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Preliminary study on heartbeats and swimming behavior of free-ranging fish, red sea bream *Pagrus major*, measured with newly developed micro data-logger

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Abstract: To estimate the physiological condition or metabolic rate simultaneously with swimming behavior, we recorded a continuous electrocardiogram (ECG) in freely swimming fish, red sea bream *Pagrus major,* in a net pen during a 24 h period. Swimming speed, swimming depth, and the bioelectric potential of the heart in the test fish were recorded with a micro data-logger. It is difficult to eliminate electric noise while recording ECGs of actively swimming fish. In the present experiment, we attached electrodes to two points on the ventral surface and successfully obtained data. Two types of micro data-loggers (one for recording ECG and another for swimming speed and depth) were attached to the dorsal side of the fish. The red sea bream swam slowly (< 1 total length/s) and stayed deeper in the net pen during most of the day, except for frequent burst of speed and vertical movements around dawn. An analysis of heartbeat variability, showed that high-frequency components, representing vagal (parasympathetic) nerve activity, rose only around midnight.

key words: ECG, electrode, heartbeat variability, autonomic nerve, swimming speed

Introduction

Since the 1980s micro data logging systems for measuring animal behavior have been developed by National Institute of Polar Research to study the ecology and physiology of Antarctic marine animals. A sophisticated system for simultaneously measuring both an electrocardiogram (ECG) and diving behavior was developed and used for Antarctic penguins late 1990s (Kuroki *et al.*, 1999). Recently this data logging system was further miniaturized for studying fish. However before using the system for free-ranging fish in Antarctica, we had to develop techniques for attaching electrodes and for noise elimination under experimental conditions.

Although a technique for recording ECGs of restrained fish in a laboratory tank has been developed (*e.g.*, Yamamori *et al.*, 1971; Nanba *et al.*, 1973; Nanba and Murachi, 1978; Nomura and Akiyama, 1984), it is still difficult to eliminate noise arising from the bioelectric potential of muscles and ambient electric potential caused by active swimming under unrestrained conditions. To overcome this problem, non-contact and

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non-invasive techniques for recording ECGs without any surgical procedure or instrumentation on the animal were developed (Laitinen and Valtonen, 1994; Hojesjo *et al.*, 1999; Altimiras and Larsen, 2000). However those trials succeeded only in narrow chamber experiments using encased electrodes, and no successful measurements have been obtained in real free-swimming conditions.

In this study, we developed a technique for recording ECGs from freely swimming fish by adjusting both the site of electrode attachment to the fish's body and the form of the electrode. We used a micro data-logger to measure swimming activities and to record the ECG data. We applied the maximum entropy method (MEM) to estimate daily activity patterns, especially in relation to possible periods of sleep.

Materials and methods

Experiment

The experiment was performed during 1 week in August 2001 at a fish farm belonging to the Marine Biological Technology Center, Nippon Suisan Kaisha Co. in Oita Prefecture, Japan. Three red sea bream (individual total lengths and weights: 52.5 cm and 2146 g, 55.0 cm and 2870 g, 56.3 cm and 3320 g) were selected for the experiment. The fish were transferred to a 2000-L aquarium until electrodes and data logging devices were attached. Bipolar electrodes to detect the bioelectronic potential of fish were constructed of a thin silver disc (approx. 5 mm in diameter) and a silver wire (approx. 20–50 mm long and 1 mm in diameter) (Fig. 1). The disc electrodes were covered with "instant glue" (KONY BOND Co., α-cyanoacrylate) to insulate them from sea-water. Before the experiment, the optimum attachment sites for the electrodes were confirmed by testing small red sea bream in a laboratory water tank. The

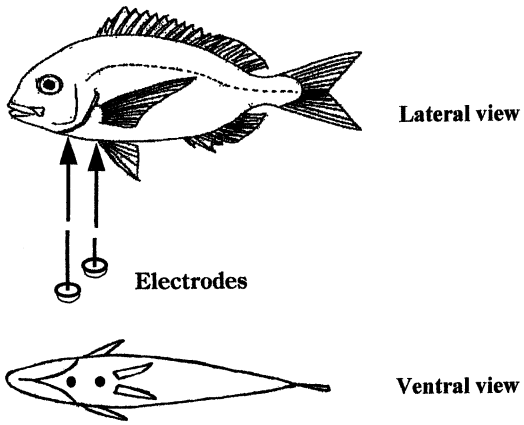

Fig. 1. Electrode-attachment sites from the lateral and ventral sides of fish. Both electrodes were on the ventral side: one electrode was inserted beneath the preopercle bones; the other was anterior to the pelvic fins. The wire of the anterior electrode was slightly longer (almost one-tenth of total length; 50–55 mm long) than the posterior one (almost 30 mm). Both silver wires were covered with thin polyethylene tubing to insulate them from the electrical potential of body muscle, except for 1–2 mm at the tips of wires.

best sites for attaching electrodes were on the ventral side, one beneath the posterior edge of the preopercle bones, and another anterior to the pelvic fins (Fig. 1). Even though the electrical potential vectors of the heart in carp are directed toward the lateral side in a horizontal plane (Ueno *et al.*, 1986), in red sea bream those vectors were directed longitudinally. The fish were anesthetized in a phenoxyethanol solution for about 2 min before surgery. First, moisture and mucus on the abdominal body surface were removed by rubbing with a paper towel. Next, at each site a small hole for attaching an electrode was made by puncturing with a needle. Finally, the silver wire was inserted into the hole, and the disc part of the electrode was attached to the body surface with instant glue (same as above). Before connecting both lead wires from the electrodes to the micro data-logger, we confirmed whether QRS waves could be detected, by connecting the wires to a bioelectronic amplifier (Nihon Koden Co., MEG-1200).

Attachment of micro data-logger

In this experiment 2 types of micro data-loggers (Little Leonardo Co.) were used. One type recorded the bioelectronic potential (model W400-ECG.SW ECG logger) and was used on all 3 test fish, and the other monitored swimming speed and depth (PD2 GT) and was attached to only 1 fish. The sampling intervals of the 3 ECG loggers were set to 5, 8, and 8 ms, and the sampling intervals of the PD2GT logger were 0.5 s for swimming speed and 1 s for depth.

Immediately after inserting and attaching the ECG electrodes to the fish and connecting them to the data-loggers, 2 loggers were sutured on both sides of the dorsal part of the fish with Nylon ties. Then each fish was released in a 2000-L tank for 14 h of recovery; then they were released into the net pen again.

Recording of swimming speed and depth was begun right after attaching the logger; however, ECG recording started 15–30 h afterwards, to allow the fish acclimate to the conditions of the net pen. The test fish swam freely in the net pen for about 24 h from the morning of 22 August to the next day. The sea-water temperature during the experiment ranged from 26 to 27℃. After 1 day of free swimming, the fish were retrieved from the net pen and anesthetized in concentrated phenoxyethanol solution while we detached the loggers.

Data analysis

The data were downloaded from the loggers to a computer and analyzed using the Igor Pro program (Wave Matrics Inc.). Heart rates (beat/min) were calculated from the R-R interval. Sets of 200 continuous beats (R-R intervals) were also measured every hour, and power spectrum densities were computed by MEM (Babloyantz and Destexhe, 1988; Lindecrantz *et al.*, 1993; Claireaux *et al.*, 1996; Kojima *et al.*, 1998, 2001).

Results and discussion

Figure 2 shows examples of ECG records obtained from 2 test fish swimming in the net pen. Noise was eliminated from ECG records exhibiting obvious QRS waves.

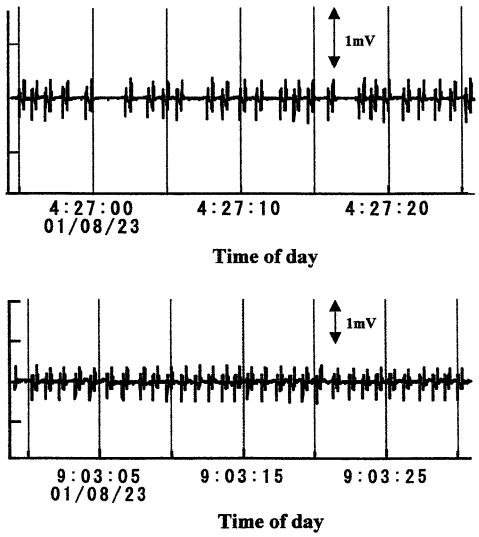

Fig. 2. Examples of ECG signal recorded with a micro data-logger from red sea bream swimming freely in a net pen.

Besides, noiseless records continued over the duration of the experiment. The logger on the third fish recorded an ECG for the longest period of time; this record was slightly inferior in terms of noise level to those shown in Fig. 2, but QRS waves were distinguishable and could be used to measure heartbeat variability.

Changes in heart rate, swimming speed, and swimming depth were monitored during a 24-h period (Fig. 3). The heart rate just after the fish was released into the net pen, was relatively high and remained between 75 and 95 bpm until about midnight. From midnight until dawn, the heart rate was still variable but slightly lower.

The swimming depth was also remarkably variable from release until about 1600. During the night, however, the fish usually stayed at a depth of about 8 m, near the bottom of the net pen. Its swimming speed was usually slow and regular, at less than 1 TL/s (or up to 30–40 cm/s). Every so often, the fish moved rapidly at a speed higher than 4 TL/s. The fish rarely swam rapidly at night. Sudden rapid movements were observed more frequently at dawn than in the daytime or at dusk.

According to Tanaka *et al.* (2001), vertical movements of chum salmon were less frequent around midnight. However, the fish made frequent vertical movements immediately before the spurts of rapid swimming at dawn, and its heart rate varied remarkably from dawn until the end of the experiment. Similarly, bumping movements by bluefin tuna reared in a net pen were observed frequently at dawn (Masuma *et al.*, 2001). There was no correlation between the heart rate and the maximum swimming speed during the preceding 5 min (Fig. 4). Whether the fish swam slowly (under 1 TL/s) or fast (over 3 TL/s), sometimes the heart rate increased, and sometimes it remained low. Figure 5 shows power spectrum analysis of heartbeat variability in one test fish, calculated by the MEM every 1–3 h from noon on August 22nd until the next morning.

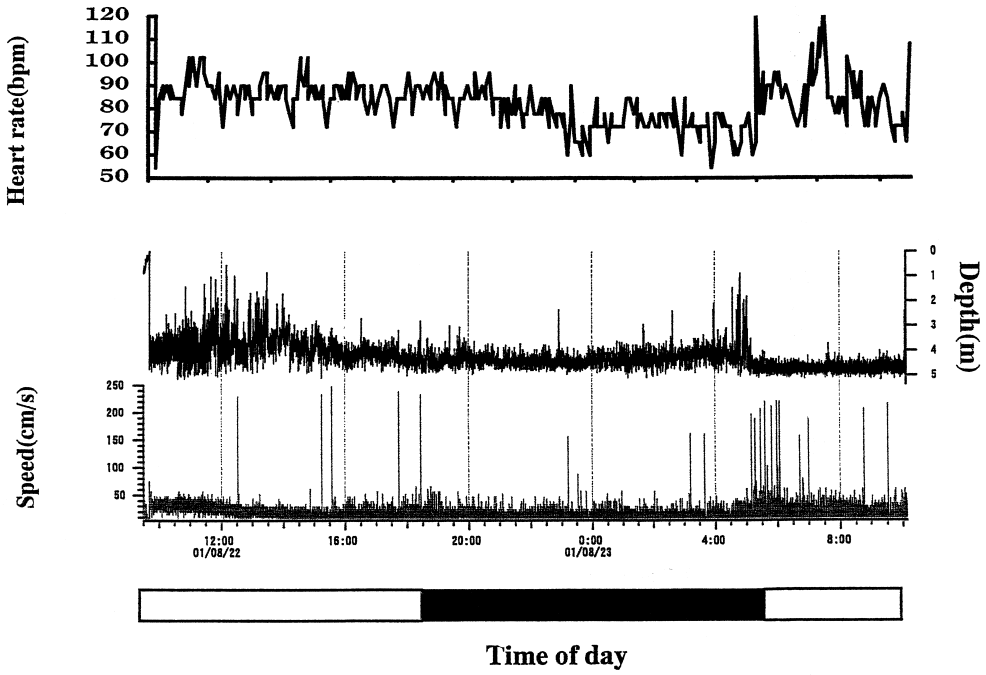

Fig. 3. Heart rates calculated from the intervals of QRS waves on an ECG, compared with swimming depth and speed recorded simultaneously in the same fish. Heart rates slightly decreased during the night. Rapid movements were observed more frequently at dawn.

As mentioned previously, a predominance of relatively high-frequency components represents vagal (parasympathetic) nerve activity, and low-frequency components represent sympathetic nerve activity. No obvious dominant component appeared in the power spectrum between noon and 2100, and between very early morning (0200) and morning (0800). However, relatively high-frequency components (0.1–0.3 cycles/beat) in the power spectrum were elevated at 2300, 0000, and 0100. In the other 2 test fish, we recorded the ECG from midnight until the next morning in one fish, and in the other from before dawn to the same time in the morning, and found relatively high-frequency components only at midnight. These findings mean that the predominant autonomic nerve activity around midnight was mainly vagal.

Although the use of submersible external electrodes on fish in an aquarium to non-invasively detect an ECG without any surgical procedure allows fish to swim freely, it was difficult to eliminate noise in those experiments (Hojesjo *et al.*, 1999; Altimiras and Larsen, 2000). Even when an electrode is directly attached to the fish's body surface and a lead wire is connected to an amplifier, it is also necessary to eliminate or avoid noise problems by using high and low pass filters. Unfortunately, the micro data-logger used in this experiment has no filtering mechanism. Thus, we must consider how to prevent noise mixing so as to detect pure ECG waves as clearly as possible. As stated previously, we carefully selected the sites for attaching electrodes in this study. Fortunately, the selected sites were also adequate for continuous detection

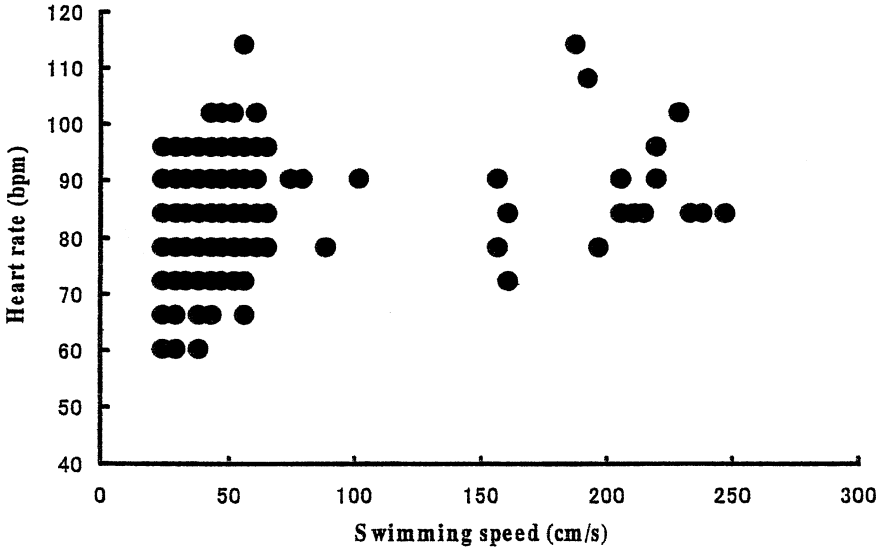

Fig. 4. Relationship between heart rate and maximum swimming speed during the 5 min before the heart rate was measured.

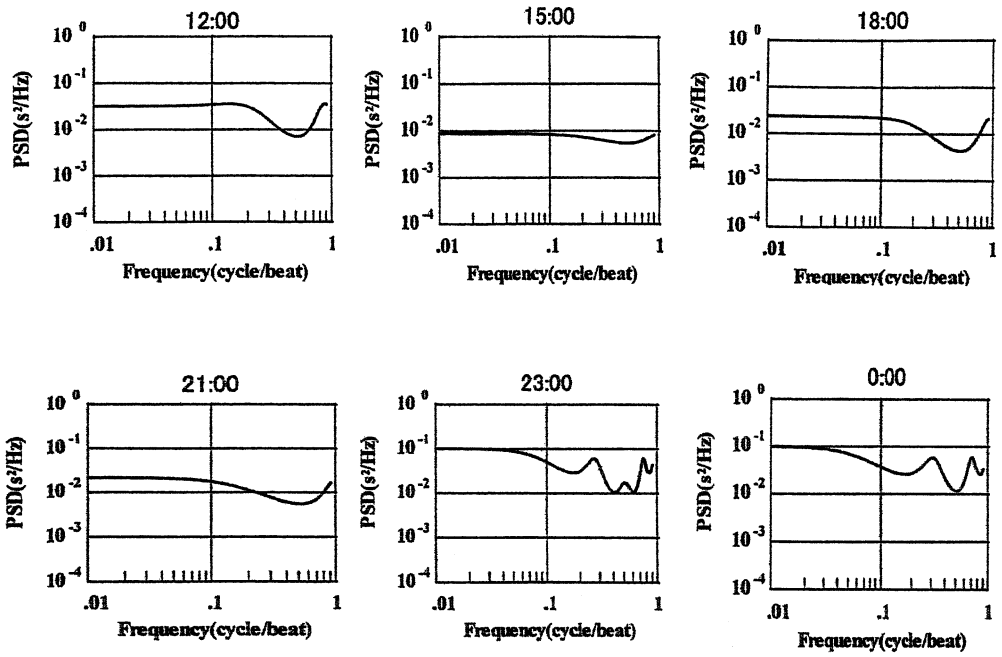

Fig. 5. Power spectrum density of heartbeat variability at various times, calculated by the MEM. During the daytime, relatively high-frequency components did not appear; however, for about 3 h around midnight, high-frequency components of about 0.2–0.3 (cycles/beat) appeared.

of clear ECG records even when the fish swam actively. For longer observations of ECG, we need to develop ways of attaching the electrodes firmly, so that they do not fall off during active swimming.

Because the heart rate was slightly decreased from midnight to dawn, we conclude that the metabolic rate of cultured fish is lower during the nighttime than in the daytime. Although both heart rate and swimming speed were variable at dawn, there was no obvious correlation between the heart rate and the maximum swimming speed during the preceding 5 min. This finding might be explained by the fact that cultured red sea bream rarely swim continuously. In addition, the heart rate may depend not only on swimming performance but also on other factors, such as the activity of the autonomic nerve system. It is not clear, from studying only the heart rate, whether the fish slept around midnight. However, because high-frequency components representing vagal nerve activity were elevated around midnight, we can suggest that the fish relaxed much at midnight.

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