Agonistic acts as possible indicator of food anticipatory activity (FAA) in rainbow trout (*Oncorhynchus mykiss*)

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Summary

Food anticipatory activity (FAA) was assessed in rainbow trout (*Oncorhynchus mykiss*) in two small raceways using demand-feeding (T2) and hand-feeding (t2). The fish of both raceways were subjected to restricted feeding (RF) at two times in two places and the fish distribution and/or trigger actuation, total agonistic behaviour between fish, and swimming speed were measured. Food anticipatory activity did not appear clearly when using the usual measures. The present study suggests that the expression of FAA is not limited to trigger actuations, fish positioning or swimming activity but may also be described by swimming speed and agonistic behaviour.

Key words: Food anticipatory activity (FAA), Self-feeders, Agonistic acts, Swimming speed, Rainbow trout

Introduction

While the circadian system of animals is able to generate self-sustained oscillations, it can also be synchronised (entrained) to the periodic variation of environmental factors (zeitgebers) such as the LD cycle or food. Mammals (such as rodents) possess an endogenous circadian clock known as a light-entrainable oscillator (LEO) which is entrained by photoperiodic signals. It is thought that the LEO of rodents is localised in the suprachiasmatic nucleus (SCN) of the hypothalamus (Sheward *et al.*, 2007; Fuller *et al.*, 2008), synchronising light-related rhythms. In fish, unlike mammals, little is known of this mechanism (Sanchez-Vazquez *et al.*, 1995), but in most fishes a circadian rhythm of locomotor activity has been proposed which appears to be feeding-entrained (Lague and Reebs, 2000).

In addition to light, there is strong evidence that food availability synchronises many behavioural rhythms in fish. In cultured fish for example, scheduled feeding (once per day at a regular time) appears to act as a zeitgeber, producing food anticipatory activity (FAA), or a pronounced increase in activity beginning several hours prior to mealtime (Mistlberger, 1994; Escobar *et al.*, 2007). The first evidence of this behaviour in fish was provided by Davis (1964), who showed that bluegill, *Lepomis macrochirus* and largemouth bass, *Micropterus salmoides* increased their locomotion 1-3 h prior to food delivery. This phenomenon has now been documented in a number of fish species, e.g. medaka, *Oryzias latipes* (Weber and Spieler, 1987), golden shiners, *Notemigonus crysoleucas* (Reebs, 1996), Inanga, *Galaxias maculatus* (Reebs, 1999), rainbow trout, *Oncorhynchus mykiss* (Chen and Tabata, 2002) and sea bass, *Dicentrarchus labrax* (Azzaydi *et al.*, 2007). It is thought that FAA is regulated by a food-entrainable circadian oscillator (FEO), which is anatomically and functionally distinct from the LEO in rats (Landry *et al.*, 2007). It is not still clear whether fish have a separate FEO in addition to a LEO. In the case of rainbow trout, the existence of a LEO has been reported by Cuenca and De la Higuera (1993) and Sanchez-Vazquez and Tabata (1998). However, the only evidence

of a FEO in addition to the LEO in rainbow trout has been suggested by Bolliet *et al.* (2001).

Restricted feeding is usually a prerequisite for FAA (Purser and Chen, 2001), which persists during food deprivation (FD) and disappears during *ad libitum* feeding (Sanchez-Vazquez *et al.*, 1997). Detection of FAA depends on the way it is measured (Pecoraro *et al.*, 2002). This study will take an approach to the detection of FAA in rainbow trout (*Oncorhynchus mykiss*) by measuring a number of behavioural methods simultaneously, i.e. fish distribution and/or trigger actuation, total agonistic behaviour between fish and swimming speed.

Materials and Methods

Fish holding conditions

The experiment was carried out for 45 days between 17 August and 30 September 2005 at the School of Aquaculture, University of Tasmania, Launceston, Tasmania, Australia. Rainbow trout were hatched and grown in captivity and then transferred to two identical raceways (T2 and t2) (3.1 m length × 0.67 m width × 0.4 m depth) with re-circulated freshwater in a temperature-controlled and insulated room. The raceways were subdivided equally into 4 sections, each 77.5 cm in length as a way of identifying the relative position of fish within each raceway. These sections were respectively numbered from section 1 (most upstream, the location of the water inlet) to section 4 (most downstream, the location of the water outlet). For convenience, sections 1 and 2 were nominated as the "upstream area" and sections 3 and 4 the "downstream area". The water temperature was set at 12 ± 1°C. Room illumination was provided by fluorescent tubes (Thorn, 36W, white light) maintained on a photoperiod of 14:10 LD (lights on at 0600 h and off at 2000 h) with a light intensity of 4 μmol/s.m^2 at the water surface during the photophase. A timer was used to turn lights on and off, with an artificial dawn and dusk of 10 min each.

One raceway (T2) was equipped with two self-feeders (ARVO-TEC T Drum) hung from the ceiling above and away from the raceway to reduce disturbance during servicing. One self-feeder was placed in section 1 (most upstream) and the other in section 4 (most downstream). The reward level was set at an average of 24 pellets (1 g) per trigger actuation, from the beginning until the end of the experiment.

The self-feeding system consisted of four parts: a microswitch, a feeder, a control unit (PLC) and a computer. Linked to the microswitch was a nylon fishing line with a black pellet-like bead, suspended about 1 cm below the water surface and used as a trigger. The bead was located in the middle of the aforementioned sections of the raceways and in the vicinity (*c*.2 cm) of the submerged outlet tube of the feeder. Once the biting and pulling action of a fish activated the trigger, a signal was generated by the PLC and the number of pellets (reward level) was delivered into the raceways, with a one-second delay between two subsequent trigger actuations. Simultaneously, the generated signal was registered and stored by a computer. The computer using the Chronolab and Citech programs (supplied by Cromarty W. A. and Co. Pty Ltd, Launceston, Australia) registered the time and number of trigger actuations (hits). Self-feeding activity was defined as a number of trigger actuations by the fish per 15 min time interval, recorded by the computer.

A self-feeder was not installed over the other raceway (t2); rather, feed was distributed relatively uniformly by hand in the sections of food delivery as in T2. Three colour cameras (Swann® C500 CCD) were mounted on the ceiling, about 2 meters above the raceways to record the fish activity. Video footage was recorded continuously on a PC hard-drive using the Chateau-XP software program, capable of recording up to 8 channels simultaneously. The fish distribution in the raceways was noted and recorded every 10 min during playback and data presented as the number of fish/10 min interval present in either the downstream area (morning meal: M) or upstream area (afternoon meal: A) in each raceway.

Procedure

Two groups of 30 rainbow trout were placed randomly in each raceway. No

training on the activation of triggers was used for fish in the self-feeding raceway (T2). Mean total length and weight of T2 fish were: 26.2 ± 16.8 cm and 264.3 ± 47.9 g and of t2 fish were: 26.1 ± 20.3 cm and 247.1 ± 56.3 g (mean ± SD). The sequential phases of the experiment were as follows:

Phase 1: Free food access (unrestricted feeding) in T2 and random feeding in t2

This phase was applied to the system as a period of acclimation for both raceways and also to find out the timing of self-feeding activity of T2 under the LD cycle of 14:10.

Phase 2: 14:10 LD and restricted feeding (RF)

The fish during this phase were subjected to restricted feeding (RF). Time of food availability during the RF was: morning (0900-1000 h) and afternoon (1600-1700 h) for both raceways. Similarly morning and afternoon feeding occurred in section 4 (most downstream) and section 1 (most upstream) respectively, for both raceways. This trial aimed to determine if feeding behaviour was synchronised to feeding time. To determine whether FAA was expressed by behaviours (other than the fish distribution and/or trigger actuation), total agonistic behaviour between fish, swimming speed, along with number of fish moving were measured on days 37, 41 and 45 of this trial, for both raceways (three h before and one h after the food delivery). Mean swimming speed, based on body length per seconds (bl/s), was measured for sample fish in the group, over a 5 sec duration every 10 min. This was done by counting the number of fish moving and calculating their swimming speeds within the first 5 sec of 10-min blocks over the 14 h period (photophase). Furthermore, on those days agonistic behaviour in the raceways was scored based on the number of aggressive acts: chasing, nips and pushing.

Data analysis

The Chronolab program was used for data acquisition and actogram construction. The software was designed to record the self-feeding activity at a resolution of 10 min, each point represents the percentage of the total trigger actuations that occurred at 24-h intervals. In addition, the rhythm profile was calculated by averaging the activity counts (or values of the y-axis) over a sample period (days).

It is well-known that the current descriptive experiment is not tank replicated and results should be considered cautiously, however trials were conducted with multiple factors describing the behaviour of a batch of trout.

Results

No mortalities occurred during the experiment. No significant differences between the total length (P = 0.9, df 29 and t = -0.120) and weight (P = 0.09, df 29 and t = -1.717) were found between raceways at the beginning of the experiment.

Phase 1: Unrestricted feeding (T2) and random feeding (t2) (days 1-20)

The actogram of self-feeding activity of the T2 fish under 14:10 LD cycle with free access to the food is shown in Figs. 1 and 2. Initially, during this period, the T2 fish were fed during the scotophase in addition to the main feeding during the photophase. However, the pattern of nocturnal feeding was extinguished, or at least became greatly damped, over time to the point where most of the self-feeding activity of the fish was confined to the photophase, showing a diurnal feeding behaviour.

Phase 2: 14:10 LD and restricted feeding (RF) (days 21-45)

By restricting feeding, food was only available at specific times of the photophase: for one h in the morning (0900-1000 h) and one h in the afternoon (1600-1700 h). After 25 days of regular restricted feeding however, self-feeding activity of the T2 fish was not fully synchronised to the hours when food was available (Figs. 1 and 2); fish rarely confined their feeding behaviour to the times of food availability. The pattern of the trigger actuations gradually damped until day 31. The trigger actuations before A (afternoon) meal strengthened on days 30 to 32, in the upstream area. Subsequent trigger

actuations before meals were more concentrated in the downstream area (M meal) than in the upstream area (A meal). These pre-feeding activities may suggest FAA, even though there was not a sustained increase in trigger actuations 2.5 times above the basal line without inflections, i.e. FAA by definition (Aranda et al., 2001).

In regard to fish spatial distribution, in most cases the fish moved to the upstream area until the M meal was available. They returned to the downstream area for the M meal, and after 1-2 h, again moved to the upstream area, until the A meal was available. As in both cases (T2 and t2) most

times the fish had appeared on the morning side of food delivery before mealtimes. This shows importance of the morning meal when measuring FAA.

Fig. 3 shows swimming speed, number of fish moving and agonistic behaviour between fish, measured during three replicate days (37, 41, and 45) of this experiment. It appeared that swimming speed of both groups of fish before the meals was low, particularly in T2, but it increased during the meals. There was however a slight increase in speed in T2 and particularly in t2 prior to the afternoon meal. The fish did not appear to consistently
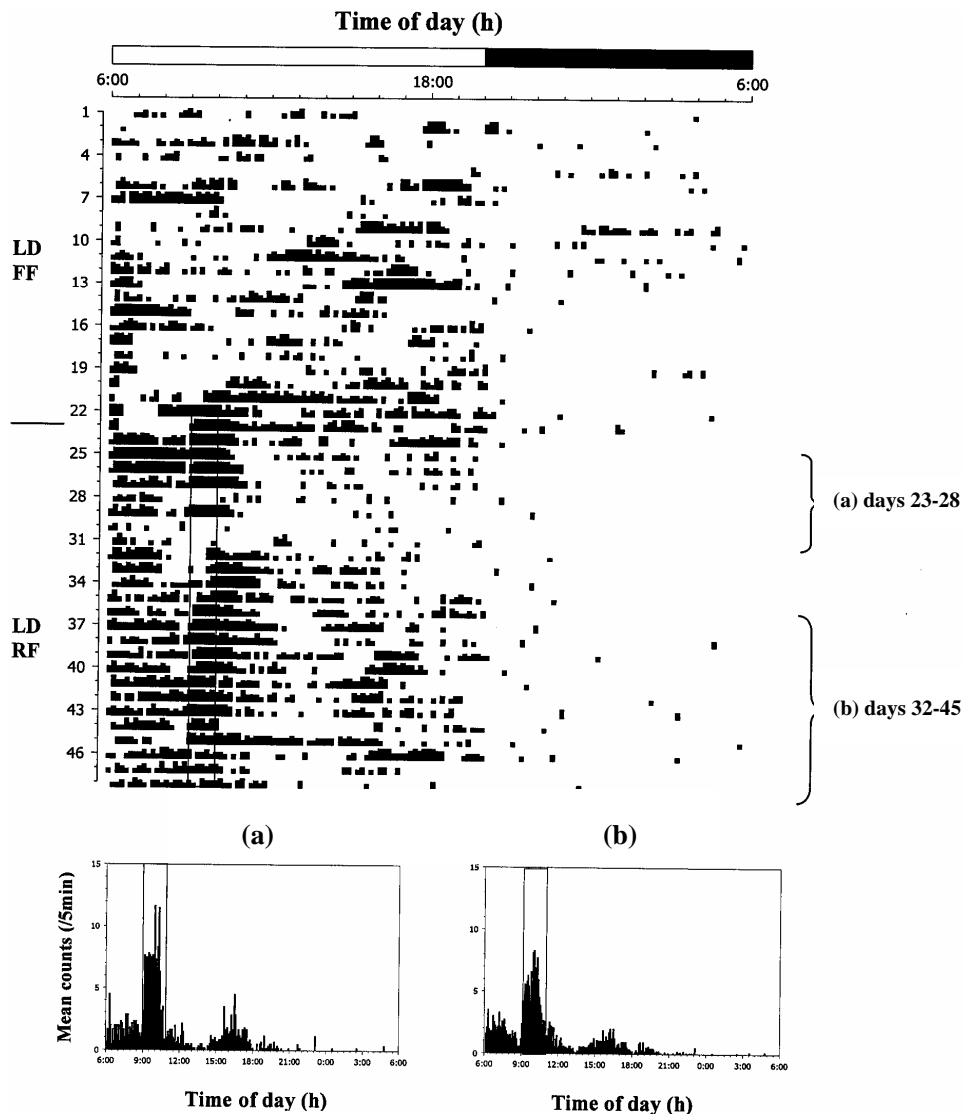

Fig. 1: Actogram of self-feeding records from T2 fish under restricted feeding (days 21-45) in the downstream area. RF, restricted feeding; FF, free food access. The light and dark phases of the LD cycle have been indicated by white and black bars respectively at the top of the graph. Rhythm profile of self-feeding activity over days 23-28 is shown in (a), over days 32-45 in (b). Mealtimes are shown by the rectangular boxes

exhibit an anticipation of the meals by an increase in swimming speed. In addition, the increase in swimming speed did not necessary correlate with the number of fish moving. This may suggest that the specific individual fish in the group were showing FAA by increasing their swimming speed. However, the agonistic behaviour of the fish may have been entrained to the meals. There was an increase in the agonistic behaviour of the fish prior to the meals which then declined during the meals. As FAA is

anticipation of food preceding a daily scheduled meal, it is suggested that the increase in agonistic behaviour between the fish was a more reliable measure of FAA for both meals than swimming speed.

Discussion

The results of the current study demonstrate that rainbow trout are not able to clearly show FAA based on the rhythm profile of self-feeding activity and/or fish
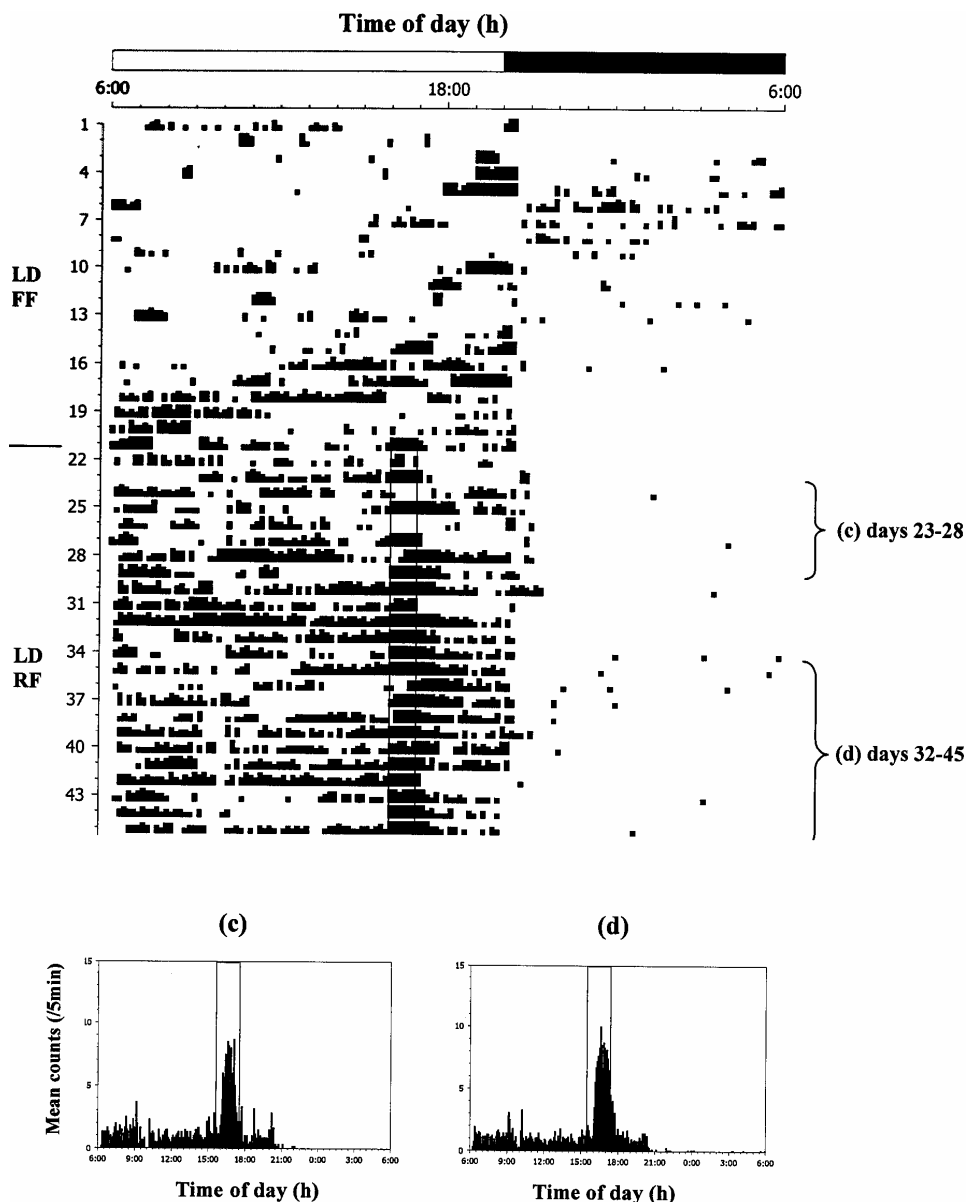

Fig. 2: Actogram of self-feeding records from T2 fish under restricted feeding (days 21-45) in the upstream area. RF, restricted feeding; FF, free food access. The light and dark phases of the LD cycle have been indicated by white and black bars respectively at the top of the graph. Rhythm profile of self-feeding activity over days 23-28 is shown in (c), over days 32-45 in (d). Mealtimes are shown by the rectangular boxes

(a) **(b)**
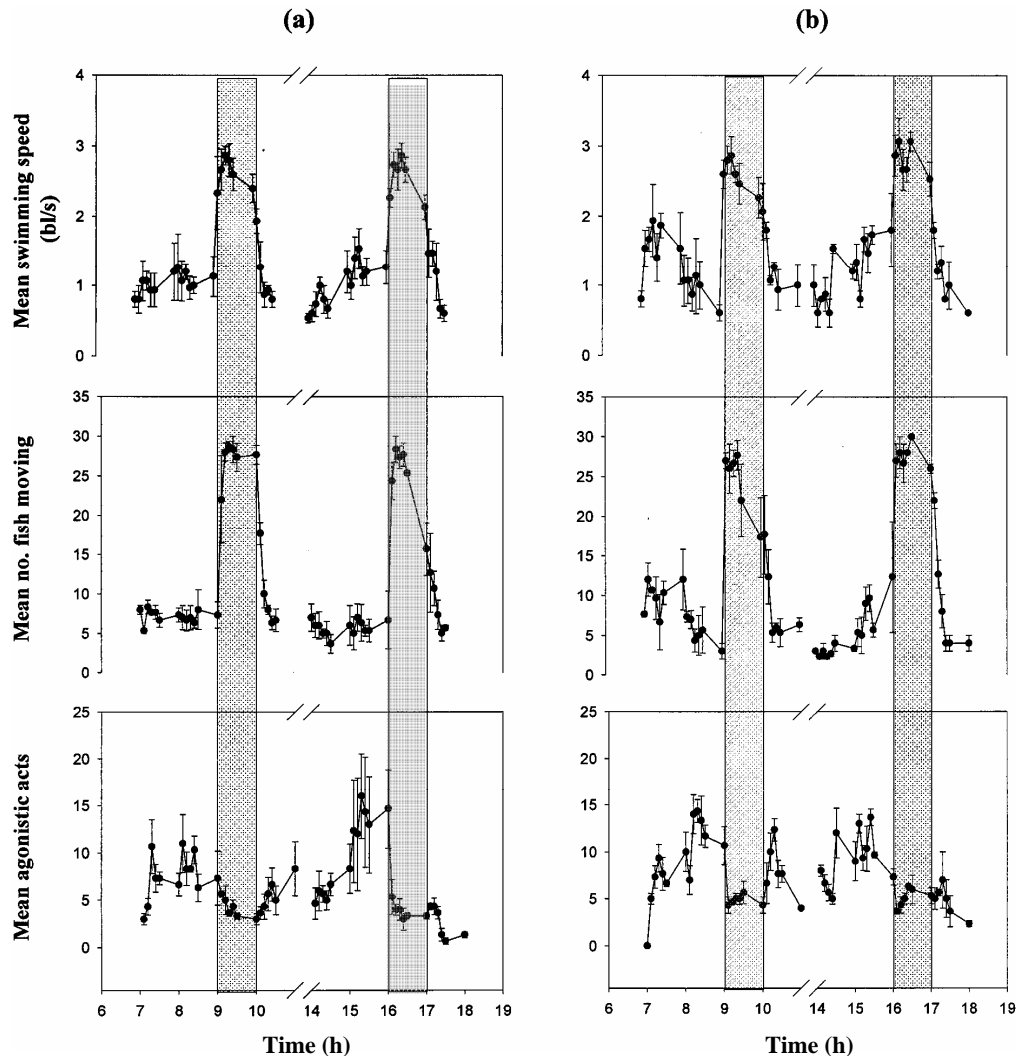
Fig. 3: Mean (X ± SE, n = 3 replicate days) agonistic acts, swimming speed (bl/s) and number of fish moving in both raceways over time of day. (a) T2, demand-feeding raceway; (b) t2, hand-feeding raceway. Bars indicate meals

spatial distribution when offered two meals per day in two locations. Instead, agonistic behaviour may be more indicative of FAA as this activity increases a couple of hours pre-feeding and decreases during feeding. The diurnal feeding behaviour of rainbow trout (Bolliet *et al.*, 2001) is evident in the actograms, the number of actuations is low during the scotophase and elevated during the photophase even during periods outside the meal time.

Chen and Tabata (2002) have demonstrated that individual rainbow trout are able to show FAA associated with two meals in one location. In addition, Bolliet *et al.* (2001) demonstrated FAA in a group of rainbow trout using a single daily meal. However, there was no evidence that shows

multiple FAAs by a group of rainbow trout. Even it has been shown that using a demand-feeder and when feeding takes place two times over one place (2T/1P), a group of rainbow trout are not able clearly to show FAA (Heydarnejad and Purser, 2008). This situation is more complicated when two different locations of food delivery are applied.

The use of two feeding locations within the raceway appeared to confuse the fish as seen, for example, in the actograms of self-feeding activity in the fish in the downstream area. On some days, there were two bands of self-feeding at the correct time of food delivery at the same area (Fig. 1, e.g. days 39-41) suggesting that the fish probably knew the time, but not the place of

food delivery (a process known as time/place learning). Maybe for this reason, the selected rainbow trout of Chen and Tabata (2002) which previously had displayed well-defined diel feeding patterns were able to anticipate multiple meals in one location. This raises the issue that FAA does not appear for all individuals in a group, even in one location of food delivery. FAA has been used as a cue for those individuals that have not learned the relationship between time and food arrival and join the experienced animals which have already shown FAA (a process called local enhancement) (Reebs and Gallant, 1997).

The results of this study suggest that agonistic behaviour may provide a better indication of FAA than self-feeding activation, as there was an indication of increased agonistic behaviour between fish before the arrival of food delivery in both raceways (T2 and t2).

This study used swimming speed as a behavioural variable to measure FAA. While other studies concerning FAA usually consider swimming or locomotor activity which exhibits daily rhythms and can be entrained by a feeding cycle to show FAA, for instance: Atlantic salmon, *Salmo salar* (Juell *et al*., 1994), loach, *Misgurnus anguillicaudatus* (Naruse`and Oishi, 1994) and goldfish, *Carassius auratus* (Sanchez-Vazquez *et al*., 1997). In addition, there is a gradual increase in agonistic behaviour between the fish until the food arrives; this behaviour then reduces during the feeding and becomes steady or increases afterwards. Therefore, it is suggested that an increasing rate of agonistic behaviour is evidence of the fish entrainment to the time of food availability. It is also suggested that the rate of agonistic behaviour is a better indication of FAA for the fish of the current study, than other common behavioural variables measuring FAA, such as fish spatial distribution (Reebs, 1993) or the rhythm of self-feeding activity (Azzaydi *et al*., 1999). As far as is known, this finding is the first evidence of the usefulness of agonistic behaviour as an indicator of FAA.

Moreover, FAA is one of the basic attributes of feeding-entrained rhythms with a single meal, denoting true synchronization to the feeding zeitgeber (Sheward *et al*.,

2007). This wills enable animals to predict the time of feeding using a biological clock. While individuals may demonstrate FAA in relation to multiple meals, it also has been suggested that different individuals in a group appear to be responsible for anticipating different meals (Chen and Tabata, 2002).

The present study suggests that FAA is not limited to trigger actuations, fish positioning or swimming activity as outlined in the literature but may also be described by swimming speed and agonistic behaviour. It has been suggested that only aggressive individuals can anticipate a situation, while non-aggressive individuals just react to environmental stimuli (Koolhaas *et al*., 1997). Based on this hypothesis and the agonistic behaviour of fish, it can be proposed that a few fish of the current study, accounting for most of the agonistic acts were those individuals capable of showing FAA. This cannot be confirmed because the fish were not individually marked. An increase in aggressive behaviour before schedule and single daily mealtimes have been reported for captive primates such as: common chimpanzees, *Pan troglodytes* (de Waal and Hoekstra, 1980), hamadryas baboons, *Papio hamadryas* (Wasserman and Cruikshank, 1993) and pigs, *Sus scrofa* (Carlstead, 1986). However, as far as it is known this finding in fish is new and differs from the findings of Weber and Spieler's (1987) on the agonistic behaviour of medaka fish, *Oryzias latipes*. These authors showed that the level of agonistic behaviour of the fish increased during a mealtime, while in the present study, this increase in the agonistic behaviour of the fish took place before the delivery of the food.

Therefore this study suggests the idea that animals may fail to show FAA using one method, while demonstrating robust anticipation as assessed by de Groot and Rusak (2004). Thus, failing to record one measure only may have mistakenly led to the conclusion that the subjects in questions are not able to show FAA. For instance, under restricted schedule feeding, mice showed robust wheel running in anticipation of mealtimes, but did not engage in anticipation of drinking (Holmes and Mistlberger, 2000). Therefore, detection of

FAA depends on the way it is measured (Pecoraro *et al*., 2002). As a consequence, in this study, FAA with an increasing number of agonistic acts preceding mealtimes was substituted for demand-feeding activity. This indicates that, our fish may have been expressing FAA through the increased number of agonistic acts, rather than by demand-feeding activity. Generally, FAA suggests an increased level of arousal prior to feeding. In this study, such behaviour appeared in the form of an increased number of agonistic acts. While in a study by Aragona *et al.* (2002) a significant correlation between the level of FAA and dopamine, a neurotransmitter involved arousal, was observed. To assess the likelihood of this hypothesis, fish must be maintained in constant conditions (e.g. constant light (LL) and FD) for several days or weeks to determine if agonistic behaviour persists.

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