Laboratory investigation of daily food intake and gut evacuation in larvae of African catfish *Clarias gariepinus* under different feeding conditions

Armando García-Ortega · Johan Verreth · Karen Vermis · Hans J. Nelis · Patrick Sorgeloos · Martin Verstegen

Abstract Temporary accumulation of ascorbic acid 2-sulfate (AAS) was measured to estimate food intake and gut evacuation in larvae of African catfish. Fish larvae were fed decapsulated cysts of *Artemia* containing AAS. In a first experiment it was found that no biosynthesis of AAS occurs in the larvae of this species. In a second experiment, the gut contents of the fish larvae fed were calculated as they changed during development. In a third experiment, the gut evacuation rate of fish larvae was determined during continuous and discontinuous feeding regimes in the first five days after the start of exogenous feeding. Food consumption by catfish larvae increased from 46.5% of their body dry weight (BDW) on day 1 after the start of exogenous feeding to 53.8% BDW on day 3. Thereafter, food consumption decreased to 27.8% BDW on day 5. A similar pattern was observed for gut evacuation, which increased during the first days of exogenous feeding and decreased as fish growth continued. The rate of gut evacuation in a continuous feeding regime was significantly higher ($P < 0.05$) than that under discontinuous feeding. On day 1 post-hatch and 7 h after first food ingestion the fish larvae evacuated 87% of the food in continuous

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feeding compared with 43% under discontinuous feeding. It was found that gut emptying differs during larval development. Under continuous feeding, on days 1 and 3 post-hatch and 11 h after the first meal 90% of the food was evacuated compared with 71% evacuated on day 5. The advantages and limitations of the AAS method for estimation of food consumption by fish larvae are discussed.

Keywords Food consumption · Feed intake · Gut evacuation · Fish larvae · Gut emptying

Abbreviations

AAS	Ascorbic acid 2-sulfate
AAS_s	Ascorbic acid 2-sulfate content of fish sample (μg)
AAS_c	Ascorbic acid 2-sulfate content of one decapsulated cyst of *Artemia*
BDW	Body dry weight
DW	Dry weight
GC	Gut content
GC_{nf}	Gut content (estimated as number of *Artemia* cysts per fish)
GC_{dwf}	Gut content (estimated as number of *Artemia* cysts per fish dry weight)
GE	Gut evacuation
FC	Food consumption

Introduction

Estimation of food consumption by fish is difficult because of the lack of accurate methods of evaluation of both ingestion and evacuation of food. Methods of quantifying food consumption are useful in nutritional studies with fish, for example they assist in estimation of their quantitative nutrient requirements. During the larval phase, fish have high growth rates and their digestive processes occur in a short time. Thus, estimation of daily food consumption is useful to elucidate the role of certain compounds in physiological processes, for example to differentiate the contribution of digestive enzymes in the food from the total enzyme activity during digestion of food in larval fish. In studies with fish larvae and juveniles, several methods of estimating food consumption have been tested with either live food or artificial diets. The methodology includes the use of radio-isotopes (Sorokin and Panov 1966; Kolkovski et al. 1993) and X-radiography with metallic markers (Talbot and Higgins 1983; Hossain et al. 1998), direct counting of food particles in experimental tanks after feeding (Fushimi 1983; Keckeis and Schiemer 1992) or in the fish digestive tract (Pedersen 1984), gravimetric (Kamler et al. 1986) and fluorescence (Morris et al. 1990; Kelly et al. 2000) techniques, and the use of ink labeling (Planas and Cunha 1999) and double markers (Teshima et al. 2000) in live food and microparticulate diets. The type of diet used in these investigations, e.g. zooplankton organisms or inert artificial diets, can affect the accuracy of estimates of food consumption. For example, zooplankton organisms are easily broken into pieces, which complicates individual counts. On the other hand, some artificial diets may lack natural attractants that are important for food acceptability, or the amount of marker substance may vary among individual food particles.

In this study, food intake and gut evacuation by fish larvae were estimated by measurement of the accumulation of ascorbic acid 2-sulfate (AAS) in the digestive tract of

larval fish when fed an AAS-containing diet. AAS is a stable derivative of ascorbic acid which occurs naturally in the encysted embryos of the brine shrimp *Artemia* (Mead and Finamore 1969). AAS is converted into ascorbic acid during embryonic development a few hours prior to hatching of the nauplii (Golub and Finamore 1972). Liberated ascorbate possibly originating from AAS in developing embryos of *Artemia* has been initially detected 16 h after cyst hydration (Dabrowski 1991). The larval and juvenile stages of some teleost fish are unable to utilize AAS as a precursor to the synthesis of ascorbic acid, and no natural occurrence of AAS has been detected in such fish (Dabrowski et al. 1990). Decapsulated cysts of *Artemia* have been successfully used as food for the larvae of several fish species (Drouin et al. 1986; Verreth et al. 1987; Vanhaecke et al. 1990). Hence AAS in *Artemia* decapsulated cysts can presumably be used as a natural marker to estimate food consumption by fish larvae. Use of decapsulated *Artemia* cysts is advantageous because of their constant particle size and acceptance by larvae of several fish species. The cysts also have a constant biochemical composition and a membrane which prevents leaching of the marker substance or other nutrients, thereby minimizing any variation in the composition of each individual food particle. In this investigation, three feeding experiments using the ascorbic acid sulfate within decapsulated cysts of *Artemia* as a natural marker were carried out to evaluate food intake and gut evacuation by fish larvae under different feeding conditions.

Materials and methods

Artemia cysts and fish larvae rearing

The same batch of cysts of the brine shrimp *Artemia* (EG type; INVE, Belgium) originating from the Great Salt Lake, USA, was used in all experiments. One day before the start of an experiment, cysts were submerged in aerated fresh water for 1 h to achieve full hydration. Once hydrated, the shells were chemically removed (decapsulation) with a chlorine solution, by the method of Sorgeloos et al. (1986). After decapsulation, the cysts were dehydrated again in saturated brine (>330 g NaCl/l) for 24 h and then stored in fresh brine at 4°C until they were fed to the fish larvae. Before being offered to the fish larvae, the cysts were immersed in fresh water for 5 min to rinse them of brine and allow hydration. The average particle size of the decapsulated cysts was 236 ($\pm$16) µm as measured with an optical ruler attached to a compound microscope.

Larvae of the African catfish *Clarias gariepinus* were obtained by induced reproduction of broodstock maintained in a hatchery. Fertilized eggs were placed in plate incubators with running water at 30°C, and hatching occurred approximately 23 h thereafter. About two days after hatching, when absorption of the yolk sac was almost complete, the larvae were counted and transferred to the experimental freshwater recirculation system consisting of rectangular (25 cm × 35 cm × 25 cm) aquaria filled up to 17 l. Larvae were reared at a constant temperature of 28°C and exposed to a light–darkness regime of 12 h:12 h. The same procedure was used for production and culture of fish larvae in all experiments. Mean weight was determined for 5–20 larvae each day and used to calculate the daily amount of food fed accounting for the predicted maximum growth (Verreth and Den Bieman 1987). When fish were fed with decapsulated cysts, the total amount of food per day was divided into five equal portions. Feeding with *Artemia* nauplii or decapsulated cysts started at the beginning of exogenous feeding (two days after hatching) and was repeated every 4 h, from 0800 to 2400 hours.

Experimental design and procedures

Accumulation of AAS in the guts of fish larvae as a result of feeding with *Artemia* decapsulated cysts was evaluated in Experiment 1. Data were obtained on the relationship between AAS content and number of cysts in the guts of the fish larva. There were two feeding treatments. In the first treatment only decapsulated cysts were fed and the number of cysts present in the digestive tract was estimated as a function of the AAS content of the fish. In the second treatment a dry artificial diet for catfish (9011; Provimi, Zwolle, The Netherlands; particle size 300–500 μm) containing no AAS was fed to the larvae to test for biosynthesis of AAS in the fish larvae. Six aquaria (three per treatment) with 1,000 larvae in each were used. Fish samples were taken on day 1 after the start of exogenous feeding.

Experiment 2 was conducted to verify whether the ingested AAS was retained or broken down by the fish. For this reason, ingestion of food as measured by the AAS content of fish larvae was followed in time. In this way the experiment would also provide indications on how consumption changed during development while the fish larvae were increasing in size. Catfish larvae were kept at an initial density of 1,200 larvae per aquarium. Four diet treatments were tested. In the first treatment catfish larvae were fed exclusively decapsulated *Artemia* cysts to measure the AAS content of fish derived from cysts (gut contents). In the second treatment combined feeding with live instar I nauplii of *Artemia* and decapsulated cysts was carried out to estimate the retention of AAS by the fish. In this treatment live nauplii were offered to the fish larvae on non-sampling days and decapsulated cysts were fed exclusively during the sampling days. In the third treatment (control 1) only live *Artemia* nauplii were fed to the fish larvae during the entire experiment. Comparison between treatments 1 and 3 should give an indication of how daily food consumption changed during development. To confirm that biosynthesis of AAS by fish larvae did not occur, even when other sources of ascorbic acid are provided, a fourth diet treatment was included using a dry artificial diet (the same as in Experiment 1) with a source of ascorbic acid different from AAS. Fish samples were taken on days 1, 3, and 5 after the start of exogenous feeding.

Besides AAS accumulation, determination of AAS evacuation rate in the fish is necessary to obtain an accurate estimate of food consumption by fish larvae ingesting decapsulated cysts of *Artemia*. Therefore, the gut evacuation rate of catfish larvae during development was estimated for two different feeding treatments in Experiment 3. Gut evacuation was measured by feeding the larvae with AAS-containing cysts only on the sampling days and exclusively for the first meal (at 0800 hours). By measuring the subsequent decrease in AAS in fish samples we can obtain an indication of the evacuation rate. Because the gut evacuation rate might be affected by meal frequency, two feeding strategies were applied during the sampling days. In the first treatment a continuous feeding regime was applied. After the first meal with cysts, subsequent feeding was with live instar I nauplii of *Artemia* which contained no AAS. In the second treatment, a discontinuous feeding strategy was followed. After the first feeding with cysts, no additional food was offered during the rest of the day. The amount of food present in fish 3 h after ingestion of the first meal was regarded as the total amount and subsequent presence of food was subtracted from this total. As control diet *Artemia* nauplii were offered to the fish during the entire experiment. In all three diet treatments the fish were fed with nauplii in between sampling days. Fish larvae samples were taken on days 1, 3, and 5 after the start of exogenous feeding.

In all experiments samples of fish were taken 15 min before the first daily meal and 3 h after each meal during the sampling days for all diet treatments. However, in the second

treatment in Experiment 1 and for the control diets in Experiments 2 and 3, fish samples were taken only 3 h after the fourth daily meal to take into account accumulation of AAS during the day. Additionally, in the discontinuous feeding treatment in Experiment 3 samples were taken before the daily first meal and 3, 7, 11, and 15 h after that first meal. Fish larvae samples were rinsed with tap water, excess water was removed and the weight and number of fish in each sample were determined. The samples were shock frozen in liquid nitrogen and stored at −80°C until AAS determination. For all experiments, the number of fish larvae in each sample for AAS analysis ranged from 80 to 120 on day 1, 40 to 50 on day 3, and 15 to 20 on day 5, depending on fish weight. The fish weight during the sampling days was determined before first feeding and after every feeding, with the exception of the last feeding. The dry matter (±10 μg) of *Artemia* cysts, artificial diet, and fish during the sampling days in all experiments were determined by drying the samples in an oven at 60°C for 24 h. Samples of decapsulated cysts of *Artemia*, hydrated for 1 and 3 h after brine storage, and instar I nauplii were also prepared for determination of AAS content.

Extracts of *Artemia* cysts, catfish larvae, and artificial microdiet were prepared as described by Nelis et al. (1994). Crude extracts were obtained from approximately 100 mg dry weight (DW) of *Artemia* decapsulated cysts homogenized in 2 ml acetate buffer (60 mmol, pH 5.0) using an all-glass Potter Elvehjem tube, and from approximately 500 mg wet weight catfish larvae. Crude extracts (6 ml) were purified by anion-exchange chromatography after transfer to a Bond Elut DEAE cartridge (Varian, USA) preconditioned with methanol (1 ml), water (1 ml), and acetate buffer (1 ml; pH 5.0) and then washed twice with 1 ml water. Reversed-phase, ion-pair, high-performance liquid chromatography (HPLC) was used for quantification of AAS. The HPLC system consisted of an LKB (Sweden) 2249 pump, an N-60 valve injector fitted with a 100-μl loop (Valco, Houston, TX, USA), a Philips Pye–Unicam PU 4025 UV detector (Pye–Unicam, UK) and a Merck–Hitachi D-7500 integrator (Hitachi, Japan). A 3-μm Adsorbosphere HS C_{18} column (150 mm × 4.6 mm; Alltech Associates, USA) was used with a mixture of acetonitrile and ammonium phosphate buffer (60 mmol pH 5.0) containing 32 mmol TBAP (1.5:8.5 $v\ v^{-1}$) as mobile phase. The flow rate was 1 ml min^{-1} and detection was performed at 254 nm.

Estimation of food consumption

The number of cysts present in the guts (GC) of catfish larvae was estimated in both absolute (GC_{nf}) and relative (GC_{dwf}) terms:

$$GC_{nf} = (AAS_s\ AAS_c^{-1})n^{-1} \tag{1}$$

$$GC_{dwf} = \left[AAS_s (DW_s\ BDW^{-1})^{-1} \right] AAS_c^{-1} \tag{2}$$

where GC_{nf} is the gut content (number of cysts per fish), AAS_s is the AAS content of the fish sample (μg), AAS_c is the AAS content of one decapsulated cyst of *Artemia* (μg), and n is the number of fish in the sample. GC_{dwf} is the gut content (number of cysts per fish DW) in the sample, DW is the dry weight of the sample (mg), and BDW is the individual body dry weight of the fish in the sample (mg). The AAS content of one cyst was calculated by dividing the mean amount of AAS in cyst samples by the number of individuals (292,398) in 1 g (DW) of decapsulated cysts. The gut evacuation (GE) rate was calculated from data from Experiment 3 as:

$$GE_{tx} = (GC_{nf})_{tx} \times 100(GC_{nf})_{ti}^{-1} \tag{3}$$

where GE_{tx} is the gut evacuation (% of initial number of cysts) at time X (7, 11, or 15 h after food deprivation), $(GC_{nf})_{tx}$ is the gut content 7, 11, or 15 h after deprivation of food with AAS, and $(GC_{nf})_{ti}$ is the initial gut content 3 h after first feeding (100% of ingested food at first feeding). Food consumption (FC) was calculated by subtracting GE from the GC and is expressed as a percentage of fish body weight. The gut contents from Experiment 2 and the gut evacuation rates from Experiment 3 were used to obtain the food consumption and gut evacuation per feeding time and per day.

Statistical analysis

Three replicates per diet treatment were used to determine fish weight, AAS content, and dry matter. Three replicates were also used for determination of dry matter and AAS content of decapsulated cysts and nauplii of *Artemia*. All data were tested for normal distribution and homogeneity of variance before analysis of variance was performed. Data on fish weight and gut content from Experiments 2 and 3 were tested separately for the effect of feeding time and diet treatment using a two-way ANOVA. When effects were found ($P < 0.05$), the Duncan multiple-range test was used to test for differences between feeding times, and least squares means were calculated to test for differences between diet treatment within feeding groups. The statistical analysis was performed separately for each of the sampling days. A *t*-test was performed to determine whether there were significant differences ($P < 0.05$) between GC calculated by use of Eqs. (1) and (2), and between the AAS content of cysts hydrated for 1 and 3 h. The required number of replicates per treatment and the power of ANOVA for estimation of gut content were tested by evaluation of the quantity ϕ according to Sokal and Rohlf (1995). The analysis was performed to test the ANOVA for a power of at least 80% at $\alpha = 0.05$ and with df (v) among treatments of $v_1 = a - 1$ and $v_2 = a(n - 1)$, where $a =$ number of treatments and $n =$ number of replicates.

Results

Fish growth

Catfish larvae displayed high feeding activity every time the food was offered. Visual verification confirmed that approximately 1 h after feeding all the food particles were consumed and the fish larvae were resting on the bottom of the aquarium until 1 h before the next feeding when they started to swim slowly. The transparency of the larvae enabled visual verification that the decapsulated cysts and nauplii were ingested. The formulated diet was not well accepted and fish growth was lower than when cysts and nauplii were fed (Table 1). Catfish larvae fed with cysts and live nauplii showed a high growth rate during the first days after the start of exogenous feeding (days 1–5), gaining approximately 40% of their initial daily weight within 12 h of feeding. The percentage dry matter of the larvae increased with fish age (Table 1). On all sampling days growth was similar for fish fed with cysts and for fish fed cysts plus nauplii (Experiment 2: first and second treatments).

Table 1 Mean (± SE) individual wet weight and dry matter content of African catfish larvae fed decapsulated cysts and nauplii of *Artemia* and an artificial diet

	Weight (mg)			
	Experiment 1	Experiment 2		
	Day 1	Day 1	Day 3	Day 5
Before first feeding (F0)				
Cysts		3.8 ± 0.1	9.8 ± 0.5	22.7 ± 0.5
Nauplii + cysts		3.6 ± 0.1	8.9 ± 1.0	24.0 ± 1.6
First feeding (F1)				
Cysts	3.8 ± 0.0	4.1 ± 0.1	10.8 ± 0.6	26.1 ± 0.7
Nauplii + cysts		3.9 ± 0.2	10.4 ± 0.8	28.0 ± 2.6
Second feeding (F2)				
Cysts	4.3 ± 0.1	4.6 ± 0.3	12.3 ± 0.8	27.5 ± 1.6
Nauplii + cysts		4.4 ± 0.3	11.4 ± 1.2	29.8 ± 1.1
Third feeding (F3)				
Cysts	4.6 ± 0.1	4.8 ± 0.4	13.5 ± 0.4	29.5 ± 2.5
Nauplii + cysts		4.5 ± 0.0	12.2 ± 1.9	31.2 ± 1.7
Fourth feeding (F4)				
Cysts	5.5 ± 0.1	5.3 ± 0.6	15.0 ± 0.8	36.0 ± 2.2
Nauplii + cysts		5.0 ± 0.2	14.3 ± 2.3	34.2 ± 3.1
Nauplii		4.8 ± 0.3	8.8 ± 0.2	22.1 ± 1.4
Artificial diet	3.8 ± 0.1	4.8 ± 0.0	6.9 ± 0.2	11.6 ± 0.7
Dry matter (%)				
Cysts	11.4 ± 0.5	11.9 ± 0.2	11.8 ± 0.1	14.6 ± 0.1
Nauplii + cysts		12.0 ± 0.1	12.5 ± 0.5	15.3 ± 0.3
Nauplii		11.0 ± 0.2	12.7 ± 0.1	15.1 ± 0.1
Artificial diet	11.0 ± 0.2	12.9 ± 0.4	13.0 ± 0.1	12.4 ± 0.1

AAS content in cysts and artificial diet and its retention in fish larvae

The mean AAS content of cyst samples after 1 h hydration was about 380 µg g^{-1} DW in Experiment 1, 470 µg g^{-1} DW in Experiment 2, and 314 µg g^{-1} DW in Experiment 3. The AAS content of one cyst was calculated to be 0.0013, 0.0016, and 0.0011 µg for Experiments 1, 2, and 3, respectively. The AAS content detected in the artificial diet was 2.7 µg g^{-1} DW.

Retention of AAS by the fish at the end of each sampling day for all experiments is shown in Table 2. With regard to the control diets in Experiment 2, the fish fed exclusively *Artemia* nauplii contained an average of 33.5 µg AAS g^{-1} DW for the three sampled days. This amount represents a small part of what was measured for other fish samples in the first and second diet treatments, and is probably because of the presence of some cysts in the nauplii solution during feeding, which were difficult to remove completely. The difference between the AAS content of *Artemia* cysts among the experiments partially explains the different AAS content of fish with the same feeding treatment (cysts) in Experiments 1 and 2 (Table 2). Moreover, the fish larvae from this diet treatment in Experiment 1 had a higher weight than those in Experiment 2 for the same day. Greater fish growth results in greater

Table 2 Ascorbic acid sulfate content of catfish samples taken 15 h after the first daily meal

Diet	Ascorbic acid sulfate (μg g^{-1} DW)		
	Day 1	Day 3	Day 5
Experiment 1			
Cysts	243.6		
Artificial diet	1.3		
Experiment 2			
Cysts (treatment 1)	192.4	257.6	189.8
Nauplii + cysts (treatment 2)	249.6	287.2	183.0
Nauplii (control diet)	39.4	38.3	22.8
Artificial diet (control diet)	0	0.3	0.5
Experiment 3			
Continuous feeding	12.0	13.5	24.6
Discontinuous feeding	17.8	18.5	31.2
Nauplii	13.7	11.8	13.8

accumulation of AAS. The small amount of cysts in the fish samples taken before first feeding in Experiment 2 indicate that the AAS is not retained for a long time in the fish. The AAS acquired from cysts was almost completely evacuated after one day of nauplii feeding as observed in the fish from the second diet treatment (Table 3). This observation provides further evidence that fish would not incorporate AAS into their body when feeding *Artemia* nauplii which lack AAS (Golub and Finamore 1972; Dabrowski 1991). It

Table 3 Daily gut contents in African catfish larvae fed decapsulated cysts and nauplii of *Artemia* from days 1–5 after the start of exogenous feeding (Experiment 2)

Diet	Number of cysts per fish			
	Experiment 1	Experiment 2		
	Day 1	Day 1	Day 3	Day 5
Before first feeding (F0)				
Cysts		26.1 ± 0.9*	159.5 ± 4.4*	206.2 ± 2.4*
Nauplii + cysts		6.0 ± 0.1*	13.3 ± 2.5*	29.6 ± 9.5*
First feeding (F1)				
Cysts	78.4 ± 9.1	39.3 ± 1.2*	175.5 ± 30.3	378.3 ± 12.1*
Nauplii + cysts		66.5 ± 4.5*	221.8 ± 22.5	474.2 ± 64.7*
Second feeding (F2)				
Cysts	81.7 ± 2.8	55.9 ± 1.6*	227.7 ± 15.5	405.4 ± 12.4*
Nauplii + cysts		81.1 ± 3.5*	248.8 ± 21.8	545.9 ± 6.5*
Third feeding (F3)				
Cysts	103.9 ± 9.4	59.7 ± 2.1*	246.6 ± 13.8	451.3 ± 28.2
Nauplii + cysts		80.6 ± 3.2*	244.5 ± 7.9	473.5 ± 28.0
Fourth feeding (F4)				
Cysts	115.6 ± 1.8	79.6 ± 0.8*	282.5 ± 4.3	573.4 ± 27.9*
Nauplii + cysts		94.1 ± 4.7*	316.5 ± 24.5	649.3 ± 11.4*

*For the same feeding period and for the same day results are significantly different ($P < 0.05$)

is unlikely that AAS in cysts digested in the fish intestine will be converted to ascorbic acid because the conditions for optimum *Artemia* embryo development and nauplii hatching (light intensity, water salinity, constant aeration) are not present inside the fish gut. In fish fed the dry artificial diet without AAS, just a small amount was detected (Table 2), probably derived from the small amount of AAS found in the artificial diet. The results for the AAS content of the two control diets in Experiment 2 confirm that no AAS biosynthesis occurs in the larvae of the African catfish.

Gut contents and feeding frequency

The amount of AAS measured in fish samples from the first diet treatment in Experiment 1 and from the first and second diet treatments in Experiment 2 were used to calculate the number of cysts in the fish larvae for the different sampling periods. The total number of cysts found in the fish samples was regarded as the gut contents. With regard to the formula used for calculation of gut contents, in the second experiment and for all sampling days, the number of cysts in the gut of catfish larvae was not significantly different ($P > 0.05$), irrespective of whether it was calculated with the exact number of fish in the sample (1) (absolute) or with the fish DW (2) (relative). When the gut contents were expressed as number of cysts per individual fish (Experiment 2), a diet effect ($P < 0.05$) was detected for most of the feeding periods on days 1 and 5 (Table 3). In the F0 samples (before first meal in each sampling day), the number of cysts per fish was lowest among the different feeding times studied, because overnight the fish were deprived of food for at least 8 h. During this time the fish digested and evacuated the nauplii. Thus, for fish larvae fed live nauplii the intestinal tract is nearly empty in the morning before the first meal. On the other hand, for fish fed decapsulated cysts, some food particles from the last daily meal were retained until the next morning. This indicates that catfish larvae take longer to digest and evacuate cysts than nauplii. The number of food particles ingested during the first meal and during the rest of the day was close to the total number of cysts measured at the end of the day (Fig. 1). This indicates a tendency in the larvae to completely fill the gut with food particles during each meal. The same observation was made for African catfish larvae (Haylor 1993) and fingerlings (Hossain et al. 1998). As fish increase in size the volume available to store food also increases. In this way the fish is able to ingest an increasing amount of food with each meal. When the gut is full, no additional ingestion of food occurs until more space is available in the gut.

Gut evacuation and food consumption

The food-evacuation rate differed significantly ($P < 0.05$) between the continuous and discontinuous feeding treatments (Fig. 2). After 7 h of ingestion of the first meal at day 1 after the start of exogenous feeding, the fish larvae evacuated approximately 87% of the amount of the food ingested in that meal compared with 43% when discontinuous feeding was applied (Table 4). Hence, 3 h after ingestion of the second meal, the newly ingested food has a significant effect on the rate of evacuation of the previous meal. During the continuous feeding regime food evacuation occurred faster than when discontinuous feeding was applied. The results from Experiment 3 further provide an indication of the time required for emptying of the gastrointestinal tract by catfish larvae. Under continuous feeding on days 1 and 3, more than 90% of the food ingested in the first meal was evacuated after 11 h compared with 71% for fish larvae on day 5 (Table 4).
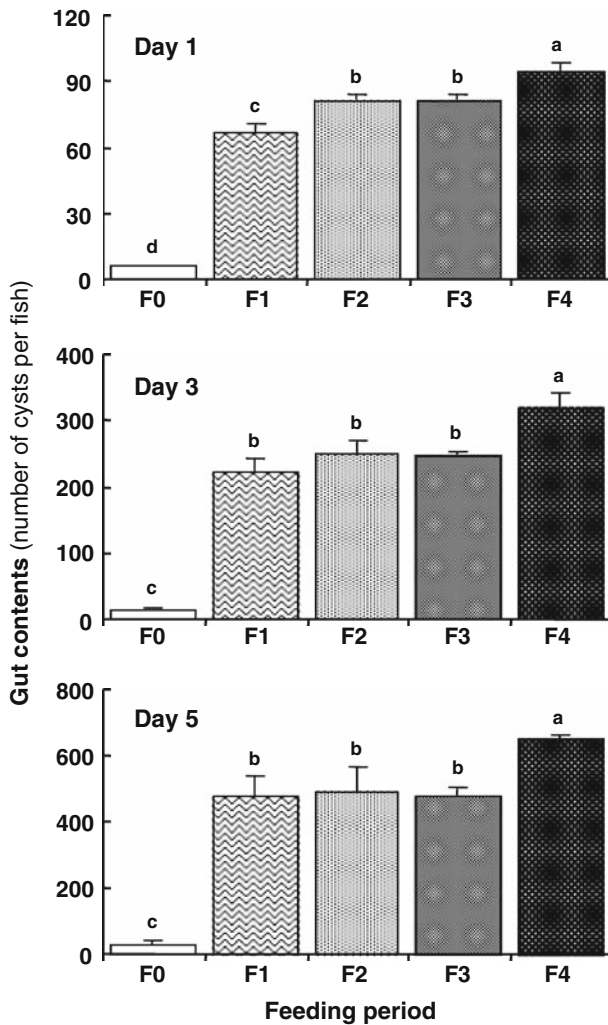
Fig. 1 Daily variation in the gut content of African catfish larvae fed decapsulated cysts of *Artemia* on days 1, 3, and 5 after the start of exogenous feeding (Experiment 2). *Bars* in the same graph with the same letter are not significantly different ($P > 0.05$). Note the different scales used for the gut contents axes among plots

The estimated daily food consumption (FC) and gut evacuation expressed as percentage of the body weight of African catfish larvae are presented in Table 5. Until day 3 the fish larvae ingested more than 45% of their body weight daily. On day 3 ingestion of food per unit dry body weight appears to reach its maximum. After this time the relative food ingestion rate decreases. This decrease has been observed to continue during the juvenile stage of African catfish from 27.9% of their body weight at a size of 0.8 g to 6.2% in fish of 37.7 g (Hogendoorn 1983; Conceição et al. 1998). The pattern for gut evacuation rate was similar to that for food consumption, increasing during the first days of exogenous feeding and decreasing as fish growth continued (Table 5).

Fig. 2 Gut evacuation by African catfish larvae during days 1, 3, and 5 after the start of exogenous feeding (Experiment 3) under continuous and discontinuous feeding regimes. *Symbols* with the *same letter* in each graph and in the same feeding treatment are not significantly different ($P > 0.05$)
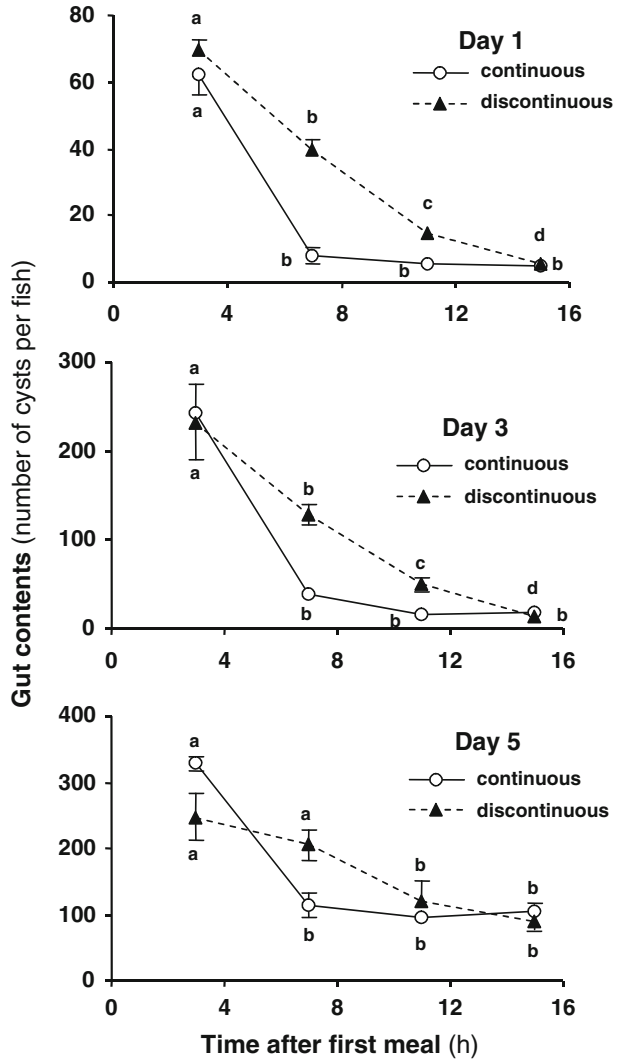
Precision of gut contents determinations

Precision in estimation of gut contents in terms of number of cysts, as the coefficient of variation, was 6.0% on day 1, 10.1% on day 3, and 8.5% on day 5 (Experiment 2). Analysis of the number of replicates and the power of ANOVA yielded a quantity ϕ of 3.1 on day 1, 7.3 on day 3, and 9.5 on day 5, for $n = 3$, representing a power of 99.9% in each case; this indicates the reproducibility of the data is good, mainly because of the small within-group mean squares. The minimum detectable difference ($1 - \beta = 0.80$, $\alpha = 0.05$) between two means in different treatments was estimated as 13 cysts per fish on day 1, 85 on day 3, and 130 on day 5. These numbers represent relative detectable differences of 17, 36, and 34% of the daily average food consumption (% body dry weight) on days 1, 3, and 5 respectively. To increase the resolution of the method and enable detection of differences of at

Table 4 Gut evacuation by catfish larvae fed decapsulated cysts of *Artemia* (Experiment 3)

Time (h)	Gut evacuation (%) of total food ingested at the first daily meal		
	Day 1	Day 3	Day 5
Continuous feeding			
3	0	0	0
7	87.0	84.1	65.6
11	91.4	93.4	71.2
15	92.1	92.7	68.3
Discontinuous feeding			
3	0	0	0
7	42.7	44.3	17.1
11	78.9	78.3	71.2
15	92.4	94.0	64.4

Table 5 Food consumption and gut evacuation rate for *Clarias gariepinus* larvae during the first days of exogenous feeding with decapsulated cysts of *Artemia*

Feeding time	Food consumption (% body dry weight)			Gut evacuation (% body dry weight)		
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5
First feeding (F1)	40.0 ± 2.2	51.4 ± 3.6	35.0 ± 3.3	–	–	–
Second feeding (F2)	48.1 ± 3.1	52.6 ± 5.6	22.4 ± 0.8	33.9 ± 2.2	42.6 ± 4.5	22.9 ± 0.8
Third feeding (F3)	46.0 ± 0.4	52.6 ± 7.3	24.4 ± 1.3	47.0 ± 0.4	52.9 ± 7.4	23.4 ± 1.2
Fourth feeding (F4)	52.2 ± 1.5	58.7 ± 8.5	29.4 ± 2.8	41.9 ± 1.2	44.8 ± 6.5	21.2 ± 2.0
Daily average	46.5	53.8	27.8	40.9	46.8	22.5

least 10% of daily food consumption, the number of replicates per treatment should be increased to 7, 22, and 20 for days 1, 3, and 5, respectively ($1 - \beta = 0.80$, $\alpha = 0.05$).

Discussion

The different AAS content of the same batch of cysts found in this study is not attributed to hydration time of the cysts because between 1 and 3 h of hydration time no significant difference was found ($P > 0.05$). Because recovery of AAS by this method was high (91–93%), the different AAS content of cysts from the same batch among the experiments might be related to a combined effect of analytical error and biological effects. The first is related to the stability of AAS standards and the storage of cysts. When the activity of the AAS standards changes with time, the calibration curves derived from such standards will reflect those changes. Moreover, although AAS is a very stable compound, it has not yet been investigated whether the AAS content of cysts changes during storage. In addition, the macro and micro-nutrient compositions of *Artemia* cysts might vary depending on cyst origin, even within a batch of cysts of the same origin (Léger et al. 1986). We therefore emphasize the need to determine the AAS content of cysts used in the feeding experiments at every analysis, by HPLC. The use of AAS as a natural marker in the method is strengthened because no biosynthesis of AAS was found in the larvae of *C. gariepinus*.

In assessment of daily food consumption and gut evacuation rates in the larval stages of fish, the high growth rate achieved during the first days of exogenous feeding must be considered. The larvae of *C. gariepinus* have higher growth rates and food conversion efficiency than the larvae of other fish species (Wieser and Medgyesy 1990; Keckeis and Schiemer 1992; Conceição et al. 1998). Within the same day and from one meal to the next the fish is growing fast, consequently the gut size rapidly increases and more capacity to store food becomes available. Evidently, the amount of food ingested by the fish larvae increased with their body weight. However, if daily food consumption is expressed as a percentage of the body weight after the start of exogenous feeding, the amount of ingested food decreases with age from day 3 onwards. Similar observations were made for larvae of three cyprinid species in which a continuous decline in food consumption from 50 to 30% of body weight was found as fish weight increased from 10 to 100 mg (Keckeis and Schiemer 1992). The AAS-based estimations of food consumption differ from those calculated with the exponential model of Elliott and Persson (1978), which considers a constant evacuation rate. Following the exponential model, Haylor (1993) predicted a constant gastric evacuation rate during the larval period in African catfish and estimated an increase in food consumption from 26.6 to 36.3% of body weight when the larvae grew from 5 to 60 mg. However, these food-consumption calculations could have been overestimated because several factors affect food-evacuation rate in fish (Persson 1981; Karjalainen et al. 1991; Bromley 1994), thus it is unlikely to have a constant gut evacuation rate under natural conditions. In our study, gut evacuation in catfish larvae was not constant, resulting in a food consumption maximum of 53.8% (body weight) at a larval weight of 15 mg and then decreasing to 27.8% for 36 mg fish (Table 5). This means that food consumption by *C. gariepinus* larvae, when expressed as a percentage of body weight, tended to decrease and not increase in larvae larger than 15 mg. Moreover, the stomach in *C. gariepinus* larvae is not functional at the start of exogenous feeding (Stroband and Kroon 1981) and only becomes functional on day 5 after the start of exogenous feeding (Verreth et al. 1992). Therefore, in fish larvae with a digestive system that is still in development, different food consumption and evacuation might be expected because of the effect of developmental and physiological processes during food digestion. In catfish larvae, the role of the stomach during food digestion might be limited to storage of food before it becomes completely functional. Food ingestion and gut evacuation rates change during development of fish larvae. Food consumption in catfish was high during the first days of exogenous feeding with a maximum level on day 3 at a fish weight of 10–15 mg; food intake then decreased as the fish approached the end of the larval period. A similar trend in feeding rate during fish larvae development was found in spotted seatrout (Wuenschel and Werner 2004). In fish larvae, after yolk sac absorption and during the first days of exogenous feeding, high food consumption rates might be expected to support the high growth rates the fish achieve during this stage of development. These high food consumption rates might also occur in the larvae of many other fish species. Indeed, in the larvae and juveniles of five cyprinids and perch, high rates of food consumption (>50% body weight per day) have been found (Marmulla and Rösch 1990).

By use of a radio-isotope technique, maximum daily food consumption by *C. gariepinus* larvae was estimated to be 51.5% of their body weight for fish on days 2 and 8 of exogenous feeding (Conceição et al. 1998). The value for day 2 is close to the result in our study for the same fish size. However, in the former study gut evacuation rate was not determined, which might account for the high food consumption rate found on day 8. The different estimates of food consumption obtained by use of different methods might be related to the number of assumptions, calculations, and procedures to obtain them in each

particular method. In the proposed AAS method, variations in food consumption by individual fish because of differences in size were reduced because of the large number of fish in each sample. Thus, a small SD was obtained in the weight measurements. The number of replicates in this study was appropriate for the statistical analysis of the results with the power of the ANOVA close to 100%. However, the precision of detection of significant differences between the number of cysts in the gut in different diet treatments was low. The method used to calculate gut contents can be improved by increasing the number of replicates per feeding treatment. Further studies are required to improve the precision of the calculations of the number of cysts in individual fish.

The feeding strategy also has an effect on the food consumption rate by fish larvae. The frequency of feeding and the type of food can affect food digestibility with effects on food consumption and evacuation rates. The evacuation rate is significantly higher in a continuous feeding regime than in discontinuous feeding for larvae of spotted seatrout (Wuenschel and Werner 2004) and juveniles of carp and turbot (Grove et al. 1985; Rösch 1987). Shorter times of gut emptying are expected in a continuous feeding regime, with rapid evacuation allowing the fish to consume food at a faster rate (Johnston and Mathias 1996). Similarly, *C. gariepinus* larvae fed multiple meals showed a faster evacuation rate than when only a single meal was given. Food-evacuation results should be compared with caution, however, because of differences between evacuation times when the analysis is performed for an individual fish or for groups of fish. A curvilinear decrease in gut contents is expected when analyzing a group of fish larvae in comparison with linear evacuation for individual fish (Karjalainen et al. 1991). The AAS method is a useful tool for determination of daily food consumption and gastric evacuation by fish larvae and enables adequate standardization. It avoids individual counting of food particles or markers by the direct analysis of the total amount of AAS in the fish and in the diet samples. Other methods might underestimate food consumption by missing food particles during counting. The negative effects on the nutritional condition of fish associated with a low food intake and poor diet digestibility are minimized by use of a natural food source that is well accepted and digested by the fish larvae. In this regard, a limitation in the method exists if the fish under study are not able to ingest decapsulated cysts of *Artemia* or any artificial microdiet which include cysts in its formulation.

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