Date
Dear Authors,

I enjoyed working on your document. In this letter, you will find some general document-related feedback as well as specific comments and notes.

Overall impressions

- The manuscript is not yet in the finalization stage. Given the extent of changes required, I recommend that you return for another round of editing before submission. Before this, please review all my changes carefully and make the necessary revisions.
- Throughout the document, the main language enhancements required were to ensure accuracy of tense and proper transition between sentences.
- Reference citations must be provided in the text for all material sourced from studies other than the present one. This includes the authors’ own previous studies as well.

Specific comments

- I have combined the results and discussion sections into one, as this seemed necessary to improve the flow.
- Figures and tables must be cited in the text in numerical order.
- At certain instances, I have deleted repetitive text or recommended that you do so, in order to make the manuscript more concise.
- Academic writing should be as specific as possible. At several points in the manuscript, you are missing this specificity. For instance, for how long were the fish anesthetized?
- Nouns followed by numbers or letters (like day 1 and group D) are not preceded by “the” since their specificity is already implied by the number/letter.

I hope you find this insight useful for future work, and I look forward to seeing your manuscript published. All the best!

Editor
Introduction

In order to detect the electrocardiogram (ECG) from the fish under unrestrained condition, several telemetry techniques such as using radio or ultrasound acoustic transmitters have been attempted. In general, fish ECG, especially from the unrestrained fish have been recorded to estimate the metabolic rate. Although these techniques can detect ECG continuously, some problems remain in using the technique. The transmitted acoustic pulses were triggered by QRS waves on the ECG. Therefore, it is very important to conduct the adjusted sensitivity of the transmitter when the fish were released to ensure that sufficient the amplitude of QRS coincides with can be reached at the trigger level. The method is also laborious and inconvenient in pursuing the subject except for the case when several fixed stations were set in closed waters. Recently, a new miniaturized micro data-logger has recently been developed to record the electric potential with high of free-ranging aquatic animals. The logger has a large memory capacity and capable of supporting frequent sampling has been developed. It can also record the ECG from free-ranging aquatic animals. The data logger can record instantaneous bioelectric potential and store the data for a given period depending on the sampling rate, allowing all the ECG wave data to be stored and memory capacity, respectively. This allows the whole ECG wave to appear after recovery and downloading. However, the miniaturized data logger has no means to confirm whether the ECG was recorded or not between the release and retrieval periods. Thus, using the logger, it is necessary to record clear waves of cardiac potential without electric noise arising from the bioelectric potential of the heart even when the fish is moving in motion.

In this study, we attempted to estimate the changes of fish in the physiological condition by means of power spectral analysis of heart rate variability conditions of fish in a net pen using a microECG data...
recorded with a micro data logger carried on attached to the dorsal portion side of the fish. The red sea bream (Pagrus major) was used as the subject species carrying the micro data logger to record ECG for certain duration and to assess fish physiological condition in the net pen. We examined the power spectrum analysis of the heart rate (HR, bpm) of red sea bream Pagrus major. Since the spectral analysis especially for the FFT methods requires certain number of data sets, we tried to record the successive ECG from recordings of free-swimming fish as long as possible. Successive ECG recording extending to almost 10 days was obtained, thus, enabling us to calculate power spectrum of heart rate variability using any duration spectra of HR data set changes at any time and for any duration at a given time. The retrieval of the fish from the net pen was easier compared to other studies where it is difficult to recapture fish carrying the logger after releasing them into the open sea.

Materials and methods

Attachment of electrodes

Six red sea bream (averaging avg. 3561 g in body weight; 54.8 cm in fork length) were reared in a net pen (8x8x8 m) and retrieved just before the experiments. The experiments were performed during August and September 2003 in the set of net pens (8x8x8 m and 5x5x3 m) set in the sea near the marine biological center Marine Biological Center of XXX. Water temperature was measured every 5 seconds during the experimental period giving an average of 23.1 °C (day time, 23.4 °C; night-time, 22.9 °C). The fish were fed with a pelletized food diet, but discontinued 72 h prior to any manipulation of animals. Feeding was suspended 72 h before the experiment to avoid any side effects.

To record cardiac activity of the fish, two stainless steel wire electrodes were implanted subcutaneously. The fish were first anaesthetized in with phenoxyethanol solution (1ml/l) and then two stainless steel wire electrodes were implanted subcutaneously. References were given for the method.

Data analysis

The records of heart activity-HR data were downloaded from the loggers to a computer and initially-analyzed using the ABC program (DEF Inc., CA, USA). Using the successive time series of bioelectric potential, the beat-to-beat intervals detected were measured when the bioelectric potential exceeded a certain trigger level. As Since the time series of instantaneous HR did not occur at regular rates but were measured in variable intervals, they were...
measured by means of heart beat counts. Frequencies of heart rate are thus expressed in cycle/beat or/beat. An approximated conversion into units equivalent to Hertz can be obtained by dividing the frequency values in cycle/beat by the mean interbeat duration. [Remainder of paragraph omitted. No statistical tests were done.]

**Results & Discussion**

Clear and distinct ECG traces were continuously recorded by the data logger for 230 h after releasing the fish into the pen. [Text omitted]

Distinct and successive ECG traces were recorded for two test fish (Fish A and B). ECG records of longer duration of ECG record reaching up to 9 days and 14 h were obtained when the sampling interval was set at 14 ms. Though the peaks of the QRS complex on the ECG trace looked downward, unlike common ECG recordings as shown in Fig. 2, downward peak trace of QRS may invert, the trace inverted upward when the connections of the lead wires from two electrodes were reversed as connected to the logger. As shown in Fig. 2, almost every QRS complex appeared like as spikes and was distinctive throughout the whole observation period. Thus each interbeat of the interbeats between QRS complexes were measured by setting a certain trigger level on the ECG trace, and were also determined by visual inspection of the trace when the QRS complexes were buried in the disturbance of the base line or electric noise.

Fig. 3 shows one record of 400,000 successive heart rates for 9 days and 14 h for Fish A. The fish was released into the net pen just after attaching the logger at 15:30 hours on 2 Sep. 2003 and retrieved at 16:00 hours on 13 Sep. 2003. The other successive record traced the ECG traces were recorded for almost about 4 days...

[The rest of the results, which have been omitted.]

**Discussion**

As shown in Figs. 3 and 4, the HR decreased from 35 to 25 bpm in the first 12 h after release. However, a clear trend of recovery following surgery and release was not noted, even though stabilizing the HR is often used as an index of recovery or acclimation from a surgery. When the test fish were released in natural conditions or into the net pen, the HR decreased and stabilized in 1 or 2 days while the time to reach resting levels was several days to 1 week when the fish were kept in a closed tank.

Clear and distinct ECG traces were continuously recorded by the data logger for 230 h after releasing because retrieval of the fish from the net pen was easier in case of the present study, while...
it may be very difficult to recapture the fish carrying the logger after releasing into the open sea. On the other hand, the micro data logger for bioelectric potential was applied similarly to record the ECG of other animals, for example, birds (W et al., 1995; B et al., 1997; E et al., 2001), penguins (K et al., 1999; G et al., 2001; G et al., 2003; F et al., 2004) and seals (M et al., 2003), of which logger were recovered with high probability after releasing. In those studies, HR change was usually used to estimate metabolic rate when animals encountered various condition or events.

As shown in Fig.3 and Fig.4, the initial changes of HR from releasing decreased from 35 to 25 bpm for. From the viewpoint of using heart beat variability to estimate the first 12 h. However, clear homophysiological conditions of recovery process in HR following surgery and releasing was not noted, even though the stabilizing of HR sometimes has been used as the index of recovery or acclimation from the surgical operation. When the test fish were released in the natural condition or net pen, the initial HR decreased to reach a stabilized level in a day or two, whereas the time course to reach resting levels tended to require several days or even 1 week if the fish were kept in the closed tank.

From the viewpoint of using heart beat variability for estimating fish condition, it was taken in the current experiment assumed that the test fish has had already recovered on the day following release (day 2) because we observed 1/f fluctuations were already observed (Fig.7) and there were some high-frequency components in the power spectral density (Fig.8) on that day.

In the power spectra of HR variability for continuous 4 days shown in Fig.6, Circadian periodicity around 24 h period was not detected in the power spectra of HR variability for 4 continuous days (Fig.6), while 10–11 h and 15–16 h periods were dominant in the spectra until the day 7. Since the period of daytime and nighttime during experiment were 12h nighttime lasted for 12 h, 50 min and 11 h 10 min, respectively, during the experimental period, one of the dominant components of the 10–11 h period almost corresponded with the night-time duration. Water temperature recordings varied widely in a distinct pattern that could be associated with the period of daytime and nighttime. Water temperature fluctuations might have been affected by the water current in flowing into the experimental area. The average duration between high and low tides and vice versa during the experimental period was approximately 6 h leading to the suggestion, suggesting that the minor peak at around 0.0001 cycle/minute (around 5 h 30 min period) reflects the tidal period.

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(1993) found that the ultradian rhythm period of rainbow trout was around 1.5 h, which was equivalent to 0.00037 cycle/beat in the present study in the present study. This result is derived from the calculation up to the 8 h period, and the observed rhythm may not be a consequence of periodic stimulation but be generated within the organism. According to the present study, it appears that there were lower ultradian periodicities at around 5 h 30 min and 10–13 h in the spectrum of red sea bream reared in the net pen, while they were lost in the spectrum of day which disappeared on days 6–9. The physiological conditions of the test fish in day on days 6–9 might have changed from the former first few days.

If the red sea bream with loggers attached loggers had not taken food for almost 7 or 8 days following surgery, it is also likely that some physiological changes occurred due to starvation. Our results may permit this study enables the assessment about the of changes and in the time course of physiological conditions of fish carrying data logger attached on their body. Some changes have definitely occurred on the fish, which was supported over time. Our results showed that some changes did occur, as demonstrated by existence of 1/f fluctuations or frequency components of the HR variability and visible injury after release to the side where the portion of attachment of logger was attached. The use of micro data-loggers that progressed as opens new avenues for research such as gathering and monitoring the days passed after releasing. Use of micro data logger offers great promises to open up various areas of research such as gathering and monitoring behavioral or physiological information from of free-ranging animals. Further study on the effect of attaching loggers and the physiological load on the animals including the associated process of physiological change are anticipated and in order should be carried out.