

## Light-Dark and Food Restriction Cycles in Red sea bream, *Pagrus major*: Effect of Zeitgebers on Demand-feeding Rhythms

Yong-Gwon Choe\*, Jae-Eun Choi, Duk-Whan Roh, Bong-Seong Bae<sup>1</sup>  
and Cheol-Young Choi<sup>2</sup>

*Myongjin Marine Biotechnology Central, Kunsan 573-701, Korea*

<sup>1</sup>*National Fisheries Research & Development Institute, Pusan 619-902, Korea*

<sup>2</sup>*Department of Biological Science, The University of Calgary, Canada*

(Received June 2001, Accepted September 2001)

Red sea bream, *Pagrus major* a fish species characterized by its dualistic feeding pattern, was investigated to study the synchronizing effect of light and food on the demand-feeding rhythm. Nocturnal and diurnal red sea bream, both in groups and individually, were exposed to restricted-feeding and Light-Dark cycles of different periods. The phase relationship between both zeitgebers was also studied. In some cases, food availability restricted to the light or dark phase contrary to that of the previous feeding phase changed a diurnal feeding pattern into nocturnal and vice versa, suggesting that food can be one of the switching factors that decides whether the circadian system of red sea bream is diurnal or nocturnal. However, the fact that the feeding pattern of some fish was unrelated with the phase in which food was available suggests that other internal and/or external factors could be involved in the temporal flexibility of red sea bream.

**Key words:** Self-demand feeder, Demand-feeding, Feeding behaviour, Food restricted-feeding, Endogenous rhythm, Red sea bream

### Introduction

The animal circadian rhythm system shows two major properties: a capacity of synchronization to periodic variations of certain environmental factors known as synchronizers or zeitgebers; and an ability to generate demand-sustained oscillations when the animal lives in a stable environment without any temporal cue. For most animals, Light-Dark cycles of around 24 hours are the principal synchronizers; however, in many species periodic access to food is able to synchronize different physiological and behavioural variables (Boulos and Terman, 1980; Fuller, 1971; Potter et al., 1968; Suda and Saito, 1979). It should not be surprising that periodic access to food has a profound influence on animal behaviour and physiology. Under natural con-

ditions, food availability is one of the most important environmental factors that affects an animal's survival. To improve food acquisition, animals have developed several systems that synchronize their behavioural activities, hormonal levels and many other physiological variables to times of feeding. Thus, when food availability is restricted to a short time period, food utilization is maximal (Fuller, 1971; Potter et al., 1968; Ruskak, 1981; Suda and Saito, 1979).

Unlike in the case of mammals, information on this topic in fish is scarce. As observed in other vertebrates, most fishes have a circadian rhythm of locomotor activity (Suda and Saito, 1979) which, in some cases, appears to be feeding-entrained. The first in depth examination of feeding entrainment in fishes was made by Davis & Bardach in the early 1960s (Davis, 1963). Since that report, feeding entrained activity rhythms have been reported in several other species of fish (Choe, 2001a). Red sea

\*Corresponding author: choeyg@hotmail.com

bream, this dualistic behaviour was observed in different individuals held under the same fish could rapidly switch from one type of phasing to another. Although the mechanisms involved in the control of such dualism in red sea bream are still unknown, this flexibility might be interpreted as an adaptative response to variable food availability in the wild (Boujard and Leatherland, 1992). Whether red sea bream are able to synchronize their daily rhythms of food-demand to periodic access to food has never been studied before. And so the objective of this paper is to investigate the characteristics of feeding-entrained demand-feeding rhythms. In addition, we try to demonstrate that food restriction can act as a switching factor in controlling the diurnal, before and after the lights and nocturnal behaviour of this fish species.

## Methods

### Subject and Housing

Six groups of eight red sea bream, *Pagrus major* of 14 cm body length and eight red sea bream of 15 cm body length were distributed in two 300 L tanks and five 35 L tanks, respectively. The fish, purchased from local dealers, were housed individually and acclimatized to laboratory conditions for at least 3 months before the beginning of the experiment. The experiment was conducted in an isolated room under strictly controlled environmental conditions. The tanks were supplied with synthetic salt water (hw-Marinemix, Wiegandt GmgH Germany) of 2.3‰ salinity and  $25 \pm 0.2^\circ\text{C}$  temperature.

Single fish were held in light-tight cylindroconical tanks with an individual light source attached to the cover. The light was provided by a daylight blue bulb (osuran, Korea, 60 W) located at the top of the tank 70 cm from the water surface (500 lux). The Light:Dark (LD) cycle was programmed by means of an electronic timer (National Model TE-331). Each single-fish tank was equipped with a demand-feeder which delivers one food pellet at each food request while a commercially available feeder (Choe et al., 2001a, b, c, d) which supplies several pellet at each operation (approx. 1 g of food) was installed on fish-group tanks. A commercial diet of the recommended pellet size for red sea bream (Tunoda Co.) was used in this experiment. The arrangement of

the demand-feeding device was essentially the same as that described elsewhere (Choe et al., 2001c). The food-demand sensor was located 1.5 cm below the water surface to facilitate the detection of the rod during darkness. Events of food requests were recorded and stored in 0.04 sec intervals by data collecting device (Keyence Model NR-350), which controlled the activation of the feeder. The timing of food availability was software-scheduled so that, when a time-restricted feeding (TRF) cycle was imposed, the food demand signal was recorded but food was not delivered in the restricted-feeding phase. Since no restriction in the demand-feeding rate (as regards quantity of food to be dispensed) was established. Fish were given free access to food during the phase of food access of the TRF cycle.

## Experiment

In this, we studied the effect of different phase relationship between the Light:Dark (LD) and time-restricted feeding schedule. Initially, fish were exposed to an LD 12:12 h (lights on at 06:00 and off 18:00) and given free access to food and demand-feeding libitum throughout the day (A). Once the daily pattern of feeding was well defined, fish were submitted to a TRF 12:12 h cycle (B). The TRF cycle was scheduled individually so that food availability was restricted to the light or dark phase contrary to that of the previous feeding phase. In other words, diurnal fish were allowed to feed only at night and nocturnal fish during the day, so that, fish were forced to change their feeding phase and thus their daily pattern of behaviour. After fifteen days under this restrictive feeding schedule, fish were allowed to feed again, so that, food was available from 10:00 to 14:00 (4 h after switching the lights off) for nocturnal fish and from 22:00 to 02:00 for diurnal fish (4 h after lights on). Twenty days later, animals were left under the same an LD 12:12 h cycle (C).

## Results

Red sea bream exposed to an LD 12:12 h cycle and demand-fed exhibited a diurnal phasing of their diel feeding pattern. One half of the fish fed

mostly during the daytime, and the other half during the nighttime. We found 3 cases (1 group and 2 single fish) of nocturnalism (Fig. 1A, Fig. 2A.), 5 of diurnalism (3 groups and 2 single fish), (Fig. 1B, Fig. 2B.) and 2 of two peaks of activity after and before the lights were switched on and off, respectively (2 single fish), Fig. 2C. The absence of alimentary reward led to an increase in the daily number of demands, defining the nocturnal or diurnal character of some fish. After the passage of several days, the majority of the diurnal fish and some of the nocturnal fish rapidly reversed their food-demand pattern (Fig. 3). When the demands were made in the phase when food was available, their total number fell to the levels which existed

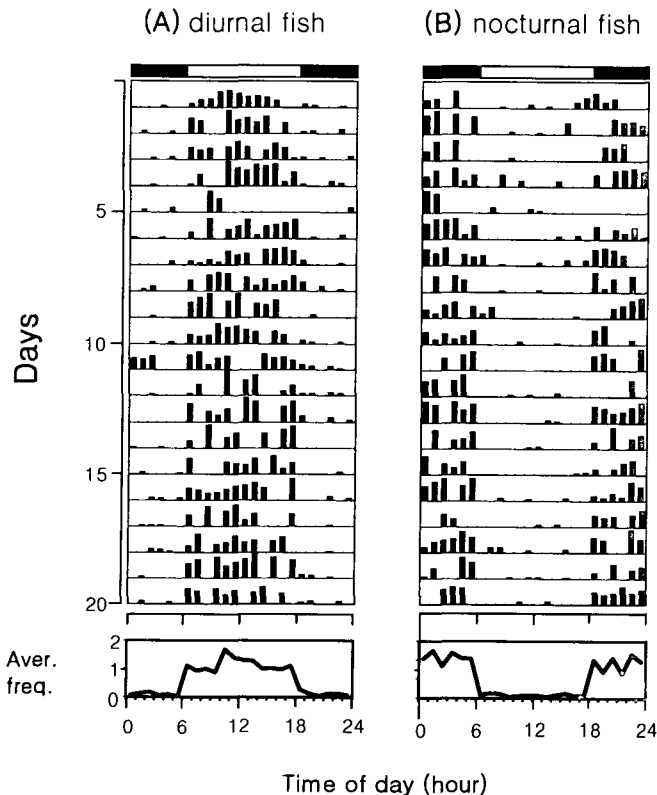


Fig. 1. Actograms of food-demand records from eight-fish groups during experiment 2. The fish were submitted to an LD 12:12 h cycle for 20 days. The number of requests for food made by fish daily (averaged daily waveform of demand-feeding activity) is plotted on the down of the actogram. Horizontal solid and open bars at the top of the graph represent the lighting schedule. Food-demand records of diurnal fish (A) and nocturnal fish (B).

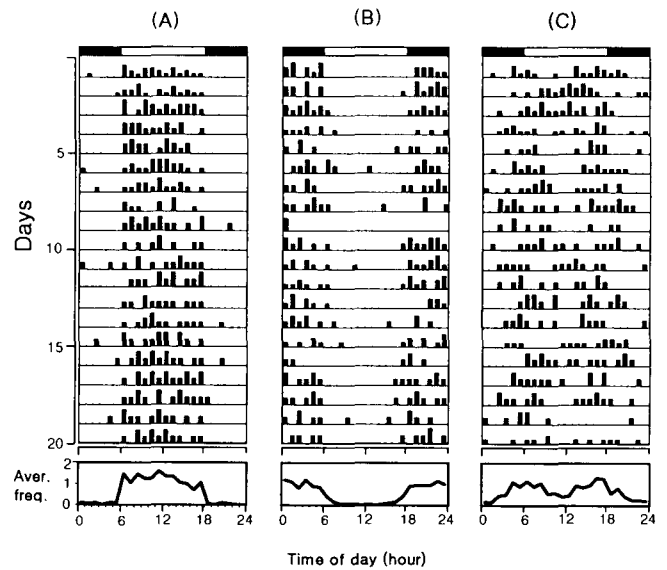


Fig. 2. Actograms of food-demand records from single fish during experiment 1. The fish were submitted to an LD 12:12 h cycle for 20 days. The number of requests for food made by fish daily (averaged daily waveform of demand-feeding activity) is plotted on the down of the actogram. Horizontal solid and open bars at the top of the graph represent the lighting schedule. Food-demand records of diurnal fish (A) and nocturnal fish (B) and two peaks of activity after and before the lights were switched on and off, respectively (C).

before the restriction was imposed. It seems surprising that most of the strongly nocturnal cases stubbornly refused to adapt to the restrictions and continued to demand food during the night throughout the period of restriction (Fig. 4). When the restriction was lifted, the fish continued to demand food during the same phase in which they were making their demands at the end of the restricted period. This means that, once they shifted from one type of phasing to another in accordance with the imposed TRF cycle, the change in the daily pattern of behaviour was stable and no cases of reversion were detected.

### Discussion

Many papers have attempted to distinguish between feeding time and light/dark alternation in fish, and to evaluate whether either or both zeitgebers

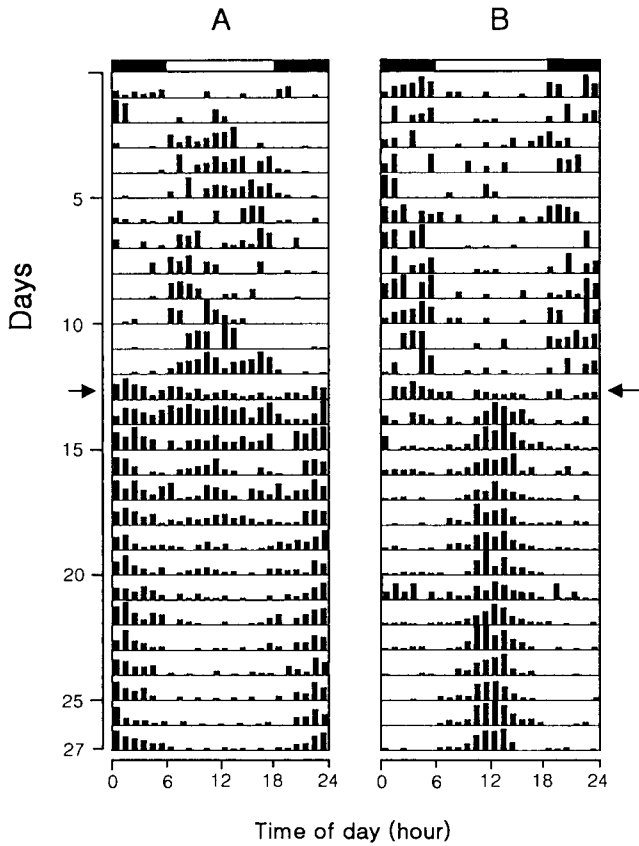


Fig. 3. Actograms of food-demand records from eight fish groups during experiment 2. On day 13, fish were submitted to an TRF 12:12 h cycle so that food was only available in the Light or dark phase contrary of their prior feeding phase. Food access times during restrictive feeding are delimited by a rectangular box. Horizontal solid and open bars at the top of the graph represent the lighting schedule. Two cases of diurnal and nocturnal fish that changed to nocturnalism and diurnalism, respectively (A and B).

can act as synchronizers, and if so, whether one of these zeitgebers has greater influence than the other (Spieler, 1992; Sanchez-Vazquez et al., 1994a; 1994b). However, those studies published to date show some different characteristics to those of our experimental design:

The flexibility of the circadian system is a general characteristic in fish. It has been suggested that the stability of aquatic environmental conditions, which is much greater than the terrestrial counterpart, should permit fish to adapt more easily to temporary variations in the food supply (Boujard et al., 1993; Daan, 1981; Potter et al., 1968). This should be particularly

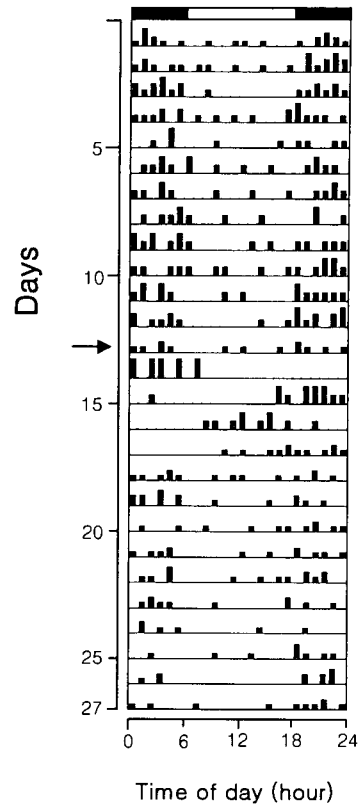


Fig. 4. Actograms of food-demand records from single fish during experiment 2. On day 13, fish were submitted to an TRF 12:12 h cycle so that food was only available in the Light or dark phase contrary of their prior feeding phase. Food access times during restrictive feeding are delimited by a rectangular box. Horizontal solid and open bars at the top of the graph represent the lighting schedule. One case of nocturnal fish in which food deprivation did not induce a shift in phasing.

true in the case of species like red sea bream with their dual phasing behaviour. The frequent phase changes of this fish might have an adaptive sense which would permit it to eat during the night or daytime with equal ease. However, our results do not entirely support this theory. The animals permitted access to food during the 12 hours in which they previously showed a minimum demand activity were not always able to reverse their pattern of behaviour even after 15 days of restricted access. This was particularly evident in nocturnal animals which displayed the strictest pattern of behaviour. This asymmetry in inverting their behaviour was less pronounced in fish kept in groups. Further evidence

of the relative weakness of restricted feeding as the only factor responsible for the phase changes in red sea bream is provided by the results obtained during the simultaneous exposure of the animals to conflicting zeitgebers (i.e. LD 12:12 h and TRF cycles). In these conditions, the phase coincidence between both cycles was fifteen days, meaning that the light phase during which food was available changed every seven days. In spite of this, no cases of inversion of feeding behaviour was observed, suggesting that the dualism of the circadian rhythm in red sea bream is not a characteristic which is given exclusively to aiding the rapid adaptation to the limited availability of food. The nocturnal and diurnal feeding patterns in red sea bream seem equally stable and food restriction, rather than encouraging an opportunistic activity at a given moment, seems to change the organization of the circadian system. When the restricted availability of food (TRF cycles) is superimposed on an Light:Dark (LD) 12:12 hours cycle, there is a very strong anticipatory activity in the 2~3 hours before the food availability, which is much stronger even than the activity which takes place during the feeding area. These results suggest that, although food has a strong synchronization effect. It cannot be concluded that the animal has an independent oscillator since red sea bream might be capable of predicting the feeding time by simply memorizing the interval of time which elapses between switching on/off the light and food availability (learning process). Another explanation of the synchronization induced by food could be that the animal uses an hourglass type mechanism based on digestive or metabolic processes which would rest every day by food. However, a variety of evidence permits us to preclude this mechanism. The results of experiment 1 show that many fish continue to demand food at the same time day after day despite the fact that they are kept food deprived.

The results reveal that red sea bream is able to synchronize its food demand activity to periodic food access, showing a partial coupling with Light:Dark (LD) synchronized activity. Moreover, restricted feeding is able, in some cases, to change diurnal behaviour in to nocturnal and vice versa, suggesting that feeding can be one of the factors which helps decide whether the circadian rhythm of red sea

bream will be nocturnal or diurnal. However, the fact that many nocturnal fish did not invert their demand feeding rhythms after food availability was restricted to daylight reveals that dualism in red sea bream may not be exclusively directed at facilitating feeding.

Finally, it should not be concluded from reading this report that fish are simply endowed with oscillators which may or may not be entrained by food or light. The flexible circadian system in fish, which is probably based on the existence of several oscillators weakly coupled to each other, shows a structural and functional complexity which is difficult to imagine. A fish group, for instance, could be seen as a population of individual oscillators that interact with each other and produce a complex output. We should set aside all previous judgements based on the study of mammals and develop new and imaginative methods to study the biological rhythms of this group of vertebrates.

## References

- Boujard, T. and J.F. Leatherland. 1992. Circadian rhythms and feeding time in fishes. *Environ. Biol. Fish.*, 35, 109~131.
- Boujard, T., S. Brett, L. Lin and J.F. Leatherland. 1993. Effect of restricted access to demand-feeders on diurnal pattern of liver composition, plasma metabolites and hormone levels in *Oncorhynchus mykiss*. *Fish Physiol. Biochem.*, 11, 337~344.
- Boulos, Z. and M. Terman. 1980. Food availability and daily biological rhythms. *Neurosci. Biobehav. Rev.*, 4, 119~131.
- Choe, Y.G., J.E. Choi and D.W. Roh. 2001a. Demand-feeding behaviour and diel cycle of feeding activity in carp *Cyprinus capio*. *J. Fish Sci. Tech.* (Submitted).
- Choe, Y.G., J.E. Choi, D.W. Roh and C.Y. Choi. 2001b. Demand-feeding and locomotor activity in red sea bream, *Pagrus major*. *J. Fish. Sci. Tech.* (Submitted).
- Choe, Y.G., J.E. Choi, D.W. Roh and C.Y. Choi. 2001c. Design and performance of an accurate selfdemand feeder and infrared sensor for the study of locomotor activity and feeding behaviour in red sea bream, *Pagrus major*. *J. Korean Fish. Soc.* (Submitted, in Korean).
- Choe, Y.G., J.E. Choi, D.W. Roh and D.G. Ryu. 2001d. Effect of water temperature on diurnal demand-feeding activity of red sea bream, *Pagrus major*. *Bull. Korean Soc. Fish. Tech.* (submitted, in Korean).
- Daan, S. 1981. Adaptive daily strategies in behavior. *In Handbook of behavioural neurobiology, Biological rhythms*. Vol. 4, J. Aschoff, ed. Plenum Press, New York, pp. 275~298.

- Davis, R.E. 1963. Daily "predawn" peak of locomotor in fish. *Anim. Behav.*, 12, 272~283.
- Fuller, R.W. 1971. Rhythmic changes in enzyme activity and their control. In *Enzyme synthesis and degradation in mammalian systems*. Basel: Karger. pp. 311~338.
- Potter, Van R., E.F. Baril, M. Watanabe and E.D. Whittle. 1968. Systematic oscillations in metabolic function in liver from rats adapted to controlled feeding schedules. *Fed. Proc.*, 27, 1238~1245.
- Ruskak, B. 1981. Vertebrate behavioural rhythms. In *Handbook of behavioural neurobiology, biological rhythms*. Vol. 4, J. Aschoff, ed. Plenum Press, New York, pp. 81~93.
- Sanchez-Vazquez, F.J., S. Zamora and J.A. Madrid. 1994a. Daily feeding activity pattern in sea bass and the effect of light-dark cycle shifting. *Eur. J. Physiol.*, 427, R35.
- Sanchez-Vazquez, F.J., S. Zamora and J.A. Madrid. 1994b. Evidence for an endogenous component in the circadian feeding rhythm of sea bass exposed to light-dark pulses. *Eur. J. Physiol.*, 427, R36.
- Spieler, R.E. 1992. Feeding-entrained circadian rhythms in fishes. In *Rhythms in fishes*. M. Ali, A., ed. Press, New York, pp. 137~147.
- Suda, M. and M. Saito. 1979. Coordinative regulation of feeding behaviour and metabolism by a circadian timing system. In *Biological rhythms and their central mechanism*. M. Suda, O. Hayashi and H. Nakagawa, eds. Elsevier, New York, pp. 263~271.