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Demand-feeding and Locomotor Circadian Rhythms in the Red sea bream, *Pagrus major*

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In the present study, the locomotor and feeding activities of single red sea bream, *Pagrus major* were simultaneously investigated to examine the existence of such dual behaviour. Seven red sea bream of 13 cm body length on average were placed individually in 35 L tanks equipped with an infrared sensor and a newly developed demand-feeding device. Fish were exposed to a light:dark 12:12 h cycle and constant darkness (DD) to study endogenous rhythmicity. Under LD 12:12 h, the daily pattern of behaviour differed between individual fish; some red sea bream were diurnal and others were nocturnal. Futhermore, some of them displayed an extraordinary flexibility in phasing because they were dark active but light feeding, and vice versa. Under DD, red sea bream showed free-running rhythms for locomotor activity and feeding. These results indicate that the type of phasing of locomotor activity did not necessarily decide the feeding phase; much of this is explained by the fact that red sea bream were demand-fed. Flexibility in phasing and a certain degree of independence between locomotor and feeding activities could be seen as an adaptative response of the highly adaptable circadian rhythms of fish.

Key words: Self-demand feeder, Demand-feeding, Locomotor activity, Feeding behaviour, Endogenous clock, Red sea bream

Introduction

In the course of evolution, and under a cyclic environment governed by the earth's rotation, an internal timing mechanism has appeared in most living organisms. The use of an endogenous clock is clearly advantageous because recurrent events such as the daily cycle of light and darkness can be predicted (Daan, 1981). Animals usually synchronize with their surroundings and, as a result, display a daily rhythmic pattern of behaviour. In the case of some fish, a particular feature has been found in their time-keeping mechanism, which is not seen in higher vertebrates: a highly flexible circadian system that is capable of changing its phasing (Eriksson, 1978). In contrasting to the common ways of life of most other vertebrates (i.e., diurnalism or nocturnalism), the daily distribution of behaviour in those fishes is not always rigidly confined to the light or dark phase. A diurnal fish may become nocturnal, and vice versa, at certain times, usually on a seasonal basis. Changes in phasing related to variations in photoperiod (Muller, 1978), light intensity (Eriksson, 1978), and water temperature (Fraser et al., 1993; Choe et al., 2001c) have been reported. A further of the dual phasing behaviour is the fact that, at least in some fish species, diurnal and nocturnal fish can coexist under identical experimental conditions (Sanchez-Vazquez et al., 1995).

The endogenous origin of the daily rhythmic pattern of behaviour is commonly seen under constant conditions (e.g., constant darkness), in which animals lose any external time reference and their circadian rhythms begin to free-run (Aschoff, 1981). In higher vertebrates, free-running rhythms of a host of behavioural (e.g., wheel running, feeding, drinking) and physiological(e.g., body temperature,

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hormones) variables have been extensively reported (Moore-Ede et al., 1978). On the other hand, although there have been many articles, describing rhythmic activities in many fish species (Choe et al., 2001a; 2001d; Sanchez-Vazquez et al., 1995; Bjornsson, 1994; Cuenca and de la Higuera, 1994; Landless, 1976), the circadian system of fish remains relatively little explored. In the case of red sea bream, *Pagrus major*, a fish of great commercial interest in Korea and Japan regions, is of particular interest for chronobiological research since a diurnal phasing capacity of diel demand-feeding rhythms has recently been studied (Choe et al., 2001a; 2001c), but little is known about the behavioural rhythms of this fish species.

In the present article, we recorded simultaneously the daily pattern of feeding and locomotor activities of single red sea bream. Demand-feeding behaviour was monitored by means of a demand-feeding device so that events of food demands, together with locomotor activity counts, were fed into a data collecting system. Special attention was paid to the timing of both rhythms to test the existence of a pattern for both activities. The phase relationship between them and the role of feeding in locomotor rhythms were also investigated. The endogenous nature of feeding and locomotor circadian rhythms was studied under constant darkness conditions.

Methods

A total of 14 (mostly female) red sea bream, *Pagrus major* with a mean body length of 13 ± 2.2 cm was used. The fish, purchased from local dealers, were housed individually and acclimatized to laboratory conditions for several months before experimental use. Solitary individuals were placed into 35 L glass tanks (60×45×45 cm) equipped with filter installed on one of the long sides of the tank. The study was conducted in two conditioned chambers in which constant ambient temperature (23℃) was maintained throughout the experiments by means of independent air cooling and heating devices. To prevent fish from seeing outside their own tank, each aquarium was covered with a dark vinyl sheet. At irregular time intervals (between 3 and 6 days and at different times), each aquarium was cleaned, and the water renewed (one-fourth of the bottom and keep the water in good condition. The cleaning process usually lasted less than 5 min and had no apparent effect on fish as they had repeatedly experienced this routine before the onset of the experiment. The glass tanks were supplied with synthetic salt water (hw-Marineemix, Wiegandt GmgH-Germany) of 2.3% salinity.

Illumination was proved by an overhead fluorescent lamp (National, 15 W) mounted above the glass ceiling of each aquarium. The mean light intensity was on average 500 lux at the water surface. An electronic timer automatically controlled the lighting schedule in each chamber.

Feeding and activity monitoring

Because both demand-feeding and locomotor activities were simultaneously investigated in the present study, each tank was equipped with a demand-feeding device and an infrared sensor. A schematic view, showing the arrangement of both systems around the aquarium, is represented in Fig. 1.

The newly developed demand-feeding device is composed of the sensor for detecting food demand and the food dispenser and, the food demand sensor consists of a hanging rod with a ball attached at the end so that fish can operate the ball in any direction (Choe et al., 2001b). To make fish feeding easier in total darkness, the rod was deliberately placed approximately 1.5 cm below the water surface, in the central of the tank and just beside the

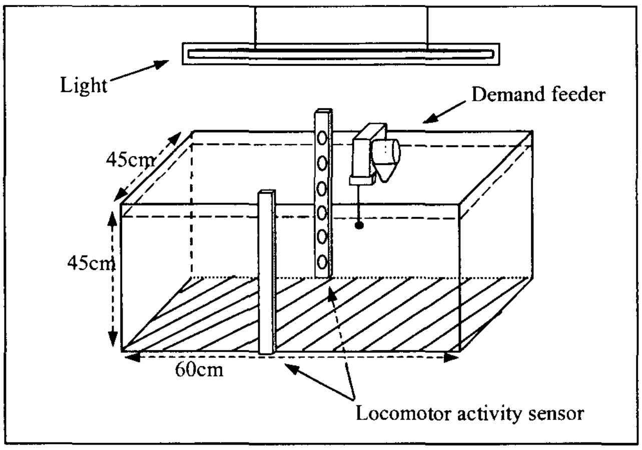

Fig. 1. Arrangement the infrared sensor for detecting locomotor activity and the sensor for food demand around the aquarium.

food dispenser. When a food request is made by fish, an type of rod switch sensor, by hitting the ball attached to the movement of the rod can activate the feeder. The food dispenser used was a modified commercial feeder for pet fish (Food Timer, Seiko Co., Japan). The feeder was adjusted to distribute approximately 0.6 g of food (around 1 pellet) at each operation. A commercial diet of the recommended pellet size for red sea bream was used in this experiment. To avoid interference with feeding activity and locomotor activity was recorded by an switch sensor and infrared sensor installed on the both of the long sides of the aquarium near the corner to the demand feeder, respectively. Each time the red sea bream swam in that zone, it interrupted a light beam, thus generating an output signal. The signals of food demand and locomotor activities were transferred via an interface to a data collecting system that simultaneously, recorded and stored the data in 0.01 sec bins.

Experimental design

The red sea bream were basically maintained under conditions similar to those described above and studied demand-feeding and locomotor activities under a regular Light:Dark (LD) 12:12 h cycle. The demand-feeding device was installed on the tank and both locomotor and feeding activities were recorded. In addition, the relative position of the rod for detecting food demand (i.e. above and below the water surface) was studied. At the end of the experiment, the rod, which hung 1.5 cm below the water, was replaced 5 mm above the surface to study the influence of its relative position on demand-feeding behaviour. After the beginning of the experiment, the circadian rhythms of feeding and locomotor activities were recorded under different light conditions to investigated the existence of endogenous rhythmicity. The fish initially exposed to a regular LD cycle were transferred to constant darkness (DD conditions) for 34 days.

Results

Demand-feeding behaviour

The performance of the newly developed demand-feeding device appeared appropriate for experimental purposes and the red sea bream rapidly learned how it functioned and operated the rod, which triggered the feeder. The usual behaviour of the fish consisted of a stroke of the rod followed by ingestion of the dropping pellets. We noted that inexperienced fish usually hit the rod with their head or back and collected food from the bottom, whereas when trained they pushed the rod with their mouth and caught the pellets near the surface. Fig. 2.

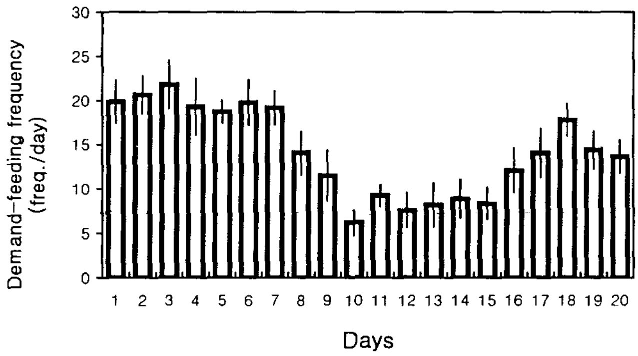

Fig. 2. Evolution of the daily demand-feeding frequency of single red sea bream, which had never experienced a demand feeder, after the installation of the feeder. Values are the mean ± S.D. of 14 red sea bream.

shows the evolution of the daily demand-feeding frequency of red sea bream that had never experienced the demand feeder. During the first 7 days, the red sea bream consistently demanded food and many food containers became empty. In the following days, they sharply decreased their daily demand-feeding frequency, probably owing to a compensatory behaviour after the previous high feeding period. From the second week on, they fed at a variable demand-feeding frequency around a half. We noticed large differences in both the mean daily number of food requests and activity counts among individual fish, ranging from 12 to 43 and from 103 to 462, respectively. Demand-feeding activity paralleled the demand-feeding frequency expect during the first 4 days, where the daily number of food to be dispensed per day and the food container became empty. A bout of activity occasionally appeared directly after the installation of the demand feeder; however, the profile of locomotor activity did not match demand-feeding activity.

When the rod, which hung 1.5 cm below the water surface, was raised to 5 mm above the surface,

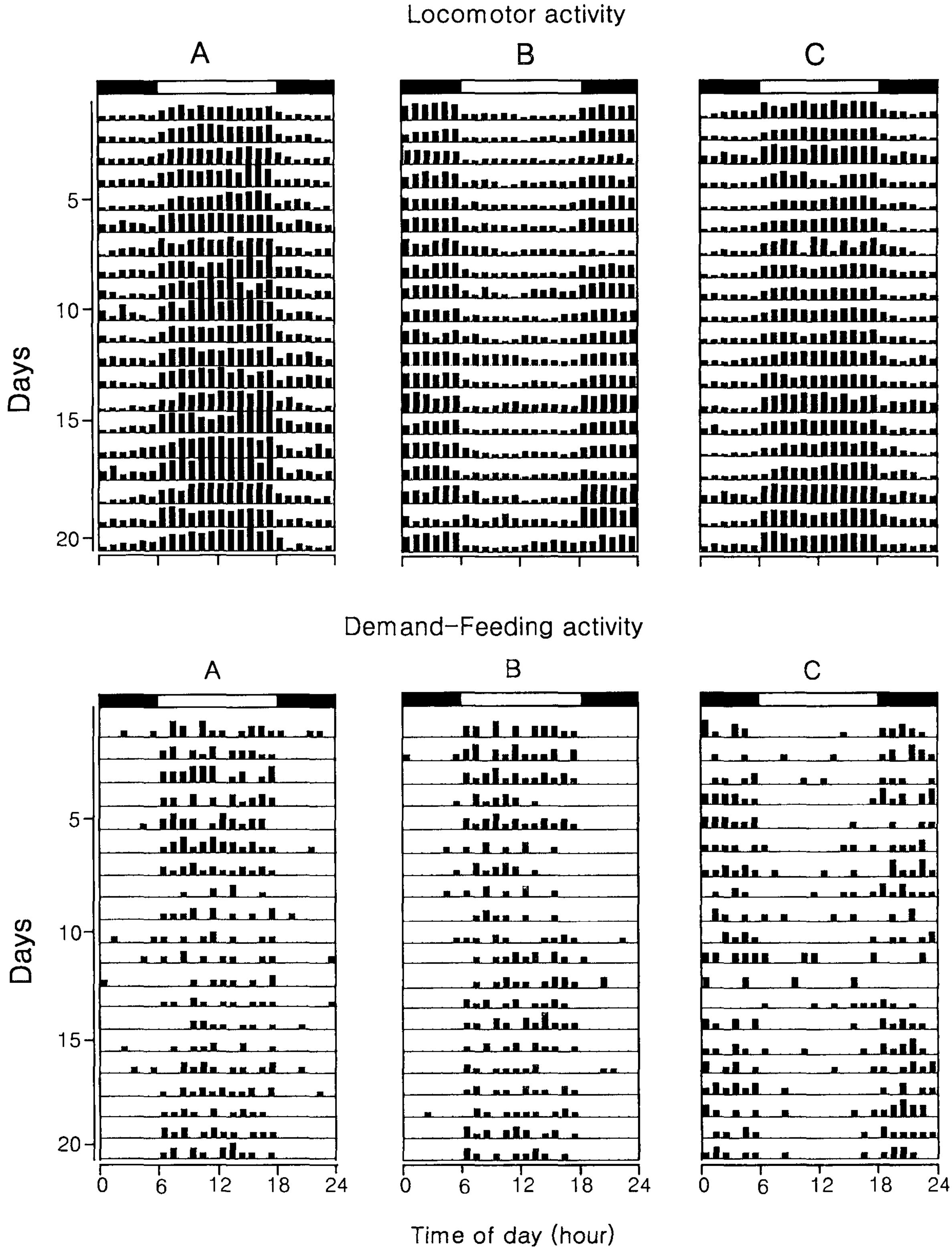

Fig. 3. Actograms of locomotor (above) and feeding (down) records of representative fish with diurnal activity and diurnal feeding (A), nocturnal activity but mostly diurnal feeding (B), and diurnal activity but nocturnal feeding (C). Horizontal solid and open bars at the top of the graph represent the dark and light phase, respectively, of the LD 12:12 h cycle.

most red sea bream dramatically reduced their daily number of food requests and some starved. Nevertheless, three of seven fish were able to operate the rod and, in addition, we observed that two of them demanded food in the dark.

Feeding and locomotor rhythms

Under a regular LD cycle, the daily pattern of fish behaviour differed because some of them exhibited diurnal behaviour whereas others were nocturnal. In addition, the red sea ream showed an extraordinary flexibility to alter their duel feeding and locomotor rhythms, with some day-active fish displaying night feeding and vice versa. Fig. 3 shows feeding and locomotor event records of representative fish with diurnal activity and diurnal feeding (A), nocturnal activity but mostly diurnal feeding (B) and diurnal activity but nocturnal feeding (C). Occasionally, some fish that showed well-defined diurnal or nocturnal locomotor activity showed an arrhythmic behaviour as to feeding and vice versa. The percentages of both feeding and locomotor activities taking place in the light phase of the regular LD cycle are represented in Fig. 4. Generally, the red sea ream tended to be day active. Ten fish out of 14 displayed more than 70% of their locomotor activity during daytime whereas only four fish had a nocturnal pattern (less than 30% of their activity occurring in the light phase). However, as regards feeding, an equal number of red sea bream appeared either diurnal or nocturnal with some cases of strict diurnalism (two fish with 91% of feeding during daytime) and strict nocturnalism (one fish with less than 4% of feeding in daytime).

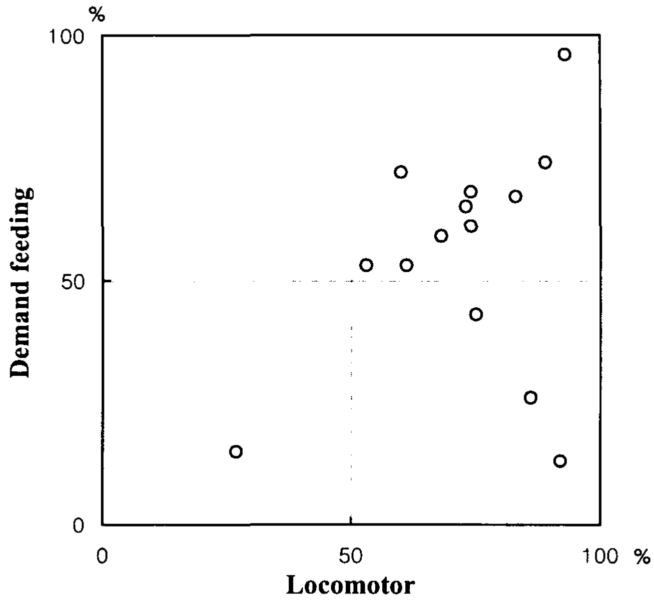

Fig. 4. Diurnal vs. nocturnal distribution of behaviour for locomotor (*x*-axis) and feeding (*y*-axis) of single red sea bream under LD 12:12 h (O, *n*=14). Values are the percentages of both locomotor and demand-feeding activities taking place in the light phase of the LD cycles over 7 consecutive days.

Circadian rhythms of feeding and locomotor activities

Under DD the endogenous period length of feeding and locomotor circadian rhythms were, on average, 23.7 ± 1.7 hours and 25.8 ± 1.6 hours, respectively, ranging from 22.2 to 26.9 hours, depending on individuals and regardless of their previous pattern of behaviour (i.e., diurnal or nocturnal). In addition, free-running rhythms lacked constancy and easily damped within few days. Occasionally, as shown in Fig. 5, circadian rhythms of feeding and locomotor activities appeared to have shorter or longer period length than 24 hours, respectively. In this fish, free-running feeding rhythms started the first day after transfer to DD with period length= 22.4 hours (Fig. 5B), whereas locomotor rhythms begun to free-run from the sixth day with period length=26.5 hours (Fig. 5A). Ultradian feeding rhythms of 2.4~4.6 hours were also detected occasionally under DD, although they lasted only a few days.

Discussion

Many researchers have reported phase changes in the daily pattern of behaviour, mostly for locomotor activity, in a variety of fish species (Eriksson, 1978; Muller, 1978). As to feeding, a number of studies have also reported dual behaviour (Daan, 1981; Moore-Ede et al., 1978) however, a few studies have linked both feeding and locomotor activities together (Eriksson, 1978). In the present study, both activities exhibited a dualistic pattern and so this fish species should be added to those with a dual phasing capacity. In addition, they displayed a particular flexibility in phasing because some of them manifest a lack of agreement in the diel timing of fee-

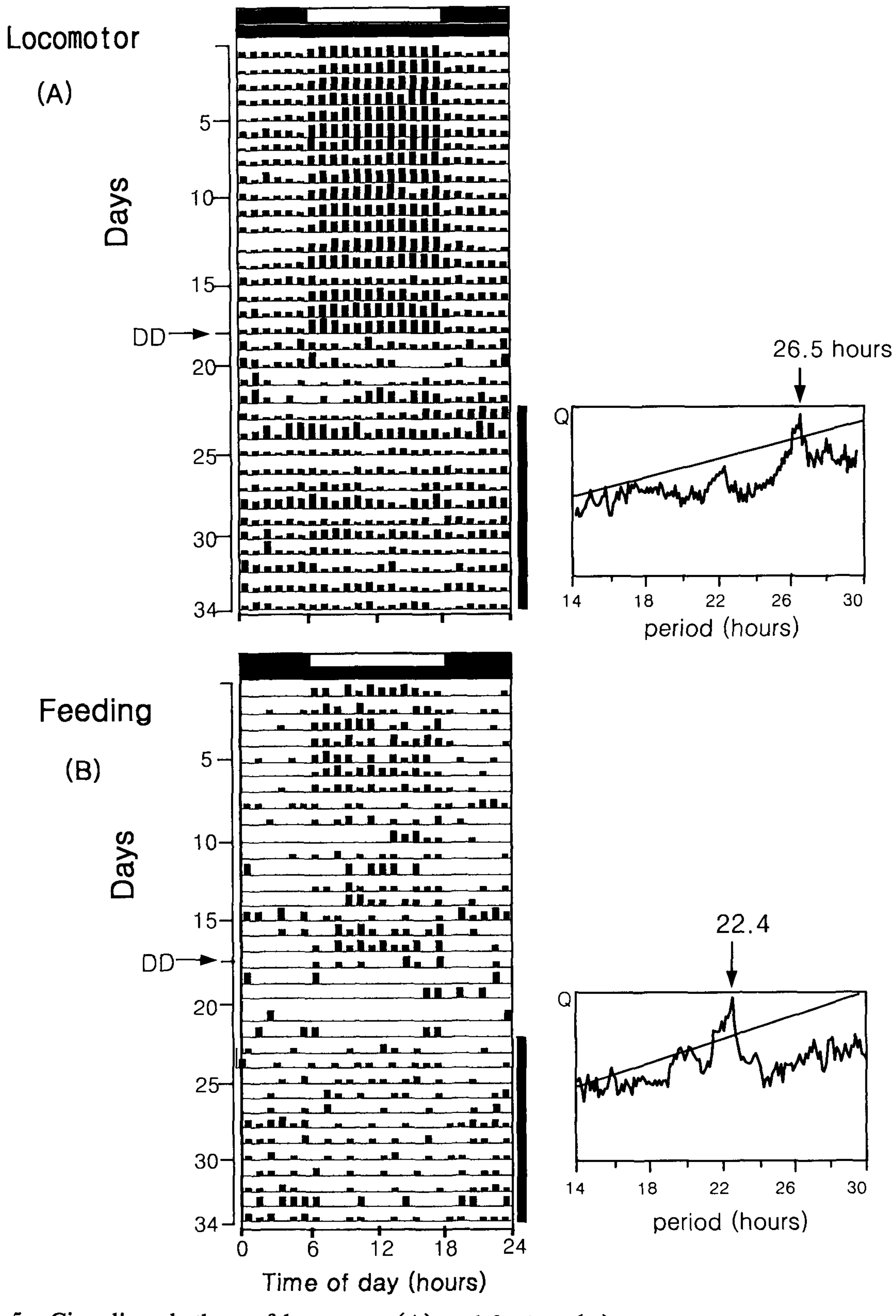

Fig. 5. Circadian rhythms of locomotor (A) and feeding (B) of one single red sea bream held under constant darkness (DD). Graph definitions are the same as given in Fig. 3. Chi-square periodgram analysis (confidence level of 95%) of free-running rhythms is shown on the right. Vertical black bars indicate the taken for analysis.

ding and locomotor activity, which contrasts with previous studies in other fish species. For instance, Hirata (1973) found a close correlation between food intake and movement patterns in Atlantic salmon. Similarly, Eriksson (1978) who studies feeding behaviour and locomotor activity in brown trout, found good correlation between both activities. Nevertheless, far from total agreement, feeding and activity diel patterns may differ in some fishes. For example, in *Alosa pseudoharengus*, Richkun and Winn (1979) observed diurnal swimming whereas Kohler and Ney (1980) observed nocturnal foraging. Support of this hypothesis is given by Eriksson (1978), who reported a crepuscular feeding pattern for diurnal as well as nocturnal bullheads. This indicates that the type of phasing of locomotor rhythms does not necessarily decide whether feeding will be diurnal or nocturnal. Assuming that the general pattern of activity is basically composed of two components (i.e., foraging and swimming unrelated to feeding), our results in red sea bream indicate that there may not always be a direct correspondence between them. In other words, when fish demand-feed they may or may not actively swim around the aquarium, and vice versa. Much of this independent behaviour could be explained by the fact that in our experiments red sea bream had free access to food by means of a demand feeder and there was, therefore, no need to actively search for food.

Since the early 1970s, laboratory studies have used demand feeders to ascertain feeding behaviour in fish (Adron et al., 1973; Eriksson, 1978). Such devices, which supply food to fish at their will coupled to continuous registration equipment provide valuable information on feeding rhythms (Cuenca and de la Higuera, 1994; Choe et al., 2001b). In this study, using a newly developed demand-feeding device, single red sea bream, which had never experienced a demand feeder before, rapidly learned how to operate the demand-feeding device soon after installation. This short learning period is consistent with findings previously reported for red sea bream (Choe et al., 2001b), Arctic charr and sea bass (Sanchez-Vazquez et al., 1995) however, these authors used fish groups whereas in the present study red sea bream were held individually.

Compared to higher vertebrates, free-running rhythms in fish are usually more unstable and are easily discarded. In spite of the relative lack of constancy, many researchers have reported circadian demand-oscillations (mostly for locomotor activity) under free-running conditions in a variety of fish species (Ebihara and Gwinner, 1992; Tabata et al., 1989). In our study, average free-running rhythms under DD were 24.9 ± 1.7 hours and 25.4 ± 1.6 hours for feeding and locomotor activities, respectively.

We cannot conclude that period length is significantly different for feeding and locomotor rhythms. On the contrary, the appearance of dissociation between feeding and locomotor free-running rhythms in the same animal (period length shorter than 24 h whereas locomotor rhythms longer, Fig. 5) suggests the existence of two independent timing mechanisms.

As a final remark, the authors wish to stress that dual phasing behaviour, which is characteristic of a highly adaptable circadian system, appears to be a common feature among fish rather than a particularity. Furthermore, flexibility in phasing and certain degree of independence between feeding and locomotor rhythms could be seen as an adaptative response of fish to a relatively stable aquatic environment but subjected to periodic changes in some biotic factors (e.g., food availability or absence of predators).

References

Adron, J.W., P.T. Grant and C.B. Cowey. 1973. A system for the quantitative study of the learning capacity of rainbow trout and its application to the study of food preference and behaviour. J. Fish Biol., 5, 625~636.

Aschoff, J. 1981. Freerunning and entrained circadian rhythms. In *Handbook of Behavioural Neurobiology, Biological Rhythms*. Vol. 4, J. Aschoff, ed. Springer-Verlag, Berlin, pp. 275~298.

Bjornsson, B. 1994. Swimming speed and swimming metabolism of Atlantic cod (Gadusmorhua) in relation to available food: A laboratory study. Can. J. Fish. Aquat. Sci., 50, 2542~2551.

Choe, Y.G., J.E. Choi, D.W. Roh, B.S. Bae and C.Y. Choi. 2001a. Light-Dark and food restriction cycles in red sea bream, *Pagrus major*: Effect of zeitgebers on demand-feeding rhythms. J. Fish Sci. Tech. (Submitted).

Choe, Y.G., J.E. Choi, D.W. Roh and C.Y. Choi. 2001b. A newly design and performance of an accurate self-demand 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. 2001c. Effect of water temperature on diurnal demand-feeding activity of red sea bream, *Pagrus major.* Bull. Korean Soc. Fish. Tech. (submitted, in Korean).

Choe, Y.G., J.E, Choi, D.W. Roh and C.Y. Choi. 2001d. Demand-feeding behaviour and diel cycle of feeding activity in carp *Cyprinus capio.* J. Fish. Sci. Tech. (Submitted).

Cuenca, E.M. and M.A. de la Higuera. 1994. Microcomputer controlled demand-feeder for the study of feeding behaviour in fish. Physiol. Behav., 55, 1135~1136.

Daan, S. 1981. Adaptative daily strategies in behaviour. In *Handbook of Behavioural Neurobiology, Biological Rhythms.* Vol 4, J. Aschoff, ed. Springer-Verlag, Berlin, pp. 275~298.

Ebihara, S. and E. Gwinner. 1992. Different circadian pacemakers control feeding and locomotor activity rhythms in Eropean starlings. J. Comp. Physiol. [A] 171, 63~67.

Eriksson, L.O. 1978. Nocturnalism vs. diurnalism-dualism within fish individuals. In *Rhythmic Activity of Fishes.* J.E. Thrope, ed. Academic Press, London, pp. 69~90.

Fraser, N.H.C., N.B. Metcalfe and J.E. Thrope. 1993. Temperature-dependent switch between diurnal and nocturnal foraging in salmon. Proc. R. Soc. Lond., 252, 135~139.

Hirata, T. 1973. Diurnal rhythms of metabolic and activity rates in juvenile Atlantic salmon, *Salmo salar* Linnaeus. Memories of the Faculty of Fisheries, Kagoshima University 22, 73~93.

Kohler, C.C. and J.J. Ney. 1980. Piscivory in a land-locked alewife (*Alosa pseudoharengus*) population. Can. J. Fish. Aquat. Sci., 37, 1314~1317.

Landless, P.J. 1976. Demand-feeding behaviour of rainbow trout. Aquaculture, 7, 1~25.

Moore-Ede, M.C., F.M. Sulzman and C.A. Fuller. 1978. Circadian timing of physiologycal system. In *The Clocks that Time Us.* M.C. Moore-Ede, F.M. Sulzman and C.A. Fuller, eds. Harvard University Press, Cambridge, pp. 91~104.

Muller, K. 1978. The flexibility of the circadian system of fish at different latitudes. In *Rhythmic Activity of Fishes.* J.E, Thrope, ed, Academic Press, London, pp. 91~104.

Richkun, W.A. and H.E. Winn. 1979. Activity cycles of adult and juvenile alewines, *Alosa pseudoharengus,* recorded by two methods. Proc. Natl. Acad. Sci. USA., 75, 6276~6280.

Sanchez-Vazquez, F.J., J.A. Madrid and S. Zamora. 1995. Circadian rhythms of feeding activity in sea bass, *Dicentrachus labrax* L.: Dual phasing cpacity of diel demand-feeding pattern. J. Biol. Rhythms, 10, 256~266.

Tabata, M., N.H. Minh-Nyo and M. Oguri. 1989. Circadian rhythms of locomotor activity in a teleost, *Silurus asotus.* Zool. Sci., 6, 367~375.