Predicting the production of waste products in the high density culture of sharptooth catfish (*Clarias gariepinus*)

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Abstract

The production of waste products by the sharptooth catfish (*Clarias gariepinus*) was measured under high density controlled laboratory conditions. The waste products that were investigated were ammonia nitrogen, nitrite nitrogen, nitrate nitrogen and suspended solids. A flow-through system with a 360 ℓ tank was used to keep 67 catfish (average mass = 550 g). Water temperature was kept constant at 24°C. The system was subjected to a continuous light cycle and the feeding level was 20 g·kg^{-1}·d^{-1}. Water samples were taken at regular intervals throughout the feeding cycle and analysed for the above-mentioned metabolic wastes. The measured concentrations were transformed mathematically into metabolic waste production per unit biomass. The resulting data compare favourably with similar data for other species published elsewhere. It was found, however, that the production was somewhat prolonged. The size distribution of the suspended solids (SS) was investigated to facilitate easier removal of this metabolic waste from systems. The results were that 52% of the SS did not pass a 300 μm sieve while 17% passed through a 100 μm sieve. The balance of the SS fell between these two sizes. This investigation also showed that there were two types of SS that had to be removed; the first type had a fine granular appearance while the second was mucoid. No attempt was made to differentiate between these two types during this investigation. A model calculation illustrates the prediction of the accumulation of wastes in high-density aquaculture systems.

Introduction

The African sharptooth catfish (*Clarias gariepinus*) is an extremely suitable aquaculture species and is successfully cultured in many parts of the world. This species has a wide tolerance for various environmental conditions, is highly fecund and high yields (12.5 to 100 t·ha^{-1}·a^{-1}) may be obtained under intensive conditions (Hecht et al., 1988). In South Africa, catfish is raised in eathern ponds with slow water exchange rates. However, the country lies in a semi-arid region in which rainfall and water bodies are unevenly distributed. Expanding demand due to rapid population increase and demographic changes will result in water becoming increasingly scarce in many parts of South Africa. Greater pollution loads and reduced flows in the country's rivers due to expanding demand, will in future place additional pressure on the already limited water resources (DWAF, 1993). In future we will have to depend to a larger extent on the recycling of water for aquacultural purposes and treatment of water from flow-through systems to comply with the effluent standards of Department of Water Affairs and Forestry. High density culture of *Clarias gariepinus* combined with the required high feeding rates may lead to severe water quality problems in high density aquaculture systems. Critical water quality variables in recirculation high-density aquaculture systems are usually low dissolved oxygen and waste products such as suspended solids (SS), ammonia and nitrite. Under such conditions it is therefore essential to manage water quality to ensure optimal survival and production of the species. If waste production levels (per biomass production unit) are known, it is possible to predict the accumulation rates of the metabolic wastes for this species in a high-density aquaculture system.

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Although the feed consumed by fish is effectively assimilated, a measurable percentage (1 to 35%) is excreted as faeces (Du Preez and Cockroft 1988). This assimilated food is then utilised for growth and reproduction while a significant portion is expended during the respiration process and excreted as urinary waste (Du Preez et al., 1990). The waste products in an effectively closed system, therefore, originate from the feed which is added to the system. Furthermore, the faeces produced as well as the feed not consumed by the fish are the major SS constituents in the high-density system.

The toxicity of the ammonia depends largely on the concentration of un-ionised ammonia (Heath, 1987). The ammonium ion is generally non-toxic to fish while un-ionised ammonia is highly toxic. Futhermore some of the ammonia may serve as substrate for the production of nitrite which is also highly toxic (Boyd, 1982). High un-ionised ammonia levels in the water affect osmoregulation of cultured fish and reduce internal iron concentrations. Ammonia may also damage gills, reduce the oxygen transport ability of the blood and the oxygen consumption rates of tissues, while sublethal concentrations may cause histopathological changes in many tissues (Boyd, 1982).

The toxic effects of nitrite are damage to gill tissue and impairment of oxygen transport. If nitrite is absorbed by fish, it reacts with haemoglobin to form methaemoglobin which is not an effective oxygen carrier. The continued absorption of nitrite can therefore lead to tissue hypoxia and cyanosis (Boyd, 1982). High SS levels may also affect the health of the fish. The gills of fish become thickened and proliferated when subjected to high SS levels. Under severe conditions this can be lethal (Alabaster and Lloyd, 1980).

The production of wastes by *Clarias gariepinus* under high density loading conditions had, as far as could be determined, not been measured systematically. Hogendoorn et al. (1983), however, studied the food utilisation efficiency of *Clarias* and using this, Bovendeur et al. (1987) concluded that when uneaten feed was

discounted, 79% of the dry matter and 74% of the nitrogen that had been taken in originally, was accounted for as faecal and non-faecal losses, the rest being used in growth processes. The progression of waste production and SS properties are not reported on.

The quantity and quality of the metabolic wastes produced by fish depend on the feeding level, the composition of the feed, the digestibility of the feed and the absorption level of digested feed (Hogendoorn, 1983; Hogendoorn et al., 1983; Henken et al., 1985). The production of ammonia, for instance, will also be influenced by gender, body mass, rate of feeding, pH and the ammonia concentration in the immediate vicinity of the fish (Paulson, 1980). Experiments done with trout (*Oncorhynchus mykiss* and *Salvelinus fontinalis*) weighing 50 to 100 g, showed that the production of ammonia reaches a maximum of 14 mg NH_4-N per kilogram unit biomass 7 to 8 h after feeding at 12°C (Paulson, 1980). The ammonia production then decreases to a basal level of 3 mg·kg^{-1} approximately 46 h after feeding at a level of 25 g·kg^{-1} biomass. Lied and Braaten (1984) found that NH_4-N production in Atlantic cod (*Gadus morhua*) reaches a maximum (9 mg·kg^{-1} biomass) 5 to 6 h after feeding with a feeding level of 20 g·kg^{-1} biomass at a water temperature of 8°C. During the above experiment the basal NH_4-N production was 1 mg·kg^{-1} biomass.

The progressive production of wastes and suspended solids was investigated in order to determine the requirements for biological and mechanical filtration of effluent water in high density *Clarias* culture systems that were to be used in experimental and production facilities at the Rand Afrikaans University.

Materials and methods

A flow-through system was used to measure the production of waste products by *Clarias gariepinus* (Fig. 1). The system was set up in a closed-environment room in which the temperature was regulated at 30°C and a continuous light cycle was provided. The system consisted of a water reservoir with a volume of 1 243 ℓ ahead of a fish tank with a volume of 360 ℓ.

Water was obtained from a borehole at a temperature of 14 to 17°C. Because of the long retention time of the reservoir (21 h) and the high temperature of the environmentally-controlled room, the water from the borehole warmed to 24±0.5°C before it reached the fish tank. The optimum temperature for *Clarias gariepinus* is given by Hogendoorn et al. (1983) as 25 to 27°C.

Water was gravity-fed from the reservoir to the fish tank at a rate of one exchange every 5½ h. The tank was stocked with 67 *Clarias gariepinus* (mean mass = 555±58 g; cumulative mass = 37 kg). The fish used in this experiment were not sexed. Water leaving the fish tank through a surface overflow was discarded. The fish were fed commercial trout pellets (Truka) at a level of 2% per unit biomass daily. The feed consisted of 90.7% dry matter of which 39.9% was protein. The other constituents of the feed were: lipids, 5.3%; ash, 9.6%; and carbohydrates, 45.2%. The energy value of the feed was 22.8 kJ·g^{-1}.

At the onset of the experiment the fish were not fed for two days in order to ensure basal production levels of metabolic wastes. After this period the fish were fed once, i.e. 72 h after the last feeding. Water samples were taken directly from the tank at regular intervals. Samples were taken at hourly intervals directly after feeding but as the experiment progressed longer intervals between samples were allowed. The experiment was terminated after 90 h. The temperature remained constant at 24°C throughout the experiment but the pH level increased slightly from 7.3 to 7.6. Water samples were analysed immediately after they had been taken.

The ammonia nitrogen levels (NH_3-N) were measured with the direct nesslerisation method. Nitrite nitrogen (NO_2-N) is seldom found in concentrations greater than 1 mg·ℓ$^{-1}$, which necessitates a sensitive analytical method. A modification of the Griess-Ilsovay diazotisation method was therefore used. The cadmium reduction method was used to determine nitrate nitrogen (NO_3-N). All these analyses, as well as the determination of SS, were carried out according to *Standard Methods* (1987).

Water samples for the size distribution analysis were taken at the same time as the samples for the chemical analysis. Each sample was filtered through preweighed 300 µm metallic sieves, 100 µm metallic sieves and GF/C Whatman-filters. The sieves and filter paper were weighed again after the samples had been dried at 105°C for 1 h.

Due to time and financial constraints, the experiment was run only once. Although the experiment should ideally have been run at least three times, the results still yield some valuable preliminary information.

Results and discussion

Every sample taken from the culture tank was analysed to determine the concentration of ammonia nitrogen, nitrite nitrogen, nitrate nitrogen and SS. This analysis gave the net concentration levels of the waste products in the system at the time of taking the samples. The data were transformed mathematically to give the excretion tempo of waste products per kilogram unit biomass as a function of time. A cumulative curve of production as a function of time was also drawn. This gives an indication of the total load of metabolic waste produced in the high density system. The results are shown in Figs. 2 to 9. The measured particle size distribution is represented in Fig. 10 which shows the mass distribution of each interval as a percentage of the total dried mass.

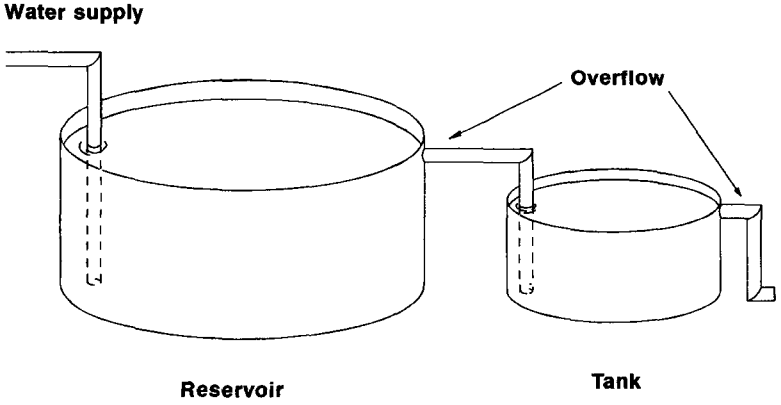

Figure 1
Experimental set-up

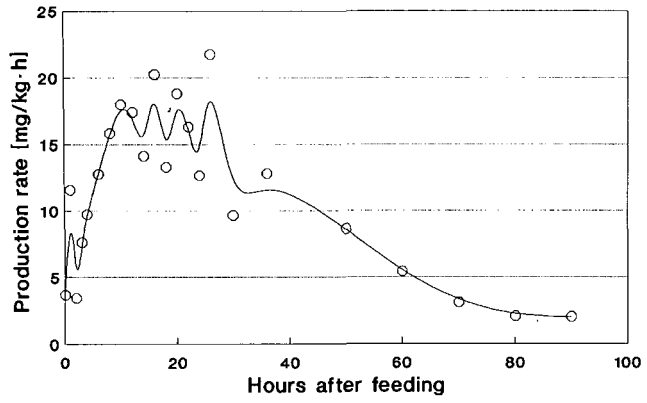

Figure 2
Production rate of ammonia nitrogen
($mg \cdot kg^{-1} \cdot h^{-1}$)

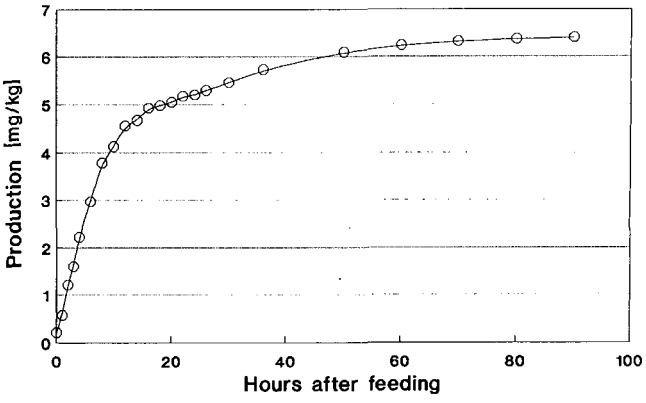

Figure 5
Cumulative production of nitrite nitrogen
($mg \cdot kg^{-1}$)

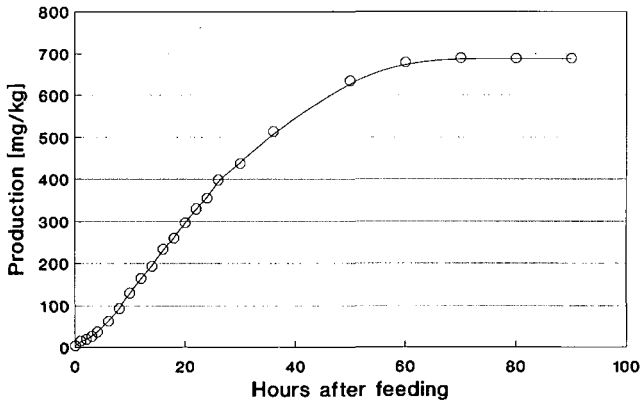

Figure 3
Cumulative production of ammonia nitrogen
($mg \cdot kg^{-1}$)

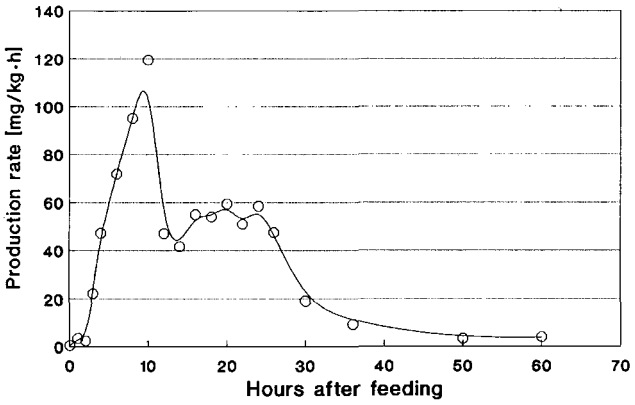

Figure 6
Production rate of nitrate nitrogen
($mg \cdot kg^{-1} \cdot h^{-1}$)

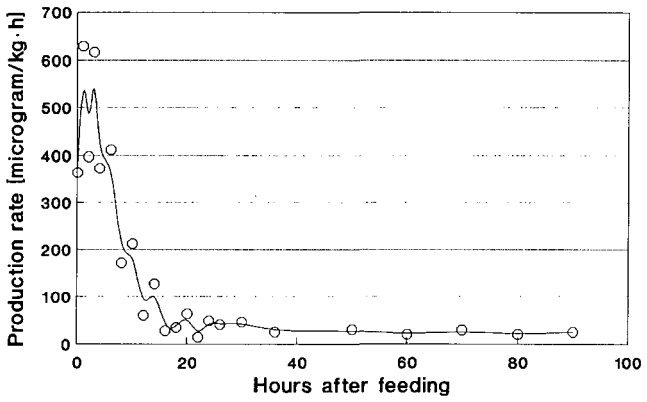

Figure 4
Production rate of nitrite nitrogen
($\mu g \cdot kg^{-1} \cdot h^{-1}$)

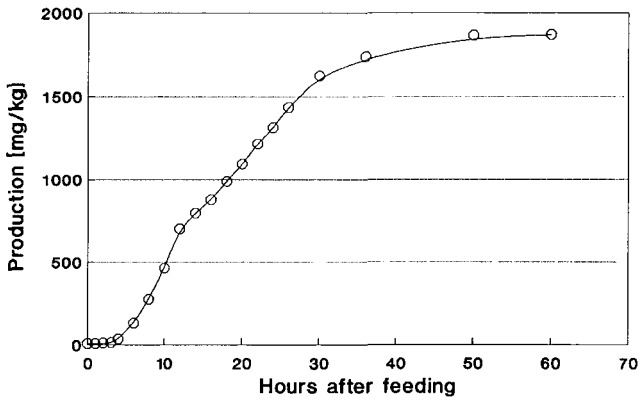

Figure 7
Cumulative production of nitrate nitrogen
($mg \cdot kg^{-1}$)

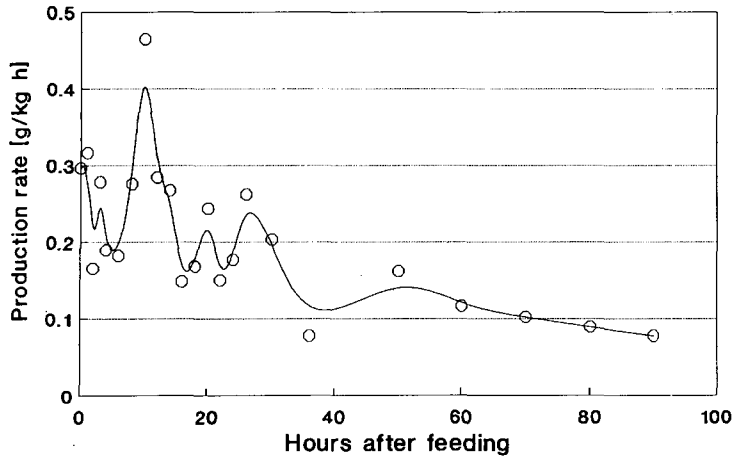

Figure 8
SS production rate ($g \cdot kg^{-1} \cdot h^{-1}$)

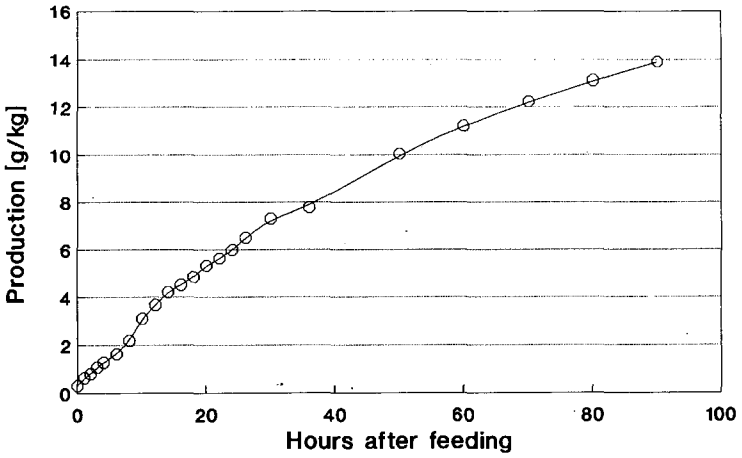

Figure 9
Cumulative SS production ($g \cdot kg^{-1}$)

TABLE 1
A COMPARISON OF AMMONIA NITROGEN PRODUCTION STUDIES

Reference	Species and weight	Basal production [$mg \cdot kg^{-1} \cdot h^{-1}$]	Maximum production [$mg \cdot kg^{-1} \cdot h^{-1}$]	Duration of production [h]	Cumulative production [$mg \cdot kg^{-1}$]	Feeding ratio
Present study	*Clarias gariepinus* (500-600 g)	2.0	17.5	60.0	700.0	2% per unit biomass daily except for the two days preceding the experiment
Lied and Braaten (1984)	*Gadus morhua* (256-2 410 g)	1.0	9.0	30.0	-	113 $kJ \cdot kg^{-1}$ of biomass five times per week except for the 48 h preceding each experiment
Paulson (1980)	*Salvelinus fontinalis* (50-92 g)	2.5	14.0	45.0	-	2.5 to 5.0% per unit biomass on the day of the experiment
	Oncorhynchus mykiss (82-142 g)	3.0	13.0	45.0	-	
Rychly and Marina (1977)	*Oncorhynchus mykiss* (29-70 g)	2.0	12.0	32.0	850.0	Ratio not reported

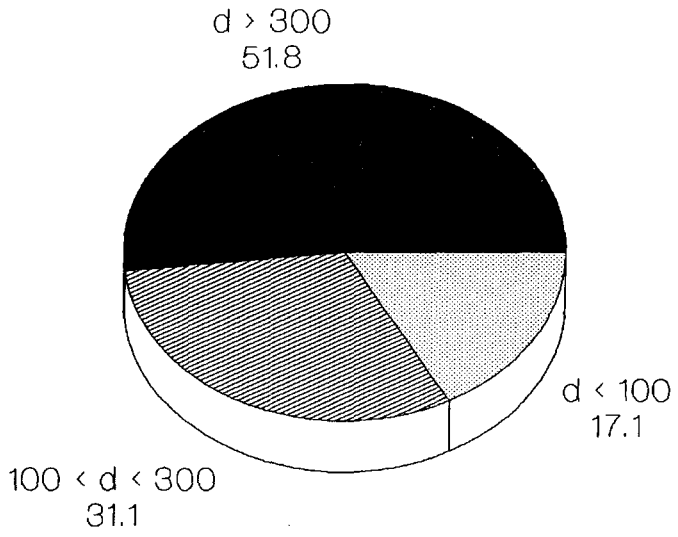

Figure 10
Particle size distribution as a percentage of the total mass of SS

The ammonia nitrogen production (Fig. 2, Table 1) is similar to the previously published production by *Gadus morhua* (Lied and Braaten, 1984) and trout (Rychly and Marina, 1977; Paulson, 1980). The production increases to a maximum value of 17.5 $mg \cdot kg^{-1} \cdot h^{-1}$ after which it decreases slowly. The production returns to basal levels (2.0 $mg \cdot kg^{-1} \cdot h^{-1}$) 60 to 70 h after feeding. The average production rate over the period is 11.7 $mg \cdot kg^{-1} \cdot h^{-1}$ while the cumulative production is 700 $mg \cdot kg^{-1}$ unit biomass (Fig. 3).

The time needed for the production levels to reach a maximum and to return to basal levels is longer than expected when compared with other studies. It has already been mentioned that the light cycle in the environmentally-controlled room was continuous. According to Rychly and Marina (1977) there is a relationship between the daily rhythm of ammonia excretion and the periodic excretion of corticosteroids, which is influenced by light-dark cycles. This lag in ammonia excretion was also noticed in the production of the other metabolic wastes in this study.

A pulse in ammonia excretion which peaks within a few hours of feeding has been recorded for various fish species (Brett and Zala, 1975; Braaten, 1979; Lied and Braaten, 1984). This pulse and associated lag in ammonia excretion are associated with the feed intake and not with the metabolic rate of fish (Brett and Zala, 1975; Lied and Braaten, 1984).

Nitrite nitrogen rarely occurs in concentrations greater than 1 $mg \cdot \ell^{-1}$. Low production rates of nitrite nitrogen were observed during this study (Fig. 4). These low levels can be ascribed to the fast conversion of nitrite to nitrate by *Nitrobacter* as well as to the fact that ammonia nitrogen and urea nitrogen account for most of the nitrogen excreted by teleosts while nitrite forms only a relatively small percentage (Elliott, 1976; Schmidt-Nielsen, 1983; Du Preez and Cockroft, 1988). The cumulative nitrite production curve is purely academic due to the fact that nitrification will take place on a continual basis in high density aquaculture systems.

The nitrate nitrogen (Fig. 6) reaches a peak of 110 $mg \cdot kg^{-1} \cdot h^{-1}$ 10 h after feeding. The cumulative production is 1.9 $g \cdot kg^{-1}$ unit biomass (Fig. 7) and the production reaches basal levels after 50 h. Although a flow-through system was used, the process of nitrification could have occurred in the tank. Assuming that all the ammonia in the system had been nitrified, it would constitute only about one half of the measured nitrate nitrogen production. It is assumed that the contribution of the uneaten feed is negligible. The only other possible source of nitrate nitrogen would then be the fish. Therefore it has to be assumed that the fish are responsible for the biggest share of the nitrate nitrogen production.

The maximum SS production is found 10 h after feeding and is 0.45 $g \cdot kg^{-1} \cdot h^{-1}$ (Fig. 8). The production levels are quite high almost immediately after feeding. If all the feed fed to the fish had not been consumed the SS concentration in the tank would be more than 2 000 $mg \cdot \ell^{-1}$. It follows that even if only a small percentage of feed is not consumed the initial SS concentration would be high. It is also true that the rummage following the addition of feed to the system or when the fish are disturbed causes settled particles to become suspended and be caught in the water samples. These reasons explain the high initial concentration as well as the other smaller peaks encountered in the SS production. This production reaches a maximum value 10 h after feeding. Due to the fact that the experiment was done under the same high-density conditions under which the fish will eventually be kept, it may be correct to assume that this apparently irregular production will also be found in full-scale systems.

The SS production had not reached steady basal levels by the time the experiment was terminated. However, when the best fit curve for the measured data is extrapolated it can be seen that the total cumulative production will be 16 to 17 $g \cdot kg^{-1}$ (Fig. 9) at an initial level of 20 $g \cdot kg^{-1}$. This means that approximately 80 to 85% of the dry material that was fed to the fish was sampled as SS. This result compares favourably with Bovendeur et al. (1987) who calculated that 73 to 79% must be excreted as SS. Furthermore, it is important to note that the feeding level differed slightly between these two experiments. The difference (maximum 12%) can also be attributed to the fact that the SS measured in the experiment with catfish included both faeces produced and uneaten food. It can therefore be concluded the extrapolation procedure is acceptable.

Figure 10 shows that 17.1% of the SS is smaller than 100 µm while 51.8% is larger than 300 µm. It has been found that there are mainly two types of SS that accumulate in high-density systems. The first is a granular type which can typically be indigestible or unconsumed feed and the second is mucoid and therefore faeces produced. Due to the high water content of the mucoid SS it contributed to the total mass to a lower degree than the granular SS although it was much more voluminous. No attempt was made to determine a quantitative ratio of mucoid to granular SS. The size distribution analysis makes it possible to investigate more efficient methods of removing SS from high density aquaculture systems.

TABLE 2
UNIT PRODUCTION OF METABOLIC WASTES BY *CLARIAS GARIEPINUS*

Metabolic waste production [mg·kg^{-1}·h^{-1}]	Time after feeding [h]					Cumulative production [mg·kg^{-1}]
	0 h	6 h	12 h	18 h	24 h	
Ammonia nitrogen	4	13	17	17	16	700
Nitrate nitrogen	5	70	90	55	55	1 900
Suspended solids	70	350	450	320	250	15 000

TABLE 3
TOTAL PRODUCTION OF WASTE PRODUCTS IN THE HYPOTHETICAL SYSTEM

Production [g·h^{-1}]	Time after feeding [h]					Cumulative production [g]
	0 h	6 h	12 h	18 h	24 h	
Ammonia nitrogen	0.2	0.65	0.85	0.85	0.8	35
Nitrate nitrogen	0.25	3.5	4.5	2.75	2.75	95
Suspended solids	3.5	17.5	22.5	16.0	12.5	750

Prediction of waste product levels in high-density systems

With the data that have been reported it is possible to predict the production of waste products by high-density aquaculture loads of *Clarias gariepinus* in a known volume of water kept under similar conditions as experienced in this study. From this the concentration of waste products in a system can be predicted if the flow rate through the system is known. The production of waste products per unit biomass can be read from the given figures. Some of the co-ordinates are given in Table 2. It is assumed that 50 kg fish are fed in a 200 ℓ tank and the flow rate through the tank is 1 ℓ·min^{-1}. The tank is connected to a flow-through system that is supplied with water that is free from SS, ammonia and nitrate. The feeding level is 20 g·kg^{-1} unit biomass.

The production of waste products in the system is calculated by multiplying the unit production of waste products by the biomass in the tank. The result of this calculation is given in Table 3.

The waste product concentration builds up in the tank because it is not washed from the tank directly after being produced. The calculation of the concentration of metabolic wastes in the tank as a function of time is illustrated with SS. The total SS mass in the system can be calculated by using Eq. (1):

$$SS_1 = \frac{SS_0(2V-Q.\Delta t)+V.\Delta t(S_{P0}+S_{P1})}{2V+Q.\Delta t} \qquad (1)$$

where:

$SS_{0,1}$ = the total SS mass at the beginning and end of a time increment [g]
V = the volume of water in the system [ℓ]
Q = the flow rate through the system [ℓ·h^{-1}]
Δt = the length of the time increment in hours [h]
$S_{P0,1}$ = the SS production rates at the beginning and end of each time increment [g·kg^{-1}·h^{-1}]

The concentration of the waste product is then calculated with Eq. (2):

$$C = \frac{SS}{V} \qquad (2)$$

To predict the concentration of a waste product as a function of time it is necessary to have an initial concentration. For this example it is assumed that this concentration will be 20 mg·$\ell$$^{-1}$. The production of SS as a function of time must also be known. This follows from Table 3. A time interval (say 0 to 6 h) is chosen. The SS production rate at the beginning and end of this increment along with all the other variables are substituted into Eq. (1). This equation produces the total mass of SS in the system at the end of the chosen time increment. By using Eq. (2) the SS concentration in the system can then be calculated.

The calculation is repeated for the next time increment. The concentration of the metabolic waste at the end of the previous time increment is used as the initial concentration for the new increment. The resultant SS concentration in the tank is tabulated in Table 4.

The concentration of the other metabolic wastes in the system can be calculated in a similar way. Basal production levels can be used as initial values for these calculations but it is important to remember that this would then simulate concentration levels that would be experienced if the fish were being starved beforehand. A smaller time increment would lead to a more accurate simulation. If the calculation leads to negative concentrations, smaller time increments should be used. The levels of the other metabolic wastes are also given in Table 4.

If it is assumed that this simulation is being done at a temperature of 20°C and a pH of 7.5 the concentration of free ammonia nitrogen can also be calculated. The equations to determine this are given by Benefield et al. (1982) and Sawyer et al. (1978).

TABLE 4
HYPOTHETICAL PRODUCTION OF WASTE PRODUCTS

Waste products	Time after feeding				
	0 h	6 h	12 h	18 h	24 h
Suspended solids [mg·ℓ⁻¹]	20.0	166.8	324.6	321.0	241.9
Ammonia nitrogen [mg·ℓ⁻¹]	2.0	6.8	12.2	14.1	13.8
Nitrate nitrogen [mg·ℓ⁻¹]	2.5	4.9	6.6	6.1	4.7
Free ammonia nitrogen [µg·ℓ⁻¹]	28.3	96.4	172.5	199	194.7

$$\ln(K) = \frac{-\Delta H^{\circ}_{reaction}}{R}\frac{T-298}{298T} + \ln(5.6*10^{-10}) \quad (3)$$

and

$$[NH_3] = \frac{S}{1+\frac{10^{-pH}}{K}} \quad (4)$$

where:

K = Dissociation constant
$\Delta H^{\circ}_{reaction}$ = -7310 cal·mol⁻¹
T = Temperature [K]
R = Universal gas constant (1.987 cal·mol⁻¹·K⁻¹)
S = Sum of the ammonium and ammonia concentrations
= $[NH_4^+] + [NH_3]$

Equation (3) is used to determine the dissociation constant K which in turn is substituted into Eq. (4) to calculate the amount of un-ionised ammonia in a given solution for a certain temperature and pH value.

Conclusions

The production of ammonia, nitrite, nitrate and SS by *Clarias gariepinus* under high-density laboratory conditions was measured. The results compare well with other published data. The absence of light-dark cycles and the effects of starvation could have influenced the production rate of waste products and this should be considered when further studies are done. From the unit biomass production rates it is possible to predict the production of and the concentrations of the waste products in high-density aquaculture systems for *C. gariepinus*. The equations presented could also be used to predict the pollution potential of waste products in effluent from high-density catfish aquaculture systems. The experiments have to be repeated for other species and mass classes in order to predict the production of waste products for those species and species mass classes under the same conditions. The method of calculation, however, will remain the same.

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