taurine concentration by 61%. Liver concentrations of glutamic acid, valine, isoleucine, leucine, and ornithine also decreased significantly when skates were acclimated to a diluted environment. Protein concentrations were 87.5 ± 3.3 mg/g liver and 31.8 ± 5.7 mg/g kidney for skates maintained in 100% seawater. Acclimation of the fish to 50% seawater significantly decreased the protein concentrations of liver to 74.8 ± 3.2 mg/g (p < .01) and kidney to 18.9 ± 1.4 mg/g (p < .05). Environmental dilution also resulted in a 10% decrease in skate liver dry weight percentage. The decrease in liver protein concentration in skates acclimated to 50% seawater can be accounted for by this change in dry weight.

Investigation of the mechanisms by which amino acid pools are altered in response to environmental salinity change can provide important information for understanding cell volume regulation. The present study revealed that oxidation of β-alanine occurs primarily in the liver and kidney of the little skate. This suggests that the dilution-induced decreases in β-alanine concentration in tissues that cannot oxidize the amino acid (e.g. muscle) may be achieved by transport of the amino acids, via the circulatory system, to the liver where deamination takes place. We found that acclimation of skates to 50% seawater caused a rise in capacity of the liver to oxidize β-alanine and alanine. This increased oxidation may have been brought about by the increased load of amino acids transported to this organ from other tissues and would facilitate a faster removal of amino acids during the acclimation period.

The inability to demonstrate taurine oxidation in any of the skate tissues suggests that this amino acid may be excreted intact. Taurine has been shown to be poorly reabsorbed by the mammalian renal tubule (Danztler and Sibernagl, Pflugers Archiv. 367:123-128, 1976). If it is handled comparably by the elasmobranch kidney, taurine released by tissues during environmental dilution would be readily excreted.

In the present study, we observed a decrease in liver NPS and an increase in liver amino acid oxidation when skates were acclimated to 50% seawater. A previous study by Goldstein and Forster (Am. J. Physiol. 220:742-746, 1971) demonstrated that acclimation to a diluted environment caused a decrease in total nitrogen excretion. Thus despite increased oxidative capacity of the liver, lowered amino acid concentrations may cause a decrease in total nitrogen excretion for skates acclimated to 50% seawater.

HYBRIDIZATION EXPERIMENTS AND SPECIATION IN *Fundulus heteroclitus* AND *Fundulus diaphanus*

Charles E. Wilde, Jr. and Mark Rosenfeld, University or Rhode Island, Kingston, Rhode Island

The discovery by one of us (M.J.R.) of what is apparently a hybrid between *Fundulus heteroclitus* (Linn.) and *Fundulus diaphanus* (Lesueur) in the estuarine waters of Narragansett Bay, Rhode Island has led to the experiments listed below. This report concerns itself with those experiments conducted at M.D.I.B.L. while a later paper will describe the natural hybrids and hybridization experiments conducted in Rhode Island. Previous reports of hybridization are few. Hubbes et al (Contrib. Lab. Vert. Biol. Univ. Mich. 23, 1943) Breder and Rosen (Modes of reproduction in fishes. Natur. Hist. Press, New York, 1966) and Newman (J. Exp. Zool. 16:447-499, 1914) have reported the event in nature and experimentally in the laboratory.

One of us (C.E.W. Jr.) has for many years been concerned with the apparent differences in developmental rate of zygotes fertilized in the laboratory at M.D.I.B.L. and those reported from experimental fertilizations at Woods Hole and other places on the south shore of Cape Cod and in Narragansett Bay. In spite of water temperature differences there remained, perhaps, the possibility that *Fundulus heteroclitus* was not the same to the north of Cape Cod as that to the south of this barrier.

A series of fertilizations, following meticulously the methods established and reported by Wilde and Crawford (Exp. Cell. Res. 44:471-488, 1966) were carried out as follows:

During the peak of the Maine breeding season, *Fundulus heteroclitus* from Narragansett Bay estuaries and *Fundulus diaphanus* from a similar southern source, both male and female, were transported to M.D.I.B.L. where they arrived in good condition.

The breeding season for *F. heteroclitus* in Maine is slightly later than that of the apparent conspecific in warmer southern waters. However there was just sufficient time to conduct two intensive experiments to assess hybridization capabilities.

The following crosses and controls were carried out. It will be apparent from the data that eggs and sperm from all animals used were viable and participated in zygote formation.

Fundulus heteroclitus (Maine) sperm was used to fertilize:

Fundulus heteroclitus (Maine) eggs (Control)	(1)
Fundulus heteroclitus (Rhode Island) eggs	(2)
Fundulus diaphanus (Rhode Island) eggs	(3)

Fundulus heteroclitus (Rhode Island) sperm was used to fertilize:

Fundulus heteroclitus (Maine) eggs	(4)
Fundulus heteroclitus (Rhode Island) eggs (Control)	(5)
Fundulus diaphanus (Rhode Island) eggs	(6)

Fundulus diaphanus (Rhode Island) sperm was used to fertilize:

Fundulus heteroclitus (Maine) eggs	(7)
Fundulus heteroclitus (Rhode Island) eggs	(8)
Fundulus diaphanus (Rhode Island) eggs (Control)	(9)

In all classes activation (fertilization?) occurred and the eggs cleaved and developed to stage 9-10 (Oppenheimer), the early blastula.

Zygotes of the following classes did not undergo development past the blastula and remained as arrested blastulae until necrosis set in.

F. heteroclitus (Maine) sperm and *F. heteroclitus* (R.I.) eggs	(2)
F. heteroclitus (Maine) sperm and *F. diaphanus* eggs	(3)
F. diaphanus sperm and *F. heteroclitus* (R.I.) eggs	(8)

Zygotes of the reciprocal classes, namely:

F. heteroclitus (R.I.) sperm and *F. heteroclitus* (Maine) eggs	(4)
F. diaphanus sperm and *F. heteroclitus* (Maine) eggs	(7)
F. heteroclitus (Rhode Island) sperm and *F. diaphanus* eggs	(6)

developed normally and hybrid fry were hatched.

Zygotes of the following three classes

F. heteroclitus (Maine) sperm and eggs	(1)
F. heteroclitus (Rhode Island) sperm and eggs	(5)
F. diaphanus sperm and eggs	(9)

all developed normally (controls) and normal fry were hatched.

The pigmentation patterns of late embryos and fry made it quite easy to distinguish between normal *heteroclitus* and *diaphanus* individuals. The pigmentation pattern of the two *heteroclitus* classes was identical. The developmental rate of the southern species at 18°C was apparently more rapid than the Maine species. This requires further analysis.

Analysis of the hybrid fry showed:

a. The cross, *diaphanus* sperm and *heteroclitus* (Maine) eggs developed with the *diaphanus* pigment pattern.
b. The *heteroclitus* (Rhode Island) sperm and *diaphanus* egg cross developed with the *diaphanus* pattern. This suggests that the genomes of both sperm and eggs in these crosses contributed to the development of fry. This will require further study.

Of the many hundreds of zygotes which made up these experiments, in <u>one</u> of the forbidden classes [*heteroclitus* (Maine) sperm and *heteroclitus* (Rhode Island) eggs], one abnormal fry was discovered. Edematous and short, its behavioral characteristics indicated a severe anomaly of its central nervous system. This case will be restudied.

It is concluded, since:

a. F. *diaphanus* male will cross with Maine but not with Rhode Island F. *heteroclitus* female

b. F. *heteroclitus* (Maine) male will not cross with F. *diaphanus* female , yet F. *heteroclitus* (Rhode Island) male will cross with F. *diaphanus* female

c. F. *heteroclitus* (Rhode Island) male will cross with F. *heteroclitus* (Maine) female but the reciprocal F. *heteroclitus* (Maine) male will not (or very rarely) cross with F. *heteroclitus* (Rhode Island) female -

that F. *h eteroclitus* (Maine) is distinct from F. *heteroclitus* (Rhode Island) at least at the sub-species level.

It is probable that the land barrier of Cape Cod which separates the colder waters of the Gulf of Maine from the warmer waters to the south has served to isolate the two *heteroclitus* groups leading to the onset of speciation.

EFFECT OF MUCOSAL pH ON PD IN DOGFISH GASTRIC MUCOSA

George W. Kidder III, Dept. of Physiology, University of Maryland, School of Dentistry, Baltimore, MD

The <u>in vitro</u> dogfish gastric mucosa produces a small PD (-5 to -10 mV, reference mucosal soln.) with sufficient O_2 and CO_2 (Kidder, AJP 231:1240, 1976). The magnitude and sign of this PD (reversed from most mucosae) raises the possibility that it arises from diffusion of HCl from the bottoms of the secretory pits (pure HCl, isosmotic, calc. pH = 0.57) to the mucosal fluid (pH 4.5 <u>in vitro</u>). To test this, 6 mucosae were mounted as before (Bulletin MDIBL 15) (hyperbaric) and PD measured as mucosal pH was varied by substitution of H^+ for Na^+. Such changes produce marked time-dependent variations in PD; I have elected to consider the PD 30 minutes after the change as representing a reasonably consistent value.

These results are shown in Figure 1. While some theoretical considerations predict a curve for such data, the large scatter precludes meaningful analysis on this basis. However, the extrapolated value at pH 0.57 (+11.7 mV) is clearly different from zero (the expected value from the diffusional proposal above), but the finite slope observed makes it likely that H^+ diffusion plays a role in the establishment of the observed PD.

In view of these results, it was decided to measure the PD and pH in dogfish stomach <u>in vivo</u>. Male dogfish of about 2 Kg were restrained on a "V-board" with their heads under running sea water. A combination pH/reference electrode was inserted into the stomach through the mouth, and PD recorded from a KCl/calomel fiber-junction electrode attached to the pH electrode and a similar electrode in the peritoneal cavity. Four fish were studied in this manner, with one additional fish monitored for pH alone. After at least 30 minutes of recording, 0.5 ml of 10^{-2} M histamine was injected into the caudal vein, and recording continued for at least an additional hour. The data are corrected for electrode asymmetries.

The results are shown in Figure 2. Stimulation by histamine caused a marked fall in lumenal pH (to 0.72 in one case) and a reversal of the PD from -4 to about +5 mV (reference lumen) during the hour following the secretagogue.