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ELECTROCARDIOGRAMS AND THEIR TELEMETRY AS PHYSIOLOGICAL INFORMATION ON RAINBOW TROUT

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1. FOREWORD

The variations in mechanical, chemical, thermal and electrical effects accompanying life activities of animals can be utilized as a source of physiological information. Not only can sophisticated modern transducers and amplifying systems detect minute information sources and amplify them to give a magnified representation, but by utilizing radio-wave carrier systems, it is possible to record and collect information at remote points. However, at present, these techniques are not in actual use. In particular, the development of the electronics technology which is readily applied to land-inhabiting animals, is greatly hindered in its application to fish because they are aquatic animals.

Present electronics technology is capable of converting most animal phenomena into electrical information. But practical experiments with fish have only begun. In this article we describe an experiment to determine the relation between the physiology of the fish and its

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2. THE ANALYSIS

The analysis is based on electrical information obtained from the activity of the heart, which plays the principal role in the life activities of a fish. In fact, we consider this experimental procedure to be of a fundamental nature, applicable to many other similar research efforts.

Since fish are cold-blooded animals, their body temperature changes with their environment. The fish heart does not have specific blood vessels to carry nutrients. Therefore, even if the heart is surgically removed from the body, the heart can be kept active for extended periods in an artificial nutrient solution. Ever since the classic work of McWilliam and Bakker, many research findings on the electrocardiography of fishes have been reported, but the only report we could find on remote recording experiments is by Frank (1968). In this article we will start our discussion from the basic operational steps of remote recording of electrocardiograms.

2. THE ELECTROCARDIOGRAM OF AN EXPOSED HEART

The characteristics of electrocardiograms of an exposed heart is described here first because they form the basis for applying the radio telemetered electrocardiograms from swimming fish to physiological and ecological research.

The electrocardiogram of a fish is similar to that of the 2 atrium -2 ventricle heart of mammals and birds, and the 2 atrium -1 ventricle heart of amphibians. The atrial P wave and the ventricular QRS-T wave can be readily identified. Also, as in the case of amphibians, the
stimulated wave from a well-developed venous cavity is present and produces a clear cut deflection preceding the atrial wave. This is generally referred to as the V-wave. However, even for those fish such as eel and catfish from which electrocardiograms are readily obtained, the electrical deflection potential that can be recorded from the body surface is at most 100 to 500 µV\(^9\) even after the effects of intermixed electromyograms and fluctuation of the baseline due to body motion is removed through anesthesia. The electrical activity of the heart to be fed into the telemetry system must not be affected by a high level of body motion and should not receive interference from the electromyogram of breathing and swimming motions. Therefore, the method of attaching electrodes to the body surface is not the best choice.

1) The Electrocardiograms Obtained Directly from the Surface of the Heart

To determine the "control pattern" of an electrocardiogram from a rainbow trout, we experimented with direct recording from the surface of the heart. Eleven rainbow trout two years of age or older with body weights 350 to 600 g were used for the experiment. They were raised by the Nikko Branch of the Freshwater District Fisheries Research Institute of the Fisheries Agency. The method of exposing the heart is as follows. The point of intersection on the forehead plane of the line connecting the left and right eyes and the central sagittal plane was drilled out shallowly and the skull pierced. A silver wire 1.2 mm in diameter was inserted and the brain destroyed. Further, the spinal cord was completely destroyed by first introducing the wire into the spinal column through
the occipital foramen in the skull and then by gently manipulating the silver wire towards the tail. Initially a momentary rigidity of the body took place and this was followed by complete relaxation due to spinal shock. Scissors were inserted along the middle of the abdominal centre line and an incision made towards the head, stopping at a point 1/3 into the isthmus of the lower jaw. From the starting point of the incision, another incision was made diagonally upwards to the edge of the gill covers, through the thoracic cavity walls to expose the heart. The electrocardiogram was taken by first inserting electrodes into the head, the rear edge of the dorsal fin and into the tail. The 3 electrodes were interconnected through 500 KΩ resistors and used as "indifferent" electrodes in the manner of Wilson. Records were taken by the so-called unipolar method. A probing electrode was constructed from a glass tube with an inner diameter of 3 mm, filled with "Ringer" fluid after the tip of the tube was tapered to a narrow diameter and a cotton thread passed through the tube to the tip.

Fig. 1. "Examples of Heart Surface Electrocardiograms of Rainbow Trout"
Figure 1 shows examples of electrocardiograms from the left and right sides of the heart surface. It was very difficult to obtain identical wave forms because of variations from specimen to specimen due to the exposure of the heart surface to the atmosphere. It was also difficult to maintain identical detection conditions. In particular, when the signal was detected from the opposite side of the exposed surface, wave forms that can be interpreted as abnormal electrocardiograms were often observed. They had raised or depressed ST-T wave sections.

**Fig. 2. "Idealized Forms of Typical Wave Forms of Heart Surface Electrocardiograms"**

Figure 2 shows idealized versions of electrocardiogram patterns considered to be typical after studying a large number of examples. If we accept these as "control patterns", it can be said that the atrial P wave is uniphased or diaphased with an amplitude of approximately 1 mV, and that when the electrode is close to the junction of the atrium and ventricle, the ventricular complex wave is composed of a diaphased transient pulse with a 10 to 15 mV amplitude and a broad negative deflection of
5 to 8 mV. We have not confirmed the stimulus transmission chain, but judging from changes in the electrocardiogram wave forms, we can deduce that the chain starts from the posterior of the atrium and propagates to the posterior of the ventricle and then to the arterial cone (conus arteriosus). This agrees with previous findings.

2) Electrocardiograms Obtained by the Bipolar Lead System

Based on the results obtained above, we undertook the design of an electrocardiogram system with bipolar leads for use in a remote telemetered recording application. The signal obtained from the body surface of the fish provides less than 100 pV potential even if the electrodes are placed in the chest section closest to the heart. Clearly, the signal is insufficient to operate a telemetry system satisfactorily. We therefore, tried the following method whereby two electrodes were placed within the thoracic cavity very close to the heart and were able to record 1.5 to 2.0 mV deflections.

First, for an electrode, a 3 mm band of insulation from a 10 strand earphone cord was removed at a point 1 cm to 1.5 cm from the end. Next, by using an implantation needle, two such electrodes were implanted on the left and right sides along the line starting from a point back of the uppermost point of the pectoral fin, directed towards the centre line of the isthmus of the lower jaw and emerging forward of the pectoral fin. The two vinyl plastic insulated leads emerging from the bottom side were fixed together to the body wall. The electrode leads were fixed at the front part of the dorsal fin. In this case the sensing sections
covered the left and right sides of the heart. It appears that electrodes implanted in such a manner do not produce a recognizable adverse reaction of the body. The implantation is carried out under anesthesia with 0.1% urethane. Recordings were taken after complete recovery from the operation. Slight differences in the electrocardiograms are observed depending on the relative positions of the heart and electrodes.

Fig. 3. "Normal Waveforms of Bipolar Lead Electrocardiograms"

Figure 3 shows normal bipolar lead electrocardiograms recorded by a Model RS-100 Direct Writing Electrocardiograph manufactured by Fukuda-Electro Company. The P wave is positive and uniphasic but on occasion it is negative-going. The amplitude is approximately 0.1 mV in both cases. The ventricular complex wave is of the rs or Rs type with an amplitude of 1.0 to 1.5 mV. The T wave is positive and uniphasic with an amplitude of 0.2 to 0.3 mV. On occasion, wave forms with a P wave which is either positive and uniphasic or diaphased, together
with a ventricular complex wave of either the qR type of the Rs type, with the addition of a T wave of the negative uniphase type are observed. In this connection, such wave forms are similar to that obtained by Oets\textsuperscript{9) who employed bipolar leads, and it may be considered that the sensitive portions of the electrodes on the left and right sides are both lying across the atrium as well as the front portion, if not the central portion, of the ventricle.

3. APPLICATION OF BIPOLAR LEAD ELECTROCARDIOGRAMS

1) The Variations in Electrocardiograms Caused by Rising Water Temperatures

A 30 litre tank filled with water at a temperature of 9.5 to 10°C was prepared and a rainbow trout of 250 to 300 g body weight with implanted electrodes was placed in the tank. The temperature was raised by a heater while aeration of the tank was maintained. Electrocardiograms were taken for every 1°C rise in water temperature and at the same time respiration rates were recorded by observing the motion of the lower jaw. Figure 4 shows an example of the results. The graph at the bottom

Fig. 4. "Variations in Electrocardiograms with Rising Water Temperature"
of the figure shows the variation in the per minute rate of the cardiac beat and the respiration count. According to the graph, the cardiac beat rate at 10°C was 60/min but as the water temperature rose, the rate increased and at 21°C, it reached 125/min. Thereafter the rate decreased rapidly and became 60/min at 25°C and at 26°C, cardiac arrest was observed. The respiration rate was 100/min at 10°C and increased with the rise in water temperature. At 15°C it became 150/min and at 20°C it reached 170/min. Just before death, it was over 180/min. If we analyze the electrocardiograms, we see that at 10°C the P wave was weak, positive and uniphased. The ventricular complex wave was of the RS type with an amplitude of 1 mV and T wave was positive and uniphased with a slight downward shift in the ST section. As the temperature was raised, the ST section remained flat but shifted negatively as a whole, and the T wave became obscured. At 20°C the negative shift of the ST section became very marked and the T wave became unidentifiable while the P wave became either diaphased or negative. Above 20°C the beat rate became irregular and not only did the ST section become further negative but a bottom portion of it rose slightly to take on a rippled shape. This portion shifted upwards with higher temperature and two negative deflections appeared in the ST section. Past 23°C, the heart beat rate became slow and at the same time irregular beats increased noticeably and the rippled portion of the ST wave stood out much clearer. These characteristics were maintained until termination by death. Neither fibrillation nor fluttering of the atrium and ventricle were observed.
2) **The Variations in Electrocardiograms Caused by Decreasing Water Temperatures**

Following a procedure similar to the preceding section, figure 5 shows the recorded results of lowering the water temperature to 10°C from 23°C. The water temperature in the tank was first raised to 23°C by direct sunlight and then cooled slowly through the addition of cold water. The electrocardiogram at 23°C shows irregular beats and shifts in the electric potential but unlike the case where the water temperature was raised rapidly to 23°C, there are no significant variations.

![Graph showing electrocardiogram variations](image)

**Fig. 5. "Variation in Electrocardiograms with Decreasing Water Temperature"**

and the electrocardiogram is near normal. The P wave is a low-potential, negative and uniphasic wave while the ventricular complex wave is of the QS type. The T wave is positive and uniphasic with a comparatively large potential. The irregular cardiac beat rate and shifts in electric potentials revert to near normal at a water temperature of around 15 to 16°C. At this temperature, the electric potentials of the ventricular
complex wave and the T wave are slightly on the high side. However, as the water temperature decreases further, the beat rate slows down and at the same time the electric potentials also decrease slowly. Finally, the waves return to the normal form of a negative and uniphasic P wave, a QR type ventricular complex wave and a positive and uniphasic T wave. The cardiac beat rate at this state is approximately 60/min and the respiratory rate is approximately 100/min.

4. WIRELESS RECORDING OF ELECTROCARDIOGRAMS BY RADIO TELEMETRY

The basic experiments relating to electrocardiography discussed above were the preliminary steps in applying radio-telemetry to further physiological and ecological research. The bipolar lead system designed by our group was considered to be quite adequate for use in radio-telemetry of electrocardiograms. Therefore, an experimental system was constructed to simultaneously record electrocardiograms and mechanical motion curves of the lower jaw through a 2-channel telemetering unit.

After implanting the electrocardiogram electrodes, two electrodes for recording the lower jaw mechanical motions were fixed on the left and right sides of the lower jaw. The two electrodes consisted of enamel-coated copper wire with exposed tips. The two enamel-coated wires were then combined with the electrocardiogram leads and tied securely with a string looped through the forward portion of the dorsal fin. The transmitter and receiver were from the model TPE-200 2-channel medical
telemetering system manufactured by Fukuda Electro Company. The commercial transmitter was slightly modified and two 9 volt stack-celled batteries were used as a power source. The transmitter and batteries were installed in a waterproof polyethylene double walled container. The existing antenna was replaced with an insulated copper wire of 30 cm length. The transmitter unit was then lashed to a 5.5 cm diameter spherical buoy with a 30 cm string. Next, a 1 m fishing line was attached to the buoy and combined with the electrode leads and secured to the forward portion of the dorsal fin. The receiver was all solid-state and weighed 40 kg. It was placed inside the laboratory and signals were received and recorded through a 1m rod antenna. A rainbow trout with electrodes installed beforehand and weighing 1,500 g was placed in a 60 litre tank. Cold water was added to lower the water temperature gradually from 10°C to 0°C and then warm water was added slowly to raise the water temperature to 25°C form 0°C over a period of approximately 2 hours and 30 minutes. During the whole period, electrocardiograms and respiratory motions were recorded continuously.

Fig. 6. "A Graph Describing the Per Minute Cardiac Beat and Respiratory Rate Variation due to Changes in the Water Temperature"
Figure 6 shows the graphic results on the per minute respiratory and cardiac beat rates continuously recorded for the entire process. At 10°C the cardiac beat rate was approximately 55/min while the respiratory rate was 90/min. With decreasing temperature, they both decreased and at 5°C the cardiac beat rate was 42/min and the respiratory rate was 78/min. At 0°C they became respectively 24/min and 48/min. The elapsed time to this point was 45 minutes. When the temperature was left at 0°C for 30 minutes, the cardiac beat rate decreased to a few per minute but the respiratory rate did not decrease as much in comparison. Next, as the water temperature was raised by mixing in warm water, at 5°C the cardiac beat rate became 25/min and the respiratory rate became 55/min. At 10°C they increased to 35/min for the cardiac beat rate and 100/min for the respiratory rate. This approximated the initial respiratory rate at 10°C before lowering of the temperature, but recovery of the cardiac beat rate was much slower when compared to the recovery of the respiratory rate. The increase in the rates continued with the rising temperature and at 15°C, the cardiac beat rate was 54/min while the respiratory rate was 114/min. At 20°C the respiratory rate increased to 120/min but the cardiac beat rate did not show any noticeable increase. At 25°C water temperature which is the limit for survival, the respiratory rate reached 150/min but the cardiac beat rate decreased in contrast. The time required to raise the temperature fully was approximately 70 minutes. Examples of electrocardiograms and respiratory motion curves during this process are shown in figure 7.
The increase or decrease of the cardiac beat rate due to the water temperature variation is observed mainly in the change of the TP interval. However, a change in the PQ interval as well as a change in the duration of the QRS complex can also be observed. But there are no observable variations in the potentials and wave forms. However, the respiratory motion curve becomes more radical and peaked with increasing respiratory activity. When the respiratory rate decreases with lower temperature, the peaking decreases as well.

In order to record accurately the cardiac beat rate of a trout in free swimming motion, an experimental miniature FM radio-telemetry system was constructed. The construction was motivated by noting that the cardiac beat rate of a rainbow trout varies considerably with water temperature according to the preceding results. Therefore, rather than recording electrocardiographic information directly with an elaborate
apparatus, it was considered desirable to record the cardiac beat remotely through concentration on miniaturization and simplified handling of the transmitter.

The transmitter was a transistorized miniature FM type experimentally constructed for underwater transmission. It consisted of two parts, an amplifier section which contained the power supply, and a transmitter section. The weight was approximately 14 g in air and the batteries were rechargeable. The standard battery voltage was 1.25 V and could be used continuously for approximately 36 hours. The transmission (oscillator) frequency was 85 to 88 MHz. A simple circuit diagram of the unit is shown in figure 8. The receiver was a commercial TRIO Model AFX-31 FM stereo-tuner and the recorder was a Model R-101 portable pen writing oscillographic recorder manufactured by the Sanei Measuring Instrument Company. A 100 m length of feeder line was used as the receiving antenna and this was arranged to be

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Fig. 8. "Circuit Diagram of the Underwater Transmitter (Oscillator)"
connected to the 75 Ω antenna terminal. Using this experimental system we investigated the correlation between water depth (water temperature) and cardiac beat rate at a practical lake site. As it is well-known, the attenuation of radio waves in water is extreme. Therefore, we restricted our objective to satisfying the fundamental concept of indirect measurement with no constraints. Thus, the trout with the transmitter installed was enclosed in a metal screen cage 36 cm in diameter and 21 cm deep. The receiving antenna was wound around the cage and the received signal was brought to the water surface through another cable.

So, a rainbow trout with the electrodes and transmitter installed was placed in this cage and experiments carried out at Mochiwada Bay and off-shore at Shishiga Abyss of Lake Chuzenji. The cage was lowered vertically into the lake from a ship and lowered in 5 m steps. At each step the cage was kept stationary for 5 minutes until a maximum depth of 50 m was reached. The process was then reversed and the cage was raised in 5 m steps. At each step the water temperature and cardiac beat rate was measured. Readings from a resistivity meter utilizing a thermistor were converted to obtain water temperature measurements.

Figure 9 presents the combined results graphically for the experimentally recorded water depths, water temperatures and cardiac beat rates. The water temperature of the shipboard tank filled with lake water was 20°C when the ship reached the centre of the lake and the cardiac beat rate was 64/min. The temperature was 21.0 to 21.3°C for
a water depth of 0 to 8 m but after passing through the jump layer at 10 to 15 m, the temperature decreased rapidly and at 25 metres it was 5.9°C. Thereafter the water temperature decreased slowly and at 30 metres it was 5.3°C and below 45 m it reached a constant 4.2°C. As soon as the cage was lowered into the lake, the cardiac beat rate rose rapidly to over 85/min, and at 10 m it became 90/min. However, thereafter the beat rate decreased in proportion to temperature in a linear fashion and at 30 m reached 58/min. After that the rate reduction slope became shallower and at 50 m the rate became approximately 40/min. Raising of the cage followed but the cardiac beat rate showed little change and up to the depth of 25 m and 8°C water temperature, the rate
remained at approximately 40/min. After passing the 20 m depth the cardiac beat rate increased rapidly and at 10 m, a rate of 84/min was recorded. From the 8 m depth to the surface, the beat rate reached 88 to 92/min. The case where the change is from low to high temperature is quite different from the opposite case of changing from high to low temperature and the considerable time lag in the former case is noticeable. This fact did not change even when sufficient recovery times, estimated from the laboratory water tank experiments, were provided for.

Figure 10 shows the experimental results for a similar experiment to a depth of 100 m using naturally grown sockeye salmon two years of age (body weight 380 to 480 g). Compared to the preceding result, the
lake surface temperature was a very low 14°C because the time was at the beginning of fall. The jump layer had also receded to a depth of 20 to 25 metres but the overall temperature distribution was similar to the preceding experiment. The variation in the cardiac beat rate with the change in water depth or water temperature was also observed in this case. However, all three specimens used for the experiment show similar results of more or less following the same curve produced during descent when they are ascending. No delayed recovery of the cardiac beat rate during ascent, as recorded in the rainbow trout, was observed. Whether this result is due to the differences in temperature adaptability arising from the broad temperature range characteristics of the rainbow trout and the narrow temperature range characteristics of the sockeye salmon or whether simply the differing environmental conditions surrounding the life history of a cultivated and natural fish are the source of the discrepancy poses an interesting question. Also, a water depth of 100 m means exposure to a high pressure of 10 atmospheres for over 5 minutes. It is interesting to note that no apparent effect of the high hydrostatic pressure could be observed on the cardiac function.

As mentioned above, we have described several experiments based on the development and application of miniature telemetering systems utilizing radio waves and the objective was restricted to electrocardiograms. By similar innovation, we hope that many other physiological effects can be utilized as information sources, and an approach towards the ecology
of fish can be made through them. In any case we hope that we have demonstrated, by practical examples, that when attempting to transmit physiological information through radio waves as a new experiment, appropriation of previous results should be avoided but fundamental knowledge must be uncovered anew from the standpoint of telemetry and that it is very important to harness a phenomenon aptly suited for telemetering.
1. Bakker, N.C. (1913): Analysis of electrocardiograms on the basis of studies performed in eel hearts.


