Individual variation in growth of African catfish *Clarias gariepinus*: a search for explanatory factors

Promotor

Prof. dr. J. A. J. Verreth

Hoogleraar in de Aquacultuur en Visserij

Wageningen Universiteit

Co-promotor

Dr. ir. J. W. Schrama

Universitair hoofddocent leerstoelgroep Aquacultuur en Visserij

Wageningen Universiteit

Promotiecommissie

Prof. dr. ir. M. W. A. Verstegen (Wageningen Universiteit)

Dr. W. G. P. Schouten (Agrotechnology and Food Innovations, WUR)

Prof. dr. Felicity Huntingford (University of Glasgow, Scotland)

Prof. dr. Anders Kiessling (Norwegian University of Life Sciences and Swedish University of Agricultural Sciences)

Dit onderzoek is uitgevoerd binnen de onderzoekschool

Wageningen Institute of Animal Sciences (WIAS)

Individual variation in growth of African catfish *Clarias gariepinus*: a search for explanatory factors

Catarina Isabel de Matos Martins

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. M. J. Kropff,
in het openbaar te verdedigen
op woensdag 12 october 2005
des namiddags om vier uur in de Aula.

Martins, C. I. M.
Individual variation in growth of African catfish *Clarias gariepinus*:
a search for explanatory factors

PhD thesis, Wageningen University, The Netherlands
With ref. – With summary in English, Dutch and Portuguese
ISBN: 90-8504-306-9

Aos meus pais, Isabel e Eduardo

À minha irmã, Ana

Ao Gonçalo

Contents

1

- CHAPTER -

General Introduction

Individual variation in the growth of fish

Growth is a complex process and is defined as an increase in size (as length, volume, mass, body composition) over time. Growth can differ between different species, strains or populations within the same species and between individuals within the same population. The higher the level of integration, the easier it is to analyse differences in growth and to understand the underlying explanatory factors (Fig. 1).

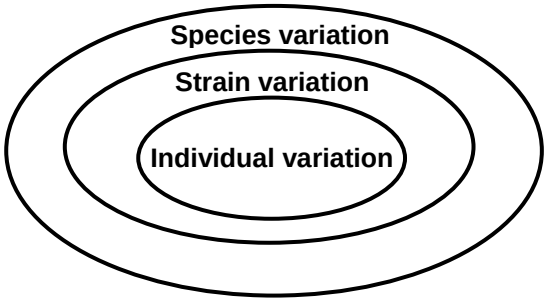


Figure 1. Different levels in which growth variation can be analysed. As the resolution increases, i.e., towards individual variation, the level of understanding decreases.

Within the present study, growth variation is studied at the level of individual animals. Among farmed animals, fish exhibit the largest individual variation in growth. According to Gjedrem (1997), the coefficient of variation (CV) for growth is 7-10% in farm animals, whereas in most fish species it is 20-35 %. In Table 1, examples of individual growth variation in various fish species are shown. Yet, most of the studies reporting data on growth do not take individual variation into account. The vast majority of growth trials involve the "feeding of the tank" and the investigation of "changes in bulk biomass" (Jobling and Baardvik, 1994). Usually a mean value is considered and although the variation around the mean is also mentioned, it is generally viewed as a sort of "statistical noise". The scarcity of data on growth performance at the level of individual organisms is strongly related to difficulty in measuring it. It requires complex techniques (e.g., individual tagging) and this implies extra labour and time. However, understanding the causes for individual variation in growth may result in the development of new management practices and improved production, contributing to the development of the aquaculture sector.

Table 1. Individual variation in growth reported for fish species. The coefficient of variation (CV = standard deviation/mean × 100) is an expression of the variability within a data set, providing a measure of individual variation.

Species	Growth expression	CV (%)	References
Brown trout (*Salmo trutta)*	Body weight (g)	18.3 - 23.6	Mambrini et al., 2004
Rainbow trout (*Oncorhynchus mykiss)*	Specific growth rate (% d^{-1})	31.1 - 166.1	Jobling and Koskela, 1996
		26.7 - 38.2	Gregory and Wood, 1998
		36.5	Dobly et al., 2004
Arctic charr (*Salvelinus alpinus)*	Specific growth rate (% d^{-1})	~16.0*	Jobling and Baardvik, 1994
Atlantic halibut (*Hyppoglossus hippoglossus)*	Specific growth rate (% d^{-1})	> 100.0 *	Kristiansen et al., 2004
		17.7	Fraser et al., 1998
Turbot (*Scophthalmus maximus)*	Specific growth rate (% d^{-1})	~30.0*	Irwin et al., 2002
	Body weight (g)	20.1 - 37.9	Imsland et al., 1996
Hybrid sunfish (*Lepomis cyanellus* x *Lepomis macrochirus*)	Specific growth rate (% d^{-1})	40.2	Wang et al., 1998
Chinese sturgeon (*Acipenser sinensis)*	Specific growth rate (% d^{-1})	31.7	Qian et al., 2002
Eel (elvers) (*Anguilla anguilla)*	Body weight (g)	37.2 - 46.6	Wickins, 1985
		27.9 - 32.2	
African catfish (*Heterobranchus longifilis)*	Body length (mm)	9.0 - 25.5	Ewa-Oboho and Enyenihi, 1999
Gilthead sea bream (*Sparus aurata)*	Body weight (g)	7.3 - 21.8	Goldan et al., 1998
Cod (*Gadus morhua)*	Specific growth rate (% d^{-1})	~100.0 *	Hart and Salvanes, 2000
		87.2	Lemieux et al., 1999
European perch (*Perca fluviatilis)*	Body weight (g)	37.7 - 54.1	Jourdan et al., 2000

* estimated using graphs

Why studying individual variation in growth?

Both for natural and farmed populations of fish, individual variation in growth may have serious consequences.

Under natural conditions, individual variation in growth may affect the survival (Letcher et al., 1996; van der Waal, 1998; Vilizzi and Walker, 1999), reproductive success (DeAngelis et al., 1979) and even the extinction risk of a population (Kendall and Fox, 2002).

Maximizing the production efficiency and producing animals of approximately the same market size are important goals of aquaculture (Noakes and Grant, 1992). The pronounced individual variation in growth observed in most of the farmed fish species can seriously hamper the achievement of these goals. Consequences of individual variation in growth for the aquaculture industry are:

- aggression and stress related to social hierarchies (Knights, 1987; Moutou et al., 1998; Nicieza and Metcalfe, 1999; Gregory and Wood, 1999);
- cannibalism (Baras, 1998; Baras et al., 2000; Baras and Fortuné d'Almeida, 2001);
- poor water quality due to an increase of food wastage (McDonald et al., 1996);
- The need for size-grading (Popper et al., 1992; Kamstra, 1993; Brzeski and Doyle, 1995, Seppä et al., 1999), which is labour intensive and stressful to fish.

However, the aquaculture industry also takes advantage of this size variation and develops selection programmes directed to the faster growing individuals. Fish show a response of 10-15 % increase in growth rate per generation to these selection programmes, resulting in a potential boost of the production (Gjedrem, 2000).

Research in fish is also affected by the pronounced individual variation in growth. A reduced inter-individual variation of measured values lowers the number of animals needed for a proper statistical experimental design (Beynen et al., 2001a). In the most common experimental animals, (e.g., mice and rats), such variability can be reduced by using genetically uniform animals (isogenetic strains) or specific pathogen free animals (SPF) (Beynen, et al., 2001b). However, in fish, isogenetic strains or SPF are still not common and therefore most of the experiments require relative high numbers of animals to enable and detect treatment effects.

Causes of individual variation in growth

Individual differences in growth may result from individual differences in **feed intake** (Koebele, 1985; Umino et al., 1997), or in **feed efficiency** (Metcalfe, 1998; Qian et al., 2002) (or a combination of both). Several factors have been suggested to influence individual variation in growth (see Table 2). However, for most fish species, it is not known to which extent individual differences in growth are caused by differences in respectively feed intake or feed efficiency. Furthermore, the knowledge on the factors influencing feed efficiency is more limited than the factors influencing feed intake.

Very often, social hierarchies are regarded as the main reason for differences in growth (Jobling, 1985; Koebele, 1985). A **social hierarchy** is typically described by the presence of dominants (usually the largest individuals) and the subordinates (usually the smallest individuals). Dominant fish can monopolize a disproportionate share of the available resources, resulting in faster growth of these fish compared to subordinates (Koebele, 1985; Metcalfe, 1986; Metcalfe et al., 1989; Jobling and Baardvik, 1994; Sloman et al., 2000). Social hierarchies can also affect feed efficiency. Abbot and Dill (1989) showed that subordinates grow slower than dominants even when both consumed equal rations, suggesting differences in feed efficiency between individuals with a different social rank. On the other hand, Sloman and Armstrong (2002) suggested that the physiological effects of dominance hierarchies are the result of laboratory artefacts and not of natural phenomena. The traditional theory that individuals grow faster because they are dominant has been questioned and a different approach has been presented: individuals compete for dominant status because they are fast growers, i.e., when the physiology of an individual has been set for fast growth then it is more likely to compete aggressively for high social status since more resources are needed to sustain a fast growth rate (Metcalfe et al., 1995). The latter hypothesis suggests that besides environmental factors (e.g., social interaction), also inherent, genetic factors may play a role in developing differences in individual growth rate. This hypothesis was further supported by the results of Wang et al. (1998) and of Qian et al. (2002) who found individual differences in feed intake and feed efficiency in fish housed individually (thus in the absence of social interaction).

Table 2. Possible explanatory factors for the individual variation in the growth of fish.

Explanatory factors	References
Maternal effects	Marteinsdottir and Steinarsson, 1998; Metcalfe and Thorpe, 1992; Kestemont et al., 2003
Feeding procedures	McCarthy et al., 1992; Jobling and Koskela, 1996; Gélineau et al., 1998; Kristiansen, 1999, Mambrini et al., 2004
Stocking density	Seymour, 1984; Jobling and Baardivk, 1994
Temperature	Imsland et al., 1996; Kestemont et al., 2003
Water currents	Jobling and Baardvik, 1994
Daylenght	Jourdan et al., 2000; Kestemont et al., 2003
Social hierarchy	Metcalfe et al., 1989; Jobling and Baardvik, 1994
Cannibalism	Baras, 1998
Standard Metabolic Rate	Metcalfe et al., 1995; Cutts et al., 1998; Cutts et al., 2001
Oxygen utilization	Nævdal et al., 1992; Salvanes and Hart, 2000; Bang et al., 2004
Protein turnover	McCarthy et al., 1994; Morgan et al., 2000; Dobly et al., 2004
Digestive enzymes	Lemieux et al., 1999
Swimming activity	Qian et al., 2002
Behaviour	Knights, 1987; Huntingford et al., 1990; Salvanes and Hart, 1998, 2000; Greaves and Tuene, 2001; Sneddon, 2003
Stress response	Tort et al., 2001; Fevolden et al., 2002
Gender	Martin-Smith and Armstrong, 2002; Saillant et al., 2001

Differences in social rank among individuals may also cause differences in feeding behaviour and stress response. It is known that subordinate fish show an altered feeding behaviour including reduced feeding rate (Abbott and Dill, 1989; Metcalfe et al., 1990; Winberg et al., 1993; Huntingford et al., 1993; McCarthy et al., 1992), positioning on the less rewarding feeding sites (Kadri et al., 1996; Harwood et al., 2003), reduced activation of the feeding trigger (Alanärä et al.,1998) and reacting later towards feed (Hart and Salvanes, 2000) as compared to dominant fish.

Further, social hierarchies induce a stress response (see Box 1). Social stress is characterised by the subordinates exhibiting higher cortisol levels than the dominant fish (Pottinger and Pickering, 1992; Fox et al., 1997). Gregory and Wood (1999) suggested that the cortisol elevation observed in subordinate fish may decrease their feed intake and feed efficiency. Not only the basal values of cortisol (reflecting a chronic, social stress) but also the

ability to cope with an acute stress seems to be affected by the social rank (Øverli et al., 1999; Sloman et al., 2002).

Individual differences in feeding behaviour and stress response may also be genetically linked. Using transgenic coho salmon (*Oncorhynchus kisutch),* Sundström et al. (2003) showed that during feeding the vertical position occupied by some individuals in the tank was a consequence of inherent differences in feeding motivation. Also the swimming behaviour and the feeding behaviour of rainbow trout *Oncorhynchus mykiss* seem to be under some genetic influence (Valente et al., 2001a). These authors suggested that behavioural differences during first feeding could explain differences in growth between fast and slow growing strains.

In the same way, some studies suggest that differences in stress response as indicated by different levels of plasma cortisol, may not necessarily be the consequence of social ranks. Consistently high and low stress responders have been identified in several species such as sea bream *Sparus aurata* (Tort et al., 2001), Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* (Fevolden et al., 1991; Pottinger et al., 1992; Pottinger and Carrick, 1999), suggesting that the stress response is a stable individual characteristic. In fact, quantitative studies have shown that the heritability of the cortisol response to stress in rainbow trout is moderate to high (Fevolden et al., 1999; Pottinger and Carrick, 1999), leading to its use in breeding programmes (Pottinger and Carrick, 1999).

In conclusion, literature shows that variation in individual growth rate is often caused by a combination of differences in feed intake and feed efficiency. Yet it is unclear to which extent both contribute to the final variation. Further, although the general assumption is that these differences in individual growth rate are related to underlying social hierarchies, literature shows that besides social interaction, genetic factors may also contribute to the final differences. Finally, it also seems that differences in feeding behaviour and stress response may play a role, either as inherent genetically linked traits of individuals or mediated through differences in social status (or a combination of both). For this reason, among the factors presented in Table 2, this thesis will focus on **(feeding) behaviour and stress response**.

Box 1. ***Stress response in teleost fish***

Teleost fish respond to stressful situations by activating two major pathways (the primary stress response): the hypothalamic-pituitary-interrenal (HPI) axis and the hypothalamic-sympathetic-chromaffin cell axis (Wendelaar Bonga, 1997). The primary stress response includes the rapid release of stress hormones, such as catecholamines and cortisol, into the circulation. The immediate and long-term actions of these hormones originates the secondary and tertiary stress responses which include changes in intermediary metabolism, hydromineral balance and immune functions (Barton and Iwama, 1991).

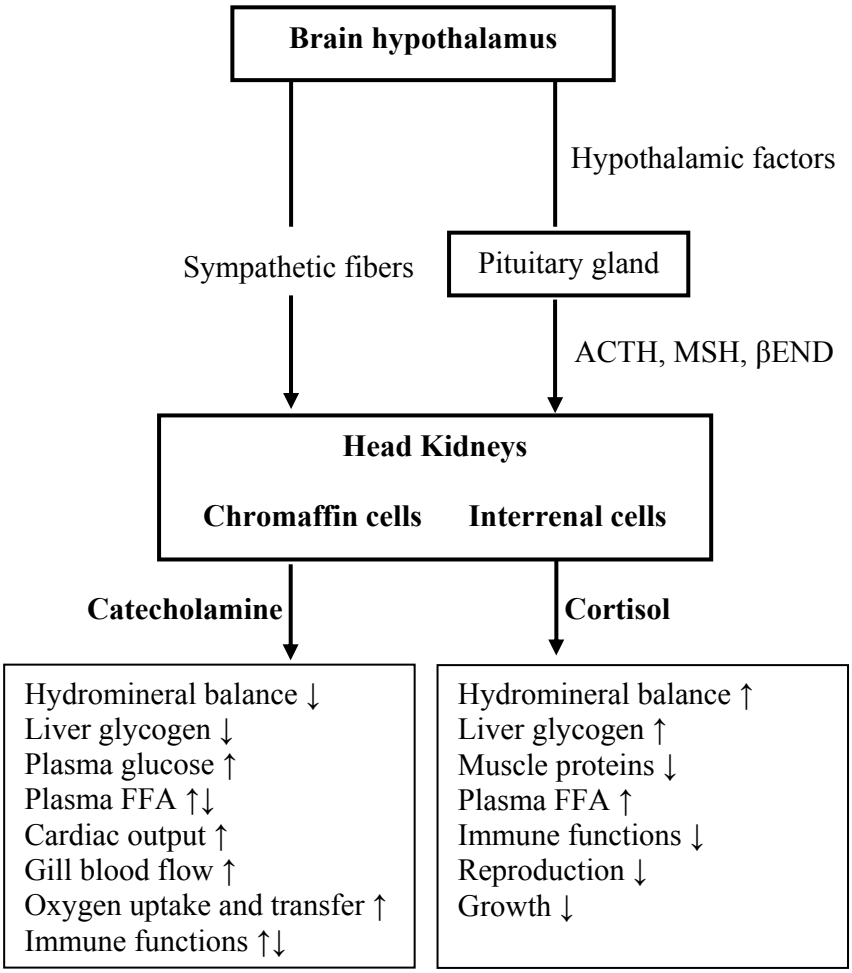


Figure Box 1. The stress response in teleost fish. ACTH, adrenocorticotropic hormone; MSH, melanophore-stimulating hormone; β-END, β-endorphin; FFA, free fatty acids; ↑, stimulatory; ↓ inhibitory (adapted from Wendelaar Bonga, 1997).

This Thesis

The general aim of this study is to understand the underlying factors responsible for the individual variation in growth of African catfish. The following factors will be investigated: 1) if individual variation in growth is mainly a consequence of social hierarchies, 2) the contribution of individual differences in feed intake and feed efficiency to the individual differences in growth and 3) the contribution of feeding behaviour and stress response in explaining individual differences in feed efficiency (Fig. 3).

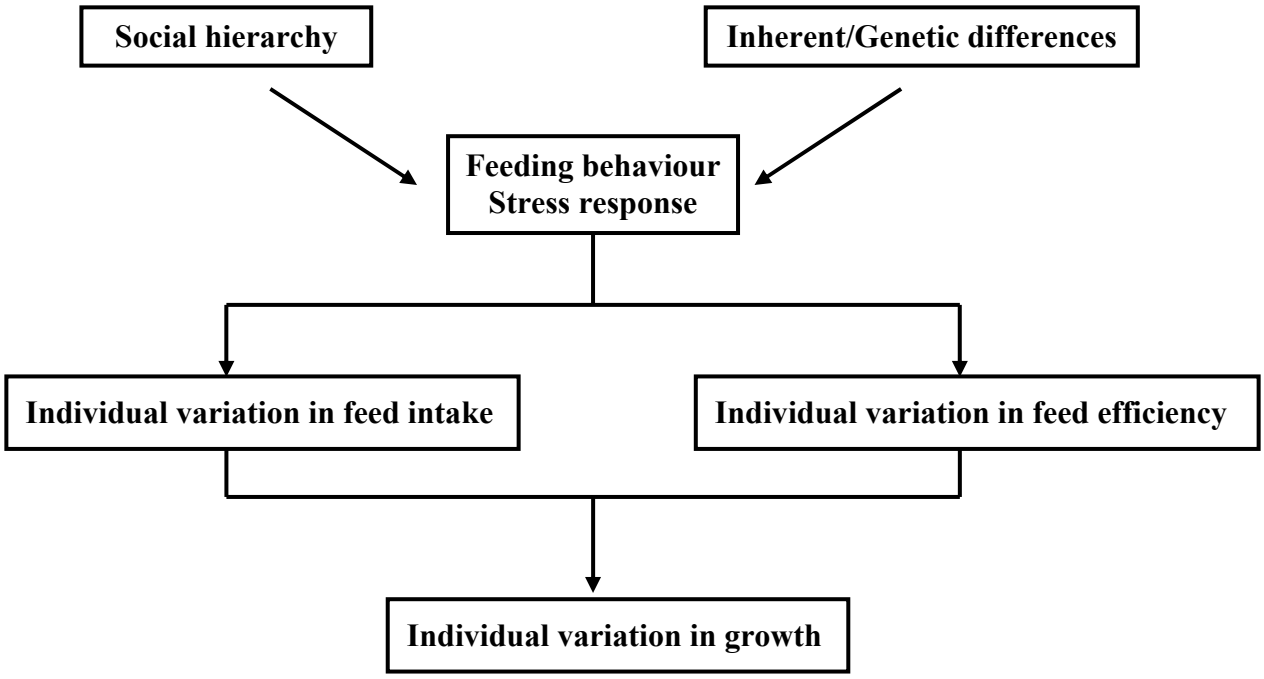


Figure 3. Summary of the factors investigated in this thesis that may explain individual variation in the growth of African catfish.

Chapters 2 and **3** investigate the importance of social hierarchy as an explanatory factor for the individual variation in growth of African catfish. This is done by comparing the growth performance (Chapter 2), behaviour (feeding behaviour: Chapter 2; aggression: Chapter 3) and stress response (Chapter 3) between groups of low-, medium- and heavy-weight fish. These chapters, by using an experimental design similar to a grading procedure allow the evaluation of grading in the farming of African catfish. Chapters 2 and 3 suggest that individual variation in growth of African catfish does not result from social hierarchies but rather from inherent differences (e.g., feeding behaviour). To further investigate the importance of inherent differences in growth variation, a set of experiments were designed using individually housed fish. The use of individually housed fish offers the advantage of studying individual differences in the absence of social interactions and to measure individual feed intake accurately. However, it raises the question whether the results obtained from housing fish individually are representative of a group housing situation. **Chapter 4** compares the growth performance, feeding behaviour and stress response of isolated and non-isolated fish and part of the fifth chapter compares the growth of fish housed individually and afterwards in a group. Chapters 5, 6 and 7 use individually housed fish to supply experimental data on inherent factors responsible for individual variation in growth. **Chapter 5** quantifies individual differences in performance traits and feeding behaviour and focuses on the repeatability of such individual differences. This experiment shows that differences in feed intake are the main determinant for growth variation but also that variation in feed efficiency is partially explaining differences in growth. This hypothesis is further investigated in **Chapter 6** using restrictively fed fish. Chapter 6 also tests if individual differences in feeding behaviour explain differences in growth by affecting feed efficiency (measured as the residual feed intake, see Box 2). In **Chapter 7**, individual differences in basal and post-stress levels of glucose, lactate and cortisol are investigated and related to individual differences in feed efficiency. In **Chapter 8**, the overall results from this thesis are discussed together with suggestions for the underlying mechanisms involved in the individual variation in growth of African catfish, implications for the aquaculture industry and suggestions for further studies.

Box 2. ***Measuring feed efficiency in fish***

There are various forms to express the efficiency at which body mass is accreted (or nutrient inputs are utilized). The notation which has the strongest roots both in ecology and physiology is the so-called K_1 or growth efficiency (Production/Intake). In physiological terms, it reflects the % retained mass (energy) per unit of mass (energy) consumed. Because in the conditions of practical fish farming or simple feeding experiments, feed consumption is hardly measurable at individual level. Therefore, in those conditions the common practice is to define feed efficiency as the gain in the wet body weight per unit of feed fed (**FCR**, feed conversion ratio). This is not a true efficiency parameter since it relates parameters with a different dimension (wet body weight gain vs dry feed intake). In addition, feed conversion ratio, being a ratio, has an irregular statistical behaviour (Iwaisaki and Wilton, 1993), hampering its use in analysing correlations with other traits (Silverstein et al., 2005), such as feeding behaviour and stress response. Therefore, Doupé and Lymbery (2004) and Silverstein et al. (2005), applied the term residual feed intake (**RFI**) which can be used as an indirect parameter to describe feed efficiency in fish populations, and which (not being a ratio) does not create statistical difficulties. Residual feed intake is defined as the difference between actual feed intake and that predicted from mean observed requirements for growth and body weight maintenance (Koch et al., 1963; see example Fig. Box 2).

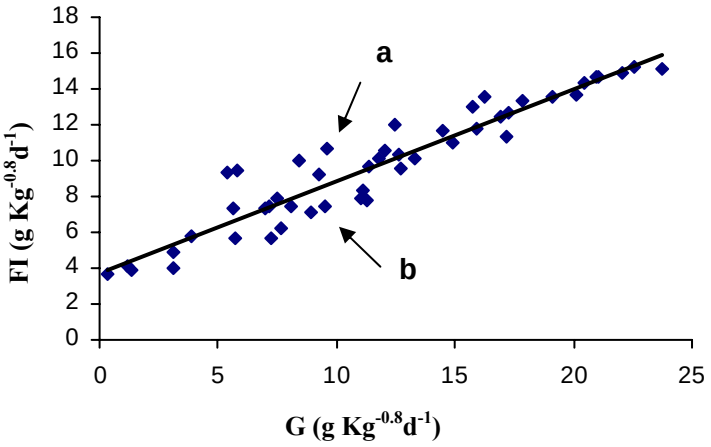


Figure Box 2. Relationship between feed intake (FI) and growth (G) for 48 juveniles of African catfish housed individually. Each point in the graph represents one fish. The line represents the expected relationship between FI and G. Fish "**a**" and "**b**" have the same G but are different regarding FI. Fish "a" has a positive RFI and fish "b" a negative RFI being respectively an inefficient and an efficient fish within the population.

The biological model: African catfish *Clarias gariepinus* (Burchell 1822)

African catfish was used as the biological model for this thesis (Fig. 4) since it is known to exhibit pronounced individual variation in growth both under natural and aquaculture conditions (van der Waal, 1998). This freshwater species has a remarkable geographical distribution from South Africa to minor Asia (de Graaf and Janssen, 1996) and combines biological characteristics that make it an ideal aquaculture species. African catfish is a fast growing species, resistant to diseases and to handling stressors. It is also an air-breather and therefore very tolerant to low oxygen levels in the water. This species is farmed on a commercial and subsistence level. Its total production is 10 471 metric tons with the main producers being Nigeria (4 024 metric tons) and the Netherlands (3 200 metric tons) (FAO, 2003).

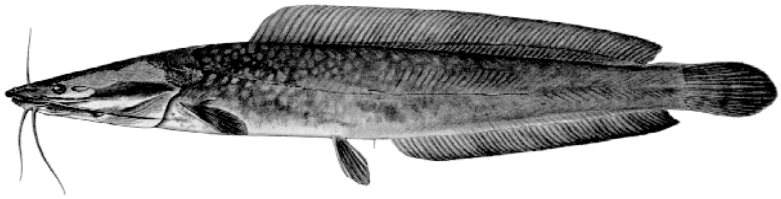
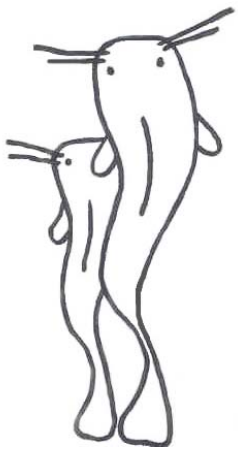


Figure 4. The biological model used in this thesis: African catfish *Clarias gariepinus* (Burchell 1822)



2

- CHAPTER -

Size distribution in African catfish (*Clarias gariepinus*) affects feeding behaviour but not growth

C. I. M. Martins

M. Aanyu

J. W. Schrama

J. A. J. Verreth

Aquaculture, 2005, in press

ABSTRACT

The goal of this study is to evaluate the effect of size distribution on growth performance and feeding behaviour in juveniles of African catfish. Two thousand sibling fish were grown for 8 weeks until the start of the experiment. Afterwards fish were individually weighed, tagged and manually selected. Four treatments were established according to similarity in weight (n=108 per treatment, mean ± SD): homogeneous low-weight (L, 83.0 ± 8.2 g), homogeneous medium-weight (M, 140.2 ± 8.7 g), homogeneous heavy-weight (H, 198.0 ± 8.1 g) and a heterogeneous group (HET, 139.7 ± 48.4 g). During 27 experimental days, fish were fed ad libitum twice per day. Fish behaviour (resting and swimming activity, waiting-in-feeding area, total feeding time, TFT and feeding rate, FR) was studied using video cameras and by direct observation on days 2, 4, 8, 11, 15, 18 and 26. Growth rate, feed intake and feed conversion ratio did not differ significantly between the treatments. There were also no significant differences between fish of similar weight reared in homogeneous and heterogeneous groups. The H treatment had significantly higher swimming activity (82.8 ± 6.4 %) and waiting-in-feeding-area behaviour (51.5 ± 11.2 %) than the L treatment (70.3 ± 9.3 % and 26.9 ± 5.7 %, respectively). The TFT (min) and FR (g min^{-1}) were lower in L (3.2 ± 0.2 min and 23.8 ± 1.05 g min^{-1}) than in M (4.7 ± 0.1 min and 29.4 ± 0.82 g min^{-1}) and H (5.5 ± 0.1 min and 29.7 ± 2.3 g min^{-1}) treatments. Overall, this study indicates that size distribution does not affect growth performance. Low-weight fish do not exhibit increased growth rates in the absence on heavier fish. The use of grading in juveniles of African catfish should therefore be re-considered. The differences in weight observed in this study seem not to be a direct consequence of social hierarchies where the larger fish suppress the growth of smaller fish. Instead, feeding behaviour seems to be an important factor, with heavier fish exhibiting feeding behaviours that may give advantage when feed is limited.

INTRODUCTION

Most of the fish species reared in aquaculture exhibit considerable growth (weight) variation. Knowing how to manage individual variation in growth and feed intake contributes to maximize the production efficiency by reducing food waste and improving water quality (McCarthy et al., 1992; Jobling and Baardvik, 1994; McDonald et al., 1996). In some species, growth heterogeneity has also been associated with cannibalism and therefore with mortality (Baras and Fortuné d'Almeida, 2001; Baras and Jobling, 2002). The establishment of social hierarchies has been identified as the most important factor responsible for the growth variation observed in group-housed fish (Metcalfe, 1986; Metcalfe et al., 1989; Johnsson 1997). Dominant fish are usually considered the larger fish which have a suppressive effect on the growth and feed intake of the subordinate (smaller) fish (Cutts et al., 1998). One procedure that is routinely performed in aquaculture to minimize growth variation is grading. Grading changes the group composition from heterogeneous to homogeneous groups of different size-classes. In this way it is assumed that social hierarchy is disrupted and the small fish have the opportunity to compensate their growth in the absence of larger fish (Jobling, 1982, 1995; Knights, 1987). Several studies have tested this assumption. The results of these studies are contradictory. For some species it has been shown that indeed small fish compensate their growth resulting in a biomass gain (Gunnes, 1976; Popper et al., 1992; Brzeski and Doyle, 1995; Seppä et al., 1999). However for several other species there was no beneficial effect on the growth of small fish (Jobling and Reinsnes, 1987; Wallace and Kolbeinshavn, 1988; Baardvik and Jobling, 1990; Kamstra, 1993; Carmichael, 1994; Sunde et al., 1998). Therefore, it has been suggested that growth heterogeneity is not necessary linked with the establishment of social hierarchies and that other factors such as differences in physiological responses may be responsible (Jobling and Reinsnes, 1986; Wickins, 1987; Sunde et al., 1998).

African catfish exhibits considerable growth variation both in aquaculture and in nature (Grobler et al., 1992; van der Waal, 1998). The causes of such variation are still not clear, although it has been suggested that inherent (i.e., genetically linked) differences in feeding behaviour may contribute to this variation (Martins et al., 2005a). Other studies have also identified the importance of inherent differences in feeding behaviour to explain differences in growth (Valente et al., 2001a; Sundström et al., 2003). Grading is commonly performed in African catfish once at a size of about 300g or twice at 150g and 350g (Verreth and Eding, 1993). However, the consequences of reducing heterogeneity in growth by grading

on performance have not yet been evaluated on this species. Moreover, to our knowledge the effect of group composition on feeding behaviour has not yet been evaluated for any species. Therefore, the aim of this study is to determine the effect on growth, feed intake, feed efficiency and feeding behaviour of changing the initial weight variation from heterogeneous to homogeneous groups of low-, medium- and heavy-weight fish. Understanding these effects will contribute to draw conclusions not only on the usefulness of grading in juveniles of African catfish but also more generally on the causes of growth variation.

MATERIAL AND METHODS

Fish stocks and rearing conditions

Two thousand sibling fish with an average weight of 8.2 ± 1.2 g (mean ± SD) were obtained from a local catfish producer (Fleuren, Someren, The Netherlands) where they experienced common housing and feeding history. On arrival at Wageningen University, the fish were transferred to 120-l tanks (90 × 45 × 45 cm), each containing 250 fish. All tanks were connected to a shared recirculation system. A 12-h light/dark photoperiod was maintained with daybreak set at 0700 h. The water flow rate (5 l min^{-1}), water temperature (24.9 ± 0.8 °C), pH (ranged between 6.95 and 7.93), O_2 concentration (>5 mg l^{-1}) and conductivity (3.5 ± 0.08 mS cm^{-1}) were checked daily. Fish were grown for a 8-week period until the start of the experiment. During this period, the fish were fed a commercial catfish diet (2 mm Trouvit; 49 % protein, 11 % fat, 1.5 % crude fibre and 11.5 % ash) using a restricted feeding level of 20 g $kg^{-0.8}$ per day. Feed was delivered using an automatic feeding belt over a period of 20 h per day starting at 0800 h.

Experimental design

Eight weeks after arrival, the fish were individually weighed and manually selected. Four treatments were established according to similarity in weight (n = 108, mean ± SD; minimum–maximum weights): homogeneous low-weight (L, 83.0 ± 8.2 g, 66.5–94.9 g), homogeneous medium-weight (M, 140.2 ± 8.7 g, 125.4–154.9 g), homogeneous heavy-weight (H, 198.0 ± 8.1 g, 184.5–214.1 g) and a heterogeneous group containing one-third of each of the groups mentioned above (HET, 139.7 ± 48.4 g, 65.2–214.3 g). The low-, medium- and heavy-weight treatment groups were homogeneous, i.e., individuals in each treatment group

had similar weight (coefficient of variation, CV < 10 %). In contrast, the heterogeneous group had a wider weight distribution (CV = 35 %). Dominance rank is often positively associated with body size (Nakano, 1995). Therefore, it was expected that, in the heterogeneous group, the social rank of each individual (if present) would be clear from the start of the experiment. Each treatment was divided into three replicates and each replicate consisted of 36 fish. Fish were individually tagged (ID 100A Micro, Trovan, Eid Aalten, The Netherlands) to examine individual growth and to determine if low- and medium-weight fish exhibited compensatory growth when reared in the absence of heavier, supposedly dominant fish. Each fish received one tag (2.12 ×11.5 mm) inserted under the skin by an implantable transponder with lancet. All fish were randomly allocated to the tanks according to their weight class. Weighing and tagging were done under anaesthesia (0.4 g l^{-1} of tricaine methanesulfonate, TMS Crescent Research Chemicals, Phoenix, Arizona, USA using 0.8 g l^{-1} of sodium bicarbonate as buffer). It was not possible to identify the tag number of fifteen fish during the experiment due to a technical failure of the reader. The total experimental period was 27 days. During this period, fish were fed ad libitum twice per day, in the morning (0800 h) and in the afternoon (1700 h). Sinking pellets of 4.5 mm were used to facilitate feeding behaviour observations (see later).

Quantification of behaviour

Fish behaviour was studied using video cameras placed in front of each tank on days 2, 4, 8, 11, 15, 18 and 26. During these days, behaviour was recorded before feeding (from 0730 to 0800 h and from 1630 to 1700 h) and during feeding (from 0800 to 0830 h and from 1700 to 1730 h). Three behaviour elements were monitored during video analysis: swimming, resting and waiting-in-feeding-area. The feeding area was defined as the area immediately under the funnel where the pellets were added. Direct observations were also done on the same days as the video recordings to assess the total feeding time (TFT, min) and feeding rate (FR, g min^{-1}). Total feeding time was defined as the time between the first and the last pellet being consumed by the group. FR was calculated as the amount of feed given divided by the TFT.

Preliminary observations of the video recordings indicated no differences in behaviour between the morning and the afternoon feeding. Therefore, only the morning feeding was selected for further behavioural analysis.

Behavioural observations were done every minute for the first 10 min of recording before and during feeding. The number of fish displaying swimming, resting and waiting-in-

feeding-area behaviours was counted as well as the total number of fish visible on the monitor. The behaviour elements were expressed as a percentage of the total number of fish counted. One sampling point consisted of the mean of ten percentage values (one percentage value was obtained every minute).

Data analysis

Larger fish have a greater absolute requirement of food for maintenance than smaller fish (Hepher, 1988). Therefore, expressing performance per metabolic body weight minimises the differences in maintenance levels between smaller and larger fish. Growth rate (GR, g $kg^{-0.8}$ d^{-1}) was calculated using the geometric body weight (BW_g) of each animal: GR=weight gain $BW_g^{-0.8}$ d^{-1} and $BW_g = \exp\{½[\ln(W_{T1}) + \ln(W_{T2})]\}$ where W_{T1} is the weight (g) at time (t) 1 and W_{T2} the weight at time 2.

Feed intake was also expressed per metabolic body weight (g $kg^{-0.8}$ d^{-1}). Feed efficiency was analysed using the feed conversion ratio (FCR) which was calculated as the feed intake (FI, g $kg^{-0.8}$ d^{-1}) divided by the growth rate (GR, g $kg^{-0.8}$ d^{-1}).

Statistical analyses were performed using SPSS 11.5 for Windows. All percentage data were arcsin transformed before further analysis. Homogeneity of variance was tested using Levene's F test (Field, 2000). Behavioural data obtained in different days were pooled. A non-parametric method was chosen to compare the swimming and resting behaviours during feeding and the TFT (Kruskal–Wallis test followed by groupwise comparisons by the Games–Howell test) since the variance was different between groups (Levene's test, $P < 0.05$). Possible differences in performance, survival and behavioural data (except the behaviours mentioned above) between treatments were tested with one-way ANOVA followed by a Tukey multiple comparison test. A Student's t- test was used to compare differences in growth rate between fish of similar weight reared in homogeneous and heterogeneous groups. Regression analysis was performed between the initial and final weight of the homogeneous (L, M and H) and heterogeneous groups. The slopes of the regression equations were compared by analyses of covariance to determine if compensatory growth took place in the homogeneous groups. Statistical significance was taken at $P < 0.05$. All the results are expressed as mean ± SD.

RESULTS

Performance

The comparison of performance data (initial and final body weight, growth rate, feed intake and feed conversion ratio) between treatments is presented in Table 1. As expected, initial body weight differed significantly between the treatments ($P < 0.001$). Multiple comparison tests showed significant differences between all the treatments, except between M (138.5 ± 1.0 g) and HET (138.7 ± 1.8 g) ($P = 0.88$). Final body weight was also significantly different between treatments ($P < 0.001$). Fish in the L treatment group had a significantly lower final body weight than all the other treatments ($P < 0.01$) while fish in the H treatment had a significantly higher final body weight than fish in the L ($P < 0.001$) and HET ($P < 0.05$) treatments. GR, FI and FCR did not differ significantly between the treatments ($P > 0.05$).

There was no significant difference in the survival rate between treatments (only two fish died, from the M and HET treatments) ($P > 0.05$).

Table 1. Performance (initial and final body weight, growth rate (GR), feed intake (FI), feed conversion ratio (FCR)) and survival data of juveniles of African catfish as affected by the treatments over a 27-day experimental period.

Performance	Treatments				*P* value
	L	M	H	HET	
Initial body weight (g)	82.4 ± 2.7^{a}	138.5 ± 1.0^{b}	192.6 ± 4.0^{c}	138.7 ± 1.8^{b}	<0.001
Final body weight (g)	202.2 ± 58.8^{a}	369.45 ± 19.5^{bc}	452.68 ± 19.4^{b}	349.6 ± 16.1^{c}	<0.001
GR (g kg$^{-0.8}$ d^{-1})	27.2 ± 1.2	28.1 ± 1.9	25.6 ± 1.8	26.2 ± 1.5	ns
FI (g kg$^{-0.8}$ d^{-1})	22.7 ± 1.0	24.4 ± 0.7	23.0 ± 0.8	23.6 ± 0.5	ns
FCR	0.8 ± 0.03	0.9 ± 0.03	0.9 ± 0.04	0.9 ± 0.04	ns
Survival rate (%)	100 ± 0.0	99.1 ± 0.5	100 ± 0.0	99.1 ± 0.5	ns

L, homogeneous low-weight; M, homogeneous medium-weight; H, homogeneous heavy-weight; HET, heterogeneous. Data are shown as the mean ± SD (n = 3 tanks/treatment). ns, not significant ($P > 0.05$). Means within a row lacking a common superscript letter differ significantly ($P < 0.05$).

The GR was also compared between similar-weight fish reared in homogeneous and heterogeneous groups (Table 2).

Table 2. Growth rates (GR, g $kg^{-0.8}$ d^{-1}) of low-, medium- and heavy-weight fish reared either in homogeneous or heterogeneous groups in juveniles of African catfish grown for 27 days.

	Homogeneous	Heterogeneous	*P* value
Low	26.3 ± 7.0 (105)	27.0 ± 7.8 (34)	ns
Medium	27.3 ± 6.0 (108)	26.5 ± 6.1 (35)	ns
Heavy	24.6 ± 5.5 (103)	24.8 ± 3.7 (32)	ns

Data are shown as the mean ± SD (n number of animals). ns, not significant ($P > 0.05$).

There were no significant differences in GR for the low- ($P = 0.885$), medium- ($P = 0.706$) and heavy-weight ($P = 0.844$) fish. Low-weight animals did not exhibit any kind of compensatory growth as shown by similar slopes of the regression equations (initial vs final body weight) obtained for the heterogeneous (Fig. 1A) and the combined homogeneous groups (Fig 1B). The estimated slopes were not significantly different (1.92 ± 1.3 for the heterogeneous group and 1.94 ± 1.4 for the combined homogeneous groups, $P > 0.05$).

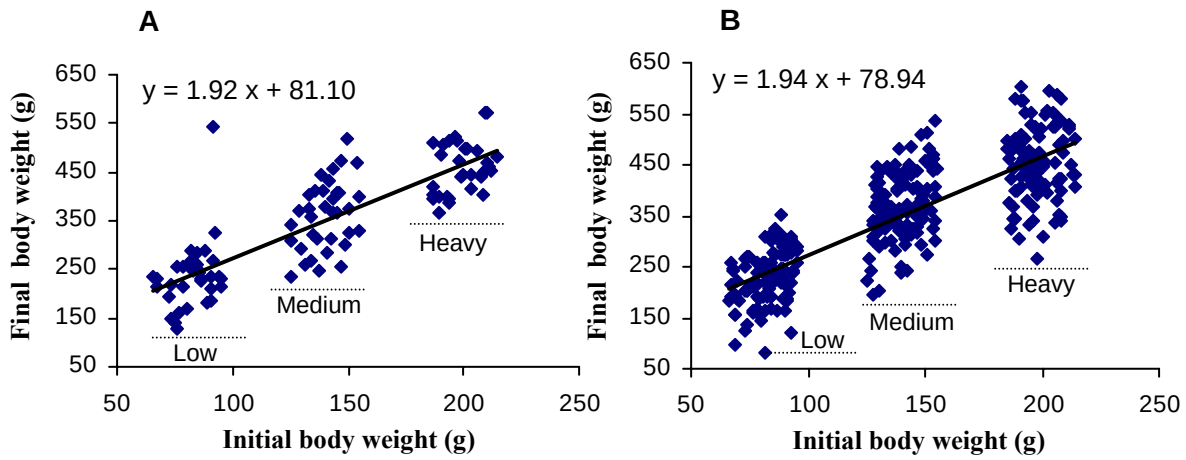


Figure 1. Relationship between initial and final body weight of the heterogeneous group (A) and the combined homogeneous groups (B) in juveniles of African catfish grown for 27 days.

Behaviour

The four experimental treatments were significantly different for all behaviour elements analysed ($P < 0.05$, Table 3). The comparison between the homogenous treatments (L, M and H) revealed that there was an increase in swimming activity and waiting-in-feeding-area behaviour with the weight increase of the treatments. The H treatment had significantly higher

swimming activity (and therefore lower resting) and waiting-in-feeding-area behaviour than the L treatment. This was verified before feeding ($P = 0.004$ for swimming activity; $P = 0.001$ for waiting-in-feeding-area) and more pronounced during feeding ($P = 0.002$ for swimming activity; $P < 0.001$ for waiting-in-feeding-area). In the H treatment, 51.5 % of the fish were present at the feeding area during the feeding period, whereas only 26.9 % of the fish in the L treatment were present at the same location.

Feed intake behaviour was also significantly different between the treatments (TFT, $P = 0.024$; feeding rate, $P = 0.003$). Fish in the L treatment had an absolute lower amount of feed intake than the M and H treatments. Therefore, the TFT in L was lower than in M ($P = 0.004$) and H ($P = 0.001$). When the feeding rate (g min^{-1}) is considered, the same differences were observed, with the low-weight animals (L) consuming lower quantities of feed per unit of time than medium- (M, $P = 0.007$) and heavy-weight fish (H, $P = 0.005$).

As shown before for the performance data, the behavioural data were also not significantly different between the M and HET treatments.

Table 3. Behaviour of African catfish as affected by the experimental treatments, before and during feeding over a 27-day experimental period

	Treatments				*P* value
	L	M	H	HET	
Before feeding					
Resting (%)	55.9 ± 11.8^{a}	56.2 ± 14.2^{a}	48.7 ± 13.3^{b}	53.7 ± 13.0^{a}	0.006
Swimming activity (%)	44.1 ± 11.8^{a}	43.8 ± 14.2^{a}	51.3 ± 13.3^{b}	46.3 ± 13.0^{a}	0.006
Waiting-in-feeding-area (%)	17.1 ± 6.6^{a}	19.5 ± 7.6^{ac}	26.8 ± 7.0^{b}	22.9 ± 8.2^{cb}	<0.001
Feeding					
Resting (%)	29.7 ± 9.3^{a}	19.0 ± 4.1^{b}	17.2 ± 6.4^{b}	20.0 ± 6.5^{b}	0.038
Swimming activity (%)	70.3 ± 9.3^{a}	81.0 ± 4.1^{b}	82.8 ± 6.4^{b}	80.0 ± 6.5^{b}	0.038
Waiting-in-feeding-area (%)	26.9 ± 5.7^{a}	43.9 ± 7.9^{b}	51.5 ± 11.2^{c}	42.8 ± 7.6^{b}	<0.001
Total feeding time (min)	3.2 ± 0.2^{a}	4.7 ± 0.1^{b}	5.5 ± 0.1^{c}	4.7 ± 0.8^{abc}	0.024
Feeding rate (g min^{-1})	23.8 ± 1.05^{a}	29.4 ± 0.82^{b}	29.7 ± 2.30^{b}	29.5 ± 1.26^{b}	0.003

Data are shown as the mean ± SD (n = 3 replicates/treatment, each replicate is the mean of days 2, 4, 8, 11, 15, 18 and 26). Means within a row lacking a common superscript letter differ significantly ($P < 0.05$).

DISCUSSION

In the present study, size distribution had no significant effect on the growth performance. Growth rates of similar-weight fish reared in both homogeneous and heterogeneous groups were not significantly different. This suggests that in this study, the presence of large fish does not affect the performance of small fish, probably because social hierarchy is not playing a major role in explaining weight (growth) variation in African catfish. Moreover, low-weight animals when reared in the absence of heavy-weight fish (in the case of the L treatment) did not exhibit any kind of compensatory growth. Therefore our study corroborates the conclusions of Doyle and Talbot (1986) which states that the increase of size variation over time is not caused by social interactions in which larger, dominant fish suppress the growth of smaller, subordinate fish. One may therefore suggest that the usefulness of grading African catfish to disrupt social hierarchies should be re-considered. However, this suggestion must be reserved for juveniles within the weight range used in this study (65.2 – 214.3 g). At the larval and early juvenile stages, African catfish is known to exhibit cannibalism (Hecht and Appelbaum, 1988). In this case, size-grading could be used as a tool to decrease growth heterogeneity and ultimately mortality.

The question still remains what causes the growth variation in African catfish. The present study suggests that feeding behaviour is an important factor responsible for these differences in weight. Although it was not possible to compare the behaviour of similar-weight fish reared in homogeneous and heterogeneous groups (like the comparison made for performance), in the heterogeneous groups the smallest animals were seen to eat from the bottom of the tank. When feeding, both low- (L) and heavy-weight fish (H) increased their swimming activity. However, heavy fish exhibited the highest swimming activities and waiting-in-feeding-area behaviour both before and during feeding. Moreover, heavy-weight fish (H) spent more time eating (higher TFT) and were faster in consuming their meal (higher FR) than low-weight fish (L). Valente et al. (2001a) obtained similar results when comparing slow and fast growing strains of rainbow trout, *Oncorhynchus mykiss*. Fish in the fast-growing strain were seen more often at the most rewarding location with respect to prey availability. The fast-growing strain also exhibited the highest swimming activity. Moreover, rainbow trout treated with growth hormone increased their feeding motivation and capacity for ingesting food (Johnsson and Björnsson, 1994; Jönsson et al., 1998). However, are these differences in behaviour related to social interaction or do they reflect inherent differences? Kadri et al. (1996) observed that dominant fish fed first, very near to the water surface, while

subordinate fish ate directly from the bottom of the cage. These differences in behaviour were also seen during the present study. Small catfish, grown in the absence of larger catfish (L treatment), exhibited the lowest swimming activity and waiting-in-feeding-area behaviour over the whole experimental period. This could reflect a carryover effect from the period before the start of the experiment. During this period small fish, if subordinate, could have spent more time on the bottom of the tank in an attempt to feed on leftover pellets. Øverli et al. (1998) suggested that the behaviour inhibition of subordinates can persist when dominants are no longer present. Alternatively, the observed differences in behaviour of African catfish may reflect inherent differences in behavioural strategies. There is now ample evidence that behavioural traits usually have a strong genetic component (Boake, 1994; Foster and Endler, 1999; Lahti et al., 2001). Studies made without the interference of conspecifics, i.e., with individually housed fish, showed that individual differences in growth and feed efficiency are related to differences in feeding behaviour (Martins et al., 2005a). This may suggest an inherent, genetic basis for the variability in feeding behaviour in juveniles of African catfish. Sundström et al. (2003) also suggested inherent differences in feeding behaviour in growth hormone transgenic coho salmon, *Oncorhynchus kisutch*. The differences in the ability to obtain the meal faster by eating faster and being at the most favourable areas of the tank, could have induced differences in growth when there is more competition for food (such as in the pre-selection period).

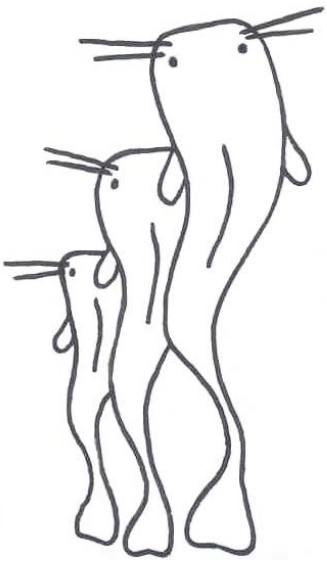
As shown by van der Waal (1998), African catfish exhibit wide growth variation in the wild. Such variation in growth seems to be related to a survival strategy of the species where fast growers can benefit under normal weather conditions and slow growers under extreme (desiccating) conditions. Although no relationship has been found in the wild between different feeding behaviour and growth rates, it has been shown that African catfish have sophisticated forms of social feeding behaviours (Bruton, 1979; Merron, 1993). If the heterogeneity in feeding behaviour observed in this study is indeed inherent, more studies should be done in the wild to understand the adaptive value of such variability.

In conclusion, this study indicates that size distribution does not affect growth performance. Low-weight fish do not exhibit increased growth rates in the absence on heavier fish. The use of grading in juveniles of African catfish is therefore questioned. The differences in weight observed in this study seem not to be a direct consequence of social hierarchies where the larger (supposedly dominant) fish suppress the growth of smaller (supposedly subordinate) fish. Instead, feeding behaviour seems to be an important factor,

with heavier fish exhibiting feeding behaviours that may give advantage when feed is limited such as being more active swimmers, spending more time at the feeding areas and eating their meal faster than low-weight fish.

Acknowledgements

This research was funded by the Foundation for Science and Technology, Portugal (grant # SFRH/BD/2946/2000). This work was also supported by the NWO (Den Haag, The Netherlands) under the project "Innovation of aquatic respirometry facilities", code 805-34.025. We would like to acknowledge Menno ter Veld, Sietze Leenstra, Aart Hutten and Wian Nusselder for their help in starting up the experiment. We also thank Ep Eding and Gonçalo Santos for technical advice during the experiment.



3

- CHAPTER -

The effect of group composition on the welfare of African catfish (*Clarias gariepinus*)

C. I. M. Martins

J. W. Schrama

J. A. J. Verreth

Applied Animal Behaviour Science, 2005, in press

ABSTRACT

Within fish farming grading, i.e., the process of sorting to approximate size, changes the group composition from heterogeneous to homogeneous. Although this procedure is considered an acute stressor, the long term consequences of grading on aggression and stress levels have not yet been investigated. The objective of this study was to quantify the consequences for welfare of rearing African catfish (*Clarias gariepinus*) in groups of homogeneous low-, medium- and heavy-weight fish as compared with a heterogeneous group. Two thousand fish were grown for 8 weeks. Afterwards, fish were individually weighed, tagged and manually selected. Four treatments were established according to weight (mean ± SD): homogeneous low- (L, 83.0 ± 8.2 g), homogeneous medium- (M, 140.2 ± 8.7 g), homogeneous heavy-weight (H, 198.0 ± 8.1 g) and a heterogeneous group (HET, 139.7 ± 48.4 g). Fish were sampled 3 times (days 13, 27 and 34) to determine aggression levels (number of skin lesions and percentage of fish bitten). Blood samples (for cortisol and glucose analysis) were taken at the end of the experiment before and after a stress test (netting). Despite all treatments exhibited high aggression levels (> 50 %), the H treatment exhibited the lowest aggression levels (58.34 ± 0.92 % of fish bitten as compared with 74.13 ± 2.52 % in L; 69.70 ± 4.38 % in M and 69.94 ± 3.29 % in HET, $P = 0.002$). Aggression significantly decreased over time in the M and H treatment but not in the L and HET treatments. In addition, heavy fish reared in heterogeneous groups exhibited higher number of skin lesions in the body (2.0 ± 0.1) than fish with similar weight reared in homogeneous groups (1.4 ± 0.1) ($P = 0.002$). Also, medium-weight fish showed lower cortisol values (before the stress test) in the homogeneous group (45.7 ± 7.5 ng ml^{-1}) as compared with medium-weight fish in heterogeneous groups (73.5 ± 13.3 ng ml^{-1}). The stress response in low-weight fish was also affected by the group composition. After a stress test, the glucose levels were higher for low-weight fish reared in homogeneous (7.1 ± 0.4 mmol l^{-1}) than in heterogeneous (5.1 ± 0.7 mmol l^{-1}) groups. This study suggested that the group composition affects welfare in African catfish, particularly in the L treatment where aggression levels were as high as 74 %. Medium- and heavy-weight fish seemed to benefit from living in homogeneous while low-weight fish from heterogeneous groups.

INTRODUCTION

Aquaculture is growing more rapidly than all other animal food production sectors (FAO, 2002). In contrast to other major forms of livestock production, there is a lack of scientific information on the welfare of fish raised under intensive aquacultural conditions (Chandroo et al., 2004). Intensive production of fish often involves factors such as grading, crowding, poor water quality and others which may hamper welfare (FSBI, 2002). Within fish farming, grading is performed for most fish species and it is identified as an important (acute) stressor (Barton and Iwama, 1991). Grading refers to the process of sorting to approximate size, i.e., fish being reared in heterogeneous groups are re-grouped into homogeneous groups according to size similarities (Conte, 2004).

Grading is believed to remove the effects of social hierarchy. Subordinate individuals are often characterized by having both chronically increased plasma cortisol levels and distinct changes in behaviour (for example, suppressed aggressive behaviour) (Adams et al., 1998; Øverli et al., 1999; Sloman et al., 2001a). It is expected that grading minimizes the stress imposed by the larger (supposedly dominants) over small individuals (supposedly subordinates), resulting in improved growth of small individuals and increased total biomass output (Gunnes, 1976; Popper et al., 1992; Brzeski and Doyle, 1995; Seppä et al., 1999). However, several studies have shown that size grading does not remove the effect of social hierarchy in all fish species (e.g., in Arctic charr (*Salvelinus alpinus)*, Baardvik and Jobling, 1990; in European eel (*Anguilla anguilla*), Kamstra, 1993; in channel catfish (*Ictalurus punctatus*), Carmichael, 1994; in turbot (*Scophthalmus maximus)*, Sunde et al., 1998; in Atlantic cod (*Gadus morhua),* Lambert and Dutil, 2001; in Atlantic halibut, Stefansson et al., 2000). This persistence of social hierarchy in spite of size grading, might be explained by a higher level of intraspecific competition and agonistic interaction in groups of fish with similar size (Knights, 1987; Baardvik and Jobling, 1990, Jørgensen and Jobling, 1993). Despite this interpretation, the studies mentioned above have focused on the consequences of grading on growth performance and not on the direct consequences on aggression and stress levels.

The African catfish (*Clarias gariepinus* Burchell, 1822) is one of the most studied species in the *Clariidae* family and is of great importance in both fisheries and fish culture (Teugels, 1996). As the majority of farmed fish, African catfish exhibits a strong differential growth rate leading the farmers to grade their fish once or twice during the production cycle (Verreth and Eding, 1993). The aim of this study is to investigate the consequences for

welfare of rearing African catfish in homogeneous size groups as compared with heterogeneous sized groups. Changes in aggression levels and stress physiology (plasma cortisol and glucose) were used as welfare indicators as an elevation of these parameters has been associated with impaired welfare (FSBI, 2002; Almazán-Rueda, 2004a).

MATERIAL AND METHODS

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

Fish stocks and rearing conditions

Two thousand sibling fish with 10 weeks of age and an average weight of 8.2 ± 1.2 g (mean ± SD) were obtained from a local catfish producer (Fleuren, Someren, The Netherlands) where they experienced common housing and feeding history. On arrival at Wageningen University fish were randomly distributed over 8 tanks of 120-l (90 × 45 × 45 cm), each containing 250 fish. All tanks were connected to a shared recirculation system. A 12L:12D photoperiod was maintained with daybreak set at 0700 h. The water flow-rate (5 l min^{-1}), water temperature (24.9 ± 0.8 ^{o}C), pH (ranged between 6.95 and 7.93), O_2 concentration (> 5 mg l^{-1}) and conductivity (3.5 ± 0.08 mS cm^{-1}) were checked daily. Fish were grown for a 8-weeks period until the start of the experiment. During this period fish were fed a commercial catfish diet (2 mm Trouvit; 49 % protein, 11 % fat; 1.5 % crude fibre and 11.5 % ash) using the restricted feeding level of 20 g $kg^{-0.8}$ d^{-1}. This feeding level was used to allow a satisfactory growth rate and to minimise the risk of ruptured intestine syndrome that may occur in small fish when higher feeding levels are used (Boon et al., 1987). Feed was delivered using an automatic feeding belt over a period of 20 h per day.

Experimental design

Eight weeks after arrival, fish were individually weighed and manually selected. Four treatments were established according to similarity in weight (n = 108 per treatment, mean ± SD; minimum – maximum weights): homogeneous low-weight (L, 83.0 ± 8.2 g, 66.5–94.9 g), homogeneous medium-weight (M, 140.2 ± 8.7 g, 125.4–154.9 g), homogeneous heavy-weight (H, 198.0 ± 8.1 g, 184.5–214.1 g) and a heterogeneous treatment (HET,

139.7 ± 48.4 g, 65.2–214.3 g). The heterogeneous treatment contained one third of the L, M and H treatments mentioned above. This was done to increase the contrast between fish inside the heterogeneous treatment and therefore facilitate the comparison of aggression and stress levels between fish of similar weight but reared in homogeneous and heterogeneous groups. The low-, medium- and heavy-weight treatments are homogeneous groups, i.e., individuals in each treatment have similar weight (coefficient of variation, CV < 10 %). In contrast the heterogeneous group has a wider weight distribution (CV = 35 %). In this way the heterogeneous group compares to a "normal" group of farmed catfish, since coefficient of variation of around 30% for final body weight has been described for groups of African catfish under commercial stocking densities (Almazán-Rueda, 2004a). Each treatment had three replicates. Each replicate consisted of a group with 36 fish. Unselected fish were killed with an overdose of anaesthesia (0.8 g l^{-1} of tricaine methanesulfonate, TMS Crescent Research Chemicals, Phoenix, Arizona, USA using 1.6 g l^{-1} of sodium bicarbonate as buffer). Selected fish (n = 432) were individually tagged (ID 100A Micro, Trovan, Eid Aalten, The Netherlands) to examine individual aggression and stress levels (see later). Each tag (2.12 ×11.5 mm) was inserted under the skin with a lancet. A portable reader (LID 500, Trovan, Eid Aalten, The Netherlands) was used to obtain a code for each tag. Tagging and blood sampling were done under anaesthesia (0.4 g.l^{-1} of tricaine methanesulfonate, TMS using 0.8 g l^{-1} of sodium bicarbonate as buffer). All fish were randomly allocated to new tanks (200-l, 87×58×46 cm) according to their weight class. During the experiment, two fish died (from the M and HET treatments). During the experiment fish were fed ad libitum (sinking pellets of 4.5 mm) twice per day, in the mornings (0800 h) and in the afternoons (1700 h).

Sampling procedures

Aggression

Aggression was determined indirectly by counting the number of skin lesions. Almazán-Rueda et al. (2004b) showed that in African catfish there is a strong correlation between the number of aggressive acts, as determined by direct observation and the number of skin lesions on the body. During the experimental period, fish were individually checked for skin lesions at day 13, 27 and 34. This was done by netting all fish from the tank, placing them on a wet surface and counting the number of skin lesions for the first and second sampling days. At the end of the experiment (day 34), skin lesions were counted after blood sampling (see latter).

Furthermore, the percentage of fish being bitten (i.e., having one or more skin lesion) was calculated per tank for each sampling day.

Stress response

Blood samples for plasma cortisol and glucose were taken from 20 fish per tank (240 samples in total) at the end of the experiment. From each tank, 10 fish were randomly selected and immediately put in anaesthesia (0.4 g l^{-1} of tricaine methanesulfonate, TMS Crescent Research Chemicals, Phoenix, Arizona, USA using 0.8 g l^{-1} of sodium bicarbonate as buffer). These samples were considered as controls and as an indicator of chronic stress. The other 10 fish were stressed by being held in a net outside the water for 1 h. This stress test represents an acute stressor and was used to investigate possible differences in the stress response between homogenous and heterogeneous groups. It should be noted that African catfish is able to breathe air, and previous experiments using emersion periods up to 3 h have been performed with no mortality reported (Buttle et al., 1996). Therefore, it is considered that 1-h air exposure is not an extreme stressor for African catfish. After the stress induction, fish were also anaesthetised for blood sampling. One ml of blood was collected from all fish by hypodermic syringe (containing 3 mg of Na_2EDTA) from the caudal blood vessels. This procedure was finalised within 3 min after fish were caught and anesthetised. The collected blood was placed in cooled 1.5 ml plastic tubes, mixed and centrifuged at 6000 *g* for 5 min at 4°C. After centrifugation plasma was collected and stored at -20°C for further analysis. All fish were killed with an overdose of anaesthesia after blood sampling and skin lesions were analysed (0.8 g l^{-1} of tricaine methanesulfonate, TMS Crescent Research Chemicals, Phoenix, Arizona, USA using 1.6 g l^{-1} of sodium bicarbonate as buffer).

Plasma analysis

Cortisol was measured in unextracted catfish plasma using the validated radio-immunoassay described by Ellis et al. (2004), but adapted by pre-heating the plasma in glutamate buffer (pH 3.3). The adaptation for direct assay originates from Dunn and Foster (1973) and Foster and Dunn (1974) and has been used widely in fish studies (e.g., Redding et al., 1984; Young, 1986; Bisbal and Specker, 1991). The catfish plasma (diluted 1:100 in glutamate buffer) was added to duplicate glass tubes for assay. Nine cortisol standards ranging from 2 to 500 pg 100 μl^{-1} were made up (in duplicate) by serial dilution in glutamate buffer. All tubes were heated in boiling water for 15 min, then cooled by standing in cold water for

5 min. The assay then proceeded as described by Ellis et al. (2004), i.e., addition of radioactive steroid and antibody, overnight equilibration, removal of unbound cortisol with dextran-coated charcoal, and scintillation counting.

Plasma glucose concentrations were measured on 20 µl aliquots using the GOD-Perid® method (GOD-PAP; Boehringer, Mannheim, Germany). Dilutions from a pure glucose solution were used to make a calibration curve. The 20 µl aliquots of plasma, standard or blanks were added to 250 µl of reagent containing GOD and incubated at 25 °C for 30 min. The absorbance of samples and standards was read against the blank at 690 nm. Plasma glucose concentration (mmol l^{-1}) was calculated using regression analysis from the linear calibration curve.

Data analysis

Tank was used as experimental unit. Therefore, statistical analysis was performed on mean values per tank. The percentage of fish bitten was arcsin transformed before further analysis. Homogeneity of variance was tested using Levene's *F* test (Field, 2000). To detect differences on the aggression parameters (mean number of skin lesions per fish and the percentage of fish bitten) between treatments, the mean values over the experimental period were analysed by one way ANOVA, followed by a Tukey test. Within treatments (group composition) the effect of time on the aggression parameters was tested with repeated measures ANOVA. The treatment effect on blood plasma values were tested separately for the control and netted fish by ANOVA, followed by a Tukey test. Within treatments the effect of netting procedure was test by a *t*-test. Additionally, the measured parameters (aggression and stress levels) in fish with similar weights but reared in homogeneous and heterogeneous groups were compared by a *t*-test. Pearson product-momentum correlation was used in analysing the relationship between the initial weight and skin lesions, cortisol and glucose levels of the heterogeneous groups. Statistical analyses were performed using SPSS 11.5 for Windows and statistical significance was taken at $P < 0.05$.

RESULTS

Aggression

Aggression levels were high in all treatments. Averaged over all sampling days, the number of skin lesions per fish tended to be different between the treatments (ANOVA, $F_{3,8} = 3.7$,

P = 0.061) with the H treatment exhibiting a trend towards a lower mean number of skin lesions per fish (1.41 ± 0.1) than the L treatment (2.04 ± 0.22, Tukey: P = 0.054). The treatment effect was significant for the percentage of fish bitten (ANOVA, $F_{3,8}$ = 13.9, P = 0.002): the H treatment exhibited significantly lower percentage of fish bitten (58.34 ± 0.92 %) than all the other treatments (L: 74.13 ± 2.52 %, Tukey: P = 0.001; M: 69.70 ± 4.38 %, Tukey: P = 0.009; HET: 69.94 ± 3.29 %, Tukey: P = 0.009).

The number of skin lesions decreased significantly over time only for the M treatment (repeated ANOVA, L: $F_{2,4}$ = 1.3, P > 0.05; M: $F_{2,4}$ = 11.5, P = 0.022; H: $F_{2,4}$ = 2.5, P > 0.05; HET: $F_{2,4}$ = 1.5, P > 0.05; Fig. 1A). The percentage of fish bitten decreased significantly over time for the M and H treatments but not for the L and HET treatments (repeated ANOVA, L: $F_{2,4}$ = 0.7, P > 0.05; M: $F_{2,4}$ = 8.7, P = 0.035; H: $F_{2,4}$ = 6.7, P = 0.053; HET: $F_{2,4}$ = 1.3, P > 0.05; Fig. 1B).

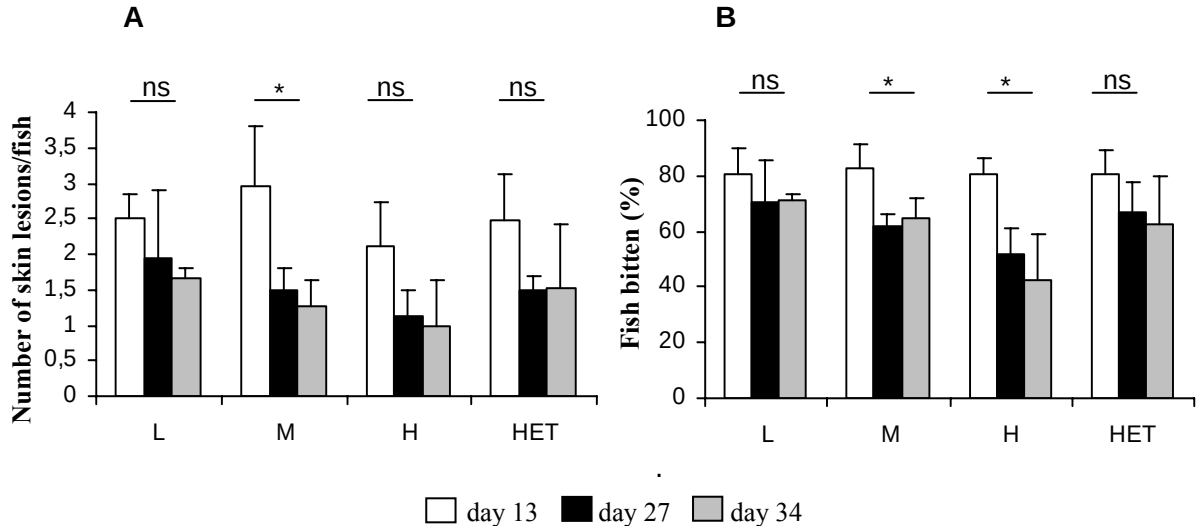


Figure 1. Time effect on the number of skin lesions per fish (A) and percentage of fish bitten (B) for all treatments (L = homogeneous low-, M = homogeneous medium-, H = homogeneous heavy-weight, HET = heterogeneous). ns means not significantly different and * significantly different at P < 0.05.

Fish with similar weight exhibited different aggression levels dependent on the group composition. In Table 1 this comparison is made.

Table 1. Means ± SD of the number of skin lesions per fish and percentage of fish bitten (averaged for all sampling days) in similar-weight fish grown in homogeneous and heterogeneous groups.

	Homogeneous	Heterogeneous	*P*-value
Skin lesions/fish			
Low	2.0 ± 0.2	1.5 ± 0.4	ns
Medium	1.9 ± 0.4	2.1 ± 0.2	ns
Heavy	1.4 ± 0.1	2.0 ± 0.1	0.002
Fish bitten (%)			
Low	74.1 ± 2.5	72.5 ± 12.6	ns
Medium	69.7 ± 4.4	70.7 ± 6.2	ns
Heavy	58.3 ± 0.9	67.9 ± 5.2	0.052

Within low and medium-weight classes, there were no significant differences in the percentage of fish bitten (*t*-test, $P > 0.05$). Heavy-weight fish tended to have a higher percentage of fish bitten in the heterogeneous than in the homogeneous group ($t_4 = 2.7$, $P = 0.052$). When skin lesions are considered, there were no significant differences in low- and medium-weight classes (*t*-test, $P > 0.05$). However, within the heavy- weight class, fish in the homogeneous group exhibited a lower number of skin lesions that in the heterogeneous group ($t_4 = 7.7$, $P = 0.002$).

There was no relationship between the initial weight and the number of skin lesions (average of day 13, 27 and 34) in fish reared on the heterogeneous group (Pearson correlation, $P > 0.05$, Fig. 2A).

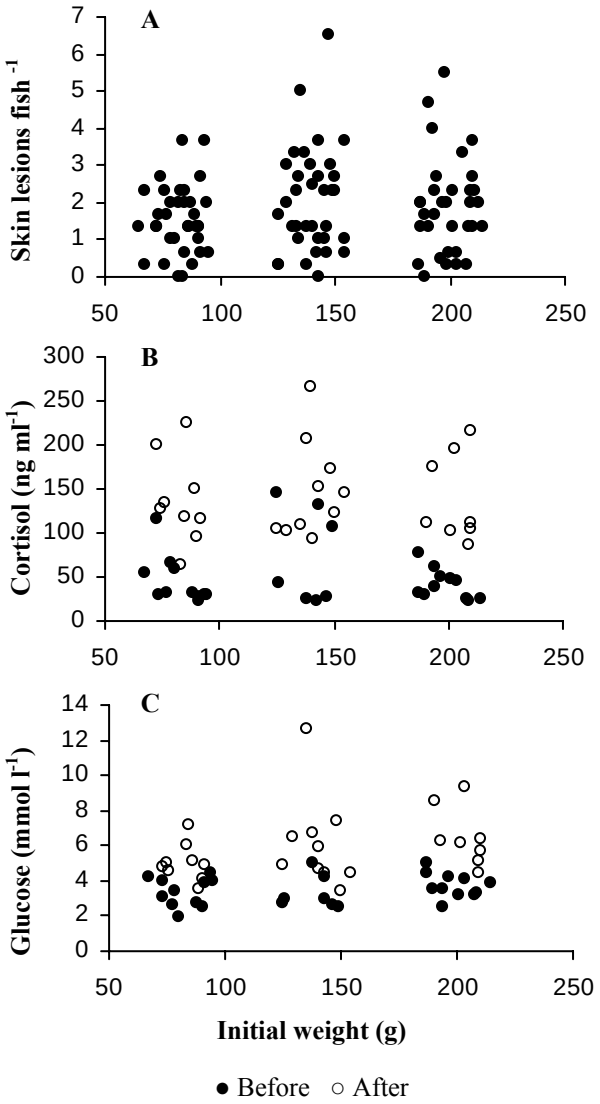


Figure 2. Relationship between initial weight and the number of skin lesions per fish (A, averaged for all sampling days), plasma cortisol (B, before and after the stress test) and plasma glucose (C, before and after the stress test) of fish reared in the heterogeneous treatment.

Stress response

There was a significant increase in cortisol levels after the stress test for all treatments, except for the L treatment (*t*-test, L: t_4 = -1.6, $P > 0.05$; M: t_4 = -6.7, $P = 0.003$; H: t_4 = -4.2, $P = 0.013$; HET: t_4 = -6.2, $P = 0.003$, Fig 3A). Plasma glucose levels increased significantly for all treatments after the stress test (*t*-test, L: t_4 = -11.8, $P < 0.001$; M: t_4 = -5.9, $P = 0.004$; H: t_4 = -7.9, $P = 0.001$; HET: t_4 = -4.1, $P = 0.015$, Fig. 3B).

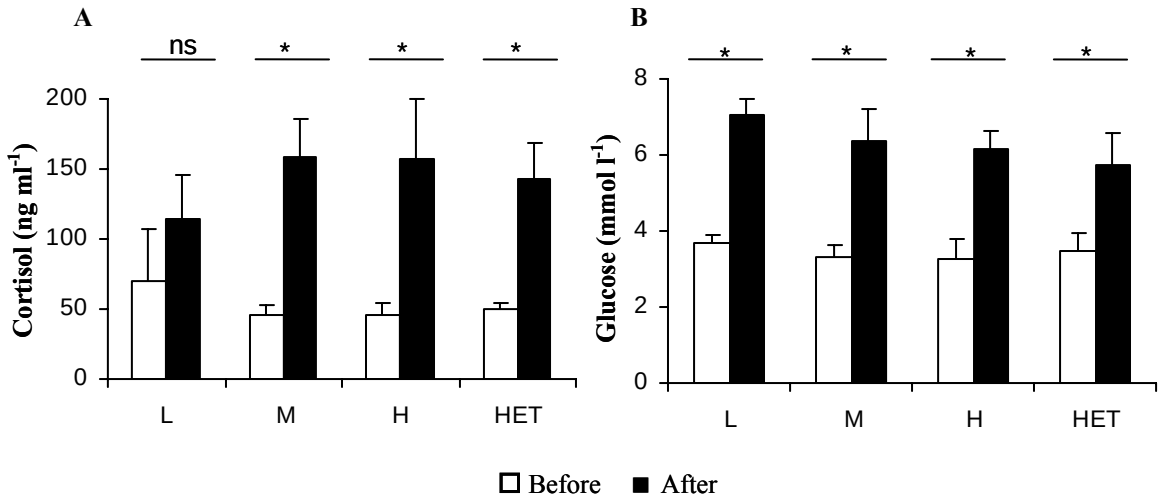


Figure 3. Means ± SD of plasma cortisol (A) and glucose (B) before and after a stress test as affected by the experimental treatments (L = homogeneous low-; M = homogeneous medium-; H = homogeneous heavy-weight; HET = heterogeneous). ns means not significantly different and * significantly different at $P < 0.05$.

Cortisol and glucose levels did not differ significantly between the treatments, either before or after the stress test (ANOVA, $P > 0.05$).

Similarly as for aggression, cortisol and glucose levels were compared within fish of similar weight reared in homogeneous and heterogeneous groups (Table 2). Cortisol levels in low-weight animals, both before and after the stress test, did not differ between homogeneous and heterogeneous groups (*t*-test, $P > 0.05$). Glucose levels also did not differ before the stress test (*t*-test, $P > 0.05$). However, after the stress test low-weight fish in homogeneous groups showed higher glucose levels (7.1 ± 0.4 mmol l^{-1}) than low-weight fish in heterogeneous groups (5.1 ± 0.7 mmol l^{-1}, $t_4 = -4.1$, $P = 0.015$). Medium-weight fish exhibited lower cortisol levels before the acute stress in the homogeneous groups (45.7 ± 7.5 ng ml^{-1}) than in the heterogeneous group (73.5 ± 13.3 ng ml^{-1}, $t_4 = -3.2$, $P = 0.034$). However, no differences were observed in cortisol after the stress test (*t*-test, $P > 0.05$) and in glucose both before and after the stress test (*t*-test, $P > 0.05$). Heavy fish had a similar stress response in homogeneous and heterogeneous groups (*t*-test, $P > 0.05$).

There was no relationship between the initial weight and the cortisol/glucose levels (both before and after the stress test) in the heterogeneous group (Pearson correlation, $P > 0.05$, Fig. 2B, C).

Table 2. Means ± SD of plasma cortisol and glucose in similar-weight fish grown in homogeneous and heterogeneous groups, obtained before and after the stress test. ( ) = number of individuals in each group.

	Before			After		
	Homogeneous	Heterogeneous	*P*-value	Homogeneous	Heterogeneous	*P*-value
Cortisol ($ng\ ml^{-1}$)						
Low	70.2 ± 37.1 (30)	44.6 ± 10.9 (11)	ns	114.9 ± 30.3 (29)	139.5 ± 43.0 (9)	ns
Medium	45.7 ± 7.5 (30)	73.5 ± 13.3 (7)	0.034	158.2 ± 28.1 (30)	147.5 ± 25.3 (10)	ns
Heavy	45.5 ± 8.9 (30)	40.0 ± 7.0 (11)	ns	156.6 ± 44.0 (30)	139.7 ± 12.0 (8)	ns
Glucose ($mmol\ l^{-1}$)						
Low	3.7 ± 0.2 (29)	3.3 ± 0.7 (11)	ns	7.1 ± 0.4 (30)	5.1 ± 0.7 (9)	0.015
Medium	3.3 ± 0.3 (30)	3.2 ± 0.8 (7)	ns	6.4 ± 0.8 (30)	5.9 ± 1.7 (10)	ns
Heavy	3.3 ± 0.5 (30)	3.6 ± 0.3 (11)	ns	6.2 ± 0.5 (30)	6.8 ± 1.9 (8)	ns

DISCUSSION

High aggression levels are often associated with the establishment of social hierarchies in which subordinates exhibit suppressed aggressive behaviour while dominants are very aggressive (Øverli et al., 1999). In this study one could expect that if social hierarchies are present in African catfish, the highest aggression levels would be found: 1) in the heterogeneous treatments where the difference in weight between the individuals is clear and therefore the heavier individuals would act as dominants and the light as subordinates (e.g., Gunnes, 1976; Popper et al., 1992; Brzeski and Doyle, 1995; Seppä et al., 1999) or 2) in the homogenous groups where the difference in weight between the individuals is low and therefore individuals would have to fight to re-establish the social rank (e.g., Baardvik and Jobling, 1990; Kamstra, 1993; Carmichael, 1994; Sunde et al., 1998; Stefansson et al., 2000).

In the present study, aggression levels were equally high for L, M and HET indicating that none of the alternatives mentioned above seems to apply for African catfish. Therefore one could wonder if social hierarchies are indeed present in this species or if present, whether the dominant-subordinate relationship is indeed determined by size differences.

The aggression level was very similar between the M and HET treatments, suggesting that a higher heterogeneity in weight is not necessarily linked with higher aggression levels and therefore with impaired welfare. Moreover, the homogeneous treatments (L, M and H) did not differ significantly in basal cortisol and glucose levels from the heterogeneous treatment, indicating that reducing the weight variation within a group by grading does not affect chronic stress levels. This may suggest that grading did not have any impact on disrupting or re-establishing social hierarchies. If a social hierarchy was present, fish with different weights reared together (as in the HET group) would also exhibit different aggression and stress levels. In this study there was no relationship between initial weight and aggression/stress levels in fish reared in the heterogeneous group. Fox et al. (1997) suggested that after the establishment of a social relationship, the cortisol levels were similar among paired cichlid fish. In the present study, plasma cortisol and glucose were measured only at the end of the experiment as an indication of chronic stress. Therefore, we can not eliminate the possibility that in the beginning of the experiment, differences in stress levels were present between fish with similar weight reared either in homogeneous or in heterogeneous groups.

Low-weight fish reared in both homogeneous and heterogeneous groups did not differ in aggression level. This suggests that low-weight fish are not acting as a subordinate fish in the HET treatment. In this study, grading the fish resulted in a lower aggression level in the H treatment. The H and M treatments were the only treatments where there was a significant decrease in the aggression level over time, suggesting a positive effect of grading on welfare for heavy- and medium-weight fish. Moreover, heavy fish exhibited more skin lesions in the heterogeneous than in the homogeneous group. One may wonder if heavy fish have to fight more in the heterogeneous group. Heterogeneous groups also did not decrease the aggression level over time, indicating a continuous high level of aggression over the whole experimental period. One may again think that this could be associated with fights to maintain an existing social hierarchy. Medium-weight fish exhibited a lower aggression level after grading and also a higher cortisol level before the stress test in the heterogeneous than in the homogeneous group. If we consider that the cortisol levels obtained before the stress test are an indicator of

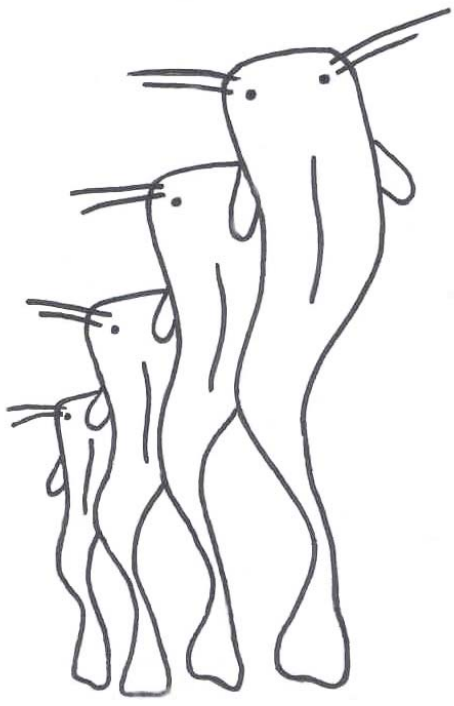
chronic stress, then medium-weight fish instead of low-weight fish would be under social dominance from the heavier fish.

The stress response seems to be affected in the L treatment. When an acute stress was induced (netting), fish in the L treatment did not show a significant increase in cortisol levels. This could raise the question whether the high levels of cortisol before applying an acute stress could hamper the stress response in the L treatment. Pottinger (1990) suggested that, in chronic stress situations or when exogenous cortisol is administrated, the attenuation of the hypothalamic-pituitary-interrenal (HPI) axis may occur by a reduction in the number of specific binding sites, and that this attenuation is a protective mechanism to avoid an over-response in situations of acute stressors.

In conclusion this study showed that grading juveniles of African catfish into homogeneous groups resulted in improved welfare for the heavy- (lower aggression levels) and medium-weight fish (lower aggression levels and lower cortisol levels before the stress test) but not for the low-weight fish. To fully understand the consequence of grading on welfare it is necessary further research on whether social hierarchies are present in African catfish and if present which type of dominant-subordinate relationship is playing a role. Behavioural observations will help achieving this goal.

Acknowledgements

This research was funded by the Foundation for Science and Technology, Portugal (grant SFRH/BD/2946/2000). This work was also supported by the NWO (Den Haag, The Netherlands) under the project “Innovation of aquatic respirometry facilities”, code 805-34.025. We would like to acknowledge Menno ter Veld, Sietze Leenstra, Aart Hutten and Wian Nusselder for their help in starting up the experiment. We also would like to thank to Alex Scott, Tim Ellis and Jonathan James for the cortisol analyses.



4

- CHAPTER -

Comparison of feed intake behaviour and stress response in isolated and non-isolated African catfish (*Clarias gariepinus*)

C. I. M. Martins

J. W. Schrama

J. A. J. Verreth

Journal of Fish Biology, 2005, accepted

ABSTRACT

The effect of isolation on feed intake behaviour and stress response of African catfish *Clarias gariepinus* was determined, by comparing isolated (n = 26**,** initial weight ± SE: 101.1 ± 4.5 g) and non-isolated fish (n = 15, initial weight ± SE: 105.9 ± 4.2 g). Fish were fed ad libitum over a total experimental period of 24 days. Two feeding behaviour parameters were studied once per week by visual observation: total feeding time and the number of feeding acts. Blood samples for plasma cortisol, glucose and lactate were taken from all fish at the start of the experiment (control, indicative of basal levels). At the end of the experiment fish were subjected to a stress test (indicative of acute stress response), followed by blood sampling. The stress test consisted of holding each fish individually in a net outside the water for 1h. Non-isolated fish exhibited higher growth rate, feed intake and final condition factor than isolated fish. Differences in maintenance level and production efficiency were not significantly different between isolated and non-isolated fish. Isolated fish spent less time eating (18.9 ± 3.5 min) than non-isolated fish (37.4 ± 3.3 min). Moreover, fish housed individually were less active during feeding, exhibiting an average number of feeding acts per hour of 2.5 ± 0.5 against 5.2 ± 0.4 of non-isolated fish. Cortisol, glucose and lactate levels did not differ significantly between the treatments both when the control and the post-stress levels are considered ($P > 0.05$). This study suggests that in African catfish feed intake is stimulated by the presence of conspecifics which will result in higher feed intake and growth rates. However, isolation per se seems not to act as a stressor in the short term or to affect the stress response, probably because periods of isolation are part of the African catfish lifestyle.

INTRODUCTION

Physiological and behavioural research in fish traditionally uses mean values and the variation around the means is often considered as a statistical noise (Kolok, 1999). However understanding individual variation, especially related to feed intake and growth parameters, is crucial for aquaculture. A reduction in variation contributes to maximize production efficiency, to reduce food wastage, and to improve water quality (McCarthy et al., 1992; Jobling and Baardvik, 1994; McDonald et al., 1996). Studies on individual variation in feed intake have been done both in grouped and individually housed fish. Monitoring individual feed consumption in groups of fish requires relatively complex techniques such as radiography (Carter et al., 1995). However, in African catfish this technique is not applicable due to vomiting reactions when fish are disturbed after feeding. Housing fish individually is therefore a good alternative to measure individual feed intake and has been used to monitor individual variation in growth (Jobling and Baardvik, 1994; Wang et al., 1998; Martins et al., 2005a), digestibility and activity levels (Qian et al., 2002). Moreover, studies aimed to investigate dominant-subordinate relationships (Øverli et al., 1998) and aggression behaviour (Winberg et al., 2001) have also used periods of isolation prior to paired tests. Most of these studies using isolation were done in socially structured species. The question arises whether depriving fish from social contact alters their behaviour and physiology. Social isolation has been shown to induce stress and to have deleterious effects on health in several vertebrates such as mice, rats, baboons, squirrel monkeys and pigs (Valzelli, 1973; Jessop and Bayer, 1989; Brown and Grunberg, 1995; Sapolsky et al., 1997; Lyons et al., 1999; Ruis et al., 2001, Späni et al., 2003; Bartolomucci et al., 2003). The effect of isolation on fish has not yet been fully investigated.

The aim of this study was to investigate the consequences of isolating African catfish *Clarias gariepinus* (Burchell 1822) in feed intake behaviour and stress response. This was done by comparing isolated and non-isolated fish.

MATERIAL AND METHODS

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

Experimental animals and housing

Fifty-six juvenile fish were obtained from a local catfish producer (Fleuren, Someren, The Netherlands) where they had experienced common housing and feeding conditions. Each fish was individually weighed and randomly allocated to one of the two treatments: isolated fish (n = 26**,** initial weight ± SE: 101.1 ± 4.5 g) and non-isolated fish (n = 15, initial weight ± SE: 105.9 ± 4.2 g). The experimental tanks consisted of glass tanks with 30 x 35 x 40 cm (40 l). Each experimental tank was divided longitudinally with a perforated screen, so that isolated and non-isolated fish would have the same space availability. Each fish of the isolation treatment was placed alone in one tank. The walls of the tanks were covered with a black plastic to eliminate any visual contact between different tanks. On the non-isolated treatment, each side of the tank contained one fish which could interact visually, chemically and physically (between the barbells only). The non-isolated fish could not bite each other or have access to the other's fish food. This type of tank allowed not only the possibility of communication between conspecifics but also the quantification of individual feed intake.

All tanks were connected to a common recirculation system. A 12L:12D photoperiod was maintained with daybreak set at 0700 h. Water temperature (25.1 ± 0.30 ^{o}C), pH (range between 7.7 and 8.2), dissolved O_2 (> 5 mg l^{-1}), conductivity (2.9 ± 0.03 mS cm^{-1}), NH_4^+ (< 2 mg l^{-1}), NO_2^- (< 0.5 mg l^{-1}) and NO_3^- (< 140.0 mg l^{-1}) were checked daily.

Feeding regimes

The fish were fed a commercial diet (floating pellets, 4.5 mm Coppens: 45 % crude protein, 12 % crude fat, 2.0 % crude fibre and 10.5 % ash) once per day by hand. Feeding started at 0800 h and continued until apparent satiation. The non-isolated fish received the first portion of feed at the same time. Whenever a pellet was eaten, it was replaced by a new one so that there would always be 5 pellets floating at the surface of the water. Feeding continued for a maximum of 1h, after which the remaining pellets were collected and counted.

Experimental procedures

Fish were allowed to adapt to the experimental conditions for 15 days, after which they were individually weighed. At the end of the experiment fish were also individually weighed. Experimental data were collected over 24 days (starting after the 15 days of adaptation). During this period, feed intake was recorded daily for each fish. Two feeding behaviour

parameters were studied once per week by visual observation: total feeding time and the number of feeding acts. Total feeding time (TFT, min) was defined as the time between the first and the last pellet being consumed. The number of feeding acts (NFA) consisted of the number of times that each fish swam towards the surface of the water and consumed one or more pellets. Only 2 researchers carried out the behavioural observations, whereby a standard protocol was followed. During the feeding period, the researchers continuously counted the number of pellets present in each tank, the time when one or more pellets were eaten and immediately replaced by new ones. In this way it was possible to determine when each portion of food was eaten.

Blood samples for plasma cortisol, glucose and lactate were taken from all fish at the start of the experiment (control, indicative of basal levels). At the end of the experiment fish were subjected to a stress test (indicative of acute stress response), followed by blood sampling. The stress test consisted of holding each fish individually in a net outside the water for 1h. It should be noted that African catfish is able to breathe air, and previous experiments using emersion periods up to 3 h have been performed with no mortality reported (Buttle et al., 1996). Therefore, it is considered that 1-h air exposure is not an extreme stressor for African catfish. Fish were anaesthetised for blood sampling (0.4 g l^{-1} of tricaine methanesulfonate, TMS Crescent Research Chemicals, Phoenix, Arizona, USA using 0.8 g l^{-1} of sodium bicarbonate as buffer). One ml of blood was collected from all fish by hypodermic syringe (containing 3 mg of Na_2EDTA) from the caudal blood vessels. This procedure was finalised within 3 min after fish were caught and anesthetised. The collected blood was placed in cooled 1.5 ml plastic tubes, mixed and centrifuged at 6000 *g* for 5 min at 4 °C. After centrifugation plasma was collected and stored at -20 °C for further analysis. All fish were killed with an overdose of anaesthesia after blood sampling (0.8 g l^{-1} of tricaine methanesulfonate, TMS Crescent Research Chemicals, Phoenix, Arizona, USA using 1.6 g l^{-1} of sodium bicarbonate as buffer).

Plasma analysis

Cortisol was measured in unextracted catfish plasma using the validated radio-immunoassay described by Ellis et al. (2004), but adapted by pre-heating the plasma in glutamate buffer (pH 3.3). The adaptation for direct assay originates from Dunn and Foster (1973) and Foster and Dunn (1974) and has been used widely in fish studies (e.g., Redding et al., 1984; Bisbal and Specker, 1991; Young, 1986). The catfish plasma (diluted 1:100 in glutamate buffer) was

added to duplicate glass tubes for assay. Nine cortisol standards ranging from 2 to 500 pg 100 μl^{-1} were made up (in duplicate) by serial dilution in glutamate buffer. All tubes were heated in boiling water for 15 min, and then cooled by standing in cold water for 5 min. The assay then proceeded as described by Ellis et al. (2004), i.e., addition of radioactive steroid and antibody, overnight equilibration, removal of unbound cortisol with dextran-coated charcoal, and scintillation counting.

Plasma glucose and lactate were determined by the GOD-Perid method (Boehringer) and using Sigma Diagnostic Kits (Sigma; Proc. No 735), respectively.

Data analysis

Growth rate (GR) and feed intake (FI) were expressed per metabolic weight units of g BW $(kg)^{-0.8}$ d^{-1}, where g represents the grams of weight gain or the grams of feed intake and BW the geometric mean: BW = exp {½ [ln (W_{T1}) + ln (W_{T2})]}, where W_{T1} is the weight (g) at time (t) 1 and W_{T2} the weight at time 2.

As described by Lupatsch et al. (2003) dietary intake can be calculated using the efficiency of utilization of dietary energy for maintenance or growth. In their study the quantification of energy and protein requirement in the growing fish is done as the sum of the need for maintenance and growth. In the present study, the same approach is used to test if there are differences in efficiency for maintenance and growth between isolated and non-isolated fish. However, instead of using energy and protein requirements, this study used absolute feed intake requirements. The following equation was used to express feed intake requirements: $FI = \beta_1 + \beta_2\ GR + e$, where β_1 represents the maintenance requirements, β_2 the requirements for production and e an error term. The maintenance requirement (β_1) is obtained by the intercept of the mentioned regression equation, which represents the amount of food being consumed when growth is zero.

The condition factor (CF, g cm^{-3}) was calculated as, CF = weight / fork $length^3 \times 100$.

The results are expressed as means (± SE). Statistical analyses were performed using Proc GLM (SAS, 1989). Possible differences in growth performance, feed intake, feeding behaviour and stress response between isolated and non-isolated fish were tested using a *t* -test for independent samples. A *t*-test for dependent samples was used, to test if the stress test induced a significant increase in plasma cortisol, glucose and lactate levels in isolated and non-isolated fish. Analysis of covariance was used to compare slopes and intercepts of the

regression lines for the feed intake requirements obtained for isolated and non-isolated fish. The experimental unit was considered the individual for the isolated fish and the average of 2 individuals for the non-isolated fish. Statistical calculations were corrected for different group sizes, using the weight statement of the GLM procedure of SAS. Statistical significance was taken at $P < 0.05$.

RESULTS

Performance, behavioural and stress parameters data are presented in Table 1. Initial body weight and the initial condition factor did not differ significantly between the treatments. However, all the other performance parameters (final condition factor, feed intake and growth rate) were significantly higher in non-isolated fish. The total feeding time and the number of feeding acts differed significantly between the two treatments. Cortisol, glucose and lactate levels did not differ significantly between the treatments, neither for the control nor for the stress tested fish.

The stress test induced a significant increase in glucose levels both in isolated and non-isolated fish (*t*-test, dependent samples; isolated: df = 24, $P < 0.001$; non-isolated: df = 14, $P < 0.001$) and in cortisol levels in non-isolated fish (*t*-test, dependent samples, df = 14, $P = 0.037$). The lactate levels were not significantly increased after the stress test for both isolated and non-isolated fish (*t*-test, dependent samples; isolated: df = 24, $P > 0.05$; non-isolated: df = 14, $P > 0.05$).

The relationship between feed intake and growth is described through the regression equation, $FI = B_1 + B_2\ GR + e$ (Fig. 1), where B_1 represents the maintenance requirements and B_2 the requirements for production. Although the maintenance requirements (B_1) in isolated fish (5.0 g $kg^{-0.8}$ d^{-1}) was higher than the maintenance requirements of non-isolated fish (4.4 g $kg^{-0.8}$ d^{-1}) they did not differ significantly (ANCOVA, df = 1,53, $P > 0.05$). The requirement for production (B_2) was also not significantly different between the two treatments (ANCOVA, df = 1,53, $P > 0.05$).

Table 1. Growth performance, behavioural and stress parameters data (mean ± SE) for isolated and non-isolated African catfish (*t*-test, independent samples, df = 39 for the performance/behavioural data and cortisol / glucose / lactate levels obtained after the stress test; df = 38 for cortisol / glucose / lactate control levels; ns = not significant, $P > 0.05$).

	Isolated	Non-isolated	*P*-value
Performance			
Initial body weight (g)	101.1 ± 4.5	105.9 ± 4.2	ns
Weight gain (g)	29.5 ± 8.5	65.9 ± 7.9	0.003
Condition factor (g cm^{-3})			
Start (day 0)	0.8 ± 0.02	0.8 ± 0.02	ns
End (day 24)	0.7 ± 0.02	0.9 ± 0.02	< 0.001
Feed Intake (g $kg^{-0.8}$ d^{-1})	7.7 ± 0.7	10.5 ± 0.7	0.007
Growth rate (g $kg^{-0.8}$ d^{-1})	5.8 ± 1.4	12.8 ± 1.3	< 0.001
Behaviour			
Total feeding time (min)	18.9 ± 3.5	37.4 ± 3.3	< 0.001
Number of feeding acts	2.5 ± 0.5	5.2 ± 0.4	< 0.001
Stress parameters			
Control			
Cortisol (ng ml^{-1})	56.6 ± 8.5	54.0 ± 6.6	ns
Glucose (mmol l^{-1})	2.1 ± 0.1	2.1 ± 0.1	ns
Lactate (mmol l^{-1})	2.5 ± 0.2	2.9 ± 0.2	ns
Stress-test			
Cortisol (ng ml^{-1})	75.2 ± 7.7	82.1 ± 8.2	ns
Glucose (mmol l^{-1})	6.3 ± 0.4	6.6 ± 0.3	ns
Lactate (mmol l^{-1})	2.9 ± 0.2	3.2 ± 0.3	ns

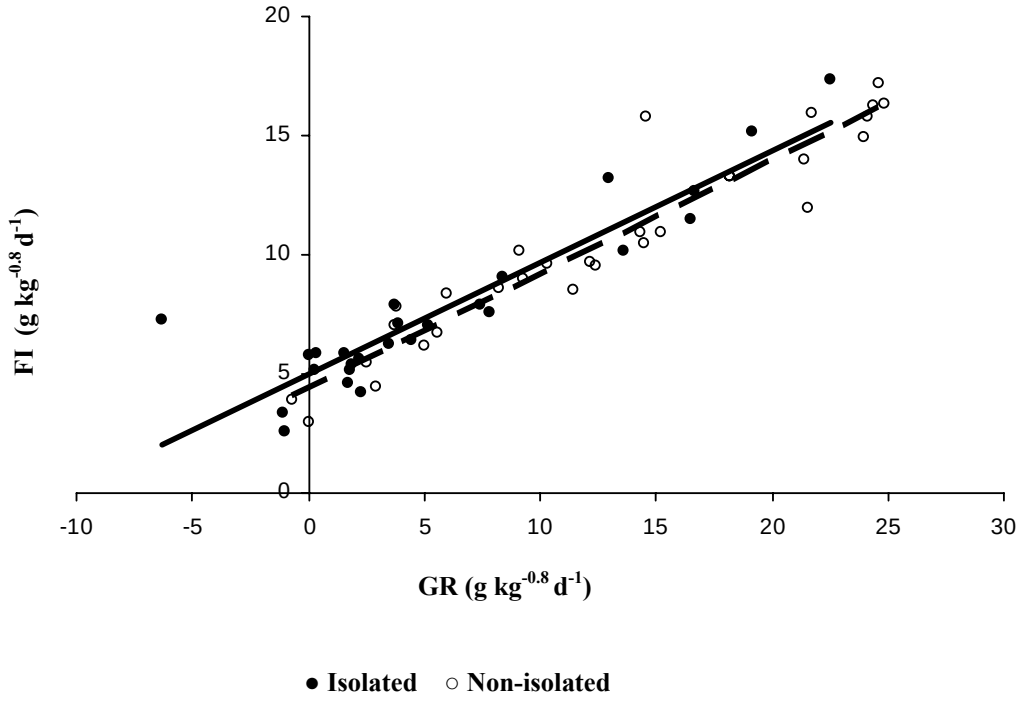


Figure 1. Relationship between feed intake (FI, g $kg^{-0.8}$ d^{-1}) and growth rate (GR, g $kg^{-0.8}$ d^{-1}) of isolated (——, $y = 0.47x + 5.0$, $R^2 = 0.83$) and non-isolated (– –, $y = 0.48x + 4.4$, $R^2 = 0.90$) fish.

DISCUSSION

The main goal of this experiment was to understand how the absence of social interaction (isolation) affects feed intake behaviour and stress response in juveniles of African catfish. The observed differences in growth rate between isolated and non-isolated fish could not be explained by differences in feed efficiency but only by differences in feed intake. In the present study, isolated fish exhibited an average feed intake of 7.7 ± 0.7 g $kg^{-0.8}$ d^{-1} and non-isolated fish of 10.5 ± 0.7 g $kg^{-0.8}$ d^{-1}. This is still much lower than reported feeding levels (24 g $kg^{-0.8}$ d^{-1}) for group-housed African catfish (Ozório, 2001). Obviously, the non-isolated fish used in this study are still not representative for a group housed situation. Possibly more complex social structures influence African catfish's performance.

The question remains whether the reduced feed intake observed in isolated fish is a result of stress or is simply due to a lack of feeding stimulation from conspecifics. It is known that stress responses typically include effects upon the whole-animal, such as reduced feed intake, appetite and growth rate (Wendelaar Bonga, 1997). Social isolation has been shown to induce

stress and to have deleterious effects on health in several vertebrates such as mice, rats, baboons, squirrel monkeys and pigs (Valzelli, 1973; Jessop and Bayer, 1989; Brown and Grunberg, 1995; Sapolsky et al., 1997; Lyons et al., 1999; Ruis et al., 2001; Späni et al., 2003; Bartolomucci et al., 2003). In this study, one could have expected a higher basal stress level in isolated fish due to the reduced feed intake, feeding motivation and poor growth performance observed. Also, the condition factor was significantly lower for fish kept in isolation than in non-isolated fish. A decline in condition factor is often indicative of a depletion of energy stores (Goede and Barton, 1990), which in this study could be explained by the fact that isolated fish ate and grew less. Despite the negative effect of isolation on growth performance, the plasma cortisol, glucose and lactate did not differ between the treatments both before (control) and after the stress test. This suggests that isolation was not acting as a stressor, at least during the first 15 days and also that isolation does not affect the stress response. However, we cannot exclude the possibility that in the long term, isolation is acting as a stressor in African catfish. However, the fact that after 15 days, isolated and non-isolated fish did not differ in cortisol, glucose and lactate levels, indicates that isolation does not act as a stressor in the short-term (15 days).

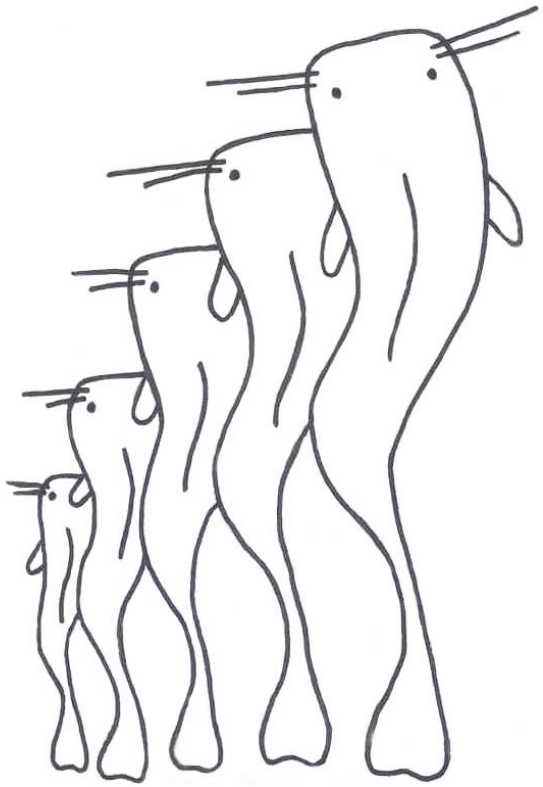
The behavioural observations done in the present study support the importance of social interaction in feeding motivation. Non-isolated fish spent more time eating and went more often to the water surface to get food than isolated fish. In fact, studies based in natural and in aquacultural condition support the view that feeding has a social context in African catfish. Under natural conditions, African catfish exhibits different feeding strategies (Bruton, 1979). Among these, social hunting, i.e., pack-hunting has been described. Pack-hunting is defined as an organized hunting strategy in which all individuals in the group benefit (Merron, 1993). This active social aggregation is considered a natural feeding strategy to take advantage of specific food supplies available only during specific times of the year. Also, under rearing conditions, African catfish exhibit an “explosive feeding frenzy” culminating in the rapid and complete consumption of the meal (Hecht and Uys, 1993). These authors suggested that African catfish kept at low densities show low feeding responses as a consequence of lack of conspecifics.

The question remains whether in African catfish isolation induces impaired welfare. On one hand, there is no indication that isolation increases basal or post-stress levels of cortisol, glucose and lactate. On the other hand, isolated fish did exhibit lower feeding motivation, leading to reduced feed intake and poorer growth than non-isolated fish.

Assessing welfare is not simple and ideally should take into account the animal's health status, its physiology and its behaviour (FSBI, 2002). The consequences of isolation in fish's well being will certainly depend on the type of social structure characteristic of the species, which can range from a completely solitary lifestyle (e.g., pike) to a synchronized schooling behaviour (e.g., herring) (Brännäs et al., 2001). Hocutt (1989) monitored radio-tagged African catfish in the wild and showed that a solitary lifestyle, although less common also exists in African catfish. This might explain the apparent contradiction of the results obtained for performance/behaviour and stress response. Feeding seems indeed to be stimulated by the presence of conspecifics which result in higher feed intake and growth rates. However, isolation per se seems not to act as a stressor in the short term, probably because periods of isolation are also part of the African catfish lifestyle.

Acknowledgements

This research was supported by a grant provided by the Foundation for Science and Technology, Portugal (grant SFRH/BD/2946/2000). We would like to acknowledge Menno ter Veld, Sietze Leenstra, Aart Hutten and Wian Nusselder for their help in starting up the experiment. We also would like to thank to Alex Scott, Tim Ellis and Jonathan James for the cortisol analyses.



5

- CHAPTER -

The consistency of individual differences in growth, feed efficiency and feeding behaviour in African catfish (*Clarias gariepinus*) housed individually

C. I. M. Martins

J. W. Schrama

J. A. J. Verreth

Aquaculture Research, 2005, accepted

ABSTRACT

Individual variation in growth, feed intake and feeding behaviour has been previously recognised in several fish species. However, there is a lack of information regarding the consistency of such individual differences, which is important to understand the probability of a certain individual trait to be inherent, i.e., genetically linked. The goal of this study is to quantify the consistency of individual differences in growth, feed intake / efficiency and feeding behaviour in African catfish *Clarias gariepinus* (Burchell 1822). Forty-eight juvenile fish (58.9 ± 0.4 g) were housed individually for 47 days and weighed every second week. The consistency of growth, feed efficiency (residual feed intake) and feeding behaviour (total feeding time, TFT) was determined using repeatability estimates. Fish exhibited pronounced individual variation in growth (CV = 52.8 %), feed intake (34.3 %) and in the total feeding time (>100 %). The repeatability estimates were 0.55 for growth, 0.70 for feed intake, 0.49 for residual feed intake and 0.81 for TFT. Individual differences in growth were mainly explained by individual differences in feed intake (~ 85 %). Individual differences in feeding behaviour were related to individual differences in residual feed intake and therefore with maintenance requirements. With increasing TFT, the maintenance requirements also increased suggesting that slow eaters were less efficient in feed / energy utilization. The results of this study indicate that individual differences in growth, feed intake / efficiency and feeding behaviour are consistent over time and therefore probably inherent. Moreover, this study may have implications on the use of feeding behaviour as a predictor of feed efficiency in juveniles of African catfish.

INTRODUCTION

Pronounced individual variation in growth and behaviour has been reported for several fish species (Grobler et al., 1992; Wang et al., 1998; Hart and Salvanes, 2000; Qian et al., 2002; Martins et al., 2005a). The establishment of dominance hierarchies has been widely accepted as a major cause of such variation, with dominant fish exhibiting superior growth and appetite relative to subordinates (Jobling and Wandsvik, 1983; Abbott and Dill, 1989; McCarthy et al., 1992; Jobling et al., 1993; Brännäs, 1998; Cutts et al., 1998; Alanärä et al., 2001). However, there is increasing awareness that individual differences in growth and behaviour are not just a consequence of social interactions, but may also be inherent and therefore genetically linked (Martins et al., 2005a; Martins et al., 2005b). Housing fish in isolation has been used to study inherent differences, and pronounced individual variations in growth rates, feed intake and feed efficiency have been found (Jobling and Baardvik, 1994; Wang et al., 1998; Qian et al., 2002; Martins et al., 2005a). The use of individual housing to study individual variation in feed intake is particularly important when the use of X-rays (which allow the measurement of individual feed intake in groups) is limited. This seems to be the case in African catfish, which exhibits vomiting reactions, when X-rays (associated with handling) are taken after feeding (Martins, unpublished data).

Individual variation in growth is of great interest for commercial purposes (e.g., breeding programs) as long as such individual variation is consistent over time. One way to study interindividuality trait variation is to partition phenotypic variation into additive genetic and environmental components by estimating heritability (h^2) and genetic correlations (Roff, 1997). However, estimation of quantitative genetic parameters requires very large sample sizes and knowledge of pedigrees (Falconer and Mackay, 1996). Boake (1989) proposed to use a trait's repeatability (consistency) as an alternative approach. In population genetics, repeatability is defined as an intra-class correlation coefficient that indicates the fraction of total phenotypic variance that is due to permanent (but not necessarily genetic) differences between individuals (Kime et al., 1998). Traits with low repeatability cannot have a high heritability and thus respond more slowly to selection than traits that are highly repeatable and highly heritable (Brodie and Russell, 1999). Although repeatability is not a proof of the genetic basis of a characteristic it places an upper limit to heritability and it quantifies how the measurement of a particular trait is representative for an individual (Kime et al., 1998; Réale et al., 2000).

The goal of this study is to analyse the consistency of individual differences in growth, feed intake / efficiency and feeding behaviour in African catfish housed individually. We also assess whether feeding behaviour can be used to predict feed efficiency. African catfish *Clarias gariepinus* (Burchell 1822) was chosen because of its wide variation in growth rate and behaviour patterns both under natural and aquaculture conditions (Bruton, 1979; Grobler et al., 1992; van der Waal, 1998; Almazán-Rueda, 2004a).

MATERIAL AND METHODS

The experiments were approved by the Ethical Committee for Animal Experiments (DEC), Wageningen University.

Preliminary experiment

Before an attempt is made to quantify the consistency of individual differences found under individual housing, the question whether the results obtained from fish housed individually can be applied to fish housed in groups must be addressed. Therefore, prior to the main experiment presented here, a preliminary experiment was conducted to test if a slow (or fast) growing fish in isolation remains a slow (or fast) growing fish under group conditions.

Fifty-five juveniles of African catfish with an initial weight of 75.0 ± 1.2 g (mean ± SE, full sibs) were divided in two treatments: individual (n = 26) and group (n = 29) housing. All fish were individually weighed and tagged. Individual and group housed fish were kept in 40 and 70-l glass aquarium, respectively. After 38 days of individual and group housing (growth phase 1), fish were individually weighted and re-grouped. All individually housed fish were placed in the same tank (70-l) and all grouped housed fish, after being individually weighed, were re-grouped in the same tank (70-l). In this way, the effect of isolation on the subsequent growth variation under group housing could be analysed. In total, the second growth phase lasted 38 days (growth phase 2). Throughout the experiment, fish were fed until apparent satiation.

A student *t*-test was made to compare the initial weight of fish housed individually and in group. Analysis of covariance was used to compare slopes and intercepts of regression lines between body weights as affected by a previous isolation or group housing.

The results from the preliminary experiment concern only the second growth phase since this reflects the effect of a previous individual housing on subsequent growth variation

under group housing. The initial weight (day 0) of the fish was not significantly different between the treatments (t_{53} = 0.32, P = 0.8). Therefore the body weight of each individual at the beginning of phase 2 (= final weight of phase 1) is indicative of how much each fish grew. The initial body weight of phase 2 was significantly different between the treatments (t_{53} = -10.1, P < 0.001). Figure 1 shows the relationship between the initial body weight and the final body weight of phase 2, for the two treatments after 38 days of group housing. When the regression lines are compared, no significant differences were found, both for the slopes and intercepts (P > 0.05). Similar results were obtained for other growth expressions such as daily growth rate (% d^{-1}), specific growth rate (% d^{-1}) and relative growth rate (g $kg^{-0.8}$ d^{-1}).

From the preliminary experiment one can conclude that although the average growth of individual housed fish is lower than of group housed fish, the individual differences in growth found in individual housing are representative of the individual differences in growth of group housing. Slow and fast growing fish under individual housing remain slow and fast growing fish under group housing. This suggests that the different growth rates observed when fish are individually housed, are a characteristic of the individual since it is maintained under subsequent group housing and not simply a consequence of isolation.

Experimental animals

Forty-eight juveniles of African catfish were used in the main experiment with an initial weight of 58.9 ± 0.4 g. All animals were obtained from a local catfish producer (Fleuren, Someren, The Netherlands) where they had experienced common housing and feeding conditions. Upon arrival at Wageningen University, each fish was housed individually in a 40-l glass aquarium within a recirculation system. A 12L:12D photoperiod was maintained with daybreak set at 0700 hours. Water temperature (24. 9 ± 0.30 ^{o}C), pH (range between 7.25 and 8.06), dissolved O_2 (> 5 mg l^{-1}), conductivity (2.57 ± 0.07 mS cm^{-1}), NH_4^+ (< 2 mg l^{-1}), NO_2^- (< 0.5 mg l^{-1}) and NO_3^- (< 75.4 mg l^{-1}) were checked daily.

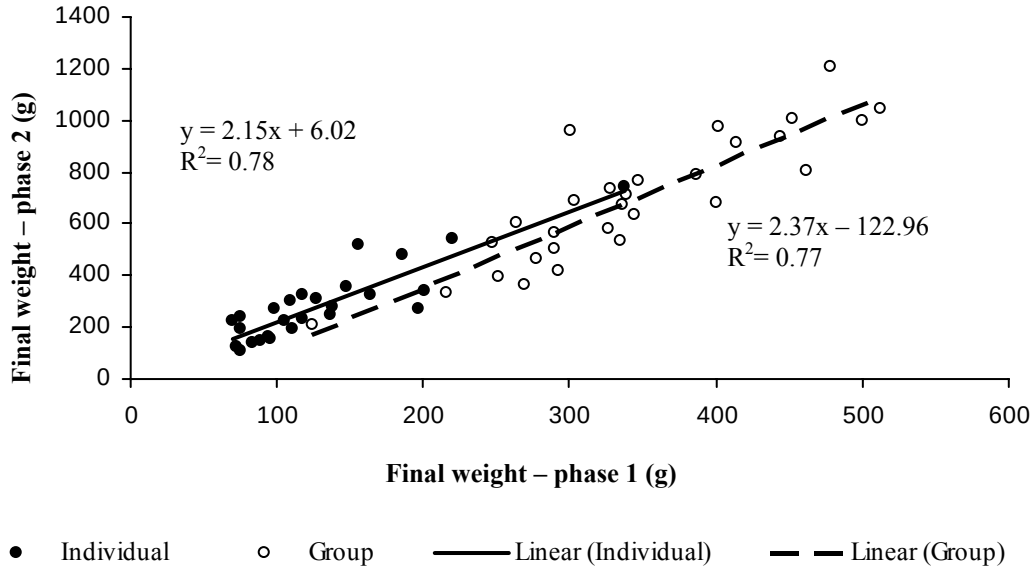


Figure 1. Relationship between the final weight at phase 1 (= initial weight at phase 2) and the final body weight at phase 2 for fish previously housed in isolation (Individual) or in a group (Group).

Feeding regimes

The fish were fed until apparent satiation with a commercial diet (floating pellets, 4.5 mm Coppens: 45 % crude protein, 12 % crude fat, 2.0 % crude fibre and 10.5 % ash) once per day by hand. The feeding procedure consisted of giving sequential portions of five pellets. New pellets were added only after the first portion had been eaten. Feeding continued for a maximum of 4 h, after which the remaining pellets were collected and counted.

Experimental procedures

For analysis of the results from the main experiment, 3 experimental periods (P) were considered: days 0-15 (P1), days 16-30 (P2) and days 31-47 (P3). At the start and end of each period, fish were individually weighed. Fish were not fed on sampling days.

Total feeding time (TFT), defined as the time from the first to the last eaten pellet, was measured visually. Only 2 experimenters carried out the behavioural observations, taking care to act similarly on all occasions and to cause minimal disturbance to the fish. During the 4 h of observation, the experimenters continuously counted the number of pellets present in each tank and added new pellets when the first portion was finished. In this way it was possible to

determine the time spent by each fish from the first to the last eaten pellet. Total feeding time was assessed once per week during P2 and P3 (2 measurements undertaken in each period).

Data analysis

As described by Lupatsch and Kissil (2003) and Lupatsch et al. (2003) dietary intake can be calculated by including the efficiency of utilization of dietary energy for maintenance or growth. In their study the quantification of energy and protein requirement in the growing fish is done as the sum of the need for maintenance and growth. In the present study the same approach is used but using the absolute feed intakes obtained. The following equation was used to express feed intake requirements: FI (g) / BW $(kg)^{0.8}$ = (M + G) / BW $(kg)^{0.8}$ + e where BW $(kg)^{0.8}$ is the metabolic body weight (BW =exp {½ [ln (W_{T1}) + ln (W_{T2})]} and W_{T1} is the weight (g) at time (t) 1 and W_{T2} the weight at time 2), M and G the coefficients describing the efficiency of utilization of feed for maintenance and growth respectively. The error term (e) of this equation can be defined as the residual feed intake (RFI), i.e., the part of the feed intake that is unexplained by food requirements for maintenance and growth. RFI can be calculated as the difference between the feed consumed by an animal and its consumption as predicted from a model involving its growth and maintenance requirements (Rauw et al., 2000). An animal with a low RFI is more efficient than one with a high RFI and vice versa (see example Fig. 2).

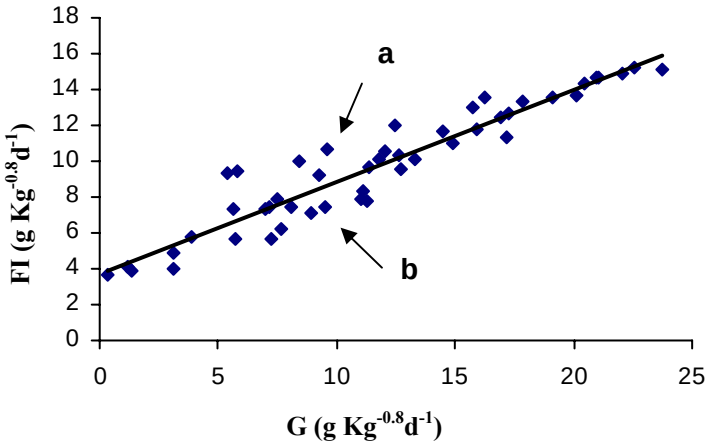


Figure 2. Relationship between feed intake (FI) and growth (G) for 48 juveniles of African catfish housed individually. Each point in the graph represents one fish. The line represents the expected relationship between FI and G. Fish "a" and "b" have the same G but are different regarding FI. Fish "a" has a positive RFI and fish "b" a negative RFI being respectively an inefficient and an efficient fish within the population.

Repeatability (R) of G, FI, RFI and TFT was calculated using Proc GLM (SAS, 1989) with individual as the main effect. The observational components of variance were calculated from the mean squares (Becker, 1984). Afterwards, repeatability was estimated as the ratio of among-individual variance (σ^2_W) to the total phenotypic variance (or the sum of within and among-individual variances, $\sigma^2_W + \sigma^2_A$): $R = \sigma^2_W / (\sigma^2_W + \sigma^2_A)$

A repeatability of zero indicates that all variance is within individuals over successive measurements, and a repeatability of one means that repeated measurements of the same individual give identical estimates (Falconer and Mackay, 1996). The standard error for repeatability was calculated according to Becker (1984).

The coefficient of variation for weight gain was calculated as:
Coefficient of variation (CV, %) = [(SD / mean)*100]

Possible differences in growth, feed efficiency and feeding behaviour over the experimental period were tested using repeated measures design. Mauchly's test was performed to assess the assumption of sphericity and the Bonferroni test for making pairwise comparisons (Field, 2000). Possible correlations between G, RFI and FI over the experimental period were investigated using Spearman ranking tests. Linear regression models were used to assess the effect of G and TFT on feed intake. Analysis of covariance was used to compare slopes and intercepts of regression lines between periods. Statistical analyses were performed using SPSS 11.5 for Windows. Statistical significance was taken at $P < 0.05$.

RESULTS

Growth, feed intake/efficiency and feeding behaviour

Body weight increased significantly during the experiment (Repeated measures ANOVA, $F_{2,47}$, $P < 0.001$, Table 1). The CV for body weight increased from 5.0 % at the start to 38.9 % on day 47. The fish also exhibited pronounced variation in feed intake (CV = 34.3 %) and growth (CV = 52.8 %). The first period of individual housing (P1) was characterized by a low feed intake and growth, indicating that the fish were still adapting to isolation conditions (Table 1).

There was no statistical difference between the mean total feeding time (TFT) during P2 and P3 (Repeated measures ANOVA, $F_{1,47}$, $P > 0.05$, Table 1). The individual variation in TFT was very high with CVs above 100 %: 120.3 % and 114.5 % after 30 and 47 days, respectively.

Table 1. Performance and feeding behavioural data of 48 juveniles of African catfish housed individually during 3 successive periods (P1: from day 0 till day 15; P2: from day 16 till day 30; P3: from day 31 till day 47). Values are mean ± SE.

	P1	P2	P3
Initial body weight (g)	58.94 ± 0.43^{a}	73.46 ± 1.66^{b}	99.09 ± 4.15^{c}
Weight gain (g)	14.52 ± 1.59^{a}	25.63 ± 2.73^{b}	47.12 ± 4.51^{c}
Feed intake (g $kg^{-0.8}$ d^{-1})	9.00 ± 0.43^{a}	9.30 ± 0.54^{a}	10.39 ± 0.53^{b}
Growth (g $kg^{-0.8}$ d^{-1})	8.21 ± 0.86^{a}	11.25 ± 1.05^{b}	13.94 ± 1.02^{bc}
Total feeding time (min)	-	67.32 ± 11.68^{a}	59.06 ± 10.67^{a}

[a,b,c] Different letters in the same line denote statistical significance at a significant level of $P < 0.05$.

Repeatability

Repeatability estimates were higher when the first 15 days (P1) were excluded (Table 2). When only P2-P3 were considered, the repeatability estimates were 0.70 for growth, 0.80 for feed intake and 0.61 for residual feed intake. Interperiod G and FI were positively correlated (Spearman correlation, $P < 0.01$, Table 3, A and B). The residual feed intake was also positively correlated between periods 1 and 3 (Spearman correlation, r = 0.38, $P < 0.01$) and between periods 2 and 3 (Spearman correlation, r = 0.54, $P < 0.01$) but not between periods 1 and 2 (Spearman correlation, r = 0.26, $P > 0.05$, Table 3, C). Total feeding time was the parameter with the highest repeatability estimate (0.81) and was strongly correlated between P2 and P3 (Spearman correlation, r = 0.63, $P < 0.01$).

Table 2. Repeatability (R) of growth (G, g $kg^{-0.8}$ d^{-1}), feed intake (FI, g $kg^{-0.8}$ d^{-1}), residual feed intake (RFI, g $kg^{-0.8}$ d^{-1}), and total feeding time (TFT, min) for 48 juveniles of African catfish housed individually between period 1, 2 and 3 (P1 – P3) and between period 2 and period 3 (P2, P3). P1: from day 0 till day 15; P2: from day 16 till day 30; P3: from day 31 till day 47.

Variable	R	SE	Confidence limits
G			
P1-P3	0.55*	0.09	0.36 , 0.71
P2,P3	0.70*	0.09	0.45 , 0.84
FI			
P1-P3	0.70*	0.06	0.56 , 0.82
P2,P3	0.80*	0.06	0.63 , 0.89
RFI			
P1-P3	0.49*	0.10	0.29 , 0.65
P2,P3	0.61*	0.11	0.27 , 0.79
TFT			
P2,P3	0.81*	0.05	0.65 , 0.90

* indicates statistical significance at a significant level of $P < 0.05$.

Relationship between performance and behaviour

The relationship between growth and feed intake is described through the regression equation, $FI = \beta_1 + \beta_2 G + e$ (Table 4). The maintenance requirement (β_1) in P1 (5.5 g $kg^{-0.8}$ d^{-1}) was significantly higher than in P2 (4.0 g $kg^{-0.8}$ d^{-1}) and in P3 (3.5 g $kg^{-0.8}$ d^{-1}, ANCOVA, $F_{2,140}$, $P < 0.01$). Such a difference was not found between P2 and P3 (ANCOVA, $F_{1,93}$, $P = 0.43$). The requirement for production (β_2) was not significantly different between P1, P2 and P3 (ANCOVA, $F_{2,140}$, $P = 0.39$). The individual differences in growth can be explained through individual differences in feed intake by 74 % in P1, 83 % in P2 and 91 % in P3. When feeding behaviour (TFT) was included in the model to explain FI ($FI = \beta_1 + \beta_2 G + \beta 3$ TFT + e), the R^2 increased by 2 % in P2, 0.4 % in P3 and 2 % when both P2 and P3 are considered.

Table 3. Spearman correlation coefficients between periods (P1, P2, P3) for growth (G, g $kg^{-0.8}$ d^{-1}; **A**), feed intake (FI, g $kg^{-0.8}$ d^{-1}; **B**) and residual feed intake (RFI, g $kg^{-0.8}$ d^{-1}; **C**) of 48 juveniles of African catfish housed individually (P1: from day 0 till day 15; P2: from day 16 till day 30; P3: from day 31 till day 47).

A

Variable (G)	P1	P2	P3
P1	1		
P2	0.63*	1	
P3	0.41*	0.70*	1

B

Variable (FI)	P1	P2	P3
P1	1		
P2	0.80*	1	
P3	0.52*	0.73*	1

C

Variable (RFI)	P1	P2	P3
P1	1		
P2	0.26	1	
P3	0.38*	0.54*	1

* indicates statistical significance at a significance level of P <0.01

The regression coefficient of TFT (β_3) significantly contributed to the model during P2 (Multiple regression, t (45) = 2.68, P < 0.01, 0.008 g $kg^{-0.8}$ d^{-1} min^{-1}) and when considering both P2 and P3 (Multiple regression, t (45) = 3.62, P < 0.001, 0.009 g $kg^{-0.8}$ d^{-1} min^{-1}). These significant positive regression coefficients of TFT indicate that with increasing TFT, the maintenance feeding levels are increased. This is also reflected in Fig. 3 which shows a significant positive correlation (Spearman correlation, r = 0.47, P < 0.01) between total feeding time and the residual feed intake. This indicates that fish taking longer to eat their meal will have higher values of RFI, i.e., will need more food to achieve the same growth as fish that eat their meal faster.

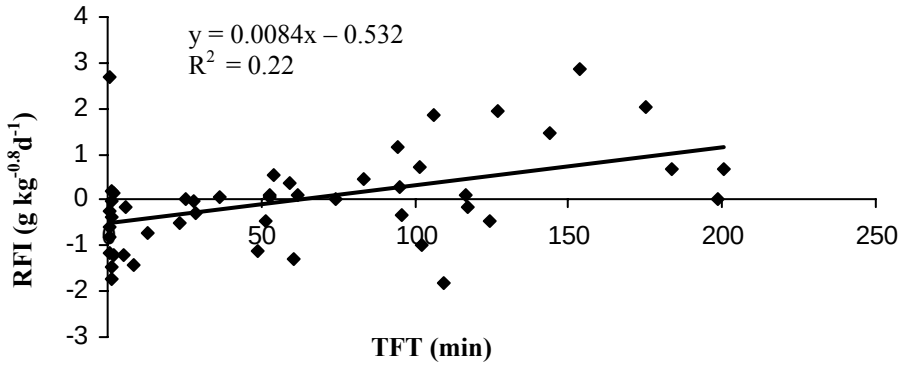


Figure 3. Relationship between the residual feed intake (RFI) and the total feeding time (TFT) for 48 juveniles of African catfish housed individually.

Table 4. Regression equations for feed intake (FI, g $kg^{-0.8}$ d^{-1} ; dependent variable), growth (G, g $kg^{-0.8}$ d^{-1}; independent variable) and total feeding time (TFT, min; independent variable) of 48 juveniles of African catfish housed individually (P1: from day 0 till day 15; P2: from day 16 till day 30; P3: from day 31 till day 47).

Period	Equation excluding TFT	R^2	Equation including TFT	R^2
P1	FI = 5.5 (±0.38)* + 0.431 (± 0.037)* G	0.74		
P2	FI = 4.0 (±0.42)* + 0.471 (± 0.031)* G	0.83	FI = 3.4 (±0.45)* + 0.475 (±0.029)* G + 0.008 (±0.003)* TFT	0.85
P3	FI = 3.5 (±0.36)* + 0.492 (±0.023)* G	0.91	FI = 3.2 (±0.41)* + 0.499 (±0.023)* G + 0.004 (±0.003) TFT	0.91
P1, P2, P3	FI = 3.8 (±0.34)* + 0.512 (±0.025)* G	0.90	FI = 3.1 (±0.35)* + 0.523 (±0.023)* G + 0.009 (±0.002)* TFT	0.92

*indicates statistical significance at a significance level of $P < 0.01$

DISCUSSION

Growth variation is common in fish species, and may result from dominant individuals consuming more food and growing faster than subordinates (Jobling and Koskela, 1996). When social hierarchies are the main factor responsible for growth variation then it is expected that in the absence of social interactions (such as in individual housing) such variation remains low. However, this was not the case in this study in which the weight variation increased over time. African catfish housed individually exhibited a pronounced individual variation in feed intake (34.3 %), growth (52.8 %) and particularly in the total feeding time (TFT, > 100 %), suggesting that these differences may be inherent. Moreover, this study showed that the individual differences in these traits are consistent over time and although this does not prove a genetic link, it suggests a high probability of being genetically linked. Wang et al. (1998) also showed significant individual variation in the growth of hybrid sunfish *Lepomis cyanellus* Rafinesque × *L. macrochirus* Rafinesque, housed individually suggesting that these differences have a genetic basis.

The development of individual differences in behaviour, especially in consistent behavioural traits, has rarely been investigated in fish (Budaev et al., 1999). Behavioural parameters often have low repeatability, given the possible sources of variation, such as fish condition, motivation and unknown aspects of the testing protocol (Brodie and Russell, 1999). To our knowledge, the present study shows for the first time that feeding behaviour (measured as the total feeding time, TFT) shows high values of repeatability (Table 2). This indicates that fast eaters remain fast eaters when reared in the same environment. Residual feed intake (RFI) also showed high levels of repeatability suggesting that individuals that have the highest feed efficiency in the beginning of the experiment will continue to be the ones with the highest feed efficiency at the end of the experiment.

Residual feed intake is positively related to feeding behaviour, indicating that fish eating their meal faster (lower TFT) have lower RFI (i.e., are more efficient in feed utilization). Rauw et al. (2000) suggested that behavioural activities could be a cause for variation in RFI. De Haer et al. (1993) suggested that pigs with a low RFI visited the feed hopper less often and spent less time eating per day than pigs with a high RFI. Besides the energy spent for feeding activity, also the energy spent for maintenance may influence the relation between TFT and feed efficiency. The maintenance levels obtained in this study are in accordance with maintenance levels reported for African catfish of 3.3 and 4 g $kg^{-0.8}$ d^{-1} by Henken et al. (1985) and Heinsbroek et al. (1989), respectively. Cutts et al. (2002) showed a

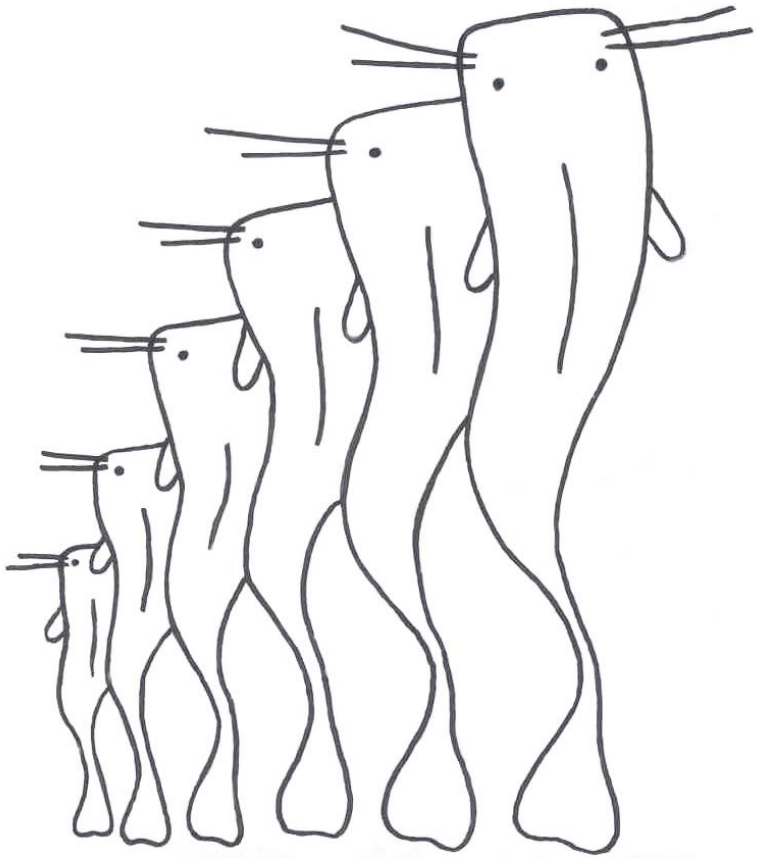
relationship between maintenance levels and feeding motivation for juveniles of Atlantic salmon *Salmo salar* L. Fish with a high standard metabolic rate had a slightly but significantly lower feeding motivation. This finding in salmon corroborates the finding of the current study in which African catfish with a high TFT have a higher RFI and also an increased maintenance feeding level (Table 4).

This study showed that individual differences in growth rate of African catfish are consistent over time. This may be related to the survival strategy exhibited by African catfish in nature. As described by van der Waal (1998), the variation in growth rate found in this species is related to a survival strategy. In normal weather conditions (normal rainfall), fast growers will have higher probabilities of survival than slow growers while under extreme conditions (lower than normal rainfall) the opposite will happen. Although different feeding patterns have already been described in nature for African catfish (Bruton, 1979), there is no information based on wild animals about individual differences in the total time spent eating. The possible adaptive value of the consistent individual differences in TFT found in this study must be further investigated.

The total feeding time was also significantly correlated to feed efficiency. This may have implications on the use of feeding behaviour as criterion for identifying fish that are efficient in feed utilization. However, before any attempt is made in the direction of the development of selection programs based on feeding behaviour, studies on heritability have to be done. Moreover, studies have to be conducted to assess the repeatability of feeding behaviour in group-housed situation and its possible relationship to feed efficiency.

Acknowledgements

This research was funded by the Foundation for Science and Technology, Portugal (SFRH/BD/2946/2000). We would like to acknowledge Menno ter Veld, Sietze Leenstra, Aart Hutten and Wian Nusselder for their help in starting up the experiment.



6

- CHAPTER -

Inherent variation in growth efficiency of African catfish (*Clarias gariepinus*) juveniles

C. I. M. Martins

J. W. Schrama

J. A. J. Verreth

Aquaculture Research, 2005, 36, 868-875

ABSTRACT

Understanding the major causes of growth variation is crucial for the success of fish farming since its reduction contributes to maximize production efficiency, reduce food waste and improve water quality. The growth variation observed in aquaculture has been associated with the establishment of social hierarchies. However, some studies suggest that this variation may not be mainly a consequence of social hierarchies but mainly a result of inherent (genetic) differences. This study investigates the magnitude of individual responses, independently of group effects (fish housed individually), in growth efficiency and feeding behaviour of African catfish *Clarias gariepinus* fed restrictively. Despite the low variation in initial body weight (CV = 6.5 %) and cumulative feed consumption (7.5 %) over the experimental period, catfish exhibited high variation in final body weight (18.1 %), specific growth rate (17.2 %) and feed conversion ratio (27.9 %), suggesting that individual variation in growth efficiency is important in determining growth rate. This individual variation may be related to individual differences in protein/fat deposition since faster growing fish deposited more protein and less fat than slower growing fish. Pronounced individual differences in feeding behaviour (reaction towards feed and time spent eating) were also observed and correlated to individual differences in growth efficiency. Fast eaters were the fast growers. We suggest that the growth variation observed in African catfish may be inherent and that the use of grading to increase uniformity should be further investigated.

INTRODUCTION

Fish exhibit widely divergent growth rates, both in natural as well as in aquaculture conditions (Larsson, 1984; Grobler et al., 1992; van der Waal, 1998; Qian et al., 2002). In aquaculture conditions, the individual variation in growth can result from external (e.g., feeding schedule, feeding frequency, social interaction) and internal factors (e.g., metabolic rate, digestibility, protein turnover, energy expenditure by swimming) (McCarthy et al., 1994; Toguyeni et al., 1997; Wang et al., 1998). Among these factors, social interaction has been identified as the major cause of individual variation in growth (Jobling et al., 1993; Cutts et al., 1998). As a consequence of social interactions, dominance hierarchies may be established. Dominant fish are usually characterised by having a better performance than subordinates (higher feed intake and growth rates) and specific behavioural strategies (swimming faster and reacting first towards feed) (Jobling and Wandsvik, 1983; Abbott and Dill, 1989; Brännäs, 1998; Cutts et al., 1998; Hart and Salvanes, 2000; Alanärä et al., 2001). The question arises whether the individual variation in growth, feed intake and behaviour, so commonly observed in group-housed fish, is mainly a consequence of social interactions or is also (or even mainly) inherent, i.e., genetically linked. Understanding the major causes of growth variation is crucial for the success of fish farming. The minimization of individual variation in food consumption and growth contributes to maximizing production efficiency, reducing food waste and improving water quality (McCarthy et al., 1992; Jobling and Baardvik, 1994; McDonald et al., 1996). Grading is commonly done in aquaculture as a way to increase size uniformity by disrupting social hierarchy (Baardvik and Jobling, 1990). However, in some species the cause of growth variation may be mainly due to genetic differences and not to the establishment of social hierarchies. In this case the use of size-grading should be re-considered. Understanding inherent causes of growth variation, without the interference of social interactions can be done when fish are socially isolated, i.e., individually-housed. The few studies done so far with fish held in isolation showed important inherent differences in feed intake and growth efficiency, suggesting that more attention should be paid to individual-housed studies (Jobling and Baardvik, 1994; Wang et al., 1998; Qian et al., 2002). Whether individual differences in feeding behaviour are also inherent or a consequence of social interaction is still not known.

African catfish *Clarias gariepinus* (Burchell 1822) is our organism of choice because it exhibits a wide variation in growth rate and behaviour patterns both under natural and aquaculture conditions (Bruton, 1979; Grobler et al., 1992; van der Waal, 1998; Almazán-

Rueda, 2004a). African catfish is also one of the most studied species in the *Clariidae* family and is of great importance in both fisheries and fish culture (Teugels, 1996). This study intends to give the first step in understanding the inherent causes of individual variation in growth of African catfish. The objectives of this study are 1) to investigate the magnitude of individual responses, independently of group effects, in growth efficiency and feeding patterns of African catfish; 2) whether individual feeding behaviour can explain possible differences in growth efficiency.

MATERIAL AND METHODS

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

Experimental animals

Thirty-one juveniles (mean initial weight ± SE, 65.5 ± 1.5 g, full sibs) were obtained from a local catfish producer (Fleuren, Someren, The Netherlands) where they experienced common housing and feeding conditions. On arrival at Wageningen University, each fish was housed individually in a glass aquarium (30 x 35 x 40 cm) of 40-l capacity, connected to a shared recirculation system. Fish had no visual contact to one another. To minimize the possible impact of stress due to isolation only fish that reached an adequate feeding level for a normal growth in this species, were used as experimental animals. After 10 days, 17 fish (68.3 ± 1.2 g) reached the same feeding level of 15g $kg^{-0.8}$ d^{-1} and were considered our experimental animals. A 12L : 12D photoperiod was maintained with day break set at 0700 h. Water quality parameters were kept as follows: pH ranged between 7.1 and 7.7, conductivity at 3.5 ± 0.06 mS cm^{-1}, NH_4^+ < 2 ppm, NO_2^- < 0.5 ppm, NO_3^- at 55 ± 5.7 mg l^{-1} and temperature at 25.5 ± 0.1 ^oC. The experiment duration was 42 days, which was considered sufficiently long for significant weight increase, based on previous experiments (Almazán-Rueda, 2004a).

Experimental procedures

Animals were hand fed an experimental diet (Table 1) once per day (0800 - 1200h). It is known that group-housed African catfish can achieve a feeding level close to satiation of 24 g $kg^{-0.8}$ d^{-1} (Ozório, 2001). In the present experiment, a feeding level of 15g $kg^{-0.8}$ d^{-1} was used to ensure that all fish would eat the same amount of feed and still realize a satisfactory

growth rate. The feeding procedure consisted of providing sequential portions of 5 pellets to each fish, randomly. New pellets were added only after the first portion was completely eaten. This procedure was repeated for 1 h, after which any remaining pellets were collected and counted. The feed intake was recorded daily since the beginning of the experiment. The observation of feeding behaviour started 10 days after the beginning of the experiment and was recorded daily since that day onwards. Two feeding behaviour parameters were studied: feeding response and total feeding time. Two feeding responses were distinguished via direct observation and were based on the fish's reactions towards the presented pellets, providing a measure of feeding motivation including movement and intent of the fish. Response (1) occurred when fish reacted at once to the feed when the first pellets were added to the tank, and ate all the pellets immediately and response (2) occurred when fish reacted at once to the feed but did not eat all the pellets subsequently, i.e., stopping several times before finishing their meal. Each fish in each day exhibited either response 1 or 2, never both responses in the same day. However some fish did not exhibited the same response every day. Therefore, the final feeding response of each fish was considered to be the feeding response with the highest frequency of occurrence (mode) over the measurement period (32 days). Total feeding time was measured using stopwatches and was defined as the time from the first to the last eaten pellet. The fish were never handled during the experiment to avoid stress induction and any modification of their feeding behaviour or consumption levels. At the end of the experiment, each fish was killed with an overdose (0.8 g l^{-1}) of tricaine methanesulfonate (TMS; Crescent Research Chemicals, Phoenix, Arizona, USA) buffered with sodium bicarbonate (1.6 g l^{-1}). All experimental fish were retained at –20 °C for subsequent proximate analyses (dry matter, ash, crude protein, fat and energy).

Analytical methods

For proximate analyses, each fish was frozen, cut into small pieces, thawed and then mixed with distilled water (25 % of the fresh sample weight). Each sample was then autoclavated at 120 °C for 2 h and homogenized with an Ultratorrax for 2 min. Triplicate sub-samples were then assayed separately. Dry matter and ash content were determined by drying the samples for 4 h at 103 °C (ISO 6496, 1983) and 550 °C (ISO 5984, 1978), respectively. Crude protein was measured by Kjeldahl according to ISO 5983 (1979) procedures and calculated as nitrogen content multiplied by 6.25. Crude fat was determined by Soxhlet extraction (EEG 18.1.84 No 15/29-30) after the samples were freeze-dried. Gross energy content was

measured by bomb-calorimetry (IKA-C-7000, Fa. IKA-Analysentechnik, Weitersheim, Germany). Due to technical problems one protein (n = 16) and two fat (n = 15) samples were not analysed.

Table 1. Experimental diet composition

Item	g kg^{-1} feed
Ingredients	
Fish meal	450.0
Soybean meal	125.0
Wheat	184.0
Wheat gluten	100.0
Fish oil	60.0
$CaCO_3$	35.0
Durabon binder	25.0
Monocalcium phosphate	7.0
NaCl	4.0
Premix [a]	10.0
Analyzed composition	
Dry matter	883.6
Crude protein (N x 6.25)	387.1
Crude fat	94.4
Ash	113.0
Gross Energy, MJ	15.9

[a] *Premix,* kg^{-1} feed : Vit B1 30 mg; Vit B2 30 mg; Vit B6 30 mg; Vit B5 100 mg; Vit B3 200 mg; Vit H 0.6 mg; B-12 0.05 mg; Folic acid 15 mg; Vit C 500 mg; Vit E 200 IU; vit A palmitate 15000 IU; D-Rovimix (D3-500) 2000 IU; K_3 K-menadione sodium bisulphate (51 %) 8 mg; Inositol, 200 mg; Choline chloride, 1000 mg; Fe 50 mg, given as Iron sulphate ($FeSO_47H_2O$); Zn 100 mg, given as Zinc sulphate ($ZnSO_47H_2O$); Co 2.4 mg, given as Cobalt sulphate ($CoSO_47H_2O$); Cu 5 mg, given as Copper sulphate ($CuSO_45H_2O$); Se 1 mg, given as Sodium selenite (Na_2SeO_3); Mn 25 mg, given as Manganese sulphate ($MnSO_44H_2O$); Mg 300 mg, given as Magnesium sulphate ($MgSO_47H_2O$); Cr 1 mg, given as Chromic chloride ($CrCl_36H_2O$); I 5 mg, given as Calcium iodate ($CaIO_36H_2O$); Anti-oxidant BHT (E300-321), 0.1 g; Calcium propionate, 1.0 g.

Data analysis

The results are expressed as means (± SE) unless otherwise stated. For each fish, the specific growth rate (SGR), feed conversion ratio (FCR) and the coefficients of variation (CV) for SGR, FCR and feed consumption were calculated as follows:

Specific growth rate (SGR, % d^{-1}) = [100 (ln W_f – ln W_i) / t] where W_f and W_i are the initial and final wet weighs (g) of the fish, respectively, after 42 days, (t); Feed conversion ratio (FCR) = (F_t / WG_t) where F_t and WG_t are the total feed consumed over the time t (g) and the weight gain during the time t (g), respectively; Coefficients of variation (CV, %) = [(SD / mean)*100].

Statistical analyses were performed using SPSS 11.5 for Windows. Specific growth rate (arc-sin transformed), cumulative consumption, feed conversion ratio and total feeding time were tested for normality (Kolmogorov – Smirnov test) and homogeneity of variances (Levene's *F* test). Differences between the feeding responses were tested using independent *t*-test using the adjusted *P*-values when equal variances could not be assumed. Possible correlation between the variables was investigated using the Spearman ranking tests. Statistical significance was taken at $P < 0.05$.

RESULTS

Individual variation in performance and feeding behaviour

Despite the low variation in initial body weight (6.5 %) and cumulative feed consumption (7.5 %) over the experimental period, fish showed a high variation in final body weight (18.1 %), SGR (17.2 %) and FCR (27.9 %). The SGR values ranged from 1.74 to 3.24 % d^{-1} and the FCR from 0.63 to 1.55 (Table 2). These values are in accordance with performance data obtained from group-housed fish (Grobler et al., 1992; Ozório, 2001; Almazán-Rueda, 2004a).

Table 2. Mean ± SE, coefficient of variation (CV), minimum (Min.) and maximum (Max.) values of variables related to performance and behaviour in juveniles of African catfish *Clarias gariepinus* (Burchell 1822) held individually for 42 days (n =17).

	Mean ± SE	CV (%)	Min.	Max.
Initial weight (g)	68.25 ± 1.24	7.50	60.60	75.00
Final weight (g)	204.72 ± 9.0	18.13	133.30	262.10
Cumulative feed intake (g)	119.87 ± 1.87	6.45	107.16	129.58
SGR (% d^{-1})	2.58 ± 0.11	17.18	1.74	3.24
FCR	0.94 ± 0.06	27.93	0.63	1.55
TFT (min)	10.37 ± 3.71	147.50	0.77	45.82

Differences in cumulative feed consumption were not correlated to SGR and FCR (Fig. 1, Spearman ranking test, $P > 0.05$), suggesting that growth efficiency was important in determining growth rate and that the observed differences in growth rate can be seen as differences in growth efficiency.

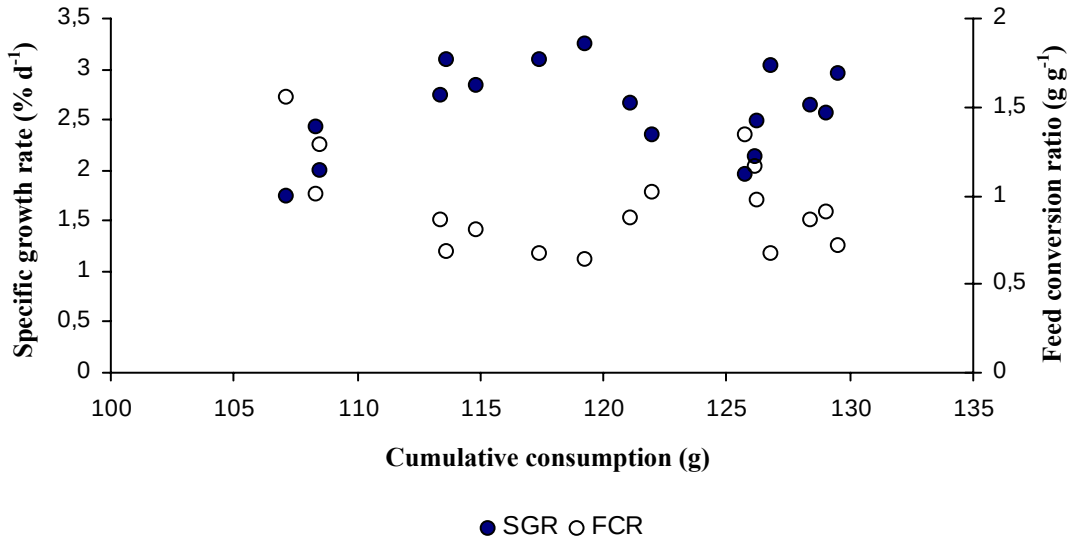


Figure 1. Relationship between cumulative feed consumption and performance (specific growth rate and feed conversion ratio) in 17 juveniles of African catfish *Clarias gariepinus* housed individually for 42 days.

Differences in growth rate, were positively correlated (Spearman ranking test, r = 0.54, $P < 0.05$) with crude body protein and negatively correlated to body fat (Spearman ranking test, r = - 0.60, $P < 0.05$). The body crude protein (y_1) and fat contents (y_2) were related to growth (x) as follows: $y_1 = 35.2\ x + 543.81$, $R^2 = 0.29$, and $y_2 = -42.127\ x + 334.52$, $R^2 = 0.36$, respectively. Faster growing fish deposited more protein and less fat than slower growing fish (Fig. 2).

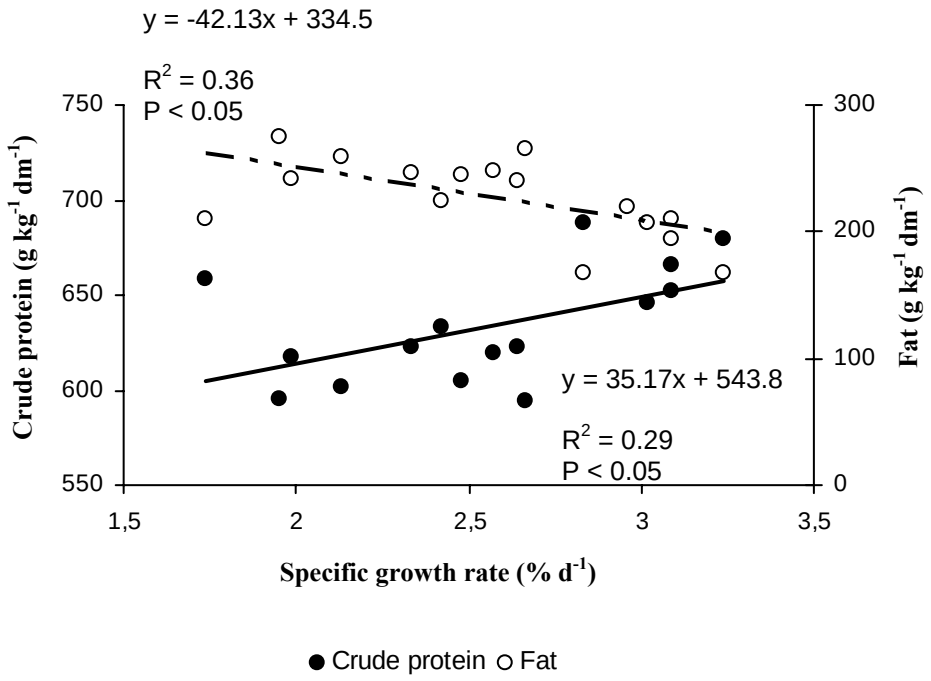


Figure 2. Relationship between the whole body protein (n = 16) and fat content (n = 15) and the specific growth rate in 17 juveniles of African catfish housed individually for 42 days.

Pronounced individual variation in feeding behaviour was observed. Feeding responses 1 and 2 were exhibited by 12 and 5 fish, respectively. Total feeding time ranged from 0.77 to 45.82 min with a CV of 147.5 % (Table 2).

Relationship between performance and feeding behaviour

The main difference between feeding responses was whether the fish ate all the pellets at once without stopping (response 1) or whether they stopped eating several times before finishing the meal (response 2). This explains why the total time spent eating differed between these feeding responses, with fish exhibiting feeding response 1 and 2 being clearly fast and slow eaters, respectively (Fig. 3A, *t*-test, $P < 0.05$). Initial body weight did not differ between the feeding responses (response 1: 67.5 ± 1.5 g, response 2: 70.0 ± 2.2 g; *t*-test, $P > 0.05$). Nevertheless, the final body weight was significantly higher in fish exhibiting feeding response 1 (221.2 ± 8.1 g) than fish exhibiting feeding response 2 (165.1 ± 10.7 g; *t*-test, $P < 0.01$). There were no significant differences in cumulative feed consumption between fish with feeding responses 1 and 2 (Fig. 3B, *t*-test, $P > 0.05$). However, fish exhibiting feeding response 1 had higher specific growth rates, and a lower feed conversion ratio (Fig. 3C and D, *t*-test, $P < 0.05$).

Moreover, total feeding time was significantly correlated to specific growth rate (Fig. 4, Spearman ranking test, $r = -0.76$, $P < 0.01$) and feed conversion ratio (Fig. 4, Spearman ranking test, $r = 0.73$, $P < 0.01$) indicating that fast eaters are the fast growers and vice-versa.

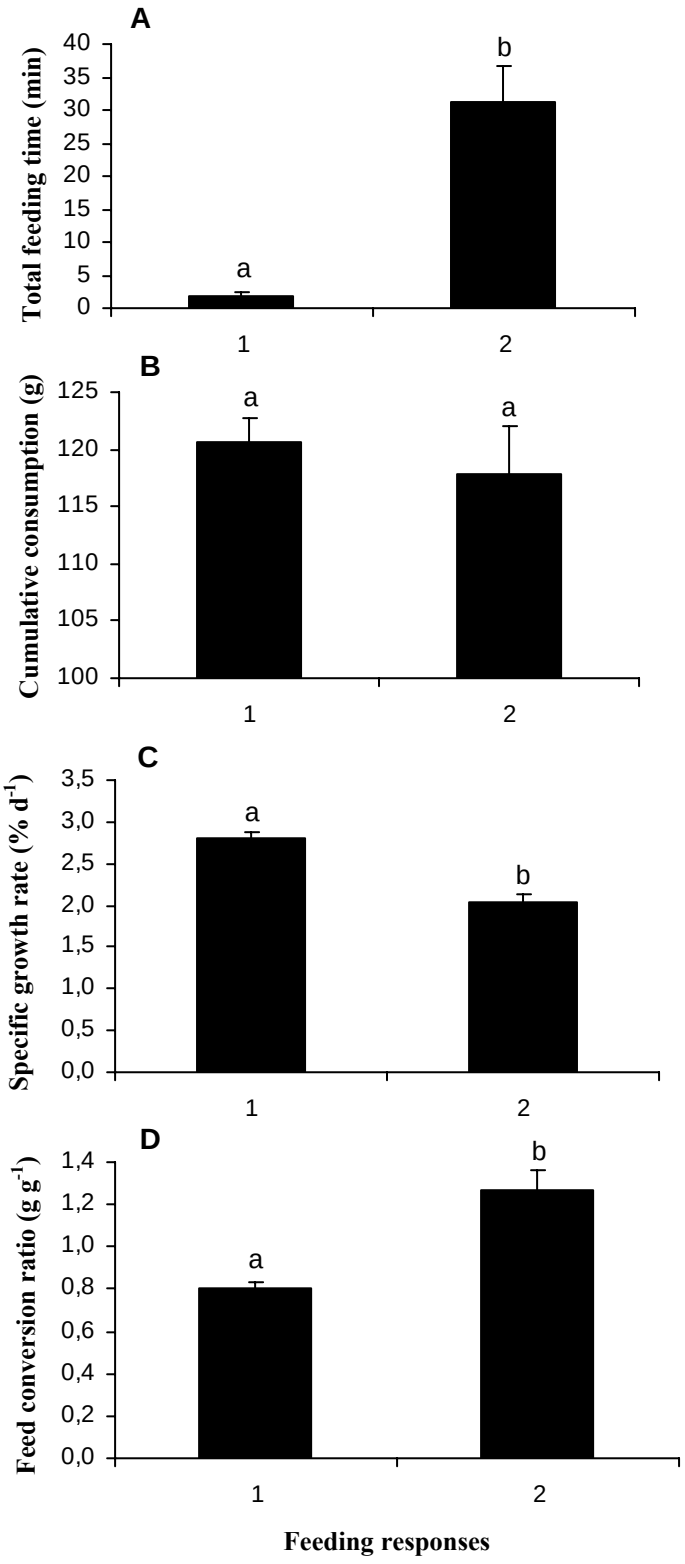


Figure 3. Total feeding time (A), cumulative feed consumption (B), specific growth rate (C) and feed conversion ratio (D) in juveniles of African catfish housed individually for 42 days. Response 1, n = 12 and response 2, n = 5. Different letters indicate significant difference, $P < 0.05$.

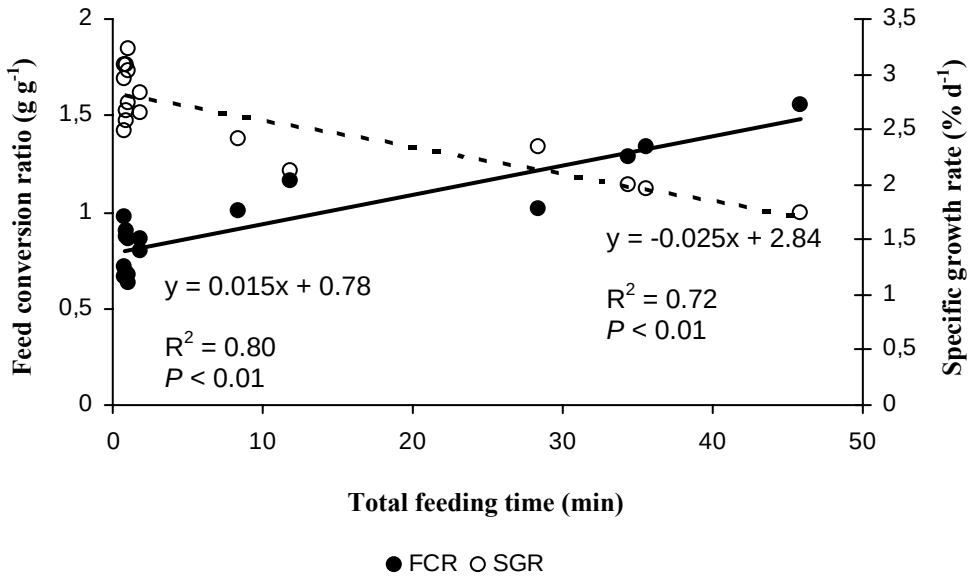


Figure 4. Relationship between total feeding time and performance (feed conversion ratio and specific growth rate) in 17 juveniles of African catfish housed individually for 42 days.

DISCUSSION

The current study showed that in the absence of social interactions *C. gariepinus* juveniles display substantial inter-individual variation in growth efficiency and feeding behaviour. This suggests that the observed individual variation may be inherent, i.e., genetically linked and not a consequence of social hierarchies.

High individual differences in growth rate for African catfish have already been reported (Grobler et al., 1992; van der Waal, 1998, Almazán-Rueda, 2004a). However, these studies have been done in group-housed fish in which the fast growers are supposedly to represent those individuals obtaining more feed through behaviour domination (Grobler et al., 1992). High individual variation in growth among individual housed fish has also been demonstrated (Wang et al., 1998; Qian et al., 2002). These studies showed that individual differences in feed intake were positively correlated to individual differences in growth, indicating that those individuals that consumed the greatest quantities of feed were also those that had the highest rates of weight gain. In our study, fish exhibited a pronounced variation in growth rates despite the small variation in feed consumption, suggesting that other factors besides differences in feed intake play an important role in determining growth rate. This

corroborates with the results obtained by Cui and Liu (1990) using six species held in isolation where the differences in growth rate were mainly explained by differences in growth efficiency and not to differences in feed intake. Differences in metabolism could be the cause for individual differences in growth efficiency (Qian et al., 2002). Smith et al. (1988) found higher body fat and lower protein levels in slow-growing rainbow trout strains, paralleling the results in our study. The significant correlations between SGR and body protein / fat content indicate that the fast growers deposit more protein per dry matter and less fat than do the slow growers, potentially as a consequence of individual differences in metabolism (e.g., different rates of protein turnover) and digestibility. As suggested by McCarthy et al. (1994) individual differences in protein turnover are important determinants of growth efficiency in fish.

African catfish exhibits different types of feeding patterns under natural conditions. These include foraging, individual shovelling, individual surface feeding and social hunting (Bruton, 1979). It has been suggested that in the presence of social interactions, individual differences in feeding strategies such as reacting first to an encountered prey, may give competitive advantage (Hart and Salvanes, 2000). This study shows a high degree of individual variation in feeding behaviour also when social interactions are absent. Although experimental fish had similar initial weights and were housed under the same housing and nutritional conditions, they exhibited pronounced differences in the reaction towards feed (feeding responses) and on the time spent eating (total feeding time). Moreover, these individual differences in behaviour were strongly correlated to individual differences in growth efficiency; the fast eaters seem to be the fast growers. This corroborates to the findings of Valente et al. (2001b) who showed in trout that a fast-growing strain exhibited a higher voluntary feed intake when compared to a slow-growing strain. Moreover, rainbow trout *Oncorhynchus mykiss* (Walbaum) treated with growth hormone increased their feeding motivation and capacity for ingesting feed (Johnsson and Björnsson, 1994; Jönsson et al., 1998). In the present study, fish ate an equivalent amount of feed over the growth period, yet they showed significant differences in growth and feed conversion ratio. Fish that ate all their meal in a few seconds grew faster than fish that needed the full meal period to eat the same amount of feed. This suggests that the total feeding time is related to the way fish utilize their feed. This difference in growth efficiency may be explained by different swimming activities. Fast eaters may have more and longer resting periods, thereby saving energy for growth. This hypothesis corroborates the results obtained by Gregory and Wood (1998, 1999) and Petrell and Jones (2000) who found a negative relationship between growth rate and swimming

activity. However, in rainbow trout, fast growing fish were instead associated with more rapid feeding and greater swimming activity (Valente et al., 2001a*;* Jönsson et al., 2003). Whether this will be the case or not for African catfish still needs to be assessed.

Feed intake and growth have a genetic basis in salmon *Salmo salar* L. and channel catfish *Ictalarus punctatus* (Rafinesque) (Thorpe, 1977; Gjerde, 1986; Metcalfe et al., 1986, 1988; Silverstein et al., 2000). If feed intake and growth differences prove to be genetic in African catfish, then the fast eaters (response 1) could be good candidates for breeding selection programs. If feeding behaviour has to be used as a selection criterion, more information will be needed on the consistency of the feeding responses and the links to growth performance.

In previous experiments, group-housed African catfish achieved a feeding level of 24 g $kg^{-0.8}$ d^{-1} (Ozório, 2001). In the present experiment, a 15g $kg^{-0.8}$ d^{-1} level was used to ensure that all fish would eat the same amount of feed and still realize a satisfactory growth rate. However, 45 % of the animals did not eat all the provided feed and therefore were not used as experimental animals. The fact that some individuals had very low feed intake levels may be related to a genetically based low appetite or to a stress response because of isolation. If the first hypothesis is true, it would mean that only a fraction of the individuals housed in a group, eat at the feeding level which was set as the group's average (restricted) level (Jobling and Koskela, 1996). This has quite some implications for research where feeding responses are measured. In those experiments, feeding levels would be better assessed using individual weights rather than tank biomass. If the second hypothesis (stress response) is true, then individual housed studies should only be used when animals that are well adapted to isolation are used. This may be achieved when species that exhibits a complete (e.g., Pike) or partial solitary lifestyle (e.g., African catfish) are used. In this study, all experimental fish exhibited SGR and FCR comparable with the ones obtained from group-housed catfish (Grobler et al., 1992; Ozório, 2001; Almazán-Rueda, 2004a). Studies with individually housed fish may also be very useful to investigate individual coping strategies when fish are exposed to stressors.

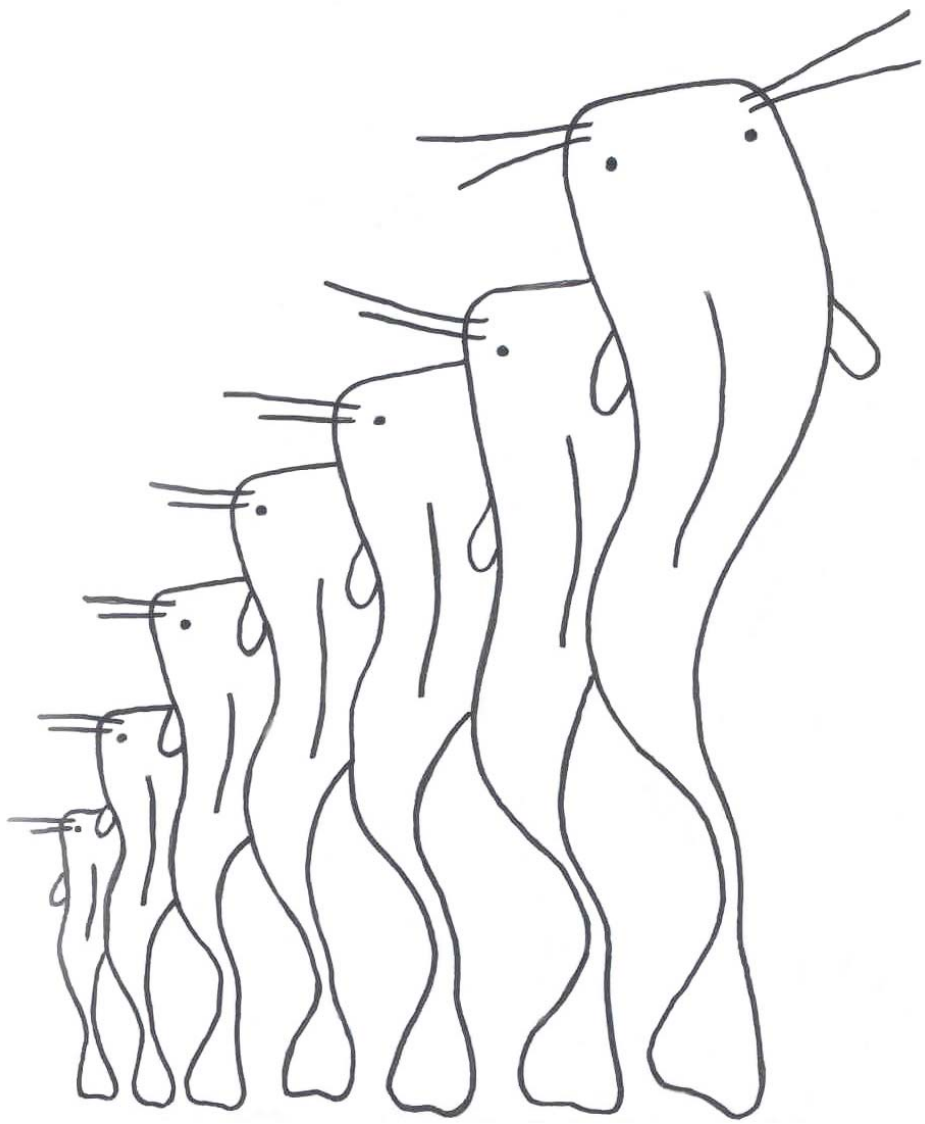
The results obtained in this study may have important consequences in farming African catfish. As the majority of farmed fish, African catfish exhibits a rather differential growth rate forcing the farmers to grade their fish once at a size of 300g or twice at 150g and 350g (Verreth and Eding, 1993). Grading is done under the assumption that small fish will grow better after larger conspecifics are removed (Baardvik and Jobling, 1990). However, if the variation in growth is not a consequence of social hierarchies but instead genetically

linked then the use of grading should be re-considered, since it will not result in increased performance of the small fish. Moreover, grading is time consuming, costly and involves handling which will cause stress to the fish.

The current study shows that in a situation of individual housing and restricted feed availability, there is a considerable individual variation in growth rate. This variation reflects individual differences in growth efficiency which seem to be related to differences in growth composition (protein / fat deposition) and feeding behaviour. We suggest that the variation in growth observed in African catfish is inherent, i.e., genetically linked and that the use of grading to increase uniformity should be further investigated.

Acknowledgements

This research was supported by the Foundation for Science and Technology, Portugal (SFRH/BD/2946/2000). We would like to acknowledge Menno ter Veld, Sietze Leenstra, Aart Hutten and Wian Nusselder for their help in starting up the experiment. We would also like to thank Ronald Booms and Tino Leffering for the technical assistance during the proximate analyses.



7

- CHAPTER -

The relationship between feed efficiency and stress response in African catfish (*Clarias gariepinus*)

C. I. M. Martins

J. W. Schrama

J. A. J. Verreth

Submitted

ABSTRACT

Despite the importance of feed efficiency and stress response in animal production, the number of studies focusing on their relationship is still very low. In fish, this relation has never been investigated. This study tests whether individual differences in the stress response of African catfish *Clarias gariepinus* can explain differences in feed efficiency.

The data used in the present study was based on two experiments. Experimental fish were juveniles of African catfish, weighing 58.9 ± 0.4 g and 75.5 ± 1.6 g (means ± SE) in experiment 1 (n = 48) and 2 (n = 26), respectively. Fish were fed until apparent satiation with a commercial diet once per day. Individual feed consumptions were registered and the residual feed intake used as a measure of feed efficiency. Blood samples for plasma cortisol, glucose and lactate were taken from all fish at the start of the experiment (control, indicative of basal levels) and after a stress test (netting) at the end of the experiment. There was a pronounced individual variation in both basal and post-stress levels. Basal levels of glucose, lactate and cortisol did not contribute significantly to explain differences in feed efficiency. However, glucose levels obtained after the stress test could explain differences in feed efficiency by 1.3 % in experiment 1 and 5.9 % in experiment 2. In experiment 2, the cortisol levels obtained after the stress test also contributed to explain differences in feed efficiency by 8.7 %. These results suggest that high stress responders are less efficient fish, indicating that individual differences in stress response explain part of the differences in feed efficiency. Stress response seems to be related to feed efficiency by explaining variance in maintenance levels.

INTRODUCTION

The pronounced individual differences in the growth of fish species are mainly attributed to differences in feed intake (Koebele, 1985; Umino et al., 1997). However, also differences in feed efficiency seem to play a role in explaining individual differences in growth (Qian et al., 2002; Martins et al., 2005a). Understanding the underlying mechanisms that control feed efficiency is fundamental in aquaculture since an improvement of feed efficiency means a decrease on 1) the amount of feed required for production and therefore on the overall cost of production and on 2) the loss of nutrients to the environment.

Variation in feed efficiency is mainly caused by variation in maintenance requirements (Luiting, 1990). Stress, by inducing defense and repair processes (e.g., heat-shock proteins, increased protein turnover) which require energy results in increased maintenance costs (Maltby, 1999). Therefore, individual differences in the mobilization and allocation of energy under a stress response are expected to be related to individual differences in feed efficiency.

Despite the importance of feed efficiency and stress response in animal production, the number of studies focusing on their relationship is still very low (e.g., in chickens, van Eerden et al., 2004; in cattle, Richardson and Herd, 2004; Richardson et al., 2004). To our knowledge such information is still lacking in fish.

Individual differences in stress response have already been identified in several fish species (Barton and Iwama, 1991, Pottinger and Carrick, 1999) but never used as explanatory factors for differences in feed efficiency. In this study residual feed intake (RFI) will be used as a measure of feed efficiency. Residual feed intake is defined as the difference between actual feed intake and that predicted from mean observed requirements for growth and body weight maintenance (Koch et al., 1963).

Plasma glucose, lactate and cortisol levels have been commonly used as a way to measure stress in fish (van Raaij et al., 1996; Pottinger, 1998; Ruane et al., 2001). In the present study, these parameters will be used to test whether individual differences in the stress response of African catfish *Clarias gariepinus* can explain differences in feed efficiency.

MATERIAL AND METHODS

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

Experimental animals and feeding procedure

The data used in the present study was based on two experiments made on different time points and for other purposes. Experimental fish were juveniles of African catfish *Clarias gariepinus* (Burchell 1822), weighing 58.9 ± 0.4 g and 75.5 ± 1.6 g (means ± SE) in experiment 1 (n = 48) and 2 (n = 26), respectively. All fish were obtained from a local catfish producer (Fleuren, Someren, The Netherlands) where they experienced shared housing and feeding conditions. On arrival at Wageningen University, each fish was housed individually in a glass aquarium (30 x 35 x 40 cm) of 40-l capacity, connected to a recirculation system. Fish had no visual contact with one another.

This study used individually housed fish because 1) in this way the possible relationships found will be independent of social interactions and therefore inherent, i.e., genetically based and 2) the study of feed efficiency implied an accurate quantification of individual feed intake which is possible in African catfish only by individual housing. It should be noted that the measure of individual feed intake by X-rays (the most common technique to measure individual feed intake in groups) is not possible in juveniles of African catfish due to emetic reactions. In addition, previous observations demonstrated that in African catfish, the ranking of growth in individual fish is not changed in group housing (Martins, unpublished data). Therefore, it is assumed that the individual differences in feed efficiency observed in this study are also representative of a group housing situation and not simply a consequence of isolation.

The experimental duration was 32 and 24 days for experiment 1 and 2, respectively. The 15 days preceding the experiment were considered as an adaptation period to isolation. A 12L:12D photoperiod was maintained with day break set at 0700 h. Water quality parameters were kept as follows: pH ranged between 7.1 and 8.1, conductivity at 3.0 ± 0.07 mS cm^{-1}, $NH_4^+ < 2$ mg l^{-1}, $NO_2^- < 0.5$ mg l^{-1}, $NO_3^- < 75.4$ mg l^{-1} and temperature at 25.2 ± 0.1 ^oC.

Fish were fed manually once per day, until apparent satiation with a commercial diet (floating pellets, 4.5 mm Coppens: 45 % crude protein, 12 % crude fat, 2.0 % crude fibre and 10.5 % ash). The feeding procedure consisted of providing sequential portions of 5 pellets to each fish, randomly. New pellets were added only after the first portion was completely eaten. The left over pellets were collected and counted after 4 hours in experiment 1 and 1 hour in experiment 2. Individual feed intake was recorded daily from the beginning of the experiment onwards.

At the end of the experiments, each fish was killed with an overdose of anaesthesia (0.8 g l^{-1} of tricaine methanesulfonate, TMS; Crescent Research Chemicals, Phoenix, Arizona, USA) buffered with sodium bicarbonate (1.6 g l^{-1}).

Stress method and sampling

Blood samples for plasma cortisol, glucose and lactate were taken from all fish at the start of the experiment (control, indicative of basal levels). At the end of the experiment fish were subdued to a stress test, followed by blood sampling. The stress test consisted of holding each fish individually in a net outside the water for 1h. It should be noted that African catfish is able to breathe air, and previous experiments using emersion periods up to 3 h have been performed with no mortality reported (Buttle et al., 1996). Therefore, it is considered that 1-h air exposure is not an extreme stressor for African catfish. Fish were anaesthetised for blood sampling (0.4 g l^{-1} of tricaine methanesulfonate, TMS Crescent Research Chemicals, Phoenix, Arizona, USA using 0.8 g l^{-1} of sodium bicarbonate as buffer). One ml of blood was collected from all fish by hypodermic syringe (containing 3 mg of Na_2EDTA) from the caudal blood vessels. This procedure was finalised within 3 min after fish were caught and anesthetised. The collected blood was placed in cooled 1.5 ml plastic tubes, mixed and centrifuged at 6000 *g* for 5 min at 4°C. After centrifugation plasma was collected and stored at -20 °C for further analysis. One plasma sample from the basal levels of experiment 2 could not be used, resulting in a total number of samples analyzed for experiment 2 of 25.

Analytical methods

Cortisol was measured in unextracted catfish plasma using the validated radio-immunoassay described by Ellis et al. (2004), but adapted by pre-heating the plasma in glutamate buffer (pH 3.3). The adaptation for direct assay originates from Dunn and Foster (1973) and Foster and Dunn (1974) and has been used widely in fish studies (e.g., Redding et al., 1984; Bisbal and Specker, 1991; Young, 1986). The catfish plasma (diluted 1:100 in glutamate buffer) was added to duplicate glass tubes for assay. Nine cortisol standards ranging from 2 to 500 pg 100 μl^{-1} were made up (in duplicate) by serial dilution in glutamate buffer. All tubes were heated in boiling water for 15 min, and then cooled by standing in cold water for 5 min. The assay then proceeded as described by Ellis et al. (2004), i.e., addition of radioactive steroid and antibody, overnight equilibration, removal of unbound cortisol with dextran-coated charcoal, and scintillation counting.

Plasma glucose and lactate were determined by the GOD-Perid method (Boehringer) and using Sigma Diagnostic Kits (Sigma; Proc. No 735), respectively.

Data analysis

The results are expressed as means (± SE). Although growth rates in aquaculture are typically described by specific growth rate (SGR) or growth rate in g per day, they do not take into account differences in metabolism. It is known that larger fish have a greater absolute requirement of food for maintenance and growth than smaller fish (Hepher, 1988). Expressing growth and feed intake per metabolic body weight will take into account theses differences. Therefore, growth (G) and feed intake (FI) were expressed per metabolic weight units of g BW $(kg)^{-0.8}$ d^{-1}, where g represents the grams of weight gain or the grams of feed intake and BW the geometric mean,

$BW = \exp\{½[\ln(W_{T1}) + \ln(W_{T2})]\}$, where W_{T1} is the weight (g) at time (t) 1 and W_{T2} the weight at time 2;

Feed efficiency was analyzed using the residual feed intake (RFI, g $kg^{-0.8}$ d^{-1}). 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: $FI = M + \beta G + e$, where M is the maintenance (g $kg^{-0.8}$ d^{-1}), FI is the feed intake (g $kg^{-0.8}$ d^{-1}) and G the growth (g $kg^{-0.8}$ d^{-1}).

Statistical analyses were performed using SPSS 11.5 for Windows. A paired *t*-test (dependent samples) was used to determine if netting induced a stress response. Plasma values for glucose, lactate and cortisol were compared between the two experiments using an independent *t*-test. Multiple regression analysis was used to determine which stress parameters influenced feed efficiency. Statistical significance was taken at $P < 0.05$.

RESULTS

The individual variation in basal and post-stress levels is depicted in Figure 1. In both experiments, the glucose levels increased significantly after the stress test (Paired *t*-test, t (47) = -20.6, $P < 0.001$, experiment 1 and t (24) = -10.1, $P < 0.001$, experiment 2). Lactate and cortisol levels increased significantly in experiment 1 (Lactate: t (47) = -11.3, $P < 0.001$; Cortisol: t (47) = -3.4, $P < 0.01$) but not in experiment 2 (Lactate: t (24) = -1.7, $P = 0.09$; Cortisol: t (24) = -1.7, $P > 0.05$). Glucose (basal and post-stress), lactate (basal) and cortisol (basal and post-stress) levels, were not significantly different between experiments 1 and 2 (Independent samples *t*-test, $P > 0.05$).

The results obtained from the multiple regression analysis are shown in Table 1.

In both experiments, feed intake requirements were mainly explained by the maintenance and the growth requirements (90 % in experiment 1 and 83 % in experiment 2). Basal levels of glucose, lactate and cortisol did not contribute significantly to explain the error term, i.e., feed efficiency ($P > 0.05$). However, the incorporation of glucose levels obtained after the stress test in the model represented an increase of accuracy by 1.3 % in experiment 1 and 5.9 % in experiment 2, suggesting that the glucose responsiveness is partly explaining differences in feed efficiency. In experiment 2, the cortisol levels obtained after the stress test also contributed to explain differences in feed efficiency by 8.7 %, suggesting that both glucose and cortisol responsiveness explain differences in feed efficiency. Therefore, the most complete model to determine feed intake requirements in experiment 1 is FI = 1.81 (± 0.69) + 0.49 (± 0.03) G + 0.28 (± 0.11) Gs ($R^2 = 0.916$) and in experiment 2 is FI = 0.91 (± 0.97) + 0.50 (± 0.03) G + 0.46 (± 0.13) Gs + 0.013 (±0.006) Cs ($R^2 = 0.914$), where FI (g kg $^{-0.8}$ d^{-1}) represents feed intake, G (g kg $^{-0.8}$ d^{-1}) the growth, Gs and Cs the glucose (mmol l^{-1}) and cortisol (ng ml^{-1}) levels obtained after the netting test.

When the relationship between residual feed intake and the basal and post-stress parameters is done, only the glucose post-stress levels in experiments 1 and 2 and the cortisol post-stress levels in experiment 2 showed significant correlations with RFI (Experiment 1- Glucose post-stress: $r_p = 0.34$, n = 48, $P = 0.02$; Experiment 2 - Glucose post-stress, $r_p = 0.56$, n = 26, $P = 0.003$, Cortisol post-stress: $r_p = 0.42$, n = 26, $P = 0.04$, Figure 2).

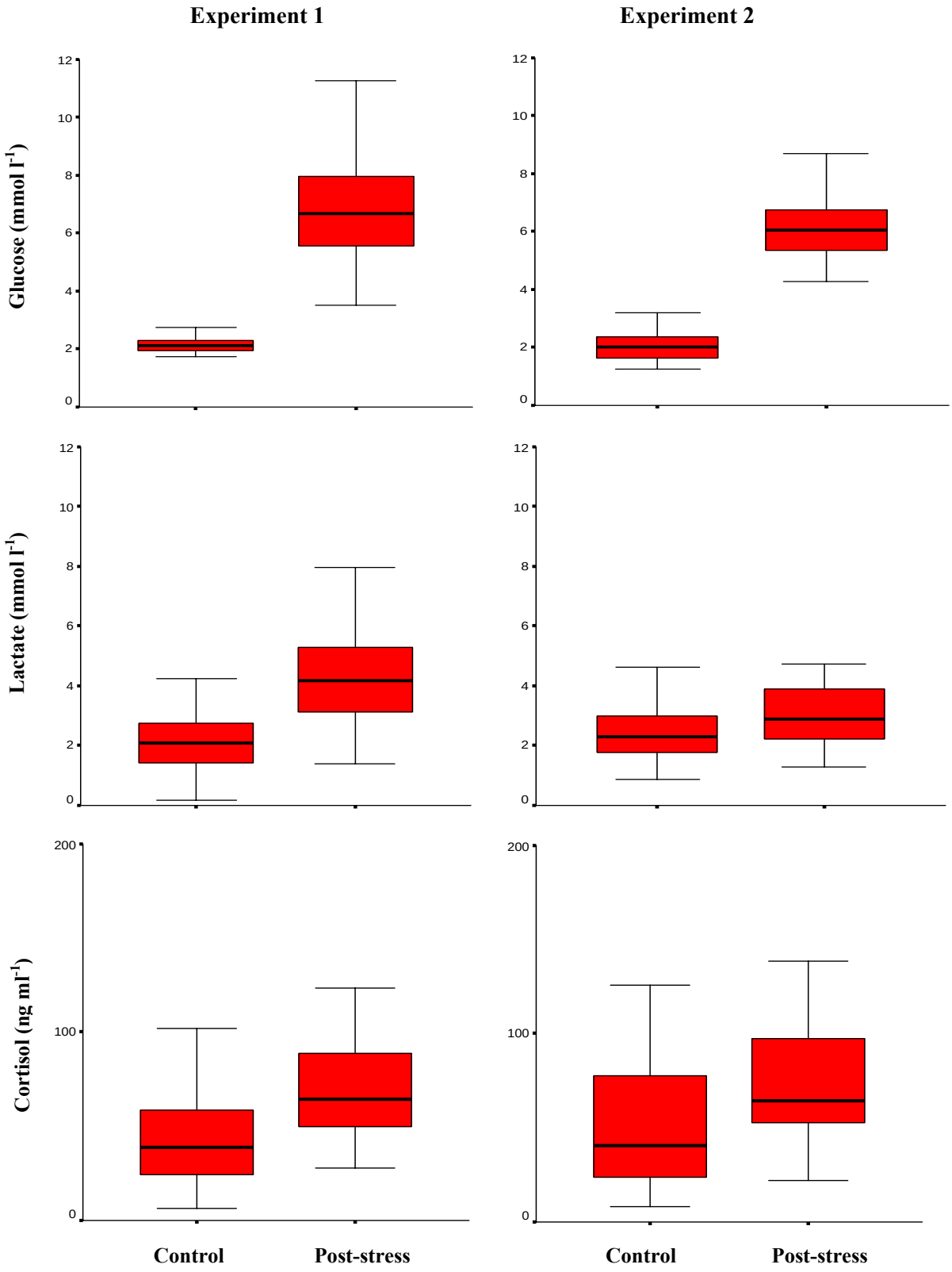


Figure 1. Box plot of plasma glucose, lactate and cortisol levels between control and post-stress values for experiment 1 and 2. The box includes observations from the 25th to the 75th percentile; the horizontal line within the box represents the median value. Lines outside the box represent the 10th and 90th percentiles.

Table 1. Regression equations between feed intake, growth rate and stress parameters for experiment 1 (Exp 1) and experiment 2 (Exp 2). Dependent variable is feed intake (FI, g $kg^{-0.8}d^{-1}$) and independent variables are growth rate (GR, g $kg^{-0.8}d^{-1}$), control and post-stress levels of glucose (Gc and Gs respectively, mmol l^{-1}), control and post-stress levels of lactate (Lc and Ls respectively, mmol l^{-1}) and control and post-stress levels of cortisol (Cc and Cs respectively, ng ml^{-1}).

Variables	n	Equations	R^2
FI , GR	a		
Exp 1	48	FI = 3.38 (± 0.36)* + 0.51 (± 0.03) GR*	0.903
Exp 2	26	FI = 4.99 (± 0.39)* + 0.47 (± 0.04) GR*	0.834
FI, GR, Gc	b		
Exp 1	48	FI = 4.54 (± 1.30)* + 0.51 (± 0.03) GR* - 0.53 (± 0.57) Gc	0.905
Exp 2	25	FI = 3.88 (± 1.05)* + 0.45 (± 0.05) GR* + 0.60 (± 0.52) Gc	0.845
FI, GR, Lc	b		
Exp 1	48	FI = 3.15 (± 0.44)* + 0.51 (± 0.03) GR* + 0.14 (± 0.15) Lc	0.905
Exp 2	25	FI = 4.78 (± 0.75)* + 0.46 (± 0.05) GR* + 0.11 (± 0.29) Lc	0.836
FI, GR, Cc	b		
Exp 1	48	FI = 2.90 (± 0.52)* + 0.53 (± 0.03) GR* + 0.006 (± 0.005) Cc	0.906
Exp 2	25	FI = 5.29 (± 0.69)* + 0.46 (± 0.05) GR* - 0.004 (± 0.008) Cc	0.837
FI, GR, Gs	b		
Exp 1	48	FI = 1.81 (± 0.69)* + 0.49 (± 0.03) GR* + 0.28 (± 0.11) Gs*	0.916
Exp 2	26	FI = 1.63 (± 0.99) + 0.51 (± 0.04) GR* + 0.49 (± 0.14) Gs*	0.893
FI, GR, Ls	b		
Exp 1	48	FI =3.18 (± 0.47)* + 0.51 (± 0.03) GR* + 0.07 (± 0.10) Ls	0.904
Exp 2	26	FI = 3.96 (± 0.97)* + 0.48 (± 0.04) GR* + 0.34 (± 0.29) Ls	0.843
FI, GR, Cs	b		
Exp 1	48	FI = 3.26 (± 0.53)* + 0.51 (± 0.03) GR* + 0.002 (± 0.005) Cs	0.903
Exp 2	26	FI = 3.85 (± 0.63)* + 0.46 (± 0.04) GR* + 0.02 (± 0.01) Cs*	0.921

*indicates statistical significance at $P < 0.05$; a, maintenance is intercept; b, maintenance is intercept + β x blood parameter

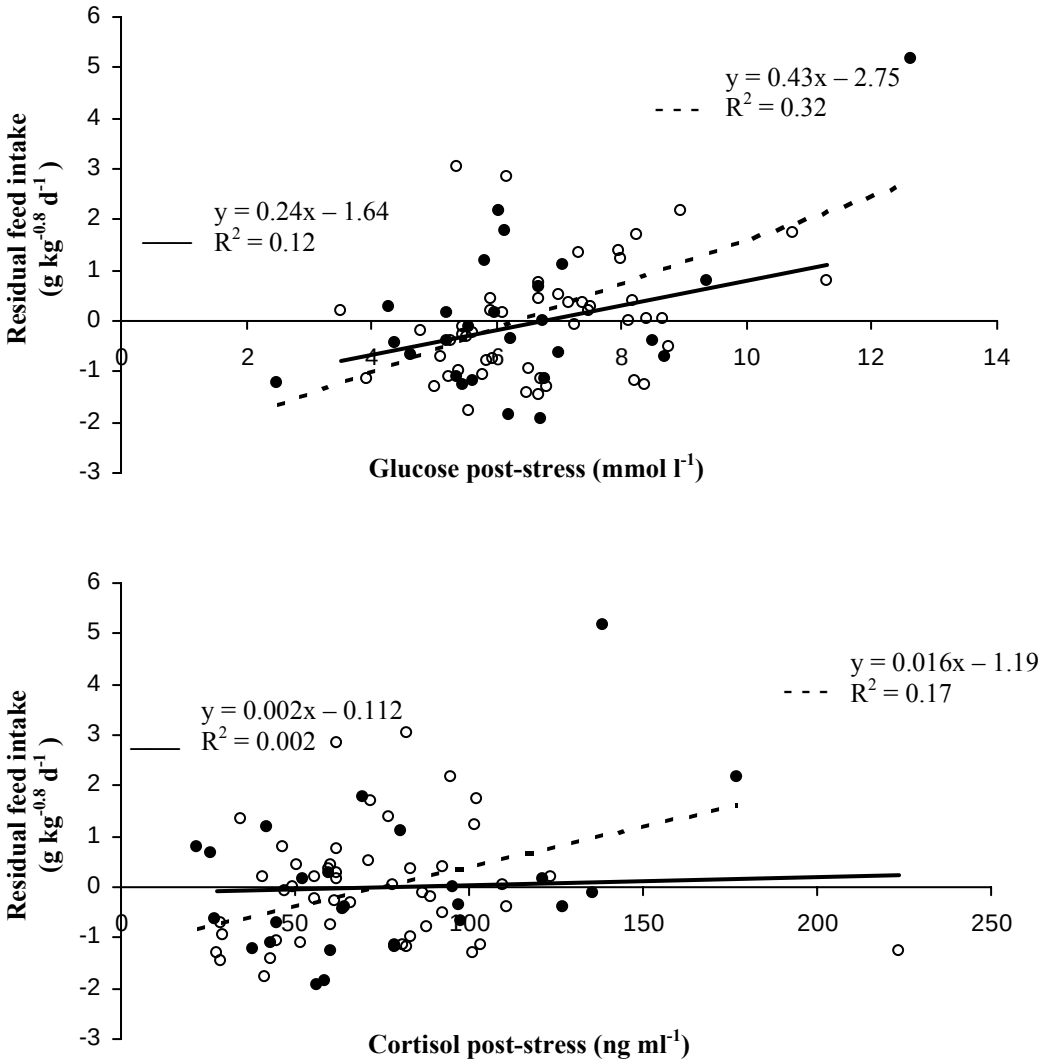


Figure 2. Relationship between glucose/cortisol levels obtained after a netting test and the residual feed intake for experiment 1 (___) and 2 (_ _).

DISCUSSION

This study suggests that individual difference in stress response contribute to explain differences in feed efficiency in fish. Few studies have focused on the relation between individual variation in performance traits and stress response and the results are often contradictory. In rainbow trout, for example selected groups for high and low post-stress values differed in growth performance: the low cortisol responding line showed a better growth performance as compared to the high responding line (Fevolden et al., 2002). Sea

bream individuals were also shown to be consistently high or low stress responders (plasma cortisol, glucose, lactate, osmolality and agglutination activity) during a 5-month experiment. However, in this case the values for specific growth rate and cortisol were significantly correlated; the fast growers were the high responders (Tort et al., 2001). Despite this relationship between stress response and growth performance no study has yet related individual differences in stress response and feed efficiency in fish. Rauw et al. (2000) suggested that animals with higher RFI have more "buffer" resources left to cope with unexpected stresses. Therefore in the present study, fish exhibiting lower RFI may spend less energy in establishing a stress response, energy that can be channeled for production. Katle et al. (1988) established a relationship between residual feed intake and stress response in chicks, the high-RFI line showed higher levels of corticosterone plasma levels, paralleling the results of this study with plasma glucose and cortisol levels. Furthermore, Richardson et al. (2004) showed that high-RFI steers tended to have a higher average blood cortisol concentration compared to low-RFI steers.

The incorporation of glucose levels, obtained after the stress test, in the model $FI = M + \beta G$ explained differences feed efficiency by explaining variance in maintenance levels. Luiting and Urff (1991) showed that differences among hens in maintenance requirements per metabolic kilogram appeared to be the major cause for the variation in residual feed intake.

The cost of activating the HPI axis (related to cortisol levels) seems not to be as clear as the activation of the sympathetic nerves-chromaffin cell axis (related to glucose levels) since the incorporation of cortisol levels obtained after the netting test was significant only in experiment 2. These differences between the two experiments may be due the higher variation in cortisol levels observed in experiment 2.

Individual differences in feed efficiency seem to be independent of basal cortisol, glucose and lactate levels since their incorporation in the model was never significant, both in experiment 1 and 2. This suggests that glucose levels, involved in metabolism (glucose turnover) are not related to individual differences in feed efficiency. This is expected since the carbodydrate catabolism appears to be of minor importance in fish under normal conditions (Hemre et al., 2002).

In this study, juveniles of African catfish exhibited a pronounced individual variation both in basal and post-stress levels of plasma cortisol, glucose and lactate. The existence of these differences in basal levels is in itself very interesting since they are usually explained as a consequence of social interactions (Fox et al., 1997). However in this study, fish were

housed individually and still displayed variation in basal glucose, lactate and cortisol level. It is generally accepted that a period of isolation of a few days is enough to eliminate the interference of previous social experiences (e.g., Oliveira et al., 2001). In this study fish were individually housed for 15 days before the blood samples (as indicative of basal levels) were taken. In this way we assume that any carry over effect of a possible social hierarchy is not playing a role on the observed individual differences. Therefore, plasma levels of cortisol, glucose and lactate are probably due to inherent differences, i.e., genetically linked.

The individual differences in basal and post-stress values observed in this study may be an indication of coping styles. They are defined as coherent sets of behaviour and physiological stress responses which are consistent over time and shaped by evolution (Koolhaas et al., 1999). Coping styles have been studied mostly in mammals but it is also accepted that they also exist in fish (van Raaij et al., 1996, Øverli et al., 2004). Differences in coping styles have been associated with individual differences in the stress response of rodents (Veenema et al., 2003), pigs (Van Erp-Van der Kooij, 2003) and even humans (Baltrusch et al., 1991; Cobb and Steptoe, 1996). Whether this was also the case in this study needs further investigation.

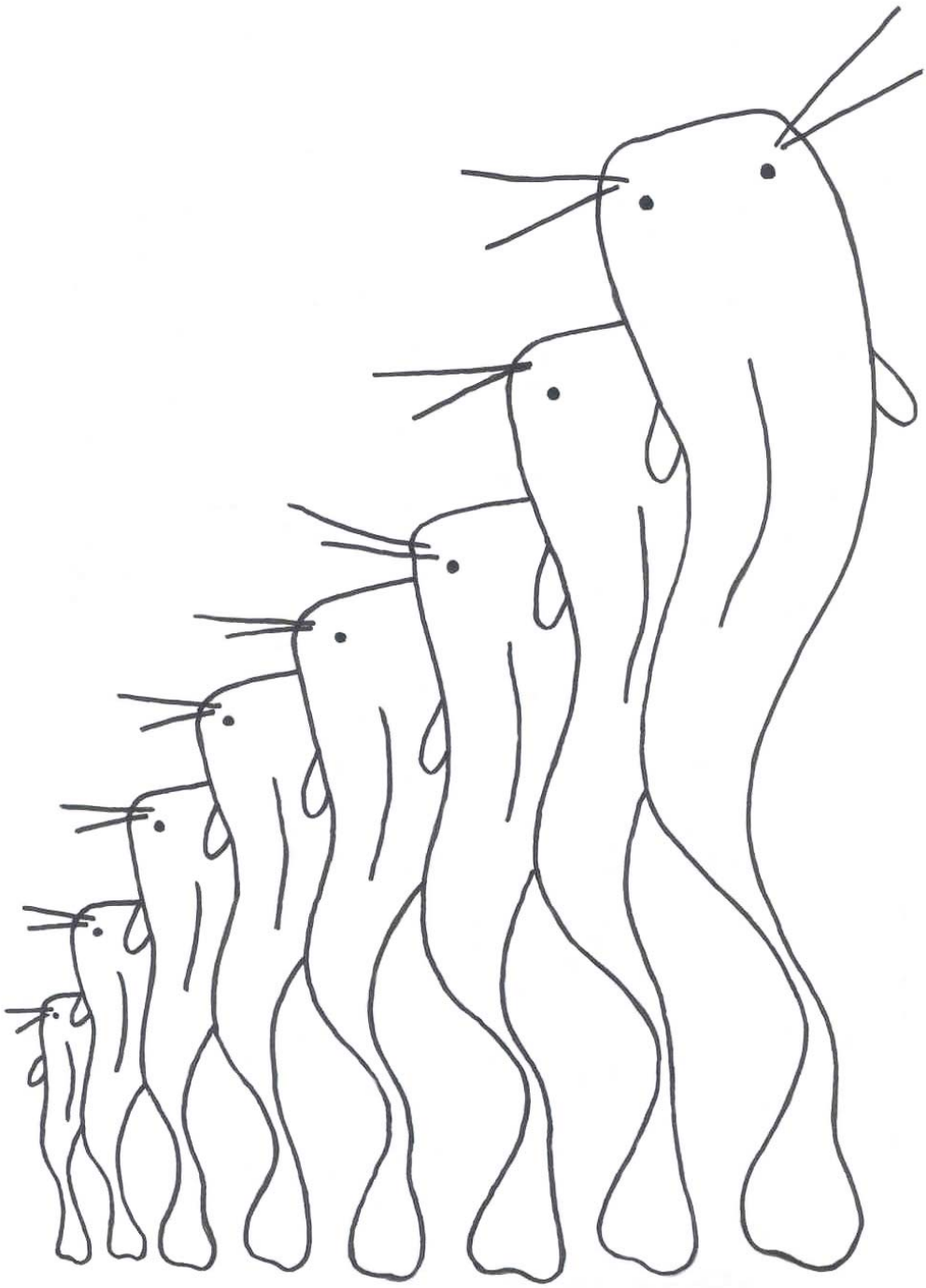
The study of feed efficiency requires the measure of individual feed intake which implied time and labor. Even in the case of species where this measurement can be done using X-rays (Carter et al., 1995), which are faster that measuring individual feed intake by individual housing, it may still not be an easy task due to equipment availability. This study showed that feed efficiency can be predicted by the levels of glucose and cortisol after a netting stress. The use of an acute stressor may therefore be useful to discriminate between fish with different feed efficiencies. The stress test used in this study was very specific for African catfish since this species is an air breather and therefore can easily handle 1 h air exposure (Buttle et al., 1996). For other species this duration of air-exposure would not be possible, however, shorter air-exposures have repeatedly been used (e.g., 30 sec, Barton et al., 2002). It would be interested to test if the same relationship between feed efficiency and stress response would be found for other species.

In conclusion, this study shows that juveniles of African catfish exhibit inherent differences in the activity and reactivity of sympathetic nerves-chromaffin cell axis (related to glucose increase) and the hypothalamo-pituitary-interrenal axis (related to cortisol increase). High stress responders were shown to be the less efficient fish in terms of resources utilization for production, suggesting that individual differences in stress response explain

part of the differences in feed efficiency. Stress response seems to be related to feed efficiency by explaining variance in maintenance levels.

Acknowledgments

This research was supported by a grant provided by the Foundation for Science and Technology, Portugal (SFRH/BD/2946/2000). We would like to acknowledge Menno ter Veld, Sietze Leenstra, Aart Hutten and Wian Nusselder for their help in starting up the experiment. We are also grateful to Alex Scott, Tim Ellis and Jonathan James for the cortisol analyses.



8

- CHAPTER -

General Discussion

The general aim of this study was to understand the underlying factors responsible for the individual variation in growth of African catfish *Clarias gariepinus*. The following factors were investigated: 1) if individual variation in growth was mainly a consequence of social hierarchies, 2) the contribution of individual differences in feed intake and feed efficiency for the individual differences in growth and 3) the contribution of feeding behaviour and stress response in explaining individual differences in feed efficiency. Chapters 2 and 3 tested the role of social hierarchies on growth variation by comparing the performance, behaviour and stress response of groups of fish with a different size distribution (homogeneous low-, medium-, heavy-weight groups and a heterogeneous group). Chapter 4 and part of Chapter 5 tested the use of individual housing as a toll to investigate individual differences in growth. Chapter 5 quantified the consistency of individual differences in performance and behaviour using individually housed fish. This chapter also determined the contribution of individual differences in feed intake and feed efficiency in explaining individual differences in growth. A relationship between feeding behaviour and feed efficiency was also established. Chapter 6 further investigated the role of feed efficiency in explaining growth variation and the relationship to feeding behaviour by using fish fed restrictively. In chapter 7, individual differences in basal and post-stress values of glucose, lactate and cortisol were investigated and related to individual differences in feed efficiency.

Individual variation in growth: role of social hierarchy in African catfish

This thesis shows that African catfish, kept under identical conditions, exhibit large individual variation in growth, paralleling the results of Grobler et al. (1992) and van der Waal (1998). It is generally assumed that growth variation within fish species, under aquaculture conditions, is the result of social hierarchies. However, this thesis on African catfish suggests that social hierarchies are not a major cause of individual variation in growth. Instead, inherent factors seem to be important for the occurrence of individual variation in the growth of African catfish.

The minor role of social hierarchies in African catfish is demonstrated by:

1) the considerable presence of individual differences in growth when African catfish is housed individually (no social interactions) (Chapters 4, 5 and 6).
2) the absence of a grading effect (i.e., disruption of social hierarchy) on growth performance, aggression and stress levels between low-, medium- and heavy-weight catfish (Chapters 2 and 3).

When social hierarchy is the major cause of individual variation in growth, we may assume that this variation is reduced in the absence of social interactions by individual housing (Jobling and Baardvik, 1994). When African catfish is housed in groups, the coefficient of variation (CV) for growth is around 30 % (Almazán-Rueda, 2004a). However, when housed individually (no social interaction), African catfish still displays a large individual variation in growth (e.g., CV = 24.1 % in Chapter 4, 52.8 % in Chapter 5). Similar results were found for individually housed hybrid sunfish *Lepomis cyanellus* × *Lepomis macrochirus* (CV = 40.2 %, Wang et al., 1998) and Chinese sturgeon *Acipenser sinensis* (CV = 31.7 %, Qian et al., 2002), suggesting that other (inherent/genetic) factors play a role in explaining individual variation in growth of fish. Wickins (1985) compared the growth variability of elvers, *Anguilla anguilla,* housed both individually and in groups, and found that a marked growth variation can occur in *A. anguilla* without physical interaction with other elvers and without competition for food (CV = 19.69 and 26.99 % for individually housed and CV = 28.99 and 53.88 % for group housed). In addition, this thesis shows that individual differences in growth are consistent over time, supporting the hypothesis of a genetic basis for the individual variation in the growth of African catfish (Chapter 5). However, there are also fish species in which the individual variation in growth is reduced when fish are housed individually, e.g., in grass carp, *Ctenopharyngodon idella* (Carter et al., 1992) and Arctic charr, *Salvelinus alpinus* (Jobling and Baardvik, 1994). Therefore, the current study combined with the literature, shows that the underlying factors for individual variation in growth are species-dependent.

Literature data on the effect of grading are contradictory when comparing various fish species. For some species it has been shown that small fish compensate their growth after grading (Gunnes, 1976; Popper et al., 1992; Brzeski and Doyle, 1995; Seppä et al., 1999). However, for several other species there was no beneficial effect of grading on the subsequent growth of small fish (Jobling and Reinsnes, 1987; Wallace and Kolbeinshavn, 1988; Baardvik and Jobling, 1990; Kamstra, 1993; Carmichael, 1994; Sunde et al., 1998). The absence of a grading effect may therefore be simply due to the fact that in certain fish species no social hierarchy is established. In other words, heavy fish are not necessarily the dominant fish. Data in Chapter 3 showed that in heterogeneous size groups, there was no increased aggression towards the smaller fish. Furthermore, the small fish in heterogeneous groups had no elevated blood stress parameters (cortisol and glucose). These data also suggest that social hierarchies were absent. However, it should be realised that the fish in Chapters 2 and 3 were fed ad

libitum. Therefore, the impact of social hierarchy on feed intake and growth might have been reduced due to the abundance of food.

In the wild, the African catfish lives in a wide variety of habitats, which can differ largely in the environmental conditions. African catfish can be found in perennial waters, in semipermanent and seasonal waters that can dry out completely for certain periods of the year (van der Waal, 1998). Harwood et al. (2003) suggested that variable environmental conditions may affect the strength of social hierarchies and determine whether dominance status is associated with growth. Sloman et al. (2001b) also suggested that hierarchies may not be established in small streams where large fluctuations in water level occur. In African catfish, the pronounced individual variation in growth observed in the wild is believed to confer survival advantages (van der Waal, 1998). According to this author, fast growing fish exhibit higher survival rates under favourable conditions while slow growing individuals survive well under desiccating conditions. Under aquaculture conditions, the environment is much more stable than in the wild, and fish often have to compete for the available resources. In tilapia (Koebele, 1985) and salmonids (Metcalfe, 1986; Metcalfe et al., 1989), such conditions have been reported to lead to social hierarchies. Competition for food seems to occur in African catfish resulting in agonistic behaviour (Hecht and Appelbaum, 1988). However, this agonistic behaviour seems to be unrelated to the establishment of social hierarchies in which large (dominant) fish suppress the growth of smaller (subordinate) fish (Chapters 2 and 3). Therefore, it may be that the pronounced individual variation in the growth of catfish under aquaculture conditions is still reflecting the survival strategy adopted in the wild.

In summary, there is strong support for the existence of social hierarchy in some species, not only under aquaculture conditions (e.g., salmonids; Abbott and Dill, 1989; Metcalfe et al., 1989; Winberg et al., 1993) but also in the wild (e.g., clownfish; Buston, 2003). However, for other species, such as African catfish, it is still not clear if social hierarchies are established under aquaculture conditions. The existence or absence of social hierarchy and its importance in explaining growth variation will probably be species-dependent and background knowledge on the biology of the species may help in understanding its potential role.

Individual variation in growth: feed intake vs feed efficiency

The feed consumed by a growing organism is used for maintenance (e.g., physical activity, protein turnover, immunological functions etc.) as well as for the growth process (i.e., protein and fat accretion). Variation in feed intake is related to variation in feed required for maintenance and for growth. However, at population level (e.g., a group of fish in a rearing tank), the variation in feed consumption between individuals cannot be fully explained by the differences in mean population estimates of costs of maintenance and growth. The unexplained fraction of the individual feed consumption, compared to the population average, is called the residual feed intake (RFI) and is defined as the difference between actual feed intake and that predicted from the mean observed requirements for growth and maintenance of the population (Koch et al., 1963). Individuals with a negative RFI are more efficient than the mean individual of the population, while individuals with a positive RFI are less efficient. RFI is a non-ratio-based measure (in contrast to the feed conversion ratio, FCR) and therefore was very useful during this study to investigate the relationship between feed efficiency and feeding behaviour / stress response (see later). The study of these relationships using a ratio-based measure such as FCR might be difficult due to the irregular statistical behaviour of ratio measures (Iwaisaki and Wilton, 1993). However, one should be aware that RFI is not a characteristic of the individual in itself since the residual of each individual is dependent on the other individual's present in the population. Both FCR and RFI are measures representing feed / growth efficiency. Both parameters have their advantages and disadvantages. Depending on the goal of the study, one or the other should be used. Particularly in selection breeding studies, the use of RFI has been shown to be advantageous due to its higher genetic variation in relation to the FCR (Silverstein et al., 2005).

In Chapters 4 and 5, feed intake was taken as the dependent variable since the interest was on the part of the feed intake variation that was not explained by the variation in maintenance and production (growth). Nevertheless, the relationship between feed intake (FI) and growth (G) has the same R^2 irrespective of which dependent variable is chosen (FI or G). Therefore, one can conclude that under ad libitum conditions and individual housing, differences in feed intake account for ~85 % of the observed individual differences in the growth of African catfish. The remaining ~15 % is related to individual differences in residual feed intake, representing differences in feed efficiency. These observations on ad libitum feeding were confirmed by the existence of a large individual variation in growth under

restricted feeding (Chapter 6). The differences in growth observed in Chapter 6 (CV = 17.2 %), with restricted feeding, fully relate to individual differences in feed efficiency.

Parallel to the current thesis, several studies have shown that most of the variation in the growth of fish is due to variation in feed intake (Koebele, 1985; Carter et al., 1992; Jobling and Baardvik, 1994; Umino et al., 1997). Furthermore, individual variation in feed efficiency has been identified as an explanatory factor for growth variation, e.g., between wild and selected Atlantic salmon (*Salmo salar*) and Japanese flounder (*Paralichthys olivaceus*) (Thodesen et al., 1999; Ogata et al., 2002), genetic strains of channel catfish (*Ictalarus punctatus*) (Silverstein et al., 1999), different families of Atlantic salmon (Thodesen et al., 2001; Kolstad et al., 2004) and individuals of the same population, e.g., hybrid sunfish (Wang et al., 1998), Chinese sturgeon (Qian et al., 2002), rainbow trout (*Oncorhynchus mykiss*) (Silverstein et al., 2005). However, despite these studies, the exact contribution of individual variation in feed intake and feed efficiency to the individual variation in growth is often omitted. Carter et al. (1992) suggested that the variation in consumption rates explained 89 % of the variation in growth rates of grass carp (*Ctenopharyngodon idella*) housed in groups, paralleling the results for African catfish. Using the data presented by Wang et al. (1998) with individually housed hybrid sunfish, it is possible to show that individual differences in feed intake explain 73 % of the individual differences in growth (when both are expressed in g $kg^{-0.8}$ d^{-1}). This value is lower than the one we obtained for African catfish, suggesting that in hybrid sunfish the contribution of feed efficiency in explaining growth differences is higher than in catfish.

Such a high contribution of feed intake differences in explaining growth variation is not common among other vertebrates, particularly in homeothermic animals. In laying hens, for example, a large fraction (5–99 %) of the variance in feed consumption among hens within stocks appears to be unexplained by differences in metabolic body weight, egg mass and body weight gain (Luiting, 1991). The higher maintenance requirements of homeotherms (e.g., 459.8 kJ of DE $kg^{-0.75}$ d^{-1} in pigs; NRC, 1998) as compared to ectotherms (e.g., 34.05, 45.38 and 47.89 kJ of DE $kg^{-0.80}$ d^{-1} in white grouper (*Epinephelus aeneus*), European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*), respectively; Lupatsch et al., 2003) certainly allow individual differences in feed efficiency to have a higher contribution in explaining growth variation.

Factors explaining residual feed intake

As mentioned above for African catfish, around 85 % of the individual variation in feed intake is explained by the average population requirements for maintenance and growth (Chapters 5 and 7). The unexplained part of the variation in feed intake (i.e., the residual feed intake, RFI) reflects the variation in feed efficiency between individuals. Luiting (1991) assessed the cause of RFI variation (i.e., feed efficiency) in laying hens by using a scheme of partitioning of energy. Figure 1 gives a schematic representation of partitioning of energy in fish, from gross energy to energy retained as fat and protein. Differences in RFI can be due to differences in: 1) digestibility of energy (i.e., faecal energy losses); 2) branchial and urinary energy losses; 3) heat production, which can be further divided into differences in energy requirements for maintenance and differences in the efficiency of the use of metabolizable energy for growth; 4) the composition of the retained energy (i.e., the ratio between fat and protein accretion).

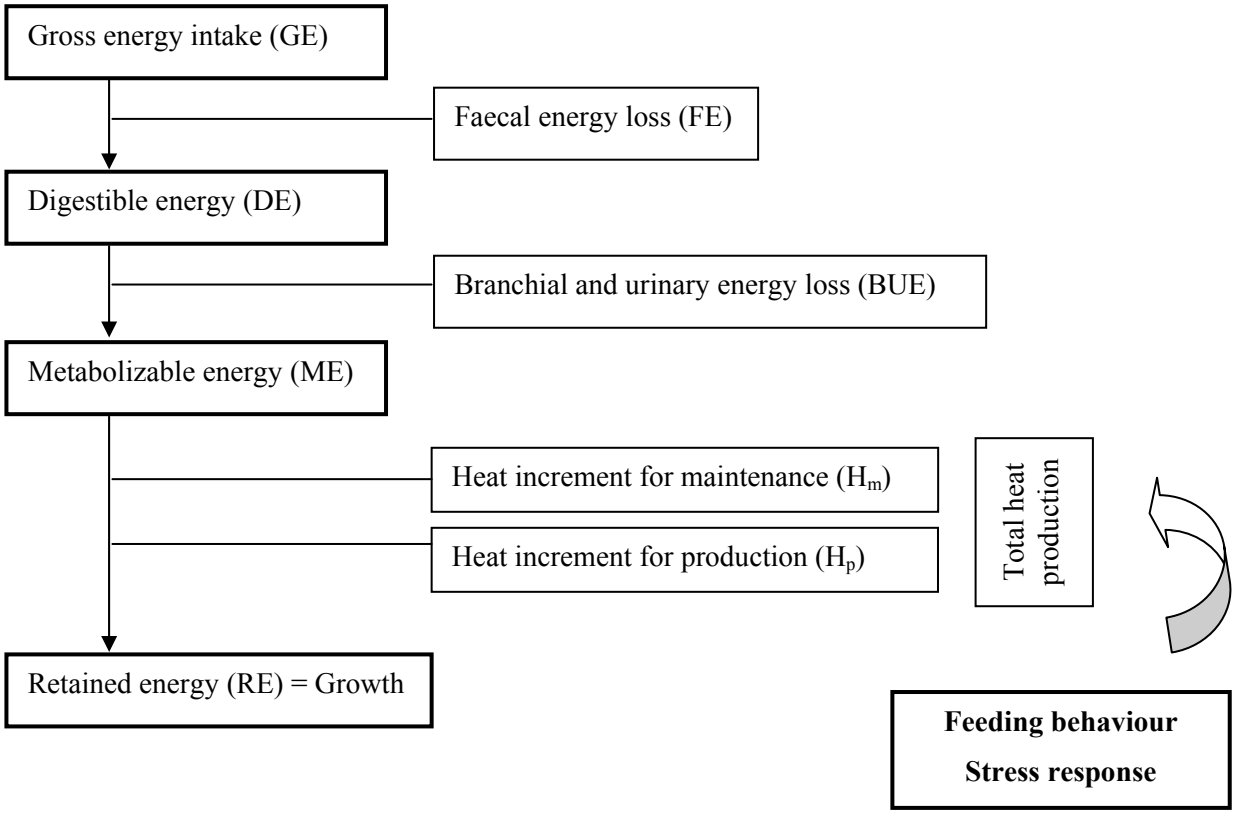


Figure 1. Proposed inclusion of feeding behaviour and stress response as factors responsible for heat production and therefore included in the maintenance costs of African catfish (adapted from Heinsbroek, 1987).

In the experiment described in Chapters 2 and 3, digestibility of nutrients was also measured (Martins, unpublished data). They did not differ between the different weight classes. Energy digestibility (apparent digestibility coefficient) was 82.5 %, 83.1 % and 83.6 % in the low-, medium- and heavy-weight classes, respectively. This parallels the findings in laying hens (Luiting, 1991), that differences between individuals in nutrient digestibility make only a minor contribution to variation in RFI. Similarly, Qian et al. (2002) showed that the individual variation in growth of Chinese sturgeon was not related to differences in digestibility. Therefore, it is most likely that in African catfish, variation in digestibility of nutrients is not contributing to the individual variation in RFI.

No data are available on the relation between variation in branchial and urinary energy losses and RFI in fish. However, in absolute terms, branchial and urinary energy losses are only a minor energy loss in the total energy partition from GE to RE (approximately 5 %; Jobling, 1994). Still, differences in protein metabolism, related for instance to protein turnover, can cause variation in BUE losses. In laying hens (Luiting, 1991) it was shown that the urine energy losses were not related to RFI. Therefore, we believe that the BUE loss will not explain the observed difference in RFI in African catfish.

In Chapter 6, it was demonstrated that in African catfish subjected to restricted feeding (all fish were fed equal amounts of feed), differences in growth on a weight basis were related to both differences in protein and fat deposition. With increasing growth rates, protein accretion increased and fat accretion decreased. This finding suggests that part of the variation in feed efficiency is due to a difference in growth composition in African catfish. However, it remains unclear whether such differences in growth composition can also explain the variation in RFI under ad libitum feeding conditions. Silverstein et al. (2005) found a (negative) significant correlation between nitrogen retention efficiency and RFI in rainbow trout, paralleling the observations for African catfish. However, in cattle and laying hens the role of variation in body composition to explain differences in RFI seems to be very small, approximately 5 % (Luiting, 1991; Richardson et al., 2004). Furthermore, the mechanism causing the differences in growth composition needs to be clarified. Possible explanations for these differences in growth composition can be individual variation in protein turnover and (genetic) variation in the optimal ratio of protein and fat deposition. The possible impact of variation in protein turnover on feed efficiency is discussed below.

Variation in heat production losses are also a possible source of variation in RFI. However, differences in heat production caused by differences in the efficiency of ME

utilization for growth (K_g), are very unlikely to be a source of variation in RFI. Heinsbroek (1987) showed that in African catfish, K_g is hardly affected by environmental factors. In laying hens Luiting (1991) and cattle (Richardson and Herd, 2004), differences in the utilization of energy for production processes hardly explain the variation in RFI. Furthermore, data in Chapters 5 and 7 suggest that the efficiency for growth is not related to RFI. In these chapters various parameters (feeding behaviour, Chapter 5, Table 4; blood stress parameters, Chapter 7, Table 1) were included in the regression model explaining the variation in feed intake of African catfish. The inclusion of these parameters had a minor impact on the feed used per unit of growth (on a weight basis). This regression coefficient of growth on feed intake requirements was on average affected by approximately 2 % when additional parameters where included in the regression model.

Therefore, as suggested for farmed animals by many researchers (Luiting, 1991; Herd and Bishop, 2000; Knap, 2000; Basarab et al., 2003), it is most likely that individual differences in RFI in African catfish are largely due to differences in maintenance requirements. This thesis also supports this hypothesis. Differences in RFI were related to variation in feeding behaviour (Chapter 5) and to the response to acute stress (Chapter 7). Inclusion of feeding behaviour (total feeding time, TFT; Chapter 5, Table 4) and stress response parameters (blood plasma cortisol and glucose levels; Chapter 7, Table 1) in the regression model of feed intake requirements increased the explained variation in feed intake up to 8.7 %.

Feeding behaviour

In Chapter 5 it was shown that in African catfish, total feeding time (the time between the first and the last consumed pellet) was correlated to RFI (i.e., feed efficiency). The most efficient individuals were the fast eaters, whereas slow eaters were less efficient. In other words, the amount of feed spent on maintenance (M, g $kg^{-0.8}$ d^{-1}) was a function of total feeding time (TFT, min):

$$M = 3.1\ (\pm 0.35) + 0.009\ (\pm 0.002) \times TFT\ (min) \quad [equation\ 1]$$

The observed mean (± SD) in TFT was 63.2 ± 59.8 min (CV = 94.6 %, Chapter 5 considering the average of periods 2 and 3). A difference of two times the variance (119.6 min) results in a 0.54 g $kg^{-0.8}d^{-1}$ difference in the amount of feed used for maintenance (12.8 % difference). Cutts et al. (2002) also showed a relationship between maintenance levels and feeding motivation in Atlantic salmon. In their study, fish exhibiting higher maintenance

levels (standard metabolic rate) had a slight but significant lower feeding motivation. This finding corroborates the results of this thesis for feeding behaviour in which African catfish with a high TFT exhibit higher maintenance levels (as predicted by Chapter 5, Table 4). De Haer et al. (1993) also related residual feed intake with feeding behaviour in growing pigs. These authors found that variation in feed intake activity (daily eating time and eating frequency) accounted for 44 % of the variation in RFI. The meal duration in cattle was also reported to be a key factor in determining the variance of RFI (Richardson and Herd, 2004).

From the current study, the underlying mechanisms causing the relation between RFI and total feeding time remain unclear. Possible mechanisms are variation in protein turnover and swimming activity.

In Chapter 6 it was shown that fast growers, which were the fast eaters, had a different body composition (more protein and less fat) than slow growers and slow eaters. This may suggest that fast and slow eaters differ in protein turnover. Protein turnover is the dynamic balance between protein synthesis and protein degradation. Protein growth or protein deposition can be achieved by increasing the rate of protein synthesis and / or by decreasing the rate of protein degradation (Houlihan et al., 1995).

Protein turnover differs not only between species but also between individuals. In fact, differences in the individual rates of protein turnover have often been related to individual differences in the growth rates of fish. Dobly et al. (2004) showed that in rainbow trout, faster growing individuals had lower rates of protein turnover. Since protein turnover is energy demanding, the low rate of protein turnover in some individuals means a lower cost of maintenance (and / or growth). McCarthy et al. (1994) found similar results in rainbow trout, supporting the hypothesis that individual differences in protein turnover are important determinants of growth efficiency in fish. Morgan et al. (2000) also related individual differences in growth to individual differences in protein turnover. They found that in juvenile Atlantic salmon, the early migrants grew faster due to a reduced rate of protein degradation. However, the contribution of protein turnover to variation in RFI in fish is still not known. The contribution of protein turnover to the variation in RFI has been studied in pigs (Knap, 2000) and cattle (Herd et al., 2004). In cattle for instance, protein turnover and ion transport are expected to be responsible for two-thirds of the variation in RFI. Further studies are needed to reveal the role of protein turnover in RFI variation of fish.

As suggested before, differences in activity may explain differences in feed efficiency. In pigs (de Haer et al., 1993), laying hens (Luiting et al., 1991) and cattle (Richardson and

Herd, 2004) a higher activity level was associated with lower feed efficiency (higher RFI). However, in our study with African catfish, individuals that ate more actively were also the most efficient. This is contradictory to the results found in other vertebrates. In this thesis, we measured behaviour always in relation to feeding. One may wonder if fast eaters exhibited lower activity levels during the non-feeding periods and in this way saved energy for more efficient resources utilization. A preliminary study on African catfish showed that individual differences in growth were not related to individual differences in swimming activity (Martins, unpublished data). Qian et al. (2002) using individually housed Chinese sturgeon, showed that individuals spending more time swimming exhibited higher growth rates, higher feed intake and a better growth efficiency. These authors hypothesized that standard metabolism or specific dynamic action, rather than activity levels was the determinant factor for differences in individual growth rate. Valente et al. (2001a) showed that swimming activity and feeding behaviour are under some genetic influence and that the fast growing strain of rainbow trout was consistently more active that the slow growing strain.

Stress response

In Chapter 7, it was shown that in African catfish, stress response parameters during acute stress (plasma cortisol and glucose levels) were correlated to RFI (i.e., feed efficiency). The most efficient individuals exhibited lower plasma cortisol and glucose levels after acute stress. In other words, the amount of feed spent on maintenance (M, g $kg^{-0.8}$ d^{-1}) was a function of plasma glucose levels (Gs, mmol l^{-1}) after an acute stress:

$M = 1.81 (\pm 0.69) + 0.28 (\pm 0.11) \times Gs$ [equation 2.1], in experiment 1, Chapter 7

$M = 1.63 (\pm 0.99) + 0.49 (\pm 0.14) \times Gs$ [equation 2.2], in experiment 2, Chapter 7

and of plasma cortisol levels (Cs, ng ml^{-1}) after an acute stress:

$M = 3.85 (\pm 0.63) + 0.02 (\pm 0.01) \times Cs$ [equation 3], in experiment 2, Chapter 7

The observed mean and variance in glucose levels obtained after the stress test (experiment 2) was 6.34 ± 1.88 mmol l^{-1} (CV = 29.7 %). A difference of two times the variance (3.76 mmol l^{-1}) results in a 0.92 g $kg^{-0.8}$ d^{-1} difference in the amount of feed used for maintenance (36.1 % difference). The same reasoning can be applied for the cortisol levels obtained after the stress test in experiment 2 (mean ± SD = 75.23 ± 38.32 ng ml^{-1}). A difference of two times the variance (76.64 ng ml^{-1}) results in a 0.77 g $kg^{-0.8}$ d^{-1} difference in the amount of feed used for maintenance (14.2 % difference).

Stress triggers a series of defence mechanisms that are energy demanding (Barton and Iwama, 1991). It is therefore expected that under stress, animals will increase their maintenance requirements and thus RFI. What is not known is how an individual's stress response relates to RFI values obtained in a stress-free environment. In Chapter 7, fish were individually stressed at the end of the experiment and the glucose and cortisol plasma values related to RFI values obtained during a stress-free period. Fish exhibiting lower RFI (more efficient) exhibited a lower stress response. In these individuals, the energetic "scope" for stress response was used for growth. Katle et al. (1988) established a relationship between residual feed intake and stress response in chicks: the high-RFI line showed higher levels of corticosterone plasma levels, corroborating the results of this study with plasma glucose and cortisol levels. In cattle, results for red and white blood cell parameters of steers selected for RFI suggested that the high-RFI steers were more susceptible to stress than the low-RFI steers (Richardson et al., 2002). High-RFI steers were also found to exhibit a tendency for a higher blood cortisol concentration compared with low-RFI steers (Richardson et al., 2004). In fact, these authors suggested that individual differences in stress susceptibility in combination with protein turnover and tissue metabolism contributed to at least 37 % of the variation in residual feed intake.

One may wonder if selection for low RFI would lead to improved welfare in catfish, since it would select for less stress-sensitive fish. The question remains if more efficient individuals are able to maintain such a low stress response if subjected to frequent (acute) stressors, which is the common situation in aquaculture. It is also not known if more efficient fish are also less sensitive to chronic stress. In addition, it may be necessary to include a combination of several stress indicators besides plasma glucose and cortisol (e.g., escape attempts, stereotypic behaviour, activity patters) to be able to answer this question.

The mechanism behind the link between feed efficiency and the stress response is still not known but it is possible that differences in protein turnover have an important role. Fish that are high stress responders at the HPI axis may also be high stress responders at the cellular level, i.e., at the production of stress proteins (heat shock proteins) and therefore exhibit a higher protein turnover.

Individual differences in feeding behaviour and stress response: a reflection of coping styles in African catfish?

One may wonder whether the observed differences in behaviour and stress response are linked to the existence of coping styles in African catfish. The study of coping styles in fish is still in its early stages as compared with mammals. However there is evidence that different coping strategies also exist in fish (e.g., Øverli et al., 2004). Coping styles seem to have a genetic base and have been defined as coherent sets of behaviour and physiological stress responses that are consistent over time and shaped by evolution (Koolhaas et al., 1999). As reviewed by these authors, the (pro)active coping style is behavioural characterised in mammals by an active attempt to counteract the stressful stimulus such as a high level of locomotor activity, active avoidance and aggression. Moreover, the (pro)active coping style exhibits a low hypothalamus-pituitary-adrenal (HPA) axis responsiveness and a high sympathetic reactivity. On the other hand, the reactive (or passive) coping style is characterised by immobility, low aggression levels, high HPA axis response and low sympathetic reactivity.

In this study, the most efficient individuals were fast eaters and low stress responders. It could well be that more efficient individuals act as active copers and less efficient animals as passive copers. However, this hypothesis needs further investigation.

Differences in personality traits (bold and shy) may also exist in fish. Examples are presented in rainbow trout (Sneddon, 2003), brown trout (*Salmo trutta*) (Sundström et al., 2004) and three-spined sticklebacks (*Gasterosteus aculeatus*) (Ward et al., 2004). Ward et al. (2004) showed that in three-spined stickbacks the position adopted by an individual within a group during feeding is related to personality traits. Bold individuals took significantly more front positions than shy conspecifics. Moreover, boldness was also positively correlated to growth. Our results seem to corroborate the results of Ward et al. (2004). The heaviest fish were found to occupy the front positions of the tanks and to eat their meal at a faster speed than smaller individuals (Chapter 2). Furthermore, in individually housed fish, fast growing individuals were fast eaters despite the absence of social interactions (Chapters 5 and 6). The individual differences in total feeding time were highly repeatable over time, suggesting that this trait may have a genetic basis. In species where dominance hierarchies are present, it is expected that bolder individuals will be competitively dominant (Sundström et al., 2004). In African catfish the possible existence of bold and shy individuals may be translated into different levels of competition that are not reflecting typical social hierarchies.

One may wonder whether the individual differences in behaviour observed in African catfish have an adaptive value in the wild by maximizing the exploration and use of available resources in an extremely variable environment.

Implications for the aquaculture industry

This thesis suggests that the individual growth variation in African catfish has a genetic basis. This may open up perspectives for breeding programmes in this species. The traits to be selected deserve further investigation but the use of residual feed intake looks very promising. Since in aquaculture the feed costs represent a large fraction of the total production costs (30 –70%) (Shang, 1990), selecting for individuals with a lower RFI (more efficient) may improve the production efficiency. However, the study of feed efficiency requires measurement of individual feed intake, which implies time and labour. Even in the case of species where this measurement can be done using X-rays (Carter et al., 1995), which is faster than measuring individual feed intake by individual housing, it may still not be an easy task due to equipment availability. This study showed that feed efficiency can be predicted by the total feeding time and the levels of glucose and cortisol obtained after a netting stress. Particularly the use of an acute stressor may be useful to discriminate between fish with different feed efficiencies in a practical situation. However, this hypothesis should be tested with individuals housed in group so that the relationship between stress response and feed efficiency is verified in the presence of social interaction, which is the case in aquaculture.

Selecting African catfish with a lower RFI would mean selecting for fish with lower maintenance requirements, which eat their meal very fast and are lower stress responders. All these aspects seem to be advantageous under aquaculture conditions. However, it is still not known whether the more efficient fish exhibit a lower stress response because they are less stress susceptible (which would be an advantage for aquaculture) or simply because they lack the capacity to react (via plasma cortisol and glucose increase) in a stressful situation. It may be that other stress parameters not measured in this thesis are affected. Furthermore, it is not known how the most efficient fish will react in the long term to repeated acute or to chronic stressors.

This thesis showed that in African catfish the pronounced individual growth variation is not caused primarily by social hierarchies in a fish tank. This observation may have important consequences for the production of this species since it suggests that during fattening (weight range between approximately 65–215 g), grading is not necessary. Grading

is done in an attempt to disrupt social hierarchies and therefore allow the smaller fish to compensate their growth in the absence of larger, dominant fish. When social hierarchies are not playing an important role in determining growth differences, then grading should be reconsidered since it is a time-consuming procedure and involves handling the fish causing skin damage, stress and even death.

Future studies

This thesis is the first step towards understanding the individual differences in the growth of African catfish. Growth is a complex trait and its variation is probably the result of interaction between many genes and environmental factors. As reviewed in the introduction there are plenty of candidate mechanisms that singularly or in combination may contribute to variation in the growth of fish. The challenge is to find the mechanisms responsible for this variation and how they are related to the individual differences in behaviour and stress response found in this thesis. Particularly interesting is the research on other factors (e.g., heat increment after feeding or specific dynamic action, protein turnover, immune response) explaining residual feed intake and therefore the maintenance requirements of African catfish. The development of divergent lines for residual feed intake in fish would help to achieve this goal.

The measurement of individual feed intake within groups of African catfish is essential to understand the factors causing individual variation in growth. However, in African catfish the use of the most common method to measure individual feed intake in groups (X-rays) is hampered due to vomiting reactions when fish are handled after feeding. This emphasizes the need for improved non-invasive techniques to measure individual feed intake in groups of African catfish.

This thesis shows that individual variation has an important biological meaning and should not be considered simply as a "statistical noise". The effect of a treatment on growth performance should therefore be tested not only on group averages but also at the individual level. It is possible that when averages of groups are used, the effect of a certain treatment on growth is not significant while its effect on the individual variation of growth is. Rice et al. (1993), using an individual-based predation model suggested that cohorts starting with similar mean growth rates but different variances may produce very different numbers and sizes of survivors, even when exposed to identical mortality mechanisms. Likewise, cohorts with very different initial mean growth rates can end up with very similar mean growth rates among survivors, if the variance in growth rates among individuals is different.

Main conclusions

- The pronounced individual variation in growth of African catfish is not a consequence of marked dominance–subordinance relationships. Instead, genetic–based differences in feed intake, feed efficiency, feeding behaviour and stress response seem to play a role in explaining growth variation in African catfish. This suggests a re-evaluation on the use of grading and stimulates the development of selection programmes in African catfish.
- Individual differences in feed intake and feed efficiency (residual feed intake) contributed ~85 and ~15 %, respectively, to explain individual differences in growth.
- Individual differences in feeding behaviour (total feeding time) explained the variation in residual feed intake (maintenance requirements) up to 2 %; more efficient fish are fast eaters.
- Individual differences in stress response (plasma glucose and cortisol after an acute stress) explained the variation in residual feed intake (maintenance requirements) up to 8.7 %; more efficient fish are low stress responders.

References

Abbott, J. C., Dill, L. M., 1989. The relative growth of dominant and subordinate juvenile steelhed trout (*Salmo gairdneri*) fed equal rations. Behaviour 108, 104-111.

Adams, C. E., Huntingford, F. A., Turnbull, J, F., Beattie, C., 1998. Alternative competitive strategies and the cost of food acquisition in juvenile Atlantic salmon (*Salmo salar*). Aquaculture 167, 17-26.

Alanärä, A., Winberg, S., Brännäs, E., Kiessling, A., Hoglund, E., Elofsson, U., 1998. Feeding behaviour, brain serotonergic activity levels, and energy reserves of Arctic char (*Salvelinus alpinus*) within a dominance hierarchy. Can. J. Zool. 76, 212-220.

Alanärä, A., Burns, M. D., Metcalfe, N. B., 2001. Intraspecific resources partitioning in brown trout: the temporal distribution of foraging is determined by social rank. J. Anim. Ecol. 70, 980-986.

Almazán-Rueda, P., 2004a. Towards assessment of welfare in African catfish, *Clarias gariepinus*: the first step. PhD thesis, Wageningen University, The Netherlands, 151pp.

Almazán-Rueda, P., Schrama, J. W., Verreth, J. A. J., 2004b. Behavioural responses under different feeding methods and light regimes of the African catfish (Clarias gariepinus) juveniles. Aquaculture 231, 347-359.

Baardvik, B. M., Jobling, M., 1990. Effect of size-sorting on biomass gain and individual growth rates in Arctic charr, *salvelinus alpinus* L. Aquaculture 90, 11-16.

Baltrusch, H. J., Stangel, W., Titze, I., 1991. Stress, cancer and immunity. New developments in biopsychosocial and psychoneuroimmunologic research. Acta Neurol. 13, 315-327.

Bang, A., Grønkjær, P., Malte, H., 2004. Individual variation in the rate of oxygen consumption by zebrafish embryos. J. Fish Biol. 64, 1285-1296.

Baras, E., 1998. Bases biologiques du cannibalisme chez les poisons. Cah. Ethol. 18, 53-98.

Baras, E., Ndao, M., Maxi, M. Y. J., Jeandrain, D., Thomé, J. P., Vandewalle, P., Mélard, C., 2000. Sibling cannibalism in dorada under experimental conditions. I. Ontogeny, dynamics, bioenergetics of cannibalism and prey size selectivity. J. Fish Biol. 57, 1001–1020.

Baras, E., Fortuné d'Almeida, A., 2001. Size heterogeneity prevails over kinship in shaping cannibalism among larvae of sharptooth catfish *Clarias gariepinus*. Aquat. Living Resour. 14, 251-256.

Baras, E., Jobling, M., 2002. Dynamics of intracohort cannibalism in cultured fish. Aquac. Res. 33, 461-479.

Bartolomucci A., Palanza P., Sacerdote P., Ceresini G., Chirieleison A., Panerai A. E., Parmigiani S., 2003. Individual housing induces altered immuno-endocrine responses to psychological stress in male mice. Psychoneuroendocrino. 28, 540-58.

Barton, B. A., Iwama, G. K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1, 3-26.

Barton, A. B., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42, 517-525.

Basarab, J. A., Price, M. A., Aalhus, J. L., Okine, E. K., Snelling, W. M., Lyle, K. L., 2003. Residual feed intake and body composition in young growing cattle. Can. J. Anim. Sci. 83, 189-204.

Becker, W. A., 1984. Manual of Quantitative Genetics. Academic Enterprises, Pullman, Washington, 188 pp.

Beynen, A. C., Gärtner, K., van Zutphen, L. F. M., 2001a. Standardization of animal experimentation. In: Principles of Laboratory Animal Science. Zutphen. L. F. M., Baumans, V., Beynen, A. C. (Eds). Elsevier, Amsterdam. 103-127.

Beynen, A. C., Festing, M. F. W., van Montfort, M. A. J., 2001b. Design of animal experiments. In: Principles of Laboratory Animal Science. van Zutphen. L. F. M., Baumans, V., Beynen, A. C. (Eds). Elsevier, Amsterdam. 219- 249.

Bisbal, G. A., Specker, J. L., 1991. Cortisol stimulates hypo-osmoregulatory ability in Atlantic salmon, *Salmo salar* L. J. Fish Biol. 39, 421-432.

Boake, C. R. B., 1989. Repeatability: its role in evolutionary studies of mating behaviour. Evol. Ecol. 3, 173-182.

Boake, C. R. B., 1994. Quantitative genetic studies of behavioral evolution. University of Chicago Press, Chicago, 400 pp.

Boon, J. H., Oorschot, R. W. A., Henken, A. M., van Doesum, J. H., 1987. Ruptured intestine syndrome of unknown etiology in young African catfish *Clarias gariepinus* Burchell 1822 and its relation to the feeding level. Aquaculture 63, 283-300.

Brännäs, E., 1998. Individual variation in distribution, activity and growth rate of Arctic charr kept in a three-tank system. J. Fish Biol. 53, 795-807.

Brännäs, E., Alanärä, A., Magnhagen, C., 2001. The social behaviour of fish. In: Social behaviour in Farm Animals. Keeling, L. J., Gonyou, H. W. (Eds). CABI Publishing, Oxon, UK. 275-303

Brodie, E. D. III, Russell, N. H., 1999. The consistency of individual differences in behaviour: temperature effects on antipredator behaviour in garter snakes. Anim. Behav. 57, 445-451.

Brown, K. J., Grunberg, N. E., 1995. Effects of housing on male and female rats: crowding stresses males but calms females. Physiol. Behav. 58**,** 1085–1089.

Bruton, M. N., 1979. The food and feeding behaviour of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with emphasis on its role as a predator of cichlids. Trans. Zool. Soc. London 35, 47-114.

Brzeski, V. J., Doyle, R. W., 1995. A test of an on-farm selection procedure for tilapia growth in Indonesia. Aquaculture 137, 219-230.

Budaev, S. V., Zworykin, D. D., Mochek, A. D., 1999. Consistency of individual differences in behaviour of the lion-headed cichlid, *Steatocranus casuarius.* Behav. Process. 48, 49-55.

Buston, P., 2003. Size and growth modification in clownfish. Nature 424, 145-146.

Buttle, L. G., Uglow, R. F., Cowx, I. G., 1996. The effect of emersion and handling on the nitrogen excretion rates of *Clarias gariepinus*. J. Fish Biol. 49, 693-701.

Carmichael, G. J., 1994. Effects of size-grading on variation and growth in channel catfish reared at similar densities. J. World Aquacult. Soc. 25, 101-108.

Carter, C.G., Houlihan, D.F., McCarthy, I.D., Brafield, A.E., 1992. Variation in the food intake of grass carp, *Ctenopharyngodon idella* (Val.), fed singly or in groups. Aquat. Living Resour. 5: 225-228.

Carter, C. G., McCarthy , I. D., Houlihan, D. F., Fonseca, M., Perera, W. M. K., Sillah, A. B. S., 1995. The application of radiography to the study of fish nutrition. J. Appl. Ichthyol. 11, 231-239.

Chandroo, K. P., Duncan, I. J. H., Moccia, R. D., 2004. Can fish suffer?: perspectives on sentience, pain, fear and stress. Appl. Anim. Behav. Sci. 86, 225-250.

Cobb, J. M., Steptoe, A., 1996. Psychosocial stress and susceptibility to upper respiratory tract illness in an adult population sample. Psychosom. Med. 58, 404-412.

Conte, F. S., 2004. Stress and the welfare of cultured fish. Appl. Anim. Behav. Sci. 86, 205-233.

Cui, Y., Liu, J., 1990. Comparison of energy budget among six teleosts - IV. Individual differences in growth and energy budget. Comp. Biochem. Phys. A 97, 551-554.

Cutts, C. J., Metcalfe, N. B., Taylor, A. C., 1998. Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. J. Fish Biol. 52, 1026–1037.

Cutts, C. J., Adams, C. E., Campbell, A., 2001. Stability of physiological and behavioural determinants of performance in Artic charr (*Salvelinus alpinus*). Can. J. Fish. Aquat. Sci. 58, 961-968.

Cutts, C. J., Metcalfe, N. B., Taylor, A. C., 2002. Fish may fight rather than feed in a novel environment: metabolic rate and feeding motivation in juvenile Atlantic salmon. J. Fish Biol. 61, 1540-1548.

DeAngelis, D.L., Cox, D. C., Coutant, C. C., 1979. Cannibalism and size dispersal in young-of-the-year largemouth bass: Experiments and model. Ecol. Model. 24, 21–41.

de Graaf, G., Janssen, J., 1996. Handbook on the artificial reproduction and pond rearing of the African catfish *Clarias gariepinus* in sub-Saharan Africa. FAO, Fisheries technical paper 362.

De Haer, L. C. M., Luiting, P., Aarts, H. L. M., 1993. Relations among individual (residual) feed intake, growth performance and feed intake pattern of growing pigs in group housing. Livest. Prod. Sci. 36, 233-253.

Dobly, A., Martin, S. A. M., Blaney, S. C., Houlihan, D. F., 2004. Protein growth in rainbow trout (*Oncorhynchus mykiss)* is negatively correlated to liver 20S proteosome activity. Comp. Biochem. Physiol. A 137, 75-85.

Doupé, R. G., Lymbery, A. J., 2004. Indicators of genetic variation for feed conversion efficiency in black bream. Aquac. Res. 35, 1305-1309.

Doyle, R. W., Talbot, A. J., 1986. Artificial selection on growth and correlated selection on competitive behaviour in fish. Can. J. Fish. Aquat. Sci. 43, 1059–1064.

Dunn, R. T., Foster, L. B., 1973. Radioimmunoassay of thyroxine in unextracted serum, by a single-antibody technique. Clin. Chem. 19, 1063-1066.

Ellis, T. James, J.D., Stewart, C., Scott, A.P., 2004. A non-invasive stress assay based upon measurement of free cortisol released into the water by rainbow trout. J. Fish Biol. 65, 1233-1252.

Ewa-Oboho, I. O., Enyenihi, U. K., 1999. Aquaculture implications of growth variation in the African catfish: *Heterobranchus longifilis* (Val.) reared under controlled conditions. J. Applied Ichthyol. 15, 111-115.

Falconer, D. S., Mackay, T. F. C., 1996. Introduction to quantitative genetics. Longman, New York, 464 pp.

FAO, 2002. The state of world fisheries and aquaculture. Food and Agriculture Organization of the United Nations, 150 pp.

FAO, 2003. Yearbook, Fisheries statistics, aquaculture production, vol 96/2.

Fevolden, S., Refstie, T., Røed, K. H., 1991. Selection for high and low cortisol response in Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture 95**,** 53–65.

Fevolden, S. E., Røed, K. H., Fjalestad, K. T., Stien, J., 1999. Post-stress levels of lysozyme and cortisol in adult rainbow trout (*Oncorhynchus mykiss*): heritabilities and genetic correlations. J. Fish Biol. 54, 900-910.

Fevolden, S. E., Røed, K. H., Fjalestad, K. T., 2002. Selection response of cortisol and lysozyme in rainbow trout and correlation to growth. Aquaculture 205, 61-75.

Field, A., 2000. Comparing several means: ANOVA (GLM 1). In: Discovering statistics using SPSS for Windows. Breakwell, G., Leeuw, J., O'Muircheartaigh, C., Saris, W., Schuman, H., van Meter, K. (Eds). Sage Publications, London. 323-374.

Foster, L. B., Dunn, R. T., 1974. Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. Clin. Chem. 20, 365-368.

Foster, S. A., Endler, J. A., 1999. Geographic Variation in Behaviour: Perspectives on Evolutionary Mechanisms. Oxford University Press, Oxford, 336 pp.

Fox, H. E., White, S. A., Kao, M. H. F., Fernald, R. D., 1997. Stress and dominance in a social fish. J. Neurosci. 17, 6463-6469.

Fraser, K. P. P., Lyndon, A. R., Houlihan, D. F., 1998. Protein synthesis and growth in juvenile Atlantic halibut, *Hyppoglossus hippoglossus* (L).: application of ^{15}N stable isotope tracer. Aquac. Res. 29, 289-298.

FSBI., 2002. Fish Welfare. Briefing Paper 2, Fisheries Society of the British Isles, Granta Information Systems. http:// www.le.ac.uk/biology/fsbi/welfare.pdf.

Gélineau, A., Corraze, G., Boujard, T., 1998. Effects of restricted ration, time-restricted access and reward level on voluntary food intake, growth and growth heterogeneity of rainbow-trout (O*ncorhynchus mykiss)* fed on demand with self-feeders. Aquaculture 167, 247-258.

Gjedrem, T., 1997. Contribution from selective breeding to future aquaculture development. J. World Aquacult. Soc. 3, 33-45.

Gjedrem, T., 2000. Genetic improvement of cold-water fish species. Aquac. Res. 31, 25-33.

Gjerde, B., 1986. Growth and reproduction in fish and shellfish. Aquaculture 57, 37-55.

Goede, R.W., Barton, B.A., 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. Am. Fish. Soc. Symp. 8, 93 -108.

Goldan, O., Popper, D., Kolkovski, S., Karplus, I., 1998. Managemenet of size variation in juvenile gilthead sea bream (*Sparus aurata)* II. Dry food and live/dry food ratio. Aquaculture 165, 313-320.

Greaves, K., Tuene, S., 2001. The form and context of aggressive behaviour in farmed Atlantic halibut (*Hippoglossus hippoglossus* L.) Aquaculture 193, 139-147.

Gregory, T. R., Wood, C. M., 1998. Individual variation and interrelationships between swimming performance, growth rate, and feeding in juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 55, 1583-1590.

Gregory, T. R., Wood, C. M., 1999. Interactions between individual feeding behaviour, growth, and swimming performance in juvenile rainbow trout (*Oncorhynchus mykiss)* fed different rations. Can. J. Fish. Aquat. Sci. 56, 479-486.

Grobler, J. P., du Preez, H. H., van der Bank, F. H., 1992. A comparison of growth performance and genetic traits between four selected groups of African catfish (*Clarias gariepinus* Burchell 1822). Comp. Biochem. Phys. A 102, 373–377.

Gunnes, K., 1976. Effect of size grading young Atlantic salmon (*Salmo salar*) on subsequent growth. Aquaculture 9, 381–386.

Hart, P. J. B., Salvanes, A. G. V., 2000. Individual variation in competitive performance of juvenile cod and its consequences for growth. J. Mar. Biol. Assoc. UK 80, 569-570.

Harwood, A. J., Armstrong, J. D., Metcalfe, N. B., Griffiths, S. W., 2003. Does dominance status correlate with growth in wild stream-dwelling Atlantic salmon (*Salmo salar*). Behav. Ecol. 14, 902-908.

Hecht, T., Appelbaum, S., 1988. Observations on intraspecific aggression and coeval sibling cannibalism by larval and juvenile *Clarias gariepinus* (Clariidae: Pisces) under controlled conditions. J. Zool. 214, 21-44.

Hecht, T., Uys, W., 1993. Effect of density on the feeding and aggressive behaviour in juvenile African catfish, *Clarias gariepinus*. S. Afr. J. Sci. 93, 537-541.

Heinsbroek, L. T. N., 1987. Effects of body weight, feeding level and temperature on energy metabolism and growth in fish. In: Energy metabolism in farm animals. Verstegen, M. W. A., Henken, A. M. (Eds). Martinus-Nijhoff, Dordrecht, The Netherlands. 478-500.

Heinsbroek, L. T. N., Thoor, R. M. H., Elizondo, L. J., 1989. The effect of feeding level on the apparent digestibilities of nutrients and energy of a reference diet for the European eel, *Anguilla anguilla* L. and the African catfish, *Clarias gariepinus* (Burchell). Proceedings of the third International Symposium on Feeding and Nutrition in Fish, Toba, Japan. 175–188.

Hemre, G. I.,Mommsen, T. P., Krogdahl, Å., 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. Aquacult. Nutr. 8, 175-194.

Henken, A. M., Kleingeld, D. W., Tijssen, P. A. T., 1985. The effect of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish *Clarias gariepinus* (Burchell, 1822). Aquaculture 52, 1-11.

Hepher, B., 1988. Nutrition of Pond Fishes. Cambridge University Press, Cambridge, 388 pp.

Herd, R. M., Bishop, S. C., 2000. Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. Livest. Prod. Sci. 63, 111-119.

Herd, R. M., Oddy, V. H., Richardson, E. C., 2004. Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Aust. J. Exp. Agr. 44, 423-430.

Hocutt, C. H., 1989. Seasonal and diel behaviour of radio-tagged *Clarias gariepinus* in lake Ngezi, Zimbabwe (Pisces: Clariidae). J. Zool. 219, 181-199.

Houlihan, D. F., Carter, C. G., McCarthy, I. D., 1995. Protein turnover in animals. In: Nitrogen Metabolism and Excretion. Walsh, P. J., Wright, P. (Eds). CRC Press, Boca Raton. 1- 31.

Huntingford, F. A., Metcalfe, N. B., Thorpe, J. E., Graham, W. D., Adams, C. E., 1990. Social dominance and body size in Atlantic salmon parr, *Salmo salar*. L. J.Fish Biol. 36, 877-881.

Huntingford, F. A., Metcalfe, N. B., Thorpe, J. E., 1993. Social status and feeding in Atlantic salmon Salmo salar Parr: The effect of visual exposure to a dominant. Ethology 94, 201-206.

Imsland, A. K., Sunde, L. M., Folkvord, A., Stefansson, S. O., 1996. The interaction of temperature and fish size on growth of juvenile turbot. J. Fish Biol. 49, 926-940.

ISO 5984, 1978. Animal feeding stuffs. Determination of crude ash. International Organization for Standardization. Geneva, Switzerland.

ISO 5983, 1979. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. International Organization for Standardization. Geneva, Switzerland.

ISO 6496, 1983. Animal feeding stuffs. Determination of moisture content. International Organization for Standardization.. Geneva, Switzerland.

Irwin, S., O'Halloran, J., FitzGerald, R. D., 2002. The relationship between individual consumption and growth in juvenile turbot *Scophthalmus maximus*. Aquaculture 204, 65-74.

Iwaisaki, H., Wilton, J. W., 1993. Regression of genotypic and phenotypic value of a ratio defined character. Biometrics 49, 1154-1163.

Jessop, J. J., Bayer, B. M., 1989. Time-dependent effects of isolation on lymphocyte and adrenocortical activity. J. Neuroimmunol. 23, 143–147.

Jobling, M., 1982. Some observations on the effects of feeding frequency on the food intake and growth of plaice, *Pleuronectes platessa* L. J. Fish Biol. 20, 431-444.

Jobling, M., Wandsvik, A., 1983. Effect of social interactions on growth rates and conversion efficiency of Artic charr, *Salvelinus alpinus* L. J. Fish Biol. 22, 577-584.

Jobling, M., 1985. Physiological and social constraints on growth of fish with special reference to Arctic charr, *Salvelinus alpinus* L. Aquaculture 44, 83–90.

Jobling, M., Reinsnes, T. G., 1986. Physiological and social constrains on growth of Arctic charr, *Salvelinus alpinus* L – an investigation of factors leading to stunting. J. Fish Biol. 28, 379-384.

Jobling, M., Reinsnes, T. G., 1987. Effect of sorting on size-frequency distributions and growth of Arctic charr, *Salvelinus alpinus* L. Aquaculture 60, 27–31.

Jobling, M., Jørgensen, E. H., Arnesen, A. M., Ringø, E., 1993. Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential. Aquacult. Int. 1, 20-46.

Jobling, M., 1994. Ingestion, absorption and excretion. In: Fish Bioenergetics. Jobling, M. (Eds). Chapman & Hall, London, UK. 99-145.

Jobling, M., Baardvik, B. M., 1994. The influence of environmental manipulations on inter- and intra-individual variation in food acquisition and growth performance of Arctic charr, *Salvelinus alpinus*. J. Fish Biol. 44, 1069-1087.

Jobling, M., 1995. Simple indices for the assessment of the influences of social environment on growth performance, exemplified by studies on Arctic charr (*Salvelinus alpinus*). Aquac. Int. 3, 60-65.

Jobling, M., Koskela, J., 1996. Interindividual variations in feeding and growth in rainbow trout during restricted feeding and in a subsequent period of compensatory growth. J. Fish Biol. 49, 658-667.

Johnsson, J. I., Björnsson, B. Th., 1994. Growth hormone increases growth rate, appetite and dominance in juvenile rainbow trout, *Oncorhynchus mykiss*. Anim. Behav. 48, 177–186.

Johnsson, J. I., 1997. Individual recognition affects aggression and dominance relations in rainbow trout. *Oncorhynchus mykiss*. Ethology 103, 267–282.

Jönsson, E., Johnsson, J. I., Björnsson, B. T., 1998. Growth hormone increases aggressive behaviour in juvenile rainbow trout. Horm. Behav. 33, 9–15.

Jönsson, E., Johnsson, V., Björnsson, B. T., Winberg, S., 2003. Central nervous system actions of growth hormone on brain monoamine levels and behaviour of juvenile rainbow trout. Horm. Behav. 43, 367-374.

Jørgensen, E. H., Jobling, M., 1993. Feeding in darkness eliminates density-dependent growth suppression in Arctic charr. Aquac. Int. 1, 90-93.

Jourdan, S., Fontaine, P., Boujard, T., Vandeloise, E., Gardeur, J. N., Anthouard, M., Kestemont, P., 2000. Influence of daylength on growth, heterogeneity, gonad development, sexual steroid and thyroid levels, and N and P budgets in *Perca fluviatilis*. Aquaculture 186, 253-265.

Kadri, S., Huntingford, F. A., Metcalfe, N. B., Thorpe, J. E., 1996. Social interactions and the distribution of food among one-sea-winter Atlantic salmon (*Salmo salar)* in a sea cage. Aquaculture 139, 1–10.

Kamstra, A., 1993. The effect of size grading on individual growth in eel, *Anguilla anguilla*, measured by individual marking. Aquaculture 112, 67–77.

Katle, J., Hamet, N., Durand, L., Rombauts, P., Mérat, P., 1988. Divergent lines for residual feed intake of layers: response of chicks to inoculation by *Eimeria* acervulina and comparison of biological parameters. Genet. Sel. Evol. 20, 387-396.

Kendall, B. E., Fox, G. A., 2002. Variation among individuals and reduced demographic stochasticity. Cons. Biol. 16, 109–116.

Kestemont, P., Jourdan, S., Houbart, M., Mélard, C., Paspatis, M., Fontaine, P., Cuvier, A., Kentouri, M., Baras, E., 2003. Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: biotic and abiotic influences. Aquaculture 227, 333-356.

Kime, N. M., Rand, A. S., Kapfer, M., Ryan, M. J., 1998. Consistency of female choice in the túngara frog: a permissive preference for complex characters. Anim. Behav. 55, 641-649.

Knap, P. W., 2000. Variation in maintenance requirements of growing pigs in relation to body composition. A simulation study. PhD thesis, Wageningen University, The Netherlands, 219pp.

Knights, B., 1987. Agonistic behaviour and growth in the European eel, *Anguilla anguilla* L., in relation to warm-water aquaculture. J. Fish Biol. 31, 265-276.

Koch, R. M., Swiger, L. A., Chambers, D., Gregory, K. E., 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22, 486-494.

Koebele, B. P., 1985. Growth and the size hierarchy effect: an experimental assessment of three proposed mechanisms; activity differences, disproportional food acquisition, physiological stress. Environ. Biol. Fishes 12, 181-188.

Kolok, A. S., 1999. Interindividual variation in the prolonged locomotor performance of ectothermic vertebrates: a comparison of fish and herpetofaunal methodologies and a brief review of the recent fish literature. Can. J. Fish. Aquat. Sci. 56, 700-710.

Kolstad, K., Grisdale-Helland, B., Gjerde, B., 2004. Family differences in feed efficiency in Atlantic salmon (*Salmo salar*). Aquaculture 241, 169-177.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt B. J., Van Reenen C. G., Hopster H., De Jong I. C., Ruis M. A. W., Blokhuis H. J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. R. 23, 925-935.

Kristiansen, H. R., 1999. Discrete and multiple hierarchy formation in juvenile salmonids. Aquac. Res. 30, 519-527.

Kristiansen, T. S., Fernö, A., Holm, J. C., Privitera, L., Bakke D., Fosseidengen, J. E., 2004. Swimming behaviour as an indicator of low growth rate and impaired welfare in Atlantic halibut (*Hippoglossus hippoglossus* L.) reared at three stocking densities. Aquaculture 230, 137-151.

Lahti, K., Laurila, A., Enberg, K., Piironen, J., 2001. Variation in aggressive behaviour and growth rate between populations and migratory forms in the brown trout, *Salmo trutta*. Anim. Behav. 62, 935–944.

Lambert, Y., Dutil, J. D., 2001. Food intake and growth of adult Atlantic cod (*Gadus morhua* L.) reared under different conditions of stocking density, feeding frequency and size-grading. Aquaculture 192, 233-247.

Larsson, P. O., 1984. Growth of Baltic *Salmon salar* in the sea. Mar. Ecol.-Prog. Ser. 17, 215-226.

Lemieux, H., Blier, P., Dutil, J. D., 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficicny in the Atlantic cod (*Gadus morhua)*. Fish Physiol. Biochem. 20, 293-303.

Letcher, B. H., Rice, J. A., Crowder, L. B., Rose, K. A., 1996. Variability in survival of larval fish: disentangling components with a generalized individual-based model. Can. J. Fish. Aquat. Sci. 53, 787 – 801.

Luiting, P., 1990. Genetic variation of energy portioning in laying hens: causes of variation in residual feed consumption. World Poultry Sci. J. 46, 133-152.

Luiting, P., 1991. The value of feed consumption data for breeding in laying hens. PhD thesis, Wageningen University, The Netherlands, 183pp.

Luiting, P., Schrama, J. W., van der Hel, W., Urff, E. M., 1991. Metabolic differences between white leghorns selected for high and low residual food consumption. Brit. Poultry Sci. 32, 763-782.

Luiting, P., Urff, E. M., 1991. Optimization of a model to estimate residual feed consumption in the laying hen. Livest. Prod. Sci. 27, 321-338.

Lupatsch, I., Kissil, G. W., 2003. Defining energy and protein requirements of Gilthead seabream (*Sparus aurata)* to optimize feeds and feeding regimes. Isr. J. Aquacult. – Bamidgeh 55, 243-257.

Lupatsch, I., Kissil, G. W., Sklan, D., 2003. Comparison of energy and protein efficiency among three fish species gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and white grouper (*Epinephelus aeneus*): energy expenditure for protein and lipid deposition. Aquaculture 225, 175-189.

Lyons, D. M., Wang, O. J., Lindley, S. E., Levine, S., Kalin, N. H., Schatzberg, A. F., 1999. Separation induced changes in squirrel monkey hypothalamic-pituitary-adrenal physiology resemble aspects of hypercortisolism in humans. Psychoneuroendocrinol. 24, 131–142.

Maltby, L., 1999. Studying stress: the importance of organism-level responses. Ecol. Appl. 9, 431-440.

Mambrini, M., Médale, F., Sanchez, M. P., Recalde, B., Chevassus, B., Labbé, L., Quillet, E., Boujard, T., 2004. Selection for growth in brown trout increases feed intake capacity without affecting maintenance and growth requirements. J. Anim. Sci. 82, 2865-2875.

Marteinsdottir, G., A. Steinarsson., 1998. Maternal influence on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. J. Fish Biol. 52, 1241-1258.

Martin-Smith, K., Armstrong, J., 2002. Growth rates of wild stream-dwelling Atlantic salmon correlate with activity and sex but not dominance. J. Ecol. 71, 413-423.

Martins, C. I. M., Schrama, J. W., Verreth, J. A. J., 2005a. Inherent variation in growth efficiency of African catfish *Clarias gariepinus* (Burchell 1822) juveniles. Aquac. Res. 36, 868-875.

Martins, C. I. M., Aanyu, M., Schrama, J. W., Verreth, J.A.J., 2005b. Size distribution in African catfish (*Clarias gariepinus)* affects feeding behaviour but not growth. Aquaculture, *in press.*

McCarthy, I. D., Carter, C. G., Houlihan, D. F., 1992. The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout *Oncorhynchus mykiss* (Walbaum). J. Fish Biol. 41, 257-263.

McCarthy, I. D., Houlihan, D. F., Carter, C. G., 1994. Individual variation in protein turnover and growth efficiency in rainbow trout, *Oncorhynchus mykiss* (Walbaum). P. Roy. Soc. Lond. B 257, 141-147.

McDonald, M. E., Tokkanen, C. A., Axler, R. P., Larsen, C. P., Host, G., 1996. Fish simulation culture model (FIS-C): a bioenergetics based model for aquaculture wasteload application. Aquacult. Eng. 15, 243-259.

Merron, G., 1993. Pack hunting in two species of *Clarias gariepinus* and *Clarias ngamensis*, in the Okavango Delta, Botswana. J. Fish Biol. 43, 575–584.

Metcalfe, N. B., 1986. Intraspecific variation in competitive ability and food intake in salmonids: consequences for energy budgets and growth rates. J. Fish Biol. 28, 525–531.

Metcalfe, N. B., Huntingford, F. A., Thorpe, J. E., 1986. Seasonal changes in feeding motivation of juvenile Atlantic salmon (*Salmo salar)*. Can. J. Zool. 64, 2439-2446.

Metcalfe, N. B., Huntingford, F. A., Thorpe, J. E., 1988. Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile Atlantic salmon *Salmo salar*. J. Anim. Ecol. 57, 463-474.

Metcalfe, N. B., Huntingford, F. A., Graham, W. D., Thorpe, J. E., 1989. Early social status and the development of life-history strategies in Atlantic salmon. Proc. R. Soc. Lond. B 236, 7–19.

Metcalfe, N.B., Huntingford, F.A., Thorpe, J. E., Adams, C. E., 1990. The effects of social status on life-history variation in juvenile salmon. Can. J. Zool. 68, 2630-2636.

Metcalfe, N. B., Thorpe, J. E., 1992. Early predictors of life-history events: the link between first feeding date, dominance and seaward migration in Atlantic salmon, *salmo salar* L. J. Fish Biol. 41: 93-99.

Metcalfe, N. B., Taylor, A. C., Thorpe, J. E., 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. Anim. Behav. 49, 431-436.

Metcalfe, N. B., 1998. The interaction between behaviour and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 55, 93-103.

Morgan, I. J., McCarthy, I. D., Metcalfe, N. B., 2000. Life-history and protein metabolism in overwintering juvenile Atlantic salmon: growth is enhanced in early migrants through lower protein turnover. J. Fish Biol. 56, 637-647.

Moutou, K. A., McCarthy, I. D., Houlihan, D. F., 1998. The effect of ration level and social rank on the development of fin damage in juvenile rainbow trout. J. Fish Biol. 52, 756-770.

Nævdal, G., Folkvord, A., Otterlei, E., Thorkildsen, S., 1992. Growth rate related to genotype of 0-group cod at three environmental temperatures. Sarsia 77, 71-73.

Nakano, S., 1995. Individual differences in resource use, growth and emigration under the influence of a dominance hierarchy in fluvial red-spotted masu salmon in a natural habitat. J. Anim. Ecol. 64, 75-84.

Nicieza, A. G., Metcalfe, N. B., 1999. Costs of rapid growth: the risk of aggression is higher for fast-growing salmon. Funct. Ecol. 13, 793-800.

Noakes, D. L. G., Grant, J. W., 1992. Feeding and social behaviour of brook and lake charr. In: Thorpe, J. E., Huntingford, F. A. (Eds). The importance of feeding behaviour for the efficient culture of Salmonid fishes. World Aquaculture Society, Baton Rouge, FL, 13-20.

NRC., 1998. Nutrient requirements of swine: 10th Revised Edition. National Academy Press, Washington D. C. 192pp.

Ogata, H. Y., Oku, H., Murai, T., 2002. Growth, feed efficiency and feed intake of offspring from selected and wild Japanese flounder (*Paralichthys olivaceus*). Aquaculture 211, 183-193.

Oliveira R. F., Lopes M.,Carneiro L. A., Canário A. V. M., 2001. Watching fights raises fish hormone levels. Nature 409, 475-475.

Øverli, Ø., Winberg, S., Damsgård, B., Jobling, M., 1998. Food intake and spontaneous swimming activity in Arctic char (*Salvelinus alpinus)*: role of brain serotonergic activity and social interactions. Can. J. Zool. 76, 1366–1370.

Øverli, Ø., Harris, C. A., Winberg, S., 1999. Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationship on brain monoamines and cortisol in rainbow trout. Brain. Behav. Evol. 54, 263-275.

Øverli, O., Korzan W., Hoglund, E., Winberg S., Bollig, H., Watt M., Forster G. L., Barton, B. A., Øverli, E., Renner, K. J., Summers,C. H., 2004. Stress coping style predicts aggression and social dominance in rainbow trout. Horm. Behav. 45, 235-241.

Ozório, R. O. A., 2001. Dietary L-carnitine and energy and lipid metabolism in African catfish (*Clarias gariepinus)* juveniles. PhD thesis, Wageningen University, The Netherlands, 133 pp.

Petrell, R. J., Jones, R. E., 2000. Power requirement of swimming in chinook salmon and Atlantic salmon and implications for food conversion and growth performance. Aquacult. Eng. 22, 225-239.

Popper, D. M., Golden, O., Shezifi, Y., 1992. Size distribution of juvenile gilthead sea bream (*Sparus aurata*), practical aspects. Isr. J. Aquac. Bamid. 44, 147–148.

Pottinger, T. G., 1990. The effect of stress and exogenous cortisol on receptor-like binding of cortisol in the liver of rainbow trout, *Oncorhynchus mykiss.* Gen. Comp. Endocrinol. 78, 194-203.

Pottinger, T. G., Pickering, A. D., 1992. The influence of social interaction on the acclimation of rainbow trout *Oncorhynchus mykiss* Walbaum to chronic stress. J. Fish Biol. 41, 435-447.

Pottinger, T. G., Pickering, A. D., Hurley, M. A., 1992. Consistency in the stress response of individuals of two strains of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 103, 275–289.

Pottinger, T. G., 1998. Changes in blood cortisol, glucose and lactate in carp retained in anglers' keepnets. J. Fish Biol. 53, 728-742.

Pottinger, T. G., Carrick, T. R., 1999. A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female rainbow trout. Aquaculture 175, 351-363.

Qian, X., Cui, Y., Xie, S., Lei. W., Xiong, B., Yang, Y., 2002. Individual variations in growth, food intake and activity in juvenile Chinese sturgeon *Acipenser sinensis* Gray. J. Appl. Ichthyol. 18, 695-698.

Rauw, W. M., Luiting, P., Bakken, M., Schuurman, T., de Veer, C. J. M., Vangen, O., 2000. Behavioural differences in non-reproductive adult females in a long term selection experiment for litter size in mice. Appl. Anim. Behav. Sci. 66, 249-262.

Réale, D., Gallant, B. Y., Leblanc, M., Festa-Bianchet, M., 2000. Consistency of temperament in bighorn ewes and correlates with behaviour and life history. Anim. Behav. 60, 589-597.

Redding, J. M., Schrek, C.B., Birks, E.K., Ewing, R.D., 1984. Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in fresh water and during seawater acclimation in yearling coho salmon *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 56, 146-155.

Rice, J. A., Miller, T. J., Rose, L. B., Marschall, E. A., Trebitz, A. S., DeAngelis, D. L., 1993. Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. Can. J. Aquat. Sci. 50, 133-142.

Richardson, E. C., Herd, R. M., Colditz, I. G., Archer, J. A., Arthur, P. F., 2002. Blood cell profiles of steer progeny from parents selected for and against residual feed intake. Aust. J. Exp. Agr. 42, 901-908.

Richardson, E. C., Herd, R. M., 2004. Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Aust. J. Exp. Agr. 44, 431-440.

Richardson, E. C., Herd, R. M., Archter, J. A., Arthur, P. F., 2004. Metabolic differences in Angus steers divergently selected for residual feed intake. Aust. J. Exp. Agr. 44, 441-452.

Roff, D. A., 1997. *Evolutionary Quantitative Genetics*. New York: Chapman & Hall.

Ruane, N. M., Huisman, E. A., Komen, J., 2001. Plasma cortisol and metabolite level profiles in two isogenic strains of common carp during confinement. J. Fish Biol. 59, 1-12.

Ruis, M. A. W., te Brake, J. H. A., Engel, B., Buist, W. G., Blokhuis, H. J., Koolhaas, J. M., 2001. Adaptation to social isolation, acute and long-term stress responses of growing gilts with different coping characteristics. Physiol. Behav. 73**,** 541–551.

Saillant, E., Fostier, A., Menu, B., Haffray, P., Chatain, B., 2001. Sexual growth dimorphism in sea bass *Dicentrarchus labrax*. Aquaculture 202, 371-387.

Salvanes, A. G. V., Hart, P. J. B., 1998. Individual variability in state-dependent feeding behaviour in three-spined sticklebacks. Anim. Behav. 55, 1349-1359.

Salvanes, A. G. V., Hart, P. J. B., 2000. Is individual variation in competitive performance of reared juvenile cod influenced by haemoglobin genotype? Sarsia 85, 265-274.

Sapolsky, R. M., Alberts, S. C., Altmann, J., 1997. Hypercortisolism associated with social subordinance or social isolation among wild baboons. Arch. Gen. Psychiat. 54, 1137–1143.

SAS, 1989. SAS/STAT® User's guide, version 6, 4th edition. SAS institute, Cary NC, USA.

Seppä, T., Peuhkuri, N., Hirvonen, H., Laurila, A., Piironen, J., Ranta, E., 1999. Narrow size regime among individuals favors rapid growth in Arctic char (*Salvelinus alpinus*) juveniles. Can. J. Fish. Aquat. Sci. 56, 1891-1897.

Seymour, E. A., 1984. High stocking rates and moving water solve the grading problem. Fish Farmer 7, 12-14.

Shang, Y. C., 1990. Aquaculture Economics Analysis: An Introduction. The World Aquaculture Society, Baton Rouge, LA, USA, 211 pp.

Silverstein, J. T., Wolters, W. R., Holland, M., 1999. Evidence of differences in growth and food intake regulation in different genetic strains of channel catfish. J. Fish Biol. 54, 607-615.

Silverstein, J. T., Wolters, W. R., Shimizu, M., Dickhoff, W. W., 2000. Ovine growth hormone treatment of channel catfish: strain and temperature effects on growth, plasma IGF-I levels, feed intake and efficiency and body composition. Aquaculture 190, 77-88.

Silverstein, J. T., Hostuttler, M., Blemings, K. P., 2005. Strain differences in feed efficiency measured as residual feed intake in individually reared rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res. 36, 704-711.

Sloman, K. A., Gilmour, K. M., Taylor, A. C., Metcalfe, N. B., 2000. Physiological effects of dominance hierarchies within groups of brown trout, *Salmo trutta*, held under simulated natural conditions. Fish Physiol. Biochem. 22, 11-20.

Sloman, K. A., Metcalfe, N. B., Taylor, A. C., Gilmour, K. M., 2001a. Plasma cortisol concentrations before and after social stress in rainbow trout and brown trout. Physiol. Biochem. Zool. 74, 383-389.

Sloman, K. A., Taylor, A. C., Metcalfe, N. B., Gilmour, K. M., 2001b. Effects of an environmental perturbation on the social behaviour and physiological function of brown trout. Anim. Behav. 61, 325-333.

Sloman, K. A., Armstrong, J. D., 2002. Physiological effects of dominance hierarchies: laboratory artefacts or natural phenomena? J. Fish Biol. 61, 1-23.

Sloman, K. A., Baker, D. W., Wood, C. M., McDonald, G. , 2002. Social interactions affect physiological consequences of sublethal copper exposure in rainbow trout, *Oncorhynchus mykiss*. Environ. Toxicol. Chem. 21, 1255-1263.

Smith, R.R., Kincaid, H. L., Regenstein, J. M., Rumsey, G. L., 1988. Growth, carcass composition, and taste of rainbow trout of different strains fed diets containing primarily plant or animal protein. Aquaculture 70, 309-321.

Sneddon, L. U., 2003. The bold and the shy: individual differences in rainbow trout. J. Fish Biol. 62, 971-975.

Späni, D., Arras, M., König, B., Rülicke, T., 2003. Higher heart rate of laboratory mice housed individually vs in pairs. Lab. Anim. 37, 54-62.

Stefansson, M. O., Imsland, A. K., Jenssen, M. D., Jonassen, T. M., Stefansson, S. O., FitzGerald, R., 2000. The effect of different initial size distributions on the growth of Atlantic halibut. J. Fish Biol. 56, 826-836

Sunde L. M., Imsland A. K., Folkvord A., Stefansson, S. O., 1998. Effects of size grading on growth and survival of juvenile turbot at two temperatures. Aquac. Int. 6, 19–32.

Sundström, L. F., Devlin, R. H., Johnsson, J. I., Biagi, C. A., 2003. Vertical position reflects increased feeding behaviour in growth hormone transgenic coho salmon (*Oncorhynchus kisutch*). Ethology 109, 701–712.

Sundström, L. F., Petersson, E., Höjesjö, J., Johnsson, J. I., Järvi, T., 2004. Hatchery selection promotes boldness in newly hatched brown trout (*Salmo trutta)*: implications for dominance. Behav. Ecol. 15, 192-198.

Teugels, G. G., 1996. Taxonomy, phylogeny and biogeography of catfishes (Ostariophysi, Siluroidei): an overview. Aquat. Living Resour. 9, 9-34.

Thodesen, J., Grisdale-Helland, B., Helland, S. J., Gjerde, B., 1999. Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (*Salmo salar*). Aquaculture 180, 237-246.

Thodesen, J., Gjerde, B., Grisdale-Helland, B., Storebakken, T., 2001. Genetic variation in feed intake, growth and feed utilization in Atlantic salmon (*Salmo salar*). Aquaculture 194, 273-281.

Thorpe, J. E., 1977. Biomodal distribution of length of juvenile Atlantic salmon (*Salmon salar* L.) under artificial rearing conditions. J. Fish Biol. 11, 175-184.

Toguyeni, A., Fauconneau, B., Boujard, T., Fostier, A., Kuhn, E. R., Mol, K. A., Baroiller, J., 1997. Feeding behaviour and food ulitisation in tilapia, *Oreochromis niloticus*: effect of sex ratio and relationship with the endocrine status. Physiol. Behav. 62, 273-279.

Tort, L., Montero, D., Robaina, L., Fernández-Palacios, H., Izquierdo, M. S., 2001. Consistency of stress response to repeated handling in the gilthead sea bream *Sparus aurata* Linnaeus, 1758. Aquac. Res. 32, 593-598.

Umino, T., Arai, K., Nakagawa, H., 1997. Growth performance in clonal crucian carp, *Carassius langsdorfii*. Effects of genetic difference and feeding history. Aquaculture 155, 271-283.

Valente, L. M. P., Saglio, P., Cunha, L. M., Fauconneau, B., 2001a. Feeding behaviour of fast- and slow-growing strains of rainbow trout, *Oncorhynchus mykiss* (Walbaum) during first feeding. Aquac. Res. 32, 471–480.

Valente, L. M. P., Fauconneau, B., Gomes, E. F. S., Boujard, T., 2001b. Feed intake and growth of fast and slow growing strains of rainbow trout (*Oncorhynchus mykiss)* fed by automatic feeders or by self-feeders. Aquaculture 195, 121-131.

Valzelli, L., 1973. The "isolation syndrome" in mice. Psychopharmacologia 31, 305–320.

van der Waal, B. C. W., 1998. Survival strategies of sharptooth catfish *Clarias gariepinus* in desiccating pans in the northern Kruger National Park. Koedoe 41, 131–138.

van Eerden, E., van den Brand, H., De Vries Reilingh, G., Parmentier, H. K. de Jong, M. C. M., Kemp. B., 2004. Residual feed intake and its effect on *Salmonella enteritidis* infection in growing layer hens. Poultry Sci. 83, 1904-1910.

Van Erp-Van der Kooij, E., 2003. Coping behaviour in pigs. Consequences for welfare and performance. PhD Thesis, Utrecht University, The Netherlands, 138 pp.

van Raaij, M. T. M., van den Thillart, G. E. E. J. M., Vianen, G. J., Pit, D. S. S., Balm, P. H. M., Steffens, A. B., 1996. Substrate mobilization and hormonal changes in rainbow trout (*Oncorhynchus mykiss,* L) and common carp (*Cyprinus carpio* L.) during deep hypoxia and subsequent recovery. J. Comp. Physiol. 166, 443-452.

Veenema, A. H., Meijer, O. C., Kloet, E. R., Koolhaas, J. M., 2003. Genetic selection for coping style predicts stressor susceptibility. J. Neuroendocrinol. 15, 256-267.

Verreth, A. J., Eding, E. H., 1993. European farming industry of African catfish (*Clarias gariepinus)*: facts and figures. Aquac. Europe 18, 6-13.

Vilizzi, L., Walker, K. F., 1999. The onset of the juvenile period in carp, *Cyprinus carpio:* a literature survey. Environ. Biol. Fish. 56, 93-102.

Wallace, J. C., Kolbeinshavn, A. G., 1988. The effect of size grading on subsequent growth in fingerling Arctic charr, *Salvelinus alpinus* (L). Aquaculture 73, 97–100.

Wang, N., Hayward, R. S., Noltie, D. B., 1998. Variation in food consumption, growth, and growth efficiency among juvenile hybrid sunfish held individually. Aquaculture 167, 43-52.

Ward, A. J. W., Thomas, P., Hart, P. J. B., Krause, J., 2004. Correlates of boldness in three-spined sticklebacks (*gasterosteus aculeatus)* Behav. Ecol. Sociobiol. 55, 561-568.

Wendelaar Bonga, S. E., 1997. The stress response in fish. Physiol. Rev. 77, 591-624.

Wickins, J. F., 1985. Growth variability in individually confined elvers, *Anguilla anguilla* (L.) J. Fish Biol. 27, 469-478.

Wickins, J. F., 1987. Effects of size, culling and social history on growth of cultured elvers, *Anguilla anguilla* (L). J. Fish Biol. 31, 71-82.

Winberg, S., Carter, C. G., McCarthy, I. D., He, Z-Y., Nilsson, G. E., Houlihan, D. F., 1993. Feeding rank and brain serotonergic activity in rainbow trout *Oncorhynchus mykiss*. J. Exp. Biol. 179, 197-211.

Winberg, S., Øverli, Ø., Lepage, O., 2001. Suppression of aggression in rainbow trout (*Oncorhynchus mykiss*) by dietary L-tryptophan. J. Exp. Biol. 204, 3867-3876.

Young, G., 1986. Cortisol secretion in vitro by the interrenal of Coho salmon (*Oncorhynchus kisutch*) during smoltification: relationship with plasma thyroxine and plasma cortisol. Gen. Comp. Endocrinol. 63, 191-200.

- APPENDICES -

SUMMARY

Among farmed animals, fish exhibit the largest individual variation in growth, yet most of the studies reporting data on growth do not take individual variation into account. Usually a mean value is considered and although the variation around the mean is also mentioned, it is generally viewed as a sort of "statistical noise". The importance of individual variation in growth should not be underestimated since it has important consequences for water quality, aggression, stress levels, farm management, selection programmes, etc. Among the factors responsible for growth variation, social hierarchy is often considered as the most important. Social hierarchies may induce behavioural inhibition and stress on subordinate fish, affecting their feed intake, feed efficiency and as a consequence reducing their growth. However, for most fish species there is no unambiguous proof that individual differences in feed intake, feed efficiency and growth result from social hierarchies. Some studies suggest that inherent (genetic) factors may also cause the variation in growth.

The general aim of this study was to understand the underlying factors responsible for the individual variation in growth of African catfish *Clarias gariepinus*. The following factors were investigated: 1) if individual variation in growth is mainly a consequence of social hierarchies, 2) the contribution of individual differences in feed intake and feed efficiency to the individual differences in growth and 3) the contribution of feeding behaviour and stress response in explaining individual differences in feed efficiency.

Chapters 2 and 3 investigated the importance of social hierarchy as an explanatory factor for the individual variation in growth of African catfish. The growth performance, behaviour (feeding behaviour, aggression levels) and stress response between groups of low-, medium- and heavy- weight fish were compared. **Chapter 2** showed that low-weight fish do not exhibit increased growth rates in the absence of heavier fish. Apparently, the growth differences were not induced by social hierarchies where the larger fish suppress the growth of smaller fish. Instead, this study suggests that feeding behaviour is a crucial factor. Heavier fish exhibit feeding behaviours that may give advantage when feed is limited, such as being more active swimmers, spending more time at the feeding areas and eating their meal faster than low-weight fish. These differences in behaviour may result in growth variation, as found in this study.

Chapter 3 showed that the aggression and stress levels did not increase in heterogeneous (weight) groups as compared with homogeneous (weight) groups.

Furthermore, low-weight fish did not exhibit a higher number of skin lesions and higher stress levels when reared in heterogeneous groups as compared with low-weight fish reared in homogeneous groups. These results suggested that low-weight fish were not behaving as subordinates and heavy fish as dominants.

To further investigate the importance of inherent differences in growth variation, a set of experiments were designed using individually housed fish. Housing fish individually enabled the study of individual differences in the absence of social interactions and to measure individual feed intake accurately. This raised the question whether the results obtained from housing fish individually could be representative of a group housing situation. **Chapter 4** compared the growth performance, feeding behaviour and stress response of isolated and non-isolated fish. This study suggested that in African catfish feed intake is stimulated by the presence of conspecifics resulting in higher feed intake and growth rates. However, isolation per se seems not to act as a stressor in the short term or to affect the stress response, probably because periods of isolation are part of the African catfish lifestyle. In addition, **Chapter 5** compared the growth of fish housed individually and afterwards in a group. The average growth of individually housed fish was lower than fish in group housing. However, slow and fast growing fish under individual housing remained slow and fast growing fish, respectively, under group housing. This suggests that the different growth rates observed when fish are housed individually are a characteristic of the individual and not simply a consequence of isolation.

Chapters 5 to 7 used individually housed fish to supply experimental data on inherent factors responsible for individual variation in growth. **Chapter 5** quantified individual differences in performance traits and feeding behaviour and focused on the repeatability of such individual differences when fish were fed ad libitum. Fish exhibited pronounced individual variation in growth (CV = 52.8 %), in feed intake (34.3 %) and in total feeding time (>100 %). The repeatability estimates were 0.55 for growth, 0.70 for feed intake, 0.49 for feed efficiency and 0.81 for total feeding time. These high repeatability estimates suggested that individual differences in growth, feed intake/efficiency and feeding behaviour are consistent over time and therefore probably inherent. Individual differences in growth were explained mainly by individual differences in feed intake (~85 %). Individual differences in feeding behaviour were shown to be related to feed efficiency, measured as residual feed intake (i.e., the difference between actual feed intake and that predicted from mean observed requirements for growth and maintenance). With increasing total feeding time,

the maintenance requirements also increased suggesting that slow eaters have higher maintenance costs. **Chapter 6** tested whether individual differences in feeding behaviour explained the differences in growth rate by affecting feed efficiency, using restrictively fed fish. This study showed that despite the low variation in initial body weight (6.5 %) and in cumulative feed consumption (7.5 %) over the experimental period, catfish exhibited high variation in final body weight (18.1 %), specific growth rate (17.2 %) and feed conversion ratio (27.9 %), suggesting that individual variation in growth/feed efficiency is important in determining growth. This individual variation may be related to individual differences in protein/fat deposition since faster growing fish deposited more protein and less fat than slower growing fish. Pronounced individual differences in feeding behaviour (reaction towards feed and time spent eating) were also observed and correlated to individual differences in growth/feed efficiency. Fast eaters were the fast growers.

Chapter 7 presented two experiments to investigate individual differences in basal and post-stress levels of glucose, lactate and cortisol and their relation to individual differences in feed efficiency. There was a pronounced individual variation in both basal and post-stress levels of plasma glucose, lactate and cortisol. Basal levels of glucose, lactate and cortisol did not contribute significantly to explain differences in feed efficiency. However, glucose levels obtained after a stress test (netting) could explain differences in feed efficiency by 1.3 % in experiment 1 and 5.9 % in experiment 2. In experiment 2, the cortisol levels obtained after the stress test also explained part of the differences in feed efficiency (8.7 %). Apparently, high stress responders are less efficient fish. The stress response probably adds to differences in maintenance costs, thereby affecting the feed efficiency.

The findings of this thesis are discussed and the main conclusions are presented in **Chapter 8**. The importance of social hierarchy in explaining individual growth variation should be considered species-dependent. In addition, social hierarchy should not be accepted a priori as the major cause of individual growth variation without previous investigation. The results of this thesis suggested that in African catfish the individual variation in growth is not the result of marked dominance-subordinance relationships. Instead, genetic-based differences in feed intake, feed efficiency, feeding behaviour and stress response seem to play a role in explaining growth variation in African catfish. In practical terms, one may question the use of grading in this species as grading is done under the assumption that it disrupts an existing social hierarchy. Furthermore, the results of this thesis called for the development of selection programmes in African catfish. Selecting for feed efficiency (residual feed intake) is a

promising direction to pursue. The most efficient fish (low residual feed intake) were shown to be fast eaters and low stress responders which may be advantageous under aquaculture conditions.

It was also concluded that individual differences in feed intake and feed efficiency (residual feed intake) contributed ~85 and ~15 %, respectively, to the individual differences in the growth of African catfish. Individual differences in feeding behaviour (total feeding time) and stress response (plasma glucose and cortisol after an acute stress) contributed to explain variation in residual feed intake (maintenance requirements) up to 8.7 %.

Despite the results obtained in this thesis, our understanding of the causes of growth variation in African catfish is far from being complete. The challenge is to find the mechanisms responsible for this variation and how they are related to the individual differences in behaviour and stress response found in this thesis.

SAMENVATTING

Onder landbouwhuisdieren vertonen vissen de grootste individuele variatie in groei. Niettemin houden de meeste studies die rapporteren over groei geen rekening met individuele variatie. Doorgaans wordt een gemiddelde waarde genomen en ondanks dat de variatie rondom het gemiddelde vermeld wordt, wordt het doorgaans gezien als een soort van "statistische ruis". Het belang van individuele variatie in groei moet niet onderschat worden, omdat het belangrijke consequenties heeft voor waterkwaliteit, agressie, stress niveaus, bedrijfsmanagement, selectie programma's, etc. Onder de verantwoordelijke factoren voor variatie in groei wordt sociale hiërarchie vaak beschouwd als de meest belangrijke. Sociale hiërarchieën kunnen stress en remming van gedrag veroorzaken in onderdanige vissen, wat de voeropname en voerefficiëntie beïnvloedt en als gevolg daarvan de groei reduceert. Echter, voor de meeste vissoorten is nog geen eenduidig bewijs dat individuele verschillen in voeropname, voerefficiëntie en groei het resultaat zijn van sociale hiërarchieën. Enkele studies suggereren dat ook aangeboren (genetische) factoren variatie in groei kunnen veroorzaken.

Het algemene doel van deze studie was de onderliggende factoren die verantwoordelijk zijn voor individuele variatie in groei van gekweekte Afrikaanse meerval *Clarias gariepinus* te begrijpen. De volgende factoren werden bestudeerd: 1) of individuele variatie in groei hoofdzakelijk een gevolg is van sociale hiërarchieën, 2) de bijdrage van individuele verschillen in voeropname en voerefficiëntie op individuele verschillen in groei en 3) de bijdrage van eetgedrag en stress respons in het verklaren van individuele verschillen in voerefficiëntie .

Hoofdstuk 2 en 3 bestudeerden het belang van sociale hiërarchieën als een verklarende factor voor de individuele variatie in groei van Afrikaanse meerval. De groeiprestatie, het gedrag (eetgedrag, agressie niveaus) en de stress respons tussen groepen vissen met laag, gemiddeld en hoog gewicht werden vergeleken. **Hoofdstuk 2** toonde dat vissen met een laag gewicht geen toegenomen groeisnelheden vertoonden in afwezigheid van zwaardere vissen. Blijkbaar werden de verschillen in groei niet veroorzaakt door sociale hiërarchieën waar grotere vissen de groei van kleinere vissen onderdrukken. Deze studie suggereert juist dat eetgedrag een cruciale factor is. Zwaardere vissen vertonen een eetgedrag dat voordelig kan zijn wanneer de hoeveelheid voer beperkt is, zoals actiever zwemgedrag, meer tijd besteden op de voerplaatsen en het sneller opeten van de maaltijd dan lichte vissen.

Deze verschillen in gedrag kunnen resulteren in groeivariatie, zoals is aangetoond in deze studie.

In **Hoofdstuk 3** werd getoond dat agressie en stress niveaus niet toenamen in heterogene (gewichts) groepen in vergelijking met homogene (gewichts) groepen. Verder vertoonden lichte vissen die gehuisvest werden in heterogene groepen geen hoger aantal littekens of wonden op de huid of hogere stress niveaus in vergelijking met lichte vissen die gehuisvest werden in homogene groepen. Deze resultaten suggereren dat lichte vissen zich niet onderdanig en zware vissen zich niet dominant gedroegen.

Om het belang van aangeboren verschillen in groeivariatie verder te onderzoeken werd een serie experimenten ontworpen waarin gebruik werd gemaakt van individueel gehuisveste vissen. Het individueel huisvesten van vissen maakte het mogelijk om individuele verschillen in afwezigheid van sociale interacties te bestuderen en om nauwkeurig de individuele voeropname te meten. Dit wierp de vraag op of de resultaten die behaald werden met individueel gehuisveste dieren representatief zouden zijn voor groepshuisvesting. In **Hoofdstuk 4** werden de groeiprestatie, het eetgedrag en de stress respons van geïsoleerde en niet-geïsoleerde vissen vergeleken. Deze studie suggereerde dat bij Afrikaanse meerval de voeropname wordt gestimuleerd door de aanwezigheid van soortgenoten, wat resulteert in een hogere voeropname en groeisnelheid. Echter, isolatie zelf lijkt niet op te treden als stressor op korte termijn of de stress respons te beïnvloeden, waarschijnlijk omdat perioden van isolatie onderdeel zijn van het leven van de Afrikaanse meerval. In aanvulling werd in **Hoofdstuk 5** de groei vergeleken van vissen die eerst individueel gehuisvest werden en daarna in een groep gehuisvest werden. De gemiddelde groei van individueel gehuisveste vissen was lager dan die van vissen gehuisvest in groepen. Echter, snel en langzaam groeiende vissen tijdens individuele huisvesting bleven snel en langzaam groeiende vissen tijdens groepshuisvesting. Dit suggereert dat verschillen in groeisnelheid van vissen tijdens individuele huisvesting een kenmerk zijn van het dier zelf en niet een gevolg van isolatie.

In **Hoofdstuk 5 tot 7** werden individueel gehuisveste dieren gebruikt om experimentele data te leveren over aangeboren factoren die verantwoordelijk zijn voor individuele groeivariatie. In **Hoofdstuk 5** werden individuele verschillen in groeikenmerken en eetgedrag gekarakteriseerd en werd geconcentreerd op de herhaalbaarheid van dergelijke individuele verschillen wanneer vissen ad libitum werden gevoerd. Vissen vertoonden sterke individuele variatie in groei (CV = 52.8 %), in voeropname (34.3 %) en in totale tijd van voeropname (>100 %). De geschatte herhaalbaarheden waren 0.55 voor groei, 0.70 voor

voeropname, 0.49 voor residuele voeropname en 0.81 voor totale tijd van voeropname. De hoge geschatte herhaalbaarheden suggereren dat individuele verschillen in groei, voeropname/voerefficiëntie en eetgedrag consistent in de tijd zijn en daardoor hoogstwaarschijnlijk aangeboren. Individuele verschillen in groei werden voornamelijk verklaard door individuele verschillen in voeropname (~ 85 %). Individuele verschillen in eetgedrag waren aantoonbaar gerelateerd aan voerefficiëntie, gemeten als residuele voeropname (i.e., het verschil tussen de daadwerkelijke voeropname en de voorspelde vooropname op basis van de gemiddelde geobserveerde benodigdheden voor groei en onderhoud). Bij toenemende totale tijd van voeropname namen de behoeften voor onderhoud ook toe, wat suggereert dat langzame eters hogere onderhoudskosten hebben. In **Hoofdstuk 6** werd met behulp van beperkt gevoerde vissen getest of individuele verschillen in eetgedrag ook de verschillen in groeisnelheid kunnen verklaren door verschillen in voerefficiëntie. Deze studie toonde dat ondanks de lage variatie in initieel lichaamsgewicht (6.5 %) en in cumulatieve voerconsumptie (7.5 %) tijdens de experimentele periode, meerval een hoge variatie toonde in uiteindelijk lichaamsgewicht (18.1 %), specifieke groeisnelheid (17.2 %) en voedsel conversie ratio (27.9%), wat suggereert dat individuele variatie in groei/voerefficiëntie belangrijk is om groei te bepalen. Deze individuele variatie kan gerelateerd zijn aan individuele verschillen in eiwit/vet opslag, omdat sneller groeiende vissen, meer eiwit en minder vet opslaan dan langzaam groeiende vissen. Sterke individuele verschillen in eetgedrag (reactie t.o.v. het voer en tijd besteed aan eten) werden ook waargenomen en gecorreleerd aan individuele verschillen in groei/voerefficiëntie. Snelle eters waren de snelle groeiers.

In **Hoofdstuk 7** werden 2 experimenten gepresenteerd om individuele verschillen in basale en post-stress waarden van plasma glucose, lactaat en cortisol te onderzoeken en de relatie met individuele verschillen in voerefficiëntie te bepalen. Er was een sterke individuele variatie in zowel basale als post-stress niveaus van plasma glucose, lactaat en cortisol. Basale niveaus van glucose, lactaat en cortisol droegen niet significant bij aan het verklaren van verschillen in voerefficiëntie. Echter, glucose niveaus na een stress test (“netting”) verklaarden verschillen in voerefficiëntie met 1.3 % in experiment 1 en met 5.9 % in experiment 2. In experiment 2 verklaarden de cortisol niveaus behaald na de stress test ook een deel van de verschillen in voerefficiëntie (8.7 %). Blijkbaar zijn sterk reagerende dieren minder efficiënte dieren. Waarschijnlijk draagt de stress response bij aan verschillen in onderhoudskosten en beïnvloed daarmee de voerefficiëntie.

In **Hoofdstuk 8** werden de bevindingen van dit proefschrift bediscussieerd en werden de belangrijkste conclusies gepresenteerd. Allereerst moet het belang van sociale hiërarchie om individuele variatie in groei te verklaren beschouwd worden als soortafhankelijk. Verder moet sociale hiërarchie niet zonder voorafgaand onderzoek a priori geaccepteerd worden als belangrijkste oorzaak van individuele variatie in groei. De resultaten van dit proefschrift suggereren dat bij Afrikaanse meerval de individuele variatie in groei niet het resultaat is van duidelijke dominatie-ondergeschiktheid verhoudingen. In plaats daarvan spelen genetisch bepaalde verschillen in voeropname, voerefficiëntie, eetgedrag en stress respons een rol in het verklaren van variatie in groei van Afrikaanse meerval. Praktisch gezien kan men zich afvragen wat het belang is van het sorteren van deze vissoort omdat dit sorteren gebeurt in de veronderstelling dat dit de bestaande sociale hiërarchie verstoort. Verder roepen de resultaten van dit proefschrift op tot het ontwikkelen van selectie programma's voor Afrikaanse meerval. Selecteren voor voerefficiëntie (residuele voeropname) is een veelbelovende richting om te volgen. De meest efficiënte vissen (lage residuele voeropname) bleken de snelste eters en hadden een lage stress respons.

Verder werd ook geconcludeerd dat individuele verschillen in voeropname en voerefficiëntie (residuele voeropname) respectievelijk ~ 85 and ~15 % bijdroegen aan het verklaren van de individuele verschillen in groei van Afrikaanse meerval. Individuele verschillen in eetgedrag (totale voer tijd) en stress respons (plasma glucose en cortisol na een acute stress test) droegen bij aan het tot 8.7 % verklaren van de variatie in residuele voeropname.

Ondanks de resultaten behaald in dit proefschrift is het inzicht in de oorzaken van groeivariatie in Afrikaanse meerval verre van volledig. De uitdaging is het vinden van de mechanismen die verantwoordelijk zijn voor deze variatie en bepalen hoe deze gerelateerd zijn aan de individuele verschillen in gedrag en stress respons zoals die in dit proefschrift gevonden zijn.

RESUMO

Os peixes são, entre os animais de produção, aqueles que exibem a maior variação individual de crescimento. No entanto, a maior parte dos estudos que apresentam dados de crescimento não consideram a sua variação individual como importante. Geralmente, nestes estudos, um valor médio e a variação em relação à média são fornecidos, sendo esta última frequentemente considerada como um "ruído estatístico". A importância da variação individual de crescimento não deve ser subestimada uma vez que a sua existência pode influenciar a qualidade da água, os níveis de agressão, *stress* e canibalismo, assim como o desenvolvimento de programas de selecção. A formação de hierarquias sociais é geralmente considerado o principal factor responsável pela variação individual de crescimento em peixes. A hierarquia social pode induzir uma inibição de certos comportamentos assim como elevar os níveis de *stress* nos peixes subordinados. Nestes peixes, não só o consumo de alimento, mas também a eficiência com que o alimento é utilizado e, consequentemente o seu crescimento final, pode ser afectado. No entanto, para a maioria das espécies ainda não se sabe se a variação individual no consumo de alimento e eficiência com que o alimento é utilizado são uma consequência de hierarquias sociais. Alguns estudos sugerem que factores genéticos também podem ser a causa da variação individual de crescimento.

O objectivo principal deste estudo foi compreender os factores responsáveis pela variação individual de crescimento no peixe gato Africano *Clarias gariepinus*. Mais especificamente, esta tese investigou: 1) se a variação individual de crescimento é uma consequência de hierarquias sociais; 2) a contribuição de diferenças individuais no consumo de alimento e na eficiência com que o alimento é utilizado para a variação individual de crescimento; 3) a contribuição do comportamento de alimentação e resposta ao *stress* para a variação individual na eficiência de utilização do alimento.

Os **Capítulos 2 e 3** investigaram a importância de hierarquias sociais como um factor responsável pela variação individual de crescimento no peixe gato Africano. O crescimento, comportamento (comportamento de alimentação e níveis de agressão) e resposta ao *stress* foram comparados entre grupos de peixe com peso baixo, médio e alto.

O **Capítulo 2** demonstrou que os peixes de peso baixo não exibem um aumento da taxa de crescimento na ausência dos peixes de peso alto. Aparentemente, as diferenças de crescimento não foram induzidas pela existência de hierarquias sociais em que os peixes maiores inibem o crescimento dos peixes mais pequenos. Este estudo sugere que o

comportamento de alimentação é um factor importante. Os peixes de peso alto exibiram comportamentos de alimentação que lhes podem ser vantajosos quando o alimento é limitado, como por exemplo, nadarem mais activamente, despenderem mais tempo na área de alimentação e consumirem o alimento mais rapidamento do que os peixes de peso baixo. Estas diferenças de comportamento podem conduzir a variações individuais de crescimento.

O **Capítulo 3** evidenciou que os níveis de agressão e *stress* não aumentaram nos grupos heterogéneos (em termos de peso) comparativamente a grupos homogéneos. Além disso, os peixes de peso baixo não exibiram um maior número de lesões na pele, nem níveis superiores de *stress* quando foram cultivados em grupos heterogéneos comparativamente aos peixes também de peso baixo mas cultivados em grupos homogéneos. Estes resultados sugerem que os peixes de peso baixo não se comportaram como subordinados, nem os peixes de peso elevado como dominantes.

A investigação sobre a importância de factores genéticos para a variação individual de crescimento foi aprofundada com o uso de peixes mantidos individualmente (isolados). O recurso a peixes mantidos individualmente possibilitou não só o estudo de diferenças individuais na ausência de interacções sociais mas também a medição precisa do consumo individual de alimento. No entanto, este procedimento experimental levantou a questão se os resultados obtidos a partir de peixes mantidos individualmente podem ser representativos de situações em que os peixes são mantidos em grupos. No **Capítulo 4**, o crescimento, o comportamento de alimentação e a resposta ao *stress* foram comparados entre peixes isolados e não-isolados. Este estudo sugeriu que no peixe gato Africano, o consumo de alimento é estimulado pela presença de outros indivíduos, resultando em taxas de crescimento mais elevadas. No entanto, o isolamento em si parece não actuar como um *stress* a curto prazo nem afectar a capacidade de resposta ao *stress,* provavelmente porque períodos de isolamento fazem parte do estilo de vida do peixe gato Africano. Por outro lado, no **Capítulo 5**, o crescimento de peixes mantidos individualmente e posteriormente, num grupo, foi comparado. O crescimento de peixes mantidos individualmente foi mais baixo do que o crescimento dos mesmos peiixes mantidos num grupo. Contudo, os peixes de crescimento rápido e lento, mantidos individualmente permaneceram rápidos e lentos quando foram mantidos num grupo. Estes resultados sugerem que as diferentes taxas de crescimento obtidas quando os peixes estão isolados são uma característica do indivíduo e não simplesmente uma consequência do isolamento.

Os **Capítulos 5 e 7** fornecem dados experimentais de factores genéticos, responsáveis pela variação individual de crescimento, usando peixes mantidos individualmente. As diferenças individuais em características de desempenho e comportamento de alimentação foram quantificadas no **Capítulo 5**. Neste capítulo também se estimou a repetibilidade dessas diferenças individuais quando os peixes são alimentados *ad libitum*. Os peixes exibiram uma elevada variação individual de crescimento (coeficiente de variação, CV = 52.8 %), no alimento consumido (34.3 %) e na duração total de alimentação (> 100 %). As estimativas de repetibilidade foram 0.55 para o crescimento, 0.70 para o alimento consumido, 0.49 para a eficiência de utilização do alimento e 0.81 para a duração total de alimentação. Estas estimativas de repetibilidade, sendo elevadas, sugerem que a variação individual de crescimento, alimento consumido, eficiência e comportamento de alimentação são consistentes ao longo do tempo e portanto, provavelmente de origem genética. A variação individual de crescimento foi explicada principalmente por diferenças individuais na quantidade de alimento consumido (~85 %). Diferenças individuais no comportamento de alimentação, foram relacionadas com eficiência de utilização do alimento, expressa como o alimento consumido residual (i.e., a diferença entre o alimento que realmente é consumido e aquele que é predito com base nas necessidades para o crescimento e manutenção). Com o aumento da duração de alimentação, as necessidades de manutenção também aumentaram, sugerindo que peixes que consomem o alimento mais lentamente possuem custos de manutenção mais elevados.

No **Capítulo 6,** usando peixes alimentados com restrição, foi investigado se as diferenças individuais no comportamento de alimentação podem explicar as diferenças nas taxas de crescimento através do seu efeito na eficiência de utilização do alimento. Este estudo demonstrou que, apesar da variação do peso inicial (6.5 %) e da quantidade total de alimento consumido (7.5 %) ao longo do período experimental serem reduzidas, o peixe gato Africano exibe uma variação elevada do peso final (18.1 %), da taxa especifica de crescimento (17.2 %) e da taxa de conversão alimentar (27.9 %), sugerindo que a variação da eficiência de utilização do alimento é importante para determinar o crescimento. Esta variação individual pode estar relacionada com variações individuais a nível da deposição de proteína/lípidos uma vez que os peixes que crescem mais rapidamente depositaram mais proteína e menos lípidos do que os peixes de crescimento lento. O comportamento de alimentação (duração total de alimentação) também exibiu uma elevada variação individual e uma correlação com a

variação individual de crescimento e eficiência de utilização do alimento. Os peixes que consumiram o alimento mais rapidamente foram os que cresceram também mais rapidamente.

O **Capítulo 7** apresenta duas experiências que investigam a variação individual dos valores basais e pós-*stress* de glucose, lactato e cortisol assim como a sua relação com a variação individual na eficiência de utilização do alimento. Verificou-se que a variação individual dos valores basais e pós-*stress* de glucose, lactato e cortisol é elevada. Os valores basais de glucose, lactato e cortisol não contribuiram significativamente para explicar a variação individual na eficiência de utilização do alimento. Pelo contrário, os valores de glucose obtidos após a aplicação de um *stress* (cada peixe foi mantido numa rede emersa durante 1 hora) explicaram 1.3 % da variação individual na eficiência de utilização do alimento na experiência 1 e 5.9 % na experiência 2. Nesta, os valores de cortisol obtidos após a aplicação do mesmo tipo de *stress* também explicou (8.7 %) da variação individual na eficiência de utilização do alimento. Aparentemente, os indivíduos que exibem uma resposta ao *stress* mais elevada são menos eficientes na utilização do alimento. Provavelmente, a resposta ao *stress* afecta a eficiência de utilização do alimento ao aumentar os custos de manutenção.

No **Capítulo 8**, os resultados obtidos nesta tese são discutidos e as principais conclusões apresentadas. A importância da hierarquia social para explicar a variação individual de crescimento é dependente de cada espécie. Adicionalmente, a hierarquia social não deve ser aceite *a priori* como a principal causa da variação individual de crescimento sem investigação prévia. Os resultados desta tese sugerem que no peixe gato Africano, a variação individual de crescimento não é o resultado de hierarquias sociais. Pelo contrário, diferenças com base genética na quantidade de alimento consumido, na eficiência de utilização do alimento, no comportamento de alimentação e na resposta ao *stress* parecem desempenhar um papel importante na explicação da variação individual de crescimento. Em termos práticos, estes resultados questionam a utilidade de se fazer triagens frequentes aquando da produção desta espécie, uma vez que este processo de selecção é feito de acordo com o pressuposto de que a hierarquia social é eliminada após os peixes serem separados por tamanho. Os resultados desta tese também estimulam o desenvolvimento de programas de selecção no peixe gato Africano. O estabelecimento de programas de selecção direccionados para a eficiência de utilização do alimento (alimento consumido residual) é um desafio para o futuro. Os peixes mais eficientes (alimento consumido residual baixo) demonstraram ser os peixes

que consomem o alimento mais rapidamente e que menos respondem a situações de *stress*, o que poderá ser vantajoso em condições de cultivo.

Esta tese também concluiu que a variação individual de alimento consumido e de eficiência de utilização do alimento (alimento consumido residual) contribuiram cerca de 85 e 15 %, respectivamente, para a variação individual de crescimento no peixe gato Africano. A variação individual no comportamento de alimentação (duração total de alimentação) e na resposta ao *stress* (glucose e cortisol obtidos após um *stress* agudo) explicaram a variação na eficiência de utilização do alimento (custos de manutenção) até 8.7 %.

Apesar dos resultados obtidos nesta tese, o conhecimento acerca dos factores subjacentes à variação individual de crescimento no peixe gato Africano ainda é limitado. O desafio para o futuro é identificar os mecanismos responsáveis por esta variação e como eles se relacionam com a variação individual de comportamento de alimentação e resposta ao *stress*, identificados nesta tese.

ACKNOWLEDGEMENTS

Remembering all the people I want to acknowledge makes me travel back during these 4 years and realise how special my time in Wageningen was. To all of you …my deepest gratitude!

First of all I would like to thank my promoter Johan Verreth for all his support, encouragement and enthusiasm about my work. Thank you for always believing! To my daily supervisor, Johan Schrama, thank you for your guidance throughout this work. Your energy and ability to process so much information is admirable! To both Johans I have to thank for teaching me the value of brainstorming! Our meetings were like participating in a rollercoaster of ideas.

To Luis Conceição I would like to express my gratitude for helping me writing the initial project and to be always available for scientific discussions by email. Your advices were always very useful. Also, thank you for reviewing my Portuguese in the *Resumo*!

I always believed that a great scientist should be not only a great researcher but also a great person as a human being. Jim Clegg you are the perfect example of such scientist. Although we have worked together for a short time I feel that your teachings and friendship will never end. Thank you for all the nice emails…they always made me laugh! Also my warmest thanks to Eileen Clegg, you made me feel like family! My regards to Bodega Bay, including the whales!

During these 4 years I was fortunate to share my office with other PhD students that were much more than colleagues, you were great friends! Thank you for all the nice discussions, tea breaks (or water breaks for me), advices and dinners. It was great fun to work with you: Pablo (thank you for all the help especially in the beginning), Paula (your willingness to help others is admirable. Your heart can embrace the entire world!) and Samad (thanks for all the jokes and for keeping asking if I would like some tea!).

I would like to thank the students who worked with me, during my PhD: Jeroen, Margaret, Lena and Bart. Thank you for the enthusiasm and hard work! I wish you all the best for your future careers.

To the Aquaculture and Fisheries group´s staff, my warmest thanks for all the support and nice chats. Thanks to Ep for his dedication on keeping my systems recirculating and for having always time to answer my questions. I also wish to express my thanks to Marcel for helping me with some existential statistical doubts.

To Pascal, thank you for the samenvatting!

To Jascha for being the most “latin-dutch” person I have ever met ;-). Dear Jascha, I will never forget your laughs in the corridor, thank you!

To the hatchery staff, Sietza, Aart, Wian, Sander and Truus: *bedankt voor het in leven houden van mijn vissen*! Aart and Carry, thank you for the horse riding and for teaching me a bit of the Dutch culture.

Menno, thank you for your excellent help during the planning and execution of the experiments! I am very grateful for your friendly way of working! *Bedankt!*

I am also grateful to Ronald and Tino for their assistance during the lab work. Thanks to Chris and Jeroen for the patience and quick answers to my computer challenges! Also, thanks to Helene, Lies and Netty for all the help with the administration procedures and for caring about the progress of my PhD.

The Aquaculture and Fisheries group is unique due to its high number of international PhDs. During these 4 years I was very lucky to meet people from all over the world. For all of you my deepest admiration for having the courage to follow your dreams: Patricia and Harrison (Kenya), Paula (Colombia), Pablo and Beatriz (Mexico), Niel (Ireland), Oliver (Germany), Titu and Ekram (Bangladesh), Samad and Mohamed-Ali (Iran), Yonas (Eritrea), An and Hanh (Vietnam), Ana and Rodrigo (Brazil). The memories of our international dinners are unforgettable!!

Friendship can be described as a "rainbow in a cloudy day". Well …in Wageningen there were many cloudy days and you, my friends, definitely brought them a lot of colour. Many thanks to: Bouda and Negar (*merci* for all the nice discussions about our cultures, for having you in Portugal, for sharing good and bad moments), Pablo, Maaike and Sissel (*gracias* for all the dinners, for coming to Portugal, for being always there!), Paula, Peter and Foxy (for the wonderful *ajiaco*, for taking care of the fish, for keeping me fit while running with Foxy!), Claudine and Sam (*obrigada* for sharing the wonderful Brazilian and English culture, for coming to Portugal and of course for the cheese breads!!!), Vagner (thank you for teaching me forró!), Elisa (for the nice time in Wageningen and Lavras), Kees (thanks for the introduction to the Haarweg´s life), Ainhoa (you can always count with my chocolate cakes!) and Raquel (your happiness is contagious!). To the Portuguese community in Wageningen a big *Obrigada*, in particular to Rui and Tania, Sónia and Filipe, João and Alexandre.

To my Haarweg *afdelingsgenoten*, Shanna, Josie, Lara, Ivar, Adam, Ben, Marieke and Ludo, thank you for all the dinners, excursions, and parties. It was a great introduction to the Dutch culture!

To the Portuguese *"Biológos"*: Tiago and Gabriela, Rui and Sara, Marisa, Zé Diogo and Parménides (are you sure?), Inês, Victor and Ana Maria, thank you for the field trips, Christmas dinners, new year parties (Açoteias forever!) and for keeping in touch!

I also want to express my warmest thanks to the family Abreu Santos, in particular to Fátima and Agostinho: *obrigada por sempre me terem recebido de portas abertas e por terem espaço no vosso coração para mais um filhote. Um enorme bem-haja ao avô Manuel e à avó Emília pelo vosso carinho e preocupação. À Orlanda, um beijinho muito especial pela ajuda no Português!*

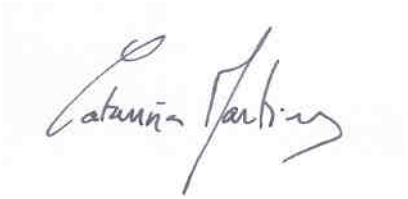
To my grandfather Joaquim Matos (in memoriam), my deepest admiration and gratitude for introducing me to me beauty of science.

I especially want to thank my grandfather (Vidaul, in memoriam), grandmother Maria do Carmo, uncle João and Zé for their understanding, support and tasty packages! *Vocês estiveram sempre presentes no meu coração, muito obrigada!*

To my sister and best friend, thank you for having always a smile and an encouragement word when I needed. *Obrigada minha linda! Pedrocas, vamos a mais uma viagem?*

My deepest gratitude goes to my Mother and Father, Isabel and Eduardo. Thank you for all your strength, love and for always supporting me in following my dreams! *Meus queridos, vocês são uns pais maravilhosos!*

Finally, my dearest Gonçalo. *Conseguimos*! Thank you SO MUCH for your passion for life, optimism and for always, always believing! *És a minha verdadeira fonte de inspiração. Obrigada!*



TRAINING AND SUPERVISON PLAN

Training and Supervision Plan **Graduate School WIAS**

Name PhD student	Catarina Isabel de Matos Martins	
Project title	Individual variation in growth of African catfish *Clarias gariepinus:* a search for explanatory factors	
Group	Aquaculture and Fisheries	
Daily supervisor(s)	Johan W. Schrama	
Supervisor(s)	Johan A. J. Verreth	
Project term	from March 2001	until March 2005
Submitted	30 March 2005	first plan / midterm / **certificate**

The Basic Package	year	cp*
. WIAS Common Course (mandatory)	2001	2,0
. Course on Philosophy of Science and Ethics (mandatory)	2001	1,0
Subtotal Basic Package		**3,0**

Scientific Exposure	year	Cp
International conferences *(minimum 2 cp)*		
. World Aquaculture 2003, Salvador, Brazil, May 19-23	2003	1,0
. 11th International Symposium on Nutrition and Feeding in Fish, Phuket, Thailand, May 2-7	2004	1,2
. Aquaculture Europe, Barcelona, Spain, October 20-23	2004	0,8
. Aquaculture Europe, Trondheim, Norway, August 5-9	2005	1,0
Seminars and workshops		
. WIAS Science day 2002, 2004, 2005, Wageningen, NL	2002-2005	0,6
. WIAS seminar plus "Fatty acids in fish", Wageningen, NL	2003	0,2
. WIAS seminar "Vitality in Fish", Wageningen, NL	2005	0,2
Presentations		
. Oral presentation, World Aquaculture 2003, Brazil	2003	0,5
. Oral and Poster presentation, 11th International Symposium on Nutrition and Feeding in Fish, Thailand	2004	1,0
. Poster presentation, Aquaculture Europe 2004, Spain	2004	0,5
. Poster presentation, Wias Science day 2004, NL	2004	0,5
. Oral presentation, Aquaculture Europe 2005, Norway	2005	0,5
Subtotal International Exposure		**8,0**

In-Depth Studies	year	Cp
. Using cannulation techniques in African catfish, Leiden, NL	2001	2,0
. Workshop on Fish Immunology, April 17-20, Wageningen, NL	2001	1,0
. Stable Isotopes in studies of nutrition dynamics: studies on stress and metabolic adaptation, Nov. 19-22, Wageningen, NL	2001	0,8
. Design and Operation of Recirculation Systems, December 3-7, Wageningen, NL	2001	1,0
. Advanced Statistics Course: Design of Animal Experiments, November 25-27, Wageningen, NL	2002	0,6
. Techniques for measuring heat shock proteins, University of California, Davis, USA	2002	1,0
. Workshop "Experimental Design and Methodologies for Nutritional Studies on Finfish and Crustaceans", Phuket, Thailand	2004	0,2
. Workshop "Challenges for Mediterranean Aquaculture" , Barcelona, Spain	2004	0,2
Subtotal In-Depth Studies		**6,8**

Professional Skills Support Courses	year	cp
. WIAS Course Techniques for Scientific Writing, July 3-6, Wageningen, The Netherlands	2001	0,8
. Use of Laboratory Animals, Wageningen, The Netherlands	2001	3,0
. Workshop on Business Plans, October 12, 13 and November 9, ICEP, Lisbon, Portugal	2001	0,8
. Laboratory Use of Isotopes, Wageningen, The Netherlands	2002	1,0
. Career Orientation, Wageningen, The Netherlands	2004	2,0
Subtotal Professional Skills Support Courses		**7,6**

Research Skills Training	year	cp
. Preparing own PhD research proposal	2001	4,0
Subtotal Research Skills Training		**4,0**

Didactic Skills Training	year	cp
Supervising practicals		
. Fish and fish production - Fish Health	2002/2003	0,5
. Aquaculture - Recirculation Systems in Aquaculture	2002/2003	0,4
. Aquaculture - Fish Behaviour	2003	0,2
Supervising MSc thesis		
. "The relationship between growth perfromance and swimming activity in African catfish", MSc thesis, Jeroen Schuphof	2003	1,0
. "The influence of social interaction in individual growth performance, feed efficiency and feeding motivation of African catfish, Erasmus thesis, Magdolna Trenovski	2004	1,0
. "The effect of within group variation in weight after grading on energy metabolism and feeding behaviour of African catfish", MSc thesis, Margaret Aanyu	2004	1,0
"The influence of feed efficiency on the agonistic motivation of African catfish", MSc thesis, Bart Hillen	2005	1,0
Subtotal Didactic Skills Training		**5,1**

Education and Training Total (minimum 21 cp, maximum 42 cp) **34,5**

*One credit point (cp) equals a studyload of approximately 40 hours

LIST OF PUBLICATIONS

Peer-Reviewed Papers

Martins, C. I. M., Schrama, J. W., Verreth, J. A. J., 2005. Inherent variation in growth efficiency of African catfish *Clarias gariepinus (*Burchell, 1822) juveniles. Aquac. Res. 36, 868-875.

Martins, C. I. M., Aanyu, M., Schrama, J. W., Verreth, J. A. J., 2005. Size distribution in African catfish (*Clarias* gariepinus) affects feeding behaviour but not growth. Aquaculture, *in press*

Martins, C. I. M., Schrama, J. W., Verreth, J. A. J., 2005. The effect of grading on the welfare of African catfish. Appl. Anim. Behav. Sci., *in press*

Martins, C. I. M., Trenovski, M., Schrama, J. W., Verreth, J. A. J., 2005. Comparison of feed intake behaviour and stress response in isolated and non-isolated African catfish. J. Fish Biol., *accepted*

Martins, C. I. M., Schrama, J. W., Verreth, J. A. J., 2005. The consistency of individual differences in growth, feed efficiency and feeding behaviour in African catfish *Clarias gariepinus* (Burchell, 1822) housed individually. Aquac. Res., *accepted*

Martins, C. I. M., Schrama, J. W., Verreth, J. A. J. The relationship between feed efficiency and stress response in African catfish *Clarias gariepinus, submitted*

Conference Proceedings

Martins, C. I. M., Schrama, J. W., Verreth, J. A. J., 2005. The effect of group composition on the welfare of African catfish *Clarias gariepinus*. Aquaculture Europe 2005. 5 – 8 August, Trondheim, Norway.

Martins, C. I. M., Aanyu, M., Schrama, J. W., Verreth, J. A. J., 2005. The effect of initial weight composition and heterogeneity on growth and behaviour of African catfish *Clarias gariepinus*. World Aquaculture 2005. 9-13 May, Bali, Indonesia.

Martins, C. I. M., Schrama, J. W., Verreth, J. A. J., 2004. The relationship between growth performance and the welfare status in African catfish. Aquaculture Europe 2004. 20-23 October, Barcelona, Spain.

Martins, C. I. M., Schrama, J. W., Verreth, J. A. J., 2004. The consistency of growth performance and feeding behaviour in African catfish housed individually and in a group. 11th International Symposium of Fish Nutrition and Feeding in Fish. 2-7 May, Phuket, Thailand.

Martins, C. I. M., Schrama, J. W., Trenovski, M., Verreth, J. A. J., 2004. The importance of social interaction on growth and feed efficiency in African catfish. 11th International Symposium on Nutrition and Feeding in Fish. 2-7 May, Phuket, Thailand.

Martins, C. I. M., Schrama, J. W., Verreth, J. A. J., 2003. Interindividual variation in feed utilization and growth rate of *Clarias gariepinus.* World Aquaculture 2003. 19-23 May, Salvador, Brasil.

Martins, C., Calado, R., Monteiro, M., Santos, O., Narciso, L., 2001. The two main problems in *Artemia* utilization: biometry and fatty acid profile. Larvi 2001. 3-6 September, Ghent, Belgium.

Calado, R., **Martins, C** ., Morais, S., Santos, O., Narciso, L., 2001. Larval development of the Mediterranean cleaner shrimp *Lismata seticaudata* (Risso, 1816) (Caridea, hippolytidae) based on different diets – costs and benefits of marketing molting. Larvi 2001. 3-6 September, Belgium.

Technical Magazines

Martins, C. I. M., Schrama, J. W., Verreth, J. A. J., 2003. Netherlands study links fish feeding behaviour with growth performance. Global Aquaculture Advocate. 6, 60-61.

Cover and chapter pages by Catarina Martins and Pablo Almazán-Rueda

Printing by Ponsen and Looijen BW, Wageningen, The Netherlands

This research was funded by Foundation for Science and Technology, Portugal