Diel Self-feeding Rhythms of the Sevenband Grouper

*Epinephelus septemfasciatus*

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**Abstract:** Daily self-feeding rhythms of the sevenband grouper were investigated under two rearing conditions, indoor tanks (0- and 1-year-old fish) and an outdoor net-pen (2-year-old fish). The fishes in the indoor tanks showed diurnal feeding pattern synchronizing with the given photoperiod with feeding peaks at artificial dawn and/or dusk. More specifically, the ratio of feeding activities during the light phase was greater in 0-year-old fish than in 1-year-old fish on the one hand and that during the crepuscular phase was greater in 1-year-old on the other. The fishes in the net-pen showed crepuscular plus diurnal feeding activities with two feeding peaks corresponding to natural dawn and dusk, respectively. Thus, a clear association between the most active time of feeding and the period of the significant change in light intensity was observed from both rearing conditions. In addition, the feeding activity of 1-year and 2-year-old sevenband grouper decreased when the water temperature was over 25°C. Therefore, the water temperature over 25°C was not appropriate for rearing sevenband grouper.

**Key words:** *Epinephelus septemfasciatus*; Self-feeding; Light regime; Water temperature

Self-feeding is a new feeding method for aquaculture based on the learning ability of fishes through operant conditioning (Alanärä 1992a). In self-feeding, a food dispenser releases a certain amount of feed into a fish tank or a net-pen according to the switch activation by fish. Namely, the fish can be exposed to a condition in which they can eat anytime as they prefer. Under this circumstance, the method of self-feeding has been applied to the basic research to determine the regulation of food intake (Rozin and Mayer 1961), the learning ability (Takahashi et al. 1981a, 1981b, 1984), the food preferences and nutrition (Adron et al. 1973; Yamamoto et al. 2000) and the social interaction among individuals (Brännäs and Alanärä 1993, 1994). In addition, studies on self-feeding activity and its implication for aquaculture have been carried out in several commercially important fishes such as rainbow trout (Landless 1976; Alanärä 1992a, 1992b, 1996; Boujard and Leatherland 1992; Boujard and Médale 1994), Arctic charr *Salvelinus alpinus* (Brännäs and Alanärä 1993, 1994), European sea bass *Dicentrarchus labrax* (Sánchez-Vázquez et al. 1994, 1995; Boujard et al. 1996; Azzaydi et al. 1999), lake trout *Salvelinus namaycush* (Aloisi 1994), and yellowtail *Seriola quinqueradiata* (Kohbara et al. 2000, 2001, 2003).

Sevenband grouper *Epinephelus septemfasciatus* attracts the attention as a new and potential culture species in Japan. The development of hatchery technology (Tsuchihashi et al.
2003) and prevention against the viral nervous necrosis by vaccination (Yamashita et al. 2009) enable a stable supply of sevenband grouper juvenile. However, further research is necessary to establish culture technology for this species, because knowledge about the physiology and ecology of this fish species is still scarce, and knowledge on proper rearing environment and feeding technology, and etc. is also limited. The present study aims to clarify the effects of the light cycle and water temperature on the self-feeding rhythms of sevenband grouper in order to improve rearing technology of this potential aquaculture fish species.

**Materials and Methods**

**Indoor Tank Experiment 1**

The experiments were performed from mid-September to mid-October 2001 at the Fisheries Research Laboratory of Mie University at Wagu, Mie Prefecture, Japan. The experimental fish were 0-year-old juveniles produced by Owase Fisheries Laboratory, Mie Prefecture Fisheries Research Institute. After being kept in a floating net-pen set off from the shore for three weeks, they were moved to the experimental room. They were anesthetized with ethyl m-aminobenzoate methanesulfonate (MS-222, Sigma-Aldrich Co. LLC., St. Louis, MO, USA) to weigh the body and were divided into 4 groups with 16 fish in each tank. The mean body weight of fish in each tank was 21.1 g, 21.3 g, 21.3 g and 21.9 g, respectively. Each group was maintained in 200 l tanks and was supplied with about 10 l/min of filtered and aerated sea water. These tanks were placed in a small experimental room and the illumination was provided by fluorescent lamps with a LD 12:12 (L; 06:00-18:00) photoperiod regime and with 25 min crepuscular periods controlled by an electric light timer (Light Controller, AQUA Co. Ltd., Tokyo, Japan). The luminance was approximately 420-440 lx measured at the surface of the water in the tanks. The water temperature ranged 22.7-26.3°C during the experimental period of 31 days. During the period of acclimatization and experiment, fish were fed with a commercial diet (Hamachi EP Special No. 2, Marubeni Nissin Feed Co., Ltd, Tokyo, Japan).

The self-feeding system consisted of four parts: a switch, a feeder, a control unit and a microcomputer. A pull-type switch and a feeder were placed above the tank. As a switch, a tactile sensor (D5B-8511, Omron Co., Kyoto, Japan) was used. At the tip of the switch string, a small orange plastic ball (5 mm in diameter) was attached. The position in the plastic ball was located 3 cm below the surface of seawater of the tank. As a feeder, an automatic food dispenser (Food Timer, Kanematsu Co, Tokyo, Japan) was used, after being modifying to be driven by the control unit (Adocom Electric Co., Ltd, Siga, Japan) according to the signal from the trigger actuation. About 5 pellets were delivered in response to each trigger actuation, and electric signals of trigger actuation were stored in the microcomputer continuously. The stored data were analyzed by using a data acquisition and analysis program (Oshima and Ebihara 1987).

**Indoor Tank Experiment 2**

The experimental procedure was almost identical with that of experiment 1. The experiments were performed from mid-May to mid-July 2002. Thirty five 1-year-old fish, originating from Owase Fisheries Laboratory, Mie Prefecture Fisheries Research Institute were used and they were divided into 5 groups. The mean body weight of fish in each tank was 94.4 g, 95.0 g, 93.9 g, 95.1 g and 98.7 g, respectively. The water temperature ranged 18.8-27.4°C during the experimental period of 68 days. During the rearing and the experimental periods, fish were fed with a commercial diet (Hamachi EP Special No. 6, Marubeni Nissin Feed Co., Ltd, Tokyo, Japan).

**Net-pen Experiment**

The experiments were performed from May to December 2003 at the Fisheries Research Laboratory of Mie University. The experimental animals were 2-year-old fish originating from Owase Fisheries Laboratory, Mie Prefecture Fisheries Research Institute, and were kept in
a floating net cage set off from the shore for about a year. Twenty fish, mean weight was 345.0 g were kept in an experimental net pen (2 × 3 m and 4 m deep). During each experimental period, fish were fed with a commercial diet for yellowtail (Hamachi EP No. 8, Marubeni Nissin Feed Co., Ltd, Tokyo, Japan).

Self-feeding device on net-pen

A pull-type switch and a feeder were placed at the center of the net-pen. As a switch, a micro switch (D2MV, Omron Co., Kyoto, Japan) was used. A small orange plastic ball (9 mm in diameter) was attached at the end of the switch string. The position of the plastic ball was located 10 cm below the surface of the sea. A vibration type feeder (Sterner Products AB, Leksand, Sweden) and the feeder control unit (AFC-03, Adocom Electric Co., Ltd, Siga, Japan) were used. The switch, feeder and feeder control unit were stored in a large plastic box in order to avoid damage by the briny air or seawater. The control unit and the battery were placed on a Styrofoam float of the net pen. The feeder and the control unit were adjusted to deliver a set quantity of pellets on trigger actuation by a fish pulling the plastic ball in its mouth. The electric signals of trigger actuation from the control unit were stored in portable data logger (HOBO Event, Onset Computer Co., MA, USA) as serial event time. The stored data were downloaded to a microcomputer to analyze the number of trigger actuations in 10 min segments and translated to a format suitable for the data analysis programs built by Oshima and Ebihara (1987) to draw actograms or periodograms.

Recording of the environmental factors

Light intensity at 1 m in depth of the net cage was recorded continuously every 10 min using a data logger (StowAway SLA, Onset Computer Co., MA, USA), and the water temperatures at 0.5 m and 4 m in depths were also recorded every hour using data loggers (Optic StowAway Temp, Onset Computer Co., MA, USA) throughout the experimental period.

Results

Indoor Experiment 1

Changes in daily trigger actuations obtained from the four fish groups are indicated in Fig. 1. Sevenband grouper in all tanks activated the feeder every day. The average number of trigger actuations at the commencement of self-feeding was 81 times/day, however it decreased to 59 times/day in the second day. After this, the number of trigger actuations gradually increased up to around 90 times/day until the middle of October while the water temperature gradually decreased from 26°C to 25°C. On the contrary, the number of trigger actuations tended to go down when the water temperature dropped less than 25°C after mid-October.

The representative actogram (double plotted procedure, 48 h time scale) obtained from one of four groups is shown in Fig. 2. The self-feeding activities were unevenly distributed throughout the light phase and were very rarely observed during the dark phase. The hourly actuation activity ratio expressed as a percentage of the daily total during the experimental period obtained from four fish groups is shown in Fig. 3. The feeding activity occurred throughout the light phase with a peak of feeding activity between 06:00 and 07:00. Also, the second peak of feeding activity occurred between
18:00 and 19:00. The periodogram analysis of the feeding activities resulted in a rhythm with a period of 24.0 h in all groups, indicating the self-feeding activities of sevenband grouper synchronized with the given LD cycle.

**Indoor Experiment 2**

Changes in daily trigger actuations obtained from the five fish groups are indicated in Fig. 4. The average number of trigger actuations at the commencement of self-feeding was 11 times/day. Although the feeding activity in the second day was scarcely observed, the number of trigger actuations on the third day rose up to 10 times/day and increased gradually up to 13-14 times/day until early June. The water temperature also gradually increased from 20 to 24°C. From early June to late June, the number of trigger actuation was stably kept from 11 to 13 times/day. On the other hand, the number of trigger actuation declined after the water temperature rose up to 24°C. The number of trigger actuations became less than 10 times/day when the water temperature exceeded 26°C.

The representative actogram (double plotted procedure, 48 h time scale) obtained from one of five fish groups is shown in Fig. 5. The self-feeding activities of 1-year-old sevenband grouper were unevenly distributed throughout the light phase with a tendency to be concentrated particularly at the beginning of the light phase. The hourly actuation activity ratio expressed as a percentage of the daily total during the experimental period obtained from five fish groups is shown in Fig. 6. The characteristics of the graph shape was almost identical with that obtained from 0-year-old fish. Namely, the feeding activity occurred throughout the light phase with a peak of feeding activity between 06:00 and 07:00. Also, the second peak of feeding

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**Fig. 2.** Representative actogram of feeding activity from a group of 0-year-old sevenband grouper under LD 12:12 condition. The data is double plotted (48-h time scale). Horizontal solid, open and hatched bars at the top of the graph represent dark, light and crepuscular phase, respectively, of the LD cycle.

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**Fig. 3.** Mean daily profile of self-feeding activity of four groups of 0-year-old sevenband grouper. Bars represent the mean values made each one hour and vertical lines indicate standard deviations. Horizontal solid, open and hatched bars at the top of the graph represent dark, light and crepuscular phase, respectively, of the LD cycle.

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**Fig. 4.** Change of the number of daily trigger actuation during 68 days experimental period of the indoor experiment 2. The change of water temperature (daily average) in an experimental tank is superimposed upon the bar graph.
activity occurred between 18:00 and 19:00. The periodogram analysis of the feeding activities resulted in a rhythm with a period of 24.0 h in all groups, indicating the self-feeding activities of sevenband grouper synchronized with the given LD cycle.

Net-pen experiments

The daily self-feeding activity of sevenband grouper in a net-cage throughout the experimental period, from May to November is shown in Fig. 7 as the mean value of activity in every half month. Also, mean value of water temperature of every half month at 0.5 m in depth of the net-pen during the experimental period was superimposed upon the self-feeding activity in the same figure. The self-feeding activity immediately after the beginning of the experiment was about six times per day, gradually increasing by the second-half of July. The number of daily feeding activities became highest, about 17 times/day in the second-half of July. Also, the water temperature gradually increased from 19.1°C in the first-half of May to 25.0°C in the second half of July. On the contrary, feeding activities began to decrease in the first-half of August when the water temperature exceeded 25°C. Moreover, self-feeding activity became remarkably low during the water temperature was over 25°C. This declining tendency in feeding activity was observed until the first-half of September in which the water temperature rose up to 28.7°C and the number of daily feeding activities became lowest.
feeding activity decreased to 7.5 times/day. At the second-half of September, the water temperature went down less than 25°C again, and the feeding activity slightly rose up, however, when the water temperature fall down less than 20°C at the second-half of October, the feeding activity remained low level, less than 10 times/day.

The actogram of self-feeding activity obtained from net-pen experiment is shown in Fig. 8. In addition, monthly changes of the daily waveform of self-feeding activity and the relationship to the changes of light intensity throughout seven months observation are indicated in Fig. 9. Throughout the experimental period, feeding activities strictly occurred for several hours just before and coinciding with dawn time. This high feeding activity seemed to coincide with the rapid increase of light intensity at dawn. On the other hand, feeding activities lay scattered during the night time from mid-July through early-August corresponding to the time of the water temperature exceeding 25°C.

Discussion

Learning of self-feeding

In the fish self-feeding experiments so far, two types of switches, namely a rod-type (a pendulum with a knob or a small plastic ball at its tip) and a pull-type (a pendulum with a string that ends in a small and pellet-like plastic ball) have been applied. The former is adopted in
the experiment of rainbow trout (Boujard and Leatherland 1992; Sánchez-Vázquez and Tabata 1998), European sea bass (Sánchez-Vázquez et al. 1994), gold fish (Sánchez-Vázquez et al. 1996) and yellowtail (Kohbara et al. 2000, 2001). The latter is adopted for rainbow trout (Alanärä 1992a, 1992b, 1996), gilthead sea bream Sparus aurata and red porgy Pagrus pagrus (Paspatis et al. 2000) and yellowtail (Kohbara et al. 2003). However little was known which type of switch was appropriate for a particular fish species. In the preliminary experiment of the present study, the self-feeding behavior of sevenband grouper was not observed for a week when we applied the rod type switch in the self-feeding system. On the contrary, the spontaneous start of self-feeding under instrumental conditioning occurred within a few days when the pull-type switch was applied. This indicates that a pull-type switch is more appropriate for self-feeding behavior of sevenband grouper than a rod-type one. This is also the case for yellowtail (Kohbara et al., 2003). The period from start to onset of demand-feeding has been reported to be within one or two days in rainbow trout (Landless 1976; Boujard and Leatherland 1992), Arctic char (Brännäs and Alanärä 1993) and European sea bass (Sánchez-Vázquez et al. 1994). On the other hand, it took 45 days to condition lake trout and this long term conditioning was considered to be due to the less aggressive or less adaptable characteristic of this species (Aloisi 1994). Under these circumstances, sevenband grouper seemed to be a fish species which can easily learn activating the switch of self-feeder within a short period.

**Feeding activity and water temperature**

In the present study, the number of self-feeding activity decreased when the water temperature exceeded 25°C in both indoor experiment using 1-year-old fish (Fig. 4) and net-cage experiment using 2-year-old fish (Fig. 7). On the contrary, the water temperature over 25°C did not give any influence on the self-feeding activity of 0-year-old sevenband grouper in out-door tanks (Kuriyama et al. 2011) as well as the case of the present indoor experiment using the fish of the same age (Fig. 1). In addition, the hatching larvae reared at 25°C showed the highest survival rate during the mass seed production (Tsuchihashi et al. 2003). These results indicate that water temperature which exceeds 25°C is not appropriate for rearing sevenband grouper older than 0-year-old, however, higher water temperature greater than 25°C is acceptable or may be necessary for the rapid growth of 0-year-old fish. The appropriate water temperature for sevenband grouper culture has been generally considered to be from 24°C to 25°C, however the sevenband grouper over 1-year-old should be reared at 24°C or less to keep a high feeding rate or to prevent sevenband grouper from being infected by viral nervous necrosis (Fukuda et al. 1996).

**Self-feeding pattern**

Both 0- and 1-year-old sevenband grouper in indoor experiments clearly showed a diurnal feeding pattern synchronizing with the given photoperiod. Although the food demands occurred throughout the light phase, a distinct peak of trigger actuations was observed from 06:00 h to 07:00 h, corresponding to the period of increasing light intensity. The detail analysis revealed that the number of trigger actuations reached the highest value within the first ten minutes (06:00-06:10) just after the onset of the light phase. This indicates that the self-feeding behavior was induced by extremely low light intensity. The second peak of trigger actuations was observed from 17:00 h to 18:00 h, during the decreasing period of light intensity. Thus, sevenband grouper showed generally crepuscular plus diurnal feeding behavior. The self-feeding experiments under artificial light conditions have been carried out using rainbow trout (Boujard and Leatherland 1992), European sea bass (Sánchez-Vázquez et al. 1994), gold fish (Sánchez-Vázquez et al. 1996), and yellowtail (Kohbara et al. 2000, 2001), and these fishes showed a feeding pattern synchronized to the given photo period with the feeding peak of just after or within 1-2 hours after the onset of light phase. In addition, sevenband grouper in outdoor net-pen also
showed high feeding activity synchronizing with crepuscular time, and this is similar to the case of indoor experiments. In rainbow trout, a clear correlation was established between the time of feeding peaks and sunset (Landless 1976). Also, two feeding peaks coinciding with sunrise and sunset were reported in yellowtail self-feeding experiment under natural light environment (Kohbara et al. 2003). Therefore, changes of luminous intensity during sunrise and/or sunset time seem to be a cue which raises the feeding activity of several fish species including sevenband grouper.

Although the food demands occurred throughout the light phase in 0 and 1-year-old sevenband grouper, the ratios of feeding activities during the light phase was greater in 0-year-old fish than in 1-year-old fish on one hand, those during the crepuscular phase was greater in 1-year-old on the other (Figs. 3, 6). This indicates that the crepuscular feeding behavior is more enhanced in the sevenband grouper older than 0-year, and 0-year-old fish show a tendency to have a diurnal feeding behavior. Similar change in self-feeding pattern relating to the fish growth has been reported in yellowtail (Kohbara et al. 2003). In addition, although its frequency was low, nocturnal feeding was observed 2-year-old sevenband grouper in the net-cage experiment. Namely, the self-feeding activities occurred several hours just before sunrise and/or sunset time throughout the experimental period except from mid-July to early-August in which the feeding pattern had changed to nocturnal (Fig. 8). This reason was not clear, but high water temperature exceeding 25°C might modify the feeding behavior of sevenband grouper. Several studies have demonstrated that species such as Atlantic salmon responded passively to change in water temperature and became nocturnal with low temperature (Fraser et al. 1993, 1995) and this phenomenon is considered to be seasonal change. Rainbow trout showed diurnal dominant feeding behavior from August to September, whereas nocturnal dominant from October to November, and this phenomenon is due to the changes of the day length (Landless 1976). European sea bass held under natural condition also showed the seasonal changes in feeding behavior, namely the fish that were diurnal in summer and in autumn changed to nocturnal in winter and returned to being diurnal in spring and this phenomenon seemed to be modulated by both monthly photoperiod and water temperature (Sánchez-Vázquez et al. 1998). Meanwhile, change in feeding pattern of sevenband grouper observed in the present study was not evident for seasonal change but for an environmental influence since it inverted again within a short period. This means that sevenband grouper took the nocturnal feeding behavior as a compensatory action, since they felt comfortable for feeding at the water temperature where water temperature was slightly higher than 25°C during night. On the other hand, the feeding activity at night has faded away when the water temperature became close to 30°C, not appropriate temperature for their feeding. Similar changes to nocturnal habit in feeding activity during summer season in which the water temperature was relatively high was reported in red sea bream.

The present study revealed that sevenband grouper have a learning ability to activate the self-feeder and daily photoperiod and water temperature are important factors which give influence on their feeding. To make self-feeding method to be a potential rearing technique for sevenband grouper culture, further study such as whole year rearing experiments in detail are necessary.

Acknowledgements

We thank Mr. H. Nakamura for help during our stay at the Fisheries Research Laboratory, Faculty of Bioresources, Mie University. The authors wish to thank all students of Laboratory of Fish Physiology, Faculty of Bioresources, Mie University for their assistance during the experiments.

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マハタの自発摂餌リズムの日周期性について

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マハタ 0 歳魚および 1 歳魚を光条件 LD12:12 の屋内水槽で、また、2 歳魚を自然光下の屋外の生け簀で自発摂餌により飼育し、摂餌活動の日周期性について光周期や水温とも関連づけて検討した。0 歳魚と 1 歳魚では、摂餌パターンはいずれも薄明時+明期摂餌型で摂餌活動のピークは明期開始直後の照度が急激に上昇する時間帯に見られた。また、明期終了直前の減光期にも比較的高い摂餌活性が認められた。一方、明期中の摂餌活動の割合は 0 歳魚の方が 1 歳魚より高く、逆に薄明時の割合は 1 歳魚の方が高かった。生け簀の 2 歳魚においても摂餌活動のピークは朝夕の薄明時に一致した。また、屋内の 1 歳魚および生け簀の 2 歳魚では水温が 25°C を超えると摂餌活性が低下する傾向があった。以上の結果から、マハタの摂餌活性と薄明時に高まり、成長と共にその傾向は強くなること、また、25°C 以上の水温は摂餌に適さない可能性が示唆された。