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Behavioral and neurophysiological responses of European sea bass groups reared under food constraint

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Abstract:

The individual food-demand behavior of juvenile European sea bass (*Dicentrarchus labrax*, L.) reared in groups under self-feeding conditions was investigated. The triggering activity on self-feeder, i.e. index of the food-demand activity, agonistic interactions and territorial behavior were monitored for periods of 42 to 68 days in six groups of 50 fish. The specific growth rate was calculated and the brain serotonergic activity was used as a stable index of social stress. Inter-individual differences appeared in triggering activity and three groups were distinguished: 3–5 high-triggering fish, 17–30 low-triggering fish and the remaining individuals were null-triggering fish. There were no significant differences in specific growth rates calculated at the end of the experiment (day 42 or day 68) between individuals with high, low, and null food-demand (ANOVA, $p > 0.05$). No territorial or agonistic behaviors were observed, however, there were significant differences in brain serotonergic activity between the three triggering groups (ANOVA, $p = 0.050$ in telencephalon and $p = 0.004$ in cerebellum). Specifically, high-triggering fish had lower serotonergic turnover than low or null-triggering fish. We put forth the hypothesis that fish with low or null-triggering activity could be stressed by the high activity of high-triggering individuals.

Keywords: Food-demand behavior; Triggering activity; European sea bass; Self-feeding; Agonistic interactions; Territorial behavior; Brain serotonergic activity; Social stress

1. Introduction

Numerous studies have investigated the social interactions between individuals within wild populations of Mammals and Birds. Information gaps still remain, however, in regard to the social organization of wild Teleost fish populations [1]. In contrast, several studies have examined the social structure of these fish reared under controlled conditions [2-9].

The development of modern techniques such as the computerized on-demand feeder coupled with individual electronic tagging (PIT-tag), have contributed to a better understanding of the individual behaviors of fish living in groups [2-7]. For example, dominance hierarchies have been described in rainbow trout, *Oncorhynchus mykiss* [2,4,8] and arctic charr, *Salvelinus alpinus* [3,4] reared under self-feeding conditions. Observations indicate that dominant fish display the most aggressive behavior, thereby gaining preferential access to food and typically display the best growth [2,3,8,9,10-12]. In addition, subordinate fish have shown higher brain serotonergic activity associated with the stress of social subordination [8,10-19]. Brain serotonergic activity is estimated using the brain 5-HIAA/5-HT ratio where 5-HIAA (5-hydroxyindoleacetic acid) is the major metabolite of 5-HT (5-hydroxytryptamine, serotonin). Furthermore, the brain serotonergic system plays a central role in the hypothalamic-pituitary-interrenal (HPI) axis regulation within the Teleost fish homologue of the mammalian hypothalamic-pituitary-adrenal (HPA) axis [12,20-25]. Specifically, 5-HT is involved in the regulation of a series of hormonal pathways related to stress. Major components include the hypothalamic corticotropin-releasing factor (CRF), pituitary adrenocorticotropin (ACTH, synthesized from a precursor protein: the pro-opiomelanocortin, POMC) and the interrenal cortisol [10,12,24-30].

Recently, Covès et al. [7] have designed a monitoring system that simultaneously records the individual triggering activity of multiple fish (at least fifty) when fed with a self-feeder. Using this system, the authors were able to provide new insight on the voluntary food-demand of the European sea bass *Dicentrarchus labrax*. Groups of 50 juvenile sea bass displayed inter-individual differences in food-demand and three sub-groups were distinguished: high-triggering, low-triggering and null-triggering fish. A few individuals within the experimental population displayed a high-triggering activity, about half fish showed a low-triggering activity and the remaining individuals had null-triggering activity of the self-feeder. Some questions have resulted from this initial investigation: Are there any social structure underlying these individual differences? Does the food-demand level reflect the social status?

In the present study, we investigate the triggering activity -i.e. the voluntary food-demand of juvenile sea bass groups reared under self-feeding conditions. To test the hypothesis of a dominance hierarchy, we investigate the agonistic interactions and territorial behavior, specific growth rate and brain serotonergic activity in fish exhibiting different triggering activity levels.

2. Materials and methods

2.1. Experimental set-up

The experiments were carried out on six groups of fifty juvenile hatchery-reared sea bass in 1 m^3 tanks at IFREMER experimental station in Palavas (France). Initial mean fish weights between experimental tanks are listed in Table 2.

Experimental tanks were supplied with sand filtered and UV-treated seawater (salinity: 38‰, pH: 8) in a flow-through system (flow rate of 1 $m^3.h^{-1}$ in each tank). Water temperature was maintained at 21.0 ± 1.0 °C and oxygen at concentration above 80% saturation in the outlet. Tanks were illuminated with 75 W lamps placed 70 cm above the water. Photoperiod was 16:8 LD (400 Lux: total darkness, light onset at 06:00 U.T. +1) with twilight transition periods of 30 min. Fish were fed a commercial sea bass diet (SICA Le Gouessant®-Grower Extrude Natura, France). Pellets were constituted by 44% crude protein and 22% lipid (according to the manufacturer) and were 4-5 mm in diameter. Feed hoppers were filled daily with uneaten pellets were counted in the sediment trap during animal care procedure, from 10:00 U.T. to 11:00 U.T.

2.2. Apparatus

Prior to the beginning of the experiment, a Passive Integrated Transponder (PIT-tag) was implanted horizontally into each fish, just behind the skull.

Each tank was equipped with one self-feeder. Each self-feeder included a food dispenser, a sensor and a control box connected to a computer [5,6]. The sensor consisted of a metal rod that was protected by a PVC cylinder set in a forward position and surrounded by the PIT-tag detection antenna [7]. Therefore, the fish entering the PVC pipe, would activate the sensor, detect the PIT-tag by the antenna and actuate the rod. Software was designed to register ID-codes that correspond to a bite on the sensor within 1 second. Every event (i.e. PIT-tag detection, rod triggering and food distribution) was counted and recorded every minute by the computer. Two French private companies designed the device: Imetronic for the computerized self-feeding system and Micro-BE for the PIT-tag detection antenna.

At each actuation, food dispenser distributed 22 pellets at the beginning of the experiment to 28 pellets at the end (mean weight of 123.5 mg/pellet), corresponding to a constant reward along the experiment of 0.5 g per kg of fish according to the expected growth. Pellets were delivered 30 cm far from the trigger.

To verify the reliability and accuracy of the monitoring system, 100% ID-codes were recognized at least once in all experiments. Furthermore, approximately 96% of the total events registered were paired with corresponding ID-code registrations.

2.3. Behavior monitoring

Similarly to Covès et al. [7], the sea bass were placed in the experimental tanks two weeks prior to the beginning of experiment. This period allowed time for the fish to adjust to the experimental environment become familiar with the triggering system. Two variables were monitored during the 42 day (tanks 1, 5, and 7) and 68 day (tanks 2, 3, and 8) experiments. These were:

- the individual food-demand estimated by the individual triggering activity: the number of actuations of each fish in each tank was daily stored on computer; and
- the total food-intake of each tank: the uneaten pellets were daily counted in the sediment trap. Then the complete number of pellets dispensed by the feeder minus the whole number of uneaten pellets was calculated to determinate the total amount of food intake by all fish in each tank.

We defined an ‘active’ day as a day when there was at least one event triggered. From this, fish were distinguished into one of three triggering activity groups at the conclusion of the experiment (day 42 or day 68):

- high-triggering fish: fish with a proportion of active days higher than 15% and a mean triggering activity higher than one per day;
- low-triggering fish: fish with a proportion of active days between 4% and 15% and less than 1 actuation per day; or with a proportion of active days greater than 15% but a mean triggering activity lower than one per day;
- null-triggering fish: fish demonstrating a percentage of active days less than 4% and a mean triggering activity lower than one per day. It was assumed that the rare actuations were involuntary.

In addition, direct observations were made of each tank throughout the duration of the experiment (from day 1 to day 42 or day 68). Video recordings were monitored for the tanks 2, 3, and 8 from day 15 to 20, day 30 to 35, and day 45 to 50 to investigate the territorial behavior and agonistic interactions. An analogical system including CCD cameras (Panasonic WV BL 200) and S-VHS recorders (Panasonic AG 6010) were used. Using the recorded triggering activity file, we determined which fish was on the screen at a precise time. Of this, 329 video sequences corresponding to 329 bites were also analysed including 278 bites from high-triggering fish and 51 bites from low-triggering fish. Finally, observations were made in effort to determine on screen whether the fish that actuate the trigger:

- were aggressive towards the other individuals;
- had a preferential access to the area where food is delivered;
- occupied a larger territory than the others.

2.4. Brain serotonergic activity

At the end of experimentation, each tank was treated individually. All fish within a tank were anesthetized simultaneously in a 0.08‰ eugenol bath and identified using a PIT-tag antenna. Then, fish of interest were collected and decapited. Brain was dissected within 2 minutes and separated into three parts: telencephalon (including olfactory bulbs), diencephalon (excluding pituitary), and cerebellum. The tissues were immediately frozen in liquid nitrogen and stored at -80°C. A maximum of 20 minutes elapsed between sampling of the first fish and last fish collected within each tank. All 6 tanks were sampled within approximately 3 hours. A total of 20 high-triggering fish, 17 low-triggering fish and 18 null-triggering fish were sampled from the six tanks.

All samples were individually homogenised in 4% (w/v) ice-cold perchloric acid containing 0.2% EDTA, using a Potter-Elvehjem homogeniser and a MSE ultrasonic disintegrator. Samples were centrifuged at 1,500 g for 10 min at 4 °C. Serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) levels were quantified in the supernatants using ELISA kits (IBL Hamburg, Germany). The plates were read with a conventional plate reader at 405 nm (ThermoLabsystems, MultiSkan EX). 5-HT and 5-HIAA levels were expressed as ng per g of brain wet weight. The 5-HIAA/5-HT ratio was calculated to evaluate the serotonergic activity in each part of the brain.

2.5. Specific growth rate

Each fish was weighed at the beginning and at the end of the experiment. Individual specific growth rates (SGR) were calculated as:

SGR = $[(\ln W_f - \ln W_i) / t] \times 100$ in % per day, where W_i and W_f are the initial and final body weight (in g) respectively, and t is the number of days between measurements.

2.6. Data analysis

Data analysis was performed with StatView 5.0 (SAS Institute Inc.). Statistical differences between mean 5-HT and 5-HIAA levels and 5-HIAA/5-HT ratios were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's post hoc test. This statistic analysis was used to test differences between initial and final weights, and specific growth rate between each tank and each activity group of fish. Linear regression analysis was used to test the relationship between the specific growth rate and the food-demand. $p<0.05$ was taken as the statistically significant threshold.

3. Results

3.1. Food-demand behavior

Within each tank, few high-triggering individuals (3 to 5) were responsible for the majority of the food-demand activity (77-84%). A mean value of 24 low-triggering fish (from 17 to 30 individuals) shared between 14 to 21% of the total actuation number. The remaining individuals were null-triggering fish and handled the trigger for less than 2% of the total food-demand (Table 1). No uneaten pellet was counted in the sediment trap during the experiment. Then, the total amount of food delivered by the self-feeder given by the complete number of trigger actuations for each whole tank is equivalent to the total food intake for each of these tanks. Also, the total number of trigger actuations and the total food intake were roughly between tanks (Table 1). At last, the total food intake per kg of fish and the total food intake per day of experiment were similar between each tank of the same experimental duration (Table 1).

3.2. Agonistic behavior and territoriality

Fish gently swam all around the tank with notable periods of gathering close to the antenna area. Individuals didn't compete for the triggering area access. After a fish actuated the trigger, the whole fish group (including the triggering fish) joined the feeding point without demonstrating any agonistic behavior.

3.3. Growth

No differences were found in mean initial weights (ANOVA, $F_{0.05(5,294)}$=0.497, p=0.778; Table 2) and in mean final weights (ANOVA, $F_{0.05(2,147)}$=0.543, p=0.582 for the 42-day tanks; $F_{0.05(2,147)}$=0.484, p=0.618 for the 68-day tanks; Table 2) between tanks. There was no significant difference in mean initial weights (ANOVA, $F_{0.05(2,46)}$=1.508, p=0.232; Table 2) and in mean final weights (ANOVA, $F_{0.05(2,46)}$=0.489, p=0.617; Table 2) between the three food-demand groups of fish in all tanks (Table 2).

No linear relationship was observed between the specific growth rate and the food-demand, e.g. in tank 2 (r^2=0.002, p=0.772; Fig. 1) and in the other tank treatments (Table 3). ANOVA analysis on the three food-demand groups of animals did not display any significant difference for specific growth rate in five tanks (ANOVA, p>0.05; Table 3).

3.4. Brain serotonergic activity: 5-HIAA, 5-HT and 5-HIAA/5-HT ratios

No significant differences in 5-HT levels between telencephalon, cerebellum and diencephalon were detected within the high, low and null-triggering fish (ANOVA, $F_{0.05(2,36)}$=0.270, p=0.765;

$F_{0.05(2,36)}$=2.758, p=0.078, and $F_{0.05(2,36)}$=0.254, p=0.777 respectively; Table 4). However, 5-HIAA levels were higher in null and low-triggering fish than in high-triggering fish in the three parts of the brain (ANOVA, $F_{0.05(2,36)}$=4.366, p=0.020 in telencephalon; $F_{0.05(2,36)}$=2.990, p=0.050 in cerebellum and $F_{0.05(2,36)}$=4.468, p=0.019 in diencephalon; Table 4). Significant differences were found in the 5-HIAA/5-HT ratio between high, low and null-triggering fish in telencephalon (ANOVA, $F_{0.05(2,36)}$=3.139, p=0.050), and in cerebellum (ANOVA, $F_{0.05(2,36)}$=6.488, p=0.004) (Fig. 2A, B). Only a moderate trend is observed in diencephalon (ANOVA, $F_{0.05(2,36)}$=1.941, p=0.158; Fig. 2C). Brain serotonergic activity is higher in null and low-triggering fish than in high-triggering fish with approximately 1.5 times more in diencephalon and telencephalon, and approximately 1.9 times more in cerebellum.

4. Discussion

4.1. Food-demand level and specific growth rate

We observed that within a group of 50 juvenile sea bass reared under self-feeding conditions, a few individuals (3-5) are responsible for the majority (about 81%) of the group food-demand, whereas the remaining fish have low and null-triggering activity. Such heterogeneity in individual triggering activity was described in rainbow trout [2] and arctic charr [3,8] reared under self-feeding conditions. Specifically, Brännäs and Alanärä [3] and Alanärä et al. [8] noticed in arctic charr, that the high-triggering fish had generally the higher specific growth rate and were the most aggressive, and labeled them as dominant fish. Moreover, subdominant and subordinate fish exhibited medium and lowest triggering activity and medium and lowest specific growth rates respectively.

In our study, no difference was found in initial and final weights between tanks. Furthermore, no significant difference was observed between high, low and null-triggering groups of fish within each tank. So, there was no linear relationship between the specific growth rate and the individual food-demand -i.e. null and low-triggering fish grew just as fast as high-triggering fish. This result is supported by Covès et al. [7] who obtained the similar results in sea bass groups tested with a similar self-feeder. Similarly, Alanärä and Brännäs [2] and Chen et al. [31] observed that the high-triggering rainbow trout, which is the dominant fish, did not always display the highest growth rates. The largest growth rate values were positive for all fish -null, low and high-triggering fish- suggesting that the food quantity delivered (near 69 to 88 g of food per day) by the high-triggering individuals seems enough to feed the whole group. We cannot say that the amount of food delivered is optimal since no waste was collected: may be the fish are fed *ad libitum*, may be their needs are not completely achieved. However, this quantity suggests that the group needs were satisfied, since between each tank, the total number of voluntary actuations displayed by the high-triggering fish -i.e. the total quantity of pellets delivered, was quite similar. The initial weights and density were equivalent between tanks and no uneaten pellet was counted during the experiment. Then, the food intake (per kg basis or per day basis) was roughly between tanks, which it explains that the final weights and the growth rates were always close. The high-triggering individuals of each tank may use information produced by the other fish to integrate and regulate the global need for food of their group. This regulated voluntary distribution of food by the high-activity fish raises the question of the underlying “social model”. Do the high-triggering fish dominate the group and what are the benefits of these animals? Do the null-triggering individuals “manipulate” them in order that they feed the group?

4.2. Territorial behavior and agonistic interactions

Throughout the experiment, each juvenile sea bass occupied the same space in a tank. High-triggering fish did not display a preferential access to the trigger or to the delivered food and no agonistic behaviors were noticed between high-triggering fish and the other individuals. No video observations were made for the first two weeks of experiment whilst the animals adjusted to and became familiar with the triggering system; so, early interactions between the individuals may have been missed. However, no scars were observed on the animal bodies at the commencement of or at anytime during experimentation. These observations confirmed those obtained by Covès et al. [7] for sea bass placed in the same conditions of temperature, density and self-feeding. In contrast, within clear dominance hierarchies, the high-ranking fish displayed agonistic behaviors [32-34]. These behaviors are generally observed during the food phases when a competition is often elevated between individuals [35,36]. In addition, dominant fish tend to occupy a more important territory, have a preferential access to food resources, and greater consumption rates than non-dominants individuals within the group [2,3,8,11,37-39].

In our experimental conditions, the absence of agonistic and territorial behaviors suggests the absence of a dominance hierarchy in our groups of juvenile sea bass. However, it is well documented that population density may directly affect the interactions between individuals [40,41]. A strong territoriality and aggressiveness were observed for the rainbow trout and the arctic charr [2,3,8] reared in groups of 8 to 15 individuals per m^3. In our study, the relatively high numerical density (50 fish per m^3) may inhibit the aggressive behaviors between individuals. The relatively large quantity of food distributed at each actuation of the self-feeder, may also explain the absence of aggressive behaviors. Indeed, McCarthy et al. [39] noticed that feeding hierarchy became less marked in rainbow trout as food availability increased.

4.3. Brain serotonergic activity

Many Vertebrates exhibit central serotonergic changes following stressful social interactions [22-25,42-44]. In Fish, individuals occupying low positions in a dominance hierarchy are characterized by a stress-induced increase in brain serotonin turnover [8,10-19,35]. Serotonin activity is commonly measured by the ratio 5-HIAA/5-HT, the 5-HIAA being the major metabolite of serotonin, 5-HT [10-12,42-45].

In our study, the 5-HIAA/5-HT ratio is higher in telencephalon and cerebellum for the null and low-triggering sea bass than for high-triggering animals suggesting that individuals exhibiting low or null food-demand display a higher stress level than fish with high food-demand. In rainbow trout and arctic charr, individuals occupying the highest ranks in dominance hierarchies displayed the highest triggering activity and the lowest serotonergic activity (5-HIAA/5-HT ratio) in telencephalon, hypothalamus and brain stem [8,10-12,35]. They displayed also aggressive behavior towards the subordinates, which is not the case in our study. So, how can we explain the observed differences in stress level in spite of no agonistic and territoriality behaviors? Social interactions induced stress may be very complex, and most likely, if the stress experienced by subordinate individuals generally results from initial loosed fights [46], it may be maintained by a continued threat from the dominant individuals. Our hypothesis is that sea bass with low or null food-demand could be stressed by the high activity and the permanent presence of the high-triggering individuals, exerting a kind of “passive” dominance on them. Additional investigation, including studies on fish densities and food management -e.g. lower rewards, will help to confirm that a dominance hierarchy really exists in juvenile sea bass groups. To explain individual food-demand differences, another hypothesis would be the existence of differences in individual learning ability to actuate the trigger. It Rubio et al. [6,47] previously reported, observations in sea bass learning abilities. In the same way, food-demand could depend on the "personality" of individuals. In rainbow trout for instance, Sneddon [48] distinguishes ”bold” from ”shy” individuals on the basis of specific behavioral tests. Applied to our study, null and low-triggering juvenile sea bass would be shy individuals according to their higher 5-HIAA/5-HT ratio. This stress may be considered either a cause of their shyness or a consequence of this feature. On the contrary, high-triggering fish would have a “bold” character. At present and under our experimental conditions, it remains difficult to make a conclusive decision on the presence of a dominance hierarchy. Nevertheless, this study is a first step in understanding the food-demand activity in juvenile sea bass groups reared under self-feeding conditions. The conjunct use of neurophysiological index and ethological markers allows raising questions on the underlying social schemes in this in-group living species. Additionally this study demonstrates that an applied investigation under laboratory conditions may provide additional insight on the social structures of wild populations of marine fish that would otherwise remain understudied.

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Table 1

Total number of trigger actuations and mean of total food intake for the whole fish in each tank; and number of high, low and null-triggering individuals for each of its. Given in parentheses in the last three columns is the relative percentage of the triggering activity -i.e. the food-demand activity.

Tank no.	Total number of actuations (nb. of days)	Mean of total food intake	Mean of total food intake/kg of fish	Mean of total food intake/day	Number of high-triggering fish	Number of low-triggering fish	Number of null-triggering fish
1	929 (42)	2868	188	68	3 (80)	27 (18)	20 (2)
5	1065 (42)	3288	215	78	3 (83)	17 (15)	30 (2)
7	829 (42)	2560	173	61	5 (77)	22 (21)	23 (2)
2	2065 (68)	6376	379	94	4 (80)	30 (19)	15 (1)
3	1893 (68)	5845	345	86	4 (84)	24 (14)	23 (2)
8	1871 (68)	5777	354	85	5 (82)	24 (17)	20 (1)

Values of total food intake are means in g.

Table 2

Initial and final mean weights (monitored during 42 days for tanks 1, 5, 7 and 68 days for tanks 2, 3, 8) of fish in all tanks, and of fish ranged into the three food-demand groups.

Tank no.	Initial weight: per tank of fish		Initial weight: per activity group	Final weight: per tank of fish		Final weight: per activity group
1	288 ± 8 (50)	Null-triggering fish	276 ± 11 (20)	306 ± 8 (50)	Null-triggering fish	297 ± 11 (20)
		Low-triggering fish	294 ± 11 (27)		Low-triggering fish	308 ± 11 (27)
		High-triggering fish	315 ± 55 (3)		High-triggering fish	345 ± 53 (3)
			ns			ns
5	284 ± 8 (50)	Null-triggering fish	284 ± 11 (30)	306 ± 7 (50)	Null-triggering fish	297 ± 9 (30)
		Low-triggering fish	276 ± 14 (17)		Low-triggering fish	308 ± 11 (17)
		High-triggering fish	332 ± 30 (3)		High-triggering fish	381 ± 29 (3)
			ns			*
7	295 ± 7 (50)	Null-triggering fish	279 ± 10 (23)	296 ± 8 (50)	Null-triggering fish	279 ± 11 (23)
		Low-triggering fish	307 ± 10 (22)		Low-triggering fish	307 ± 12 (22)
		High-triggering fish	310 ± 15 (5)		High-triggering fish	330 ± 16 (5)
			ns	ns		ns
2	279 ± 7 (49)	Null-triggering fish	263 ± 13 (14)	343 ± 9 (49)	Null-triggering fish	335 ± 19 (14)
		Low-triggering fish	282 ± 9 (30)		Low-triggering fish	344 ± 10 (30)
		High-triggering fish	304 ± 23 (4)		High-triggering fish	370 ± 39 (4)
			ns			ns
3	287 ± 9 (51)	Null-triggering fish	291 ± 13 (23)	332 ± 10 (51)	Null-triggering fish	331 ± 17 (23)
		Low-triggering fish	278 ± 12 (24)		Low-triggering fish	328 ± 12 (24)
		High-triggering fish	315 ± 34 (4)		High-triggering fish	362 ± 40 (4)
			ns			ns
8	282 ± 7 (50)	Null-triggering fish	284 ± 12 (20)	333 ± 8 (49)	Null-triggering fish	331 ± 13 (20)
		Low-triggering fish	278 ± 10 (25)		Low-triggering fish	329 ± 11 (24)
		High-triggering fish	296 ± 28 (5)		High-triggering fish	359 ± 27 (5)
	ns		ns	ns		ns

All values are means ± S.E.M. in g and *n*, number of fish in parentheses.
Analysis of variance (ANOVA). Significance levels are denoted by asterisks: * $p < 0.05$, ns = no significance.

Table 3

Relationship between the specific growth rate (SGR) and the individual food-demand in all experimental tanks; and mean SGR of the three food-demand groups of fish.

Tank no.	Number of fish	r^2	*p*-Values		Mean SGR
1	50	0.067	0.070	Null-triggering fish	0.118 ± 0.026 (20)
				Low-triggering fish	0.081 ± 0.028 (27)
				High-triggering fish	0.160 ± 0.075 (3)
					ns
2	49	0.002	0.772	Null-triggering fish	0.247 ± 0.056 (14)
				Low-triggering fish	0.231 ± 0.027 (30)
				High-triggering fish	0.150 ± 0.062 (4)
					ns
3	51	0.006	0.580	Null-triggering fish	0.134 ± 0.030 (23)
				Low-triggering fish	0.187 ± 0.033 (24)
				High-triggering fish	0.154 ± 0.021 (4)
					ns
5	50	0.017	0.370	Null-triggering fish	0.083 ± 0.029 (30)
				Low-triggering fish	0.192 ± 0.035 (17)
				High-triggering fish	0.222 ± 0.084 (3)
					*
7	50	0.018	0.350	Null-triggering fish	-0.003 ± 0.033 (23)
				Low-triggering fish	-0.012 ± 0.025 (22)
				High-triggering fish	0.098 ± 0.023 (5)
					ns
8	50	0.016	0.389	Null-triggering fish	0.177 ± 0.022 (20)
				Low-triggering fish	0.193 ± 0.027 (25)
				High-triggering fish	0.224 ± 0.072 (5)
					ns

Linear regression analysis. r^2 and *p*-Values are noted.
Values of SGR are means ± S.E.M. in %/day and *n*, number of fish in parentheses.
Analysis of variance (ANOVA). Significance levels are denoted by asterisks: * $p < 0.05$, ns = no significance.

Table 4

Mean levels of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in telencephalon, cerebellum and diencephalon of the three food-demand groups of fish.

		5-HT	5-HIAA
Telencephalon	Null-triggering fish	55.0 ± 5.6 (15)	6.9 ± 0.7 (15)
	Low-triggering fish	61.8 ± 12.2 (9)	5.6 ± 1.1 (9)
	High-triggering fish	62.9 ± 10.1 (15)	4.2 ± 0.5 (15)
		ns	**
Cerebellum	Null-triggering fish	38.2 ± 4.3 (15)	4.5 ± 0.3 (15)
	Low-triggering fish	56.9 ± 8.6 (9)	4.2 ± 0.6 (9)
	High-triggering fish	53.6 ± 6.2 (15)	3.2 ± 0.3 (15)
		ns	*
Diencephalon	Null-triggering fish	19.1 ± 1.7 (15)	2.4 ± 0.1 (15)
	Low-triggering fish	21.4 ± 3.5 (9)	2.1 ± 0.3 (9)
	High-triggering fish	19.5 ± 1.8 (15)	1.6 ± 0.2 (15)
		ns	**

Values are in ng/g of wet weight for 5-HT and in 10^{-3} ng/g of wet weight for 5-HIAA.
All data are means ± S.E.M. and *n*, number of fish in parentheses.
Analysis of variance (ANOVA). Significance levels are denoted by asterisks: * $p < 0.05$, ** $p < 0.01$, ns = no significance.

Legends for figures

Fig. 1. Relationship between the specific growth rate and the number of trigger actuations -i.e. the individual food-demand in tank 2. Linear regression analysis: r^2, *p-Values*.

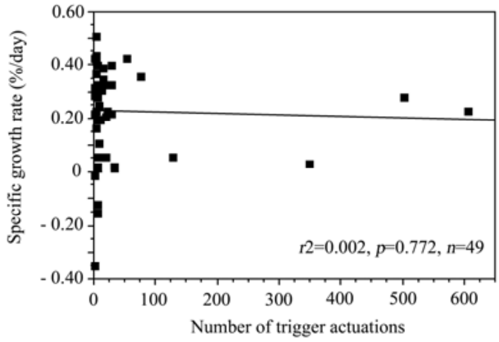

Fig. 2. 5-HIAA/5-HT ratios in telencephalon (A), cerebellum (B) and diencephalon (C) of the three food-demand groups of fish. Values are means ± S.E.M. and *n*, number of fish in parentheses. Analysis of variance (ANOVA) followed by Fisher's post hoc test with significance levels denoted by asterisks: * $p < 0.05$, ** $p < 0.01$.