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Article

Measurement of Diurnal Body Tilt Angle Distributions of Threeline Grunt *Parapristipoma trilineatum* Using Micro-Acceleration Data Loggers

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Abstract: The body tilt angle of a fish has a large effect on the acoustic target strength. For an accurate estimation of fish abundance using acoustic methods, it is necessary to measure body tilt angles in free-ranging fish. We measured diurnal body tilt angle distributions of threeline grunt (*Parapristipoma trilineatum*) while swimming in schools in a fish cage. Micro-acceleration data loggers were used to record (for 3 days) swaying and surging accelerations (at 16 Hz intervals) of 10 individuals among 20 forming a school in a fish cage. Time series analysis of 1-h mean body tilt angles revealed significant differences in body tilt angles between day (−7.9 ± 3.28°) and night (0.8 ± 5.89°), which must be taken into account when conducting acoustic surveys. These results will be useful for calculating the average dorsal aspect target strength (TS) of threeline grunt for accurate estimations of fish abundance.

Keywords: body tilt angle; data loggers; bio-logging; fish behaviour; fisheries acoustics; fish abundance; target strength; threeline grunt; *Parapristipoma trilineatum*

1. Introduction

Quantitative echo-sounding has been used effectively to estimate fish abundance since the development of the echo-integration method based on the proportional relationship between fish abundance and acoustic backscattering strength of the animals (e.g., [1]). However, the target strength (TS) of an individual fish depends greatly on its body tilt angle (e.g., [2,3]). Consequently, body tilt angle distributions in swimming fish must be taken into account for accurate estimation of abundance.

Optical instruments, such as underwater cameras, have been widely applied to measure the body tilt angles of swimming fish [4,5]. However, these instruments cannot be used at night because natural light is insufficient and artificial light may change fish behaviors, affecting normal diurnal patterns. Bio-logging systems [6] can be used to record animal behaviors in light and in darkness.

Accelerations of animals with attached loggers can be simultaneously recorded along two axes, measuring both tail-beat frequency and body tilt angle. Tanaka *et al.* [7] applied acceleration data loggers to measure body tilt angle distributions in chum salmon (*Oncorhynchus keta*) for their homing migrations. Loggers have since been used for measurements of not only commercial species (e.g., the Splendid alfonsino [8]) but also threatened fish (e.g., Japanese lates [9]). The earlier studies measured behavior in single-swimming fish equipped with loggers. However, behavior in fish swimming in schools is poorly known.

The threeline grunt (*Parapristipoma trilineatum*) is a commercial species that swims in schools [10]. It is benthopelagic, inhabiting rocky coastal seascapes where they make an important fishing ground. Komatsu *et al.* [11] revealed that body tilt angle is closely associated with the movement of the fish. Therefore, because the swimming activity generally differs in day and night, we expect the body tilt angles of the fish to differ in day and night.

The aims of this paper is to test if accelerometers will give good estimates on the tilt angles of schooling threeline grunts, if the tilt angle varies diurnally and what eventual consequences such variations will have for fish abundance estimation using acoustic techniques.

2. Material and Method

Experiments were conducted in October 2007 in a fish cage (4.0 × 4.0 m long, 3.5 m deep) deployed off a fishing port on Mishima, an island (34°45′N, 131°09′E) in the Sea of Japan. Threeline grunt were caught by hook and line at a depth <30 m above the Hachirigase sea hill situated 10 km north of Mishima. Caught fish were transported for <2 h from Hachirigase to the fish cage in a boat tank continually supplied with fresh seawater. They were acclimated in the cage for 3 days before experimentation.

Wild fish schools usually consist of similarly-sized individuals [12,13]. Suzuki *et al.* [10] reported that threeline grunts form schools and swim together when >10 individuals are held in a circular tank. Accordingly, we selected a group of 20 individuals with a mean total length of 30.3 cm ± 2.45 (±SD) and a mean weight of 340 g ± 90.93. None of the fish had surface damage from handling. School formation and group swimming were recorded in the fish cage using an underwater camera (FM-4100, QI Co.) set at 2 m depth, and by surface observation from a gantry around the fish cage. (We use here the term school in a broad sense as a group of fish based on a mutual attraction.)

The micro-acceleration data loggers (128 Mbit M190L-D2GT accelerometer; Little Leonardo Co., Tokyo, Japan) used in this study measured 15 mm in diameter, 53 mm in length, and 6 g in weight (underwater). The loggers recorded accelerations along two axes (sway and surge) at 16 Hz, and they recorded depth and temperature at 1 Hz for 3 days. To measure body tilt angle of fish accurately, the acceleration data logger must be firmly attached to the fish body. We used plastic cable ties to reduce handling time during logger attachment. Two ties were used to fix the logger to the dorsal side of the fish parallel to the body axis. The ties were inserted through the dorsal muscle tissue of the fish and quickly fastened (<1 min). Before returning each fish to the cage after logger attachment, we measured (for 10 s) its starting acceleration parallel to the water surface in a shallow tank of 0.5 m depth; this allowed us to determine the logger attachment angle relative to the body axis.

Gallepp and Magnuson [14] showed the effects of negatively buoyant load for fish. Our preliminary experiment to investigate how the fish would react to being equipped with a logger showed that threeline grunts swam diagonally, or pitched and rolled near the bottom. The negative buoyancy of the logger is believed to have caused this abnormal behavior because tags >2% of fish body weight in air or 1.25% in water are known to affect swimming performance [15]. Accordingly, we attached a copolymer foam float (Nichiyu Giken Kogyo Co., Saitama, Japan) to the data logger, making the apparatus almost neutrally buoyant in seawater. The float was box shaped (4.0 cm in length, 2.0 cm in width, 1.0 cm in height) and had a density of 0.26 $g \cdot cm^{-2}$.

Seawater temperature and salinity were recorded at 3 m depth with an auto-logging water temperature and conductivity sensor (Compact CT, Alec Electronics Co.); light intensity was recorded at 3 m depth with an auto-logging light sensor (MDS-MkV/L, Alec Electronics Co.). These physical variables were measured at 1-min intervals through the experiment. Average water temperature, salinity, and light intensity during the experiment were 23.3 ± 0.24 °C, 33.9 ± 0.14‰, and 117 ± 107.6, μmol photons $m^{-2} \cdot s^{-1}$ respectively. We categorized mean light intensities for each hour of the experiment into day or night periods. Daytime was defined by periods with 1-h mean light intensities of >0 μmol photons $m^{-2} \cdot s^{-1}$. Periods during the night had light levels <0 μmol photons $m^{-2} \cdot s^{-1}$.

The population was divided into two groups of 10 individuals with a partition across the cage in order to examine whether even 10 individuals form a school. Subsequently, micro-acceleration data loggers were attached to all the fish in one group. After confirming that the 10 individuals with and without loggers had formed a school in the cage, we removed the central partition, allowing the whole group of fish to swim together. Fish with attached loggers were retrieved from the cage 3 days after release. Acceleration data were downloaded from the logger to the computer. We excluded data from four loggers that were no longer snug on the fish bodies on retrieval.

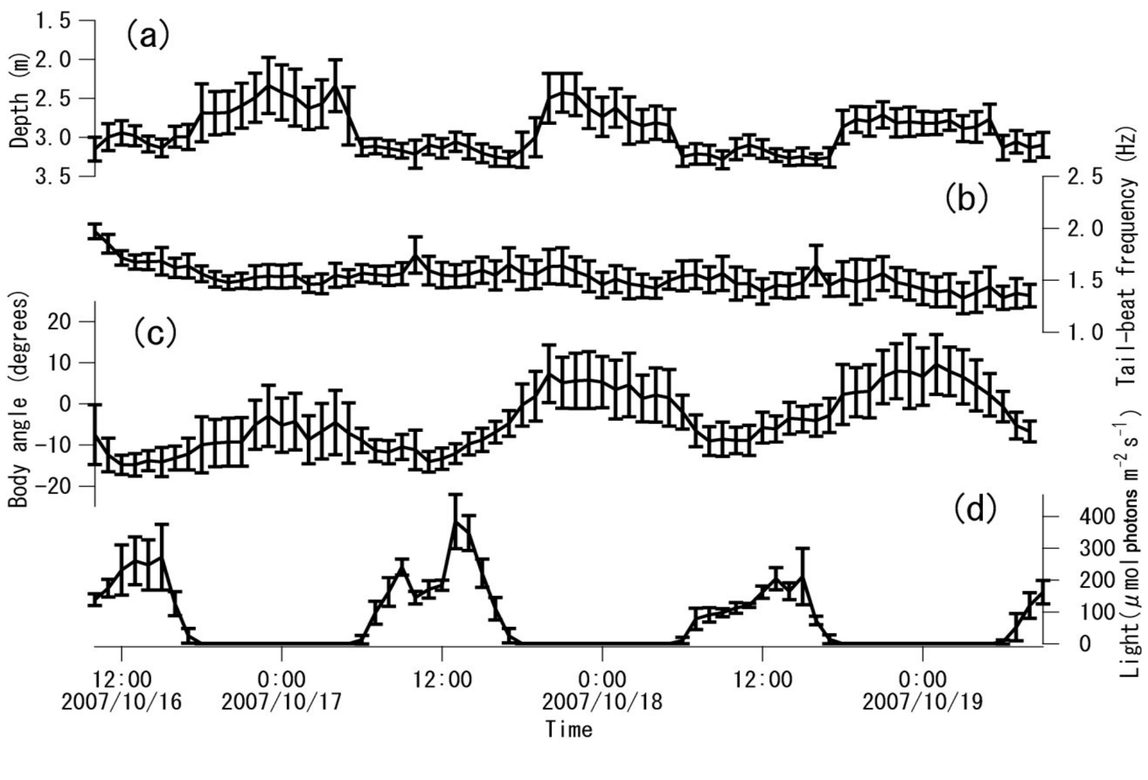
Power spectral densities (PSD) were calculated from swaying acceleration records from six loggers to determine the dominant stroke cycle frequency using fast Fourier transformation with IGOR Pro ver. 6.03 (WaveMetrics, Lake Oswego, OR, USA) following Ropert-Coudert *et al.* [16]. Tail beats were derived by high-pass filtering (less than 0.98 Hz) the swaying acceleration. The body angle was extracted by low-pass filtering the surge acceleration [7]. To remove higher frequency acceleration caused by tail beats, a low-pass filter was applied, with the threshold being the predominant frequency of tail beats to surging acceleration.

3. Results

Video movies recorded with the underwater camera showed that threeline grunt with and without data loggers formed a single school and swam together during the experiment. The tail beat frequency of the tagged fish was higher than for the untagged fish in the first 9 h of the experiment.

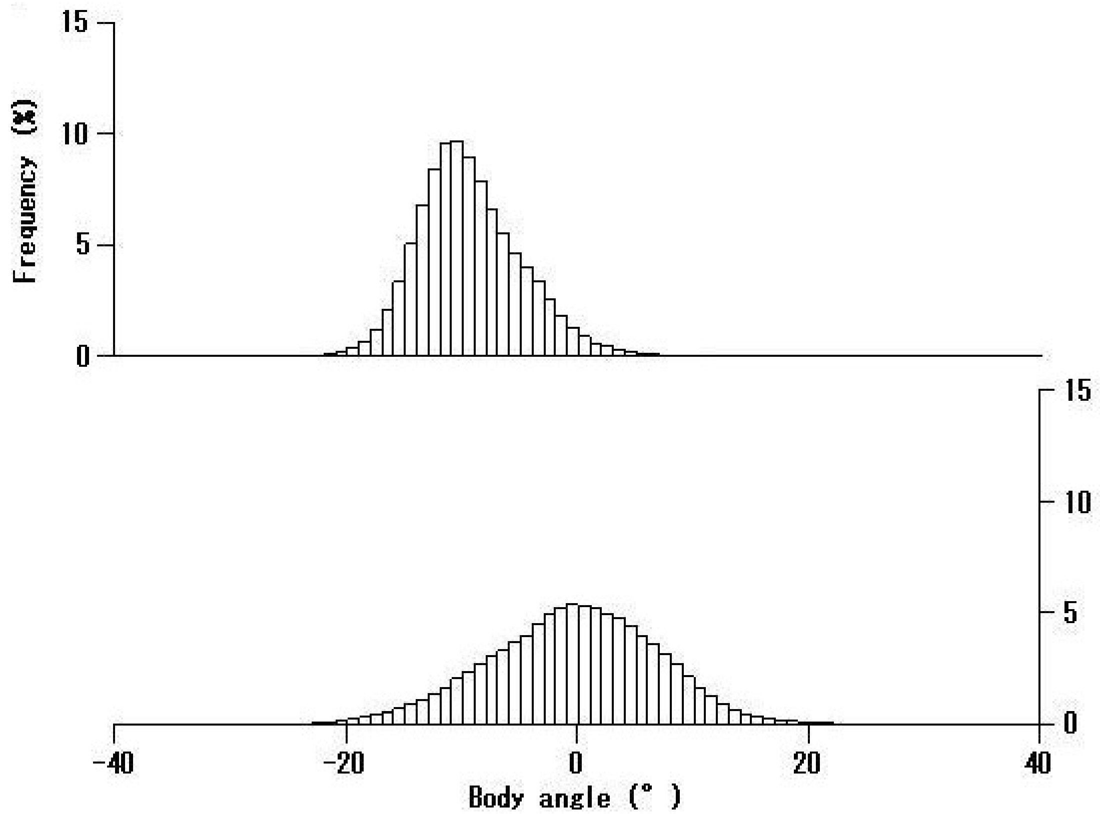
The 1-h mean swimming depth, tail-beat frequency, and body tilt angle of one individual displayed in Figure 1 is typical of the accumulated time-series data. Mean tail-beat frequency from the start of the experiment to 9 h later (1.73 ± 0.11 Hz, mean ± SD) was significantly greater than that (1.51 ± 0.08 Hz) from hour 9 until the end of the experiment (Mann-Whitney U, $p < 0.05$). During the latter period, mean 1-h tail-beat frequency in the daytime (1.53 ± 0.09 Hz) was not statistically different from that at night (1.49 ± 0.07 Hz) (Mann-Whitney U, $p > 0.05$). One-hour mean body tilt angle fluctuated diurnally, with larger tilt angles in daytime (−7.9 ± 3.28°) than at night (0.8 ± 5.89°) (Mann-Whitney U, $p < 0.01$). One-hour mean swimming depth also varied diurnally, with the fish staying deeper during daytime (3.18 ± 0.07 m) than at night (2.71 ± 0.19 m) from hour 9 onward through the experiment (Mann-Whitney U, $p < 0.01$).

Figure 1. Example (for one individual) of 1-h means of depth (**a**), frequency of tail beat (**b**), tilt angle (**c**), and light intensity (**d**). Bars represent standard deviations.



Body tilt angle data measured at 1-s intervals in day and at night (for six individuals) from hour 9 onward through the experiment were normally distributed (Kolmogorov-Smirnov test, $p < 0.01$) (Figure 2). Mean body tilt in daytime was −9.3 ± 5.0°, indicating that fish heads were oriented downward. At night, the mean angle was −0.5 ± 8.2°, indicating that fish heads were horizontal and significantly higher than during the day (*t*-test, $p < 0.01$).

Figure 2. Tilt angle distributions in day (upper panel) and night (lower panel) measured for six individuals among a school of 20 individuals.



4. Discussion

Procedural artefacts of tagging must be determined in behavioral experiments (e.g., [17]). To reduce stress on the fish we attached a float to each of the data loggers to make them almost neutrally buoyant in seawater. Time-series data showed that 1-h mean tail-beat frequency of logger-equipped individuals changed less from hour 9 onward to the end of the experiment. The higher tail-beat frequencies before hour 9 may therefore be attributed to handling stress.

The standard deviation of both the distribution of body tilt angle and the swimming depth of the six selected individuals was greater at night than during the day (Figure 1) and these vertical movements at night indicate that the fish were more active at night. According to Matsumiya and Takahashi [18], threeline grunt in coastal waters feed on zooplankton at night. Kawano [19] reported the presence of zooplankton in the stomachs of threeline grunt caught over Hachirigase sea hill. Since our experimental specimens were caught over Hachirigase sea hill, we believe that the caged fish with and without loggers foraged in a manner similar to wild fish over the sea hill and that the greater variance of body tilt angle at night in the fish cage suggests a foraging behavior of threeline grunt.

The average body tilt angle was below horizontal in the day, with heads pointed downward (Figure 2). Komatsu *et al.* [11] reported that body tilt angle averaged −19.5 ± 7.5°, when threeline grunt did not move. Thus it is suggested that the fish tilt their heads downward were resting in the day.

5. Conclusion

This study demonstrates that body tilt angle of schooling fish can be measured with micro-acceleration data loggers. Moreover, the study revealed significant differences in body tilt angles between day and night, which must be taken into account when conducting acoustic surveys. These results will be useful for calculating the average dorsal aspect target strength (TS) of threeline grunt for accurate estimations of abundance, which are necessary for sustainable use of resources.

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Conflict of Interest

The authors declare no conflict of interest.

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