physiology, vol. 3, no. viii, pp. 383-396.
1881. MATHER, F. Fishes which can live in both salt and fresh water. Transactions of the American Fish Cultural Association, pp. 65-75.
1890. MOSSO, A. Ueber verschiedene Resistenz der Blutkörperchen bei verschiedenen Fischarten. Biologisches Centralblatt, Bd. X, p. 570.
1900. QUINTON, R. Communication osmotique chez l'Invertébré marin normal, entre le milieu intérieur de l'animal et le milieu extérieur. Comptes rendus de l'Académie des Sciences, t. 131, pp. 905-908.
1900. ————. Perméabilité de la paroi extérieure de l'Invertébré marin, non seulement à l'eau mais encore aux sels. Comptes rendus de l'Académie des Sciences, t. 131, pp. 952-955.
1904. ————. L'eau de mer milieu organique. Constance du milieu marin original comme milieu vital des cellules à travers la série animale. Paris, 1904, 503 pp.
1899. RODIER, E. Observations et expériences comparatives sur l'eau de mer, le sang et des liquides internes des animaux marins. Travaux des laboratoires de la Station Zoologique d'Arcachon, pp. 103-123 (cited by Fredericq, 1904).
1900. ————. Sur la pression osmotique du sang et des liquides internes chez les poissons Sélaciens. Comptes rendus de l'Académie des Sciences, t. 131, pp. 1008-1010.
1902. RUTTER, C. Studies in the natural history of the Sacramento salmon. Popular Science Monthly, July, 1902, pp. 195-211.
1904. ————. Natural history of the quinnat salmon. Bulletin of the U. S. Fish Commission 1902, vol. xxii, pp. 67-142.
1903. SIEDLECKI, M. Sur la résistance des épinoches aux changements de la pression osmotique du milieu ambiant. Comptes rendus de l'Académie des Sciences, t. 137, pp. 469-471.
1903. ————. L'action des solutions des sels alcalins et alcalino-terreux sur les épinoches. Comptes rendus de l'Académie des Sciences, t. 137, pp. 525-527.
1897. SMITH, EUGENE. The fishes of the fresh and brackish waters in the vicinity of New York City. Proceedings of the Linnæan Society of New York, pp. 9-51.
1902. ————. The Home Aquarium. New York, 1902.
1892. DE VARIGNY, H. Experimental evolution. London, 1892.