Hearing Response to Sweeping Sound in Bastard Halibut *Paralichthys olivaceus*

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The hearing sensitivities of bastard halibut *Paralichthys olivaceus* decreased sharply at the frequency band between 200 Hz and 400 Hz. The hearing response of bastard halibut was measured by sweeping sound which the center frequency moves from non-hearing frequency (300–1600 Hz) to hearing frequency (100–200 Hz), and discussed the upper limit frequency of hearing.

The classical conditioning was reinforced by the tone burst sound in a continuous time of 1 s, a frequency of 100 Hz and a sound pressure level of 131 dB. The sweeping sound was projected in five steps at intervals of 5 dB. The sweeping speed of eight types was adjusted with a combination of a start and end of sweeping frequency. The conditioning response was measured as suppression in the respiratory activity by a piezoelectric sensor fitted on the gill cover.

The response to sweeping sound was 82% at the frequency band between 200 Hz and 340 Hz, which agreed with the boundary of the hearing area. The upper limit frequency of hearing of bastard halibut existed under 500 Hz. The hearing response to sweeping sound did not agree with the audiogram of bastard halibut because the sound pressure level fluctuated in the small water tank. The sweeping speed is inversely proportional to the individual difference of the response time.

Key words: bastard halibut, audiogram, sweeping sound, hearing area

Auditory thresholds of the tilapia *Tilapia niloticus*,^{1,2)} the Japanese horse mackerel *Trachurus japonicus*³⁾ and the bastard halibut *Paralichthys olivaceus*²⁾ were measured by the authors according to the method of limits using tone burst sounds. But for bastard halibut, the responses to sounds were unstable at 200 Hz or more, therefore it could not detect the audiogram. After this experiment, the auditory threshold was measured by the up-down method, but the result showed that the individual threshold had significant difference in the frequency band between 200 Hz and 400 Hz.⁴⁾ This may be caused by the hearing sensitivity of bastard halibut which sharply decreases to 13–40 dB/octave at the frequency band. If the fish really has such a characteristic at the frequency band, the boundary between the non-hearing frequency area and hearing frequency area will agree with the threshold of the sound pressure level. In the present study, I wish to report on the hearing response of bastard halibut produced by sweeping sound in which the center frequency moves from non-hearing area to hearing area, to consider the upper limit frequency of the fish's hearing, and to suggest the possibility of new sound stimulus.

Methods

Seven bastard halibut, 386 mm in average total length, were used in this experiment. They were obtained from an intermediate breeding supplier (Kiire Kagoshima, Japan) and maintained in a communal tank for over five months in the laboratory. The fish were randomly selected from 20 fish.

The experimental equipment, classical conditioning and response detecting method were utilized in exactly the

same as our previous study⁴⁾ in measuring the audiogram of bastard halibut. The block diagram of the apparatus and equipment used in the experiments is shown in Fig. 1. Sweeping sounds were produced by a function generator (NF1930; NF Elec. Ins.). The test was carried out in a water tank (56 × 80 × 48 cm). The tank was enclosed with a thick blanket hung from the ceiling in an acoustic chamber, a ten feet container. The water temperature of the experimental tank was equal to that of the rearing tank at 20.5°C (±0.8°C). The water level was maintained at 40 cm. The sound produced by function generator was emitted from two loudspeakers placed face to face 10 cm away from the tank. The gill movements by respiratory activity were measured by piezoelectric sensor (Ldt2-028k; Kyner) (hereinafter referred to as "sensor") fitted on the gill cover by a wooden arm. The method of fitting the sensor to the test fish is shown in Fig. 2. The fish was restrained on the fish size's bottom with a height of 80 mm enclosed with a net, so that the body and gill movements were unrestrained. This method applies the principle that bastard

Fig. 1. Block diagram of the apparatus and equipment used in the experiments.

Fig. 2. Method of fitting the piezoelectric sensor to the test fish.

PS: Piezoelectric Sensor, SA: to Signal Analyzer, ES: Electric Shock Generator).

Fig. 3. Upper figure shows the projecting method of sweeping sound (1000 Hz $\rightarrow$ 100 Hz) and lower figure shows the relation between the audiogram of bastard halibut⁴⁾ (bold solid line) and sound pressure level's curves (solid lines) of sweeping sounds to five sound steps. Sound pressure level shows in dB (0 dB re 1 μ Pa).

halibut lie covered in the sea bottom. The signal from the sensor was pre-amplified, then low-pass filtered at 20 Hz (E-3201B; NF Elec. Ins.). The filtered signal was displayed on a monitor of a signal analyzer (SA74; Rion).

Conditioning stimulus used a tone burst sound with a continuous time of 1 s, a frequency of 100 Hz and a sound pressure level of 131 dB. An electric shock with a continuous time of 0.1 s and an amplitude of 15 V (DC) was generated across two steel electrodes at wooden arm and bottom. It was given to the fish 0.1 s after the sound was projected. The sound and electric shock were repeated ten times at five minute intervals. The sweeping sound which the center frequency moves from non-hearing area to hearing area was used to measure the conditioned response. A

Table 1. Sweeping speed of sweeping sound

Sweeping start frequency (Hz)	Sweeping end frequency (Hz)	Sweeping speed (octave/20 s)
400	200	1
300	100	$1\frac{2}{3}$
400	100	2
500	100	$2\frac{1}{3}$
600	100	$2\frac{2}{3}$
800	100	3
1,000	100	$3\frac{1}{3}$
1,600	100	4

projecting method of sweeping sound and sound pressure level curves of five sound steps and the audiogram of bastard halibut are shown in Fig. 3. The sweeping speed was adjusted with a combination of sweeping start frequency (non-hearing frequency; 300–1600 Hz) and sweeping end frequency (hearing frequency; 100–200 Hz). It has eight types of one to four octave bands per 20 s as shown in Table 1. The measurements were carried out five times at the same sweeping speed and in five sound level steps at 5 dB intervals, shown in Fig. 3. It refers to the sound level step as "Sound step" and the lowest sound level is No. 1 in a set. The underwater sound pressure level (0 dB re 1 μ Pa) of sweeping sound was measured at the fish occupied position. The sweeping sound indicates that the sound pressure level curve crosses the audiogram from non-hearing area to hearing area. These curves have no relation to the sweeping speed. The peaks of the sound pressure level exist at 190 Hz and 320 Hz for the acoustic characteristics of the experimental tank.

In measurement, after the continuous sound of the sweeping start frequency has reached over 20 s, the sweeping sound was started when the respiratory periods were confirmed stable. The first 20 s of continuous sound stage was named "first term" and the next 20 s of sweeping sound stage was "second term". The signals from the sensor were converted to digital data by signal analyzer, and saved to a floppy disk during the first and second term. A computer was used to control the sound (conditioned) and electric shock (unconditioned) stimuli, and analyze the response.

The conditioned response was decided by the change of respiratory period which was obtained from the peak interval of recorded 40 s signal wave. The peak indicates the either + or - signal above half of maximum peak height over first term. The value (mean $\pm$ 1.96 $\times$ s.d.) over first term was used as the control reference. The first interval within the second term which is greater than the control reference value was accepted as a positive response. The front peak time of that suggests a positive response point.

Results

The respiratory signal waves to the sweeping sound measured by the sensor is shown in Fig. 4. The waves indicate the differential waves of respiratory activity and the peak levels are the maximum acceleration of opening and

Fig. 4. The example of conditioned response to sweeping sound in the respiratory activity measured by the piezoelectric sensor. The closed triangles indicate the response point.

closing movements because the sensor is acceleration output type. The peak levels often differ according to the fitting position of the sensor on the gill cover and slightly change with the measurement steps. But the peak interval doesn't generally change for 40 s. The spread peak intervals were indicated between 8 s and 15 s after the sweeping start. It confirms that suppression occurs in the respiratory activity as a conditioned response to sweeping sound even if the tone burst sound is used as the conditioning stimulus. After this suppression continued for 2–3 s, the respiratory activity recovered a constant period. This sensor was able to measure other responses, body motions *etc.*, but like burst sounds, they didn't occur for sweeping sound.

The relation between the responded frequencies and sound pressure level measured by all sweeping sound is shown in Fig. 5. The positive response to sweeping sound over 500 Hz were not confirmed and occupied 82% in 200–340 Hz range. The maximum frequency of positive response was present at two frequency bands, 240–260 Hz and 300–340 Hz. Each positive response was not directly proportional to a sound step set. In Fig. 5, the audiogram of bastard halibut produced by the tone burst sound is shown at the same time. The responded sound pressure level indicates a greater distribution than the thresholds examined by the tone burst sound. The main positive responses occurred in both frequency band from 220 Hz to 280 Hz and from 340 Hz to 370 Hz in which the sound pressure level tends to increase, so that the inclination of these tend to be contrary to the audiogram. But the average sound pressure level increased from 95 to 117 dB according to the rise in the sound step. These accorded substantially with the audiogram. Consequently, there was a boundary of hearing

Fig. 5. Relation between the audiogram of bastard halibut⁴⁾ (bold solid line) and the responded frequency and sound pressure level to sweeping sound.

The dotted area indicates the range of auditory threshold in the previous study.⁴⁾ Sound pressure level is shown in dB (0 dB re 1 μ Pa).

Fig. 6. Relation between the response time to sweeping sound and sweeping speed.

Solid lines indicate eight sweeping speeds and the closed circles indicate response time.

area for the frequency between 200 Hz and 400 Hz, and the upper limit frequency of hearing of bastard halibut existed under 500 Hz.

The relation between the sweeping speed and the interval from sweeping start time until a positive response time

is shown in Fig. 6. However, the sound pressure level was excluded from the factor because the change by sweeping was larger than the sound steps. If the sweeping speed is slow, the difference of response time in each fish becomes significant. It indicates that the individual difference of response is amplified by the slow frequency change, and the frequency change in a respiratory period becomes greater since the sweeping speed is faster. For example, to take a respiratory period (1.05 s) as shown in Fig. 4, the frequency change in that period is 10.5 Hz in case of the sweeping speed is 1 octave/20 s, and 79 Hz in case of 4 octave/20 s.

Discussion

The lower limit frequency which could be heard an elastic wave is called "lower limit of hearing".⁵⁾ For the fish, it was difficult to discriminate the hearing of either the sound pressure level or particle motion. On the other hand, the upper limit frequency which is an elastic wave is called "upper limit of hearing".⁵⁾ It generally differs depending on the fish, but the results could be obtained easier. For example, the measured upper limit of hearing of the following fish was 400 Hz in bastard halibut,⁴⁾ 250 Hz in flatfish *Pleuronectes platessa* and *Limanda limanda*,⁶⁾ 520 Hz in cod *Gradus morhua*,⁷⁾ and 1 kHz in walleye pollock *Theragra chalcogramma*.⁸⁾

For fish which lack a gas-filled swim-bladder such as a bastard halibut, the hearing area is narrower,^{4,6,9)} and the sensitivity decreases more sharply and at lower frequency than fish with a gas-filled swim-bladder.⁶⁾ The hearing sensitivity sharply decreased at over 300 Hz (29 dB/octave) in bastard halibut,⁴⁾ and over 1.2 kHz (30 dB/octave) in herring *Clupea harengus*.¹⁰⁾ In this experiment, it was revealed that the boundary of hearing area for frequency existed at between 200 Hz and 400 Hz, and that the upper limit frequency of hearing of bastard halibut was under 500 Hz. This almost agreed with the audiogram of the previous study.⁴⁾ The psychophysiological measurements on the auditory threshold of fish are generally performed in a small water tank. The small dimensions of the tank in comparison with the wavelength of the sound frequencies of relevance gives rise to a sound field with many peaks and troughs due to resonance and multiple reflections. And the vibration proof rubbers were inserted between the floor of the acoustic chamber and the bottom of the experimental tank, but in this fish fitting method, the influence of particle motion was unavoidable because the fish's lateral line touched a setting floor. In the method of measurement of the upper limit frequency using sweeping sound in the small tank, the sound pressure level of the sweeping sound and the influence of particle motion raised questions of accuracy. Therefore the relation between the responded frequency and sound pressure level is in contrast with the tendency of the audiogram. It is necessary for an accurate boundary area by frequency axis that the limit is measured by the gain controlled sweeping sound or swept burst sound with a constant sound pressure level. The gain-controlled frequency sweep generator with feedback system is utilized in auditory research,¹¹⁾ but isn't suitable for high speed sweeping. The sound pressure level was more difficult to fix than frequency in the transmission path and

mechanical conditions. In this study, only the occurrence of the response for some sweeping sounds was confirmed.

A tone burst sound of 300 Hz has been selected as an uninfluenced frequency by a background noise in the experimental nursery of bastard halibut using acoustic conditioning. But it did not achieve sufficient results, since the 300 Hz sound stimulus belongs to the frequency band in which the hearing sensitivity of the fish is sharply decreased.^{12,13)} For a bastard halibut in which the hearing area is narrow, the sound stimulus needs to satisfy the demands for both background noise and hearing characteristics. It has been reported that the conditioned fish is attracted to any intermittent sound after release^{14,15)} and the key component of the sound is the raising pressure.¹⁵⁾ Since the sweeping sound is able to move the center frequency up and down between hearing and non-hearing area, it suggests that it is possible to make up for a weak point of single frequency sound. For high speed sweeping, the response tends to occur intensively over a period after sweeping starts. If the sweeping sound is used in the experimental nursery, it will be able to produce an intensive or sporadic response. The high speed sweeping sound is capable of reducing the individual difference of response. Should an intensive response by sound stimulus occur, it should project a high speed sweeping sound. Conversely, should a sporadic response occur, it should project a slow speed sweeping sound. But generally, since a fish with a gas-filled swim-bladder has a wide hearing area and a gentle curve audiogram, the upper limit frequency of hearing will not become clearer. This effect would be limited to fish in which hearing sensitivity sharply decreases in a narrow frequency band such as a bastard halibut. But there are reports that bastard halibut do not have the ability to connect sound and behavior.⁴⁾ The fish did not exhibit an escaping behavior for sweeping sound. We can not draw the conclusion that the sweeping sound is effective for sound stimulus instead of tone burst sound. This remains to be solved in the future.

In nature, a broad-band noise can be heard to sweep when a ship approaches a sound receiver,^{16,17)} or a sound receiver approaches a low frequency sound source¹⁸⁾ or conversely in shallow water. Thus the frequency interference is continuously changed by the phase difference between a direction wave and reflected waves on the sea surface and sea bottom. This phenomenon occurs particularly in a heavy traffic area such as a harbor¹⁹⁾ and coastal fishing grounds in shallow water.¹⁶⁾ The emitted noise by a ship is shown by an interference striped pattern in the spectrogram.

In order to solve the relationship between the noise emitted by a ship and fish behavior in shallow water, one must not only study the change of the sound pressure level and the frequency characteristics of the emitted sound but also the hearing ability and response for the sweeping sound of the fish and the characteristics of the sweeping sound in nature.

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