WELFARE OF AFRICAN CATFISH

EFFECTS OF STOCKING DENSITY

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PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
OP GEZAG VAN DE RECTOR MAGNIFICUS
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My home's noisy.   I'm not. I'm mute
in this dwelling place.   A deity shaped
our twinned journey.   I'm more turbulent than he,
at times stronger.   He's tougher - durable.
Sometimes I come to rest.   He always runs on ahead.
For as long as I shall live   I shall live in him.
If we undo ourselves,   death's due claims me.

10th century riddle
Translation: Chris McCully

ABSTRACT

In the Netherlands, the welfare of farmed fish is subject of growing public and governmental concern. Comparable to concerns regarding the intensity of terrestrial livestock production, stocking densities applied in fish farming have been highlighted as an important area of welfare concern. The general aim of this thesis was to determine the impact of stocking density on welfare indicators in African catfish, *Clarias gariepinus*, cultured in a recirculating aquaculture system. The following factors were studied: 1) the effects of stocking density on physical, physiological, and behavioural responses of African catfish, 2) how age mediates the effects of stocking density on physical, physiological, and behavioural responses of African catfish, 3) the potential effects of chemical alarm cues on the welfare of farmed African catfish, and 4) the fitness consequences of different coping strategies in intensive husbandry systems. Stocking density influenced the welfare of African catfish. Strongly generalised, African catfish responded to increasing density with the formation of dense clusters of fish with constant movement and low aggression. However, factors as an acute stressor and fish age modulated the effects of stocking density. Applying a challenge test (net stressor) resulted in a strong increase in agonistic behaviour of juvenile African catfish, especially in catfish housed at high density. At increasing age, the effects of stocking density changed. For African catfish of 10-100 gram, both high and low densities had detrimental effects on fish welfare. For African catfish of 100-300 gram, welfare improved at increasing stocking density. For catfish of 1000-1500 gram no effects of stocking density on a range of welfare indicators were shown. Aggression in African catfish is often associated with improper stocking densities. The current study showed that individually housed African catfish respond to chemical alarm cues that might be released as a result of this aggression. A large individual variation in the response to the alarm cue was observed, related to feed efficiency. Fish with a lower feed-efficiency showed a relative active response to chemical alarm cues while more feed-efficient animals showed a more passive response. Study of the effects of a single chemical alarm cue administration on the behaviour and growth performance of group-housed African catfish indicated that although a short-term behavioural response was shown, chemical alarm cues, at the concentration applied, had no long-term effects on both behaviour and growth performance of the fish. The large individual variation in behavioural responses to chemical alarm cues found in this study gave an indication for the existence of coping strategies in African catfish. Study of the consequences of different coping strategies in intensive husbandry systems indicated that coping strategy did not seem to affect the fitness of African catfish. The current study confirmed that stocking density does indeed affect the welfare of African catfish. However, stocking density is an area with many interacting factors that affect welfare. In this study, a number of possible factors have been highlighted, i.e. age, individual variation in behavioural stress responses and acute additional stressors. This complexity suggests that concentrating on stocking density alone will not allow us to control welfare accurately.

CONTENT

Appendices

1

GENERAL INTRODUCTION

INTRODUCTION

The global production of farmed fish and shellfish has more than doubled in the past 15 years and it seems that growth is set to continue (Naylor et al., 2000). Total world demand for fish and fishery products is projected to expand to 179 million tonnes by 2015 (FAO, 2006). At the same time, ocean fisheries stocks are maximally exploited and stocks of a number of fish species are in decline mainly due to over-fishing. As wild stocks of fish species such as cod (*Gadus morhua*), halibut (*Hippoglossus hippoglossus*), turbot (*Psetta maxima*), and tuna (*Thunnus sp.*) become pressurised they become more and more attractive as potential aquaculture species. Farming of such species can fulfil consumer demand that can no longer be met by wild capture fisheries alone. It is therefore expected that the anticipated expansion of the consumer demand for fish and fishery products will predominantly be met by aquaculture, which was projected to account for 41 percent of global fish production in 2015 (FAO, 2006).

Alongside the growth of the aquaculture sector, and perhaps partly due to this rapid expansion, the welfare of farmed fish is a subject of growing public concern (FSBI, 2002). For the consumer the issue of welfare in fish has long been less important compared to the intensive terrestrial livestock production. This may partly be related to the fact that fish are sometimes considered to be inferior in evolutionary terms when compared to mammals and birds (Rose, 2002). Furthermore, the husbandry of fish is less visible than that of farm animals and the aquatic environment makes comparison with humans less easy. Nowadays, consumer, but also industry and governmental awareness for fish welfare is undoubtedly increasing. Fish farming is more and more associated with problems which hitherto were only known from the intensive farming of for instance cattle and poultry. Unfortunately, our knowledge of the welfare of farmed fish is still very limited. Due to a growing public attention towards fish welfare, there is an increasing pressure on legislators to protect the welfare of farmed fish. Often, the pressure for protection is outpacing scientific understanding of the nature of welfare, its assessment and control (Turnbull et al., 2008).

At present, the welfare of farmed fish in the Netherlands is covered by the Animal Health and Welfare Act (1993), which provides general rules concerning animal health, animal welfare, transportation, slaughter, and housing. This legislation covers all farmed animals, including fish. However, this legislation does not specifically promote fish welfare. The Council of Europe has recently introduced new laws which do specifically relate to farmed fish welfare. The Standing Committee of the European Convention for the Protection of Animals Kept for Farming Purposes first started drafting fish welfare conditions in 1998 and these were finally adopted on December 2005 and entered force in June 2006. Nonetheless, these regulations currently still lack recommendations on species-specific requirements regarding for instance stocking density, water quality, feeding, social behaviour, and environmental structures.

ASSESSING FISH WELFARE

Animal welfare is by no means a simple concept. Two major issues in the study of animal welfare have been (1) to define what is meant by animal welfare and (2) how best to assess it (Ashley, 2007; Dawkins, 1998). Producing a definition of animal welfare has not been easy, particularly because the concept of animal welfare is complex and the word has been used in different contexts. Generally, most definitions can be divided into three broad categories (Duncan & Fraser, 1997; Fraser et al., 1997): (1) Feelings-based definitions, based on subjective mental states, (2) function-based definitions, based on the physical state of an animal, and (3) nature-based definitions, based on the biological functioning of an animal. However, none of the three different concepts of animal welfare offer a comprehensive view on the major ethical concerns over the quality of life of animals. To overcome the problems of producing a comprehensive definition of animal welfare, criteria were defined which should be fulfilled in order for an animal to experience good welfare. These 'five freedoms' were elaborated by the Brambell Committee of the U.K. Parliament (Brambell, 1965). Freedom from hunger and thirst; freedom from discomfort, freedom from pain, injury, or disease, freedom of fear and distress, as well as the freedom to express normal behaviour, provides a logical framework with which to assess welfare issues.

In research a wide array of physiological, biochemical, and behavioural parameters are used to asses fish welfare. Unfortunately, none of these parameters can be considered reliable in isolation. For example, a physiological stress response such as cortisol release is an autonomic response indicating activity or arousal rather than being a specific welfare indicator. Therefore, this measurement may be affected by a number of different parameters (Dawkins, 1998; FSBI, 2002). Similar problems arise when interpreting single behavioural measures (Dawkins, 1998). Although single measurements can to a certain extent provide valid information, appropriate combinations of measurements on, for example, physiology, behaviour, disease status, and growth provide a more compelling assessment of welfare (Broom, 1997). Nonetheless, assessing a range of different parameters by univariate analyses may also have its limitations since it is difficult to quantitatively relate such measurements to the welfare status of the fish and the relationship between indicators is not always constant (Turnbull et al., 2008).

Recently the use of multivariate analyses was proposed to overcome these problems (Turnbull et al., 2005). Multivariate analysis combines a range of welfare indicators, based on the observed statistical relationships between them (Manly, 1994). Principal components analysis (PCA) allows trends in multivariate data to be reduced to scores that can then be used as dependent variables in subsequent analyses, without requiring a priori judgements of the relative value of individual variables. These scores may possibly provide an integrated and objective reflection of diverse measured welfare indicators. Two recent studies in Atlantic salmon (*Salmo salar*) used a PCA to combine four commonly used measures of fish welfare (condition of body and fins and plasma concentrations of glucose and cortisol) into a single welfare score (Adams et al., 2007; Turnbull et al., 2005). This score proved to be biologically consistent with welfare and was consistent with the evaluation of welfare by experienced farmers (Turnbull et al., 2005). Future studies should determine the applicability of a PCA as a welfare assessment tool when a wider range of welfare indicators, including fish behaviour, is applied.

STOCKING DENSITY

Comparable to concerns over the intensity of terrestrial livestock production, stocking densities applied in fish farming have been highlighted as an important area of welfare concern, generally associated with problems as reductions in feed conversion efficiency, condition factor and growth at increasing density (Anonymous, 1996; Ellis et al., 2002; Lymbery, 1992, 2002). Setting welfare guidelines or legislation may therefore be required. Compared to terrestrial livestock production, determining minimal spatial requirements for fish is, however, somewhat more complex (Ashley, 2007; Conte, 2004; FSBI, 2002).

Stocking density is normally being referred to as the weight of fish per unit volume or per unit volume in unit time of water flow through the holding environment (Ashley, 2007). In contrast to most terrestrial animals, fish utilise a three dimensional medium and depend on this medium for both behavioural (spatial) and physiological (e.g. oxygen supply, dilution of metabolic waste products) needs (Ellis et al., 2002). The relationship between fish welfare and stocking density is therefore strongly mediated through factors as water quality and social interactions.

INTER-SPECIES VARIATION

Due to a pronounced interspecies variation in behavioural and physiological requirements, it may come as no surprise that the way in which stocking density affects various aspects of the welfare of farmed fish is strongly species-specific. High densities have been shown to reduce the survival of coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawyischa*; Ewing & Ewing, 1995). In seabream (*Sparus aurata*), high stocking densities result in a chronic stress situation, with increased plasma cortisol levels, haematocrit, haemoglobin concentration, and red blood cell count (Montero et al., 1999). Furthermore, high densities in rainbow trout (*Oncorhynchus mykiss*) may lead to increased fin erosion and reduced feed intake and feed conversion efficiency, nutritional condition and growth (Ellis et al., 2002). In contrast, Arctic charr (*Salvelinus alpinus*) and African catfish (*Clarias gariepinus*) show a depressed feed intake and/or growth rate at low densities but grow well and suffer less physical damage at high densities (Almazán Rueda, 2004; Jørgensen et al., 1993). This indicates that knowledge on the effects of stocking density in one fish species can not and should not be directly applied to other fish species.

INTER-INDIVIDUAL VARIATION: EFFECTS OF AGE

Not only differences between species in their response to stocking density are marked, differences among fish of the same species can also be pronounced. This may be due to for instance changes in behaviour, morphology, and environmental requirements throughout their life history. For example, aggressive interactions and cannibalism are mainly found in early life stages of a number of fish species, but this behaviour is rare in older fish (e.g. Atlantic cod, *Gadus morhua*, Baskerville-Bridges & Kling, 2000; Atlantic halibut, *Hippoglossus hippoglossus*; Greaves & Tuene, 2001). Such differences may strongly affect the response of fish to stocking density. Studies in African catfish suggest that fish age has a pronounced effect on their response to stocking density. For instance, African catfish larvae have shown negative effects of increasing density, reflected by decreased growth performance and increased cannibalism (Haylor, 1991, 1993; Hecht & Appelbaum, 1988; Hossain et al., 1998). In contrast, studies of juvenile African catfish showed a positive effect of increasing density, reflected by increased growth performance and decreased aggression (Almazán Rueda, 2004; Hecht & Appelbaum, 1988; Hecht & Uys, 1997; Kaiser et al., 1995a, b). The circumstances under which these studies were performed were highly variable. This raises the question to what extent in these studies the age effects are really caused by stocking density. To test if and how age affects the response to stocking densitie, a series of experiments under similar environmental conditions is necessary.

INTER-INDIVIDUAL VARIATION: EFFECTS OF COPING STRATEGIES

To make matters even more complex, fish of the same species and age are not all identical, often showing physiological and behavioural variation in the way they cope with certain stressors. Although such individual differences are common, these differences have for long been interpreted as the result of inaccurate measurements or as non-adaptive variation around an adaptive mean (Groothuis & Carere, 2005). At present, it is increasingly more recognised that these individual differences are caused by stable biological traits instead of having a situation-specific origin. In literature, these consistent differences between individuals have been referred to as differences in personality, temperament, coping styles/ strategies, boldness, neophobia, or behavioural syndromes/ profiles (Gosling & John, 1999; Greenberg & Mettke-Hofmann, 2001; Groothuis & Carere, 2005; Koolhaas et al., 1999; Sih et al., 2004b; Wilson et al., 1994).

A basic feature of all these labels is that they reflect differences in behaviour and physiology, which are stable over time and consistent over situations. Although debatable, individual differences tend to be categorised into two opposite strategies, e.g. 'boldness' versus 'shyness' (Coleman & Wilson, 1998; Wilson et al., 1994), neophobic versus neophilic (Cavigelli & McClintock, 2003; Greenberg & Mettke-Hofmann, 2001), or proactive versus reactive coping strategies (Koolhaas et al., 1999). Important features of the proactive response are territorial control, aggression, low hypothalamus-pituitary-adrenal (HPA) axis activity, and high sympathetic reactivity (Benus et al., 1989; Benus et al., 1990; Driscoll et al., 1990; Fokkema et al., 1995; Ruis et al., 2000; Schouten & Wiegant, 1997; Van Oortmerssen & Bakker, 1981). Immobility, low levels of aggression, high HPA-reactivity, and low sympathetic reactivity characterize the reactive response.

These consistent differences may have important consequences. For example, across a range of situations proactive animals are considered to be more aggressive than reactive animals. Depending on the situation, all animals shift their aggression levels up and down. However, proactive animals remain consistently more aggressive than reactive animals. Proactive animals should do well in competitive situations where aggression is important. However, in situations where caution or care are more appropriate proactive animals may be unsuitably aggressive (e.g. in an anti-predator or parental care context). In contrast, reactive copers might do well in situations where low aggression is favoured, but poorly in competitive situations (Sih et al., 2004a).

The value of these individual differences was recently shown by Dingemanse and colleagues (2004). Their study showed that bold males and shy females of the Great tit (*Parus major*) flourish after winters when food is abundant, while shy males and bold females profit from meagre winters. According to the authors, since females are subordinate to males, females are expected to be more affected by competition for winter food, while males are expected to be more affected by competition for territorial space. Apparently, bold, fast exploring personalities are successful when competition is high: bold males survive better when breeding population densities are high, while bold females perform better when food is limiting in the winter. But when the sex-specific resource is not limiting, boldness is likely to be costly and shy, slow exploring great tits then appear to be favoured. Selection pressure on the two different coping strategies thus changed between years, depending on food availability. Coping strategies seem to give an obvious advantage to a population confronted with fluctuations in environmental factors.

Not only do these coping strategies play an important role in nature, they may also have consequences for the fitness of different copers in intensive husbandry systems. However, at the moment it is largely unknown if and how coping strategies affect the welfare of fish in intensive fish farms. In a recent paper, Huntingford and Adams (2005) reviewed the evidence for fitness consequences of coping strategies in wild salmonids, in particular Atlantic salmon. Based on a comparison of the behaviour of wild and farmed fish they hypothesised that domestication of Atlantic salmon (especially when accompanied by selection for fast growth in the farm environment) has resulted in the simultaneous selection for high aggression and risk taking (i.e. proactive fish). This in turn suggests that risk-avoiding fish (representing a reactive coping strategy) fare poorly in intensive husbandry systems. This may be due to a lower competitive ability of risk-avoiding fish when housed at high densities and with a predictable food source, as is usually the case in these husbandry systems. However, these hypotheses have yet to be experimentally validated.

RECIRCULATING AQUACULTURE SYSTEMS

In the Netherlands, fish are mainly farmed in recirculating aquaculture systems (RAS). RAS are intensive, indoor tank-based systems which allow fish to be grown in any climatic region and offer complete control over the rearing environment. RAS allow a high percentage of water reuse by utilising a combination of mechanical, biological and chemical filtration. A growing environmental awareness concerning the use of water resources, and the discharge of wastes from fish farms has led to a world-wide interest in recirculation technology for freshwater and marine species. The RAS' complete control over the rearing environment, including water quality, facilitates a further intensification of the husbandry process. As a result, stocking densities applied in RAS are considerably higher than those used in cages or raceways (Table 1). To what extent

Table 1. Commercially applied stocking densities in cages, raceways, and tanks.

System	Fish species	Stocking density (kg/m^3)	References
Cages	Salmon	15-20	Lymbery, 2002
	Salmon	9-21	Adams et al., 2007
	Sea bass	20-30	pers. comm.
Raceways	Rainbow trout	15-40	Ellis et al., 2002
	Rainbow trout	30-60	Lymbery, 2002
RAS	African catfish	200-500	Van de Nieuwegiessen et al., 2008
	European eel	≤ 100	pers. comm.
	Sea bass	>100	Blancheton, 2000
	Rainbow trout	>150	pers. comm.

these exceptionally high densities affect the welfare status of farmed fish is still largely unknown. Considering the world-wide interest in recirculation technology it is crucial that the consequences of culturing fish at the stocking densities applied in RAS are thoroughly studied.

Recirculating aquaculture systems do not stand apart from other husbandry systems because of the applied stocking densities only. The closed character of a RAS, in terms of water re-use, may also result in the unwanted accumulation of a wide range of compounds. A compound that may be of particular importance is the Ostariophysan chemical alarm cue or Schreckstoff.

CHEMICAL ALARM CUES

Chemical alarm cues (alarm pheromones) have been demonstrated in a wide variety of aquatic vertebrates and invertebrates (Chivers & Smith, 1998; Smith, 1992). In fish, these chemicals can be released as a result of mechanical skin damage, e.g. through a predator attack. Chemical alarm cues may also be released voluntarily (disturbance pheromones), as is the case in the Iowa darter, *Etheostoma exile* (Wisenden et al., 1995). When detected by nearby conspecifics or sympatric heterospecifics, these chemical alarm cues generally elicit species specific anti-predator responses, e.g. increased shoaling, freezing, and refuging (Brown & Godin, 1999; Brown et al., 1995; Chivers & Smith, 1998; Mathis & Smith, 1993). Damage-released chemical alarm cues have been demonstrated in a number of fish taxa, including Ostariophysans, darters, gobies, sculpins, sticklebacks, poecilids, cichlids, and salmonids (Chivers and Smith, 1998).

Restricting ourselves to Ostariophysans (to which also our model fish, *Clarias gariepinus*, belongs), the skin of members of this superorder has large numbers of specialised epidermal club cells that contain damage-released chemical alarm cues (Smith, 1992). These cells have no outlet to the surface, indicating that the alarm cue is only released when the skin is damaged. It is suggested that the damage-released chemical alarm cue is hypoxanthine 3-N-oxide or a similar molecule with a nitrogen-oxide functional group

(Brown et al., 2000). Lawrence & Smith (1989) showed that each club cell of the fathead minnow (*Pimephales promelas*) contained enough damage-released chemical alarm cues to create an active space of roughly 80 litres. One square centimetre of skin would provide an active signal in about 58.000 litres. The alarm signal may be transmitted even further by visual signalling (Brown et al., 1999).

Although debatable (see Irving & Magurran, 1997; Magurran et al., 1996), fright responses to damage-released chemical alarm cues are commonly considered to be adaptive (Smith, 1997). Laboratory studies have shown benefits to both signallers and receivers of damage-released chemical alarm cues. Housed with pike (*Esox lucius*), fathead minnows survived 40% longer when exposed to chemical alarm cues. This was largely due to increased shelter use and increased shoaling (Mathis & Smith, 1993). In addition, fathead minnows learned to avoid areas based on damage-released chemical alarm cues present in the faeces of pike fed on minnows (Brown et al., 1996). Signaller benefits to alarm cue release have also been shown. Mathis and co-workers (1995) showed that pike are attracted to chemical alarm cues of fathead minnows. In aquaria, when a second pike was released once a first pike had a minnow in it's mouth, the second pike interfered, increasing the handling time for the first pike and often allowing the minnow to escape (Chivers et al., 1996).

It is not surprising that in aquatic environments, public information about risk often takes the form of these chemical alarm cues. Water is the universal solvent and ideal for the distribution of a wide range of chemical compounds (Chivers et al., 2007). Furthermore, the aquatic environment is the oldest of all environments, leaving a long evolutionary opportunity for aquatic life forms to exploit this information (Chivers et al., 2007; Wisenden, 2003). Surprisingly, the potential effects of chemical communication in a farming situation are largely unexplored. Nonetheless, chemical alarm cues are likely to be present in aquaculture systems. Factors as handling, grading, agonistic behaviour or high stocking densities may all lead to skin damage, possibly resulting in the release of damage-released chemical alarm cues. The effects of these chemical alarm cues on the welfare of farmed fish are currently unknown and require further study.

THIS THESIS

In The Netherlands, the welfare of farmed fish is a subject of growing public and governmental concern. The Dutch government, through the Ministry of Agriculture, Nature and Food quality, has supported the current study to increase scientific knowledge on fish welfare, in particular on the effects of stocking density on the welfare of African catfish (*Clarias gariepinus*).

The current knowledge in this field is far from complete and often difficult to extrapolate to Dutch farm conditions. Most of the work has focused on the effects of relatively low stocking densities, making it incomparable to the situation in a modern RAS. Moreover, most studies assessed maximal 3 different stocking densities, making it difficult to come to a proper dose-response curve for the effects of stocking density on the welfare of African catfish.

While it is well possible that the relationship between fish welfare and stocking density is mediated through factors as fish age and coping strategies, it is yet unclear to what extent these factors confound the relationship between stocking density and the welfare of African catfish. Finally, although aggression is often associated with improper stocking densities, it is not known if this aggression results in the release of chemical alarm cues and, if released, what the consequences of these cues are for the welfare of African catfish.

The general aim of this thesis is to determine the impact of stocking density on welfare indicators in African catfish cultured in a recirculating aquaculture system. The following factors will be studied: 1) The effects of stocking density on physical, physiological, and behavioural responses of African catfish, 2) How age mediates the effects of stocking density on physical, physiological, and behavioural responses of African catfish, 3) The potential effects of chemical alarm cues on the welfare of farmed African catfish, 4) The fitness consequences of different coping strategies in intensive husbandry systems.

OUTLINE OF THE THESIS

The focus of this thesis is to study how stocking density affects the welfare of African catfish. Physical, physiological and behavioural parameters are studied on both individual and group level.

The first part of this thesis (**Chapter 2** and **3**) focuses on group responses to stocking density of African catfish at 3 different size classes. In **Chapter 2**, physical, physiological, and behavioural responses of African catfish in a growth stage of 10-100 grams are studied. **Chapter 3** describes the effects of stocking density in African catfish of 100-300 and 1000-1500 gram. Furthermore, in **Chapter 3** it is discussed how the impact of stocking density changes as the size/age of African catfish increases.

One important finding of **Chapter 2** and **3** is that the number of skin lesions increases at decreasing stocking density. This increased aggression may potentially lead to the release of chemical alarm cues. To gain insight into the possible consequences of this alarm cue release in a farming situation, the effects of a conspecific skin extract administration on the behaviour and growth performance of African catfish are studied in **Chapter 4 and 5**. **Chapter 4** assesses if individually housed African catfish respond to conspecific skin extract. **Chapter 5** examines the effects of a single conspecific skin extract administration on the behaviour and growth performance of group-housed African catfish.

Chapter 4 indicates a pronounced individual variation in the behavioural response to conspecific skin extract. Such individual behavioural variation suggests differences in coping strategies in African catfish. **Chapter 6** focuses on the possible consequences of these coping strategies for the fitness of the different behavioural phenotypes in intensive husbandry systems. The objective of this study is to assess if and how coping strategies affect the welfare of African catfish housed at low and high stocking density.

Chapter 8 discusses the overall results and implications.

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2

Assessing the effects of a chronic stressor, stocking density, on welfare indicators of juvenile African catfish, *Clarias gariepinus* Burchell

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ABSTRACT

Fish welfare is an area of increasing interest and stocking densities used in commercial aquaculture have been highlighted as a subject of specific welfare concern. The present study assessed how stocking density affects growth performance (final body weight, SGR, FCR), physiological (plasma cortisol, glucose, lactate) and behavioural (swimming activity, stereotypes, escapes, aggression, airbreathing) responses of farmed African catfish (*Clarias gariepinus*). We stocked African catfish (10 gram) in triplicate in 120L tanks at densities of 500, 1125, 1750, 2375, and 3000 animals/m^3. After exposure to stocking density, responses (plasma cortisol, glucose, lactate, number of lesions) to an acute stress challenge were studied. Growth performance, physiological and behavioural data showed little indications of impaired African catfish welfare within the range of densities examined in this study. However, the acute stress challenge gave indications that African catfish were in fact stressed. Catfish housed at densities of 500 and 3000 fish/m^3 showed signs of chronic stress, reflected by the absence of a cortisol response after an acute net stressor. Furthermore, fish housed at high densities (2375 and 3000 fish/m^3) showed a strong increase in the amount of skin lesions after the 1-hour group housing after net-stress. In conclusion, an interaction effect between stocking density and additional stress was shown. Since additional stressors (e.g. handling, grading) are not uncommon in a farming situation, this means that the total farming situation may influence the effect of stocking density. Welfare of African catfish seemed impaired at both the lowest and highest density. Due to opposing data, the situation of fish housed at densities between 1125 and 2375 fish/m^3 is less clear. The higher aggression at the low end of this density range and increased escape attempts at the high end of this density range seem to be balancing each other. Which stocking density within this range should be regarded optimal depends on the relative weight assigned to the different parameters.

INTRODUCTION

In recent years fish welfare has been a much debated topic. Many animal rights associations have publically expressed their concerns on the welfare of farmed fish. Because of this, public, commercial, and governmental concern on the subject has grown (Anonymous, 1996; FSBI, 2002; Lymbery, 1992, 2002). Stocking densities used in commercial aquaculture have been highlighted as an area of specific welfare concern. Fish farmers are keen to rear fish at high densities, partly because operating at higher stocking densities can reduce production costs. However, stocking density has been demonstrated to affect various aspects of the welfare of farmed fish, although differences between species are distinct. High densities may impair the welfare of some fish species (e.g. trout, Ellis et al., 2002; salmon, Ewing & Ewing, 1995; seabream, Montero et al., 1999), while for instance Arctic charr grow more rapidly at high density (Jørgensen et al., 1993).

Published data on the effect of stocking density on growth performance of African catfish is contradictory. Several studies find decreasing growth performance with increasing density (Haylor, 1991, 1993; Hecht & Appelbaum, 1988; Hossain et al., 1998).

Other studies show increasing growth performance with increasing density (Almazán Rueda, 2004; Hecht & Uys, 1997) or no effect of density (Hengsawat et al., 1998). Data on the effect of stocking density on behaviour show a positive effect of high densities on the behaviour of African catfish. Increasing densities reduce the occurrence of agonistic behaviour in African catfish larvae and juveniles (Almazán Rueda, 2004; Hecht & Appelbaum, 1988; Hecht & Uys, 1997; Kaiser et al., 1995a, b).

Information on the effects of stocking density on later life stages of African catfish is scarcely available. Furthermore, the stocking densities under investigation are generally low, making them hardly comparable to situations in modern aquaculture. Moreover, most studies focus on only 2-3 different stocking densities, making it difficult to come to a proper dose-response curve for the effect of stocking density on African catfish.

Therefore, the purpose of this study was to assess the influence of stocking density on possible welfare indicators of juvenile African catfish. A range of five stocking densities, comparable to densities applied in modern aquaculture, will be studied. Stocking density is often considered a chronic stressor, potentially leading to a suppression of stress responses. Therefore, a acute stress challenge (netting stress) was applied to reveal such a chronic stress situation. An acute stress challenge has been shown to give valuable, additional information on the effects of stocking density in fish (Di Marco et al., 2008). A range of dose-response relationships will be established for the effects of stocking density on growth performance, physiology, and behaviour with the ultimate goal to come to an optimal stocking density for this fish species at a weight range of 10-100 grams.

MATERIALS AND METHODS

ANIMALS, ADAPTATION PERIOD AND EXPERIMENTAL CONDITIONS

All of the procedures involving animals were conducted in accordance with the Dutch law on experimental animals and were approved by the Wageningen University Animal Experimental Committee (DEC). Juveniles of mixed sex African catfish were obtained from a commercial fish farmer (Fleuren & Nooijen viskwekerijen BV, Someren, The Netherlands). Prior to the experiment, fish were held at the central fish facility (De Haar Vissen, Wageningen University, The Netherlands) in two aquaria (120L; stocking density 62.5 and 60.2 kg/m^3) to enable adaptation to the recirculating water system. During this 2-week adaptation period floating pelleted feed (Skretting ME-2, Fontaine les Vervins, France) was provided at a feeding level of 16.8 gram food/kg metabolic fish weight (g/kg$^{0.8}$ per day). Feed was presented by belt feeding, which gave the fish continuous access to food between 8.00-16.00h and 17.00-01.00h.

At the start of the 49-day experimental period (day 0), the fish had an average weight (± S.D.) of 7.52 ± 0.35 g. Fish were randomly assigned to one of 15 glass aquaria (90 cm x 45 cm x 45 cm) with a water volume of 120L. Treatments were randomly assigned to the aquaria. During both the adaptation period and the experimental period, the aquaria were filled with recirculating UV-treated tap water with a water temperature of ± 25 °C, pH 7.0-7.5, NH_4^+ <1 mg/l, NO_2^- <1 mg/l, NO_3^- <1000 mg/l and the conductivity ranged between 2500 and 5000 µs/cm. The photoperiod was 12L:12D. Flow rate was set at 8 l/min. The given flow rate ensured that NH_4^+, NO_2^-, and NO_3^- levels were independent of stocking density. Furthermore, it ensured that oxygen saturation remained well above 40%. It has been shown that air breathing in African catfish increased only (but insignificantly) at saturation levels below 40% (Oellerman, 1996). Therefore, the possible effects of reduced oxygen saturation levels at higher densities were discounted.

Experimental procedures and measurements

African catfish culture in the Netherlands typically consists of two growth phases. Phase 1, which we tried to imitate in the current study, consists of growing 10-gram fingerlings to an average size of 100 gram. At the end of the first growth period densities range between 100 and 300 kg/m^3 (i.e. 1000-3000 fish/m^3). After grading, fish are restocked and grown to an average weight of 1500 gram. At the end of this second growth period densities range between 200 and 500 kg/m^3. To study the welfare effects of the first growth period (i.e. 10-100 gram) each of the 15 aquaria was assigned to one of five experimental treatments: 500 fish/m^3, 1125 fish/m^3, 1750 fish/m^3, 2375 fish/m^3, and 3000 fish/m^3. Expressed in kg/m^3 this corresponds with densities of 50, 112.5, 175, 237.5, and 300 kg/m^3 at the end of the experimental period. Our treatments included a lower density than used in aquaculture; a higher density was not selected. Fish farmers do not use stocking densities higher than 3000 fish/m^3 because practical experience of farmers has shown that densities above 3000 fish/m^3 lead to skin problems and subsequent infections.

During the experimental period fish were fed floating pelleted feed (Skretting ME-2, Fontaine les Vervins, France) at a feeding level of 20.0 gram food/kg metabolic fish weight (g/$kg^{0.8}$ per day). Feed was presented by an automated feeder, which gave the fish continuous access to food between 8.00-16.00h and 17.00-01.00h. Fish in this study were fed restrictively. The reason for this was that young catfish can experience a feed-related problem known as “Ruptured Intestine Syndrome” (RIS) or “Open Belly Syndrome”, which is caused by high feed loads (Hariati et al., 1994).

The total weight of the fish per aquaria was measured at day 0 and day 48. From this data, specific growth rate (SGR), expressed as a percentage, was calculated by subtracting the natural logarithm of the mean initial weight from the natural logarithm of the mean final weight and dividing this by the total numbers of experimental days. Feed conversion ratio (FCR) was calculated per aquaria by dividing the total feed fed by the total final biomass minus the total initial biomass.

At day 48, 20 fish/tank were blood sampled. From each tank, we simultaneously caught 20 fish of which 10 fish were directly anaesthetized and blood sampled to asses the effect of stocking density on plasma cortisol, glucose, and lactate. Directly after the first 10 fish were put in the anaesthetic, the other 10 fish were exposed to an additional net stressor, to asses the stress reactivity to an acute stressor. For this net stressor fish were randomly distributed over 10 nets (size 14 cm x 20 cm x 12 cm) hanging just above the water surface. Fish were removed from the nets after 10 minutes and placed in a 30 l tank (10 fish/tank) for 1 hour (a previously performed pilot study showed that a maximum stress response is seen at one hour after netting stressor). At the end of this hour, fish were anaesthetised and blood sampled. Prior to blood sampling of both control and netted fish, fish were anaesthetised for 3 minutes with 0.4 g/l tricaine methanesulfonate (MS-222, Crescent Research Chemicals, Phoenix, AZ, USA) and 0.8 g/l of $NaHCO_3$. One ml of blood was collected by hypodermic syringe (containing 3 mg Na_2EDTA) from the caudal blood vessels. The procedure of catching, anaesthetisizing and blood collection was completed for all fish within 5 minutes. We recorded the sequence at which control and stressed fish were blood sampled and could not detect a significant effect of sample sequence on plasma cortisol, glucose, and lactate. The collected blood was placed in cooled 1.5 ml plastic tubes, mixed and centrifuged at 6000 g for ten minutes at 4°C. The collected plasma was stored at -20°C for further analyses.

As an indicator of aggression, the number of scars and wounds on the body of the 20 blood sampled fish per tank were determined. From the unsampled fish in the tank, 40 additional fish were selected to determine scars and wounds after anaesthesia (anaesthesia was similar to that of the blood sampled fish). After sampling, all fish were euthanatized by 0.8 g/l tricaine methanesulfonate (MS-222, Crescent Research Chemicals, Phoenix, AZ, USA) and 1.6 g/l of $NaHCO_3$.

PLASMA ANALYSES

Plasma analysis was performed on blood plasma of five stressed and five unstressed fish/tank. Plasma cortisol levels were measured by enzyme immunoassay (Cortisol ELISA, RE52061, IBL, GmbH), a kit developed for the quantitative measurement of cortisol in human serum and plasma. Results of African catfish plasma samples measured by this kit were compared with the validated radio-immunoassay described by Ellis et al. (2004) and showed a correlation of R=0.96 (n=30). The cross-reactivity of the antiserum to other steroids in the cortisol production pathway are progesterone (<0.1%), 17α-hydroxyprogesterone (<0.5%), 11-deoxycortisol (<1.0%), cortisone (3.0%), and corticosterone (29%). Due to the high corticosterone cross-reactivity corticosterone levels were determined in African catfish plasma levels (Corticosterone Immunoassay Kit no. 901-097, Assay Designs Inc., USA) and were (± S.D.) 3.02 ± 1.53 ng/ml (n=10; five unstressed and five stressed fish). Because of these relative low corticosterone concentrations the results of the cortisol analysis were not corrected for corticosterone. Plasma glucose was determined by the GOD-Perid® method (Boehringer). Plasma lactate was determined enzymatically using Sigma Diagnostic Kits (Sigma; Proc. No. 735).

QUANTIFICATION OF BEHAVIOUR

Fish behaviour was recorded both on video and by direct observation. Video recordings were made of each aquarium on the front side and took place at days 14, 28 and 42. From each tank, 30 minutes of video (12.00-12.30h) were analysed. Table 1 shows the ethogram used in this study. Furthermore, every 5 min (12.00, 12.05, 12.10, 12.15, 12.20, 12.25, 12.30h) the total number of fish swimming and total number of fish rest-

Table I. The ethogram used for behavioural observations.

Behavioural element	Definition
Agonistic behaviour	Chasing or biting a fish, or being chased or bitten by another fish
Air breathing	The animal moves to the water surface and takes a gulp of air. This was checked by escaping air of the gills, when it was swimming back to the bottom of the tank
Escape attempts	The animal moves to the water surface for at least past it's gill cover
Resting	Moving passively through the water or lying still at the bottom of the tank
Stereotypic behaviour	Continuous and compulsice swimming under a fixed, repetitive pattern for at least 10 seconds
Swimming	A displacement of the body, while browsing, moving, eating, and airbreathing

ing were counted as well as the total number of visible fish. The activity patterns were expressed as a percentage of the total number of fish counted. Escape attempts (frequency) and stereotypic behaviour (frequency and duration) were recorded by all occurrence sampling. Both escape attempts and stereotypic behaviour were recorded per tank per period. A correction was made for the difference in number of fish visible per tank between the treatments by dividing the frequencies of escape attempts and stereotypic behaviour by the average number of fish visible per tank per treatment.

Direct observations were made at day 28, 35 and 42, starting at 9.00h. Per tank, an individual fish was followed throughout the five-minute period. Whenever the fish was out of sight another fish was immediately selected from the position where the first fish was last observed and the observations continued. Resting time was expressed as a percentage of the total time of observation. Swimming time was defined as 100 minus the percentage of resting. Air-breathing and agonistic behaviour were quantified by counting the number of times this behaviour took place, and were expressed as a frequency.

STATISTICAL ANALYSES

In this study, aquaria were used as experimental unit for analysing growth performance and behavioural data. For physiological data, fish were used as experimental unit. The plasma cortisol data of 36 fish was excluded from our analyses because plasma cortisol levels of these fish were below the detection limit of our assay. All calculations were made using the SAS System (SAS, 1999). Growth performance and physiological data was analysed using a one-way analysis of variance (ANOVA) followed by the Tukey's HSD post hoc test. The error terms of these ANOVA analyses were tested for homogeneity of variances and normality, using respectively the Levene's test and the Shapiro-Wilk test. Due to significant non-normality, plasma glucose, plasma lactate, and behavioural data were analysed using a Kruskal-Wallis ANOVA followed by pair wise comparisons by the Mann-Whitney U-test. Results are considered statistically significant when P-values are below 0.05.

RESULTS

GROWTH PERFORMANCE

Growth performance responses of African catfish to stocking density are presented in Table 2. Highest and lowest final body weight were observed at stocking densities of 1750 fish/m^3 (93.7 g) and 3000 fish/m^3 (86.7 g), respectively. However, final body weight did not differ significantly between treatments, F(4,10)=0.57, P=0.690. Stocking density had no influence on SGR (F(4,10)=0.97, P=0.464) and FCR (F(4,10)=2.35, P=0.124). Mortality was not different between treatments, being 2.5% over all treatments.

Table 2. Final body weight, daily feed intake, specific growth rate (SGR), and feed conversion ratio (FCR) in African catfish (*Clarias gariepinus*).

	Stocking density (fish/m^3)					S.E.M.	P-value
	500	1125	1750	2375	3000		
Final body weight (g)	89.0	89.2	93.7	90.3	86.7	3.37	0.690
SGR (%/day)	5.1	5.1	5.1	5.1	4.9	0.07	0.464
FCR (g/g)	0.59	0.60	0.61	0.63	0.65	0.015	0.124

STRESS PARAMETERS

Physiological responses of African catfish are presented in Figure 1-3. Plasma cortisol of control fish was unaffected by stocking density. With increasing density, plasma glucose levels decreased until a density of 2375 fish/m^3 at which an increase was observed, $\chi^2(4)=17.23$, P=0.002. Plasma lactate levels increased at increasing density, $\chi^2(4)=12.37$, P=0.015. The net stressor elicited a significant increase in both glucose and lactate, $\chi^2(1)=77.26$, P<0.001 and $\chi^2(1)=48.54$, P<0.001, respectively. However, this increase was unaffected by stocking density. Although control and net-stressed levels of plasma cortisol did not seem to differ between different densities, animals housed at the two ex-

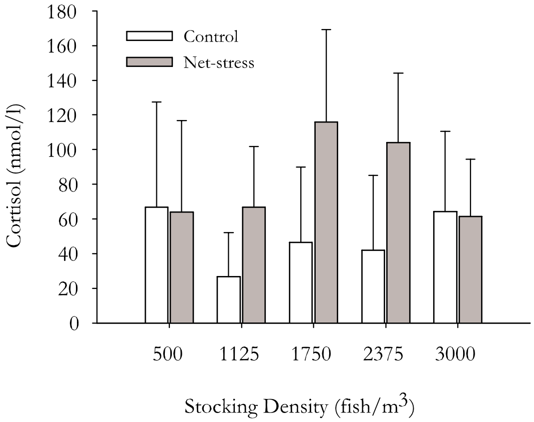

Figure 1. Mean control and net-stressed plasma cortisol responses of African catfish (*Clarias gariepinus*) for different stocking densities. Values are ± S.D.

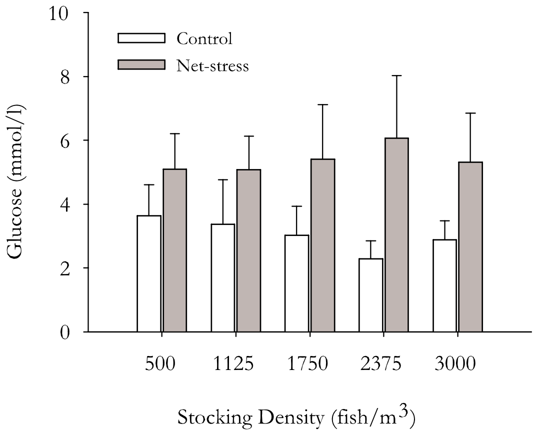

Figure 2. Mean control and net-stressed plasma glucose responses of African catfish (*Clarias gariepinus*) for different stocking densities. Values are ± S.D.

tremes of the density range failed to show an acute stress response resulting in an interaction effect between treatment (control vs. netted) and density, F(4,94)=3.01, P=0.022. Furthermore, fish housed at higher densities showed a more pronounced acute plasma glucose response, F(4,130)=4.58, P=0.002. No interaction effect between treatment and density was found for plasma lactate.

Behaviour

The behavioural responses of African catfish to stocking density are presented in Table 3. Escape attempts increased at increasing density, with significantly higher numbers of escape attempts at densities of 2375 and 3000 fish/m^3, $\chi^2(4)=15.79$, P=0.003. Furthermore, the data shows that escape attempts occurred mainly during the first stages of the

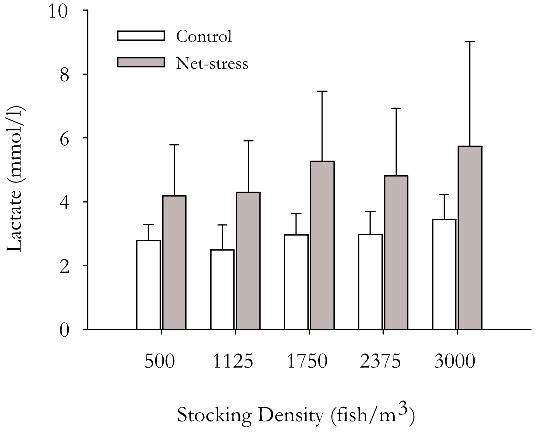

Figure 3. Mean control and net-stressed plasma lactate responses of African catfish (*Clarias gariepinus*) for different stocking densities. Values are ± S.D.

Table 3. Behavioural responses of African catfish (*Clarias gariepinus*) to stocking density. Mean values are expressed as percentage of fish for video observations, percentage of time for direct observations and frequencies for stereotypes, escape attempts, air breathing and agonistic behaviour.

	Stocking density (fish/m^3)					S.E.M.	P-value
	500	1125	1750	2375	3000		
Lesions (#)[1]	5.1^{a}	4.0^{bc}	5.3^{ab}	3.7^{c}	2.0	1.29	<0.001
Video							
Swimming activty day 14 (%)	75.2	74.6	80.5	90.2	91.0	3.67	0.095
Swimming activty day 28 (%)	85.0	88.5	93.7	98.7	94.8	1.83	0.034
Swimming activty day 42 (%)	96.0	99.0	95.8	94.4	86.5	4.63	0.304
Swimming activty T (%)[2]	85.4	87.4	90.0	94.4	90.8	3.04	0.330
Stereotypes day 14 (#/fish/h)	0.00	0.04	0.00	0.01	0.00	0.020	0.519
Stereotypes day 28 (#/fish/h)	0.02	0.00	0.00	0.00	0.00	0.008	0.406
Stereotypes day 42 (#/fish/h)	0.00	0.01	0.00	0.00	0.00	0.007	0.406
Stereotypes day T (#/fish/h)	0.01	0.02	0.00	0.00	0.00	0.007	0.423
Escape attempts day 14 (#/fish/h)	0.16	0.02	0.00	1.73	0.09	0.600	0.465
Escape attempts day 28 (#/fish/h)	0.00	0.01	0.01	0.51	0.94	0.267	0.085
Escape attempts day 42 (#/fish/h)	0.00	0.00	0.00	0.04	0.38	0.114	0.106
Escape attempts T (#/fish/h)	0.05^{a}	0.01^{a}	0.09^{a}	0.64^{b}	0.47^{b}	0.206	0.003
Direct observation							
Swimming activity day 28 (%)	52.7	87.0	92.9	99.2	96.8	8.82	0.030
Swimming activity day 35 (%)	96.8	100.0	96.3	96.2	99.6	1.80	0.323
Swimming activity day 42 (%)	89.9	89.4	91.0	91.4	96.2	5.74	0.941
Swimming activity day T (%)	79.8	92.2	93.4	95.6	97.5	4.48	0.290
Air breathing day 28 (#/fish/h)	32.0	104.0	76.0	72.0	124.0	33.99	0.251
Air breathing day 35 (#/fish/h)	12.0	44.0	28.0	80.0	44.0	15.90	0.092
Air breathing day 42 (#/fish/h)	20.0	48.0	48.0	96.0	160.0	21.39	0.045
Air breathing T (#/fish/h)	21.3^{a}	65.3^{bc}	50.7^{b}	82.7^{c}	109.3^{bc}	15.71	0.001
Aggressive acts day 28 (#/fish/h)	12.0	8.0	0.0	0.0	0.0	5.70	0.222
Aggressive acts day 35 (#/fish/h)	0.0	0.0	0.0	0.0	0.0	0.00	1.000
Aggressive acts day 42 (#/fish/h)	0.0	4.0	0.0	0.0	0.0	1.79	0.406
Aggressive acts T (#/fish/h)	4.0	4.0	0.0	0.0	0.0	2.00	0.067

[1] Lesions of net-stressed animals are not included.

[2] T represents pooled data of the three separate observations.

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

experiments, declining gradually during subsequent observations. Frequencies of agonistic behaviour tended to decrease at increasing density, $\chi^2(4)=8.76$, $P=0.067$. Again, the occurrence of agonistic behaviour was most prominent during the first behavioural observation and declined during following observations. However, the amount of lesions nonetheless show that aggression was also present in later stages of the experiment.

Stocking density also influenced swimming activity. During the first and second video observation swimming activity increased at increasing density, $\chi^2(4)=7.90$, $P=0.095$ and $\chi^2(4)=10.43$, $P=0.034$, respectively. No effect of stocking density on swimming activity was observed during the third video observation and for the overall swimming activity. A similar pattern was observed for the swimming activity during direct observations. During the first direct observation swimming activity increased at increasing density, $\chi^2(4)=8.82$, $P=0.030$. The swimming activity during period two and three of direct observations was not significantly influenced by stocking density and overall swimming activity during direct observations was not affected by stocking density.

Air breathing increased at increasing density during the third direct observation, $\chi^2(4)=9.77$, $P=0.045$. Overall amounts of air breathing also increased at increasing density, $\chi^2(4)=17.65$, $P=0.001$.

Possible chronic effects of stocking density are observed for the amount of skin lesions. Fish housed at a stocking density of 3000 fish/m^3 showed lower amounts of lesions in comparison with the four lower densities, $\chi^2(4)=86.33$, $P<0.001$ (Figure 4). Because we observed an increase in aggression during the 1-hour group housing after net stress, the increase in skin lesions of these animals was also determined. A density-dependent increase in skin lesions was observed with a significantly higher increase in the amount of lesions for fish originally housed at densities of 1125, 2375 and 3000 fish/m^3.

DISCUSSION

In this study a range of welfare indicators, consisting of physiological, performance and behavioural parameters, was used to assess the effects of stocking density on fish welfare. Without additionally stressing the fish both performance and physiological indicators gave little indication of impaired fish welfare within the range of densities examined in this study. However, stocking density did influence the behavioural response of African catfish. In general, African catfish responded to increasing density (from 500 - 3000 fish/m^3) with the formation of dense clusters of fish with constant movement and low aggression. This is in accordance with previous studies that show an increase in activity and/or decrease of aggression at higher densities (Appelbaum & Kamler, 2000; Hecht & Appelbaum, 1988; Hecht & Uys, 1997; Kaiser et al., 1995b). Hecht & Uys (1997) suggested that the formation of dense shoals interferes with the cues for initiation of agonistic behaviour. In low densities (500-1125 fish/m^3), agonistic behaviour was observed for four weeks. After four weeks, agonistic behaviour ceased to be observed. However, at these lower densities, agonistic behaviour was also present

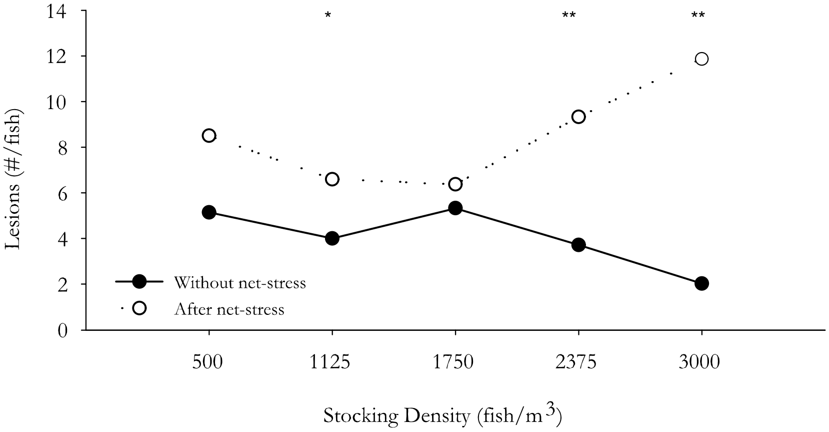

Figure 4. Lesion scores of unstressed (control) and net-stressed African catfish (*Clarias gariepinus*) for different stocking densities. Lesions of unstressed fish were scored directly after the 6-week group housing. Lesions of net-stressed fish were scored after an additional net stress, followed by a 1-hour group housing. Asterisk (*) indicates a significant difference between unstressed and netstressed fish (*: $P<0.05$, **:$P<0.0001$).

in later stages of the experiment, as indicated by the amount of lesions. At the highest densities we observed a higher incidence of escape behaviour, which may be the result of crowding stress (FSBI, 2002; Lambooij et al., 2002).

Without additionally stressing the fish we only got a behavioural indication that fish welfare is impacted at the lower range of densities examined in this study. However, challenging the fish with an additional stressor (10 minute netting, followed by a 1-hour group housing) revealed that fish at the higher range of densities may be stressed even if this was not clear from the basic physiological and behavioural recordings. Fish housed at densities of 500 and 3000 fish/m^3 failed to show a cortisol response after net stress. This impaired cortisol response may be partially related to down regulation of ACTH or cortisol receptors, due to chronic stress (Wendelaar Bonga, 1997). Enhanced cortisol clearance could also explain an impaired cortisol response (Wendelaar Bonga, 1997), although the accompanying increase in energy use was not reflected by a lower growth performance of fish housed at densities of 500 and 3000 animals/m^3. Furthermore, although we observed little to no aggression between fish housed at high densities, Figure 4 shows that fish housed at these high densities (2375 and 3000 fish/m^3) showed a strong increase in the amount of skin lesions after the 1-hour group housing after net-stress. The exact reason for the higher aggression at high densities is unclear and needs further study. Possibly the crowding stress at these densities affected their behaviour. However, we cannot exclude an effect of the relative changes in stocking density going from the normal group housing to the 1-hour group housing after net-stress. For fish housed at 3000 fish/m^3 the density dropped with a factor 9, while for fish housed at 500 fish/m^3 this was only a factor 1.5. In conclusion, these results show that there exists an interaction effect between stocking density and acute additional stress. Since additional stressors (e.g. handling, grading) are not uncommon in a farming situation, this means that the total farming situation may influence the effect of density. Furthermore, it shows that a challenge test gives additional and crucial information about the basic welfare situation.

When studying fish welfare under laboratory conditions as done in the current study we should always ask ourselves to what extent the results can be extrapolated to normal farm conditions. Although we have used stocking densities comparable to commercial conditions, the actual group sizes and tank sizes are considerably lower then those used under commercial conditions. In a study to quantify the effects of increasing both group size and tank size while keeping stocking density fixed, Almazán Rueda (2004) found that performance improved when group and tank size increased. Furthermore, overall activity increased at increasing group and tank size. He concluded that increasing group and tank size while keeping stocking density fixed had a positive effect on behaviour and growth performance. These results are in contrast to data on trout which show that larger group sizes reduce growth and increase size variation (Ellis et al., 2002). This discrepancy can probably be explained by differences in biology and environmental requirements for each fish species. We assume that our results found under laboratory conditions can be extrapolated to commercial conditions, although the magnitude of the effects of stocking density might be lower under farming conditions. Unfortunately, no reference data is available on comparable studies in intensive African catfish farms and further study in this field is required.

In conclusion, this study showed that a challenge test can provide crucial information about the welfare status of an animal in a chronic stress situation. It also showed that the total farming situation, through acute stressors, can have an impact on the effects of stocking density. Welfare of African catfish seemed impaired at both the lowest and highest density, due to high aggression or crowding stress, respectively. This was further backed by cortisol data, which showed an impaired cortisol response of fish housed at a density of 500 and 3000 fish/m^3. Due to opposing data, the situation of fish housed at densities between 1125 – 2375 fish/m^3 is less clear. The aggression at the low end of this density range and increased escape attempts at the high end of this density range are balancing each other. Which stocking density within this range should be regarded optimal depends on the relative weight assigned to the different parameters.

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3

EFFECTS OF AGE AND STOCKING DENSITY ON THE WELFARE OF AFRICAN CATFISH, *CLARIAS GARIEPINUS* BURCHELL

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ABSTRACT

Information on the effects of stocking density on especially later life stages of the African catfish (*Clarias gariepinus*) is scarcely available. Therefore, the purpose of this study was to assess the influence of density on the welfare of juvenile African catfish in a growth stage of 100-1500 gram. Furthermore, the changing impact of stocking density on welfare indicators at different stages throughout the growth cycle (from 10-1500 gram) of African catfish are discussed. In experiment I, 15 groups of African catfish (100 gram) were assigned to one of five final densities (20, 40, 80, 160, and 320 kg/m^3). In experiment II, 12 groups of African catfish (1000 gram) were assigned to one of four final densities (100, 233, 366, and 500 kg/m^3). Welfare was studied by recording both growth physical, physiological and behavioural parameters. A combination of univariate and multivariate analysis was applied in order to acquire an integrated and objective reflection of the effects of stocking density on a range of welfare indicators. In experiment I, a significant effect of stocking density was found. Factor scores for the first component increased with increasing density. Low factor scores on the first component appeared to be indicative for decreased welfare (e.g. elevated cortisol, increased aggression), suggesting that fish welfare improved with increasing density. In experiment II, no effects of stocking density were found. These results confirm that stocking density does indeed affect the welfare of fish. However, they also show that the effects of stocking density are not uniform throughout the growth cycle. These differences need to be taken into account by husbandry practices to assure that high welfare standards are maintained throughout the life cycle of the fish being cultured.

INTRODUCTION

The African catfish (*Clarias gariepinus*) is the second most important cultured fish in the Netherlands and known for it's ability to be cultured at high densities up to 500 kg/m^3. Fish farmers are keen to rear fish at such high densities, because operating at higher stocking densities can reduce production costs. However, densities used in commercial fish production have been highlighted as an area of welfare concern (Anonymous, 1996; Lymbery, 1992, 2002). Stocking density has been demonstrated to affect various aspects of the welfare of farmed fish, although differences between species are distinct. High densities may impair the welfare of some fish species (e.g. trout, Ellis et al., 2002; salmon, Ewing & Ewing, 1995; seabream, Montero et al., 1999), but for other species positive effects of high densities have been shown (e.g. Arctic charr, Jørgensen et al., 1993; African catfish, Almazán Rueda, 2004).

Not only differences between species are marked, differences among fish of the same species can also be pronounced, due to for instance changes in behaviour, morphology, and environmental requirements throughout their life history. These differences may strongly affect the response of fish to stocking density. Studies of African catfish larvae have shown negative effects of increasing density, reflected by decreased growth performance and increased cannibalism (Haylor, 1991, 1993; Hecht & Appelbaum, 1988; Hossain et al., 1998). In contrast, studies of juvenile African catfish showed a positive effect of increasing density, reflected by increased growth performance and decreased aggression (Almazán Rueda, 2004; Hecht & Appelbaum, 1988; Hecht & Uys, 1997; Kaiser et al., 1995a, b). Such differences need to be taken into account by husbandry systems and practices to assure that high welfare standards are maintained throughout the life cycle of the fish being cultured.

African catfish in the Netherlands is exclusively reared in intensive recirculation systems. The culture typically consists of 2 growth phases. Phase 1 consists of growing 10-gram fingerlings to an average size of 100-150 gram after which they are graded and restocked. At the end of this growth period densities range between 100-300 kg/m^3. During Phase 2 African catfish are further grown to a market size of 1500 gram. At the end of this second growth period densities range between 200-500 kg/m^3. Although information is available on how stocking density affects welfare indicators of African catfish in the first phase of the grow-out (Van de Nieuwegiessen et al., 2008), the situation in the second phase of the grow-out is largely unclear.

The purpose of this study was to assess the influence of stocking density on welfare indicators of African catfish in a growth stage of 100-1500 gram, with the ultimate goal to come to an optimal stocking density for this fish species at a weight range of 100-1500 grams. Furthermore, these results were compared with those of earlier studies in fish of 10-100g, to assess the changing impact of stocking density on welfare indicators at different stages throughout the growth cycle (from 10-1500 gram) of African catfish. A combination of univariate and multivariate analysis was applied in order to acquire an integrated and objective reflection of the effects of stocking density on a range of welfare indicators .

MATERIALS AND METHODS

ANIMALS, ADAPTATION PERIOD AND EXPERIMENTAL CONDITIONS

All of the procedures involving animals were conducted in accordance with the Dutch law on experimental animals and were approved by the Wageningen University Animal Experimental Committee (DEC). Mixed sex African catfish were obtained from a commercial fish farmer (Fleuren & Nooijen viskwekerijen BV, Someren, The Netherlands). Prior to the experiments, fish were held at the central fish facility (De Haar Vissen, Wageningen University, The Netherlands) to enable adaptation to the recirculating water system. During this 2-week adaptation period floating pelleted feed (Skretting ME-3, Experiment 1; Skretting ME-4.5, Experiment 2, Fontaine les Vervins, France) was provided ad lib by pendulum feeding.

During both the adaptation period and the experimental period, the aquaria were filled with recirculating UV-treated tap water with a water temperature of ± 25 ºC, pH 7.0-7.5, NH_4^+ <1 mg/l, NO_2^- <1 mg/l, NO_3^- <1000 mg/l and the conductivity ranged between 2500 and 5000 µs/cm. The photoperiod was 12L:12D. Flow rate was set at 8 litre/minute. The given flow rate ensured that NH_4^+, NO_2^-, and NO_3^- levels were independent of stocking density. Furthermore, it ensured that oxygen saturation remained well above 40%. It has been shown that air breathing in African catfish increased only (but insignificantly) at saturation levels below 40% (Oellerman, 1996). Therefore, the possible effects of reduced oxygen saturation levels at higher densities were discounted.

EXPERIMENTAL PROCEDURES AND MEASUREMENTS

The second growth phase of African catfish culture in the Netherlands is characterised by a 15-fold increase in stocking density, starting with relatively low densities at the start of the growth period (13.3-33.3 kg/m^3) and ending at high densities at the end of the growth period (200-500 kg/m^3). To study both the start and the end of this growth period in detail, we decided to perform two separate experiments. Experiment I studied a growth phase of 100-300 gram, while Experiment II studied a growth phase of 1000-1500 gram. Table 1 shows the applied densities in both experiments.

At the start of Experimental I and II , the fish had an average weight (± S.D.) of 102.1 ± 3.49 g and 1044.6 ± 31.6 g, respectively. Fish were randomly assigned to one of 15 120L-aquaria in Experiment I and assigned to one of 12 200L-aquaria in Experiment II. Treatments were randomly assigned to the aquaria. Fish were fed a floating pelleted feed (Skretting ME-3, Experiment 1; Skretting ME-4.5, Experiment 2, Fontaine les Vervins, France) by pendulum feeding and had ad lib access to the feed.

Fish were weighed at day 0 and day 28. Specific growth rate (SGR), expressed as a percentage, was calculated by subtracting the natural logarithm of the mean initial weight from the natural logarithm of the mean final weight and dividing this by the total numbers of experimental days. Feed conversion ratio (FCR) was calculated per aquaria by dividing the total feed fed by the total final biomass minus the total initial biomass.

At day 28, a maximum of 10 control fish per tank were caught and immediately

Table 1. Stocking densities applied in Experiment 1 (tank size 120L) and Experiment 2 (tank size 200L).

Fish tank	Stocking density (fish/m^3)	Start density[1] (kg/m^3)	End density[2] (kg/m^3)	End density[3] (kg/m^3)
Experiment I				
8	67.7	6.7	20	100
16	133.3	13.3	40	200
32	267.7	26.7	80	400
64	533.3	53.3	160	800
128	1067.7	106.7	320	1600
Experiment II				
13	67	67	100	100
31	155	155	233	233
49	244	244	366	366
67	333	333	500	500

[1] Stocking density at the start of the experiment

[2] Stocking density at the end of the experiment

[3] Theoretical stocking density at market size (i.e. 1500 gram)

blood sampled to asses the effect of stocking density on plasma cortisol, glucose, and lactate. A maximum of 10 other fish were caught simultaneously with the control fish, but were first exposed to an additional net stressor, to asses the stress reactivity to an acute stressor. For this net stressor fish were randomly distributed over nets hanging just above the water surface. Fish were removed from the nets after 10 minutes and placed in a tank (5 fish/tank) for 1 hour (a previously performed pilot study showed that a maximum stress response is seen at one hour after netting stress). At the end of this hour, fish were anaesthetised and blood sampled. Prior to blood sampling of both control and netted fish, fish were anaesthetised for 3 minutes with 0.4 g/l tricaine methanesulfonate (MS-222, Crescent Research Chemicals, Phoenix, AZ, USA) and 0.8 g/l of $NaHCO_3$. One ml of blood was collected by hypodermic syringe (containing 3 mg Na_2EDTA) from the caudal blood vessels. The procedure of catching, anaesthetisizing and blood collection was completed for all fish within 5 minutes. We recorded the sequence at which the fish were blood sampled and could not detect a significant effect of sample sequence on plasma cortisol, glucose, and lactate. The collected blood was placed in cooled 1.5 ml plastic tubes, mixed and centrifuged at 6000 g for ten minutes at 4°C. The collected plasma was stored at -20°C for further analyses.

As an indicator of aggression, the number of scars and wounds on the body of the 20 blood sampled fish per tank was determined. From the unsampled fish in the tank, a maximum of 40 additional fish were selected to determine scars and wounds after anaesthesia (anaesthesia was similar to that of the blood sampled fish). After sampling, all fish were euthanazized by 0.8 g/l tricaine methanesulfonate (MS-222, Crescent Research Chemicals, Phoenix, AZ, USA) and 1.6 g/l of $NaHCO_3$.

PLASMA ANALYSIS

Plasma cortisol levels were measured by enzyme immunoassay (Cortisol ELISA, RE52061, IBL, GmbH), a kit developed for the quantitative measurement of cortisol in human serum and plasma. Results of African catfish plasma samples measured by this kit were compared with the validated radio-immunoassay described by Ellis et al. (2004) and showed a correlation of R=0.96 (n=30). The cross-reactivity of the anti-serum to other steroids in the cortisol production pathway are progesterone (<0.1%), 17α-hydroxyprogesterone (<0.5%), 11-deoxycortisol (<1.0%), cortisone (3.0%), and corticosterone (29%). Due to the high corticosterone cross-reactivity plasma corticosterone levels were determined (Corticosterone Immunoassay Kit no. 901-097, Assay Designs Inc., USA) and were (± SD) 3.02 ± 1.53 ng/ml (n=10; 5 unstressed and 5 stressed fish). Because of these relative low corticosterone concentrations the results of the cortisol analysis were not corrected for corticosterone. Plasma glucose was determined by the GOD-Perid® method (Boehringer). Plasma lactate was determined enzymatically using Sigma Diagnostic Kits (Sigma; Proc. No. 735).

BEHAVIOUR ANALYSIS

Fish behaviour was recorded both on video and by direct observation. Video recordings were made of each aquarium on the front side and took place at day 0, 2, 7, 14, and

Table 2. The ethogram used for behavioural observations.

Behavioural element	Definition
Agonistic behaviour	Chasing or biting a fish, or being chased or bitten by another fish
Air breathing	The animal moves to the water surface and takes a gulp of air. This was checked by escaping air of the gills, when it was swimming back to the bottom of the tank
Escape attempts	The animal moves to the water surface for at least past it's gill cover
Resting	Moving passively through the water or lying still at the bottom of the tank
Stereotypic behaviour	Continuous and compulsice swimming under a fixed, repetitive pattern for at least 10 seconds
Swimming	A displacement of the body, while browsing, moving, eating, and airbreathing

28. From each tank, 30 minutes of video (12.00h-12.30h) were analysed. Table 2 shows the ethogram used in this study. Furthermore, every five minutes (12.00, 12.05, 12.10, 12.15, 12.20, 12.25, 12.30h) the total number of fish swimming and total number of fish resting were counted as well as the total number of visible fish. The activity patterns were expressed as a percentage of the total number of fish counted. Escape attempts (frequency) and stereotypic behaviour (frequency and duration) were recorded by all occurrence sampling. Both escape attempts and stereotypic behaviour were recorded per tank per period. A correction was made for the difference in number of fish visible per tank between the treatments by dividing the frequencies of escape attempts and stereotypic behaviour by the average number of fish visible per tank per treatment.

Direct observations were made at day 7, 14, 21, and 28, starting at 9.00h. Per tank, an individual fish was followed throughout the five-minute period. Whenever the fish was out of sight another fish was immediately selected from the position where the first fish was last observed and the observations continued. Resting time was expressed as a percentage of the total time of observation. Swimming time was defined as 100 minus the percentage of resting. Air-breathing and agonistic behaviour were quantified by counting the number of times this behaviour took place, and were expressed as a frequency.

STATISTICAL ANALYSES

In this study, aquaria were used as experimental unit. All calculations were made using the SAS System (SAS, 1999). Data was first analysed using a one-way analysis of variance (ANOVA) followed by the Tukey's HSD post-hoc test. The error terms of these ANOVA analyses were tested for homogeneity of variances and normality, using respectively the Levene's test and the Shapiro-Wilk test. When the assumptions of an ANOVA were not met, a Kruskal-Wallis ANOVA followed by pair wise comparisons by the Mann-Whitney U-test was applied. Subsequently, data from experiments I and II were subjected to principal component analysis (PCA) to detect trends observed between simultaneously measured parameters (see Turnbull et al., 2005). To gain insight into the changing impact of stocking density on welfare indicators at different stages throughout the growth cycle of African catfish, data of experiments I (100-300 gram) and II (1000-1500 gram) were combined with data of a previous study (10-100 gram; Nieuwegiessen, 2008) and also subjected to PCA. The variables included in the PCA incorporated physical indicators of welfare (feed intake, FCR, SGR, skin lesions, mortality) as well as physiological (cortisol, glucose, lactate) and behavioural (swimming, airbreathing, stereotypes, aggression, escapes) indicators. Plasma cortisol after net stress showed a inexplicable pattern (i.e. a decline in plasma cortisol after a stressor) and was therefore not included in the PCA analyses. Furthermore, because we observed no stereotypic behaviour, this parameter was also excluded from the PCA analyses. The relative contribution of each variable to the different principal components (PC) was obtained along with factor scores for each of the PC's for every tank within the dataset. The factors scores of the different PC's were then included as dependent variables in a one-way analysis of variance (ANOVA) followed by the Tukey's HSD post-hoc test. Results are considered statistically significant when P-values are below 0.05.

Results

Experiment 1. Growth stage 100-300 gram.

Low stocking densities promote agonistic behaviour in juvenile African catfish. Stocking densities applied at the start of this growth period are relatively low (i.e. 13.3-33.3 kg/m^3). To asses if higher densities could control the occurrence of agonistic behaviour, we applied 2 densities in this experiment which are higher than used commercially (i.e. theoretically resulting in final densities of 800 and 1600 kg/m^3).

Table 3 presents the physical, physiological, and behavioural responses of African catfish (weight range 100-300 gram) to stocking density. Daily feed consumption ranged between 3.60 g/d and 4.90 g/d. Daily feed intake at stocking densities of 80 and 320 kg/m^3 was significantly higher compared to a density of 20 kg/m^3, F(4,10)=5.79, P=0.0112. A trend was observed for the effect of stocking density on SGR, with a highest SGR at a density of 160 kg/m^3, F(4,10)=3.01, P=0.0719. No significant effects of stocking density on FCR and mortality were observed (P>0.05). Physiological responses of African catfish (weight range 100-300 gram) were unaffected by stocking density (P>0.05). Surprisingly, African catfish did not respond with an increase in plasma cortisol after net stress, fish housed at the two lowest densities even showed a decline in plasma cortisol after net stress. Stocking density did affect the behavioural responses of African catfish (weight range 100-300 gram). Swimming activity significantly increased at increasing stocking density ($\chi^2(4)=52.45$, P=0.0001). Highest percentage of swimming fish was observed at a density of 320 kg/m^3, whereas the lowest percentage of swimming fish was observed at a density of 40 kg/m^3 (93.87% vs. 47.42%). The amount of airbreathings also increased at increasing density ($\chi^2(4)=15.13$, P=0.0044). Stocking density did not influence both the occurrence of escape behaviour and agonistic behaviour. However, skin lesions, an indirect measurement of agonistic behaviour, did indicate an effect of stocking density ($\chi^2(4)=10.74$, P=0.0297). Numbers of lesions decreased at increasing density, ranging between 1.0 at a density of 320 kg/m^3 and 2.1

Table 3. Physical, physiological, and behavioural responses of African catfish (*Clarias gariepinus*), weight range 100-300 gram.

	Stocking density (fish/m^3)					S.E.M.	P-value
	20	40	80	160	320		
Physical parameters							
Feed intake (g/d)	3.60^a	4.25^{ab}	4.90^b	4.50^{ab}	4.65^b	0.207	0.011
SGR (%/d)	1.85	2.05	2.22	2.28	2.14	0.097	0.072
FCR (g/g)	0.78	0.81	0.81	0.71	0.81	0.048	0.550
Mortality (%)	0.00	2.08	3.13	2.08	2.60	1.292	0.527
Physiological parameters							
Cortisol control (nmol/l)	358.60	373.56	177.46	235.14	199.91	64.752	0.171
Cortisol net-stress (nmol/l)	158.23	201.65	191.23	180.40	134.71	24.643	0.224
Glucose control (mmol/l)	2.97	3.32	3.09	2.77	3.77	0.304	0.248
Glucose net-stress (mmol/l)	6.63	7.62	6.30	6.67	6.55	0.341	0.145
Lactate control (mmol/l)	4.67	4.21	4.33	3.75	4.36	0.567	0.858
Lactate net-stress (mmol/l)	5.96	6.69	5.18	4.75	5.08	0.474	0.083
Behavioural parameters							
Lesions control (#)	1.4^a	2.1^a	1.7^a	1.5^a	1.0^b	0.34	0.030
Lesions net-stress (#)	5.2^a	4.5^a	3.3^a	3.0^a	1.1^b	0.81	0.002
Swimming activity (%)	50.4^a	47.4^a	68.7^b	80.7^b	93.9^b	3.99	<0.001
Air breathing (#/fish/h)	43.0^a	55.0^{ab}	72.0^{bc}	83.0^c	80.0^{bc}	8.63	0.004
Escape attempts (#/fish/h)	0.2	0.0	0.0	0.5	0.2	0.14	0.143
Aggressive acts T (#/fish/h)	8.0	3.0	2.0	1.0	0.0	2.45	0.409
Stereotypic behaviour (#/fish/h)	0.0	0.0	0.0	0.0	0.0	0.00	1.000

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

at a density of 40 kg/m^3. After the net stressor, fish housed at 320 kg/m^3 showed less lesions than fish housed at lower densities ($\chi^2(4)=16.60$, $P=0.0023$).

Principal component analysis (Table 5) showed that the first component (accounting for 33.2% of the variability of the data) was primarily related to swimming time (0.85 factor loading) and agonistic behaviour (-0.82 factor loading), and secondarily to airbreathing (0.77 factor loading), SGR (0.76 factor loading), control levels of cortisol (-0.68 factor loading), and the increase in plasma glucose levels after the netstressor (-0.61 factor loading). Tanks with high levels of swimming time, airbreathing, and SGR and low levels of agonistic behaviour, low control levels of plasma cortisol, and a low increase in plasma glucose after net stress thus had high factors scores on the first component. The main variables contributing to the second component (accounting for 21.4% of the variability of the data) were feed intake, mortality, and plasma lactate levels before and after net stress. Positive factor coordinates for feed intake, mortality, and the increase in levels of plasma lactate after net stress and a negative factor coordinate

Table 4. Physical, physiological, and behavioural responses of African catfish (*Clarias gariepinus*), weight range 1000-1500 gram

	Stocking density (fish/m^3)				S.E.M.	P-value
	100	233	366	500		
Physical parameters						
Feed intake (gd)	11.68	12.04	12.25	11.86	0.143	0.101
SGR (%/d)	1.27	1.22	1.17	1.23	0.057	0.691
FCR (g/g)	0.81	0.86	0.90	0.83	0.039	0.443
Mortality (%)	0.00	0.00	0.00	0.50	0.249	0.441
Physiological parameters						
Cortisol control (nmol/l)	255.11	301.11	251.41	383.34	25.896	0.533
Cortisol net-stress (nmol/l)	151.47	136.73	80.18	128.23	36.515	0.571
Glucose control (mmol/l)	2.73	3.18	3.49	3.67	0.194	0.055
Glucose net-stress (mmol/l)	6.74	6.07	6.23	6.58	0.246	0.275
Lactate control (mmol/l)	2.91	2.75	2.89	3.18	0.294	0.737
Lactate net-stress (mmol/l)	4.69	3.64	4.68	4.93	0.56	0.419
Behavioural parameters						
Lesions control (#)	1.4	2.1	2.0	1.7	0.35	0.540
Lesions net-stress (#)	2.2	2.3	2.4	2.1	0.31	0.894
Swimming activity (%)	42.1	48.1	45.0	43.0	2.65	0.599
Air breathing (#/fish/h)	52.0	50.0	64.0	73.0	9.34	0.330
Escape attempts (#/fish/h)	0.8	0.8	0.6	1.0	0.35	0.933
Aggressive acts T (#/fish/h)	13.0	1.0	0.0	0.0	4.47	0.172
Stereotypic behaviour (#/fish/h)	0.0	0.0	0.0	0.0	0.00	1.000

for control plasma lactate levels suggest that tanks with high feed intake, high mortality, and high increase in plasma lactate levels after net stress combined with low control levels of plasma lactate had high factor scores for the second component.

For both the first and second component, factor scores of individual tanks were used as dependent variables in a one-way ANOVA, with stocking density as independent variable. A significant effect of stocking density on the first component was found, F(4,10)=8.54, $P<0.05$ (Figure 1). Factor scores for the first component increased with increasing density. Low factor scores on the first component appear to be indicative for decreased welfare (e.g. elevated cortisol, increased aggression), suggesting that fish welfare improved with increasing density. No significant effect of stocking density on the second or any of the other components was found (Figure 1).

EXPERIMENT II. GROWTH STAGE 1000-1500 GRAM.

Table 4 presents the physical, physiological, and behavioural responses of African cat-

Table 5. Factor coordinates of parameters included in the principal component analysis

Growth stage	10-100 gram		100-300 gram		1000-1500 gram	
	PC1	PC2	PC1	PC2	PC1	PC2
Variability explained (%)	27.0	15.3	33.2	21.4	25.7	19.4
Physical parameters						
Feed intake	0.67	-0.03	0.57	0.66	-0.08	0.40
SGR	-0.43	-0.47	0.76	0.28	-0.94	0.00
FCR	0.89	0.21	-0.39	0.48	0.83	0.09
Mortality	0.45	-0.29	0.17	0.63	-0.07	0.14
Physiological parameters						
Cortisol control	0.16	0.59	-0.68	0.43	-0.04	-0.32
Δ Cortisol	-0.31	-0.59	-	-	-	-
Glucose control	-0.63	0.33	0.42	-0.33	0.25	0.57
Δ Glucose net-stress	0.47	-0.71	-0.61	0.45	-0.52	-0.60
Lactate control	0.29	-0.09	-0.12	-0.78	0.24	0.28
Δ Lactate net-stress	-0.05	-0.77	-0.55	0.61	0.64	-0.20
Behavioural parameters						
Lesions control	-0.72	-0.15	-0.30	0.58	0.50	-0.58
Lesions net-stress	0.58	-0.08	-0.51	-0.40	-0.58	0.38
Swimming activity	0.47	-0.30	0.85	0.06	-0.28	0.54
Air breathing	0.65	0.19	0.77	0.28	0.30	0.75
Escape attempts	0.56	-0.30	-0.49	-0.05	0.58	-0.52
Aggressive acts	-0.56	-0.15	-0.82	-0.13	-0.64	-0.46
Stereotypic behaviour	-	-	-	-	-	-

fish (weight range 1000-1500 gram) to stocking density. Average daily feed consumption ranged between 11.68-12.25 g/d. No significant effects of stocking density on feed consumption were observed, F(3,8)=2.91, P=0.1012. Furthermore, stocking density had no influence on SGR (F(3,8)=0.50, P=0.6912) and FCR (F(3,8)=0.99, P=0.4432). Physiological and behavioural responses of African catfish (weight range 1000-1500 gram) were unaffected by stocking density (P>0.05). Surprisingly, African catfish did not respond with an increase in plasma cortisol after net stress. In contrast, plasma cortisol levels declined after net stress.

Principal component analysis (Table 5) showed that the first component (accounting for 25.7% of the variability of the data) was primarily related to SGR (-0.94 factor loading) and FCR (0.83 factor loading), and secondarily to agonistic behaviour (-0.64 factor loading) and the increase in plasma lactate levels after the netstressor (0.64 factor loading). Tanks with high levels of FCR and increase in plasma lactate levels after a

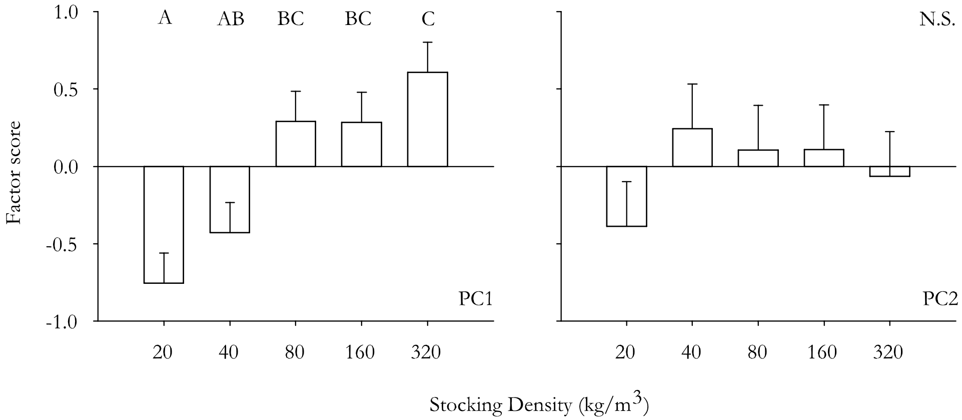

Figure 1. Factor scores for the first (PC1) and second (PC2) factor of a principal component analysis applied to physical, physiological, and behavioural parameters measured in African catfish (growth range 100-300 gram) housed at densities of 20, 40, 80, 160, and 320 kg/m^3. Bars lacking of a common character differ significantly ($P<0.05$).

netstressor and low levels of agonistic behaviour and SGR thus had high factors scores on the first component. The main variables contributing to the second component (accounting for 19.4% of the variability of the data) were airbreathing and the increase in plasma glucose levels after the netstressor. A positive factor coordinate for airbreathing and a negative factor coordinate for increase in plasma glucose levels after the netstressor suggest that tanks with high airbreathing combined with a low increase in plasma glucose levels after net stress had high factor scores for the second component.

For both the first and second principal component, factor scores of individual tanks were used as dependent variables in a one-way ANOVA, with stocking density as independent variable. No relation was found between stocking density and the first or second component, nor was there a relation between stocking density and any of the other principal components ($P>0.05$; Figure 2). This suggests that other factors than stocking density were related to the trends observed in the data. We were unable to find an explanatory factor for the trend indicated by the first component. A correlation analysis indicated that the second component was related to the distance between the tanks and the main aisle ($r_p=0.68$, $N=12$, $P=0.0145$). Tanks located further from the aisle (less disturbance) responded with a higher activity, but lower amounts of escape attempts, lesions, and increase in plasma glucose levels after net stress.

DISCUSSION

EXPERIMENT I

In this study a range of welfare indicators, consisting of physical, physiological, and behavioural parameters, was used to assess the effects of stocking density on the welfare of African catfish. For a growth range of 100-300 gram, both univariate and multivariate analyses indicated a significant effect of stocking density. At increasing density we

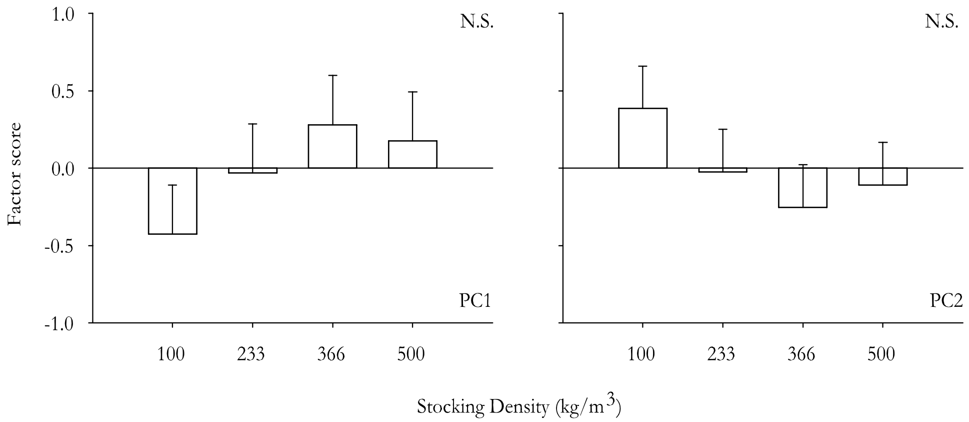

Figure 2. Factor scores for the first (PC1) and second (PC2) factor of a principal component analysis applied to physical, physiological, and behavioural parameters measured in African catfish (growth range 1000-1500 gram) housed at densities of 100, 233, 366, and 500 kg/m^3.

observed an increase in activity and SGR combined with a decrease in plasma cortisol and agonistic behaviour, suggesting an improved welfare at increasing stocking density. This is in accordance with previous studies that showed increased growth performance and an increase in activity and/or decrease of aggression at higher densities (Almazán Rueda, 2004; Appelbaum & Kamler, 2000; Hecht & Appelbaum, 1988; Hecht & Uys, 1997; Kaiser et al., 1995a, b). Hecht and Uys (1997) suggested that the formation of dense shoals interferes with the cues for initiation of agonistic behaviour. Surprisingly, increasing stocking density well above commercially used densities did not seem to have any negative effects on fish welfare. On the contrary, a density of 320 kg/m^3 resulted in the highest factor scores on PC1, although not significantly higher than factor scores of 80 and 160 kg/m^3. These results indicate that a higher density at the start of this growth period may be applicable, thereby controlling agonistic behaviour.

The second principal component indicated a trend, common to all densities, for tanks combining high feed intake, high mortality, high amount of lesions, and high control levels of plasma lactate with a low increase in plasma lactate levels after net stress. We were unable to find an explanatory factor for this trend, although it may have been caused by differences in details of housing or by group composition. Nonetheless, this variability within density indicates that other factors than stocking density affect fish welfare and that focusing solely on stocking density is insufficient to control welfare. Similar results were found in a study of broilers showing that differences among producers in the environment that they provide for chickens had more impact on welfare than had stocking density itself (Dawkins et al., 2004).

EXPERIMENT II

This experiment showed little indications for adverse effects of stocking density on the used range of welfare indicators. Experiment II indicates that it is possible to grow African catfish at densities up to 500 kg/m^3 without affecting growth or mortality. Furthermore, no effects of stocking density on physiological and behavioural parameters were found. The reason why we find no effects of density in this growth stage, in contrast to previous growth stages, is unclear. A possible explanation may lie in the major changes many (cultured) fish undergo in morphology, behaviour and environmental requirements through their life history. For example, Atlantic salmon transform from freshwater parr (territorial, aggressive fish) to smolts (non-aggressive fish) and then to ocean-going adult salmon (non-territorial, shoaling fish). Unfortunately little is known about such changes or natural behaviour in general of African catfish. The little information that is available suggests that African catfish transform from highly cannibalistic, aggressive fry (Haylor, 1991, 1993; Hecht & Appelbaum, 1988; Hossain et al., 1998) to more social, shoaling, and packhunting adults (Bruton, 1979; Merron, 1993). In our own experiments we observed that both swimming activity and agonistic behaviour (assessed by the amount of skin lesions) declined at increasing age. Further research should elucidate which factors cause these behavioural changes. As in experiment I, the results of experiment II further indicate that other factors than stocking density, in this case position of the tank, affected physical, physiological, and behavioural responses of African catfish.

Plasma cortisol responses

A striking result of both Experiment I and II is the plasma cortisol response. First of all, the control levels of plasma cortisol appear to be quite high in comparison to other fish species (i.e. 177.5-383.3 nmol/l). However, previous studies in the African catfish (e.g. Almazán Rueda, 2004; Martins et al., 2006) have shown comparable cortisol levels, indicating that relatively high plasma cortisol levels may be common in this fish species. The African catfish in this study also showed an unusual cortisol response after net stress. In Experiment I, fish responded to a net stressor with either a decline in plasma cortisol or no response. In Experiment II, all fish responded to the net stressor with a decline in plasma cortisol. The reason for this response is unclear, and further study of the effects of age on both the height and kinetics of the cortisol response is necessary.

Implications for aquaculture

The current study confirms that stocking density does indeed affect the welfare of fish. However, they also show that the effects of stocking density are not uniform throughout the growth cycle. A previous study on the effects of stocking density of African catfish of 10-100 gram (Nieuwegiessen, 2008) indicated that both high and low densities had detrimental effects on fish welfare. At low densities we observed increased aggression and skin lesions, while an acute stressor resulted in increased aggression in fish housed at high density. A PCA (Table 5) reflected these findings, showing a significant linear relation between factor scores for the first component and stocking density (Figure 3). At increasing age/size (100-300 gram) welfare concerns shift to low stocking densities, where we observed increased plasma cortisol levels and increased aggression. Finally, there was no apparent effect of stocking density on welfare indicators measured in African catfish of 1000-1500 gram.

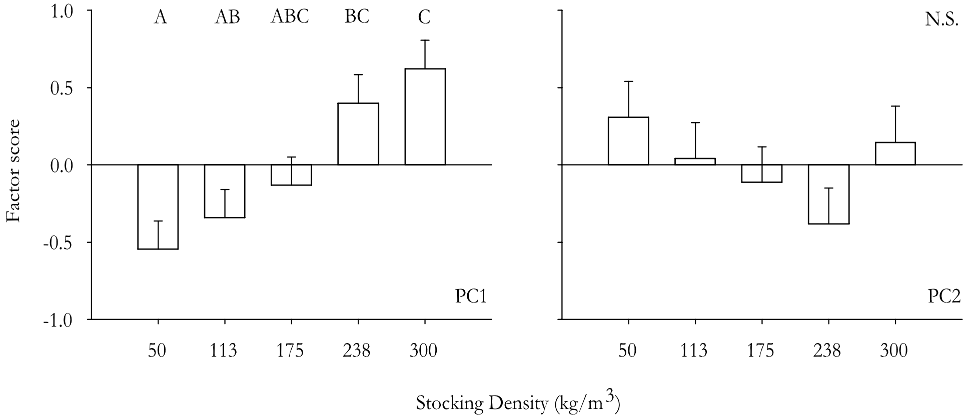

Figure 3. Factor scores for the first (PC1) and second (PC2) factor of a principal component analysis applied to physical, physiological, and behavioural parameters measured in African catfish (growth range 10-100 gram) housed at densities of 50, 113, 175, 238, and 300 kg/m^3.

Such differences need to be taken into account by husbandry practices to assure that high welfare standards are maintained throughout the life cycle of the fish being cultured. While focus for early juveniles should lie on defining both minimum and maximum limits to stocking density, for fish around 100-300 a lower density limit appears to be more useful than limiting maximum stocking densities. At further increasing age, the impact of stocking density appears to become negligible.

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4

Alarm cues in juvenile African catfish, *Clarias gariepinus* Burchell: indications for opposing behavioural strategies

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Applied Animal Behaviour Science 113, 270-275

ABSTRACT

Following exposure of African catfish to alarm cues, a relationship ($r^2_p=0.60$, $N=10$, $P=0.07$) between feed efficiency (measured as residual feed intake) and the change in percentage of time spent swimming in response to damage-released alarm cues was observed. Feed-efficient animals responded with a decrease in percentage of time spent swimming ($P<0.05$) whereas feed-inefficient animals responded with an increase in percentage of time spent swimming ($P<0.05$). This study shows opposing behavioural strategies in African catfish in response to damage-released alarm cues being related to feeding efficiency.

INTRODUCTION

The Ostariophysan alarm cue or Schreckstoff system has been demonstrated in a wide variety of fish species. The system is characterised by distinct epidermal club cells that contain the alarm pheromone. These cues may be released as a result of mechanical skin damage. Other ostariophysans detect it and perform a species specific alarm response (Smith, 1992). Studies on alarm cues typically report average group responses to alarm cues. However, animals often show a pronounced variation in behavioural response to numerous factors, and this individual variation is sometimes more instructive than a group average. Surprisingly, individual variation in the response to alarm cues has not been studied. At present, however, it is increasingly more recognized that behavioural (and physiological) differences between individuals of the same species may be caused by stable biological traits instead of having a situation-specific origin. In literature, these consistent differences between individuals have been referred to as differences in personality, temperament, coping styles/strategies, boldness, neophobia, or behavioural syndromes/profiles (Sih et al., 2004). A basic feature of all these labels is that they reflect individual differences in behaviour and/or physiology, which are stable over time and consistent over situations.

Recent work in African catfish (*Clarias gariepinus*) has shown stable behavioural differences between individual African catfish which were related to feed efficiency (Martins et al., 2005). The objective of the present study was to assess whether individual differences in behavioural response to alarm cues are related to feed efficiency of African catfish.

MATERIALS AND METHODS

ANIMALS, ADAPTATION PERIOD AND EXPERIMENTAL CONDITIONS

All of the procedures involving animals were conducted in accordance with the Dutch law on experimental animals and were approved by the Wageningen University Animal Experimental Committee (DEC). Juvenile *Clarias gariepinus* (N=10, mixed sex) were obtained from a commercial fish farmer (Fleuren & Nooijen viskwekerijen BV, Someren, The Netherlands). At day 1, each fish was individually weighed and randomly allocated to one out of ten 30L aquaria. Each tank was equipped with a single air-stone attached to the tank outlet. An extra length of airline tubing was attached to the air-stone, allowing for the injection of experimental stimuli from behind a black plastic blind.

All tanks were connected to a common recirculation system. Water temperature (± 25 °C), pH (7.0-7.5), NH_4^+ (<2.0 mg/l), NO_2^- (<0.5 mg/l), NO_3^- (<500 mg/l), and conductivity (2500-3000 µs/cm) were checked daily. A 12L:12D photoperiod was maintained. The fish were fed a commercial pelleted feed (Skretting ME-3, Fontaine les Vervins, France) once a day by hand. Feeding started at 09:00h and continued until apparent satiation.

EXPERIMENTAL PROCEDURES AND MEASUREMENTS

African catfish skin extract was prepared from one donor (81.8 gram; male). The donor was euthanatized with 0.8 g/l tricaine methanesulfonate (MS-222, Cresent Research Chemicals, Phoenix, USA) and 1.6 g/l $NaHCO_3$. Skin sections were immediately removed from either side of the donor and placed in 50 ml of chilled demineralised water. Skin sections were then homogenized, the solution filtered through glass wool (to remove any remaining tissue), and the final volume adjusted by adding demineralised water. A dilution of 0.1 cm^2 skin/ml of demineralised water was used (dilution factor based on Lawrence and Smith, 1989).

After 13 days of individual housing a total of 10 individual catfish were tested to both conspecific skin extract and a control (demineralised water). One hour before the control trial the tanks were disconnected from the recirculation system, to avoid diluting the skin extract. Control trials started at 10.00h, skin extract trials started at 10.30h. Control and skin extract trials consisted of a 15 min pre-stimulus and 15 min post-stimulus observation period. Prior to the pre-stimulus period, 50 ml of tank water was withdrawn and discarded and an additional 50 ml of tank water was withdrawn. At the start of the post-stimulus period, either 5 ml of demineralised water or 5 ml of skin extract was injected and slowly flushed into the tanks using the retained 50 ml of tank water. Dye tests showed that this procedure results in a homogeneous distribution of the chemical stimuli throughout the tank within 30 seconds. Control trials were conducted before skin extract trials to exclude a possible masking of the response to the control trial by the skin extract stimuli. Swimming behaviour (a displacement of the body by more than one body length, while browsing, moving, eating and air-breathing; expressed as a percentage of time) during both the pre-stimulus and post-stimulus observation periods was studied. Pre-stimulus observation periods were analysed for 15 minutes, post-stimulus periods were analysed in 4 subsequent blocks of 2.5 minutes. Studying the post-stimulus response in four subsequent blocks was done to get an indication on possible temporal changes in the response to conspecific skin extract. Behavioural response to either the control or conspecific skin extract trial was calculated by subtracting the pre-stimulus response from the post-stimulus response. After the control and skin extract trials, the fish remained in the tanks for 2 more days, after which all fish were individually weighed.

Feed efficiency was analyzed using the residual feed intake (RFI, $g/kg^{0.8}$ per day).

RFI was calculated as the difference between the feed consumed by an animal and its consumption as predicted from a regression model involving the maintenance requirements and growth as independent variables (Luiting and Urff, 1991), and is therefore the error term in the model: $FI=M + \beta G + \varepsilon$, where FI is the feed intake (g/kg$^{0.8}$ per day), M is the maintenance (g/kg$^{0.8}$ per day) and G the growth (g/kg$^{0.8}$ per day). Animals with a low RFI are more feed-efficient than animals with a high RFI. Based on their RFI, animals were arbitrarily assigned to an feed-efficient type ($RFI<0$) or feed-inefficient type ($RFI>0$; Fig. 1A).

STATISTICAL ANALYSES

The results are expressed as means (± S.E.). Preliminary analysis on the effects of gender on the behavioural response to alarm cues indicated that gender did not have an effect on the behavioural response to alarm cues. In this study, fish were considered the experimental unit. Changes in swimming behaviour were analysed using a repeated measures analysis. The error terms of these analyses were tested for homogeneity of variances and normality, using respectively the Levene's test and the Shapiro-Wilk test. Results were considered statistically significant when P-values were below 0.05; P-values between 0.05 and 0.10 were called trends.

RESULTS

No change in time spent swimming was observed between control and skin extract trial, from which can be concluded that the African catfish shows no response to conspecific skin extract. However, further analysis showed a correlation between RFI and mean change in time spent swimming (%) of individual African catfish exposed to conspecific skin extract (Fig. 1B; $r^2_p=0.60$, $N=10$, $P=0.07$; first 5 minutes of exposure to the conspecific skin extract). Animals were therefore assigned to an feed-efficient RFI-type ($RFI<0$) or a feed-inefficient RFI-type ($RFI>0$). Inclusion of the RFI-type in our statistical model, showed an interaction between RFI-type and response to control or skin extract trial (repeated measures ANOVA; $F(1,56)=9.69$; $P<0.01$). Both feed-efficient and feed-inefficient animals showed no response to the control. However, feed-efficient and feed-inefficient animals showed an opposing response to the conspecific skin extract. Feed-efficient animals responded to conspecific skin extract with a decrease in time spent swimming (Fig. 1C; repeated measures ANOVA; $F(1,21)=5.30$, $P<0.05$), whereas feed-inefficient animals responded to conspecific skin extract with an increase in time spent swimming (Fig. 1D; repeated measures ANOVA; $F(1,35)=4.59$, $P<0.05$). In contrast to studies in other fish African catfish do no show the typical freezing or immobile behaviour.

Behavioural responses to conspecific skin extract were not only correlated with feed efficiency (measured as RFI), we also found a correlation between daily feed intake and behavioural responses to conspecific skin extract (Table I). In general, we observed a gradual increase of feed intake during the experiment, which seemed to reach a plateau around day 11 (data not presented). Animals with a more active behavioural response to

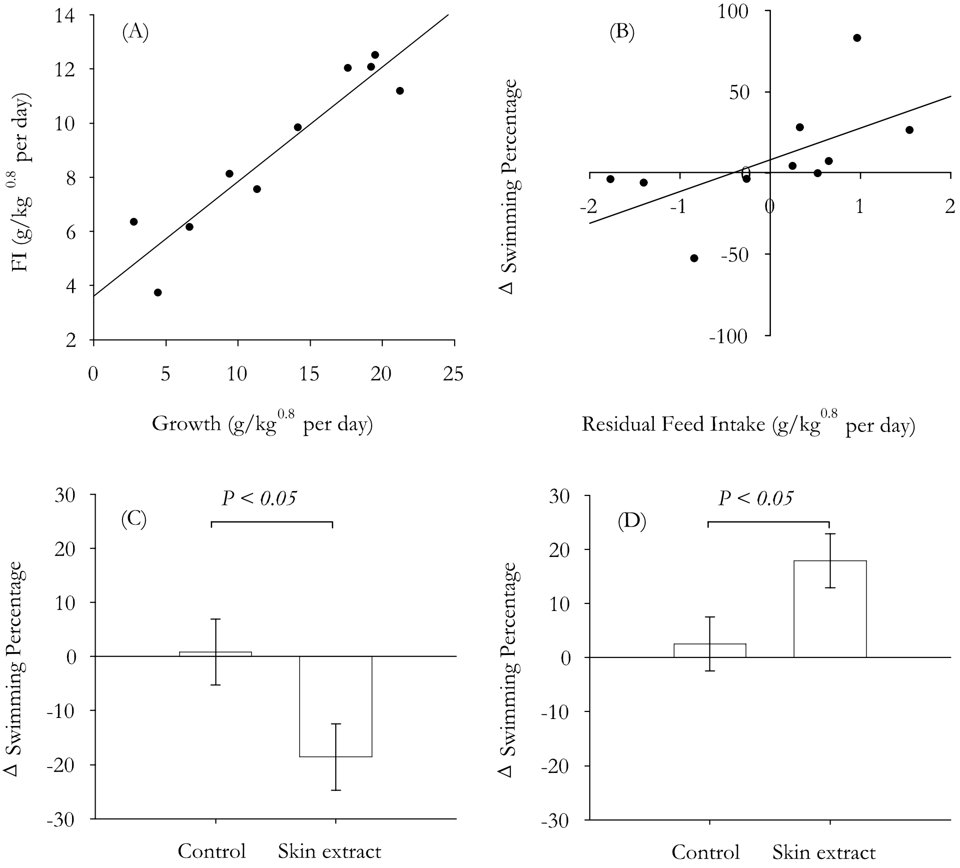

Figure 1. Relationship between metabolic growth and metabolic feed intake (1A). Relationship between metabolic feed intake and the change in percentage of time swimming of individual African catfish exposed to conspecific skin alarm cue (1B). Changes in percentage of time swimming of feed efficient (1C) and feed inefficient (1D) African catfish exposed to conspecific alarm cue.

conspecific skin extract showed a faster increase in feed intake than animals with a more passive behavioural response to conspecific skin extract

Discussion

The results of this study demonstrate that African catfish show a large individual variation in the response to conspecific skin extract. Changes in percentage of time spent swimming in response to conspecific skin extract ranged from -68.4% to 85.1%. Such differences in activity levels suggest differences in coping strategies. Recent work on African catfish has shown stable behavioural differences between individual African catfish (Van de Nieuwegiessen et al., unpublished data). Furthermore, Martins et al. (2006) suggested that variation in feed efficiency (measured as RFI) in African catfish may be related to differences in coping strategies. Could coping strategies have affected

Table I. Pearson correlation coefficients between daily feed intake and mean change in percentage of time spent swimming of individual African catfish exposed to conspecific skin extract. Behavioural responses after exposure to conspecific skin extract were analyzed from 0-5 minutes and 0-10 minutes.

	Daily feed intake (g/kg$^{0.8}$ per day)												
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
Swimming percentage (0-5 min)	-0.17	0.14	0.59$^{\#}$	0.85*	0.66*	0.58$^{\#}$	0.73*	0.55$^{\#}$	0.50	0.64*	0.39	0.61$^{\#}$	-0.40
Swimming percentage (0-10 min)	-0.30	-0.04	0.47	0.78*	0.52	0.42	0.60$^{\#}$	0.45	0.43	0.58$^{\#}$	0.30	0.55	-0.46

[*] $P<0.05$

[#] $P<0.10$

the behavioural response of African catfish to conspecific skin extract?

Looking at the pooled response to conspecific skin extract no apparent behavioural response was observed. However, when (subjectively) observing the behavioural response to conspecific skin extract, a clear, but variable behavioural response was noticed. In general, animals showed an anxious response, with one of the fish regurgitating its last meal, an often observed acute stress response in African catfish (personal observations). Could different coping strategies have masked the response to conspecific skin extract? Following Martins' (2006) suggestion on the relation between coping strategies and RFI, the relationship between RFI and the behavioural response of African catfish to conspecific skin extract was examined. A relationship (Fig. 1B; r^2_p=0.60, N=10, P=0.07) between feed efficiency (measured as residual feed intake) and the change in percentage of time spent swimming in response to damage-released alarm cues was found. Feed-efficient and feed-inefficient animals appeared to use opposing strategies in their response to damage-released alarm cues. These results suggest that the behavioural response of feed-inefficient animals to conspecific skin extract is more dominated by a pro-active coping strategy than that of feed-efficient animals.

Moreover, differences in behavioural response to conspecific skin extract showed to be related to feeding behaviour in isolation, reflected by a positive correlation between daily feed intake and behavioural response to conspecific skin extract. Stressful situations like individual housing (Øverli et al., 2002) have been shown to lead to a reduction in feed intake, which is confirmed by the current study in which animals showed a continuous increase in feed intake during individual housing. The speed at which animals resume feed intake after transfer to a novel environment has been linked to differences in coping strategies, with representatives of the pro-active coping strategy showing a faster resumption of feed intake (Øverli et al., 2005). Our results suggest that the behavioural response of feed-inefficient animals to transfer to a novel environment is more dominated by a pro-active coping strategy than that of feed-efficient animals.

With the limited knowledge this study provides, we can only hypothesize on the implications of these behavioural strategies in natural contexts. The alarm cue response shown by the feed-efficient animals appears to correspond with the typical response of fish towards damage-released alarm cues (e.g. decreased movement and foraging; (Chivers & Smith, 1998). This response may benefit both signal senders and receivers, amongst others by increasing survival probability (Mathis and Smith, 1993). The question than is why feed-inefficient animals showed an increase in swimming time, a response that a first sight does not appear to be an optimal response? Following the theory of behavioural syndromes, of which coping strategies are an example, a behavioural response does not need to be optimal (Sih et al., 2004). One of the implications of behavioural syndromes is that they might represent constraints on optimal behaviour. Although in behavioural ecology it is usually assumed that behaviour is plastic and appropriate behaviour can be shown in different situations, the behavioural syndromes view stresses the possibility of suboptimal behaviour in any given context. In other words, individuals have a general tendency to behave in a similar way in different contexts. So, in the current study, animals showing an active response to conspecific skin extract may simply show an active response towards all stimuli. However, further study will need to clarify the behavioural responses of feed-efficient and feed-inefficient animals in different contexts.

The objective of the present study was to assess whether individual differences in behavioural response to alarm cues are related to feed efficiency of African catfish. In conclusion, this study shows opposing behavioural strategies in African catfish in response to damage-released alarm cues being related to feeding efficiency.

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Chemical alarm cues in juvenile African catfish, *Clarias gariepinus* Burchell: a potential stressor in aquaculture?

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ABSTRACT

Previous studies on the effects of stocking density on, among others, the behaviour of African catfish have shown that at low densities, especially directly after restocking of tanks, increased aggression might occur. This aggression may directly affect the welfare of the fish. In addition, the resulting skin damage may also lead to the release of chemical alarm cues from the skin of the fish, possibly acting as a secondary stressor in a farming situation. Moreover, in a recirculating aquaculture system, a build-up of chemical alarm cues might occur. The objective of this study was to examine the effects of a single chemical alarm cue administration on the behaviour and growth performance of group-housed African catfish. Furthermore, we tested if a single passage over a biofilter could affect the biological activity of chemical alarm cues. Although African catfish responded to chemical alarm cues with a short-term increase in swimming activity, no long-term effects were observed on both behaviour and growth performance of the fish. Furthermore, our results indicated that a single passage over a biofilter did not affect the biological activity of the alarm cue, indicating no short-term breakdown of the alarm cue. In conclusion, the results of this study indicate that chemical alarm cues, at the concentration applied in this study, cannot be considered a stressor for African catfish, although the effects of higher cue concentrations need further study. In addition, further study into the effects of chemical alarm cues on other, non-predatory, farmed fish is recommended.

INTRODUCTION

The Ostariophysan chemical alarm cue or Schreckstoff system has been demonstrated in a wide variety of fish species (Smith, 1992), including the African catfish, *Clarias gariepinus* (Van de Nieuwegiessen et al., 2008a). The system is characterised by distinct epidermal club cells which contain the chemical alarm cue. These cues may be released as a result of mechanical skin damage, e.g. through a predator attack. When detected by nearby conspecifics, these chemical alarm cues generally elicit species specific antipredator responses, e.g. increased shoaling, freezing, and refuging (Brown & Godin, 1999; Brown et al., 1995; Mathis & Smith, 1993), although some (predatory) species may use these signals as foraging cues (Chivers et al., 1996; Mathis et al., 1995).

Although there is an extensive knowledge on the ecological role of chemical alarm cues, in a farming situation the potential effects of these cues are largely unexplored. Chemical alarm cues are likely present in aquaculture systems, released through agonistic behaviour, handling, or high stocking densities. Especially in recirculating aquaculture systems (RAS) chemical alarm cues may pose a problem. In contrast to flow-through systems, RAS are closed systems which re-use water with mechanical and biological treatment between each use. Because of the closed character of a RAS a range of compounds, including chemical alarm cues, may accumulate.

Previous studies on the interaction between stocking density and behaviour of African catfish revealed that at low densities, especially directly after stocking, increased aggression occurs (Van de Nieuwegiessen et al., 2008b). This may potentially lead to the release of chemical alarm cues. To gain insight into the possible consequences of this alarm cue release in a farming situation, the objective of this study was to examine the effects of a single alarm cue administration on the behaviour and growth performance of group-housed African catfish. Since it is unclear if the water treatment within a RAS affects the biological activity of chemical alarm cues, we also assessed if a single passage over a biofilter could affect this biological activity.

MATERIALS AND METHODS

ANIMALS, ADAPTATION PERIOD AND EXPERIMENTAL CONDITIONS

All of the procedures involving animals were conducted in accordance with the Dutch law on experimental animals and were approved by the Wageningen University Animal Experimental Committee (DEC). Full sib juveniles of *Clarias gariepinus* Burchell (N=160, mixed sex) were obtained from a commercial fish farmer (Fleuren & Nooijen viskwekerijen BV, Someren, The Netherlands). Prior to the experiment, fish were held at the central fish facility (De Haar Vissen, Wageningen University, The Netherlands) in 16 70L-aquaria (10 fish/tank) to enable adaptation to the recirculating water system.

Each tank was equipped with a single air-stone attached to the tank outlet. An extra length of airline tubing was attached to the air-stone, allowing for the injection of experimental stimuli from behind a black plastic blind.

During both the adaptation period and the experimental period, the aquaria were filled with recirculating UV-treated tap water with a water temperature of ± 25 °C, pH 7.5-8.0, NH_4^+ 0 mg/l, NO_2^- <0.03 mg/l, NO_3^- <150 mg/l and the conductivity ranged between 2000 and 4000 µs/cm. The photoperiod was 12L:12D. Flow rate for each aquarium was set at 8 litre/minute. Fish were fed a commercial pelleted feed (Skretting ME-3, Fontaine les Vervins, France) twice a day by hand. Feeding started at 09:00h and 17.00h and continued until apparent satiation.

STIMULUS PREPARATION

African catfish skin extract was prepared from a single donor fish (female, 217.9 gram). The donor fish was euthanatized with 0.8 g/l tricaine methanesulfonate (MS-222, Cresent Research Chemicals, Phoenix, USA) and 1.6 g/l $NaHCO_3$. Skin sections were immediately removed from either side of the donor and placed in 50 ml of chilled demineralised water. Skin sections were then homogenized, the solution filtered through glass wool (to remove any remaining tissue), and the final volume adjusted by adding demineralised water. A dilution of 0.1 cm^2 skin/ml of demineralised water was used (Lawrence & Smith, 1989). We applied 15 ml of this stimulus in a 70L tank. Given the area of skin typically damaged during predation, this concentration is most likely an ecologically valid one (G.E. Brown, personal communication).

EXPERIMENTAL PROCEDURES AND MEASUREMENTS

After 14 days of adaptation, four experimental treatments were studied with a 2 by 2 factorial design. Factor 1 was directly adding conspecific skin extract in the tank of the fish (yes or no). Factor 2 was the housing system (RAS or flow-through). Four replicates per treatment were applied. The experimental period lasted for 14 days.

African catfish were first tested to a control (demineralised water) and subsequently tested to either conspecific skin extract or a second control (demineralised water). One hour before the control trial the tanks were disconnected from the recirculation/flow-through system, to avoid diluting the skin extract. Control trials and skin extract/second control trials were 60 minutes apart. Control and skin extract/second control trials consisted of a 15 min pre-stimulus and 15 min post-stimulus observation period. Prior to the pre-stimulus period, 50 ml of tank water was withdrawn and discarded and an additional 50 ml of tank water was withdrawn. At the start of the post-stimulus period, either 15 ml of demineralised water or 15 ml of skin extract was injected and slowly flushed into the tanks using the retained 50 ml of tank-water. Dye tests showed that this procedure resulted in a homogeneous distribution of the chemical stimuli throughout the tank within 30 seconds. Control trials were conducted before skin extract trials to exclude a possible masking of the response to the control trial by the skin extract stimuli. During both the pre-stimulus and post-stimulus observation period we continuously studied the percentage of animals swimming. Furthermore, we measured the number of escape attempts (defined as an animal moving to the water surface and it's head emerges from the water surface for at least past it's gill cover). Behavioural response to either the control or conspecific skin extract/second control trial was calculated by subtracting the pre-stimulus response from the post-stimulus response.

Following these behavioural observations, all tanks were reconnected to the recirculation/flow-through system. The chemical alarm cues added to the tanks either entered the RAS or were flushed out of the flow through system. The tanks receiving the double control either came in contact with chemical alarm cues after the cues passed the biofilter (in the RAS treatment) or never came in contact with chemical alarm cues (in the flow-through system). By comparing the behavioural response of these tanks we assessed the biological activity of chemical alarm cues after passing a biofilter. The behaviour of the fish was therefore studied, in blocks of 5 minutes, for 3 hours after the tanks had been reconnected to the systems. For long-term behavioural changes, additional behavioural video recordings were made at Day 1, 3, 7, and 12 from 12.00h - 12.30h. We recorded the percentage of animals swimming (a displacement of the body, while browsing, moving, eating and air-breathing), percentage of animals resting (moving passively through the water or lying still at the bottom of the tank) and numbers of escape behaviour (the animal moves to the water surface and it's head emerges from the water surface for at least past it's gill cover). Every minute the total number of fish swimming and total number of fish resting were counted as well as the total number of visible fish. The activity patterns were expressed as a percentage of the total number of fish counted. Escape attempts (frequency) were recorded by all occurrence sampling and expressed as number of escape attempts per fish per hour. The quality of the video recordings did not allow for a proper quantification of agonistic behaviour. Therefore, as an indicator of aggression, the number of bite wounds on the body of the fish were determined at the end of the experiment. This indirect measurement of agonistic be-

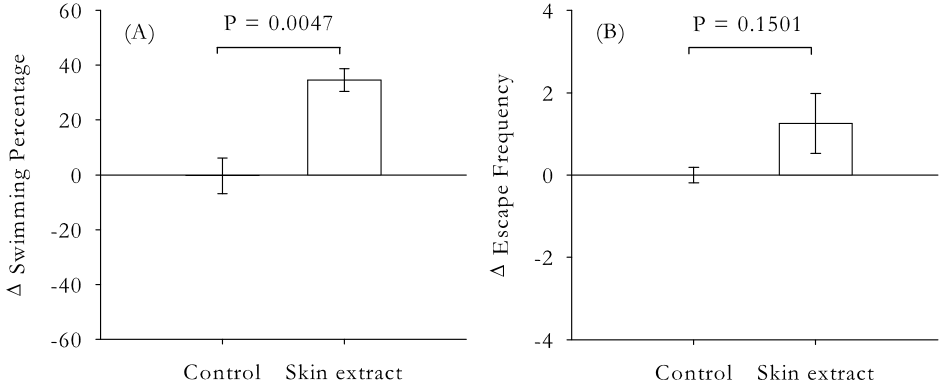

Figure 1. Mean (± S.E.) change in percentage of swimming fish and escape attempts in response to chemical alarm cues.

haviour was shown to have a strong correlation with the number of aggressive acts of African catfish (Almazan-Rueda et al., 2004). At the end of the 2-week experimental period, all fish were individually weighed.

Statistical analysis

The results are expressed as means (± S.E.M). In this study, tanks were considered the experimental unit. All data was analysed using a t-test or 2-way ANOVA. The error terms of these ANOVA analyses were tested for homogeneity of variances and normality, using respectively the Levene's test and the Shapiro-Wilk test. Results were considered statistically significant when P-values were below 0.05; P-values between 0.05 and 0.10 were called trends.

Results

Behavioural responses to chemical stimuli and the housing system

African catfish responded to chemical alarm cues with an increase in swimming activity (T(7)=4.07, P=0.005; Figure 1). No significant changes in the amount of escape attempts were observed (T(7)=1.62, P=0.150). African catfish receiving a second control showed no changes in swimming activity (T(7)=-0.53, P=0.612) or in the amount of escape attempts (T(7)=-0.88, P=0.407). Housing system did not affect the behavioural response to chemical alarm stimuli (P>0.05, data not shown).

Chemical alarm cues had no long term effect on the behaviour of African catfish (Table 1). Neither the percentage of fish swimming (F(1,12)=0.06, P=0.809), the amount of escape attempts (F(1,12)=0.38, P=0.549), or the amount of bite wounds (F(1,12)=1.62, P=0.228) were affected by chemical alarm cues. The behaviour of the fish was affected by the housing system. Fish housed in the flow through system showed a slightly higher swimming activity (F(1,12)=5.02, P=0.045). A similar trend was observed for amount of escape attempts (F(1,12)=3.43, P=0.089). No interaction effects

Table 1. Long term behavioural responses of African catfish to chemical alarm cues and the housing system.

	Experimental treatment								
Housing system	Flow through		RAS			Alarm cue		Housing system	
Alarm cue	Control	Alarm cue	Control	Alarm cue	SEM	F(1,12)	P	F(1,12)	P
Swimming activity (%)	51.1	50.4	44.9	44.2	2.77	0.06	0.809	5.02	0.045
Escape attempts (#/fish/hr)	1.4	1.6	0.4	0.8	0.51	0.38	0.549	3.43	0.089
Bite wounds (#/fish)	3.1	4.6	3.6	3.8	0.71	1.62	0.228	0.06	0.814

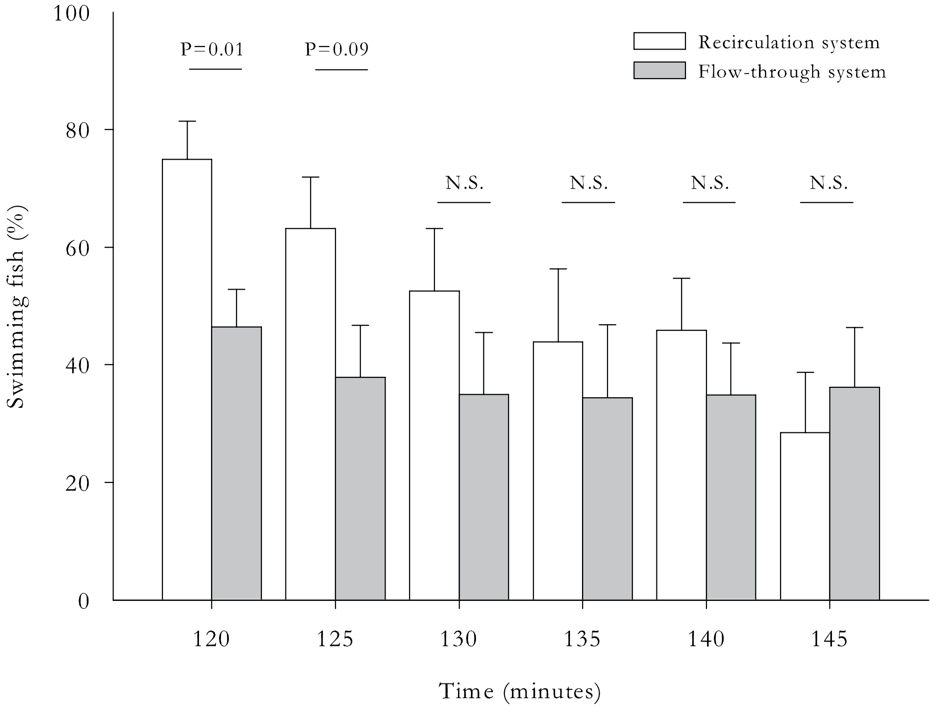

Figure 1. Mean (± S.E.M.) percentage of fish swimming in response to chemical alarm cues after passage over the biofilter.

between chemical alarm cue and housing system were found (P>0.05).

GROWTH PERFORMANCE IN RESPONSE TO CHEMICAL STIMULI AND THE HOUSING SYSTEM

Table 2 shows the growth performance of African catfish in response to the four experimental treatments. No effect of experimental treatment was observed for final body weight, specific growth rate or feed conversion ratio (P>0.05). Furthermore, daily metabolic feed intake did not differ between the experimental treatments (P>0.05). Considering that chemical alarm cues might only have short term effects on feed intake, we also tested for differences in feed intake for every individual day following the administration of the alarm cue. No differences in feed intake between the experimental treatments in any of the 13 days following the administration of the alarm cue were present (P>0.05, data not presented).

BIOLOGICAL ACTIVITY OF CHEMICAL ALARM CUES AFTER PASSING THE BIOFILTER

To test if the biofilter could potentially affect the biological activity of chemical alarm cues, we compared the behavioural responses of fish in a RAS with those of fish in a flow-through system. Both groups of fish previously received a second control and thus had no previous contact with the chemical alarm cue. During the first two hours following the introduction of the chemical alarm cue to the RAS no behavioural differ-

Table 2. Growth performance of African catfish in response to chemical alarm cues and the housing system.

	Experimental treatment								
Housing system	Flow through		RAS			Alarm cue		Housing system	
Alarm cue	Control	Alarm cue	Control	Alarm cue	SEM	F(1,12)	P	F(1,12)	P
End weight (g)	244.5	245.8	252.5	240.7	9.51	0.30	0.592	0.03	0.876
SGR (%)	3.20	3.38	3.31	3.32	0.139	0.48	0.501	0.03	0.863
FCR (g/g)	0.79	0.75	0.77	0.79	0.020	0.30	0.592	0.06	0.805
Feed intake (g/kg$^{0.8}$/d)	18.0	19.1	19.0	18.3	1.01	0.03	0.863	0.00	0.972

ences were apparent between fish in a RAS and fish in a flow-through system. However, 120-125 minutes after introducing chemical alarm cues to the RAS, fish in this recirculation system became more active than fish in a flow-through system (F(1,6)=9.91; P=0.01; Figure 2). During the next 5 minutes a similar trend was observed (F(1,6)=4.13; P=0.09) after which the activity levels of fish in the RAS slowly returned to those of fish in the flow-through system. Despite an approximate 10-fold dilution of the chemical alarm cue in the RAS, the magnitude of the behavioural response to these cues was only marginally lower compared to the response of fish receiving the cues directly. Fish receiving the cues directly showed a 36% increase in activity (Table 1A), while fish receiving the cues after passage over the biofilter showed a 25% increase in activity (data not presented).

DISCUSSION

A chemical alarm cue concentration as applied in the current study has been shown to elicit anti-predator responses in a range of fish species, e.g. fathead minnows (*Pimephales promelas*; Brown et al., 2000), finescale dace (*Chrosomus neogaeus*; Brown et al., 2000), green sunfish (*Lepomis cyanellus*; Brown & Brennan, 2000), channel catfish (*Ictalurus punctatus*; Brown et al., 2003), and several others. However, the response of African catfish in this study, i.e. an increase in swimming activity, indicates that these fish do not perceive this concentration of chemical cue as an alarm cue, but rather as a foraging cue. From a welfare point of view, one can still argue though that providing the fish with a foraging cue but not 'rewarding' them with food may lead to frustration, at which animals have been shown to respond to with frustration-induced-aggression (FIA; Dollard et al., 1944). However, the amount of laesions do not give us indications of FIA in tanks receiving chemical alarm cues.

A possible explanation for the fact that African catfish perceived this chemical alarm cue concentration as a foraging cue may lie in the omnivorous feeding habits of this species. Previous studies have shown that a variety of predators use chemical alarm cues as a foraging response. For instance, Mathis et al. (1995) and Chivers et al. (1996) showed that northern pike (*Esox lucius*) and diving beetles (Dytiscidae) are attracted to chemical alarm cues of the fathead minnow (*Pimephales promelas*). Nonetheless, in some situations chemical alarm cues may still elicit anti-predator responses in predatory fish. Several predatory fish species show a threat-sensitive shift in their response to chemical alarm cues, shifting from a foraging to an anti-predator response depending on amongst others alarm cue concentration, age/size, and feeding motivation (Brown et al., 2001). Personal observations (still to be confirmed) made during the course of our experiments, suggest that African catfish show a similar threat-sensitive shift. So although the present results indicate that chemical alarm cues do not elicit an anti-predator response in cultured African catfish, at the concentration used in this study, higher chemical alarm cue concentrations may possibly do so.

Although further research is required, we can speculate if higher cue concentrations are likely to be present in a RAS. The chemical alarm cue concentration within a RAS is influenced by a number of factors, e.g. the amount of fish in the system (i.e. stocking density), aggression levels (i.e. the number of bite wounds/fish), and possibly the build-up of chemical alarm cues. The density used in this study (≈ 142 fish/m^3) is comparable to densities used in a Dutch farming situation, although some farms may go higher than 300 fish/m^3. Regarding aggression, our personal experience with African catfish has shown that now and then these fish may respond to restocking of tanks with a strongly increased aggression. In the past this has led to 15.7 ± 1.47 bite wounds (± S.E.) per fish (Van de Nieuwegiessen et al., unpublished data). This number of bite wounds compares to a punctured skin area much higher than the one we used (i.e. ≈ 0.15 cm^2/fish). Hence it is very reasonable to assume that in such situations the alarm cue concentrations reach a level which may elicit an anti-predator response.

The build-up of chemical alarm cues in a RAS, strongly increasing chemical alarm cue concentrations, also needs to be considered. Our results have indicated that a single passage over a biofilter did not affect the biological activity of the alarm cue, indicating no short-term breakdown of the alarm cue. The biological activity of the chemical alarm cues thus seems mainly dependent on the chemical stability of the alarm cue. The landmark study of Von Frisch (1941) indicated that chemical alarm cues kept their full biological potential for at least 3 days. Pfeiffer et al. (1985) observed a biological activity for 24-26 hours.

Although chemical alarm cues, at the concentration applied in this study, do not appear to be a stressor for African catfish, the effects of these cues might be different for other cultured fish species. Non-predatory fish, generally use chemical alarm cues to a lesser extent as foraging cues and may show an anti-predator response at much lower alarm cue concentrations than the African catfish. Since we have no indications for a short term breakdown of chemical alarm cues by a RAS, once released these cues may remain present in stressful concentrations for up to several days. Further study into the effects of chemical alarm cues in non-predatory cultured fish, e.g. Nile tilapia (*Oreochromis niloticus*), is therefore necessary.

In conclusion, the results of this study indicate that chemical alarm cues, at the concentration applied in this study, cannot be considered a stressor for African catfish, although the effects of higher cue concentrations need further study. In addition, further study into the effects of chemical alarm cues on other, non-predatory, farmed fish is recommended.

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6

COPING STRATEGIES IN FARMED AFRICAN CATFISH, *CLARIAS GARIEPINUS* BURCHELL - DOES IT AFFECT THEIR WELFARE?

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ABSTRACT

A number of studies have provided evidence for the existence of coping strategies among several species of cultured fish. However, up to now these studies have provided little insight into the consequences of these coping strategies for the fitness of the different behavioural phenotypes in intensive husbandry systems. The objective of this study was to assess if and how coping strategies affect the welfare of African catfish housed at low and high density. To determine the coping characteristics of our fish, 560 juvenile African catfish were confronted twice with an escape test. Based on the amount of escape attempts and general activity during the first escape test, fish were assigned to a proactive, intermediate, or reactive coping type. Fish were group housed for five weeks following a 2*3 factorial design. The first factor was stocking density (low density, 100 kg/m^3 (8 fish/tank); high density, 500 kg/m^3 (40 fish/tank)), the second factor was group composition (pro-active, re-active, mixed). During group housing physical and behavioural data were collected. Group composition influenced feed intake, SGR, FCR, and energy retention ($P<0.05$). The latter was fully due to differences in feed intake ($P<0.05$), since energy partitioning (on % GE intake basis) was similar among the group composition treatments. Stocking density influenced FCR, energy retention, agonistic behaviour and feeding speed ($P<0.05$). None of the measured parameters was influenced by the interaction effect. In mixed groups, SGR and amount of skin lesions seemed to be affected by different behavioural phenotypes at low stocking density, but not at high density. These results indicate that both stocking density and group composition affect physical and/or behavioural responses of African catfish. Furthermore, physical and behavioural data of individual fish housed in mixed groups suggests that coping strategy affects the fitness of different behavioural phenotypes at low, but not at high stocking density.

INTRODUCTION

Humans show consistent individual differences in personality, e.g. people who are generally more bold versus people who are generally more shy. These individual differences are not restricted to humans, comparable individual differences have been shown in a broad range of species, including mammals, birds, amphibians, etc. While in humans, these differences have been termed personality types (Pervin & John, 1999), in other taxa these consistent differences between individuals have been referred to as differences in personality, temperament, coping styles/ strategies, boldness, neophobia, or behavioural syndromes/ profiles (Sih et al., 2004). All these terms reflect individual differences in behaviour and/or physiology, which are stable over time and consistent over situations. When challenged, animals on the proactive (i.e. "bold" animals) side of the spectrum are characterized by being more aggressive and showing a higher activity (fight/flight reaction). In contrast, animals on the reactive (i.e. "shy" animals) side of the spectrum respond to a similar challenge with immobility and lack of initiative (a conservation/withdrawal reaction; Bohus et al., 1987; Koolhaas et al., 1999).

Studies in a number of animal species (e.g. mice, *Mus musculus*, Koolhaas et al., 1999;

rats, *Rattus norvergicus*, Koolhaas et al., 1999; and great tits, *Parus major*, Drent et al., 2003) have provided us with knowledge on the causation, development and function of these individual differences in behaviour and/or physiology. Such individual variation often appears to be inherited and variation in selection regimes results in different phenotypes performing best in different conditions (Dingemanse et al., 2004).

A number of studies have provided evidence for the existence of coping strategies among several species of cultured fish, including rainbow trout (Øverli et al., 2005) and brown trout (Brelin et al., 2005). However, up to now these studies have provided little insight into the consequences of these coping strategies for the fitness of the different behavioural phenotypes in intensive husbandry systems. Huntingford and Adams (2005) suggested that fish at the reactive end of the spectrum may gain little food over long periods when housed at high densities and with a predictable food source, as is usually the case in intensive husbandry systems. Possible remedial actions may include selecting specific behavioural phenotypes for farming purposes or altering husbandry systems to create a farm environment in which a range of behavioural phenotypes may flourish. However, before such actions are carried out, the exact consequences of coping strategies in a farm environment need to be examined. Therefore, the objective of this study was to assess if and how coping strategies affect the welfare of African catfish housed at low and high stocking density.

Materials and methods

Animals and experimental conditions

All of the procedures involving animals were conducted in accordance with the Dutch law on experimental animals and were approved by the Wageningen University Animal Experimental Committee (DEC). Juveniles of *Clarias gariepinus* Burchell (N=560, mixed sex) were obtained from a commercial fish farmer (Fleuren & Nooijen viskwekerijen BV, Someren, The Netherlands). Fish were held at the central fish facility (De Haar Vissen, Wageningen University, The Netherlands). Tanks were connected to a common recirculation system. The water flow rate (7 l/min), water temperature (25 ± 1.03°C), pH (7.1 ± 0.27), NH_4^+ (0.25 ± 0.388mg/l), NO_2^- (0.23 ± 0.125mg/l), NO_3^- (370 ± 163mg/l), oxygen concentration (6.7 ± 0.44mg/l; 83 ± 4.8%) and conductivity (2639 ± 429 µs/cm) were checked daily. A 12L:12D photoperiod was maintained. During the adaptation period fish were fed a pelleted feed (Skretting ME-3, Fontaine les Vervins, France) twice a day by hand. Feeding started either at 09:00h or 16.00h and continued until apparent satiation.

Experimental procedures and measurements

To determine the coping characteristics of our fish, the animals were confronted twice with an escape test (before and after a group-housed period), a test successfully used to determine coping characteristics of the African catfish (Van de Nieuwegiessen, unpublished results). The escape test consisted of draining the tank (30L before the group-housed period; 70L after the group-housed period) of an individually housed fish by

Table 1. The ethogram used for behavioural observations.

Behavioural element	Definition
Novel environment test	
Swimming	A displacement of the body, while browsing, moving, eating, and airbreathing
Resting	Lying still in the water or at the bottom of the tank
Air breathing	The animal moves to the water surface and takes a gulp of air. This was checked by escaping air from the gills of the fish, when it was swimming back to the bottom of the tank
Escape attempts	The animal moves to the water surface and it's head emerges from the water surface for at least past it's gill cover
Total feeding time	The time elapsed between the moment the first pellet is eaten, and the moment the fish are fully satiated
Total feeding speed	The amount of food eaten divided by the total feeding time
Latency time to feeding	The time elapsed between the first pellet added to the tank, and the moment the first pellet is eaten.
Escape test	
Moving	A displacement of the body
Resting	Lying still at the bottom of the tank
Escape attempts	The head of the animal emerges from the water surface for at least past it's gill cover

means of a siphon. This drained the tank with about 1.5 cm of water remaining in the tank. The fish remained in this tank for 5 minutes. During the escape test moving time, resting time, and escape attempts were observed (see Table 1). Moving and resting time were expressed as a percentage of the total time of observation. Escape attempts were quantified by counting the number of times this behaviour took place, and were expressed as a frequency. Based on the amount of escape attempts and general activity during the first escape test, fish were assigned to a proactive coping type (25% most active and escape willing fish; 7.8 ± 4.57 escape attempts) or a reactive coping type (25% least active fish with no escape behaviour; 0.0 ± 0.00 escape attempts). The rest of the population was characterised as an intermediate coping type.

For individual identification, all fish were individually tagged using PIT tags (Trovan, East Yorkshire, United Kingdom). PIT tags were implanted dorsally, directly behind the head. Dimensions of the cylindrical, glass tags were 12 mm long, a diameter of 2 mm, and a weight of 0.09 gram. After both escape tests, standard length, weight, and skin lesions were determined. After the first escape test, we randomly sampled 10 pro-active and 10 re-active fish for initial body composition analysis. After the second escape test, we randomly sampled 8 fish/tank for final body composition analysis. These fish were killed with an overdose of MS-222 (0.8 g/l buffered with 1.6 g/l sodium bicarbonate), placed in a plastic bag, sealed and stored at -20 °C for further processing and analysis.

The period of group housing had a 2*3 factorial design. The first factor was stocking density (low density, 100 kg/m^3 (8 fish/tank); high density, 500 kg/m^3 (40 fish/

Table 2. Experimental diet formulation on % dry matter basis.

Ingredient	Amount
Wheat	17
Wheat gluten	10
Fishmeal	40
Fish oil	6
Soybean meal	20
Premix	1
Binder (Durabon)	1
$CaCO_3$	1.5
$CaPO_4$	1.5
Diamol (AIA)[1]	2.0

[1] AIA = acid insoluble ash. To allow for digestibility studies, Diamol was added as inert marker.

tank)), the second factor was group composition (pro-active, re-active, mixed). High density treatments had 2 replicates, low density treatments had 3 replicates. The period of group housing lasted 5 weeks.

At the start of the group housing (day 0), the fish had an average weight (± S.E.) of 192.8 ± 3.1 g. Fish were randomly assigned to one of 15 glass aquaria (90x45x45 cm) with a water volume of 120L. Each of the 15 aquaria was randomly assigned one of four experimental treatments. Fish were fed an experimental diet twice a day by hand (Table 2). Feeding started either at 09:00h or 16.00h and continued until apparent satiation. Diamol (diatomaceous cell powder, product of Franz Bertram, Hamburg, Germany) was added to the diet as an acid insoluble ash (AIA) marker to determine apparent digestibility coefficients (ADC).

Every day, before each meal, faeces were collected using a commercially available settling tank (AquaOptima AS,Trondheim, Norway) with a volume of 17L and a column height of 44.0 cm and a diameter of 24.5 cm. At the bottom of the settling tank, a tap was placed at which a bottle was attached. The sampling bottles were continuously submerged into ice water to prevent bacterial decay of faeces during the collection period. Faeces were stored at -20 °C until analyses.

BEHAVIOURAL ANALYSES

During the group housing fish behaviour was recorded both on video and by direct observation. Video recordings were made of each aquarium on the front side and took place at day 1, 2, 5, 8, 15, 22, 29, and 36. From each tank, 30 minutes of video (12.00h-12.30h) were analysed. Table 1 shows the ethogram used in this study. The activity patterns were expressed as a percentage of the total number of fish counted. Escape attempts (frequency) were recorded by all occurrence sampling and were recorded per tank per period. A correction was made for the difference in number of fish visible per tank between the treatments by dividing the frequencies of escape attempts by the average number of fish visible per tank per treatment. Furthermore, every five minutes

(12.00, 12.05, 12.10, 12.15, 12.20, 12.25, 12.30h) the total number of fish swimming and total number of fish resting were counted as well as the total number of visible fish.

Direct observations were made at day 7, 14, 21, 28, and 35, starting directly after the morning feeding. Per tank, an individual fish was followed throughout a five-minute period. Whenever the fish was out of sight another fish was immediately selected from the position where the first fish was last observed and the observations continued. Resting time was expressed as a percentage of the total time of observation. Swimming time was defined as 100 minus the percentage of resting. Air-breathing and agonistic behaviour were quantified by counting the number of times this behaviour took place, and were expressed as a frequency. In addition, once every week latency to feeding, total feeding time (TFT) and total feeding speed (TFS) were measured.

At the end of the period of group housing skin laesions were measured, as an indicator of agonistic behaviour. This indirect measurement of agonistic behaviour has been shown to have a strong correlation with the number of aggressive acts of African catfish (Almazan-Rueda et al., 2004). Previous studies in juvenile African catfish have shown a decrease of aggression at higher densities (Hecht & Uys, 1997; Kaiser et al., 1995a; Van de Nieuwegiessen et al., 2008b).

Digestibility and energy metabolism measurements

Before chemical analysis, the sampled fish were cut into small pieces, which were homogenized by passing them through a 4.5 mm-screen grinder two times. The sampled feed was ground twice using a 1 mm-screen grinder. Samples for dry matter determination were taken from the homogenates of fish and feed before the remaining materials were freeze-dried. All faeces were freeze-dried. Each freeze-dried sample of fish was thoroughly mixed in a blender before further analysis. This was also done for each freeze-dried sample of feed and of faeces.

All chemical analyses were done in triplicate. Dry matter content was determined as weight loss after drying the samples for 4 h at 103 °C until constant weight (ISO, 1983). Crude protein content was determined using the Kjeldahl method and multiplying the nitrogen content by 6.25 (ISO, 1997). Crude fat content was determined after petroleum–ether extraction using a Soxhlett system (ISO, 1999). Gross energy content was determined using a bomb calorimeter (IKA-C7000, IKA-analysentechnik, Weitersheim, Germany). Ash was determined by burning the oven-dried samples in a muffle furnace at 550 °C (ISO, 1978). Acid insoluble ash (AIA) was determined by treating the residue obtained after ash determination with hydrochloric acid. This mixture was then filtered to obtain the insoluble residue (ISO, 1981).

Calculations

Feed intake of the fish expressed per metabolic weight unit (FI_{MBW}) was calculated as FI_{MBW} (g/kg$^{0.8}$ per day) = FI / (W_{mean} / 1000)$^{0.8}$, where FI (g/d) is the average feed intake per fish per day and W_{mean} is the geometric mean body weight, which was calculated as W_{mean} (g)= $\sqrt{}(W_i*W_f)$, where W_i and W_f are the initial and final average individual fish weight (g).

Specific growth rates (SGR) were calculated as SGR (%/d) = $[(\ln W_f - \ln W_i) / t] \times 100$, where t is the experimental duration (days). Feed conversion ratio (FCR) was calculated as FCR = $FI_{tot} / (W_f - W_i)$, where FI_{tot} (g) is the total average feed intake per fish during the experimental period.

Apparent digestibility coefficients of nitrogen and energy in the diets were calculated as $ADC_X = (1 - AIA_{diet} / AIA_{faeces} \times X_{faeces} / X_{diet}) \times 100$, where X represents nitrogen or energy, AIA_{diet} and AIA_{faeces} are the AIA content (% dry matter) in the diet and faeces, respectively and X_{diet} and X_{faeces} are the quantity of X in 1 g dry matter of the diet and faeces, respectively.

Total digestible nitrogen (DN; mg/fish) was calculated as the product of total gross nitrogen intake (GN; mg/ fish) and ADC of nitrogen (in %), where GN was calculated as the product of total feed intake (g/fish) and nitrogen content of the diets (mg/g). Total faecal nitrogen losses (FN; mg/fish) were calculated as the difference between GN and DN. Total retained nitrogen (RN; mg/fish) was calculated as the difference between the final and initial nitrogen mass (mg/fish). Total branchial and urinary nitrogen losses (BUN; mg/fish) were calculated as the difference between DN and RN. The above-calculated values of GN, FN, DN, BUN and RN were divided by $[t \times (W_{mean} / 1000)^{0.8}]$ to be expressed in mg/kg$^{0.8}$ per day.

Total digestible energy (DE; kJ/fish) was calculated as the product of total gross energy intake (GE; kJ/fish) and ADC of energy (in %), where GE was calculated as the product of total feed intake (g/fish) and energy content of the diets (kJ/g). Total faecal energy losses (FE; kJ/fish) were calculated as the difference between GE and DE. Total metabolizable energy (ME; kJ/fish) was calculated as the difference between DE and the energy in the total branchial and urinary excretory products (BUE; kJ/fish), which was estimated as BUE = (BUN × 24.9) / 1000, where 24.9 is the amount of kJ equivalent to 1 g excreted nitrogen, assuming that all nitrogen is excreted as NH_3–N (Bureau et al., 2002). Total retained energy (RE; kJ/fish) was calculated as the difference between the final and initial energy quantities (kJ/fish). Total heat production (HP; kJ/fish) was calculated as the difference between ME and RE. The above-calculated values of GE, FE, DE, BUE, ME, HP and RE were divided by $[t \times (W_{mean} / 1000)^{0.8}]$ to be expressed in kJ kg$^{0.8}$/d.

Statistical Analyses

All calculations were made using the SAS System (SAS, 1999). Behavioural consistency in escape attempts and moving time in the escape test was determined by the Spearman correlation test, with individual fish as the experimental unit. Effects of coping type and stocking density on growth performance were analysed using PROC GLM, with individual tanks as the experimental unit. Error terms of the statistical model were tested for homogeneity of variances and normal distribution, using respectively the Levene's test and the Shapiro-Wilk test. Due to significant non-normality, effects of coping type and stocking density were analysed using a Kruskal-Wallis ANOVA followed by pair wise comparisons by the Mann-Whitney U-test. Results are considered statistically significant when P-values are below 0.05; P-values between 0.05 and 0.10 are considered

Table 4. Main effects of group composition and stocking density on growth performance of African catfish.

	Experimental treatment						Stocking density (kg/m^3)				
	Proactive	Mixed	Reactive	SEM	F(2,9)	P	100	500	SEM	F(1,9)	P
FI$_{MBW}$ (g/kg$^{0.8}$/d)	17.3^a	17.3^a	15.4^b	0.462	5.62	0.0260	16.2	17.2	0.364	3.66	0.0880
SGR (%/d)	3.0^a	3.0^a	2.6^b	0.085	6.87	0.0155	2.9	2.9	0.067	0.00	0.9454
FCR (g/g)	0.66^a	0.68^{ab}	0.71^b	0.007	9.40	0.0062	0.66	0.71	0.006	31.01	0.0003

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

Table 5. Main effects of group composition on energy partitioning in African catfish.

	Experimental treatment					
	Proactive	Mixed	Reactive	SEM	F(2,9)	P
ADC Energy (%)	87.8	88.2	88.4	0.277	1.15	0.3593
GE (kJ/kg$^{0.8}$/d)	354^a (100%)	354^a (100%)	315^b (100%)	9.430	5.62	0.0260
FE (kJ/kg$^{0.8}$/d)	43.2^a (12.2%)	41.9^{ab} (11.8%)	36.7^b (11.7%)	1.350	6.48	0.0180
DE (kJ/kg$^{0.8}$/d)	310^{ab} (87.8%)	312^a (88.2%)	278^b (88.4%)	8.555	4.94	0.0357
BUE (kJ/kg$^{0.8}$/d)	15.6 (4.4%)	15.5 (4.4%)	14.6 (4.6%)	0.366	2.38	0.1477
ME (kJ/kg$^{0.8}$/d)	295^{ab} (83.4%)	296^a (83.8%)	263^b (83.7%)	8.273	4.97	0.0351
HP (kJ/kg$^{0.8}$/d)	103 (29.3%)	96 (27.2%)	92 (29.3%)	5.293	1.13	0.3654
RE (kJ/kg$^{0.8}$/d)	191^{ab} (54.1%)	200^a (56.6%)	171^b (54.4%)	6.711	4.85	0.0372
Maintenance (kJ/kg$^{0.8}$/d)	55.6 (15.7%)	46.2 (13.1%)	49.4 (15.7%)	5.655	0.71	0.5163

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

( ) Energy balance expressed as a percentage of GE.

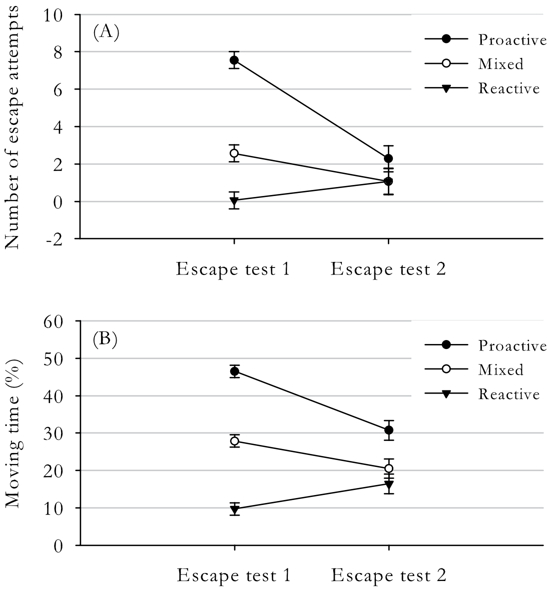

Figure 1. Mean number of escapes (A) and moving time (B) of African catfish housed in proactive, mixed, and reactive groups at escape test 1 and 2. Group composition significantly affected the changes in escape attempts (1A; F(2,9)=33.32, P<0.0001) and moving time (1B; F(2,9)=14.21, P=0.0016) between the first and second escape test.

trends.

RESULTS

ESCAPE TEST

During the first escape test, African catfish showed on average a moving activity of 27.7 ± 1.32 % and an average number of escape attempts of 3.1 ± 0.26. During the second escape test, African catfish showed on average a moving activity of 21.3 ± 0.93 % and an average number of escape attempts of 1.1 ± 0.17. This resulted in correlation coefficients of r_s=0.36 (n=288, P<0.0001) for moving activity and r_s=0.31 (n=288, P<0.0001) for escape attempts.

Although in general the behavioural response to the second escape test was characterised by a lower overall activity, the changes in behavioural response of fish in between both escape test were also significantly affected by the group composition the fish were previously housed in. Fish housed in proactive or mixed groups showed an average decrease in number of escape attempts of 5.3 and 1.5 attempts, respectively (Figure 1A). In contrast, fish housed in reactive groups on average showed 1.0 more escape attempt in the second escape test (F(2,9)=33.32, P<0.0001). A similar effect of group composition was found for moving activity (Figure 1B; F(2,9)=14.21, P=0.0016). Fish housed in proactive and mixed groups lowered their moving activity by 15.8% and

Table 6. Main effects of stocking density on energy partitioning in African catfish.

	Experimental treatment				
	100	500	SEM	F(2,9)	P
ADC Energy (%)	88.8	87.4	0.218	17.49	0.0024
GE (kJ/kg$^{0.8}$/d)	330 (100%)	351 (100%)	7.421	3.66	0.0880
FE (kJ/kg$^{0.8}$/d)	37.0 (11.2%)	44.1 (12.6%)	1.062	20.93	0.0013
DE (kJ/kg$^{0.8}$/d)	293 (88.8%)	307 (87.4%)	6.732	1.92	0.1998
BUE (kJ/kg$^{0.8}$/d)	14.3 (4.3%)	16.3 (4.6%)	0.288	23.23	0.0009
ME (kJ/kg$^{0.8}$/d)	279 (84.5%)	291 (82.8%)	6.510	1.49	0.2532
HP (kJ/kg$^{0.8}$/d)	97 (29.5%)	97 (27.7%)	4.165	0.00	0.9793
RE (kJ/kg$^{0.8}$/d)	182 (55.0%)	193 (55.1%)	5.281	2.33	0.1613
Maintenance (kJ/kg$^{0.8}$/d)	51.9 (15.7%)	48.8 (13.9%)	4.450	0.23	0.6442

( ) Energy balance expressed as a percentage of GE.

Table 7. Effects of group composition and stocking density on the behaviour of African catfish.

	Experimental treatment						Stocking density (kg/m^3)				
	Proactive	Mixed	Reactive	SEM	$\chi^2(2)$	P	100	500	SEM	$\chi^2(1)$	P
Laesions (#/fish)	1.6	1.2	1.2	0.533	F(2,9)=0.17	0.8426	1.6	1.1	0.420	F(1,9)=0.72	0.4188
Aggression (#/fish/hr)	16.5	8.0	11.5	5.503	0.88	0.6444	20.0	4.0	4.773	6.56	0.0104
Air Breathing (#/fish/hr)	36.3	43.0	38.0	4.146	1.49	0.4755	40.7	37.5	3.487	0.17	0.6767
Swimming Time (%)	58.1	65.8	73.2	4.878	2.24	0.3263	59.8	71.5	4.713	3.56	0.0593
Resting Time (%)	42.0	34.2	26.9	4.878	2.24	0.3263	40.2	28.5	4.713	3.56	0.0593
Escapes (#/fish/hr)	0.26	0.09	0.10	0.123	0.59	0.7447	0.06	0.24	0.100	0.22	0.6377
Latency Feeding (s)	0.13	0.10	0.07	0.093	0.45	0.7990	0.20	0.00	0.076	4.45	0.0348
TFT (s)	200^a	234^b	175^a	10.791	8.54	0.0140	198	209	8.767	0.50	0.4795
TFS (g/s)	0.89	0.84	1.00	0.051	0.26	0.8781	0.34	1.45	0.055	10.13	0.0015

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

7.4%, while fish housed in reactive groups increased their activity by 6.7% in the second escape test. Stocking density did not affect the behavioural response of African catfish in both escape tests.

Group housing - performance

The effects of group composition and stocking density on growth performance of African catfish are presented in Table 4. Group composition affected feed intake (F(2,9)=5.62, P=0.0260), SGR (F(2,9)=6.87, P=0.0155), as well as FCR (F(2,9)=9.40, P=0.0062). Re-active groups (comprised of 100% reactive fish) had a 15% lower SGR, a 12% lower feed intake and a 4% higher FCR than pro-active groups. Stocking density only had an effect on the FCR (F(1,9)=31.01, P=0.0003), with fish housed at high density showing a 8% higher FCR than fish housed at low density. None of the measured parameters were influenced by the interaction between group composition and stocking density (P<0.05).

Group housing - energy partitioning

Table 5 present the effects of group composition on energy partitioning of African catfish. Reactive groups had a lower energy intake, measured as GE (F(2,9)=5.62, P=0.0260). The lower energy intake of reactive fish also resulted in a lower FE (F(2,9)=6.48, P=0.0180), DE (F(2,9)=4.94, P=0.0357), ME (F(2,9)=4.97, P=0.0351), and RE (F(2,9)=4.85, P=0.0372). However, expressing FE, DE, ME, and RE as a percentage of GE indicated that partitioning of energy was not affected by group composition. The differences in energy balance between the three group compositions were fully due to differences in feed intake. Table 6 present the effects of stocking density on energy partitioning of African catfish. Fish housed at high density showed a higher FE (F(1,9)=20.93, P=0.0013) and BUE (F(1,9)=23.23, P=0.0009) than fish housed at low density. Furthermore, the ADC of energy was higher for fish housed at low density (F(1,9)=17.49, P=0.0024). None of the measured parameters was influenced by the interaction effect.

Group housing - behaviour

The effects of group composition and stocking density on the behaviour of African catfish are presented in Table 7. Group composition only affected TFT, with mixed groups having a higher TFT than proactive and reactive groups ($\chi^2(2)=8.54$, P=0.0140). Stocking density also affected the behaviour of African catfish. Fish housed at high density showed lower aggression levels ($\chi^2(1)=6.56$, P=0.0104), a lower latency of feeding ($\chi^2(1)=4.45$, P=0.0348) and a higher TFS ($\chi^2(1)=10.13$, P=0.0015). Furthermore, a trend for higher swimming activity at higher density was present ($\chi^2(1)=3.56$, P=0.0593).

DISCUSSION

ESCAPE TEST

Correlations in escape attempts and moving activity were found between the two escape tests at different ages. This suggest that the individual differences in behavioural response in African catfish are at least partly caused by stable biological traits. A previous study corroborated with these results, showing consistent behavioural responses across time and situation (Van de Nieuwegiessen et al., unpublished results). This corresponds to other studies demonstrating fairly consistent behavioural traits in fish. Francis (1990) demonstrated distinct and stable behavioural profiles in Midas cichlids, *Amphilophus citrinellus*. Budaev (1997; 1999) indicated consistent individual differences in several behavioural patterns in the guppy, *Poecilia reticulata* (r 0.3-0.8) and lion-headed cichlid, *Steatocranus casuarius*. Studies on adult *Gasterosteus aculeatus* showed consistency in bold or shy behaviour (r 0.37 - 0.73 (Ward et al., 2004).

Although in the current study we see, to a certain extent, behavioural consistency towards an escape test, correlation coefficients were substantially lower than 1 (r_s=0.31/0.36), implying that other factors also affected the behavioural responses. One factor affecting the correlation coefficient is the behavioural change of African catfish fish during the escape tests. Not only was the behavioural response to the second escape test in general characterised by a lower overall activity, the behavioural response of fish in the second escape test was also affected by the group composition the fish were previously housed in. While fish housed in mixed or proactive groups showed a decrease in swimming activity and number of escape attempts, fish housed in reactive groups showed an increase. This suggests that animals can to a certain extent change their behavioural phenotype. These findings corroborate with those found in a study on the effects of group composition on the behavioural response of piglets to a backtest (Van Erp-Van der Kooij et al., 2003). Mean backtest results for piglets housed in HR pens (HR: high responders, suggested to represent a proactive coping strategy) decreased in time. In contrast, mean backtest results for piglets housed in LR pens (LR: low responders, suggested to represent a reactive coping strategy) increased in time. A possible explanation for the behavioural change could be that behavioural variation is beneficial for the group. Hessing (1994) stated that the complementary individual behavioural characteristics of the active and the reactive pigs under stress will result in a better socially integrated group. A socially well integrated group will be more successful in solving their problems, which is beneficial for each individual and for the group as a whole. How exactly fish benefit from increased behavioural variation in a group is unclear and requires further study.

Following Hessing's hypothesis, one would expect that fish housed in both proactive and reactive groups would show a low behavioural consistency because a number of animals would have changed their behavioural phenotype in order to come to a better socially integrated group. In contrast, fish housed in a mixed group would show an above average behavioural consistency, since no shifting of behavioural phenotype should be required. To a certain extent the results in the current study back Hessing's hypothesis. Animals housed in proactive and reactive groups do indeed show a below average correlation coefficient for escape attempts (r_s=0.18, N=91, P=0.09 and r_s=0.16, N=99, P=0.13, respectively) and moving time (r_s=0.21, N=91, P=0.05 and r_s=-0.10, N=99, P=0.34, respectively). However, although fish housed in mixed groups show higher correlation coefficients for escape attempts and moving time than fish housed in proactive and reactive groups do, these correlations are not far above average (r_s=0.30, N=98, P=0.003 and r_s=0.30, N=98, P=0.003, respectively). As a result, other unknown factors must also have affected the behavioural response to our escape tests.

Group housing

In the current study, two factors, stocking density and group housing, affected the physical and/or behavioural responses of African catfish. Fish housed at high density responded with an increased FCR, TFS, and activity and with lower aggression and a lower latency of feeding. This is in accordance with previous studies which show an increase in activity and/or decrease of aggression at higher densities (Appelbaum & Kamler, 2000; Hecht & Appelbaum, 1988; Hecht & Uys, 1997; Kaiser et al., 1995b; Van de Nieuwegiessen et al., 2008b). It is suggested that the formation of dense shoals interferes with the cues for initiation of agonistic behaviour (Hecht & Uys, 1997).

Group composition strongly affected the growth performance of African catfish, which was fully due to differences in feed intake. Reactive animals showed a 19% lower feed intake than proactive copers, which is in accordance with previous work in rainbow trout. Øverli et al. (2005) observed a significantly higher incidence of feed intake in LR trout (suggested to represent the reactive coping strategy) when held in observation tanks (40% versus 0% of the fish took food when in isolation), suggesting that these fish acclimated more successfully to the experimental conditions than HR fish (suggested to represent the proactive coping style) did. Similarly, a study in African catfish gave indications that animals suggested to represent the reactive coping strategy took more time to resume feed intake after transfer to a novel environment than proactive animals did (Van de Nieuwegiessen et al., 2008a).

While these results indicate that both stocking density and group composition affect physical and behavioural responses of African catfish, it does not directly answer the main question. What are the consequences of coping strategies for the fitness of different behavioural phenotypes in intensive husbandry systems? The current study showed that reactive copers have a lower growth performance when housed with other reactive copers, however this does not necessarily mean that reactive copers have a lower growth performance in intensive husbandry systems where they are housed in groups with a larger variation in behavioural phenotypes. To answer our main question we should focus more closely on behavioural and physical responses of individual fish housed in mixed groups in this study. Unfortunately, due to technical limitations, it is currently not possible to collect individual behavioural data on large groups of fish. Collecting data on individual feed intake of group-housed African catfish, using radiography, has been attempted in the past (unpublished results). Due to regurgitation of food while handling the fish, this method could not be used. Therefore, we could only use SGR

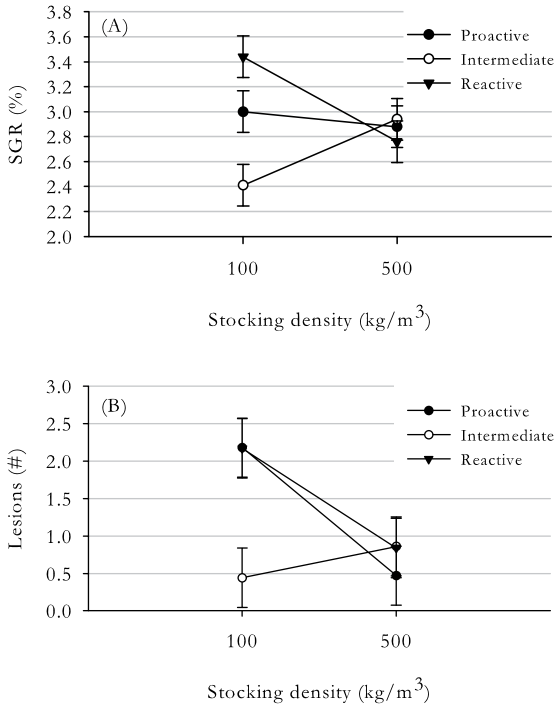

Figure 2. Mean SGR (A) and number of skin lesions (B) for proactive, intermediate, and reactive African catfish housed in mixed groups. An interaction effect between coping strategy and stocking density was present for SGR (2A; F(2,98)=6.04, P=0.0034) and amount of lesions (2B; F(2,98)=4.36, P=0.0153).

and the amount of skin lesions to assess the effects of coping strategies for the fitness of different behavioural phenotypes (Figure 2). Figure 2 indicates an interaction between coping strategy and stocking density. While at low density SGR appears to be affected by coping strategy, at high density this does not seem to be the case (Figure 2A; F(2,98)=6.04, P=0.0034). For the number of lesions a similar interaction is present (Figure 2B; F(2,98)=4.36, P=0.0153). In contrast to predictions of Huntingford and Adams (2005), the fitness of African catfish does not seem to be affected by different behavioural phenotypes at high stocking density, but at low density. Previous studies have demonstrated that in African catfish low rather than high stocking densities affect various aspects of their welfare, reflected by decreased growth performance and increased aggression at decreasing densities (Almazán Rueda, 2004; Hecht & Appelbaum, 1988; Hecht & Uys, 1997; Kaiser et al., 1995a, b; Van de Nieuwegiessen et al., 2008b). In other fish species, coping strategies may more profoundly impact the fitness of fish housed at high stocking density. More study in this field is required. In addition, Figure 2 also suggest that both proactive and reactive fish are actively competing for food (i.e. higher SGR and higher amount of skin lesions), while intermediate fish seem to avoid the competition (i.e. lower SGR and lower amounts of skin lesions) and consequently gain less food. Remarkably, while reactive copers show the lowest growth performance when housed in groups fully comprised of reactive fish, this is no longer the case when housed in mixed groups. At high density reactive copers in mixed groups show a comparable SGR to intermediate and proactive copers. At low density reactive copers in mixed groups even show the best growth performance. Apparently the presence of intermediate/proactive copers stimulates the feeding motivation of reactive copers. Behavioural observations on individual fish are necessary to get a better insight into the (feeding) behaviour of different behavioural phenotypes in mixed groups.

In conclusion, this study has shown that both stocking density and group composition affect physical and/or behavioural responses of African catfish. Furthermore, physical and behavioural data of individual fish housed in mixed groups suggests that coping strategy affects the fitness of different behavioural phenotypes at low, but not at high stocking density.

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7

GENERAL DISCUSSION

The general aim of this thesis was to determine the impact of stocking density on welfare indicators in African catfish cultured in a recirculating aquaculture system (RAS). The following factors were studied:

1) The effects of stocking density on physical, physiological, and behavioural responses of African catfish (Chapter 2 and 3);

2) How age mediates the effects of stocking density on physical, physiological, and behavioural responses of African catfish (Chapter 3);

3) The potential effects of chemical alarm cues on the welfare of farmed African catfish (Chapter 4-6);

4) The fitness consequences of different coping strategies in intensive husbandry systems (Chapter 7);

RELATION BETWEEN WELFARE AND STOCKING DENSITY

The intensive farming of fish inevitably involves holding animals at stocking densities that are greater than they normally experience in the wild. This applies even more so for the densities used in recirculating aquaculture systems. Because of this, public, scientific, and governmental attention to fish has focused on stocking density as a key factor that may impair the welfare of fish in intensive husbandry systems. In 1996, the Farm Animal Welfare Council identified stocking density as an area where scientific research was necessary to provide information upon which to base welfare guidelines and legislation (Anonymous, 1996). Unlike terrestrial farm animals, for which minimum stocking densities are generally set to provide for an animal's welfare needs there are currently no regulations regarding the densities at which fish can be farmed.

Chapter 2 and 3 of the current study showed that in African catfish stocking density influenced the welfare of African catfish. Strongly generalised, juvenile African catfish (10-300 gram) responded to increasing density with the formation of dense clusters of fish with constant movement and low aggression. This is in accordance with other studies in African catfish showing an increase in activity and/or decrease of aggression at higher densities (Almazán Rueda, 2004; Appelbaum & Kamler, 2000; Hecht & Appelbaum, 1988; Hecht & Uys, 1997; Kaiser et al., 1995a). It is suggested that the formation of dense shoals interferes with the cues for initiation of agonistic behaviour (Hecht & Uys, 1997).

Although in general our results corroborate with those of earlier studies, an interesting new finding of the current study is that the effects of stocking density on the behaviour of African catfish seem to be strongly time-dependent. The most noticeable effects of stocking density were found in the first weeks of the experiments. For example, Chapter 2 showed that agonistic behaviour was most prominent in the first four weeks of the experiment (Table 3). After four weeks, no relation between stocking density

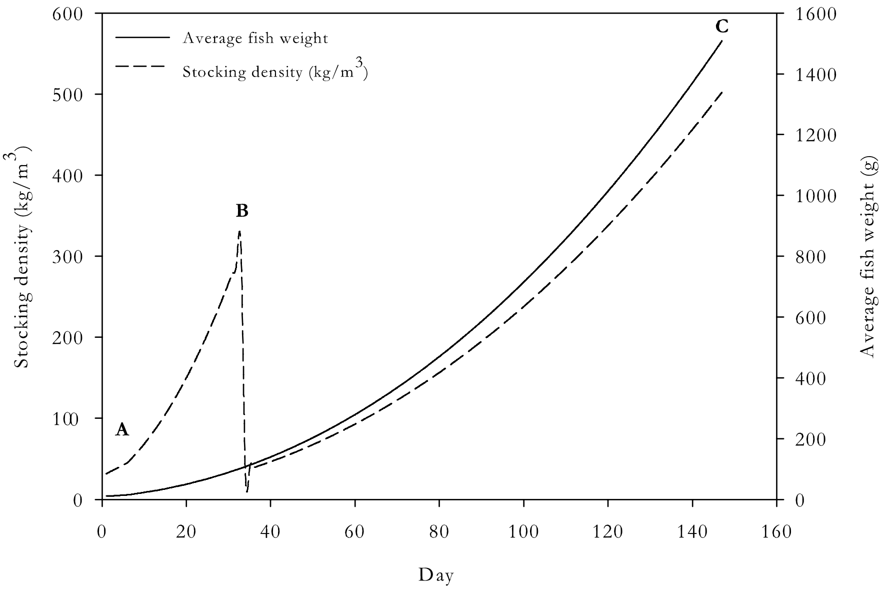

Figure 1. The stocking density experienced by African catfish during a production cycle: (A) fish stocking; (B) first grading reducing stocking density; (C) harvest.

and agonistic behaviour was found. Fish in growth ranges of 100-300 and 1000-1500 gram only showed agonistic behaviour in the first two weeks of the experiment (data not presented). Similar results were obtained for parameters as swimming activity and escape behaviour. Apparently, the effects of stocking density are not static within time. What could have caused these time-dependent changes? Two possible mechanisms are proposed that might have affected the effects of stocking density on the behavioural responses of African catfish. First, it should be realised that stocking density is far from a static factor, both fish growth and grading have a strong effect on the concentration of fish within a tank (Figure 1). It is clear that how fish experience a certain stocking density may change dramatically over the production cycle. In our experiments, the increase in stocking density through fish growth might have caused a suppression of agonistic behaviour over time. Secondly, the behavioural effects of stocking density might depend on other aspects of husbandry practice. At the start of the experiment fish most likely experience increased levels of acute stress, due to a new environment and a new group composition. We suggest that the acute stress experienced by the fish in these first weeks of the experiment modulates the effects of stocking density by triggering aggression. A positive feedback mechanism between stress and aggression has been previously shown in for instance humans and rodents (Guerra et al., 1995; Hayden-Hixson & Ferris, 1991; Kruk et al., 2004; Sanson et al., 1993).

The experiments discussed in Chapter 2 and 3 pinpointed another factor that modulates the effects of stocking density. Chapter 2 showed low levels of aggression at high stocking densities. However, a challenge test (netting stress) gave additional and crucial information about the basic welfare situation. When this acute stressor (netting stress) was applied, a strong increase in agonistic behaviour, especially in fish housed at high density, occurred (Chapter 2, Figure 4). The exact reason for the increased aggression at high densities is unclear and needs further study. It is possible that the crowding stress at high density suppressed agonistic behaviour even though the welfare of the animals was impaired. An additional stressor combined with less crowding after the nestressor may have just been the trigger to reveal this situation of impaired welfare. However, we cannot exclude an effect of the relative changes in stocking density going from the normal group housing to the 1-hour group housing after net-stress. For fish housed at 3000 fish/m^3 the density dropped with a factor 9, while for fish housed at 500 fish/m^3 this was only a factor 1.5. Whatever the exact reason for the density-dependent increase in agonistic behaviour may be, an important conclusion to be drawn from these results is that there exists an interaction effect between stocking density and acute additional stress. Since additional stressors (e.g. handling, grading) are not uncommon in a farming situation, this means that the total farming situation may influence the effects of stocking density.

In conclusion, stocking density is a prime example of an area that demonstrates the significance of taking into account the existence of a complex web of interacting factors that affect welfare. Our experiments indicated that acute stressors may act as such possible factors. Two other factors, fish age and coping strategies will be discussed below.

EFFECTS OF AGE

Differences in the effects of stocking density among fish of the same species can be pronounced. For instance, in halibut (*Hippoglossus hippoglossus*), a non-social flatfish that spends most of its time resting on the sea floor, tolerance for high stocking density appears to be life stage dependent. Small juvenile halibut had a better growth performance and suffered fewer injuries from aggressive encounters at high densities (Greaves & Tuene, 2001). In contrast, adult halibut showed abnormal activity, reduced food consumption, and reduced growth rates at increasing stocking density (Kristiansen et al., 2004).

Chapter 2 and 3 of the current study gave indications that the effects of stocking density in African catfish are also life stage dependent. Chapter 2 indicated that for African catfish of 10-100 gram, both high and low densities had detrimental effects on fish welfare. At low densities we observed increased aggression and skin lesions, while an acute stressor (netting stress) resulted in increased aggression in fish housed at high density. Chapter 3 showed that for African catfish of 100-300 gram, increasing density led to an increase in both activity and SGR. Plasma cortisol and agonistic behaviour decreased at increasing density, suggesting an improved welfare at increasing stocking density. For catfish of 1000-1500 no effects of stocking density on a range of welfare indicators was shown.

Taking together all available literature data on the effects of stocking density in African catfish a similar life stage-dependency can be deduced. African catfish larvae have shown negative effects of increasing density, reflected by decreased growth performance and increased cannibalism (Haylor, 1991, 1993; Hecht & Appelbaum, 1988; Hossain et al., 1998). In contrast, studies of early juveniles showed a positive effect of increasing density, reflected by increased growth performance and decreased aggression (Almazán Rueda, 2004; Hecht & Appelbaum, 1988; Hecht & Uys, 1997; Kaiser et al., 1995a, b). The current study indicated that such life stage dependent changes do not only occur at young (larval/ early juveniles) life stages of African catfish, but also occur at later life stages.

What exactly causes the life stage dependent effects of stocking density in the African catfish is unclear. A possible explanation may lie in the major changes many fish undergo in morphology, behaviour and environmental requirements through their life history. For example, Atlantic salmon transform from freshwater parr (territorial, aggressive fish) to smolts (nonaggressive fish) and then to ocean-going adult salmon (non-territorial, shoaling fish). Unfortunately, little is known about such changes in natural behaviour of African catfish. The little information that is available suggests that African catfish change from highly cannibalistic, aggressive fry (Haylor, 1991, 1993; Hecht & Appelbaum, 1988; Hossain et al., 1998) to more social, shoaling, and packhunting adults (Bruton, 1979; Merron, 1993). In our own experiments we observed that both swimming activity and agonistic behaviour (assessed by the amount of skin lesions) declined at increasing age. These results suggest that with age African catfish become less active and aggressive, which results in a decreased influence of stocking density. Further research should elucidate exactly which factors cause these behavioural changes.

These results demonstrate the importance of taking into account that the effects of stocking density strongly depend of the life stage of an animal. If recommendations on a suitable stocking density are to be made, these recommendations should be adjusted according to the life stage of the animal.

EFFECTS OF COPING STRATEGIES

To make matters even more complex, fish of the same species and age are not all identical, often showing physiological and behavioural variation in the way they cope with certain stressors. Although such individual differences are common, these differences have for long been interpreted as the result of inaccurate measurements or as non-adaptive variation around an adaptive mean (Groothuis & Carere, 2005). At present, it is increasingly more recognised that these individual differences are caused by stable biological traits instead of having a situation-specific origin. In literature, these consistent differences between individuals have been referred to as differences in personality, temperament, coping styles/ strategies, boldness, neophobia, or behavioural syndromes/ profiles (Gosling & John, 1999; Greenberg & Mettke-Hofmann, 2001; Groothuis & Carere, 2005; Koolhaas et al., 1999; Sih et al., 2004; Wilson et al., 1994).

Although debatable, individual differences tend to be categorised into two opposite strategies, a proactive and reactive strategy, both aimed at successful control over the environment and other individuals (Koolhaas et al., 1999). Important features of the proactive response are territorial control, aggression, routine formation, low hypothalamus-pituitary-adrenal (HPA) axis activity, and high sympathetic reactivity (Benus et al., 1989; Benus et al., 1990; Driscoll et al., 1990; Fokkema et al., 1995; Ruis et al., 2000; Schouten & Wiegant, 1997; Van Oortmerssen & Bakker, 1981). Immobility, low levels of aggression, high levels of behavioural flexibility, high HPA-reactivity, and low sympathetic reactivity characterize the reactive response. Such individual variation often appears to be inherited and variation in selection regimes results in different phenotypes performing best in different conditions (Dingemanse et al., 2004). The consequences of these coping strategies for the fitness of the different behavioural phenotypes in intensive husbandry systems are yet unclear. Huntingford and Adams (2005) suggested that that domestication of Atlantic salmon (especially when accompanied by selection for fast growth in the farm environment) has resulted in the simultaneous selection for high aggression and risk taking (i.e. proactive fish). This in turn suggests that risk-avoiding fish (representing a reactive coping strategy) fare poorly in intensive husbandry systems. This may be due to a lower competitive ability of risk-avoiding fish when housed at high densities and with a predictable food source, as is usually the case in these husbandry systems.

The large individual variation in behavioural responses to chemical alarm cues found in Chapter 4 of this study gives an indication for the existence of coping strategies in African catfish. Chapter 4 showed a large individual variation in the response to conspecific skin extract; changes in percentage of time spent swimming in response to conspecific skin extract ranged from -68.4% to 85.1%. Such differences in activity levels suggest differences in coping strategies. Chapter 4 also showed that the large individual variation in the response to conspecific skin extract was related to feed efficiency. While feed-efficient animals decreased their activity in response to conspecific skin extract, feed-inefficient animals responded to conspecific skin extract with an increase in time spent swimming. Martins et al. (2006) suggested that variation in feed efficiency (measured as residual feed intake, RFI) in African catfish may be related to differences in coping strategies. These results suggest that the behavioural response of feed-inefficient animals to conspecific skin extract is more dominated by a pro-active coping strategy than that of feed-efficient animals.

Additional personal observations, which still need to be confirmed, suggested that these opposing behavioural strategies could be threat-sensitive. At high concentration of conspecific skin extract, fish with a lower feed-efficiency seemed to show a more active, foraging response while more feed-efficient animals seemed to shift to a more passive (anti-predator) response. Such a difference in behavioural response could no be observed at lower alarm cue concentration, suggesting there might be a dependence on the level of risk. As mentioned, these observations still need to be confirmed. Although consistent variation in risk-taking behaviour of fish has been previously shown in other studies (e.g. Huntingford, 1976), the possible link between coping strategies and feed efficiency is intriguing.

The exact relation between feed efficiency and the behavioural response of African catfish to chemical alarm cues remains unclear. Previous studies have shown a link between variation in RFI and variation in physical activity. Luiting et al. (1991) observed a higher activity related heat production in a high RFI (low efficiency) laying hen line than in a low RFI line (high efficiency). Other studies supported these results: inefficient laying hens were more active than efficient laying hens (Braastad & Katle, 1989; Morrison & Leeson, 1978). Similar results were found in pigs: efficient pigs visited a feed hopper less often and spent less time eating per day than inefficient pigs (de Haer et al., 1993). Based on Beilharz's Resource Allocation Theory (1993), Rauw et al. (2000) proposed a biological explanation to the relation between RFI and activity levels. They suggested that animals with higher RFI have more "buffer" resources left for e.g. physical activity and the ability to cope with unexpected stresses. In the current study, fish exhibiting a higher RFI may have had more energy available to spent on fight-flight behaviour, and may therefore have been able to show a more active behavioural response to chemical alarm cues. However, very little information is available regarding individual variation in RFI of fish. Therefore, future research should further investigate the biological basis for variation in residual feed intake in fish to elucidate the consequences residual feed intake might pose on variation in behavioural and/or physiological strategies.

Although Chapter 4 gave indications for the existence of coping strategies in African catfish, no evidence was given for a consistent behavioural variation in the way these fish cope with certain stressors. Chapter 6 did provide evidence for such a consistent behavioural variation. In Chapter 6, animals were confronted twice with an escape test to determine the coping characteristics of each fish. This resulted in correlation coefficients of r_s=0.36 for moving activity and r_s=0.31 for the number of escape attempts. A previous study in which the escape test was used to determine coping characteristics of African catfish showed comparable correlation coefficients (Van de Nieuwegiessen, unpublished results). This corresponds to other studies demonstrating fairly consistent behavioural traits in fish. Francis (1990) demonstrated distinct and stable behavioural profiles in Midas cichlids, Amphilophus citrinellus. Budaev (1997, 1999) indicated consistent individual differences in several behavioural patterns in the guppy, *Poecilia reticulata* (r 0.3-0.8) and lion-headed cichlid, *Steatocranus casuarius*. Studies on adult *Gasterosteus aculeatus* showed consistency in bold or shy behaviour (r 0.37 - 0.73; Ward et al., 2004).

However, further analysis of data in Chapter 6 indicated that behavioural responses to the escape tests changed according to the group composition the animals were housed in. In proactive groups, average moving activity and average number of escape attempts decreased while in reactive groups average moving activity and average number of escape attempts increased. In both groups moving activity and the number of escape attempts in the escape tests were not correlated. In mixed groups, both average moving activity and average number of escape attempts in both escape tests were correlated. These findings corroborate with those found in a study on the effects of group composition on the behavioural response of piglets to a backtest (Van Erp-Van der Kooij et al., 2003). This suggests that in some circumstances animals can, to some extent, change their behavioural coping style. A possible explanation for the behavioural change could be that behavioural variation is beneficial for the group. Hessing (1994b) stated that the complementary individual behavioural characteristics of active and reactive pigs under stress will result in a better socially integrated group. A socially well integrated group will be more successful in solving their problems, which is beneficial for each individual and for the group as a whole. The fact that in our study, group composition only changed the behavioural responses of proactive and reactive groups, and not of mixed groups, favours this explanation. We conclude that the response of African catfish to an escape test is a fairly consistent trait in a socially stable environment, but that African catfish can change their behaviour if environmental conditions require so.

The next question coming to mind is if and how the welfare of different behavioural phenotypes is differently affected in intensive husbandry systems? Both coping strategies may be equally successful in coping with stress, but the relative success of a coping strategy may be context dependent (the match between individual coping characteristics and the actual environmental conditions; Benus et al., 1991; Van Oortmerssen et al., 1985). The higher tendency of active copers to develop routines suggests that this strategy may be more advantageous under the stable conditions provided by intensive husbandry systems (Benus et al., 1991; Koolhaas et al., 1999; Van Oortmerssen et al., 1985). In fish, the possible fitness consequences of different coping strategies in intensive husbandry systems is a largely untouched subject. Studies in other farmed animal species give little indications that either a proactive or reactive coping strategy is more beneficial for the animals than the other. Proactive pigs seem to be better performers in mixed groups. However, selection for proactive animals is complicated by the fact that these animals also display higher levels of aggression (Hessing et al., 1994a). Do fish at the risk avoiding end of the spectrum (i.e. reactive fish) gain less food at high densities, as Huntingford and Adams (2005) suggested? Chapter 6 showed an impaired growth performance of reactive fish when housed with other reactive fish, but no such effects are seen in mixed populations, which is how these fish are housed in a farming situation. The impaired growth performance therefore seems to be more dependent on group composition than on coping strategy itself. Based on these results, coping strategies seem to be only weakly associated with the welfare of African catfish. We should however realize that the conditions under which this experiment was performed were relatively stress free. In a study on pigs, Geverink and co-workers (2002) found no effects of coping strategy on group performance under stable conditions. However, during the first week following relocation or relocation plus splitting up groups, proactive copers displayed a higher weight gain than reactive copers (Geverink et al., 2004). Therefore, if stressful conditions may arise in a farming situation, for example due to inappropriate water quality conditions, impaired health status, bad stockmanship, etc, the effects of coping strategy may become more pronounced. Therefore, further studies on the effects of coping strategies under more stressful conditions are required.

CHEMICAL ALARM CUES

Chapter 4 of the current study has indicated the presence of the Ostariophysan chemical alarm cue in African catfish, adding a new member to a steadily growing list of fish species possessing a chemical alarm signaling system. While feed-efficient animals decreased their activity in response to conspecific skin extract, feed-inefficient animals responded to conspecific skin extract with an increase in time spent swimming.

A range of situation-specific factors have been shown to influence a threat sensitive trade-off between foraging and anti-predator behaviour. Brown et al. (2001) proposed the following non-mutually exclusive factors which may influence this threat-sensitive trade-off; (1) the form and frequency of predation pressure, (2) the degree of habitat complexity, (3) allometric relationships (e.g. condition factor), and (4) the individual's feeding motivation (i.e. hunger level). The results from Chapter 4 and some, yet unconfirmed, observations from later experiments, where individual differences in feed efficiency seemed to affect the behavioural response to alarm cues only at higher alarm cue concentrations, suggest that perhaps also feed efficiency should be added to Brown et al. (2001)'s list. Future studies should provide conclusive evidence for a threat sensitive trade-off between foraging and anti-predator behaviour in the African catfish.

Although there is an extensive knowledge on the ecological role of chemical alarm cues, the potential role of these cues in a farming situation is largely unknown. The current study has made a first step in exploring the role of chemical communication in a closed farming situation. Chapter 5 indicated that group housed African catfish respond to a chemical alarm cue concentration of 0.1 cm^2 skin/ml. The response they showed, an increase in swimming activity, indicated a foraging response rather than an alarm response (Brown et al., 2002). A likely explanation for the fact that African catfish perceived this chemical alarm cue concentration as a foraging cue may lie in the omnivorous feeding habits of this species. Previous studies have shown that a variety of predators use chemical alarm cues as a foraging response. For instance, Mathis et al. (1995) and Chivers et al. (1996) showed that northern pike (*Esox lucius*) and diving beetles (Dytiscidae) are attracted to chemical alarm cues of the fathead minnow (*Pimephales promelas*). Does this mean that chemical alarm cues should not be considered a stressor for African catfish? Of course the issue is not that straightforward. Several predatory fish species show a threat-sensitive shift in their response to chemical alarm cues, shifting from a foraging to an anti-predator response depending on amongst others alarm cue concentration, age/size, and feeding motivation (Brown et al., 2001). The latter observations once more suggest that it would really be valuable to check whether also *Clarias* sp.would shift from a foraging to an anti-predator response depending on alarm cue concentration.

With our current knowledge we can only speculate on the practical implications of chemical alarm signalling within a closed farming system as a RAS. Future studies should give more insight on several questions. At which chemical alarm cue concentration do group-housed African catfish shift to an anti-predator response? Do ontogenetic changes in the response of African catfish to chemical alarm cues occur? What is the faith of chemical alarm cues in a RAS in terms of removal, chemical breakdown, etc? What concentrations of chemical alarm cues are present in commercial farms? Answering these questions should hopefully shed more light on the relationship between chemical alarm cues and the welfare of farmed African catfish.

ASSESSING FISH WELFARE

Although the evaluation of methods with which the welfare of fish is assessed was not a specific objective of the current study, the possibilities and impossibilities of the methods applied in the current study will be shortly discussed.

In research a wide array of physiological, biochemical, and behavioural parameters are used to asses fish welfare. However, none of these parameters can be considered reliable in isolation. Because most of these parameters are influenced by factors other than welfare, they are often imprecise or noisy parameters. Chapter 2 and 3 provide a number of examples that indicate that the use of single parameters can not be considered reliable. For example, let's focus on two commonly used welfare parameters, plasma cortisol and the number of skin lesions. Based purely on basal levels of plasma cortisol, often considered to be one of the most important welfare indicators, we did not detect an effect of stocking density in fish of 10-100 gram. Nonetheless, other parameters indicated that stocking density did in fact affect the fish. For the other two growth stages (100-300gram/1000-1500 gram), basal levels of plasma cortisol did give a relialble reflection of the effects of stocking density. Skin lesions (measured before netstress), an indicator that would be well suited for on-farm welfare assessments, indicated a situation of impaired welfare at the lowest densities for fish in a range of 10-100 gram. In contrast, it did not provide indications for impaired welfare at the highest densities. For the other two growth ranges, skin lesions did not indicate a situation of impaired welfare. While this was a proper reflection of the welfare situation of fish of 1000-1500 gram, it failed to show the effects stocking density had on fish of 100-300 gram. Therefore, if we would have based our conclusions regarding the effects of stocking density on only a single indicator, these conclusions would not have always been fully reliable. Although single measurements can to a certain extent provide valid information, appropriate combinations of measurements on, for example, physiology, behaviour, diseases status, and growth provide a more compelling assessment of welfare (Broom, 1997). In the current study we have therefore chosen to assess a range of physical (e.g. feed intake, SGR, FCR), physiological (i.e. cortisol, glucose, lactate), and behavioural (e.g. swimming, airbreathing, escape behaviour, agonistic behaviour, stereotypic behaviour) parameters. Nonetheless, assessing a range of different parameters by univariate analyses may also have its limitations since it is difficult to quantitatively relate such measurements to the welfare status of the fish and the relationship between indicators is not always constant (Turnbull et al., 2008).

The use of multivariate analysis to combine a range of welfare indicators, based on the observed statistical relationships between them, might be a possible solution to overcome these problems (Manly, 1994). Principal components analysis (PCA) allows trends in multivariate data to be reduced to scores that can then be used as dependent variables in subsequent analyses, without requiring a priori judgements of the relative value of individual variables. These scores may provide an integrated and objective reflection of diverse measured welfare indicators. This method may provide a more robust and/or sensitive measure of welfare than univariate measures could provide individually. Two recent studies in Atlantic salmon (*Salmo salar*) used a PCA to combine four commonly used measures of fish welfare (condition of body and fins and plasma concentrations of glucose and cortisol) into a single welfare score (Adams et al., 2007; Turnbull et al., 2005). This score proved to be biologically consistent with welfare; high scores were given to fish with a high condition of body and fins and low plasma concentrations of glucose and cortisol. Furthermore, the welfare score was consistent with the evaluation of welfare by experienced farmers (Turnbull et al., 2005).

In the current study we used this multivariate statistical approach to combine data derived from a range of welfare indicators of three distinct functional systems (physical, physiological, and behavioural responses) into a single index of welfare. For both a growth range of 10-100 gram and 100-300 gram factor scores of the first principal component indicated an effect of stocking density. For a growth range of 1000-1500 gram factor scores were not affected by stocking density. For a growth range of 100-300 gram the first component produced by the PCA was biologically consistent with welfare, with highly positive loadings for feed intake, specific growth rate, and swimming activity and highly negative loadings for plasma cortisol and agonistic behaviour. High factor scores indicated a situation of good welfare, whereas low factor scores indicated a situation of impaired welfare. However, for a growth range of 10-100 gram the picture was somewhat more complex. The first principal component loaded feed intake, feed conversion ratio, lesions after netstress, airbreathing and escape behaviour positively and plasma glucose, lesions before netstress, and agonistic behaviour negatively. Because indicators of impaired welfare load both negatively (e.g. agonistic behaviour) and positively (e.g. lesions after netstress, escape behaviour), both high and low factor scores for the first principal component may be a sign of a situation of impaired welfare in this study. This example indicates that the results of a PCA analysis should be interpreted with great care. Although it is tempting to consider high and low factor scores as indication of a situation of good or bad welfare (or vice versa), this is certainly not always the case.

Despite the fact that interpretation of factor scores is not always that straightforward, multivariate analyses offer some interesting possibilities. Chapter 3 showed that both univariate and multivariate analyses on welfare indicators measured in fish in a growth range of 1000-1500 gram did not indicate an effect of stocking density. Furthermore, a series of univariate analysis showed no effects of other environmental factors (e.g. disturbance of the tank, sex ratio) on the used welfare indicators. However, a correlation analysis indicated that the first principal component generated by a PCA was related to the sex ratio within the tank (r_p=0.68, N=12, P=0.0153). Tanks with a high male:female ratio showed low SGR, low aggression, low amount of lesions after netstress, and high FCR and increase in plasma lactate levels after the netstressor. The second component was related to the distance between the tanks and the main aisle (r_p=0.68, N=12, P=0.0145). Tanks located further from the aisle (less daily disturbance) responded with a higher activity, but lower amounts of escape attempts and lesions. A study on the effects of stocking density in Atlantic salmon showed that the effects of stocking density on welfare depended in complex ways on disturbance of the tanks (Adams et al., 2007). By combining a range of welfare indicators, the PCA provided a moe powerfull approach to detect subtle effects of environmental factors as sex ratio and disturbance of tanks that could not have identified by univariate analyses.

In conclusion, the current study indicated that the interpretation of a welfare score generated by multivariate analyses is not always as straight forward as suggested in other studies. Judgements of the relative value of individual variables may still be necessary. Nonetheless, multivariate analyses may be a very powerful approach to identify important trends in datasets combining a large range of welfare indicators and to identify factors influencing fish welfare that cannot be identified by univariate analyses alone.

IMPLICATIONS FOR AQUACULTURE

When studying fish welfare under laboratory conditions as done in the current study we should always ask ourselves to what extent the results can be extrapolated to normal farm conditions. Although the stocking densities we have used are comparable to commercial conditions, the actual group sizes and tank sizes we have used are considerably lower then those used under commercial conditions. In a study to quantify the effects of increasing both group size and tank size while keeping stocking density fixed, Almazán Rueda (2004) found that performance improved when group and tank size increased. Furthermore, overall activity increased at increasing group and tank size. He concluded that increasing group and tank size while keeping the stocking density fixed had a positive effect on behaviour and growth performance. These results are in contrast to data on trout which show that larger group sizes reduce growth and increase size variation (Ellis et al., 2002). However, this discrepancy can probably be explained by differences in biology and environmental requirements for each fish species. Therefore, we assume that the results we found under laboratory conditions can be extrapolated to commercial conditions. However, the magnitude of the effects of stocking density will be lower under farming conditions, which makes our recommendations on appropriate stocking densities to be on the safe side. Unfortunately, no reference data is available on comparable studies in intensive African catfish farms and further study in this field is required.

The current study confirms that stocking density does indeed affect the welfare of African catfish. Based on the results of this study, recommendations can be made regarding minimum or maximum densities. Both Chapter 2 and 3 of this study provide such recommendations. However, can focussing on stocking density alone guarantee the welfare of farmed African catfish? The curent study has shown that stocking density is an area with many interacting factors that affect welfare. In this study, a number of possible factors have been highlighted, i.e. age, individual variation in behavioural stress responses, acute stressors, etc. These, however, are not the only possible interacting factors. For instance, stockmanship, water quality parameters, and health management are also likely to interact with stocking density.

Other studies on the effects of stocking density on farmed animals have given further evidence that the effects of stocking density are strongly mediated by a range of factors. Almazán Rueda et al. (2004) showed that the behavioural effects of stocking density in African catfish were affected by light intensity and photoperiod. Compared to continuous light, a 12L:12D photoperiod decreased the number of bite wounds at low (250 fish/m^3) density, but not at high (2500 fish/m^3) density. In addition, compared to a high light intensity (150 lux), a low light intensity (15 lux) increased the swiming activity at low density, but not at high density. A study of cage-reared salmon showed that, although density influenced welfare, the variability within cages, sample periods, and density ranges did not allow the authors to predict the welfare of the animals based on stocking density alone (Turnbull et al., 2005). Another study on the effects of stocking density on the welfare of salmon showed that stocking density compromised welfare in some conditions, but that the observed variation in welfare depended much more on exposure to disturbance during routine fish farm husbandry than on stocking density (Adams et al., 2007). Finally, a study on the effects of stocking density in broilers indicated that differences among producers in the environment that they provide for their chickens, e.g. litter moisture, ventilation, temperature, etc., had more impact on welfare than had stocking density itself (Dawkins et al., 2004).

In conclusion, the complex relation between stocking density and fish welfare, shown by the current and other studies, suggests that concentrating on stocking density alone will not allow us to control welfare accurately. Both low and high densities may be applied without adverse effects as long as the rest of the farm environment is optimal. However, if elements within the farm environment are sub-optimal, even the most optimal stocking density might not ensure fish welfare. As Ellis et al. (2002) suggested, maybe the best way to promote the welfare of farmed fish is to develop simple welfare indicators which can be used on farms to reliably and transparently monitor the welfare status of any group of fish. Setting acceptable levels of for instance water quality parameters (e.g. DO, CO_2, NH_3), physical condition (e.g. SGR, condition factor), and behavioural indicators (e.g. bite wounds) might be (1) easier to achieve and (2) easier to transfer to other fish species than specific density limits. Although stocking density may be an easy target for animal rights groups, setting limits to stocking density alone will not guarantee the welfare of farmed African catfish.

MAIN CONCLUSIONS

Objective 1: Does stocking density affect physical, physiological, and behavioural responses of African catfish?

Stocking density affects the welfare of African catfish. Especially in the earlier life stages (10-300 gram) low densities appeared to be more problematic than high densities. Strongly generalised, juvenile African catfish (100-300) responded to increasing density with the formation of dense clusters of fish with constant movement and low aggression. However, the relationship between stocking density and the welfare of African catfish was strongly mediated through factors as acute stress experienced by the fish at stocking the tanks or through a netting stressor. Such stressors (restocking tanks, grading, handling) are common in a farming situation, indicating that the total farming situation may influence the effects of stocking density.

Objective 2: Does age mediate the effects of stocking density on physical, physiological, and behavioural responses of African catfish?

Age mediated the effects of stocking density. For African catfish of 10-100 gram, both the highest and lowest density had detrimental effects on fish welfare. At low densities we observed increased aggression and skin lesions, while an acute stressor (netting stress) resulted in increased aggression in fish housed at high density. For African catfish of 100-300 gram, increasing density led to an increase in both activity and SGR. Plasma cortisol and agonistic behaviour decreased at increasing density, suggesting an improved welfare at increasing stocking density. For catfish of 1000-1500 no effects of stocking density on a range of welfare indicators was shown. These results demonstrate the importance of taking into account that the effects of stocking density strongly depend of the life stage of an animal. If recommendations on a suitable stocking density are to be made, these recommendations should be adjusted according to the life stage of the animal.

Objective 3: What are the potential effects of chemical alarm cues on the welfare of farmed African catfish?

African catfish respond to conspecific skin extract. The behavioural response to conspecific skin extract was affected by the feed efficiency of an animal. Fish with a lower feed-efficiency showed an active response to chemical alarm cues while more feed-efficient animals showed a more passive response. Our experiments did not provide indications that conspecific skin extract has a profound impact on the welfare of African catfish under farm conditions. However, the welfare consequences of conspecific skin extract concentrations exceeding the one used in Chapter 5 require further study.

Objective 4: What are the fitness consequences of different coping strategies in intensive husbandry systems?

African catfish show a consistent individual variation in the behavioural response to an escape test, suggesting the existence of coping strategies in African catfish. However, this consistent individual variation seems to be only weakly associated with the welfare of farmed African catfish.

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Appendices

Summary
Samenvatting
Acknowledgements
List of publications
Training and Supervision Plan
About the author

SUMMARY

In the Netherlands, the welfare of farmed fish is subject of growing public and governmental concern. The Dutch government, through the Ministry of Agriculture, Nature and Food quality, has supported the current study to increase scientific knowledge on fish welfare, in particular on the requirements of African catfish (*Clarias gariepinus*) for stocking density. Comparable to concerns regarding the intensity of terrestrial livestock production, stocking densities applied in fish farming have been highlighted as an important area of welfare concern. In contrast to most terrestrial animals, fish utilise a three dimensional medium and depend on this medium for both behavioural (spatial) and physiological (e.g. oxygen supply, dilution of metabolic waste products) needs. The relationship between fish welfare and stocking density is therefore strongly mediated through factors as water quality and social interactions. However, factors as age, individual variation in behavioural stress responses (i.e. coping strategies) and acute additional stressors have also been suggested to confound the relation between fish welfare and stocking density.

In general, African catfish seem to respond to increasing density with increased growth performance and the formation of dense clusters of fish with constant movement and low aggression. However, the current knowledge in this field is far from complete and often difficult to extrapolate to Dutch farm conditions. Most of the work has focused on the effects of relatively low stocking densities, making it incomparable to the situation in a modern recirculating aquaculture system (RAS). Moreover, most studies assessed a maximum of 3 different stocking densities, making it difficult to come to a proper dose-response curve for the effects of stocking density on African catfish. While it is well possible that the relationship between the welfare of African catfish and stocking density is mediated through other factors (e.g. fish age, coping strategies, acute additional stressors) it is yet unclear to what extent these factors may confound this relationship. Finally, although aggression in African catfish is often associated with improper stocking densities, it is not known if this aggression results in the release of chemical alarm cues and, if released, what the consequences of these cues are for the welfare of African catfish.

The general aim of this thesis was to determine the impact of stocking density on welfare indicators in African catfish, *Clarias gariepinus*, cultured in a recirculating aquaculture system. The following factors were studied: 1) The effects of stocking density on physical, physiological, and behavioural responses of African catfish, 2) How age mediates the effects of stocking density on physical, physiological, and behavioural responses of African catfish, 3) The potential effects of chemical alarm cues on the welfare of farmed African catfish, and 4) The fitness consequences of different coping strategies in intensive husbandry systems.

Chapter 2 and **3** studied the effects of stocking density on a range of welfare indicators. Physical, physiological, and behavioural responses to a range of 4-5 densities were studied at 3 growth stages (10-100, 100-300, and 1000-1500 gram). **Chapter 2** and

3 showed that stocking density influenced the welfare of African catfish. Strongly generalised, African catfish responded to increasing density with the formation of dense clusters of fish with constant movement and low aggression. Interestingly, the effects of stocking density on the behaviour of African catfish seemed to be strongly time-dependent. The most noticeable effects of stocking density were found in the first weeks of the experiments. Possibly, the acute stress experienced by the fish in these first weeks of the experiments modulates the effects of stocking density by triggering aggression. **Chapter 2** pinpointed another factor that modulates the effects of stocking density. Low levels of aggression were observed at high stocking densities. However, a challenge test (netting stress) gave additional and crucial information about the basic welfare situation. After applying this acute stressor (netting stress), a strong increase in agonistic behaviour, especially in fish housed at high density, occurred. An important conclusion to be drawn from these results is that there exists an interaction effect between stocking density and acute additional stress. Since additional stressors (e.g. handling, grading) are not uncommon in a farming situation, this means that the total farming situation may influence the effects of stocking density. In conclusion, stocking density is a prime example of an area that demonstrates the significance of taking into account the existence of a complex web of interacting factors which affect welfare.

Chapter 2 and **3** of the current study also gave indications that the effects of stocking density in African catfish were mediated by age. **Chapter 2** indicated that for African catfish of 10-100 gram, both high and low densities had detrimental effects on fish welfare. At low densities we observed increased aggression and skin lesions, while an acute stressor (netting stress) resulted in increased aggression in fish housed at high density. **Chapter 3** showed that for African catfish of 100-300 gram, increasing density led to an increase in both activity and SGR and a decrease in plasma cortisol and agonistic behaviour. This suggests an improved welfare at increasing stocking density. For catfish of 1000-1500 no effects of stocking density on a range of welfare indicators was shown. These results demonstrate the importance of taking into account that the effects of stocking density strongly depend of the life stage of an animal. If recommendations on a suitable stocking density are to be made, these recommendations should be adjusted according to the life stage of the animal.

Chapter 4-5 studied the behavioural responses of African catfish to chemical alarm cues and the consequences these alarm cues may have in a farming situation. **Chapter 4** studied if and how individually housed African catfish respond to chemical alarm cues (i.e. conspecific skin extract). This study showed that African catfish do respond to chemical alarm cues. A large individual variation in the response to the alarm cue was observed; changes in percentage of time spent swimming in response to conspecific skin extract ranged from -68.4% to 85.1%. The large individual variation in the response to conspecific skin extract was related to feed efficiency; feed-efficient animals decreased their activity in response to conspecific skin extract while feed-inefficient animals responded to conspecific skin extract with an increase in time spent swimming.

Chapter 5 assessed the effects of a single chemical alarm cue administration on the behaviour and growth performance of group-housed African catfish. African catfish responded to chemical alarm cues with a short-term increase in swimming activity, indicating a foraging response rather than an anti-predator response. No long-term effects were observed on both behaviour and growth performance of the fish. A possible explanation for the fact that African catfish perceived this chemical alarm cue concentration as a foraging cue may lie in the omnivorous feeding habits of this species. Previous studies have shown that a variety of predators use chemical alarm cues as a foraging response. Nonetheless, many predatory fish species show a threat-sensitive shift in their response to chemical alarm cues, shifting from a foraging to an anti-predator response depending on amongst others alarm cue concentration. It can therefore be assumed that at increasing chemical alarm concentration African catfish will shift from a foraging to an anti-predator response. The current study has made a first step in exploring the role of chemical communication in a closed farming situation. Future studies should give more insight on the practical implications of chemical alarm signaling within a closed farming system as a RAS.

The large individual variation in behavioural responses to chemical alarm cues found in **Chapter 4** of this study gave an indication for the existence of coping strategies in African catfish. The results of **Chapter 4** suggested that the behavioural response of feed-inefficient animals to conspecific skin extract is more dominated by a pro-active coping strategy than that of feed-efficient animals.

Although **Chapter 4** gave indications for the existence of coping strategies in African catfish, this chapter did not provide evidence for a consistent behavioural variation in the way these fish cope with certain stressors. **Chapter 6** did provide evidence for such a consistent behavioural variation. **Chapter 6** assessed if and how group composition (groups composed of only proactive animals, only reactive animals, or a random selection of animals) affected the effects of stocking density. In **Chapter 6**, animals were confronted twice with an escape test to determine the coping characteristics of each fish. This resulted in correlation coefficients of r_s=0.36 for moving activity and r_s=0.31 for the number of escape attempts. However, correlations for moving activity and the number of escape attempts were only found in fish housed in mixed groups. In proactive groups, swimming activity and number of escape attempts decreased while in reactive groups swimming activity and number of escape attempts increased. In both groups swimming activity and the number of escape attempts in the escape tests were not correlated. A possible explanation for the behavioural changes of fish housed in proactive or reactive groups could be that behavioural variation is beneficial for the group. We conclude that the response of African catfish to an escape test is a fairly consistent trait in a socially stable environment, but that African catfish can change their behaviour if environmental conditions require so.

Chapter 6 found no indications for fitness consequences of different coping strategies in intensive husbandry systems. Although an impaired growth performance of reactive fish housed in reactive groups was shown, no such effects were seen in reactive fish housed in mixed populations, which is how these fish are housed in a farming situation. The impaired growth performance therefore seemed to be more dependent on group composition than on coping strategy itself. Based on these results, coping strategies seem to be only weakly associated with the welfare of African catfish.

The current study confirmed that stocking density does indeed affect the welfare of African catfish. However, stocking density is an area with many interacting factors that affect welfare. In this study, a number of possible factors have been highlighted, i.e. age, individual variation in behavioural stress responses and acute additional stressors. These, however, are not the only possible interacting factors. For instance, stockmanship, water quality parameters, and health management are also likely to interact with stocking density. This complexity suggests that concentrating on stocking density alone will not allow us to control welfare accurately. Maybe the best way to promote the welfare of farmed fish is to develop simple welfare indicators which can be used on farms to reliably and transparently monitor the welfare status of any group of fish. Setting acceptable levels of for instance water quality parameters (e.g. DO, CO_2, NH_3), physical condition (e.g. SGR, condition factor), and behavioural indicators (e.g. bite wounds) might be (1) easier to achieve and (2) easier to transfer to other fish species than specific density limits. Although stocking density may be an easy target for animals rights groups, setting limits to stocking density alone will not guarantee the welfare of farmed African catfish.

SAMENVATTING

In Nederland is de publieke en politieke aandacht voor het welzijn van vis groeiende. De Nederlandse overheid, middels het ministerie van Landbouw, Natuur en Voedselkwaliteit heeft de huidige studie ondersteund om de wetenschappelijke kennis op het gebied van viswelzijn in het algemeen, en het effect van visdichtheid op de Afrikaanse meerval *Clarias gariepinus* in het bijzonder, te vergroten. Vergelijkbaar met de aandacht voor de intensivering van de veehouderij zijn dichtheden toegepast in de aquacultuur een belangrijk aandachtspunt. In tegenstelling tot landdieren gebruiken vissen een driedimensionaal medium. Zij zijn zowel qua gedrag als fysiologisch (e.g. als zuurstof bron, verdunning van metabole producten) sterk van dit medium afhankelijk. De relatie tussen viswelzijn en visdichtheid wordt daardoor sterk beïnvloed door factoren als waterkwaliteit en sociale interacties. Echter, ook factoren als leeftijd, individuele variatie in de reactie op stress (i.e. coping strategieën) en acute stressoren zouden de relatie tussen viswelzijn en visdichtheid kunnen beïnvloeden.

Algemeen gesteld reageert Afrikaanse meerval op toenemende visdichtheid met een toenemende groei en het formeren van dichte clusters vis die zich kenmerken door continue beweging en lage agressie. Echter, de huidige kennis op dit gebied is verre van compleet en vaak moeilijk te extrapoleren naar omstandigheden in de Nederlandse aquacultuur. Veel onderzoek heeft zich gericht op de effecten van relatief lage visdichtheden, waardoor de resultaten van dit onderzoek maar moeilijk toe te passen zijn op de situatie zoals die zich voordoet in een modern recirculatiesysteem (RAS: recirculating aquaculture system). Verder vergelijken veel studies maximaal 3 visdichtheden, waardoor het opstellen van een dosis-respons relatie bemoeilijkt wordt. Ondanks dat het zeer wel mogelijk is dat de relatie tussen het welzijn van Afrikaanse meerval en visdichtheid beïnvloedt wordt door overige factoren (e.g. leeftijd, coping strategieën, acute stressoren) is het momenteel niet duidelijk in welke mate deze factoren de relatie tussen viswelzijn en visdichtheid beïnvloeden. Tot slot, ondanks dat agressie bij Afrikaanse meerval vaak geassocieerd wordt met de toepassing van ongeschikte visdichtheden is het niet duidelijk of deze agressie ook resulteert in het vrijkomen van chemische alarmstoffen en, indien deze alarmferomonen vrijkomen, welke consequenties dit heeft op het welzijn van Afrikaanse meerval.

Het doel van dit proefschrift was het bepalen van de invloed van visdichtheid op welzijnsindicatoren van in recirculatiesystemen gekweekte Afrikaanse meerval, *Clarias gariepinus*. De volgende factoren werden bestudeerd: 1) Het effect van visdichtheid op groei, fysiologie en gedrag van Afrikaanse meerval, 2) Hoe leeftijd het effect van visdichtheid op groei, stressfysiologie en gedrag van Afrikaanse meerval beïnvloedt, 3) De mogelijke effecten van chemische alarmstoffen of het welzijn van Afrikaanse meerval en 4) de consequenties van coping strategieën op het welzijn van Afrikaanse meerval in intensieve vishouderij systemen.

Hoofdstuk 2 en **3** bestudeerden de effecten van visdichtheid op uiteenlopende welzijnsindicatoren. De effecten van series van 4-5 dichtheden op groei, stressfysiologie en gedrag werden bestudeerd tijdens 3 groeitrajecten (10-100 gram, 100-300 gram en 1000-1500 gram). **Hoofdstuk 2** en **3** toonden dat visdichtheid het welzijn van Afrikaanse meerval beïnvloedt. Sterk gegeneraliseerd reageerden Afrikaanse meerval op toenemende visdichtheid met een toenemende groei en het formeren van dichte clusters vis die zich kenmerken door continue beweging en lage agressie. Het effect van visdichtheid op het gedrag van Afrikaanse meerval bleek sterk tijdsafhankelijk. De meest merkbare effecten van visdichtheid werden geobserveerd tijdens de eerste weken van de experimenten. Het is mogelijk dat de acute stress die de dieren tijdens deze eerste weken ervoeren, door agressie te veroorzaken, het effect van visdichtheid beïnvloedde. **Hoofdstuk 2** toonde nog een factor aan die het effect van visdichtheid beïnvloedt. In aquaria met een hoge visdichtheid werd weinig agressief gedrag waargenomen. Echter, een "challenge test" (een acute stressor waarvoor het dieren voor korte duur in een net boven het wateroppervlak werd geplaatst) gaf belangrijke, additionele informatie omtrent de welzijnssituatie van deze dieren. Na het toepassen van de acute stressor trad een sterke toename van agressie op, met name bij dieren die bij hoge dichtheid waren gehuisvest. Een belangrijke conclusie die we uit deze resultaten kunnen trekken is dat er een interactie bestaat tussen visdichtheid en acute stressoren. Omdat acute stressoren (e.g. sorteren van vis) niet ongebruikelijk zijn in een vishouderij, betekent dit dat de gehele vishouderij de effecten van visdichtheid kan beïnvloeden. In conclusie, visdichtheid is een belangrijk voorbeeld van een onderwerp dat aantoont dat rekening moet worden gehouden met het bestaan van een complex web van factoren die viswelzijn beïnvloeden.

Hoofdstuk 2 en **3** van deze studie gaven ook aanwijzingen dat de effecten van visdichtheid beïnvloed worden door de leeftijd van de vis. **Hoofdstuk 2** toonde dat zowel lage als hoge visdichtheden een negatief effect hebben op Afrikaanse meerval in een groeifase van 10-100 gram. Bij lage dichtheid werd een verhoogde agressie en een verhoogd aantal bijtwonden waargenomen. Een acute stressor resulteerde in een toename in agressief gedrag bij vissen gehuisvest bij hoge dichtheid. **Hoofdstuk 3** toonde dat bij Afrikaanse meerval in een groeifase van 100-300 gram een toenemende dichtheid leidde tot een toename in activiteit en groeisnelheid en een afname in plasma cortisol en agressief gedrag. Dit suggereert dat het welzijn van Afrikaanse meerval in dit groeitraject toeneemt bij toenemende visdichtheid. Visdichtheid had geen effect op uiteenlopende welzijnsindicatoren gemeten in Afrikaanse meerval in een groeitraject van 1000-1500 gram. Deze resultaten tonen aan dat er rekening mee moet worden gehouden dat de effecten van visdichtheid sterk afhankelijk zijn van de leeftijd van het dier. Wanneer aanbevelingen met betrekking tot een optimale visdichtheid moeten worden gedaan, zullen deze aangepast moeten worden aan de leeftijd van een dier.

Hoofdstuk 4-5 bestudeerden de gedragsrespons van Afrikaanse meerval op chemische alarmstoffen en welke consequenties deze alarmstoffen kunnen hebben in een vishouderij. **Hoofdstuk 4** bestudeerde of en hoe individueel gehuisveste Afrikaanse meerval reageerde op chemische alarmstoffen (i.e. huidextract van soortgenoten). Dit onderzoek toonde dat Afrikaanse meerval reageert op chemische alarmstoffen. Er werd een grote individuele variatie in gedragsrespons waargenomen; veranderingen in het percentage van de tijd die vissen zwemmend doorbrachten, varieerden van -68.4% tot 85.1%. Deze grote individuele variatie in de gedragsrespons op huidextract van soortgenoten was gerelateerd aan de voerefficiëntie. Efficiënte dieren verlaagden hun activiteit in reactie op het huidextract terwijl inefficiënte dieren hun activiteit juist verhoogden.

Hoofdstuk 5 bestudeerde de effecten van een enkele alarmstof toediening op het gedrag en de groei van Afrikaanse meerval in groepshuisvesting. Afrikaanse meerval reageerde op de alarmstof met een tijdelijke verhoging van de zwemactiviteit, duidend op een voerrespons in plaats van een anti-predator respons. Langetermijneffecten op gedrag en groei van de vissen werd niet waargenomen. Het feit dat Afrikaanse meerval deze concentratie van alarmstoffen gebruikten als voersignaal kan mogelijk verklaard worden door de omnivore levensstijl van deze dieren. Voorgaande studies hebben aangetoond dat een verscheidenheid aan predatoren chemische alarmstoffen als voersignaal gebruiken. Desalniettemin vertonen veel van deze predatoren een risicoafhankelijke verschuiving in hun respons op chemische alarmstoffen, verschuivend van voersignaal naar anti-predator signaal. Het is daarom niet onwaarschijnlijk dat bij toenemende concentratie van alarmstoffen ook de Afrikaanse meerval van voergedrag overgaat op anti-predator gedrag. De huidige studie heeft een eerste stap gezet in het onderzoeken van de rol van chemische communicatie in een gesloten vishouderij. Toekomstig onderzoek moet meer inzicht bieden in de praktische implicaties van chemische communicatie in gesloten systemen als een recirculatiesysteem.

De sterke individuele variatie in gedragsrespons op chemische alarmstoffen zoals gevonden in **Hoofdstuk 4** van deze studie geeft een indicatie voor het bestaan van coping strategieën bij Afrikaanse meerval. De resultaten van **Hoofdstuk 4** suggereren dat de gedragsrespons van voerinefficiënte dieren op huidextract van soortgenoten meer gedomineerd wordt door een proactieve coping strategie dan de reactie van voerefficiënte dieren.

Ondanks dat **Hoofdstuk 4** indicaties geeft omtrent het bestaan van coping strategieën in Afrikaanse meerval, geeft het geen bewijs voor consistente variatie in de manier waarop deze dieren gedragsmatig met stressoren omgaan. **Hoofdstuk 6** gaf wel bewijs voor een dergelijke consistente variatie. **Hoofdstuk 6** bestudeerde of en hoe groepssamenstelling (groepen samengesteld uit enkel proactieve dieren, enkel reactieve dieren of een willekeurige selectie van dieren) het effect van visdichtheid beïnvloedde. In **Hoofdstuk 6** werden dieren tweemaal geconfronteerd met een "escape test" om de coping karakteristieken van elk dier te bepalen. Dit resulteerde in correlatie coëfficiënten van r_s=0.36 voor activiteit en r_s=0.31 voor het aantal ontsnappogingen. Echter, correlaties voor zowel activiteit als het aantal ontsnap pogingen werden alleen vastgesteld bij groepen bestaande uit een willekeurige selectie van dieren. In proactieve groepen daalden activiteit en het aantal ontsnappogingen terwijl in reactieve groepen dit juist toenam. In beide groepen werden geen correlaties tussen beide "escape tests" gevonden. Een sterke variatie in gedrag kan voordelen voor de groep bieden. Dit verklaart mogelijk de veranderingen in gedrag van vissen gehuisvest in proactieve en reactieve groepen. Wij concluderen dat de reactie van Afrikaanse meerval op een “escape test” een redelijk consistente eigenschap is in een stabiel milieu, maar dat de Afrikaanse meerval zijn gedrag kan aanpassen als de omstandigheden daarom vragen.

Hoofdstuk 6 gaf geen indicaties voor mogelijke consequenties van coping strategien in intensieve vishouderij systemen. Reactieve dieren, gehuisvest in reactieve groepen, vertoonden een verminderde groei. Echter, wanneer reactieve dieren gehuisvest werden in een gemixte groep, representatief voor de huisvesting in een vishouderij, vertoonden zij een vergelijkbare groei als proactieve dieren. De verminderde groei van reactieve dieren lijkt daarom meer afhankelijk van groepssamenstelling dan van coping strategien. Gebaseerd op deze resultaten lijken coping strategieën daarom slechts in beperkte mate gerelateerd aan het welzijn van Afrikaanse meerval.

Deze studie bevestigde dat visdichtheid het welzijn van Afrikaanse meerval beinvloedt. Echter, vele factoren beinvloeden de relatie tussen visdichtheid en viswelzijn. In deze studie wordt aandacht besteed aan een aantal van deze factoren, i.e. leeftijd van de vis, individuele variatie in stressrespons en acute, additionele stressoren. Dit is echter slechts een klein deel van de mogelijke factoren. Hoogstwaarschijnlijk spelen ook factoren als kwaliteiten van de vishouder, waterkwaliteit en ziektepreventie een belangrijke rol. Deze complexiteit toont aan dat concentreren op alleen visdichtheid niet voldoende zal zijn om het welzijn van vis te garanderen. Ontwikkeling van eenvoudige welzijnsindicatoren, waarmee op transparante en betrouwbare wijze het welzijn van vis bepaald kan worden, is wellicht een meer geschikte manier om viswelzijn te garanderen. Het opstellen van acceptabele niveaus voor bijvoorbeeld waterkwaliteit (bijvoorbeeld O_2, CO_2, NH_3), fysieke parameters (groeisnelheid, conditie factor) en gedragsindicatoren (bijvoorbeeld bijtwonden) zou 1) makkelijker te bereiken zijn en 2) makkelijker te extrapoleren zijn naar andere vissoorten dan het geval is met limitering van visdichtheden. Ondanks dat visdichtheid een gemakkelijk doelwit is voor dierrechtenorganisaties kan alleen limitering van visdichtheid het welzijn van Afrikaanse meerval niet garanderen.

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LIST OF PUBLICATIONS

PEER REVIEWED PAPERS

Nieuwegiessen, P.G. van de, Schrama, J.W., Verreth, J.A.J., 2008. A note on alarm cues in juvenile African catfish, *Clarias gariepinus* Burchell: Indications for opposing behavioural strategies. Applied Animal Behaviour Science 113, 270-275.

Nieuwegiessen, P.G. van de, Boerlage, A.S., Verreth, J.A.J., Schrama, J.W., 2008. Assessing the effects of a chronic stressor, stocking density, on welfare indicators of juvenile African catfish, *Clarias gariepinus* Burchell. Applied Animal Behaviour Science 115, 233-243.

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Nieuwegiessen, P.G. van de, Schrama, J.W., Verreth, J.A.J., 2008. The combined effects of stocking density and coping strategy on welfare indicators in African catfish (*Clarias gariepinus)*. In: Aquaculture Europe 2008, Krakow, Poland.

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Nieuwegiessen, P.G. van de, Schrama, J.W., Verreth, J.A.J., 2005. Individual variation in behaviour and HPI-axis activity, coping styles, in African catfish (*Clarias gariepinus)*. In: Aquaculture Europe 2005, Trondheim, Norway.

Training and Supervision Plan	**Graduate School WIAS**
Name PhD student	Pascal G. van de Nieuwegiessen
Project title	Welfare of African catfish - effects of stocking density
Group	Aquaculture and Fisheries
Daily supervisor	Dr. Johan W. Schrama
Supervisor	Prof. dr. Johan A.J. Verreth

EDUCATION AND TRAINING	Year	ECTS
The Basic Package		
WIAS Introduction Course	2005	1.5
Course on philosophy of science and/or ethics	2005	1.5
Subtotal Basic Package		**3.0**
Scientific Exposure		
International conferences		
Aquaculture Europe 2005	2005	1.5
Aquaculture Europe 2006	2006	1.5
Aquaculture Europe 2007	2007	1.5
Seminars and workshops		
WIAS Science Day	2005-7	0.9
Workshop on the recovery and the artificial reproduction of eel	2006	0.3
Presentations		
2 Oral presentations at Aquaculture Europe 2005	2005	2.0
Oral presentation Aquaculture Europe 2006	2006	1.0
Oral presentation at WIAS Science Day 2006	2006	1.0
Poster presentation at Aquaculture Europe 2007	2007	1.0
Poster presentation at WIAS Science Day 2007	2007	1.0
Subtotal Scientific Exposure		**11.7**
In-Depth Studies		
Disciplinary and interdisciplinary courses		
Fish Immunology/Vaccination Workshop	2006	1.5
Aqualabs 3 Training Course - Fish Welfare	2005	2.0
Advanced statistics courses		
Design of Animal Experiments	2005	1.0
Advanced Statistics	2006	1.5
Subtotal In-Depth Studies		**6.0**
Statutory Courses		
Use of Laboratory Animals	2004	4.5
Subtotal Statutory Courses		**4.5**
Professional Skills Support Courses		
Course Techniques for Scientific Writing	2006	1.2
Course Supervising MSc thesis work	2006	0.8
Project And Time Management	2007	1.5
Subtotal Professional Skills Support Courses		**3.5**
Research Skills Training		
Preparing own PhD research proposal	2004	6.0
Subtotal Research Skils Training		**6.0**
Didactic Skills Training		
Supervising practicals and excursions		
Practical: AFI-32306 - Nutrition, Welfare and Reproduction in Aquaculture	2005-8	0.8
Practical: AFI-20306 - Aquaculture and Fisheries	2005-6	0.6
Supervising theses		
MSc thesis Annette Boerlage, Wageningen University	2004	2.0
MSc thesis Jacob Olwo, Wageningen University/Ghent University	2005	2.0
MSc thesis Sophoan Khong, Wageningen University/Ghent University	2006	2.0
MSc thesis Sydney Kabembwa, Wageningen University/Ghent University	2006	2.0
MSc thesis Heling Zhao, Wageningen University/Ghent University	2007	2.0
MSc thesis Bastiaan Knegtel, Wageningen University	2007	2.0
MSc thesis Norulhuda Mohamed Ramli, Wageningen University	2007	2.0
Subtotal Didactic Skills Training		**15.4**
Management Skills Training		
Membership of boards and committees		
Board member of the European Aquaculture Society Student Group	2007-8	2.0
Subtotal Management Skills Training		**2.0**
Education and Training Total		**52**

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Pascal G. van de Nieuwegiessen was born on the 23rd of December, 1978, in Waalwijk, The Netherlands. He studied Animal Sciences at Wageningen University, Wageningen, The Netherlands from 1997 to 2003. During his study he specialized in aquaculture and fisheries. For his minor thesis at the Cell Biology and Immunology Group of Wageningen University he studied the use of a WEHI-164 bioassay to measure the activity of tumor necrosis factor in common carp (*Cyprinus carpio* L.). For his major thesis at the Aquaculture and Fisheries Group of Wageningen University he worked on the development of a test to distinguish coping styles in African catfish (*Clarias gariepinus*). In 2004 he started his PhD research at the Aquaculture and Fisheries Group of Wageningen University. This research resulted in the current thesis.

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