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Discrimination between endogenous and exogenous water sources in juvenile rainbow trout fed extruded dry feed

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Abstract − A tracer (^{51}Cr-EDTA) study was undertaken with juvenile (20 g) freshwater rainbow trout, *Oncorhynchus mykiss*, using an experimental design that minimized stress effects during feeding and drinking trials. A calculation procedure was developed, where feed intake (pellet number), tracer intake (mL), water in stomach contents (g) and drinking rate ($mL \cdot kg^{-1} \cdot h^{-1}$) are essential to discriminate between the major stomach water sources prandially and postprandially: water bound in food; initial water absorption of pellets; prandial water intake; postprandial water intake and endogenous stomach secretion. We put forward the hypothesis that intake of dry food with a minor water content (10 %) may impose a demand for water to moisture the feed up to the level in natural feed (75 %) as preparation for gastric emptying, whereafter food is ready to pass from the stomach through the pyloric sphincter. Moisture content of pellets increased from 9.4 to 24.9 % in the pre-meal period. The pellets were ingested with 4.0 to 19.3 μL water per pellet, reflecting high inter-individual variation. Prandially moisture content rose to 52 % and further increased to 56 % in the delay period. Moisture content was ca. 65 %, when pellets began to disintegrate and move through the sphincter in accordance with the hypothesis. Stomach secretion contributed 34–44 % of the stomach water and ingested water 25–35 %. The sampling and calculation procedure gave convincing evidence for the detailed stomach water budget and this individual approach can be very useful in comparisons of artificial and natural diets.

drinking rate / water sources / feeding / moisturizing / stomach secretion / rainbow trout

Résumé − Distinction entre l'origine endogène et exogène des sources d'eau dans l'alimentation de truites juvéniles, nourries d'aliments secs extrudés. Une étude effectuée au moyen d'un traceur radioactif, ^{51}Cr-EDTA (éthylène diamine tétra-acétate), a été entreprise avec des juvéniles de truites d'eau douce, *Oncorhynchus mykiss* (20 g), en utilisant un protocole expérimental qui minimise les effets de stress durant des tests d'alimentation et d'absorption de liquide. Un mode de calcul est développé, où la prise de nourriture (nombre de granulés), la quantité de traceur absorbée (mL), l'eau du contenu stomacal (g) et le taux de liquide absorbé ($mL \cdot kg^{-1} \cdot h^{-1}$) sont essentiels pour permettre la distinction entre les sources d'eau les plus importantes avant ou après les repas : les quantités limites d'eau dans les aliments, l'absorption d'eau initiale des granulés, l'absorption d'eau prandiale et postprandial, avant et après le repas, et la sécrétion stomacale endogène. Nous avons émis l'hypothèse que la prise d'aliment sec avec un contenu d'eau minimum (10 %) peut imposer une demande d'eau pour humidifier l'aliment au niveau aqueux d'un aliment naturel (75 %) ainsi préparé pour l'évacuation gastrique, à la suite de quoi la nourriture est prête à passer de l'estomac vers le sphincter pylorique. Le contenu en eau des granulés a augmenté de 9,4 à 24,9 % lors de la période pré-repas. Les granulés sont ingérés avec 4,0 à 19,3 μL d'eau par granulé, ce qui reflète une variation inter-individuelle élevée. Le taux d'humidité a augmenté jusqu'à 52 % après les repas et a continué d'augmenter jusqu'à 56 % pendant la période de délai. La partie aqueuse est d'environ 65 %, lorsque les granulés commencent à se désintégrer et se diriger vers le sphincter conformément à l'hypothèse émise. La sécrétion stomacale contribue 34–44 % de l'eau stomacale et l'eau ingérée 25–35 %. L'échantillonnage et le mode de calcul donnent un résultat convaincant pour le budget précis de l'eau stomacale et cette approche individuelle peut être très utile dans les comparaisons entre aliments artificiels et naturels.

taux d'absorption de liquide / sources en eau / nutrition / humidification / sécrétion stomacale / truite arc-en-ciel

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1. INTRODUCTION

Water is quantitatively the most important body constituent in fish, and as a rule of thumb salmonids contains 70–75 % water, e.g. in rainbow trout, *Oncorhynchus mykiss* and brook trout, *Salvelinus fontinalis* (Rasmussen and Ostenfeld, 2000). A fish in a balanced nutritional state must be supplied with enough water to compensate for losses to the surrounding water. Anadromic fish face the problem of obtaining body water homeostasis in a fluctuating aquatic environment. Osmoregulation is necessary, when salinity is higher or lower in the surrounding water compared with the salinity of the blood. Fish tend to hydrate in freshwater and the kidney compensates for this by producing large volumes of dilute urine as shown in eel (*Anguilla anguilla* L. – Chester Jones et al., 1969; Gaitskell and Chester Jones, 1971). Some water is also lost with faeces, and some is gained as metabolic water during the oxidation of fats, proteins and carbohydrates. The water budget in relation to digestive functions in teleosts has recently been reviewed (Smith, 1989).

Although body water is taken directly from the aquatic environment, water is also provided with the food. Many natural food items contains 70–80 % water (Kristiansen, unpubl. results) and besides energy, vitamins and minerals etc., diet is a potentially important water source. However, extruded dry pellets contains less than 10 % water with an energy content of more than 20 $kJ \cdot g^{-1}$ wet wt (Jobling, 1986) in contrast to moist feeds (35–40 %, 15–20 $kJ \cdot g^{-1}$ wet wt) and wet feeds (70 %, 4–8.5 $kJ \cdot g^{-1}$ wet wt) that was used in the past (Jobling, 1986). In the preceding 30 years, there has been some attempts to compare feed intake, feed conversion and growth of commercial aquaculture species fed moist and dry feed (Poston, 1974; Bromley, 1980; Bromley and Smart, 1981; Chou, 1984; Hughes, 1989). The well known result from these typical feeding-growth studies is that fish compensate diets with increasing water content by increasing feeding rate (Bromley, 1980; Bromley and Smart, 1981) and the performance on a dry matter basis is virtually identical. However, there are few detailed gastro-intestinal tract (GIT) studies on how this dramatic decrease in water fraction and increase in energy density will affect aquaculture fish with a carnivore stomach that during evolution was adapted to function with a heavy load of water from the natural feed items (Buddington et al., 1997). There is limited evidence that the GIT of sea bass, *Dicentrarchus labrax*, fed formulated feeds are less diverse due to the uniform formulated diet (Deplano et al., 1991). There is also weak evidence of interspecific differences in the ability to moisture dry feed in the fish stomach (Hughes and Barrows, 1990) without further measurements of GIT physiology.

Windell et al. (1969) wrote "providing artificial foods containing 90–95 % digestible organic matter may pose special physiological problems, when one considers that trout are adapted to natural foods containing approximately 5–20 % digestible organic matter. A fish stomach may hold its content until a certain degree of liquefaction and digestion is reached, and then the contents are moved into the intestine". Summarizing 'state of the art' scientific literature, there is still limited physiological evidence related to the interactions between drinking and feeding (Usher et al., 1988; Tytler et al., 1990; Ruohonen et al., 1997). We used an individual approach to this physiological contribution by developing an experimental design with minimal stress effects and a calculation procedure that can be used to discriminate between the major stomach water sources during a meal and postprandially in the delay period before gastric emptying begins. Furthermore, we put forward the hypothesis that intake of dry food with a minor water content (10 %) may impose a demand for water to moisture the feed sufficiently to raise the stomach's water content up to the level in natural feed (75 %) as preparation for gastric emptying, whereafter moisturized food is ready to move from the stomach through the pyloric sphincter and towards the pyloric caeca and intestine. This hypothesis is a further development of the work by Ruohonen et al. (1997).

2. MATERIALS AND METHODS

2.1. Husbandry and experimental procedure

Rainbow trout were used for the study because this carnivore salmonid eats fish and invertebrates in the wild (Kristiansen, pers. obs.), but concurrent with selective breeding during the last century's commercial trout farming, the diet has changed gradually towards less and less water. Juvenile trout (20.0 ± 3.0 g (95 % confidence limits)) were obtained from the Dront Mølle freshwater hatchery located in Ørum-Højslev in Northern Jutland, Denmark. The fish were acclimated for 2 weeks in freshwater (16 $mOsm \cdot kg^{-1}$, pH 7.45) at 14–16 °C and fed by hand (BioMar Ecolife 18^{90}; 2-mm pellets, gross energy 22.6 $kJ \cdot g^{-1}$, protein 45 %, fat 22.5 %, nitrogen-free extracts 16 %) about 1 % body weight per day. Feeding response was good and no mortality occurred.

Experiments were designed to discriminate between stomach water sources based on the intake of feed pellets and the drinking tracer, ^{51}Cr-EDTA (ethylene diamine tetra-acetic acid). Trials were conducted in three cylindrical opaque tanks (height 65 cm; diameter 50 cm) with lid and covered with black plastic. This minimized the risk of stressing the fish. Twenty litres oxygenated water was used with ten or fifteen fish in the tanks (with two centrally positioned air stones) and the densities thus ranged between 10–15 $kg \cdot m^{-3}$. The number of fish per group was chosen according to previous experience with interindividual variation in meal size and numbers of non-feeding fish (Kristiansen, 1998, 1999). Water volume in the tanks was a compromise to avoid detrimental effects and ensure a

strong and immediate feeding response, while at the same time maintaining a high tracer concentration, when the volume of this was kept to a minimum. Because feeding and drinking may vary during a 24-h period, experiments were always conducted at the same time of day and experiments were completed within 14 d.

Fish were not fed the day before transfer from acclimation tanks in an attempt to increase hunger. Fish were always put into the experimental tanks 24 h before ^{51}Cr-EDTA was added as a security against stress effects. Temperature was 16–18 °C (mean 17 °C) during the experiments. A couple of feed pellets were added to check the response before adding the tracer. If an immediate response was observed ^{51}Cr-EDTA (Amersham 37.0 Mbq in 10-mL ampoules) was squirted into the centre of the tanks and allowed to mix by aeration for 10 min. Then an initial water sample was collected to estimate the radioactivity of the external medium (R_m). A tracer concentration of 2 kBq·mL^{-1} was used based on experiments of Usher et al. (1988) adjusted for the different exposure times used in the present work.

Feed was offered carefully from a point source without disturbing the fish, until the feeding response ceased after a few minutes. The groups were left undisturbed for 20, 40, 60, 120 and 240 min postprandially, respectively. In one additional short lasting feeding experiment (prandial), pellets were dropped intensively for 2 min and then the fish were immediately killed. The exact weight of the feed ration for each tank was recorded and uneaten pellets were counted at the end. Vomiting was never observed. A second water sample was collected at the end to check if mixing was complete at the time of feeding. Common for prandial and postprandial experiments was the transfer of fish to a rinsing tub for 15 min (the rinsing period was only 2 min in the prandial experiment). This was to ensure transfer of tracer residues from the buccal cavity and the pharynx and to clean the body surface before dissecting. Then a lethal dose of benzocaine (500 ppm final concentration) was added.

The dead fish were immediately stored on ice and postmortem dissections carried out by carefully opening the belly. The gut was ligatured at the oesophagus and rectum and the entire gastro-intestinal system (incl. liver and spleen) was removed. After a short freezing period of 2 h, the samples were dissected into stomach, pyloric caeca region and intestine by cutting at the pyloric sphincter and the intestine posterior to the caeca region. The samples were counted in vials in a Wallac gamma counter for 1 200 s to maximum 10 000 counts and corrected for background radiation. Drinking rate was also estimated with starved fish, but because reported FW drinking rates are low, the whole gastro-intestinal system was counted after ligaturing without freezing and dissecting. The body weight (M) was recorded to transform absolute drinking rates to weight specific units.

After counting, the stomachs of fed fish were opened and the number of eaten pellets recorded. This was possible after short time exposure (2–40 min), but after 1 h some of the pellets close to the pyloric sphincter were partly homogenized and the food bolus was surrounded by mucus. Because the number of pellets eaten is an important parameter in the calculation procedures used to discriminate between water sources, results obtained after 40 min postprandially are not reported in this context. The stomach content was removed carefully without visible mucus residues and weighed (± 0.001 g). After drying at 80 °C until constant weight, the water content (W_{st}) was calculated.

The passive water absorption before capture (W_a) of the dry pellets was estimated three times by keeping 100 pellets in water for 5 s. This duration was based on experience from the point source feedings. Then the mean water fraction in the pellets after absorption was calculated.

2.2. Calculations and statistics

The calculation procedure (*table I*) is based on the idea that stomach water may originate from various exogenous and endogenous sources. The exogenous sources are water bound in the food (W_f), water absorption of the food before it is captured (W_a), water swallowed with the food (W_p) and water drank after feeding (W_d). Although it is reasonable to believe that the endogenous source (W_s) is a mixture of pre-meal secretion and postprandial secretion it was not possible to discriminate between these two fractions.

The absolute ingestion of tracer medium was calculated from the equation:

$$W_{51Cr} = R_g \times R_m^{-1}$$

where R_g is the radioactivity of the gut.

The weight specific drinking rate (D, mL·kg^{-1}·h^{-1}) was calculated from the equation:

$$D = 1\,000\, R_g \times (R_m\, Mt)^{-1}$$

where M is the body weight of the fish (g).

The radioactivity counts were corrected for background radiation (B). In the calculation of postprandial drinking (equation 5 in *table I*), it was necessary to use drinking rates for starved fish. There has not been any convincing evidence that this should be wrong, but for completeness a multiplication factor to convert starvation into postprandial drinking has been included for general application in other studies.

Calculations were based on individual fish and because this type of data has not previously been published, feed intake and water sources is given for each individual. Mean or median values were calculated to compare prandial and postprandial water budget in the stomachs. Normal and non-normal distributed data were tested with a single factor ANOVA and a single factor Kruskal-Wallis analysis of variance

Table I. Calculation procedures used to discriminate between water sources.

Equations	Definitions
1 (total water content of ingested dry food)	$W_{st} = W_f + (W_a + W_p + W_d) + W_s$
2 (total volume of ^{51}Cr-EDTA water)	$W_{51Cr} = W_a + W_p + W_d$
3	$W_f = P_n \times P_{m1} \times P_{f1}$
4	$W_a = (P_n \times P_{m2} \times P_{f2}) - W_f$
5	$W_d = (R_g - B) \times (R_m - B)^{-1} \times t^{-1} \times k \times t_{pp}$
6	$W_p = W_{51Cr} - (W_a + W_d)$
7	$W_s = W_{st} - (W_f + W_a + W_p + W_d)$
W_{st}	water content of food bolus in the stomach (g or mL)
W_f	water bound in the food (g)
W_a	initial water absorption of pellets for 5 s in water column before capture (g)
W_p	water intake during the prandial period (swallowing food) (mL)
W_d	drinking during the postprandial period (mL) ($W_d = 0$ for prandial period)
W_s	secretion from the stomach tissue into the food bolus (mL)
W_{51Cr}	absolute volume of ^{51}Cr-EDTA water in the stomach (mL)
P_n	number of pellets eaten
P_{m1}	weight of dry pellet (0.0222 g)
P_{f1}	water fraction bound in pellets (0.094)
P_{m2}	weight of dry pellet after pre-meal absorption (0.0268 g)
P_{f2}	water fraction in pellets after pre-meal absorption (0.249)
R_g	radioactivity in the gut of starved fish (cpm)
B	background radioactivity (cpm)
R_m	radioactivity of 1 mL of the external medium (cpm)
t	duration of the drinking period for starved fish (h)
k	multiplication factor for converting starvation rate into postprandial rate (1.0)
t_{pp}	duration of the post prandial drinking period (h)

on ranks, respectively. If there were significant differences, Tukey's and Dunn's pairwise multiple comparison procedures were used to isolate groups that differed for normal and non-normal distributions, respectively. The statistical software package Sigma Stat 2.0 (Jandel Scientific) and biostastistical analysis (Zar, 1984) was used.

3. RESULTS

Feeding response was good and in most feeding fish, the pellet number was high enough to ensure accurate calculations of the water sources (*tables II*, *III*). As expected the water fraction increased with postprandial time, but water fractions were fairly similar within the groups. As expected from the calculation procedure, water bound in the food and water absorbed in the food increased in proportion to the number of pellets eaten. Water drunk after feeding was the smallest contribution and this was calculated based on a drinking rate estimate of 1.9 ± 0.6 mL·kg^{-1}·h^{-1} ($\pm$ 95 % C.L.). In contrast there was high inter-individual variation in water swallowed with the food reflecting differences in feeding behaviour. The range was between 4.0 and 19.3 μL per pellet, when the fish that only ate three pellets was excluded. The estimated mean values were 5.5 ± 1.2 and 9.1 ± 2.2 ($\pm$ 95 % C.L.) and median value 10.1 (95 % confidence interval from 7.5 to 11.3) μL per pellet for the prandial, 20-min postprandial and 40-min postprandial groups, respectively. Mean number of pellets eaten were not significantly different (single factor ANOVA, $P = 0.245$), but the prandial intake per pellet was significantly lower for the prandial group than the postprandial groups (single factor Kruskal-Wallis, $P = 0.006$ followed by Dunn's pairwise comparison procedure, $P < 0.05$). There was a high inter-individual variation in stomach secretion in the postprandial groups, but not in the prandial group. The range was between 20.3 and 48.2 % of the water content in stomachs, when the fish that only ate three pellets was excluded. The estimated mean values were 44.2 ± 2.7, 33.6 ± 5.8 and 38.4 ± 5.0 ($\pm$ 95 % C.L.) for the prandial, 20-min postprandial and 40-min postprandial groups, respectively. There was a significant difference between the prandial and the 20-min postprandial groups (single factor ANOVA, $P = 0.012$ followed by Tukey's pairwise comparison procedure, $P < 0.05$).

An overview of the percentage distributions of water sources in stomachs is given in *figure 1*. The temporal variation of water in the dry feed depicts the water absorption in the pre-meal period before feed intake took place (*figure 2*). Early in the delay period the rate of increase was fast followed by a slowly increasing phase until gastric emptying began 1 h postprandially. The transition between delay period and gastric emptying period was directly seen, because feed pellets began to disintegrate and move through the pyloric sphincter after 1 h.

Table II. Feed intake and prandial water sources in eight juvenile rainbow trout fed extruded dry feed.

Feed intake		Prandial water sources (mL)				
Stomach contents (g wet wt)	Pellet number (P_n)	Stomach contents (W_{st})	Bound in food (W_f)	Absorbed in food (W_a)	Swallowed with food (W_p)	Stomach secretion (W_s)
0.158	3	0.0101 (63.9)	0.006	0.014	0.003 (1.0)	0.078 (77.2)
0.501	11	0.283 (56.5)	0.023	0.050	0.090 (8.2)	0.119 (42.0)
1.146	26	0.566 (49.4)	0.054	0.119	0.120 (4.6)	0.273 (48.2)
1.123	27	0.576 (51.3)	0.056	0.124	0.160 (5.9)	0.236 (41.0)
1.233	29	0.655 (53.1)	0.061	0.133	0.154 (5.3)	0.307 (46.9)
1.272	31	0.631 (49.6)	0.065	0.142	0.156 (5.0)	0.268 (42.5)
1.429	34	0.718 (50.2)	0.071	0.156	0.187 (5.5)	0.304 (42.3)
1.572	38	0.754 (48.0)	0.079	0.174	0.151 (4.0)	0.350 (46.4)

W_{st} ($W_{st} \times$ wet $wt^{-1} \times 100$ %); W_p ($W_p \times P_n^{-1} \times 1\,000$ µL); W_s ($W_s \times W_{st}^{-1} \times 100$ %).

Table III. Feed intake and postprandial water sources in ten juvenile rainbow trout fed extruded dry feed 20 min and 40 min postprandially.

Feed intake		Postprandial water sources (mL)					
Stomach contents (g wet wt)	Pellet number (P_n)	Stomach contents (W_{st})	Bound in food (W_f)	Absorbed in food (W_a)	Swallowed with food (W_p)	Water drank after feeding (W_d)	Stomach secretion (W_s)
				Water sources 20 min postprandially (mL)			
1.228	27	0.653 (53.2)	0.056	0.124	0.191 (7.1)	0.012	0.270 (41.3)
1.354	28	0.762 (56.3)	0.058	0.128	0.343 (12.3)	0.012	0.220 (28.9)
1.244	30	0.641 (51.5)	0.063	0.138	0.170 (5.7)	0.012	0.259 (40.0)
1.459	31	0.784 (53.7)	0.065	0.142	0.292 (9.4)	0.012	0.273 (34.8)
1.420	31	0.749 (52.7)	0.065	0.142	0.212 (6.8)	0.012	0.319 (42.6)
1.187	31	0.614 (51.7)	0.065	0.142	0.135 (4.4)	0.012	0.260 (42.3)
1.464	32	0.835 (57.0)	0.067	0.147	0.370 (11.6)	0.012	0.239 (28.6)
1.681	39	0.913 (54.3)	0.081	0.179	0.330 (8.5)	0.012	0.311 (34.1)
2.093	45	1.167 (55.8)	0.094	0.206	0.589 (13.1)	0.012	0.266 (22.8)
2.008	47	1.122 (55.9)	0.098	0.216	0.568 (12.1)	0.012	0.228 (20.3)
				Water sources 40 min postprandially (mL)			
1.201	26	0.683 (56.9)	0.054	0.119	0.237 (9.1)	0.024	0.249 (36.5)
1.323	26	0.721 (54.5)	0.054	0.119	0.196 (7.5)	0.024	0.327 (45.4)
1.583	28	0.898 (56.7)	0.058	0.128	0.290 (10.4)	0.024	0.398 (44.3)
1.443	30	0.798 (55.3)	0.063	0.138	0.269 (9.0)	0.024	0.305 (38.2)
1.353	31	0.747 (55.2)	0.065	0.142	0.208 (6.7)	0.024	0.308 (41.2)
1.732	31	0.980 (56.6)	0.065	0.142	0.349 (11.3)	0.024	0.400 (40.8)
1.606	32	0.934 (58.2)	0.067	0.147	0.324 (10.1)	0.024	0.372 (39.8)
1.983	34	1.185 (59.8)	0.071	0.156	0.655 (19.3)	0.024	0.279 (23.5)
2.238	46	1.243 (55.5)	0.096	0.211	0.468 (10.2)	0.024	0.444 (35.7)

4. DISCUSSION

Because the experiments were undertaken in small tanks, it is reasonable to assume that stress could be an important factor and affect feeding and drinking. Stress effects were minimized by excluding visual stimuli from the surroundings, and by avoiding subsampling procedures from the tracer tanks as used by Usher et al. (1988) and Tytler et al. (1990), and by always acclimating the fish in the tracer tanks at least 24 h before the tracer was added. A lack of feeding response is critical, but the hunger level was always high and the feed delivery time could be reduced to a few minutes.

The proposed calculation procedure may be a valuable working tool in quantifications of exogenous and endogenous water sources in the stomach of feeding fish or monogastric animals. Rainbow trout are perfect animal models, because of the J-shaped stomach with enlarged lumen and muscular closures at each end – the cardiac sphincter anteriorly and the pyloric sphincter (pylorus) posteriorly (Smith, 1989). The present animal model could be used in further comparative tests of industrial pellet manufacturing procedures (Hilton et al., 1981), optimal water content (Jobling, 1986) and water binding properties of binders in fish feeds (Storebakken, 1985). The maximum information is obtained when the number of pellets eaten and the

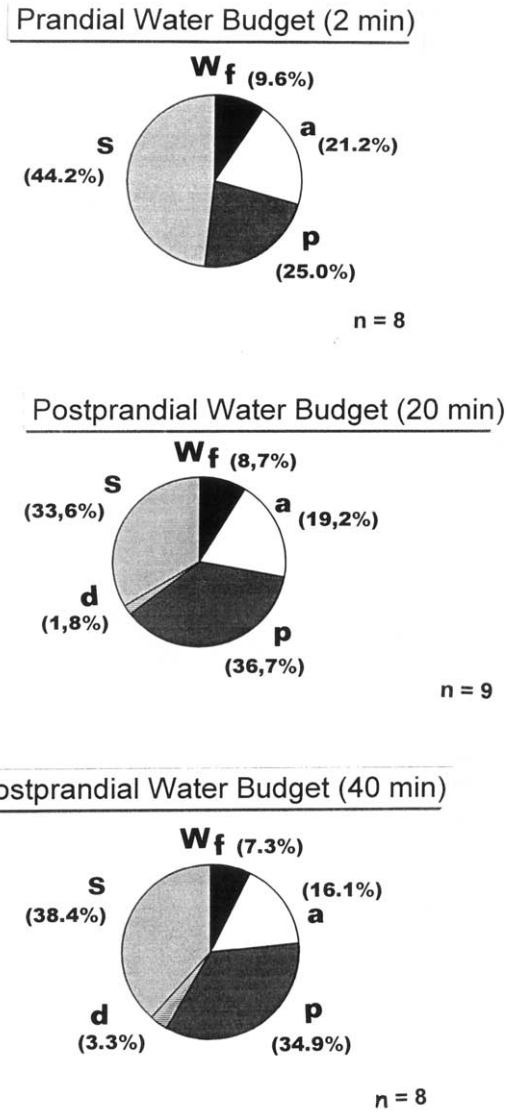

Figure 1. Prandial and postprandial water budget in juvenile rainbow trout fed extruded dry feed: W_f water bound in food, W_a water absortion of food, W_p water swallowed with food, W_d water drunk after feeding, W_s stomach secretion.

water content of the pellets is determined by killing each individual (present paper). Number of pellets eaten could have been estimated repetitively in vivo by radiography with glass beads as markers (Kristiansen, 1998, 1999) or with radioisotopes mixed into the feed (Storebakken et al., 1981). However, this would preclude a detailed discrimination between the water sources unless the total water content in the stomachs could be estimated separately. Several methods have been useful to collect stomach contents in vivo (Damsgård and Kristiansen, 2001) and it should be possible to remove the pellets before they are disintegrated and estimate the water content.

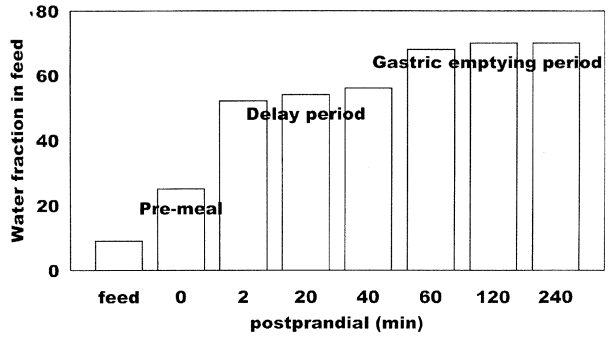

Figure 2. Temporal variation of water fraction in feed from pre-meal to postprandially in juvenile rainbow trout.

The initial water absorption of the pellets (W_a) may vary depending on the industrial pellet manufacturing procedure. Hilton et al. (1981) found that water absorption properties differed between extruded pellets (EP) and standard pressure steam pellets. They showed that water content of EP increased from 7.5 to 32 % after 10 s water exposure, which is very similar to the present results (9.4 to 24.9 %) after 5 s exposure. Besides the pellet structure and composition, fish feeding behaviour can affect W_a. This is only important if the pellets are left uneaten at the bottom of the tracer tank for more than 1 min, because Hilton et al. (1981) reported that water content of EP increased slightly from 32 to 35.4 % after 1 min exposure and then further to 43.5 % after 3 min. If studies are performed with natural food items, W_a will approximate zero and this will increase the precision of the prandial water intake estimate (W_p). W_p gave a high inter-individual variation in the present study, but when expressed as water intake per pellet the results stabilized around 5.5 μL per pellet for the prandial intake. The reason for larger differences in the other trials is possibly related to differences in feeding behaviour, which in turn may be dependent on social interactions in the tank.

There are only few published data that discriminates between water sources or mechanisms for obtaining water. Tytler et al. (1990) presented a diagram for 31-d post-larval rainbow trout that is interpreted here with approximate figures. One curve for unfed fish represents starvation drinking (0.55 $mL \cdot kg^{-1} \cdot h^{-1}$), whereas the other curve for fed fish represents prandial intake (W_p = 2.34 $mL \cdot kg^{-1} \cdot h^{-1}$) followed by postprandial drinking (W_d = 0.33 $mL \cdot kg^{-1} \cdot h^{-1}$). This information corroborates the present study that a major part of the water is ingested with the food, but W_d is rather low compared with 1.9 $mL \cdot kg^{-1} \cdot h^{-1}$ in the present study. Since the larvae were directly transferred to the tracer bath followed by subsampling every hour the low W_d may be an effect of stress. Regarding W_d, there are contradictions in the literature as to whether drinking increases (Usher et al., 1988) or decreases (Tytler et al., 1990) after a meal (compared with starvation).

Hassan (1985) found that drinking in juvenile turbot (3–7 g) in full strength Sw increased from a low value 3 h after feeding to a significantly ($P < 0.001$) higher value 9 h postprandially with a further significant ($P < 0.001$) increase between 9 h and 3 d. Because of this uncertainty, it was assumed in the present calculation procedure that fish drank at the same rate during starvation and postprandially. W_d could be estimated more accurately, but this would require additional studies of unstressed fish fed in the tracer tank and then exposed to the tracer after the food has been swallowed.

There has been much dispute as to whether fish can secrete sufficient gastric juice to digest a full meal of pellets (Windell et al., 1969; Hughes and Barrows, 1990). Norris et al. (1973) studied rates of gastric acid and pepsin secretion in response to force-feeding glass beads, together with measurements of hydrolytic activity of secreted pepsin in bluegill sunfish, *Lepomis macrochirus*. With two glass-beads making up 1.24 % of the body weight (10 g) the authors stated that the secretory capability of the stomach was exhausted, which implies that feed assimilation and feed conversion efficiency would be low, when relative feed intake exceeds ca. 1 % bw. Smit (1967) approached the problem more directly by estimating secretory rate using force-fed pieces of sponge in the brown bullhead, *Ictalurus nebulosus* (Le Sueur). The rate for 390-g fish at 20 °C decreased from 3.7 $mL \cdot h^{-1}$ after the meal to 2.4 after 2 h and decreased further to 1.7 after 4.5 h. Since the composition of the gastric juice (acid, buffer and pepsin) was found to be influenced by the secretory rate (SR), the author assumed that the digestive power increased with increasing SR due to the higher acidifying power of the gastric juice. The present study gave convincing evidence that stomach secretion is of paramount importance in the prandial period and in the delay period. There was no indication that the secretory capacity was exhausted since food started to disintegrate in all stomachs 1 h postprandially. In the present study, it was not possible to calculate accurate SRs in relation to gastro-intestinal parameters such as gastric emptying rate, digestibility and feed conversion efficiency.

The secretion estimate depends upon the accuracy of five model parameters. However, the estimate is probably more accurate than the others during the first hour, when postprandial drinking and gastric emptying are negligible, because there is no need to discriminate between the ^{51}Cr-EDTA sources ($W_a + W_p + W_d$). One problem may arise from the mucus layer surrounding the food bolus, which may stick to the stomach wall. This layer is also present in starved fish (pers. obs.) and thus may not be part of the moisturizing of the food (maybe a reserve for the subsequent meal). Prandially, it was observed that a high fraction of the stomach water originated from secretion. This is possibly a pre-meal effect (Boujard and Leatherland, 1992), where secretion begins in a regular rhythm before food is offered. Other studies have associated the stomach secretion with Mg^{2+} concentration changes (Hickman, 1968; Parmelee and Renfro, 1983); the latter study estimated that endogenous secretion and passive osmotic water movement contributed 30 % of the water in the stomach for starved Sw winter flounder, *Pseudopleuronectes americanus*. This sounds very high, but the water may serve to dilute the hypertonic seawater, although the oesophagus effectively reduced the NaCl content substantially.

5. CONCLUSION

The overall conclusion is that a number of marine aquaculture species such as turbot, *Scophthalmus maximus* (L.) (Bromley, 1980) and sea bass, *Lates calcarifer* (Chou, 1984) and anadromic species such as brown trout, *Salmo trutta* (Poston, 1974), rainbow trout (Bromley and Smart, 1981) and Atlantic salmon, *Salmo salar* (Hughes, 1989) can obtain their nutritional requirements from dry feed. It is still a matter of discussion if formulated feeds are used as effectively as natural feeds (Jobling, 1986). More studies on the digestibility and feed conversion efficiency in wild fish is needed to clarify this with the limitation that confident comparative estimates are very difficult to obtain. The importance of the diet has been investigated by Ruohonen et al. (1997). W_d for rainbow trout (1–1.5 kg) was very low (0.07 $mL \cdot kg^{-1} \cdot h^{-1}$), when the fish were fed a natural diet (herring, 77.3 % water) compared with 0.44, when the diet was artificial (5.8 % water). Moreover, there was indirect evidence that stomach secretion contributed 42 % of the water in the stomach 3–4 h after a dry feed meal, but stomach secretion was almost absent after a herring meal (Ruohonen et al., 1997). Since dry feed induced drinking as well as secretion, the delay period of 1 h in the present study is presumably a necessary moisturing period, as suggested by Windell et al. (1969). In that period the moisture level increased to 56 %. Interestingly this is the same level as Hilton et al. (1981) found in juvenile rainbow trout 30 min postprandially. Our study thus supports the hypothesis of Ruohonen et al. (1997) that dry food may impose a demand for water and the ability of the fish to meet this demand forms one of the constraints, which might be offset by drinking. Comparative studies with fish fed artificial and natural diets may clarify this.

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