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Daily rhythms of locomotor and demand-feeding activities in *Schizothorax pelzami* (Kessler, 1870)

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ABSTRACT

A study was carried out to investigate the daily rhythms of locomotor and feeding activity of Khajoo, *Schizothorax pelzami*, a candidate species for freshwater aquaculture. Using self-feeder juvenile Khajoo were exposed to a 12/12 LD cycle to determine the rhythms of locomotor and feeding activity. The effects of feeding on locomotor and feeding activity of fish were also examined. Finally, the endogenous rhythmicity under different lighting condition tested. Fish displayed a strictly diurnal feeding and locomotor activities with 98% and 84% of the total activity occurred in the photophase, respectively. In scheduled feeding, both the L-group (fed in light) and the D-group (fed in the dark) showed a diurnal locomotor activity pattern. However, the L-group had a peak of locomotor activity near the feeding time, but the D-group had a scarce locomotor activity in the scotophase with no significant change at the mealtime. Most of the individuals display free-running rhythms when exposed to different lighting condition including, constant darkness, ultradian 45:45 min LD cycle and reversed DL photo cycle. Taken together the results of this study showed that both locomotor and feeding activity have diurnal rhythms in Khajoo *S. pelzami*, even fish feeding had taken place at night. Additionally, the free-running locomotor activity of the fish in the absence of external light stimuli, suggests the existence of an endogenous timing mechanism in this fish species.

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circadian rhythm; feeding activity; free-running rhythms; locomotor activity; *Schizothorax pelzami*

Introduction

For a successful aquaculture program, it is important to optimize the feeding management, as about 50% of aquaculture production cost is referred to feeding practices (Molina-Garcia and Claver-Farias 1999). Further, it is necessary to understand the feeding and activity rhythms of the species to improve aquaculture production (Boujard and Leatherland 1992; Madrid et al. 2001).

The schizothorax genus has been shown to be widely distributed in China, India, Afghanistan and some other countries (Coad 2016). Some schizothorax species considered as the potentially important species for aquaculture industry with respect to their nutritional and trade values, but the farming of these species is still in the experimental stage (Gharaei et al. 2010). Khajoo, *Schizothorax pelzami*, a species with great potential for aquaculture, is distributed in the northeast of Iran. The information on the domestication

requirements and reproduction and production bionormative indices (including absolute and working fecundities, rearing conditions of nursery, fingerling and growing stages etc.) of this invaluable species is in infancy. Despite the market demand, there is no farm for culturing this species yet. Some studies were previously conducted to address different issues such as reproductive hormones, distribution and ecological aspects (Abdoli et al. 2007; Coad and Ville 2004; Safari et al. 2014). To date, little published data exists about the locomotor and feeding activity rhythms in Khajoo.

Feeding and locomotor activities are widely studied to understand the circadian rhythms in fish (Kotrschal and Essler 1995; Madrid et al. 2001). Both locomotor and feeding activity is mainly affected by light and feeding time (Sanchez-Vazquez and Madrid 2001). The timing mechanism of fishes is more flexible than that of other vertebrates, (Ali 1992; Sánchez-Vázquez et al. 2001).

There are relative similarities in the physiology of biological rhythms in mammals that could not be perceived in fishes. Across other reasons, it could be explained that more than 400 million years of evolutionary history and over 25000 fish species are not comparable with a history of 40 million years and 2000 species for mammals (Madrid et al. 2001). Between, and even within, individuals of the same fish species, there is a remarkable variability in feeding patterns. This variability may partly be explained by a lack of homogeneity in experimental design, but it is also likely to be a characteristic of feeding rhythms in fish (Madrid et al. 2001). Furthermore, some fish species may change their phasing (Sánchez-Vázquez et al. 2001).

Circadian rhythms are controlled by an endogenous mechanism and continue under constant external conditions, in which they usually start to free-run with an internal period (τ) of about 24 h (Aschoff 1981).

To investigate the endogenous origin of daily locomotor and feeding rhythms, fish have to be reared in a constant condition that separates them from any external time cues. In such situation that there is no external time cues to synchronize with behavioral rhythms, if there is an endogenous oscillator, circadian rhythms are expected to begin to free-run.

Providing the basic knowledge of feeding and movement of this species can improve future studies of the Khajoo farming program. To our best knowledge, the feeding and activity rhythms of Khajoo *S. pelzami*, however, have not yet been described. The aim of this study is to investigate the daily rhythms of locomotor and feeding activity of Khajoo *S. pelzami*.

Materials and methods

Animals and housing

Juvenile fish were collected from the rivers in the northeast of Iran (i.e. Khorasan-razavy Province), using electrofishing (Samus-725G). Fish were transferred to the Ferdowsi University of Mashhad. To acclimate to the captivity conditions, fish were kept in 120 liters glass aquaria for 12 weeks. Thereafter,

individuals were transferred to 64 liters aquariums (40 × 40 × 50 cm) and fed using automatic feeder (commercial tout feed, Biomar, France). Fish were kept in well-aerated tap water and the temperature ($17 \pm 1^\circ\text{C}$) was maintained throughout the experiments by means of independent air cooling/heating devices. To remove any external cue, each aquarium was covered with a black plastic. At random time with 3–5 days interval, uneaten food and debris were siphoned from the bottom of the aquarium and new aerated water replaced. The light was set to turn on and off at 08:00 and 20:00 respectively using an automatic electronic timer. The light intensity at the water surface was set to an average 400 lx and provided with LED lamps.

Feeding and activity monitoring

To record feeding activity, each aquarium was provided with an automatic demand-feeder which store feeding activities during the experiment. The feeder consisted of a self-feeder (Classica food timer- China) connected to an infrared photocell (E18-D80NK_China) that was placed under the self-feeder. Feed consumption was recorded to a microcomputer, and feed was released by the feeder in response to detection of the fish approaching more than 3 cm to photocell. To record locomotor activity, another infrared photocell was placed in the middle of the aquarium, 12 cm below the water surface and the infrared beam covered the width of the aquarium. The interruptions of the infrared beam were detected by the sensor and stored in the microcomputer.

Experimental design

Three experiments were conducted to investigate the demand-feeding rhythms and light entrainment and endogenous rhythmicity of locomotor activity.

Experiment 1: daily rhythms of locomotor and feeding activities

Fish were exposed to a 12/12 LD cycle (lights on at 10:00 and off at 20:00) and each aquarium equipped with a demand-feeder. As fish approached to the feeder by less than 3 cm, the

photocell detected the fish and the feeder released food (0.1% BW/day) to the water. The locomotor and feeding activities recorded for 45 days.

Experiment 2: Locomotor and feeding activities under scheduled feeding

12 fish were weighted and divided into the two groups. The light was set to 12/12 LD photoperiod (lights on at 10:00 and off at 22:00). In the first phase, fish were fed once a day (1.5% BW/day), using an automatic food dispenser for 30 days. Uneaten food was removed and weighted 1 h after feeding time determining the food consumption. Feeding time was set to 20:00 for the L-feeding group and at 08:00 for the D-feeding group. In the second phase of the first experiment, fish were fed twice a day at the same time as in the first phase (20:00 and 08:00) for 30 days.

At the end of each phase, fish were weighted to calculate the growth performance through specific growth rate (SGR) and food conservation ratio (FCR). SGR is the average percentage of body weight added per day and FCR is the ratio of the added wet body weight of the fish to the amount of eaten food.

Specific growth rate (SGR) and food conversion ratio (FCR) were calculated according to the following formulas:

$$\text{SGR} = (\text{Ln} [\text{final body weight}] - \text{Ln} [\text{initial body weight}]) / \text{days} * 100$$

$$\text{FCR} = \text{Food intake (g)} / \text{Increment of biomass}$$

Experiment 3: Endogenous timing of locomotor activity

The circadian rhythms of locomotor activity of 12 individual fish were recorded under different light

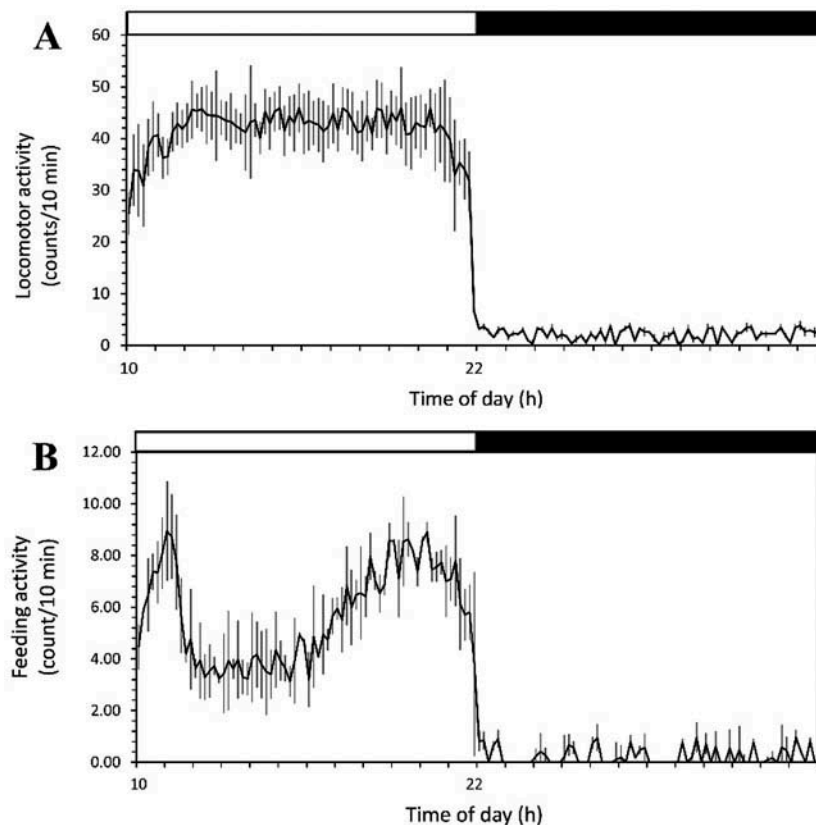


Figure 1. Average daily locomotor and feeding activity waveforms for individual Khajoo kept under 12/12 LD photo cycle. The black bars at the top of the graph indicate the length of the dark phase. Values represent the mean \pm S.E.M of 12 fish during the 45 days of experiment.

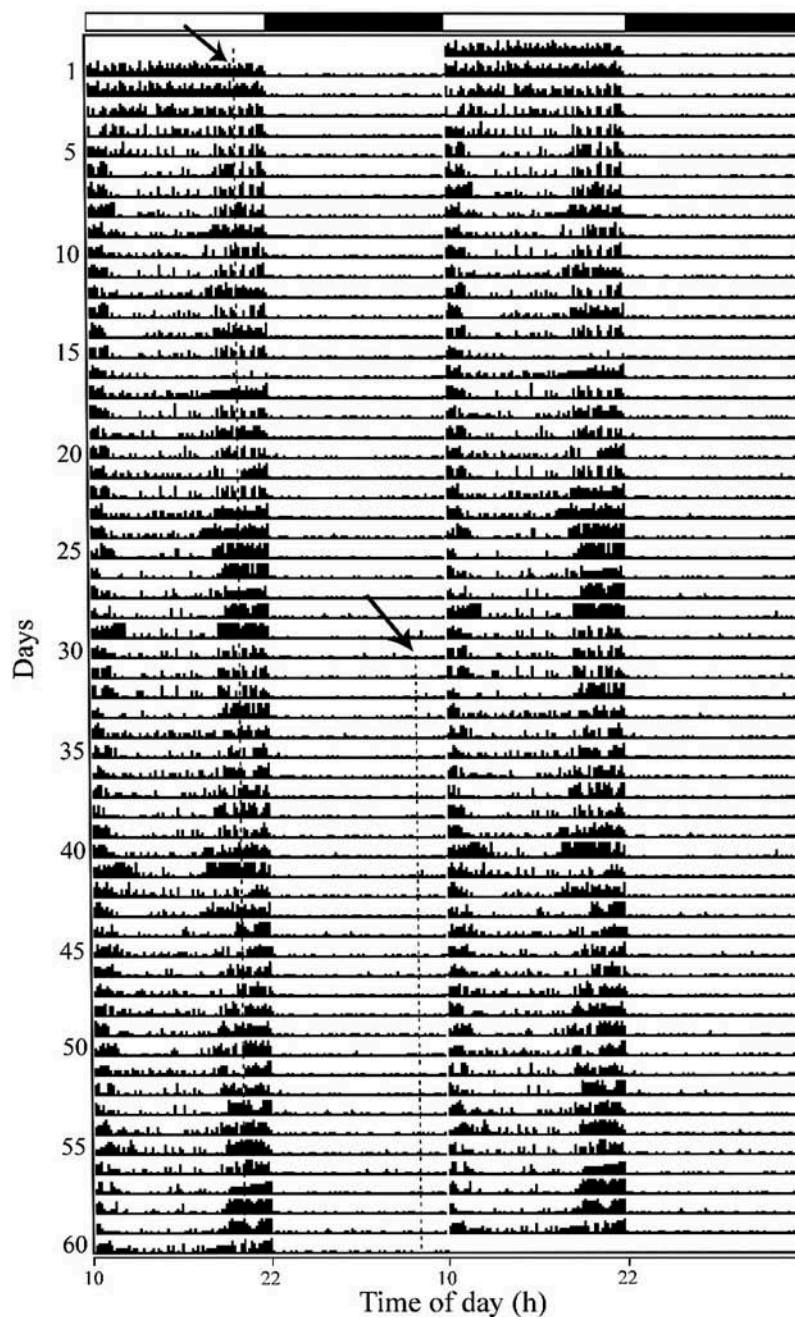


Figure 2. Double plotted actogram of locomotor activity of representative Khajoo in the L-group under scheduled feeding condition. The arrows indicate the feeding time. In the days 1–30 they fed once a day in the photophase and from day 30 to 60 another feeding time added in the scotophase. The black bars at the top of the graph indicate the length of the dark phase.

condition to investigate the existence of endogenous rhythmicity. Initially, fish were exposed to a regular 12/12 LD cycle (lights on at 10:00 and off at 20:00) for two weeks then they transferred to DD condition (darkness) for two weeks thereafter the two weeks of regular LD cycle repeated to resynchronize fish to regular lighting condition

and then they exposed to an ultradian 45/45 min LD cycle for two weeks. Subsequently, the regular LD cycle was restored again and finally reversed for two weeks. In addition to DD condition, ultradian lighting was used to stabilize free-running rhythms in fish (Eriksson 1978; Sánchez-Vázquez et al. 1995). During the experiment, fish were fed

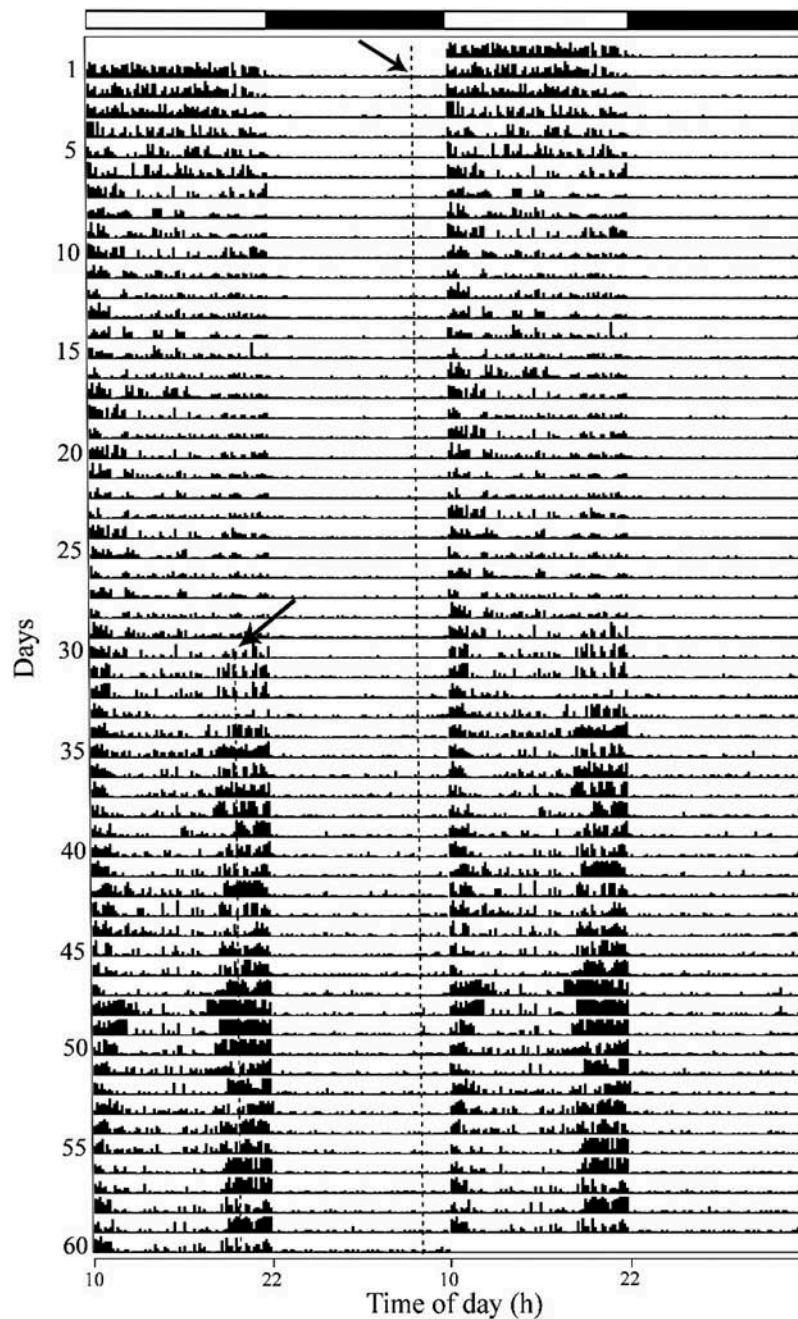


Figure 3. Double plotted actogram of locomotor activity of representative Khajoo in the D-group under scheduled feeding condition. The arrows indicate the feeding time. In the days 1–30 they fed once a day in the scotophase and from day 30 to 60 another feeding time added in the photophase. The black bars at the top of the graph indicate the length of the dark phase.

every two days at random time to prevent the interfering of feeding schedule.

Data analysis

Feeding activity data as well as locomotor activity data were saved in a microcomputer that was connected to the both demand-feeder and photocell. All data were transferred to a

computer and exported to Microsoft Excel (ver. 2016) for further analysis, and then using Actogramj (ver. 1.0) for plotting. Statistical differences between mean of diurnal and nocturnal activities were analyzed using t-Student test at the significant level of $P < 0.05$. SPSS package ver, 2013 was used for statistical analyses in the current study. The period length (tau) of free-running rhythms was determined by

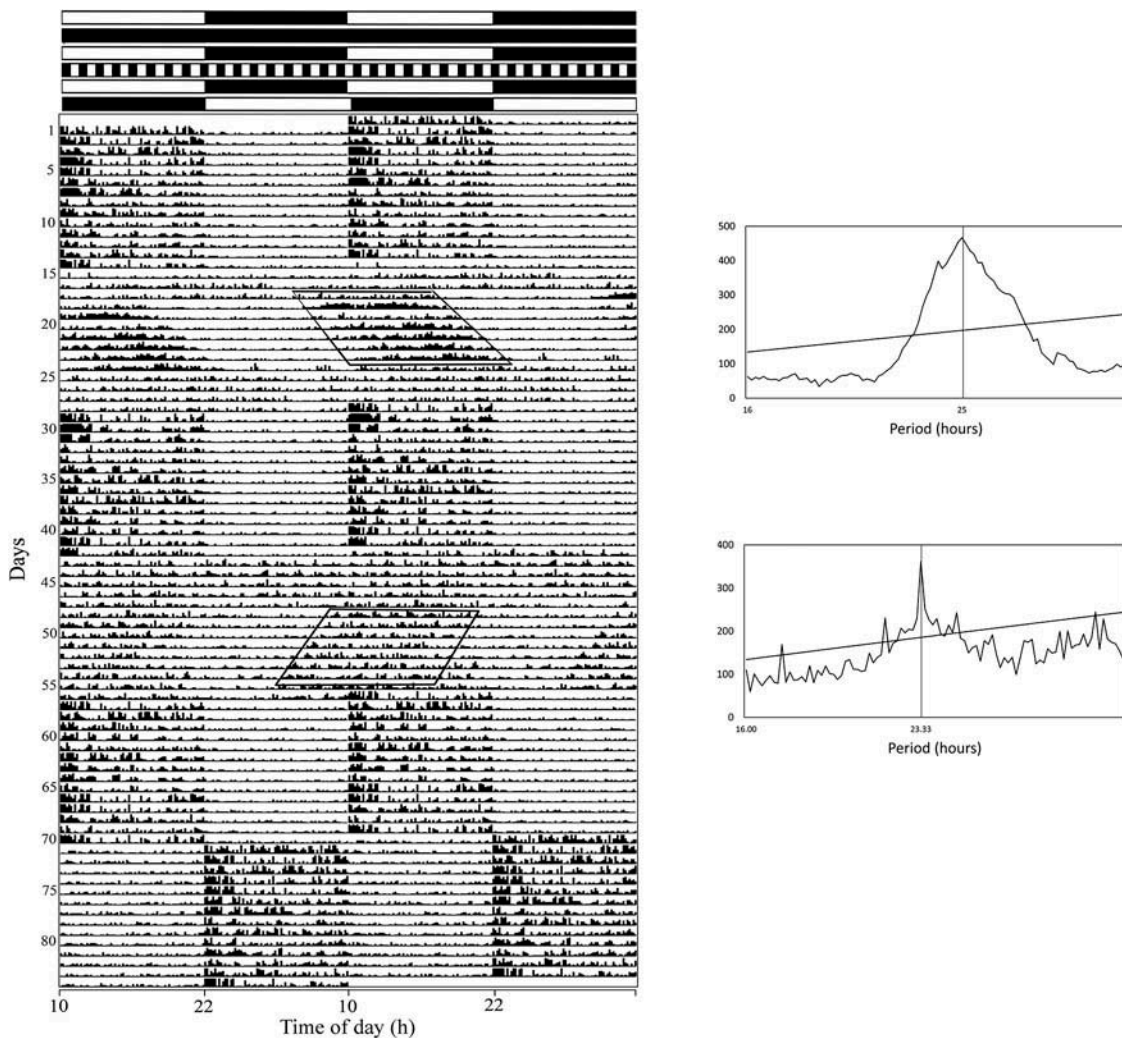


Figure 4. Double plotted actogram of locomotor activity of a representative individual Khajoo kept under different lighting condition for 84 days. Two weeks for each lighting condition including 12/12 LD, DD (constant darkness), 12/12 LD, 45:45 min LD, 12/12 LD and 12/12 DL. The black bars at the top of the graph indicate the length of the dark phase. Chi-square periodograms analyses (confidence level of 95%) of free-running rhythms are shown in the right. Periodograms were analyzed using data of those days when the rhythms were free-run.

periodogram analysis at a confidence level of 95% using Actogramj software.

Results

Experiment 1: Daily rhythms of locomotor and feeding activities

Juvenile Khajoo exposed to 12/12 LD cycle and experienced the demand-feeder, were able to learn how to approach to and activate the demand-feeder sensor after 4–5 day. Fish displayed a strictly diurnal

feeding activity with 98% of the total feeding occurred in the photophase. The majority of individuals (i.e. 9 out of 12) exhibited a similar feeding pattern with a peak after the lights on and another before the lights off (Figure 1A). Very few feeding demand were recorded in the dark.

With respect to the locomotor activity, juvenile Khajoo displayed a diurnal activity rhythms with 84% of the total locomotor activity occurred in the photophase (Figure 1B). The activity arose after the lights on and continued to the end of the photophase.

Experiment 2: Locomotor and feeding activities under scheduled feeding

Locomotor activity in two groups with different feeding time was investigated. In the first phase of the second experiment, both the L-group and the D-group showed a diurnal locomotor activity pattern as seen in the first experiment (Figure 2). The L-group had a peak of locomotor activity near the feeding time, but the D-group had a scarce locomotor activity in the scotophase with no significant change at the mealtime. In the L-group, each individual seems to anticipate the feeding time since the locomotor activity increased 2–3 h before mealtime (t test, $P = 0.02$) and decrease 1–2 h after that. In the second phase, when fish fed twice a day, the diurnal locomotor activity was observed for both groups (Figure 3). Fish in the L-group did not change their feeding activity, even though individuals in the D-group synchronized their locomotor activity to the daytime feeding and displayed feeding anticipatory activity (FAA) (t test, $P = 0.03$), as in the L-group.

When fish fed once a day, for few days the feeding rate was higher in the L-feeding group than that of the other group. Nonetheless, overall feeding rate in the second phase was similar in both groups and no significant difference was observed between SGR and FCR of the experimental groups (SGR t test, $P = 0.7$; FCR t test, $P = 0.63$).

Experiment 3: Endogenous timing of locomotor activity

Endogenous rhythmicity of locomotor activity was examined in the dark and ultradian of 45/45 min photo cycle. Locomotor activity of fish began to free-run Under DD condition. Five fish after 3 days and 3 fish after 5 days of transferring to the dark show free-running in rhythms with an average $\tau \pm \text{SEM}$ of 24.3 ± 0.4 h, ranging from 23.3 to 25.6 h (Figure 4). However, it did not continue and disappeared after few days.

Eight out of 12 individuals displayed circadian rhythmicity after exposing to ultradian of 45/45 min LD cycle. Six fish began to free-run 4 days after exposing to ultradian LD cycle, whereas two other fish exhibited circadian

rhythms after 7 days and four fish show arrhythmic activity in this phase. Locomotor activity free-run with an average $\tau \pm \text{SEM}$ of 23.8 ± 0.3 h, ranging from 22.7 to 24.6 h and lasted for few days (Figure 4).

In the last phase of the third experiment, juvenile Khajoo exposed to reversed LD cycle to examine the ability of resynchronization to new rhythm of zeitgeber. All fish were able to adapt to new photoperiod, but the duration of adaptation was different between individuals. Six fish throughout the first day, two fish after 3 days and the remaining after 6 days resynchronized to new circadian rhythm (Figure 4).

Discussion

The results of this study for the first time showed the locomotor and feeding activity rhythms of Khajoo *S. pelzami*, an endemic species with the great potential for aquaculture. Both locomotor and feeding activity rhythms were diurnal and had same phase. They increased at the beginning of the photophase and declined at the end of the daytime. Feeding activity rhythms were bimodal and had two peak, namely at the early daytime and before the lights off. However, the locomotor activity had no significant concentration in any time of the day. Feeding and locomotor activities may have the same rhythmicity (Vera et al. 2006) and occur in same time of the day. In sharpsnout seabream, *Diplodus puntazzo*, the daily rhythms of feeding and locomotor activities were investigated under LD cycle. Sharpsnout seabream display a diurnal behavior for both feeding and locomotor activities (Vera et al. 2006). In the similar study on locomotor and feeding activities of tench, *Tinca tinca*, they displayed a strict nocturnal behavior for both feeding and swimming (Herrero et al. 2005).

Although these behavioral rhythms are not in phase in all fish species (Herrero et al. 2005; Sánchez-Vázquez et al. 1996). Same individual can display dual phasing, in which it may display diurnal locomotor activity and nocturnal feeding activity or vice versa (Sánchez-Vázquez et al. 1995; Vera et al. 2006). For instance, *Salmo salar* (Fraser et al. 1995), *Salvelinus alpinus* (Alanärä and Brännäs 1997), *Dicentrarchus labrax* (Javier Sánchez-Vázquez et al. 1995; Sánchez-Vázquez

et al. 1995), *Sparus aurata* (López-Olmeda et al. 2009) showed dual phasing system.

In the second experiment, two groups of fish were fed in the day and night to investigate the effect of feeding time on the locomotor activity rhythms. The L-group feed and swim in the daytime, but the D-group swim in the daytime and feed at the night. While food intake observations indicated the food intake in the D-group, no change in locomotor activity was detected. It seems that fish in the D-group consume the available food at the nighttime, but no significant change was found in locomotor activity before or after the feeding time. Our results suggested that Khajoo is a strict diurnal species, which is evolutionary adopted for swimming in the daytime but it may feed during night time depending upon food availability. A previously published study on another cyprinid fish, *Tinca tinca*, showed similar result of consistency in locomotor activity rhythms (Herrero et al. 2005). In tench, *T. tinca*, nocturnal activity behavior did not change even when food was delivered in the daytime. In contrast, locomotor activity of goldfish (Sánchez-Vázquez et al. 1997) and golden shiner (Reebs and Lague 2000), was revealed to be more flexible and concentrated to the mealtime, regardless of the light phase in which the food is delivered.

The L-group exhibited the food anticipatory activity (FAA) in the daytime feeding. In scheduled feeding tasks, when animal are subjected to time-restricted feeding, they can anticipate the mealtimes. Food anticipatory activity (FAA) usually is the synchronization of locomotor activity and feeding behavior which increases just prior to mealtimes, and its timing is regulated by circadian mechanisms (Mistlberger 1994). Anticipation of the mealtime allows animals to be the first ones for food intake, which is beneficial for them both in nature as well as in captivity (Reebs and Gallant 1997). FAA were demonstrated for some fish species, such as bluegill and largemouth bass (Davis 1964), goldfish (Aranda et al. 2001; Gee et al. 1994; Sánchez-Vázquez et al. 2001, 1997; Spieler and Noeske 1984); golden shiner (Lague and Reebs 2000; Laguë and Reebs 2000; Reebs 1996; Reebs and Gallant 1997; Reebs and Lague 2000); loach (Naruse 1994); rainbow trout (Chen and Tabata 2002); european catfish (Bolliet et al. 2001);

greenback flounder (Chen and Purser 2001; Purser and Chen 2001); arctic charr (Brannas et al. 2005); tench (Herrero et al. 2005); inanga (Reebs 1999) and sea bass (Azzaydi et al. 1998; Sánchez-Vázquez et al. 1995). Fish in the D-group did not display FAA, and it may be due partly to the inhibitory effects of the dark on the locomotor activity. Nevertheless, in the second phase of the experiment, the D-feeding group displayed FAA for the daytime feeding, when they were fed twice a day.

DD condition is used as a way to exclude any external light stimuli and testing the endogenous rhythmicity in animals, because according to the theory light entrainable circadian oscillator (LEO) is the main responsible in the regulation of circadian rhythms (Mistlberger 1994). In addition an ultradian 45:45 min LD cycle is proposed to stabilize circadian oscillators in fish (Eriksson 1978; Javier Sánchez-Vázquez et al. 1995).

Juvenile Khajoo reared under DD and ultradian LD cycle had circadian rhythmicity in locomotor activity, suggesting the existence of an endogenous timing mechanism. Free-running rhythms are mainly known to be more labile in comparison with higher vertebrates. However, this mechanism has been reported in many fish species (Herrero et al. 2003; Hurd et al. 1998; Kavaliers 1979; Lissmann and Schwassmann 1965; Nishi 1989; Sanchez-Vazquez and Tabata 1998). Some fish may show arrhythmic activity while others may lose free-running shortly after establishing constant condition (Davis 1964; Godin 1981; Nishi 1990). Fish exhibited free-running of locomotor activity under DD condition 66.6% and under 45:45 min LD cycle 75%. The levels of free-running rhythms has been variably reported for different fish species, e.g. tench 41% (Herrero et al. 2003 in DD condition), sharpsnout seabream 33% (Vera et al. 2006), Nile tilapia 58.6% (Vera et al. 2009) and in zebra 62.5% (Del Pozo et al. 2011). However, the ratio of exhibiting circadian rhythmicity is usually higher in mammals and also lasts for a longer period (Benstaali et al. 2001).

Finally, in the last phase of the third experiment we used phase shifting as another way to investigate the existence of endogenous timing mechanism. Eight out of 12 fish were immediately

resynchronize to new LD cycle. This also has been reported in the hagfish *Eptatretus burger* (Sadako and Kabasawa 1995), golden shiners *Notemigonus crysoleucas* (Lague and Reeb 2000) and Nile tilapia *Oreochromis niloticus* (Vera et al. 2009).

In conclusion, our finding revealed that locomotor and feeding activity have diurnal rhythms in Khajoo. Notwithstanding restricted feeding at night, the locomotor activity did not change and displayed diurnal rhythms. Locomotor activity was driven by an endogenous clock and last in constant darkness and ultradian LD pulses. These results may be applicable for feeding management of this newly-introduced aquaculture-candidate species.

Regarding to this results, scheduled daytime feeding is suggested for this species. Food may be delivered twice a day, one at the beginning of the day and other at the last hours of the day. In addition, the ability to display FAA in daytime feeding revealed that feeding at the day may improve food efficiency and has economic as well as environmental implications. Moreover, anticipation of a forthcoming meal affecting some physiological factors which allow the animals to optimize their digestive and metabolic processes (Davidson and Stephan 1999; Stephan 2002). Further studies are needed to determine the social interaction on behavioral rhythms of Khajoo.

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