ELECTROCARDIOGRAM OF THE RAINBOW TROUT
AND ITS RADIO TRANSMISSION

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Since McWilliams (1885) and Bakker (1913), a considerable number of researches on fish electrocardiogram (ECG) have been published (Kisch 1948, 1949, 1950; Oets 1950; Otis 1957; Garey 1962; Noseda 1962). Because stability and consistency of amplitude in the cardiac action potential is necessary as a minimal requirement in telemetering ECG from free-swimming fish, any ordinarily available method of picking up the cardiac action potential from the body surface cannot be used. From this point of view, Garey's method of detecting undistorted ECG configuration of higher amplitude from within the body will be recommended.

The earlier investigators found that the fish ECG has a pattern similar to those of mammals and birds, showing the auricular P wave, the ventricular QRS complex in depolarization, and the T wave in repolarization in the heart. In addition, the fish ECG often has a notch on the beginning of the P wave which is equivalent to that of amphibians. This notch is produced by excitatory action of the venous sinus and is indicated as a V wave or Bakker's excitatory wave. In general, the amplitude of fish ECG is fairly small. Taking the fish's largest ECG in eels and catfishes for example, the amplitude of QRS complex recorded by the use of surface electrodes can hardly exceed more than 100 µV, despite the base line wandering and EMG disturbance are completely eliminated by administration of narcotic drug.

The present report describes procedures necessary to register the ECG of unrestrained fishes and some primary experiments, and briefly illustrates the possibility of using ECG telemetry to study fish ecology.

ECG DIRECTLY RECORDED ON THE SURFACE OF RAINBOW TROUT'S HEART

Two methods of electrode emplacement were tested: directly applied to the surface of the heart (epicardial), and inserted through the body wall into the thoracic cavity. The epicardial attachments were useful in studying the characteristics of the fish ECG, but are not feasible for studies of unrestrained fish. The body wall attachment is suitable for monitoring ECG of free swimming fish.

1. Material and Methods

Eleven rainbow trout, more than 2 years old, from a hatchery pond of Nikko Branch Office of Freshwater Fisheries Research Laboratory were used for the study. The fish was immobilized by the method of cerebro-spinal destruction. In order to prevent an unexpected occurrence of distortion in ECG configuration due to temperature

difference between constant 9.5°C in the hatchery pond and approximate 20°C in the laboratory room, half of the trout's body was immersed in water at 9.5 to 10°C and water of the same temperature was poured into the mouth. An incision was made with scissors starting mid-ventrally at the anterior third of the chest wall and extending dorso-craniad along a curved line. The dissected flesh was folded over towards the head without cutting it off in order not to damage the blood vessel to the chest fin.

A cotton thread inserted into a slender glass tube which was filled with Ringer's solution was used as the exploring electrode. The sites of Wilson's terminal electrodes were selected as on the head, dorsal and caudal fin respectively. Finally, the ground electrode was immersed in the water.

2. Result and Discussion

Although much care was taken, we could not prevent occasional exposure of parts of the heart to warm air, thus giving rise to distortion of the ECG configuration. As the temperature in the laboratory deviated from fish to fish, similar configurations were difficult to record in the different trout. Figures 1 and 2 show two examples of epicardiac ECG and Figure 3 is a schematic illustration of wave forms, reconstructed from several epicardiac records. As seen in Fig. 3, Bakker's excitatory wave from the venous sinus was composed of a monophasic, positive depolarization deflection of 0.5 mV, and an intermediate segment of equipotential and monophasic, negative repolarization wave of 0.5 mV. On the left side the auricular wave, P, was composed of a positive deflection of approximate 1 mV and a monophasic negative repolarization wave, without any equipotential segment. On the right side the P wave was registered as a diphasic de- and repolarization wave. According to Einthoven's membrane theory, these features of the auricular complex would be explained as follows: cardiac excitation was initiated at the left side of the sino-auricular junction and radiated along the auricular wall to reach as far as the auriculo-ventricular junction. The ventricular wave was composed of a spiky positive deflection of more than 20 mV and a vasin-like monophasic negative depression of less than 10 mV. On the left side of the heart, this wave appeared as an Rs type at the auriculo-ventricular junction, and changed to an Rs or Rs type, gradually increasing the height of the R wave until the cardiac apex.

Fig. 1. Epicardiac ECG of the Heart

left side right side
1. sinus venosus 3. ventricle
2. auricle 4. bulbus arteriosus
was reached. Recording ventrad along the longitudinal center line on the left-side free wall, the ventricular complex appeared as an rS or RS type. Recording ventrad along the center line on the left-side free wall longitudinally, the ECG appeared as rS or RS type. The repolarization wave of the ventricle gradually decreased in its negative-potential deflection, moving from the atrio-ventricular junction, and finally changed into rest- or platform-shaped positive deflection at the cardiac apex. A similar change in the shape of the ECG occurred on the fore and/or hind margin of the left side wall, as well as on that of the right side. This was equivalent to the patterns of the ventricular complex which were observed by Noseda in his experiment on fish ECG7). The following assumption might be permitted. The repolarization wave radiated over the ventral wall from caudal-upward to cranial-downward and the cardiac gradient occurred as the epicardium repolarized much earlier than the endocardium. The characteristics of respective ECG patterns in different sites on the surface of heart can be easily interpreted under the membrane theory.
DETECTION OF CARDIAC ACTION POTENTIAL BY BIPOLAR LEADS FROM WITHIN THE BODY

The amplitude of ECG deflections on the body surface scarcely exceeds 100 µV in rainbow trout, even when the exploring electrode is placed at the point of the chest wall nearest to the heart where the largest deflection occurs. This signal is not sufficient for radio transmission.

The author's method of implanting electrodes is similar to that proposed by Garey, in which the bared parts of the electrodes are inside the chest cavity. If this method is practiced appropriately, it is possible to register ECG potentials up to 1.5 to 3.0 mV.

1. Material and Methods

A number of rainbow trout weighing 150 to 200 gm raised at Nikko Branch Office were used. A pair of plastic insulated electric wires, 20 cm long, were used as the electrode leads. About an 8 mm length of insulation was removed 1.5 to 2.0 cm from the end of each lead. The leads were symmetrically inserted into the both sides of chest cavity using a thick sewing needle at a point 1.0 to 1.5 cm dorso-caudal to the pectoral fin. The leads ran obliquely along the inside of the chest wall and came out at the median line of the isthmus (between the mandibular arcs) approximately 1 cm in front of the pectoral fin. The bared parts of the leads were positioned to face the heart. The left and right electrodes were tightly tied together at their ends with a cotton thread. Other parts of both cord wires were attached in front of the dorsal fin with a thread stitched through the dorsal margin of the body. Placement of the electrode was done under anesthesia by being free the fish in MS222 (1/15,000 to 1/20,000 in concentration) or 0.1% urethane solution.

2. Result and Discussion

Usually the ECG appeared in either one of two forms depending on the position of the bared parts of the electrodes relative to the surface of the heart. If the electrode faced to the lateral side of atria and ventricle, the ECG was similar to Fig. 4a. If the grid electrodes stood only against the ventricle, the result was as in Fig. 4b. The features of respective ECG were described as follows: 1) P wave was monophasic, negative and sometimes positive, and 0.1 mV in amplitude. Ventricular complex was rS or RS type being 1.0 to 1.8 mV in its size. T wave was monophasic and positive of 0.2 to 0.3 mV. These patterns were similar to those which Oets recorded on an isolated heart of an eel by the use of bipolar leads placing the grid electrode on the venous sinus and the ground on a filter paper moistened by Ringer's solution. Occasionally, Bakker's excitatory V wave appeared as a notch at the beginning of P wave. Comparing with the epicardiac ECG already mentioned, it was supposed that the bared

Fig. 4. ECG Configuration by Bipolar Leads

[Diagram of ECG configurations]
portions of electrodes passed diagonally across the lateral side of both the atria and ventricle. 2) P wave was monophasic, positive or sometimes diphasic. Ventricular complex was Rs type. T wave was monophasic-negative. These patterns were similar to those which Oets took from the cel's isolated heart by means of the bipolar leads. In this case, the grid electrode was connected with the atrio-ventricular junction. Reference to the epicardiac ECG above-mentioned, it was clear that these patterns were presented by the bared parts of electrodes passing through diagonally across the ventricular wall.

EFFECT OF WATER TEMPERATURE ON ECG AND HEART RATE

The water temperature in the hatchery pond remains almost constant throughout the year at about 9.5°C. The ECG and heart rate were recorded from fish in an aquarium while the water temperature was caused to vary from 0°C to 25°C.

1. Material and Methods
Ten rainbow trout, weighing 150 to 180 gm were used. The trout equipped with electrodes were freed in a 30 l aquarium, filled with fresh water of 9.5°C. Two pieces

Fig. 5. ECG Changes, Resulted from the Increase of Water Temperature, from 10°C to 26°C

![ECG graph showing changes in heart rate and respiratory rate with water temperature.]
of electric heater element (100 W) were used in order to warm the water, and ECG was registered at each 1-degree increment. Respiratory rate was simultaneously registered by counting the mandibular movements during timed intervals.

Decrease of temperature to 0°C was caused by pouring cold and/or ice water into the aquarium. ECG and respiration counts were recorded continuously.

2. Result and Discussion

Figure 5 shows the change in heart and respiration rate. At the beginning, in 10°C water, the heart rate was 60 beats per minute. The rate increased in response to the rise of water temperature to 12°C at 21°C and abruptly decreased thereafter to 60 at 25°C. The heart stopped at 25°C. The respiration rate was 100 per minute at 10°C, 150 at 15°C and 176 at 20°C. Immediately before the death, the rate was 187 per minute.

The ECG pattern at 10°C characteristically has a small monophasic and positive P wave, Rs type of ventricular complex of 1 mV, and monophasic positive T. The ST segment was depressed evenly and slightly in comparison with the zero-base (or isoelectric) line. This pattern suggested that the bared portion of electrodes passed through from the center of the auricular lateral wall to the dorso-cranial corner of the ventricle. Following an increase in water temperature, the level ST segment

Fig. 6. Successive ECG Changes, Resulted from the Decrease of Water Temperature, from 23.2°C to 10°C

[Graph showing ECG changes from 23.2°C to 10°C]

- Heart rate
- Respiratory rate
descended gradually and the T wave became inconspicuous. Starting at 20°C, the ST segment went down extremely, and the T wave changed to become negative. Beyond 20°C, the heart beat became arrhythmic. The base of the ST segment ascended at its center to show a peculiar elevation. Meanwhile, the ascent of middle part of ST developed to present a depression at its both sides.

Temperature higher than 23°C caused lengthening of heart beat intervals, irregularity of heart beat and marked ascent of the ST, followed up by gradual cessation of the cardiac activity. Pathological distortion of the ECG configuration, such as flutter and/or fibrillation in the auricle and ventricle, was not recognized. All of the experimental animals showed the same phenomena as in the example above.

An example of ECG change resulting from a decrease in water temperature is presented in Fig.6. The normal ECG configuration at 10°C was composed of a negative-monophasic P, an rS type ventricular complex of 500 μV and a positive-monophasic T wave. These characteristics in ECG configuration suggested that the exploring electrodes passed diagonally through both sides of the ventricle.

The water was naturally warmed by sunshine to 23°C over a 5-hour period. In this case, no marked distortion of ECG occurred such as that in a rapid temperature rise mentioned above. There was no amplitude fluctuation and/or arrythmia. The P wave had a negative deflection of low amplitude. The QRS complex appeared as a Qr or QS type and the T wave as positive-monophasic. By adding cold water to lower the temperature to about 15°C, the slightly distorted ECG returned to normal except for a high deflection in both the P and ventricular complex waves, similar to that occurring with further decrease in water temperature. The heart rate was reduced and the activity potential gradually diminished to return to its original normal state. At 10°C the heart rate was 60 beats per minute. Although the water temperature was further reduced down to the usual 9.5°C of hatchery water, neither distortion of the ECG configuration nor fluctuation of the deflection amplitude occurred. But the rate of heart beat dropped to 25 beats per minute.

WIRELESS TRANSMISSION OF ECG BY RADIO TELEMETER

The primary purpose of the ECG study was to determine whether the ECG could be used as one of the physiological signals in wireless transmission for ecological research in rainbow trout. One- or two-channel telemeter commercially available were used here for transmitting the ECG and/or respiration signals.

1. Material and Methods
   
   Electrodes for ECG tracing mentioned above were set up in large rainbow trout more than 1,500 gm in body weight. Electrodes for detecting respiration movement were a pair of slender enamel-insulated copper wires, 200 μ in diameter. A small part near the ends was stripped of insulation. The wires were inserted at either side of the mid-line of the isthmus between the mandibular arcs, and so fixed with the bared part under the skin. Respiration was sensed as an impedance change between the electrodes as a result of the mandibular movement. Transmission and reception of radio signals were performed by the use of a 2-channel medical telemeter TPE 200, Fukuda Electro Co. Ltd., Tokyo.

   The experimental trout thus heavily equipped was freed in a 30 l aquarium at 10°C. Cold water mixed with ice was gradually poured into the container until the temperature reached 0°C. Then warm water was added slowly to raise the water
temperature to 25°C. It took about two hours and a half for this entire course of water temperature change. Figure 7 shows the changes in heart and respiration rates.

2. Result and Discussion

Under normal conditions at the start, heart beat and respiration rates were 45 and 90 per minute respectively. They decreased in response to the fall of temperature, presenting respective value of 42 and 78 at 5°C and 24 and 48 at 0°C. During the water was kept at 0°C, the heart rate showed slightly to less than 24.

When temperature of the water was gradually increased to 5°C, the heart beat and respiration rates increased to 30 and 54 respectively.

During the course of return to respective rates of 36 and 104 at 10°C, approximately the normal temperature, the heart rate showed a slower increase than that of the respiration. As the water temperature went up gradually, the rate of heart beat and respiration increased, thus reaching 54 and 114 at 15°C respectively. While the
respiratory rate became 120 at 20°C, the heart beat was approximately the same value of 110. At the temperature of 25 to 26°C, at which death occurred, the respiration rate was as high as 150, but the heart beat slowed abruptly with severe arrhythmia. The temperature rise covered a period of one hour and 10 minutes.

The changes of ECG configuration and respiration are shown in Fig.8. As clearly depicted, an increase or decrease in the heart rate was caused mainly by lengthening of the T-P interval reinforced by prolongation of the P-R and QRS times. The amplitude of deflections in the P wave and ventricular complex did not change conspicuously, but the T wave responded regularly to temperature change, increasing at high and diminishing at low temperatures. The respiration movement increased its amplitude and assumed a more rounded shape in warm water.

HEART RATE CHANGE CORRESPONDING TO THE DEPTH OF LAKE WATER

1. Material and Methods

Six rainbow trout weighing 1,000 gm to 1,500 gm and completely equipped with the ECG electrodes were transported to the center of Chuzenji-Lake by motor boat. The depth of water at the selected location was estimated to be about 100 to 120 m.

The experimental animals, housed individually in cylindrical metal, mesh cages, 90 cm in height and 70 cm in diameter, were lowered vertically into the water from the boat. Successive changes of water temperature with depth was measured by a thermister fixed on the cage and wire-transmitted to the boat. The cage was lowered by 5 m stages to a depth of 50 m. At each 5 m stage, the heart rate was counted by the use of an FM heart beat telemeter. The transmitter was placed on the bow of the boat and the receiver was fixed in the cabin. At each 5 m stage the cage was held two minutes to allow the fish to adjust to the temperature before the heart rate was recorded. The temperature of water in respective places was measured at the same time.

When the cage was pulled up, the same procedure of measurement was repeated in reverse order.

2. Result and Discussion

Figure 9 shows the changes in heart rate with increase and decrease of temperature at various depths.

On the way from the laboratory to the lake, the water in the fish tank went up to 15°C, but the heart rate stayed at 60 beats per minute.

The temperature of the lake water was 21.2 to 21.3°C at the 0 to 5 m depth. Beyond 10 m, the water temperature fell rapidly to 12.3°C at 15 m, 5.9°C at 25 m and 5.3°C at 35 m. In the stratum deeper than 45 m, the water remained constantly at 4.2°C, as expected under the law of specific gravity in the water.

Immediately after the cage was immersed into the surface stratum, the heart rate rose quickly to 85/min and reached 90/min at 10 m. However, with temperature decrease, the heart rate decreased to 75 at 20 m and 58 at 30 m depth. Deeper than 30 m, the heart rate decreased less rapidly, reaching 50 at 40 m and 46 at 49 m. During a five-minute wait at 49 m, the deepest station in this procedure, the heart rate was reduced to 40/min. Thereafter, the cage was pulled up by 2 m stages, allowing a few minutes pause at each station.

At first, the heart rate did not increase, keeping 40/min until the cage reached
Fig. 9. Diagram, Showing Correlative Changes in Depth and Temperature of Chuzenji-lake Water, Plus Heart Rate of Trout, Housed in a Cage

Fig. 10. Diagram, Showing Correlative Change in Heart Rate and Water Temperature, in Trout's Free Swimming
at 25 m (8°C). Approaching the surface, the heart rate rose abruptly to 46/min at 20 m, 60/min at 15 m and 86/min at 10 m in depth and then continued at more than 90/min until the cage reached the surface of the lake.

One trout was released in the lake without the cage. First it dived immediately to a depth of 15 m, passing through the thermocline. The thermocline is a stratum just beneath the surface in which the temperature of the water declines more rapidly with depth than in other strata. Then, the trout surfaced and again dived within a few minutes to an approximate 20 m deep water. Five minutes after this dive the animal gradually swam up and stayed in the stratum of warmer temperature of approximate 10°C. After about 15 minutes, the animal dived again to the 20 m-depth and remained there. At this time, a slight arrhythmia appeared in the heart beat and the heart rate slowed to less than 50 beats per minute. When picked up from the water, the animal was very weak, and breathed with great difficulty (Fig. 10).

The results obtained from the series of limited experiments described above lead to the following tentative conclusions. 1) Heart rate is an increasing function of water temperature. That is, a decline in water temperature is accompanied by a decrease in heart rate and vice versa. 2) Heart rate is directly correlated with the vertical distribution of water temperature in the lake. However, if a trout is held for a while at a stratum where the temperature is lower than the fish normally experiences, the recovery of heart rate during passage through warmer strata is considerably delayed. 3) When set free in the lake, the animal dives through the thermocline into a depth where the temperature is far lower than that habitually experienced. It takes approximately twenty minutes to return to the stratum, in which the animal behaves usually.

A PRELIMINARY STUDY OF THE DIURNAL AND NOCTURNAL VARIATION IN HEART RATE IN FREE SWIMMING RAINBOW TROUT

This is the final goal of the series of experiments described above. Such field experiments demonstrate the usefulness of radiotelemetry in studies of the ecology and behavior of fish under natural and laboratory conditions.

In this experiment, the heart rate of a larger rainbow trout, equipped with a radio-buoy, was measured at two-hour intervals throughout one full day to obtain information on daily changes in heart rate.

1. Material and Methods
   A two-year old rainbow trout, weighing approximately 600 gm, was equipped with electrodes to sense the ECG. The leads were attached to a buoy which the trout towed. The buoy was a waterproof plastic case, 10 × 6 × 4 cm, containing a 220 gm radio transmitter with a 9 volt battery. A 30 cm bare copper wire antenna was fixed perpendicularly on the case. The trout was freed in a 50 cm deep and 45 m² wide hatchery pond, in which a large school of young trout, about 150 gm in average body weight, was being raised.

2. Result and Discussion
   Figure 11 shows one 24 hour cycle of the heart rate in the experimental animal. Actual numbers of heart beats per minute are plotted at two-hour intervals through a 24 hour period.
The pond was located at an altitude greater than 1000 m above sea level. The mountain weather in mid-summer was changeable, clear in the early morning and rainy with thunder and abrupt fall of air temperature in the afternoon. In the night it was cloudy with brief showers. The temperature of the water in the pond remained constant at a bit lower than 10°C.

The trout's average heart rate was about 64 beats per minute for the entire 24 hour period. Two peaks of activity appear, a high peak arising about 65 beats per minute at 21:00 and a lower one (about 60 beats per minute) at noon. Similar two periods of slower heart rate occurred the lowest (55 beats per minute) at 8.00, and the other (61 beats per minute) at 16:00.

This method should be most useful in studies of experimental ecology or behavioristics of the rainbow trout, if the experiment is limited to a small area.

Although our main objective in this experiment was to study the effect of the instrumentation on the trout's behavior and to test the equipment, we obtained in addition a gratifying amount of biological information.

RESUME

1) These preliminary experiments were accomplished to test the applicability of radiotelemetry in research on ECG, heart rate, and respiratory movement in free-swimming rainbow trout.

2) A simple method of applying electrodes for sensing ECG was devised and several experiments to develop ECG telemetry were performed and the following objectives were achieved: 1. Described the configurations of ECG using epicardiac electrodes. 2. Described the positive correlation of ECG and/or heart rate and ambient water temperature. 3. Described the vertical distribution of water temperature in high-altitude Chuzenji Lake and its relation to the heart beat in rainbow trout. 4. Described circadian rhythm of heart rate in a free-swimming trout towing a radiotelemetry buoy. 5. Both ECG and respiratory movement were recorded by the use of a two-channel telemeter. 6. Based upon the results obtained, the authors demonstrated the feasibility of using radiotelemetry to study the ecology and physiology research of the rainbow trout.

REFERENCES

ニジマスの心電図とその無線搬送

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ニジマスの心電図と呼吸運動を無線的に記録し、魚類の生産研究に新しい技術を導入することを企図し、その基礎的な実験を行なった。成績の概要はつきの通りである。

1) 髄腔穿刺により不動化した後、閉胸し、心表面誘導による波形を記録した。穿刺による出血は、従来の記載に反し、極めて微量であった。静脈洞、心房、心室波の波形はBakker (1913), Kisch (1948), Ottis (1950), Noseda (1963)らの記載とほぼ一致した。
この成績に基づき、ラジオ・テレメーターの入力として用いる心電図の誘導方法を決定した。この誘導法は、一円の胸部内誘導であるが、電極装着後、2ヶ月以上を経過しても生存した。波形は哺乳類、鳥類などと同様であったが、まれにBakkerのV波を観察した。

2) 水温と心拍数の相関関係はおおよそ直線的であった。水温の低下に比例して、心拍数は増加した（水槽内）。

3) 中禅寺湖で水温の垂直分布に従って、計測した心拍数は温度の下降に比例して減少したが、水温の上昇に追従できなかった。実験湖で行った実験結果から考えられる適応時間を与えても同様であった。

4) 中禅寺湖に放流したニジマスにつき、水深50m、水温4.3℃までの心拍数をFM式ビート・メーターにより計測した。水温の変化による心拍数の変動は、実験室内でえた成績とはほぼ同様であった。

5) 愛鰭池に放流したニジマスにつき、心拍数の日周期変化を計測した。心拍数は朝晩に少なく、午後ないし夜間に増加した。因みに水温は9.5℃を維持していた。