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Development of the Startle Response to Sound Stimuli in the Red Sea Bream, *Pagrus major* TEMMINCK *et* SCHLEGL

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The startle response of the red sea bream was examined in order to clarify the development of behavioral change upon application of audio-stimulation.

The sound stimuli applied were pure tones which were generated electrically and projected from an underwater speaker placed in an FRP experimental tank. Frequencies used were 200, 300, 400, 500 and 700 Hz. Projecting duration was 100 ms and sound amplitude ranged from 40 to 90 dB.

Pre-larvae did not respond to the audio-stimuli even at the maximum amplitude by our apparatus. After metamorphosis from pre-laval to larval stage, however, the startle response appeared and was found to depend on the amplitude of the stimulus. The threshold was between 55 and 60 dB at the examined frequencies. Although the response was remarkable at the larval stage, this gradually became less marked in larger fish above 18 cm in body length. These behavioral changes to audio-stimulation may indicate a change in adaptability in order to avoid predation.

No distinct effect of frequency of sound stimulus to the startle response was observed in the present experiments.

It has been confirmed that fish are able to respond sensitively to sound stimuli. The red sea bream also responds to pure tones and the occurrence of startle behavior depends on the sound quality, which includes the amplitude of the sound and its frequency spectrum (ISHIOKA *et al.* 1985).

It would be interesting to know how such behavior caused by audio-stimulation of these fish has developed, since this information would contribute to the evaluation of the effect on fishery production of audio-stimulation generated by construction or underwater explosions. Despite such interest in the relationship between developmental stage and the behavioral changes caused by audio-stimulation, the earlier literature is very fragmented. In the herring, BLAXTER *et al.* (1981) described that the appearance of a startle response to noise was closely assciated with the development of the bullae-swimbladder system in late-stage larvae.

In the present study, the startle response to audio-stimulation was examined at each developmental stage of the red sea bream.

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Materials and Methods

Larval red sea bream supplied for the experiment was hatched in April, 1982 at our laboratory and reared for a few months under ordinary culture conditions. Larger fish were obtained from a fisheries farm, Nomi Suisan Co., Hiroshima, one month before the experiment and were maintained in a 1-ton experimental tank.

Larvae with a body length of less than 10 mm were kept in a plankton-net cage in order to facilitate easier observation of the response at precise position. The cage was placed at a distance of 5 cm from the sound source in an 800 l FRP experimental tank. These fish were kept in the cage for one day before the experiment and all fish were sacrificed for body size measurement soon after the experiment.

Fish larger than 150 mm in body length were kept in the experimental tank reported previously (ISHIOKA *et al.* 1985) and the response of the fish was observed at a position marked 5 cm from the sound source, when the fish were exposed to pulses of sinusoidal sound generated in the tank through an underwater speaker. Larger fish were repeatedly supplied for experiments, since it was difficult for the fish to become accustomed to the experimental conditions in only a few week. Details of the respective experimental conditions are indicated in Table 1.

Table 1. Size of the fish and experimental conditions

	Period	No. of Exps.	No. of fish examined	Body length (cm) mean ± S. D.	Range of Temperature (°C)	Remarks
I	May 25—31	9	132	0.32 ± 0.019	17.9—19.9	Hatched May 19～June 5
II	June 9—16	12	216	0.59 ± 0.069	18.7—20.0	Hatched May 19～May 25
III	July 20—21	10	98	3.34 ± 0.34	21.4—23.5	Hatched May 19～May 25
IV	July 27—Aug. 2	6	10	18.3 ± 1.34	22.2—25.2	1 years old
V	July 27—Aug. 4	5	10	23.6 ± 1.96	22.2—24.6	2 years old
VI	Aug. 9—8	10	10	33.5 ± 1.58	23.3—25.8	3 years old

The sound projection system was the same as that described in the previous paper (ISHIOKA *et al.* 1985) except for the underwater speaker. This was a type-FC-123 speaker (SHIMADA SCI. IND. CO.) which was able to produce stable sound pressure between 0.15 and 4.5 KHz of frequency. Maximum power input and maximum depth usable was 30W on pulse wave and 50 m, respectively.

The behavioral response observed was the startle response of the fish which were exposed to the intended pulse at a precise position, where the sound pressure had been predetermined.

A range of frequencies between 200 and 700 Hz was used and the projecting time was 100 ms for each sound pressure and frequency.

The rate of startle response was expressed as:

(Number of experiments in which a fish startle reflex was observed)/(Total number of experiments) × 100 % for each audio-stimulus.

Results

Experimental results are shown in Table 1 and in Fig. 1. Fish 3.2 mm in body length did not respond to the sound stimuli at a range of sound pressure between 50 and 80 dB at each frequency examined. Above 80 dB, only a few larvae moved in random directions soon after sound projection.

Larvae with a body length of 5.9 mm were sensitive to audio-stimulation. The behavioral changes observed involved swimming speed and rapid turning. The behavioral change of a single larva was quickly reflected in the entire group and all fish responded simultaneously to the audiosignal. The behavioral change was observed at a range of sound pressure between 55 dB and 60 dB at the frequencies examined.

Larvae 33 mm in body length responded sensitively to sound stimuli above 40 dB, although the variation in the response of each individual was greater at this stage.

In the experiment using fish 183 mm and 236 mm in body length, the rate of occurrence of startle behavior increased with increase of sound pressure at each frequency.

No distinct relationship between the rate of occurrence of startle response and sound amplitude was observed in the fish 335 mm in body length at any of the frequencies examined and at this stage, the activity of the fish in responding to the stimuli was very low.

The effect of frequency of audio-signal to fish behavior was not so distinct in the present experiment.

Discussion

In order to facilitate on understanding of the relationship between behavioral response and sound stimuli in life history, the 50% level of sound amplitude for which behavioral change was observed was estimated by logit analysis based on the data of cumulative rate of response calculated by Behrend's method. The result is shown in Table 2 and the development of the startle response in the fish exposed to a sound of 200 Hz is illustrated in Fig. 2. In this figure, the data for fish 85 mm in body length were

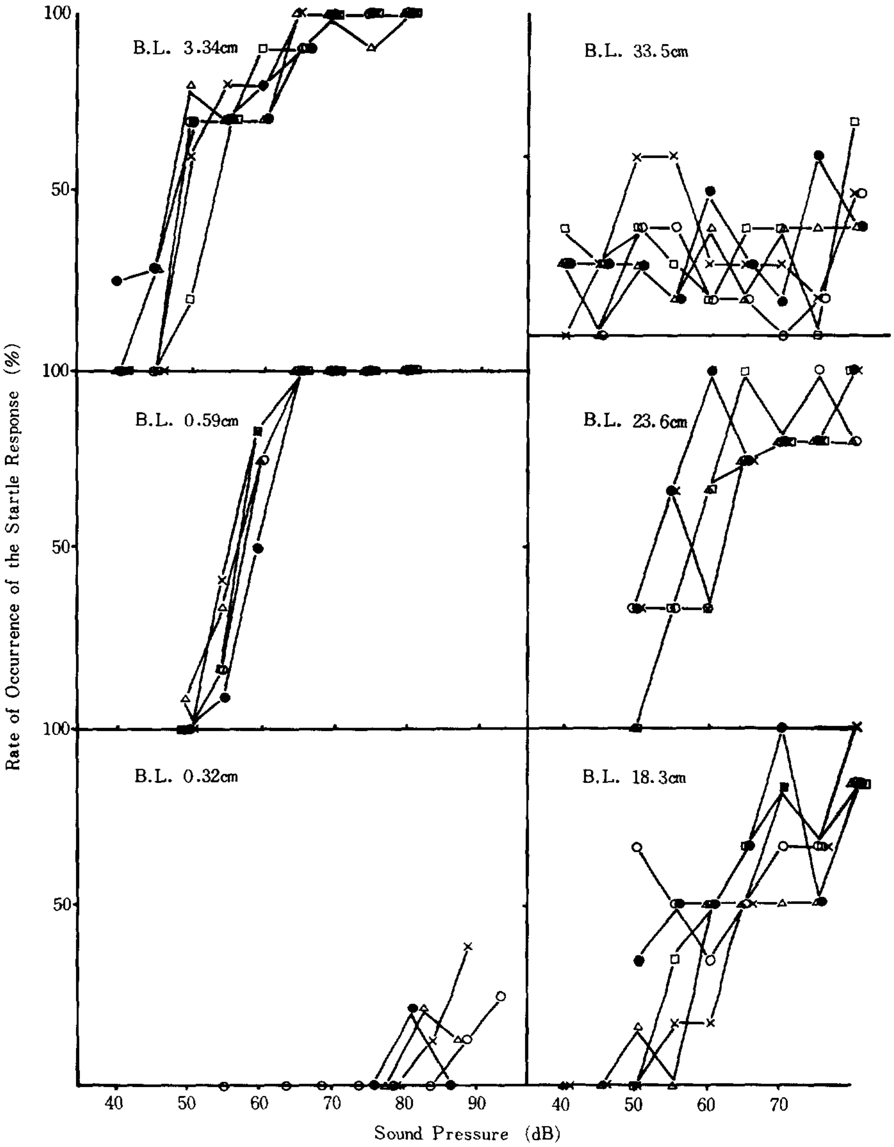

Fig. 1 The relationship between the rate of occurrence of the startle response and sound amplitude.

○: 200 Hz ×: 300 Hz △: 400 Hz ●: 500 Hz □: 700 Hz

Table 2. 50% point of sound pressure with 95% reliable range (dB) estimated by logit analysis on the basis of cumulative rate of occurrence of startle response by Behrend.

Body length (cm)	Frequency (Hz) 200	300	400	500	700
0.32	85.9≦105.1≦128.5	82.0≦96.0≦112.4	81.0≦98.7≦120.2	77.3≦105.1≦143.0	—
0.59	54.3≦57.8≦61.4	53.3≦56.6≦60.1	53.2≦56.5≦60.1	55.6≦59.4≦63.5	53.9≦57.4≦61.1
3.34	48.7≦51.2≦54.0	48.1≦51.4≦55.0	48.4≦51.0≦53.8	47.4≦50.0≦52.0	51.3≦54.1≦57.0
18.3	55.6≦58.8≦62.2	62.4≦65.6≦69.0	64.6≦68.0≦71.6	55.6≦58.7≦62.1	56.4≦59.7≦63.1
23.6	57.1≦61.1≦65.4	55.8≦59.7≦64.0	53.7≦58.7≦64.2	53.0≦57.5≦62.4	53.5≦57.8≦62.4
33.5	69.1≦75.0≦81.5	66.9≦71.1≦75.5	69.6≦74.6≦80.1	67.1≦71.5≦76.2	66.3≦71.1≦76.3

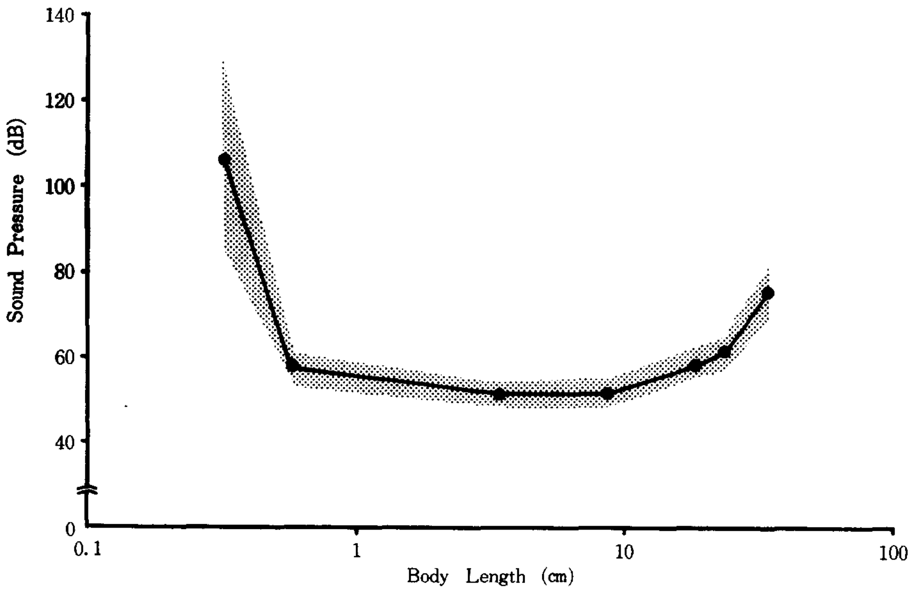

Fig. 2 Change of 50% in the level of the occurrence of the startle response in sound amplitude in the life history of red sea bream.

calculated using experimental data obtained in 1981 (Ishioka *et al.* 1985).

As the audio-stimulation is received by the inner ear and lateral organ, it is important that the relationship between the appearance of the startle response to audio-stimulation and the development of receptive organs is clarified. The developmental stage of fish with a body length of 3.2 mm is a pre-larva with a fin-fold and in which the yolk in the sack is mostly absorbed. As Okamoto *et al.* (1982) reported, they are still unable move actively, although they are distributed in a normal manner over the upper surface of the water, and the auditory vesicle has developed in a canal-like structure. Also, the air-bladder which will share the auditory function does not contain air. There are 8-13 pairs of free neuromasts, which will subsequently develop into the lateral organ, in upper orbit, otocyst and trunk extruding the cupula from the surface of the fin-fold.

These morphological studies strongly support the experimental results suggesting that the pre-larva is not able to hear and respond to audio-stimulation because both the receptor of the stimulus and the muscle structures effecting the response are undeveloped.

Larvae 5.9 mm in body length reared for 20 days after hatching are strongly sensitive to audio-stimulation and all respond simultaneously. By this stage, the auditory organ has developed. The air-bladder contains air, the pneumatic duct is closed, and on the lateral organ, increased numbers of neuromasts are distributed in a complicated fashion in the head region and in each myotome of the trunk. With these morphological changes, behavioral activity is remarkably increased in response to the motion of other fish and prey. These typical forms of autonomous behaviors of the fish make observation of response to stimuli easy and indicate the development of muscular and sensory organ function. The remarkable response ocurring in unison may depend on both the development of audiofunction and large numbers of free neuromasts that respond to every stimulus from any direction.

Recently, BLAXTER *et al.* (1981), and ALLEN and BLAXTER (1976) reported in detail the development of the auditory system of herring larvae and described that the appearance of the startle response was associated with the introduction of gas into the auditory bullae from the gut. In their paper, they regarded the pneumatic duct as playing an important role in gas introduction. These data resemble the results of our present experiment in that the startle response was clear after the closure of the pneumatic duct.

Fish 33 mm in body length are typical larvae and with regard to the lateral orgen, a canal-structure is formed in the trunk region and a few free neuromasts remain in the head region. The response to audio-stimuli is more sensitive than in the former stage, although differences in indidual response become larger.

Fish reared for 3 months after hatching, which are about 80 mm in body length, have perfectly formed adultlike audio-receptor organ structures. Ecologically, the fish feeds actively and the habitat expands vertically and horizontally. At this stage the behavioral response is very sensitive to audio-stimulation, although differences in behavioral response in each experiment increase. The fish begin to attain the ability to distinguish the direction of audio-stimulation, which makes them less vulnerable to predation.

In larger fish the occurrence of the startle response was observed to become gradually reduced as shown in Fig. 2. It is supposed that this does not indicate a decline in the hearing ability of the fish, but a change in the behavioral response to audio-stimulation. The fish lives in schools in the larval stage and rseponds in unison to stimuli, which may be advantageous for the survival of the fish, while the startle response may have no advantage for survival in the adult stage, when the fish has a solitary mode of life.

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音刺激に対するマダイの驚愕行動の発達

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マダイの発育段階毎の音刺激に対する驚愕行動を調べた。用いた音刺激は電気的に発生させ，これを水中スピーカーによって放声し，定位置におけるマダイの驚愕行動を観察した。音刺激は周波数200, 300, 400, 500, 700 Hz，放声時間は 100 ms の純音で，音圧は 40 から 90 dB の間で変化させた。

前期仔魚期には最高音圧でも全く反応がみられなかったが，稚魚期に入ると一斉に反応を起し，反応を起こす最低音圧は 55 から 60 dB の範囲にあった。稚魚期から若魚期にかけて，この反応は個体差は大きくなるものの明瞭であるが，体長18cm以降では見かけ上，反応が起りにくくなる。これは生態に合せた反応の在り方の変化と考えられた。本実験範囲では周波数の反応惹起に対する効果は明らかではなかった。