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Specific Individuals of Rainbow Trout (*Oncorhynchus mykiss*) Are Able to Show Time-Place Learning

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Abstract: Using demand-feeders, time-place learning association of food was investigated in rainbow trout (*Oncorhynchus mykiss*) in 2 small raceways (TR1 and TR2). In order for the fish to be visually identified, they were tagged individually and their behaviour and distribution were monitored by cameras. Equipping with a demand-feeder, TR1 was assigned to test 2 times/1 place (2T/1P) while 2 demand-feeders were used in TR2 assigned to test 2 times/2 places (2T/2P). After 26 days of restricted feeding (RF) the fish were deprived of food for 3 days. The results of the present study indicate that, at the group level, rainbow trout are not able to clearly demonstrate time-place learning except for some fish in the group. These fish were identified as dominants, in terms of a higher rate of agonistic acts or a greater amount of food eaten. Feeding behaviour was largely influenced by social dominance of the group, especially through territoriality.

Key Words: Time-place learning, aggressiveness, social behaviour, rainbow trout

Bazı Alabalık (*Oncorhynchus mykiss*) Bireyleri Zaman-Yer Öğrenme Özellikleri Gösterebilir

Özet: Yemlik talebi kullanarak besin ile ilişkili zaman-yer öğrenme konusu küçük tanklarda (TR1 ve TR2) yetiştirilen alabalıklarda (*Oncorhynchus mykiss*) incelenmiştir. Balıkların görsel olarak izlenebilmesi için her birey etiketlenmiş, davranışları ve dağılımları kameralar ile gözlenmiştir. TR1 tankında yemlik, 2 zaman/1 yer (2T/1P), TR2 de ise 2 zaman/2 yer (2T/2P) şeklinde deney tasarlanmıştır. Balıklara 26 gün boyunca çok düzenli bir beslenme uyguladıktan sonra üç gün boyunca aç bırakılmışlardır. Bu çalışmanın sonunda balıkların grup olarak zaman/yer öğrenme yeteneğinin olmadığı fakat gruptaki bazı balıkların zaman-yer öğrenme yeteneğine sahip olduğu gözlenmiştir. Bu balıklar çok fazla besin yeme açısından dominant olarak tanımlanmıştır. Beslenme davranışı özellikle belirli bölgelerde grubun sosyal baskınlığından oldukça etkilenmiştir.

Anahtar Sözcükler: Zaman-yer öğrenme, saldırganlık, sosyal davranış, alabalık

Introduction

The phenomenon in which animals learn to appear at the correct time and place of food delivery is known as time-place learning. In fact, time-place learning is a kind of response to daily spatio-temporal patterns of food availability. This behaviour has been demonstrated in a variety of organisms (1-6), birds (7-10), and rats (11-19).

In fish, however, our knowledge of time-place learning is generally limited. The only evidence of this phenomenon has been demonstrated by Reebs (20,21) in 2 species, namely *Notemigonus crysoleucas* (golden shiners) and *Galaxias maculatus* (inanga), and in another cichlid fish, the angelfish *Pterophyllum scalare* by Gomez-Laplaza and Morgan (22). In addition, as food is presented periodically on a daily feeding cycle in time-place learning, the fish may develop some activity (e.g., by increasing their swimming speed) in anticipation of the forthcoming meal within a few days of the feeding cycle being established. This pre-feeding behaviour is known as feeding anticipatory activity (FAA) (23).

Experimental studies in fishes show that there is an individual variability in behavioural responses (24). For example, it has been suggested that only aggressive individuals can anticipate a situation, while non-aggressive individuals just react to environmental stimuli (25). Likewise, Sneddon (26) was able to classify individual rainbow trout into bold (sociable) and shy (fearful/timid). He found that bold individuals spend more time in the feeding area and therefore appear to learn more quickly than the shy individuals, due to the fact that personality traits (e.g., bold/shy) influence learning ability (27).

Despite the existence of a large body of knowledge on the ecology, physiology, and nutrition of rainbow trout, one of the most studied aquaculture species of fish, time-place learning has not been examined individually in this species.

Materials and Methods

Fish holding conditions

The experiment was carried out over 52 days between 6 March and 26 April 2005 at the School of Aquaculture, University of Tasmania, Launceston, Tasmania, Australia.

Rainbow trout, hatched and grown in captivity, were transferred to 2 identical raceways (TR1 and TR2) (length × width × depth; 3.1 × 0.67 × 0.4 m) with re-circulated freshwater in a temperature-controlled and insulated room. The raceways were positioned within a recirculating system and each was supplied with filtered and aerated freshwater at a rate of 20 l min^{-1}. 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). Depth of water in both raceways was about 22 cm, with a surface current velocity of 1.2 ± 0.2 cm s^{-1} (measured by a floating object in the water current along the length of the raceways). The water temperature was set at 12 ± 1 °C. The submerged water inlet was positioned towards the wall of the raceways to enable a high level of water exchange without significant longitudinal water velocity. For convenience, sections 1 and 2 were nominated as the 'upstream area' and sections 3 and 4 as the 'downstream area'. Room illumination was provided by fluorescent tubes (Thorn, 36 W, 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^{-1} 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.

Three demand-feeders (ARVO-TEC T Drum) were used. Hanging them from the ceiling enabled them to be placed above and away from the raceway to reduce disturbance during servicing. The bottoms of the demand-feeders were connected to a funnel, linked to the submerged outlet tubes (25 mm in diameter) in the raceways. One raceway (TR1; assigned to test 2 times/1 place) (2T/1P) was equipped with 1 demand-feeder and the other raceway (TR2; assigned to test 2 times/2 places) (2T/2P) was equipped with the 2 demand-feeders. In TR1, the demand-feeder was placed in section 1 (most upstream), and in TR2 1 of the 2 demand-feeders was placed in section 1 (most upstream) and the other in section 4 (most downstream).

The self-feeding system consisted of 4 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 position of 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. To minimise accidental activation of the trigger, fish needed to bite and pull the trigger. Once the biting and pulling action of a fish activated the trigger, a signal was generated by the PLC and a number of pellets (reward level) were delivered into the raceways, with a one-second delay between 2 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. & 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.

Three colour cameras (Swann® C500 CCD) were mounted on the ceiling, about 2 m above the raceways to record the fish activity. The first camera filmed section 1 (most upstream), the second section 4 (most downstream), and the third sections 2 and 3 of both raceways. On sample days, recordings were made continuously during the photophase (14 h). Video footage was recorded continuously on a PC hard-drive using Chateau-XP software, capable of recording up to 8 channels simultaneously. The recorded footage was stored on DVDs. The fish distribution in the raceways was noted and recorded every 10 min. Data are presented as the number of fish/10 min interval present in either the downstream area (morning: M) or upstream area (afternoon: A) in each raceway.

Procedure

In order for the fish to be visually identified, they were tagged individually, by means of the button technique (Noble, pers. comm.) in which 2 buttons were attached behind, or just in front of the dorsal fin by means of a strong silk thread and to distinguish fish individually different combinations of coloured buttons were applied. A little antibiotic solution (0.1% Acriflavin) was used to heal the wound area. Thus each fish was given an ID code, by which it could be identified (see Table 1).

Table 1. Fish ID based on their coloured button tags.

TR1 (2T/1P)		TR2 (2T/2P)	
Fish No.	Fish ID	Fish No.	Fish ID
1	B/R	1	B/W
2	R/W	2	R/W
3	W/R	3	R/B
4	R/B	4	B/R
5	B/B	5	B/B
6	W/B	6	W/W
7	R/R	7	W/R
8	W/W	8	W/B

W, White; B, Black; R, Red

The reward level was set at an average of 1 pellet (0.041 g) per trigger actuation for the first 12 days of the experiment. Later, the reward level was increased to an average of 24 pellets (1 g) per trigger actuation until the end of the experiment. The feed used was a 3 mm Nutra Tranfer salmon pellet (50% protein, 23% lipid, Skretting, Pty Ltd. Cambridge, Tasmania).

Prior to the experiment, the fish were acclimated to the trigger to bite and pull the trigger in order to obtain food. During the training period (35 days), the fish were fed around the trigger at least once a day by hand, so that the fish could learn to associate pulling the trigger with the release of food. After this period, it took an additional 25 days until the pattern of trigger actuations by the fish stabilised. During the training period, the fish had free access to food throughout the photophase (14 h). Sequential phases of the experiment were as follows:

Phase 1: Free food access (unrestricted feeding) with a low reward level (LRL) (1 pellet/trigger actuation) during the photophase (days 1-12)

Phase 2: Free food access (unrestricted feeding) with a high reward level (HRL) (24 pellets/trigger actuation) during the photophase (days 13-23)

Phase 3: Restricted feeding (RF) under a 14L:10D photoperiod (days 24-49)

Phase 4: Food deprivation (FD) (days 50-52)

Phase 1: Free food access (unrestricted feeding) with a low reward level (LRL)

In order for the fish to be acclimatised to the LD cycle of 14:10 and establish a daily pattern of feeding activity, they were subjected to an ad libitum feeding regime. However, the time during which food was available was limited to the photophase (14 h). The amount of food released per trigger actuation by the fish (reward level) was set at an average of 1 pellet (0.041 g) (using a feeder drum with 24 holes, each hole 4 mm in diameter). This trial continued for 12 days (6-17 March 2005). At the end of this trial, the effects of the LRL on the fish social interaction, size variations, and growth performance were calculated as follows:

Condition Factor (K) = 100 Weight (g) / Length^3 (cm)

Specific Growth Rate (SGR) (% day^{-1}) = 100 [ln (W_f) – ln (W_i)]/t (the period of the trial), where W_f and W_i are final and initial weights, respectively

Coefficient of Variation of weight (CVw (%)) = 100 (standard deviation/mean)

Growth depensation (ΔCVw (%)) = 100 [(CV_{wf}/CV_{wi}) – 1], where ΔCVw is the change in the CVw during the course of the trial, and CV_{wf} and CV_{wi} are final and initial coefficient of variation for body weights, respectively (Carter et al., 1996).

Phase 2: Free food access (unrestricted feeding) with a high reward level (HRL)

The conditions of this phase of the experiment, which extended for 11 days (18-28 March 2005), were exactly the same as those in the above trial, except that in this period the reward level was increased to an average of 24 pellets (1 g) per trigger actuation by the fish (using a feeder drum with 12 holes, each hole with dimensions of 450 × 20 mm). This approach was used because a marked dominance hierarchy developed in phase 1, particularly for TR1, during which most of the fish were not able to feed, and lost weight. The feeding regime was increased because of animal ethics considerations. The level of 24 pellets/trigger actuation was the next largest drum available to the project after the 1 pellet/trigger actuation drum.

Phase 3: Restricted feeding (RF) under a 14L:10D photoperiod (days 24-49)

In this phase, the fish were subjected to restricted feeding (RF). Food was restricted to 2 feeding periods (meals), during which food was freely available: morning (0900-1100 h) and afternoon (1600-1800 h) meals. The morning and afternoon meals in TR1 were in section 1, whereas those in TR2 were in sections 1 and 4, respectively. This trial aimed to entrain (synchronise) the timing of demand-feeding activity of the fish with that of food availability (RF). Consequently, TR1 was designed to test the learning ability of fish in relation to 2 times/1 place and TR2 in relation to 2 times/2 places. The phase was conducted for 26 days (between 29 March and 23 April 2005).

Phase 4: Food deprivation (FD) (days 50-52)

After 26 days of feeding, the fish were deprived of food for 3 days (24-26 April 2005). This phase was used to determine whether the fish had learned to appear at the correct time and correct place of food delivery.

Data analysis

The Chronolab program was used for data acquisition, displayed as actograms. The software was designed to record the self-feeding activity at a resolution of 10 min, each point representing the percentage of the total trigger actuations that occurred in 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).

Results

Phase 1: Unrestricted feeding with a LRL

The actogram of the self-feeding behaviour of rainbow trout under 14L:10D with free access to food at the photophase, with a low reward level of an average 1 pellet/trigger actuation in TR1, is shown in Figure 1. The spatial distribution of fish recorded on days 1, 5, 8, and 10 indicates that few fish occupied sections 1 and 2, with many in 3 and most in 4 (Figure 2). Results of the trigger actuations on the sampling days revealed that in TR1 just 1 fish (White/White or W/W) accounted for more than 95% of the total trigger actuations (hits) (see Table 2). This led to a marked dominance hierarchy and increased aggression between the fish. Competition between individuals for food particles increased also. The fish W/W formed a territory and by monopolising the feeding area (section 1, most upstream) inhibited access to the food by the other fish. Consequently, most of the fish stayed and starved in section 4 for most of the time (most downstream) (see Figure 2). Therefore, it was decided that the reward level should be increased to an average of 24 pellets (1 g)/trigger actuation (using the next size of feeding drum). Tables 3 and 4 respectively demonstrate the weight comparison and growth performance between the fish at the start and end of this trial. All fish except the WW lost weight.

The actogram of the self-feeding behaviour in TR2 fish is shown in Figures 3 and 4. The pattern of trigger

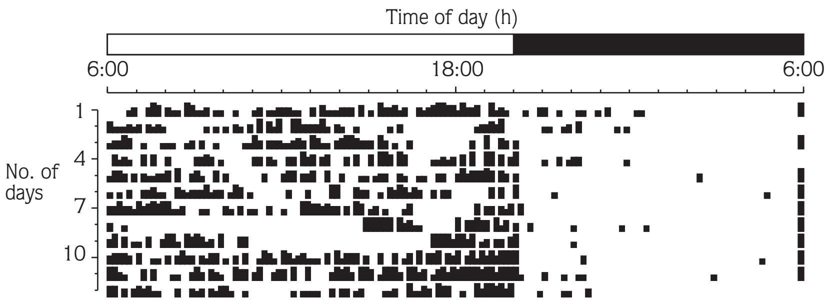

Figure 1. Actogram of self-feeding records from TR1 (2T/1P) fish during unrestricted feeding with a low reward level (LRL). Food was only available in the light phase. The horizontal bar on the top of the figure represents the LD cycle (open for the light phase, solid for the dark phase).

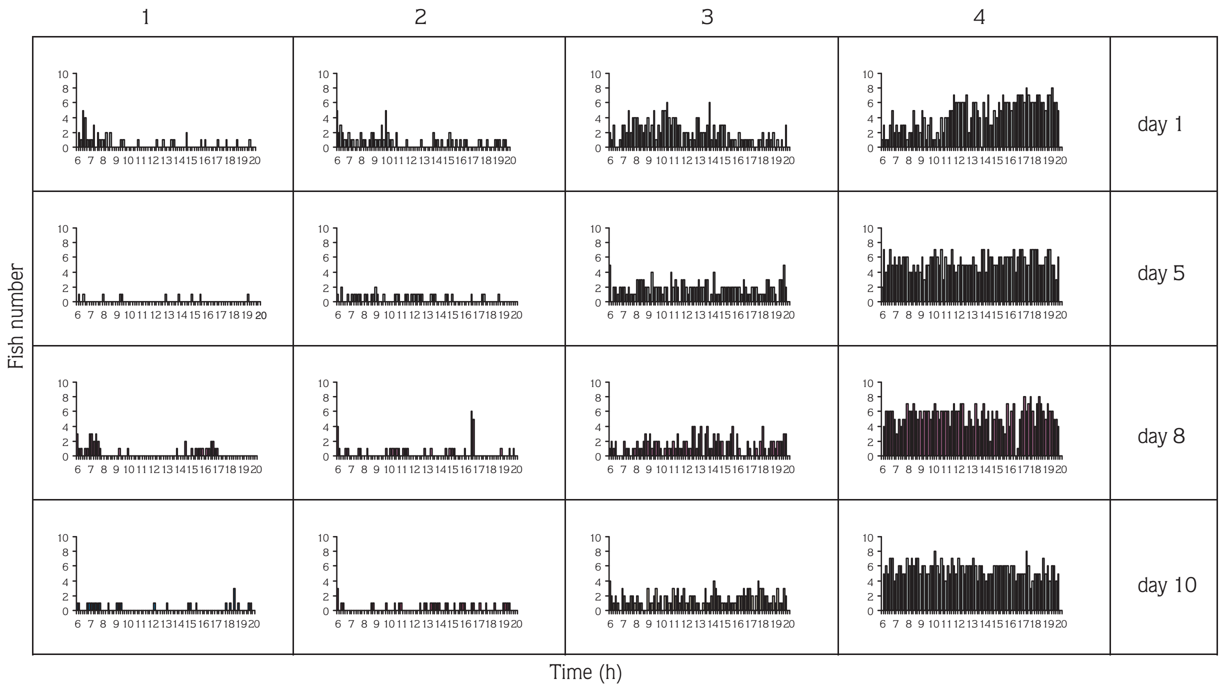

Figure 2. Fish spatial distribution of TR1 (2T/1P) under unrestricted feeding with a low reward level (LRL). The fish numbers were recorded every ten minutes throughout the photophase (14 h). Numbers at the top of figure refer to the sections of the raceway from most upstream (1) to most downstream (4), respectively.

actuations in TR2 was not confined to 1 fish. There were 3 fish that accounted for more than 89% of combined total actuations of the 2 demand-feeders (up- and downstream), namely W/B (White/Black), W/R (White/Red), and W/W (White/White) (Table 5). The contribution of the fish W/B in the 2 demand-feeders was far greater than that of the fish W/R or W/W. All the fish gained weight (see Table 6 and also Table 7 for growth performance). Figure 5 depicts the fish distribution on the sampling days of this trial showing that fish used the 2 feeding areas and tended to stay in sections 1 and 4, rather than the other 2 sections, i.e. 2 and 3.

Phase 2: Unrestricted feeding with a HRL

The increase in the reward level to an average of 24 pellets (1 g)/trigger actuation from day 13 onwards resulted in availability of more food and therefore a decreased self-feeding activity in both raceways (Figures 6 and 7). In TR1, because defence of the feeding area was no longer economical, the feeding territory of the fish W/W was broken down. This was due more to the outweighing cost than to the benefit of sustaining a territory by the fish W/W, when the amount of food availability increased during the HRL. Therefore, the social interactions between the fish were ameliorated. Visual observations proved that not only the fish W/W but also the other individuals of the group were able to consume food particles. Nevertheless, fish distribution did not change perceptibly compared to LRL (Figure 8). Sampling occurred on days 19 and 22. Interestingly, activations of the trigger were carried out mostly by the other fish (e.g., R/R, Red/Red or R/B, Red/Black) rather than W/W and the proportion of biting activity by the latter decreased greatly (Table 2).

In TR2, the pattern of trigger actuations did not vary greatly and the 3 fish (W/B, W/R, and W/W) mastered the biting action of 2 demand-feeders with a major contribution by the fish W/B (Table 5). The fish preferred to stay in the downstream area (especially section 3) during most of the photophase period (Figure 9).

In both raceways, the total number of trigger actuations (hits) was reduced compared to the LRL (Tables 2 and 5).

Phase 3: Restricted feeding (RF) under a 14L:10D photoperiod

By restricting the times during which food was available, demand-feeding activity of the fish became more

Table 2. Number of trigger actuations for each fish in TR1 (2T/1P) at different phases of the experiment.

PHASES		Fish ID								
		DAYS	W/W	R/R	B/B	B/R	R/B	W/R	R/W	W/B
Open access to food	LRL	1	142	1	0	0	0	0	0	2
		5	123	0	0	0	0	0	0	0
		8	112	1	1	0	1	1	0	0
		10	195	17	1	0	0	0	0	0
	HRL	19	1	13	0	0	31	0	0	0
		22	3	22	0	0	19	0	0	0
RF & LD		26	1	45	9	4	9	3	0	0
		29	14	85	0	9	11	4	0	2
		33	13	48	0	4	7	1	0	0
		38	11	9	0	1	2	3	0	4
		40	18	7	7	3	0	0	0	0
		45	20	5	3	0	7	0	1	2
		47	8	5	0	2	7	d	0	3
		49	15	9	0	1	0	d	0	1
FD		50	135	26	4	2	0	d	0	0
		52	146	31	1	0	0	d	1	2

Table 3. Initial and final body weight (g) during a low reward level (LRL) trial for TR1 (2T/1P) fish.

Fish ID	TR1 (2T/1P)		
	W(i)	W(f)	ΔW
W/W	259.1	310.6	51.5
W/R	146.4	145.1	-1.3
B/B	164.3	161.1	-3.2
W/B	188.1	182.2	-5.9
R/W	234.5	227.6	-6.9
B/R	213.3	206.1	-7.2
R/R	174.6	166.6	-8
R/B	155.3	147.1	-8.2

i: initial weight (g)
f: final weight (g)
ΔW: W (f) – W (i)

Table 4. Growth performance of TR1 (2T/1P) fish during a low reward level (LRL).

	TR1 (2T/1P)
Initial body weight	191.9
Final body weight	193.3
SGR%	0.06
K (initial)	1.51
K (final)	1.46
ΔCV_w %	37

SGR: Specific growth rate
K: Condition factor
ΔCV_w: Growth depensation (the change in the coefficient variation for weight)

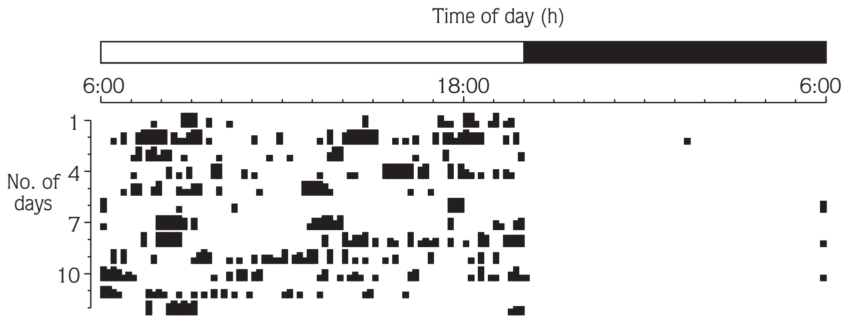

Figure 3. Actogram of self-feeding records from TR2 (2T/2P) fish in the most upstream during unrestricted feeding with a low reward level (LRL). Food was only available in the light phase. The horizontal bar at the top of the figure represents the LD cycle (open for the light phase, solid for the dark phase).

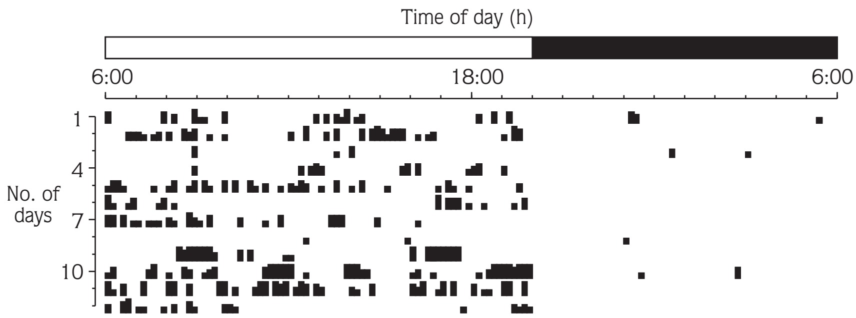

Figure 4. Actogram of self-feeding records from TR2 (2T/2P) fish in the most downstream during unrestricted feeding with a low reward level (LRL). Food was only available in the light phase. The horizontal bar at the top of the figure represents the LD cycle (open for the light phase, solid for the dark phase).

or less synchronized to the mealtimes. After 1 week, TR1 fish synchronised their demand-feeding activity to food availability and probably learned to confine their activity to the restricted feeding times, as evidenced by most of the trigger actuations occurring at the time of food availability. This synchronisation, however, occurred on days 32-35 but was less defined thereafter (Figure 10). Most individuals in the group participated in the trigger actuations to some extent (Table 2). Progressively, the fish became less congregated in the most downstream area (section 4), as the number of fish in each section increased and a more even fish spatial distribution in each section appeared (days 40 through 49) (Figure 11).

In TR2, synchronisation of the feeding activity to the feeding times varied in the 2 locations, so that the fish in the downstream area (Figure 12) achieved it more quickly than those in the upstream area (Figure 13). The synchronization took place after a week downstream, but upstream it rarely, if ever, happened except for the last 3 days of this trial after 24 days. Trigger actuations were still carried out by the 3 fish; however, towards the end of this trial, the contribution of the fish W/R to trigger actuation was reduced (Table 5). Sections 2 and 3 were most popular throughout this trial (Figure 14).

Phase 4: Food deprivation (FD)

Figure 15 shows fish distribution on days of food deprivation (50-52) for TR1 fish, which did not appear as a group at the correct time and correct place of food delivery (section 1). This indicates that with the exception of maybe a few fish (in particular W/W) they did not show time-place learning. A continuous self-feeding activity occurred for the whole photophase during the days of food

Table 5. Number of trigger actuations for each fish in TR2 (2T/2P) at different phases of the experiment.

				Fish ID						
PHASES		DAYS	Stream	W/B	W/W	W/R	B/B	R/B	B/R	R/W	B/W
Open access to food	LRL	1	Up	36	1	19	0	0	0	0	0
			Down	9	11	18	0	0	0	0	0
		5	Up	19	0	33	4	0	0	0	0
			Down	0	1	0	35	0	5	0	0
		8	Up	44	0	69	0	0	0	0	0
			Down	0	1	0	0	0	1	0	0
	HRL	10	Up	2	0	53	0	0	0	0	0
			Down	109	33	0	2	1	3	0	0
		19	Up	9	0	3	0	0	0	0	0
			Down	15	14	0	0	0	0	0	0
		22	Up	16	0	16	0	0	0	0	0
			Down	22	26	0	0	0	0	0	0
RF & LD		26	Up	5	0	10	0	0	0	0	0
			Down	19	45	0	0	0	0	0	0
		29	Up	4	0	30	0	0	0	0	0
			Down	45	24	0	0	0	1	0	0
		33	Up	22	2	14	0	0	0	0	0
			Down	7	29	0	0	0	0	0	0
		38	Up	0	15	2	0	0	0	0	0
			Down	40	11	1	0	0	0	0	0
		40	Up	9	4	0	0	0	0	0	0
			Down	38	6	0	0	0	0	0	0
		45	Up	0	1	3	0	0	0	0	0
			Down	26	8	0	0	0	0	0	0
		47	Up	24	0	0	2	0	2	0	0
			Down	2	6	0	1	0	0	0	0
		49	Up	17	0	3	0	0	0	0	0
			Down	36	31	0	0	1	1	0	0
FD		50	Up	0	0	4	0	0	0	0	0
			Down	37	13	0	0	0	2	0	0
		52	Up	73	0	18	0	1	0	0	0
			Down	100	7	0	0	0	0	0	0

LRL, low reward level; HRL, high reward level; FF, free food access
RF, restricted feed; LD, light-dark cycle; FD, food deprivation
i, severely fungus-infected fish was removed; §, this fish (dominant) was removed

Table 6. Initial and final body weight (g) during a low reward level (LRL) trial for TR2 (2T/2P) fish.

Fish ID	TR2 (2T/2P)		
	W(i)	W(f)	ΔW
W/B	189.8	225.8	36
W/R	234.9	253.2	18.3
W/W	253.5	264.1	10.6
B/B	193.8	201.5	7.7
B/R	184.3	191.8	7.5
R/B	153.6	156.8	3.2
R/W	191	192.5	1.5
B/W	213.4	214.2	0.8

i: initial weight (g)
f: final weight (g)
ΔW: W (f) – W (i)

Table 7. Growth performance of TR2 (2T/2P) fish during a low reward level (LRL).

	TR2 (2T/2P)
Initial body weight	201.8
Final body weight	212.5
SGR%	0.43
K (initial)	1.51
K (final)	1.45
ΔCV_w %	6

SGR: Specific growth rate
K: Condition factor
ΔCV_w: Growth depensation (the change in the coefficient variation for weight)

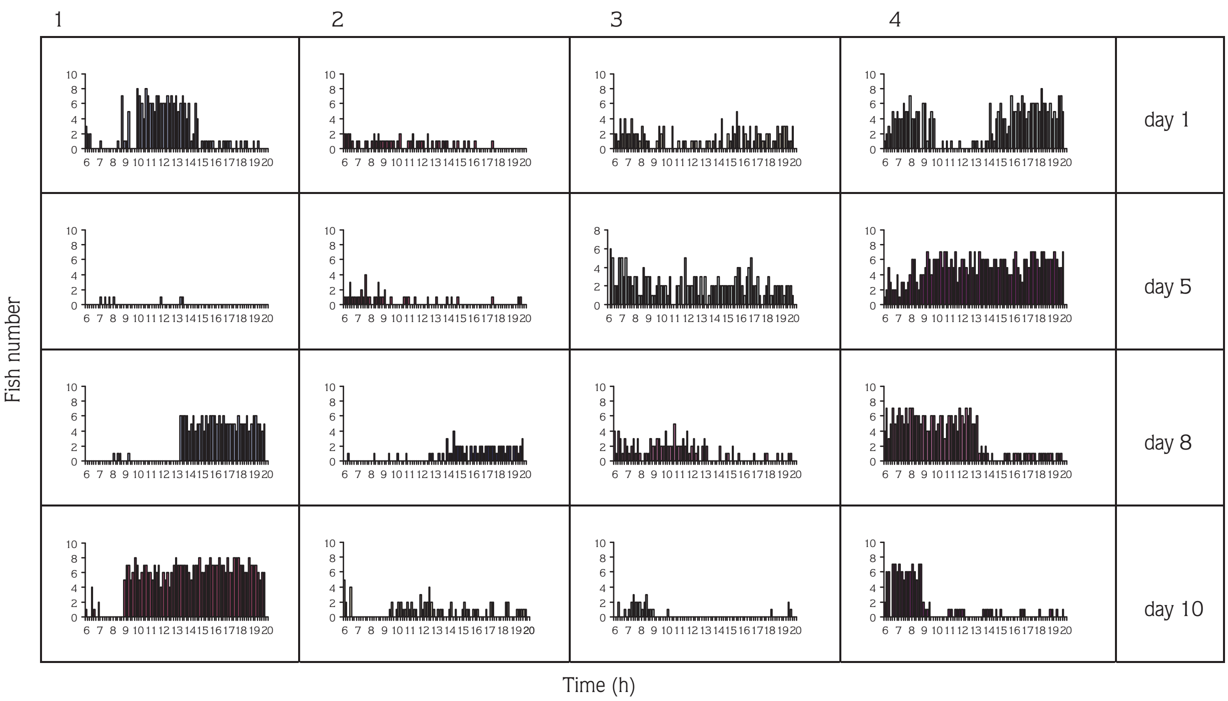

Figure 5. Fish spatial distribution of TR2 (2T/2P) under unrestricted feeding with a low reward level (LRL). The fish numbers were recorded every ten minutes throughout the photophase (14 h). Numbers at the top of the figure refer to the sections of the raceway from most upstream (1) to most downstream (4), respectively.

deprivation (Figure 10) with the fish W/W accounting for more than 80% of the trigger actuation. Fish R/R was the second highest instigator (16%) of trigger actuation (see Tables 2 and 8). Spatial distribution demonstrated no evidence of the occurrence of time-place learning, as the fish did not appear in the feeding area (section 1) at the correct time.

A good pattern of trigger actuations was found for TR2 fish with a more pronounced pattern in section 1 (most upstream) than in section 4 (most downstream) (Figures

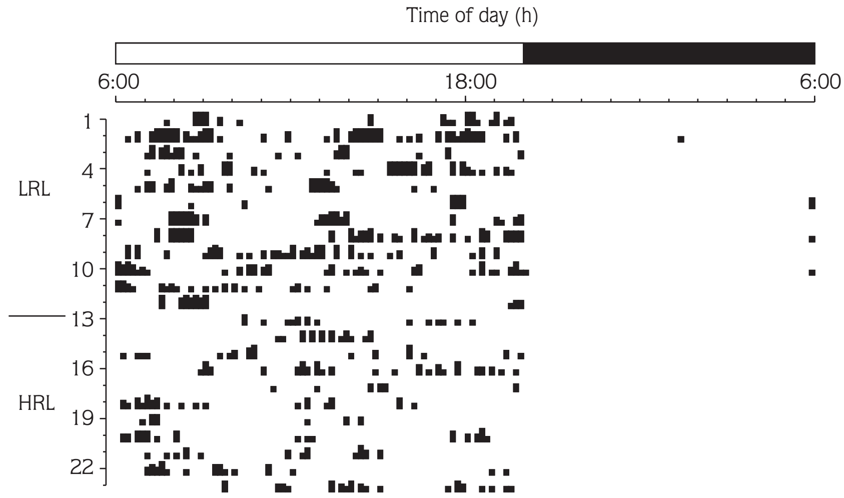

Figure 6. Actogram of self-feeding records from TR1 (2T/1P) fish during unrestricted feeding with a high reward level (HRL) (days 13-23). Food was only available in the light phase. The horizontal bar at the top of the figure represents the LD cycle (open for the light phase, solid for the dark phase). Note: a clear reduction of hits during the HRL relative to the LRL trial.

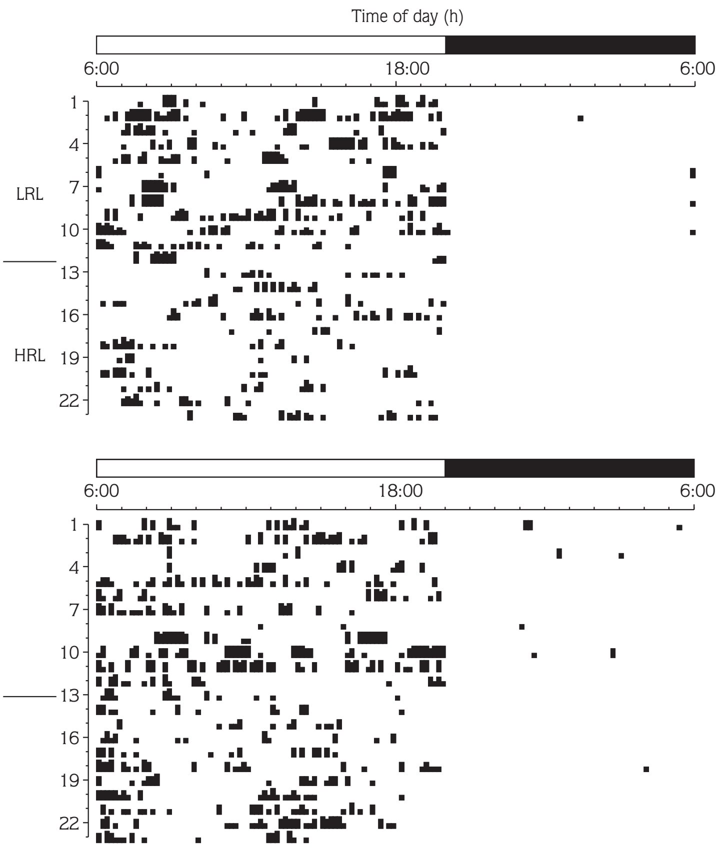

Figure 7. Actogram of self-feeding records from TR2 (2T/2P) fish in the most upstream (upper figure) and the most downstream (lower figure) during unrestricted feeding with a high reward level (HRL) (days 13-23). Food was only available in the light phase. The horizontal bar at the top of the figure represents the LD cycle (open for the light phase, solid for the dark phase).

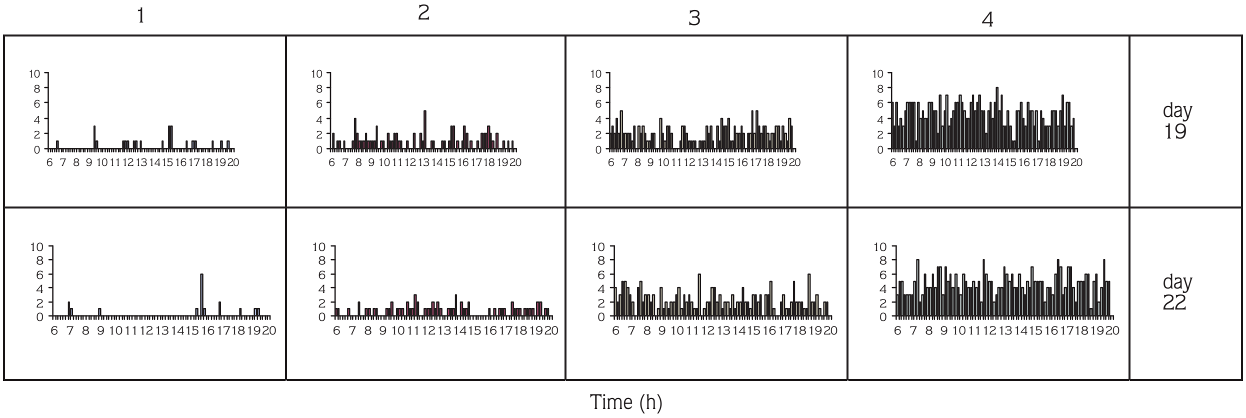

Figure 8. Fish spatial distribution of TR1 (2T/1P) under unrestricted feeding with a high reward level (HRL). The fish numbers were recorded every ten minutes throughout the photophase (14 h). Numbers at the top of the figure refer to the sections of the raceway from most upstream (1) to most downstream (4), respectively.

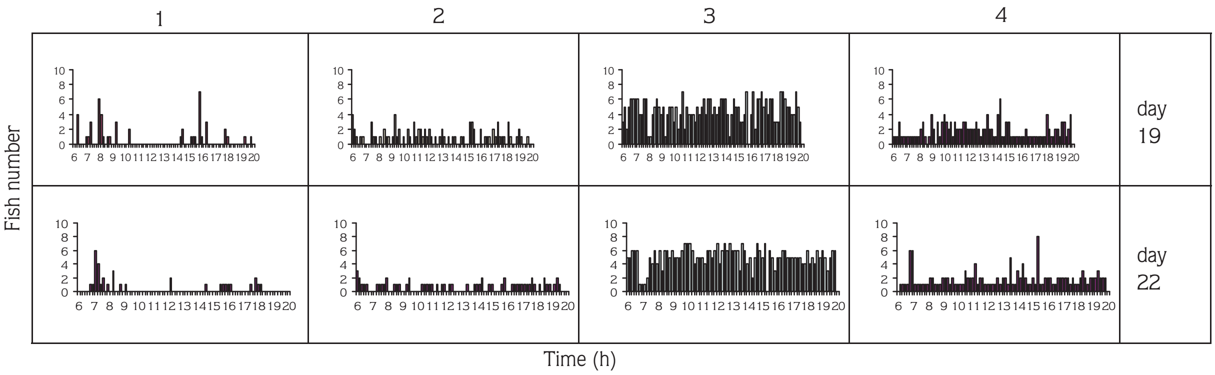

Figure 9. Fish spatial distribution of TR2 (2T/2P) under unrestricted feeding with a high reward level (HRL). The fish numbers were recorded every ten minutes throughout the photophase (14 h). Numbers at the top of the figure refer to the sections of the raceway from most upstream (1) to most downstream (4), respectively.

13 and 14). Most of the trigger actuations in both areas were carried out by fish W/B (more than 80%) (Table 5). To a lesser extent, other fish participated in the trigger actuations on the days of food deprivation (e.g., W/R and W/W). In addition, the Schematic Figure on day 52 revealed that fish W/B (of TR2) and WW (of TR1) learned time-place association of food due to their appearance at the correct time and place of food delivery. Fish spatial distribution on these days showed that the fish tended to stay in sections 2 and 3 rather than the feeding areas, i.e. sections 1 and 4 (Figure 16).

Discussion

The results of the present study indicate that at the group level, under the conditions tested, rainbow trout are not able to clearly demonstrate time-place learning except for some fish in the group. These fish were identified as dominants, in terms of a higher rate of agonistic acts or a greater amount of food eaten, when the fish were tagged individually. In fact, feeding behaviour was largely influenced by social dominance of the group, especially through territoriality.

A number of studies have shown that in demand-feeding systems relatively few fish within a group account for the majority of the trigger actuations (28-32). This is more likely to be related to the development of dominance hierarchies, rather than to differences among fish in learning abilities (33). Thus, behavioural differences may be ascribed to the social rank of each fish within the group. It has been shown that the reward level can have an effect

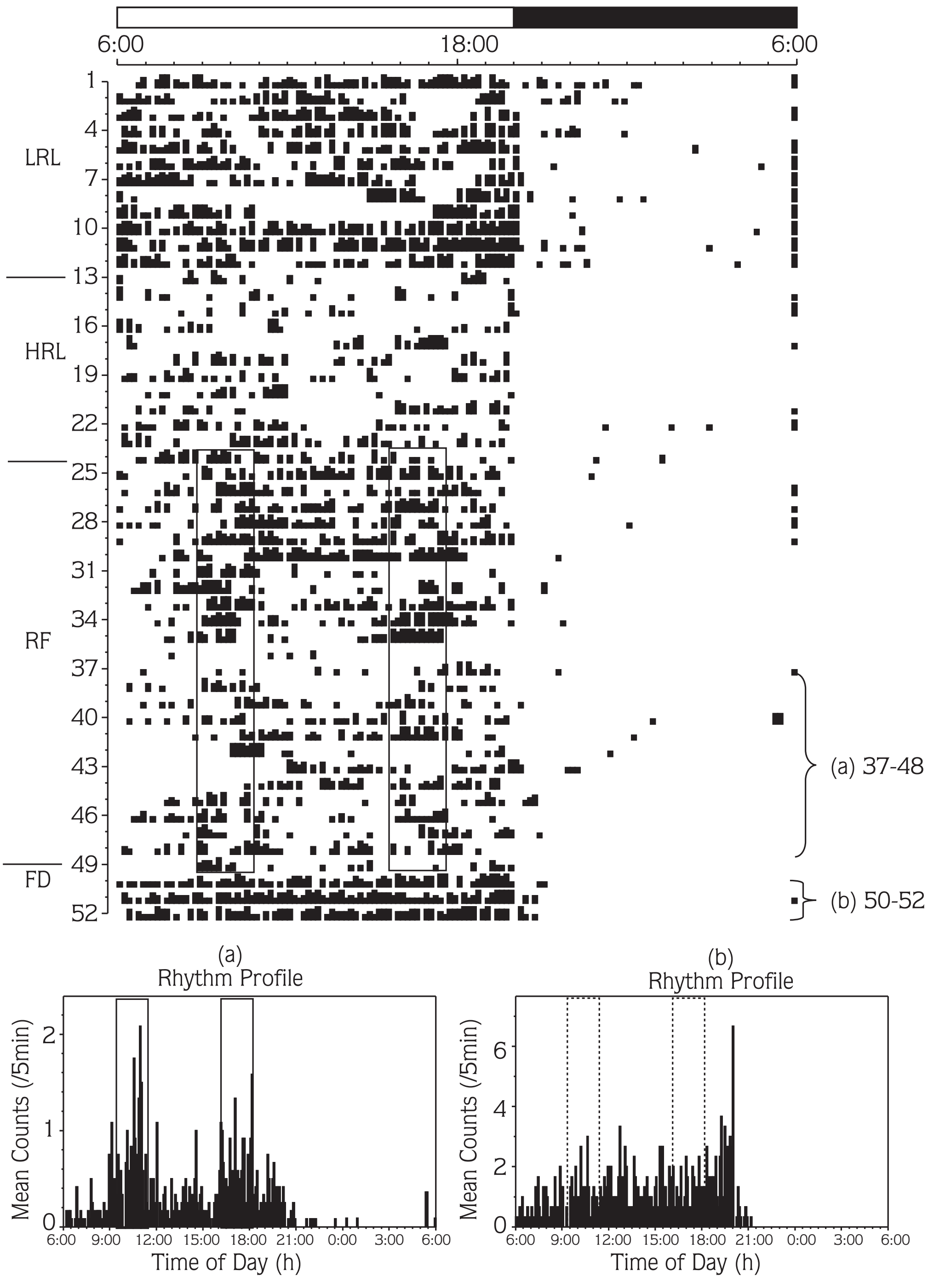

Figure 10. Actogram of self-feeding records from TR1 (2T/1P) fish (upper figure) during restricted feeding (days 24-49). The horizontal bar at the top of the figure represents the LD cycle (open for the light phase, solid for the dark phase). RF, Restricted feeding; FD, food deprivation. Rhythm profile of self-feeding activity over days 37-48 is shown in (a) and over days 50-52 (food deprivation) in (b). Mealtimes are shown by the rectangular boxes. The dotted rectangular boxes show subjective mealtimes.

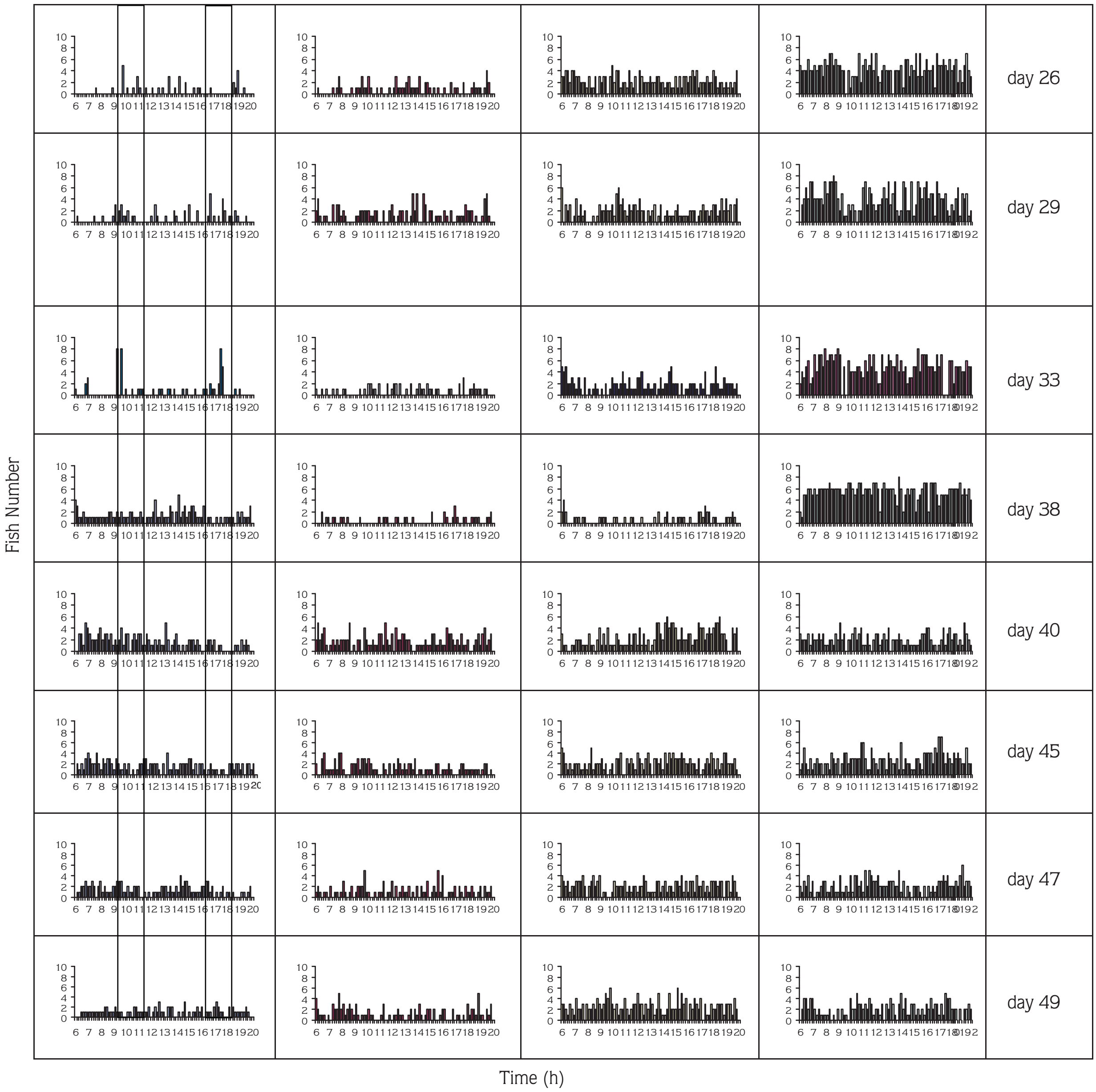

Figure 11. Fish spatial distribution of TR1 (2T/1P) under restricted feeding (RF). The fish numbers were recorded every ten minutes throughout the photophase (14 h). Numbers at the top of the figure refer to the sections of the raceway from most upstream (1) to most downstream (4), respectively. Mealtimes are shown by the rectangular boxes.

on individual demand feeding activity and the strength of social hierarchies (29). Establishment of a pronounced social hierarchy between the fish (in particular TR1) during a LRL in the present study is in agreement with McCarthy et al. (34) and Jobling and Koskela (35), who showed that, in rainbow trout under feed restriction, feeding hierarchies were established, whereby dominant individuals had preferential access to food, compared with subordinates. This led to an unequal fish distribution within the sections of the raceway (Figure 2). Chen et al. (31) also reported the effect of reward level on the fish spatial distribution, in which, at a lower reward level, an uneven fish spatial

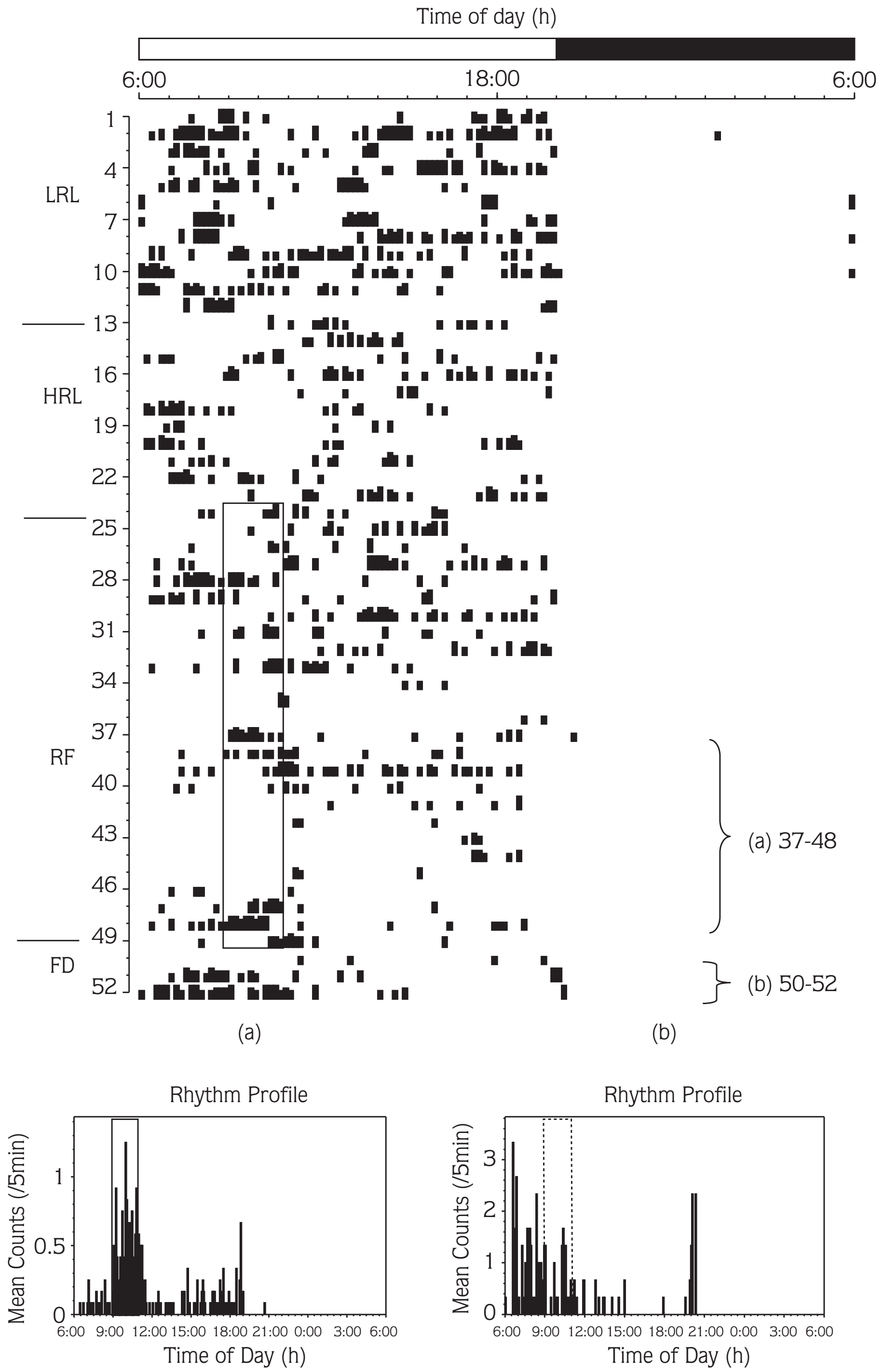

Figure 12. Actogram of self-feeding records from TR2 (2T/2P) fish (upper figure) in the most upstream during restricted feeding (days 24-49). The horizontal bar at the top of the figure represents the LD cycle (open for the light phase, solid for the dark phase). RF, Restricted feeding; FD, food deprivation. Rhythm profile of self-feeding activity over days 37-48 is shown in (a) and over days 50-52 (food deprivation) in (b). Mealtimes are shown by the rectangular boxes. The dotted rectangular box shows subjective mealtime.

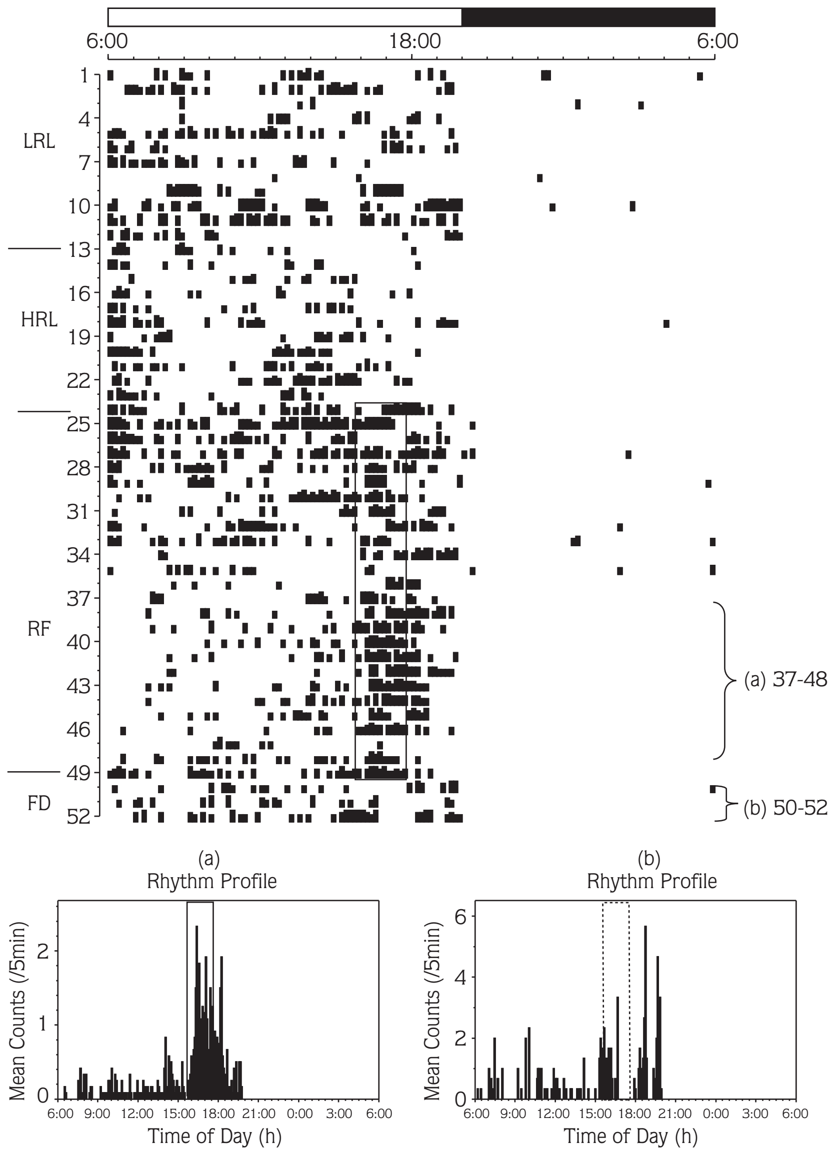

Figure 13. Actogram of self-feeding records from TR2 (2T/2P) fish (upper figure) in the most downstream during restricted feeding (days 24-49). The horizontal bar at the top of the figure represents the LD cycle (open for the light phase, solid for the dark phase). RF, Restricted feeding; FD, food deprivation. Rhythm profile of self-feeding activity over days 37-48 is shown in (a) and over days 50-52 (food deprivation) in (b). Mealtimes are shown by the rectangular boxes. The dotted rectangular box shows subjective mealtime.

distribution favouring dominant individuals was achieved. It could be suggested that the occurrence of trigger actuations at night during a LRL in TR1 was due to alternate strategies of feeding by subordinate individuals, when the dominant fish were less aggressive (36). Generally, feeding fish with restricted rations has led to monopolisation of the food supply by some individuals and therefore starvation and weight loss in others (37) and consequently increased variation in size (38-41). Table 3 shows that in TR1 all fish except W/W lost weight. The

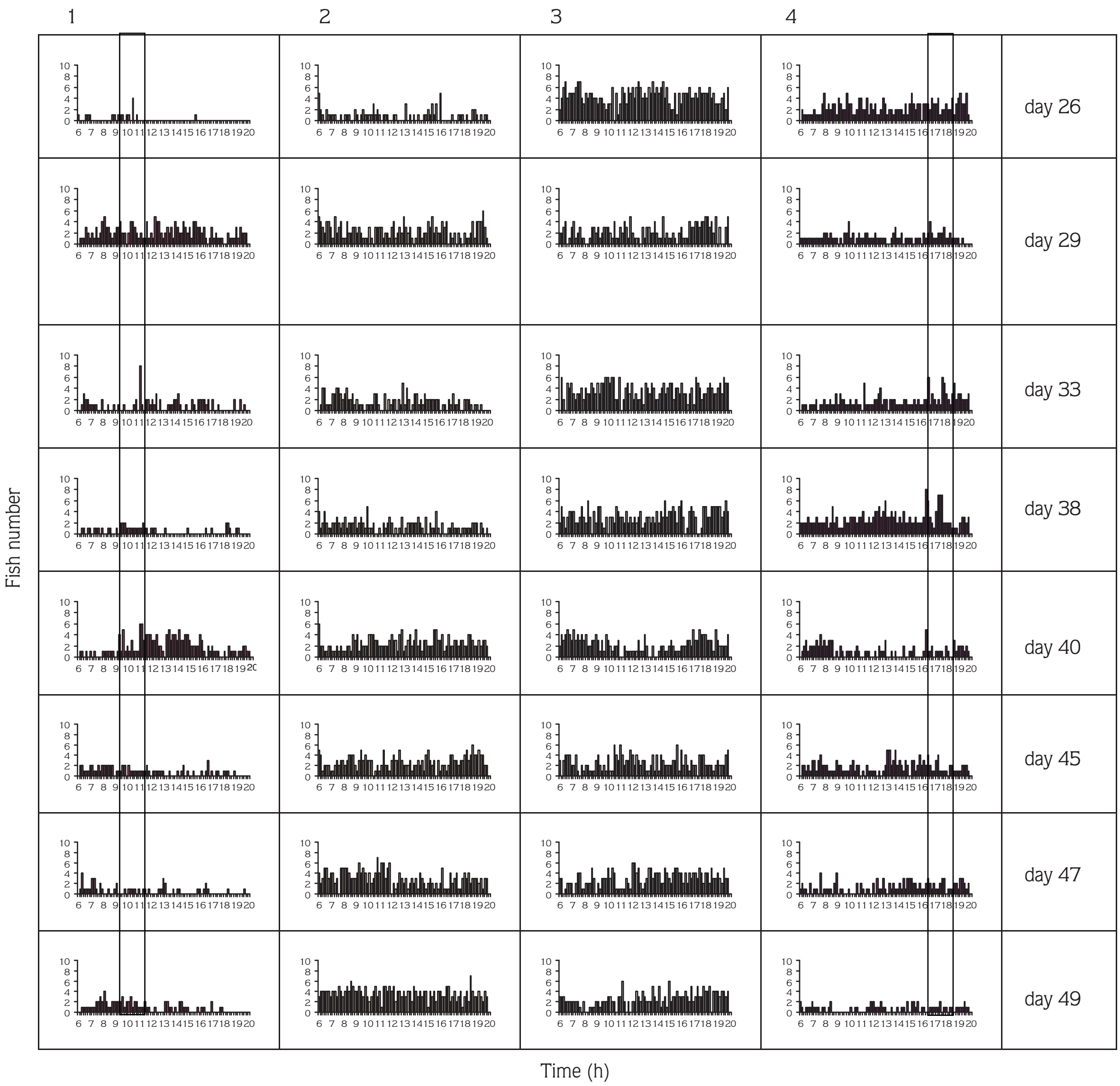

Figure 14. Fish spatial distribution of TR2 (2T/2P) under restricted feeding (RF). The fish numbers were recorded every ten minutes throughout the photophase (14 h). Numbers at the top of the figure refer to the sections of the raceway from most upstream (1) to most downstream (4), respectively. Mealtimes are shown by the rectangular boxes.

increased coefficient of variation (CV) in the weight of TR1 fish indicates the strength of the social interactions between the fish (see Table 4). This is in parallel with what other authors observed during a low food ration in rainbow trout (34), chum salmon (38), and Arctic charr (42). They found an increase in CV of weight, suggesting that CV could be used as an indicator of social interaction.

By shifting from a LRL to a HRL and an increase in food availability, the feeding territory of fish W/W (of TR1) was broken down, as the other fish had an opportunity to feed. Based on the theory of economic dependability (43), whenever the benefit of holding a territory exceeds its cost, or net benefits of defence of territory exceed the net benefits of not defending, the territory will not be defended. This is consistent with the findings reported by Jobling and Koskela (35) in rainbow trout, in which previously subordinate and suppressed fish that had difficulty gaining access to food were able to feed when

Table 8. Number of trigger actuations of each fish during food deprivation (FD) (days 50-52) in relation to mealtimes in TR1 (2T/1P) fish.

			Time (h)				
Total trigger actuations	Day	Fish ID	0600-0900	0900-1100	1100-1600	1600-1800	1800-2000
135	50	W/W	20	18	49	26	22
146	52		21	24	57	27	17
26	50	R/R	4	4	12	0	6
31	52		5	1	21	1	3
4	50	B/B	3	1	0	0	0
1	52		0	0	1	0	0
2	50	B/R	1	0	1	0	0
0	52		0	0	0	0	0
0	0	W/B	0	0	0	0	0
2	52		1	1	0	0	0
0	50	R/W	0	0	0	0	0
1	52		0	0	1	0	0
0	0	R/B	0	0	0	0	0
0	52		0	0	0	0	0

food availability increased. Likewise, Moutou et al. (44) reported the establishment of strong feeding hierarchies by means of low ration in rainbow trout, which was relaxed by an increase in the ration level. In demand-feeders, Jobling and Koskela (35) showed that with increasing reward level most trigger actuations were carried out by other fish ranks, apart from the top rank, but no attempt was made to explain the reason. It is suggested that a switch in contribution of trigger actuations from fish W/W to the other fish during a HRL for the TR1 fish in the present study could be due to the cost and benefit of holding a territory, as the size of a territory decreases with increasing food density (45). In fact, it was difficult for the fish W/W to sustain the feeding territory when the reward level was increased. In contrast, Chen and Tababa (46) found that in order for the territoriality of rainbow trout to be broken down, merely increasing the reward level is not sufficient, which suggests that behavioural characteristics of some individuals in a group could play a role in the outcome of the territoriality.

It is noteworthy that the fish W/W of TR1 and W/B of TR2 were the dominant fish of the group, based on the highest proportion of trigger actuations, as well as the number of agonistic acts and their spatial position in relation to the feeder (data not shown). This is because it has been shown that the most aggressive fish are individuals with priority access to preferred resources, such as food (47), and social dominance is usually associated with increased levels of aggression (48). In addition, Fausch (50), Metcalfe et al. (51), and Johnsson (52) identified spatial positioning of fish in the tank in relation to access to food, or profitable feeding positions, as a reliable ranking of dominance in salmonids. This is because dominant individuals can contest and obtain food items more easily than the other individuals in the social hierarchy. Likewise, Brännäs (unpublished data as reported in Brännäs et al. (53)) classified individual Arctic char into dominant, subdominant, and subordinate fish, according to their position in the aquarium and aggressiveness of their interactions. In this study, dominant fish were also the

Table 9. Number of trigger actuations of each fish during food deprivation (FD) (days 50-52) in relation to mealtimes in TR2 (2T/2P) fish.

Total trigger actuations	Day	Fish ID	Stream	Time (h)				
				0600-0900	0900-1100	1100-1600	1600-1800	1800-2000
37	50	W/B	Up	0	0	0	0	0
			down	0	0	1	1	35
173	52		Up	56	14	3	0	0
			down	10	8	38	19	25
13	50	W/W	Up	0	0	0	0	0
			down	1	7	5	0	0
7	52		Up	0	0	0	0	0
			down	1	3	3	0	0
4	50	W/R	Up	0	0	2	1	1
			down	0	0	0	0	0
18	52		Up	8	9	1	0	0
			down	0	0	0	0	0
2	50	B/R	Up	0	0	0	0	0
			down	1	0	1	0	0
0	52		Up	0	0	0	0	0
			down	0	0	0	0	0
0	50	R/B	Up	0	0	0	0	0
			down	0	0	0	0	0
1	52		Up	1	0	0	0	0
			down	0	0	0	0	0
0	50	B/B	Up	0	0	0	0	0
			down	0	0	0	0	0
0	52		Up	0	0	0	0	0
			down	0	0	0	0	0
0	50	R/W	Up	0	0	0	0	0
			down	0	0	0	0	0
0	52		Up	0	0	0	0	0
			down	0	0	0	0	0
0	50	B/W	Up	0	0	0	0	0
			down	0	0	0	0	0
0	52		Up	0	0	0	0	0
			down	0	0	0	0	0

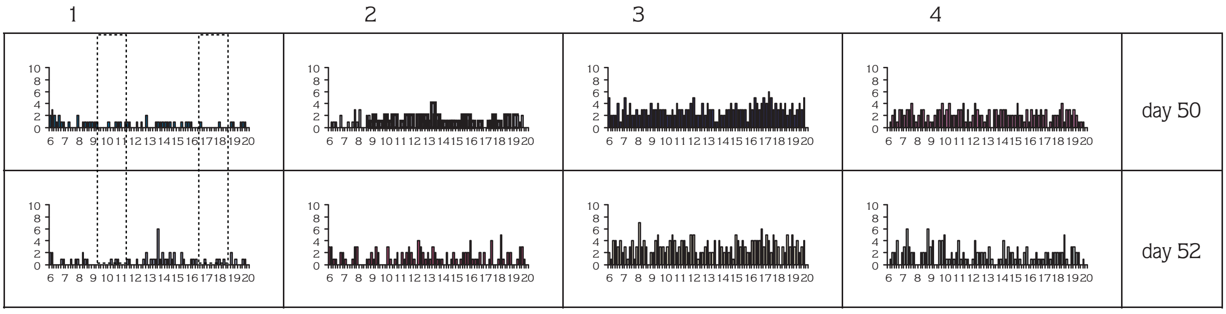

Figure 15. Fish spatial distribution of TR1 (2T/1P) under food deprivation (FD). The fish numbers were recorded every ten minutes throughout the photophase (14 h). Numbers at the top of the figure refer to the sections of the raceway from most upstream (1) to most downstream (4), respectively. Subjective mealtimes are shown by the dotted rectangular boxes.

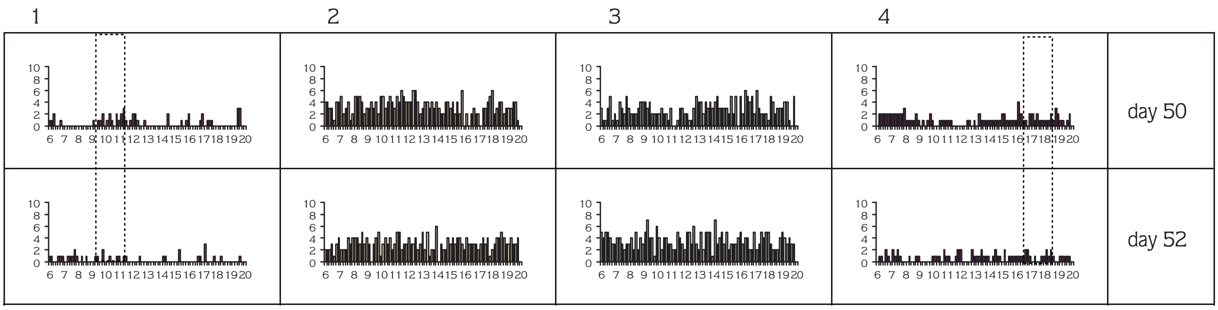

Figure 16. Fish spatial distribution of TR2 (2T/2P) under food deprivation (FD). The fish numbers were recorded every ten minutes throughout the photophase (14 h). Numbers at the top of the figure refer to the sections of the raceway from most upstream (1) to most downstream (4), respectively. The dotted rectangular boxes show subjective mealtimes.

most aggressive and accounted for the majority of trigger actuations. However, other fish in the group may not fall into the simple categories; there existed some fish that were less aggressive but more successful competitors in the trigger actuations or vice versa (data not shown).

Lack of consistent demand-feeding activity when food availability was restricted can be attributed to inter-individual variability and social interactions within the group. The effect of social interactions on the differences in feeding rhythms between isolated and grouped rainbow trout has been reported (54). For the TR2 downstream feeder most of the self-feeding activity occurred within the feeding window. Thus it can be postulated that the fish might have had a good sense of timing, because, based on Ferster and Skinner (55), those animals having a perfect sense of time should not respond before the feeding time at all. In addition, it may be postulated that the fish have no sense of timing but this can be disregarded since when the fish were deprived of food (days 50-52) most of trigger actuations appeared to have taken place at the subjective mealtime for the TR2 downstream feeder (see Figure 5), suggesting that they had a reasonable sense of timing, rather than none.

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