has been shown that the abrupt potential transition at −45 mv in stage B occurs as a result of the negative resistance region in the steady-state *I-V* curve, which is possibly caused by the abrupt decrease in K conductance, as discussed in connection with the eel electroplaque membrane (*6*). Both of the presumed Na and Ca inflections should be overwhelmed by the abrupt potential shift resulting from a decrease in K conductance even if they may exist.

The possible participation of Na was indicated by the fact that the duration of the plateau outlasting a current pulse became shorter in Na-free ASW (Fig. 1B). A somewhat clearer demonstration of the existence of Na and Ca components is illustrated in Fig. 2B. These records were obtained from the same embryo in different media with current pulses of the same intensities. While there was an inflection at −45 mv in 1/10 Ca, Na-free ASW, there was a steeper rise of the potential above −20 mv in 10 Ca, Na-free ASW. There was only a slight increase of the slope of the rising phase from −45 to −20 mv in 10 Ca ASW. It will be necessary to examine further the Na contribution at stage B.

At a later stage in B, the response to an outward current showed a steep potential rise with an inflection at approximately −10 mv and a sharp peak (not shown here). The response is a transitional one to the very long action potential at early stage C.

At stage C, the resting potential was in the range −70 to −55 mv, and an action potential with an overshoot of about +30 mv was elicited in all-or-none fashion with a critical membrane potential of approximately −15 mv in the muscle cells of young tadpole larvae (Fig. 1C). The action potential consisted of an initial steep rise followed by a plateau and then a slowly falling phase. The total duration of the action potential tended to shorten from the longest value of 10 seconds to about 100 msec with the advancement of stages within C. In Na-free ASW the resting potential usually remained unchanged or slightly increased. The action potential was still elicited, and the initial steep rise and the overshoot were not altered, but the slowly falling phase was almost eliminated. The latter indicates that, even at stage C, Na ions probably participate in the later phase of the action potential. When the external Ca concentration was raised ten times, the overshoot was increased by 20 mv.

A spike potential with an overshoot of 0 to +10 mv was obtained at a critical membrane potential of −30 mv in muscle cells at stage D (Fig. 1D). The spike duration was short, less than 50 msec at half amplitude. The resting potential was in the range −50 to −40 mv in standard ASW, and the spike was always followed by a large positive afterpotential. In Na-free ASW, although the resting potential increased to −60 to −70 mv, the afterpotential became a negative one, and the threshold and the overshoot of the spike were the same as those in standard ASW. As shown in Fig. 1D, 10 Ca, Na-free ASW increased the overshoot by 15 mv. The spike potentials were abolished by 20 m*M* Co ions in standard ASW, while tetrodotoxin (5×10^{-6} g/ml) had no effect on them. Therefore, it can be said that the fully differentiated muscle cell shows an almost pure "Ca spike."

In conclusion, the egg cell membrane even before the two-cell stage is capable of producing a regenerative potential change due to an increase in permeability to Ca and probably to Na ions. This is true even of mature but unfertilized eggs. A "Ca spike" is observed in the fully differentiated muscle, while the Na component in the action potential disappears in young tadpoles some time before hatching. It is possible that in some other tissues, such as nerve tissues, the Na component may remain and the Ca component disappear (*7*).

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8. The authors wish to express their indebtedness to Prof. S. Hagiwara and Dr. A. D. Grinnell for help in preparing the manuscript, and to Prof. A. Takeuchi and Dr. Y. Kidokoro for their comments on the manuscript. The authors thank the Laboratory of Physiology, Ocean Research Institute, University of Tokyo, for supply of natural seawater; the Marine Biological Laboratory at Asamushi of Tohoku University, the Marine Laboratory of Fishery at Onagawa of Tohoku University, and Otaru City Aquarium and Wakkanai City Aquarium in Hokkaido for collecting materials. Supported by a grant from the Japan Educational Ministry.

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Behavioral Thermoregulation by Fishes: A New Experimental Approach

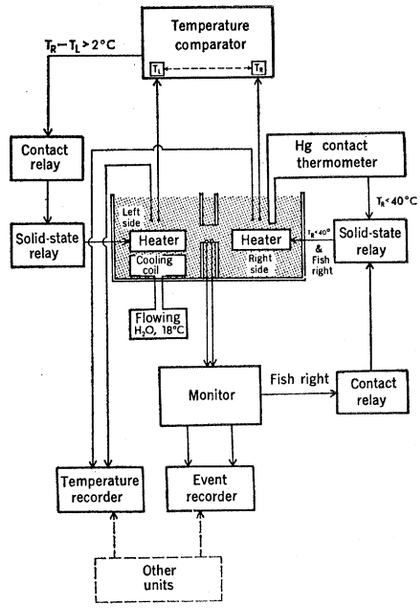
Abstract. *Electronic equipment allows fishes, by their spatial movements, to regulate the temperature in experimental tanks. Swimming into warmer water causes the temperature of the entire tank to increase; conversely, swimming into cooler water causes the temperature to decrease. The technique may be adapted for studying simultaneous behavioral regulation of temperature and nonthermal factors.*

Fishes, as mobile organisms living in heterothermal environments, can exercise substantial behavioral control over the temperatures they experience. They achieve this by swimming into, or remaining in, areas with certain temperatures and leaving, or avoiding, parts of the habitat with other temperatures.

Understanding how different fishes behave in heterothermal environments is crucial in predicting the ecological impact of heated effluents. Discharges from the rapidly expanding steam–electric power industry not only heat the receiving waters but do so unevenly, causing strong spatial variation in tempeature. Where fishes live in such waters largely determines what metabolic and ecological effects actually accrue.

Behavioral thermoregulation by fishes has been investigated primarily through two experimental techniques. The more

Fig. 1. An experimental system for study of behavioral thermoregulation in fishes. The right half of the tank warms at 3° to 5°C an hour (potentially to 40°C) whenever the fish is in the right half and cools at 3° to 5°C an hour whenever the fish is in the left half. The left half of the tank is always 2°C cooler than the right half. Thus, the fish always has available alternative temperatures 2°C apart.



conventional approach is to allow fish to "choose" temperatures by positioning themselves in a horizontal or vertical gradient of temperature (*1*). The second technique is to permit conditioned fish to control either the upper or the lower limit of temperature by activating a trigger (*2*); this method, in generalized form, was first applied for studying behavioral thermoregulation in terrestrial vertebrates (*3*).

Our technique incorporates features of both the earlier methods, but it is unique in several respects. Neither maintenance of temperature gradients in large volumes of water nor elaborate conditioning of experimental subjects is required. Data can be collected continuously and automatically, even in near-darkness; the form of the data is appropriate for direct processing by computer.

A fish can control environmental temperature by dividing his time between the halves of an appropriately equipped aquarium (Fig. 1). The fish is able to swim from one half to the other by passing through a tunnel in the partition that divides the tank. Passage of the fish is sensed by a pair of photocells in the tunnel; the direction of the passage is determined by the sequence in which the fish interrupts dim beams of light passing across the tunnel from positions opposite the photocells. The signal from the photocells is interpreted by a monitor (based on the bistable multivibrator of computer circuitry), recorded, and used to control heat transfer in the tank (*4*). Passage of the fish into one side (say, right) of the tank causes that side to begin warming at 3° to 5°C an hour. The warming of the right side ceases only when the fish swims to the other side; then the right side begins cooling at 3° to 5°C an hour. The temperature of the left side is kept 2°C lower than that of the right side by a comparator-relay circuit. Accordingly, the fish always has available alternative temperatures 2°C apart. Sequential testing and choosing of one temperature over the other results in thermoregulation; thus, the fish serves as its own thermostat.

Young fishes of six species have successfully regulated temperature in the apparatus described above. The record of one specimen of each species is presented in Fig. 2 (*5*, *6*) to exemplify, without typifying, regulatory performances during trials lasting 3 days. Temperatures between 18° and 40°C were potentially available in each case. Sufficient data were collected to demonstrate that regulated temperatures varied significantly among species and were in accord with temperature distributions of the same species in a lake receiving heated effluent (*7*).

In a subsequent experiment, a naive bluegill (*Lepomis macrochirus*) was required to regulate the temperature of a tank with no differential between halves. The fish maintained a temperature re-

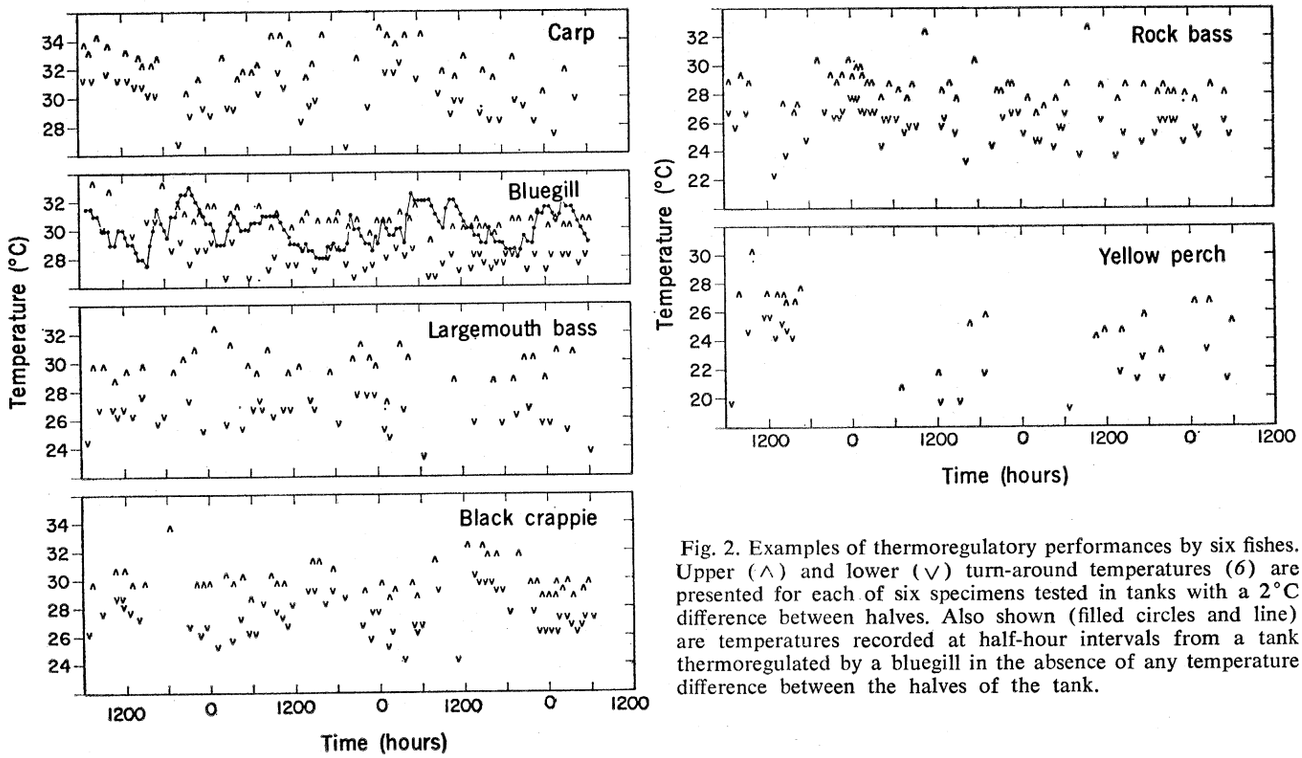


Fig. 2. Examples of thermoregulatory performances by six fishes. Upper (∧) and lower (∨) turn-around temperatures (*6*) are presented for each of six specimens tested in tanks with a 2°C difference between halves. Also shown (filled circles and line) are temperatures recorded at half-hour intervals from a tank thermoregulated by a bluegill in the absence of any temperature difference between the halves of the tank.

gime similar to that regulated by other bluegills in tanks with a 2°C difference between sides (Fig. 2). Successful regulation in a tank without a temperature differential indicated that the bluegill learned to associate changes in its spatial position with eventual (but not immediate) changes in temperature.

We believe that our experimental system is amenable to several other modifications that may increase its versatility. If the tunnel is essentially curved on itself to form a doughnut-shaped swimming space, behavioral thermoregulation by continuously swimming fishes of the open sea, such as tunas (*8*), can be studied. Swimming direction (clockwise, counterclockwise) can control the direction of temperature change; swimming speed can control the rate of temperature change. Thus, fishes could be required to thermoregulate in continuous, gentle (0.1°C per 100 m) gradients of temperature in space that are simulated by changes in temperature through time.

Simultaneous behavioral regulation of temperature and a second, nonthermal factor may be investigated by using a tank with four compartments and two monitors. Concentrations of soluble substances (such as O_2, salts, pollutants) can be regulated by a fish-controlled mixing valve; light intensity can be regulated by fish-controlled dimmer circuitry. Movements of the fish in one dimension would regulate temperature and, in the other, the second variable (*9*). Such an approach would make it possible to measure the joint preferendum for two independently varying factors.

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4. The circuit diagram of the monitor and other details are available on request from the authors.
5. The specimens 50 to 100 mm long that provided the data for Fig. 2 were taken from waters near Madison, Wisconsin, and were acclimated to 22°C prior to experiments.
6. The upper turn-around temperatures were local temperature maximums recorded from the warmer side of the tank. The *i*th local maximum, U_i, was the highest temperature recorded along the time sequence such that U_i was separated from adjacent maximums, U_{i-1} and U_{i+1}, by temperatures at least 0.5°C less than $\min(U_{i-1}, U_i)$ and $\min(U_i, U_{i+1})$, respectively. The lower turn-around temperatures were the corresponding local minimums recorded from the cooler side of the tank. The *i*th local minimum, L_i, was the lowest temperature recorded along the time sequence such that L_i was separated from adjacent minimums, L_{i-1} and L_{i+1}, by temperatures at least 0.5°C greater than $\max(L_{i-1}, L_i)$ and $\max(L_i, L_{i+1})$, respectively.
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8. Work proposed by the first two authors and A. Dizon for execution at the Honolulu Laboratory of the Southwest Fisheries Center, National Marine Fisheries Service.
9. Systems analogous to those described here may be used to study behavioral regulation of nonthermal factors at various constant temperatures.
10. Development of the method described here and its initial application were part of a thesis submitted by W.H.N. to the graduate faculty of the University of Wisconsin, Madison, in 1971, in partial fulfillment of the requirements for the Ph.D. degree. The work was done at the Laboratory of Limnology and was supported in part by funds from the Wisconsin Utilities Association and the Office of Water Resources Research, Department of the Interior (MG OWRR B-028-Wis—WRC 70-010M). We gratefully acknowledge the technical assistance of Thomas C. Byles and Bruce K. Quirk.

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Rearing Complexity Affects Branching of Dendrites in the Visual Cortex of the Rat

Abstract. *Higher-order dendritic branching is considerably greater in Golgi-stained neurons from the occipital cortex of rats reared in groups in a complex environment than in similar neurons of littermates reared individually in laboratory cages. Littermates reared in pairs in cages have intermediate amounts of branching, while lower-order branching did not appear to be affected by any rearing environment.*

Alterations in the fine structure and ultrastructure of the cortex have been reported to follow extremes of sensory deprivation. Light deprivation has been found to alter the number of apical dendritic spines on pyramidal cells in the visual cortex of rodents (*1*) and to diminish higher-order dendritic branching in cats (*2*). Electron microscopic studies have indicated alterations in the size and density of individual synapses in the visual cortex after deprivation (*3*). Further, changes in spine density may be brought about by excess sensorimotor stimulation. Schapiro and Vukovich (*4*), for example, reported increased spine density and altered Golgi staining patterns after intense multimodal stimulation was given during the early postnatal period.

The above-mentioned changes have generally been reported only after animals were given differential stimulation far greater than that which the normal laboratory environment might provide. However, Rosenzweig, Bennett, and Diamond (*5*), along with others (*6*), have suggested that similar effects may be generated by milder forms of deprivation and enrichment. Increases in cortical weight and thickness, glial numbers, perikaryonal size, and enzyme activity have been found in rats subjected to a complex environment as compared to deprived littermates. Electron microscopic studies have indicated that average synaptic thickenings in some areas are longer after enriched rearing, but whether they also increase in number

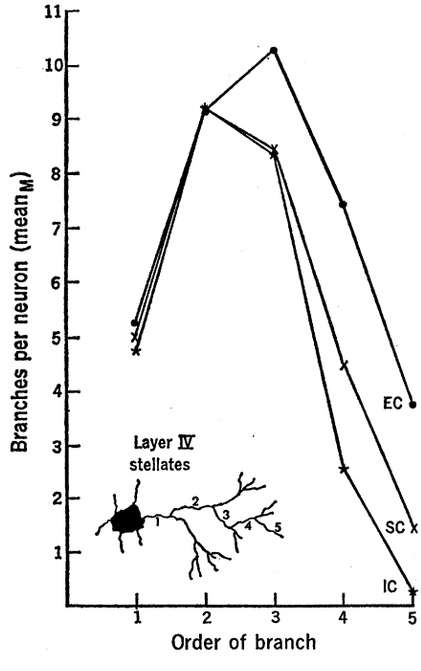

Fig. 1. Branches per layer IV stellate cell in the three rearing groups: *EC*, enriched; *SC*, social; and *IC*, isolated. Differences are confined to higher-order (3, 4, 5) branches. The diagram in lower left indicates scoring procedure. *Mean*$_M$ is the mean of mean scores for individual animals.