Ph.D. Thesis

Studies on the Feeding Behavior and Growth Performance of Nile Tilapia (*Oreochromis niloticus)* using Self-feeding System

（自発摂餌システムを活用したナイルティラピアの摂餌行動と成長に関する研究）

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Abstract

The Nile tilapia (*Oreochromis niloticus*) is one of the most important aquaculture species groups especially in South East Asia, Africa, South America and China. The gross production of Nile tilapia increases year by year because of the strong worldwide demands for this potential freshwater fish as a food resource for humans. Therefore, further research is necessary for the establishment of the culture technology for this species such as appropriate rearing conditions and feeding technology. The development of self-feeding system has led to a new perspective for future Nile tilapia culture and alternative dietary selection. The purpose of this study is to clarify the feeding characteristics, such as daily feeding pattern and growth performance of Nile tilapia using a self-feeding technique.

The self-feeding activity experiment was conducted under two rearing conditions for individual test, insulated room treatments under a controlled light regime (LD 12:12) and constant water temperature (25ºC), and indoor treatments under uncontrolled light regime and monitored water temperature (25ºC) by cooler and heater. For group experiment, the trials were conducted by outdoor treatments under natural conditions which consisted of Experiment one period one (Expt1-P1), Experiment one period two (Expt1-P2), Experiment two period one (Expt2-P1), and Experiment two period two (Expt2-P2). The outdoor treatment was carried out from early summer through late autumn in Mie, Central Japan. Another experiment was conducted to determine whether feeding activities of Nile tilapia are regulated by internal circadian oscillator, the feeding activity of individual Nile tilapia was recorded under controlled light regime and water temperature conditions and within a light-dark (LD) cycle, as well as under constant lightness (LL) or constant darkness (DD) conditions.

The results of self-feeding activity experiment showed that the daily self-feeding activity of Nile tilapia in indoor treatments was nearly daytime synchronizing with the given photoperiod (24hr). In outdoor treatments, when the water temperature was between 25-30ºC, the feeding profile showed daytime feeding activity. However, in Expt2-P2, the growth performance diminished when the water temperature dropped below 20ºC and Nile tilapia shifted to a nighttime feeding profile. The results revealed that Nile tilapia has a dualistic capacity for demand-feeding both in light and dark phases. In addition, under the circadian rhythm experiments with a constant environmental condition, one individual showed a free running phenomenon in its feeding activity. The result indicates that the feeding behavior of Nile tilapia is under control of biological clock. Interestingly, the individual showed a circadian rhythm longer than 24 hours (τ =24.4) when it exposed to constant darkness on one hand, it showed a rhythm shorter than 24 hours (τ =23.6) when it exposed to constant lightness on the other. Thus, Nile tilapia seemed to have a dual biological clock system and this circadian system may affect the seasonal change of diel feeding activity synchronizing with the change of natural photoperiod.

In growth performance aspects, in indoor treatments, the value of mean specific growth rate (SGR), feed efficiency (FE), and the rate of daily self-service food supply/body weight (BW) were 2.49%/day, 103.23%, and 2.26%BW/day. The results of the SGR, FE, and BW in insulated room experiment were 2.81%/day, 109.67%, and 2.38%BW/day. In group treatments, the highest result of SGR, FE, and BW was in Expt2-P1 with 3.45%/day, 118.90% and 2.62%/BW/day, respectively. The weight gain was significantly greater ($P<0.05$) in males than that in females in both group experiments.

In conclusion, it became clear that Nile tilapia has a dualistic feeding pattern and assumed has a dual system of biological clock relating to the dualistic phase of feeding. In addition, my finding suggested that the self-feeding could be an effective method for the tilapia culture. Moreover, information obtained from self-feeding experiments will enable us to identify the physiological condition of farmed fishes through their expression of appetite. Therefore, we will be able to give to Nile tilapia suitable amounts of feed which best fit the environmental conditions based on this knowledge.

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General Introduction

A major determinant of successful growth and intensification of aquaculture production is on feeding supply and feeding technology. In 2015, global aquaculture production reached 106 million tons, and 76.6 million tons was increased during the period 1995-2015. Among them, the production of feed-dependent aquaculture increased over fourfold from 12.2 to 50.7 million tons, largely through intensification of production methods (FAO, 2017). The use of aquatic species/species groups such as tilapias, carps, shrimp and salmonids with established aquaculture technologies provided firm market opportunities for increasing production and driving production efficiency and it was dependent upon the supply of external nutrient inputs provided in the form of fresh feed ingredients, farm-made feeds or commercially manufactured feeds.

The Nile tilapia (*Oreochromis niloticus*) is one of the most important aquaculture species groups especially in South East Asia, Africa, South America and China. Several species of tilapia are cultured commercially, but Nile tilapia is the predominant cultured species and its global production has reached to nearly 3.67 million tons in 2014. Including the production of Nile tilapia, cichlid fish production contributes about 5.6% of total aquaculture production (FAO 2017). The gross production of Nile tilapia increases year by year because of the strong worldwide demands for this potential freshwater fish as a food resource of human being.

Therefore, tilapia is likely to be higher rank in global aquaculture production next to carp production. According to El-Sayed (2006), tilapia is an ideal candidate for aquaculture, especially in developing countries because of rapid growth, omnivorous fish which can use high proportion of inexpensive plant sources in their feeds, high tolerance in wide range of environmental conditions, such as temperature, salinity, low dissolved oxygen, resistance against stress and diseases, and so on. In order to optimize the production of Nile tilapia, management system in aquaculture production such as the feeding technology and feeding nutrition are needed.

In most commercial fish farms at present, one of feeding technology such as scheduled automatic-feeding has been employed to supply the diet. Alternatively, some studies have recently introduced self-feeding to fish farming (Alanärä, 1992; 1996). In terms of fish farming, self-feeding is a feeding method which utilizes fish appetite and leads to better feed conversion and growth rates, and reduces the amount of uneaten feed pellets. Besides that, dietary protein also leads the major and most expensive component of fish feed supply (Wilson, 2002).

Self-feeding can be seen as an instrumental learning by delivering food each time a trigger is actuated based on the learning ability of fish (Alanärä, 1996). When fish are fed using self-feeders, growth and feeding efficiency are expected to be improved because fish can regulate distribution relating with their feeding rhythms and behavior (Boujard and Leatherland, 1992). Several studies have been carried out to support this statement, such as European sea bass *Dicentrarchus labrax* (Sánchez-Vázquez et al., 1995), Yellowtail *Seriola quinqueradiata*

(Kohbara et al., 2000), and seabream *Sparus aurata* (Sánchez et al., 2009). The adaptation of self-feeding system may differ in some species and further research is necessary for the establishment of the culture technology for Nile tilapia because knowledge about the physiology and ecology on this fish species is still lacking and there are many obscure points such as appropriate rearing conditions and feeding technology. Moreover, learning the feeding behavior of Nile tilapia is necessary for a better understanding of the physiological mechanism and a better applying this feeding method for tilapia culture in the future.

Chapter 1. Daily Feeding Pattern of Nile Tilapia Rearing under Self-feeding System

1.1 Background

The self-feeding system delivers food based on the learning ability of fish and it is considered to be a new feeding technique for aquaculture that might be controlled by a biological clock (Alanärä, 1992) with food demands depending on appetite (Amano et al., 2007). Therefore, the feeding method agrees with the physical state of the cultured fish and can be used to identify trophic patterns in feeding behavior. Indeed, self-feeding systems have been studied in several culture fish species such as rainbow trout *Oncorhynchus mykiss* (Landless, 1976; Alanärä, 1992, 1996; Boujard and Leatherland, 1992; Chen et al., 2002), European sea bass *Dicentrarchus labrax* (Sánchez-Vázquez et al., 1994, 1995; Boujard et al., 1996; Azzaydi et al., 1998, 2000; Millot and Bégout, 2009), yellowtail *Seriola quinqueradiata* (Kohbara et al., 2000, 2001, 2003), greater amberjack *Seriola dumerili* (Chen et al., 2007), and ayu *Plecoglossus altivelis altivelis* (Amano et al., 2007) to investigate feeding behavior and the appropriate feeding regime of fish culture under self-feeding system.

These practical studies also revealed that the self-feeding pattern of fish varied according to differences in rearing conditions, even within the same species. For example, rainbow trout showed a feeding peak at dusk and also a high level of nocturnal feeding activity in the field condition (Landless, 1976), however a clear diurnal pattern synchronizing with the given LD cycle in laboratory conditions

(Boujard and Leatherland, 1992). European sea bass showed a dualistic feeding behavior, nocturnal and diurnal feeding pattern under controlled experimental condition (Sánchez-Vázquez et al., 1995), and a seasonal phase inversion of the diel feeding pattern was found in field conditions (Sánchez-Vázquez et al., 1998). Yellowtail also showed the inversion of the diel feeding pattern between indoor experimental tanks and outdoor sea cages (Kohbara et al., 2000). Based on the information of self-feeding systems, study about the utility of a self-feeding system for Nile tilapia culture is important to compare with different results of previous studies mentioned above. This study aims to clarify if Nile tilapia has the ability to learn self-feeding under different experimental conditions, indoor and outdoor tanks.

1.2 Materials and Methods

Indoor experiment

The trials were performed from mid-May until mid-June and late June until early August at the Fish Physiology Laboratory of Mie University, Mie Prefecture, Japan. The experimental fish, Nile tilapia *Oreochromis niloticus* were obtained from a wild stock in substream of the Nikko River, Kanie Town, Aichi Prefecture, Japan.

They were anesthetized with 0.01% ethyl m-aminobenzoate methanesulfonate (MS-222, Sigma-Aldrich Co., MO, USA) to weigh the initial body and 6 fish were individually kept in tanks. The body weight of fish in each tank was 63.4 g, 47.1 g, 70.0 g, 46.4 g, 47.5 g, and 75.3 g, respectively. Each fish was maintained in 24*l* tanks (W24 cm x D40 cm x H25 cm) and was supplied with about 6-7 *l*/min of filtered and aerated water. These tanks were placed in a small experimental box (W180 cm x D90 cm x H90 cm) and the illumination was provided by fluorescent lamps with an LD 12:12 (L:06:00-18:00) photoperiod regime with 25 min crepuscular periods (06:00-06:25 and 17:35-18:00) controlled by an electric light timer (Automatic light controller, Aqua Co. Ltd., Tokyo, Japan). The luminance was approximately 200-300 lx (0.6 W/m^2) at the surface of water in the tanks (measured by Digital Photometer Type T1, Konica Minolta Tokyo, Japan and RAD irradiance probe, Delta OHM Padova, Italy). The water temperature was maintained using a water cooler (Coolway 100, GEX Co. Ltd. Osaka, Japan) and heater at 25ºC during the trial periods (approximately 35 days). The self-feeding system in the indoor experiment consisted of a self-feeder (developed by Yamaha Motor Co., Ltd., Shizuoka, Japan) with pull type switch (D2MV, Omron, Kyoto, Japan) which was placed above each tank (Fig. 1.1). At the tip of the stainless rod of switch, a small orange plastic ball (5 mm in diameter) was attached. The position of the plastic ball was located 1 cm below the surface water of the tank. During the trial periods, fish were fed with a commercial diet (Hikari mini, CP min 32%, Kyorin Food Ind., Ltd, Himeji, Japan). The feeder delivered approximately 5 pellets each time a fish activated the switch, and the self-feeder sent signals to a microcomputer that continuously stored the event time data of the feeder actuations. The stored data were treated by a chronobiology data acquisition and analysis program (Analyze 98, Stanford Software Systems, Santa Cruz, CA, USA).

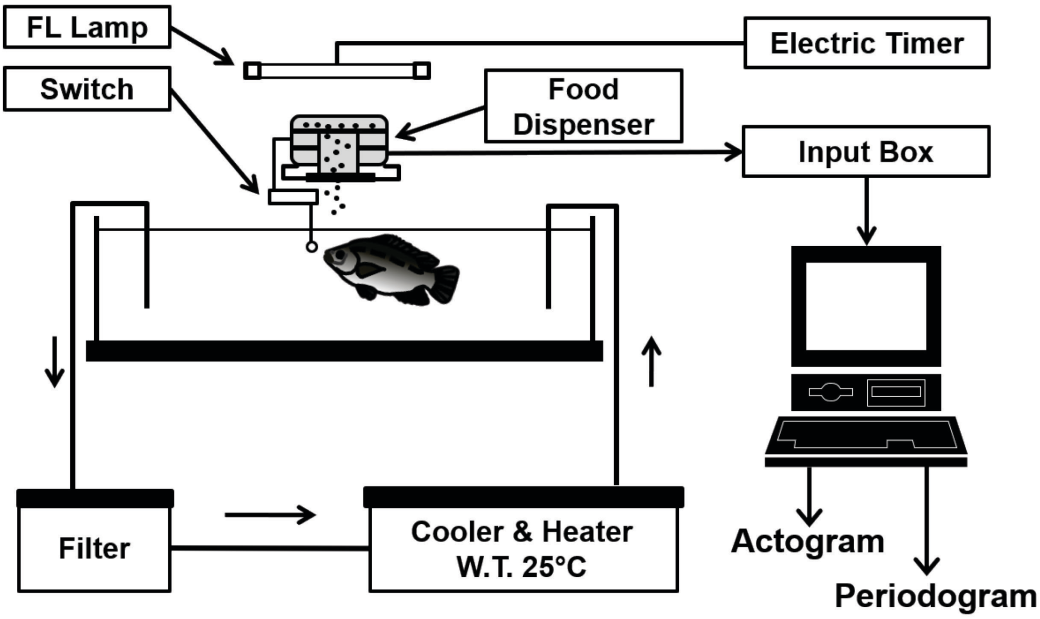

Fig. 1.1 Schematic view of the self-feeding apparatus for indoor experiments (artificial light condition).

Outdoor experiment

The Nile tilapia used for outdoor trials had the same origin as explained for the indoor experiment. After being kept in an outdoor concrete tank of the experimental water tank facility for several weeks, they were immobilized under MS-222 anesthesia and fish around 100 g of body weight were selected and individually tagged using a microchip PIT tag for identification proposes. Fish were divided into triplicate groups each consisting of thirty fish and being almost same biomass, and each group was kept in an experimental concrete tank (2 m x 2 m and 1 m deep), respectively.

The equal trials were repeated twice for analysis. Namely, Experiment 1-Period 1 (Expt1-P1, June 13-July 16), Period 2 (Expt1-P2, July 19-August 22), and Experiment 2-Period 1 (Expt2-P1, August 30-October 4), and Period 2 (Expt2-P2, October 5-November 5). The initial mean weight ± standard deviation for Expt1-P1 was 98.26 ± 0.83 g reared for 34 days, Expt1-P2 was continued and used the same group of fish as Expt1-P1 which had an initial mean body weight of 216.17 ± 18.72 g and were reared for 35 days. Ex2 used different fish groups with period Ex1, initial mean body weight was 64.30 ± 0.00 g reared for 36 days, and Expt2-P2 was continued and used the same group of fish with Expt2-P1 which had an initial mean body weight of 211.00 ± 6.98 g and were reared for 35 days.

An automatic food dispenser (developed by Shin-Nihon Ventures Co., Ltd., Osaka, Japan) was used, after being modified to be driven by a control unit (AFC-3 Adocom Electric Co., Ltd., Shiga, Japan) according to the signal from the switch actuation. As a switch, the same pull-type switch (D2MV, Omron, Kyoto, Japan) was used as for the indoor treatments. At the tip of the stainless rod of switch, a small orange plastic ball (9 mm in diameter) was attached. The position of the plastic ball was located 3 cm below water surface of the tank. A pull-type switch and a feeder were placed at the center of each tank. The event time, when the feeder was driven by the signal from the control unit, was stored in a portable data logger (Hobo Event, Onset Computer Co., MA, USA). The event time data collected by the logger were downloaded to a computer to analyze the number of feeder actuations in 10 min segments and translated to a format suitable for the data analysis programs to draw actograms or periodograms following the method detailed in Oshima and Ebihara (1988).

Light intensity at about 1m above of the experimental tank was recorded continuously every 10 min using a data logger (StowAway, SLA, Onset Computer Co., MA, USA), and the water temperatures of each tank were also recorded every hour using a data logger (Hobo Pendant Temp, Onset Computer Co., MA, USA) throughout each experimental period.

1.3 Results

Changes in daily feeding activity in indoor treatment

At the beginning of the experiments, 2 fish (Tank 1 and 2) activated the self-feeder within four days and the fish in Tank 6 also activated within a short time, of about two days. However, the other three fish had a different response, self-feeding was not observed within a short time. To overcome this problem, the self-feeder was artificially activated by pulling the switch to stimulate the feeding activity. After this treatment for about 8-9 days, fish in Tank 4 and Tank 5 started to activate the feeder while the fish in Tank 3 took the longest period to activate the feeder of about 18 days. These fish needed a longer time to activate the feeder than the two fish in Tank 1 and 2.

Self-feeding activity showed important variation throughout the trials. Daily variations in feeding activity differed between the experimental fish. A representative actogram (double plotted procedure, 48 hr time scale) obtained from one of the six fish is shown in Fig. 1.2a. The self-feeding activity profile was somewhat unstable at the initial phase of the treatment. Namely the tendency of diurnal feeding pattern was observed, however, night feeding activities occurred at a relatively high rate for 3 days from the beginning of the experiment. After 15-17 days rearing, the self-feeding pattern became stable and showed a clear diurnal feeding profile for 20 days until the end of trial.

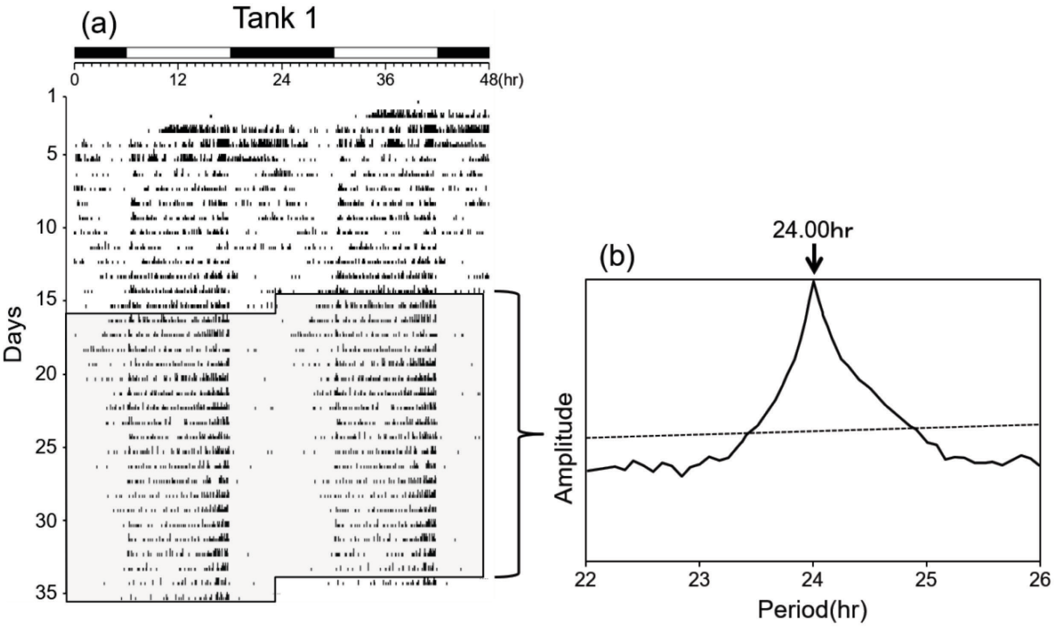

Fig. 1.2 (a) Temporal distribution of self-feeding records from a representative individual (Tank 1) during the experimental period. The feeding pattern shows a clear daytime profile after 15-17 days rearing under the controlled laboratory condition until the end of experiment. The thin vertical bars placed on x-axis indicate the time of activating the self-feeder activation, and the height of the bar, frequency of the feeding activity within 5 minutes. The black and white bar at the top of bar graph indicates the light regime; black bar, dark phase: white bar, light phase; shaded bar, crepuscular phase, respectively. (b) Periodogram analysis of the feeding rhythms under the LD 12:12 cycle, calculated for the shaded area of actogram. The highest variance obtained at 24.0 hr period indicates the phase of feeding completely synchronizes with daytime. The leaning dotted line indicates 95% confidence limit of variance.

Another case of Tank 2 is shown in Fig. 1.3a. Nocturnal feeding activity was observed for 7 days at the early period of the experiment. The diurnal feeding activity was low in number. After 7 days, however, the end of feeding activity corresponding with the end of the dark phase had moved forward day by day showing a somewhat free running like rhythm and the activity profile changed from nocturnal to diurnal taking about 8 days for this alteration. Thus, after 15-17 days rearing, the self-feeding pattern resynchronized to given photoperiod resulting in a clear and stable diurnal pattern.

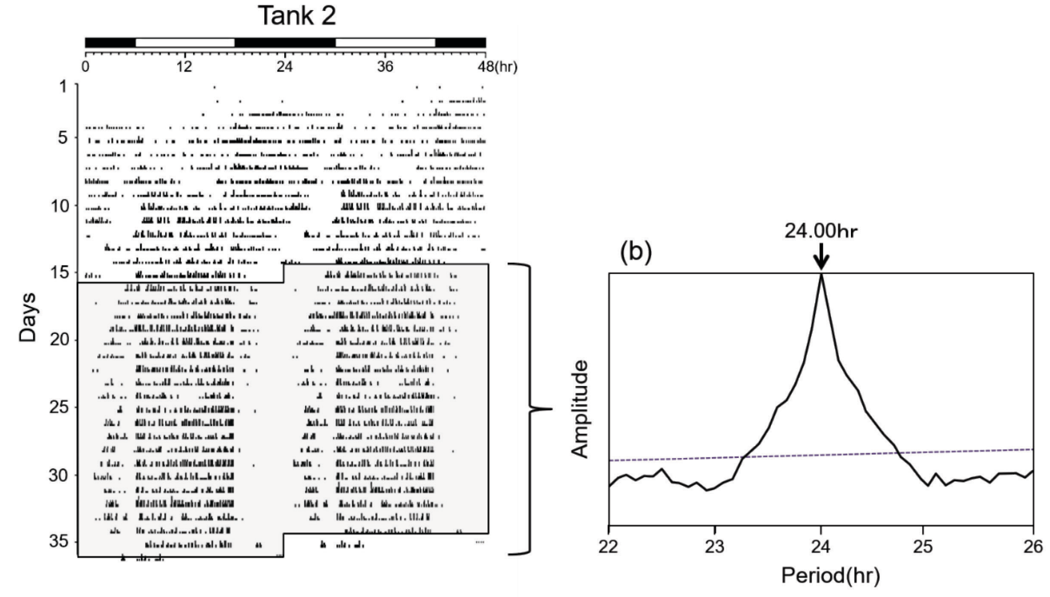

Fig. 1.3 (a) Temporal distribution of self-feeding records from a representative individual (Tank 2) during the experimental period. The feeding pattern shows nighttime feeding profile for 7 days at the early period, however a clear daytime feeding profile appears after 15-17 days rearing until the end of the experiment through free running-like shifting activities. (b) Periodogram analysis of the feeding rhythms under the LD 12:12 cycle calculated from the shaded area of actogram. Additional explanations, see Figure 1.2.

Five fish demanded feed before the lights-on and lights-off, whereas the other fish showed an unstable feeding activity. In regard to the fish in Tank 6, two days after the feeders were activated, a transition phenomenon appeared. The nighttime activity was altered into the diurnal and it was observed within 13 days. The nocturnal activity was altered into the diurnal and it was observed within 13 days.

The nocturnal activity corresponding with the end of the dark phase as observed in Tank 2. The self-feeding activity of the other four fish has begun on the way of the experiments after 4-18 days from the first day of experiments, therefore the transition phenomenon from nocturnal to diurnal was not observed and the self-feeding profile obtained from these four fish showed a diurnal activity, except for the fish in Tank 3 which showed a somewhat unstable feeding activity. The actogram in Fig. 1.3a represents the feeding pattern of the fish in Tank 1 and had a similar stable feeding pattern as fish in Tank 4 and 5. In summarized, the way individual fish start self-feeding is highly variable, but after a 2-3 week adaptation/learning period a specific feeding pattern was established.

The analysis of periodic activity by the chi-square periodogram (Fig. 1.2b and 1.3b) was done using the clear and stable diurnal feeding period marked by the hatched area in Figs. 1.2a and 1.3a. The feeding rhythms under the LD 12:12 cycle resulted in a rhythm of 24.0 hr which means the phase of feeding completely synchronized with the given of photoperiod In the case of fish in Tank 3-6, the clear and stable diurnal feeding period occurred after 13-26 days rearing. The chi-square periodogram analyses showed the same results with the representative fish as shown in Figs. 1.2 and 1.3. Thus, the phase of feeding activity completely synchronized with the given LD 12:12 with a periodicity of 24 hr.

Changes in daily feeder actuation obtained from the six groups of the fish are shown in Fig. 1.4. Nevertheless, Nile tilapia in all tanks activated the feeder every day. The figure made from 20 days representative data of stable period of the six individuals. The changes of self-feeding activity during the experimental period for 20 days were almost stable from day to day ranging 94-159 times/day (Fig. 1.4).

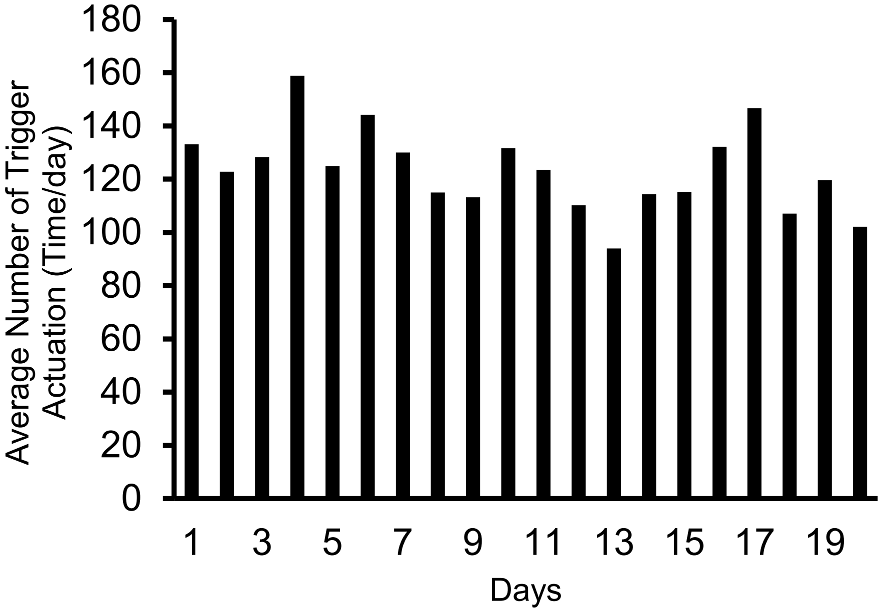

Fig 1.4 Changes the number of feeder actuations of fish from 20 days of representative data of the stable period under the controlled laboratory condition. Each bar indicates the mean value of daily number of feeding activity from six individuals.

The hourly self-feeding activity ratio data expressed as a percentage of the number of daily feed trigger actuations during the experimental period obtained from six fish is shown in Fig. 1.5. This figure was based on 20 days representative data from stable period, and the self-feeding activity of Nile tilapia showed a tendency to be concentrated during the light phase with a peak of feed demands at both increased and reduced light phases at the beginning and end of daytime, between 06:00-07:00 (11 times/day) and 17:00-18:00 (14 times/day).

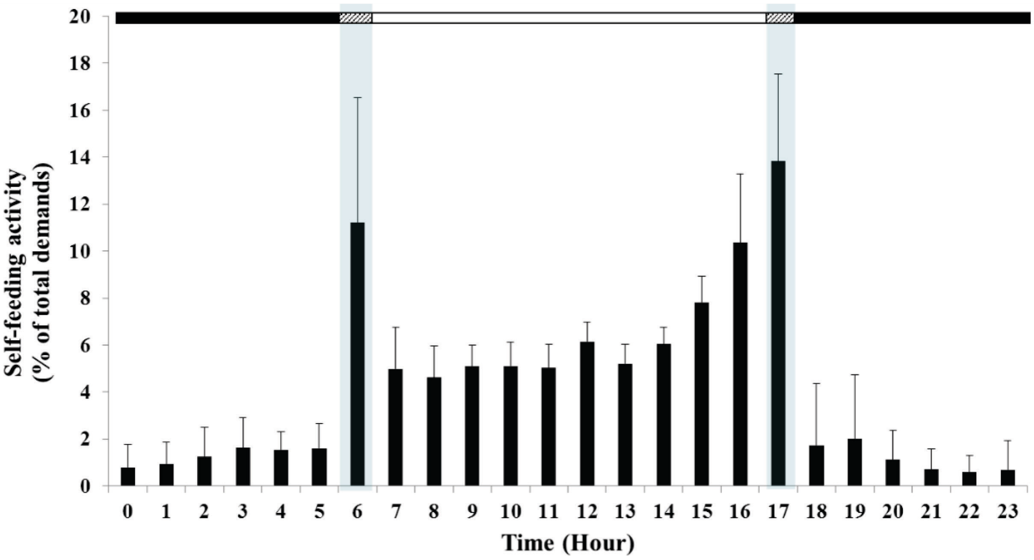

Fig. 1.5 Hourly percentages of total feeding activities during the experimental period. Two clear activity peaks were observed at both increased and reduced light phases of beginning and end of daytime. Each bar indicates mean value from six individuals and thin line on each bar indicates standard deviation. The black and white bar at the top of bar graph indicates the light regime; black bar, dark phase: white bar, light phase; shaded bar, crepuscular phase, respectively.

Changes of daily feeding activity in outdoor treatment

All groups in the outdoor treatment showed stable self-feeding activity during the experimental period, all of the fish started pulling the switch on day one without any artificial induction. Thus, self-feeding was observed on the first day of the experimental period without any artificial induction. This means that the fish groups in the outdoor tanks could adapt to self-feeding within a short of time on their own.

Throughout Expt1-P1 until Expt2-P1 (mid-June through early-October), self-feeding activity mostly occurred during daylight time and showed mainly a diurnal feeding profile (Figs. 1.6a, 1.6b and 1.7a).

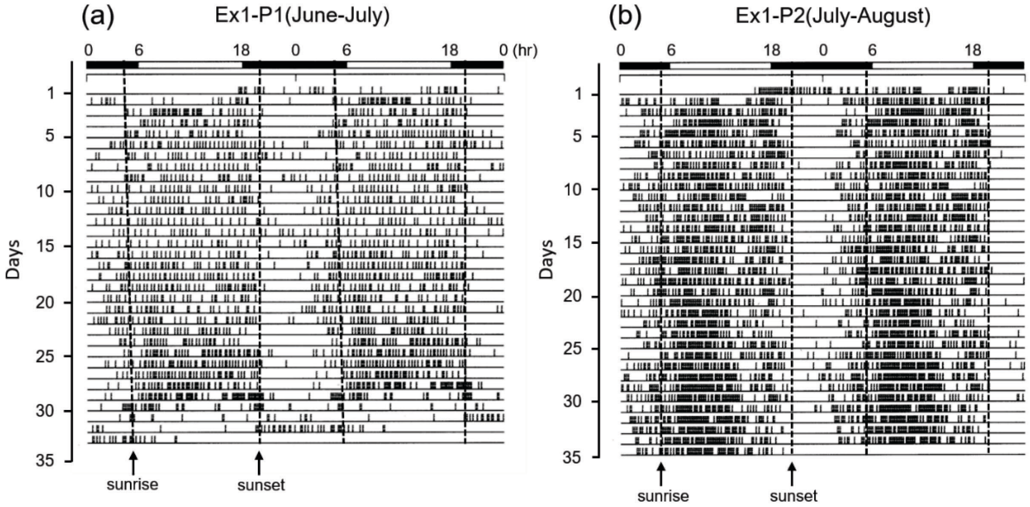

Fig. 1.6 Temporal distributions of self-feeding records from one representative group (30 fish) of outdoor experiments. (a) Actogram of experiment one-period one (Ex1-P1). (b) Actogram of experiment one-period two (Ex1-P2). The feeding pattern shows almost a daytime profile in natural conditions. For convenient visualization, the data have been single plotted (24hr time scale), the thin vertical bar indicates the occurrence of self-feeding activity within 10 minutes. Two dotted lines indicate sunrise time and sunset time, respectively.

On the other hand, during Expt2-P2 (early-October through early-November) feeding activities were spread throughout the day during the first seven or eight days from the beginning of the experiment. After day 10, the feeding activities distributed during night time increased and the actogram showed a clear nocturnal profile (Fig. 1.7b).

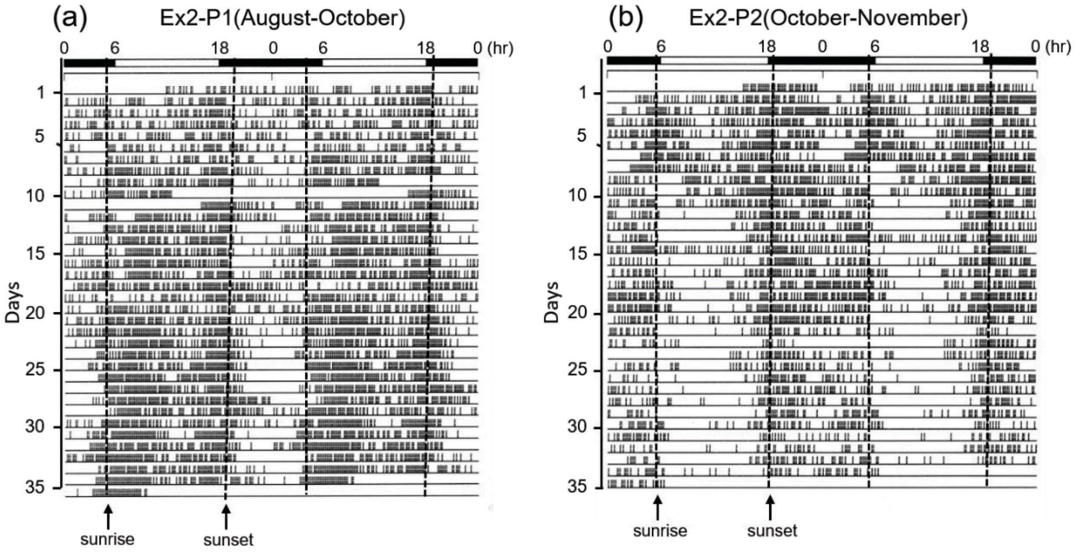

Fig. 1.7 Temporal distributions of self-feeding records from one representative group (30 fish) of outdoor experiments. (a) Actogram of experiment two-period one (Ex2-P1). (b) Actogram of experiment two-period two (Ex2-P2). The feeding pattern shows almost a daytime profile in figure (a), however it has shifted to a nighttime profile in figure (b). Additional explanations, see Figure 1.6.

Percentages of the hourly feeding activity from during the experimental periods in outdoor trials reached about 7-8% in each hour of the total demands (Fig. 1.8). The activities practically occurred mostly during the light phase, but time of peak activity was different between the experimental periods.

Two obvious peaks of feeding activity were observed around 6:00 - 8:00 and 16:00 - 18:00 except in Expt2-P2, when the water temperature became lower and the light phase became shorter than the other periods. Nevertheless, the peaks of feeding activities occurred just several hours before dawn and after dusk. The number of the feeding activities in Expt2-P2 during the light phase also decreased while that in the night phase increased.

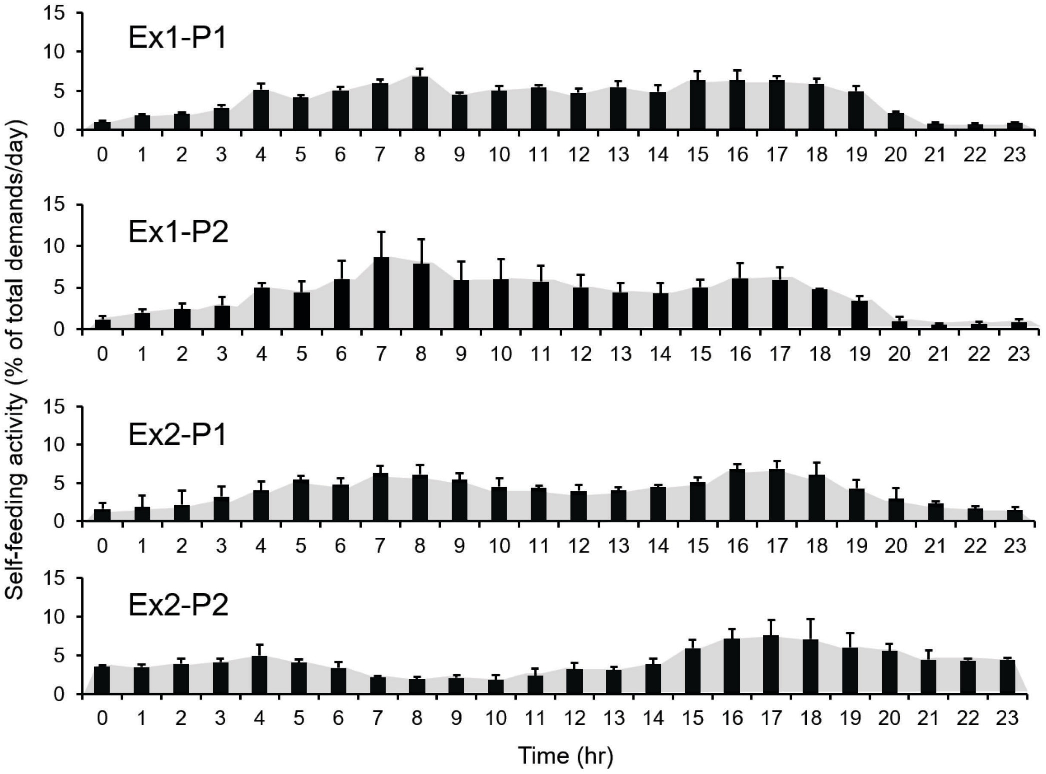

Fig. 1.8 Hourly percentages of total self-feeding activities during the four experimental periods under natural conditions. Each single graph indicate the percentage of self-feeding activity in each period of Experiment one and Experiment two. The vertical bar indicates mean value and the thin line on each bar, standard deviation (n=3). The shaded background which shows the outline of seasonal transition of feeding profile is added for convenient visualization.

The relationship between the percentage of feeding activity that occurred during the daylight time and the water temperature is shown in Fig. 1.9. The decreasing percentages of feeding activity during the daylight time synchronized clearly with the declining of water temperature. In Expt2-P2, when the water temperature dropped below 20ºC, the percentage of feeding activity that occurred during the daylight time also decreased to less than 50%.

1.4 Discussion

Adaptation of fish to self-feeding systems can be influenced by environmental conditions and social interactions (Alanärä, 1996; Kohbara et al., 2000). The present study showed that tilapia learned and adapted to the self-feeding system quickly and most of the pellets distributed were consumed. The period of starting of self-feeding activity has been reported to be within a couple of days in rainbow trout (Boujard and Leatherland, 1992), European sea bass (Sánchez-Vázquez et al., 1994), and yellowtail (Kohbara et al., 2000). Nile tilapia itself has been reported to require various lengths of time to activate the feeder. Toguyeni et al. (1997) reported that tilapia can adapt to operate a self-feeding system within a short time and groups of tilapia completely adapted to the self-feeder within four days. Fortes-Silva et al. (2010) recorded that self-feeding activity of individual tilapia starting on day one but some fish required a few weeks to adapt to a self-feeding system and show a stable feeding pattern. There was a different result between a single fish and group fish to activate the switch. In this study, most of a single tilapia that were kept in indoor tanks took 2-9 days to activate the self-feeder while the groups of tilapia that were kept in outdoor tanks activated it within 1-2 days. In the case of rainbow trout, Landless (1976) reported that learning of self-feeding for a group of fish took 2 days, much faster than for a single fish (7 days). This result indicated that Nile tilapia has a dualistic feeding pattern under different condition of self-feeding system. Self-feeding pattern in indoor trials clearly showed a diurnal feeding pattern and synchronizing with the given photoperiod. The feeding activities of tilapia present greater activity at dawn and dusk. During 34-36 days of the experimental period in indoor tanks, the number of trigger actuation varied with days. There was observed a clear peak of feeder actuation from 06:00 hr to 07:00 hr, with the increasing period of light intensity and from 17:00 hr to 18:00 hr during the induced dark phase. The results demonstrated that the number of feeder actuations reached to the highest within the first ten minutes just after the light intensity increased or decreased. This indicates that the self-feeding behavior of Nile tilapia was influenced by the change of light regime. Therefore, Nile tilapia showed generally crepuscular plus diurnal feeding behavior under the controlled laboratory condition. Self-feeding experiments under artificial light conditions have been carried out for rainbow trout (Boujard and Leatherland, 1992), European sea bass (Sánchez-Vázquez et al., 1994), yellowtail (Kohbara et al., 2000, 2001), and tilapia (Toguyeni et al., 1997; Fortes-Silva et al., 2010). In the case of tilapia, feeding activity increased at dawn, peaked during the half-hour of artificial dawn and during the last hours of the light period (Toguyeni et al., 1997).

In outdoor treatments, the daily self-feeding activity of Nile tilapia showed different results between each experimental period and synchronized with natural changes of light regime and/or water temperature. Basically, based on the daily phase of behavior, most fish could generally be categorized into one of three patterns: diurnal, nocturnal, or crepuscular (Eriksson, 1978). Nevertheless, Nile tilapia seems to be flexible in the phasing of diel rhythms related to the change of natural light regime and/or water temperature. For example, an uncertain feeding pattern has been reported in tilapia, which is considered a diurnal species (Toguyeni et al., 1997) with the percentage of feeding activity in daylight time being more than 80%. On the contrary, Fortes-Silva et al. (2010) reported that feeding activity of tilapia was almost exclusively nocturnal which represented 93% of the daily feeding activity occurred during the night time. Both of those inversions have been examined under controlled light regimes and water temperature. Eriksson (1978) mentioned that such a discrepancy in the experimental results using the same fish species originated from the seasonal difference of the experimental period. Indeed, it should be noted that the present study recorded the feeding activity of Nile tilapia not only under the controlled laboratory condition but also under natural conditions which the change of light regime and water temperature seem to influence on the phase of feeding activity in this species. In the case of outdoor treatments, the trials of Expt1-P1, Expt1-P2, and Expt2-P1 were observed from early summer until early autumn and the day length ranged 12-14 hr light phase. The light phase in Expt2-P2 in late autumn was about 10 hours and day length decreased extremely. The data of daily feeding activity indicated that the feeding profile has a strong association with the ratios of daytime and nighttime. Water temperature might have the same essential role on the feeding profile of Nile tilapia. The results of this study showed that the feeding profile changed from diurnal to nocturnal when the water temperature decreased below 20ºC at the end of Expt2-P2 (Fig. 1.9).

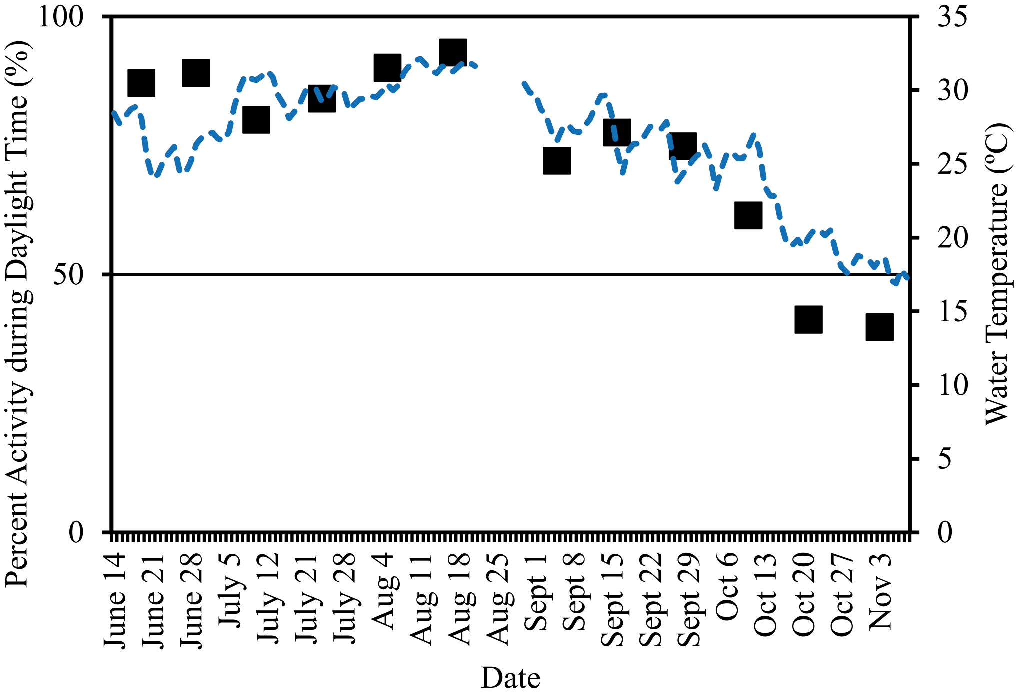

Fig. 1.9 Relationship between the percentage of seasonal change of self-feeding activity during daylight time and the change of water temperature throughout the outdoor experimental period. Each closed square represents the percentage of mean value obtained seven days feeding activity during the daylight time from Experiment one through Experiment two. Seasonal change of water temperature represented by dotted line is superimposed. When the temperature was lower than 20ºC, the daytime activity decreased below 50% at the end of Experiment two. This means the feeding profile of Nile tilapia could be changed from daytime to nighttime when the water temperature become less than 20ºC.

Thus, the comparison of self-feeding pattern of tilapia between under artificial LD conditions and natural conditions were basically similar, almost self-feeding occurred during the light phase except in Expt2-P2 which showed a more nocturnal feeding activity than the other three periods. There must be some tendency of feeding activity corresponding to the light regime and/or water temperature in the case of Nile tilapia. As shown in Fig. 1.3, low feeding activities appeared during the day time and this seems related to the initial feeding behavior of tilapia under natural conditions before rearing under the controlled laboratory condition. In addition, the transient feeding phase like a free-running rhythm was observed. This suggests that the diel feeding rhythm of Nile tilapia seems to be regulated by endogenous circadian oscillators of the biological clock. Eriksson (1978) suggested that the biological clock controlled the seasonal inversion of the several activities of fish.

Feeding during the daytime has been suggested for optimal growth performance of Nile tilapia, however recently, nocturnal pattern of feeding as well as diurnal patterns in tilapia under LD condition have been reported (Vera et al., 2009; Fortes-Silva et al., 2010). Meanwhile, to make self-feeding as a potential system for Nile tilapia culture, especially in outdoor tanks, it should be noted that the daily photoperiod and water temperature are important factors which might give some influences on the feeding behavior of this species. Therefore, the feeding pattern obtained in this study is a particular laboratory result under specific treatments and conditions. The characteristics of feeding behavior in the present study do not always show in nature or large scale aquaculture of this species. However, clearly Nile tilapia have an ability to feed in both light and dark phases of indoor and outdoor conditions.

1.5 Conclusion

As presently reported, the utilization of self-feeding system has been successfully applied for culture of several fish species. Finally, my findings that the Nile tilapia can use self-feeders quickly and efficiently to adjust food intake according to diet consumption, can contribute to the improvement of fish production and conservation of the water environment and aquaculture aspects.

Moreover, we will be able to give to the fish suitable amounts of feed which best fit the environmental conditions based on this knowledge. Information obtained from self-feeding experiments will enable us to identify the physiological condition of farmed fishes through their expression of appetite such as the dualistic feeding pattern which was appeared in Nile tilapia suggested that this species have an ability to feed in daytime and nighttime. Furthermore, to test whether this dualistic feeding behavior of Nile tilapia is controlled by a biological clock, some experiments relating with circadian rhythm of feeding activity in this species were conducted and explained in chapter two.

Chapter 2: Circadian Rhythms of Feeding Activity in Nile Tilapia

2.1 Background

Most animals, including fish, show daily behavioral rhythms and active either during the day or at night. Such rhythmic behavioral patterns appeared from long-term evolution under influence of cyclic selective forces by the rotation on earth, this biological rhythms related to physiological parameters which is driven by endogenous oscillators within the brain or sensory organs (Sánchez, et al. 2009), specifically behavioral activity of fish such as feeding and locomotion behavior which is synchronized with the change of this kind of environmental variations (Chen et al., 2007). The most important entertaining factors are light-dark cycle and food ability. For example, the biological clock continuous to operate and circadian rhythms persist with their own free-run period, diverging slightly from the environmental cycle to which they are normally synchronized (Sánchez-Vázquez et al., 1997).

It has also been suggested that feed utilization could be improved by better understanding circadian feeding behavior (Smith et al., 1989; Boujard and Leatherland, 1992; Madrid et al., 1997). Daily rhythms of feeding activity have been found in many species under controlled environmental conditions, such as; silver carp, *Hypophthalmichthys molitrix* (Smith et al., 1989); *Oncorhynchus mykiss* (Landless, 1976; Boujard and Leatherland, 1992); and European sea bass, *Dicentrarchus labrax* (Sánchez-Vázquez et al., 1995; Boujard et al., 1996; Madrid et al., 1997).

Based on chapter one, Nile tilapia has a dualistic feeding pattern correspondence to the seasonal change of water temperature and/or light regime. There was a tendency that Nile tilapia of outdoor experiments showed diurnal feeding pattern during summer time, while it changed gradually into nocturnal one from the end of autumn to winter. Eriksson (1978) suggested that such a seasonal inversion in fish activity is controlled by biological clock. To determine whether feeding activities of Nile tilapia are regulated by biological clock (internal circadian oscillator) or not, the feeding activity of Nile tilapia was recorded under controlled laboratory conditions and within a light-dark (LD) cycle, as well as under constant lightness (LL) or constant darkness (DD) conditions.

2.2 Materials and Methods

Experimental fish and condition

The experimental fish, Nile tilapia *Oreochromis niloticus* were obtained from a wild stock of substream of the Nikko River, Kanie Town, Aichi Prefecture, Japan. After being kept in an outdoor experimental concrete tank (2 m x 2 m and 1 m deep) for a several months, the experimental fish were divided into two groups, the autumn group and the winter one. The experimental fish of autumn group (Fish ID; NT-1, NT-2, NT-3, NT-4) was took out from outdoor concrete tank and housed individually in 24 *l* PVC tanks which were placed in a small experimental box (W180 cm x D90 cm x H90 cm) on September 6th, 2016. The experimental fish of winter group (Fish ID; NT-5, NT-7, NT-8, NT-9) was housed on November 17th, 2016 with the same procedure. They were anesthetized with

0.01% ethyl m-aminobenzoate methanesulfonate (MS-222, Sigma-Aldrich Co., MO, USA) to weigh the initial body weight which ranged 40-55 g and initial total length 13-15 cm, respectively. Each fish was maintained in 24*l* experimental tank described before and was supplied with about 6-7 *l*/min of filtered (EHEIM Professionals Co., Ltd.) and aerated water. The illumination was provided by fluorescent lamps controlled the light/dark cycle by digital timer (Automatic light controller, Aqua Co. Ltd., Tokyo, Japan). The luminance was approximately 200-300 lx (0.6 W/m^2) at the surface of water in the tanks (measured by Digital Photometer Type T1, Konica Minolta Tokyo, Japan and RAD irradiance probe, Delta OHM Padova, Italy). The water temperature was maintained using a water cooler (Coolway 100, GEX Co. Ltd. Osaka, Japan) and heater at 25ºC during the trial periods.

Light-dark experiment (LD 12:12)

The experimental trials were divided into two periods with four replicates each. Namely, the autumn group experiment (A-Expt, Sept 6-Oct 10) and the winter group experiment (W-Expt, Nov 8- Dec 10, except one trial Dec 28-Jan 16). These experiments were kept under constant light regime with LD 12:12 during the trial periods. After these experiment, A-Expt fish were exposed to constant lightness cycle (LD 24:0) and W-Expt fish were exposed to constant darkness cycle (LD 0:24) at least 10 days described below.

Constant lightness experiment (LD 24:0)

The experimental trials were used the same fish as LD 12:12 experiment of A-Expt. The fish were exposed to continuous light conditions (LD 24:0) to examine the biological clock of self-feeding rhythms.

Constant darkness experiment (LD 0:24)

The experimental trials were used the same fish as LD 12:12 experiment of W-Expt. The fish were exposed to continuous dark conditions (LD 0:24) to examine the biological clock of self-feeding rhythms. After the constant darkness experiment, fish were exposed again to the LD 12:12 cycle for re-entertainment for at least 10 days. After this entrainment, LD 12:12 cycle was replaced with LD 24:0 (constant lightness condition) to determine the influence of biological clock on the feeding behavior.

Self-feeding apparatus

The self-feeding system were used the same apparatus with the insulated indoor experiment in chapter one, consisted of a self-feeder (developed by Yamaha Motor Co., Ltd., Shizuoka, Japan) with pull type switch (D2MV, Omron, Kyoto, Japan) which was placed above each tank (Fig. 1.1). At the tip of the stainless rod of switch, a small orange plastic ball (5 mm in diameter) was attached. The position of the plastic ball was located 1 cm below the surface water of the tank. During the trial periods, fish were fed with a commercial diet (Hikari mini, CP min 32%, Kyorin Food Ind., Ltd, Himeji, Japan). The feeder delivered approximately 5 pellets each time a fish activated the switch, and the self-feeder sent signals to a microcomputer that continuously stored the event time data of the feeder actuations. The stored data were treated by a chronobiology data acquisition and analysis program (Analyze 98, Stanford Software Systems, Santa Cruz, CA, USA).

2.3 Results

Differences in daily feeding activity between autumn group and winter group in a constant environmental condition

At the beginning of the experiments, there were four individual of Nile tilapia of A-Expt reared separately throughout the experimental periods under constant environmental condition (LD 12:12). All individuals activated the self-feeder within a short time. In W-Expt, four individuals reared separately throughout the experimental periods under constant environmental condition (LD 12:12). Three of four fish were activated the self-feeder within a short time. However, one individual fish (NT-6) showed no spontaneous self-feeding activity during the experimental period. To overcome this problem, a new fish (NT-9) was used as a supplemental experiment.

The self-feeding activity profile was somewhat unstable at the initial phase of both experiments. A representative actogram from A-Expt and W-Expt are shown in Fig. 2.1.

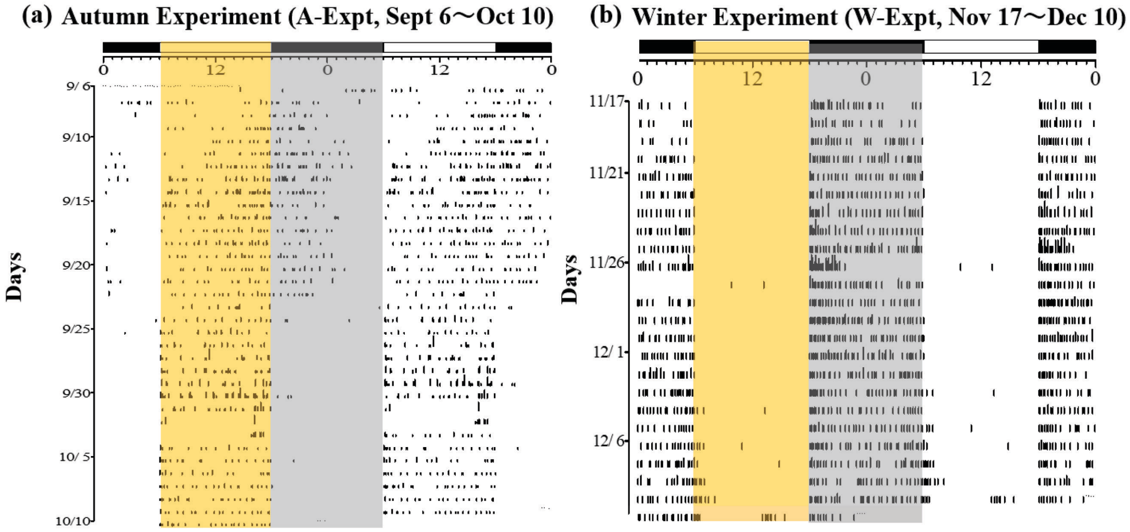

Fig. 2.1 Temporal distributions of self-feeding records from one representative individual under constant cycle (LD 12:12) experiments. (a) Actogram of one individual (NT-1) from autumn experiment (A-Expt). The feeding pattern shows an indifferent and changed to a diurnal feeding profile. (b) Actogram of one individual (NT-7) from winter experiment (W-Expt). The feeding pattern shows almost a nocturnal profile from beginning until the end of experiment. For convenient visualization, the data have been double plotted (24 hr time scale). The bar which is painted with the white and black color on the upper side of the figure shows the light/dark cycle, schematically. The thin vertical bar indicates the occurrence of self-feeding activity within 10 minutes, and the grey-yellow hatched area indicated the time of light-dark cycle, respectively.

In A-Expt, most of fish generally showed a diurnal feeding pattern under LD 12:12 light regimes. There was interesting phenomenon from one representative trial (NT-1), the frequency of feeding in the dark period tended to be high at the beginning of the experiment but decreased at the middle of experimental period. For about 17 days, the end of feeding activity corresponding with the end of dark phase had moved forward day by day showing somewhat free running like rhythm and the activity profile had changed from nighttime to daytime feeding pattern taking about several days for this alteration. Thus, from day 23 rearing period until the end of experiment, the self-feeding pattern resynchronized to the given of photoperiod resulting in clear and stable diurnal feeding pattern (Fig. 2.1a).

In case of W-Expt, one representative fish trial (NT-7) showed a clear and constant feeding activity from the beginning until the end of experiment. Feeding activity was consistently performed only during the dark period throughout the experiment (Fig. 2.1b).

Circadian rhythms of Nile tilapia under self-feeding system

After all trials reared in constant environmental condition (LD 12:12), several experiments such as; light-dark cycle (LD 12:12) with moving backward the onset, constant lightness cycle (LD 24:0), and constant darkness cycle (LD 0:24) were conducted to examine the biological clock of self-feeding rhythms. Most of fish confined their feeding activity and synchronized with the given LD 12:12 photoperiod condition. However, the self-feeding of all Nile tilapia in A-Expt was unevenly distributed throughout the constant light phase in LD 24:0 condition and that of all individuals in W-Expt, unevenly distributed throughout the constant dark phase in LD 0:24 condition except one individual (NT-5).

At the beginning of experiment (phase 1), NT-5 fish was exposed with constant light-dark cycle (LD 12:12) and started to activate the feeder within 13 days. The self-feeding pattern synchronized with the given photoperiod resulting a clear and stable nighttime feeding activity for about 20 days. Then, the LD 12:12 cycle was replaced by constant darkness LD 0:24 cycle (phase 2). Under this condition, the phase transient of self-feeding activity was occurred, the onset of diel self-feeding activity was progressively shifted backwards to become indifferent feeding profile. It was occurred after 2-3 days and displayed a clear free-running rhythms persisted for about 10 days. Consequently, the feeding profile showed somewhat unstable for several days. The transient free-running rhythm of feeding activity are shown in Fig. 2.2. The actogram at left side represent feeding pattern of NT-5 fish and the chi-square periodogram analysis for the self-feeding activity is shown at the right area of Fig 2.2, respectively. The period (τ) of free-running rhythm was 24.4 hr.

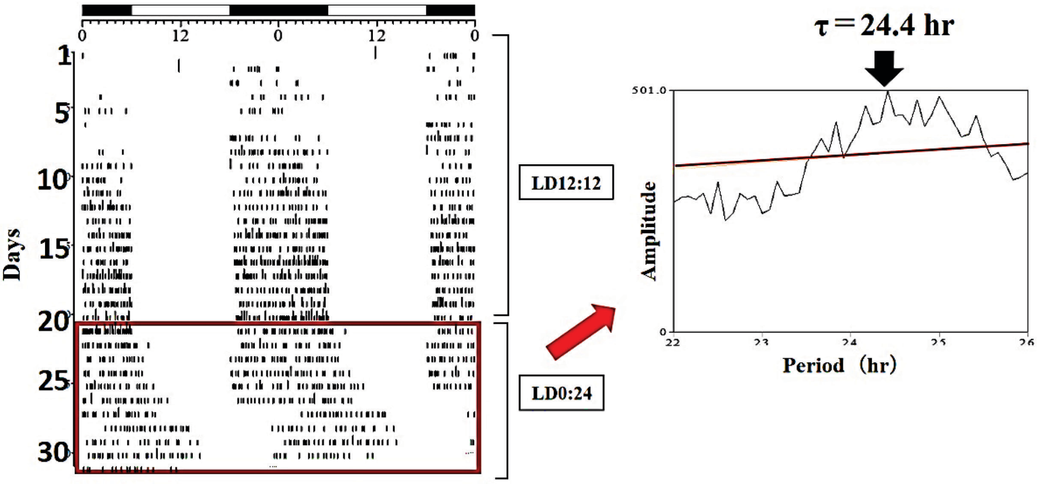

Fig. 2.2 (a) Temporal distribution of self-feeding records from a representative individual (NT-5) of winter experiment (W-Expt). The feeding pattern shows nighttime (nocturnal) feeding profile for 20 days under the LD 12:12 at the early period, however a free-running rhythm appeared immediately after shifting to continuous constant darkness (LD 0:24) cycle. The thin vertical bars placed on x-axis indicate the time of activating the self-feeder activation, and the height of the bar, frequency of the feeding activity within 5 minutes. The black and white bar at the top of bar graph indicates the light regime; black bar, dark phase: white bar, light phase; shaded bar, crepuscular phase, respectively. (b) Periodogram analysis of the feeding rhythms throughout the period calculated from the boxed area of actogram and showed the feeding rhythm period (τ=24.4) longer than 24 hours. The leaning dotted line indicates 95% confidence limit of variance.

The fish were exposed again to the LD 12:12 cycle (phase 3) for about 10 days and immediately resynchronized with the given photoperiod, the feeding activity occurred mostly in the light phase only. Then, the time of light on and off were set 6 hours forwards, the feeding pattern also moved towards the onset of the light within 15 days. When the fish re-entertained again with the LD 12:12 (phase 4), a free-running feeding activity was directly entrained by the light phase and after several days, the activity showed a clear daytime feeding activity within 20 days. Under the constant lightness (LD 24:0) cycle (phase 5), the phase transient of self-feeding activity was observed. Unlike previous free-running rhythm under constant darkness (LD 0:24) cycle (phase 2), this circadian rhythm showed forwards movement with a rhythm shorter than 24 hours (τ = 23.6). The transient free running rhythm during constant lightness condition (LD 24:0) is shown in Fig 2.3.

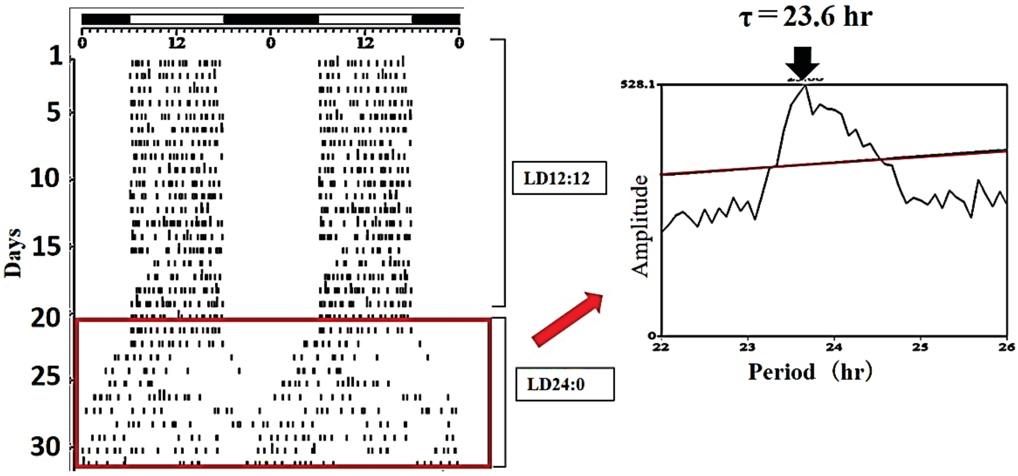

Fig. 2.3 (a) Temporal distribution of self-feeding records from a representative individual (NT-5) of winter experiment (W-Expt). The feeding pattern shows daytime (diurnal) feeding profile for 20 days under the LD 12:12 at the early period, however a free-running rhythm appeared immediately after shifting to continuous constant lightness (LD 24:0) cycle. (b) Periodogram analysis of the feeding rhythms throughout the period calculated from the boxed area of actogram and showed the feeding rhythm period (τ=23.6) shorter than 24 hours. Additional explanations, see Figure 2.2.

2.4 Discussion

The present study showed that the individual Nile tilapia learned to activate the self-feeding device quickly under constant environmental condition. Similar with individual fish rearing under insulated room experiment in Chapter 1. Since the study of Chapter 1 assumed that Nile tilapia have a dual phasing capacity of feeding profile corresponding to the seasonal change, the present study in this chapter revealed that Nile tilapia has a dualistic capacity for self-feeding both in light and dark phases under controlled environmental condition. Interestingly, the feeding profile varied throughout the experimental periods. According to Eriksson (1978), feeding profile classified into diurnal, indifferent, and nocturnal feeding activity. In autumn experiment (A-Expt), NT-1 fish showed the shifting feeding profile from indifferent to diurnal, NT-2 showed the shifting from diurnal to nocturnal, NT-3 showed the shifting form indifferent (nocturnal tendency) to indifferent (diurnal tendency), and aNT-4 showed a stable feeding profile of indifferent (diurnal tendency) throughout the experiment. In case of winter experiment (W-Expt), NT-5, NT-7 and NT-8 showed clear nocturnal feeding profile and NT-9 showed an indifferent (nocturnal tendency) until the end of experimental periods (Fig. 2.4). Nile Tilapia showed the tendency of diurnal feeding in the experiment which started in early autumn (A-Expt), while it showed the tendency of nocturnal feeding which started in early winter (W-Expt). Thus, the results of outdoor experiment observed in chapter 1 were able to be reproduced by the insulated room experiment and it seemed that a specific phase of the seasonal feeding variation in Nile tilapia was reflected for the feeding pattern of the insulated room experiments.

The revelation of phase transients and persistent free-running self-feeding rhythms in the present study suggests that Nile tilapia has a dual feeding profile. Exclusively, the free-running feeding rhythms started when the LD 12:12 cycle was replaced with the constant darkness (LD 0:24) or constant lightness (LD 24:0), this indicates that feeding activity controlled under a biological clock by the endogenous circadian oscillators. This phenomenon also reported feeding rhythms of greater amberjack *Seriola dumerili* (Chen et al., 2007).

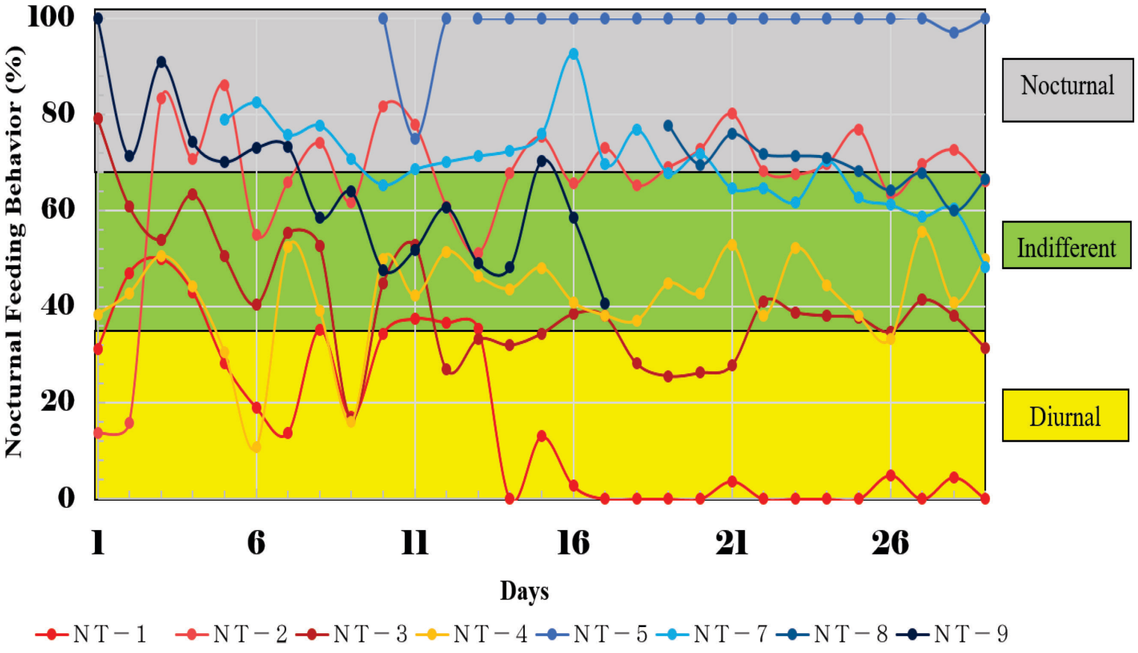

Fig. 2.4 Summary of feeding profile of Nile tilapia under controlled light condition (LD 12:12) of self-feeding system. The figure shows the period of nighttime (nocturnal) feeding activity and classified as nocturnal, indifferent, and diurnal, respectively. Based on Eriksson (1978).

Although Nile tilapia was firstly described as a diurnal animal, present study in line with previous studies have also reported nocturnal patterns under some condition (Toguyeni, et al. 1997; Vera, et al. 2009; Fortes-Silva, et al. 2010). This ability to display either diurnal or nocturnal behavior in the same species is known as dualism, which in fish is related to a flexible circadian system (López - Olmeda, et al. 2012). Moreover, the present study revealed this flexible circadian rhythm occurred in one individual fish. As far as is known, in fish, as well as in mammals, the circadian system may contain two independent oscillators, one entrained by a Food-entrainable Oscillator (FEO) and the other by a light-entrainable oscillator (LEO) as reported in goldfish and rainbow trout (Sánchez-Vázquez et al., 1995; Bolliet et al., 2001).

In addition, the feeding rhythm period in the present study showed two periods with difference length, one was longer than 24 hr when exposed to the constant darkness and another one was shorter than 24 hr when switched to the constant lightness. It also have been investigated in locomotor activity of Nile tilapia reported by Vera, et al. (2009), under constant darkness, males showed circadian period (τ) 24.1 hr and after switched into ultradian LD (45: 45 min) cycle some fish showed 23.9 hr. Different of circadian period also occurred in other species, such as catfish, the feeding rhythm period one from nine catfish which were exposed under continuous lightness condition (LD 24:0) showed less than 24 h and other were 24 h (Kasai et al., 2009).

Dual feeding rhythm period seemed related with dual capacity of feeding activity which is influenced by the endogenous pacemaker mechanism, which rhythms belongs to the type of system that are capable of self-sustaining oscillator and free-running rhythms persists for many periods (Aschoff, 1981). Fish might have two possible mechanisms, first, the existence of separate couple light and food oscillators or a single oscillator entrainable by both light and food. One of oscillators which entrained by light (LEO) might be located in pineal organ, since the main function of the fish pineal organ is to integrate light information and elaborate messages that will impact on physiology and behavior (Falcon et al., 2010).

2.5 Conclusion

In conclusion, the results of present study showed a dual capacity of feeding activity in single fish with the periodicity less than 24 hours (τ =23.6) under the continuous lightness and longer than 24 hours (τ=24.4) under the continuous darkness, suggesting that there may be two independent oscillators. Further study is necessary to investigate this possibility.

Chapter 3: Growth Performance of Male and Female Nile Tilapia Rearing under Self-feeding System

3.1 Background

In most commercial fish farms recently, automatic timer-scheduled feed dispensers have been introduced to supply the diet in order to compensate for the manpower shortage. The automatic feed dispenser is convenient, however, it does not deal directly with the appetite of the fish, and consequently, it may cause not only low feed efficiency and low growth performance but also the deterioration of the water environment quality by feed waste of aquaculture site. Alternatively, some studies have been carried out for a new method such as self-feeding system. Self-feeding is a new feeding method for aquaculture based on the learning ability of fishes through operant conditioning (Alanärä, 1992). In self-feeding, a feed dispenser releases a certain amount of feed into a fish tank or a net-pen according to the switch activation by fish. The fish can be exposed to a condition in which they can eat anytime as they prefer. First observations of self-feeding system as an aquaculture method have been done in rainbow trout *Onchorhynchus mykiss* (Landless, 1976). Development of self-feeding system have been progressed in many years such as utilities of recording device by computer (Boujard et al., 1992; Sánchez-Vázquez et al., 1994), individual self-feeding activity monitoring under group rearing condition (Alanärä and Brännäs, 1993), relationship between seasonal pattern of feeding and water temperature in net cages (Kohbara et al., 2003). Most of that, have been used for important commercial fish such as rainbow trout (Landless, 1976; Alanärä, 1992; Sánchez-Vázquez and Tabata, 1998; Chen et al., 2002), Florida pompano *Trachinotus carolinus* (Heilman and Spieler, 1999), yellowtail *Seriola quinqueradiata* (Kohbara et al., 2003), Sea bream *Sparus aurata* (Sánchez et al., 2009), European Sea bass *Dicentrarchus labrax* (Sánchez-Vázquez et al., 1994) and Ayu *Plecoglossus altivelis altivelis* (Amano et al., 2007) and so on.

In order to develop an efficient and intensive culture method with low environmental impact for Nile tilapia is important in world aquaculture activity. The aim of this study is to investigate the growth performance of Nile tilapia under self-feeding system and to make a valid application of self-feeding method to tilapia culture.

3.2 Materials and Methods

Experimental fish and condition

Nile tilapia were obtained from a substream of the Nikko River, Kanie town, Aichi Prefecture, Japan and reared in an outdoor concrete tank at the tank facility of the Graduate School of Bioresources, Mie University, Japan for acclimatization. After several weeks, fish were individually immobilized under 0.01% ethyl m-aminobenzoate methanesulfonate (MS-222, Sigma-Aldrich Co., MO, USA) anesthesia to weigh the body and to implant a passive integrated transponder tag (PIT-tag, Destron Fearing Co., TX, USA) to be identified individual fish. During each experimental period, fish were fed with a commercial diet for carp (Hikari, crude protein, 32%; crude lipid, 4%; crude fiber, 4%; moisture, 10%; ash, 12% , Kyorin Co., Ltd., Hyogo, Japan).

Individual experiment 1

The experiment was performed at the Fish Physiology Laboratory of Mie University, Mie Prefecture, Japan. The mean initial body weight (mean±SD) of fish was 58.28 ± 12.93g (n=6). Each fish was maintained in a 24l tanks (W24 cm x D40 cm x H25 cm) and was supplied with about 6-7 l/min of filtered and aerated water. These tanks were placed in a small insulated room (W180 cm x D90 cm x H90 cm) and the water temperature was regulated by a water cooler (Coolway 100, GEX Co. Ltd. Osaka, Japan) and a heater at 25ºC during the experimental period for 34-36 days. The light regime was controlled by fluorescent lamps with a LD 12:12 (L:06:00-18:00) photoperiod regime with 25 min crepuscular periods controlled by an electric light timer (Automatic light controller, Aqua Co. Ltd., Tokyo, Japan). The luminance was approximately 200-300 lx.

Individual experiment 2

The experiments were performed at the indoor tank facility of the Graduate School of Bioresources, Mie University, Japan. The initial body weight of fish was 73.37 ± 16.86 g (n=6). Each fish was individually reared in a 100*l* volume polyethylene cylinder tank for about 34-38 days. Each tank was supplied with 6～7 l/min of filtered and aerated water. Temperature was monitored during the entire trial and regulated by a water cooler (Coolway 100, GEX Co. Ltd., Osaka, Japan) and a heater at 25ºC during the experimental period 34-38 days.

Group experiment

The group experiments were conducted in an outdoor concrete tank and associated with the natural change of water temperature and light regime. The experiments were divided into triplicate groups consisting of thirty fish and each group was kept in an experimental concrete tank (2 m x 2 m and 1 m deep), respectively with consisting of two experimental seasons with two periods each. Experiment one (Expt1) was carried out from June 13- July 16 for first period (Expt1-P1) with the initial body weight was 98.26 ± 0.83 g reared for 34 days and continued to the second period (Expt1-P2) from July 19-August 22 which has initial body weight 216.17 ± 18.72 g reared for 35 days. In experiment two (Expt2), the period was started from August 30-October 4 for the first period (Expt2-P1) and was used different fish groups with period in Expt1, the initial body weight was 64.30 ± 0.00 g reared for 36 days, and continued to the second period Expt2-P2 from October 5-November 5 which has the initial body weight 211.00 ± 6.98 g reared for 35 days.

Self-feeding system and experimental set-up

In the individual insulated room experiment, a self-feeder (Yamaha Motor Co., Ltd., Shizuoka, Japan) with pull type switch (D2MV, Omron, Kyoto, Japan) was used and placed above each tank. At the tip of the stainless rod of switch, a small orange plastic ball (5 mm in diameter) was attached. The position of the plastic ball was located 1 cm below the surface of the water. The feeder delivered approximately 5 pellets in each time when a fish activated the switch, and the self-feeder sent signals to a microcomputer that continuously stored the event time data of feeder actuations. The stored data were treated by a chronobiology data acquisition and analysis program (Chronobiology Kit, Stanford Software Systems, Santa Cruz, CA, USA).

In the indoor experiment, an automatic feed dispenser (Food timer, Seiko Clock Inc., Tokyo, Japan) was used, after modifying to be driven by the control unit according to the signal from the switch actuation. The feeder delivered approximately 5 pellets in each time when the fish activated the switch. The pull type switch was the same as used in the insulated room experiment. The event time, during the feeder was activated by the signal from the switch, was stored in a portable data logger (HOBO Event, Onset Computer Co., MA, USA). The stored data was analyzed with the exclusive software (Boxcar Pro, Onset Computer Co., MA, USA) for the data logger.

In the group experiments the self-feeders (Shin-Nihon Ventures Co., Ltd., Osaka, Japan) with additional driven by the control unit (AFC-3 Adocom Electric Co., Ltd., Shiga, Japan) according to the signal from the trigger actuation was used. The pull-type switch was the same described in the insulated room experiment except the small orange plastic ball was 9 mm in diameter. The event time data from the switch was stored and analyzed as mentioned in the indoor experiment.

Growth performance analysis

The food demand was recorded during each period based on the fish adaptation to the self-feeder. The total amount of feed consumed by fish was obtained by deducting remainder feed from the total amount of loaded feed to dispenser. Fish were individually weighed after each experiment. Exclusively in group experiments, the final mean body weight (Wf) of Expt1-P1 were used for initial mean body weight (Wi) for Expt1-P2 and final mean body weight of Expt2-P1 were used for initial mean body weight for Expt2-P2. At the end of period Expt1-P2 and Expt2-P2, fish were dissected and sexed by both visual analysis of the gonads and genital papilla based on PIT-tag individual identification to separate the growth performance in male and female Nile tilapia.

Feed efficiency (FE) and specific Growth Rate (SGR) were calculated with the following equations:

FE (%) = ((final body weight (Wf) – initial body weight (Wi) / (total amount of feed consumed)) x 100

SGR (%) = ((ln (Wf)-ln(Wi))x100/number of days in one period

As the statistical analysis, t-test was used to determine if the body weight gain between males and females was significantly different using SPSS software.

3.3 Results

Growth performance

The results of growth performance of individual Nile tilapia in the insulated room experiment tended to be almost similar to that observed in the indoor experiment. The results showed that daily food supply of fish reared in the insulated room experiment was 2.38 ± 0.39% BW/day with FE and SGR were 109.67±21.38% and 2.81±0.61%/day. Daily food supply of fish in indoor experiment was 2.26±0.44% BW/day with FE and SGR were 103.23 ± 12.71% and 2.49 ± 0.58%/day (Table 3.1).

Table 3.1 Growth performance of individual Nile tilapia rearing under controlled conditions of self-feeding system (mean ± SD).

	Insulated room Experiment (24 *l* tank, W.T. 25.0ºC, LD 12:12)	Indoor Experiment (100 *l* tank, W.T. 25.0ºC)
Rearing period (days)	34-36	34-38
Initial BW (g)	58.28 ± 12.93	73.37 ± 16.86
Final BW (g)	154.32 ± 16.62	176.40 ± 23.99
FE (%)	109.67 ± 21.38	103.23 ± 12.71
SGR (%/day)	2.81 ± 0.61	2.49 ± 0.58
Self-service food supply (%BW/day)	2.38 ± 0.39	2.26 ± 0.44

In Expt1-P1, the initial body weight obtained from triplicate fish groups was 98.26 ± 0.83 g and the final body weight was 216.17 ± 18.72g. The body weight gain was almost 220%. In Expt1-P2, the initial body weight was 216.17 ± 18.72 g and the final body weight was 358.28 ± 28.97 g. The body weight gain was almost 166%. The mean value of SGR of Expt1-P1 and Expt1-P2 were 2.31 ± 0.24 %/day and 1.44 ± 0.09 %/day, respectively. The mean value of FE in Expt1-P1 and Expt1-P2 were 115.37 ± 20.42 % and 77.12 ± 3.02 %, respectively (Table 3.2).

In Expt2-P1, the initial body weight obtained from triplicate fish groups was 64.30 ± 0.00 g and the final body weight was 222.10 ± 14.89 g. The body weight gain was almost 345%. In Expt2-P2, the initial body weight was 222.10 ±

14.89 g and the final body weight was 272.70 ± 2.67 g. The body weight gain was almost 123%. The mean value of SGR of Expt2-P1 and Expt2-P2 were 3.45 ± 0.18%/day and 0.70 ± 0.16%/day, respectively. The mean value of FE in Expt2-P1 and Expt2-P2 were 118.90 ± 21.48 % and 72.94 ± 13.26%, respectively. The mean value of self-service food supply of Expt2-P1 and Expt2-P2 were 2.62 ± 0.35 %/BW/day and 0.95 ± 0.14 %/BW/day, respectively (Table 3.2).

Table 3.2 Growth performance of group Nile tilapia rearing under natural condition of self-feeding system (mean ± SD).

	Experiment1-Period1 (W.T. 24-32ºC)	Experiment1-Period2 (W.T. 29-33ºC)	Experiment2-Period1 (W.T. 25-30ºC)	Experiment2-Period2 (W.T. 17-25ºC)
Rearing period (days)	34 (Jun 13-Jul 16)	35 (Jul 19- Aug 22)	36 (Aug 30-Oct 4)	35 (Oct5-Nov8)
Initial BW (g)	98.26 ± 0.83	216.17 ± 18.72	64.30 ± 0.00	211.00 ± 6.98
Final BW (g)	216.17 ± 18.72	358.28 ± 28.97	222.10 ± 14.89	272.70 ± 2.67
FE (%)	115.37 ± 20.42	77.12 ± 3.02	118.90 ± 21.48	72.94 ± 13.26
SGR (%/day)	2.31 ± 0.24	1.44 ± 0.09	3.45 ± 0.18	0.70 ± 0.16
Self-service food supply (%BW/day)	1.97 ± 0.49	1.85 ± 0.06	2.62 ± 0.35	0.95 ± 0.14

Relationship between self-feeding activity and water temperature

Since the group experiments were carried out in outdoor, the relation between self-feeding activity and water temperature became clear under the course of the experiment. The daily number of feeder actuations was fairly constant and a gradual increment in the frequency of actuation was occurred according to the fish growth (Fig. 3.1). This constant feeding activity was observed when the water temperature higher than about 20ºC (Expt1-P1, Expt1-P2, Expt2-P1), however the feeding activity gradually decreased when the water temperature declined less than 20ºC at the end of Expt2-P2 (Fig. 3.2).

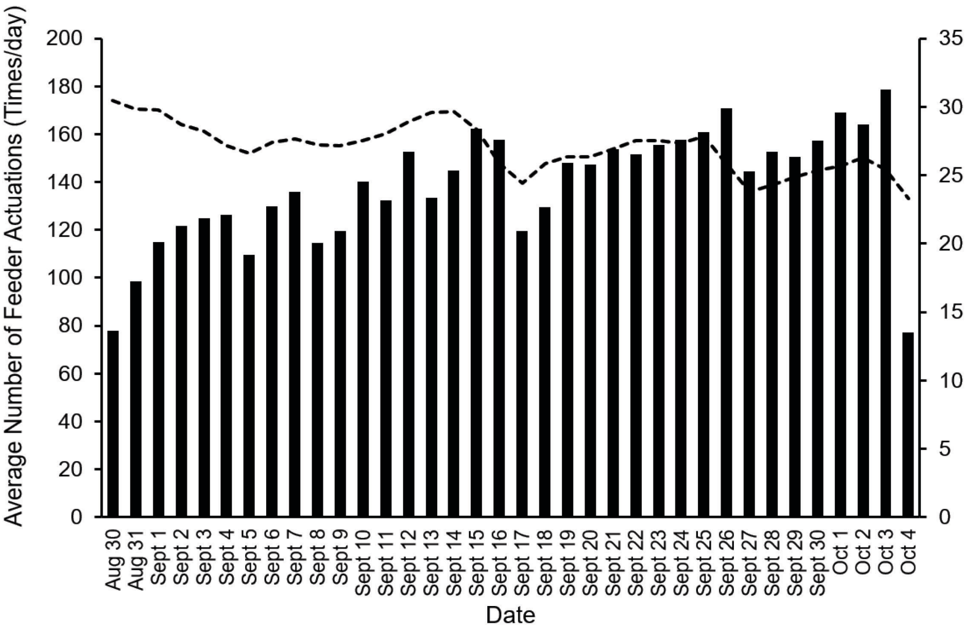

Fig. 3.1 Changes of the average number of daily feeder actuations by Nile tilapia in experiment two, period one (Expt2-P1) obtained from 3 groups. The changes of water temperature are superimposed upon the bar graph.

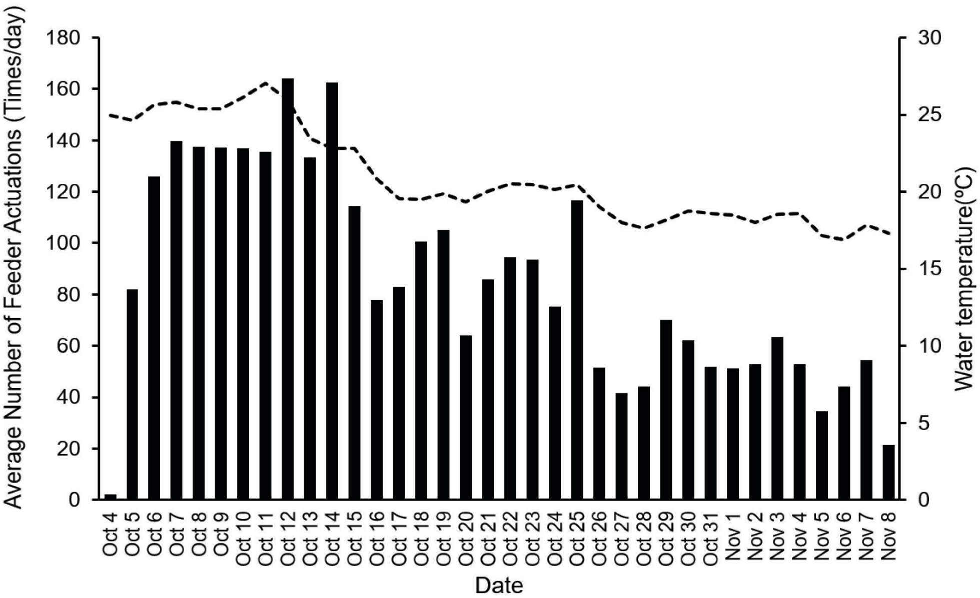

Fig. 3.2 Changes of the average number of daily feeder actuations by Nile tilapia in experiment two, period two (Expt2-P2) obtained from 3 groups. The changes of water temperature are superimposed upon the bar graph.

Difference between male and female in growth performance

The SGR of males and females were shown separately in Table 3.3. The initial body size was not different between males and females, however, SGR of males were significantly greater than those of females in both experiments (paired t-test, $p<0.05$). The body weight gain of males and female in group experiments is shown in Fig.3.3.

Table 3.3. Difference of specific growth rate (SGR) between male and female of Nile tilapia in group experiment (mean ± SD)

	Experiment1(n=3) (W.T. 24-33°C)	Experiment2(n=3) (W.T. 17-30°C)
Rearing period (days)	71 (Jun 13-Aug 22)	73 (Aug 30-Nov 8)
Male		
Initial BW (g)	96.81 ± 7.18	69.69 ± 1.61
Final BW (g)	403.44 ± 39.43	318.91 ± 16.71
SGR (%/day)	2.03 ± 0.21^{a}	2.18 ± 0.05^{a}
Female		
Initial BW (g)	99.03 ± 4.44	62.77 ± 1.28
Final BW (g)	315.76 ± 6.30	228.58 ± 4.03
SGR (%/day)	1.65 ± 0.06^{b}	1.75 ± 0.04^{b}

Different letter of SGR in each experiment indicates significant differences between males and females (paired *t*-test, $P<0.05$). Sex ratio of males and females in experiment 1 was 1:1.25 and in Experiment 2, 1:1.11

The increase of body weight observed during the period of Expt1 showed that in males which have initial body weight 96.81±7.18 g (W0) after being reared 34 days, the end of Expt1-P1 (W1), the body weight increased to 239.16±20.27 g. And thirty five days later, the end of Expt1-P2 (W2), the body weight reached to 403.44±39.43 g. In the case of females, the body weight also increased day by day. At the beginning of Expt1-P1 (W0), the initial body weight was 99.03±4.44 g and became 194.91±12.85 g after being reared for 34 days, at the end of Expt1-P1 (W1), it then further increased to 315.76±6.30 g at the end of Expt1-P2 (W2).

The increment of body weight also occurred in fish of Expt2. The experiment used differed groups of Nile tilapia from Expt1. The initial body weight of males was 64.69±1.61 g and that of females was 62.77±1.28 g (W0). These initial body weights were smaller than those fish in Expt1. At the end of Expt2-P1 (W1), the body weight of males increased to 243.04 ± 14.11 g and females became 182.19 ± 4.97 g. Even though the weights gain was significantly different (P<0.05) between males and females of fish in Expt2, the body weight gain of both males and females from the end of period one and/or the beginning of period two (W1) decreased until the end of Expt2-P2 (W2). Thus, the mean body weight were significantly greater (*P*<0.05) in males than in females in all treatments in group experiments (Fig. 3.3).

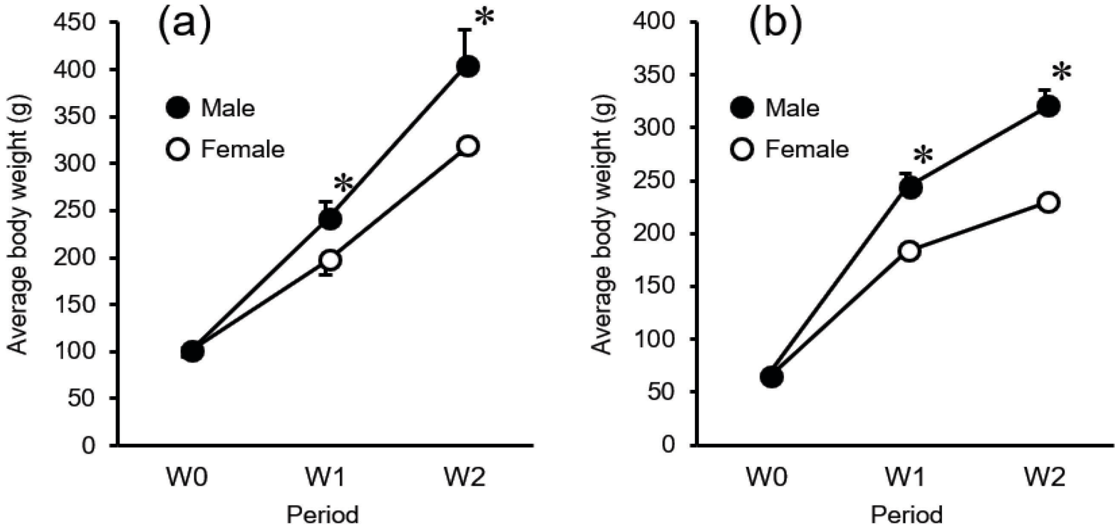

Fig. 3.3 Average body weight of male and female Nile tilapia from experiment one (a) and from experiment two (b). It showed that male body weight was greater than females at the middle (W1) and the end (W2) stage in both experiments.

Asterisks indicate the body weight of male is significantly greater than that of female (*t*-test, $P<0.05$).

3.4 Discussion

Nile tilapia can adapt and activate the self-feeder within a short period. Basically, the body weights of fish were increased during the experimental period. Comparing with the studies about the growth of tilapia, Essa (2000) investigated the difference of growth performance of hybrid tilapia (*O. niloticus x O. aureus*) by feeding methods that automatic feeding gave higher SGR (1.40%/day) than manual feeding (1.18 - 1.31%/day). Deen et al. (2010) used Nile tilapia with initial body weight of 100 ± 20 g under controlled water temperature (28ºC) by hand feeding within 45 days, and the SGR was about 0.90%/day. Workagegn (2012) investigated juvenile Nile tilapia (initial B.W. 4.19 g) under different water temperature conditions (24-34ºC) resulted range of SGR was 2.16-2.93%/day.

Thus, concerning about the factors which may give some influence on the growth performance in Nile tilapia including the present study, it is depending on size of initial body weights (Toguyeni et al., 1997), feed quality (Fortez-Silva et al., 2010) and environmental factors such as water temperature (Deen et al., 2010; Pandit and Nakamura, 2010; Workagegn, 2012). For example, the body weight and growth rate of young tilapia are higher than adult tilapia (Xie et al., 1997) and feed intake relatively decrease as the body weight increase (El-Sayed, 2006).

As shown in Table 3.1, in artificial condition with controlled water temperature and light regime (24*l* tank, 25ºC, LD 12:12), SGR was 2.81 %/day and FE was 109.67%. Another experiments in controlled water temperature (100*l* tank, 25ºC) showed similar SGR and FE with the results of 2.49%/day and 103.23 %, respectively. Therefore, the results of the growth performance obtained from the present study suggested that the self-feeding is a potential rearing method to grow Nile tilapia efficiently since the fish size used in the present study was larger than that used in the studies previously reported.

Another demonstration was showed in group experiment. During Expt1-P1 period, FE was ranging about 99.73-138.47%, while FE decreased to range about 73.85-79.80% in Expt1-P2. This seemed to originate for the increase of fish body weight and is reasonable result as mentioned above. However, SGR in Expt2-P2 were much lower than in Expt2-P1. The obvious difference in SGR in Expt2 might be strongly influenced by the environmental factors such as declining of water temperature which caused low feeding rate. As shown in Fig. 3.3, the number of daily feeding activity decreased gradually, when the water temperature became 20 °C or less. It possibly correlated that low temperature (lower than 20ºC) might be involved in the growth rate of this species and decrease the growth performance of Nile tilapia. Thus, the water temperature ranging 27-32ºC seemed to be appropriate for tilapia (Pandit and Nakamura, 2010; Workagegn, 2012).

During the outdoor experimental periods of Expt1 and Expt2, males and females tilapia were reared in the same tank and at the end of each period, the fish were individually weighed. In generally, sexual maturity of Nile tilapia is known to be reached at a size of 20-30 cm in natural conditions and under aquaculture conditions, tilapia mature at smaller sizes (El-Sayed, 2006). The minimum range of water temperature required for spawning is 22ºC and the optimum temperature for reproductions range 25-30ºC (Popma and Lovshin, 1995), therefore the experimental fish used in the outdoor experiments considered to be the mature fish. As shown in Fig. 3.3, it is clear that the final body weight of females is comparatively smaller than males, both in Expt1 and Expt2 ($P<0.05$). In addition, SGR of males obtained from both Expt1 and Expt2 is greater than that of females (Table 3.3). Toguyeni et al. (1997) reported that males may have a higher capacity to metabolize and digest than females which is related to the difference in growth rate between males and females. In addition, Bhatta et al. (2012) mentioned that the weight gain of females in spawning period became smaller than males due to a weak appetite which might be caused by the gonad development. However, the body weight gain of both males and females from the end of period one and/or the beginning of period two (W1) decreased until the end of Expt2-P2 (W2) (Fig. 3.3(b)). This is obvious that the low water temperature during Expt2-P2 gave a negative effect for the growth rate.

3.5 Conclusion

In conclusion, feeding diets by self-feeding system proved to attain growth performance comparable to other methods and seems to have the potential to be successful feeding method for Nile tilapia culture. Moreover, information obtained from self-feeding experiments enables us to identify the influence of environmental changes on a physiological condition of farmed fish through their expression of appetite. Interestingly, Endo et al. (2002) reported that the immune responses of Nile tilapia under self-feeding condition with range temperature 24ºC-26ºC showed that fish reared under self-feeding condition had a less stressful physiological status than in scheduled feeding and had a possibility for practical commercial fish farming. Further study such as determination of the most important environmental factors, such as the seasonal change of water temperature and/or day length, or a relationship between these two factors under self-feeding system in detail is necessary.

General Discussion

The present study showed that Nile tilapia has a capability to activate the self-feeder, both in individual and group treatments. Nile tilapia learned to operate the self-feeding device quickly. The findings suggest the possibility of applying a self-feeding system to deliver foods in Nile tilapia farming operations. As mention in chapter 1, adaptation of fish to the self-feeding system might be influenced by social interactions and environmental factors (Alanärä 1996). Results of present study showed that group of tilapia could activate the feeder on the first day without any artificial induction while individual treatments took about 2-9 days. Time to activate the feeder was different between individual and group rearing conditions. For instance, Landless (1976) also reported that groups of rainbow trout took 2 days to activate the feeder while a single rainbow trout took 7 days This is such a common results that assumed group of fish are quickly to learn self-feeding device than a single fish. Therefore, knowledge about feeding activity of Nile tilapia in details under a self-feeding system is needed if a self-feeding system is considered to be used for feeding the fish.

A long with a capability of adaptation to self-feeding system, this task can be difficult in fish since individuals from the same species can play a high flexibility in their daily feeding activity patterns. Some fish species can display diurnal and nocturnal feeding behaviors, in between, or shifting from one phase to the other, along their life cycle. This ability is known as “dualism”, and has been reported to be a common feature among fish (Eriksson, 1978). Many studies revealed about this flexibility such as; seabass (Sánchez-Vázquez et al., 1995), yellowtail (Kohbara et al., 2000), and sharpsnout seabream (Vera et al., 2006). The mechanism which drive dualism still are unknown, but Sánchez-Vázquez (1996) stated it has been suggested to be related to a high flexibility of circadian system, which allows animals to adapt quickly to environmental changes.

In line with the reports presented above, the present study also revealed that tilapia has a dual capacity corresponding to the seasonal change of light regime and/or water temperature. Our first report, the dual capacity happened in groups of experiment which exposed with the natural light regime and water temperature under self-feeding system. The feeding profile have shifted from diurnal to nocturnal when the daytime shorter than 10 hr and the water temperature decreased below 20°C. To better understanding about the environmental changes which may give some influences to the change of feeding profile of this species, detail observations about circadian rhythm system has been done and explained in chapter 2.

Under laboratory conditions, Nile tilapia from the same origin, maintained under self-feeding system individually. The results displayed diurnal feeding behavior in some tanks reared in the first season (late summer- early autumn) whereas, simultaneously, in all fish from the second season (late autumn-early winter) displayed nocturnal feeding behavior. Under this controlled environmental conditions (W.T 25°C; LD 12:12), it was possible to reverse the phase of feeding behavior in some fish to the opposite feeding phase selected spontaneously. It seems related with the feeding phase in wild condition, since these experimental fish taken from a wildlife condition near the hot spring spot. Conceivably, some fish maintained their demands in the phase of no-reward and indicated to feed out of their spontaneously selected feeding phase (Sánchez-Vázquez et al., 1995). Dualistic feeding pattern in wild conditions is related to the seasons (López-Olmeda and Sánchez-Vázquez, 2010) as explained in outdoor tank experiments of chapter 1, which showed the feeding profile could be changed when the light regime became shorter and the water temperature decreased rapidly.

In addition, the fish shifted from one behavior to the other while the photoperiod was reversed. It showed a different feeding rhythm period in one individual fish when constant light-dark condition (LD 12:12) was manipulated to different long-short photoperiod (LD 24:0 and LD 0:24). Although the mechanisms that regulate the phase inversions of feeding pattern are still unknown, it is possible to hypothesize that a biological clock which driven by two independent circadian oscillators may be involved. According to Eriksson (1978), dualism has been suggested to be related to a highly flexible of circadian rhythm, the ability to shift the behavioral patterns; change from diurnal to nocturnal and *vice versa* at some stages of life history.

Beside the behavioral approach, the growth performances of Nile tilapia under self-feeding system are also important. As described in chapter 3, the growth performance of Nile tilapia rearing under self-feeding system were much more greater than under manual feeding system based on previous reports. Furthermore, the experiments were simulated the difference of male and female in growth performance terms. Males in natural condition of light regime and water temperature, obtained from both seasons (Expt1 and Expt2) are greater than females (Table 3.3 and Fig. 3.3). This study provided such a common evidence of growth performance in Nile tilapia. Supporting this, Toguyeni et al. (1997) and Bhatta et al. (2012) demonstrated this hypothesis that male tilapia seemed a good for fish farming. Besides that, additional nutritive aspects may give influence to improve the growth performance of this species, such as reported by Pratiwy et al. (2018) by adding *Sargassum* meal as a feed supplement 8% give the best growth performance without negative effect.

Most of all, in a self-feeding system, fish are assumed to be able to precisely control the feeding, simply activating a trigger sensor inserted into the water (Alanärä and Brännäs, 1996). In addition, attachment of the system of self-feeders to a computer system allows the feeding activity of the fish though seasons to be quantified, characterizing the feeding circadian rhythms, as well as the nocturnal, diurnal, crepuscular or even dual feeding habits.

Based on the present study, it suggested that Nile tilapia can use self-feeder quickly and efficiently. In Indonesia, mostly Nile tilapia cultured in cages and sometimes a feeding practice may cause a high level of feed waste and a severe self-pollution of aquaculture environment. Since, we found that tilapia has a dualistic feeding profile and this species may change the feeding profile corresponding to the biological clock, learning of utilization of this method may help the farmers to adapt self-feeding system for Nile tilapia farming. Besides that, nutritional preferences for micronutrients, such as vitamins, minerals, essential amino acids, or food additives are necessary to investigate.

Summary

As presently reported, the utilization of self-feeding system has been successfully applied for culture of several fish species. Finally, our findings Nile tilapia can use self-feeder quickly and efficiently to adjust food intake according to diet consumption, contribution to improve fish production and conservation of the water environment and aquaculture aspects. This method could potentially improve the water quality around the aquaculture stations and reduce an economic burden on fish culturists.

Information obtained from self-feeding experiments enables us to identify the influences of environmental changes on a physiological condition of farmed fishes through their expression of appetite, such as the feeding pattern which revealed on the present study showed that Nile tilapia has a dualistic feeding pattern depends on the change of environmental factors. The seasonal change of light photoperiod and/or water temperature gave some influence on the appetite of Nile tilapia. The feeding pattern changed from diurnal to more nocturnal under 10 hr day time and water temperature less than 20ºC. Moreover, circadian rhythm experiments showed that Nile tilapia has a dual feeding capacity which may controlled by different oscillators. This feeding technique also could increase the growth performance which suggested that male seemed fit for aquaculture production. Therefore, based on this knowledge, we will be able to give a proper feeding management for Nile tilapia.

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