Towards assessment of welfare in African catfish, *Clarias gariepinus*: the first step

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Towards assessment of welfare in African catfish, *Clarias gariepinus*: the first step

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A mis padres, Francisca y Pablo

A Maaike

Contents

Chapter 1

General Introduction

Overview

Usually, farming implies that animals are kept in small areas in densities above the ones found in natural populations. These conditions may evoke reactions of impaired welfare when the animal is not well adapted to cope with its surroundings and conspecifics. In such cases, different behavioural patterns may occur, from reduced feed intake, to aggression and death. Information about the animal in such conditions may help us to understand its welfare. The overall objective of this thesis was to determine the effect of different husbandry conditions on social behaviour of the African catfish (*Clarias gariepinus*) and the results of this social behaviour on stress, feed intake and growth. A series of experiments assessing feeding method, photoperiod, light intensity and stocking density (Chapters 2 - 5) were carried out. Based on the results of the previous experiments, a detail assessment of the effect of photoperiod and stocking density on behaviour, stress parameters and growth was carried out (Chapter 6 and 7).

World fisheries and aquaculture production

World fish production from fisheries and aquaculture is currently at record level (FAO, 2002). Fish products are very important for food security, providing more than 15 % of total animal protein supplies (FAO, 2002). According to FAO, the contribution of aquaculture to the world fish supplies (including crustaceans and molluscs) continues to grow, increasing from 3.9 percent of total production by weight in 1970 to 27.3 percent in 2000. Aquaculture is growing more rapidly than all other animal food producing sectors. The annual growth rate of the aquaculture sector is estimated to be between 9 - 10 % since 1970, compared with only 2.8 percent for terrestrial farmed meat production systems. More than half of the total world aquaculture production in 2000 was finfish (FAO, 2002).

During the past three decades, aquaculture has expanded, diversified and intensified. To increase production under farm conditions, the most common practice is to increase the number of fish per culture unit and to increase growth rate by improved feeds and feeding to optimised water quality. Secondary practices are to manipulate photoperiod and/or light intensities. All these efforts focused on increasing growth in an attempt to improve productivity, but did not pay much attention to the question how the animal would cope with these new husbandry conditions. Welfare gained only very recently some interest in fish farming.

Welfare, behaviour and stress

Through the past years, public concern about animal behaviour and animal welfare increased, especially regarding pig and poultry farming. Extensive research has been done in these species (for example, Barnett and Newman, 1997; Den Ouden et al., 1997; Bracke et al., 2002; v. Erp-v. d. Kooij et al., 2002; Duncan, 2002; Geverink et al., 2003). At present, public apprehension about intensive fish culture has not reach the high levels of concern as it has for the pig and poultry industry. However, animal welfare pressure groups have suggested that current farming practices may compromise fish welfare as well (Lymbery, 2002).

What is meant by behaviour and by welfare? Animal behaviour and animal welfare are different disciplines. Animal behaviour refers to all sorts of activities animals engage in (Jensen 2002). Animal welfare refers to its quality of life, and this implicates many different aspects such as health, happiness and longevity (Duncan and Fraser, 1997). As a result of improvement of welfare in farmed animals, the Brambell Committee (1965) and later on, the British Farm Animal Welfare Council (in Keeling and Jensen, 2002) declare five freedoms that the animals must have: 1) Freedom from thirst, hunger and malnutrition. 2) Appropriate comfort and shelter. 3) Prevention, or rapid diagnosis and treatment of injury and disease. 4) Freedom to display most normal patterns of behaviour, and 5) freedom from fear. In the case of farmed fish, these five freedoms can be translated into: sufficient quantity and quality of food, suitable temperature, good oxygen level, water free of pollution, a stocking density which takes into account normal swimming and social interaction, and good possibilities to avoid perceived danger and good monitoring of the health status of the fish (FAWC, 1996).

There are several factors that may affect welfare in fish. The animal will respond at different levels to impaired welfare conditions, varying from endocrine changes to atypical behaviour (Table 1). Not all responses to stressors are unique for impaired welfare and do not occur at the same intensity. As a result, it is still a matter of research how to assess (impaired) welfare in fish. The term welfare is thereby often regarded as synonym to stress. Even though high stress is related to impaired welfare, both terms are different and complement each other. While welfare is related to the feeling of well-being, happiness and/or enjoyment; stress is related to the feeling of pain and fear (Broom, 1998).

In fish, stress response to different stimuli is well documented (see for example Barton and Iwama, 1991; Iwama et al., 1997; Wendelaar Bonga, 1997; Iwama et al., 1999; Ruane, 2002). Wendelaar Bonga (1997) stated that “stress is a condition in which the dynamic equilibrium of animal organisms, called homeostasis, is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli, commonly defined as stressors”. At organism level, the response to a stressor is divided into primary, secondary, and tertiary response; and depending on the duration in time of the stressor, stress can be divided into acute (short term perturbation) and chronic (long term perturbation).

Table 1 Examples of environmental factors that have been shown to influence fish performance, behaviour and/or blood parameters.

Environmental factors	affect	References
Fish density [a]	blood parameters, behaviour, growth, survival, mortality, body composition	Diana and Fast, 1989; Haylor, 1991, 1992; Siddiqui, et al., 1993; Hecht and Uys, 1997; Khwuanjai Hengsawat et al., 1997; Hossain, et al., 1998; Hecht and Appelbaum, 1987, 1988; Appelbaum and McGeer, 1998;
Photoperiod [a]	behaviour, feed intake, growth, body composition	Stickney and Andrews, 1971; Boujard et al., 1991; Boujard and Leatherland, 1992; Deacon and Hecht, 1996; Appelbaum and McGeer, 1998; Downing and Litvak, 1999; Hossain et al., 1999; Boeuf and Le Bail, 1999; Nwosu and Holzlöhner, 2000; Appelbaum and Kamler, 2000;
Light intensity [a]	growth	Nwosu and Holzlöhner, 2000; Boeuf and Le Bail, 1999;
Nutrition [b]	blood parameters, growth, body composition	Barton et al., 1988; Vijayan and Moon, 1992; Reddy et al., 1995;
Water quality [b]	blood parameters, growth,	Carmichael et al., 1984, Barton et al., 1985; Pickering and Pottinger, 1987
Temperature	blood parameters, growth, mortality	Clap et al., 1997; Kieffer et al., 1994; Tank, 2000.

[a] References are reported for a clariid fish and some especially for the African catfish (*Clarias gariepinus*)

[b] Data from Barton, 1997.

The primary response is at the endocrine system level. Following perception of a stressful stimulus by the central nervous system, corticotrophin releasing factor from the hypothalamus stimulates the pituitary to release adrenocorticotropic hormone (ACTH). ACTH is circulated to the interrenal cells in the anterior kidney and stimulates them to secrete cortisol. The chromaffin tissue of the anterior kidney is stimulated by the sympathetic nervous system to release adrenaline and other catecholamine hormones (Wedemeyer, 1996). The secondary response is reflected in blood and tissue alterations. Adaptive changes in blood and tissue chemistry occur, such as increased gill perfusion and elevated sugar in the plasma (Wedemeyer, 1996). They influence a range of physiological processes such as metabolism and immune capacity. Mobilization of energy substrates, such as glucose and free fatty acids (FFA), is required as a result of the metabolic cost of stress response (Ruane, 2002). Hormonal control (such as cortisol) helps the body to regulate all these processes (Wendelaar-Bonga, 1997). The tertiary response is at whole-animal level e.g. reduced growth, impaired resistance to infectious diseases, and behavioural changes such as impaired reproductive behaviour may lead to reduced survival (Schreck et al., 2001)

A range of indicators based on primary, secondary and tertiary responses are used to measure stress levels in fish (Barton et al., 2002; Ruane, 2002). To measure the acute stress response, physiological parameters such as cortisol levels are a good indicator (Barton and Iwama, 1991) followed by FFA, glucose and lactate levels in plasma. These plasma parameters are however less suitable for assessing chronic stress, because physiological responses tend to adapt to long-term stressors (Pickering and Stewart, 1984). Generally, chronic stress has often an impact on behaviour, e.g. sometimes chronic stress can lead to atypical behaviour, such as stereotypes. Therefore, for assessing welfare in relation to chronic stressors, measuring the fish behaviour may be very instrumental. In addition, to assess degree of “feeling well “, so far no other indicators than behavioural responses are known. Therefore in the present study a combined approach of analysis of behavioural, physiological and performance parameters was used.

Hypothesis and the objectives of this thesis

The overall objective of this thesis is to determine the effect of different environmental factors on social behaviour of the African catfish (*Clarias gariepinus*) and the results of this social behaviour in relation to stress response, feed intake, and growth. In this thesis we hypothesise that environmental factors play a major role in the expression of behaviour and performance of the African catfish (*Clarias gariepinus*).

Outline of the thesis

The focus of this thesis was to investigate the effects of common husbandry conditions on stress response, growth and behaviour of the African catfish (C. gariepinus). As mentioned earlier, husbandry conditions such as feeding method, stocking density, photoperiod and light intensity may play an important role in growth and welfare of the fish. The general outline of the thesis is given in Figure 1.

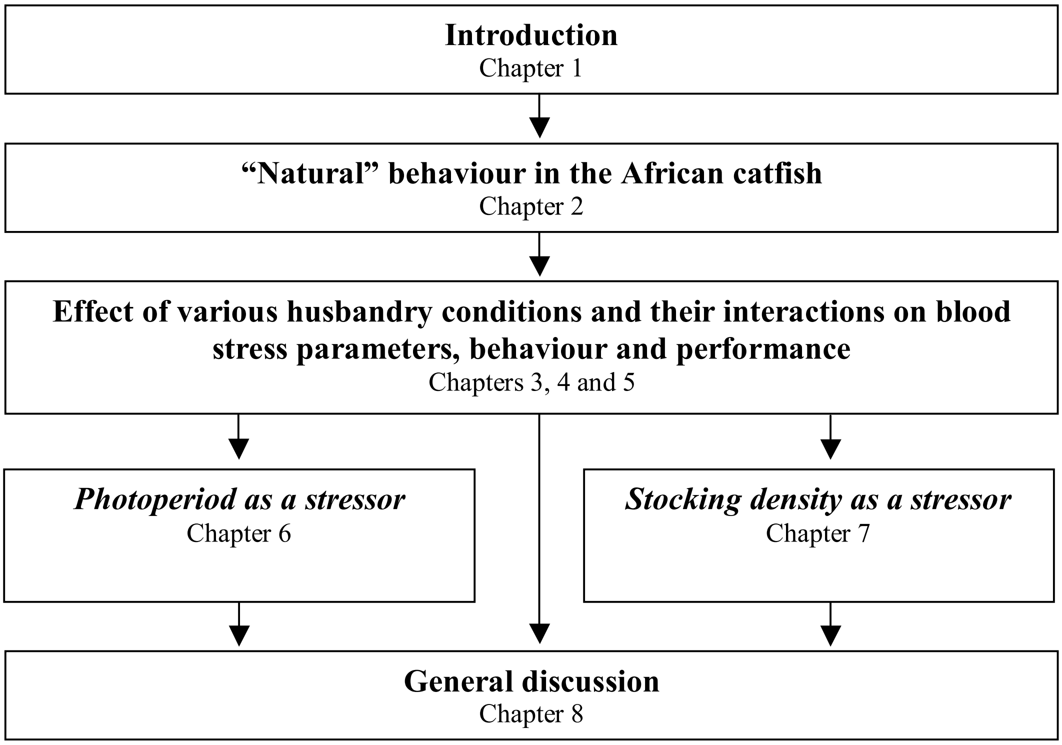

Figure 1. General outline of the thesis

The first part of this thesis (**Chapter 2**) focuses on the development of base line data on the behaviour of the African catfish (Clarias gariepinus) under "normal" and standardised conditions. In this part, behaviour patterns are described and used as a reference for the following chapters.

In the second part of this thesis (**Chapters 3, 4** and **5**), various husbandry conditions were screened for being potential stressors and thus affecting the fish welfare. In this assessment also attention was paid to occurrence of interaction effects between different husbandry conditions. In **Chapter 3** and **4**, feeding method, photoperiod and light intensity were assessed. In **Chapter 5**, stocking density, photoperiod and light intensity were assessed. These factors were studied by creating rather large contrast in each of the factors.

Based on the results (impact on behaviour, stress response and growth) in **Chapters 3**, **4** and **5**, stocking density and photoperiod were selected for detailed assessment in **Chapter 6** and **7**, respectively. Different photoperiod regimes (24D:0L, 18D:06L, 12D:12L and 06D:18L) were studied in **Chapter 6**. Both group size (number of fish) and available swimming area (tank/aquarium size) determine the actual stocking density. In **Chapter 7**, the response effects of group size as well as available swimming area on the fish's behaviour, stress response and growth was assessed. Furthermore it was studied whether stocking density is a proper parameter for characterising husbandry conditions for both group size and available swimming area. In **Chapter 8**, the overall results and its implications are discussed.

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Chapter **2**

Behaviour patterns of the African catfish (*Clarias gariepinus*) under controlled conditions

Abstract

Behavioural problems such as reduced feed intake, aggression, stereotypes and death are examples of stress caused by intensification of farming conditions. Under farming conditions, abnormal behaviour will appear in combination with normal or natural behaviour caused by restriction of the area. Results of the present study showed that the African catfish (*Clarias gariepinus*) is diurnally active, spending most of its time swimming and most of this activity was done during the night. In contrast to the swimming activity, fish demanded nearly two-thirds of the daily feed ration during the light periods of the day. The percentage of fish bitten (number of fish with one or more scars and/or wounds) was on average 84 %. This study also suggests that African catfish may develop stereotypes; higher incidence of stereotypic behaviour occurred during the dark period of the day, when the fish were actively swimming and browsing.

Keywords: African catfish, behaviour, swimming activity, stereotypes.

This chapter has been submitted

P. Almazán Rueda, J.A.J. Verreth and J.W. Schrama. *Applied Animal Behaviour Science.*

Introduction

The African catfish *Clarias gariepinus* (Burchell, 1822), ranges from the Orange River in Cape Province of South Africa throughout Africa and into eastern Turkey (Hocutt, 1989). The broad geographical distribution of the species can be attributed to several aspects of its biology and ecology and by its ability to tolerate physiologically adverse environmental conditions (Hecht and Appelbaum, 1988). The presence of an accessory breathing organ enables this species to breath air when active or under very dry conditions (Teugels, 1986). *Clarias gariepinus* is an aggressive fish (Hecht and Appelbaum, 1988; Britz and Pienaar, 1992; Hecht and Pienaar, 1993; Kaiser et al., 1995a; Hossain et al., 1998). Because this fish species grows quickly, is omnivore and desirable as food, it is valuable for aquaculture worldwide (Khwuanjai Hengsawat et al., 1997).

Fish farming conditions, from extensive to highly intensive, may cause the same sort of behavioural problems are known from other animal production systems such as for pig and poultry. Behavioural problems such as reduced feed intake, aggression, stereotypes and death are examples of stress caused by intensification of farming conditions. These behavioural problems can be solved or at least reduced if we understand their causes. Therefore first we have to understand the “normal” behaviour of the fish under farming conditions. Normal behaviour is the one that has developed during evolutionary adaptation (Keeling and Jensen, 2002). Normal or natural behaviour should promote biological functioning in the sense of survival, health and reproductive success (Duncan and Fraser, 1997). Under farming conditions, normal or natural behaviour in combination with abnormal behaviour will appear caused by restriction of the area. To study fish behaviour under farming conditions is extremely difficult due to the high number of fish, making unambiguous observations almost impossible. Therefore studying fish behaviour needs to be done under experimental conditions, which mimic farming conditions.

The objective of this study is to develop base line data of the behaviour of the African catfish under “normal” and standardised conditions. Furthermore, in this study within day variation in behaviour is described.

Material and methods

Animals, adaptation period and experimental conditions

Juveniles of African catfish were obtained from a commercial hatchery (Maatschap Fleuren en Nooijen, Someren, The Netherlands) and held in four aquaria during an adaptation period, which lasted two weeks. During this period fish were fed a pelleted feed (with a size of 2 mm) by means of self-feeders. During the adaptation period, he photoperiod was 14L:10D (lights on at 07:00 hr), and light intensity was 150 lux (measured at the water surface).

At the start of the 6 week experimental period, fish weighed 69.2 ± 6.8 g (SD). Fish were randomly assigned to one of 4 aquaria (90x45x45 cm). Each aquarium was stocked with 30 fish. The aquaria were within a recirculation system and the average water flow through each aquarium during the experiment was maintained at approximately 5 $l.min^{-1}$. Water depth was maintained at approximately 30 cm. Water temperature was 24.9 ± 0.9 ºC; oxygen concentration 7.2 ± 0.3 $mg.l^{-1}$, and pH ranged between 6.5 and 7.3.

Experimental set-up

The experimental treatment was 12 hours darkness and 12 hours light (12D:12L) where lights were on from 07:00 to 19:00 h. Light was provided by 60 watt bulbs (frosted-mat; Rodulphus B.V, Scheepselktro, Duiven, The Netherlands) providing 150 lux of light at the water surface. Fish were allowed free access to feed by means of self-feeders that were connected to a computer. The self-feeders released approximately 0.8 g of feed every time the pendulum was hit, from this the total feed demanded at day and at night was calculated. During the experimental period, formulated feed (commercial catfish diet, Trouvit ME-2 meerval, Trouw Nutrition, Nutreco, The Netherlands) was fed in the form of floating 3-mm pellets. The dry matter of the diet was 91.2 %, and on dry matter basis the content of crude protein content was 48.3 %, crude fat 9.7 %, ash 9.8 % and gross energy of 19.0 $kJ.g^{-1}$.

Quantification of behaviour

Fish behaviour was recorded by digital video recording system (Net DVR-5016H LG, Seoul, Korea) four days before the end of the experiment at day 38. Digital video recordings were made during 24 hrs for each of the aquaria. During the video recordings, red light bulbs (15 W, darkroom lamp, red, Phillips, The Netherlands) together with the normal lighting system were used during the 24 h. Low intensity red illumination was chosen with the intention of observing the fish behaviour during the dark period of the day.

The fish's behaviour was divided into two categories: swimming (while browsing, moving, eating and air breathing), and resting (lying on the bottom of the tank). Furthermore, air breathing and eating activity were recorded. Every five min, the number of fish in each of these categories was counted as well as the total number of visible fish on the monitor. The activity patterns were expressed as a percentage of the total number of fish counted.

Aggressive behaviour could not be quantified by video recordings, because aggressive acts could not be clearly distinguished. In the current study aggressive behaviour was quantified by counting the number of scars and wounds on the body of individual fish at the end of the experiment at day 42. The number of scars and/or wounds was divided according to the different location on the body: on the head, abdomen, side, fins and tail of the fish. From these data, the average numbers of scars plus wounds per fish was calculated per aquarium. Furthermore, the percentage of fish being bitten (i.e., having one or more scars or bites) was calculated per tank.

Literature data on stereotypic behaviour in fish is scarce. Stereotypic behaviour is characterised by a repetitive behaviour pattern in animals (often seemingly non-functional behaviour). In the current study, stereotypic behaviour of African catfish was defined as continuously and compulsive swimming under a fixed pattern (often in circles) for a period of time. In this study fish stereotypes were divided in the two following categories according to the time involved: Type I, fish that were continuously and compulsive swimming under a fixed pattern between 10 to 59 seconds; and Type 2, fish that were continuously and compulsive swimming under a fixed pattern between 60 to 240 seconds. The distinction between Type I and Type II stereotypic behaviour was made on the assumption that Type I stereotypic behaviour would be the early development stage of the more severe Type II stereotypic behaviour. Stereotypic behaviour was recorded by all occurrence sampling. The frequency of the occurrence of both classes of stereotypic behaviour was recorded per tank during the total 24 h of recording. Furthermore, escaping behaviour was recorded by all occurrence sampling. This behaviour was defined as follows: when the fish tried to go out of the tank and more than half of its body was above the water level and exposed to air.

Statistics

The effect of light versus dark periods within a day (phase) on behaviour and feed demanded was assessed by repeated measurement analysis (PROC GLM, SAS, 1990). The error terms of these analyses were tested for homogeneity of variance and normality. Percentages were arcsine transformed before repeated measurement analysis was carried out.

Results

Behaviour of the fish

Results of the present study showed that the African catfish (*Clarias gariepinus*) spent most of their time swimming. This is reflected in the average percentage of fish swimming during the overall observation period of 24 h (75 %) (Table 1). Air breathing activity was 1.3 % of the total activity of the fish. The average number of injuries per fish was 1.8 (Table 1). The vast majority of these scars and/or wounds were found on the side of the fish (1.34) followed by the head (0.20), abdomen (0.17) and fins (0.05). No scars and/or wounds on the tail were found. The percentage of fish bitten (number of fish with one or more scars) was on average 84 %. At the end of the experiment the survival rate was in average 98.3 %.

Table 1. Behaviour patterns of the African catfish (*Clarias gariepinus*) under conditions of 12L:121D photoperiod. Mean values are expressed as percentage of active or resting fish, eating and air breathing. The escaping behaviour, scars and/or wounds on the fish body and stereotypes are expressed as frequency. All mean values were taken from the 24 h cycle.

	Average	SD
Escaping behaviour (*frequency per tank*)	12.0	3.6
Behaviour		
Swimming activity (%)	75.7	11.4
Air breathing (%)[a]	1.3	0.92
Eating (%)[a]	1.6	1.05
Resting (%)	24.3	11.4
Injuries per fish *(aggression)*		
Total scars on body	1.76	0.36
Head	0.20	0.05
Tail	0.0	0.00
Abdomen	0.17	0.06
Side	1.34	0.33
Fins	0.05	0.05
% of fish bitten	84	5.6
Stereotypes (*frequency per tank*)		
Type 1	17.0	2.9
Type 2	6.25	2.2
Survival rate (%)	98.3	3.3

[a] Percentage of contribution of eating or air breathing to swimming activity

Stereotypes were recognised by the continuously and compulsive swimming under fixed pattern (often in circles) for a period of time. Figure 1 shows the patterns that were recognised during the stereotypic behaviour. Generally, the fish follow a circle pattern in one of the quadrants (corner) of the aquarium (Figure 1a); or the same circle pattern in two quadrants of the aquarium (Figure 1b). Other stereotypic behaviour was continuously swimming describing a triangle shape (Figure 1c) using three quadrants of the aquarium. Stereotypic behaviour was divided in two types: type I and type II. The average time that the fish spent in type I stereotype was 0.35 sec (minimum 10 and maximum 57 sec). The average time that the fish spent in type II stereotype was 1 min with 55 sec (minimum 1:10 min and maximum 3:05 min). The occurrence of type I stereotype was almost three times higher compared with type II stereotype (17 vs 6.2 times) (Table 1). The average frequency of escaping behaviour was 12 attempts per tank during a 24 cycle. This escaping behaviour was performed during times where high swimming activity was observed in the aquaria.

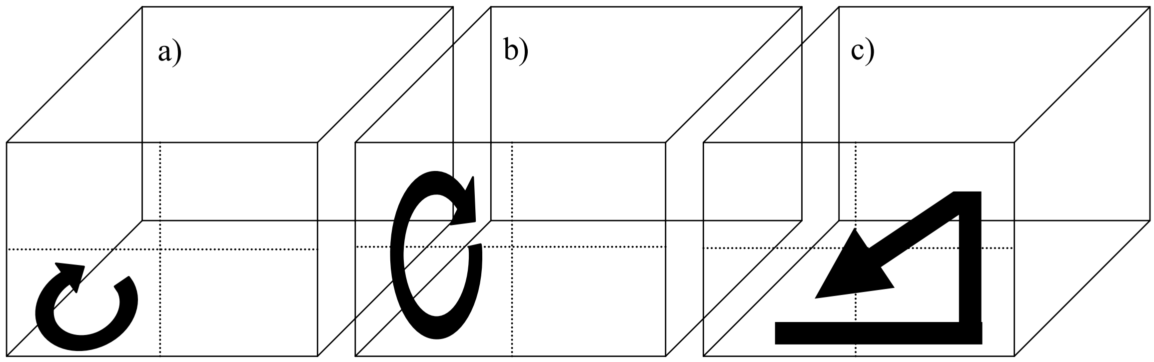

Figure 1. Stereotypic behaviour of the African catfish (*Clarias gariepinus*). Stereotypic behaviour was defined as continuously and compulsory swimming under fixed pattern often in a circles (a and b) or in a triangle (c) for a period of time. The arrows show the line of the fish movements during the stereotypic behaviour.

Day/night behaviour

The number of active fish was higher at night (84.5 %) than at day (58.8%) (*P*=0.01) (Table 2). Air breathing activity was different between dark and light periods. During the light phase of the day, fish air-breathed nearly 3.5 times more than at night (0.33 and 1.19 % respectively; *P*=0.04) (Table 2). A similar effect was found on the percentage of fish eating recorded by the video. The percentage of fish eating was higher in the light (3.04%) compared to the dark phase of the day (0.18%) (*P*=0.04; Table 2). This eating behaviour was also reflected by the results of the demand feeders. Fish demanded more feed during the light period (62.5 %) compared with the dark period (37.5 %) (*P*=0.02; Table 2). Stereotypic behaviour was also influenced by periods of the day. Numerically (but not statistically), type I stereotype occurred more during the light compared to the dark phase (9.5 vs. 6.5 times per tank, respectively). Type II stereotype was higher during the dark (5.0) compared to the light phase of the day (1.2) (*P*=0.01, Table 2). Escaping behaviour was not affected by dark or light periods.

Table 2. Behaviour patterns of the African catfish (*Clarias gariepinus*) at day and at night under conditions of 12L:121D photoperiod. Mean values are expressed as percentage of the total active or resting fish, eating or air breathing. The feed demanded was calculated from the total feed demanded and expressed as percentage at day and at night.

Conditions	12D:12L			
Period	Light	Dark	SEM	***P***
Escaping behaviour *(frequency per tank)*	6.25	5.75	1.49	n.s.
Behaviour				
Swimming activity (%)	66.9	84.5	2.19	0.0105
Air breathing (%)[a]	2.19	0.33	0.37	0.0405
Eating (%)[a]	3.04	0.18	0.61	0.0472
Resting (%)	33.1	15.4	2.19	0.0107
Stereotypes *(frequency per tank)*				
Type I	9.5	6.5	1.5	n.s.
Type II	1.25	5.0	0.53	0.0154
Feed demanded (%)	62.53	37.47	10.2	0.0248

Light period was from 07:00 to 19:00 h and dark period was from 19:00 to 07:00 h.

[a] Percentage of contribution of eating or air breathing to swimming activity

n.s. = not significant (P>0.05)

Within day variation of behaviour

Results of the present study showed that the African catfish (*Clarias gariepinus*) is active both at night and at day. This is reflected in the observations done every 5 min (Figure 2). Figure 2 shows a constant activity after the lights switched off (19:00 h). This swimming activity remained rather constant until the lights switched on (07:00 h). At this time, fish gradually had more resting periods until noon and from this, activity increased again till 16:00 h. At this point fish had more resting time and activity started to rise after 18:00 h. (Figure 2). Breathing activity of the fish changed during the 24 h (Figure 3). The percentage of fish that were air breathing during the beginning of the dark period was decreasing through the night to zero just prior to the start of the light period. At the beginning of the light period, air breathing increased again until the end of the light period when the highest percentage of air breathing fish was recorded (Figure 3).

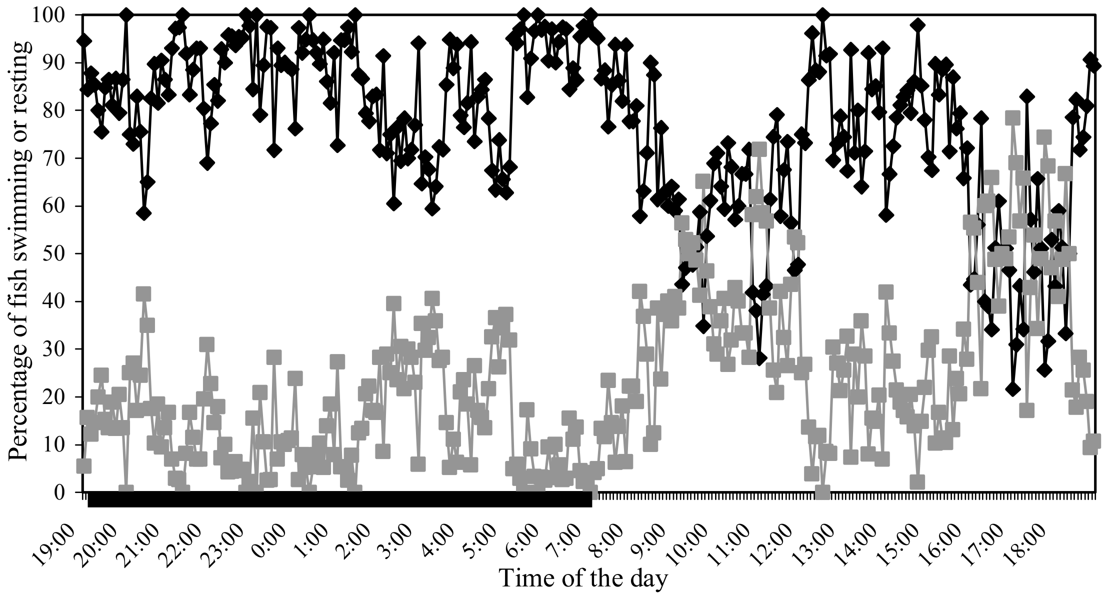

Figure 2. Fish activity during 24 hours cycle. Mean values were calculated from the observation period. Black bar at the bottom of the graph represents the dark period. Observations were made every five min and the total number of active and resting fish was counted, from this the total number of fish was calculated and presented as percentage of fish active or resting. Black lines referred to swimming and gray lines referred to resting activity.

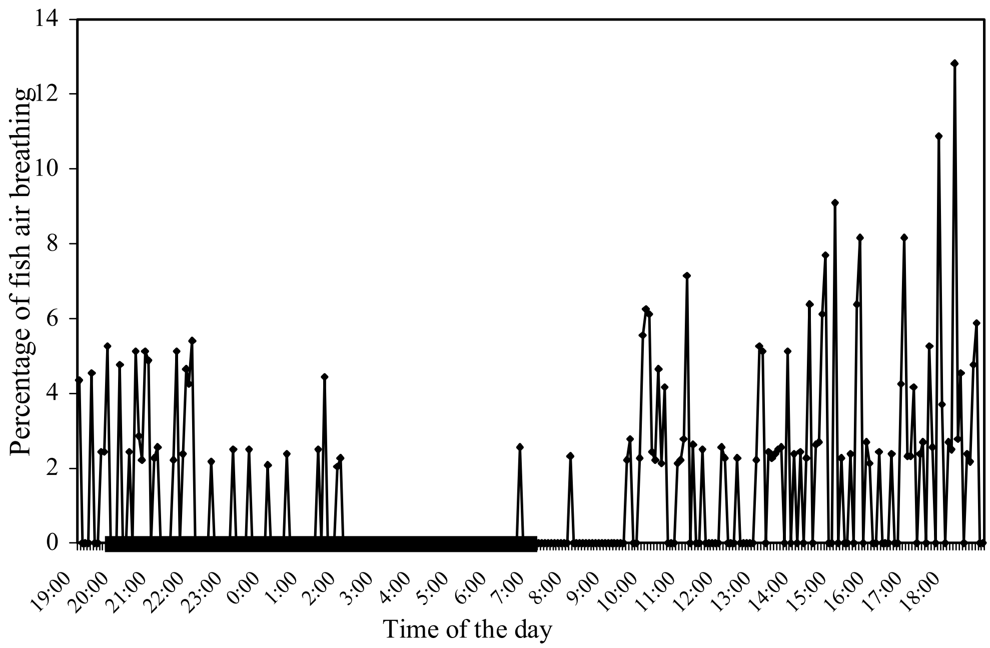

Figure 3. Fish air breathing activity during 24 hours cycle. Mean values were calculated from the observation period. Black bar at the bottom of the graph represent the dark period. Observations were made every five min and the number of fish air breathing was counted and expressed as a percentage of the total observed fish.

Discussion

All animals, either wild or domesticated, are social. In domesticated animals the expression of some characteristic behaviour depends on two things: the species characteristics, and the management that they are submitted to. In studies with African catfish larvae, territorial and aggressive behaviour was recorded (Britz and Pienaar, 1992; Kaiser, et al., 1995 a, b; Hecht and Uys, 1997). *C. gariepinus* juveniles also are aggressive, at least under the conditions of the stocking density (250 $fish.m^{-3}$) and light (12D:12L similar to natural conditions) of the present study. This aggression was reflected in the number of bites and/or wounds on the fish body and the percentage of fish being bitten. Aggression results in skin lesions and fin damage. This in turn increases their susceptibility to disease and weakens the fish, making them more liable to cannibalism or death as a consequence of their wounds (Kaiser et al, 1995a). This aggression may therefore results in stock losses, and in turn to depressed food conversion efficiency and growth due to the higher energy requirement (Hecht and Uys, 1997). It seems that light provokes an indirect effect on juvenile *C. gariepinus* aggression by increasing swimming activity which in turn promotes multiple encounters between individuals and enhances aggressive behaviour (Appelbaum and McGeer, 1998). In the present study, swimming activity was the predominant activity during the 24 h cycle. 75 % of the fish were engaged in swimming and this enabled more encounters between fish. These encounters resulted in a higher number of bites and/or wounds on the side of the fish.

The African catfish is considered as both a nocturnal and a day light feeder (Appelbaum and McGeer, 1998). The results of the present study showed that *C. gariepinus* demanded almost two-thirds of the daily ration during the light periods of the day. These results are contrary to those of Hossain et al., (1999), who found that *C. gariepinus* ate two-thirds of the daily ration at night. This contradiction may suggest that husbandry history of the fish prior to the experiment can play an important role in the future development of their behaviour. Another plausible explanation however is the presence of other stimuli, which triggered the feeding demand during the day, such as: checking the self-feeders and refilling them by the staff of the experimental facility. In both cases, further research must be done to elucidate the precise mechanism behind these observations. Nevertheless, swimming activity was higher during dark compared to light periods of the day. This finding agrees with Hossain et al., (1999) who reported that African catfish are nocturnally active. Britz and Pienaar (1992) reached a similar conclusion on studies in African catfish larvae.

Air breathing activity in *C. gariepinus* is similar as in other clariid catfish (Chapman and Chapman, 1994; Singh and Hughes, 1971). Fish before reaching the water surface generally released bubbles of air prior to the breathing bout, as the fish was turning to return to the bottom of the aquarium. This air breathing activity parallels the feeding activity and it appeared not to be associated with the fish swimming activity. This activity was increased from the moment when lights were switched on, reaching its maximum point at the last hour of light. This activity was gradually decreased to zero from the start of the dark period to almost the middle of the night. It is possible that air-breathing activity compensates for a reduced level of O_2 in the water, which is associated with feeding. During the day, fish demanded more feed. While fish eat more, they need more oxygen for their metabolism and at the same time less oxygen is available due to the excretion of metabolites.

Literature data on stereotypic behaviour in fish is scarce. Stereotypic behaviour is characterised by a repetitive behaviour pattern in animals (often seemingly non-functional behaviour). Stereotypes might be the inevitable outcome of a restricted environment (Jensen, 2002) and have often been related to the exposure to chronic stress induced by sub-optimal environments (Masson, 1991; Mench and Mason, 1997; Robert, et al., 1997). The development of stereotypes is often a response to frustration, thwarting or a conflict of motivations (Würbel and Stauffacher, 1997). The results of the present study suggest that African catfish may develop stereotypes. In the present study, higher incidence of stereotypes (Type II stereotype) was performed during the dark period of the day, when the fish were actively swimming and browsing but not eating. Stereotypes in captive animals often seem to derive from normal foraging behaviour (Mason and Mendel, 1997). Regarding the frequency of stereotypes, it should be realised that the absolute frequency of stereotypic behaviour was low: Type 1 and Type 2 stereotypes occurred about 17 and 6.25 per 30 fish during 24 h, respectively.

Conclusion

The African catfish *Clarias gariepinus* behaviour is variable during the 24 h. Day or night increased or depressed some activities. Fish spent more time swimming during the dark and performed more stereotypes (Type II). During the day, fish spent less time swimming, had more air-breathing activity and demanded more feed.

Acknowledges

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Chapter 3

Effect of feeding method on water quality, feed consumption and growth of the African catfish (*Clarias gariepinus*) under different light regimes

Abstract

This study assessed the effect of feeding method (continuous by self-feeders vs. twice a day hand feeding), light intensity (15 vs. 150 lux) and photoperiod (continuous light vs. 12D:12L) on water quality and growth of juvenile African Catfish (*Clarias gariepinus*). Sixteen aquaria, each with 30 fish (average initial weight of 55 g) were used in a 6 week experiment, and measurement of total ammonia nitrogen (TAN) and suspended solids (SS) were used as indicators of water quality. Feeding method did not affect growth rate. However, self-fed fish demanded 16.3% more feed that was provided by hand feeding. Light intensity did not affect performance (growth, feed consumption and feed efficiency), whereas photoperiod did. Fish under the 12D:12L photoperiod had higher feed consumption and growth rate than fish exposed to continuous light. Feeding method influenced water quality, with SS production being almost doubled in self-fed fish compared with those fed by hand fed, whereas TAN production was higher under 15 lux compared to 150 lux. TAN tended to increase during the night and decrease during the day, whereas SS tended to increase during the day and decrease during the night. Despite of the similar growth, self-fed fish demanded more feed compared to hand fed fish. Based on performance data, juvenile African catfish (*C. gariepinus*) seem to be insensitive to light intensity but are influenced by photoperiod. A 12D:12L photoperiod increases growth and feed consumption compared with 0D:24L .

Keywords: Claridae, feed intake, weight gain, light intensity, photoperiod, water quality.

This chapter has been submitted.

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Introduction

Aquaculture inevitably leads to the production of wastes. It discharges water, rich in nutrients and biodegradable wastes. In fish farming, water quality is affected by various factors: animal related factors (e.g., species, age), environmental factors (e.g., rearing conditions), and nutritional factors (e.g., diet formulation). The major part of the waste produced by fish farms originates from feed (Cho et al*.,* 1994). The amount of feed ingested by the fish will depend on the husbandry conditions and the method of feed delivery; the combination of both may result in differences in the production of waste feed (Thorpe et al., 1990, 1995).

Environmental conditions can affect feed intake, growth and water quality. Factors, such as photoperiod and light intensity, may be important in this context. Fish can behave in a variety of ways under different conditions and on time of day. The mechanisms underlying the interactions (feeding method, photoperiod and light intensity) are not well understood, but many physiological pathways may be influenced (Boeuf and Le Bail*,* 1999). Boujard et al*.,* (1991) suggest that the circadian rhythms in fish are synchronised by both photoperiod and light intensity, with circadian variations influencing the metabolic utilisation of feed by the fish. A synchronisation of the rearing conditions with the biological rhythms of the fish can improve the efficiency of production (Boujard et al*.,* 1990). Effects of photoperiod on growth, feed demand, and circulating metabolites have been studied in several species (see for example Stefansson et al*.,* 1989; 1991; Boujard et al., 1990; 1991; Berg et al*.,* 1992; Jørgensen et al*.,* 1992; Baras et al*.,* 1998; Houlihan *et. al*. 2001). Light intensity also has been study but mainly in marine species, such as Atlantic salmon (Stefansson et al*.,* 1990; Oppedal et al., 1997).

The fresh water *Clarias gariepinus*, is widely cultivated world wide, especially in Europ, Africa and Middle East, but little is known about the effect of different husbandry conditions on feed intake and growth. Most of the work, which has been done on the African catfish (*C. gariepinus*) under such conditions, has focused on larval stages (Appelbaum and McGeer, 1998; Hossain et al., 1999; Appelbaum and Kamler, 2000; Nwosu and Holzlöhner, 2000). Moreover, the indirect effect of husbandry conditions (e.g., feeding method, photoperiod and light intensity) on water quality and feed wastage has not been well documented for juveniles *Clarias gariepinus* kept in recirculation systems.

The aim of the present study was to assess the influence of feeding method, light intensity and photoperiod on feed demand and growth of juvenile African catfish reared in recirculation systems. A secondary aim was to investigate the effects of the above factors on water quality by monitoring total ammonia-nitrogen (TAN) and suspended solids (SS) production during 24 hours cycle.

Material and Methods

Animals, adaptation period and experimental conditions

Juveniles of African catfish were obtained from a commercial hatchery (Maatschap Fleuren en Nooijen, Someren, The Netherlands) and held in four aquaria (two weeks) for adaptation to the recirculating water system of the experimental facilities at Wageningen University. During this period they were fed pelleted feed (pellet size, 3 mm) at 3% of bw.d^{-1} (two equal meals) at 09:00 and 17:00 h. The photoperiod was 14L:10D (lights on at 07:00 h), and light intensity was 150 lux measured at the water surface. Light intensity was measured by a lux meter (model zu 104766, AEG, Frankfurt, Germany).

At the start of the 6-week experimental period, fish were 47-weeks old and weighed 55 ± 11.3 g (SD). Fish were randomly assigned to 16 aquaria (90x45x45 cm, 120-L) with 30 fish per aquarium. The aquaria were within a recirculation system and water flow through each aquarium was approximately 5 l.min^{-1} on average over the total period of the experiment. Average water depth was maintained at approximately 30 cm. Water temperature was 26.6 ± 0.2 ºC; oxygen concentration 6.5 ± 0.3 mg.l^{-1}, and pH ranged between 6.2 and 7.1.

Experimental set-up

Each of the 16 aquaria was assigned to one of eight experimental treatments according to a 2x2x2 factorial design to assess the effect of photoperiod, light intensity and feeding method (two replicates per treatment). Two levels of light intensity were compared: 15 versus 150 lux. Light was provided by bulbs (frosted-mat; Rodulphus B.V, Scheepselktro, Duiven, The Netherlands); 15 and 60 Watt bulbs providing respectively 15 and 150 lux of light at the water surface. The two photoperiods were continuous light (0D:24L) versus 12 h darkness and 12 h light (12D:12L). Lights were on from 08:00 to 20:00 h using a timer. All fish were fed to satiation by two different feeding methods, either continuously available or twice daily provided by hand. Commercial self-feeders (model: CJ feed automat 1 P, pendel type. 5 liter. Catvis. s'Hertogenbosh, The Netherlands) were used for the continuous feeding and comprised of a hopper fitted with a pendulum. The self-feeder was calibrated to deliver on average 0.8 gram of 4-mm pellets per strike of the pendulum. The pendulum was placed 2 cm above the water surface. For the hand-feeding method, fish were fed at 09:00 and 17:00 h to apparent satiation. During the experimental period, a commercial catfish diet was fed (Dana Feed A/S. Horsens, Denmark) in the form of floating 4-mm pellets. The proximate analysis of the diet (dried basis) was 90% dry matter, 48.3% crude protein, 5% crude fat and 7 % ash and gross energy of 18.9 $kJ.g^{-1}$.

Measurements

Fish were sampled at days 0, 14, 28 and 42. At each sampling time, individual fish weight was measured per aquaria. Specific growth rate (SGR) was calculated from the natural logarithm of mean final weight minus the natural logarithm of the mean initial weight and divided by total number of experimental days expressed as percentage. The coefficient of variation (C.V.) was calculated from individual body weight of each aquarium to study the influence of the treatments on the variation in weight. Feed intake per aquaria was recorded daily over the total experimental period. Additionally, in aquaria under hand feeding treatment, the amount of feed consumed during the first 5 min of feeding was registered; from this and the total feed intake, the percentage of feed consumed during these 5 min was calculated. Feed conversion ratio (FCR) was calculated per aquaria from feed intake data and weight gain.

Water quality was assessed by measuring total ammonia (TAN) and suspended solids (SS) during a 24 hour cycle. This was done at 38 of the experiment.

Feed demanded at the hand feeding method during the sampling day was as follows: treatment 150 lux and 12D:12L: 227.3 g, treatment 15 lux and 12D:12L: 251.3 g, treatment 150 lux and 00D:24L: 194.3 g, and treatment 15 lux and 00D:24L: 154.1 g. Water samples were taken from the water outlet of each aquarium and from the inlet of one aquarium (assuming identical input for the rest of the aquaria). Samples were taken every two hours. Water flow through each aquarium was measured at each sampling time. TAN concentration was measured immediately after sampling by Ammonia Electrode Model 95-12 and pH/ISE meter Model710A (both Orion Research Incorporated. Massachusetts, U.S.A.). The SS water samples were stored at –20°C, and thawed samples was measured by filtering 50 ml of water under vacuum through pre-weighted glass microfibre filters (7.0 cm. GF/C Whatman, Whatman International Ltd. Maidstone, England). Filters were dried in an air oven at 75ºC to constant weight. Total daily TAN and SS production were calculated for each aquarium over the total measuring period. Within day variation of TAN and SS production was also calculated for each two-hour period according to the following formula:

$$P_{t1-t2}=Q*((Co_{t1}+Co_{t2})/2-(Ci_{t1}+Ci_{t2})/2)*t+V*(Co_{t2}-Co_{t1})$$

Where: P_{t1-t2} = TAN or SS production during the time period, Q = flow rate through the aquarium ($L.h^{-1}$), Co = concentration of TAN or SS at the outlet (in $g.L^{-1}$), Ci = concentration of TAN or SS at the inlet (in $g.L^{-1}$), t = time interval between samples, t1 = sampling moment 1, t2 = sampling moment 2, and V = aquaria volume (in L).

Statistics

In this study, aquaria were used as experimental unit. Mean performance data (including the calculated C.V. in final body weight per aquaria), total TAN and SS production over the total 24 h measurement period were analysed for the effects of photoperiod, light intensity and feeding method using a three-way ANOVA (using PROC GLM, SAS, 1990) which included interaction effects. For each 2 hour sampling interval within the 24 hour measuring cycle, TAN and SS production were subjected to the same statistical analysis.

Results

Performance

Final body weight and specific growth rate (SGR) were not influenced by both feeding method and light intensity. Fish held under 12D:12L at tended to be heavier and tended to have higher SGR than fish kept at continuous light (0D:24L) (P<0.10; Table 1), and this coincided with higher feed demand (P<0.01, Table 1). Feed consumption was 4.84 $g.d^{-1}$ and 5.96 $g.d^{-1}$ for fish kept at 0D:24L and at 12D:12L, respectively. Feed consumption (including feed waste) was affected by feeding method (P<0.05). Continuous feeding by self-feeders resulted in a higher demand than hand feeding (5.78 and 5.06 $g.d^{-1}$, respectively). Despite the differences in feed consumption and SGR between treatments, feed conversion ratio (FCR) was not significant different among treatments (Table 1). Survival rate and the variation in final body weight within aquaria were similar among treatments (Table 1).

Table 1. Final body weight, coefficient of variance in final body weight within aquaria (C.V.), specific growth rate (SGR), feed conversion ratio (FCR), feed consumption and survival rate in African catfish as affected by the experimental treatments.

	Experimental treatment											
Feeding method	Continuous feeding				Hand feeding (twice a day)					P-value main effects		
Photoperiod	0D:24L		12D:12L		0D:24L		12D:12L			Feeding	Photo-	Light
Light intensity [a]	Low	High	Low	High	Low	High	Low	High	SEM	method	period	intensity
Final body weight (g)	401.9^{wx}	359.8^{yz}	432.1^{w}	429.3^{w}	328.3^{z}	366.5^{xyz}	415.3^{w}	392.2^{wxy}	36.29	ns	0.0713	ns
C.V. in final body weight (%)	24.5	25.5	21.8	27.6	30.3	25.6	21.8	22.3	2.61	ns	ns	ns
SGR (%.d^{-1})	4.6	4.5	4.8	4.9	4.3	4.5	4.7	4.7	0.23	ns	0.0991	ns
FCR (g.g^{-1})	0.68	0.67	0.73	0.73	0.62	0.66	0.67	0.70	0.04	ns	ns	ns
Feed demanded (g.d^{-1})	5.5^{wx}	4.9^{wx}	6.4^{w}	6.3^{w}	4.0^{x}	4.9^{wx}	5.1^{wx}	5.6^{w}	0.47	0.0496	0.0099	ns
Survival (%)	100	100	100	100	97	98.5	98.5	100	1.29	ns	ns	ns

[a] Low light intensity was 15 lux and high light intensity 150 lux both measured at the water surface.

^{wxyz}Means within a row lacking of a common superscript differ significantly ($P < 0.05$).

Table 2. Average values per experimental treatment of daily production of total ammonia (TAN) and suspended solids (SS) per aquaria with a stocking density of 30 African catfish.

	Experimental treatment											
Feeding method	Continuous feeding				Hand feeding (twice a day)					P-value main effects		
Photoperiod	0D:24L		12D:12L		0D:24L		12D:12L			Feeding method	Photo-period	Light intensity
Light intensity [a]	Low	High	Low	High	Low	High	Low	High	SEM			
TAN ($g.d^{-1}$)	15.2 ^{a}	8.9 ^{b}	25.8^{a}	8.2 ^{b}	9.4 ^{b}	9.3 ^{b}	10.9 ^{ab}	8.6 ^{b}	4.97	n.s.	n.s.	0.0519
SS ($g.d^{-1}$)	63.2 $^{a b}$	77.3 ^{a}	102.7 ^{a}	109.3 ^{a}	52.2 ^{b}	53.3 ^{b}	42.2 ^{b}	49.5 ^{b}	13.29	0.0021[b]	n.s.[b]	n.s.

[a] Low light intensity was 15 lux and high light intensity 150 lux both measured at the water surface.

[b] Suspendid solids (SS) was influenced by the interaction effect between photoperiod and feeding method (*P*<0.0410)

[xyz] Means within a row lacking of a common superscript differ significantly (P < 0.05).

The feeding behaviour of fish under hand feeding was assessed by recording the amount of feed consumed during the first five minutes of feeding. This amount of feed was calculated as a percentage of the total feed consumption. Fish ate more of the morning meal within the first five minutes than was the case for the afternoon meal (P<0.001; Figure 1), and this differed between photoperiod treatments in the morning (P<0.0003) but not in the afternoon (P>0.1; Figure 1). Furthermore, an interaction between photoperiod and light intensity was present (P<0.0018) during the morning meal. The effect of photoperiod and feed intake in the morning meal was larger at low light intensity. The feed intake during the first five minutes of the morning meal at the low light intensity was 85 and 95.7 % for the continuous and 12D:12L photoperiods respectively, and at the high light intensity 90.1 and 92.5 % respectively. Fish under 12D:12L and low light intensity responded eagerly to eat compared with fish under continuous light and high light intensity.

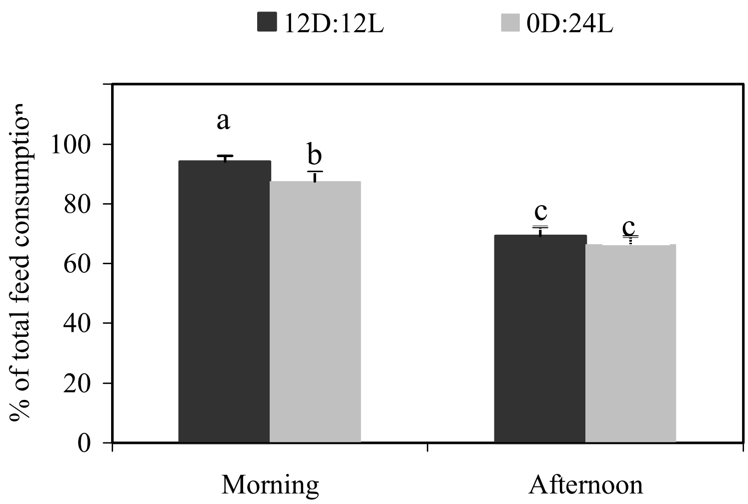

Figure 1. Feed consumption during the first 5 minutes of feeding as percentage of the total feed intake in African catfish, which were hand fed twice a day (morning and afternoon) to satiation feeding level. Bars with different letters are significantly different (P<0.05).

Total ammonia nitrogen and suspended solids.

Total production of TAN and SS was affected by treatments (Table 2). The total production of TAN was not affected by feeding method or photoperiod, but was affected by light intensity (P<0.0519). TAN values were higher in aquaria under 15 lux compared to those at 150 lux. The total production of SS under continuous feeding was almost double compared to the hand feeding regime (P<0.002). The total production of SS was also affected by an interaction between photoperiod and feeding method (P>0.05). Within day variation in the production of TAN was markedly influenced by light intensity, slightly affected by feeding method but not by photoperiod (Figure 2). Within day variation in the production of SS was markedly influenced by slightly affected by light intensity but not by photoperiod (Figure 3). TAN tended to increase during the night and decrease during the day (Figure 2), whereas SS tended to increase during the day and decrease during the night (Figure 3).

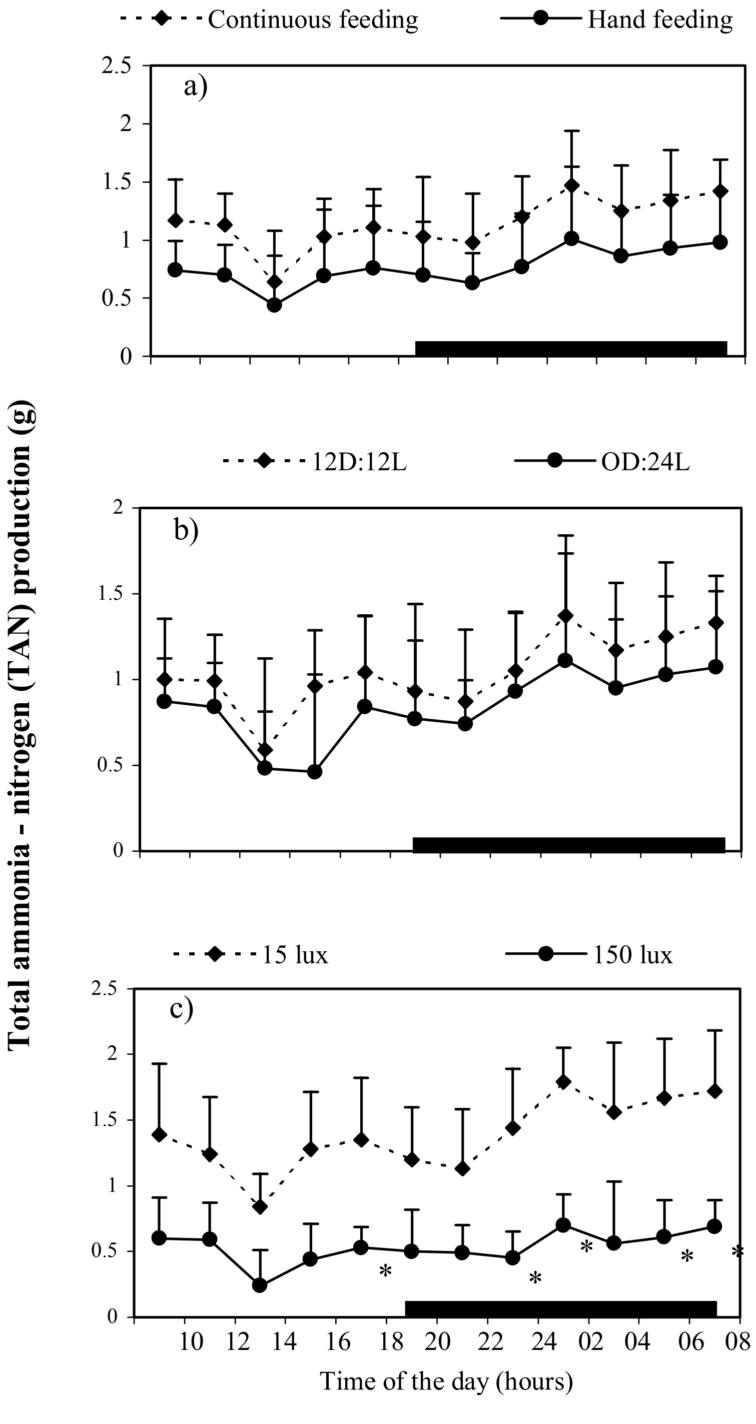

Figure 2. Within day variation of the total ammonia-nitrogen (TAN) production of the African catfish, as affected by feeding method (part a), photoperiod (part b) and light intensity (part c). The vertical arrows indicate the feeding moment for the hand feeding method and the dark line indicates the 12 h of darkness period of the 12D:12L treatments. Asterisk (*) indicates a significant difference between means within a 2 hours sampling period.

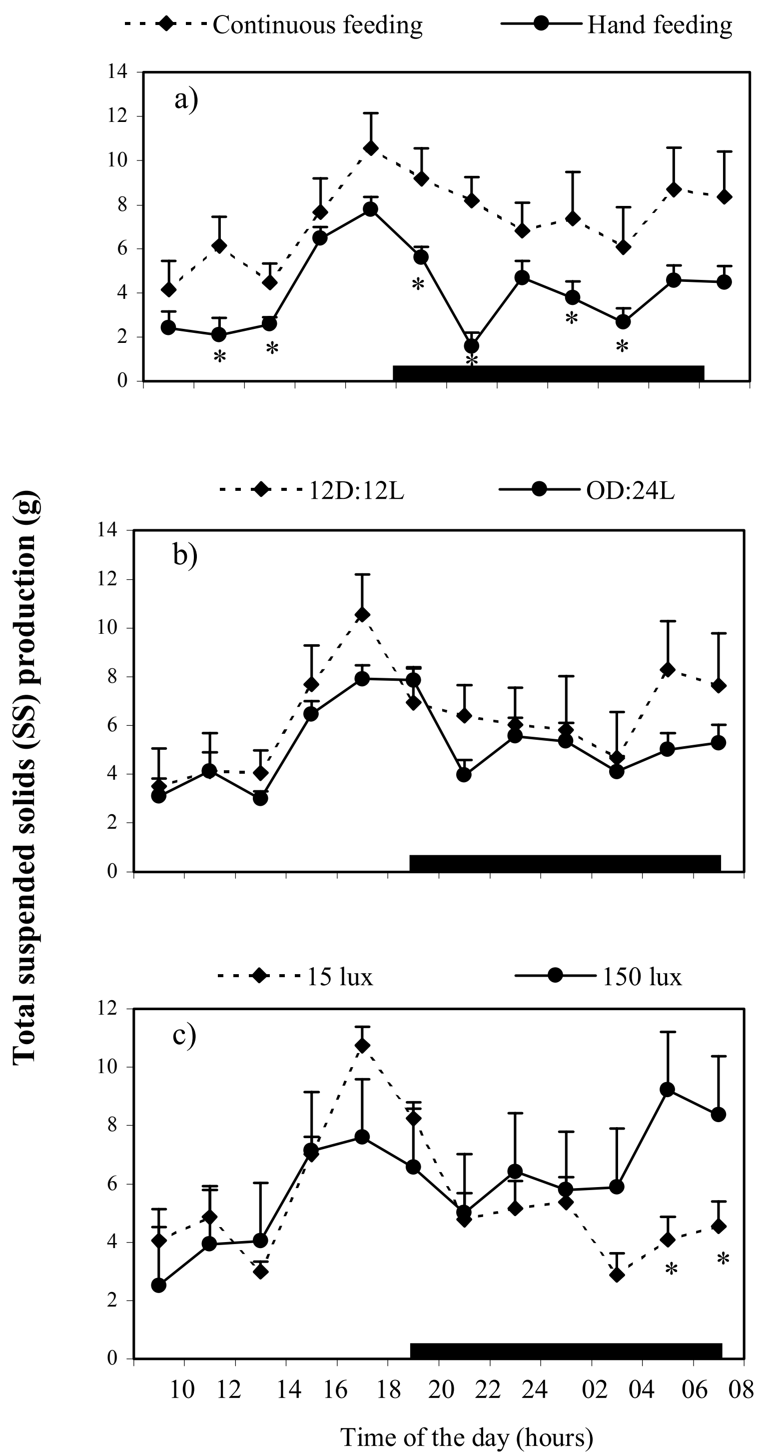

Figure 3. Within day variation of the total suspended solids (SS) production of the African catfish, as affected by feeding method (part a), photoperiod (part b) and light intensity (part c). The vertical arrows indicate the feeding moment for the hand feeding method and the dark area indicates the 12 h of darkness period of the 12D:12L treatments. Asterisk (*) indicates a significant difference between means within a 2 hour sampling period.

Discussion

Nowadays, there are several ways to measure feed demand in fish, from the most sophisticated systems (Brännäs and Alanärä, 1993), to the simplest ones. One of the simplest and commonly used in the past is by hand feeding to satiation during one or more meals a day. Hand feeding give a reliable results due to the direct observation of the feed demand by fish; on the other hand, hand feeding is time consuming and in most of the fish farms do not used. Feed demand by demand feeders is widely studied (see for example Thomassen and Fjæra*,* 1996; Paspatis and Boujard, 1996; Fast et al*.,* 1997; Houlihan, et al., 2001). One of the reasons for using demand feeders is to reduce losses of uneaten food, thereby saving food costs and reducing the wastewater load (Alanärä et al*.,* 1994). In the current experiment the use of self-feeders nearly doubled the production of suspended solids in the aquarium effluents, compared with the hand feeding method. The measured SS coincided with only a 15.1 % higher feed demanded from the self-feeders. It seems that most of the excess of feed demanded by fish under the self-feeder regime was wasted, or at least, less efficiently used. This is also reflected in the similar growth performance under the two feeding methods; as well as the slightly higher FCR under continuous feeding (0.70, compared to 0.65 under the hand-feeding method). This suggests that the fish may have been playing with the pendulum.

The average concentration of TAN and SS was increased by 34.9% and 5.8% respectively, under a 12L:12D regime compared to continuous light. This increase of TAN and SS is related to the amount of feed that the fish demand under these conditions. TAN concentrations increased during the night and decreased during the day, while the opposite pattern was observed for SS concentrations in the water. These may be related to the excretion time for ammonia and SS. First, ammonia is released to the water (highest peak between 6-12 hours after feeding) (Fraser, et al., 1998), and then faeces, which peaks several hours later. In relation to SS production, an important interaction was found between photoperiod and feeding method. The production of these two parameters is higher at 12D:12L compared to continuous light (both under continuous feeding). Hand feeding twice daily gave no difference, irrespective of the photoperiod.

Calculation of SS and TAN production can have some discrepancies. To calculate production (of SS or TAN) using a “curvilinear model” can be used when a static system is used; that means higher concentration of SS and/or TAN is going out of the aquarium at the beginning compared to the end of a certain time. A "linear model" as it was used to calculate the SS and TAN in the present study, assumes that the concentration of SS and/or TAN is going out of the aquarium is constant due to the interaction of the constant production by the fish and the movement of the fish. In both cases, the time involve between samples is very important. In the present study, the linear equation gave reasonable values. For example, fish were fed 250 g at the sampling day, the digestibility of dried matter is 80%, one should find back around 50 g of faeces and that is approximately the values that are found under hand-feeding method. This 250 g were fed the fish at the day of the sampling time, not the average value of feed intake of the total experiment as it showed in the tables.

The photoperiod of 12L:12D influenced the overall performance of *C. gariepinus*. Fish grew better, and had a better SGR, compared with those under continuous light. Similar results were found by Stickney and Andrews (1971), in studies with channel catfish (*Ictalurus punctatus*) where a slight tendency for improved growth and somewhat lower food conversion ratios under a 12D:12L photoperiod was demonstrated, but no significant differences were found among treatments. The mechanism of this photoperiod effect on growth is not well understood in fish. Physiological mechanisms, such as increased hormone production (e.g. somatotropin), this increased in hormones may improve feed conversion efficiency (Purchase et al*.,* 2000). According to Britz and Piennar (1992) photoperiod may induce changes in the level of aggressive behaviour, and thus fish waste less energy in territorial behaviour. *C. gariepinus* is a photophobic species, Appelbaum and Kamler (2000) found that *C. gariepinus* larvae reared in the dark, showed an increased survival and growth. It seems that juveniles of *C. gariepinus* also need a dark phase during 24 hours. The better growth under a 12L:12D photoperiod, is also related with the feed consumption, an increase of 18.8% of feed demand compared to the continuous light was found. This is in contrast to the findings of Boujard et al., (1991), who reported that *Hoplosternum littorale* (also a nocturnal fish) showed no differences in feed intake when light period was artificially increased. For some other species day length may indirectly modify growth by increasing food consumption or muscle mass by exercise (Boeuf and Le Bail, 1999). Based on performance data of the present experiment, juvenile African catfish (*C. gariepinus*) seem to be insensitive to light intensity but are influenced by photoperiod.

Photoperiod also plays an important role in fish activity. Fish under 12D:12L showed more activity and quicker demand of feed in the first five minutes of feeding (two times a day) compared to continuous light. Afternoon meal did not show difference in feed demand. Hossain et al., (1999) found similar results, fish that were deprived of food during the night, showed increased feeding activity once the feed was made available, and feeding activity was decreased during the day.

Conclusion

Hand-feeding twice a day reduces suspended solids production, by almost 100%, compared with continuous feeding by self-feeders.

Based on performance data, juvenile African catfish (*C. gariepinus*) seem to be insensitive to light intensity but are influenced by photoperiod. A 12D:12L photoperiod increases growth and feed consumption compared with 0D:24L .

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Chapter **4**

Behavioural responses under different feeding methods and light regimes of the African catfish (*Clarias gariepinus*) juveniles.

Abstract

Little is known about the behaviour of fish under culture conditions. Several factors may have a direct effect on fish behaviour and its variations during the day. This study assessed the effect of feeding method (continuous by self-feeders vs. twice a day hand feeding), light intensity (15 vs. 150 lux) and photoperiod (continuous light vs. 12D:12L) on behaviour of juvenile African Catfish (*Clarias gariepinus*). Sixteen aquaria, each with 30 fish (average initial weight of 55 g) were used during a 6 week experimental period. Fish behaviour was recorded by direct observation. Fish were more active under hand-feeding regime than under self-feeding. Fish spent more time swimming under continuous light than under a 12D:12L. Furthermore, the time that fish spent swimming was higher at high light intensity (150 lux) than under low light intensity (15 lux). Aggression was affected by photoperiod and light intensity. Continuous light resulted in 41.6 % more scars and wounds than the 12D:12L photoperiod, while high light intensity resulted in 2.46 times more scars and wounds than low light intensity. Fish that spent more time swimming and browsing, were more aggressive and they had more scars and wounds on the body. Fish under the hand-feeding method showed a higher activity during the morning compared to the afternoon. Fish also showed a higher activity before each meal (morning and afternoon meals) than afterwards. These activities were more evident during the mornings. The current study demonstrated that for juveniles of the African catfish, swimming activity and agonistic behaviour are strongly affected by husbandry conditions.

Key words: behaviour, African catfish, husbandry conditions

This chapter has been accepted.

P. Almazán-Rueda, J.W. Schrama and J.A.J. Verreth. Aquaculture.

Introduction

Little is known about the behaviour of fish under culture conditions. Like other animals, fish often defend their food and territory (Grant, 1997). These activities find their origin in the need to balance the need to eat and the need to avoid being eaten (Thorpe and Cho, 1995). In fish, activities, such as aggression, results in skin lesions and in fin damage. This in turn increases their susceptibility to disease and weakens the fish making them more liable to cannibalism, or death as a consequence of their wounds (Kaiser et al., 1995a). Aggressive activities also cost a lot of energy which otherwise would be used for growth (Hecht and Uys, 1997). Thus, aggression can result in stock losses, reduced food conversion efficiency and in slower growth.

Several factors may have a direct effect on fish behaviour and its variations during the day. These factors include food availability, light intensity and photoperiod. Hecht and Pienaar (1993) showed that in the African catfish, *Clarias gariepinus*, a gradual reduction in food availability resulted in an increase in territorial behaviour and the number of aggressive acts. On the other hand, photoperiod and light intensity are strong factors synchronising the endogenous cycles of metabolism and activity (Boeuf and Le Bail, 1999). The inherited behaviour of fish is not suppressed under culture conditions (Thorpe and Cho, 1995), but could be influenced by manipulating some factors such as feeding method, photoperiod and light intensity. In larvae of the African catfish feeding frequency is a major factor affecting behaviour (Hecht and Pienaar, 1993). Furthermore, behaviour is influenced by light intensity, photoperiod and density (Page and Andrews, 1975; Britz and Pienaar, 1992; Thorpe and Cho, 1995; Kaiser et al., 1995b; Appelbaum and McGeer, 1998; Hossain et al., 1998; Boeuf and Le Bail, 1999; Appelbaum and Kamler, 2000; Nwosu and Holzlöhner, 2000). The majority of these studies have tried to quantify cannibalism and aggression in larvae and juveniles (Hecht and Appelbaum, 1988; Britz and Pienaar, 1992; Hecht and Pienaar, 1993; Kaiser et al., 1995b; Hossain et al., 1998). Only a few studies have been done regarding cannibalism and aggression in larger juveniles (Hecht and Uys, 1997).

Therefore, the aim of the present study was to assess how feeding method, photoperiod and light intensity, and their interactions, would affect the behaviour (activity and agonistic behaviour) of juvenile African catfish. Furthermore, in one of the feeding methods (hand feeding), the effect of light intensity and photoperiod on within day variation in behaviour was assessed.

Material and Methods

Animals, adaptation period and experimental conditions

Juveniles of African catfish were obtained from a commercial hatchery (Maatschap Fleuren en Nooijen, Someren, The Netherlands) and held in four aquaria for adaptation to the recirculating water system of the experimental facilities at Wageningen University. During this period they were fed pelleted feed (pellet size, 3 mm) at 3% of bw.d^{-1} (two equal meals) at 09:00 and 17:00 h. The photoperiod was 14L:10D (lights on at 07:00 h), and light intensity was 150 lux measured at the water surface. Light intensity was measured by a luxmeter (model zu 104766, AEG, Frankfurt, Germany).

At the start of the 6-week experimental period, fish were 47-weeks old and weighed 55 ± 11.3 g (SD). Fish were randomly assigned to one of 16 aquaria (90x45x45 cm, 120-L) with 30 fish per aquarium. The aquaria were within a recirculation system and water flow through each aquarium was approximately 5 l.min^{-1} on average over the total period of the experiment. Average water depth was maintained at approximately 30 cm. Water temperature was 26.6 ± 0.2 ºC; oxygen concentration 6.5 ± 0.3 mg.l^{-1}, and pH ranged between 6.2 and 7.1.

Experimental set-up

Each of the 16 aquaria was assigned to one of eight experimental treatments according to a 2x2x2 factorial design to assess the effect of photoperiod, light intensity and feeding method (two replicates per treatment). Two levels of light intensity were compared: 15 versus 150 lux. Light was provided by bulbs (frosted-mat; Rodulphus B.V, Scheepselktro, Duiven, The Netherlands); 15 and 60 watt bulbs providing respectively 15 and 150 lux of light at the water surface. The two photoperiods were continuous light (0D:24L) versus 12 h darkness and 12 h light (12D:12L). Lights were on from 08:00 to 20:00 h using a timer. All fish were fed to satiation by two different feeding methods, either continuously available or twice daily provided by hand. Commercial self-feeders (model: CJ feed automat 1 P, pendulum type. 5 liter. Catvis. s'Hertogenbosh, The Netherlands) were used for the continuous feeding and comprised of a hopper fitted with a pendulum. The self-feeder was calibrated to deliver on average 0.8 gram of 4-mm pellets per strike of the pendulum. The pendulum was placed 2 cm above the water surface. For the hand feeding method, fish were fed at 09:00 and 17:00 h to apparent satiation. During the experimental period, a commercial catfish diet was fed (Dana Feed A/S. Horsens, Denmark) in the form of floating 4-mm pellets. The proximate analysis of the diet (dried basis) was 90% dry matter, 48.3% crude protein, 5% crude fat and 7 % ash and gross energy of 18.9 $kJ.g^{-1}$.

Quantification of behaviour

Fish behaviour was recorded by direct observation. These behavioural observations were carried out after and before feeding time (09:00 and 17:00 h) for the hand feeding method. One observation (around noon) was recorded for the self-feeding method. Each aquarium was observed for 8 days throughout the experimental period. Two stopwatches were used; one was set up to countdown for five minutes and the other was used to record the swimming activity time. The first stopwatch was turned on when the observation started, and the second stopwatch was switched on or off according to the swimming activity of the fish. For the purposes of this experiment, fish activities were divided into three categories: swimming (movement of the fish for swimming, browsing and air breathing), resting (lying on the bottom of the tank) and aggressive activities (chases, body bites, barbel fights and lateral displays). During the observation time, one fish was selected and observed throughout the five-minute period. As it was not always possible to observe the selected fish over the entire period, another fish was immediately selected from the position where the first fish was last observed and the observation period continued. Swimming activity was measured in seconds. Resting time was defined as 300 seconds (five minutes) minus the swimming activity time. Aggressive behaviour was quantified by counting the number of aggressive acts, and was expressed as a frequency. Furthermore, the effect of aggression was quantified by counting the number of scars and wounds on the body of each fish at the end of the experiment per tank. From these data, the average numbers of scars per fish was calculated per tank. Furthermore, the percentage of fish being bitten (i.e., having one or more scars or bits) was calculated per tank.

Statistics

In this study, aquaria were used as the experimental units. Mean behaviour data were tested for the effect of photoperiod, light intensity and feeding method using a three-way ANOVA (using PROC GLM, SAS, 1990), which included all interaction effects. The error terms of these ANOVA analyses were tested for homogeneity of variances and normality. Spearman's correlation coefficients between average swimming activity, average number of aggressive acts and average number of scars per treatment were calculated.

Results

Feeding Method

Feeding method influenced the overall daily activities of the fish. Fish activity under the hand-feeding regime was slightly but significantly higher (Figure 1 and Table 1; P=0.017) than under self-feeding. Feeding method did not influence the average number of bites per fish, but the percentage of fish bitten was higher under the hand-feeding regime (P=0.034), compared to the self-feeding (47.4% vs. 39.6%, respectively). Survival was not affected by feeding method (Table 1).

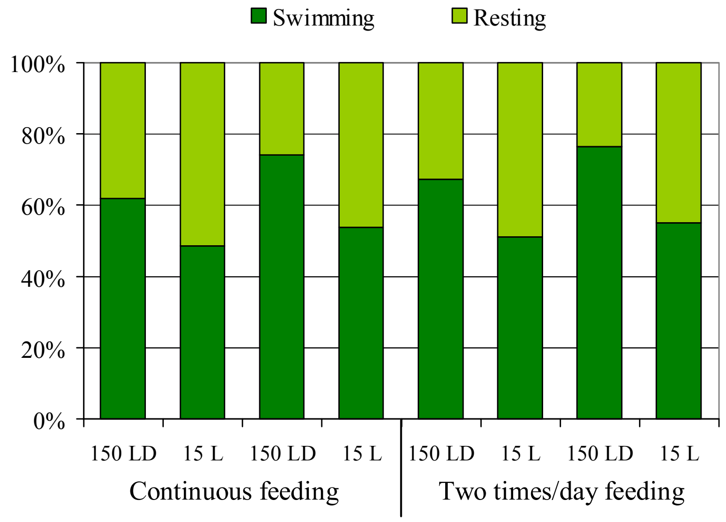

Figure 1. Temporal behavioural patterns of African catfish juveniles (*Clarias gariepinus*) according to treatments Mean value is for each treatment, with 60 fish per treatment, and expressed as a percentage of the time observed. 150 and 15 means 150 and 15 lux respectively. LD means photoperiod of 12L:12D. L means continuous light.

Photoperiod and light intensity

Swimming activity was affected by photoperiod (*P*=0.001, Table 1). Fish spent more time swimming under continuous light than under a 12D:12L. The frequency of aggressive acts was 13.7% higher under continuous light conditions than under a 12D:12L regime. These aggressive acts were also reflected in the number of scars and wounds on the body. Continuous light resulted in 41.6 % more scars and wounds than the 12D:12L photoperiod (Table 1); most of these scars and wounds were located on the side of the fish body (*P*=0.020). Photoperiod did not affect the number of fish bitten and in addition had no effect on the survival rate.

Table 1. Behaviour patterns of the African catfish as affected by the experimental treatments. Mean values are expressed as seconds for the activities; frequency for the aggressions acts during five minutes observation and number for the scars on body.

	Experimental treatment											
Feeding method	Continuous feeding				Hand feeding (twice a day)					P-value main effects		
Photoperiod	0D:24L		12D:12L		0D:24L		12D:12L			Feeding	Photo-	Light
Light intensity [a]	Low	High	Low	High	Low	High	Low	High	SEM	method	period	intensity
Activity												
Swimming activity	160.7^{w}	221.5^{z}	145.9^{v}	185.1^{x}	165.4^{w}	229.2^{z}	153.3^{vw}	201.5^{y}	4.25	0.0172	0.0001^{b}	0.0001^{b}
Resting	139.3^{w}	78.5^{z}	154.1^{v}	114.9^{x}	134.6^{w}	70.8^{z}	146.7^{vw}	98.5^{y}	4.25	0.0172	0.0001^{c}	0.0001^{c}
Aggression												
Aggressive acts	11^{z}	35.5^{x}	6^{z}	25^{y}	9^{z}	35.5^{x}	10^{z}	29.5^{y}	1.49	ns	0.0012^{d}	0.0001^{d}
Number of scars on body per fish												
Total scars	0.53^{yz}	1.33^{vw}	0.35^{z}	1.16^{vwx}	0.72^{xyz}	1.66^{v}	0.49^{yz}	0.98^{wxy}	0.18	ns	0.0143	0.0001
On the head	0.10^{yz}	0.20^{yz}	0.12^{yz}	0.08^{z}	0.10^{yz}	0.27^{y}	0.14^{yz}	0.13^{yz}	0.05	ns	ns^{e}	ns^{e}
On the side	0.42^{yz}	1.13^{vw}	0.23^{z}	1.00^{vwx}	0.55^{xyz}	1.39^{v}	0.30^{z}	0.85^{wxy}	0.16	ns	0.0200	0.0001
Fish bitten (%)	33.3^{yz}	46.6^{wxy}	28.3^{z}	48.3^{wx}	35.0^{xyz}	64.9^{v}	31.6^{z}	55.0^{vw}	4.17	0.0344	ns	0.0001
Survival (%)	100	100	100	100	97	98.5	98.5	100	1.99	ns	ns	ns

Note: on the total scars on body are included bitten fins and ventral scars of the fish, not showed in the results.

[a] Low light intensity was 15 lux and high light intensity was 150 lux, both measured at the water surface.

^{bcde} Interaction effect between photoperiod and light intensity: ^{b}(P=0.015); ^{c}(P=0.015); ^{d}(P=0.018) and ^{e}(P=0.047);

^{vwxyz} Means within a row lacking of a common superscript differ significantly (P < 0.05).

ns = not significant (P>0.005).

In general, all fish activities were influenced by light intensity (Figure 1, Table 1). Fish spent more time swimming under high light intensity (150 lux) than under low light (15 lux; Table 1). The frequency of aggressive acts was 3.4 times higher in fish under 150 lux than 15 lux. These aggressive acts were also reflected in the number of scars and wounds on the body. High light intensity resulted in 2.46 times more scars and wounds than low light intensity. Most of these scars and wounds were on the side of the fish body (*P*=0.001; Table 1). The number of fish bitten was 67% higher under 150 lux comparing to 15 lux. Light intensity did not affect the survival rate (Table 1).

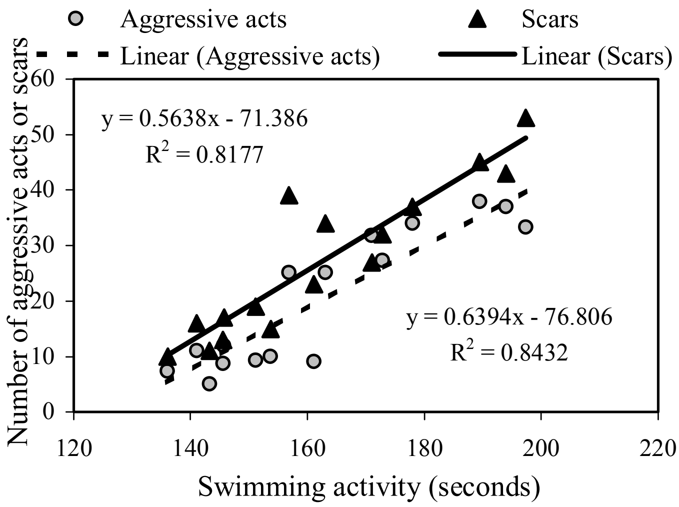

Figure 2. Spearman's correlation between average swimming activity, average numbers of aggressive acts and average number of scars ($P<0.05$).

Aggression of the fish was also affected by an interaction between light intensity and photoperiod (*P*=0.018, Table 1). More aggressive acts were recorded under high light intensity and continuous light; whereas fish showed 3.4 times less aggressive acts under 12D:12L and low light intensity.

A correlation was found between the swimming activity, the number of aggressive acts and the total number of scars and wounds on the fish body (*P*=0.018, Figure 2). In tanks where fish spent more time swimming and browsing, fish were more aggressive and they had more scars and/or wounds on the body. A similar correlation was found between total number of scars and/or wounds on the fish body and the number of aggressive acts per treatment (Figure 3).

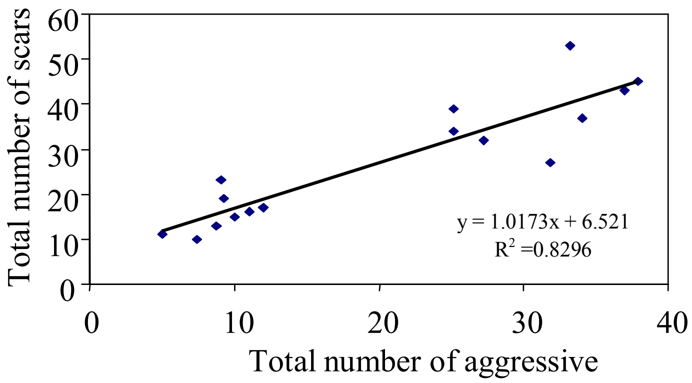

Figure 3. Relation between the average total number of aggressive acts and average total number of scars on body (P<0.05).

Within day variation

Feeding method

Fish under the hand-feeding method showed a higher activity during the morning compared to the afternoon (Figure 4). Fish also showed a higher activity before each meal (morning and afternoon meals) than afterwards. However, the increase in activity before feeding was higher in the morning than in the afternoon (P=0.041).

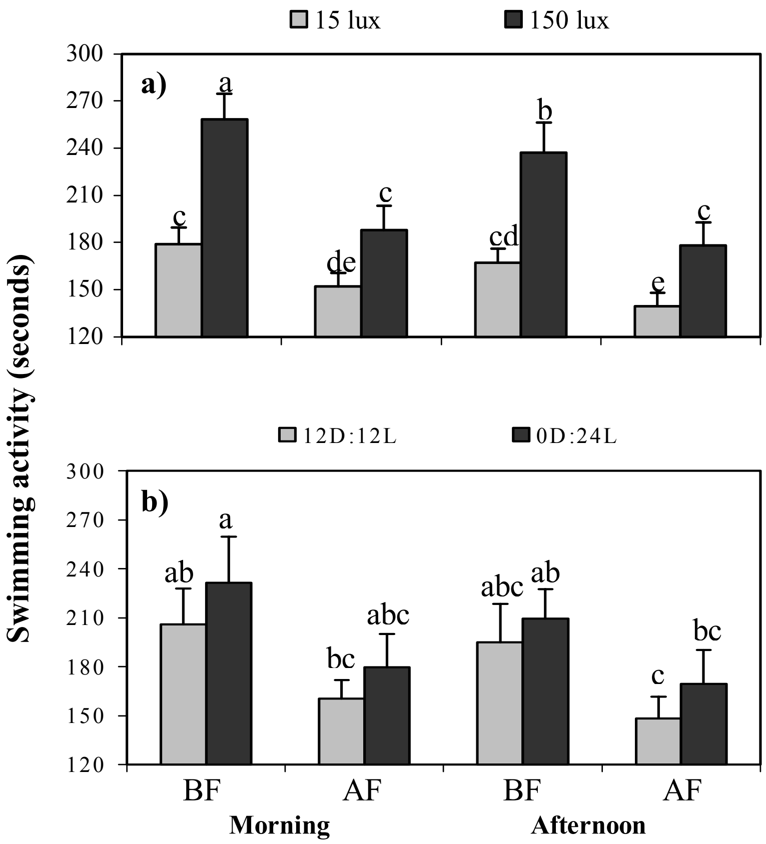

Figure 4. Swimming activity patterns of the African catfish (*C. gariepinus*) from the treatments of hand feeding; a) according to light intensity; and b) according to photoperiod. Mean values are expressed as seconds of the observation period (300 seconds). BF= before feeding; AF= after feeding. Different letters indicates significant difference (P<0.05).

Photoperiod and light intensity

Fish activity is not constant within a daily cycle and seems to be correlated with feeding times. Swimming activity was always higher before feeding than after feeding and this difference between pre and post feeding was larger in the morning than in the afternoon. It occurred at all treatments, however it was affected by the light treatments (intensity or photoperiod). Fish swimming activity under the hand-feeding method showed similar patterns under both photoperiods and light intensities, although activity was higher under continuous light than under a 12D:12L regime

(P=0.001, Figure 4). Aggression was not affected by the interaction between photoperiod and feeding time (09:00 and 17:00 h) (Figure 5).

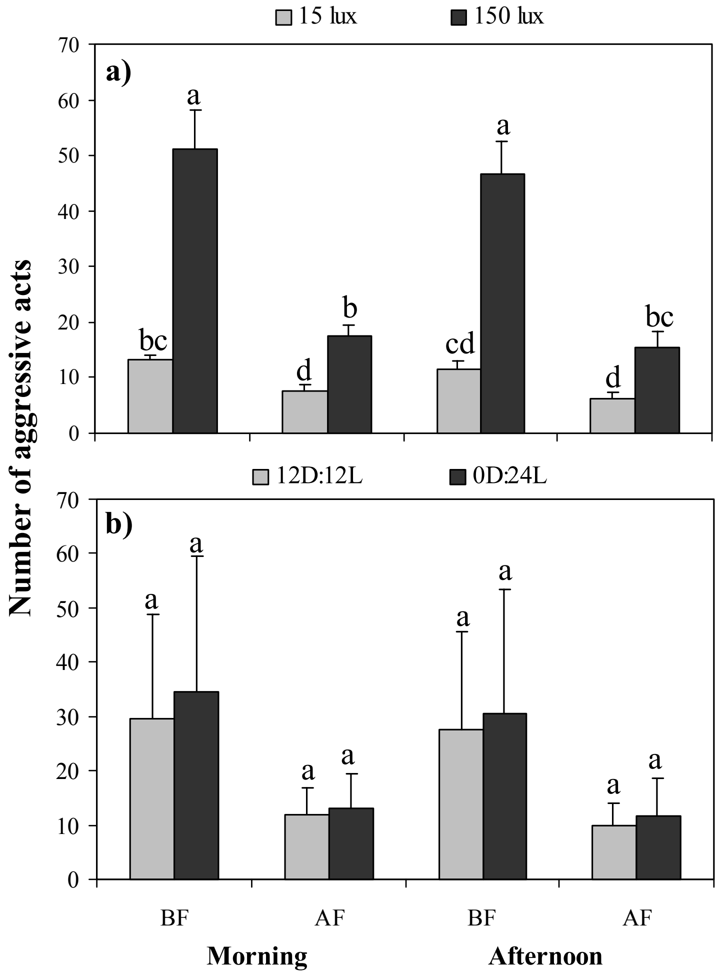

Figure 5. Number of aggressive acts of the African catfish (*C. gariepinus*) from the treatments of hand-feeding. a) according to light intensity; and b) according to photoperiod. Mean values are expressed as frequency of the observation period (300 seconds). BF= before feeding; AF= after feeding. Different letters indicates significant difference (P<0.05).

In the hand-fed fish, the swimming activity pattern within the day was affected by light intensity (Figure 4) indicated by the interaction between light intensity and feeding (meal) time (09:00 and 17:00 h) (P=0.001). Swimming activity of fish under 150 lux was higher in the morning than in the afternoon, whereas swimming activity of fish under 15 lux did not differ between the morning and afternoon (Figure 4).

Furthermore, the increase in swimming activity during the pre-feeding observation compared with post-feeding was influenced by light intensity (P=0.001). The incline in swimming activity pre-feeding was higher in fish under 150 lux than under 15 lux (Figure 4). The within day variation in aggression was also influenced by light intensity (Figure 5). The increase in aggressive acts during the pre-feeding observation compared with post-feeding was influenced by light intensity (P=0.001). The increase in aggressive acts pre-feeding was higher in fish under 150 lux than under 15 lux (Figure 5). The number of aggressive acts was however unaffected by the interaction between light intensity and feeding (meal) time (09:00 and 17:00 h). The difference in aggressive acts between fish under 15 and 150 lux were similar during the morning and afternoon (Figure 5).

Discussion

Results from this study, regardless of the treatments, showed that a higher swimming activity leads to an increase in the incidence of aggressive acts and as a result, fish had higher number of scars and/or wounds on the body. As swimming activity is increased, a higher number of encounters appears, making the fish more susceptible to agonistic behaviour. A higher number of aggressive acts leads to skin lesions and fin damage, making the fish vulnerable to disease or even death. The present result showed conclusively that aggressive behaviour in African catfish is correlated with the number of scars and /or wounds on the body. Since direct observation of aggression behaviour is timing consuming, the incidence of scars and wounds provides a useful indicator of the level of aggressive behaviour.

Feeding method

In comparison to the other experimental factors (light intensity and photoperiod), feeding method had a relatively small effect on the activity of the catfish and seemingly no impact on their aggressive behaviour. A fish's day appears to be spent either pursuing food or avoiding predator (Helfman, 1993). The higher swimming activity in hand feed fish compared to fish fed by self-feeders in the current study might be related to the difference in availability of feed within the day. Helfman (1993) suggested that activity patterns in fish are strongly determined by the activity patterns of their prey. The constant availability of feed for the fish fed by self-feeding may have reduced the time spend on browsing and foraging and thereby increased the time spend resting. Differences in feeding level (i.e., food abundance) can affect both activity and aggressive behaviour in fish (Grant, 1997; Hecht and Pienaar, 1993). Despite the fact that the catfish at the hand feeding method were fed to satiation, the restriction of feed intake in time (twice a day) may have resulted in a lower feed intake, which consequently may have caused the increased swimming activity in the current study. However, such a confounding effect of feeding level with the feeding method treatment is unlikely, because there were no differences observed in growth rate between both feeding methods in the current study (data not shown).

The current data on the influence of feeding method on aggressive behaviour in African catfish seem to be conflicting. Although, feeding method had a significant effect on the percentage of fish bitten, it did not result in a significant difference in the total number of aggressive acts or scars per fish. The higher percentage of fish bitten at the hand feeding method can be explained by the increased time spend swimming. Consequently, the probability of contacts between fish and thereby the risk of being bitten is increased at the hand feeding method. On the other hand, the self-feeding method resulted in a lower percentage of fish bitten, but with a higher number of bites on the body of fish, which have been bitten. This implies that feeding method did influence the intensity of encounters. At the continuous feeding method by self-feeders the change of encounters, which resulted in aggressive behaviour is reduced but the intensity of the agonistic encounters is increased compared to the hand feeding method. These more intense aggressive encounters may be due to direct competition for feed when the pendulum is been hit.

Photoperiod and light intensity

This study showed that photoperiod affects the behaviour of African catfish juveniles. Fish exhibit a 24 h cycle in their activities, which may often be a matter of simple photokinesis, and this cycle can affect the fish behaviour (Boeuf and Le Bail, 1999). Fish under continuous light showed more swimming activity and aggressive behaviour and less resting time compared to a 12D:12L regime. Similar results were found by Appelbaum and McGeer (1998) where larvae of *C. gariepinus* grew better under continuous darkness while continuous illumination resulted in increased aggression, probably due to stress. This was also found in early juveniles of *C. gariepinus.* Under constant light conditions they were stressed and displayed a cover-seeking behaviour and low growth rates, whereas those in the dark conditions appeared normally active (Britz and Pienaar, 1992). These results are contrary to the suggestion by Hecht and Pienaar (1993) that continuous light would be better to keep fish dispersed throughout the water column thereby reducing cannibalism. Indeed, results from the current study indicate that continuous light keeps fish more active, but also more aggressive. The number of wounds and scars found on the fish body were affected by the light regime, confirming that continuous light is a factor that affects agonistic behaviour in *C. gariepinus*.

Also the intensity of the light was found to affect fish activity. Under 150 lux, fish were more active and high numbers of aggressive acts were recorded compared to a 12D:12L regime. As a result of this high rate of aggressive behaviour, a higher number of wounds and scars were found on the fish's' body at the end of the experiment. Britz and Pienaar (1992) found similar results. Their studies showed that avoidance of light and preference for dark areas is an innate behaviour among *C. gariepinus* larvae. Light, which is too intense, may be stressful or even lethal for fish (Boeuf and Le Bail, 1999). However, in the present study, light intensity did not influence mortality rates. Yet under high light intensity, especially under continuous illumination, fish showed a high stress response when the feed was spread over the tank. If a lot of food was given, fish moved immediately to the bottom of the tank and started to eat minutes later and only in small amounts.

It seems that interactions between husbandry conditions (feeding method, photoperiod and light intensity) have a major effect on the behaviour of the African catfish. The interactions between light intensity and photoperiod resulted in higher swimming activities and a higher incidence of aggressive acts. On the other hand, photoperiod and feeding method resulted in significantly higher aggressive acts. Light intensity seems to strongly affect the overall activity, resting and aggressive behaviour of *C. gariepinus*, followed by the photoperiod, and last by the feeding method.

Within day variation

Fish displayed more aggressive behaviour and activity before feeding in treatments under the hand-feeding method. This is in agreement with Kaiser et al., (1995a), who stated that fish become increasingly active around one hour before feeding (after getting used to the times at which they were fed). During the morning more activity and aggression was observed than in the afternoon, probably due to long period (16 h) of no feed availability from 17:00 to 09:00 h. Fish, in both cases, reduced activity and aggressive acts after feeding. Photoperiod and light intensity influenced fish behaviour in similar way. Continuous light and high light intensity made the fish more active and aggressive, particularly in the morning and before feeding, whereas swimming activity and aggressive acts decreased sharply in the afternoon and after feeding. These behaviour patterns could also be related to hunger or other internal physiological process (as opposed to the physical presence of feed). Furthermore, it might be related to the circadian rhythm of the fish.

Conclusions

In agreement with the literature on behavioural studies in larvae, the current study suggested that for juveniles of the African catfish, swimming activity and agonistic behaviour are affected by husbandry conditions (feeding method, photoperiod and light intensity). Continuous feeding seemed to reduce the swimming activity and increase the intensity of aggressive encounters between fish compared with the hand-feeding method. It seems that increasing the period of light and light intensity, and their interactions (light intensity*photoperiod) make the fish more active and aggressive.

With-in day variations showed that swimming activity and aggression was higher pre-feeding, compared to post-feeding. It is therefore extremely important for behaviour studies to establish the time and duration of the observation period. The effect of light intensity on aggression and swimming activity was also more pronounced at pre-feeding than post-feeding.

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Chapter 5

Stocking density affects growth and behaviour of the African catfish (*Clarias gariepinus*) reared under different light regimes

Abstract

Increasing the number of fish per culture unit is one of the most common practices under farm conditions to increase production. Stocking density is known to have a strong influence on growth, survival and behaviour of fish. This study assessed the effect of stocking density (250 vs. 2500 fish.m^{-3}), light intensity (15 vs. 150 lux) and photoperiod (continuous light vs. 12D:12L) on growth and behaviour of juvenile African Catfish (*Clarias gariepinus*). Sixteen aquaria were used during a 6 week experimental period. Fish kept at high stocking density were heavier and had a higher SGR than fish kept at low stocking density. FCR was lower at high than at low stocking density (0.62 versus 0.74; $P<0.05$). Photoperiod did not affect any performance trait except for the SGR, where it was higher in fish kept under continuous light than at 12L:12D (3.7 versus 3.5%; $P<0.05$). Light intensity had no influence on the performance of the catfish. Feed consumption and survival was not affected by the treatments. Swimming activity was higher in fish at high stocking density as compared to low stocking density. At low stocking density, the effect of light regime on activity diminished with time. Fish at the high stocking density showed a lower number of total scars and/or wounds on the body than at the low stocking density (5.3 versus 6.5 scars and/or wounds per fish; $P<0.05$). The influence of stocking density on stereotypic behaviour changed with time during the experimental period. Incidence of stereotypic behaviour was higher at low stocking density and at the end of the experimental period as compared to high stocking density and at the beginning of the experiment. Stereotypic behaviour was unaffected by photoperiod and light intensity. Glucose levels in plasma of fish at high stocking density were higher than in fish at low stocking density. Cortisol levels had a tendency toward higher values in fish at photoperiod of 12D:12L. Lactate levels in plasma were not affected by treatments. High stocking density (2500 fish.m^{-3}) in juvenile African catfish (*C. gariepinus*) increased growth and swimming activity, and reduced FCR, aggression (determined by means of scars on body) and stereotypic behaviour compared to low stocking density (250 fish.m^{-3}). This suggests that the low stocking density induced chronic stress. The occurrence of stereotypic behaviour is a potential indicator for measuring chronic stress in African catfish.

Key words: Husbandry conditions, behaviour, *Clarias gariepinus*.

This chapter has been submitted.

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Aquaculture.

Introduction

The annual growth rate of the aquaculture sector is estimated to be between 9 - 11% (FAO, 2002). This rapid growth is partially based on a continuous intensification of the production process, in which the improvement of husbandry conditions plays a critical role. Increasing the number of fish per culture unit is one of the most common practices under farm conditions to increase production. Although high stocking densities may be good for the revenue of the farm, stocking density is known to have a strong influence on growth and survival of fish (Hecht and Appelbaum, 1988; Haylor 1991, 1993; Siddiqui et al., 1993; Kaiser et al., 1995a, 1995b; Khwuanjai Hengsawat et al., 1997; Hossain et al., 1998). In addition, when the density of fish is increased, agonistic behaviour may occur. This agonistic behaviour may lead to severe wounds on the body and even to death. Thus aggression can result in stock losses, reduced food conversion efficiency and to lower growth because of the higher energy requirement (Hecht and Uys, 1997). Generally in fish, performance declines with increasing stocking density and aggressive behaviour increases. However, in larvae of the African catfish (*C. gariepinus*), stocking density has the opposite effect (i.e. high stocking densities reduce agonistic behaviour) (Hecht and Appelbaum, 1988; Kaiser et al., 1995b). Data on the effect of stocking density in juveniles catfish (> 20 g.) are scarce (Hecht and Uys, 1997).

In addition to density, light regime affects growth and behaviour (Britz and Pienaar, 1992; Thorpe and Cho, 1995; Appelbaum and McGeer, 1998; Hossain, et al., 1998; Boeuf and Le Bail, 1999). Until recently, little attention has been paid to light as a stressor affecting animal functions (Fanjul-Moles et al., 1998). Light acts as a powerful directive factor synchronising cycles of metabolism and activity in fish and other organisms (Hossain et al., 1999). Photoperiod not only limits the time searching for food but also can stimulate growth. Despite the limited information on the effects of light regime, it is assumed that growth and rate of food ingestion are maximised at certain ranges of light intensity, depending on the species (Nwosu and Holzlöhner, 2000). For example, stress, aggression and cannibalism were reduced and growth enhanced (Britz and Pienaar, 1992) when *C. gariepinus* larvae were reared under conditions of continuous darkness or low light intensity.

Whereas the factors mentioned above, stocking density, photoperiod or light intensity, may have an influence on the inhibition of growth and behaviour, other physiological processes are also disturbed, such as plasma cortisol, glucose and lactate. Fish under stress characteristically show elevated cortisol levels in the blood. Cortisol is known to promote gluconeogenesis in teleost fish. Thus energy reserves are mobilised to cope with the increased metabolic demand at the expense of growth (Vijayan et al., 1990).

Relatively little is known about the effect of husbandry conditions such as density and light regime, on agonistic behaviour and stress parameters in African catfish juveniles, especially in older juveniles (> 50 g). Therefore, the purpose of the present study was to assess the influence of stocking density, light intensity and photoperiod (and their interactions) on food intake, growth, social behaviour (swimming and resting activities), aggressive behaviour (measured by means of wounds and/or scars on body), stereotypic behaviour, and stress parameters (plasma cortisol, glucose and lactate) of the African catfish (*Clarias gariepinus*) juveniles.

Material and Methods

Animals, adaptation period and experimental conditions

Juveniles of African catfish were obtained from a commercial hatchery (Maatschap Fleuren en Nooijen, Someren, The Netherlands) and held in four aquaria to enable adaptation to the recirculating water system. During this period they were fed pelleted feed (pellet size was 3 mm) at 3% of bw.d^{-1}. Feed was given by hand in two equal meals at 08:00 and 16:00 h. The photoperiod was 14L:10D (lights on at 07:00 h), and the measured light intensity at the water surface was 150 lux. Light intensity was measured with a lux meter (model zu 104766, AEG, Frankfurt, Germany).

At the start of the 47-day experimental period (day 0), the fish were 14-weeks old and weighed 23 ± 0.9 g (SD). Fish were randomly assigned to one of 16 glass aquaria (90x45x45 cm) with a water volume of about 120 L. Treatments were randomly assigned to the aquaria. The aquaria were within a recirculation system and water flow through each aquarium was approximately 5 $l.min^{-1}$ on average over the total period of the experiment. Water depth was maintained at approximately 30 cm. In the aquaria, water temperature was 24.2 ± 0.8 ºC, oxygen concentration 6.5 ± 0.3 $mg.l^{-1}$, and pH ranged between 6.4 and 7.1.

Experimental set-up

Each of the 16 aquaria was assigned to one of eight experimental treatments according to a 2x2x2 factorial design to assess the effect of light intensity, photoperiod and stocking density (and their interactions). Two levels of light intensity were compared: 15 versus 150 lux. Light was provided by bulbs (frosted-mat; Rodulphus B.V, Scheepselktro, Duiven, The Netherlands); 15 and 60 Watt bulbs providing respectively 15 and 150 lux of light at the water surface. The two photoperiods were: continuous light (0D:24L) versus 12 h darkness and 12 h light (12D:12L). Lights were on from 07:00 to 19:00 h using a timer. Two different stocking densities were used: a high stocking density of 300 fish per tank (2,500 $fish.m^{-3}$) versus a low stocking density of 30 fish per tank (250 $fish.m^{-3}$). The high stocking density is equal to the stocking densities applied in recirculation systems of Dutch catfish farms. Consequently the low stocking density in this experiment was only 10% of the commonly applied density in Dutch catfish farms. All fish were fed to apparent satiation level by hand feeding. Fish were fed twice a day at 08:00 and 16:00 h. Feed was provided to the fish, until the fish stopped eating and two pellets were left in the tanks. During the experimental period, a commercial catfish diet was fed (Dana Feed A/S. Horsens, Denmark) in the form of floating 3-mm pellets. The dry matter content of the diet was 94.2% and on dry matter basis the content of crude protein content was 49%, crude fat 11%, ash 11% and gross energy 19.6 $kJ.g^{-1}$.

Sampling procedures and measurements

Fish were sampled at days 0, 14, 28 and 42. At each sampling time, the total fish biomass per aquaria was measured. Specific growth rate (SGR) was calculated from the natural logarithm of mean final weight minus the natural logarithm of the mean initial weight and divided by total number of experimental days expressed as percentage. Feed intake per aquaria was recorded daily over the total experimental period. Feed conversion ratio (FCR) was calculated per aquaria from feed intake data and weight gain.

At each sampling time, five fish per tank were anaesthetised with tricaine methanesulfonate (MS-222, 0.35 $g.l^{-1}$ (Crescent Research Chemicals, Phoenix, AZ, USA) and 0.7 $g.l^{-1}$ of $NaHCO_3$). One ml of blood was collected by hypodermic syringe (containing 3 mg Na_2EDTA) from the caudal blood vessels, within 3 min after being caught and anaesthetised. The collected blood was placed in cooled 1.5 ml plastic tubes, mixed and centrifuged at 6000 *g* for five min at 4ºC. The collected plasma was stored at minus 20ºC for further analysis. After blood sampling, these fish were put in fresh water for recovery from the sedation before being returned to the aquaria.

Plasma analysis

Plasma cortisol levels were measured by radioimmunoassay using commercial antibody (cortisol-3-OCMO-antiserum, Bioclinical Services Ltd.) as described by Ruane et al., (2001). Plasma glucose was determined by the GOD-Perid® method (Boehringer). Plasma lactate was determined enzimatically using Sigma Diagnostic Kits (Sigma; Proc. no. 735).

Quantification of behaviour

The behaviour of the fish was recorded on video. Video recordings were made of each aquarium on the front side (45 x 45 cm) on Days 10, 24 and 38 (sampling periods 1, 2 and 3 respectively); four days before each sampling time for plasma. In each sampling period, behaviour was recorded around the morning (from 7:30 till 9:00 h) and around the afternoon feeding (from 15:30 till 17:00 h). In total, 180 min of behaviour were recorded per tank per period.

The fish's behaviour was divided into two categories: swimming (while browsing, moving, eating and air breathing), and resting (lying on the bottom of the tank). Furthermore, air breathing was recorded. Every five min., the number of fish in each of these categories was counted as well as the total number of visible fish on the monitor. The activity patterns were expressed as a percentage of the total number of fish counted.

Literature on stereotypic behaviour in fish is scarce. Stereotypic behaviour is characterised by a repetitive behaviour pattern in animals (often seemingly non-functional behaviour). In the current study, stereotypic behaviour of African catfish was defined as continuous and compulsive swimming in a fixed pattern, often in circles, for a period of time. In this study fish stereotypes were divided in the two following categories according to the time involved: Type I, fish that were continuous and compulsive swimming under fixed pattern between 10 to 59 seconds; and Type 2, fish that were continuous and compulsive swimming under fixed pattern between 60 to 240 seconds. The distinction between Type I and Type II stereotypic behaviour was made on the assumption that Type I stereotypic behaviour would be the early development stage of the more severe Type II stereotypic behaviour. Stereotypic behaviour was recorded by all occurrence sampling. The frequency of the occurrence of both classes of stereotypic behaviour was recorded per tank per period during the total 180 min of recording. No correction for the difference in number of fish visible per tank between the treatments (especially between high en low stocking density) was made because the total number of visible fish was not recorded.

Aggressive behaviour could not be quantified by video recordings, because aggressive acts could not be clearly distinguished. Almazan Rueda et al., (submitted) showed that there is a strong correlation between the number of aggressive acts and the number of scars and wounds on the body of African catfish. In the current study aggressive behaviour was quantified by counting the number of scars and wounds on the body of individual fish at the end of the experiment. At the low stocking density, scars and wounds were counted in all fish and at the high stocking density it was counted in 100 fish, which were randomly selected. The number of scars and/or wounds was divided according to the different location on the body: on the head, abdomen, side, fins and tail of the fish (Almazán Rueda et al., submitted). From these data, the average numbers of scars plus wounds per fish was calculated per tank.

Statistics

In this study, aquaria were used as experimental unit. Mean performance and behaviour data were tested for the effect of photoperiod, light intensity and stocking density using a three-way ANOVA (using PROC GLM, SAS, 1990), which included all interaction effects. The error terms of these ANOVA analyses were tested for homogeneity of variance and normality. Preliminary analysis of all parameters, which were repeatedly measured in time, showed that there were only time related changes in the effects of treatments regarding swimming activity as well as both types of stereotypic behaviour. Therefore, these parameters were also analysed by the above mentioned ANOVA model separately for each period

Results

Performance

Stocking density affected final body weight, specific growth rate (SGR) and food conversion ratio (FCR) (Table 1). Fish kept at high stocking density were heavier ($P<0.05$) and had a higher SGR ($P<0.001$) than fish kept at low stocking density. FCR was lower at high than at low stocking density (0.62 versus 0.74; $P<0.05$). Photoperiod did not affect any performance trait except for the SGR (Table 1). SGR was higher in fish kept under continuous light than at 12L:12D (3.7 versus 3.5%; $P<0.05$). Light intensity had no influence on the performance of the catfish (Table 1). Feed consumption was not affected by the treatments. Survival rate was unaffected by the experimental treatments (Table 1).

Table 1. Final mean body weight, specific growth rate (SGR), feed conversion ratio (FCR), individual feed consumption per day and survival rate in African catfish as affected by the experimental treatments.

	Experimental treatment											
Stocking density [a]	High stocking density				Low stocking density					P-value main effects		
Photoperiod	0D:24L		12D:12L		0D:24L		12D:12L			Stocking	Photo-	Light
Light intensity [b]	Low	High	Low	High	Low	High	Low	High	SEM	density	period	intensity
Final body weight (g)	132.2^{w}	126.6^{wx}	120.8^{wxy}	124.3^{wx}	109.6^{xy}	109.6^{xy}	102.4^{y}	107.2^{xy}	5.524	0.0014	ns	ns
SGR ($\%.d^{-1}$)	3.9^{w}	3.8^{wx}	3.6^{wxy}	3.8^{wx}	3.6^{wxy}	3.4^{xy}	3.3^{y}	3.3^{y}	0.101	0.0009	0.0449	ns
FCR ($g.g^{-1}$)	0.62^{w}	0.64^{w}	0.63^{w}	0.62^{w}	0.70^{wx}	0.76^{xy}	0.78^{y}	0.74^{xy}	0.025	0.0002	ns	ns
Feed consumption ($g.d^{-1}$)	1.50	1.44	1.35	1.40	1.35	1.45	1.35	1.35	0.071	ns	ns	ns
Survival rate (%)	93.0	90.6	95.0	93.0	98.3	98.3	95.0	95.0	2.751	ns	ns	ns

[a] High stocking density was 300 fish per tank and low stocking density was 30 fish per tank.

[b] Low light intensity was 15 lux and high light intensity 150 lux both measured at the water surface.

[wxy] Means within a row lacking of a common superscript differ significantly ($P < 0.05$).

[c] interaction between stocking density and light intensity (*P*=0.0082).

ns = not significant (*P*>0.05).

Table 2. Behaviour and stress parameters of the African catfish as affected by the experimental treatments. Mean values are expressed as percentage of active fish for the activities; frequency for the scars on body, and concentration for the stress parameters.

	Experimental treatment											
Stocking density [a]	High stocking density				Low stocking density					P-value main effects		
Photoperiod	0D:24L		12D:12L		0D:24L		12D:12L			Stocking density	Photo-period	Light intensity
Light intensity [b]	Low	High	Low	High	Low	High	Low	High	SEM			
Behaviour												
Swimming activity (%)	87.4^{w}	86.5^{w}	86.2^{w}	86.7^{w}	77.9^{x}	62.6^{z}	71.6^{xy}	67.0^{yz}	2.62	0.0001^{d}	ns^{d}	0.0071^{d}
Air breathing (%)[c]	4.8^{w}	4.9^{w}	4.9^{w}	5.0^{w}	0.6^{x}	0.9^{x}	0.9^{x}	1.5^{x}	0.34	0.0001	ns	0.0962
Resting (%)	12.6^{w}	13.5^{w}	13.8^{w}	13.3^{w}	22.1^{x}	37.4^{z}	28.4^{xy}	33.0^{yz}	2.62	0.0001^{d}	ns^{d}	0.0071^{d}
Injuries (aggression)												
Total scars on body	5.4^{xyz}	7.2^{x}	4.1^{yz}	4.4^{yz}	5.7^{xyz}	7.0^{xy}	6.6^{xyz}	6.9^{xy}	0.74	0.0462^{e}	ns^{e}	ns
Head	0.3^{yz}	0.4^{yz}	0.2^{z}	0.2^{z}	0.6^{yz}	0.5^{yz}	0.5^{yz}	0.7^{y}	0.11	0.0140	ns	ns
Tail	0.04^{yz}	0.02^{z}	0.01^{z}	0.0^{z}	0.02^{z}	0.02^{z}	0.10^{y}	0.00^{z}	0.01	ns	ns	ns
Abdomen	0.1^{y}	0.2^{y}	0.3^{y}	0.3^{y}	0.8^{z}	1.1^{z}	1.0^{z}	0.8^{z}	0.15	0.0001	ns	ns
Side	4.3^{yz}	5.5^{y}	2.8^{z}	3.0^{z}	3.9^{yz}	4.5^{yz}	4.7^{yz}	5.0^{yz}	0.62	ns^{f}	ns^{f}	ns
Fin	0.7	1.1	0.8	0.9	0.4	0.9	0.3	0.4	0.21	0.0386	ns	ns
Stress parameters												
Glucose ($mmol.l^{-1}$)	1.8	1.8	1.8	1.7	1.6	1.6	1.7	1.7	0.06	0.0371	ns	ns
Lactate ($mmol.l^{-1}$)	2.6	2.6	2.3	2.8	3.1	2.8	2.8	3.0	0.24	ns	ns	ns
Cortisol ($ng.ml^{-1}$)	61.1	64.9	72.3	91.8	65.1	90.2	96.1	82.1	11.1	ns	0.0879	ns

[a] High stocking density was 300 fish per tank and low stocking density was 30 fish per tank. [b] Low light intensity was 15 lux and high light intensity 150 lux both measured at the water surface. [c] % of contribution of air breathing to swimming activity [wxyz]Means within a row lacking of a common superscript differ significantly ($P < 0.05$). [def] Interaction between factors: [d]stocking density, photoperiod and light intensity (P=0.0184); [e] stocking density and photoperiod (P=0.0512) and [f] photoperiod and stocking density (P=0.0187). ns = not significant (P>0.005).

Behaviour

Stocking density influenced the behaviour of the fish (Table 2). Fish spent more time swimming at the high compared with the low stocking density (86.7 % versus 69.7%; $P<0.001$). Air breathing increased from 0.95 % at low stocking density to 4.8 % at high stocking density ($P<0.001$). Swimming and resting were affected by the three way interaction between light intensity, photoperiod and stocking density (Table 2). Differences between the light intensity and photoperiod treatments regarding these activity pattern parameters were larger at the low stocking density compared with the high stocking density.

Fish at the high stocking density showed a lower number of total scars and wounds on the body than at the low stocking density (5.3 versus 6.5 scars plus wounds per fish; $P<0.05$, Table 2). However, this effect of stocking density on the total number of scars plus wounds tended to be dependent on the photoperiod; indicated by the significant interaction effect ($P=0.051$). Compared with continuous light, the 12L;12D photoperiod did not influence the number of scars plus wounds at the low stocking density, but decreased the number of scars and wounds at the high stocking density. This interaction effect between photoperiod and stocking density on the total number of scars and wounds was due to the interaction effect on the number of scars and wounds on the side of the fish ($P<0.05$; Table 2). Fish at the high stocking density had a lower number of scars on the head and on the abdomen than fish at the low stocking density ($P<0.05$, Table 2). Fish at the high stocking density had a higher number of scars and/or wounds on their fins as compared to the low stocking density.

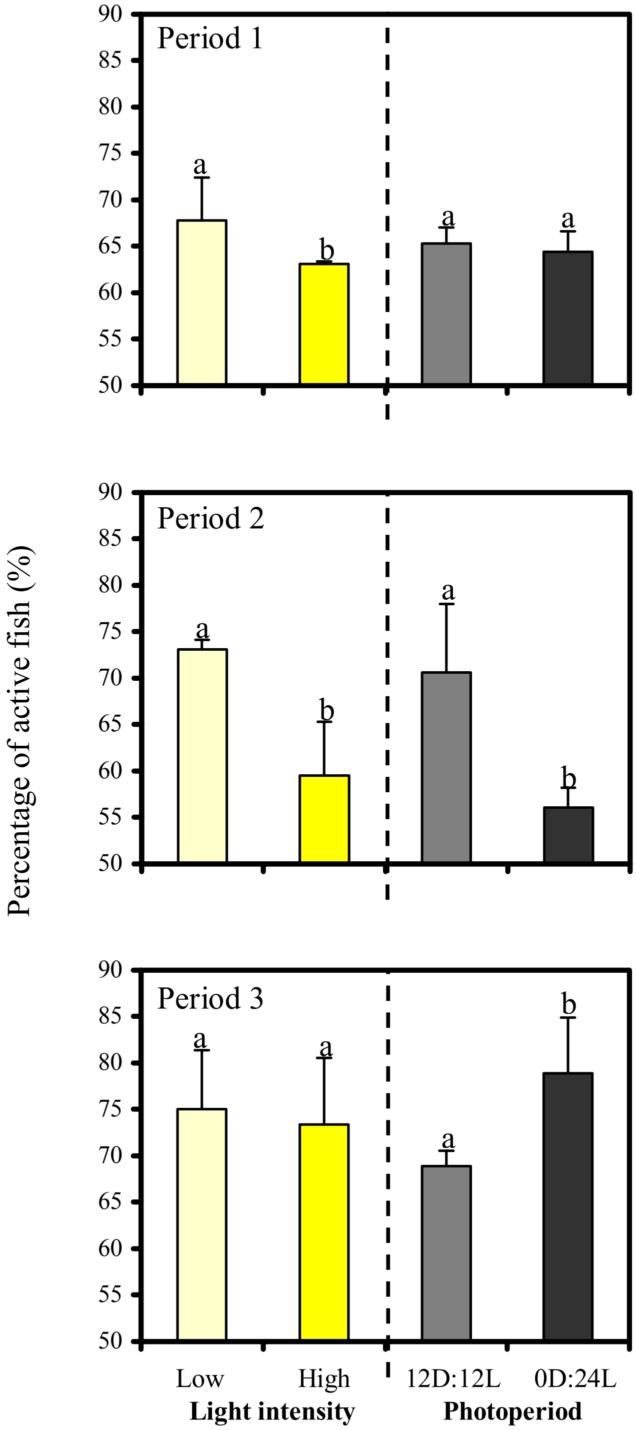

Figure 1. Percentage of fish active under low stocking density as affected by light intensity and photoperiod and period of observation. Each period was separated by 15 days from each other. Period 1 is related to young fish (23 g). Period 2 is related to medium age fish (65 g) and Period 3 is related to fish at the end of the experiment (117 g).

The influences of light intensity and photoperiod on swimming activity changed with time over the experiment, depending on the stocking density. During all sampling periods, swimming activity at the high stocking density was unaffected by light intensity and photoperiod. However, at the low stocking density these light regime effects changed between the various periods (Figure 1). The effect of light intensity on activity diminished with time. Swimming activity during Sampling Periods 1 and 2 was influenced by light intensity (P=0.02). Fish at the low light intensity were more active than fish at high light intensity. During Sampling Period 3, the effect of light intensity on activity was no longer present. The changed over time of the effect of photoperiod on activity was less consistent. Photoperiod had no affect on activity during Sampling Period 1. During Sampling Period 2, the continuous lighting schedule reduced the swimming activity of the fish (P=0.04), whereas during sampling period 3 it increased the swimming activity of the fish (P=0.05).

Stereotypic behaviour was unaffected by photoperiod and light intensity. However, the influence of stocking density on stereotypic behaviour changed with time during the experimental period (Figure 2). The incidence of both Type 1 and Type 2 stereotypic behaviour was highest during sampling period 3. During Sampling Period 1, fish at the low stocking density showed higher incidence of Type 1 stereotype than at the high stocking density. During Period 1, no Type 2 stereotypes occurred. During both Sampling Periods 2 and 3, there were no significant difference between the stocking densities regarding Type 1 stereotypes, but the number of Type 2 stereotypes were increased at the low stocking density compared with the high stocking density.

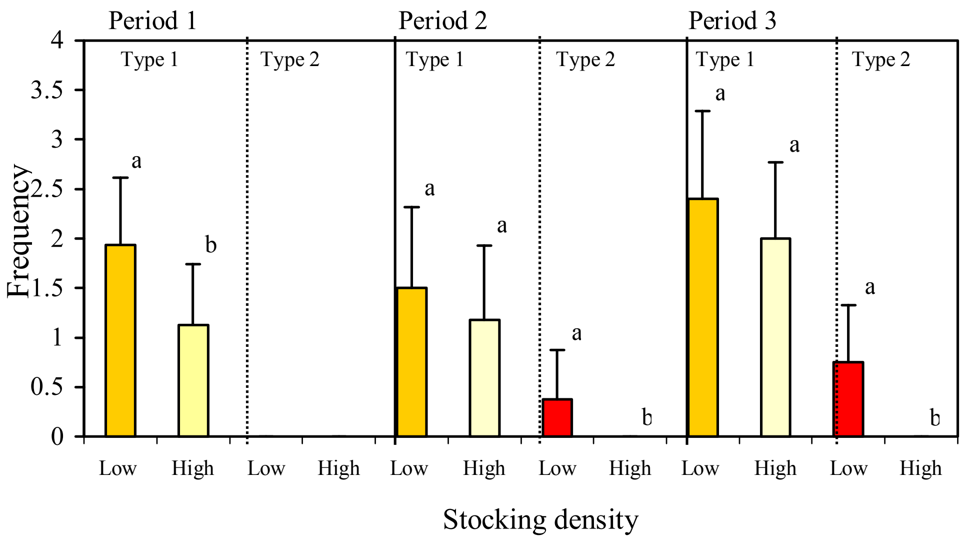

Figure 2. Average incidence of stereotypes according to stocking density during three hours observation time for each period per treatment. Each peridod was separated by 15 days. The duration of Type 1 stereotype was between 10 to 60 seconds. The duration of Type 2 stereotype was between 60 to 240 seconds. Low stocking density was 30 fish per tank (Low) and high stocking density was 300 fish per tank (High).

Stress parameters

Plasma analysis showed higher levels of glucose (P<0.05, Table 2) in fish at high stocking density than in fish at low stocking density. Photoperiod showed a tendency (P<0.1) for an effect on the level of cortisol in plasma. Fish at continuous light had lower levels of cortisol (average 70.3 ng.ml^{-1}) than fish at the 12D:12L photoperiod (70.3 versus 85.6 ng.ml^{-1}). Plasma lactate levels were unaffected by the experimental treatments (Table 2).

Discussion

Generally it is believed that stocking density has a negative affect on fish performance (growth, feed intake, survival and feed conversion), because increasing stocking density results in stress, which has a metabolic cost, leading to the reduction in growth and food utilization (Khwuanjai Hengsawat et al., 1997). Literature on African catfish (*C. gariepinus*) is in line with this general assumption that performance is decreased when stocking density is increased (Hecht and Appelbaum, 1988; Haylor 1991, 1993; Hossain, et al., 1998). However, in the current study the performance of African catfish improved when the stocking density was increased from 250 to 2500 fish.m^{-3} (Table 1). The most likely reason for the difference between literature data and the current study is the difference in stocking density used in the various experiments. The highest stocking density applied in earlier catfish studies were mostly lower or similar to the low stocking density (250 fish.m^{-3}) applied in the current study (658 fish.m^{-3}; Hecht and Uys, 1997; 320 fish.m^{-3}, Siddiqui et al., 1993; 15 fish.m^{-3}, Eglal, 1996; 200 fish.m^{-3}, Khwuanjai Hengsawat et al., 1997). This suggests that the relation between stocking density and performance is curvilinear in African catfish. Low stocking densities are negatively related to performance, but beyond an inflection point, low stocking densities are positively related to performance. Another possible explanation for the difference with past data is that the effect of stocking density on performance changes with the age of the fish. Most literature data on stocking density has been carried out on catfish with an initial weight smaller than 9 g (Siddiqui et al., 1993; Kaiser et al., 1995a, 1995b; Appelbaum and McGeer, 1998; Hossain et al., 1998), whereas the fish in the current study had an initial weight of 23 g.

The possible mechanism by which stocking density influences performance is related to the impact of stocking density on the behaviour of the fish, thereby inducing different levels of chronic stress in the fish. In the current study, both aggressive behaviour (number of scars and wounds) and stereotypic behaviour were reduced when the stocking density increased form 250 to 2500 fish.m^{-3} (Table 2 and Figure 2). The display of aggressive behaviour is influenced by several internal factors as well as environmental stimuli and social experience (Jönson et al., 1998). Kaiser et al., (1995b) demonstrated that high stocking densities reduced agonistic behaviour in African catfish larvae. In larvae and early juveniles of *C. gariepinus* (< 40 days old) the level of aggression was reduced with an increase in density (Hecht and Appelbaum, 1988; Hecht and Uys, 1997). Results on aggressive behaviour in the current study concur with those in larvae and early juveniles. This finding was indicated by the reduction in the total number of scars and fresh wounds on the body when the stocking density was increased (Table 2). At a high stocking density some fish form a dense shoal that interferes with the cues for initiation of aggressive events (Hecht and Uys, 1997) and consequently defending a particular territory becomes inefficient or impossible (Ellis et al., 2002). The location of the scars and wounds on the body of the catfish in the current study seems to confirm this (Table 2). Increasing the stocking density from 250 to 2500 fish.m^{-3} increased the number of scars and wounds on the fins but decreased the number on the head, side and abdomen. It might be that in the current study at the high stocking density catfish had insufficient space to perform the natural pattern of aggressive behaviour. As reviewed by Ellis et al. (2002) decreasing the stocking density (i.e., increasing the space availability of the fish) facilitates 'face to face' confrontation among individuals (explaining the difference in bites on the head) followed by escapes and chasing (explaining the difference in bites on side and abdomen).

The current study confirms the practical experience in the Dutch intensive farming of African catfish, that for juveniles, stocked at a high stocking density of about 2500 fish.m^{-3} give optimal performance results. Furthermore, the current results suggest that this density is also optimal regarding decreasing chronic stress, based on observation of aggressive and stereotypic behaviour. However, further research is needed to determine optimal stocking densities in African catfish especially in relation to other factors influencing the optimal densities (e.g., the fish age and other husbandry conditions).

Light regime (photoperiod and light intensity) is considered an important factor affecting both performance and behaviour in fish (Britz and Pienaar, 1992; Appelbaum and McGeer, 1998; Hossain, et al., 1998; Boeuf and Le Bail, 1999). In experiments with *C. gariepinus* larvae under conditions of continuous darkness or low light intensity, which approximated to the conditions of natural light regime (Hossain et al., 1998), stress, aggression and cannibalism were reduced and performance was improved (Britz and Pienaar, 1992; Hecht and Pienaar, 1993). Previous experiments in African catfish juveniles (250 fish.m^{-3}) (Almazán Rueda et al., submitted) supported the observations made in catfish larvae with respect to photoperiod and light intensity (Hecht and Appelbaum, 1988; Britz and Pienaar, 1992; Kaiser et al., 1995b; Appelbaum and McGeer, 1998; Hossain et al., 1998). However, in the current experiment, the effect of both photoperiod and light intensity on performance as well as behaviour characteristics was rather small or even absent (Table 1 and 2). Some parameters such as swimming activity and total number of scars and wounds on the body, exhibited an interaction between stocking density and photoperiod (Table 2). In the current study the effects of light regime depended on the stocking density, being more pronounced at the low stocking density. Another reason for the relatively small effect of the light regime in the current study is that the age of the fish might have interfered. The current study shows that both the effects of photoperiod and light intensity on swimming activity varied during the experimental period (Figure 1). From the present study it cannot be concluded whether this time effect (differences between periods) is due to an age or size effect of the fish. Moreover, it might also be related to the adaptation of the fish to the experimental conditions. Fish light receptivity changes with the development stage (age/weight). During very young stages, light direction and intensity play a major role in most species (Boeuf and Le Bail, 1999). The results of this study are in accordance with Boeuf and Le Bail (1999) that light intensity is an important factor affecting activity (growth and survival) at young stages. Fish activity at a medium age was affected by photoperiod and light intensity; it seems that there is a transition period between young and older stages. When fish are getting older, photoperiod seems to play a major role in activity and gonadal development (Boeuf and Le Bail, 1999; Boeuf and Falcón, 2001).

In addition to physiological and physical responses to stress, behavioural responses are important to assess welfare of animals. Abnormal behaviour, such as stereotypes, has been used as indicator of poor welfare and has often been related to the exposure to chronic stress induced by sub-optimal environments (Masson, 1991; Mench and Mason, 1997; Robert et al., 1997). Stereotypic behaviours are repetitive, invariant behaviour patterns with no obvious goal or function, which are often displayed by captive animals (Masson, 1991). The source behaviour patterns from which stereotypies develop are often a response to frustration, thwarting or a conflict of motivations (Würbel and Stauffacher, 1997). The present study showed that African catfish can develop stereotypic behaviour. This stereotypic behaviour was exhibited by repetitive swimming in the same pattern, mainly in circles. The stereotypic behaviour was classified into two types based on its duration: Type 1 between 1 to 59 seconds and Type 2 between 60 and 240 seconds. It is suggested that Type 1 is the early developmental stage of the final developmental stage of stereotypic behaviour exhibited in Type 2. The increase in Type 1, but especially in Type 2, stereotypes suggest that these fish were subject to chronic stress. The development of stereotypes was more common in fish kept at the low stocking density than at the high stocking density. However, it should be realized that in Figure 2 the frequency of stereotypes are given per tank during the total recording time of 180 min. Consequently when considering the higher number of fish at the high stocking density the incidence of stereotypes on a fish base is even lower at the high stocking density. The lower number of stereotypes might suggest that African catfish at the density of 2500 fish.m^{-3} had a relatively lower exposure to chronic stress than the fish at the density of 250 fish.m^{-3}. However, the lower level of stereotypic behaviour at the high stocking density might also simply be the consequence of the inability to perform these behaviour types due to space limitation for movement. In mammals it has been demonstrated that stereotypic behaviour is important for the animal to be able to cope with the exposure to chronic stress (Cronin et al., 1986, Schouten and Wiepkema, 1991) by e.g., the release of ß-endorphines acting as opiates. The better performance (SGR and FCR, Table 1) and the reduced aggressive behaviour (indicated by the number of scars and wounds, Table 2) at the high stocking density as compared to the low stocking density, suggests that the higher number of stereotypes at the low stocking density is an indication of exposure to chronic stress. Further research is required regarding the physiological purpose of stereotype for these catfish, to clarify factors that trigger it as well as whether this behaviour reflects an exposure to chronic stress. Regarding the latter it should be realized that the absolute frequency of stereotypic behaviour was low: at the low stocking density, Type 1 and Type 2 stereotypes occurred about 2.5 and 0.75 times per 30 fish during 180 min., respectively.

In farm animals the occurrence of stereotypes depends on the observation time in relation to the feeding time. Some farm animals performed stereotypes especially before feeding times, whereas its occurrence declined once the meal has been provided (Mason and Mendl, 1997). African catfish also appear to exhibit a similar behavioural pattern. A higher incidence of stereotypes (Type 2) was found pre- than post-feeding (data not shown). This is related with the motivational systems of controlling feeding and foraging (Mason and Mendl, 1997).

The data on performance and behaviour (especially aggressive behaviour and stereotypic behaviour) suggest that at the low stocking density African catfish were subject to chronic stress. However, no differences in plasma cortisol and lactate were observed between the two stocking density treatments. Only the glucose levels were higher at the high stocking density. The higher glucose levels at the high stocking density might be related to the higher swimming activity of the fish at this treatment compared with the low stocking density. Increased plasma cortisol levels have been shown in rainbow trout *Oncorhynchus mykiis* at high stocking density (Kebus at al., 1992) as well as in red porgy (Rotllant and Tort, 1997). The absence and relatively small difference in blood stress parameters in the current study may be due the 'acclimation' of the stress response over time to high stocking density (Pickering and Stewart, 1984). This parallels the suggestion of Ruane (2002) that blood stress parameters like glucose, cortisol and lactate are mainly good indicators of acute stress but less appropriate for chronic stress. Therefore, for assessing the impact of husbandry conditions, which may act as chronic stressors, types of welfare indicators like behaviour, performance, and blood stress parameters should be combined.

Conclusions

High stocking density (2500 fish.m^{-3}) in juvenile African catfish (*C. gariepinus*) increased growth and swimming activity, and reduced FCR, aggression (determined by means of scars on body) and stereotypic behaviour compared to low stocking density (250 fish.m^{-3}). This suggests that the low stocking density induced chronic stress. The occurrence of stereotypic behaviour is a potential indicator for measuring chronic stress in African catfish.

In contrast to the major effect of stocking density, light regime (photoperiod and light intensity) had no or minor impact on performance or behaviour. However, regarding the behaviour characteristics (swimming activity and aggressive behaviour) the effects of light intensity and photoperiod were dependent upon the stocking density. This was indicated by the interaction effect with stocking density. Furthermore, both the effects of photoperiod and light intensity varied over time during the experiment. This suggests that either age (i.e., weight) or adaptation to the experimental conditions plays an important role in the behaviour of juvenile African catfish.

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Chapter **6**

Photoperiod affects growth, behaviour and stress parameters in the African catfish (*Clarias gariepinus*).

Abstract

Photoperiod can affect the performance and behaviour of the African catfish (*Clarias gariepinus*). Short periods of light or no light (6L:18D and 0L:24D) resulted in an increased growth compared to extended periods of light (18L:6D and 12L:12D). Fish under longer periods of light (12L:12D and 18L:6D) showed higher swimming activity, more aggression (injuries on the body), higher lactate, free fatty acids, and cortisol levels compared to those who were reared at shorter periods of light (0L:24D and 6L:18D). Feeding activity during light and dark periods in this experiment shows that *C. gariepinus* has both night and day feeding activities, with a preference to diurnal feeding in the 12L:12D photoperiod. The results showed that light plays an important role in the African catfish behaviour and its well being. As the hours of light increased during the 24 h cycle, data suggests that the fish were more stressed and aggressive, compared to those under a reduced number of light hours.

Keywords: *Clarias gariepinus*, photoperiod, growth, stress parameters, behaviour.

This chapter has been submitted.

P. Almazán Rueda, van Helmond, A.T.M, J.A.J. Verreth and J.W. Schrama. *Journal of Fish Biology.*

Introduction

The growing awareness in society about animal welfare puts increasing demands on the housing conditions in fish farming facilities. To enable and develop welfare friendly housing systems, more information is needed on the social behaviour and the factors that can affect the social interactions in fish, especially in fish which exhibit aggressive behaviour and cannibalism (for example, the African catfish, *Clarias gariepinus*). Such information on behaviour could contribute to the assessment of animal welfare.

Like in other fish, stocking density of African catfish is considered the most important factor affecting cannibalism (Hecht and Appelbaum, 1988; Kaiser et al., 1995) and aggression (Hecht and Uys, 1997; Almazán Rueda et al, submitted a, b). However, in African catfish aggressive behaviour is also affected by factors other than stocking density such as photoperiod (Almazán Rueda et al, accepted c). Photoperiod requirements are species specific and vary for each developmental stage. Light and dark alternation is generally thought to be the main synchronizer of feeding activity (Boujard et al., 1991; Hossain et al., 1999). Photoperiod not only affects feeding activity, but also plays a decisive role in growth, survival and social behaviour (Boeuf and Le Bail, 1999; Boeuf and Falcón, 2001). Such influences can be caused by physiological mechanisms; such as altered hormone production, which may improve feed conversion efficiency (Purchase et al, 2000).

The effect of photoperiod on growth, survival and social behaviour of *Clarias gariepinus* was demonstrated by several authors (Hecht and Appelbaum, 1988; Britz and Pienaar, 1992; Hecht and Pienaar, 1993; Kaiser et al, 1995; Appelbaum and McGeer, 1998; Hossain et al., 1998) predominantly during the larval stages. Only a few studies have assessed the effect of photoperiod on growth, food intake, and behaviour during the juvenile or semi adult stage of the African catfish (Almazán Rueda et al., accepted c, submitted d). Almazán Rueda et al., (accepted c) showed the effect of continuous light versus 12D:12L under hand feeding and self-feeding regime on behaviour of the African catfish. Almazán Rueda et al., (submitted d) showed the effect of 12D:12L regime on diurnal behaviour of the African catfish. Therefore, the aim of the present study was to assess the influence of different photoperiods

(24D:0L, 18D:06L, 12D:12L and 06D:18L) on growth, feed demand, survival, behaviour and stress parameters of the African catfish (*C. gariepinus)* juveniles. Furthermore, the effect of these photoperiods on diurnal variations in feed demand and behaviour was assessed.

Material and Methods

This experiment was approved by the Ethical Committee judging Animal Experiments (DEC) of the Wageningen University.

Animals, adaptation period and experimental conditions

Juveniles of African catfish (*Clarias gariepinus*) were obtained from a commercial hatchery (Maatschap Fleuren en Nooijen, Someren, The Netherlands) and held in four aquaria during an adaptation period, which lasted two weeks. During this period fish were fed a pelleted feed (with a size of 2 mm) by means of self-feeders and the photoperiod was 14L:10D (lights on at 07:00 hr). Light intensity was 150 lux (measured at the water surface). At the start of the 6-week experimental period, fish weighed 69.2 ± 6.8 g (SD). Fish were randomly assigned to one of 16 aquaria (90x45x45 cm). Each aquarium was stocked with 30 fish. The aquaria were within one water recirculation system. The average water flow through each aquarium over the total period of the experiment was 5 $l.min^{-1}$. Water depth was maintained at approximately 30 cm. Water temperature was 24.9 ± 0.9 ºC; oxygen concentration 7.2 ± 0.3 $mg.l^{-1}$, and pH ranged between 6.5 and 7.3.

Experimental set up

The 16 aquaria were assigned to one of four experimental treatments (four replicates per treatment) to assess the effect of photoperiod. The four applied photoperiods were: continuous darkness (24D:0L); 18 hours dark and 6 hours light (18D:6L); 12 hours darkness and 12 hours light (12D:12L) and 6 hours dark and 18 hours dark (6D:18L). For the photoperiod of 24D:0L, lights were off all the time; for 18D:6L, lights were on from 10:00 to 16:00 hrs; for 12D:12L, lights were on from

07:00 to 19:00 hrs, and for 6D:18L, lights were on from 04:00 to 22:00 hrs. Light was provided by 60-watt bulbs (frosted-mat; Rodulphus B.V, Scheepselktro, Duiven, The Netherlands) providing 150 lux of light at water surface.

All fish were fed by means of self-feeders connected to a computer, which was comprised of a hopper fitted with a pendulum. The self-feeder was calibrated to deliver on average 0.8 g of 3 mm pellets per strike of the pendulum. The pendulum was placed 2 cm above the water surface. Each time the fish hit the pendulum, feed fell from the hopper into the aquaria and a pulse was sent to the computer. A computer recorded the pulses per minute as hits per minute for each self-feeder.

During the experimental period, a formulated feed (commercial catfish diet, Trouvit ME-2 meerval, Trouw Nutrition, Nutreco, The Netherlands) was fed in the form of floating 3-mm pellets. The dry matter content of the diet was 91.2 %. On dry matter basis, the content of crude protein content was 48.3 %, crude fat 9.7 %, crude ash 9.8 % and gross energy 19.0 $kJ.g^{-1}$.

Sampling procedure

During the experimental period, fish were sampled three times (at day 16, 31 and 42) for blood and growth performance. Five fish per aquarium were anaesthetised with tricaine methanesulfonate (MS-222, 0.35 $g.l^{-1}$ (Crescent Research Chemicals, Phoenix, AZ, USA) and 0.7 $g.l^{-1}$ of $NaHCO_3$). One ml of blood was collected by hypodermic syringe (containing 3 mg Na_2EDTA) from the caudal blood vessels, within 3 min after being anaesthetised. The collected blood was placed in cooled 1.5 ml plastic tubes, mixed and centrifuged at 6000 *g* for five min at 4ºC. The collected plasma was stored at -20ºC for further analysis. After blood sampling, fish were weighed, measured and after depuration in fresh water they were returned to the aquaria. After blood samples were taken, the other fish in each aquarium were individually weight and returned to aquaria.

Measurements

Specific growth rate (SGR) was calculated from the natural logarithm of mean final weight minus the natural logarithm of the mean initial weight and divided by the total number of experimental days expressed as a percentage. Total feed intake (TFI) per aquaria was recorded daily over the total experimental period. Feed conversion ratio (FCR) was calculated per aquaria from feed intake data and weight gain.

Plasma analysis

Plasma cortisol levels were measured by radioimmunoassay using a commercial antibody (cortisol-3-OCMO-antiserum, Bioclinical Services Ltd.) as described by Ruane et al., (2001). Plasma glucose was determined by the GOD-Perid® method (Boehringer). Plasma lactate was determined enzimatically using Sigma Diagnostic Kits (Sigma; Proc. no. 735).

Quantification of behaviour

Fish behaviour was recorded by a digital video recording system (Net DVR-5016H LG, Seoul, Korea) four days before each sampling time (at days 12, 27 and 38). Digital video recordings were made during 24 hrs for each of the tanks.

For the purposes of this experiment, fish activities were registered every 30 min and the number of visible fish were counted and categorised according to two categories: swimming (browsing, eating and air breathing), and resting (lying on the bottom of the tank). Each of these categories was expressed as a percentage of the total number of fish counted. Furthermore the number of fish air breathing and eating was recorded and expressed as a percentage of the total number of fish observed. Aggressive behaviour was difficult to quantify on the video recordings. Almazán Rueda et al., (accepted c) showed that there is a strong correlation between the number of aggressive acts and the number of scars and wounds on the body of African catfish. In the current study, aggressive behaviour was quantified by counting the number of scars and wounds on the body of individual fish at the end of the experiment. To study the influence of the photoperiods on the variation in body weight, coefficient of variation (C.V.) of final body weight within aquaria was calculated from individual body weight.

Within day variation of feed demand

Feeding activity was recorded every minute as the total number of hits per aquarium. For each aquarium, the mean amount of feed released into the water per hit was calculated by dividing the total daily feed intake (TFI) by the total number of recorded hits. The average hourly feed intake demanded ($g.h^{-1}$) was calculated by multiplying the total amount of hits per hour by the amount of feed released per hit. Furthermore, feeding activity during light and dark periods was calculated from the mean feed intake per hour for each of these periods and expressed as grams per hour.

Statistics

In this study, aquaria were used as the experimental unit. Mean performance data, mean plasma data, mean behaviour data and feeding activity were analysed for the effect of photoperiod using ANOVA (PROC GLM, SAS, 1990). The error terms of these ANOVA analyses were tested for homogeneity of variance and normality. Percentages were arcsine transformed before ANOVA was carried out. To assess the differences in feed demanded in periods of light and dark repeated measurement analysis was used. A difference of $P < 0.05$ was accepted as significant.

Results

Performance

Photoperiod affected the performance of the African catfish juvenile. Fish kept at photoperiods of 24D:0L and 18D:6L were heavier (P=0.002) and tended to have a higher SGR (P=0.068) than fish kept at 12D:12L and 6D:18L. FCR, feed consumption, C.V. in final body weight and survival rate was not affected by photoperiod (Table 1).

Table 1. Final mean body weight, coefficient of variation in final body weight (C.V.), specific growth rate (SGR), feed conversion ratio (FCR), individual feed consumption per day and survival rate in African catfish as affected by photoperiod.

	Photoperiod				SEM	*P*
	0L:24D	6L:18D	12L:12D	18L:6D		
Final body weight (g)	223.9[a]	237.1[a]	178.5[b]	192.2[b]	10.6	0.002
C.V. (%)	57.0	51.8	54.5	48.0	3.33	n.s.
SGR ($\%.d^{-1}$)	2.7	2.9	2.4	2.6	0.12	0.068
FCR ($g.g^{-1}$)	0.72	0.72	0.87	0.80	0.04	n.s.
Feed consumption ($g.d^{-1}$)	2.5	2.7	2.1	2.4	0.18	n.s.
Survival rate (%)	100	98.3	98.3	96.6	2.04	n.s.

n.s. = not significant (P>0.005)

Behaviour

Photoperiod influenced the behaviour of the fish (Table 2). Fish tended to spend more time swimming at 6D:18L and 12D:12L compared with 18D:6L and 24D:0L (81.9 and 80.4 % versus 72.1 and 72.2 %; P=0.051). Air breathing was not affected by the photoperiods. The percentage of fish eating was higher at the continuous darkness (24D:0L) than in the other three photoperiods (Table 2). The number of scars and/or wounds on the body gradually increased from 1.4 to 2.2 as the number of light hours increased (P=0.001; Table 2). Fish at 6D:18L photoperiod had the highest number of scars and/or wounds on the body.

Table 2. Behaviour patterns of the African catfish as affected by photoperiods. Mean values are expressed as percentage of active fish for the different activities; frequency for the scars on body, and concentration for the stress parameters.

	Photoperiod					
	0L:24D	6L:18D	12L:12D	18L:6D	SEM	*P*
Behaviour						
Swimming activity (%)	72.2 [a]	72.1 [a]	80.4 [ab]	81.9 [b]	2.835	0.0512
Eating (%)	6.7 [a]	2.6 [b]	2.5 [b]	2.1 [b]	0.885	0.0093
Air breathing (%)	2.3	2.3	2.0	1.2	0.669	n.s.
Resting (%)	27.8 [a]	27.9 [a]	19.6 [ab]	18.1 [b]	2.835	0.0512
Injuries (aggression)						
Total scars on body	1.4 [a]	1.5 [ab]	1.7 [b]	2.2 [c]	0.114	0.0001
% of fish bitten	73.0	66.7	84.0	82.0	7.459	n.s.
Stress parameters						
Glucose ($mmol.l^{-1}$)	3.6 [a]	3.1 [b]	3.6 [a]	3.9 [a]	0.124	0.0008
Lactate ($mmol.l^{-1}$)	3.2 [a]	3.1 [a]	3.2 [a]	3.7 [b]	0.122	0.0110
FFA ($mmol.l^{-1}$)	0.20 [a]	0.23 [bc]	0.21 [ab]	0.24 [c]	0.009	0.0166
Cortisol ($ng.ml^{-1}$)	133.5 [a]	119.6 [a]	138.3 [ab]	158.9 [b]	8.141	0.0088

FFA = plasma total non-esterified fatty acids.

n.s. = not significant (P>0.005).

Figure 1 shows a comparison of within day variation in activity between light and dark periods of the day. Activity patterns of the African catfish were different between the light and dark phases of the day. However this difference was influenced by the applied photoperiods. When the duration of the light phase increased, the difference in air breathing between the light and dark phase decreased (Figure 1a). Similar effects were observed for eating activity recorded in video.

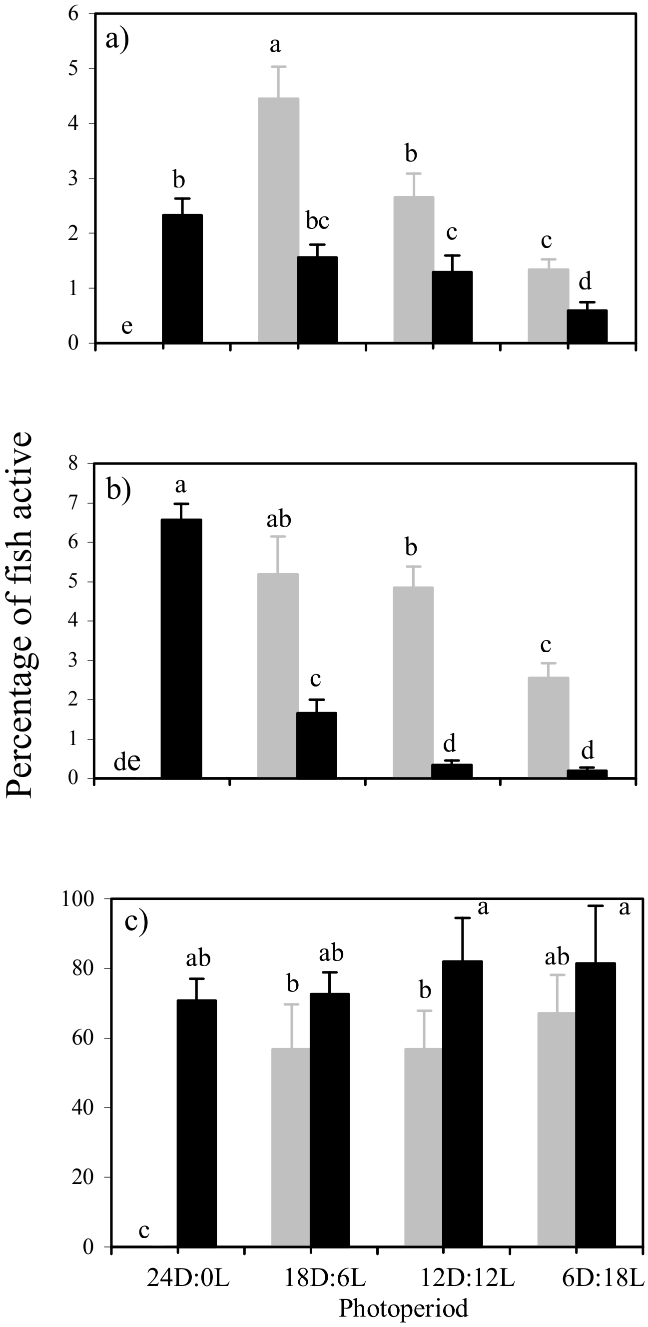

Figure 1. Activity patterns of the African catfish (*Clarias gariepinus*): a) air breathing; b) eating and c) swimming activity. Grey and black bars represent the mean percentages (± S.D.) of activity during the light and dark periods of the day respectively. Bars with the same letter in each graph and in the same activity are not significantly different (*P*>0.05).

During continuous darkness (24D:0L), the percentage of fish eating was higher than during the dark phases of the other photoperiods. For the light phases, the percentage of fish that were eating decreased as the number of hours of light increased

(Figure 1b). The percentage of fish swimming was slightly different according to the dark and light phases of the different photoperiods. The highest percentage of fish swimming was found at 6D:18L and 12D:12L. The percentage of fish swimming increased when the dark phases of the different photoperiods became shorter (Figure 1c). Swimming activity during the light period was constant for each treatment, with a slight increase at the 6D:18L photoperiod.

Stress parameters

Plasma analysis showed higher levels of glucose (P=0.008, Table 2) in fish at 6D:18L and12D:12L than in fish at 18D:6L and 24D:0L. Plasma lactate levels were affected by the different photoperiods (Table 2). Fish at the photoperiod of 6D: 18L had the highest levels (3.7 mmol.l^{-1}). An increased concentration of FFA (plasma total non-sterified fatty acids) was observed as the hours of light increased in the photoperiod treatments (P=0.016, Table 2). The FFA values increased from 0.20 to 0.24 mmol.l^{-1} at the photoperiods 24D:0L and 6D:18L, respectively. Photoperiod affected the level of cortisol in plasma (P=0.008, Table 2). The highest cortisol levels were observed in the 6D:18L photoperiod (158.9 ng.ml^{-1}) and the lowest at the 18D:6L photoperiod (119.6 ng.ml^{-1}).

Within day variation in feeding activity

The amount of feed demanded by the fish was not significant different in the four applied photoperiods during 24 h. (Figure 2). However, in all of the treatments, feed demand started around midday and decreased around 20:00 h. Fish feeding activity was higher during the light phases of the photoperiods. This feed demand increased as the number of hours of light was longer, and during the dark periods of these photoperiods, feed demanded was reduced.

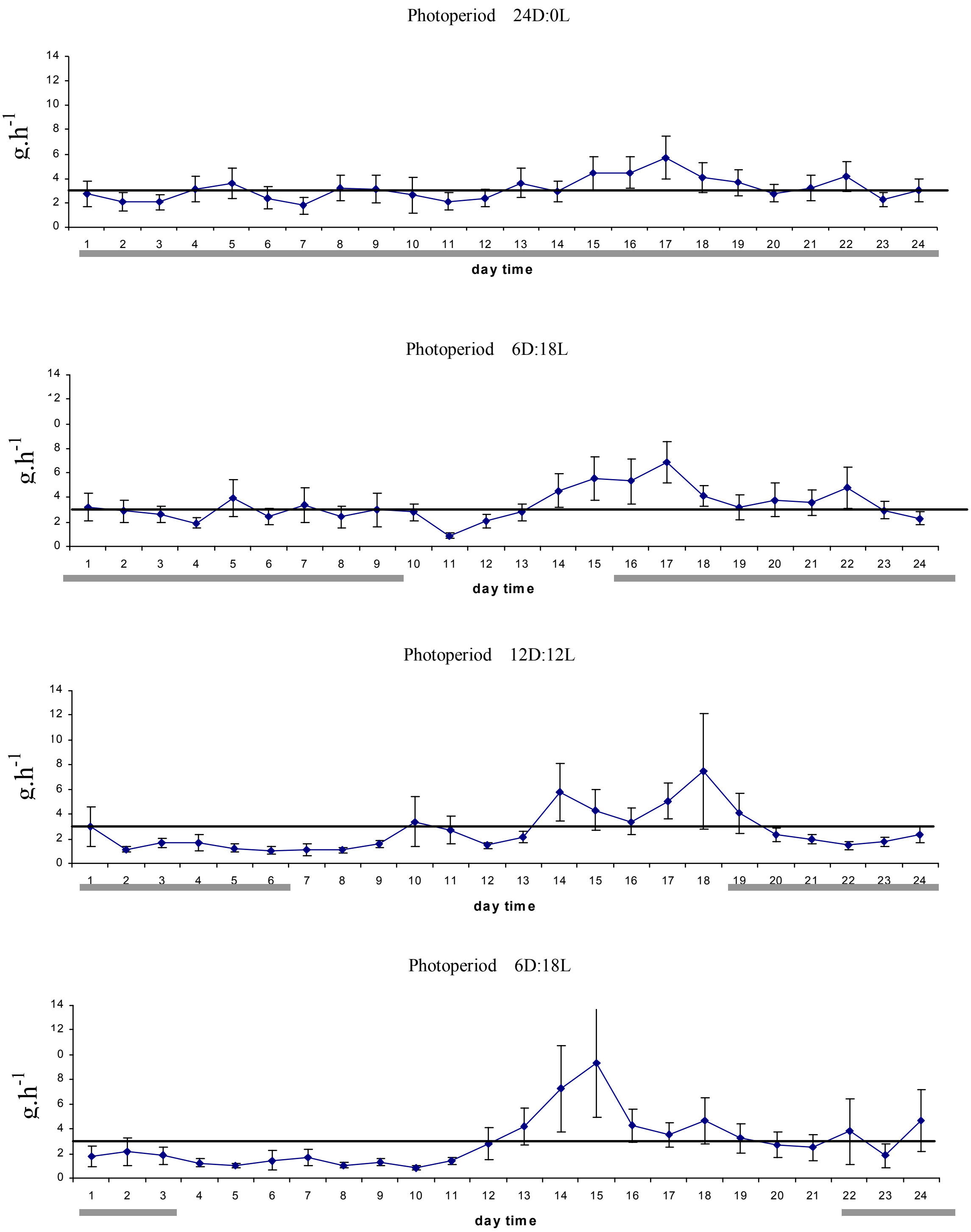

Figure 2. Feed demanded per hour ($g.h^{-1}$) during 24 hours cycle. Mean values (± S.D.) were calculated from the average feed intake and the number of hits in each of the aquaria. Horizontal lines in the graphs represent the average feed demand per hour during the whole day. Grey bars below each of the graphs represent the dark period within the day.

According to the dark and light periods of the day, the amount of feed demanded was rather constant. There were no differences of feed demand according to the two periods of the day, only during the dark period of the day at 12L:12D photoperiod the amount of feed demanded was significantly lower (Figure 3).

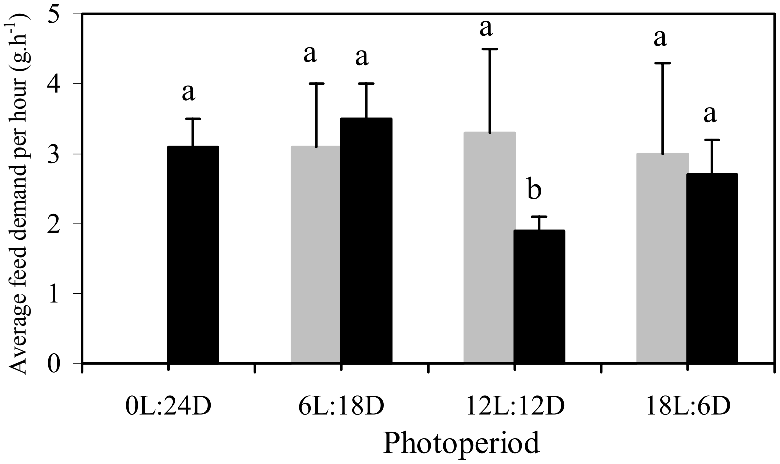

Figure 3. Mean feed demand (in g.h^{-1}) (± S.D.) during the dark (black bars) and light (grey bars) periods of the day as affected by photoperiod in the African catfish (*Clarias gariepinus*). Bars with the same letters are not significantly different (*P*>0.05).

Discussion

Performance

Results from the present study showed that photoperiod had an impact on the performance and behaviour of the African catfish (*Clarias gariepinus*) juveniles. Short periods of light or no light at all resulted in an increased growth (6L:18D and 0L:24D) compared to extended periods of light (18L:6D and 12L:12D). Britz and Pienaar (1992) and Almazán Rueda et al (submitted e) found similar results, i.e. growth rates of African catfish *Clarias gariepinus* decline as the length of light period increase. Similar results were found in *C. gariepinus* larvae, indicated by the tendency that larvae grew to a larger size when reared at dark conditions (Appelbaum and Kamler, 2000). In studies on channel catfish (*Ictalurus punctatus*) a slight tendency for higher growth and somewhat lower food conversion ratios under a 12D:12L photoperiod was demonstrated compared to other photoperiods; but no significant differences were found among treatments (0, 6, 12, 18 and 24 hours of light per treatment) (Stickney and Andrews, 1971).

According to the fish species, increasing the hours of light per 24 h, has the potential for increasing growth rates without an increased in food consumption (Purchase et al., 2000). Opposite results were found in this study with reference to photoperiod. Results of the present study showed that decreasing the photoperiod (shorter light phase) had the potential to increase growth without increasing food consumption. This confirms the preference of African catfish for dark conditions (Britz and Pienaar, 1992; Appelbaum and McGeer, 1998). However, the current study suggests that African catfish need a short light period during a 24 h cycle. This was indicated by the highest growth rate at the 18D:6L photoperiod. The later confirms the statement of Boeuf and Falcón, (2001), "most of the fundamental rhythms in nature, such as growth, are related to the periodicity of light". Lower performance might be related to the innate behaviour and the stress caused by longer periods of light (Appelbaum and Kamler, 2000) as more time is spent searching for cover and display of aggression in territorial behaviour (Appelbaum and McGeer, 1998). However, during darkness more energy is channelled into growth and less spent on activity related to metabolism (Appelbaum and Kamler, 2000).

Behaviour

Over a full diel 24 h cycle, fish swimming activity seems to be variable and no consistent pattern according to photoperiod was found. This is in accordance to Hocutt (1989), where radio-tagged *Clarias gariepinus* in the lake Ngezi, Zimbabwe, did not show crepuscular nor nocturnal habits and individual activity occurred by day or night with no apparent pattern. In the present study, fish under longer periods of light (12L:12D and 18L:6D) showed higher swimming activity compared to those who were reared at shorter periods of light (0L:24D and 6L:18D). Fish under longer periods of light exhibited more aggression (injuries on the body). This is in accordance with the results of Almazán Rueda et al, (accepted c), who demonstrated that swimming activity and aggression are related in the African catfish. Fish that spent more time swimming, had more scars and/or wounds on the body. Therefore, the observed effects of photoperiod in the current study on blood stress parameters (cortisol, FFA, lactate and glucose) might be related to the increased aggression levels.

Stress parameters

In all photoperiods, constant swimming activity resulted in similar plasma glucose. Increases of plasma lactate levels are primarily due to anaerobic glycolysis in the white muscle from burst-type activity (Kieffer et al., 1994). High levels of lactate were found in fish under the longer light periods (18L:6D), where those fish spent more time swimming and were more aggressive. Burst-type activity occurred at the moment that fish attacked each other and tried to escape (data not shown). This is also reflected in the higher number of injuries on the body at this photoperiod compared to the fish under the other photoperiods. These aggression acts are also related to levels of plasma cortisol and FFA. Fish under a photoperiod of 18L:6D showed the highest level of plasma cortisol and FFA. The increase of cortisol can be due to the stress of a high swimming activity, searching for cover and/or aggression. A secondary response of the high levels of cortisol is the increased in plasma FFA. This increase in plasma FFA levels may be in part due to elevated concentration of cortisol in the fish (Ruane et al., 2002) as lipolysis is stimulated by cortisol and catecholamines. The results showed that light plays an important role in the African catfish behaviour and its well being. As the hours of light increased during the 24 h cycle, data suggests that the fish were more stressed and aggressive, compared to those under a reduced number of light hours.

Feed demand

No consistent effect of photoperiod on the within day variation in feed demand was found in the present study. The results showed a constant feeding pattern around the average feed intake for the photoperiods with continuous darkness and 6 h of light (0L:24D and 6L:18D). In photoperiods 12L:12D and 18L:6D during the light periods, feed demand per hour was increased above the average level. The feed intake per hour during dark periods was below the average feed intake (12L:12D and 18L:6D). This results indicate that *Clarias gariepinus* has a diurnal feeding pattern, also in the most natural photoperiod, 12L:12D. These results are in contrast with those found by Hossain et al., (1999). According to Hossain et al. (1999) feeding activity of African catfish, it is basically done at night. Although more studies suggest that *C. gariepinus* is an active nocturnal species (Britz and Piennar, 1992; Appelbaum and McGeer, 1998) it has been noted by Hecht and Uys (1997) that *C. gariepinus* is not only active at night. *C. gariepinus* will show feeding and browsing behaviour if feed is available during the light period. The results of feeding activity during light and dark periods in this experiment shows that *C. gariepinus* has both night and day feeding activities, with a preference to diurnal feeding in the 12L:12D photoperiod. Feeding activity can be entrained according to feeding schedules (Pavlidis et al., 1999), which are synchronised by environmental periodic factors, e.g. light or temperature. In the case of this study, checking the self-feeders between 10 A.M and 11 A.M. could also be an environmental periodic factor, which could explain the increase of feeding activity around 12 A.M. Checking self-feeders is a environmental factor not taken into account during this study.

In summary, increasing the period of light during 24 h had a negative impact on the performance and behaviour of the African catfish *Clarias gariepinus*. Fish reared under shorter periods of light (0L:24D and 6L:18D) showed an increase in growth compared to fish under longer periods of light (12L:12D and 18L:6D). Increasing the period of light increased fish activity and the number of injuries on the body. Longer periods of light result in stress, as evidenced by increased levels of plasma cortisol and FFA.

Feeding demand of *Clarias gariepinus* under conditions of free access to feed results in both a night and a day feeding activity.

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Chapter 7

Stocking density is not a proper parameter to define husbandry conditions in studies on welfare in *Clarias gariepinus*

Abstract

Stocking density can be altered either by changing: a) group size; b) tank/aquarium dimensions and c) a combination of both. Different stocking densities may evoke reactions of impaired welfare when the animal is not well adapted to cope with its surroundings and conspecifics. This study showed that in African catfish (*Clarias gariepinus*) increasing group size (fish per tank) improved growth, increased swimming activity, and reduced aggression, stereotypes and cortisol levels. Reducing the available swimming area reduced growth and swimming activity and increased cortisol levels. Differences in nutrient partitioning and behaviour suggested that fish's maintenance cost is greater in fish at restricted space and/or low stocking density due to agonistic behaviour and stress factors. The term "stocking density", defined as the number of fish per unit of space, cannot be used as a criterion to evaluate or compare systems.

Keywords: Group size, available swimming area, behaviour, stress, growth.

This chapter has been submitted.

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Introduction

Recently, there are increasing concerns about the welfare of farmed fish. Animal welfare is difficult to define, but refers to the quality of life (relationship between the physical and mental state of the animal and its environment) (Ellis et al., 2002). Fish farm conditions may lead to a confinement of the animals in small areas, resulting in a high number of fish per cultivated unit. Obviously stocking densities play an important role in the welfare of the fish. The term stocking density refers to the concentration at which fish are initially stocked into a system (Ellis et al., 2002). Stocking densities can be calculated according to the number of fish or kilograms (kg) per unit volume (m^3). Thus stocking density can be altered either by changing: a) group size, b) tank/aquarium dimensions and c) a combination of both. It can be hypothesised that group size and tank dimensions have a different impact on the fish. Group size will affect the rate of fish-fish encounters but has a minor influence on the available space where fish can swim, which is important e.g. for escape possibilities. Tank size will affect both fish-fish encounters and the available swimming area.

Different stocking densities may evoke reactions of impaired welfare when the animal is not well adapted to cope with its surroundings and conspecifics. In such cases, different behavioural patterns may occur, from reduced feed intake, to aggression and death. Physiological changes in cortisol, glucose and lactate levels in the blood may also occur.

Literature data show contradictory results concerning fish performance responses to changes in stocking density. Usually, increasing stocking density results in stress that leads to enhanced energy requirements causing reduced growth and food utilisation (Hecht and Appelbaum, 1987; Siddiqui, et al, 1993; Khwuanjai Hengsawat et al, 1997; Hecht and Uys, 1997). However, in a number of species, growth rate is not affected by an increase in fish density (Diana and Fast, 1989). In some other studies, increasing stocking densities had a positive effect on growth (for example, some cichlid species showed an increased growth rate with increasing density) (Hecht and Uys, 1997). Almazán Rueda et al., (submitted a) showed that growth of *C. gariepinus* increased at higher stocking densities as compared to low stocking densities.

Furthermore, several studies have demonstrated that high stocking densities can affect behaviour. Kaiser et al., (1995) showed that high stocking density reduced agonistic behaviour in African catfish. Most of these studies dealt with larvae and early stages in African catfish only (Hecht and Appelbaum, 1987; Hecht and Appelbaum, 1988; Kaiser et al., 1995). In larval and early juveniles of *C. gariepinus* (< 40 days old) the level of aggression was reduced as density increased (Hecht and Appelbaum, 1987; Hecht and Appelbaum, 1988). Almazán Rueda et al (submitted a) demonstrated that also for juvenile *C. gariepinus* the number of fish in a tank had a large impact on their agonistic behaviour. At a low stocking density (250 fish.m^{-3}) the numbers of scars and/or wounds on the body were higher and more agonistic behaviour occurred compared to the high stocking density (2500 fish.m^{-3}). Since the latter density is similar to the ones in actual Dutch farming conditions, these results are of practical importance. So far, no clear explanation is known why *C. gariepinus* aggressive behaviour reduces with increasing stocking density, or which factors affect this response.

As mentioned above, altering either group size or swimming space (tank size) or a combination of both can vary stocking density. Different conditions may lead to exactly the same stocking density (e.g. 300 fish m^{-3} can be achieved by either stocking 30 fish in an aquarium of 100 L or by 300 fish in an aquarium of 1000 L). The living conditions or "environment" in both examples is quite different and may lead to different responses by the fish. It is therefore hypothesised that the precise conditions of the living environment (tank size and group size) rather than stocking density determine the reaction of the fish. In the present study, the effect of these factors on growth, nutrient partitioning, behaviour and various blood parameters was assessed.

Material and methods

Animals, adaptation period and experimental conditions

Juveniles of African catfish were obtained from a commercial hatchery (Maatschap Fleuren en Nooijen, Someren, The Netherlands) and held in four aquaria to enable adaptation to the recirculating water system. During this period they were fed pelleted feed (pellet size was 3 mm) at 3% of bw.d^{-1}. Feed was given by hand in two equal meals at 09:00 and 17:00 h. The photoperiod was 14L:10D (lights on at 07:00 h), and the measured light intensity at the water surface was 150 lux. Light intensity was measured with a lux meter (model zu 104766, AEG, Frankfurt, Germany).

At the start of the 42-day experimental period (day 0), fish were 19-weeks old and weighed 29.5 ± 1.5 g (SD). Fish were randomly assigned to one of 14 aquaria (90x45x45 cm) with a water volume of about 120 L. Treatments were randomly assigned to the aquaria. The aquaria were within a recirculation system and water flow through each aquarium averaged approximately 5 $l.min^{-1}$ over the total period of the experiment. Water depth was maintained at approximately 30 cm. In the aquaria, average water temperature was 25.6 ± 0.9 ºC; oxygen concentration 7.2 ± 0.2 $mg.l^{-1}$, and pH ranged from 6.4 to 7.1.

Experimental set-up

Stocking density can be altered either by changing: a) group size; b) tank/aquarium dimensions and c) a combination of both (group size and tank dimensions). The present study had the following objectives: 1. to assess the effect of stocking density by altering the tank/aquarium dimensions at a constant group size; 2. to assess the effect of stocking density by altering the group size at a constant tank/aquarium dimension and 3. to assess the effect of two housing conditions while differing in tank/aquarium dimensions and group size, but at similar stocking density (number of $fish.m^3$).

Each of the 14 aquaria was assigned to one of four experimental treatments according to the following: Treatment 1 consisted of 30 fish with an available swimming area of 0.12 m^3 (four replicates); treatment 2 consisted of 30 fish with an available swimming area of 0.06 m^3 (four replicates); treatment 3 consisted of 30 fish with an available swimming area of 0.012 m^3 (four replicates) and treatment 4 consisted of 300 fish with an available swimming area of 0.12 m^3 (two replicates). Thus treatments 1, 2, 3 and 4 had a stocking density of 250, 500, 2500 and 2500 $fish.m^3$ respectively. Comparison A (objective 1) compared treatments 1, 2 and 3.

Comparison B (objective 2) compared treatments 1 and 4. Comparison C (objective 3) compared treatments 3 and 4.

Tank/aquarium dimensions do have an effect on water quality due to the volume buffering capacity, this in turn can affect performance and behaviour. Therefore, to study the pure effect of dimensions on performance and behaviour, the aquarium dimensions were kept equal but the available swimming area within the aquarium was changed. The area allowance in the tanks was induced by means of movable screens. These screens consisted of a rubber-edged metal frame with nylon mesh, which did not hinder water movement or waste removal. All fish were kept at a continuous light photoperiod (24 h light). Light was provided by 60 watts bulbs (frosted-mat; Rodulphus B.V, Scheepselktro, Duiven, The Netherlands) providing 150 lux of light at the water surface. All fish were fed by hand to apparent satiation (feed was provided to the fish until two pellets were left in the tanks). Fish were fed at 09:00 and 17:00 hrs every day. Formulated feed (commercial catfish diet, Trouvit ME-2 meerval, Trouw Nutrition, Nutreco, The Netherlands) was ground and mixed with 2% of diamol (Acid Insoluble Ash, as a digestibility marker), re-pelleted and fed to the fish during the experimental period. The dry matter of the diet was 92.8%, and on dry matter basis the content of crude protein was 48.8%, crude fat 10%, ash 10% and gross energy of 19.2 $kJ.g^{-1}$. Automatic faeces collectors (Choubert et al, 1982) continuously collected faeces from day 1 to the end of the experiment at day 42. Choubert collectors were connected to two tanks of each treatment. The faeces samples were collected from the collectors twice a day, in the morning and in the afternoon, both before feeding time. The collected faeces were stored at –20ºC for further analysis.

Sampling procedures and measurements

Fish were sampled at days 0, 14, 28 and 42. At each sampling time, the total fish biomass per aquaria was weighed. Specific growth rate (SGR) was calculated from the natural logarithm of mean final weight minus the natural logarithm of the mean initial weight and divided by total number of experimental days expressed as percentage. Feed intake per aquarium was recorded daily during the experimental period. Feed conversion ratio (FCR) was calculated per aquaria from feed intake data and weight gain. To study the influence of the treatments on the variation in weight, the coefficient of variation (C.V.) was calculated from individual body weight of each aquarium. Apparent digestibility coefficient (ADC) of energy and protein was calculated according to the following equation:

$$\text{ADC of a nutrient in diet (\%)} = 100\left[1 - \frac{[\%MD][\%NF]}{[\%MF][\%ND]}\right]$$

Where: MD is the marker in diet; MF is the marker in faeces; ND is the nutrient in the diet, and NF is the nutrient in faeces. The marker (AIA) was determined using acid-based digestion method. Energy determination of the samples was calculated by bomb calorimetry (IKA-calorimeter C7000, IKA-analysentechnik, D-79423, Weitersheim, Germany). Crude protein determination was made by Kjeldahl method (ISO 5983, 1979). Energy and protein analyses from freeze-dried material were done in duplicate.

At each sampling time, five fish per tank were anaesthetised with tricaine methanesulfonate (MS-222, 0.35 $g.l^{-1}$ (Crescent Research Chemicals, Phoenix, AZ, USA) and 0.7 $g.l^{-1}$ of $NaHCO_3$). One ml of blood was collected by hypodermic syringe (containing 3 mg Na_2EDTA) from the caudal blood vessels, within 3 min after being caught and anaesthetised. The collected blood was placed in cooled 1.5 ml plastic tubes, mixed and centrifuged at 6000 *g* for five min at 4ºC. The collected plasma was stored at minus 20ºC for further analysis. After blood sampling, these fish were put in fresh water to recover from the sedation before being returned to the aquaria.

Plasma analysis

Plasma cortisol levels were measured by radioimmunoassay using commercial antibody (cortisol-3-OCMO-antiserum, Bioclinical Services Ltd.) as described by Ruane et al., (2001). Plasma glucose was determined by the GOD-Perid® method (Boehringer). Plasma lactate was determined enzimatically using Sigma Diagnostic Kits (Sigma; Proc. no. 735).

Quantification of behaviour

The behaviour of the fish was recorded on video. Video recordings were made of each aquarium on the front side (45 x 45 cm) on days 10, 24 and 38; i.e., four days before each sampling time for plasma. Behaviour was recorded around the morning (from 08:30 till 10:00 h); around midday (from 12:30 to 14:00 h); around the afternoon feeding (from 15:30 till 17:00 h) and around midnight (00:30 t0 02:00 h). In total, 360 min were recorded per aquarium per period. The fish's activity behaviour was divided into two categories: swimming (while browsing, moving, eating and air breathing), and resting (lying on the bottom of the tank). Furthermore, air breathing was recorded. Every five min., the number of fish in each of these categories was counted as well as the total number of visible fish on the monitor. The activity patterns were expressed as percentages of the total number of fish counted.

Literature data on stereotypic behaviour in fish is scarce (Almazán Rueda et al, submitted a, b). Stereotypic behaviour is characterised by a repetitive behaviour pattern in animals (often seemingly non-functional behaviour). In the current study, stereotypic behaviour of African catfish was defined as continuously and compulsive swimming under a fixed pattern for a period of time (see Almazán Rueda et al, submitted b). In this study fish stereotypes were divided into two of the following categories according to the time involved: Type I, fish that were continuously and compulsive swimming in circles between 10 to 59 seconds; and Type 2, fish that were continuously and compulsive swimming in circles between 60 to 240 seconds. The distinction between Type I and Type II stereotypic behaviour was made on the assumption that Type I stereotypic behaviour would be the early development stage of the more severe Type II stereotypic behaviour (Almazán Rueda et al, submitted a). The frequency of the occurrence of both classes of stereotypic behaviour was recorded per tank per period during the total 360 min of video recording. No correction for the difference in number of fish visible per tank between the treatments (especially between high and low stocking density) was made because the total number of visible fish was not recorded.

Aggressive behaviour could not be quantified by video recordings, because aggressive acts could not be clearly distinguished. Almazán Rueda et al. (accepted c) showed that there is a strong correlation between the number of aggressive acts and the number of scars and wounds on the body of African catfish. In the current study aggressive behaviour was quantified by counting the number of scars and wounds on the body of individual fish at the start and at the end of each period of the experiment. From these data, the average numbers of scars plus wounds per fish was calculated per tank.

Statistics

In this study, tank was used as the experimental unit. Mean performance data and mean behaviour data were analysed by One-way ANOVA (PROC GLM, SAS, 1990) as follows: A) between treatments 1, 2, and 3 to analyse the effect of swimming space at the same group size; B) between treatments 1 and 4 to analyse the effect of group size in the same swimming space C) between treatments 3 and 4 to analyse the effect of different group size and different swimming space at the same stocking density. The error terms of these ANOVA analyses were tested for homogeneity of variance and normality. Percentages were arcsine transformed before ANOVA was carried out.

Results

Objective 1. Effect of stocking density by altering available swimming area at a constant group size (n=30)

In table 1 and 2, the P value of comparison A reflects whether treatment 1 is equal to treatment 2 and is equal to treatment 3. This comparison reflects the effect of stocking density by only altering the available swimming area at a constant group size. Results from this study showed that performance of the fish was affected when the available swimming area was reduced (comparison A, Table 1). Fish showed a decrease in growth and SGR as available swimming area decreased (P=0.028 and P=0.001 respectively). FCR increased from 0.8 to 1.0 when the available swimming area decreased (P=0.009, Table 1). Differences on apparent digestibility or body composition were not found. The available swimming area (comparison A, Table 2) affected swimming activity of the fish. Fish spent less time swimming when the available swimming area was decreased (Table 2). Air breathing activity was not different among treatments. Injuries on the body (scars and/or wounds) were lower at the smallest available swimming area (*P*=0.001). This was reflected in the percentage of fish with scars and/or wounds per aquarium. At the smallest available swimming area, a lower percentage of fish bitten (*P*=0.003, Table 2) was found. A similar relation was found for the stereotypic behaviour. Type 1 stereotype was reduced as the available swimming area was reduced (*P*=0.003, Table 2). Type 2 showed no significant difference. The available swimming area (comparison A, Table 2) also affected stress parameters in the blood. Fish showed an increase in cortisol levels when the available swimming area decreased (*P*=0.004). No differences in glucose or lactate were found.

Objective 2. Effect of stocking density by altering group size at a constant swimming area (0.12 m^{-3})

In table 1 and 2, the *P* value of comparison B reflects whether treatment 1 is equal to treatment 4. This comparison reflects the effect of stocking density by only altering the group size at the same available swimming area. Fish performance differed among the treatments (comparison B, Table1). Fish showed a significant increase in growth and a tendency for a higher SGR as group size increased (*P*=0.024 and *P*=0.088 respectively). FCR was not affected by group size, but feed consumption was. Fish at a large group size had a tendency to demand more feed than fish at small group size (*P*=0.094, Table 1). Differences in apparent digestibility or body composition were not found between both group sizes. Fish air breathing was affected by group size (comparison B, Table 2). Fish in the large group size air-breathed more times than fish in small group size (*P*=0.001). Aggression of the fish, by means of scars and/or wounds on the body, was lower at large group size (*P*=0.002). This was also reflected in the percentage of fish with scars and/or wounds per tank. At large group size, the percentage of fish bitten was the lowest (*P*=0.045, Table 2). A similar relation was found for the stereotypic behaviour. Type 1 stereotype was reduced as the group size was increased (*P*=0.023, Table 2). Type 2 stereotype tended to be higher in fish at small group size compared with fish at large group size (*P*=0.064). Plasma lactate was affected by group size (comparison B, Table2). Fish at small group size tended to have higher lactate levels than fish at big group size (*P*=0.069, Table 2). No differences in glucose or cortisol were found.

Objective 3. Comparison of two housing conditions with equal stocking density but different group size and available swimming area.

Stocking density can be altered by group size and by the available swimming area. Consequently, housing conditions can still change (different group size and available swimming area) at the same stocking density. In comparison C treatment 3 and treatment 4 were compared to assess this hypothesis. Treatments 3 and 4 had a group size of 30 and 300 fish and an available swimming area of 0.012 and 0.12 m^{-3}, respectively. Despite the same stocking density, performance and behaviour were different between treatments 3 and 4 (comparison C, Table 1 and 2). At treatment 3, final body weight and SGR were lower compared with treatment 4 (*P*=0.005 and *P*=0.003 respectively). FCR increased from 0.8 and 1.0 at treatment 3 and 4, respectively (*P*=0.026, Table 1). Feed consumption was higher at treatment 4 than at treatment 3 (*P*=0.005, Table 1). Apparent digestibility and body composition were not different between treatments 3 and 4. Both time spent swimming and air breathing activity ware lower at treatment 3 that at treatment 4 and (*P*=0.001 and *P*=0.001, respectively, Table 2). The number of scars and/or wounds on the fish body, the percentage of fish bitten per tank, as well as stereotypic behaviour, was not different between treatment 3 and 4. Plasma lactate was different between treatments (comparison B, Table2). Fish at treatment 3 had higher lactate levels than fish at treatment 4 (*P*=0.008, Table 2). No differences in glucose or cortisol were found.

Table 1. Final mean body weight, coefficient of variation (C.V.), specific growth rate (SGR), feed conversion ratio (FCR), individual feed consumption per day, survival rate, apparent digestibility and body composition in African catfish as affected by the experimental treatments.

	Experimental treatments								Comparison		
Treatment	1		2		3		4		A	B	C
Group size (fish.tank^{-1})	30		30		30		300				
Available swimming area (m^{-3})	0.12		0.06		0.012		0.12				
Stocking density (fish.m^{-3})	250	S.D.	500	S.D.	2500	S.D.	2500	S.D.	*P*	*P*	*P*
Final body weight (g)	186.2	6.2	181.4	13.9	163.2	9.5	207.1	10.4	0.028	0.024	0.005
C.V. (%)	32.6	3.1	34.0	3.2	31.4	3.6	28.6	3.5	n.s.	n.s.	n.s.
SGR (%.d^{-1})	4.5	0.1	4.2	0.2	4.0	0.1	4.7	0.1	0.001	0.088	0.003
FCR (g.g^{-1})	0.8	0.05	0.9	0.14	1.0	0.19	0.8	0.01	0.009	n.s.	0.026
Feed consumption (g.d^{-1})	2.9	0.1	3.0	0.2	2.9	0.1	3.2	0.1	n.s.	0.094	0.005
Survival rate (%)	92.5	4.2	94.2	3.2	96.7	4.7	92.3	4.2	n.s.	n.s.	n.s.
Apparent digestibility											
Protein (%)	89.4	1.4	86.4	1.9	90.3	1.7	90.3	0.3	n.s.	n.s.	n.s.
Energy (%)	87.6	0.6	84.5	2.2	87.8	1.9	87.9	0.4	n.s.	n.s.	n.s.
Body composition											
Dry matter (g.kg^{-1})	251.1	0.8	250.1	8.3	245.4	6.4	251.6	0.4	n.s.	n.s.	n.s.
Crude protein (g.kg^{-1})	642.6	8.7	633.7	10.3	647.6	12.1	639.2	15.4	n.s.	n.s.	n.s.
Crude fat (g.kg^{-1})	234.1	6.4	232.0	16.7	232.8	6.9	239.3	7.97	n.s.	n.s.	n.s.
Ash (g.kg^{-1})	115.9	5.9	114.5	1.7	114.8	4.8	113.3	0.4	n.s.	n.s.	n.s.
Gross energy (kJ.kg^{-1})	24.4	0.2	24.3	0.3	24.4	0.2	24.7	0.1	n.s.	n.s.	n.s.

n.s.= not significant (P>0.005). Comparison A: ANOVA performed between treatments 1,2 and 3. Comparison B: ANOVA performed between treatments 1 and 4. Comparison C: ANOVA performed between treatments 3 and 4.

Table 2. Behaviour patterns of the African catfish as affected by the experimental treatments. Mean values are expressed as percentage of active fish for the activities; frequency for the scars on body, and concentration for the stress parameters.

	Experimental treatments								Comparison		
Treatment	1		2		3		4		A	B	C
Group size (fish.tank^{-1})	30		30		30		300				
Available swimming area (m^{-3})	0.12		0.06		0.012		0.12				
Stocking density (fish.m^{-3})	250	S.D.	500	S.D.	2500	S.D.	2500	S.D.	*P*	*P*	*P*
Behaviour											
Swimming activity (%)	63.2	4.9	64.5	9.4	49.4	2.9	72.1	1.3	0.001	n.s.	0.001
Air breathing (%)	2.1	0.3	2.3	0.3	2.9	0.4	4.9	1.0	n.s.	0.001	0.001
Resting (%)	36.8	4.9	35.5	9.4	50.6	2.9	27.9	1.3	0.001	n.s.	0.001
Injuries (aggression)											
Total scars on body	1.78	0.5	1.73	0.7	0.66	0.2	0.67	0.05	0.001	0.002	n.s.
% of fish bitten	82.2	11.7	84.1	11.8	44.1	15.3	56.6	2.9	0.003	0.045	n.s.
Stereotypes											
Type 1	20.3	10.4	18.0	3.7	0.0	0	0.5	0.7	0.003	0.023	n.s.
Type 2	2.3	0.5	3.3	3.8	0.0	0	0.5	0.7	n.s.	0.065	n.s.
Stress parameters											
Glucose (mmol.l^{-1})	6.48	0.6	6.34	0.4	6.34	0.2	6.20	0.5	n.s.	n.s.	n.s.
Lactate (mmol.l^{-1})	2.53	0.6	2.48	0.4	2.70	0.1	2.04	0.3	n.s.	0.069	0.008
Cortisol (ng.ml^{-1})	139.9	23.4	182.7	37.3	183.0	29.8	162.7	15.1	0.004	n.s.	n.s.

n.s. = not significant (P>0.005). Comparison A: ANOVA performed between treatments 1,2 and 3. Comparison B: ANOVA performed between treatments 1 and 4. Comparison C: ANOVA performed between treatments 3 and 4

Discussion

Literature data show contradictory results concerning African catfish performance in relation to stocking densities: negative correlation between performance and stocking density have been reported by Hecht and Appelbaum (1987), Siddiqui et al, (1993), Khwuanjai Hengsawat et al, (1997) and Hecht and Uys, (1997). No correlation was found by Diana and Fast (1989); and positive correlation between performance and stocking density have been reported by Hecht and Uys (1997) and Almazán-Rueda et al, (submitted a). This contradiction may be related to the experimental conditions that were used in the different studies. Stocking densities can be varied by altering either group size or available swimming area (tank dimensions) or a combination of both. The current study shows that stocking density is not a good indicator for characterising the husbandry conditions. At the same stocking density, the performance was affected by changing both group size and available swimming area (treatment 3 vs. 4 Table 1). This may be as explanation for the different reported studies in literature on the relation between stocking density and performance.

Studies of stocking density under laboratory conditions usually are done at a reduced scale compared to normal farm conditions. The current results imply that one should be careful regarding the extrapolation of the results to normal farm conditions. For setting regulations regarding stocking density for farm conditions more research is required on this scale effect.

In this study, increasing the stocking density by increasing group size (number of fish per tank) had positive results for performance of the African catfish with no effect on the size variation of the fish. This is in accordance with the results found by Almazán-Rueda et al., (submitted a), that under similar conditions (fish.m^{-3}), fish showed improved growth rates when reared in tanks with a higher number of fish. These results are contrary to the general belief for fish that larger group sizes reduce growth and increase size variation (see review of Ellis et al., 2002). Variations among these results were probably caused by differences in biology and environmental requirements for each species. Behaviour is also affected by different group size. Fish in a large group showed a lower number of scars and/or wounds on the body than fish in a small group. This is in accordance with results found in fish (Dill, 1983; Brown, et al., 1992; Alanärä and Bränäs, 1996), e.g., aggression is decreased as the group size is increased. At the big group size, fish form a dense shoal that interferes with their innate aggressive behaviour and consequently defending a territory becomes inefficient (Ellis et al., 2002; Almazán Rueda et al., submitted a).

The results of the present study showed that growth in the African catfish (*C. gariepinus*) was increased by increasing the available swimming area (m^3) and the latter had no effect on the size variation of the fish. Reducing the available swimming area for the fish can affect normal functions such as swimming. Swimming activity was reduced as the available swimming area was reduced. In this way, growth may be related to the ability of the fish to transform feed into muscle as the space allows them to swim. The increased growth in fish that swim more may be due three factors: 1) the chronic exercise may cause protein synthesis to increase relatively more than protein degradation (Houlihan and Laurent, 1987). 2) Swimming may increase growth hormone levels (Barret and McKeown, 1988); or swimming may reduce stress levels (Woodward and Smith, 1985). The lack of space also affected aggression and stereotypic behaviour in the African catfish. Fish with a larger swimming area showed a higher number of injuries (scars and/or wounds) on the body compared with the fish that had a reduced swimming area. In a similar experiment with rabbits, Morisse and Maurice (1997), showed that social behaviour was reduced as the available area was reduced. Those rabbits also exhibited a reduced locomotor behaviour. In the present study, the lack of space influenced the stress level of the fish. Fish under small available swimming area had higher levels of cortisol. Indeed crowding may induce stress, as is illustrate by literature (Pickering and Stewart, 1984; Ruane et al, 2002). On the other hand, continuous swimming may also reduce cortisol levels (Woodward and Smith, 1985). In the present study, fish that spent more time swimming had lower values of cortisol than those who spent less time swimming did.

The results of the present study suggest that at fixed group size, increasing the available swimming area had negative effects in terms of swimming activity and aggression, but positive effects in terms of growth. Swimming activity will be reduced and aggression will be increased when increasing available swimming area. In this case, poor growth is expected due to the increased of aggression and low swimming activity.

The development of stereotypes in the present study suggests that fish were subjected to chronic stress (Almazán Rueda et al., submitted a) or sub-optimal environment (Masson, 1991). The present results showed that available swimming area and group size are important factors for the development of stereotypes in the African catfish. Fish under a high stocking density (either by increasing group size or by reducing the available swimming area) reduced the frequency of stereotypes and this may be related to the lack of space (Almazán Rueda et al., submitted a). Stereotypic behaviour is known to develop without apparent purpose. The development of stereotypes can be due to frustration, unavoidable stress or fear and restraint and lack of stimulation (Masson, 1991). The fish may develop stereotypes as an answer to adverse factors.

Eglal (1996), in experiments with (*C. lazera = gariepinus*), found that increasing the stocking density reduced fish body composition in terms of DM%, CP%, EE% and energy content and also reduced growth and feed intake. Opposite results were found in this study, body composition was not affected in any of the treatments, but it did for final body weight. If growth was affected and body composition and apparent digestibility values were not affected, it means that maintenance levels of the fish were different among the treatments. These results suggest that when stocking density is reduced (by available swimming area, group size or both), the maintenance cost is bigger than the one at high stocking density. African catfish is an aggressive fish (as one can see by the number of scars and/or wounds on the body) and increasing the stocking density reduces aggression. This suggests that agonistic behaviour is energetically costly, and by reducing the agonistic behaviour (by increasing the stocking density) the energy that fish expend in defending territories goes to growth. Fish under high stocking densities had better performance (better growth, SGR, and FCR) and less agonistic behaviour (injuries on the body) and less stereotypes than those who grew under low stocking density.

General public opinion is that high stocking density is related to poor welfare. Welfare pressure groups have suggested that current farming practices may compromise fish welfare (Lymbery, 2002). The current study shows that high stocking density improves growth, reduces aggression and some stress parameters in the African catfish. These observations cannot be extrapolated to other species, since each species has their own requirements.

Conclusions

The term “stocking density”, defined as the number of fish per unit of space, cannot be used as a criterion to evaluate or compare systems.

The above conclusion was substantiated in the present study by the observation that increasing stocking density did not impair welfare in the African catfish, as measured by better growth, SGR and FCR. Fish also showed more swimming activity and fewer injuries on the body (aggression); reduced frequency of stereotypes and lower plasma cortisol levels.

High number of the African catfish per unit ($fish.m^{-3}$) is not a problem as long as there is sufficient available swimming area. However, reducing the available swimming area irrespectively of the stocking density affects growth and behaviour.

Differences in nutrient partitioning and behaviour suggested that fish’s maintenance cost is greater in fish at restricted space and/or low stocking density due to agonistic behaviour and stress factors.

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Chapter 8

General discussion

Animal welfare and legislation

After World War II, federal policies (in U.S.A. and Europe) encouraged intensified agricultural production practices. The first attempt to regulate animal welfare was done by the Branbell Committee in 1965 in the United Kingdom. This commission stated that animals should be reared in such a way that they can perform their natural and species-specific behaviour. By the 1990's, the public opinion pressed governments to consider legislation regarding animal welfare in farming systems. In the UK, the first report published regarding fish farming was “Compassion In World Farming” (CIWF) in 1992, in which serious welfare problems in the rearing systems were highlighted. In the same year the UK Farm Animal Welfare Council (FAWC), updated the five freedoms for animals stipulated in the Branbell Committee. In 1996, FAWC reported that, in certain areas, welfare conditions were even worse than first documented by CIWF (Lymbery, 2002). The Committee is presently in the process of drafting E.U. regulations regarding welfare in fish farming. Some of the most important factors affecting welfare are stocking density, feeding methods, disease and parasitism, handling and transport, and starvation and slaughter. In 2003, the Dutch Advisory Board regarding Animals (Raad voor Dieren Aangelegenheden, RDA) submitted a formal advice to the Dutch government regarding criteria to assess whether new fish species can be admitted to the list of animals, which are allowed to be kept for production purposes (positive list). Welfare criteria are, besides health concerns, the most important criteria to accept the fish species on this list

Welfare in fish

According to the Cambridge Dictionary of English the term welfare refers to the physical and mental health and happiness of a person. A similar term can be used for animals. Duncan and Fraser (1997) refer to welfare as a status of good life for animals. This quality of life may involve several aspects, such as health, happiness and longevity (Duncan and Fraser, 1997). It is generally agreed that farmed animals should not go through unnecessary suffering (Morzel et al., 2002). The term welfare is applied to the whole life of the animal, from egg development, growth, reproduction to death. In farm animals, the production processes affect this course of life. As in any type of animal production, fish production involves the transformation of feed into fish in the least time possible. Welfare is composed of two parts: welfare related to production, that means keeping the animal alive and growing; and the welfare related to the quality of life, that means keeping the animal from suffering as a result of poor husbandry conditions (Lymbery, 2002). Common farming practices usually score well on welfare related to production, but face serious problems regarding welfare related to quality of life. The latter is due to inherent characteristics of most farming systems, e.g. massive and unnatural overcrowding of the animals. As indicated above, the public awareness about welfare issues referred to the quality of live is increasing. In the last decade attention to welfare also increased regarding the aquaculture sector. Therefore it is inevitable that the fish farming industry must address the concerns raised by the consumer, and the animal welfare agencies.

How to assess welfare?

Parameters for assessing the impact of husbandry conditions on welfare can be classified into: 1) Performance responses, 2) Physiological responses, and 3). Behavioural responses

These responses (performance, physiological and behaviour) cannot be seen apart from each other because of the functional interaction between these responses (Figure 1). To study welfare in terrestrial farm animals, normally all three classes of responses are assessed. However, in aquatic animals, welfare assessment is still in its early stages. To date, the potential impact of husbandry conditions, which might act as stressors, on fish welfare are assessed by studying the performance responses and/or by studying the physiological responses. Growth response to different feeding systems, feed ingredients, and husbandry conditions was the main focus of aquacultural research in the past 20 years. The physiological response of fish to stress has been widely studied (Barton and Iwama, 1991; Iwama et al., 1997; Wendelaar Bonga, 1997; Iwama et al., 1999; Perry and Berbier, 1999; Ruane, 2002). Regarding the behavioural response of fish to husbandry stressors, not much is known to date, and this area really constitute a gap in the knowledge for welfare studies. Only very little was published regarding the combined effect of performance, physiology, and behaviour (see for example, Britz and Pienaar, 1992; Kaiser et al., 1995 a, b; Hecht and Uys, 1997; Appelbaum and Kamler, 2000; Ellis et al., 2002).

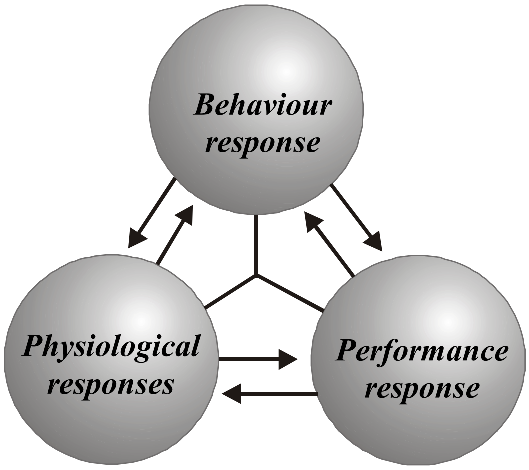

Figure 1. Relationship between behavioural, physiological and performance responses

In case of acute stress (short term reaction) measuring the physiological response (e.g. plasma cortisol, lactate and glucose levels) in combination with performance responses (e.g. feed intake) is often sufficient for clearly assessing welfare. However, in case of chronic stress, only measurements of physiological and performance responses are not sufficient for welfare assessment in fish. In chronic stress situations, fish will try to cope and adjust to the new situation. For example, when fish are forced to swim, plasma cortisol increases in the first week of the exercise, but reduces to normal levels after a few weeks (Woodward and Smith, 1985). In the present study, African catfish (Chapter 7) grew bigger when a large swimming area was provided. When performance alone would be used as welfare indicator, the results would suggest that a large swimming area is beneficial for the welfare of *Clarias gariepinus*. This conclusion would be further substantiated by the lower cortisol levels found in this fish, compared to the ones kept in a restricted area. However, when we also take the behavioural response into account, the picture becomes more complicated. Fish kept in a large swimming area exhibited more aggressive behaviour (scars and/or wounds on body), than the ones in a restricted area. The question whether a large swimming area is beneficial for welfare of African catfish or not, is less clear when all parameters are taken into account.

In conclusion, for assessing welfare in fish, measurements of behavioural, physiological as well as performance responses are needed. Behaviour tends to react slowly and to persist longer to changes in the environment. Therefore, behavioural responses may be especially suitable to assess chronic stress and cannot be omitted.

Behaviour

This study is a first step in a wider and badly needed research on welfare and welfare assessment in farmed fish. The current study focused thereby on the impact of various husbandry conditions on the behaviour of the African catfish, *Clarias gariepinus*. The following behavioural types were identified as potentially parameters for assessing welfare:

1) Swimming activity: when fish were moving, browsing, eating and air breathing.
2) Air breathing.
3) Agonistic behaviour by direct observation.
4) Agonistic behaviour by indirect observation (by counting the number of scars and/or wounds on the fish body).
5) Occurrence of stereotypes.
6) Escaping behaviour.
7) Feeding behaviour

Table 1 presents the summarised effects of various husbandry conditions on the behaviour of African catfish. From this table it becomes clear that various husbandry conditions influence the behaviour of the fish. Consequently, this indicates that, some husbandry conditions might impair the natural behaviour of the fish. On the other hand, there is also evidence that husbandry conditions can be used to reduce undesirable (e.g. agonistic behaviour). To assess welfare of fish in farming conditions, the key question is which parameter is really suitable as indicator for welfare.

Table 1. Behaviour patterns of the African catfish (*Clarias gariepinus*) as affected by different husbandry conditions.

	Swimming activity (%)	Air breathing (%)	Eating (%)	Stereotypes Type I	Stereotypes Type II	Scars and/or wounds	Fish bitten (%)
Feeding method							
Hand feeding (C3)	+ +	NM	(-)	NM	NM	0	+ + +
Photo period							
Increasing light period (C4, C5, C6)	+ +	0	0	0	0	+ + +	+ +
Light intensity							
Increasing intensity (C4, C5)	+ +	+	0	0	0	+ +	+ + +
Stocking density							
Increasing group size (C5, C7)	+ + +	+ + +	+	- -	- -	- - -	- - -
Increasing swimming area (C7)	+ + +	0	0	+ + +	+	+ + +	+ + +

Cn = chapter number. (NM) = not measured. (0) = No impact. (-) = Reduction. (+) = Increment. (-) or (+) low; (- -) or (+ +) intermediate, and (- - -) or (+ + +) high reduction or increment, respectively. Hand feeding method (control = self-feeding).

Which behaviour parameters are good for measuring welfare?

Swimming activity

Swimming activity increased as the light hours increased (Chapter 4 and 6). Also high light intensity and feeding fish by hand increased the swimming activity. The significance of these results may be derived from the correlation, which was found between swimming activity and aggression (Chapter 4). There is a strong relation between swimming activity and aggression (Figure 2). Apparently activity levels and aggression seems to respond similarly to environmental variables.

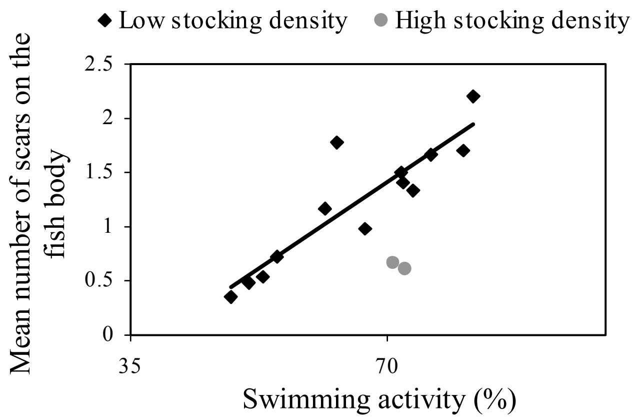

Figure 2. Relationship between the percentage of swimming activity and aggression (as scars and/or wounds on the fish body). For low stocking density data from chapters 4, 6 and 7 were used; and for high stocking density data from chapter 6 were used.

Based on the relation between activity and aggression, one may hypothesise that any husbandry condition or management measure, which potentially stirs or stimulates swimming activity, could also induce higher aggression levels. The present study shows that reality is less straightforward. When we consider the effect of stocking density (Chapter 7), we found that high stocking densities resulted in higher percentage of fish swimming, yet also in lower levels of aggression, stereotypes and cortisol. Following these results, one can hypothesise that fish at high stocking density may maintain aggression constant, even if the fish swim more (Figure 3). Therefore, swimming activity in *Clarias gariepinus* is not a good parameter for welfare assessment.

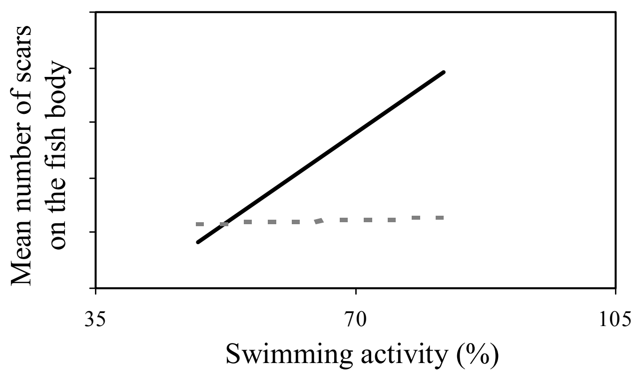

Figure 3. Hypothetical relationship between the percentage of swimming activity and aggression (as scars and/or wounds on the fish body). Solid line is for low stocking density and dotted line for high stocking density.

Aggressive behaviour (direct observation)

From the human point of view aggression among fish is not desirable. On the other hand, if aggression is part of the natural behaviour of the species, one can argue about the previous anthropomorphic view to fish behaviour. Whatever is the case, excessive aggression is not desirable. Therefore, measuring aggressive behaviour by direct observation might be a good parameter for welfare assessments. However, there are some potential practical limitations to the observation of agonistic behaviour: 1) it is very laborious; 2) at high stocking densities, agonistic behaviour by direct observations or video recordings are very difficult to observe. At high stocking density and due to the high number of fish in a small area, it is impossible to distinguish agonistic behaviour. However, this argument also exists for other types of behaviour; 3) Under more practical conditions, behaviour observations are difficult to perform due to factors such as low light intensity and water turbidity. Again this is also a problem for measuring other types of behaviour and thus not unique for aggressive behaviour. In this thesis, agonistic behaviour was only studied in chapter 4 when fish were kept at a low stocking density.

Aggressive behaviour (indirect observation)

Aggressive behaviour can also be measured indirectly by counting the number of scars and/or wounds on the fish body. This parameter seems to be a good candidate for assessing welfare. Indeed, the number of scars had a good relation with the agonistic behaviour of the fish (Chapter 4). Furthermore, it does not take much time to sample the fish and count the number of scars and/or wounds on the body. But also this type of observation must be taken cautiously. Scars and wounds can heal between sampling times; this can mask part of the effects. Another problem for measuring scars can be the sort of stressors that the fish are submitted to. Fish will react in a different way, if they are submitted to acute or chronic stressors. Acute stressors may result in a higher number of scars and/or wounds as a response to the spontaneous stress, such as housing of new groups, sampling times, different feeding method, etc. Therefore, measurement of chronic stressors might still be strongly determined by the initial acute phases of the response to the chronic stressor.

Stereotypes

The results of the present study suggested that the African catfish (*C. gariepinus*) might develop stereotypes. In general, stereotypic behaviour is related to poor or sub optimal conditions. High stocking density seems to reduce the incidence of stereotypes. The reduction in the frequency of stereotypes and in aggression, at high stocking density, can be just the effect of lack of space to allow the fish to perform the behavioural response. Stereotypes must be good indicator of welfare in fish. However, in future studies, the observed behaviour, which was classified as stereotypic behaviour, should be verified as a true stereotype.

Escaping behaviour

Escaping behaviour, e.g. attempt of the fish to escape from de tank with more than half of the body outside the water level, was only assessed in one study in the present thesis. Escaping behaviour can be considered as an attempt of the African catfish to flee from undesirable conditions. This type of behaviour is rather simple and can be observed at the water surface (either by direct observation or by video recordings). Escaping behaviour might be a good candidate for assessing welfare or stress response in fish. However, one should be conscious whether this escape behaviour might be hampered in its expression by various husbandry conditions. For example, at very high stocking density, the individual fish might not have the ability to create sufficient momentum for lifting its body out of the water due to the limited space availability.

Can the current study provide a basis for criteria concerning welfare in fish?

The answer to this question is negative. To give some criteria concerning fish welfare, one must understand the nature of the fish, from the reaction at the senses and brain level to the whole system performance and the relation among conspecifics, and then relate the possible responses to changes in the environment. There is still a large need for knowledge on which parameters/responses should be measured and also how and when these parameters should be measured in order to generate objective conclusions.

In the present study, we mostly create large contrasts between husbandry conditions, to measure the response of the fish to those conditions. Actually, we just reproduced unacceptable levels of tension. Furthermore, it is difficult to draw conclusions concerning fish welfare from this study, due to some contradictory results. However, concerning the light regime, the African catfish (*C. gariepinus*) tended to behave in a similar way through all the experiments. Data suggest that the fish preferred shorter photoperiods (Table 1), as seen by the lower aggression levels, swimming activity, some physiological parameters and by the better performance. Similar results were also found concerning light intensity, at high light intensity made the fish more aggressive compared to low light intensity. Another reason why is difficult to formulate criteria for welfare is the occurrence of interactions effects between husbandry conditions. For instance, stocking density overshadows the effect of light regime in the expression of behaviour and performance (Chapter 5). In this study, stocking density seems to be the strongest factor affecting the overall response of the fish. Stocking density depends on two factors: group size and available swimming area (tank size) (Chapter 7). Data suggest that group size is more important than available swimming area concerning performance, behaviour and physiological responses, but as long as the fish has enough swimming area available. When swimming area is limited, this has such a negative impact on performance and physiological responses that it overrules the effects of group size. A proper analysis of the effect of stocking density on the welfare of fish would require more densities on the scale of low to high to be tested. Only in this way a “density response” pattern and/or optimal stocking density level may be found. Because in the present study we tested only three stocking densities, the results are not suitable to draw conclusions about the optimal density for farming *Clarias gariepinus.*

Future research must focus on series of welfare indicators for practical conditions for everyday use, e.g. certain behavioural response, which are easy to detect and it do not involve sophisticated tools or analysis.

Conclusions

The results of the present thesis proved that small changes in husbandry conditions, such as reduced hours of light during a 24 hours cycle and low light intensity may improve the general conditions for the African catfish (*Clarias gariepinus*). Contrary to the general belief, higher stocking density reduced aggression and improved performance. The results of the present thesis may be helpful to solve some problems of aggression, improve growth and reduce negative physiological responses to stress in the African catfish culture.

On the other hand, assessing the welfare of the African catfish is a hard task, especially because results can be contradictory. Using a single parameter, such as behaviour, physiological levels or performance, is not enough to assess welfare. A combination of the three types of parameters mentioned above may help to understand fish welfare, but precautions must be taken in order not to draw erroneous conclusions.

This thesis is the first step towards assessment of the welfare in the African catfish.

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Summary

During the past three decades, aquaculture has expanded, diversified and intensified. To increase production under farm conditions, the most common practice has been to increase the number of fish per culture unit and/or to increase growth rate by improving feeds, feeding strategies and optimising water quality. Secondary practices are to manipulate photoperiod and/or light intensities. All these efforts strongly focused on the improvement of productivity, but little attention has been paid on the question how and if the animal could cope with these new husbandry conditions. Welfare issues gained only very recently some interest in fish farming. Public apprehension about intensive fish culture has not reach the high levels of concern as it has for the pig and poultry industry. However, animal welfare pressure groups have suggested that current farming practices may compromise fish welfare as well. There are several factors that may affect welfare in fish. Animals will respond at different levels when they are subject to environmental conditions which impaired their welfare status: varying from endocrine changes to atypical behaviour. The overall objective of this thesis is to determine the effect of different environmental factors on social behaviour of the African catfish (*Clarias gariepinus*) and the results of this social behaviour in relation to stress response, feed intake, and growth. In this thesis we hypothesise that environmental factors play a major role in the expression of behaviour and performance of the African catfish (*Clarias gariepinus*).

The first step of this study (Chapter 2) focused on the development of base line data on the behaviour of the African catfish (*Clarias gariepinus*) under "normal" and standardised conditions. In this part, behaviour patterns, such as swimming activity, air breathing, aggression (as scars and/or wounds on body), and stereotypes were described and used as a reference for the following chapters.

In the second part of this thesis (Chapters 3, 4 and 5), various husbandry conditions were screened for being potential stressors and thus affecting the fish welfare. In addition to the main effect of the studied husbandry factor, there was also attention paid to the occurrence of interaction effects between different husbandry factors. In **Chapter 3**, the effects of feeding method (continuous by self-feeders vs. twice a day hand feeding), light intensity (15 vs. 150 lux), photoperiod (continuous

light vs. 12D:12L) and their interactions on water quality and growth of juvenile African Catfish (*Clarias gariepinus*) were studied. Feeding method influenced water quality. Suspended solid production was almost doubled in self-fed fish compared with those fed by hand fed. TAN production was influenced by light intensity, being higher under 15 lux compared to 150 lux. Despite of the similar growth, self-fed fish demanded more feed compared to hand fed fish. Based on the performance data, juvenile African catfish (*C. gariepinus*) seemed to be insensitive to light intensity but were influenced by photoperiod. A 12D:12L photoperiod increases growth and feed consumption compared with 0D:24L.

In **Chapter 4** the same husbandry factors as in Chapter 3 were assessed but now in relation to the behaviour of the fish. Fish under continuous light and at high light intensity spent more time swimming, were more aggressive and consequently had more scars on the body than fish under 12D:12L photoperiod and at low light intensity. A good correlation between swimming activity and aggression and/or scars on the body was found.

In **Chapter 5** stocking density, photoperiod and light intensity were assessed. High stocking density (2500 fish.m^{-3}) increased growth, swimming activity, and reduced FCR, aggression and the occurrence of stereotypes compared to low stocking density (250 fish.m^{-3}). High stocking density also showed to have an influence on plasma cortisol and glucose levels.

Based on the results in **Chapters 3**, **4** and **5** (behavioural, physiological and performance responses to the imposed husbandry conditions), stocking density and photoperiod were selected for more detailed assessment in Chapter 6 and 7, respectively. Different photoperiod regimes (24D:0L, 18D:06L, 12D:12L and 06D:18L) were studied in Chapter 6. Short periods of light or no light (6L:18D and 0L:24D) resulted in an increased growth compared to extended periods of light (18L:6D and 12L:12D). Fish kept at longer periods of light (12L:12D and 18L:6D) showed an increased swimming activity, more aggression (injuries on the body), and higher blood plasma levels of lactate, free fatty acids, and cortisol compared to fish kept at shorter periods of light (0L:24D and 6L:18D). As the hours of light increased during the 24 h cycle, data suggested that the fish became more stressed and aggressive.

In **Chapter 7**, the effects of group size as well as available swimming area on the fish's behaviour, stress response and growth were assessed. African catfish (*Clarias gariepinus*) improved growth, increased swimming activity, and reduced aggression, stereotypes and cortisol levels when the group size increased (fish per tank). Reducing the available swimming area reduced the growth and the swimming activity and increased blood plasma cortisol levels. Differences in nutrient partitioning and behaviour suggested that fish's maintenance cost was higher in fish at the restricted space and/or low stocking density due to agonistic behaviour and/or exposure to stress. The term "stocking density", defined as the number of fish per unit of space, cannot be used as a criterion to evaluate or compare systems.

This thesis proved that small changes in husbandry conditions, such as reduced hours of light during 24 h and low light intensity might improve general conditions for the African catfish (*Clarias gariepinus*). Furthermore contrary to the general belief, "high" stocking density reduced aggression and improved growth in African catfish. The current results may be helpful to solve some problems of aggression, improve growth and reduce physiological responses to stress in the African catfish culture. On the other hand, assessing the welfare of the African catfish is a hard task, especially because results can be contradictory. Measurement of a single parameter, such as behaviour, physiological levels or performance parameter is not sufficient for an objective assessment of welfare. A combination of these three types of parameters mentioned above might help to understand fish welfare, but precautions must be taken in order to prevent erroneous conclusions.

This thesis is the first step towards assessment of welfare in the African catfish.

Samenvatting

Gedurende de afgelopen drie decennia heeft de visteelt zich uitgebreid, veranderd en geïntensifeerd. De stijging in productie op praktijkbedrijven is voornamelijk gerealiseerd door verhoging van het het aantal vissen per teelteenheid en/of door stijging van de groeisnelheid door het verbeteren van voer, voederstrategieën en optimalisatie van de water kwaliteit. Daarnaast zijn ook fotoperiode en lichtintensiteit gebruikt om de productie te sturen. Alle deze inspanningen richtten zich sterk op de verbetering van de productiviteit. Er is echter weinig aandacht besteed aan de vraag óf en hoe de vissen zich konden aanpassen aan deze nieuwe houderijcondities. Pas sinds kort krijgt het vraagstuk van dierwelzijn aandacht in de aquacultuursector. Daarbij is de aandacht en zorg voor dierenwelzijn in de visteelt nog lang niet zo intens als in de varkens- en pluimveeindustrie. Echter dierwelzijnsgroeperingen suggereren dat onder de huidige teeltcondities ook het welzijn van de vissen mogelijk niet optimaal is. Er zijn meerdere factoren die het welzijn van vissen kunnen beïnvloeden. Dieren reageren op verschillende manieren wanneer zij onderworpen worden aan omstandigheden die hun welzijnsstatus nadelig beinvloedt: variërend van endocriene veranderingen tot atypisch gedrag. Het algemene doel van dit proefschrift is te bepalen in welke mate verschillende omgevingsfactoren het (sociale) gedrag van de Afrikaanse meerval (*Clarias gariepinus*) beïnvloeden en hoe dit (sociale) gedrag bijdraagt aan de reactie op stress, voer opname en groei. De onderliggende werkhypothese was dat omgevingsfactoren een belangrijke rol spelen in de uitdrukking van gedrag en groeiprestatie van de Afrikaanse meerval (*Clarias gariepinus*).

Het eerste deel van deze studie (**Hoofdstuk 2**) concentreerde zich op de ontwikkeling van een set referentiegegevens m.b.t. het gedrag van de Afrikaanse meerval (*Clarias gariepinus*) onder “normale” en gestandaardiseerde omstandigheden. In dit deel werden gedragsparameters, zoals zwem-activiteit, lucht ademhalen, aggressie (als littekens en/of wonden op het lichaam), stereotiep gedrag, beschreven en gebruikt als een referentie voor de daaropvolgende hoofdstukken.

In het tweede deel van dit proefschrift (**Hoofdstukken 3, 4** en **5**) werden verschillende houderijcondities doorgelicht en werd onderzocht of deze mogelijk een

stressor zijn voor de vis en dus het welzijn van de vis treffen. In **Hoofdstuk 3** werden de effecten van voedermethode (continue voedering door middel van pendel voedering versus tweemaal per dag met de hand voederen), lichtintensiteit (15 versus 150 lux), fotoperiode (continu licht versus 12D:12L) en hun onderlinge interactie op waterkwaliteit en groei van jonge Afrikaanse meerval (*Clarias gariepinus*) bestudeerd. De methode van voederen beïnvloedde de waterkwaliteit. De productie van zwevende stofdeeltjes in het water werd bijna verdubbeld bij pendel voedering in vergelijking tot de handgevoederde vissen. TAN productie werd beïnvloed door lichtintensiteit en was hoger bij 15 lux dan bij 150 lux. Ondanks een vergelijkbare groei, was het voerverbruik bij pendel gevoerde vissen hoger dan bij handgevoerde vissen. Gebaseerd op de productieparameters, bleek jonge Afrikaanse meerval (*C. gariepinus*) ongevoelig te zijn voor lichtintensiteit, maar wel voor verandering in de fotoperiode. Een fotoperiode van 12D:12L resulteerde in een hogere groei en voerverbruik ten opzichte van 0D:24L (continue belichting)..

In **Hoofdstuk 4** werden dezelfde houderijfactoren als in Hoofdstuk 3 bestudeerd, maar nu ten aanzien van hun invloed op het gedrag van de vis. Vissen verkerend in continu licht en in hoge lichtintensiteit besteedden meer tijd aan zwemmen, waren agressiever en hadden meer littekens op het lichaam dan vissen verkerend in een 12D:12L fotoperiode en lage lichtintensiteit. Zwemactiviteit bleek sterk gecorreleerd te zijn met de mate van aggressie en/of littekens op het lichaam.

In **Hoofdstuk 5** werden dichtheid, fotoperiode en licht intensiteit bestudeerd. Bij een hoge dichtheid (2500 fish.m^{-3}) was de groei en zwemactiviteit hoger en de voedselconversie, agressie en de frequentie van stereotiep gedrag lager dan bij lage dichtheid (250 fish.m^{-3}). De dichtheid beïnvloedde ook de bloedplasma concentraties van cortisol en glucose.

Gebaseerd op de resultaten in Hoofdstukken 3, 4 en 5 werden dichtheid en fotoperiode geselecteerd voor een meer gedetailleerde studie in respectievelijk **Hoofdstukken 6** en **7**. In **Hoofdstuk 6** werden verschillende fotoperiodes (24D:0L, 18D:06L, 12D:12L en 06D:18L) bestudeerd. Korte periodes van licht of geen licht (6L:18D en 0L:24D) resulteerden in een toename van de groei vergeleken met langere periodes van licht (18L:6D en 12L:12D). Bij deze langere periodes van licht vertoonden de vissen ook een toename in zwemactiviteit, meer aggressie (verwondingen op het lichaam) en hogere bloedplasma niveaus van lactaat, vrije vetzuren en cortisol vergeleken met vissen die gehouden werden onder kortere periodes van licht (0L:24D en 6L:18D). Wanneer het aantal uren licht gedurende een etmaal stijgt, leken de vissen meer gestresst en agressief te worden.

In **Hoofdstuk 7** werden de effecten van groepsgrootte en van de beschikbare zwemruimte bestudeerd op het gedrag van de vissen, de stressresponse en de groei. Bij Afrikaanse meerval (*Clarias gariepinus*) verbeterde de groei, nam zwemactiviteit toe en verminderde aggressie, stereotiepe gedragingen en cortisol niveaus naarmate de groepsgrootte toenam (aantal vissen per tank). Het verminderen van beschikbare zwemruimte verminderde de groei en de zwemactiviteit en liet bloedplasma cortisol niveaus toenemen. Verschillen in nutriëntbesteding en gedrag suggereerden dat de onderhoudsbehoeften aan voer van de vissen hoger waren bij vissen in beperkte ruimte en/of lage teeltdichtheid tengevolge van agonistisch gedrag en/of blootstelling aan stress. De benaming "teeltdichtheid", gedefinieerd als het aantal vissen per eenheid ruimte, kan niet gebruikt worden als een criterium om systemen te evalueren of te vergelijken.

Dit proefschrift heeft aangetoond dat kleine veranderingen in houderijcondities, zoals een verminderd aantal uur licht gedurende 24 uur en lage lichtintensiteit, de algemene omstandigheden voor de Afrikaanse meerval (*Clarias gariepinus*) kunnen verbeteren. In tegenstelling tot de algemeen heersende opvatting, verminderde een "hoge" teeltdichtheid aggressie en verbeterde het de groei in Afrikaanse meerval. De hier gepresenteerde resultaten kunnen nuttig zijn om sommige aggressieproblemen op te lossen, groei te verbeteren en fysiologische reacties op stress te verminderen in de teelt van deze soort. Aan de andere kant is het bepalen van welzijn van de Afrikaanse meerval een moeilijke opgave, voornamelijk omdat resultaten tegenstrijdig kunnen zijn. Het meten van een enkele parameter, zoals gedrag, een fysiologische bloedparameter of een productieparameter, is niet voldoende om tot een objectieve beoordeling van het welzijn van vissen te komen. Een combinatie van deze drie soorten parameters zou echter wel kunnen helpen om het welzijn van vissen te begrijpen. Voorzichtigheid blijft echter noodzakelijk om geen verkeerde conclusies te trekken.

Dit proefschrift kan daarom beschouwd worden als een eerste stap in de richting van de beoordeling van het welzijn van Afrikaanse meerval.

Resumen

Durante las últimas tres décadas, la producción acuicola se ha expandido, diversificado e intensificado. La práctica más común para aumentar la producción bajo condiciones comerciales, ha sido el aumento en el número de peces por unidad de cultivo y/o aumentar la tasa de crecimiento mediante: una alta calidad de los alimentos, las estrategias de alimentación y mejorando la calidad de agua. Las prácticas secundarias para aumentar la producción son por medio de la manipulación en el fotoperiodo e intensidades de luz. Todos estos esfuerzos están centrados fuertemente en el aumento de la productividad, pero poca atención se ha prestado al animal mismo y cómo el animal puede hacer frente a estas nuevas condiciones de cultivo. Recientemente, los aspectos de bienestar animal han ganado un cierto interés en la piscicultura. La preocupación pública sobre cultivo intensivo de peces no ha alcanzado los altos niveles de preocupación como lo tiene la industria del cerdo y de las aves de corral. Sin embargo, los grupos de presión a favor del bienestar animal han sugerido que las prácticas acuícolas actuales también pueden comprometer el bienestar de los peces. Hay varios factores que pueden afectar el bienestar en peces. Los peces reponderán a diferentes niveles cuando son sometidos a diferentes condiciones ambientales, las cuales, afectan su bienestar: estos niveles van desde cambios endocrinologicos hasta comportamiento atípico. El objetivo general de esta tesis es determinar el efecto de diversos factores ambientales en el comportamiento social del bagre africano (*Clarias gariepinus*) y de los resultados de éste comportamiento social en relación con los niveles de estres, con la tasa de alimentación y con el crecimiento. En esta tesis se presume que los factores ambientales juegan un papel importante en la expresión del comportamiento y del funcionamiento del bagre africano (*Clarias gariepinus*).

El primer paso de esta tesis (**Capítulo 2**) se centró en el desarrollo de datos basicos del comportamiento del bagre africano (*Clarias gariepinus*) en condiciones normales y estandarizadas. En esta parte, diferentes tipos de comportamiento, por ejemplo tiempo de natación, respiración de aire, agresión (cicatrices y/o heridas en cuerpo), y los estereotipos fueron descritos y utilizados como referencia para los capítulos siguientes.

En la segunda parte de esta tesis (los **Capítulos 3**, **4** y **5**), varias condiciones de cultivo fueron seleccionadas por ser posibles factores estresantes. Además del efecto principal de los factores estudiados, también se prestó atención a los efectos producidos por la interacción entre dichos factores. En el **Capítulo 3**, se estudio los efectos del método de alimentación (continuo, usando alimentadores de demanda, y alimentación a mano dos veces al día), intensidad de luz (15 contra 150 lux), y el fotoperiodo (luz continua contra 12D:12L) en la calidad del agua y crecimiento del bagre africano (*Clarias gariepinus*). El método de alimentación influenció la calidad del agua. La producción de sólidos suspendidos casi se duplico bajo el regimen de alimentadores de demanda comparados con los alimentados a mano. La producción de amonia fue influenciada por la intensidad de luz, siendo más alta bajo condiciones de 15 lux comparados con 150 lux. Aunque los peces tuvieron crecimiento similar, los peces bajo alimentadores de demanda consumieron mas alimento. De acuerdo con los datos de crecimiento, el juvenil del bagre africano (*Clarias gariepinus*) parecería ser insensible a la intensidad de luz pero fue influenciado por el fotoperiodo. El fotoperiodo de 12D:12L incremento el crecimiento y la demanda de alimento comparado con el fotoperiodo de 0D:24L

En el **Capítulo 4**, los mismos factores ambientales que se utilizaron en el capítulo 3, fueron analizados pero ahora en lo referente al comportamiento de los peces. Los peces bajo luz continua y alta intensidad de luz pasaron más tiempo nadando, fueron más agresivos y por lo tanto tuvieron más cicatrices en el cuerpo que los peces bajo fotoperiodo de 12D:12L y baja intensidad de luz. Se encontró una alta correlación entre la actividad de natación y agresión y/o las cicatrices en el cuerpo.

En el **Capítulo 5**, la densidad de siembra, el fototoperiodo y la intensidad de luz fueron analizados. La alta densidad de siembra (2500 fish.m^{-3}) aumentó el crecimiento, el tiempo de natación, y redujo el FCR, la agresión y la ocurrencia de los estereotipos comparados con los peces bajo una densidad de siembra baja (250 fish.m-3). La alta densidad de siembra también demostró tener influencia en los niveles de cortisol y glucosa en el plasma.

De acuerdo a los resultados en los capítulos 3, 4 y 5 (respuestas de comportamiento, respuestas fisiológicas y respuestas de crecimiento a los factores ambientales de cultivo), la densidad de siembra y fotoperiodo fueron seleccionados para un analisis más detallado en el **Capítulo 6** y **7**, respectivamente. En el **Capítulo 6**, diversos fotoperiodos (24D:0L, 18D:06L, 12D:12L y 06D:18L) fueron estudiados.

Los períodos cortos de luz o de ninguna luz (6L:18D y 0L:24D) dieron lugar a un incremento en el crecimiento de los peces comparado a los períodos largos de luz (18L:6D y 12L:12D). Los peces bajo períodos largos de luz (12L:12D y 18L:6D) demostraron un incremento en la actividad de natación, más agresión (lesiones en el cuerpo) y niveles mas altos de ácido láctico, de ácidos grasos libres y de cortisol en plasma comparado con los peces bajo períodos más cortos de la luz (0L:24D y 6L:18D). Estos resultados demostraron que mientras se aumenta las horas de luz en un ciclo de 24 horas, los peces estuvieron mas estresados y agresivos.

En el **Capítulo 7**, se determinaron los efectos del tamaño del grupo así como el área disponible para la natación en el comportamiento de los peces, la respuesta al estres y su crecimiento. El bagre africano (*Clarias gariepinus*) incrementó su crecimiento, aumentó la actividad de natación y redujo la agresión, estereotipos y niveles de cortisol cuando el tamaño del grupo aumentó (número de peces por tanque). La reducción del área disponible de natación redujo el crecimiento y la actividad de natación y aumentó los niveles de cortisol en plasma. Las diferencias en uso de nutrientes de los peces, así como su comportamiento sugieren que el costo de mantenimiento de los peces era más alto en peces bajo espacio reducido y/o la densidad de siembra baja debido al comportamiento agonístico y/o a la exposición a estres. El termino "densidad de siembra" definido como el número de peces por unidad de siembra (espacio), no puede ser utilizado como criterio para evaluar o comparar sistemas.

Esta tesis probó que cambios pequeños en las condiciones de cultivo, tales como horas reducidas de luz durante 24 horas y una intensidad baja de la luz pudieron mejorar en general las condiciones para el bagre africano (*Clarias gariepinus*). Además contrariamente a la creencia general, "alta" densidad de simbra redujo la agresión y mejoró el crecimiento en el bagre africano. Estos resultados pueden ser provechosos para solucionar algunos problemas de agresión, mejorar el crecimiento y reducir las respuestas fisiológicas al estrés en el cultivo del bagre africano. Por otra parte, la determinación del bienestar del bagre africano es una tarea ardua, especialmente porque los resultados pueden ser contradictorios. Medir un solo parámetro, tal como comportamiento, niveles fisiológicos o respuesta de crecimiento no es suficiente para un analisis objetivo de bienestar animal. La combinación de estos tres tipos de parámetros puede ayudar a entender el bienestar de los peces, pero se debe de tener cuidado para prevenir conclusiones erróneas.

Esta tesis es el primer paso hacia la apreciación del bienestar en el bagre africano.

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Almazán Rueda, P., Verreth, J.A.J., Schrama., J.W. Behaviour patterns of the African catfish (*Clarias gariepinus*) under controlled conditions. Submitted to *Applied Animal Behaviour Science.*

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Curriculum Vitae

Pablo Almazán Rueda was born on June 17th , 1970 in Cd. Juarez, Chihuahua, México as the third son of Francisca Rueda Salazar and Pablo Almazán Luviano. His elementary studies were completed at the Centro Escolar Chilpancingo (1983) in Chilpancingo, Guerrero. In 1986, he graduated from the Instituto Valladolid (secondary school) in Morelia, Michoacán. He obtained his Bachelor of Science degree in Biochemical Engineering focus on aquatic resources in December of 1993 at the Instituto Tecnologico y de Estudios Superiores de Monterrey, Campus Guaymas. After graduation he began to work as a specialist for the Bank of México, FIRA, in Mérida Yucatán. His main task was developing and evaluating aquaculture projects. In 1995, he enrolled the University of Stirling, Scotland. One year later, he was granted the diploma of Master of Science in Aquaculture. In September of 1996, he started to work as a research assistant at the Research Center for Food and Development (CIAD, A.C.) in Mazatlán, México (Research Station on Aquaculture and Environmental Management). At the end of 1998 he started his PhD studies at the Wageningen University, The Netherlands. After his graduation he will continue his work at CIAD, Mazatlán.

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