Influence of Displacement Behavior on Nitrogen Excretion by Starved Flounder *Paralichthys olivaceus*

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Key words: displacement behavior, nitrogen excretion, starved flounder

Abstract

It is well known that the group effect of fish has an influence on the metabolic physiology by solitary or group formation in different ecological conditions (Itazawa 1991)[1]. However, most of the group effect is on the free living processes. Honda (1988)[2] found that the overlapping behavior of the flounder, observed when the bottom of aquarium is not covered with sand, is one of displacement behavior for the natural behavior of burrowing themselves into the sand. From the viewpoint of overlapping behavior, this displacement behavior of flounder is considered as a kind of group effect. Accordingly, the displacement behavior grasp the action for bottom area of culture tank. The purpose of this paper is to obtain the rate of fish occupation estimated by the proportions of half body surface area of flounder for the bottom area of the tank, and is to present the effects of displacement behavior on the rate of nitrogen excretion in the cultured japanese flounder.

Materials and Methods

Experiments on nitrogen excretion of Japanese flounder were carried out in flounder culture tanks (Yamashita Fisher Co.) in Azuma-cho, Kagoshima, Japan. The octagonal, indoor tank with a circulation system was 33.7 m² in area and about 0.5m deep, and was constructed from glass fiber reinforced polyester with a number of fish fed on pelleted artificial flounder feed (Higashimaru Food Inc.) in a group during more than two months acclimation period in before experimentation was 750 to 5,600 individuals. Each experimental fish, after 48 hours starvation for young fish (14 to 59 g) and 72 hours starvation for adult fish (160 to 633 g), was placed in a square polyethylene tank which was 0.282 m² in bottom area and 0.38 m deep with flowing water system. The light intensity in surface of the indoor tank was reduced to about 0.5% of an open field. The rate of water exchange was about one tank volume 100 L per hour, so the retention time of culture water was about one hour. Dissolved oxygen content of the culture water was maintained by aeration at more than 5 mg per one litter.

Experiments were conducted at mean water temperature of 16±0.5℃, 19±0.5℃, and 23±0.5℃. The number and body weights of japanese flounder used in this experiments were 8 to 107 individuals ranging from 14 g to 24 g at 16℃, 4 to 80 individuals 53 g to 638 g at 19℃, and 2 to 18 individuals 160 g to 410 g at 23℃. As an indicator of the stocking density of flounder, the rate of fish occupation in half body surface area of fish for the bottom area of the culture tanks can be expressed as follows;

$$\beta = 100 \cdot S / B \qquad (1)$$

Where, β: rate of fish occupation, in percentage
  S : half body surface area of fish in m²
  B : bottom area of the culture tank in m²

Rate of fish occupation ranged from 15% (1.51 kg/m²) to 203% (22.8 kg/m²) in the experiments at 16℃, from 24%

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(1.51 kg/m²) to 237% (22.8 kg/m²) at 19℃, from 26% (2.93 kg/m²) to 206% (23.2 kg/m²) at 23℃. Metabolic rate of most animals is generally indicated by the rate of oxygen consumption. As a series of studies on the excretion rate of nitrogen of japanese flounder, the excretion rate of nitrogen (E_N) was estimated by summing the nitrogen excreted in the forms of ammonia (E_{NH4}) and urea (E_{urea}) nitrogen. It has been confirmed that the relationship between the excretion rate of nitrogen and body weight of fish can be shown in an exponential equation proposed by Jobling (1981)[3] and Hepher (1988)[4] as follows;

$$E_N = \alpha_\theta \cdot W^r \qquad (2)$$

Where, E_N: mean rate of nitrogen excretion in mg N/fish/h

α_θ: nitrogen excretion rate at water temperature θ℃

W: mean body weight of fish in wet g/fish

γ: constant

Therefore, α_θ can be computed by rewriting the equation using the value of $\gamma = 0.57$ obtained by Tanaka and Kadowaki (1995)[5] as follows;

$$\alpha_\theta = E_N / W^{0.57} \qquad (3)$$

Inlet and outlet water of fish cultured was sampled every hour for 24 hours. The sampling time of outflow was delayed to account for the retention time. Water samples were filtered through 0.45μm membrane filters and stored in frozen for chemical analyses. Total ammonia nitrogen analyses were performed using the indophenol method (Liddicoat et al, 1975)[6]. The concentration of urea nitrogen was determined using the method reported by Newell et al (1967)[7].

Results and Discussion

Experimental conditions, rates of nitrogen excretion by starved flounders, and the results are shown in **Table 1**. The nitrogen excreted in the forms of ammonia (E_{NH4}) and urea (E_{urea}) nitrogen range from 0.0253 to 0.0450 mg N / fish /h and 0.0032 to 0.0042 mg N/fish/h at 16℃, 0.126 to 0.644 mg N / fish/ h and 0.0137 to 0.214 mg N/fish/h at 19℃, 0.185 to 0.610 mg N/fish/h and 0.0574 to 0.217 mg N/fish/h at 23℃.

Diurnal changes in the nitrogen excretion rate at water temperature θ℃ (α_θ) for each rate of fish occupation are shown in **Figs. 1**, **2**, and **3**. Clear diurnal rhythm was not observed for α_θ in starved flounder. these results are similar to those reported by Kikuchi et al (1991)[8] who found that the photoperiod did not seem to affect the rates of nitrogen excretion in starved flounder.

The relationship between the nitrogen excretion rates (α_θ) at three different temperature and the rates of fish occupation are shown in **Fig. 4**. Higher values of α_θ are shown as water temperature becomes higher. The value of α_{23} (α_θ value at 23℃) is high in low and high stocking density corresponding to the rate of fish occupation (β) at 26% and 173% to 206%, but is low in middle stocking density corresponding to the values of β at 41% to 100%. The value of α_{19} (α_θ value at 19℃) is high in low stocking density corresponding to the rate of fish occupation is shown 24%, but is low in middle and high stocking density which the value of β is shown 44% to 237%. The reason why the value of α_θ becomes high when the value of β is less than 30% is a result of dissatisfied displacement behavior. This displacement behavior is considered a kind of group effect expressed psychological process pointed out by Itazawa (1991)[1] and Honda (1988)[2], working on the rate of oxygen consumptions per individual of one, two, three, and five flounder groups at 20℃, stated the positive group effect that the decrease of group formation conduces the increment of oxygen consumption per individual. These are consistent with the results obtained in this study.

It was considered that high rate of nitrogen excretion by The cultured flounder in more than 170% of β values at 23℃ was caused by restless behavior due to small living space per fish (Itazawa et al, 1978)[9]. Itazawa (1991)[1] and Imabayashi (1992)[10] working on juveniles and young of red sea bream concluded that this phenomenon was a negative group effect meaning that the metabolic rate per body weight of fish increases accordingly, as the number of cultured fish increases. Therefore, cultured flounder display a negative group effect under the conditions of high water temperature and stocking density.

When the water temperature fell to at 16℃, the rate of nitrogen excretion by cultured flounder declined suddenly. The values of α_{16} (α_θ values at 16℃) were low at 15% and 74% and high at 148% and 203% of β values. These results show that the small living space per fish rather than displacement behavior promotes increased metabolism of flounder at 16℃.

The relationship between the range of fluctuation in

Table 1. Experimental conditions and data on rates of nitrogen excretion and E_{NH4} / E_N by starved flounders.

Date	Water temperature (°C)	Body weight (g/fish)	Number of fish	Rate of fish occupation (%)	Stocking density (kg/m²)	E_{NH4}	E_{urea}	E_N	E_{NH4} / E_N	α_θ
						(mg N/fish per h)			(%)	
19 April,1993	16	24	8	15	0.686	0.0281	0.0036	0.0317	88.6	0.00518
		17	39	74	2.37	0.0253	0.0042	0.0295	85.8	0.00587
		14	78	148	3.90	0.0367	0.0032	0.0399	92.0	0.00887
		20	107	203	7.64	0.0450	0.0034	0.0484	93.0	0.00878
27-28 May,1993	19	59	20	64	4.21	0.126	0.0137	0.140	90.2	0.0137
		59	39	126	8.22	0.155	0.0194	0.174	88.9	0.0171
		59	60	193	12.6	0.129	0.0233	0.152	84.7	0.0149
		59	80	237	16.9	0.136	0.0310	0.167	81.4	0.0163
1-2 June,1992		53	8	24	1.51	0.207	0.116	0.323	64.1	0.0336
		53	15	44	2.84	0.162	0.0631	0.225	72.0	0.0234
		53	30	89	5.68	0.178	0.0331	0.211	84.3	0.0220
		633	4	62	9.04	0.644	0.214	0.858	75.1	0.0217
27-28 November,1992		468	6	79	10.0	0.486	0.154	0.640	75.9	0.0192
		491	13	180	22.8	0.486	0.121	0.607	80.1	0.0177
30-31 July,1992	23	160	6	41	3.43	0.214	0.0730	0.287	74.6	0.0159
		166	10	68	5.93	0.225	0.0654	0.290	77.5	0.0158
		162	14	88	8.10	0.185	0.0574	0.242	76.3	0.0133
15-16 October,1992		410	2	26	2.93	0.574	0.243	0.817	70.2	0.0265
		357	14	173	17.9	0.482	0.212	0.694	69.5	0.0243
		361	18	206	23.2	0.610	0.217	0.827	73.8	0.0288
12-13 October,1993		380	4	47	5.43	0.353	0.118	0.471	74.9	0.0159
		400	8	100	11.4	0.422	0.102	0.524	80.5	0.0172
		354	14	154	17.7	0.507	0.0820	0.589	86.1	0.0208
		350	18	196	22.5	0.531	0.156	0.687	77.3	0.0244
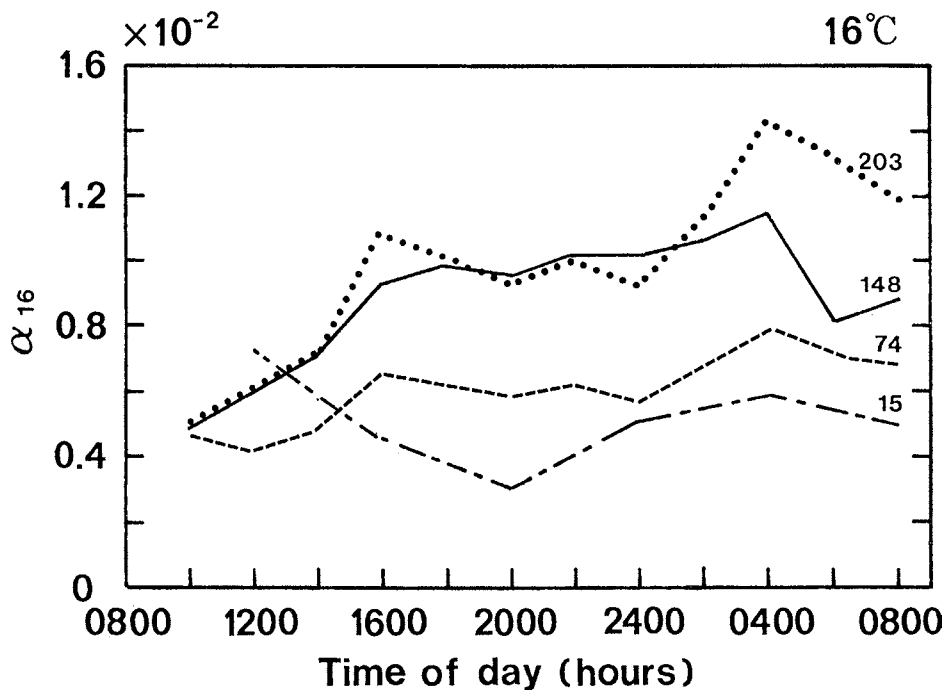
Fig. 1. Diuranal changes in the α_{16} value for cultured flounder at 16°C. Numeral show rate of fish occupation.

nitrogen excretion rates $(\alpha_{max} - \alpha_{min})$ at three different temperatures the values of β is shown in **Fig. 5**. The range of fluctuation in nitrogen excretion rates become wide and narrow at 23°C as the value of β become low and high, respectively. The range of nitrogen excretion rates becomes 0.016 to 0.032 at 19°C as the value of β becomes 44% to 24%, and becomes stable at less than 0.01 with β value greater than 80%. The range of fluctuation in nitrogen excretion rates becomes stable less than 0.01 at 16°C regardless of the value of β.

As shown in Table 1, the proportion of E_{NH4} to E_N by starved flounder amounts to 89.9% at 16°C, 79.7% at 19°C, and 76.1% at 23°C. The average is 81.9% for all temperatures. Hepper (1988)[4] has shown that the percentages of ammonia nitrogen to total nitrogen excreted by fed and starved rainbow trout were 84.7% to 92.5% and 69.8%, respectively. Jobling (1981)[3] found that the greatest excretion product of plaice was ammonia nitrogen which accounts for 75% to 85% of the
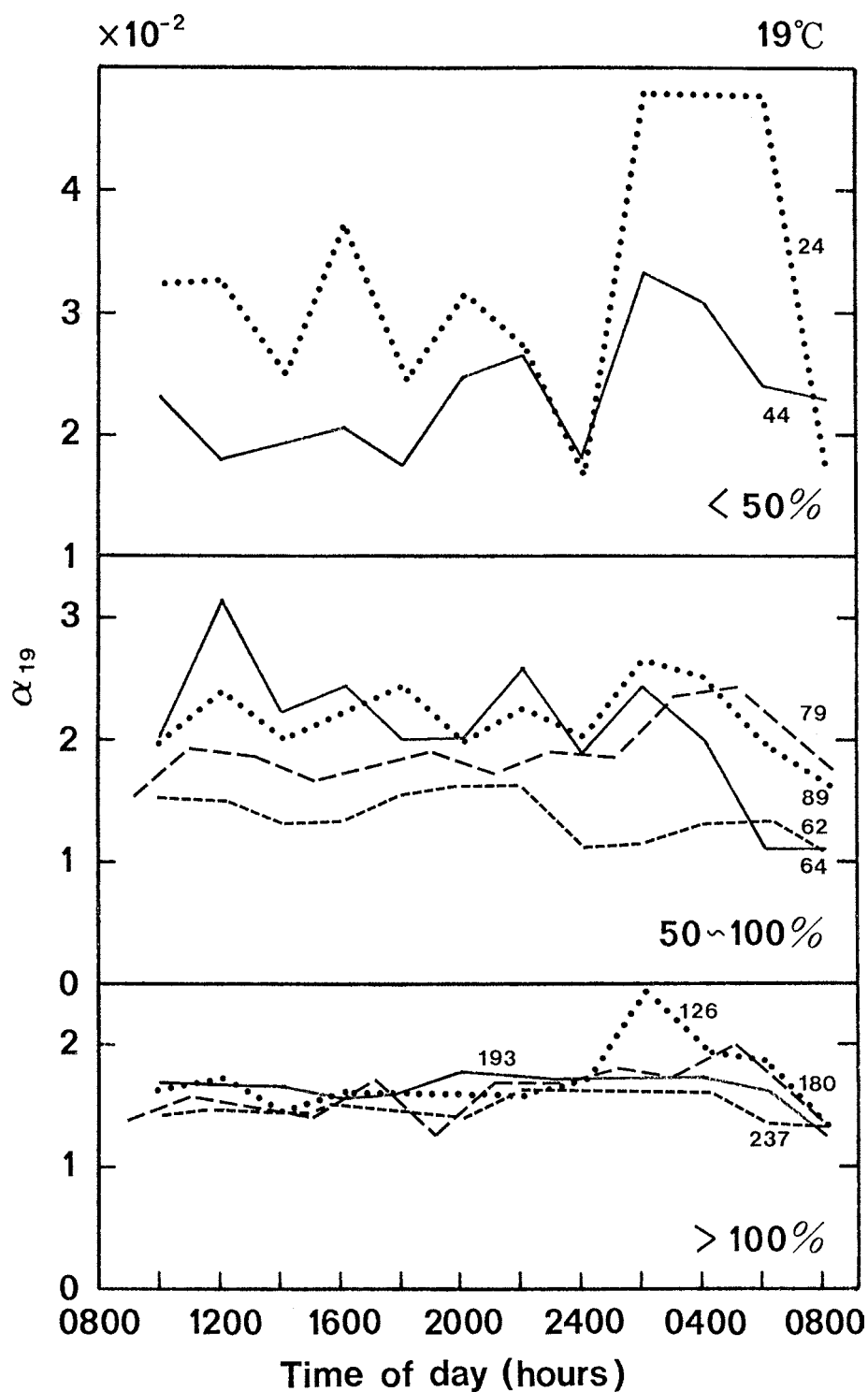
Fig. 2. Diuranal changes in the α_{19} value for cultured flounder at 19°C. Numeral show rate of fish occupation.

nitrogen excretion. Wood (1958)[11] working on nitrogen excretion of starry flounder, has shown that the proportion of E_{NH4} to E_N by solitary flounder amounts 81.4% to 86.2% at 12°C. The results obtained in this study are similar to previous studies.

Fig. 6 shows the relationship between ratio of excreted ammonia to total nitrogen and α_0. It has been proposed that there is relationship between the utilization of cultured water and the form of nitrogen excretion which shows a characteristic of adaptation for the environmental conditions
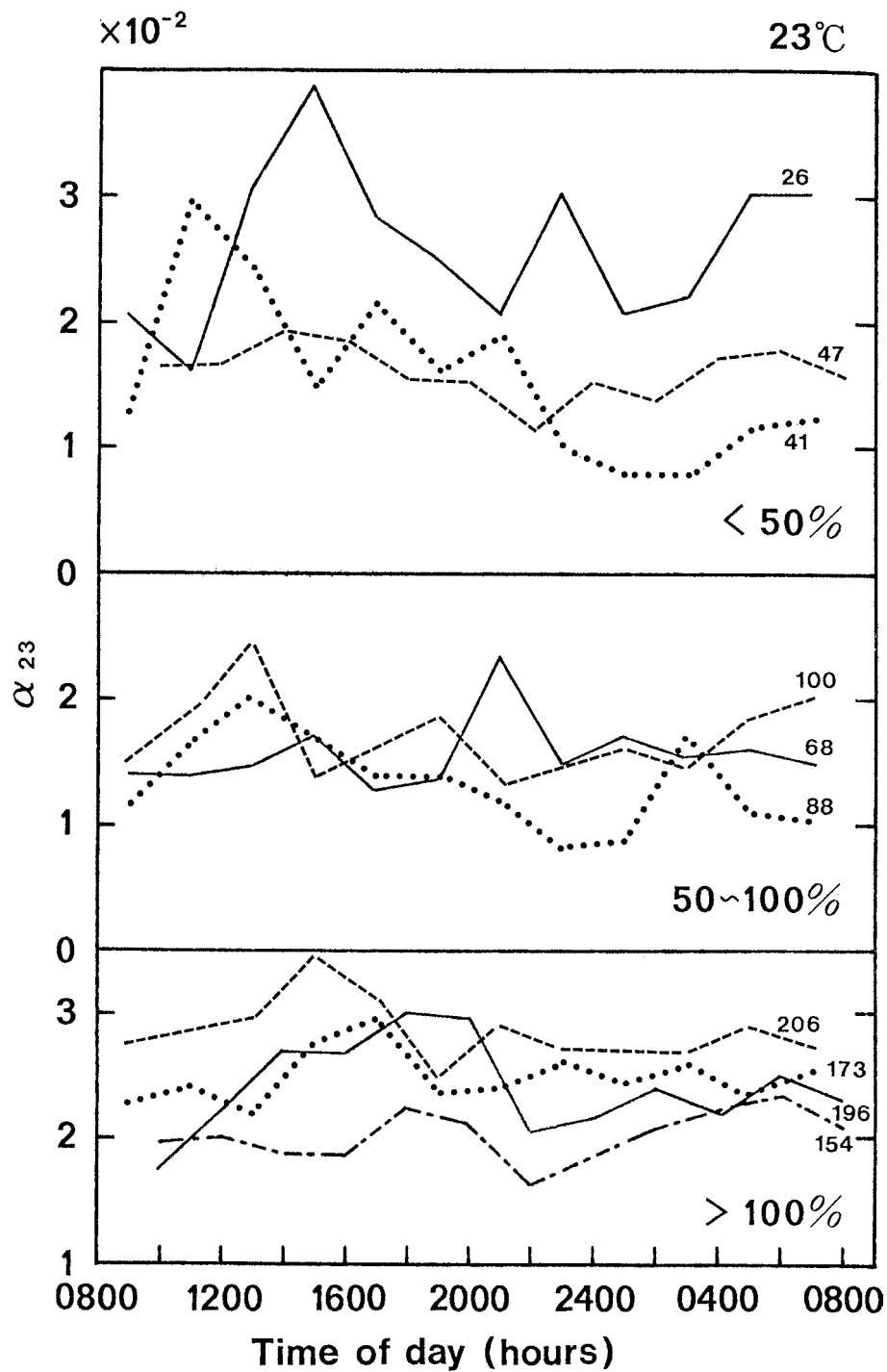
Fig. 3. Diuranal changes in the α_{23} value for cultured flounder at 23°C. Numeral show rate of fish occupation.

(Ozaki, 1977)[12]. Furukawa and Ogasawara (1955)[13] working on nitrogen excretion of starved carp and goldfish on oxygen deficiency, was shown that urea excretions which low toxicity for fish was higher than the ammonia excretion. Even to the optimum water temperature for flounder at 19°C to 23°C (Harada, 1981)[14], the ratio of urea nitrogen excretion for total nitrogen excretion tend to increase in less than 30% and more

than 170% of β values. These results show the value of β has an effect on the forms of nitrogen excretion of cultured flounder. The α_θ plotted of the right side X axis shows the basal metabolism of nitrogen excretion. and the values of α_θ were in the range 0.0050 to 0.0060 at 16°C.

On the other hand, at the same temperature of culture water, significant differences existed between the proportion of E_{NH4}
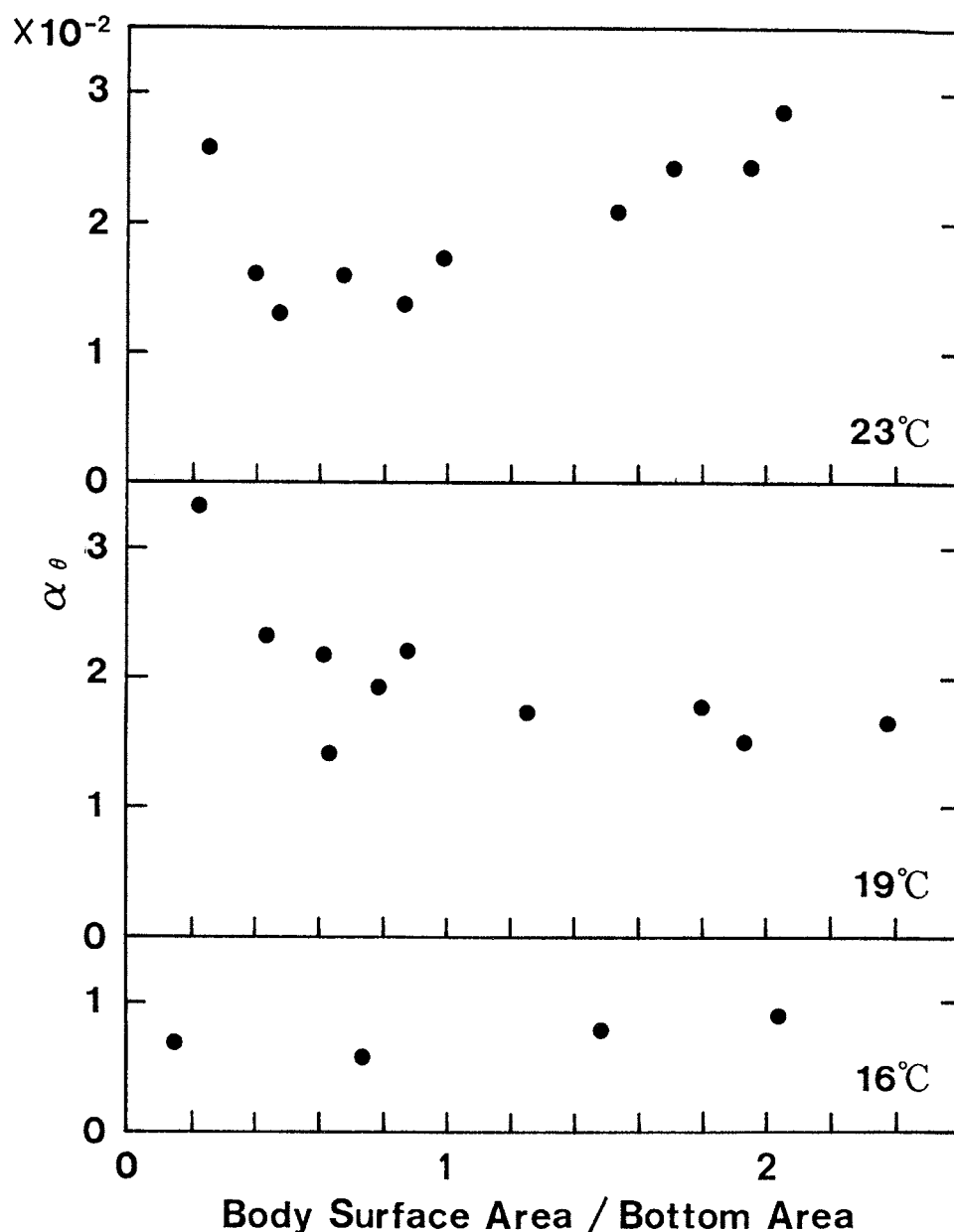
Fig. 4. Relationship between half body surface area per bottom area and α_θ values at 16°C, 19°C and 23°C.

to E_N at 74.8% in the wide range of α_θ fluctuation and 81.6% in the narrow range of fluctuation in α_θ, respectively. The cultured flounder at the condition of wide range fluctuation in α_θ increased the rate of urea nitrogen excretion as compared with the condition of narrow range α_θ fluctuation. Therefore, the proportion of E_{NH4} to E_N is considered a quantitative indicator that expresses the psychological phenomenon of fish as the physiological phenomenon.

At 19°C to 23°C, the value of β, when the values of α_θ are low and the range of fluctuation in α_θ is narrow, are from 44% (2.8 kg/m²) for body weight of 53 g to 154% (17.7 kg/m²) for

body weight of 354 g. These values are similar to the results reported by Harada (1981)[14] at optimum stocking density of 5 kg/m² to 15 kg/m² with 0.5 per one hour of water exchange at 20°C to 25°C. Nakamura et al (1986)[15] working on effects on different stocking densities on growth and survival of cultured flounder, has reported that 92% of β value (13 kg/m²) showed the best growth and survival rate for body weight of 180 g at 22°C to 26°C. Different water temperatures and exchange rates considered, it was suggested that the values of 50% to 150% of fish occupation rate obtained in this study are applied in the case of optimum stocking density from 19°C to 23°C .
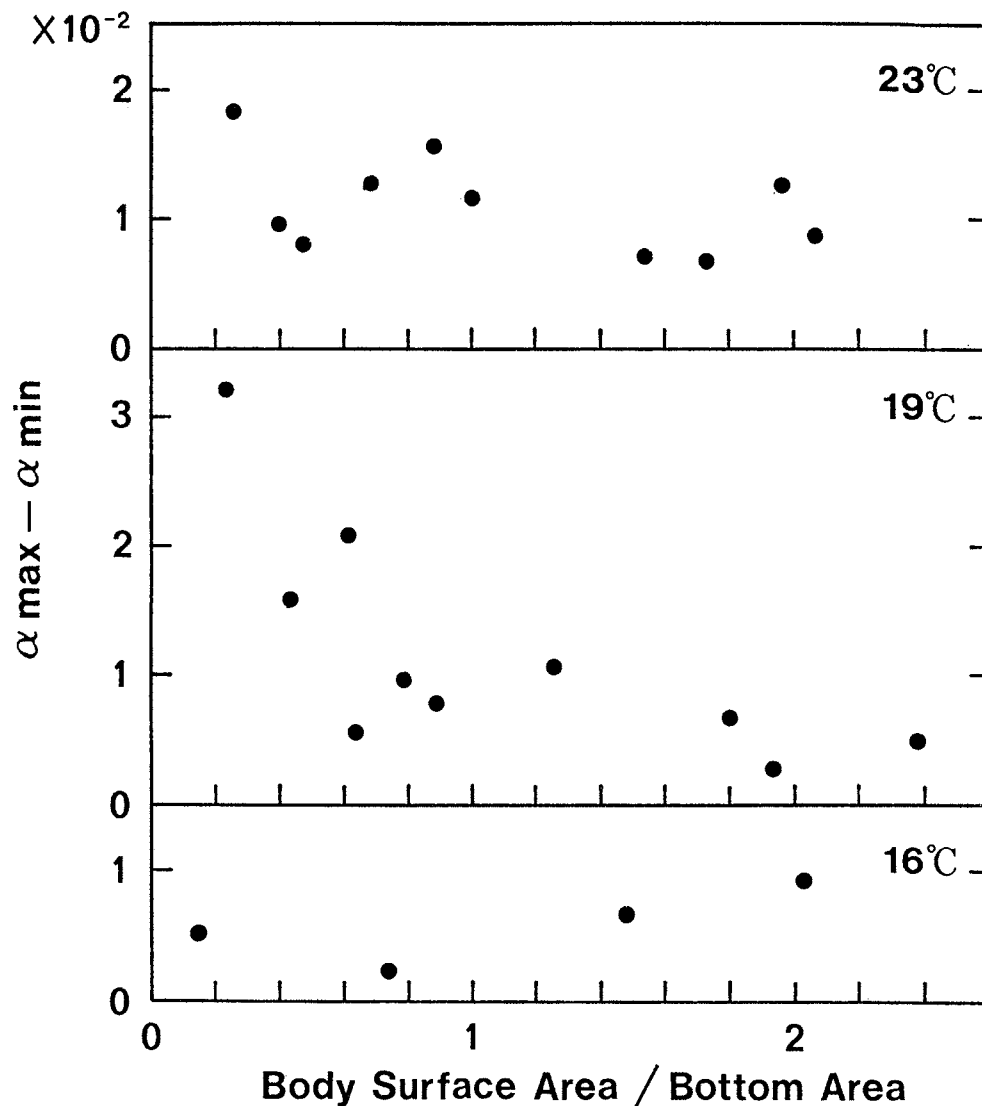
Fig. 5. Relationship between half body surface area per bottom area and $\alpha_{max} - \alpha_{min}$ values at 16°C, 19°C and 23°C.

Therefore, the behavior of grouped flounder overlapping each other instead of burrowing themselves into the sand is a type of compensation behavior to make the metabolism saving physiologically. It is necessary to secure the optimum stocking density for flounder cultured in a tank with no sandy bottom, because of compensating for displacement behavior.

Literature Cited

1) Itazawa Y. (1991). Respiration. Fish Physiology. (Itazawa Y. and I. Hanyu editors), Kouseishakouseikaku, Tokyo, Japan. (in Japanese).

2) Honda H. (1988). Displacement behavior of Japanese flounder *Paralichthys olivaceus* estimated by the difference of oxygen consumption rate. Nippon Suisan Gakkaishi, 54, 1259.

3) Jobling, M. (1981). Some effects of temperature, feeding and body weight on nitrogenous excretion in young plaice *Pleuronectes platessa* L. Journal of Fisheries Biology 18, 87–96.

4) Hepher, B. (1988). Nutrition of pond fishes. Cambridge University Press, New York, USA.

5) Tanaka Y. and S. Kadowaki. (1995). Kinetics of nitrogen excretion by cultured flounder *Paralichthys olivaceus*. Journal of the World Aquaculture Society, 26, 188–193.
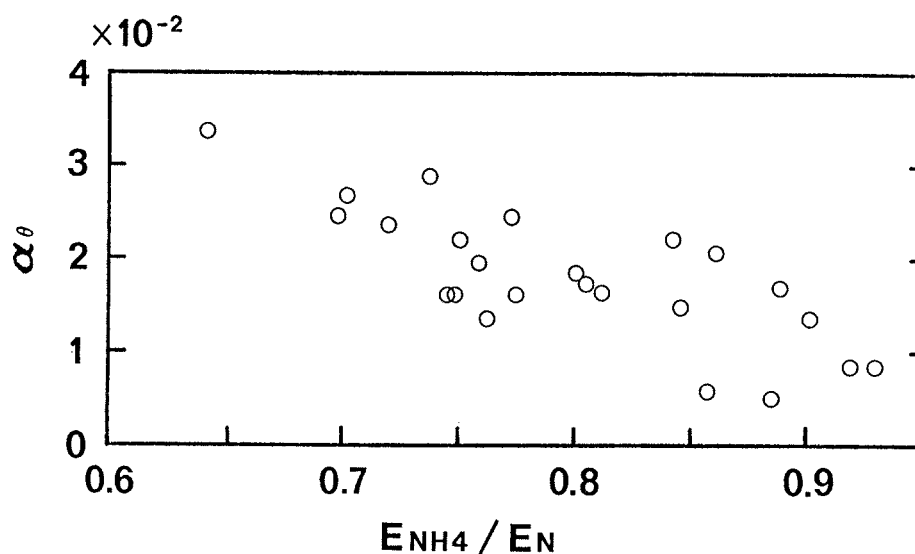
Fig. 6. Relationship between E_{NH4} / E_N and α_θ values.

6) Liddicoat, M. I., S. Tibbitts and E.I. Butler. (1975). The determination of ammonia in seawater. Limnology and Oceanography, 20, 131–132.

7) Newell, S., B. Morgan, and J. Cundy. (1967). The determination of urea in seawater. Journal of Marine Research, 25, 201–202.

8) Kikuchi K., S. Takeda, H. Honda, and M. Kiyono. (1991). Effect of feeding on nitrogen excretion of Japanese flounder *Paralichthys olivaceus*. Nippon Suisan Gakkaishi, 57, 2059–2064.

9) Itazawa Y.,Y. Matumoto, and T. Kanda. (1978). Group effects on physiological and ecological phenomena in fish - I. Group effect on the oxygen consumption of the rainbow trout and the Medaka. Bulletin of the Japanese Society of Scientific Fisheries, 44, 965–969. (in Japanese).

10) Imabayashi H., M Kumasaki, and M. Takahashi. (1992). Group effect on oxygen consumption of the red sea bream *Pagrus major* from postlarval to young stages. Journal of the Faculty of Applied Biological Science, Hiroshima University, 31, 127–136.

11) Wood, J. D. (1958). Nitrogen excretion in some marine teleosts. Canadian Journal Biochemistry and Physiology, 36, 1237–1242.

12) Ozaki H. (1977). Fish Physiology, volume 6, VI Physiology of Kidney. Midorishobou, Tokyo, Japan. (in Japanese).

13) Furukawa A. and Y. Ogasawara. (1955). Studies on nutrition of fish - V. The excretion of NH3 - N and Urea - N by carp and goldfish. Bulletin of the Japanese Society of Scientific Fisheries, 21, 119–122.

14) Harada T. (1981). Land culture of flounder. Yoshoku, 18, 44–48. (in Japanese).

15) Nakamura T., T Takahashi, and H. Hayakawa. (1986). Studies on culture of flounder - V. Effects of different stocking densities on growth and survival. Bulletin of Chiba Prefectual Fisheries Experimental Station. 44, 67–70. (in Japanese).