J. exp. Biol. ***179***, *197–211 (1993)*
Printed in Great Britain *© The Company of Biologists Limited 1993*

FEEDING RANK AND BRAIN SEROTONERGIC ACTIVITY IN RAINBOW TROUT *ONCORHYNCHUS MYKISS*

SVANTE WINBERG[1], CRIS G. CARTER[2], IAN D. McCARTHY[2], ZHONG-YANG HE[2], GÖRAN E. NILSSON[1] and DOMINIC F. HOULIHAN[2]

[1]*Department of Zoophysiology, Uppsala University, Norbyvägen 18A, S-752 36 Uppsala, Sweden* and [2]*Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen, AB9 2TN, Scotland, UK*

Accepted 2 March 1993

Summary

Two methods for assessing the status of an individual rainbow trout *Oncorhynchus mykiss* within a group hierarchy, radiographic determination of individual food intake and analysis of brain serotonergic activity, were compared. The results showed that individual food intake, measured as the average share of the group meal, and brain serotonergic activity, measured as brain levels of 5-hydroxyindoleacetic acid (5-HIAA) or as 5-HIAA/5-HT (serotonin) ratios, were inversely correlated with each other, suggesting that both methods could be used as indicators of the position of the rainbow trout in a dominance hierarchy. In addition, specific growth rate correlated significantly with brain 5-HIAA/5-HT ratios.

The results indicate that the increase in brain 5-HIAA/5-HT ratios in subordinate individuals is caused by an increased use (release) of 5-HT in these fish, and not by an increase in the level of tryptophan, the amino acid precursor of 5-HT. The relationships between social rank, food intake, growth, stress and brain serotonergic activity of fish in a social hierarchy are discussed.

Introduction

The social structure of juvenile salmonid fish is characterized by the formation of dominance-based social hierarchies (Yamagishi, 1962; Symons, 1968; Jenkins, 1969; Abbott and Dill, 1989; Metcalfe *et al.* 1989). Dominant fish tend to have higher growth rates than subordinates and growth depensation is common (Jobling and Reinsnes, 1986; Davis and Olla, 1986). Differential food intake is the major cause of differences in the growth rates of individual fish (Carter *et al.* 1992*a*,*b*; McCarthy *et al.* 1992*b*), although other factors, such as genetic differences (Magnuson, 1962), differences in activity (Metcalfe, 1986) and energy expenditure attributable to different levels of stress (Jobling and Wandsvik, 1983; Abbott and Dill, 1989), may also have an influence. In salmonids, dominant fish within pairs or small groups have been observed to consume a greater proportion of the group meal than subordinates (Fausch, 1984; Metcalfe *et al.* 1989; Huntingford *et al.* 1990). The amount of food consumed by rainbow trout [*Oncorhynchus*

Key words: feeding rank, hierarchy, serotonin, tryptophan, rainbow trout, *Oncorhynchus mykiss*.

mykiss (Walbaum)] in larger groups (24 individuals) has been studied using radiography and the result revealed that feeding hierarchies were formed (McCarthy *et al.* 1992*a*).

Experiments on Arctic charr [*Salvelinus alpinus* (L.)] have shown that there is a strong negative correlation between social rank and the brain level of 5-hydroxyindoleacetic acid (5-HIAA), the major serotonin (5-HT) metabolite (Winberg *et al.* 1991, 1992*a*). Additionally, the ratio 5-HIAA/5-HT, an index of serotonergic activity (Shannon *et al.* 1986), was found to be higher in low-ranking than in dominant Arctic charr (Winberg *et al.* 1991, 1992*a*). Differences in brain levels of 5HIAA and in 5-HIAA/5-HT ratios are the first neurochemical markers of social rank to be demonstrated in salmonids. The correlation between social rank and brain serotonergic activity has, however, only been studied in small groups (four individuals) of Arctic charr because of the practical problems involved in conducting the behavioural observations required to determine individual social rank in larger groups of fish (Winberg *et al.* 1991, 1992*a*).

The aim of this study was to compare the two methods, measurement of food intake using radiography and analysis of brain serotonergic activity, for assessing the status of an individual rainbow trout within a group hierarchy.

Materials and methods

Fish

Thirty rainbow trout were divided into two groups, each consisting of fifteen fish of approximately equal size (group A 132.0±4.7g and group B 89.2±2.2g). After 2 days without food, the fish were anaesthetized in MS 222 ($0.1\,\mathrm{g\,dm^{-3}}$) weighed and individually marked by Alcian Blue injection on the ventral surface using a Panjet. The fish were held in 350l outdoor tanks supplied with fresh Aberdeen tapwater at a rate of approximately $40\,\mathrm{l\,h^{-1}}$. The fish were fed a nominal daily ration of 1% initial body mass per day of a commercial trout diet (Aqualine Trout Grower, North Eastern Farmers). A low tank ration was chosen in order to promote a pronounced feeding hierarchy (McCarthy *et al.* 1992*a*). Food was supplied over a period of up to 60min between 11:00 and 12:00h every day. Single pellets were dropped into the tanks until all the daily ration had been eaten or the last five pellets to be supplied were rejected. The experiment lasted for 75 days, from 27 November 1991 until 7 February 1992. The fish were exposed to natural variation in photoperiod and water temperature (6.6±1.7˚C: range 3.0–9.0˚C).

Measurement of individual consumption rates

Consumption rates of individual fish were determined four times for both groups (days 1, 22, 43, 72). Radiography was used to measure consumption rates. Pellets containing X-ray-opaque Ballotini glass beads (size 8, 400–520 μm, Jencons Scientific Ltd) were given in the same way as the normal food, radiographs were taken 2h after feeding and the fish were then weighed before being returned to the tanks (McCarthy *et al.* 1992*a*). None of the X-rays was blurred and the number of glass beads present in the alimentary canal of each fish could be counted on each occasion when food intake was monitored. Individual consumption rates [$\mathrm{mg\,g^{-1}\,day^{-1}}$: dry mass (mg) food per gram wet fish per day] were calculated as described previously (Carter *et al.* 1992*a*; McCarthy *et al.* 1992*a*). Share of meal was calculated for each fish as the proportion of the total food consumed by that fish on the particular day and this was used to calculate the overall mean share of meal (MSM). The day-to-day variation in consumption for an individual fish was assessed using the coefficient of variation for consumption (CV_C):

$$CV_C\,(\%) = (100 \times \text{S.D.})/C_m\,,$$

(McCarthy *et al.* 1992*a*), where C_m is the mean consumption rate for individual fish and S.D. is its standard deviation. Specific growth rate (SGR) was calculated as:

$$\text{SGR}\ (\%\text{day}^{-1}) = 100[\log_e (W_f/W_s)]/t\,,$$

where W_s and W_f are the wet masses of the fish at the start and finish of the experiment, respectively, and t is the time in days (75). Two fish were not included in further analysis; one fish from group A showed abnormal weight loss and one fish from group B died after 22 days.

Tissue sampling

The fish were killed on day 75, a minimum of 72h after the last application of MS 222. Fish were removed from the tank individually, decapitated and the brain (excluding olfactory bulbs and pituitary gland) was removed and divided into two parts: the telencephalon and the remainder, denoted here as the brain stem (Winberg *et al.* 1991). All samples were removed and frozen within 150s of decapitation. The samples were wrapped in aluminium foil, frozen in liquid nitrogen and kept at −70˚C until shipment. The samples were flown from Aberdeen to Uppsala on dry ice. The time it took to catch each fish and the order of removal of the fish from the tank was recorded.

Assay of 5-HT, 5-HIAA and tryptophan

The frozen brain samples were homogenized in 4% (w/v) ice-cold perchloric acid containing 0.2% EDTA, 0.05% sodium bisulphite and 40ngml^{-1} epinine (deoxyepinephrine, the internal standard), using a Potter-Elvehjem homogenizer (brain stem) or an MSE 100W ultrasonic disintegrator (telencephalon).

5-HT and 5-HIAA were quantified using high performance liquid chromatography (HPLC) with electrochemical detection as described by Nilsson (1989). Tryptophan was analyzed using the same HPLC system by setting the detector on a higher oxidizing potential (1V instead of 750mV). As a measure of serotonergic activity, the 5-HIAA/5-HT ratio was calculated for each individual (Shannon *et al.* 1986; Winberg *et al.* 1991). Owing to a technical problem, two telencephalon samples from group A were lost.

Statistics

All values presented are means ± standard error (S.E.M.). The coefficient of variation (CV) was also used as a measure of variability since this is expressed relative to the magnitude of the mean (Zar, 1984). The data for the mean share of the group meal (MSM) and specific growth rate (SGR) were subjected to arcsine transformation prior to analysis. Thereafter, step-wise multiple linear regression analyses (model computed) were performed on pooled data from both groups with MSM, SGR and CV_C as independent variables. In this way, the food consumption and growth variables that explained most of the observed variation in different neurochemical variables were determined. Relationships indicated in this analysis were examined further, for individual groups and pooled data, using two-variable linear regressions by means of the Pearson correlation coefficient. Statistical differences at the 5% level were considered significant. The relationships between growth and food consumption variables and the brain levels of monoamines and monoamine metabolites were examined both for the two groups separately and for pooled material. The rationale for the pooling of the data from the two groups of fish was that specific neurochemical responses to hierarchical position (feeding rank) of individuals were being sought.

Results

Consumption, growth and feeding hierarchies

The experiment was carried out over the winter when consumption rates and growth rates were relatively low. The level of growth depensation, indicated by the change in the CV for wet mass, was small for both groups (Table 1), but nevertheless a wide range of individual growth rates was found in both groups (Table 1). Consumption rates varied between individuals and the fact that MSM varied between 0.8 and 16.6% in group A and between 2.8 and 13.4% in group B (Table 1) provides evidence for feeding hierarchy formation. The higher CV value for MSM in group A suggested a stronger feeding hierarchy between these fish, since differential consumption was more pronounced. The intra-individual variation in consumption rate (CV_C) varied between 23.5 and 173.3 % and showed more variation in group A since the CV for CV_C was 52.5% compared with

Table 1. *Mean, ranges and coefficient of variation (CV) for initial and final wet masses, specific growth rate (SGR), individual daily rate of food consumption, mean share of meal (MSM) and coefficient of variation for consumption (CV_C) for the two groups of rainbow trout (*N*=14)*

	Group A		Group B	
	Mean ± S.E.M. (range)	CV	Mean ± S.E.M. (range)	CV
Initial mass (g)	132.50±4.97 (109.70–166.10)	14.05	89.17±2.23 (74.90–102.20)	9.37
Final mass (g)	161.60±6.73 (122.60–204.00)	15.58	118.20±4.06 (95.60–146.80)	12.85
SGR (%day^{-1})	0.276±0.033 (0.018–0.478)	45.07	0.393±0.040 (0.171–0.754)	38.3
Consumption (mg g^{-1} day^{-1})	2.97±0.57 (0.36–7.47)	71.84	5.80±0.48 (2.28–9.53)	30.74
MSM (%)	7.14±1.39 (0.75–16.60)	73.00	7.14±0.70 (2.79–13.42)	36.75
CV_C (%)	88.44±12.41 (40.11–173.30)	52.50	76.68±8.93 (23.50–128.80)	43.58

Table 2. *Pearson correlation coefficients for relationships between specific growth rate (SGR), mean share of meal (MSM), coefficient of variation for food consumption (CV_C), start mass (W_s), final mass (W_f), telencephalon 5-HIAA/5-HT ratio and brain stem 5-HIAA/5-HT ratio of rainbow trout in group A and group B (N=14, except for telencephalon 5-HIAA/5-HT ratio in group A, where N=12)*

	SGR†	MSM†	CV_C	W_s	W_f	Telencephalon 5-HIAA/5-HT	Brain stem 5-HIAA/5-HT
Group A							
SGR†		0.152	−0.263	−0.048	0.481	−0.772**	−0.455
MSM†			−0.642*	0.471	0.431	−0.496	−0.657*
CV_C				−0.309	−0.307	0.434	−0.346
W_s					0.837***	−0.245	−0.356
W_f						−0.591*	−0.543*
Telencephalon 5-HIAA/5-HT							0.739**
Group B							
SGR†		0.583*	−0.691**	−0.164	0.695**	−0.595*	−0.371
MSM†			−0.434	0.420	0.786***	−0.419	−0.372
CV_C				0.164	−0.427	0.306	−0.037
W_s					0.581*	0.074	0.209
W_f						−0.537*	−0.112
Telencephalon 5-HIAA/5-HT							0.572*

*$P<0.05$; **$P<0.01$; ***$P<0.001$.

†Correlation analysis was performed on arcsine-transformed data.

43.6% in group B (Table 1). MSM was used to estimate the feeding rank of individual fish within each group. It was not correlated with initial wet mass in either group (Table 2) or with final wet mass in group A (Table 2), but was significantly correlated with final wet mass in group B (r=0.786, P<0.001).

The correlation between CV_C and MSM has been used to indicate the strength of feeding hierarchy in rainbow trout (McCarthy *et al.* 1992*a*). In both groups, fish which secured a greater share of the group meal tended to have a lower CV_C, but the correlation was only significant for group A (Fig. 1A).

In group B, SGR was significantly correlated with MSM (r=0.583, P=0.029) and final wet mass (r=0.695, P=0.006) and inversely correlated with CV_C (r=−0.691, P=0.006). However, SGR did not correlate significantly with MSM, final wet mass or CV_C in group A (Table 2).

Brain levels of 5-HT, 5-HIAA and tryptophan

Neither brain tryptophan, 5-HT or 5-HIAA concentrations nor 5-HIAA/5-HT ratios correlated with the time needed to catch individual fish, or the order in which the fish were caught.

The effects of MSM, SGR and CV_C, on telencephalon and brain stem 5-HIAA/5-HT ratio was tested using a step-wise multiple linear regression analysis on pooled data from

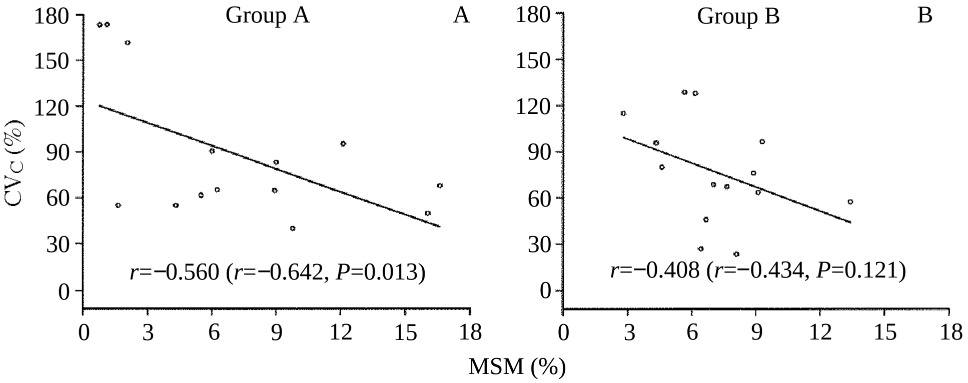

Fig. 1. The relationship between coefficient of variation in mass-specific consumption (CV_C) and the mean share of the group meal (MSM) for individual rainbow trout in (A) group A (*N*=14) and (B) group B (*N*=14). Values and the least-square regression line are original non-transformed data. Before correlation analysis the MSM data were subjected to arcsine transformation and *r*- and *P*-values for the correlation analyses on transformed data are given within parentheses.

both groups with MSM, SGR and CV_C as independent variables. The variation in telencephalon 5-HIAA/5-HT ratio was best explained by SGR (*P*=0.0022) and MSM (*P*=0.0499). The resulting multiple linear regression only included two independent variables, SGR and MSM, and accounted for 46.4% of the variation in telencephalon 5-HIAA/5-HT ratio. Similarly, the variation in brain stem 5-HIAA/5-HT ratio was best explained by a multiple linear regression including MSM (*P*=0.0005) and SGR (*P*=0.003) as independent variables. In this case, the resulting multiple linear regression accounted for 48.0% of the variation in brain stem 5-HIAA/5-HT ratio.

Corresponding multiple linear regression analyses were performed with the levels of 5-HIAA, 5-HT and tryptophan as outcome variables. The variation in telencephalon 5-HIAA levels was best explained by SGR (*P*=0.071), and the final multiple linear regression, which only included SGR as independent variable, accounted for 12.9% of the variation in telencephalon 5-HIAA level. By contrast, MSM was the only independent variable that had a significant effect on brain stem 5-HIAA level (*P*=0.003), accounting for 29.5% of the variation. None of the independent variables (MSM, CV_C and SGR) tested had any significant (*P*>0.05) effect on telencephalon or brain stem 5-HT and tryptophan levels.

The relationships between neurochemical variables and MSM and SGR indicated in the multiple linear regression analysis were further examined in the individual groups and in pooled data using two-variable linear regressions. When the data for groups A and B were pooled, significant negative correlations were found between MSM and 5-HIAA/5-HT ratio in both telencephalon (Fig. 2A) and brain stem (Fig. 2B). The fish that were subordinate in the feeding hierarchy showed increased 5-HIAA/5-HT ratios. A similar trend was found in the individual groups, although the relationships were not significant at the 5% level.

In both groups, the telencephalon 5-HIAA concentration was higher in low-feeding

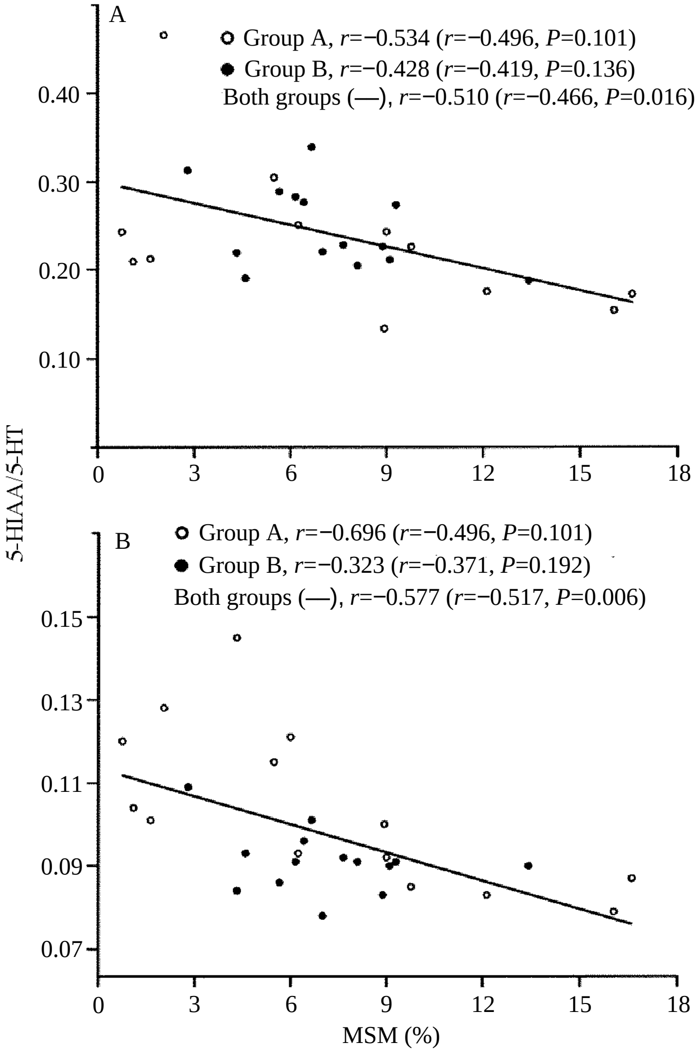

Fig. 2. The relationship between mean share of meal (MSM) and 5-HIAA/5-HT ratio in telencephalon (A) and brain stem (B) of rainbow trout. Relationships are presented for group A (telencephalon, *N*=12, and brain stem, *N*=14) and group B (*N*=14) separately, as well as for both groups taken together. Values and least-square regression lines are original non-transformed data. Before correlation analysis, the MSM data were subjected to arcsine transformation and *r*- and *P*-values for the correlation analyses on transformed data are given within parentheses.

fish, although the relationship was not significant (pooled data $r=-0.297$, $P=0.141$). However, the level of 5-HIAA in the brain stem was significantly correlated with MSM in group A ($r=-0.567$, $P=0.035$) and in pooled data ($r=-0.543$, $P=0.003$), with low-ranking fish showing increased 5-HIAA levels. A similar relationship was seen in group B, although this did not attain statistical significance ($r=-0.527$, $P=0.053$).

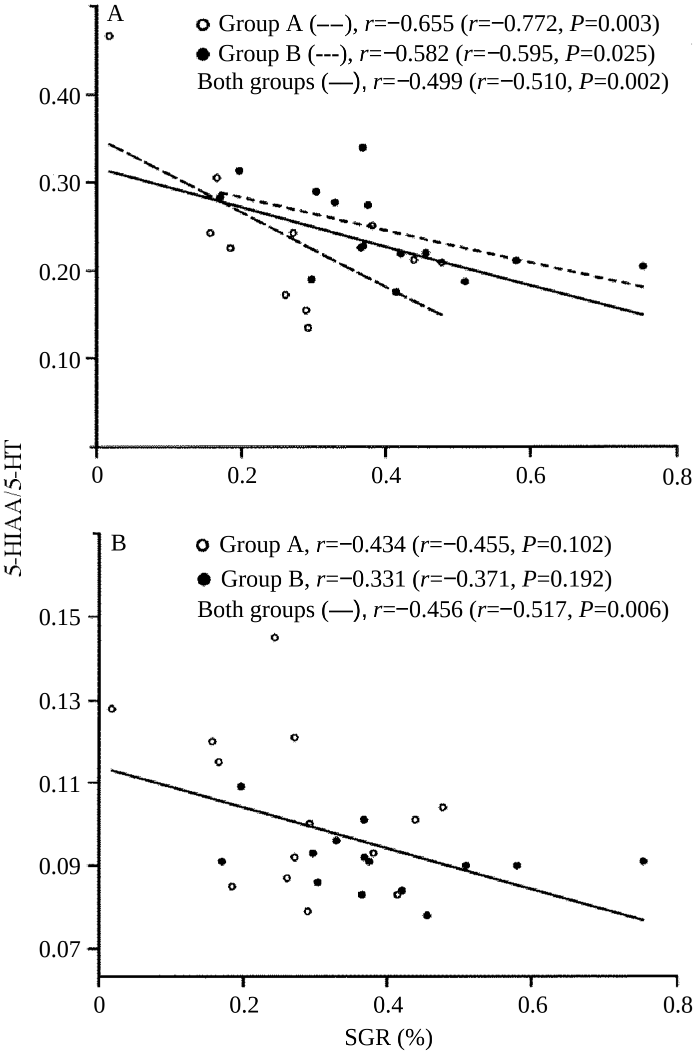

Fig. 3. The relationship between specific growth rate (SGR) and 5-HIAA/5-HT ratio in telencephalon (A) and brain stem (B) of rainbow trout. Relationships are presented for group A (telencephalon, *N*=12, and brain stem, *N*=14) and group B (*N*=14) separately, as well as for both groups taken together. Values and least-square regression lines are original non-transformed data. Before correlation analysis, the SGR data were subjected to arcsine transformation and *r*- and *P*-values for the correlation analyses on transformed data are given within parentheses.

Significant negative correlations were found between telencephalon 5-HIAA/5-HT ratio and SGR in individual groups as well as in pooled data (Fig. 3A). A similar relationship was found between brain stem 5-HIAA/5-HT ratio and SGR, although this was only significant in pooled data (Fig. 3B). Telencephalon 5-HIAA levels were significantly correlated with SGR in group A ($r=-0.746$, P=0.005) but not in group B ($r=-0.141$, P=0.632). A tendency towards a correlation between SGR and 5-HIAA level

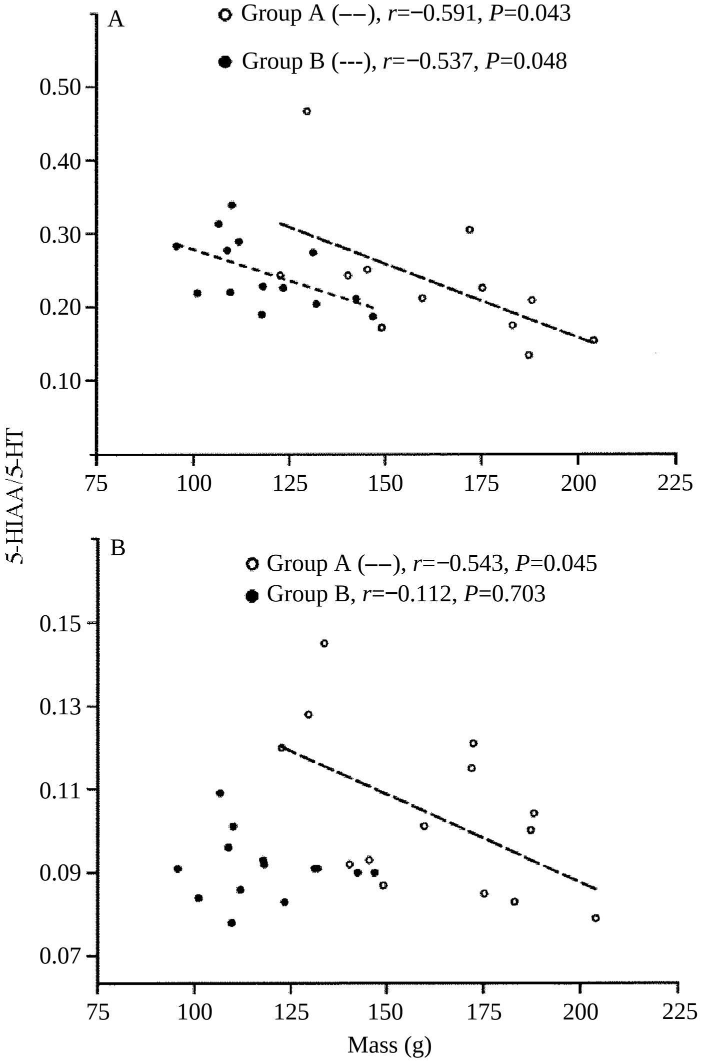

Fig. 4. The relationship between final wet mass and 5-HIAA/5-HT ratio in telencephalon (A) and brain stem (B) of rainbow trout. Relationships are presented for group A (telencephalon, N=12, and brain stem, N=14) and group B (N=14) separately. Lines are least-square regression lines.

in the telencephalon was found in pooled data (r=−0.360, P=0.071). Furthermore, a relationship between brain stem 5-HIAA level and SGR was indicated in group A (r=−0.455, P=0.102) and, when data from both groups were pooled, a significant negative correlation was found between brain stem 5-HIAA level and SGR (r=−0.395, P=0.037).

Significant correlations between telencephalon 5-HIAA/5-HT ratio and final wet mass were found in both groups (Fig. 4). Furthermore, brain stem 5-HIAA/5-HT ratio correlated significantly with final mass in group A (Fig. 4). However, there were no

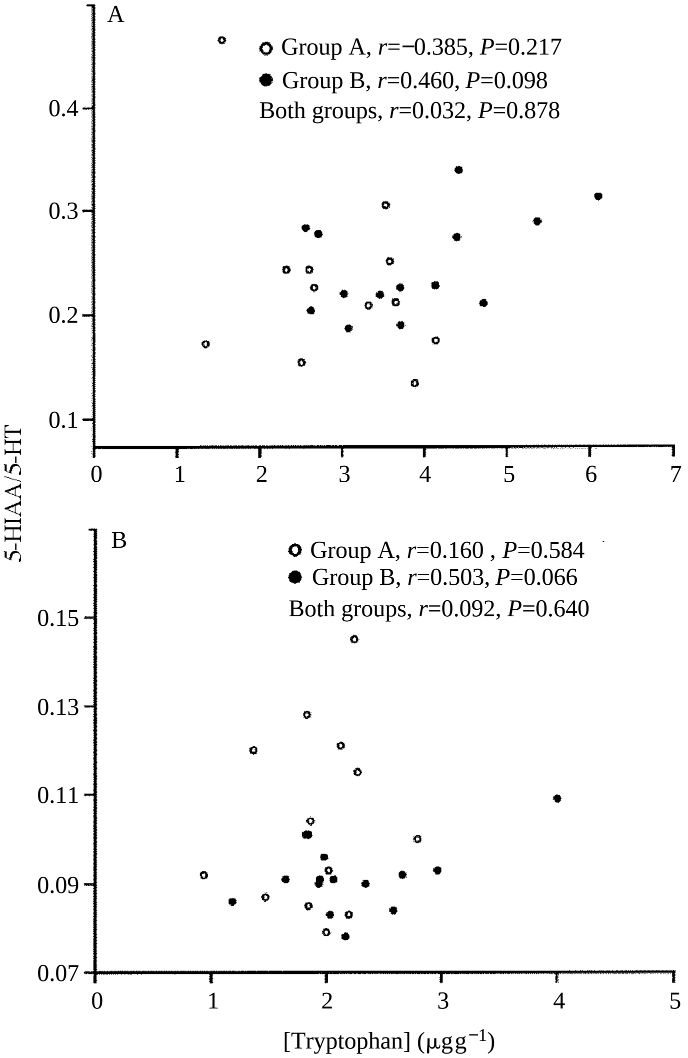

Fig. 5. The relationship between tryptophan concentration and 5-HIAA/5-HT ratio in telencephalon (A) and brain stem (B) of rainbow trout. Relationships are presented for group A (telencephalon, N=12, and brain stem, N=14) and group B (N=14) separately, as well as for both groups taken together.

significant correlations between 5-HIAA level, in either telencephalon and brain stem, and final mass in any of the groups. Because of the difference in start mass between the groups, relationships between final mass and the neurochemical variables were not examined in pooled data.

Tryptophan concentrations did not correlate significantly with 5-HIAA/5-HT ratios, in any of the groups or in the pooled data (Fig. 5).

Discussion

The development of feeding hierarchies, in which high-ranking individuals consume a greater proportion of the group meal, is a well-known phenomenon in salmonid fish (Jenkins, 1969; Fausch, 1984; Metcalfe, 1986). The results of the present study show that the average meal size of an individual fish (MSM) is inversely correlated with brain 5-HIAA/5-HT ratios. The 5-HIAA/5-HT ratio in the brain has previously been shown to correlate with behavioural rank in groups of four Arctic charr (Winberg *et al.* 1992*a*). Thus, the results of the present study suggest that the position of a fish in a feeding hierarchy is correlated with its position in the dominance hierarchy. The relationships found between individual food intake and 5-HIAA/5-HT ratio were seen in both of the brain areas studied, telencephalon and brain stem (Fig. 2). Similarly, previous studies on Arctic charr have shown that both subordinate experience (Winberg *et al.* 1991, 1992*a*) and repeated artificial stress (Winberg *et al.* 1992*b*) increase serotonergic activity in telencephalon as well as in brain stem. Thus, the effect of stress and subordinate experience on serotonin utilization seems to be widely distributed in the brain.

The correlations discussed above were weaker and not significant in group B, which may be indicative of a weaker hierarchy in this group. In group A, the CV values for consumption rate, for MSM, for growth rate and for CV_C were all large, suggesting a strong hierarchical structure. Additionally, the correlation between CV_C and MSM, an indicator of hierarchy strength (McCarthy *et al.* 1992*a*), was weaker in group B.

Fish occupying seemingly similar tanks can develop hierarchies of different strengths. For example, Atlantic salmon parr in six replicate tanks formed dominance hierarchies of different strengths (Symons, 1968), possibly reflecting individual differences in competitive ability between fish in each tank. In order to promote pronounced hierarchy formation, each of the two groups used in the present study was composed of fish that were approximately equal in size. Consequently, a size difference in mean mass between the two groups was unavoidable. The fish in group A, where the strongest hierarchy was indicated, were larger than the fish in group B. This size difference may have affected competitive ability and the strength of the hierarchy formed.

Disproportional food acquisition explains a large part of the variation in growth rates of rainbow trout (McCarthy *et al.* 1992*b*) and other salmonids (Jobling *et al.* 1989; Carter *et al.* 1992*a*). Individual differences expressed during the formation of hierarchies will result in disproportionate food acquisition and higher growth rates of dominant fish, so that final mass and growth rate are often found to correlate with final rank (e.g. Yamagishi, 1962; Winberg *et al.* 1992*a*). In the present study, feeding rank correlated with final mass in group B, the group that appeared to have the weaker hierarchy, but not in group A.

Although it is obvious that MSM and SGR must be interrelated, their relationship in a hierarchy is complicated by factors such as aggression and stress. Interestingly, in group A, the group where the strongest hierarchy was indicated, neither SGR nor final mass correlated significantly with food intake (MSM). Thus, some of the fish in this group appeared to have a reduced growth efficiency and did not grow in proportion to the amount of food consumed. This may be related to a stress-induced increase in the rate of

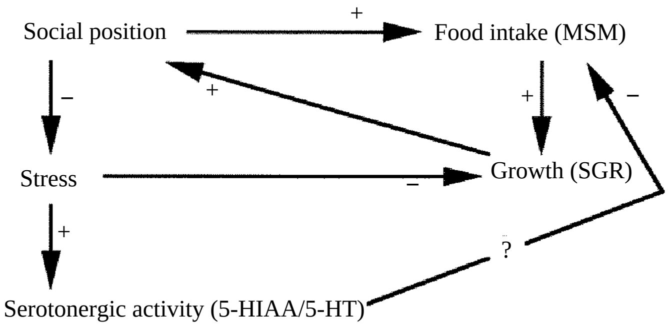

Fig. 6. Possible relationships between social position, stress, growth, food intake and brain serotonergic activity of fish in a dominance hierarchy. An arrow indicates that an increase in the variable from which the arrow originates will stimulate (+) or inhibit (−) the other variable. A question mark indicates an uncertain connection. See text for further explanation.

catabolism at the expense of the rate of anabolism (Jobling and Wandsvik, 1983; Abbott and Dill, 1989; Winberg *et al.* 1992*b*). Physiological signs of stress have previously been reported in subordinate fish (Noakes and Leatherland, 1977; Ejike and Schreck, 1980; Peters *et al.* 1980; Scott and Currie, 1980), and Abbott and Dill (1989) showed that the dominant individual in a size-matched pair of steelhead trout (*Oncorhynchus mykiss*) had a higher growth rate than the subordinate, even if both fish were fed equal amounts. Since subordinates were less active, Abbott and Dill (1989) suggested that the reduced growth rate in subordinate fish was caused by an increased metabolic rate induced by stress. This was also indicated in one of our previous studies, where we found that Arctic charr that had been both starved and stressed lost more weight than fish that had been subjected to starvation alone, even though stress caused a striking reduction in locomotor activity (Winberg *et al.* 1992*b*). The increased brain serotonergic activity, indicated by increased brain 5-HIAA/5-HT ratios, in low-ranking fish is probably a reflection of the stress experienced by these individuals (Winberg *et al.* 1992*b*). In the present study, the variation in 5-HIAA/5-HT ratio in the telencephalon was best explained by growth rate (SGR), a variable that, apart from being related to food intake, is also affected by metabolic rate and, therefore, by stress. By contrast, starvation alone does not increase the 5-HIAA/5-HT ratio (Winberg *et al.* 1992*b*), so there is probably no direct link between MSM and serotonergic activity. The probable and possible relationships between factors such as social position, food intake, growth, stress and brain serotonergic activity of fish in a dominance hierarchy have been tentatively summarized in Fig. 6.

In Fig. 6, we have indicated the possibility that increased serotonergic activity could cause a reduction in food intake and, in this way act, to reinforce the hierarchical structure. Increased brain serotonergic activity in mammals has been found to have an inhibitory effect on food intake (Fernstrom, 1981; Samanin, 1989). Recently, Johnston *et al.* (1992) reported that stimulation of the brain serotonergic activity by tryptophan administration resulted in reduced food intake in rainbow trout fed a high-carbohydrate diet. In the same study, inhibition of serotonergic activity by the 5-HT synthesis inhibitor *p*-chlorophenylalanine (*p*-CPA) gave a seemingly contradictory result, since this treatment also resulted in a decreased food intake (Johnston *et al.* 1992). However, we recently found that inhibition of brain serotonergic activity by *p*-CPA treatment causes a considerable increase in spontaneous locomotor activity in Arctic charr (Winberg *et al.* 1993), an effect that may interfere with feeding.

In mammalian studies, stress has been found to increase tryptophan levels in both blood plasma and brain (Curzon *et al.* 1972; Neckers and Sze, 1975; Dunn, 1988). Tryptophan is the amino acid precursor of 5-HT and increased brain tryptophan concentrations may increase the rate of 5-HT synthesis and turnover since the rate-limiting step in 5-HT synthesis seems to be restricted by tryptophan availability (Boadle-Biber, 1982). In rats, stress-induced increase in brain 5-HIAA levels can be inhibited by valine, a large neutral amino acid that competes with tryptophan for uptake into the brain (Kennett and Joseph, 1981). However, the increase in brain serotonergic activity in low-feeding fish observed in the present study does not appear to be related to a concomitant increase in tryptophan availability. First, it is hard to believe that the increase in brain 5-HIAA concentrations in subordinate fish could be an effect of increased brain tryptophan availability, since the 5-HT concentration remained unchanged or even showed a tendency to decrease in the telencephalon of subordinate individuals. Second, in the present study, brain tryptophan concentrations did not correlate with either 5-HIAA/5-HT ratios or individual food intake. Consequently, there is little reason to believe that the increases in brain 5-HIAA/5-HT ratios observed in low-feeding individuals had any direct connection with tryptophan availability. Thus, it appears more likely that the increase in brain 5-HIAA/5-HT ratios reflects an increase in 5-HT release caused by an activation of the serotonergic system.

In conclusion, the results of the present study suggest that both individual food intake, measured as MSM, and brain serotonergic activity, measured as brain 5-HIAA/5-HT ratios, could be used as indicators of the position of individual rainbow trout in a dominance-related feeding hierarchy. Indeed, of the variables measured, MSM, SGR and 5-HIAA/5-HT ratios correlated with each other and are probably all reflections of the hierarchical position of an individual, although the relationships between these variables are complicated by other factors, such as stress.

The authors are grateful to an unknown referee for comments on the manuscript. This work was supported by The Swedish Council for Forestry and Agricultural Research (to G.E.N.), the Carl Trygger Foundation and the the foundation of Helge Ax:son Johnsons stiftelse, the Ministry of Agriculture, Fisheries and Food (UK) and the Science and Engineering Research Council (UK).

References

Abbott, J. C. and Dill, L. M. (1989). The relative growth of dominant and subordinate juvenile steelhead trout (*Salmo gairdneri*) fed equal rations. *Behav*. **108**, 104–113.

Boadle-Biber, M. C. (1982). Biosynthesis of serotonin. In *Biology of Serotonergic Transmission* (ed. N. N. Osborne), pp. 63–87 Chichester, New York, Brisbane, Toronto, Singapore: John Wiley and Sons.

Carter, C. G., Houlihan, D. F. and McCarthy, I. D.(1992*a*). Feed utilization efficiencies of Atlantic salmon (*Salmo salar* (L.)) parr: effect of a supplementary enzyme. *Comp. Biochem. Physiol.* **101**A, 369–374.

CARTER, C. G., HOULIHAN, D. F., MCCARTHY, I. D. AND BRAFIELD, A. (1992*b*). Variation in the food intake of grass carp, *Ctenopharyngodon idella* (Val.), fed singly or in groups. *Aquat. Liv. Res*. (in press).

CURZON, G., JOSEPH, M. H. AND KNOTT, P. J.(1972). Effects of immobilization and food deprivation on rat brain tryptophan metabolism. *J. Neurochem.* **9**, 1967–1974.

DAVIS, M. W. AND OLLA, B. L. (1986). Aggression and variation in growth of Chum salmon, *Oncorhynchus keta*, juveniles in seawater: effects of limited rations. *Can. J. Fish. aquat. Sci.* **44**, 192–198.

DUNN, A. J. (1988). Changes in plasma and brain tryptophan and brain serotonin and 5-hydroxyindoleacetic acid after footshock stress. *Life Sci.* **42**, 1847–1853.

EJIKE, C. AND SCHRECK, C. B. (1980). Stress and social hierarchy rank in coho salmon. *Trans. Am. Fish. Soc.* **109**, 423–426.

FAUSCH, K. D. (1984). Profitable stream positions for salmonids: relating specific growth rate to net energy gain. *Can. J. Zool.* **62**, 441–451.

FERNSTROM, J. D. (1981). Physiological control of brain serotonin synthesis: relevance to physiology and behaviour. In *Serotonin Neurotransmission and Behavior* (ed. B. L Jacobs and A. Gelperin), pp. 75–96. Cambridge, MA; London, England: The MIT Press.

HUNTINGFORD, F. A., METCALFE, N. B., THORPE, J. E., GRAHAM, W. D. AND ADAMS, C. E.(1990). Social dominance and body size in Atlantic salmon parr, *Salmo salar* (L.). *J. Fish Biol.* **36**, 877–881.

JENKINS, T. M., JR (1969). Social structure, position and microdistribution of trout species (*Salmo trutta* and *Salmo gairdneri*) resident in mountain streams. *Anim. Behav. Monogr.* **2**, 56–123.

JOBLING, M., BAARDVIK, B. M. AND JORGENSEN, E. H.(1989). Investigation of food–growth relationships of Arctic charr, *Salvelinus alpinus* L. using radiography. *Aquaculture* **81**, 367–372.

JOBLING, M. AND REINSNES, T.-G.(1986). Physiological and social constraints on growth of Arctic charr, *Salvelinus alpinus* L.: An investigation of factors leading to stunting. *J. Fish Biol.* **28**, 379–384.

JOBLING, M. AND WANDSVIK, A. (1983). Effect of social interactions on growth rates and conversion efficiency of Arctic charr, *Salvelinus alpinus* L. *J. Fish Biol.* **22**, 577–584.

JOHNSTON, W. L., ATKINSON, J. L. AND GLANVILLE, N. T.(1992). Effect of PCPA or tryptophan on brain serotonin and on consumption of high protein or high carbohydrate diet by rainbow trout, *Oncorhynchus mykiss. J. nutr. Biochem.* **3**, 421–428.

KENNET, G. A. AND JOSEPH, M. H. (1981). The functional importance of increased brain tryptophan in the serotonergic response to restraint stress. *Neuropharmacology* **20**, 39–43.

MAGNUSON, J. J. (1962). An analysis of aggressive behaviour, growth and competition for food and space in medaka (*Oryzias latipes* (Pisces, Cyprinodontidae)). *Can. J. Zool.* **40**, 313–363.

MCCARTHY, I. D., CARTER, C. G. AND HOULIHAN, D. F. (1992*a*). The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Biol*. **41**, 257–263.

MCCARTHY, I. D., CARTER, C. G. AND HOULIHAN, D. F. (1992*b*). Individual variation in consumption in rainbow trout measured using radiography. In *Fish Nutrition in Practice* (ed. S. J. Kaushik and P. Luquet). Paris: INRA (in press).

METCALFE, N. B. (1986). Intraspecific variation in competitive ability and food intake in salmonids: consequences for energy budgets and growth rates. *J. Fish Biol.* **28**, 525–531.

METCALFE, N. B., HUNTINGFORD, F. A., GRAHAM, W. D. AND THORPE, J. E. (1989). Early social status and the development of early life-history strategies in Atlantic salmon. *Proc. R. Soc. Lond*. *B* **236**, 7–19.

NECKERS, L. AND SZE, P. Y. (1975). Regulation of 5-hydroxytryptamine metabolism in mouse brain by adrenal glucocorticoids. *Brain Res.* **93**, 123–132.

NILSSON, G. E. (1989). Regional distribution of monoamines and monoamine metabolites in the brain of the crucian carp (*Carassius carassius* L.). *Comp. Biochem. Physiol.* **94**C, 223–228.

NOAKES, D. L. G. AND LEATHERLAND, J. F. (1977). Social dominance and interrenal activity in rainbow trout, *Salmo gairdneri* (Pisces, Salmonidae). *Envl Biol. Fish* **2**, 131–136.

PETERS, G., DELVENTHAL, H. AND KLINGER, H.(1980). Physiological and morphological effects of social stress in the eel (*Anguilla anguilla* L.). *Arch. Fischereiwissenschaft* **307**, 157–180.

SAMANIN, R. (1989). Serotonin and feeding. In *Behavioural Pharmacology of 5-HT* (ed. P. Bevan, A. Cools and T. Archer), pp. 259–281. Hillsdale, NJ; Hove, London: Lawrence Erlbaum Associates.

SCOTT, D. B. C. AND CURRIE, C. E. (1980). Social hierarchy in relation to adrenocortical activity in *Xiphophorus helleri* Heckel. *J. Fish Biol.* **16**, 265–277.

SHANNON, N. J., GUNNET, J. W. AND MORE, K. E. (1986). A comparison of biochemical indices of 5-hydroxytryptaminergic neuronal activity following electrical stimulation of dorsal raphe nucleus. *J. Neurochem.* **47**, 958–965.

SYMONS, P. E. K. (1968). Increase in aggression and in the strength of the social hierarchy among juvenile salmon deprived of food. *J. Fish Res. Bd Can.* **25**, 2387–2401.

WINBERG, S., NILSSON, G. E. AND OLSÉN, K. H. (1991). Social rank and brain levels of monoamines and monoamine metabolites in Arctic charr, *Salvelinus alpinus* (L.). *J. comp. Physiol.* A **168**, 241–246.

WINBERG, S., NILSSON, G. E. AND OLSÉN, K. H. (1992*a*). Changes in brain serotonergic activity during hierarchic behavior in Arctic charr (*Salvelinus alpinus* L.) are socially induced. *J. comp. Physiol.* A **170**, 93–99.

WINBERG, S., NILSSON, G. E. AND OLSÉN, K. H. (1992*b*). The effect of stress and starvation on brain serotonin utilization in arctic charr (*Salvelinus alpinus*). *J. exp. Biol.* **165**, 229–239.

WINBERG, S., NILSSON, G. E., SPRUIJT, B. E. AND HÖGLUND, U. (1993). Spontaneous locomotor activity in arctic charr measured by a computerized imaging technique: role of brain serotonergic activity. *J. exp. Biol.* **179**, 213–232.

YAMAGISHI, H. (1962). Growth relations in some small experimental populations of rainbow trout fry, *Salmo gairdneri* Richardson, with special reference to social relations among individuals. *Jap. J. Ecol.* **12**, 43–53.

ZAR, J. H. (1984). *Biostatisical Analysis.* London: Prentice Hall.