Studies on Burying Ability in Sand and Selection to the Grain Size for Hatchery-reared Marbled Sole and Japanese Flounder

Minoru Tanda^{*1}

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The burying ability and grain size selection of hatchery-reared marbled sole and Japanese flounder juveniles were studied using sand of various grain sizes.

Sand obtained from fishing areas were categorized into six grain-sizes: ~0.125 mm, 0.125~0.25 mm, 0.25~0.5 mm, 0.50~1.0 mm, 1.0~2.0 mm and 2.0~2.83 mm. The buried area was visually measured. Marbled sole juveniles from all three size groups (The means of the total lengths were 35.0, 58.7 and 80.6 mm.) were able to bury themselves in sand consisting of grains smaller than 1 mm in diameter. However, in sand with a diameter larger than 1 mm, only the 80.6 mm group was able to bury themselves. Thus, the burying ability increased with growth.

Sands of two different grain sizes were laid on both halves of the bottom of the container. Marbled sole (35.0 mm TL) and flounder (55.7 mm TL) preferred medium grain sizes, 0.125~0.5 mm in diameter. A positive correlation between the preference index and the buried area was found in both species. It is suggested that the juveniles preferred the type of sand in which they can bury themselves most easily.

The marbled sole *Limanda yokohamae* and the Japanese flounder *Paralichthys olivaceus* are distributed in waters around Japan and both species are commercially important. The juveniles are mass-produced for enhancement of stock and are released into the sea. These juvenile fish must be released at an optimum time and place to give them the best chance of survival and recruitment to the adult stock. However, mainly due to lack of fundamental information on behavior of the juveniles, this has not yet been fully realized.

Among the factors concerning successful release, the condition of the seabed is particularly important for flatfishes. Although we can find various papers on the ecology of these two species,¹⁻³⁾ ^{*2-4} substratum preference remains poorly understood. The main purpose of this study is

to examine the burying ability in sand and preference for grain size in marbled sole and Japanese flounder juveniles.

Materials and Methods

Materials

1. Fish: We obtained marbled sole juveniles, averaging 21 mm in total length, from Hyogo Prefectural Mariculture Center. The rearing period started on March 3 and ended on September 27, 1988. They were reared in a 1-ton polycarbonate tank ($\phi 138$ cm $\times$ 82 cm depth) and fed on *Artemia* sp. and pellets. Sand was not used on the bottom during the preliminary rearing. The flounder juveniles were hatched and reared in Kobe Municipal Mariculture Center. They were

^{*1} Hyogo Prefectural Fisheries Experimental Station, Akashi, Hyogo 673, Japan (反田 實: 兵庫県立水産試験場).

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Table 1. Grain size divisions of sand

Grain size (mm)
~0.125
0.125~0.250
0.250~0.500
0.500~1.00
1.00 ~2.00
2.00 ~2.83

transferred to the laboratory on July 6, 1988 and were reared until September 27, 1988. The rearing conditions were the same as for the marbled sole.

2. Sand: Sand, collected from the fishing area, was passed through a sieve to obtain 6 different sizes (Table 1) using sieves in conformity with JIS (Japanese Industrial Standards). Sixty three percent of the total weight of the ~0.125 mm category consisted of granules smaller than 0.063 mm. The sand was washed in fresh water and dried at 130°C twice. The size categories of ~0.125 mm and 0.125~0.25 mm appeared grayish and the others brownish in color but they were all free of odor. In a preliminary experiment on marbled sole two different colors of sand (whitish and brownish) with 0.25~0.5 mm grain size were used. There was no significant difference in the results obtained when using sand of two colors (Table 2). Therefore, it was assumed that there was no significant influence on the preference index when using sand of different colors.

Methods

1. Degree of concealment: This experiment was performed only with the marbled sole, since the same experiment had been performed with the flounder.⁴⁾ The marbled soles were divided into three size classes according to total length: 35.0 mm (S.D.=4.3 mm, n=73), 58.7 mm (S.D.=4.2 mm, n=24) and 80.6 mm (S.D.=5.6 mm, n=21). Sand was laid on the bottom of 10 l plastic containers (36 cm long, 23 cm wide and 13 cm depth), to a thickness of 1~2 cm. Three to ten juveniles were transferred from the 1-ton stock tank to each container. The degree of concealment was measured visually one hour after transfer. This was defined as a percentage of buried area to the whole area of the ocular side of a juvenile and was measured to the nearest 10%. The experiment was repeated 5~8 times with each grain size for each size group. The experimental tanks were placed in the laboratory under 150~1500 lx at the water surface. Water temperatures were not controlled

and ranged between 11.0~25.0°C.

2. Preference for grain size: The six grain-size divisions which were used in the size preference experiments were the same as for the burying experiment. Five 10 l plastic containers (same size as in the burying experiments) were placed in the laboratory. Sand of 0.25~0.5 mm grain size was laid on half the area of the bottom of each container in a 1~2 cm layer. The preference for this grain size was compared with that for other grain sizes. It was found, after the preliminary observation, that medium size sand of 0.25~0.5 mm was the most attractive. The other halves of the containers were covered with sand other than the medium size. Six marbled sole juveniles (four flounder) were transferred to each container, three fish on each grain size. The number of fish located on each substratum were counted 2 hours after the transfer of juveniles. The experiment was repeated 10~21 times for each combination. The light intensity was 200~1500 lx at the water surface, and to reduce the light-intensity effects the orientation of the container was alternately turned 180° for every observation. The 35.0 mm size group was used for this experiment. Water temperatures were not controlled and fluctuated between 11.1~11.9°C. A similar experiment was also performed for flounder juveniles averaging 55.7 mm (S.D.=4.5 mm, n=34) in total length.

In another experiment for the preference of grain size, the right and left halves of the container bottoms (60 cm long, 40 cm wide, 20 cm depth) were covered with two different grain sizes of sand, 0.25~0.5 mm and 1~2 mm. Two groups consisting of six individuals of marbled sole and flounder were used for the first experiment, and ten juveniles for the second experiment. Half of the individuals of each species were carefully transferred to one side and the others to the opposite side of the container. The number of juveniles located on each side was counted 2 or 3 hours after transfer. The first and second experiments consisted of 21 and 16 observations, respectively. The size of juveniles used in the experiments were 70.7 mm TL (S.D.=3.6 mm, n=25) with marbled sole and 74.3 mm TL (S.D.=5.3 mm, n=22) with flounder. Juveniles of both species treated here did not show any apparent aggressive or exclusive behavior.

Fig. 1. Change in time of buried area of marbled sole juveniles averaging 35.0 mm TL. 0.25~0.5 mm grain size sand was used. The experiment was repeated three times. Vertical bars represent standard deviation.

Results

Degree of Concealment

The time-related changes in buried area of 35 mm-size marbled sole juveniles, examined under 0.25~0.5 mm grain size, are shown in Fig. 1. A majority of the juveniles buried their body into the sand immediately after transfer to the container. The average buried area was 53% 30 min. after transfer, but decreased gradually with time: 47% (2 hrs), 39% (4 hrs) and 14% (8 hrs). This change with time differed from that of flounder juveniles,⁴⁾ in which the degree of concealment reached a maximum three or four hours after transfer with gradual decreases later. From this result, we decided to measure the degree one hour after transfer in the subsequent experiments.

The relation between the buried area and grain size is shown in Fig. 2. The buried area for grain

Fig. 2. Relationship between grain size and buried area in three size-group marbled sole juveniles: 35.0 mm, 58.7 mm and 80.6 mm TL. Vertical bars represent standard deviation.

sizes of 0.125~0.25 mm and 0.25~0.5 mm indicated the maximum value among all divisions in every size-group. Although the buried area for ~0.125 mm grain size was low in 35.0 mm and 58.7 mm size-groups, the general trend exhibited was a negative relationship between the buried area and grain size in the three size-groups. A positive

Table 2. Preference for grain size of sand in marbled sole juveniles averaging 35.0 mm TL. Number of juveniles located in the two divisions was examined by χ^2 test. Expected ratio is 50:50.

Combination of grain sizes (mm)	Total number examined	Number located in		Statistical significance (P)
		0.25~0.5 mm	Other size	
0.25~0.5 vs. ~0.125	125	80 (64)* ¹	45 (36)	0.01
0.25~0.5 vs. 0.125~0.250	100	51 (51)	49 (49)	ns
0.25~0.5 vs. 0.500~1.00	106	66 (62)	40 (38)	0.05
0.25~0.5 vs. 1.00~2.00	74	67 (91)	7 (9)	0.01
0.25~0.5 vs. 2.00~2.83	59	51 (86)	8 (14)	0.01
0.25~0.5 vs. 0.25~0.5* ²	122	59 (48)	63 (52)	ns
(whitish) (brownish)				

*¹ Figures in parentheses show percentage.

*² Result in a preliminary experiment (different sand color).

Table 3. Preference for grain size of sand in flounder juveniles averaging 55.7 mm TL. Number of juveniles located in the two divisions was examined by χ^2 test. Expected ratio is 50:50.

Combination of grain sizes (mm)	Total number examined	Number located in		Statistical significance (P)
		0.25~0.5 mm	Other size	
0.25~0.5 vs. ~0.125	79	56 (71)*	23 (29)	0.01
0.25~0.5 vs. 0.125~0.250	79	39 (49)	40 (51)	ns
0.25~0.5 vs. 0.500~1.00	82	47 (57)	35 (43)	ns
0.25~0.5 vs. 1.00 ~2.00	81	57 (70)	24 (30)	0.01
0.25~0.5 vs. 2.00 ~2.83	82	69 (84)	13 (16)	0.01

* Figures in parentheses show percentage.

Table 4. Preference for grain size of sand when the marbled sole (70.7 mm TL) and flounder (74.3 mm TL) were put together in an experimental container. In case A 6 juveniles of each species were put into the container, and in B 10 juveniles each. If the data for both case A and B experiments are combined in a Mantel-Haenszel test, the result is significant ($p=0.021$).

	Grain size (mm)		Total number
	0.25~0.5	1.0~2.0	
Marbled sole	88	38	126
Flounder	77	49	126
Total number	165	87	252

	Grain size (mm)		Total number
	0.25~0.5	1.0~2.0	
Marbled sole	117	43	160
Flounder	101	59	160
Total number	218	102	320

relationship between the buried area and fish size was recognized. In the largest size group of 80.6 mm TL, the buried areas of more than 40% were found in 1~2 mm grain size in contrast to nearly 0% in the other two size-groups. These facts show that the burying ability becomes more developed with growth.

Preference for Grain Size

1. Marbled sole: The results obtained in this experiment for preference of marbled sole for grain size are summarized in Table 2. Significant differences of 1% level by χ^2 test were recognized in the combinations of the 0.25~0.5 mm and ~0.125 mm, 1~2 mm, 2~2.83 mm. The difference was significant at the 5% level in the 0.25~0.5 mm and 0.5~1 mm combination. Mean-

while, there was no significant difference in the 0.25~0.5 mm and 0.125~0.25 mm combination.

2. Japanese flounder: The results obtained in this experiment for preference of flounder for grain size are summarized in Table 3. Significant differences from an expected ratio of 50:50 were observed with the combinations of 0.25~0.5 mm with ~0.125 mm, 1~2 mm, 2~2.83 mm (χ^2 test, $p<0.01$). No significant differences were found with the combinations of 0.25~0.5 mm with 0.125~0.25 mm, 0.5~1 mm.

3. Marbled sole and flounder: The results for this experiment, which aimed to examine the species-specific difference between the two species are shown in Table 4. Experiments with 6 juveniles (case A) and 10 juveniles (case B) of each species both show a slight preference of marbled sole for the finer sand (0.25~0.5 mm in grain size). This difference between the two species was significant (Mantel-Haenszel test, $p=0.021$).

Discussion

In the present experimental conditions, 35 mm-size and 58.7 mm-size marbled sole juveniles were able to bury themselves in sand of less than 1 mm in diameter and, except for less than ~0.125 mm grain size, a negative relationship between the buried area and grain size was found. A similar tendency was observed in Japanese flounder juveniles.⁴⁾ The increase in burying ability with growth was inferred from the experimental result that the degree of concealment was higher in larger-size groups, and that the largest group of 80.6 mm TL was able to bury themselves even in 1~2 mm sand. This trend seems to show an enlargement of habitat of juvenile marbled soles as they grow. From the tendency above, mentioned juveniles should be released at an appropriate place for their size-related burying abilities.

In the Seto Inland Sea, juvenile and immature

Fig. 3. Relationship between buried area and preference index in marbled sole (solid circle) and flounder juveniles (open circle). These figures were obtained from results cited in Fig. 2, Table 2, Table 3 and the buried area of flounder juveniles obtained in a previous experiment.⁴⁾

marbled soles are more abundant in areas covered with coarse and medium sands than in areas with fine sand and silt in Suo-nada.^{*1} Meanwhile, juveniles are observed most frequently in fine sand areas during the period from April through June in the coastal areas of Okayama Prefecture.^{*2} In the northern part of Japan, many of the juveniles inhabit a sandy area, most of which is composed of grains smaller than 0.15 mm, in Mangoku inlet,^{*3} whereas the juveniles have been found to be distributed in a silty area of Sendai Bay.^{*4} There seems to be considerable discrepancies of substratum types for juvenile marbled sole habitats, according to locality. Some of the discrepancies may be caused by the size or growth differences between locations. In connection with the relationship between the grain size and juvenile distribution in flounder, Ishida *et al.*⁵⁾ reported that the juveniles are most abundantly distributed in fine sand ($\phi=0.17$ mm) and are scarce on both coarse ($\phi=1.0$ mm) and very fine ($\phi=0.09$ mm) sand areas.

Kosaka⁶⁾ demonstrated that young marbled soles settled more easily on sand than on gravel substratum. This trend was observed more clearly in marbled sole than in stone flounder,

Kareius bicoloratus under experimental conditions. The results also support the possibility that substratum grain size may be an important factor in habitat selection. In the present experiments marbled sole and flounder juveniles clearly exhibited preferences related to sand grain size. The relationships between preference index and buried area in both species are shown in Fig. 3. The positive correlations found in both species suggest that those juveniles prefer sand in which they can bury themselves more easily.

Yasunaga and Koshiishi⁷⁾ showed that Japanese flounder juveniles are less vulnerable to predation by black sea bream *Acanthopagrus schlegelii*, when the bottom of the tank was covered with sand. This means that burying behavior is ecologically important in minimizing predation risk. Therefore it was concluded that the substratum was a very important factor for survival of flatfish juveniles in relation to their burying ability.

The tendency of marbled soles to choose sand-grain smaller than those preferred by flounders was demonstrated in the present experiment. The difference was small but statistically significant. As previously cited, the marbled sole exhibits a stronger preference for sand than gravel substrata as compared with stone flounder, under the same experimental conditions.⁶⁾ In his reviews for the early life history of pleuronectiforms, Minami⁸⁾ shows species-specific nursery habitats. This habitat segregation may be attributed to the physical conditions of the seabed, such as the grain size of the substratum.

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