development to a 5-celled stage. The cultures, however, had to be abandoned before the production of antheridia and oogonia could be demonstrated. Satisfactory methods were worked out for the culture of the gametophytes, which should lead to results quite promptly when this study can be resumed.

NOTE ON THE BEHAVIOR OF THE BARNACLE

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Attempts to use the barnacle, *Balanus balanoides,* for studies on stimulation during the summer of 1933 were unsuccessful due to the almost continuous irregularity of the cirral movements. Previous work has shown that irregularity is caused by mild stimulating agents in the sea water, and that some substances are very effective in extremely low concentrations. Similar irregularity has been encountered in past years at certain times during the summer, especially after the middle of August, but it has never continued for more than a week or two. Preliminary experiments designed to identify the causes of the irregularity clearly showed that some constituent of the sea water was responsible. Shaking the water with charcoal and filtering through paper resulted in a marked improvement of the cirral movements. Filtering through paper only gave a temporary improvement which was just noticeable. These and other experiments seem to indicate that the factor responsible for irregularity is some substance in solution in the sea water, perhaps contributed by the disintegration of micro-organisms. Systematic studies on the fluctuations of the micro-organic population, of the hydrogen ion concentration, of the specific gravity and of the chemical composition of the sea water correlated with the behavior of the barnacle will be necessary before the problem can be solved.

STUDIES ON STIMULATION BY HYDROCHLORIC, SULFURIC AND NITRIC ACIDS AND THEIR SODIUM SALTS IN *FUNDULUS**

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The investigation of chemical stimulation in aquatic organisms was continued during the summer of 1933 by testing the stimulating efficiencies of hydrochloric, sulfuric and nitric acids, and the corresponding sodium salts in *Fundulus heteroclitus.* The experimental procedure was the same as that described in previous reports. The temperature was 17.6 $\pm$ 0.2°C. Each solution was tested 10 times on each of two fish. The hydrogen ion concentrations of the solutions

* A correction should be made to the research summary on stimulation of Fundulus by hydrochloric acid and by fatty acids published on page 30 in the 1933 report. The sentence, beginning on line 11 of paragraph 1 should read: "To give a reaction time of approximately 10 seconds, the following hydrogen ion concentrations were necessary for each acid: caproic, 1.123 x 10^{-7}; heptylic, 2.188 x 10^{-7}; valeric, 2.692 x 10^{-7}; butyric 6.457 x 10^{-7}; propionic, 8.318 x 10^{-7}; acetic, 11.23 x 10^{-7}; formic and hydrochloric, 15.15 x 10^{-7}."

were measured by a quinhydrone electrode method for all solutions of pH = 6 or less; for solutions of higher pH as well as for sea water a colorimetric method employing phenol red was used. Table I presents the substances tested, the concentration and the pH limits. The corrected pH of the sea water varied from 8.1 to 8.2 during the summer.

TABLE I

Substance	*Number of concentrations*	*Limits of concentrations (N)*	*Limits of pH of solutions*	*Number of observations*
HCl	21	0.0014 - 0.005	2.70 - 5.80	420
H_2SO_4	16	0.0014 - 0.005	2.68 - 5.87	320
HNO_3	16	0.0015 - 0.005	2.70 - 5.85	320
NaCl	22	0.08 - 0.19	pH of sea water	440
Na_2SO_4	9	0.08 - 0.18	pH of sea water	180
$NaNO_3$	21	0.08 - 0.18	pH of sea water	420

A recognizable response of the opercula was observed when the hydrogen ion concentration of sea water was increased above a certain limiting value (approximately 1×10^{-6}) by the addition of one of the mineral acids to sea water. The time for this response to appear (reaction time) decreased as the hydrogen ion concentration increased. Equation (1) describes the relationship between these two

$$\text{R.T.} - 2 = \frac{1}{3.716(H^+)^{0.292}} \tag{1}$$

variables over the experimental range. Two seconds is subtracted from each reaction time to correct for a latent period inherent in the experimental procedure and in the receptor-response mechanism. Since equation (1) is applicable to the data obtained from hydrochloric, sulfuric, or nitric acids, equivalent concentrations of these strong acids are equally effective as stimulating agents. There is no apparent effect due to the differing nature of the anion added to the environment. An analysis of the data demonstrates that the per cent variation in response is independent of the change in hydrogen ion concentration and that the mechanism of the reaction is unchanged within the limits imposed.

Experiments performed to determine the stimulating efficiencies of the sodium salts of these acids prove that the anions do not produce disturbances which can be correlated with their structural nature even when present in much higher concentration than in the acid solutions. Equation (2) gives the characteristics of the data obtained for sodium chloride, sodium sulfate, and sodium nitrate

$$\text{R.T.} - 2 = \frac{1}{4.498C^{1.78}} \tag{2}$$

where c represents equivalent concentration of the salts added to the sea water environment. Per cent variation is independent of the change in the salt concentration of the environment and there is no apparent break in the relationship stated by equation (2) over the range of concentrations tested.

STIMULATION BY THE DICARBOXYLIC ACIDS AND THEIR DERIVATIVES IN *FUNDULUS HETEROCLITUS*

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The stimulating efficiency of the first six dicarboxylic acids was investigated on *Fundulus heteroclitus* in both salt and fresh water media. Eleven hydroxy or double bond derivatives of these acids were also studied in salt water only. The experimental procedure was the same as that described last year (BULL. MT. DESERT IS. BIOL. LAB., 1933, pp. 30-32). The temperature was 18°C.$\pm$0.3°, and the pH of the experimental solution varied from 2.7 to 7.0 inclusive. Since it was known from previous studies that tests on single individuals are as reliable as the averages from several animals, only one fish was used for all the experiments with salt water solutions. The number of observations was the same for each solution, so that the percentage probable error of the mean could be used as a measure of the variability of response. In view of the fact that every animal is limited to a maximum rate of response, determined by the neuro-muscular mechanism, the actual reaction time in seconds should be corrected by subtracting the minimum time in which the animal can respond under the given experimental conditions. For these experiments four seconds is subtracted. Four thousand three hundred observations were made on 215 different concentrations of the 18 acids.

The data obtained indicate that a parabolic relationship exists between the rate of reaction and the H^+ concentration for the dicarboxylic acids and their derivatives when *Fundulus* is stimulated by these acids in salt or fresh water. This means that the course of receptive processes which leads to stimulation is the same in both media, but differs quantitatively. Stimulation of *Fundulus* by these acids may be expressed by the following equation:

$$(\text{rate of reaction}) = K\ (H^+)^n$$

where K and n are constants.
Expressing this in terms of the corrected reaction time and the actual H^+ concentration the equation becomes:

$$\frac{100}{\text{R.T.} - 4} = K\ (H^+ \times 10^7)^n$$

The constants in the equation are characteristic of the particular acid, the fish, and the environment.

It may be pointed out that if the corrected reaction time instead of its reciprocal had been considered then a hyperbolic relationship between (R.T. –4) and ($H^+ \times 10^7$) would appear of the type: