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Cultivation of African catfish (*Clarias gariepinus* Burchell, 1822) in Recirculating Aquaculture Systems (RAS) in Northern Germany

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Zusammenfassung

Die Produzenten von Afrikanischen Raubwelsen in Norddeutschland haben große Probleme ihre Betriebe gewinnbringend zu betreiben. Hohe Betriebskosten, geringe Verkaufspreise und suboptimale Produktionsbedingungen haben dazu geführt, dass Betriebe bereits geschlossen haben, geplant haben zu schließen oder damit kämpfen kostendeckend zu wirtschaften. Im Folgenden wird auf die Optimierungsmöglichkeiten der Produktionsbedingungen eingegangen. Hierfür wurden der Einfluss von Besatzdichten, Sortierungsstrategien bei Jungfischen, Wasserqualität, Futtermittel und die potenzielle Nutzung des Kreislaufwassers für die aquaponische Pflanzenproduktion auf den Produktionserfolg untersucht.

Zur Bestimmung des optimalen Besatzes wurden drei verschiedene Besatzdichten (finale Besatzdichten am Ende der Mastperiode: semi-intensiv = 100 kg m^{-3}, intensiv = 200 kg m^{-3} und super-intensiv = 400 kg m^{-3}) über eine gesamte Mastperiode (circa 12 – 1700 g) hinweg miteinander verglichen. Im Rahmen dieser Untersuchung wurden das Wachstum, die Mortalitäten sowie das Wohlbefinden der Fische berücksichtigt. Es zeigte sich, dass man Welse bis zu einem Durchschnittsgewicht von ca. 1300 g in super-intensiven Besatzdichten halten kann, ohne dass das Wachstum signifikant eingeschränkt ist. Ab 1300 g sollten die Fische hingegen in semi-intensiven Besatzdichten gehalten werden, um ein maximales Wachstum erreichen zu können.

In einem weiteren Versuch mit juvenilen Welsen (circa 0,1 – 0,3 g), wurden verschiedene Besatzdichten (10, 20 und 30 Fische L^{-1}) und Sortierregime (Intervalle: täglich, alle zwei Tage, nach fünf Tagen; Methoden: Handsortierung, Selbstsortierung) unter Berücksichtigung des Wachstums, Mortalitäten und Verhalten miteinander verglichen. Es zeigte sich, dass diese ohne Einschränkungen im Wachstum oder Verhalten mit 30 Fischen pro Liter gehalten werden können. Zusätzlich hat sich gezeigt, dass sich die durchschnittliche Mortalität von 25 % auf unter 5 % reduzieren lässt, wenn alle 5 Tage Kannibalen durch Sortierung entfernt werden.

Hinsichtlich der Wasserqualität sollte herausgefunden werden, ob es möglich ist, die pH-Anpassung in einer Wels-Kreislaufanlage an eine Kalium Aufdüngung (zum Beispiel mit Hilfe von KOH) des Kreislaufwassers zu koppeln, um es dadurch für eine aquaponische Nutzung aufwerten zu können. Dafür wurden vier Gruppen von Welsen verschiedenen Kalium Wasserkonzentrationen (0, 200, 400 und 600 mg L^{-1}) ausgesetzt, wobei das Wachstum, Wohlbefinden, die Körperzusammensetzung, und Mortalitäten als Bewertungsparameter

dienten. Die Ergebnisse haben gezeigt, dass die Zugabe von Kalium das Nährstoffprofil des Wassers in Hinblick auf eine gekoppelte Pflanzenproduktion verbessert und dass das Wohlbefinden der juvenilen Fische bei 200 – 400 mg L^{-1} Kalium gesteigert werden kann.

Bezogen auf das Futtermittel wurde untersucht, ob der regional abgebaute Futtermittelzusatzstoff Montmorillonite–illite/Muscovite (1g557) positive Auswirkungen auf das Wachstum, die Überlebensrate oder Wohlbefinden der Welse hat. Dabei hat sich gezeigt, dass diejenigen Fische, die den Zusatzstoff erhalten haben (0,5 %), signifikant weniger Bisswunden und etwas höhere Endgewichte als die Kontrollgruppe hatten.

Zur Beurteilung der Nutzung des Kreislaufwassers der Welse für die aquaponische Pflanzenproduktion wurde in einem entkoppelten System mit Ebbe-Flut-Tischen das Wachstum von Minze untersucht. Das Wachstum der Pflanzen wurde bei der Gabe von 1. kommerziellem Flüssigdünger, 2. intensivem Fisch-Kreislaufwasser und 3. extensivem Fisch-Kreislaufwasser miteinander verglichen. Es hat sich gezeigt, dass Minze in einem entkoppelten aquaponischen System ohne Zudüngung mit intensivem Fisch-Kreislaufwasser erfolgreich produziert werden kann ohne, dass das Wachstum der Fische eingeschränkt wird.

Zuletzt wurden im Rahmen der Diskussion die potenziellen Ausmaße verschiedener Produktionsbedingungen (Erstfutter, pH, Wassertemperatur, Futtermenge, Größensortierung, Besatzdichte, Ammonium- und Nitritkonzentrationen im Wasser) auf das Überleben und die Wachstumsrate der Afrikanischen Raubwelse modellhaft berechnet und bewertet. Es hat sich gezeigt, dass sich je nach Management der Ertrag um das 8-Fache unterscheiden kann. So können unter guten Bedingungen ausgehend von 300 000 Larven in 200 Tagen 295 Tonnen produziert werden und unter schlechten Bedingungen nur 33 Tonnen Fischbiomasse.

Die durchgeführten Untersuchungen haben gezeigt, dass verschiedene Besatzdichten, Sortierregime sowie Stoffe im Wasser und Futter, Einfluss auf das Wachstum, Verhalten und Wohlergehen von Afrikanischen Raubwelsen haben können. Zudem wurde bestätigt, dass es möglich ist, erfolgreich aquaponische Minze zu produzieren und damit eine zusätzliche Einkommensquelle zu generieren. Die Ergebnisse demonstrieren, wie facettenreich die Einflussmöglichkeiten auf die Produktion in einer Kreislaufanlage sind, aber auch wie essenziell eine kontinuierlich gute Fischbetreuung ist und wie gravierend die Auswirkungen beim nicht Einhalten verschiedener Grenzwerte sind.

In dieser Dissertation werden wichtige Einflussfaktoren auf den Erfolg der Produktion in Wels-Kreislaufanlagen untersucht und dementsprechend Empfehlungen ausgesprochen. Weiterhin wird die Relevanz einer einwandfreien Anlagenbetreuung modellhaft dargestellt und diskutiert.

Abstract

African catfish producers in Northern Germany have difficulty running their operations profitably. High running costs, low sales prices, and suboptimal production conditions have led to farms already closing, planning to close, or struggling to cover expenses. In the following, the optimization possibilities of the production conditions are discussed. For this purpose, the influence of stocking densities, grading strategies for juvenile fish, water quality, feed, and the potential use of process water for aquaponic plant production on production success was investigated.

Three different stocking densities (final stocking densities at the end of grow-out: semi-intensive = 100 kg m^{-3}, intensive = 200 kg m^{-3}, and super-intensive = 400 kg m^{-3}) were compared over an entire grow-out period (approximately 12 – 1700 g) to determine optimal stocking conditions. Thereby, growth, mortality, and welfare of the fish were considered. It was found that catfish up to an average weight of about 1300 g can be kept at super-intensive stocking densities without significant growth restrictions. Above 1300 g, the fish should be kept at semi-intensive stocking densities to achieve maximum growth.

In another experiment with juvenile catfish (approximately 0.1 – 0.3 g), different stocking densities (10, 20, and 30 fish L^{-1}) and grading regimes (intervals: daily, every two days, after five days; methods: manual grading, self-grading) were compared considering growth, mortality rates, and behavior. The results showed that the juveniles could be held at stocking densities of 30 fish L^{-1} without restrictions on growth or behavior. In addition, it has been shown that average mortality can be reduced from 25 % to less than 5 % if cannibals are removed by grading every five days.

Regarding water quality, the aim was to find out if it is possible to couple pH adjustment in a catfish recirculating aquaculture system with potassium fertilization (for example, using KOH) of the process water, thereby upgrading it for aquaponic use. For this purpose, four catfish groups were exposed to different potassium water concentrations (0, 200, 400, and 600 mg L^{-1}) while growth, welfare, body composition, and mortality rates were compared. The results showed that the addition of potassium, on the one hand, improved the nutrient profile of the water in terms of coupled plant production. On the other hand, the welfare of juvenile fish could be increased between 200 – 400 mg L^{-1} of potassium.

Concerning the feed, it was investigated whether the regionally mined feed additive Montmorillonite-illite/Muscovite (1g557) positively affects the growth, survival rate, or

welfare of catfish. The results showed that fish receiving the additive (0.5 %) had significantly fewer biting wounds and slightly higher final weights than the control group.

Mint growth was studied in a decoupled system with ebb-and-flood tables to evaluate the usability of catfish process water for aquaponic plant production. Plant growth was compared when 1. commercial liquid fertilizer, 2. process water from intensively cultured fish, and 3. process water from extensively cultured fish were applied. It was shown that mint could be successfully produced in a decoupled aquaponic system in process water from intensively cultured fish without supplemental fertilizer and without limiting fish growth.

Lastly, as part of the discussion, the possible extents of various production conditions (type of first feed, water pH, water temperature, amount of feed, grading of young fish, stocking density, ammonium and nitrite concentrations in the water) on the survival and growth rate of African catfish were calculated and evaluated as a model. It has been shown that yield can differ by a factor of 8 depending on management. Thus, starting from 300 000 larvae, 295 tons of fish biomass can be produced in 200 days under good conditions and only 33 tons under poor conditions.

The studies have shown that different stocking densities, grading regimes, and substances in the water and feed can influence the growth, behavior, and welfare of African catfish. In addition, it was confirmed that it is possible to successfully produce aquaponic mint, thus generating an additional source of income. The results demonstrate how multifaceted the possibilities for influencing production in a RAS are, how essential continuous good fish care is, and how severe the effects are when specific parameters are unmet.

This dissertation investigates important factors influencing production success in African catfish RAS and makes recommendations accordingly. Furthermore, the relevance of proper operational management is exemplified and discussed.

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List of Publications

To ensure uniform formatting throughout this dissertation, the studies listed below show some formal differences from the published versions in this thesis. No changes were made to the content.
Moreover, citations of one of the listed publications are marked in bold in the text.

Effects of Stocking Density on Larval and Adult Growth and Behavior

I. Baßmann, B., Hahn, L., Rebl, A., Wenzel, L. C., Hildebrand, M.-C., L., Verleih, M., Palm, H. W. (2023). Effects of Stocking Density, Size, and External Stress on Growth and Welfare of African Catfish (*Clarias gariepinus* Burchell, 1822) in a Commercial RAS. *Fishes, 8*(2), 74. https://doi.org/10.3390/fishes8020074

II. Wenzel, L. C., Berchtold, E., Palm, H. W. (2022). Effects of stocking density and grading on behavior, cannibalism, and performance of African catfish (*Clarias gariepinus*) fry. *Aquaculture Reports*, *27*, 101400. https://doi.org/10.1016/j.aqrep.2022.101400

Influence of Water Quality and Feed Additives on Growth Performance and Welfare

III. Wenzel, L. C., Strauch, S. M., Eding, E., Presas-Basalo, F. X., Wasenitz, B., Palm, H. W. (2021). Effects of Dissolved Potassium on Growth Performance, Body Composition, and Welfare of Juvenile African Catfish (*Clarias gariepinus*). *Fishes*, *6*(2), 11. https://doi.org/10.3390/fishes6020011

IV. Palm, H. W., Berchtold, E., Gille, B., Knaus, U., Wenzel, L. C., Baßmann, B. (2022) Growth and Welfare of African catfish (*Clarias gariepinus* Burchell, 1822) under Dietary Supplementation with Mixed-Layer Clay Mineral Montmorillonite–Illlite/Muscovite in Commercial Aquaculture. *Aquaculture Journal, 2*(3), 227-245. https://doi.org/10.3390/aquacj2030013

Growth and Performance of African catfish in Aquaponics Production Systems

V. Knaus, U., Wenzel, L. C., Appelbaum, S., Palm, H. W. (2020). Aquaponics (*s.l.*) Production of Spearmint (*Mentha spicata*) with African Catfish (*Clarias gariepinus*) in Northern Germany. *Sustainability, 12*(20), 8717. https://doi.org/10.3390/su12208717

Additional Publications

Strauch, S. M., Wenzel, L. C., Bischoff, A., Dellwig, O., Klein, J., Schüch, A., Wasenitz, B., Palm, H. W. (2018). Commercial African catfish (*Clarias gariepinus*) Recirculating Aquaculture Systems: Assessment of Element and Energy Pathways with Special Focus on the Phosphorus Cycle. *Sustainability, 10*(6), 1805. https://doi.org/10.3390/su10061805

Prüter, J., Strauch, S. M., Wenzel, L. C., Klysubun, W., Palm, H. W., Leinweber, P. (2020). Organic Matter Composition and Phosphorus Speciation of Solid Waste from an African Catfish Recirculating Aquaculture System. *Agriculture, 10*(10), 466. https://doi.org/10.3390/agriculture10100466

Contribution of the Authors to the Individual Publications

I. Effects of Stocking Density, Size, and External Stress on Growth and Welfare of African catfish (*Clarias gariepinus* Burchell, 1822) in a Commercial RAS.

Baßmann, B.: Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing - original draft preparation, writing - review & editing, visualization, project administration.

Hahn, L.: Investigation, data curation.

Rebl, A.: Formal analysis, investigation, data curation, writing - review & editing, visualization.

Wenzel, L. C.: Conceptualization, investigation, resources, writing - review & editing.

Hildebrand, M.-C.: Formal analysis, investigation, writing - review & editing.

Verleih, M.: Investigation, data curation, writing - review & editing.

Palm, H. W.: Writing - review & editing, supervision, funding acquisition.

II. Effects of stocking density and grading on behavior, cannibalism, and performance of African catfish (*Clarias gariepinus*) fry.

Wenzel L. C.: Conceptualization, methodology, validation, formal analysis, investigation, data curation, main writing, visualization, supervision.

Berchtold E.: Software, investigation, methodology, visualization.

Palm H. W.: Conceptualization, methodology, resources, writing, project administration, funding acquisition.

III. Effects of Dissolved Potassium on Growth Performance, Body Composition, and Welfare of Juvenile African Catfish (*Clarias gariepinus*).

Wenzel, L. C.: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing.

Strauch, S. M.: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing, supervision.

Eding, E.: Writing—review and editing, supervision.

Presas-Basalo, F. X.: Methodology, software, validation, formal analysis, investigation, resources, data curation, writing—review and editing.

Wasenitz, B.: Investigation, resources, data curation, writing—original draft preparation, writing—review and editing.

Palm, H. W.: Conceptualization, resources, writing—review and editing, supervision, funding acquisition.

IV. Growth and Welfare of African catfish (*Clarias gariepinus* Burchell, 1822) under Dietary Supplementation with Mixed Layer Clay Mineral Montmorillonite–Illlite/Muscovite in Commercial Aquaculture.

Palm, H. W.: Conceptualization, methodology, validation, writing–review and editing, supervision, funding acquisition.

Berchtold, E.: Validation, investigation, data curation.

Gille, B.: Investigation, data curation.

Knaus, U.: Conceptualization, validation, formal analysis, statistics.

Wenzel, L. C.: Validation, investigation, resources, data curation.

Baßmann, B.: Validation, investigation, resources, data curation, writing–original draft preparation, writing–review and editing, visualization, project administration.

V. Aquaponics (*s.l.*) Production of Spearmint (*Mentha spicata*) with African Catfish (*Clarias gariepinus*) in Northern Germany.

Knaus, U.: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing—original draft preparation, writing—review and editing, visualization, supervision, project administration, funding acquisition.

Wenzel, L. C.: Methodology, validation, formal analysis, data curation, writing—original draft preparation, writing—review and editing.

Appelbaum, S.: Conceptualization, validation, formal analysis, data curation, writing—original draft preparation, writing—review and editing, supervision.

Palm, H. W.: Conceptualization, validation, formal analysis, investigation, data curation, writing—original draft preparation, writing—review and editing, supervision, project administration, funding acquisition.

Additional publications

Commercial African catfish (*Clarias gariepinus*) recirculating aquaculture systems: Assessment of element and energy pathways with special focus on the phosphorus cycle.

Strauch, S. M.: Main writing, analyses of nutrient fluxes and budgets.

Wenzel, L. C.: Element analyses, nutrient calculations, main sampling, material and methods, figures.

Bischoff, A.: Nutrient cycles.

Dellwig, O.: Element analyses, material, and methods.

Klein, J.: Vermifiltration.

Schüch, A.: Energy content and fiber analyses, interpretation of results.

Wasenitz, B.: Fish quality analyses, interpretation of results.

Palm, H. W.: Planning of experiments, manuscript structure, and writing.

Organic Matter Composition and Phosphorus Speciation of Solid Waste from an African Catfish Recirculating Aquaculture System.

Prüter, J.: Conceptualization, methodology, validation, formal analysis, investigation, resources, writing—original draft preparation.

Strauch, S. M.: Methodology, resources, writing—review and editing.

Wenzel, L. C.: Methodology, resources, writing—review and editing.

Klysubun, W.: Methodology, validation.

Palm, H. W.: Validation, resources, writing—review and editing.

Leinweber, P.: Conceptualization, methodology, validation, resources, writing—review and editing, supervision, funding acquisition.

1 Introduction

Within 40 years, the world's population increased by 75 %, from 4.45 billion people in 1980 to almost 7.8 billion in 2020 (Statista, 2022d). At the same time, the world population is predicted to still increase to over 10 billion people by 2060 (Statista, 2019), which means a further increase of around 28 % in the next 40 years. One of the biggest problems is securing the human population's food supply while not overstretching the ecosystems (Josling, 2019).

1.1 Aquaculture Development

Among the food production sector, aquaculture is the world's fastest growing industry (FAO, 2018). The average worldwide per capita consumption of fish increased from 18.5 kg in 2010 to 19.8 kg in 2020 (Statista, 2022c), and even if the consumption dropped from 2019 to 2020 by 0.7 kg, the increase of 1.4 kg since 2010 shows the overall rising demand for fisheries products (Statista, 2022c). 174.6 million metric tons (MMT) of fish were produced worldwide in 2020, an increase of 26.5 MMT since 2010, while capture fishery increased by 1.4 and aquaculture by 25.1 MMT over this time (FAO, 2022c) (Figure 1.1).

As wild fish is limited and exposed to the risk of overfishing the natural populations, aquaculture is gaining more and more importance in order to be able to meet human demand for fish consumption. To clearly distinguish the term aquaculture, it was defined by FAO (1988): "*Aquaculture is the farming of aquatic organisms, including fish, mollusks, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated. For statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture, while aquatic organisms which are exploitable by the public as a common property resource, with or without appropriate licenses, are the harvest of fisheries.*"

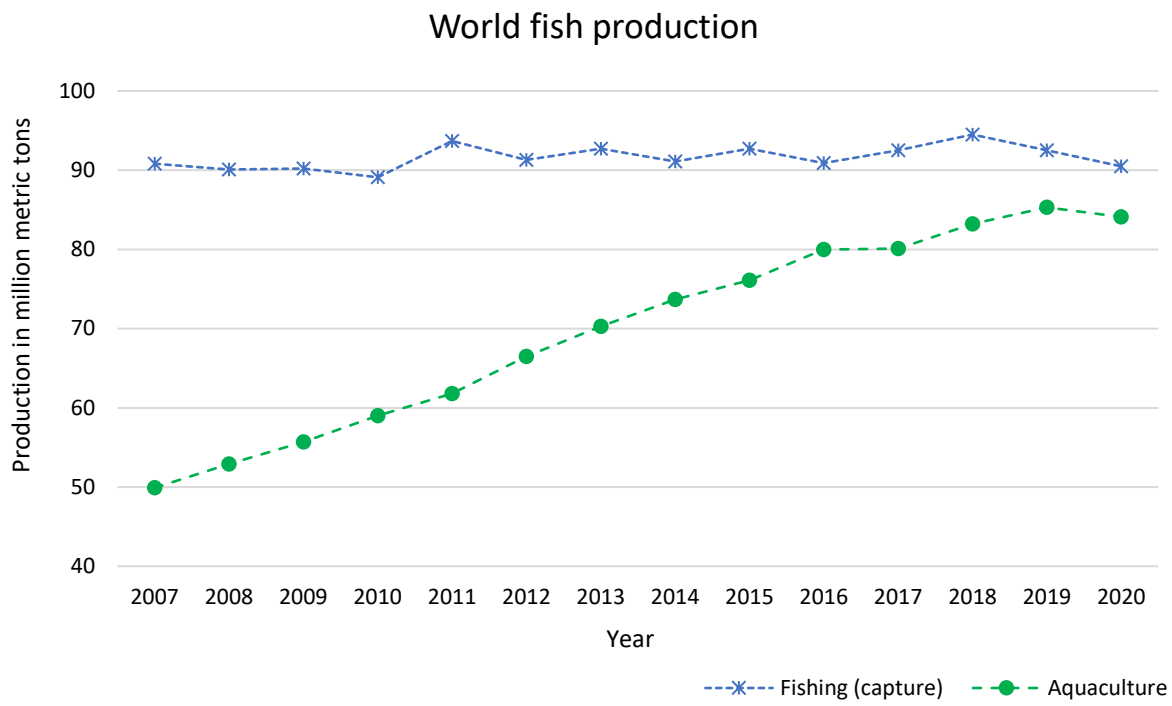

Figure 1.1: *World fish production from 2007 to 2020, divided into capture fisheries and aquaculture (FAO, 2022c)*

There are scenarios in which it is assumed that the demand for fish will increase by 36 – 74 % by 2050 (Naylor et al., 2021b). Since it has to be expected that the world population will also increase in the next decades as well as the per capita fish consumption, it can be assumed that the demand for fish products will also rise dramatically. Besides the production for nutrition (around 88 % in 2018), aquaculture organisms are also produced for non-food applications like the production of fish meal/oil, ornamental fish, cosmetics, pharmaceuticals, and biofuels (FAO, 2020; Leal et al., 2018). The main aquaculture species in 2020 were carps, barbels, and other cyprinids that accounted for almost 25 % of the world aquaculture production by live weight, followed by red and brown seaweed that accounted for almost 15 and 14 % (FAO, 2022b). Marine shrimps (and prawns), oysters, tilapia (including other cichlids), and catfishes accounted for about 5 % each, while clams, cockles, and ark shells as a group accounted for 4.7 %, and salmons, trouts, and smelts for 3.3 %. Thereby, China was by far the largest producer with 49.6 MMT, followed by India (8.6 MMT), Indonesia (5.2 MMT), Vietnam (4.6 MMT), Bangladesh (2.6 MMT), Egypt (1.6 MMT), and Norway (1.5 MMT) as the only European country in the worldwide top 10 (Statista, 2022a).

1.2 Closed Recirculating Aquaculture Systems

Fish farming in ponds is the oldest form of aquaculture and dates back to the Xia Dynasty in China (around 2000 BC) (Nash, 2011). Even today, fish production in earthen ponds is still the most commonly used production system for inland aquaculture (FAO, 2020). However, the highest commercial value is attributed to the salmonids mainly kept in net cage mariculture (FAO, 2020; Gardner Pinfold Consultants Inc., 2019). Both production systems release a high number of nutrients into the environment and can even cause environmental damage if the ecosystem is stressed beyond its natural carrying capacity (Truong et al., 2017). Therefore, the development of new aquaculture systems that limit the waste released into the environment while maximizing the output is required. Closed recirculation aquaculture systems (RAS) can produce highly valuable fish indoors under controlled conditions. It is one of the most promising production methods for the future, having complete control over wastewater treatment and disposal (Ahmed and Turchini, 2021). Closed RAS are indoor systems that enable intensive fish (or other aquatic organisms) production under controlled environmental conditions independent of the location (Ahmed and Turchini, 2021). At the simplest, it consists of a cultivation unit (fish tanks), a mechanical filtration unit to remove suspended solids, a unit for biological water treatment to remove ammonia/ammonium by nitrification, a device to ensure appropriate ventilation and one or more pumps to circulate the water (Bovendeur et al., 1987). Additional components for disinfection (Summerfelt et al., 2009), denitrification (Müller-Belecke et al., 2013), feeding (Helfrich and Libey, 1991), monitoring, and controlling (Fowler et al., 1994) are possible. They are often used in larger operations depending on the species and intensity of the production. The systems are not entirely closed as some waste products or accumulations have to be removed, and water evaporates and has to be substituted. Freshwater requirements in “innovative” RAS can be below 0.1 m^3 per kg feed (Martins et al., 2010). In RAS, it is possible to fully control the environmental conditions, including water quality, wastewater treatment and disposal, and pathogen control through appropriate hygiene measures (Nazar et al., 2013).

Mechanical and gravitational methods are the most popular used for solids removal in aquaculture (Cripps and Bergheim, 2000). The solid separation unit is usually placed directly behind the fish tanks to remove suspended solids as quickly as possible, preventing leaching and associated deterioration in water quality (Summerfelt and Vinci, 2008). Besides that, the removal is essential to avoid direct negative effects on the fish, like alterations of gill

morphology (Au et al., 2004; Hatem et al., 2013). Typical filters for bigger particles in aquaculture are sedimentation tanks, screen filters like drum or disc filters, granular filters like sand filters, or swirl separators (Steicke et al., 2009). For smaller particles, foam fractionation, ozonation, or membrane filtration is applied (Steicke et al., 2009). When the solids are removed, sludge thickening as a further wastewater treatment step can be done, for example, by using settling basins, geotextile bags, belt filters, or membrane reactors (Van Rijn, 2013).

The biological treatment unit needs aerobic conditions and a large surface area for the nitrifying bacteria to colonize. The bacteria convert the fish-damaging ammonium to nitrite and further to the less harmful nitrate (Steicke et al., 2009). Big surface areas are created by different kinds of biocarriers, usually made of plastic in commercial-scale systems (Dezotti et al., 2018). However, porous natural materials can also be used (Lekang and Kleppe, 2000).

There are many different filter types; three well-known types are trickling filters (Steicke et al., 2009), rotating biological contactors, and moving-bed biofilm reactors (Xiao et al., 2019). In trickling filters, the water is distributed at the upper end of the filter and drips down over the filter material, while the ammonium is broken down by the colonizing bacteria (Crab et al., 2007; Steicke et al., 2009). The advance of trickling is that it is simple in design and functions as degassing and oxygenation columns. A disadvantage is that the active surface area is rather low, so the filter must be relatively big. Rotating biological contactors consist of discs or a drum to increase the surface area (Crab et al., 2007; Steicke et al., 2009). The discs/drum rotates slowly, and approximately 40 % submerges in the water. In a RAS, the process water flows through a tank containing the filter, and the rotation of the discs/drum ensures the contact of the bacteria with the ammonium-rich water and with atmospheric oxygen ensuring nitrification by the bacteria. A moving-bed biofilm reactor consists of a separate container filled with single loose biocarriers that are constantly moved (hydraulically or mechanically) to prevent clogging and building of anaerobic zones. In this filter type, the water has to be aerated (often combined with the movement of biocarriers) to ensure aerobic conditions for the nitrifying bacteria (Dezotti et al., 2018). A denitrification unit is another possible biological water treatment step (Hamlin et al., 2008). It can remove nitrate by converting it into nitrogen gas under anoxic conditions (Knowles, 1982). Moreover, a carbon source is needed, like methanol, acetic acid, molasses, cereloseTM (Hamlin et al., 2008), ethanol, or even fish organic waste (Letelier-Gordo et al., 2020). This treatment step can, for example, be

performed in a moving bed biofilm reactor under the exclusion of oxygen and the possibility of degassing the nitrogen (Chu and Wang, 2011).

The pump sump can contain one or several pumps based on the system design and the need for pumps. Depending on the cultivated species, additional oxygen is introduced by aeration or technical oxygen by, for example, a u-tube (Wood, 1991), a counter-current flow injector, or a micro-bubble device (Helfrich and Libey, 1991). Degassing carbon dioxide is as crucial as introducing enough oxygen (Mota et al., 2019). That can be done, for example, by submerged aerators, paddle wheels (Eshchar et al., 2003), or trickling filters (Karimi et al., 2020). UV-C lights (Lakeh et al., 2013) or ozone (Gonçalves and Gagnon, 2011) are often used to disinfect the process water and reduce the pathogen contamination in the water. Depending on the species and their needs, additional technical components such as sensors and controllers for temperature, pH, dissolved oxygen, water flow rate, water level, and feed can be installed to reach almost entirely automated fish management (Fowler et al., 1994).

In short, the pumps provide a continuous water flow to circulate the water from the fish tanks to the various filters and water treatment units and back again. The flow rate ensures that the water in each fish tank is exchanged about 1.5 – 6 times per hour (Obirikorang et al., 2019; Sun et al., 2016). Common RASs can have very different daily water exchange rates lying between 30 and 300 L per kg feed and day, depending on the species and system components (Martins et al., 2009). Nowadays, even sensible species such as pikeperch (*Sander lucioperca*) can be kept in RAS; however, requiring a certain amount of investment into the RAS (Steinberg et al., 2018). On the other hand, also highly tolerant fish species, such as the African catfish, can be commercially produced in RAS (Pasch and Palm, 2021).

1.3 African catfish (*Clarias gariepinus* Burchell, 1822)

The African catfish (*Clarias gariepinus* Burchell, 1822), a promising species for RAS production, is native to Lebanon, Israel, Jordan, Turkey, and most parts of Africa except for Maghreb, Upper and Lower Guinea, and the Cape provinces (FAO, 2022a). It was found in flood zones, creeks, rivers, and marshes (FAO, 2022a). Over time it has also been introduced to several countries in South America, Asia, India, and Europe (FAO, 2022a).

1.3.1 Biology and Ecology

The African catfish is an air-breathing catfish and belongs taxonomically to the class Teleostei, the order Siluriformes, and the family Clariidae (Van der Laan et al., 1998). It has an

elongated body without scales, a long anal and dorsal fin, and can vary in coloration from black to light brown and grey, while its belly side is always lighter than the darker back side that can also be marbled (Gunder, 2004; Iswanto et al., 2015). The head is strong and bony with a wide mouth, four pairs of barbels, and small lateral eyes. Its brain is embedded in fatty tissue, surrounded by strong bones (Hörnig, 2017), which makes it very difficult to stun larger specimens (Figure 1.2). It is an omnivorous warm water fish that eats zooplankton, insects, worms, mollusks, other fish, plants, detritus, birds, and small mammals (FAO, 2022a; Tesfahun, 2018). Larvae eat zooplankton during the first week of feeding, and it takes five days until the digestive system has fully developed (Verreth et al., 1992). After the first week, they start eating insects and later eat the food mentioned above (Holl, 1968). They are highly cannibalistic, especially in early life stages, which is a challenge in fingerling production as it can lead to extremely high mortality rates (Abdelhamid et al., 2010). Therefore, young catfishes are usually graded regularly to remove shooters and potential cannibals (Abdelhamid et al., 2010).

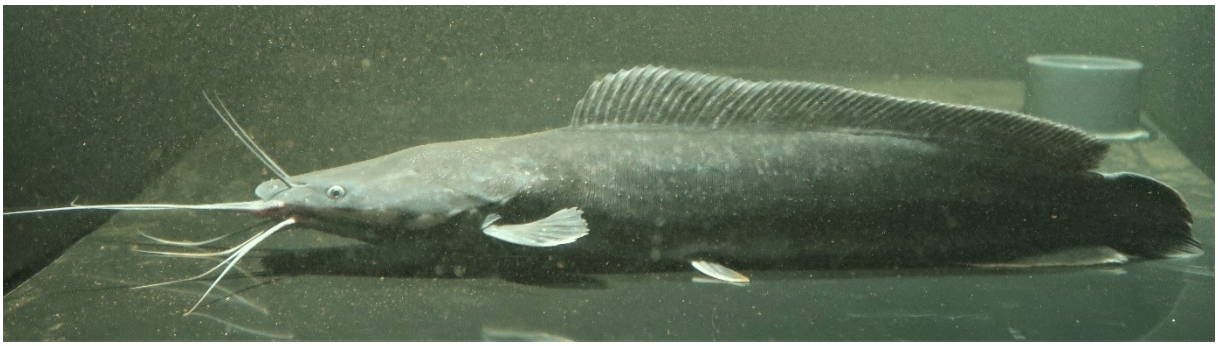

Figure 1.2: *African catfish (Clarias gariepinus)* (Hildebrand, 2022).

It has excellent growth potential, while the maximum documented weight is 60 kg, and the maximum reported age is 15 years (Froese and Pauly, 2022). Growth can strongly vary and is influenced by feed, illumination (Appelbaum and Mc Geer, 1998), feeding frequency (Aderolu et al., 2010), water temperature (Verreth and Bieman, 1987), stocking density (Wei et al., 2011), and genetic predisposition (Martins et al., 2005b). Depending on age and culture conditions, specific growth rates (SGRs) in aquaculture can strongly vary, ranging from 85 % of body weight d^{-1} in fry starting exogenous feeding (Hogendoorn, 1980) to 0.92 % for fish between 500 and 860 g (Pasch, 2022). Under ideal conditions, they can reach 1.3 – 1.5 kg within 140 – 150 days (Baßmann et al., 2023; EUROFISH Magazine, 2019).

Under natural conditions, African catfish usually reach sexual maturation after one year (FAO, 2022a). The annual reproduction naturally occurs during the rainy season when not only the water rises but also the temperature and the photoperiod, which influence gonadal maturation (Olaleye, 2005). Under aquaculture conditions, the fish usually reach sexual maturity after 6 – 9 months, while the final egg maturation is missing due to the absence of the appropriate environmental stimuli (Olaleye, 2005). Reaching spontaneous spawning under captivity is only possible by imitating the corresponding environmental conditions (El Naggar et al., 2006). However, this does not allow a reliable production of fingerlings for aquaculture purposes since only 5 – 11 fingerlings m^{-2} can be produced by this method (Christensen, 1981). Therefore, at a commercial scale, different hormones such as carp pituitary extract, human chorionic gonadotropin, luteinizing hormone-releasing hormone analogs, and gonadotropin-releasing hormone analogs are injected to reach final maturation (El-Hawarry et al., 2016). Sadly, the males cannot be stripped, and even if it is possible to obtain sperm surgically (Adebayo et al., 2012; Majhi et al., 2020) or by puncture (Tkacheva et al., 2020), in commercial production, the males are usually sacrificed, as the other two methods are very complex and in the case of puncture only 66 % successful.

Considering environmental conditions, the African catfish is tolerant towards high temperatures (Klyszejko et al., 1993) and pH (Palm et al., 2018b) differences as well as high concentrations of ammonia (Schram et al., 2010), nitrite (Roques et al., 2015), nitrate (Schram et al., 2014), and phosphate (Strauch et al., 2019). Moreover, it has a paired arborescent suprabranchial chamber in the gill cavity, forming an accessory air-breathing organ that makes it possible to breathe atmospheric oxygen as a facultative air breather under hypoxic conditions (Belão et al., 2011; Teixeira et al., 2015). Air-breathing commences before the accessory air-breathing organ starts to develop, while both are temperature dependent and start 2 – 4 weeks (at 25 – 35 °C) after the first feeding (Haylor and Oyegunwa, 1993). Due to its air-breathing organ, it can survive at very low oxygen concentrations and even months in parched areas if it is kept moist (Păpuc et al., 2019). During the dry periods in these arid areas, the African catfish can sit tightly packed together with its conspecifics at high densities waiting for the next rain. In the wild, this helps it to survive under adverse conditions with low oxygen content (EUROFISH Magazine, 2019). In aquaculture, this enables intensive production under very high stocking densities of up to 500 kg m^{-3} (Van de Nieuwegiessen et al., 2009). However, even if they can survive in such harsh conditions, the influences on mortality (Kaiser et al.,

1995b), growth performance (Wei et al., 2011), and welfare (Van de Nieuwegiessen et al., 2008) have to be considered in determining a suitable stocking density for each life stage in aquaculture fish production.

1.3.2 Welfare and Behavior under Aquaculture Conditions

In animal production, in general, besides ethical reasons, welfare is also important because it can influence product quality (Blokhuis et al., 2008; Poli et al., 2005). According to the terrestrial code (Ch. 7.1, Art. 7.1.1), "*Animal welfare means the physical and mental state of an animal in relation to the conditions in which it lives and dies*", while "*Good animal welfare requires desease prevention and appropriate veterinary care, management and nutrition, a stimulating and safe environment, humane handling and humane slaughter or killing*". Another generally accepted guideline for minimum legal standards of farm animal welfare is the following *Five Freedoms:*

> *"**Freedom from thirst, hunger, and malnutrition** by ready access to fresh water and a diet to maintain full health and vigour.*
> ***Freedom from discomfort** by providing a suitable environment, including shelter and a comfortable resting area.*
> ***Freedom from pain, injury, and desease** by prevention or rapid diagnosis and treatment.*
> ***Freedom from fear and distress** by ensuring conditions that avoid mental suffering.*
> ***Freedom to express normal behaviour**, by providing sufficient space, proper facilities, and company of the animal's own kind"* (Webster, 2008).

To evaluate animal welfare status, behavioral, physical, physiological, and production intended indicators can be used (Veerasamy et al., 2011).

Little attention has been paid to the welfare of fish in aquaculture for a very long time. As this topic is gaining more and more importance not only among animal welfare organizations but also among consumers and is also required by law (EU, Council Directive 98/58/EC), researchers have addressed this subject in recent decades (Huntingford et al., 2006; Noble et al., 2018; Prunet et al., 2012). In this regard, the African catfish is particularly important as it is generally considered very robust and is often kept under extreme conditions compared to other fish species. Therefore, it is interesting to find out under which conditions it not only

survives but also its welfare is ensured. As in terrestrial animals, the welfare status of fish is commonly assessed by behavioral, physical, and physiological parameters. When evaluating the behavior of African catfish, both individual and group observations can be performed. Thereby, air-breathing events, escape attempts, resting, swimming, aggressive actions, stereotypic behavior, and skin lesions are assessed (Van de Nieuwegiessen et al., 2009). Baßmann et al. (2017, 2020), for example, observed fewer skin lesions when fish were held in coupled aquaponic systems compared to systems without plants. In the case of aquaponic basil cultivation, they additionally detected more agonistic behavior in the system without plants.

Physical parameters like body weight, feed intake, growth, and mortality are also used to evaluate well-being (Strauch et al., 2019; Van de Nieuwegiessen et al., 2009; **Wenzel et al., 2021)**. Also, blood concentrations of cortisol, glucose, and lactate can be used (Silbergeld, 1974; Sumpter et al., 1986; Van de Nieuwegiessen et al., 2009; Van der Vyver et al., 2013). Newer methods to evaluate fish welfare include transcriptomics, metabolomics, or proteomics (Seibel et al., 2021). Even if physiological parameters give information about the welfare status of the fish, regular data collection of such parameters is usually done in research institutions because the realization of sampling is rather difficult in commercial production systems.

1.3.3 Production Systems

The commercial production of *Clarias gariepinus* can take place in many different systems. For example, in earthen ponds in the form of monoculture or polyculture as it is common in Africa, in RAS the way it is mainly done in Europe due to the colder climate, or in aquaponic production systems in combination with the production of plants. In German RAS, the waste heat of biogas plants is very often used to temper the fish systems, allowing farmers to save on expensive heating costs. In Hungary, heating costs are saved by using geothermal waters (Gál, 2022).

1.3.3.1 Traditional Production Systems

Aquaculture in flooded ponds is the traditional African catfish production system and has existed for centuries (Pouomogne et al., 2008). Here, the naturally occurring swimming into the flooded ponds is promoted, and then the fish are left there for ongrowing, with varying degrees of human intervention, until harvest. They usually feed on what is growing in the

ponds, but some farmers also supply additional food. The fish are typically harvested after 1 – 2 years of growth (FAO, 2022a). In Bangladesh and Nepal, where the species has been introduced, it is produced in ditches/ponds called “Catfish holes”, where they are stocked with around 1 g and are harvested after 5 – 7 months of ongrowing, with a survival rate of about 40 %. They are usually held together with *C. batrachus* and fed various food wastes. African catfish are also kept as “police-fish” in polyculture with Nile tilapia in earthen ponds at a ratio of catfish:tilapia from 1:1 to prevent an uncontrolled propagation of Nile tilapia (Pouomogne et al., 2008). Traditional production systems are extensive systems that require only a small amount of supervision, labor, and know-how. Due to rising demand, aquaculture production in Africa is also developing to an increasingly intensive form (Adeleke et al., 2020). Besides traditional pond aquaculture, fish are produced in artificial tanks and at higher densities. The high demand for fingerlings has led to their production in intensive RAS (Komugisha and Rajts, 2021).

1.3.3.2 RAS

Intensive warm water RAS for African catfish have been developed in the Netherlands, where the catfish was introduced to Europe by Dutch scientists in 1977 (Verreth and Eding, 1993). After that, commercial production in the Netherlands evolved and later in other European countries like Belgium, Hungary, Poland, Slovakia, Czechia, Russia, and Germany (Piria et al., 2019). To ensure a constant fingerling availability of good quality, the Dutch also developed a breeding strain based on wild fish from Central Africa, Israel, and the Republic of South Africa (Rosendaal, 2022, personal communication). The broodstock selection is based on rapid growth, late egg maturity, absence of deformities, tranquil behavior, and stress resistance. It took around ten generations until the fish were adapted to the culture conditions in RAS.

The African catfish RAS developed in the Netherlands were rather simple and low-tech, originally consisting of fish tanks, a sedimentation tank with lamella inserts, and a trickling filter for biological treatment and gas exchange (Bovendeur et al., 1987). Nowadays, all the systems usually have UV-C lights for disinfection and start to be equipped with drum filters instead of sedimentation tanks with lamella inserts to reduce labor, but also because the lamella filters tend to clog and, therefore, are not practical (Haage, 2021, personal communication). Reduced water consumption cannot be achieved (Scheibe, 2020, personal communication).

Fingerlings are also produced in RAS and are usually separated from the grow-out farms to guarantee appropriate hygiene measures. The construction of the systems for fry and larvae are typically held similarly to those for out-growing but on a smaller scale and under much better water conditions, as the young fish are still much more vulnerable. The most crucial thing in such hatcheries besides water quality is regular grading, as the highest mortalities at young life stages are due to cannibalism (Al-Hafedh and Ali, 2004). Compared to the traditional production in tropical and subtropical regions, the production in European closed RAS is more expensive as heating and more technical equipment are needed (Pasch and Palm, 2021). That is also why these systems must be run under the highest densities to be profitable.

1.4 Optimized Cultivation Conditions in African catfish Aquaculture

In warm water RAS for African catfish, where production costs and the production per area are very high, a yield increase or decrease of a few percent can enormously influence a company's profitability (Pasch and Palm, 2021). There are different areas where small modifications can have a significant influence on cultivation conditions and, thus, on yield. For example, water quality parameters (Person-Le Ruyet et al., 1995; Maclntyre et al., 2008) or feed additives (Bharathi et al., 2019; Herrera et al., 2019) can influence survival, growth performance, physiology, and welfare of the fish, showing that RAS management has a significant impact on the success of production. Additionally, due to the increasing environmental pollution caused by humans, the sustainable use of waste products is also moving more and more into focus when optimizing aquaculture facilities.

1.4.1 Water Quality

As the whole body of the fish is constantly exposed to the water, its quality is one of the most important parameters in aquaculture. For example, when water oxygen concentrations, temperature, and pH are inadequate, it can negatively affect the fish. Oxygen depletion, for example, can lead to an increase in mortality rates (Carlson et al., 1974), a change in behavior resulting in more frequent air-breathing events (Randle and Chapman, 2005), or aquatic surface respirations (Gee et al., 1978; Kramer and McClure, 1982; Kramer and Mehegan, 1981; Lewis Jr, 1970) and in a reduced feed uptake, which leads to poorer growth (Thorarensen et al., 2017). Fish can also show reduced growth if the temperature is too high or low (Hogendoorn et al., 1983; Nytrø et al., 2014). The water pH can influence fish's hatching rate, survival (Marimuthu et al., 2019), and growth performance (Rebouças et al., 2015).

Additionally, the pH is responsible for whether the excreted nitrogen is present as ammonium (NH_4^+) or as ammonia (NH_3), which is toxic to fish (Lekang, 2019). Besides that, the concentrations of the nutrients can also strongly influence the fish. If, for example, water ammonia (Schram et al., 2010), nitrite (Roques et al., 2015), and nitrate (Schram et al., 2014) concentrations in African catfish RAS are too high, it can result in reduced feed intake and growth or, in the case of ammonia, damages the gills (Benli et al., 2008; Milne et al., 2000). At the same time, elevated water phosphate concentrations up to 80 mg L^{-1} seem to positively influence fish growth, while 120 mg L^{-1} leads to increased biting wounds and reduced activity (Strauch et al., 2019). Consequently, the recommended values for the different water parameters in which the African catfish can grow ideally are shown in Table 1.1.

Table 1.1: *Water parameter threshold values for the African catfish (Clarias gariepinus).*

Fish size/age	Parameter	Recommended values	Reference
34.7 g	Oxygen	≥ 3.0 mg L^{-1}	(Oellermann, 1995)
4 days after the hatch 6 weeks old (0.45 g)	Temperature	25 – 33 °C	(Britz and Hecht, 1987)
0.5 g	Temperature	27.5 – 32.5 °C	(Hogendoorn et al., 1983)
125 g	Temperature	25 °C	(Hogendoorn et al., 1983)
Egg incubation	pH	6.7 – 7.6	(Marimuthu et al., 2019)
Just hatched larvae	pH	6.5 – 7.5	(Marimuthu et al., 2019)
141 g	Ammonia	≤ 0.34 mg L^{-1} NH_3–N	(Schram et al., 2010)
219.7 g	Nitrite	≤ 0.6 mg L^{-1} NO_2^-–N	(Roques et al., 2015)
154.3 g	Nitrate	≤ 140 mg L^{-1} NO_3^-–N	(Schram et al., 2014)
30.81 g	Phosphate	≤ 80 mg L^{-1} PO_4^{3-}–P	(Strauch et al., 2019)

1.4.2 Feed Additives

Besides water conditions, substances in the feed can also influence the fish. The regular fish feed used in RAS consists of proteins, lipids, carbohydrates, minerals, and vitamins. The different ingredient proportions depend on the fish species' requirements and their respective life stage/age (Craig et al., 2017). Apart from the required ingredients, special feed additives can be added. According to the regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition, "*'feed additives' means substances, micro-organisms or preparations, other than feed material and premixtures, which are intentionally added to feed or water in order to perform, in particular, one or more of the functions mentioned in Article 5(3);"* (Art. 2), where feed additives *"… shall:*

(a) favourably affect the characteristics of feed,

(b) favourably affect the characteristics of animal products,

(c) favourably affect the colour of ornamental fish and birds,

(d) satisfy the nutritional needs of animals,

(e) favourably affect the environmental consequences of animal production,

(f) favourably affect animal production, performance or welfare, particularly by affecting the gastro-intestinal flora or digestibility of feedingstuffs, or

(g) have a coccidiostatic or histomonostatic effect…" (Regulation (EC) No 1831/2003, Art. 5). According to this definition feed additives are divided into 5 categories:

"(a) technological additives: any substance added to feed for a technological purpose;

(b) sensory additives: any substance, the addition of which to feed improves or changes the organoleptic properties of the feed, or the visual characteristics of the food derived from animals;

(c) nutritional additives;

(d) zootechnical additives: any additive used to affect favourably the performance of animals in good health or used to affect favourably the environment;

(e) coccidiostats and histomonostats." (Regulation (EC) No 1831/2003, Art. 6).

Thereby antioxidants and emulsifiers are examples for technological additives, flavors and colorants for sensory additives, vitamins and amino acids for nutritional additives and digestibility enhancer for zootechnical additives (Regulation (EC) No 1831/2003, ANNEX 1).

In fish feed, additives like phytogenics, microalgae, yeast, enzymes, organic acids, mycotoxin binders, probiotics, prebiotics, seaweeds, and mushrooms are used to improve the resistance and increase the productivity (Bharathi et al., 2019). Depending on the substances, phytogenics can, for example, stimulate the appetite, stabilize the microbiome or stimulate the production of digestive enzymes (Caipang, 2020). Microalgae can stimulate and improve the immune system in fish (Shah et al., 2018). In fish and shrimp, it has been shown that yeast can enhance growth, survival, and gut maturation and improve the immune system (Navarrete and Tovar-Ramrez, 2014). It is also possible to directly add enzymes that support the digestive system (Zheng et al., 2020). The most commonly used in aquaculture are phytase treatments, which can increase the digestibility of plant-derived phosphate. Organic acids decrease the pH in the stomach, whereby the activity of digestive enzymes is improved. Additionally, they can positively influence growth and disease resistance (Encarnação, 2016).

The fishmeal contained in fish feed is increasingly replaced by plant proteins, which also introduce mycotoxins into the feed. These secondary metabolites of fungi mainly grow on agricultural products (Gonçalves et al., 2018). Mycotoxin binders like bentonite and montmorillonite (clay minerals) can bind these agents, reducing the fish's contamination (Hussain, 2018). Probiotics are microorganisms administered by the feed or the process water and positively influence the host (Hai, 2015). In fish, it can improve health, growth, stress response, and the digestive system, as well as feed utilization. Prebiotics can improve the microbiome by stimulating the growth/activity of healthy gut bacteria (Ringø et al., 2010). Substances in seaweeds and mushrooms are diverse and can improve the fish's growth and immune response (Van Doan et al., 2019).

1.4.3 Management Measures

Besides water quality and feed, especially farm management can strongly influence the success of a fish production facility. The main influencing factors are water exchange rates (Ajiboye et al., 2015), feeding strategies (Eyo and Ekanem, 2011; Hogendoorn et al., 1983; Okomoda et al., 2019), light exposure (Almazán-Rueda et al., 2004; Britz and Pienaar, 1992), and grading intervals (Abdelhamid et al., 2010; Biu et al., 2015; Mwangi et al., 2020). Adequate cleaning intervals and water exchange rates are simple interventions but can strongly influence fish performance as they directly influence water quality and, thus, fish growth (Ajiboye et al., 2015). Ajiboye et al. (2015), for example, could improve the weight gain of African catfish by 38 % within 12 weeks by increasing the water exchange rate. At the same time, finding the right feeding strategy for each fish size/age can also influence catfish growth. In this regard, Hogendoorn et al. (1983) found better growth for fish between 0.5 and 10 g when they were fed continuously the whole day (24 h) or continuously 12 h during the night (between 08:00 p.m. – 08:00 a.m.) compared to continuously 12 h during daytime (between 8:00 a.m.– 08:00 p.m.) or as 2 or 4 portions during daytime. Eyo and Ekanem (2011) found more than twice as much weight gain when fish were fed twice a day compared to once within 56 days, while Okomoda et al. (2019) found the best growth for fry (approximately fish weight: 0.022 g) when fed 5 or 6 times a day and for fingerlings (approximate fish weight: 4.22 g) 3 or 4 times a day compared to less feeding intervals. Another point is that African catfish are strongly negative phototactic (Britz and Pienaar, 1992), so illumination plays an important role in their keeping. Almazán-Rueda et al. (2004) showed that continuous illumination could lead

to 41.6 % more scars and wounds than under 12 h light: 12 h dark conditions in African catfish, while Appelbaum and Mc Geer (1998) observed better growth in complete darkness compared to continuous light. Britz and Pienaar (1992) additionally observed increased territorial aggression in constant light while under darkness, the territorial aggression was low, and swimming activity, air-breathing, and browsing were higher. Hence, daily farm management should be organized to keep the illumination as short as possible to reduce stress for the fish. Moreover, young African catfish are highly cannibalistic (Biu et al., 2015; Mollah et al., 1999). Therefore, excessive mortality rates of more than 98 % can occur within four weeks if no measures are taken (Abdelhamid et al., 2010). Regular grading every third day could increase the survival rate by 41 %. Mwangi et al. (2020) and Biu et al. (2015) reached a reduction of 38.4 % and 25 % in mortality rates within eight weeks for fish with an initial weight of approximately 0.7 – 0.9 g and 2 g when grading was conducted every two weeks and twice weekly.

The above-mentioned management measures should be optimized for the respective production systems to ensure ideal fram management and production. In Germany especially, the management of African catfish RAS has great potential for improvement as they are a relatively new production branch operated mainly by farmers with no previous experience in fish farming.

1.4.4 Sustainable Aquaculture Production and Aquaponics

Besides improving the cultivation conditions of the fish, sustainability is increasingly important in aquaculture production because of the rising nutrients released into the environment by humans worldwide (Gowen, 1994; Malone and Newton, 2020). The *European Green Deal* and the *Circular Economy Action Plan* of the European Commission also demand the development of aquaculture systems that provide more sustainable fish production and the reuse of by- and waste-products to reduce the environmental impact.

The waste products of RAS can generally be divided into “solid waste” and “dissolved waste” (Dauda et al., 2019). Strauch et al. (2018) could show in African catfish RAS that a high amount of phosphorous (9.7 – 19.3 %) accumulates inside the solid waste. This phosphorous is exclusively bound as calcium phosphate, possibly useful as a soil amendment **(Prüter et al., 2020**). As mentioned in 1.2, aquaculture solid waste can also be recycled as a carbon source for a denitrification reactor (Gao et al., 2020). Besides that, fish carcasses unsuitable for

human consumption can be used to produce sustainable fish meals or oil (Afreen and Ucak, 2020). Additional conceivable applications are the pet industry as dog snacks or similar, where people are generally willing to spend more money (Albert and Bulcroft, 1987; Reid and Anderson, 2009). That shows the broad range these by-products can be used for, while the major problem bringing them into the industry is, adapting them to commercial systems and making them profitable

The process water is a liquid by-product (= dissolved waste) of RAS and can be used as fertilizer in aquaponic plant production. As fish cannot digest 100 % of the food, a part of it always ends up in the process water, resulting in a nitrogen release of approximately 75 % (Hargreaves, 1998) and a phosphorous release of 60 – 80 % (Yogev et al., 2020). These feed remains contain nutrients that are partly dissolved in the process water. Consequently, if this water is discharged into the environment, it can lead to eutrophication and the associated consequences (Porrello et al., 2003). Aquaponic combines fish production with plant cultivation, as the word creation from aquaculture and hydroponic (soilless plant cultivation) indicates. The advantage of this is a more sustainable use of resources because it can reduce the environmental impact due to more efficient water and nutrient use (Lennard and Goddek, 2019). Ten years ago, aquaponics was defined as RAS incorporating soilless plant production (Rakocy, 2012). Later Lennard (2015) added that at least 80 % of the nutrients for plant growth must come from the fish to prevent the term from being used solely for advertising purposes. Newer definitions say it is the combination of the production of aquatic organisms and plants, while the majority (> 50 %) of nutrients ensuring optimal plant growth must come from the fish (Palm et al., 2018a). An advantage of this definition is the possibility of producing aquaponic plants in soil. This makes it easier to sell the plants and makes them more attractive for commercial production. Another benefit is that the process water can be applied to agricultural land for fertilization, called “aquaponics farming”. On the other hand, recent definitions try to define aquaponics in a new way and restrict the term “aquaponic farming” to hydroponic production systems combined with tank-based animal aquaculture and additionally include microbiological processes. At the same time, “trans-aquaponics” extends aquaponics to tankless aquaculture and non-hydroponic plant production (Baganz et al., 2022). However, as the newest definitions show (Palm, 2023, personal communication), the attempt to redefine aquaponics by Baganz et al. (2022) has deficiencies in its content. This newest (Palm 2023, personal communication) clarification largely refers to Palm et al. (2018a),

where at least 50 % of the nutrients used for plant production must come from the aquatic organism/fish. Additionally, this definition introduces the term “aquaorganoponics” (as a better description of the principle of aquaponics) to clarify that natural organic compounds in the process water of the aquatic organisms are transferred to the plants for nutrition. Regardless of these different definitions, aquaponics or aquaorganoponics, in the broadest way, describe the use of nutrient-rich aquaculture effluents for plant production with the aim of more sustainable resource utilization.

Aquaponic systems can be operated in 2 ways, as coupled or decoupled systems (Monsees et al., 2016). In coupled systems, fish and plants stay in contact via a common circuit. The advantage of these systems is that less technical effort is required. The disadvantage is that a compromise must be found between the needs of the plants and the needs of the fish. That often leads to the fact that aquaponic plants are not competitive with those produced in traditional production systems, where they are grown under optimal conditions. In decoupled systems, the fish and plant systems are separated. The advantage is that plants and fishes can be cultured under their respective preferred conditions, where the plants benefit through an optimal pH of around 6 (Da Silva Cerozi and Fitzsimmons, 2016; Zou et al., 2016). The disadvantage is that if the plants are produced in a greenhouse with an automatic pump and distribution device for the fish water, the technical effort for this is often very high and expensive. Besides focusing on the yield of edible plants, aquaponics can also serve primarily for water purification, where plant mass production is secondary (Endut et al., 2016). The focus here is on the removal of the strongly accumulating nitrate.

During the intensive production of African catfish, a considerable amount of feed is introduced into the production systems (**Strauch et al., 2018**). As the African catfish is very tolerant towards environmental conditions, it seems to be a good candidate for aquaponics as higher nutrient concentrations can be reached and low oxygen concentrations are not a problem (Oellermann, 1995). The nutrients in African catfish RAS that are the most deficient for optimal plant growth are K as a macronutrient and Fe, Mn, and Mo as micronutrients (**Strauch et al., 2018**). Nonetheless, practical studies showed that basil (*Ocimum basilicum*) (Knaus et al., 2020a; Pasch et al., 2021a), lettuce (*Lactuca sativa* L.) (Yeşiltaş et al., 2021), and pumpkin (Oladimeji et al., 2020) could be grown very well in the process water of African catfish.

1.5 Aquaculture Production Systems in Germany

Even if there are some commercial aquaponic production systems in Germany, aquaculture generally plays a minor role compared to other agricultural sectors. EU and national regulations on nature and species conservation prevent the expansion and establishment of new businesses, particularly net cages and flow-through systems whose environmental impact is high and can be influenced little (AFC Consulting Group AG and COFAD GmbH, 2017). Especially, the competitiveness of relatively small farms is impaired by restrictions and conditions imposed by environmental, water, nature conservation, and veterinary law, which leads to considerable additional bureaucracy and high costs, irrespective of restrictions on production (AFC Consulting Group AG and COFAD GmbH, 2017). In the case of flow-through systems, obtaining the necessary water rights and other requirements for constructing a new farm is almost impossible. Therefore, the existing farms are often family-owned for generations, and hardly any new farms are built. Additionally, damages due to predators increased while prevention, especially in pond farms, is almost impossible, which makes an acquisition unattractive for subsequent generations (AFC Consulting Group AG and COFAD GmbH, 2017). In 2020 the per capita fish consumption (including crustaceans and mollusks) in Germany was 14.1 kg (Statista, 2022b), while the self-supply of fish products in Germany was below 30 % (Ahrens, 2021). Only the self-supply for common carp was around 80 % in 2016 (AFC Consulting Group AG and COFAD GmbH, 2017).

Despite all the obstacles for several years, projects concerning the aquaculture industry are funded by the government also in Germany, with the goal of making it more sustainable but also to improve its competitiveness (ABl. MV 2018, 701; MBl. NRW. 2016 S. 406; ABl. BB 2016, 21). The overall aquaculture fish production increased by 327 tons from 2011 to 2020, with a total production volume of 18,596 tons in 2020 (Statistisches Bundesamt BMEL, 2021; Statistisches Bundesamt DESTATIS, 2022). Trout makes up the largest share with 45 %, followed by carp (*Cyprinus carpio* L.) with 26 %, mainly produced in ponds and flow-through systems (Statistisches Bundesamt BMEL, 2021; Statistisches Bundesamt DESTATIS, 2022). Additional species produced are char, European eel (*Anguilla anguilla* L.), African catfish (*Clarias gariepinus* Burchell, 1822), European catfish (*Silurus glanis* L.), Siberian sturgeon (*Acipenser baerii* Brandt, 1869), tench (*Tinca tinca* Gersault, 1764), Pike perch (*Sander lucioperca* L.) and pike (*Esox lucius* L.) (Statistisches Bundesamt DESTATIS, 2022) (see Figure 1.3).

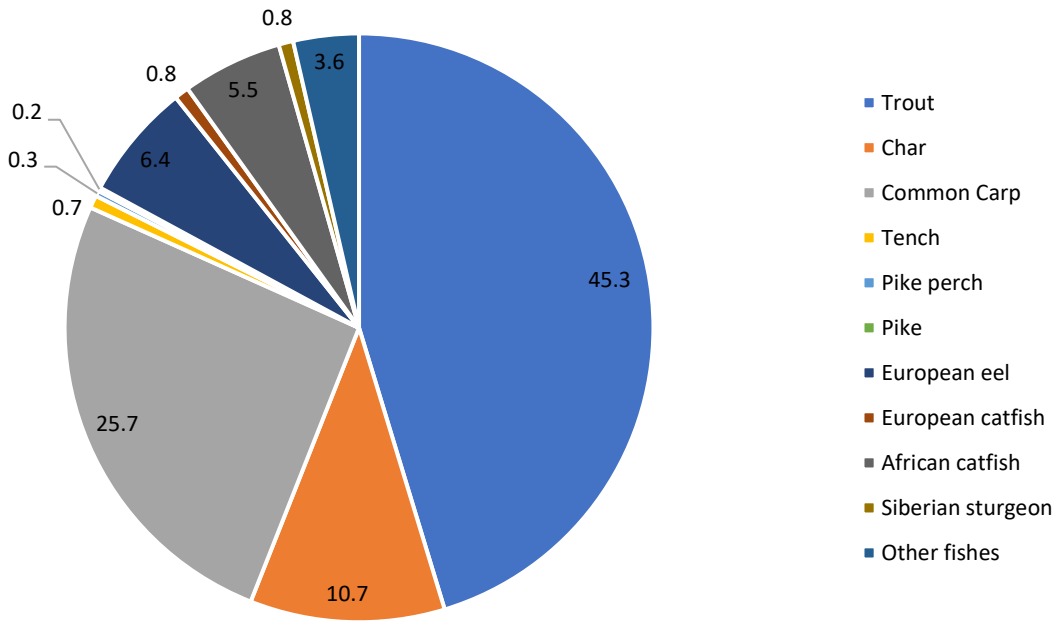

Figure 1.3: *Percentage of fish species produced in Germany in 2020 (Statistisches Bundesamt DESTATIS, 2022).*

1.5.1 Traditional Aquaculture Systems

Traditional aquaculture in Germany consists primarily of trout and carp farming, which is described in more detail in this chapter with reference to AFC Consulting Group AG and COFAD GmbH (2017). The pond farming of common carp has a thousand years of tradition in Germany, where carp is cultured as the main species together with several co-species like tench, pike perch, and sturgeon. Typical for those systems are big pond areas where the fish are produced extensively in a near-natural environment. Especially in Bavaria, Saxony, and Brandenburg, they represent a special cultural landscape. Small producers usually buy their fish for stocking, while bigger farms tend to produce the fish by themselves. The fish are usually fed by cereals or other generated agricultural products. Often, the naturally occurring food in the ponds also makes up a large part of the diet, sometimes promoted by fertilization.

The traditional trout production in Germany is conducted outside in flow-through systems, fed by running waters via bypass systems. At the same time, the use of water from springs and wells is also possible. The fish are usually held in flow channels, gutters, or earthen

ponds. Some systems are covered by nets or are roofed to prevent losses from predators. The degree of mechanization often depends on the size of the farms, while usually, only the big farms are equipped with automated water and feeding management systems. Here also often grading and harvesting are automated. The fish for stocking is usually produced in separate farms or obtained from abroad.

Additionally, to those traditional production systems, since 1970, a small amount of trout has been produced in net cages. For around 30 years also, RASs were tested and used to culture fish in fresh water. Species that came into use were eel, tilapia, European catfish, pike perch, sturgeon, trout, African catfish, and a few more. The African catfish was introduced about ten years ago and is the most successful species in RAS. At least a few marine species, such as trout, shrimps, yellowtail kingfish, and sea bream, have been produced in net cages and RAS.

1.5.2 African catfish Aquaculture

The first African catfish grow-out farm in Germany was Tessiner Edelfisch GmbH in Mecklenburg-Western Pomerania (Northern Germany) which started to produce fish in 2007, followed by a second one (Bioenergie Lüchow GmbH & Co. KG) in 2008/2009 and a hatchery (Fischzucht Abtshagen GmbH & Co. KG), which started in 2011.

The company “Fleuren and Nooijen” (today “AquacultureID” and “Zebcare”), which was founded in 1985 as a spin-off business from Wageningen University (Ley, 2017) in the Netherlands, developed a customized broodstock. The broodstocks used in Germany today go back to this Dutch strain.

The third and last big grow-out farm in Northern Germany started in 2012 (Sukower Bioenergie und Welsfarm Verwaltungs GmbH) (from Pasch, 2022). Besides this, in 2015, the University of Rostock built the FishGlassHouse, Europe’s most modern research facility for aquaponics, where African catfish are produced in three RASs that are constructed in the same way as commercial RAS in this geographical area. Since then, a lot of research in this field has been conducted (**Baßmann et al.**, 2017, **2023**; Palm et al., 2014; Knaus et al., 2020b, 2021; Pasch and Palm, 2021; **Prüter et al., 2020**; **Strauch et al., 2018**, 2019; **Wenzel et al., 2021, 2022**). The reason why so many catfish farms were founded in such a short time is that they were subsidized by the European Fisheries Fund and received increased feed-in tariffs for

electricity from biogas plants through the Renewable-Energy-Sources-Act (Erneuerbare-Energien-Gesetz = EEG) if the waste heat was used for fish production.

In 2020, 1025 tons of African catfish were produced in German warm water RASs, a threefold increase since 2011 (Statistisches Bundesamt DESTATIS, 2022). Still very low compared to the world's production of 235,580 tons in 2019 (FAO, 2021) and unstable in the last few years (Figure 1.4) (Statistisches Bundesamt DESTATIS, 2022) since “Tessiner Edelfisch GmbH” in 2018 had temporarily stopped the production due to a disease outbreak. Nonetheless, the government still wants and supports its successful production as the catfish is one of Germany's most promising candidates for local and sustainable RAS production.

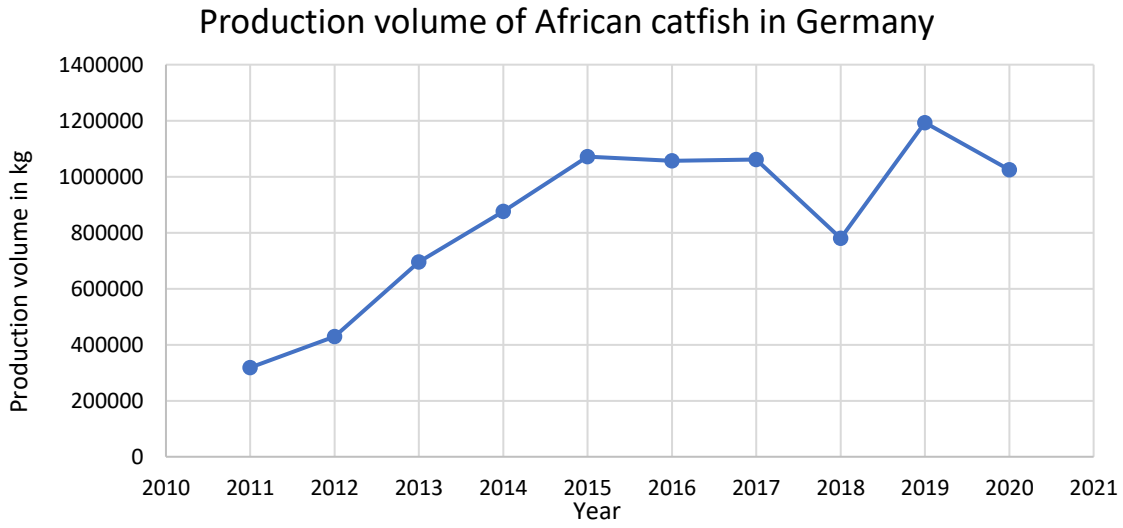

Figure 1.4: *Production volume of African catfish in Germany from 2011 to 2020 (Statistisches Bundesamt DESTATIS, 2022).*

The biggest problems nowadays are very low selling prices for the fish (< 6 € kg^{-1} for frozen fillet for delivery to wholesalers, own experiences Dec. 2021), high production costs, a lack of qualified personnel that wants to work at the respective locations and a high work effort as the RASs were developed low tech and most work has to be done by manpower, which raises labor costs. Most producers are farmers with biogas plants that use the waste heat to temper the fish systems. Therefore, they do not know much about fish and depend mostly on the knowledge of their employees.

1.6 Objectives of this Work

Due to the abovementioned problems, this thesis intends to improve different cultivation parameters in order to make Northern German catfish aquaculture more sustainable. One problem, amongst others, is the optimization of production processes, which can significantly influence production success. Therefore, this study aimed to investigate the possible influence of stocking density, grading, water contents, and feed additives on the performance and welfare of African catfish. Additionally, the suitability of African catfish process water for aquaponic plant production as a further income opportunity was examined. Based on the results, the potential to improve production success by adjusting management strategies was evaluated. For this purpose, a scenario for ideal and poor production conditions has been calculated as a model to demonstrate how much production success can vary. The following working hypotheses were addressed:

H1: Stocking density influences growth and performance in African catfish RAS.

H2: Grading regimes influence growth and survival in African catfish RAS.

H3: Selected macronutrients inside the process water and feed ingredients positively affect African catfish's growth performance and welfare in RAS.

H4: Regular African catfish growth in RAS enables successful plant production using the resulting process waters.

H5: Optimized cultivation conditions determine successful African catfish RAS production in Northern German localities.

1.7 Structure of the Dissertation

Chapter 1 summarizes the existing literature and gives an overview of the worldwide aquaculture development, the technical components of RAS, the biological and ecological background of African catfish, and possible production methods. Moreover, different cultivation conditions and their influences on RAS and aquaculture production in Germany are discussed.

Chapter 2 investigates different stocking densities during the grow-out of African catfish from approximately 12 – 1600 g. Samples were collected at the beginning and every six weeks over

a 6-month experimental period to be able to differentiate between the various age stages. Final stocking densities of approximately 100, 200, and 400 kg m^{-3} were compared in triplicate groups considering growth and welfare (**Baßmann et al., 2023**).

Chapter 3 deals with the optimization of the stocking density and grading of African catfish fry. It was investigated if different stocking densities (10, 20, and 30 fish L^{-1}) and grading regimes (intervals: daily, every two days, after five days; methods: manual grading, self-grading) influence behavior, cannibalism, and growth performance (**Wenzel et al., 2022**).

Chapter 4 investigates the influence of different water potassium concentrations (2, 218, 418, and 671 mg L^{-1} K^{+}) on juvenile African catfish. Therefore, the fish were cultured for 42 days in triplicate groups in process waters containing the respective potassium concentrations before growth performance, body composition, and welfare were evaluated (**Wenzel *et al.*, 2021**).

Chapter 5 describes the influence of mixed layer clay mineral Montmorillonite-illite/Muscovite (1g557) as a feed additive on the growth and welfare of African catfish in RAS. In the study, 0, 0.5, and 2 % of dietary supplementation were compared in two experiments at two different locations. In the first experiment, all three concentrations were compared, while in the second experiment, only 0 and 0.5 % were compared in triplicate groups (**Palm et al., 2022**).

Chapter 6 examines the suitability of nutrient-rich process water from African catfish production for spearmint (*Mentha spicata*) cultivation in decoupled aquaponic systems. Therefore, plant growth was compared between a commercial hydroponic fertilizer and the process water of an extensively (35 fish $tank^{-1}$) and an intensively (140 fish $tank^{-1}$) stocked RAS over 70 days (**Knaus *et al.*, 2020**).

Chapter 7 provides a discussion of the results that were obtained throughout the experimental trials described in chapters 2 – 6. Additionally, a mathematical model determines production success under ideal and poor production conditions considering survival rate and growth.

Chapter 8 summarizes the knowledge gained from the discussion in Chapter 7.

Chapter 9 provides a short outlook on important upcoming topics in the field of African catfish aquaculture in Northern Germany.

Chapter 10 provides the references used in this dissertation.

Chapter 11 provides the affidavit (Eidesstattliche Erklärung) about the preparation of this dissertation.

Chapter 12 lists and answers the hypotheses of this dissertation.

Chapter 13 provides a curriculum vitae of the author of this dissertation.

Chapter 14 acknowledges the people that supported the author during the development of this dissertation.

Chapter 15 provides supplementary materials (Appendix).

2 Effects of Stocking Density, Size, and External Stress on Growth and Welfare of African catfish (*Clarias gariepinus* Burchell, 1822) in a Commercial RAS

Abstract

The effects of semi-intensive (100 kg m^{-3}), intensive (200 kg m^{-3}), and super-intensive (400 kg m^{-3}) stocking densities on the growth and welfare of African catfish (*Clarias gariepinus*) were investigated under commercial production conditions. Plasma cortisol, glucose, and selected transcripts following a stress challenge, lactate, as well as skin lesions, were analyzed at regular intervals (from 12 g juveniles to 1.5 – 2.0 kg). The fish grew well, but after 23 weeks, the semi-intensively stocked fish had a mean final weight of 1830.5 g, significantly higher than the super-intensively stocked fish with 1615.4 g and considerably higher than the intensively stocked fish with 1664.8 g ($p > 0.05$). Cortisol and glucose responses significantly differed between stressed and unstressed fish but not between treatment groups. An unforeseen external stressor (nearby demolition noise) caused stress responses among all treatment groups but was similarly coped with. Mortality ranged between 3.8 – 9.2 %. In the juveniles, skin lesions were reduced under intensive or super-intensive densities, with the least under semi-intensive densities in outgrown fish. Expression profiles of 22 genes were compared in the spleen at semi-intensive and super-intensive densities. The transcript concentrations of most genes remained unchanged, except for *slc39a8* and *mtf1*, which were significantly downregulated in stressed catfish under semi-intensive conditions. We demonstrated that African catfish growth performance and welfare depend on age and stocking density, also reacting to demolition noise. This supports farm management to optimize stocking densities during the grow-out of African catfish in RAS and suggests avoiding external stress.

Keywords

Cortisol; Demolition noise; Fish well-being; Grading; Growth performance; Recirculating aquaculture; Mortality

2.1 Introduction

In finfish aquaculture, stocking density is a pivotal factor and an issue of frequent debate, as it may be a source of chronic stress, leading to physiological alterations, including stress responses, growth reduction, and impairment of health (Carbonara et al., 2020; Ellis et al., 2002; Montero et al., 1999). Based on these indicators, fish welfare can be considered

diminished (Ashley, 2007; Baldwin, 2011; Conte, 2004; Ellis et al., 2002; Oké and Goosen, 2019). According to Ellis et al. (2002) (p. 494), "... the term 'stocking density' refers to the concentration at which fish are initially stocked into a system" (*sensu strictu*). However, most often, the term is used to describe the density of fish at any time. It may thus be understood as a dynamic factor since the actual density increases or decreases as the fish grow or are removed from the rearing volume. Fish species in aquaculture are stocked at very different densities, typically ranging from < 10 to 100 kg m^{-3} (Conte, 2004; Ferreira et al., 2010). This widely varies due to the different needs and/or tolerances of the respective species. Similarly, the impact of a specific stocking density on the species' welfare may differ as well (Baldwin, 2011). In Europe, fish farming is often industrialized and highly intensive (Lymbery, 2002). According to Naylor et al. (2021a) (p. 1017), "intensification implies increasing the density of individuals, which requires greater use and management of inputs, greater generation of waste products, and increased potential for the spread of pathogens". In this respect, particularly high but also low stocking densities may result in stress, poor fish health, and welfare (North et al., 2006; Ortuno et al., 2001; Yarahmadi et al., 2016), subsequently having a negative impact on the fish performance, which in turn may also have economic consequences (Debnath et al., 2022; Farhaduzzaman et al., 2020).

A species whose global production in aquaculture has distinctly increased in recent years is the African catfish (*Clarias gariepinus* Burchell, 1822). In Germany, production in recirculating aquaculture systems (RASs) increased from 318,575 kg $year^{-1}$ to 1,193,137 kg $year^{-1}$ between 2011 and 2019 (Destatis, 2012, 2020). In moderate climates, this warmwater species is farmed in RASs, while ponds are predominantly used in tropical and subtropical regions (Dauda et al., 2018). The African catfish has a very good feed conversion ratio (FCR), a high growth potential, and is highly tolerant towards adverse environmental conditions, such as low oxygen or elevated nitrogen compounds in the water (Păpuc et al., 2019; Roques et al., 2015). This is based on its biological adaptations to its natural habitats, which are often characterized by shrinking water bodies or complete drainage during the dry season. Especially favorable is their ability to utilize atmospheric oxygen with their arborescent organs (Belão et al., 2011). For this reason, African catfish are adapted to survive for some time, densely packed inside smaller pools or even in moist substrates and mud (Păpuc et al., 2019). This attribute is utilized in commercial aquaculture by stocking this species up to super-intensive densities (max. 500 kg m^{-3}) (Van de Nieuwegiessen et al., 2008).

Reduced growth, alteration of physiological processes, increased stress responses, and/or rise in mortality were described under high stocking densities (Liu et al., 2017; Long et al., 2019; Montero et al., 1999; Oyarzún et al., 2020; Refaey et al., 2018; Schram et al., 2006). According to Van de Nieuwegiessen et al. (2009), final densities between approximately 100 – 500 kg m^{-3} did not affect the growth and welfare of African catfish in a size range from approximately 1.0 – 1.5 kg $fish^{-1}$. In contrast, wellbeing improved with increasing stocking density in fish between approximately 100 – 300 g at final densities between 16.7 – 315 kg m^{-3}, as significantly fewer skin lesions occurred at the highest density. According to Van de Nieuwegiessen et al. (2008), African catfish juveniles between approximately 10 – 100 g showed a comparable growth performance and mortality at all the tested densities, but there was evidence that growth may be best at medium densities (175 kg m^{-3}) and worst at the higher densities (300 kg m^{-3}). Plasma cortisol, glucose, and lactate remained unaffected by stocking densities, and fish from both low (50 kg m^{-3}) and high densities (300 kg m^{-3}) showed no cortisol response to an additional acute stress challenge. The authors suggested an impaired cortisol response related to the downregulation of components of the hypothalamic-pituitary-interrenal (HPI) axis in fish as a result of chronic stress: the adrenocorticotropic hormone (ACTH) or cortisol receptors (Van de Nieuwegiessen et al., 2008; Wendelaar Bonga, 1997). African catfish fry between approximately 0.1 – 0.3 g showed no significant differences in growth performance at stocking densities between 10 – 30 fish L^{-1}, but aggressive behavior was less frequent at higher stocking densities (**Wenzel et al., 2022**). In juvenile African catfish (approximately 10 – 100 g) under extensive stocking densities (500 and 1125 fish m^{-3}), increased agonistic interactions during the first weeks were described. In contrast, mature African catfish from higher stocking densities showed the highest number of skin lesions after stress induction. In general, however, aggression and the number of lesions were described to decrease with age. It was suggested that both low and high stocking densities might impair welfare (Van de Nieuwegiessen et al., 2008, 2009).

So far, most findings have originated from experiments in aquaria and not under commercial conditions. Therefore, the present study presents the growth performance and welfare of African catfish throughout an entire grow-out period in a commercial RAS under intensive conditions. We evaluate the dependence of current fish welfare indicators and the expression of several genes on fish size under three different stocking densities. In addition, the effect of an unforeseen external stressor during the experiment is discussed.

2.2 Materials and Methods

2.2.1 Production System, Maintenance, and Water Quality

A commercially scaled RAS at the aquaculture research facility 'FishGlassHouse' of the University of Rostock (Germany) was used for this experiment. It consisted of nine identical rearing tanks (each measuring (L × W × H) 1.8 × 1.0 × 0.7 m, 1.26 m^3), a settling tank (1.76 m^3, total effective surface area: 108.08 m^2) to remove suspended solids from the water, a trickling filter (5.6 m^2, specific surface area: 125.00 m^2 m^{-3}, total volume: 11.80 m^3, total specific surface area: 1474.20 m^2) for biological water treatment, and a sump (4.41 m^3). The total system contained approximately 16.90 m^3 of water (Palm et al., 2018b). An automatic temperature control/heater and a float switch for water level regulation were located in the sump. Tap water was used to replace evaporated water.

The water parameters of temperature, pH, oxygen concentration and saturation, electric conductivity (EC), salinity, and redox potential were controlled each day after trickling filtration (before the rearing tanks) with a portable multimeter (Hach-Lange HQ40D, Germany). Weekly water samples were taken and analyzed in triplicate with an automatic photometric-analyzer (Gallery™, Thermo Fisher Scientific) to monitor concentrations of ammonium/ammonia (NH_4^+/NH_3), nitrite (NO_2^-), and nitrate (NO_3^-). If the pH dropped below a threshold of 5, lime hydrate was added to the RAS or the water was changed in order to accordingly adjust the water quality. After starting the experiment, the settling tank was cleaned once a week; later, it was cleaned much more frequently to ensure system stability. The water quality parameters in the course of the experiment are summarized in Table 2.1.

Table 2.1: *Water parameters as mean ± standard deviation (SD) and minimum/maximum values between samplings (T0 – T1, T1 – T2, etc.). The parameters were measured every day (or weekly*).*

	Mean	±	SD	Min / Max (T0 – T1)	Min / Max (T1 – T2)	Min / Max (T2 – T3)	Min / Max (T3 – T4)	Min / Max (T4 – T5)
Temperature in °C	26.1	±	1.6	24.2 / 26.1	23.8 / 26.7	19.0 / 27.5	23.6 / 28.0	25.7 / 28.6
O_2 in mg L^{-1}	6.0	±	0.9	7.1 / 8.1	6.1 / 8.3	4.1 / 8.7	4.7 / 6.8	4.4 / 6.2
O_2 in %	74.0	±	10.9	90.4 / 96.5	74.4 / 103.5	50.8 / 97.1	60.1 / 82.6	53.1 / 80.2
pH	6.7	±	0.9	7.7 / 8.2	3.7 / 8.1	6.1 / 7.7	6.1 / 7.8	5.0 / 7.2
EC in µS cm^{-1}	1382	±	451	762 / 883	906 / 1610	672 / 1520	814 / 2230	1371 / 2480
Salinity in ‰	0.7	±	0.2	0.4 / 0.4	0.4 / 0.8	0.4 / 0.7	0.4 / 1.1	0.7 / 1.2
RedOx in mV	160.1	±	31.7	173.6 / 194.5	158.8 / 276.2	120.2 / 192.0	105.7 / 191.2	60.8 / 174.3
NH_4* in mg L^{-1}	13.60	±	3.88	9.62 / 18.99	9.03 / 14.73	8.8 / 13.41	8.48 / 16.50	8.78 / 13.21
NO_2* in mg L^{-1}	0.19	±	0.17	0.03 / 0.03	0.09 / 0.49	0.06 / 0.36	0.05 / 0.35	0.07 / 0.37
NO_3* in mg L^{-1}	475	±	154	591 / 606	511 / 518	339 / 346	325 / 750	344 / 349

2.2.2 Fish stocking, Feeding, and Growth

Three different treatment groups were compared with a semi-intensive (100 kg m^{-3}), intensive (200 kg m^{-3}), and super-intensive (400 kg m^{-3}) stocking density. On 18 January 2021, a total of 1848 African catfish juveniles of mixed sex were obtained from a local farmer (Fischzucht Abtshagen GmbH & Co. KG, Abtshagen, Germany). The fish were randomly stocked with either 88, 176, or 352 fish in 3 of the 9 tanks (randomized block-design), respectively, to reach an approximate final weight of 100, 200, or 400 kg m^{-3} at a slaughter weight of 1.5 kg. From each stocking density, 100 individual weight and length measurements were recorded as representative samples. Since newly added individuals are often harassed, fish in this study were not graded in order to avoid affecting the respective group compositions and thus possibly influencing the results.

The fish were fed with a catfish diet (Alltech Coppens, pellet sizes of 1.5 – 4.5 mm) according to a commercial feeding protocol from Alltech Coppens for African catfish of 10 – 2000 g at 5.62 – 0.84 % BW d^{-1} (BW: body weight). Feeding took place every two hours between 07:00 p.m. – 05:00 a.m. by using automatic feeders. The feed conversion ratio (FCR) [2.1], the specific growth rate (SGR) [2.2], and the condition index (CI) [2.3] were calculated for each sampling date.

$$FCR = \frac{TFI}{W_f - W_i} \quad [2.1]$$

$$SGR = \frac{ln\,(W_f) - ln\,(W_i)}{t} \times 100\% \quad [2.2]$$

$$CI = \frac{fish\ mass\ in\ g}{fish\ length\ in\ cm^{\,3}} \times 100 \quad [2.3]$$

with TFI = total feed intake in g, W_i = initial fish weight in g, W_f = final fish weight in g, SGR = specific growth rate in % BW (body weight) per day, t = time in days, CI = condition index.

After final sampling, the individual weights and total body lengths of 100 fish per stocking density were recorded again. The experiment was expected to last 5 to 6 months, depending on when the fish would reach their slaughter weight.

2.2.3 Sampling

All treatments were carried out in accordance with the EU guidelines 2010/63/EU for animal experiments and were approved by the relevant ethics committee.

The fish of the three treatment groups (semi-intensive, intensive, and super-intensive) were sampled at the beginning (i.e., after stocking and a one-week adaptation period) and regularly afterwards at 6-week intervals throughout the entire growth phase. In each sampling, three fish per tank (nine fish per treatment group) were anesthetized (eugenol bath, dosage: 50 mg L^{-1}), and blood was sampled over their caudal blood vessels within 5 min. Another three fish per tank (nine fish per treatment group) were anesthetized and blood sampled after additional induced stress, i.e., after netting and 30 min confinement in a tub without water but under humidification. According to Martins et al. (2006b), air exposure up to 1 h is a rather moderate stressor for African catfish. In each case, blood glucose and lactate levels were determined *in situ* using test strips (Roche, Accu Check Aviva/Accutrend Plus, Mannheim, Germany). The remaining blood was anticoagulated (BD Vacutainer, 5.4 mg K-EDTA, Franklin Lakes, NJ, USA) and stored on ice for later centrifugation (Hettich Universal 320 R, 10 min at 10,000 rpm and 4 °C, Tuttlingen, Germany). The plasma was separated from the cells. Cortisol was analyzed in the plasma by enzyme-linked immunosorbent assay (ELISA, Cusabio fish cortisol, sensitivity: 0.0023 ng mL^{-1}, Wuhan, China) according to the manufacturer's instructions using a micro-plate reader at 450 nm (iMark, Bio-Rad, Hercules, CA, USA). The cortisol concentrations were then calculated using a standard curve with Curve Expert 1.4.

The number and area of skin lesions were recorded for each fish. In order to determine the lesion area, a clear template with a 0.25 cm^2 grid was placed over each lesion, and the smallest possible area was recorded. All lesion areas per fish were then calculated. Sex, total body length, and weight were recorded. Each sampled fish was also implanted with a microchip (Mini Star ID, 1.4 × 8 mm) at an identical position, subcutaneously, close to the dorsal fin, in order to distinguish 'experimental fish' from accompanying fish, which were not sampled later on. The latter were kept among the experimental fish to achieve the respective stocking densities. After sampling, the anesthetized fish were transferred to an aerated recovery tank and returned to their respective tanks after reflexes and reactivity were regained.

The final sampling was preponed by one week (in week 23 instead of week 24), because the fish had already reached their slaughter weight. Afterwards, all fish were weighed (in groups) and double-checked for microchips. All fish with microchips were separated from the accompanying fish, properly stunned, killed, and separately disposed.

2.2.4 External Stressor

During the course of the experiment, at about the time of the third sampling, renovation work began in the adjacent building of the RAS facility used. This resulted in an unexpected stressor for the fish due to considerable noise impact. The demolition noise occurred for several hours each day and continued for several weeks, especially covering samplings 3 and 4. Therefore, the experiment can be divided into three different phases, the regular experiment between sampling 1 and 2, the time where the demolition noise took place (samplings 3 – 4), and a phase without any further disturbance (sampling 5). The noise level was not acoustically measured, but as it involved chiseling off ceilings and rebuilding exterior facades with heavy machinery, it can be assumed that > 90 dB was reached (Haufe, 2022).

2.2.5 RNA Isolation and Multiplex Quantitative PCR

Total RNA was extracted from individual spleens (n = 3 per group: stressed or unstressed fish under semi-intensive or super-intensive stocking densities from sampling 3 or sampling 5) using 1 mL TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA). After RNA purification using the RNeasy Mini Kit (Qiagen, Venlo, The Netherlands), the RNase-free DNase I (Qiagen) was used to digest residual DNA. Then, the concentration and the purity of the extracted RNA were assessed using a NanoDrop OneC spectrophotometer (NanoDrop Technologies, Wilmington, NC, USA).

The expression of four reference genes (*rna18s, rpl, actb, gapdh*) and twenty-two target genes (Table 2.5; Hildebrand et al., 2023) was profiled in the extracted RNA specimens from all of the groups using the integrated fluidic circuit (IFC) technology of the Standard BioTools Gene Expression biochips. The multiplex quantitative PCR (qPCR) analyses were performed on one 48.48 IFC chip (Standard BioTools, South San Francisco, CA, USA) using the BioMark HD system (Standard BioTools). To this end, the total RNA was adjusted at a concentration of 10 ng μL^{-1} and reverse-transcribed (42 °C, 30 min) using the Reverse Transcription Master Mix (Standard BioTools). The resulting cDNA aliquots were mixed with primers (100 µM) and the PreAmp master mix (Standard BioTools) and preamplified in 15 cycles (95 °C, 15 s; 60 °C, 4 min)

in a TAdvanced thermocycler (Biometra, Jena, Germany). After this pre-amplification step, exonuclease I (New England BioLabs, Ipswich, MA, USA) was added to degrade single-stranded oligonucleotide primers, followed by a 30-min incubation period at 37 °C. Then, 43 µL TE buffer (Sigma, St. Louis, MO, USA) was added per sample, and each 50-µL-cDNA sample was diluted in SsoFast EvaGreen Supermix with Low ROX (Bio-Rad) and 20 × DNA Binding Dye Sample Loading Reagent (Standard BioTools) to produce the sample mixes. After priming the 48.48-IFC chip in the MX Controller (Standard BioTools), the primers and the sample mixes together with one no-template (water) control were transferred to the assay and sample inlets on the primed 48.48-IFC chip. Finally, multiplex qPCR was conducted following the manufacturer's thermal protocol 'GE Fast 48.48 PCR + Melt v2.pcl'.

2.2.6 Statistics

Statistical tests were conducted with the Statistical Package for the Social Sciences (SPSS, v. 25, IBM Corp., 2017, Armonk, NY, USA) statistical software package. First, the resulting data were tested for distribution. Then, the non-parametric Kruskal-Wallis test by ranks and post hoc multiple range tests were conducted; Tukey's-HSD test for variance homogeneity and Dunnett-T3 test for variance inhomogeneity. Significance values were adjusted for several tests by the Bonferroni correction. All tests were performed with a significance level of $p < 0.05$.

For plasma cortisol and glucose, significances were only indicated within one sampling between stressed and unstressed fish of one single treatment group, as well as between stressed or unstressed fish of different treatment groups. No distinction was made between stressed and unstressed specimens for weight, length, CI, lactate, lesion number, and lesion area. Significances were indicated in this regard between treatment groups of one single sampling, as well as between similar treatment groups of different samplings.

The qPCR data were analyzed using the Fluidigm RealTime PCR Analysis Software (v. 4.5.2, South San Francisco, CA, USA) and normalized against the geometric mean of three suitable normalizer genes (*rna18s, actb, gapdh*). Copy numbers were calculated on the basis of ideal standard curves assuming a primer efficiency of 100 %. GraphPad Prism software (v. 9.1.0, San Diego, CA, USA) was used for the statistical analysis of the normalized qPCR data. Significant differences ($p < 0.05$) between the different groups were assessed using a two-way analysis of

variance (ANOVA) followed by a Holm-Šídák's post hoc test to correct for multiple comparisons.

2.3 Results

2.3.1 Fish Growth Performance

The mean initial and final weights and lengths of African catfish (n = 100) from the different stocking densities are given in Table 2.2. Before stocking, the weights of the representative sampling (n = 100) were statistically the same ($p > 0.05$). However, the lengths of the 100 fish measured in the intensive stocking density were found to be significantly different from both other groups. After 23 weeks, the mean weights in the semi-intensive density were about 200 g higher than in the higher stocking densities and significantly different from the super-intensive density ($p < 0.05$). In one semi-intensive and two super-intensive tanks, a few fish were caught due to organ sampling. That is why the stocking density is slightly lower at the end in these respective tanks. A comparative growth performance, including the CI of the fish from the three stocking densities over the course of the experiment based on sampling after 6 weeks each (n = 18), is given in the Appendix (Figures 2.6 – 2.8).

Table 2.2: *Initial and final weights and total body lengths of African catfish (mean ± standard deviation, SD, n = 100), p < 0.05, superscripts indicate significant differences between the groups.*

	Intended Stocking density	**n Fish Tank^{-1}**	**Mean Weight Fish^{-1} in g**	**±**	**SD**	**Mean Length Fish^{-1} in cm**	**±**	**SD**	***da facto* Density Tank^{-1} in kg m^{-3}**
Before stocking	Semi-intensive (0.84 kg m^{-3})	88 88 88	12.5	±	1.9	12.1	±	0.7[a]	0.87 0.87 0.87
	Intensive (1.68 kg m^{-3})	176 176 176	12.4	±	1.9	11.6	±	0.8[b]	1.72 1.72 1.72
	Super-intensive (3.35 kg m^{-3})	352 352 352	12.2	±	1.6	12.0	±	0.6[a]	3.41 3.41 3.41
After 23 weeks	Semi-intensive (0.84 kg m^{-3})	55 73 73	1830.5	±	596.7[a]	57.3	±	6.3	89.15* 106.40 106.85
	Intensive (1.68 kg m^{-3})	161 161 163	1664.8	±	588.5[a,b]	55.6	±	7.8	230.62 212.34 214.96
	Super-intensive (3.35 kg m^{-3})	284 313 309	1615.4	±	451.7[b]	56.1	±	5.1	364.52* 401.13 396.60*

* Deviation due to organ samplings.

The FCR tended to increase in all three stocking densities. Between the second-last and final sampling (T4 and T5, Table 2.3), the intensive stocking density showed the lowest FCR and, therefore, the best feed conversion rate. Throughout the entire experiment, the super-intense stocking density showed the comparatively weakest FCR. The SGR decreased with increasing age or weight of the fish. Comparing the three stocking densities, the highest SGR values were recorded under an intensive stocking density between T0 and T1, T2 and T4, and between T4 and T5 (Table 2.3).

2.3.2 Evaluation of Fish Welfare

Mortality varied between the rearing tanks, ranging from 2.59 % to 14.62 % (Table 2.4). The intensive stocking density showed the lowest mortality in total, while the super-intensive stocking density showed the highest mortality on average ($p > 0.05$). No increased mortality was observed during the phase of demolition noise.

Table 2.3: *The feed conversion ratio (FCR) and specific growth rate (SGR) of African catfish 1 week after stocking (T0–T1), after 6 weeks (T1–T2), after 12 weeks (T2–T3), after 18 weeks (T3–T4), and after 23 weeks, at the final weighing of all fish (T4–T5). Calculations are based on sub-samplings (n = 18 per group), except totals (right column).*

Stocking Density		**T0 – T1**	**T1 – T2**	**T2 – T3**	**T3 – T4**	**T4 – T5**	**T0 – T5 (in Total)**
Semi-intensive	FCR	0.7	0.7	0.8	0.7	1.1	0.77
(100 kg m^{-3})	SGR in % BW d^{-1}	7.0	6.0	2.2	1.7	0.9	3.0
Intensive	FCR	0.6	0.7	0.7	0.9	0.8	0.75
(200 kg m^{-3})	SGR in % BW d^{-1}	7.8	5.8	2.4	1.4	1.2	3.0
Super-intensive	FCR	0.7	0.7	0.8	0.8	1.2	0.83
(400 kg m^{-3})	SGR % BW d^{-1}	6.8	6.0	2.3	1.6	0.7	2.9

BW: Body weight

Table 2.4: *Mortality in the three treatment groups with semi-intensive, intensive, and super-intensive stocking densities (p > 0.05).*

Stocking Density	Mortality Tank^{-1} in %	Mean in %	±	SD
Semi-intensive (100 kg m^{-3})	2.59 9.56 9.20	7.12	±	3.92
Intensive (200 kg m^{-3})	3.89 4.32 3.16	3.79	±	0.59
Super-intensive (400 kg m^{-3})	14.62 7.71 5.40	9.24	±	4.80

The mean plasma cortisol responses of samplings 1, 2, 4, and 5 were in a range of 2.7 to 24.1 ng mL^{-1} (Figure 2.1). Significant differences between unstressed and stressed fish, but not between the different stocking densities, were found within the respective samplings. At the first sampling, the cortisol responses were identical between stressed and unstressed fish kept at the super-intensive stocking density. The highest cortisol levels during the experiment, with most values between 17.1 and 32.2 ng mL^{-1}, were recorded in the third sampling, meaning the beginning of the second phase of the experiment, when the demolition noise likely affected the fish. However, during the fourth sampling, cortisol levels had already returned to the normal range, although the demolition noise was still ongoing. All relevant significances within a single sampling between unstressed and stressed fish in a treatment group, as well as between unstressed or stressed fish in a treatment group between different samplings, are given in Table 2.6.

Blood glucose mainly ranged between 3.8 and 8.1 mmol L^{-1}. There were significant differences between unstressed and stressed fish within the sampling groups. However, there were no significant differences between different stocking densities within a single sampling. Notably, the mean glucose values were mainly higher at the third sampling, and the interquartile ranges and upper Whiskers were mostly wider compared to the majority of boxplots at other samplings (Figure 2.9).

The lactate values mainly ranged between 2 and 8 mmol L^{-1} (Figure 2.2). There were neither significant differences between the different stocking densities within one sampling nor between the samplings. Nevertheless, in the second phase of the experiment (under the influence of the demolition noise), i.e., from the third to the fourth sampling, all of the lactate

values noticeably decreased and increased again to approximately their initial level at the fifth sampling.

Skin lesions due to agonistic interactions occurred during the entire experiment (Figures 2.3, 2.4, and 2.10). At the first and third sampling, several skin lesions per individual were found under all stocking densities, whereas at the second and fourth sampling, the number of lesions decreased in general. At the first sampling, the semi-intensively stocked juveniles had the highest number of lesions, while at the fifth sampling, the adult fish had more lesions at intensive and super-intensive stocking densities compared to the semi-intensive stocking density. In particular, during the third sampling, meaning the beginning of the second phase (with noise impact), the highest number and largest skin lesions were determined in total. Significant differences ($p > 0.05$) were not found.

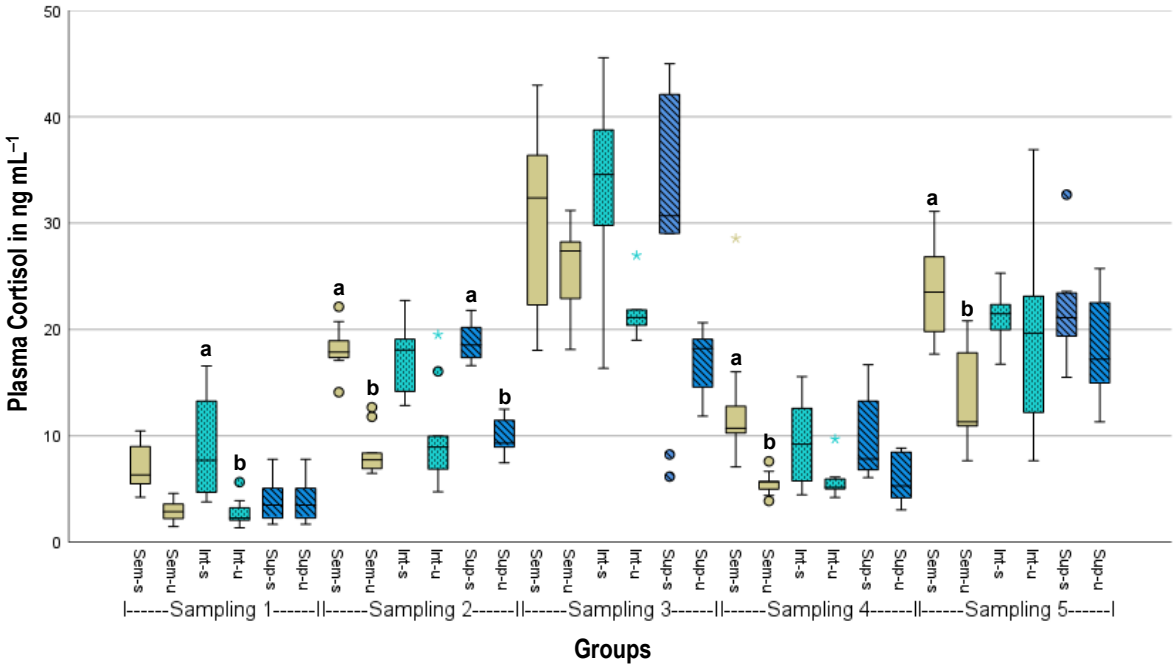

Figure 2.1: *Plasma cortisol responses of stressed (s) or unstressed (u) African catfish (n = 9) under three different stocking densities (Sem = semi-intensive, Int = intensive, Sup = super-intensive). Kruskal-Wallis test with Tukey-HSD or Dunnett-T3 post hoc test, significances ($p < 0.05$) are only given within the respective samplings, marked by letters. Circlets = outliers; asterisks = extreme values.*

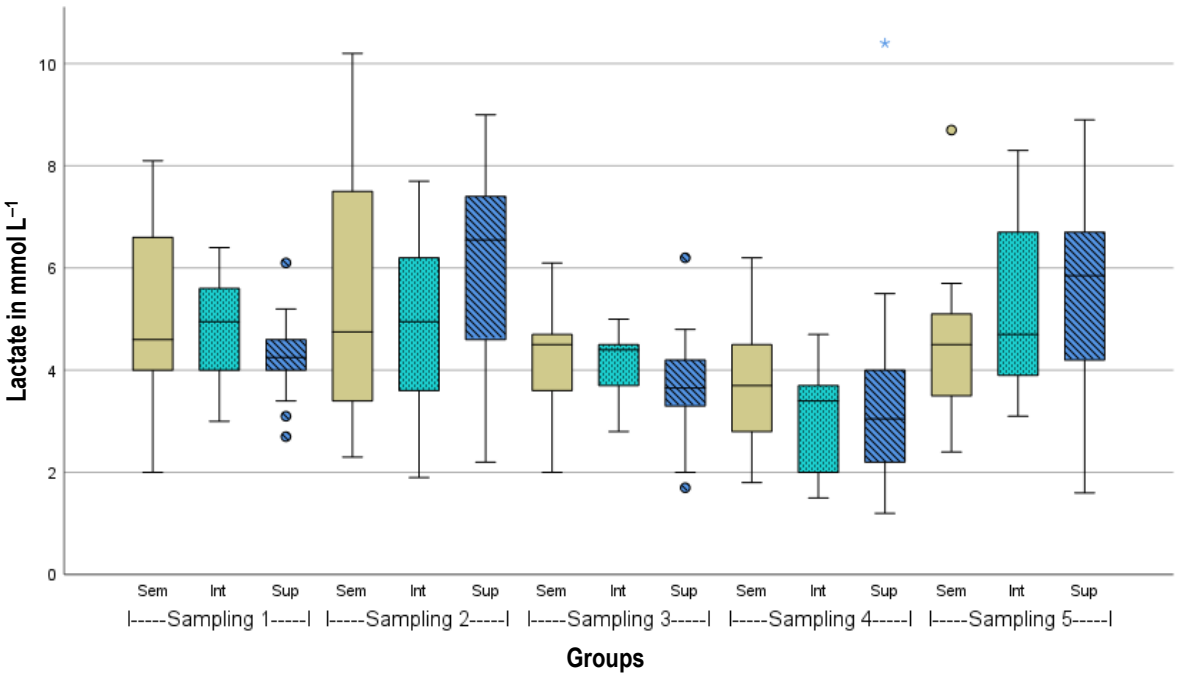

Figure 2.2: *Lactate level of African catfish (n = 18) in three different stocking densities (Sem = semi-intensive, Int = intensive, Sup = super-intensive), p > 0.05. Circlets = outliers; asterisk = extreme value.*

Figure 2.3: *Skin lesions (bite marks) on an African catfish in the typical jaw form of this species as result of agonistic interactions. The photo was contrast-enhanced by 25 %.*

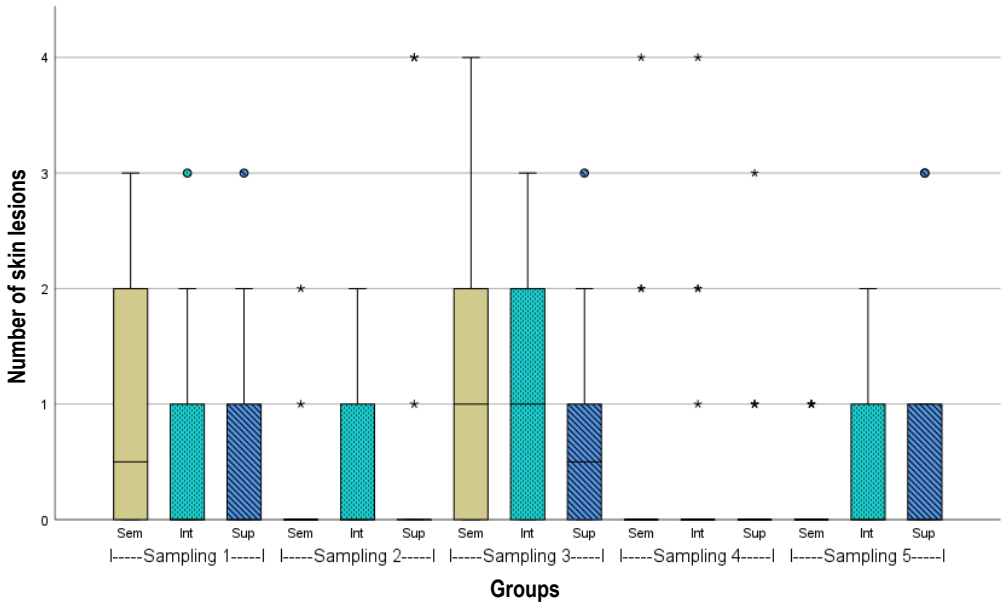

Figure 2.4: *Number of external injuries (skin lesions) of African catfish (n = 18) in three different stocking densities (Sem = semi-intensive, Int = intensive, Sup = super-intensive), p > 0.05. In sampling 3, in Sem, a value of 6, and in Sup a value of 8 is not illustrated. Circlets = outliers; asterisks = extreme values.*

Gene profiling focused on the third and fifth samplings, which were noticeable due to the high number of injured individuals. We hypothesized that skin lesions might entail not only stress but also immune responses. Therefore, we quantified the transcript number of 22 selected genes in the spleen tissue of either unstressed or stressed African catfish kept under semi-intensive or super-intensive conditions (Figure 2.5). The spleen was chosen as the target tissue because it responds to both immune and stress-related stimuli. For instance, it has been well proven that the spleen stores red blood cells under stress conditions and/or during metabolic alterations.

The obtained qPCR data revealed a moderate impact on the level of the selected transcripts. At the third sampling, only *kmt2a* was slightly (1.2- to 1.3-fold, $p < 0.002$) downregulated in both unstressed and stressed African catfish kept under super-intensive versus semi-intensive conditions and also in stressed versus unstressed individuals kept under both semi- and super-intensive conditions.

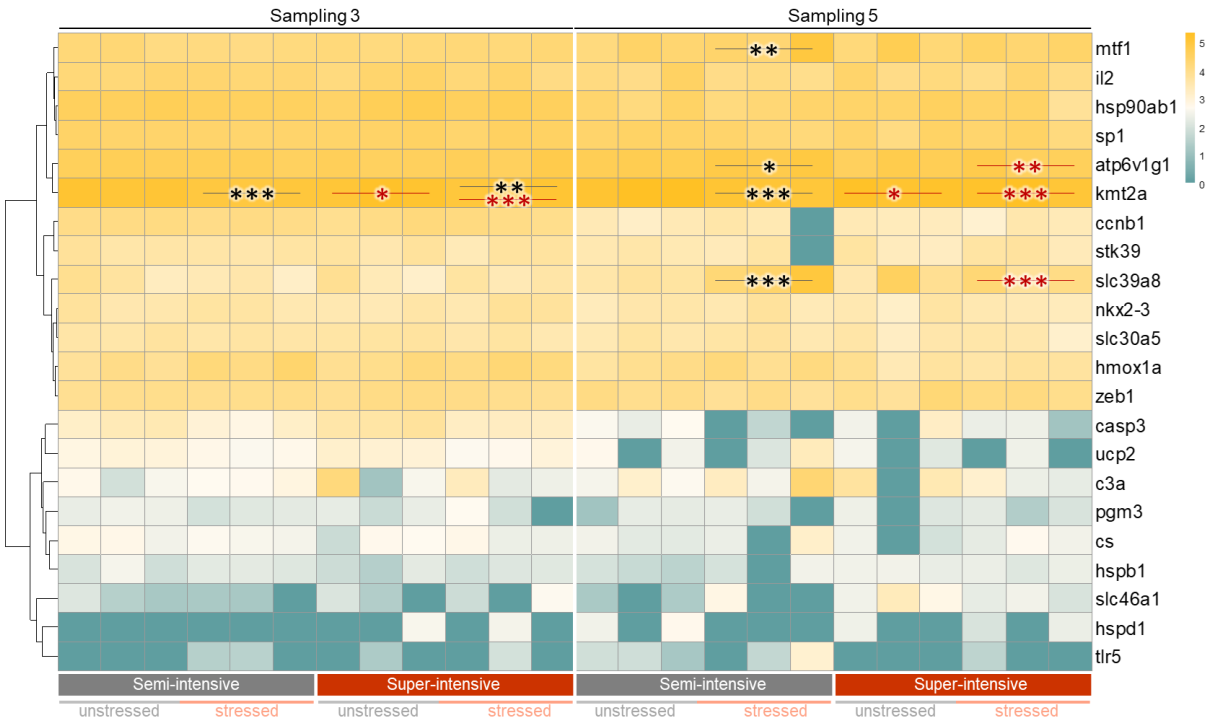

Figure 2.5: *Hierarchical clustering of log10-transformed transcript numbers measured in the spleen of individual African catfish. The fish were kept under semi-intensive or super-intensive conditions, and individuals of both groups were exposed to stress, while a cohort remained unstressed (indicated below the heatmap). The sampling time points are indicated above the heatmap. The transcripts quantified are listed as gene symbols on the right margin; transcript levels are colored according to the scale on the left. Black asterisk(s) indicate a different expression in the stressed group compared to the matching unstressed group of the same stocking density and at the same sampling timepoint; red asterisk(s) indicate a different expression in the super-intensive group versus the semi-intensive group under the same conditions (either unstressed or stressed) and at the same sampling timepoint (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).*

At the fifth sampling, *kmt2a* was again differentially expressed between both unstressed and stressed African catfish kept under super-intensive versus semi-intensive conditions, and also in stressed versus unstressed individuals kept under semi-intensive conditions; however, in each case, the deviation was only between 1.1 and 1.5 (with $p < 0.03$). Similarly low (1.4- to 1.6-fold) but statistically significant ($p < 0.02$) were the deviations of the *atp6v1g1* levels between stressed versus unstressed African catfish kept under semi-intensive conditions and stressed individuals kept under super-intensive versus semi-intensive conditions.

In contrast, *slc39a8* was this study's most strongly regulated differentially expressed gene. In stressed African catfish kept under semi-intensive conditions, *slc39a8* was 9.7-fold down-regulated (with $p = 0.008$) compared to the unstressed cohort, in stressed individuals

kept under super-intensive conditions, *slc39a8* was 3.9-fold up-regulated (with $p < 0.0001$) compared to the stressed catfish kept under semi-intensive conditions.

The levels of *mtf1* were moderately down-regulated by 2.2 (with $p = 0.008$) in stressed African catfish kept under semi-intensive conditions compared to unstressed fish. The transcript concentrations of the remaining 18 genes examined showed no significant alterations. Nevertheless, the 24-fold decrease in *casp3* transcripts (with $p > 0.05$) in stressed catfish kept under semi-intensive conditions compared to unstressed individuals may be worth mentioning.

2.4 Discussion

In the present study, the stocking density influenced the growth performance of African catfish under commercial rearing conditions. In addition, the effects of stocking density in relation to fish size, as well as external stress due to construction noise on different fish welfare indicators, were demonstrated.

In commercial RASs, African catfish can reach slaughter weight in about 6 months; in ponds, this may take longer (Hecht, 2013). The production time depends, in particular, on the water temperature and feed quality and quantity. In the present study, African catfish at all stocking densities reached appropriate slaughter weights after 23 weeks; 1 week earlier than expected. The water quality in the RAS was also found to provide suitable conditions for African catfish aquaculture without much potential to impair fish welfare. However, due to the very high stocking densities, this required an increased effort. Consequently, the cultivation conditions, as well as the growth performance, can be described as very good in each of the sampled groups.

Van de Nieuwegiessen et al. (2009) indicated no growth difference between African catfish from different stocking densities (96.9 – 485.6 kg m^{-3}) after the final growth phase from approximately 1000 – 1500 g $fish^{-1}$. In addition, partially contradictory findings have been reported. For instance, Toko et al. (2007) described the influence of rather extensive stocking densities (4 – 8 fish m^{-3}) on juvenile African catfish (approximately 35 – 150 g) in ponds, whereby growth performance significantly increased with increasing densities. However, other studies have reported lower growth with increasing densities in juveniles of approximately 30 – 100 g in tanks at 35 – 125 kg m^{-3} (Dai et al., 2011; Wang et al., 2013; Wang et al., 2017) or in pond cages at max. 63 kg m^{-3} (Hengsawat et al., 1997). According to Hecht

and Appelbaum (1987), particularly in early developmental stages (larvae) of African catfish, stocking density can have an impact on growth. Li et al. (2021) pointed out the heterogeneity of studies regarding the effect of stocking densities on growth.

In the present study, the super-intensively stocked fish showed the weakest growth performance, significantly different to the growth performance of semi-intensively stocked fish, which was approx. 13 % better. This was also visible when comparing the FCR or CI (Table 2.3, Figure 2.8). Consequently, this study comes to similar conclusions regarding the influence of stocking density on growth as several previous studies; it is the case, even in commercial-scale RAS culture, that African catfish tend to grow slightly less under high stocking densities than under extensive conditions. This clearly contradicts the study by Van de Nieuwegiessen et al. (2009).

Mortality or survival of African catfish, with respect to stocking density, has again been differently described. Van de Nieuwegiessen et al. (2008) reported a mortality rate of 2.5 % under aquarium conditions, regardless of stocking density. Akinwole and Faturoti (2007) described a survival rate of 75 – 93 % (conversely, 7 – 25 % mortality) for different developmental stages in commercial RASs. Palm et al. (2018b) found survival rates of 96 % for extensively (50 kg m^{-3}) to 81 % for intensively (200 kg m^{-3}) stocked fish. In the present study, mortality was not directly correlated with the increase in stocking density. The highest mortality was found in a super-intensive density tank, while it was overall lower in intensive and semi-intensive densities. However, African catfish survival rates can vary between different tanks, possibly due to differences in group composition, a factor that is difficult to control. Based on our experience, we see a higher risk of a greater percentage of African catfish dying under higher stocking densities. Unfortunately, in the present study it was not possible to investigate at what time how many fish died at each stocking density because the carcasses were quickly eaten by conspecifics.

Boerrigter et al. (2016) described the physiological stress-related indicators, plasma cortisol, glucose, and lactate, prior to and post stress in juveniles of African catfish (8 – 10 g). The basal levels (unstressed) for cortisol were at 6.5 – 15.3 ng mL^{-1}, for glucose at 3.7 – 5.4 mmol L^{-1}, and for lactate at 1.3 – 2.2 mmol L^{-1}, respectively. Directly, as well as 30 and 60 min after stressing, the fish plasma cortisol was at 12 – 48.5 ng mL^{-1}, glucose at 4.2 – 6.8 mmol L^{-1}, and lactate at 1.1 – 2.3 mmol L^{-1}, respectively. Martins et al. (2006a) described, in particular after stress induction, higher levels of more than 100 ng mL^{-1} for

plasma cortisol and up to 8 mmol L^{-1} for glucose. Manuel et al. (2014) depicted basal levels of cortisol for adults of African catfish (1 – 1.5 kg) at < 10 ng mL^{-1}; after handling, stress cortisol increased to 35 ng mL^{-1}, and after 3 h transportation to 50 ng mL^{-1}. These authors reported plasma glucose between approximately 3.5 – 6.5 mmol L^{-1}, with only minor elevations after stress induction. Van de Nieuwegiessen et al. (2009) reported basal lactate levels in adult African catfish (1 – 1.5 kg) with approximately 3 mmol L^{-1} and elevated levels of 3.6 – 4.9 mmol L^{-1} after stress.

In the present study, the mean plasma cortisol and glucose levels of fish from each stocking density were in comparable ranges. Significant or at least trended differences with about two-fold elevated cortisol levels were frequently found between stressed and unstressed fish within one sampling. Both the basal levels and the elevations of cortisol due to induced stress were considered regular, according to the observations of Boerrigter et al. (2016); thus, the different stocking densities had at least no effect on this stress indicator. This is in contrast to the findings of Van de Nieuwegiessen et al. (2008), according to which an impaired cortisol response was observed under the lowest and highest stocking densities and a down-regulation of ACTH or a depletion of the cortisol receptors due to chronic stress was suspected.

The overall glucose level can be considered relatively high in the measured ranges, but we assume that this could also be related to feeding, as earlier described by Polakof et al. (2012). In fact, feeding in our experiment was aimed at promoting excellent growth, which was achieved. In addition, we detected significant increases due to induced (acute) stress compared to basal levels and no differences between semi-intensively and super-intensively stocked fish, suggesting feeding was the reason for an overall high glucose level. The mean lactate values were still within the normal range, albeit some fish had slightly elevated values compared to the abovementioned references. The induced stress was inappropriate to affect lactate levels because no physical activity or hypoxic stress was imposed by it. Lactate, as a gluconeogenic precursor, is directly related to blood glucose (also elevated) and generally increases with increased physical activity and/or oxygen deficiency. Both may have occurred under the high stocking densities tested.

The number of skin lesions on African catfish has been recorded in several studies (Baßmann et al., 2017; Baßmann et al., 2020; Martins et al., 2006b; Palm et al., 2022; Van de Nieuwegiessen et al., 2008; Van de Nieuwegiessen et al., 2009). The number largely varied

between 0 – 5 per fish, depending on developmental stage and group composition. Studies addressing the area of skin lesions in African catfish are not known to the authors to date. In the present study, both the stocking densities and the respective developmental stage/fish size were relevant for the number and area of skin lesions. While early juveniles under semi-intensive stocking density had twice as many skin lesions on average as under intensive or super-intensive stocking density, it was reversed towards the end of the production period. Here, the matured fish under semi-intensive stocking densities showed very few skin lesions, while the number and area of bite wounds remained similar under intensive and super-intensive stocking densities. In any case, the occurrence of skin lesions may impair welfare. If possible, this should be considered for the different growth stages, also with regard to their current stocking density (Huntingford et al., 2006). This behavioral change was already evident from other studies. According to Kaiser et al. (1995a) and Van de Nieuwegiessen et al. (2008), juvenile African catfish frequently show agonistic interactions, particularly under low stocking densities. This decreases under higher densities where the fish form “dense clusters [. . .] with constant movement and low aggression” (Van de Nieuwegiessen et al., 2008) (p. 241). Information on adult African catfish is scarce, particularly at intensive or super-intensive stocking densities of commercial facilities. However, there is evidence that overall aggression decreases, resulting in fewer skin lesions (Van de Nieuwegiessen et al., 2008, 2009).

Van de Nieuwegiessen et al. (2008) described that skin problems and, consequently, infections might increase at stocking densities above 300 kg m^{-3}. Similar findings could not be generally confirmed in our study. However, a few fish, in fact, had to be removed from the super-intensive stocking density due to very severe and partially infected skin lesions.

The recording of somatic indices and the quantification of metabolic parameters of African catfish was complemented by gene-expression analysis to simultaneously quantify 22 genes associated with metabolism, immunity, and stress physiology. The transcript concentrations of most of these genes slightly changed or did not change significantly across the groups investigated, except for two genes. Both *slc39a8* and *mtf1* were significantly at least two-fold down-regulated in stressed African catfish kept under semi-intensive conditions compared to the unstressed fish. *Mtf1* encodes a transcription factor that is involved in cellular adaptation to a range of stress conditions (Günther et al., 2012). *Slc39a8* codes for a transporter-like protein that maintains zinc homeostasis (Jeong and Eide, 2013) and regulates

cell migration (Wu et al., 2017). It would be highly speculative to suggest possible functions of both gene products in African catfish, but the reduced levels of both *mtf1* and *slc39a8* might indicate an impending maladaptation of African catfish to adverse conditions. Previous single-gene expression analyses indicated few transcriptional parameters that could serve as potential indicator genes of stress responses in *Clarias sp*. (Kari et al., 2022; Swaleh et al., 2020)]. The present study adds *mtf1* and *slc39a8* to the list of potential indicator genes that might be included in future studies on the welfare of African catfish.

Coppola et al. (2006) described the noise as a physical stressor on animals leading to physiological, behavioral, and anatomical responses. It is well-known that anthropogenic noise affects many species (Kunc and Schmidt, 2019). Anthropogenic noise, which may include the background and partly loud noises of a mechanized aquaculture system, as well as other anthropogenic noise impacts (including construction and demolition noise), can negatively affect fish behavior, health, and welfare (Bart et al., 2001; Hang et al., 2021; Wysocki et al., 2006). This presupposes that acoustic waves are transmitted from air into water. In the second phase of the experiment, particularly during the time of the third sampling, all treatment groups showed an increase in plasma cortisol and glucose levels, with noticeably larger interquartile ranges and upper Whiskers, slightly flattened lactate levels, and a larger number of skin lesions (including an increase in injured surface area), indicating that the fish were generally affected or stressed. Apparently, endogenous stress responses occurred more frequently in this second phase under noise exposure. The tentatively decreased lactate levels may indicate altered behavior, such as a reduction in swimming activity. At the same time, the African catfish reacted more aggressively, as evidenced by the increased incidence of skin lesions. Most likely, the demolition noise raised the stress levels in the African catfish and led to behavioral alterations. Therefore, it is recommended that external stressors in aquaculture, including loud noises, need to be avoided, especially if they persist for an extended period, preventing a chronic stress situation for the fish. It could have negative consequences for their wellbeing, health, and growth.

2.5 Conclusions

The growth of African catfish was very high at all stocking densities. However, at the end of the experiment, the semi-intensively stocked fish had significantly higher weights (by about 13 %) than the super-intensively stocked fish. This study further demonstrates that, particularly for early juvenile life stages of African catfish, intensive or super-intensive stocking density, whereas for matured fish, semi-intensive stocking density results in less aggression and fewer skin lesions. In addition, the fish welfare investigation revealed evidence of the influence of the demolition noise that occurred. We, therefore, suggest an adaptation of stocking density during the growth phases of African catfish after size-grading and the avoidance of external stressors in aquaculture to enhance production efficiency and fish welfare.

2.6 Appendix

Table 2.5: *Oligonucleotide-primer sequences derived from Clarias gariepinus, Ictalurus punctatus, or Pangasianodon hypophthalmus.*

Gene Symbol	Gene Product	Function	Sense Primer (5'→3'), Antisense Primer (5'→3')	Source (Species; Accession Code)	Amplicon Length (bp)
Reference genes:					
rna18s	18S ribosomal RNA	Structure of eukaryotic ribosomes	CTCTGCTGGACGATGGCTTAC, TCGATGAAGAACGCAGCCAGC	*C. gariepinus*; GQ465239	94
actb	Actin-beta	Cell structure and motility, intercellular signaling	ACCACCACAGCCGAGAGAGAA, CTTCCAGCCATCTTTCCTTGGT	*C. gariepinus*; EU527191	204
gapdh	Glyceraldehyde-3-phosphate dehydrogenase	Carbohydrate metabolism	TATGAAGCCCGCTGAGATCCC, GCCTCTTCTCACTTGCAGGGT	*C. gariepinus*; AF323693	106
rpl	Ribosomal protein, large subunit	Structure of eukaryotic ribosomes	ACTAAATAGCAACTGATCCCTATC, GAATATCTGACCACTAAGATCCG	*C. gariepinus*; MW080924	134
Target genes:					
atp6v1g1	ATPase H+ transporting v1 subunit g1	Intercellular Fe homeostasis	CGGAAAAACCGCCGCTTGAAG, GACCAAGGAAGCCGCGGCAC	*P. hypophthalmus*; XM_026922532	106
c3a	Complement component 3, variant a	Bacteria opsonisation and destruction	ATGTCTTTCGATGTCACGGTTTAT, TCGAACCAAGAGTAACGGCATG	*I. punctatus*; XM_017457024	114
casp3	Caspase 3	Apoptosis	CTCTTTATCATTCAGGCTTGTCG, GTACTCTACTGCTCCAGGTTATT	*I. punctatus*; XM_017473312	139
ccnb1	Cyclin b1	Control of the G2/M transition phase of the cell cycle	TCAAAAATCGGAGAGGTTACAGC, TGCACTTTGCTCCCTCTCTGG	*I. punctatus*; NC_030443	103
cs	Citrate synthase	Aerobic metabolism	GGTGGTGAAGTGTCCGATGAAA, GCTATGGGCATGCTGTCCTGA	*I. punctatus*; XM_017487510	94

Gene Symbol	Gene Product	Function	Sense Primer (5'→3'), Antisense Primer (5'→3')	Source (Species; Accession Code)	Amplicon Length (bp)
hmox1a	Heme oxygenase 1	Cellular response to xenobiotic stimulus	GATTCTTCTGTGTTCCCTGTATG, CCATCTACTTCCCTCAGGAGC	*I. punctatus*; XM_017491622	104
hsp90ab1	Heat-shock protein 90 alpha family class b member 1	Chaperone function, stress response	GAACATCAAGCTGGGCATCCAT, TTACTACATCACTGGTGAGAGCA	*I. punctatus*; XM_017456214	167
hspb1	Heat-shock protein family b (small) member 1	Differentiation of cell types, stress response	ACAGGACAACTGGAAGGTGAAC, GATTATCGGAAACCATGAGGAGA	*Clarias batrachus*; KT359728	107
hspd1	Heat shock protein family d (hsp60) member 1	Chaperone function, stress response	GCACGCTTGTCCTCAACAGGTT, AGACATGGCGATTGCTACTGGA	*I. punctatus*; XM_017469365	113
il2	Interleukin-2	Activation and proliferation of lymphocytes	GTCGGCCTGGGAAAAAGCCAAT, TTATGTGTTTGCACCAGACAACG	*I. punctatus*; XM_017474923	162
kmt2a	Lysine-specific methyltransferase 2a	Regulation of early development and hematopoiesis	ATTGGGTCGAAATCGTGCTGTAT, ATGATAAGTCTTCAGTGGCAGGT	*I. punctatus*; XM_017490460	121
mtf1	Metal regulatory transcription factor 1	Catabolic regulation of cartilages	GTAGGAGGGCATTCAGGGAAC, AGTCAGAACGCTGCCCCCTC	*I. punctatus*; XM_017475296	146
nkx2-3	NK2 homeobox 3	Cell differentiation	TACAGGACAACCTGGTGGAAAG, ACAACTCTTGGTTTCCTGCTCTT	*I. punctatus*; XM_017464595	119
pgm3	Phosphoglucomutase 3	Carbohydrate metabolism	GACACAGGCAGGGCTGAATCT, CTTCGTACAGCACACTGTAACC	*I. punctatus*; XM_017494096	112
slc30a5	Solute carrier family 30, member 5	Zinc transportation	AATAGTCACCAAAAGACAGTGGAT, CATCGTTGTGCTCGAACAACAG	*I. punctatus*; XM_017459891	134
slc39a8	Solute carrier family 39, member 8 alias ZIP8	Cellular zinc uptake, protection from inflammation-related injury and death	TTTAACCTGATCTCAGCCATGTC, TATGTTCCCTGAGATGAATGCCA	*I. punctatus*; XM_017489708	151
slc46a1	Solute carrier family 46, member 1	Folate transportation	AATGGCGACATGCACAAGGGTAT, AGAACAGCCTTGCCCCAGGG	*I. punctatus*; XM_017491375	129
sp1	SP1 transcription factor	Cell growth, apoptosis, differentiation, and immune responses	AGCACAGCAGGTGATCAGGGA, GAGAAGCGTGCACATGTCCATA	*I. punctatus*; XM_017450095	119
stk39	Serine/threonine kinase 39	Stress response	TGTAGTTGTTGCTGCTAACCTTC, AGATCCCTGACGAGGTGAAGC	*I. punctatus*; XM_017469076	116
tlr5	Toll-like receptor 5	Detection of bacteria	GGCAGCATGGGAAAGGGAGTT, GTTAAGGCTCTGGATCTGTCCA	*I. punctatus*; NM_001200229	103
ucp2	Uncoupling protein 2	Regulation of production of reactive oxygen species, function of mitochondria	GGCTCCAGATCCAAGGGGAGA, CCACGTAGTCTCTACAACGGG	*I. punctatus*; XM_017489367	131
zeb1	Zinc finger e-box binding homeobox 1	Repression of interleukin-2 function	GCAGAGACCAGCGGCATGTAA, ATACGAGTGCCCCAACTGTAAAA	*I. punctatus*; XM_017483097	156

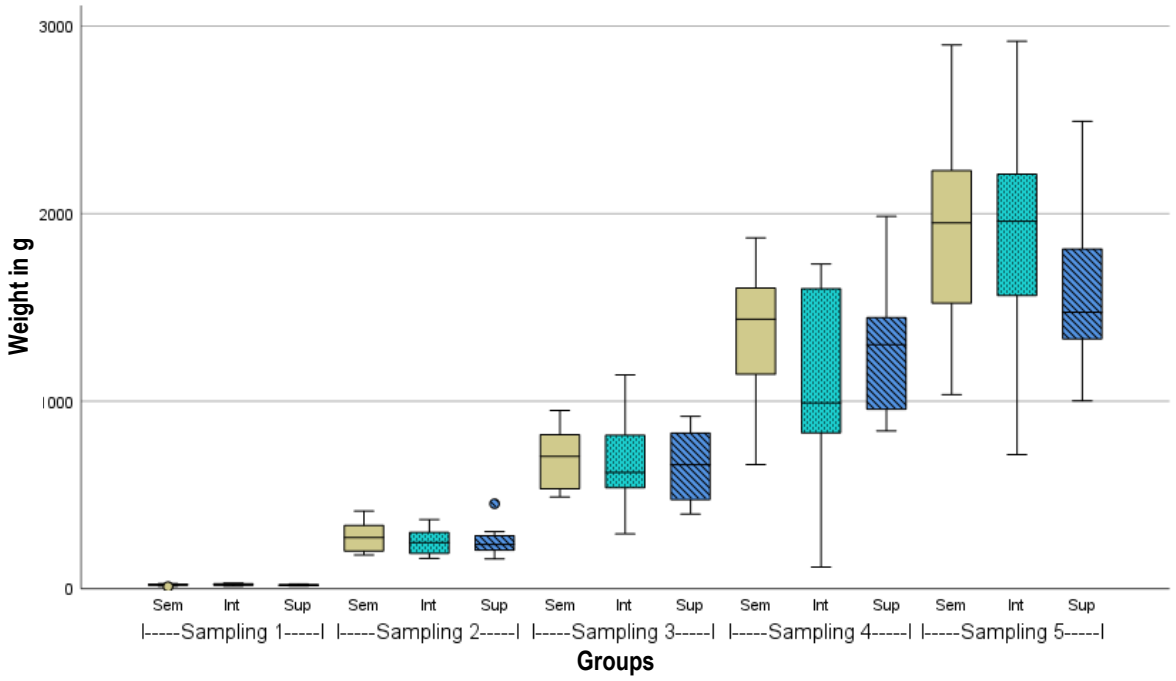

Figure 2.6: *Weight gain of African catfish from three different stocking densities (Sem = semi-intensive, Int = intensive, Sup = super-intensive) over 23 weeks, n = 18, Circlet = outlier.*

Table 2.6: *Cortisol: Relevant significances within a single sampling between unstressed and stressed fish in a treatment group and between unstressed or stressed fish in a treatment group between different samplings. Sem = semi-intensive; Int = intensive; Sup = super-intensive; the number before the stocking density abbreviation indicates the respective sampling; u = unstressed, s = stressed.*

Group Comparison	*p*-Value	Group Comparison	*p*-Value	Group Comparison	*p*-Value
Within a sampling		**Within a sampling**		**Within a sampling**	
2Sem-u-2Sem-s	0.022	1Int-u-1Int-s	0.047	2Sup-u-2Sup-s	0.038
4Sem-u-4Sem-s	0.043	**Between samplings**		**Between samplings**	
5Sem-u-5Sem-s	0.032	1Int-u-2Int-u	0.021	1Sup-u-2Sup-u	0.040
Between samplings		1Int-s-2Int-s	0.026	1Sup-s-2Sup-s	0.000
1Sem-u-2Sem-u	0.042	1Int-u-3Int-u	0.000	1Sup-u-3Sup-u	0.000
1Sem-s-2Sem-s	0.006	1Int-s-3Int-s	0.000	1Sup-s-3Sup-s	0.000
1Sem-u-3Sem-u	0.000	1Inti-u-5Int-u	0.000	1Sup-s-4Sup-s	0.049
1Sem-s-3Sem-s	0.000	2Int-u-3Int-u	0.003	1Sup-u-5Sup-u	0.000
1Sem-u-5Sem-u	0.001	2Int-u-5Int-u	0.043	1Sup-s-5Sup-s	0.000
1Sem-s-5Sem-s	0.000	4Int-s-2Int-s	0.048	4Sup-s-2Sup-s	0.031
2Sem-u-3Sem-u	0.000	4Int-u-3Int-u	0.000	4Sup-u-3Sup-u	0.002
4Sem-u-3Sem-u	0.000	4Int-s-3Int-s	0.000	4Sup-s-3Sup-s	0.002
4Sem-s-3Sem-s	0.004	4Int-u-5Int-u	0.001	4Sup-u-5Sup-u	0.001
4Sem-u-5Sem-u	0.018	4Int-s-5Int-s	0.004	4Sup-s-5Sup-s	0.008
4Sem-s-5Sem-s	0.013				
5Sem-u-3Sem-u	0.021				

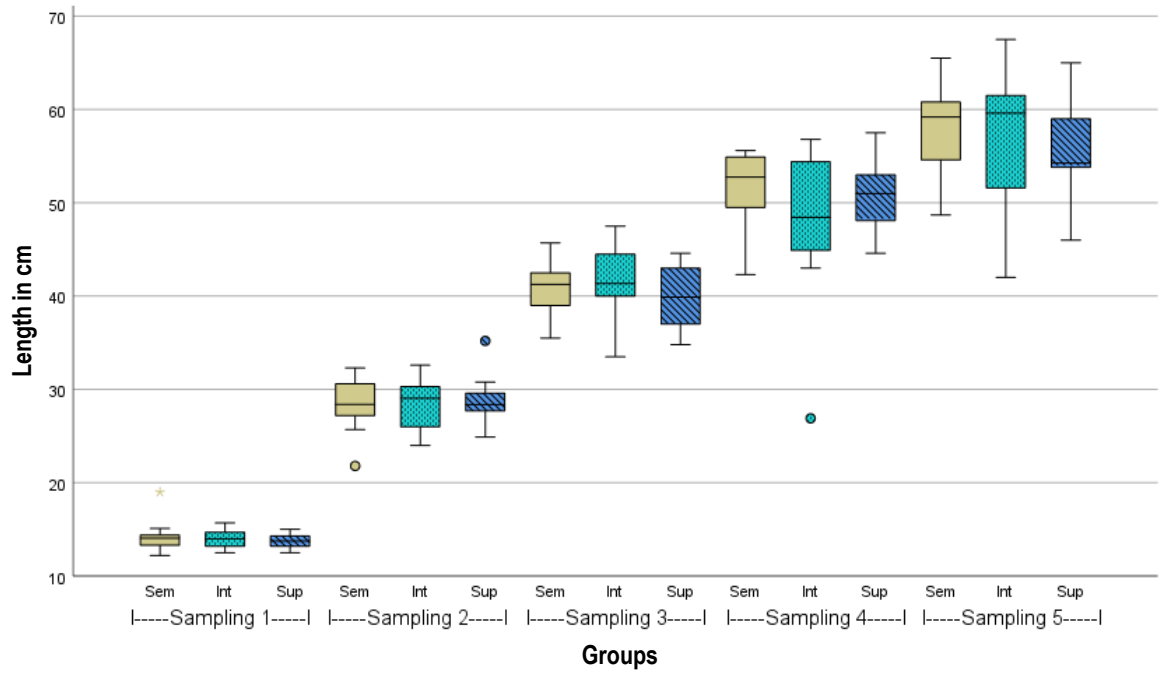

Figure 2.7: *Total body length of African catfish from three different stocking densities (Sem = semi-intensive, Int = intensive, Sup = super-intensive) over 23 weeks, n = 18, p > 0.05 in respective samplings. Circlets = outliers, asterisk = extreme value.*

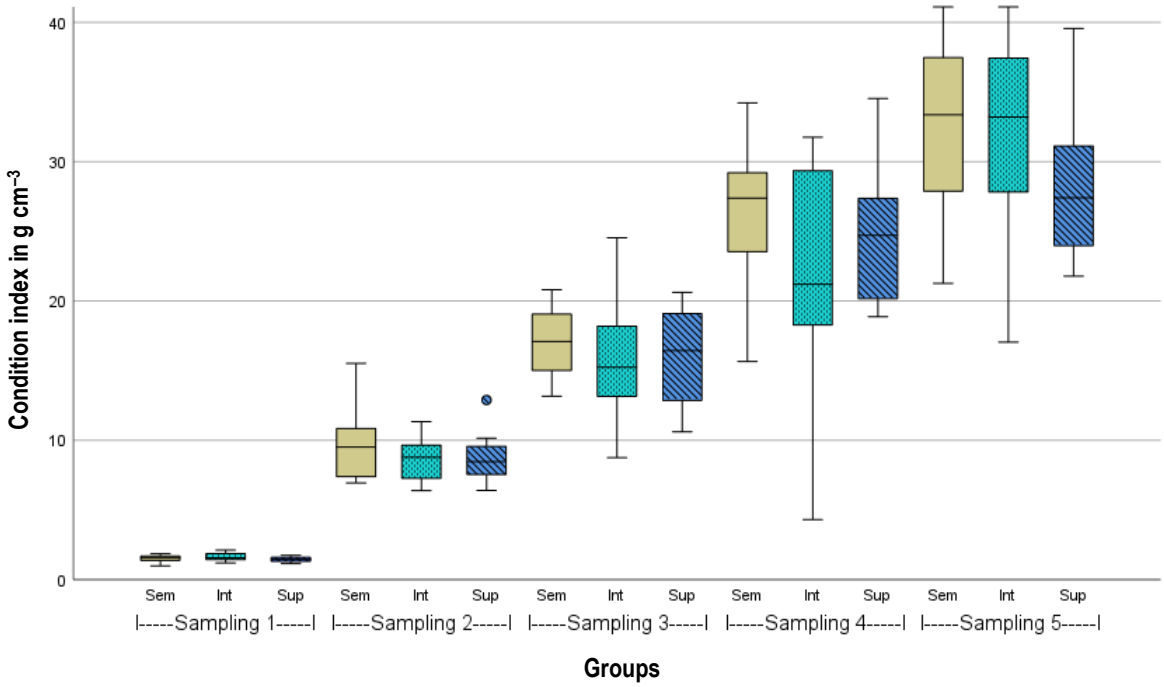

Figure 2.8*: Condition indices of African catfish from three different stocking densities (Sem = semi-intensive, Int = intensive, Sup = super-intensive) over 23 weeks, n = 18, p > 0.05 in respective samplings. Circlet = outlier.*

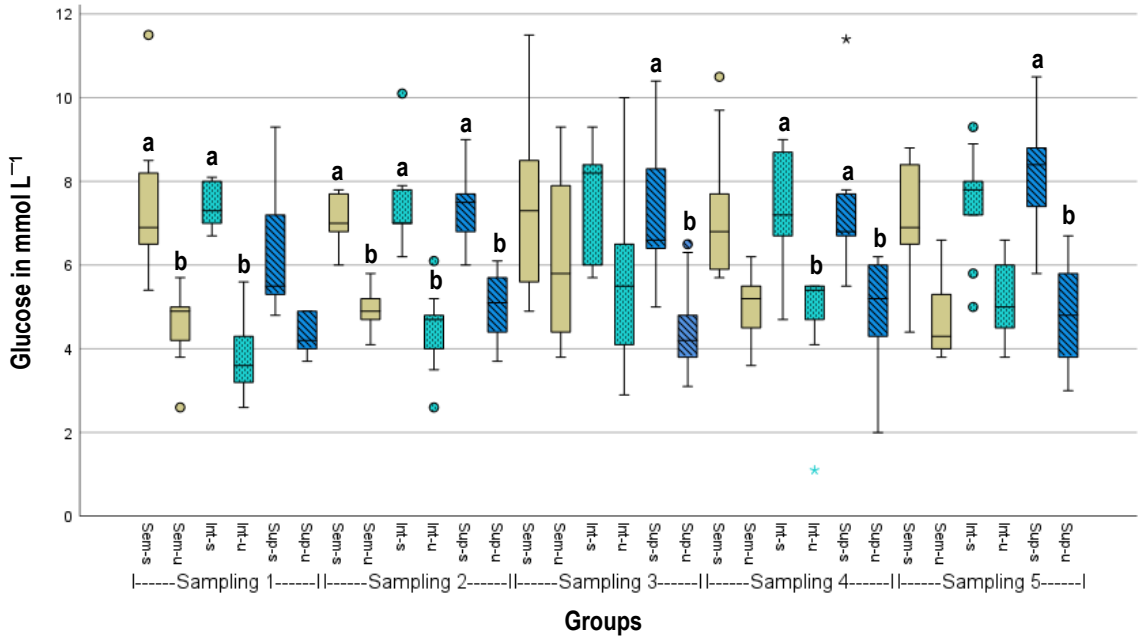

Figure 2.9: *Glucose levels of stressed (s) or unstressed (u) African catfish (n = 9) under three different stocking densities (Sem = semi-intensive, Int = intensive, Sup = super-intensive). Kruskal-Wallis test with Tukey-HSD or Dunnett-T3 post hoc test, significances (p < 0.05) are only given within the respective samplings, marked by letters. Circlets = outliers; asterisks = extreme values.*

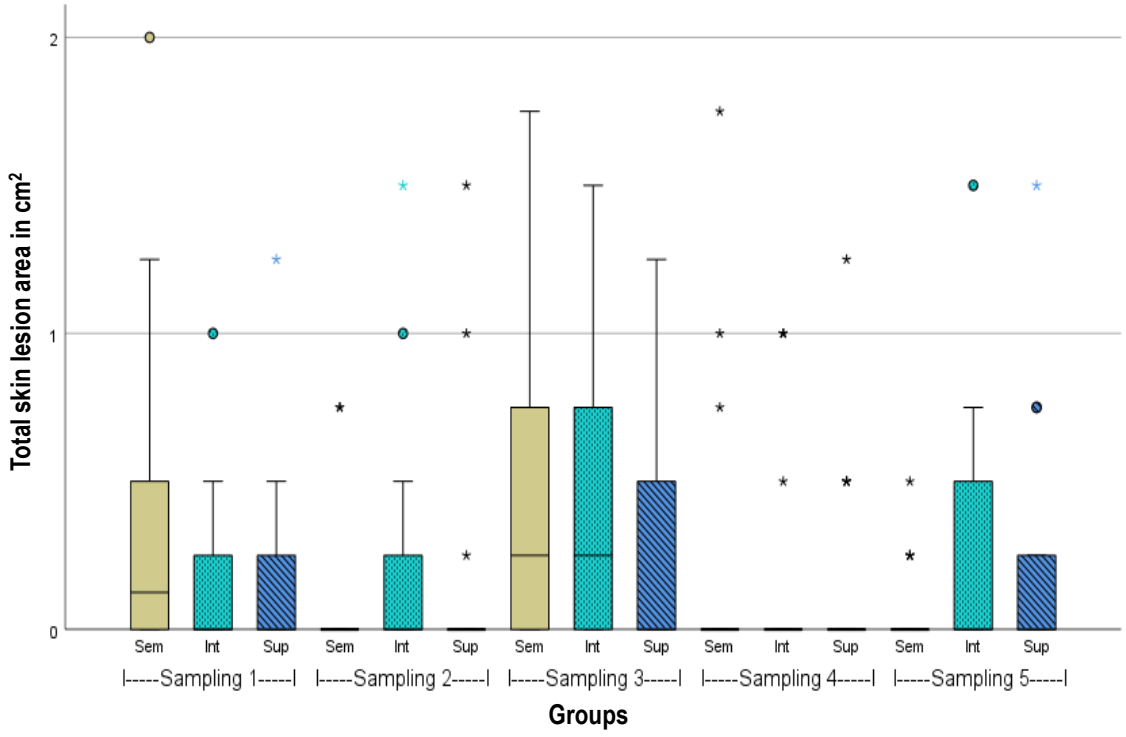

Figure 2.10: *Total skin lesion area of African catfish in three different stocking densities (Sem = semi-intensive, Int = intensive, Sup = super-intensive), n = 18, p > 0.05. In sampling 3, in Sem, a value of 3, and in Sup a value of 4 is not illustrated. Circlets = outliers; asterisks = extreme values.*

3 Effects of Stocking Density and Grading on African catfish (*Clarias gariepinus*) Fry

Abstract

African catfish hatcheries in Europe require economical fry availability throughout the year. The purpose of this study was to identify the best suitable stocking density and appropriate grading regime for African catfish fry of around 0.1 g in 3 experiments (E1, E2, and E3). In E1, 3 stocking densities (10, 20, and 30 fry L^{-1}) without grading were compared for behavior, mortality, cannibals, and growth. In E2, additional daily manual grading removed shooters or potential cannibals. In E3, 3 different handling procedures (manual grading on days 2, 4, 6, and 8: 2dH, manual grading on day 5: 5dH, self-grading on day 5: 5dS) under highest stocking density (30 fish L^{-1}) were applied. Each experiment lasted 10 days, and the fish had a mean initial weight of 0.08, 0.06, and 0.06 g $fish^{-1}$, respectively. In E1 and E2, stock activity increased with increasing stocking density, but aggressive behavior was lowest at the higher stocking densities. The stocking density had no significant effect on mortality rates or growth performance from 10 to 30 fish L^{-1} between groups. However, regular removal of cannibals reduced the total mean mortality rate from 25 % in E1 to less than 5 % in E2 and E3. The mean feed conversion ratio (FCR) improved from 0.68 in E1 to ≤ 0.55 in E2 and E3. The tested handling procedures revealed no significant differences in fry behavior and performance, while final stocking densities increased with decreasing handling stress. This suggests that African catfish fry of about 0.1 g are kept optimally at high stocking densities of 30 fish L^{-1} or 30,000 fry m^{-3} inside regular breeding containments, with effective self-grading every 5 days in order to reduce total mortality, maximize production and minimize the amount of labor.

Keywords

African catfish fry, Stocking density, Grading, Cannibalism, Mortality, Behavior

3.1 Introduction

African catfish hatcheries in Europe face an uphill struggle to remain economically viable. The underlying reasons are scarcity of customers, a low selling price, and the lack of qualified personnel. Feed accounts for 42.14 % of the total costs and 61.42 % of the variable costs (Pasch and Palm, 2021). In addition, all hatcheries in Europe are operated as warm water recirculating aquaculture systems (RAS). In combination with insufficient technological standards and

temperature requirements of around 28 °C, this results in relatively high production costs. In Germany, the fish normally remain inside the hatchery up to a weight of 10 g before being sold to the grow-out farms. In order to minimize production costs, maximum output has to be guaranteed permanently and reliably. For this purpose, operators must maximize growth and survival by working with optimal stocking densities and consideration of the fish behavior.

According to Van de Nieuwegiessen et al. (2008, 2009), different stocking densities are best suitable for different age groups in older African catfish (10 – 1500 g). For fish with a mean weight between 10 and 100 g, he observed best results at medium stocking densities of around 1750 fish m^{-3} (tested range 500 – 3000 fish m^{-3}) (Van de Nieuwegiessen et al., 2008). Fish at this age showed more aggressive behavior at lower stocking densities, while after induced netting stress, more biting wounds occurred at highest densities. By contrast, fish between 100 and 300 g showed an improved welfare status with increasing stocking density (tested final stocking densities 20 – 320 kg m^{-3}). This is expressed by an increased activity, improved specific growth rate, and reduced aggressive behavior. In fish between 1000 and 1500 g, stocking density (tested stocking densities 100 – 500 kg m^{-3}) showed no effect on growth, physiological and behavioral responses (Van de Nieuwegiessen et al., 2009). Kaiser et al. (1995b) showed that stocking density linked to the bottom surface area influences aggressive behavior of African catfish larvae. He observed highest aggression at medium stocking densities of 0.6 larvae cm^{-2}. Considering African catfish fry, Haylor (1992) investigated stocking densities (initial weight: 0.02 g $fish^{-1}$) between 50 and 250 fish L^{-1}. He found a negative density dependence for growth but no influence on mortality. Hossain et al. (1998) ran an experiment with stocking densities of 5 and 10 fish L^{-1} (0.79 g $fish^{-1}$) and observed that the growth rate was lower at higher stocking densities. Though several studies examined the stocking densities of African catfish larvae and fry (Almazán-Rueda et al., 2004; Haylor, 1991; 1993; Hecht and Appelbaum, 1988; Hossain et al., 1998; Josiah et al., 2012; Kaiser et al., 1995a, 1995b; Kareem and Olanrewaju, 2015; Nwipie et al., 2015), data are still lacking for the early life cycle stages, and grading has not been considered.

Survival rates of African catfish in hatcheries are highly influenced by cannibalism, that can reduce the survival rate to less than 2 % within 4 weeks (Abdelhamid et al., 2010). The first cannibalism already occurs 3.5 d after exogenous feeding (Hecht and Appelbaum, 1988). Cannibalism is influenced by genetics (Truong et al., 2020; Yang et al., 2015), feeding (Katavić et al., 1989), light (Appelbaum and Arockiaraj, 2010), refuges (Qin et al., 2004) and stocking

densities (Corrêa and Cerqueira, 2007). Abdelhamid et al. (2010) could demonstrate that grading of African catfish fry with an initial weight of 0.38 g $fish^{-1}$ every 3 days to remove shooters can increase the survival rate by 41 % within 4 weeks. Mollah et al. (1999) found a 49.6 % lower cannibalism-induced mortality within 15 days for fish with an initial mean weight of 3.98 mg when grading was conducted every 5 days. For fish with an initial weight between 0.7 and 0.9 g, Mwangi et al. (2020) observed a reduction of 34.9 % cannibalism-induced mortality within 8 weeks when fish were graded every 2 weeks. Besides the simple removal of shooters, grading is also used to divide one fish group into 2 or more size-sorted groups. That leads to a smaller size heterogeneity within a group, resulting in less cannibalism (Umanah, 2019).

Evaluation of fish behavior is a useful tool to assess appropriate stocking densities that influence fish activity, air-breathing events, or aggressive interactions. Aggression, for example, may be accompanied with a higher number of biting wounds (Baßmann et al., 2020), skin lesions (Almazán-Rueda et al., 2004), and increased mortality (Kaiser et al., 1995b). Regular grading increases survival rates but also results in stress for the fish, is very time-consuming and costly, and therefore must be applied as rarely as possible but as often as necessary. Though different stocking densities and grading influence fish behavior, growth, and mortality of African catfish, there is no generally available protocol for optimal hatchery management in intensive RAS production in Northern Germany so far. We, therefore, tested the following hypotheses in 3 consecutive experiments using African catfish fry with initial weights between 0.06 and 0.08 g:

1. The stocking density affects behavior, survival, and growth, without having an effect on the percentage of shooters or potential cannibals in African catfish fry. (E1)
2. Constant removal of shooters or potential cannibals influences mortality rates of the fry, independent of stocking density. Mortality rates decrease with cannibals' removal. (E2)
3. Different handling procedures (to remove shooters or potential cannibals) of the fry under optimal stocking densities influence survival and growth because the success of removing shooters or potential cannibals differs. (E3)

3.2 Materials and Methods

3.2.1 System Design

African catfish hatcheries require hatching tanks for the hatch and larval development until 0.1 g, breeding tanks or grooves from 0.1 g to 0.5 – 1 g, and juvenile tanks until 5 – 10 g. The experimental breeding tank RAS (total water volume: 1290 L) at the University of Rostock consisted of a sump (120 L) with 3 identical pumps (AQUA MEDIC DC-Runner 5.3, AB Aqua Medic GmbH, Bissendorf, Germany) pumping water to 9 identical aquaria (130 L water volume per aquarium) in triplicates. The water returned passively through a solid separation compartment into the sump, constructed with filter pads (15 L, 20 ppi). The water passed the pads before it was pumped back into the aquaria. A flow-through heater (Hydor HEATER 300–16, Ferplast S.p.A, Castelgomberto, Italy) and a UV light (JBL AquaCristal UV-C II 36 W, JBL GmbH & Co. KG, Neuhofen, Germany) were connected to the sump as bypass. In addition, the sump contained a pump (AQUA MEDIC DC-Runner 3.3, AB Aqua Medic GmbH, Bissendorf, Germany) connected with the drain for water exchange. The biological treatment (Hamburger Mattenfilter) was conducted by filter pads (20 ppi) inside the aquaria (29 L filter material per aquarium). In each aquarium, 30 L were delimited with the help of the mats to create the cultivation area (l x w x h = 21 × 47 × 31 cm). Each aquarium was additionally equipped with an aquarium heater (EHEIM 3616 Aquarium Heater, EHEIM GmbH & Co. KG, Deizisau) and 2 aeration stones (1 ×50 mm Ø, 1 ×80 mm Ø), connected to a membrane pump (HIBLOW HP-200, Techno Takatsuki CO., Japan). One additional aquarium (water volume 80 L) served for pre-temperation for water exchange (AQUA MEDIC DC-Runner 3.3, AB Aqua Medic GmbH, Bissendorf, Germany) into the emptied sump. 25 cm above each aquarium, an artificial light bar was attached (SolarStinger SunStrip 70 FRESH, Econlux GmbH, K˙oln, Germany). All pumps and lights were connected to a computer (ProfiLux® 4 Aquariencomputer, GHL, Kaiserslautern, Germany) and automatically controlled, resulting in an automated water exchange and light regime (Figure 3.1).

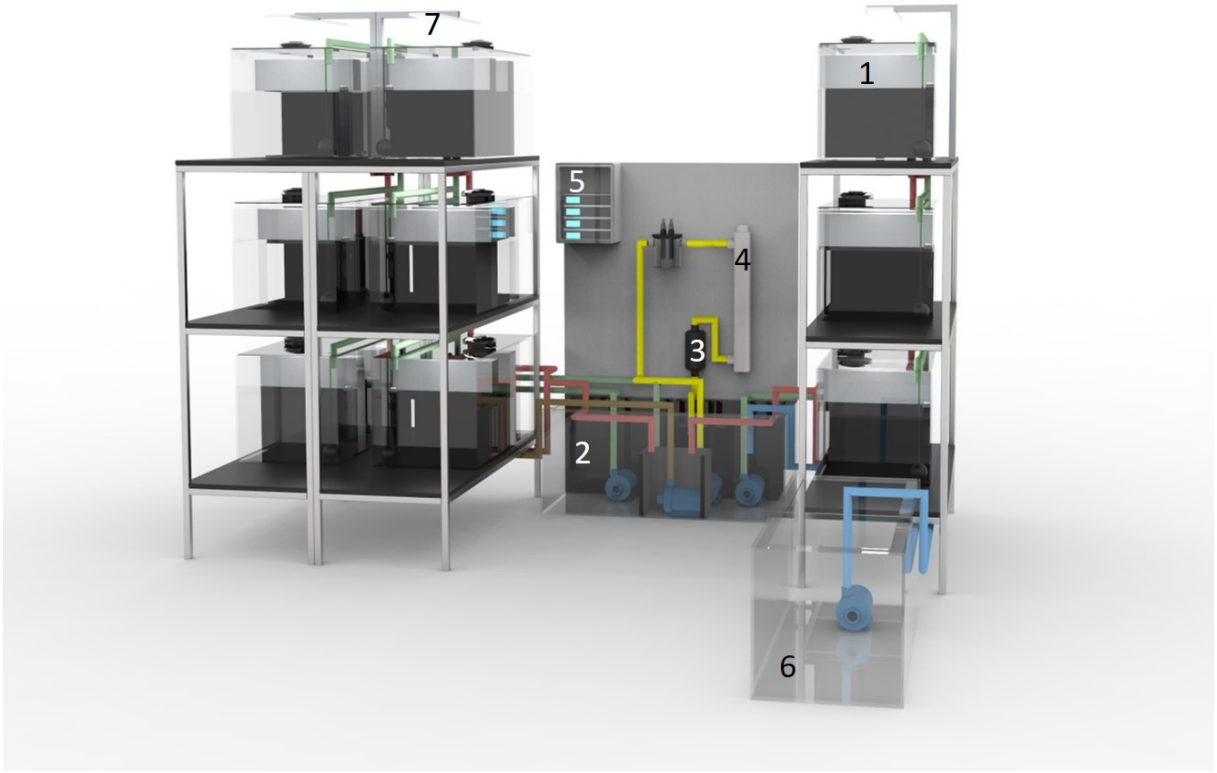

Figure 3.1: *Overview of the experimental recirculating aquaculture system. 1. Aquarium, 2. Sump with pumps and solid separation compartment, 3. Flow through heater, 4. UV-C light, 5. Computer to control the light and water exchanges, 6. Separate aquarium for water exchange, 7. Light bar.*

3.2.2 Fish

African catfish fry was bought from two commercial suppliers. The fish for E1 were supplied by ZEBCARE (Nederweert, Netherlands), and fish for E2 and E3 by Bioenergie Lüchow GmbH & Co. KG (Altkalen, Germany). In E1, E2, and E3, the initial mean weights were 0.079, 0.059, and 0.057 g fish^{-1}. At this time, the fish were 17, 19, and 18 days old. At stocking, the experimental holding compartments were stocked in turn 3 times with one-third of the total fish amount to make sure the average weight in each tank was the same (all fish were taken out from one presorted tank; removal of shooters if necessary). The initial average weight and length were determined by weighing and measuring 3 times 33 fish from the pre-sorted tank (at the beginning, after the first third of each aquarium, and after the second third of each aquarium). These fish were not used for stocking but were seen as representatives for the average weight and length. That was done to avoid weighing all fish which would have resulted in a high mortality, possibly influencing the subsequent experiments.

3.2.3 Experimental Conditions

The water temperature was around 29 °C, and the flow through of each aquarium was set to 3.1 L min^{-1} in E1 and 5 L min^{-1} in E2 and E3. The light bars simulated natural sunrise and sunset (8 h complete darkness, 8 h increase in light intensity, at 12 p.m. strongest light intensity with 2915 lux at 50 cm distance Ra = 82, CCT = 6902 K), 8 h decrease in light intensity). Each Experiment lasted for 10 days with a water exchange of 70 L on days 3, 6, and 9. The fish were first fed 12 h after stocking with a commercial diet for African catfish (Advance 0.3 – 0.5 mm, Alltech Coppens) between 6 % and 7 % of body weight per day. Daily feeding was done by an automated feeder (Grässlin, Rondomatic 400) every 6 h at 6:00 and 12:00 a.m. and p.m.

3.2.4 Experimental Setup

All experiments lasted for 10 days (day 1: stocking, day 10: final sampling) and were performed in triplicates and randomized block design. In E1 fish behavior, fish performance (mortality rate, percentage of shooters or potential cannibals, feed conversion ratio = FCR, Fulton's condition factor = K, specific growth rate = SGR, final weight, final length) and final stocking densities were compared between 3 groups, differing in stocking densities: 10, 20 and 30 fish L^{-1} (≙ 300, 600 and 900 fish per aquarium). E2 was carried out in the same way as E1, consisting of 3 groups with stocking densities of 10, 20, and 30 fish L^{-1}. In contrast to E1, all aquaria were graded daily from day 2 – 9 between 9 and 10 am, at least 3 h after the last feeding. Grading was conducted manually with a 4 mm bar grader to remove shooters or potential cannibals. Those were defined as faster-growing fish individuals that reached distinctly larger fish size and weight (approximately ≥ 30 %) than the remainder of the fish inside the aquaria and could be separated by a simple grading regime. The grader was newly designed with a CAD software (Solid Edge, Siemens PLM Software) and printed with PETG in a 3D printer (RAISED3D Pro2, Production ToGo, Karlsbad, Germany). The grader had the same width as the holding compartment and an angle at the lower end and was drawn through the aquarium, similar to a shovel Figure 3.2. The smaller fish swam or fell through the gaps, while the big ones remained inside the grader and were shoveled out and removed. In E3, all aquaria were stocked with 30 fish $L^{-1,}$ and 3 different grading regimes were performed: 2dH: Manual grading every 2 days (day 2, 4, 6, and 8), with the same method as in E2; 5dH: Manual grading on day 5 with the same method as in E2; 5dS: Grading on day 5 with a self-grading system. For

self-grading, a standing grader was newly designed with the CAD software and printed with PETG in the 3D printer. The self-grader had trap-shaped elongated 4 mm openings that the smaller fish were able to pass in one direction, and the bigger fish were not able to pass at all Figure 3.3. The self-grader had the same width as the holding compartment of the aquarium and was drawn from one side to the other, stopping 5 cm before reaching the other side, resulting in an accumulation of the fish in the 5 cm zone. Then the rear area was darkened with a light impermeable plastic plate to attract the fish to swim into this compartment, as African catfish fry prefers dark conditions (Britz and Pienaar, 1992). The self-grader was left untouched for 30 min. The rest of the fish in the 5 cm zone was removed with a net and brought through the normal bar grader to separate the left small fish from the shooters or potential cannibals, joining them with the other fish in the aquarium.

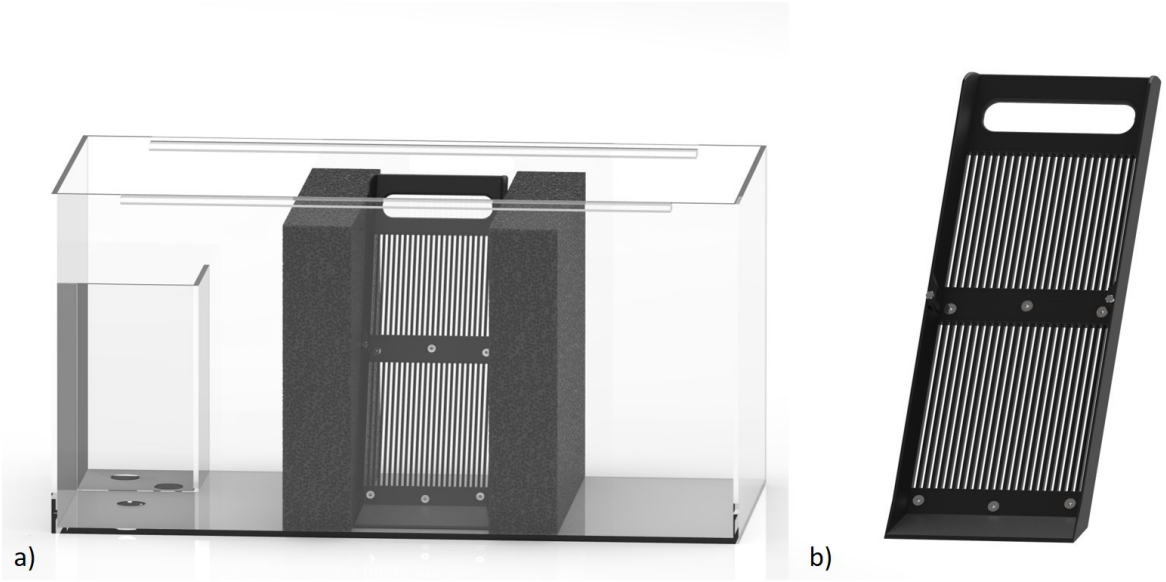

Figure 3.2: *a) Manual grader inside the cultivation area of the aquarium during grading. b) Total view of the manual grader.*

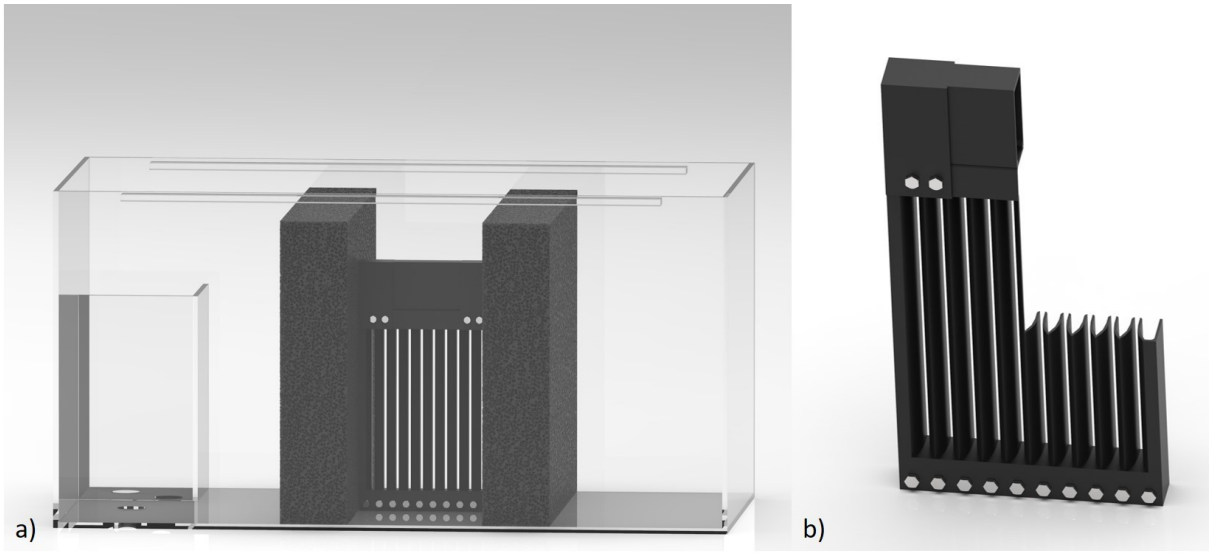

Figure 3.3: *a) Self-grader inside the cultivation area of the aquarium during grading. b) Overview with a cross-section of the grading bars of the self-grader.*

3.2.5 Sampling

Physicochemical water parameters (oxygen concentration, temperature, pH, electrical conductivity (EC), and salinity) were measured daily in each aquarium by using a multimeter (HQ40d, HACH®, Düsseldorf, Germany). Additionally, water samples were taken 3 times during each experiment directly before the water exchange. The samples were stored at – 18 °C in a freezer until they were analyzed for the nitrogen compounds NH_4^+ (analytical method includes NH_3 and NH_4^+), NO_2^-, and NO_3^- (in this method, the analyzer calculates the theoretical NO_3^- concentration from the available N that is bound as NO_3^- and NO_2^-) with an automated photometric analyzer (Gallery™ Discrete Analyzer, Thermo Fisher Scientific, Waltham, MA, USA). The analyses were performed according to the manufacturer's protocols. From these results, TAN (total ammonia nitrogen) [3.1], TON (total oxidized nitrogen) [3.2], NO_2^-–N [3.3], NO_3^-–N [3.4], and TDN (total dissolved nitrogen) were calculated [3.5].

Total ammonia nitrogen (TAN):

$$c(TAN) = \frac{c(NH_4^+/NH_3)}{M(NH_4^+)} \times M(N) \quad [3.1]$$

Total oxidized nitrogen (TON):

$$c(TON) = \frac{c(NO_3^-)}{M(NO_3^-)} \times M(N) \quad [3.2]$$

Nitrite nitrogen (NO_2^-–N):

$$c(NO_2^- - N) = \frac{c(NO_2^-)}{M(NO_2^-)} \times M(N) \quad [3.3]$$

Nitrate nitrogen (NO_3^-–N)

$$c(NO_3^- - N) = c(TON) - c(NO_2^- - N) \quad [3.4]$$

Total dissolved nitrogen (TDN):

$$c(TDN) = c(TON) + c(TAN) \quad [3.5]$$

Where c: concentration, M: molar mass.

For behavioral analyses, 15 min video recordings were made from each aquarium on days 6, 7, and 8 (GoPro HERO 4, Go Pro Inc. USA). The recordings were taken at 9 a.m., 3 h after feeding, and before grading. That was done to exclude influences of hunger or acute grading stress on behavior. For recordings, the camera was placed at a distance of 30 cm in front of the aquarium. The first 5 min of each record were discarded to prevent influences of handling in front of the aquarium on the behavior of the fish. The last 10 min were used for evaluation according to the ethogram described in Table 3.1. One randomly chosen fish from each aquarium was observed for 10 min, and the durations of swimming, resting, and browsing were determined (VLC media player, 3.0.14 Vetinari). These time intervals were then used to calculate the percentage of time that one of these behavioral patterns was exhibited [3.6]. Additionally, the number of disturbed rests, non-aggressive contacts, aggressive contacts, and air-breathing events during this time were counted. When the fish disappeared from the field of view, the closest one, with a similar behavioral pattern, was observed instead. To evaluate the behavior of the stock, the activity was determined by creating a 3 s video loop at the beginning, after 5 min, and at the end of the 10 min interval. These video loops were used to determine the stock activity by counting all visible resting and active (swimming or browsing) fish (DotDotGoose, version 1.5.2) and calculating with these values the percentage of active fish considering all counted fish [3.7]. Moreover, all visible fight events of the stock and stock air-breathing events within the 10 min intervals were counted.

Behavioral pattern (BP):

$$BP = \frac{t_{BP}}{t_{total}} \times 100\% \quad [3.6]$$

Stock activity:

$$Stock\ activity = \frac{n_{active}}{n_{total}} \times 100\% \quad [3.7]$$

Where BP: behavioral pattern (swimming, resting or browsing) in %, t_{BP}: the duration in s in which one behavioral pattern was shown, t_{total}: the duration in s of the entire observation (10 min = 600 s), n_{active}: number of active fish, n_{total}: number of all counted fish (active and resting fish).

Table 3.1: *Ethogram describing the behavioral patterns that were used for evaluation.*

Behavior (single fish)	**Definition**
"Resting"	Moving passively through the water or lying still at the bottom of the tank.[2]
"Swimming"	Random swimming in the water column or up and down the sides of the tank.[1]
"Browsing"	Moving along the bottom of the aquarium, usually searching for food.[1]
"Disturbed rests"	Contacts between two fish, where the resting fish would temporarily swim away from its position but immediately afterwards resume a resting mode.[1]
"Non-Aggressive contacts"	Contact between two fish that does not lead to biting or chasing.[1]
"Aggressive contacts"	Contact between two fish where at least one of the two fish clearly bit or attacked the other fish on any part of its body.[1]
"Air-breathing events"	The fish moves to the water surface and takes a gulp of air. This was checked by escaping air from the gills of the fish when it was swimming back to the bottom of the aquarium.[2]
Behavior (stock)	
"Stock activity"	Percentage of active (not resting) fish in the stock.
"Fight events"	Visible "aggressive contacts" between fish considering the whole stock.
"Stock air-breathing events"	Visible "air-breathing events" considering the whole stock.

Adapted from Kaiser et al. (1995b)[1] and Van de Nieuwegiessen et al. (2009)[2].

To determine fish performance and final stocking densities, at the end of each experiment after 10 days, the weights (scale: Kern KB, KERN und SOHN GmbH, Balingen, Germany) and lengths (millimeter paper) of 147 fish in E1 and 265 fish in E2 and E3 from each aquarium were determined. The remaining fish were counted and weighed together to record the total biomass. In E1, also all shooters and potential cannibals were counted and weighed for each aquarium. In E2 and E3, the fish that were removed through grading were counted. From these data, the mortality rate [3.8], percentage of shooters and potential cannibals (P_{SC}) [3.9], FCR

[3.10], SGR [3.11], K [3.12], and stocking density as fish L^{-1} [3.13] and g L^{-1} [3.14] were calculated.

Mortality (Mo) in %:

$$Mo = \frac{n_{dead}}{n_0} \times 100\% \quad [3.8]$$

Percentage of shooters or potential cannibals (P_{SC}):

$$P_{SC} = \frac{n_{SC}}{n_0} \times 100\% \quad [3.9]$$

Feed conversion ratio (FCR):

$$FCR = \frac{TFI}{BW_F - BW_0} \quad [3.10]$$

Specific growth rate (SGR) in % BW (body weight) d^{-1}:

$$SGR = \frac{lnBW_F - lnBW_0}{d} \times 100\% \quad [3.11]$$

Fulton's condition factor (K) in g cm^{-3}:

$$K = \frac{BW_F}{L_F^3} \times 100 \quad [3.12]$$

Stocking density (sd):

$$sd_{fish} = \frac{n_{fish}}{V_{HC}} \quad [3.13]$$

$$sd_{mass} = \frac{m_{fish}}{V_{HC}} \quad [3.14]$$

Where n_{dead}: number of dead fish, n_0: initial number of fish, n_{SC}: number of shooters or potential cannibals, TFI: Total feed intake over the run of the experiment, BW_F: Final body weight in g, BW_0: Initial body weight in g, d: duration of the experiment in days, L_F: Final fish length in cm, sd_{fish}: Stocking density as fish L^{-1}, n_{fish}: Number of fish in the holding compartment, V_{Hc}: Water volume of the holding compartment in L, sd_{mass}: Stocking density as fish biomass L^{-1}, m_{fish}: Fish biomass per holding compartment in g.

3.2.6 Statistics

For statistical evaluation (IBM SPSS Statistics, Version 27.0.1.0), the physicochemical water parameters, nitrogen compounds, individual and group behavioral observations, fish performance parameters (mortality rates, P_{SC}, FCRs, SGRs, Ks, final fish weights, and final fish lengths) and final stocking densities were compared between the groups of each experiment. First, all data sets were tested for normal distribution (Shapiro-Wilk-Test). In the case of normal distribution, the data were tested for homogeneity of variance (Levene's Test). If the

data were homogeneous in variance, they were compared with each other using ANOVA and Tukey-HSD as post-hoc test (E1: oxygen (% and mg L^{-1}), temperature, pH, EC, TON, NO_3–N, TDN, FCR, K, disturbed rest, air-breathing, stock activity, stock fighting, stocking densities (fish L^{-1}, g L^{-1}); E2: TAN, NO_2–N, oxygen (%), pH, SGR, non-aggressive contacts, air-breathing, stock activity, stock air-breathing events, stocking densities (fish L^{-1}, g L^{-1}); E3: pH, FCR, K, SGR, individual resting, individual swimming, air-breathing, stock activity, stock fighting events, stocking densities (fish L^{-1}, g L^{-1})). If they were not homogenous in variance, Welch's Test and Dunnett's T3 was used as post-hoc test (E1: NO_2–N, mortality, SGR, non-aggressive contacts; E2: mortality, P_{SC}, stock fighting events; E3: NO_2–N). In case of not normal distributed data, they were compared with a Kruskal Wallis test using a Bonferroni correction (E1: salinity, TAN, fish weights, fish lengths, P_{SC}, individual resting, individual swimming, individual browsing, aggressive contacts, stock air-breathing events; E2: oxygen (mg L^{-1}), temperature, EC, salinity, TON, NO_3–N, TDN, fish weights, fish lengths, FCR, K, individual resting, individual swimming, individual browsing, disturbed rest, aggressive contacts; E3: oxygen (% and mg L^{-1}), temperature, EC, salinity, TAN, TON, NO_3–N, TDN, fish weights, fish lengths, mortality, P_{SC}, individual browsing, disturbed rest, non-aggressive contacts, aggressive contacts, stock air-breathing events). Additionally, for each experiment in each aquarium, the correlation between mortality rate and the percentage of shooters or potential cannibals was tested using Spearman's rank correlation coefficient.

3.3 Results

3.3.1 Water Quality Parameters

In E1, the mean oxygen concentrations ranged from 89.7 % at 30 fish L^{-1} to 93.1 % at 10 fish L^{-1}, showing significant differences between those two groups ($p < 0.05$). The mean temperature was between 29.7 and 29.8 °C, the pH around 8.2, EC between 973 and 976 µS cm^{-1}, and salinity around 0.44 ‰, all of them showing no significant differences between the groups. In E2, the mean oxygen concentrations ranged from 95.1 % at 20 fish L^{-1} to 96.5 % at 10 fish L^{-1}, showing significant differences between those two groups ($p \leq 0.05$). The mean temperature was between 29.0 and 29.2 °C, the pH around 7.3, EC between 1158 and 1164 µS cm^{-1}, and salinity around 0.53 ‰, all of them showing no significant differences between the groups. In E3, the mean temperature ranged from 28.4 °C in 5dH to 28.7 °C in 2dH, showing significant differences between those two groups ($p \leq 0.05$). The mean oxygen

concentrations were between 94.4 and 95.7 %, the pH around 8.2, EC between 1116 and 1123 µS cm^{-1}, and salinity around 0.52 ‰, all of them not significantly different between the groups (see Table 3.2). In all three experiments, the mean NH_4–N/NH_3–N concentrations were below 0.08 mg L^{-1}, the NO_2–N concentrations below 0.07 mg L^{-1}, and the NO_3–N, TON and TDN concentrations below 15.5 mg L^{-1} (see Table 3.3).

Table 3.2: *Mean values of the physicochemical water parameters of the different groups in the 3 experiments.*

	Physicochemical water parameters (mean ± CV)																	
	Group	**O_2 in mg L^{-1}**			**O_2 in %**			**T in °C**			**pH**			**EC in µS cm^{-1}**			**Sal in ‰**		
Experiment 1	10 fish L^{-1}	6.9^{a}	±	2.5	93.1^{a}	±	3.0	29.8^{a}	±	2.6	8.2^{a}	±	1.8	976^{a}	±	4.5	0.44^{a}	±	2.0
	20 fish L^{-1}	6.9^{a}	±	2.9	91.5^{ab}	±	3.0	29.7^{a}	±	2.6	8.2^{a}	±	1.6	973^{a}	±	4.5	0.44^{a}	±	2.2
	30 fish L^{-1}	6.7^{b}	±	3.6	89.7^{b}	±	4.2	29.7^{a}	±	2.6	8.2^{a}	±	1.7	973^{a}	±	4.4	0.44^{a}	±	2.1
Experiment 2	10 fish L^{-1}	7.3^{a}	±	1.2	96.5^{a}	±	1.3	29.2^{a}	±	0.9	7.3^{a}	±	1.5	1164^{a}	±	1.3	0.53^{a}	±	1.8
	20 fish L^{-1}	7.2^{b}	±	1.7	95.1^{b}	±	1.9	29.0^{a}	±	0.8	7.3^{a}	±	1.5	1158^{a}	±	1.2	0.53^{a}	±	1.8
	30 fish L^{-1}	7.2^{ab}	±	1.2	95.5^{b}	±	1.4	29.1^{a}	±	0.8	7.3^{a}	±	1.4	1161^{a}	±	1.3	0.53^{a}	±	1.8
Experiment 3	2dH	7.3^{a}	±	3.9	94.4^{a}	±	3.5	28.7^{a}	±	0.8	8.2^{a}	±	1.7	1123^{a}	±	2.3	0.52^{a}	±	2.2
	5dH	7.4^{a}	±	1.8	95.7^{a}	±	1.6	28.4^{b}	±	0.6	8.2^{a}	±	1.4	1116^{a}	±	2.1	0.52^{a}	±	2.4
	5dS	7.4^{a}	±	2.1	95.4^{a}	±	1.9	28.5^{b}	±	0.6	8.2^{a}	±	1.5	1118^{a}	±	2.1	0.52^{a}	±	2.3

Different superscript letters show significant differences between the groups within one experiment considering one parameter ($p \leq 0.05$). T: Temperature, EC: Electrical conductivity, Sal: Salinity.

Table 3.3: *Mean values of the concentrations of the nitrogen compounds of the different groups in the 3 experiments.*

	Nitrogen concentrations (mean ± CV)															
	Group	**TAN**			**NO_2–N**			**NO_3–N**			**TON**			**TDN**		
Experiment 1	10 fish L^{-1}	0.02^{a}	±	205	0.03^{a}	±	25	10.2^{a}	±	18	10.3^{a}	±	18	10.3^{a}	±	19
	20 fish L^{-1}	0.03^{a}	±	124	0.02^{a}	±	24	10.8^{a}	±	19	10.9^{a}	±	19	10.9^{a}	±	19
	30 fish L^{-1}	0.07^{a}	±	171	0.04^{a}	±	36	10.2^{a}	±	19	10.2^{a}	±	19	10.3^{a}	±	19
Experiment 2	10 fish L^{-1}	0.04^{a}	±	48	0.04^{a}	±	36	11.6^{a}	±	15	11.7^{a}	±	15	11.7^{a}	±	15
	20 fish L^{-1}	0.04^{a}	±	48	0.03^{a}	±	44	11.3^{a}	±	15	11.3^{a}	±	15	11.4^{a}	±	15
	30 fish L^{-1}	0.06^{a}	±	40	0.04^{a}	±	39	11.5^{a}	±	13	11.6^{a}	±	13	11.6^{a}	±	13
Experiment 3	2dH	0.06^{a}	±	102	0.06^{a}	±	5	15.3^{a}	±	18	15.4^{a}	±	18	15.4^{a}	±	17
	5dH	0.05^{a}	±	110	0.05^{a}	±	17	15.3^{a}	±	17	15.4^{a}	±	17	15.4^{a}	±	17
	5dS	0.06^{a}	±	98	0.05^{a}	±	13	15.2^{a}	±	17	15.2^{a}	±	17	15.3^{a}	±	17

Different superscript letters show significant differences between the groups within one experiment considering one parameter ($p \leq 0.05$).

3.3.2 Behavioral Observations

In all experiments, there were no significant differences in mean individual swimming, resting, and browsing between the groups. While swimming was the most common behavior ranging from 71.9 to 80.8 % in E1, from 82.8 to 87.7 % in E2, and from 89.1 to 91.9 % in E3. Resting ranged from 14.7 to 24.2 % in E1, from 10.0 to 14.9 % in E2, and from 7.2 to 9.0 % in E3. Browsing ranged from 3.9 to 4.6 % in E1, from 1.4 to 2.3 % in E2, and from 0.9 to 1.8 % in E3. Considering individual behavioral events, mean disturbed rests only showed significant differences between 10 and 30 fish L^{-1} in E2, while air-breathing events showed no significant differences between the groups in any of the experiments. Mean disturbed rests ranged from 9.4 to 11.9 in E1, from 5.5 to 8.4 in E2, and from 3.0 to 5.2 in E3. Mean air-breathing events ranged from 4.2 to 6.6 in E1, from 3.3 to 4.3 in E2, and from 3.2 to 5.0 in E3. Non-aggressive contacts during individual observations showed significant differences in E1 and E2 but not in E3. In E1, mean non-aggressive contacts ranged from 28.3 at 10 fish L^{-1} to 55.0 at 30 fish L^{-1} and showed a significant difference between those two groups. In E2, mean non-aggressive contacts showed values of 20.6 at 10 fish L^{-1}, 39.7 at 20 fish L^{-1}, and 53.2 at 30 fish L^{-1} and were significantly different between all groups. In E3, mean values for non-aggressive contacts ranged from 22.1 to 27.7. Aggressive contacts during individual observations showed significant differences in E1 and E2 but not in E3. In E1, mean aggressive contacts showed values of 5.6 at 10 fish L^{-1}, of 3.7 at 20 fish L^{-1}, and of 1.3 at 30 fish L^{-1} and were significantly higher at 10 and 20 than at 30 fish L^{-1}. In E2, mean aggressive contacts ranged from 0.4 at 20 fish L^{-1} to 1.7 at 10 fish L^{-1} and only showed significant differences between those 2 groups. In E3, mean aggressive contacts ranged from 2.1 to 3.1 (see Table 3.4).

Stock activity increased with increasing stocking density. In E1, mean stock activity was 73.9 % at 10 fish L^{-1}, 85.4 % at 20 fish L^{-1}, and 89.4 % at 30 fish L^{-1}, resulting in significant differences between 10 fish L^{-1} and 20 as well as 30 fish L^{-1}. In E2, mean stock activity was 79.5 % at 10 fish L^{-1}, 82.6 % at 20 fish L^{-1}, and 85.4 % at 30 fish L^{-1}, resulting in significant differences between 10 and 30 fish L^{-1}. In E3, mean stock activity ranged from 78.7 % in 5dS to 82.6 % in 2dH, showing no significant differences between the groups. Stock fighting events within 10 minutes increased with decreasing stocking density. In E1, mean stock fighting events were 43.2 at 10 fish L^{-1}, 26.9 at 20 fish L^{-1}, and 17.0 at 30 fish L^{-1}, resulting in significant differences between 30 fish L^{-1} and 20 as well as 10 fish L^{-1}. In E2, mean stock fighting events were 15.2 at 10 fish L^{-1}, 8.3 at 20 fish L^{-1}, and 8.1 at 30 fish L^{-1}, resulting in significant

differences between 10 fish L^{-1} and 20 as well as 30 fish L^{-1}. In E3, mean stock fighting events ranged from 14.1 in 5dH to 19.0 in 2dH, showing no significant differences between the groups. In E1, mean stock air-breathing events within 10 minutes were 173.8 at 10 fish L^{-1}, 128.9 at 20 fish $L^{-1,}$ and 117.9 at 30 fish L^{-1}, resulting in significant differences between 10 fish L^{-1} and 20 as well as 30 fish L^{-1}. In E2, mean stock air-breathing events ranged from 118.6 at 30 fish L^{-1} to 142.8 at 10 fish L^{-1}, showing no significant differences between the groups. In E3, mean stock air-breathing events ranged from 192.9 in 2dH to 208.4 in 5dS, also showing no significant differences between the groups (see Table 3.5).

3.3.3 Survival and Fish Performance

Mean mortality rates showed no significant differences between the groups within the experiments, while the total mean value in E1, with 25 %, was more than 5 times higher than in E2 and E3, with 4.1 and 4.6 % (see Table 3.6). The percentage of shooters or potential cannibals showed no significant differences between the groups within the experiments but showed a very large overall variation, with the highest total mean value in E2 with 1.59 % and the lowest in E3 with 0.21 % (see Table 3.7).

Table 3.4: *Mean values of the individual behavioral observations of the different groups in the 3 experiments*

		Individual behavioral observations (mean ± CV)																					
		Resting in %			Swimming in %			Browsing in %			Disturbed Rests			Non-aggressive contacts			Aggressive contacts			Air-breathing events		
Experiment 1	10 fish L^{-1}	24.2^{a}	±	75	71.9^{a}	±	28	3.9^{a}	±	97	9.4^{a}	±	82	28.3^{a}	±	43	5.6^{a}	±	66	4.2^{a}	±	64
	20 fish L^{-1}	16.7^{a}	±	51	78.7^{a}	±	14	4.6^{a}	±	100	11.9^{a}	±	83	37.3^{ab}	±	26	3.7^{a}	±	93	5.9^{a}	±	35
	30 fish L^{-1}	14.7^{a}	±	73	80.8^{a}	±	14	4.5^{a}	±	79	11.3^{a}	±	92	55.0^{b}	±	51	1.3^{b}	±	145	6.6^{a}	±	54
Experiment 2	10 fish L^{-1}	14.9^{a}	±	122	82.8^{a}	±	24	2.3^{a}	±	91	6.1^{a}	±	143	20.6^{a}	±	37	1.7^{a}	±	71	3.4^{a}	±	72
	20 fish L^{-1}	12.4^{a}	±	141	86.2^{a}	±	20	1.4^{a}	±	76	5.5^{ab}	±	57	39.7^{b}	±	24	0.4^{b}	±	182	3.3^{a}	±	57
	30 fish L^{-1}	10.0^{a}	±	48	87.7^{a}	±	7	2.3^{a}	±	108	8.4^{b}	±	38	53.2^{c}	±	18	0.9^{ab}	±	137	4.3^{a}	±	42
Experiment 3	2dH	7.2^{a}	±	94	91.9^{a}	±	8	0.9^{a}	±	228	3.3^{a}	±	80	24.9^{a}	±	31	2.7^{a}	±	95	3.6^{a}	±	80
	5dH	7.9^{a}	±	92	90.9^{a}	±	10	1.2^{a}	±	156	5.2^{a}	±	79	27.7^{a}	±	48	2.1^{a}	±	98	3.2^{a}	±	88
	5dS	9.0^{a}	±	71	89.2^{a}	±	9	1.8^{a}	±	173	3.0^{a}	±	131	22.1^{a}	±	47	3.1^{a}	±	75	5.0^{a}	±	81

Different superscript letters show significant differences between the groups within one experiment considering one parameter ($p \leq 0.05$).

Table 3.5: *Mean values of the group behavioral observations of the different groups in the 3 experiments.*

		Group behavioral observations (mean ± CV)								
		Stock activity in %			Stock fighting events*			Stock air-breathing events*		
Experiment 1	10 fish L^{-1}	73.9^{a}	±	10.0	43.2^{a}	±	34.0	173.8^{a}	±	33.7
	20 fish L^{-1}	85.4^{b}	±	5.0	26.9^{a}	±	24.7	128.9^{b}	±	17.3
	30 fish L^{-1}	89.4^{b}	±	5.5	17.0^{b}	±	31.2	117.9^{b}	±	20.6
Experiment 2	10 fish L^{-1}	79.5^{a}	±	5.6	15.2^{a}	±	33.1	142.8^{a}	±	24.9
	20 fish L^{-1}	82.6^{ab}	±	4.9	8.3^{b}	±	36.8	122.5^{a}	±	27.4
	30 fish L^{-1}	85.4^{b}	±	3.7	8.1^{b}	±	36.3	118.6^{a}	±	22.0
Experiment 3	2dH	82.6^{a}	±	4.3	19.0^{a}	±	70.7	192.9^{a}	±	50.2
	5dH	80.6^{a}	±	4.6	14.1^{a}	±	51.2	200.0^{a}	±	36.7
	5dS	78.7^{a}	±	4.8	15.7^{a}	±	66.9	208.4^{a}	±	39.2

*: within 10 minutes, in experiments 1 and 2, always in relation to 300 fish. Different superscript letters show significant differences between the groups within one experiment considering one parameter ($p \leq 0.05$).

Table 3.6: *Mortality rates of the different groups in the 3 experiments as percentage of stocked fish. Shown as single values per aquarium, mean value per group, and mean value per experiment.*

	Mortality rate in %			
Experiment 1	**10 fish L^{-1}**	**20 fish L^{-1}**	**30 fish L^{-1}**	**Total***
single values	10.7/20.0/15.3	16.3/66.8/12.0	16.0/40.1/27.6	
mean ± CV	15.3^{a} ± 24	31.7^{a} ± 78	27.9^{a} ± 35	25.0 ± 68
Experiment 2	**10 fish L^{-1}**	**20 fish L^{-1}**	**30 fish L^{-1}**	**Total***
single values	2.7/3.0/8.3	3.8/2.8/4.0	4.0/5.8/2.4	
mean ± CV	4.7^{a} ± 56	3.6^{a} ± 14	4.1^{a} ± 33	4.1 ± 14
Experiment 3	**2dH**	**5dH**	**5dS**	**Total***
single values	6.0/5.6/4.4	4.1/5.2/4.1	3.3/3.2/5.7	
mean ± CV	5.3^{a} ± 12	4.5^{a} ± 12	4.1^{a} ± 28	4.6 ± 21

Different superscript letters show significant differences between the groups within one experiment considering one parameter ($p \leq 0.05$). *: Mean value of all 9 tanks within one experiment.

Table 3.7: *Counted (at the end of E1) or removed shooters and potential cannibals (E2 and E3) of the different groups in the 3 experiments as percentage of stocked fish as single values per aquarium, mean value per group and mean value per experiment.*

	Percentage of shooters and potential cannibals in % (P_{SC})			
Experiment 1	**10 fish L^{-1}**	**20 fish L^{-1}**	**30 fish L^{-1}**	**Total***
single values	0/0.67/0.67	0.17/0.83/0.17	0.11/0.67/0.44	
mean ± CV	0.44^{a} ± 71	0.39^{a} ± 81	0.41^{a} ± 56	0.41 ± 70
Experiment 2	**10 fish L^{-1}**	**20 fish L^{-1}**	**30 fish L^{-1}**	**Total***
single values	2.3/1.0/3.3	2.0/1.8/1.5	1.1/0.7/0.6	
mean ± CV	2.22^{a} ± 43	1.78^{a} ± 12	0.78^{a} ± 31	1.59 ± 53
Experiment 3	**2dH**	**5dH**	**5dS**	**Total***
single values	0.1/0.4/0.8	0/0.2/0.2	0/0.1/0	
mean ± CV	0.44^{a} ± 61	0.15^{a} ± 71	0.04^{a} ± 141	0.21 ± 116

Different superscript letters show significant differences between the groups within one experiment considering one parameter ($p \leq 0.05$). *: Mean value of all 9 tanks within one experiment.

In E1, there was a significant correlation between the number of fish that died in an aquarium and the number of shooters or potential cannibals (Spearman's rank correlation coefficient, $p = 0.033$). In E2 and E3, there was no correlation between the number of dead fish and the number of shooters or potential cannibals. The mean weight of shooters or potential cannibals in E1 was 0.9 g fish^{-1} at 10 fish L^{-1}, 3.7 g fish^{-1} at 20 fish L^{-1}, and 3.4 g fish^{-1} at 30 fish L^{-1}. In E2, the first shooters or potential cannibals could be removed between day 2

and 7, regardless of stocking density. Growth performance parameter showed no significant differences between the groups within the experiments, while the total average values for the FCR, K, and SGR were highest in E1 (0.68, 0.85, and 11.9 % BW d^{-1}) and lowest in E3 (0.53, 0.79 and 10.1 % BW d^{-1}) (Table 3.8).

Table 3.8: *Mean values of growth performance parameters of the different groups in the 3 experiments.*

	Growth performance parameters (mean ± CV)								
	Group	**FCR**			**K**			**SGR in % BW d^{-1}**	
Experiment 1	10 fish L^{-1}	0.63^{a}	±	10.1	0.80^{a}	±	5.6	10.3^{a}	± 4.1
	20 fish L^{-1}	0.68^{a}	±	19.9	0.89^{a}	±	7.2	13.5^{a}	± 23.8
	30 fish L^{-1}	0.74^{a}	±	11.3	0.85^{a}	±	3.7	12.0^{a}	± 5.6
	Total*	0.68	±	15.7	0.85	±	7.4	11.9	± 19.3
Experiment 2	10 fish L^{-1}	0.57^{a}	±	10.7	0.76^{a}	±	1.0	10.2^{a}	± 5.8
	20 fish L^{-1}	0.54^{a}	±	8.6	0.82^{a}	±	9.9	10.3^{a}	± 5.0
	30 fish L^{-1}	0.52^{a}	±	9.9	0.81^{a}	±	1.5	10.4^{a}	± 3.8
	Total*	0.55	±	10.4	0.80	±	6.9	10.3	± 5.0
Experiment 3	2dH	0.57^{a}	±	7.0	0.79^{a}	±	2.2	10.0^{a}	± 5.8
	5dH	0.54^{a}	±	3.0	0.79^{a}	±	1.1	9.9^{a}	± 2.2
	5dS	0.49^{a}	±	4.3	0.79^{a}	±	1.0	10.3^{a}	± 3.7
	Total*	0.53	±	8.5	0.79	±	1.6	10.1	± 4.4

Different superscript letters show significant differences between the groups within one experiment considering one parameter ($p \leq 0.05$). FCR: Feed conversion ratio, K: Fulton's condition factor, SGR: Specific growth rate. *: Mean value of all 9 tanks within one experiment.

In E1, E2, and E3, the mean initial total lengths of the fish were about 2.0, 1.9, and 2.0 cm, with an average initial weight of 0.08, 0.06, and 0.06 g $fish^{-1}$. In E1, the mean final total lengths of the fish ranged from 3.0 cm at 10 fish L^{-1} to 3.3 cm at 20 fish L^{-1}, showing significant differences between all three groups. The mean final weights ranged from 0.22 g $fish^{-1}$ at 10 fish L^{-1} to 0.32 g $fish^{-1}$ at 20 fish L^{-1}, also showing significant differences between all three groups. In E2, the mean final total lengths of the fish ranged from 2.7 cm at 20 and 30 fish L^{-1} to 2.8 cm at 10 fish L^{-1}, showing significant differences between 20 and 10 fish L^{-1}. The mean final weights ranged from 0.163 g $fish^{-1}$ at 10 fish L^{-1} to 0.166 g $fish^{-1}$ at 30 fish L^{-1}, showing no significant differences between the groups. In E3, the mean final total lengths of the fish in all three groups were around 2.7 cm, showing no significant differences between the groups. The mean final weights ranged from 0.154 g $fish^{-1}$ in 5dH to 0.159 g $fish^{-1}$ in 5dS, showing no significant differences between the groups (Table 3.9). The final weight differences in E1 were

considerably higher than in E2 and E3. Within E1, the final weight differences at 20 and 30 fish L^{-1} were, in each case, in 2 aquaria much higher than at 10 fish L^{-1} (see Figure 3.4).

Mean final fish numbers per liter in E1 ranged from 8.5 (10 fish L^{-1}) to 21.6 (30 fish L^{-1}), only showing significant differences between those 2 groups. Mean final fish biomass per liter ranged from 1.8 g L^{-1} (10 fish L^{-1}) to 5.1 (30 fish L^{-1}) and was significantly different between all 3 groups. Mean final fish numbers per liter in E2 ranged from 9.5 (10 fish L^{-1}) to 28.8 (30 fish L^{-1}), showing significant differences between all three groups. Mean final fish biomasses per liter ranged from 1.5 g L^{-1} (10 fish L^{-1}) to 4.7 (30 fish L^{-1}) and were also significantly different between all groups. Mean final fish numbers per liter in E3 were around 28.5 in all groups, showing no significant differences, while mean final fish biomasses per liter ranged from 4.3 g L^{-1} in 2dH to 4.8 in 5dS, showing significant differences between these 2 groups (Table 3.10).

Table 3.9: *Mean initial and final fish lengths and weights of the different groups in the 3 experiments.*

	Initial fish lengths in cm	**Final fish lengths in cm**		
Experiment 1		**10 fish L^{-1}**	**20 fish L^{-1}**	**30 fish L^{-1}**
mean ± CV	2.0 ± 12.8	3.0^{a} ± 15.3	3.3^{b} ± 19.4	3.1^{c} ± 16.4
Experiment 2		**10 fish L^{-1}**	**20 fish L^{-1}**	**30 fish L^{-1}**
mean ± CV	1.9 ± 13.9	2.8^{a} ± 14.5	2.7^{b} ± 15.6	2.7^{ab} ± 14.0
Experiment 3		**2dH**	**5dH**	**5dS**
mean ± CV	2.0 ± 10.4	2.7^{a} ± 13.7	2.7^{a} ± 13.3	2.7^{a} ± 14.2
	Initial fish weights in g	**Final fish weights in g**		
Experiment 1		**10 fish L^{-1}**	**20 fish L^{-1}**	**30 fish L^{-1}**
mean ± CV	0.079 ± 24.2	0.222^{a} ± 48.1	0.320^{b} ± 138.0	0.263^{c} ± 107.6
Experiment 2		**10 fish L^{-1}**	**20 fish L^{-1}**	**30 fish L^{-1}**
mean ± CV	0.059 ± 39.0	0.162^{a} ± 41.9	0.164^{a} ± 43.0	0.166^{a} ± 39.8
Experiment 3		**2dH**	**5dH**	**5dS**
mean ± CV	0.057 ± 29.3	0.154^{a} ± 40.9	0.154^{a} ± 39.5	0.159^{a} ± 41.7

Different superscript letters show significant differences between the groups within one experiment considering one parameter ($p \leq 0.05$). CV = Coefficient of variation

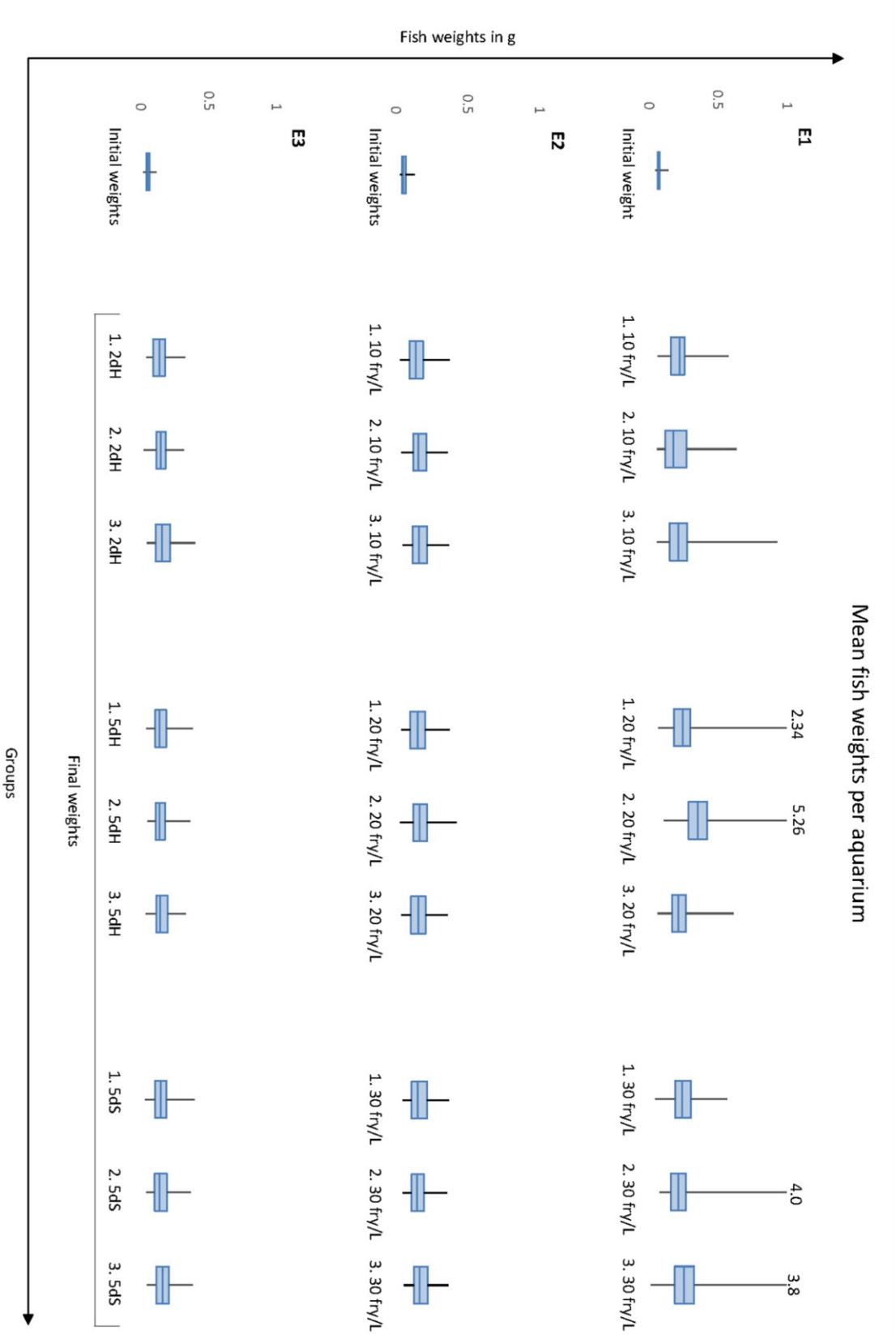

Figure 3.4: *Boxplot of the initial and final weights of the different aquaria in the 3 experiments. The triplicates of the groups are labeled 1, 2, and 3.*

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Table 3.10: *Mean stocking densities at the beginning (initial) and end (final) of the different groups in the 3 experiments.*

		Mean initial stocking densities		Mean final stocking densities	
Experiment	**Group**	**Fish L^{-1}**	**g L^{-1}**	**Fish L^{-1}**	**g L^{-1}**
E1	10 fish L^{-1}	10	0.79	8.5^{a}	1.8^{a}
	20 fish L^{-1}	20	1.58	13.7^{ab}	3.6^{b}
	30 fish L^{-1}	30	2.37	21.6^{b}	5.1^{c}
E2	10 fish L^{-1}	10	0.59	9.5^{a}	1.5^{a}
	20 fish L^{-1}	20	1.18	19.3^{b}	3.1^{b}
	30 fish L^{-1}	30	1.77	28.8^{c}	4.7^{c}
E3	2dH	30	1.71	28.3^{a}	4.3^{a}
	5dH	30	1.71	28.6^{a}	4.5^{ab}
	5dS	30	1.71	28.8^{a}	4.8^{b}

Different superscript letters show significant differences between the groups within one experiment considering one parameter ($p \leq 0.05$).

3.4 Discussion

We could demonstrate that stock activity rose with increasing stocking density and decreased aggressiveness at higher stocking densities for African catfish fry with a mean initial weight of 0.06 – 0.08 g, held at stocking densities of 10, 20, and 30 fish L^{-1}. Higher stocking densities did not have negative effects on the mortality rates, percentage of cannibals, and growth performance parameters. By regular grading, the total mean mortality rate was reduced from 25 % in E1 to less than 5 % in E2 and E3 within 10 days; likewise, the total mean FCR was reduced from 0.68 in E1 to ≤ 0.55 in E2 and E3. Additionally, we observed a significant correlation between the mortality rates and the percentage of shooters or potential cannibals in E1 when fish were not graded.

3.4.1 Water Quality Parameter

The mean oxygen concentrations in all experiments were above 89 % (6.7 mg L^{-1}). Even if they showed minor differences between the groups in E1 and E2, they were within normal range (Beingana et al., 2016; Musiba et al., 2014; Saleh, 2020), and no decrease in growth or increase in air-breathing events were observed. Therefore, we can expect that these differences did not influence our results. There were minor differences in temperature of E3, but the temperature varied between 28 and 30 °C during all experiments, in the optimum range for African catfish fry where no significant differences in growth can be expected (Britz and Hecht, 1987). The pH (Ndubuisi et al., 2015), salinity (Gbulubo and Erondu, 1998), and EC

(Palm et al., 2018b; Strauch et al., 2018) were within common ranges for African catfish and showed no significant differences between the groups in all three experiments. The nitrogen concentration of NH_3–N/NH_4^+–N (Schram et al., 2010), NO_2^-–N (Roques et al., 2015), and NO_3^--N (Schram et al., 2014) did not exceed recommended threshold values and showed no significant differences between the groups. Therefore, it can be assumed that water quality had no influence on the observed fish behavior or fish performance of the different groups during the experiments.

3.4.2 Behavioral Observations

Most of the individual observations coincided with group observations. Nonetheless, as individual observations only reflected the behavior of one single fish in an aquarium while group observations that of all fish, in cases of inconsistencies, higher importance should be given to the results of the group observations. The main behavioral differences between stocking densities (E1, E2) were an increasing stock activity with increasing stocking densities and most aggressiveness (aggressive contacts, stock fighting events) at lowest stocking density. The higher activity was also reflected by the tendentially increasing individual swimming activity and decreasing individual resting time with increasing stocking densities. The higher swimming activity at higher stocking densities and higher aggression at lower stocking densities in African catfish confirms the observations by Kaiser et al. (1995a) and Almazán-Rueda et al. (2004). The higher swimming activity seemed to be a consequence of less relative resting space at higher stocking densities, so that the fish were forced to swim. Such constant movement under high stocking densities results in a higher number of random meetings between conspecifics, which was also reflected by the tendentially higher number of disturbed rests at higher stocking densities. However, even though the fish were permanently swimming and had constant contact to other conspecifics, it seemed that they did not develop territorial behavior and therefore showed less aggressiveness at higher stocking densities. Haylor (1991) also described reduced territoriality at higher stocking densities in African catfish fry. Additionally, in E1 but not in E2 stock air-breathing events were significantly higher at 10 fish L^{-1} than at 20 and 30. A reason therefore might be that the cannibals perform a more aggressive searching for food when there is less fish available because they need more time to catch prey. That in turn leads to more stress for potential prey, what consequently leads to a higher oxygen demand and air breathing frequency. In E3,

where all aquaria were stocked with 30 fish L^{-1} behavioral observations showed no significant differences between the groups, but the fish showed a tendency to reduce activity (individual swimming, stock activity) with decreasing handling stress (2dH > 5dH > 5dS). Laitinen and Valtonen (1994) already showed that handling stress in brown trout resulted in an increased activity of the fish for 24 to 48 h. But because the differences in our study were not significant, the extent of the stress, even at 2dH, did not seem to cause stress to a great extent. Nonetheless, because grading every 5 days showed the same result compared with grading every 2 days and less grading always means less stress, work, and labor costs, grading every 5^{th} day should be preferred. Abdelhamid et al. (2010) did a similar experiment lasting for 4 weeks with fish having an initial weight of 0.38 g and reported that grading in a 3-day rhythm results in a better survival than grading in a 7-day rhythm.

3.4.3 Survival and Fish Performance

Even though the mortality rates showed no significant differences between the groups, total mean mortality rates ranged from 25 % in E1 to less than 5 % in E2 and E3. Consequently, regular grading considerably decreased mortality rates. Also, Hengsawat et al. (1997) who investigated African catfish with a mean initial weight of 32 g in net cages, Hossain et al. (1998), who examined African catfish with a mean initial weight of 0.70 g in a RAS, and Haylor (1992) who studied African catfish larvae also did not observe a relation between mortality and stocking density. Although there were no significant differences between different handling procedures (E3), a clear trend of decreasing mortality rates with decreasing handling stress (2dH > 5dH > 5dS) until a minimum value of 3.2 % in a single tank occurred. This tendency is in accordance with the observations by Haylor (1992), who found a higher mortality due to handling stress in African catfish larvae. In order to be able to clearly determine possible differences under the influence of the different handling regimes, another experiment would be required over a longer period of time.

Also, the number of shooters or potential cannibals showed no significant differences between the groups at different stocking densities (E1 and E2) and handling procedures (E3) but varied between experiments and fish tanks. Even though the number of potential shooters did not depend on stocking density, in total, they grew faster at higher stocking densities. This was most likely due to a higher possibility to meet potential conspecific prey and spending less time searching for it. In addition to stocking density, however, different genetic prerequisites

or other factors also seemed to have an influence on cannibalism because not all aquaria at higher stocking densities showed higher maximum weights. The phenomenon of a higher cannibalism rate at higher stocking densities has been earlier demonstrated for juvenile *Orechromis niloticus* (Fessehaye et al., 2006), juvenile *Lates calcarifer* (Ribeiro et al., 2015), *Wallago attu* (Sahoo et al., 2002) and *Clarias gariepinus* (Hecht and Appelbaum, 1988).

FCRs, Ks, and SGRs showed no significant differences between the groups of the experiments. Nonetheless, the total mean FCR in E1 with a value of 0.68 was reduced to ≤ 0.55 in E2 and E3 by grading, reducing energy loss by multiple digestions (feed > fish > cannibal) or hunting conspecifics. Additionally, there was a clear tendency of decreasing FCR with decreasing handling stress (2dH > 5dH > 5dS), probably due to a lower energy consumption with less stress because they were less active. The herewith observed average FCR values between 0.49 and 0.74 are comparable with the results of Jamabo and Dienye (2016), who observed FCRs between 0.41 – 0.73 for African catfish juveniles and were tendentially lower than those observed by Fauji et al. (2018) who recorded FCRs between 0.72 and 0.97 for African catfish with an initial fish weight of 0.96 g. By removing the shooters, mean total K sank from 0.85 in E1 to 0.80/0.79 in E2/E3, indicating differences in body proportions between cannibals and normally grown fish. The K values of around 0.8 for E2 and E3 were a bit higher than those observed by Gabriel et al. (2019), who found values of around 0.7 for 3.1 g African catfish juveniles but lower than those found by Britz and Pienaar (1992) who observed values between 0.94 and 1.22 for African catfish larvae. The mean total SGR in E1 was close to 12 and was reduced to around 10.2 % BW d^{-1} in E2 and E3. The SGRs in this study were in the range of those observed by Appelbaum and Mc Geer (1998), who found values between 4 and 30 % BW d^{-1} at around 20 days old African catfish fry. Hogendoorn et al. (1983) found SGRs between 2 and 12 % BW d^{-1} for 0.3 – 3 g African catfish juveniles depending on the feeding level.

In E1, mean final fish weights and lengths significantly reached the highest values at 20 fish L^{-1}, followed by 30 fish L^{-1}, and 10 fish L^{-1}, showing the lowest values (Table 3.9). At the same time, maximum individual final fish weights in 2 aquaria at 20 and 30 fish L^{-1} were 2 to 5 times higher than the maximum weights at 10 fish L^{-1} (Figure 3.4). It seems that the weight differences between the groups in E1 were due to the above-discussed faster growth of cannibals at higher stocking densities, subsequently confirmed by the results of E2, where shooters were removed, and no differences in final weights occurred. In contrast to our

results, Coulibaly et al. (2007) observed a better growth at lower stocking densities of *Heterobranchus longifilis* with a mean initial weight of 0.8 g in cage culture after 90 days of experiment (tested stocking densities: 50, 100, 200, 500 and 1000 fish m^{-3}). Also, in *C. gariepinus* experiments by Dai et al. (2011), Hengsawat et al. (1997), Suleiman and Solomon (2017), Hossain et al. (1998), Akinwole et al. (2014) and Dasuki et al. (2013) indicated an improved growth at lower stocking densities, while different stocking densities, experimental periods (between 4 weeks and 150 days) and age classes (mean initial weights between 0.79 and 32 g) were tested. In contrast to that, Van de Nieuwegiessen et al. (2008) did not observe density-dependent (tested stocking densities: 500, 1125, 1750, and 2375 fish m^{-3}) differences in growth of African catfish with an initial weight of 7.5 g which were held in a RAS for 49 days. Toko et al. (2007) even observed an improved growth with increasing stocking density at a tested range of 4, 6, and 8 fish m^{-3} in fish with a mean initial weight of 34.8 g held in ponds for 70 days. As literature presents contradictory results and our experiments only lasted for 10 days which is a very short period of time to evaluate growth differences, it cannot be excluded that after a longer period of time differences might have been detected. In E2, the only significant difference in length was detected between 10 and 20 fish L^{-1}. This higher fish length in relation to the body weight was also reflected by a slightly lower K at 10 fish L^{-1}. Most probably, the relatively higher length at lowest stocking density was due to less caudal fin erosions at low stocking densities, like it had been observed in Rainbow trout (North et al., 2006) or European perch (Stejskal et al., 2020). However, because this parameter has not been taken into account during the present study, this theory cannot be verified. In E3 no significant differences in fish length and weight were observed.

Referring to the number of fish, final stocking densities in E1 only differed significantly between 10 and 30 fish L^{-1}. It would have been expected to find differences between all 3 groups as they were initially stocked with 10, 20, and 30 fish L^{-1}. It seems that the absence of differences is due to the high variation in mortality rates within each group. This suggestion is confirmed by the results of E2, where all 3 groups showed significant differences at the end of the experiment, and strong differences in mortality have been avoided by grading. In E3, contrary to the other performance parameters, the stocking density as biomass per volume in 5dS was significantly higher than in 2dH, indicating that the fish grew better with decreasing handling stress. One possible reason that the other parameters, like FCR, SGR, or final weights,

did not differ significantly is that the experiments only lasted for 10 days, and the time was too short to show differences for these parameters.

In summary, the 3 experiments demonstrate that African catfish fry between 0.06 and 0.32 g mean weight is best kept at high stocking densities up to 30 fish L^{-1}. Calculated at a commercial scale, a total of 30,000 fish m^{-3} can be kept inside adequate breeding tanks or grooves. Regular grading, manually or by self-grading, considerably increases the survival rate, improves the FCR, and ensures a more even growth of the cohort. Comparing different grading regimes, self-grading every 5 days is the best possible option in terms of reducing stress and improving growth for the fish and reducing the workload for the personnel.

3.5 Conclusion

The results of this study allow the following conclusions. 1) The stocking density affected the behavior but not the survival and growth performance parameters in African catfish fry and had no effect on the percentage of shooters or potential cannibals in the respective cohort. The recorded differences in final weights and lengths referred to a different growth of the recorded cannibals. 2) The constant removal of shooters or potential cannibals reduced the mortality rates of the fry, independent of stocking density. 3) Different handling procedures and grading regimes (to remove shooters or potential cannibals) of the fry under optimal stocking densities did not influence survival and growth parameters but final fish biomass per L^{-1}, indicating a slightly improved growth with decreasing handling stress. Future studies must focus on evaluating the necessary grading intervals for other age classes as well as the most efficient low-budget grading methods that can reduce work effort and labor costs under commercial conditions. So far, for fry with a mean weight of around 0.1 g, the hatcheries should keep their fish at optimal stocking densities of 30 fish L^{-1} and perform regular grading, self-grading at best, every 5 days to stay economical.

4 Effects of Dissolved Potassium on Growth Performance, Body Composition, and Welfare of Juvenile African catfish (*Clarias gariepinus*)

Abstract

Optimal crop production in aquaponics is influenced by water pH and potassium concentrations. The addition of potassium hydroxide (KOH) into the recirculating aquaculture system (RAS) may benefit aquaponics by increasing the water pH for better biofilter activity and supplementing K for better plant growth and quality. We investigated the growth, feed conversion, body composition, and welfare indicators of juvenile African catfish (*Clarias gariepinus*) treated with four concentrations of K (K0 = 2, K200 = 218, K400 = 418, and K600 = 671 mg L^{-1}). While growth, feed conversion, and final body composition were unaffected, the feeding time and individual resting significantly increased with increasing K^+. The swimming activity and agonistic behavior were reduced significantly under increased concentrations of K^+. Leftover feed and the highest number of skin lesions were observed under K600. We suggest that K^+ concentrations between 200 and 400 mg L^{-1} can improve the welfare status of juvenile African catfish. This enables the application of KOH in RAS to supply alkalinity to achieve optimum nitrification at minimum water exchange and improve the nutritional profile of the process water with benefits for the welfare status of African catfish and aquaponics plant production and quality.

Keywords

African catfish; *Clarias gariepinus*; aquaponics; nutrient management; potassium; plant growth; plant quality; animal welfare; water quality

4.1 Introduction

Potassium (K) is vital to plant and animal nutrition, as it is involved in many different physiological processes. In plants, K takes part in osmoregulation (Dhindsa et al., 1975; Ford and Wilson, 1981), influences enzyme activities (Murata and Akazawa, 1968; Sodek et al., 1980), and is involved in energy metabolism (Gajdanowicz et al., 2011). In animals, K plays a crucial role in nerve functioning (Hodgkin and Horowicz, 1959; Takeuchi and Takeuchi, 1960), also influences enzyme activities (Garrahan et al., 1969; Gruener and Avi-Dor, 1966; Reuben and Cohn, 1970; Schwartz and Laseter, 1964; Schweet et al., 1957) and affects the acid-base balance (Dersjant-Li, 2000). To fulfill the needs of fish in freshwater aquaculture, commercial

diets for the African catfish (*Clarias gariepinus*) contain about 0.94 – 0.97 % of K **(Strauch et al., 2018**).

Recommended values of K^+ concentrations in hydroponic solutions range between 156 and 300 mg L^{-1} (Trejo-Téllez and Gómez-Merino, 2012). K^+ concentrations inside the process water of African catfish (*C. gariepinus*) in RAS are 2 – 13 times lower (Palm et al., 2018b; Strauch et al., 2018), requiring fertilization before its use for hydroponics. In coupled aquaponics (Palm et al., 2018a), however, high K^+ concentrations benefit the plants but may impair the fish. In terrestrial animals, overdose symptoms of K^+ have been well investigated (Emmens and Marks, 1942; Finch et al., 1946; Neathery et al., 1979), leading, e.g., to excessive salivation, muscular tremor, and even to collapse (Neathery et al., 1979). In aquatic animals, the 50 % mortality (TL_m) after 24 h KCl exposure occurred in snail eggs (*Lymnaea sp*.) at 1941 mg L^{-1}, water fleas (*Daphnia magna*) at 343 mg $L^{-1,}$ and the bluegill (*Lepomis macrochirus*) at 5500 mg L^{-1} (Dowden and Bennett, 1965). The latter showed no mortality for 96 h at 871 mg L^{-1} of K (Trama, 1954), demonstrating high tolerance levels. The Channel catfish (*Ictalurus punctatus*) had a dietary K requirement of 2.6 g K per kg feed if the fish gets the K solely through the feed (Wilson and El Naggar, 1992). At water K^+ levels of 4 mg L^{-1}, the Channel catfish had no need for dietary K due to the ability of uptake from the rearing water. On the other hand, Shearer (1988) observed anorexia, tetany, and death under deficient dietary K supply for Chinook salmon (*Oncorhynchus tshawytscha*) fry in freshwater, indicating optimum feed K concentrations of 0.6 – 1.2 % for this species. The effects of elevated K^+ water concentrations (K^+ > 200 mg L^{-1}) on commercial aquaculture fish species have not yet been described.

Water pH is critical in aquaculture and is often controlled to optimize the water quality for the fish and the nitrifying biofilter. In commercial African catfish RAS, usually with high stocking densities and feed input, pH regulation can reduce freshwater usage due to better microbial nitrification. In hydroponics, inadequate water pH negatively affects nutrient availability. Consequently, for African catfish aquaponics, pH control is recommended. Alkalinity agents for aquaculture contain sodium ($NaHCO_3$, NaOH) or calcium ($CaCO_3$, $Ca(OH)_2$, CaO) (Masser et al., 1999) with the disadvantage of Na^+ and Ca^{2+} accumulation. In hydroponics (and can be assumed for aquaponics), Na^+ interferes with the uptake of the essential plant nutrients K^+, H^+, and NH_4^+–N, although high Ca^{2+} concentrations can reduce these negative effects (Shabala et al., 2003). Too high Ca^{2+} concentrations, especially combined with a high

pH, can lead to unwanted precipitation with PO_4^{3-} (**Prüter et al., 2020**; Rijck and Schrevens, 1998), making this macronutrient unavailable for the plants. This suggests the use of KOH for pH regulation in aquaponics (Pantanella et al., 2010; Rakocy et al., 2006).

Commercial RAS production of African catfish in Germany has been practiced since 2007, with production volumes of around 1000 tons per year since 2015 (Destatis, 2020). First studies on its application in coupled and decoupled aquaponics are described in the literature (Palm et al., 2018a). However, water K^+ concentrations in aquaponics do not meet plant requirements for optimal growth and quality if the only source of K^+ in the aquaponics system is fish feed (**Strauch et al., 2018**). Rakocy et al. (2004) suggested the addition of KOH in coupled aquaponics to control water pH, improve biofilter efficiency while promoting plant growth. However, no information is available on the effects of the elevated K^+ concentrations on the performance and welfare of African catfish. While the toxicity of the nitrogenous compounds NH_3 (Schram et al., 2010), NO_2^- (Roques et al., 2015), and NO_3^- (Schram et al., 2014) and the safe use of PO_4^{3-} (Strauch et al., 2019) with African catfish have been assessed, the effects of elevated K^+ concentrations are still unknown. The purpose of the present study was to better understand the effects of high K^+ concentrations in the rearing water on the growth and welfare of African catfish. Chemical analyses of the proximate body composition have been done to identify possible effects of elevated K^+ concentrations in the rearing water on fish. Future potential applications of the KOH addition in aquaponics are discussed.

4.2 Results

4.2.1 Water Quality

The physicochemical water parameters (mean ± CV) over the run of the experiment are given in Table 4.1. Significant differences ($p < 0.05$) were detected for DO, temperature, conductivity, NO_2^-–N, and K^+. No differences ($p \geq 0.05$) were detected for pH, TAN (total ammonia nitrogen), TON (total oxidized nitrogen), NO_3^-–N, TDN (total dissolved nitrogen), PO_4^{3-}–P, and Mg^{2+}. In K200, the mean DO (dissolved oxygen) was lower than in the other groups. The temperature was lowest in K200 and highest in K600. Conductivity was lowest in K200 and highest in K0 and K400. NO_2^-–N concentrations were highest in K400 and lowest in K200. The mean values of the physicochemical parameters were DO = 6.3 – 6.5 mg L^{-1}, temperature = 29.1 – 29.8 °C, pH ~ 6.8, conductivity = 2344 – 2411 µS cm^{-1}, TAN = 0.66 – 1.61 mg L^{-1}, NO_2^-–N = 0.35 – 0.59 mg L^{-1}, TON = 29.5 – 32.9 mg L^{-1},

NO_3^--N = 29.1 – 32.3 mg L^{-1}, TDN = 30.4–34.5 mg L^{-1}, PO_4^{3-}–P = 3.3 – 3.6 mg L^{-1} and of Mg^{2+} = 9.6 – 10.8 mg L^{-1}.

Table 4.1: *Physicochemical water parameters (mean ± CV).*

		Group			
Parameter	**Unit**	**K0**	**K200**	**K400**	**K600**
DO	mg L^{-1}	6.5 ^{a} ± 4.6	6.3 ^{b} ± 5.1	6.4 ^{a} ± 7.2	6.2 ^{a} ± 5.0
DO	%	84.9 ^{a} ± 5.8	81.6 ^{b} ± 5.3	84.1 ^{a} ± 6.9	84.8 ^{a} ± 4.9
Temperature	°C	29.5 ^{ab} ± 2.4	29.1 ^{c} ± 2.3	29.5 ^{a} ± 2.5	29.8 ^{b} ± 2.4
pH		6.8 ± 9.6	6.8 ± 7.9	6.8 ± 9.0	6.8 ± 7.1
Conductivity	µS cm^{-1}	2382 ^{a} ± 4.7	2344 ^{b} ± 4.3	2411 ^{a} ± 4.6	2376 ^{ab} ± 3.6
TAN	mg L^{-1}	1.48 ± 128.9	0.98 ± 122.2	1.61 ± 133.9	0.66 ± 106.2
NO_2^-–N	mg L^{-1}	0.48 ^{ab} ± 56.9	0.35 ^{b} ± 44.4	0.59 ^{a} ± 63.0	0.52 ^{a} ± 49.1
TON	mg L^{-1}	30.0 ± 46.3	29.5 ± 43.9	32.9 ± 43.4	31.3 ± 40.6
NO_3^-–N	mg L^{-1}	29.6 ± 47.0	29.1 ± 44.4	32.3 ± 44.2	30.8 ± 41.4
TDN	mg L^{-1}	31.5 ± 48.4	30.4 ± 45.0	34.5 ± 46.3	32.0 ± 40.9
K^+	mg L^{-1}	11.7 ^{a} ± 34.7	217.7 ^{b} ± 27.4	418.5 ^{c} ± 30.6	671.0 ^{d} ± 23.5
PO_4^{3-}–P	mg L^{-1}	3.4 ± 50.1	3.3 ± 61.1	3.6 ± 51.6	3.4 ± 50.8
Mg^{2+}	mg L^{-1}	9.6 ± 34.9	10.1 ± 29.5	10.2 ± 29.8	10.8 ± 22.7

Superscript letters indicate significant differences between the experimental groups ($p < 0.05$).

4.2.2 Fish Performance and Welfare Indicators

Fish growth and feed efficiency parameters (mean ± CV) from stocking to final sampling are given in Table 4.2. No significant differences were detected for initial weight, final weight, final total length, final standard length, growth, SGR, FCR, and TFI. No significant mortality occurred.

Table 4.3 shows the welfare indicators of the fish from the different groups (mean ± CV). Significant differences ($p < 0.05$) were observed for the number of skin lesions, with the highest numbers in K600 and the fewest numbers in K200. Significant differences for swimming (individual), agonistic behavior (individual), and fight events (group) were determined, which were most frequent in K0 and least frequent in K600. Significant differences were observed for resting (individual) and stock resting (group), most frequent in K600 and least frequent in K0. No differences ($p \geq 0.05$) were detected for air-breathing (individual), stereotypic behavior (individual), and aggregation behavior (group). Feeding time showed significant differences and was longest in K600 and shortest in K0. Additionally, K600 was the only group with uneaten feed during the 30 min period of feeding. The total amount of uneaten feed in K600 over the run of the experiment was 124 g.

Table 4.2: *Fish growth and feed efficiency of C. gariepinus (mean ± CV). Final sampling was performed on day 42.*

		Group											
Parameter	**Unit**	**K0**			**K200**			**K400**			**K600**		
Initial weight (W0)	g fish^{-1}	28.6	±	20.5	28.3	±	22.0	29.6	±	24.8	30.5	±	23.6
Final weight (Wt)	g fish^{-1}	135.0	±	22.3	138.7	±	28.3	145.6	±	32.7	140.7	±	28.5
Final total length	cm	26.4	±	8.3	26.5	±	9.1	26.6	±	10.2	26.7	±	8.4
Final standard length	cm	23.7	±	8.5	23.8	±	9.1	23.9	±	10.4	24.0	±	8.5
Growth (G)	g fish^{-1}	106.6	±	6.8	110.4	±	2.1	116.0	±	4.5	110.3	±	13.5
SGR	% BW d^{-1}	3.4	±	3.0	3.5	±	0.6	3.5	±	1.3	3.3	±	5.6
FCR		0.80	±	5.91	0.76	±	1.28	0.75	±	2.50	0.80	±	7.36
TFI	g fish^{-1}	84.5	±	1.2	83.7	±	1.3	87.4	±	2.0	87.6	±	7.5

Table 4.3: *Welfare indicators of C. gariepinus challenged with different amounts of dissolved potassium (*: n = 48 (– mortalities); **: n = 6; ***: n = 51; means ± CV).*

		Group											
Parameter	**Unit**	**K0**			**K200**			**K400**			**K600**		
Skin lesions (biting wounds) *	n fish^{-1}	3.3 ^{ab}	±	68.7	3.0 ^{b}	±	78.9	4.1 ^{ab}	±	77.7	4.7 ^{a}	±	66.9
Swimming (individual) **	%	64.5 ^{a}	±	7.4	33.9 ^{ab}	±	20.6	53.8 ^{a}	±	15.9	23.1 ^{b}	±	3.3
Resting (individual) **	%	24.2 ^{a}	±	19.6	62.4 ^{bc}	±	12.4	42.5 ^{ab}	±	26.4	74.2 ^{c}	±	3.6
Agonistic behavior (individual) **	%	7.5 ^{a}	±	10.1	1.1 ^{ab}	±	70.7	1.1 ^{ab}	±	141.4	0.0 ^{b}	±	N/A
Air-breathing (individual) **	n fish^{-1} h^{-1}	30	±	28.3	8	±	93.5	10	±	102.0	16	±	77.1
Stereotypic behavior (individual)	%	11.3	±	141.4	0.0	±	N/A	1.6	±	141.4	0.0	±	N/A
Stock resting (group) **	%	15.1 ^{a}	±	52.7	69.4 ^{b}	±	11.6	39.3 ^{ab}	±	36.8	71.0 ^{b}	±	21.9
Fight event (group) **	%	7.5 ^{a}	±	20.2	1.1 ^{b}	±	70.7	3.2 ^{ab}	±	40.8	0.0 ^{b}	±	N/A
Aggregation behavior (group) **	%	43.6	±	15.1	87.6	±	5.3	45.2	±	44.0	78.5	±	17.0
Feeding time ***	min	2.8 ^{a}	±	27.9	4.1 ^{b}	±	56.1	3.7 ^{b}	±	28.5	15.1 ^{c}	±	78.7

Superscript letters indicate significant differences between the experimental groups ($p < 0.05$).

4.2.3 Proximate Body Composition

The proximate body compositions (mean ± CV) of the whole fish are given in Table 4.4. No significant differences ($p \geq 0.05$) in moisture, protein, fat, ash, calcium, phosphorous, sodium, magnesium, and potassium were observed between the groups.

Table 4.4: *Final proximate compositions and mineral contents in whole fish of C. gariepinus (n = 3, each sample of 8 pooled fish) (mean ± CV). Final sampling was performed on day 42.*

		Group											
Parameter	**Unit**	**K0**			**K200**			**K400**			**K600**		
Moisture	%, ww	73.4	±	1.6	74.0	±	0.6	75.2	±	2.2	73.3	±	0.2
Protein	%, ww	14.8	±	16.3	14.9	±	14.6	14.7	±	11.9	15.0	±	12.9
Fat	%, ww	5.8	±	5.1	5.2	±	8.7	5.2	±	13.1	5.7	±	3.8
Ash	%, ww	3.7	±	5.8	3.6	±	11.3	4.1	±	10.9	3.6	±	6.0
Calcium	g kg^{-1}, dm	37.0	±	6.3	39.7	±	27.6	47.1	±	24.0	42.7	±	6.9
Phosphorus	g kg^{-1}, dm	24.4	±	5.7	26.8	±	16.3	29.6	±	19.0	27.5	±	4.7
Sodium	g kg^{-1}, dm	4.4	±	10.5	5.2	±	36.7	4.7	±	2.2	5.0	±	18.8
Magnesium	g kg^{-1}, dm	1.5	±	1.4	1.5	±	9.0	1.6	±	9.8	1.5	±	3.2
Potassium	g kg^{-1}, dm	12.2	±	7.1	13.2	±	18.5	13.1	±	5.1	11.5	±	0.5

ww: wet weight, dm: dry matter.

The apparent net nutrient utilization (ANNU) of protein, P, and K by the fish showed no significant differences between the treatments (Table 4.5). The ANNU of protein and K increased with a trend from K0 to K200, and the ANNU of P increased from K0 to K400.

Table 4.5: *Apparent net nutrient utilization (ANNU) of C. gariepinus in % (n = 3; mean ± CV).*

	Groups											
Parameter	**K0**			**K200**			**K400**			**K600**		
Protein	35.6	±	21.9	37.6	±	18.4	37.3	±	17.0	36.2	±	16.0
Phosphorous	63.4	±	4.3	71.5	±	16.0	76.2	±	22.2	72.9	±	7.7
Potassium	50.0	±	18.4	55.6	±	20.7	52.1	±	9.7	46.1	±	6.8

4.3 Discussion

This study demonstrates that in African catfish RAS, the addition of K^+ to the process water reaching a concentration of 600 mg L^{-1} has no negative effects on growth performance and proximate body composition of the juvenile *C. gariepinus*. The best apparent animal welfare was reached at concentrations of 200 – 400 mg L^{-1}, with lower concentrations resulting in more fight events and higher concentrations reducing feed intake and increasing skin lesions (biting wounds). Consequently, K^+ concentrations inside the process water up to 400 mg L^{-1} are tolerated and even beneficial for African catfish under RAS and possibly aquaponics production conditions.

4.3.1 Water Quality

To ensure that any differences between the treatment groups are exclusively attributable to the applied K^+ concentrations, all other influencing parameters were kept constant during the run of the experiment. The experiment was carried out in four identical RAS, positioned in the same room at a constant temperature, all treated in the same way (stocking procedure, feed input, water exchange, and data acquisition/sampling) and using the same-sized fish from the same batch and producer.

The water pH values, TAN, NO_3^-–N, and PO_4^{3-}–P water concentrations, showed no significant differences between the treatment groups and were within the recommended range for African catfish (pH: 5.2 – 8.5 (Palm et al., 2018b); NO_3^-–N: < 140 mg L^{-1} (Schram et al., 2014); TAN: toxic, unionized form NH_3 nearly absent at pH values < 7 (Lekang, 2019; Thurston et al., 1981)). Still, except for the intended different K^+ concentrations, minor differences in DO, temperature, conductivity, and NO_2^-–N concentrations were observed. However, these differences were small and within the tolerance level of African catfish (DO: > 3.0 – 4.5 mg L^{-1} (Oellermann, 1995; Palm et al., 2018b); temperature:~ 25 – 30 °C (Britz and Hecht, 1987; Hogendoorn et al., 1983); conductivity: < 2.5 ppm (Britz, 1988) (< 5 mS cm^{-1} at 29 °C); NO_2^-–N: < 0.6 mg L^{-1} (Roques et al., 2015)). According to the observed values, in the case of DO, no differences in air-breathing events (Oellermann, 1995; Strauch et al., 2019), and in the case of temperature (Hogendoorn et al., 1983) and NO_2^-–N (Roques et al., 2015), no difference in growth can be expected. In all treatment groups, the NO_3^-–N concentrations constantly increased over the run of the experiment, while the NO_2^-–N concentrations remained low around 0.5 mg L^{-1}, confirming a regular functioning of the nitrifying biofilter.

The TAN concentrations also remained low < 1 mg L^{-1} for the most part of the experiment and started to increase (max. 3 – 8 mg L^{-1}) only during the last two weeks, indicating a beginning overload of the biofilter. Consequently, the four test systems performed as intended during the run of the experiment, revealing comparable water parameters, with the main difference between the treatment groups remaining the four differently applied levels of potassium (K^+).

4.3.2 Fish Performance and Welfare Indicators

The evaluation of fish performance is usually straightforward and reliable, as it is based on clearly measurable and calculable parameters, such as weight, length, growth, FCR, SGR, and TFI. The determination of the actual welfare status of the animal is rather difficult, as the interpretation is based on indirect parameters. Therefore, welfare indication in fish is usually evaluated by a combination of different behavioral (Van de Nieuwegiessen et al., 2008), performance (Santos et al., 2010), or immunological parameters (Ortuno et al., 2001).

The present study assessed the performance indicators final weight, final total length, final standard length, growth, SGR, FCR, TFI, and the behavioral welfare indicators skin lesions (biting wounds, proof of aggressive behavior), swimming (individual), resting (individual), agonistic behavior (individual), air-breathing (individual), stereotypic behavior (individual), stock resting (group), fight events (group) and feeding time (group) in African catfish reared under different water K^+ concentrations.

Considering weight, length, growth, SGR, FCR, and TFI, the fish performance in this study was not significantly different under the four K^+ water concentrations. The SGRs and FCRs in the present study were in accordance with Strauch et al. (2019), who worked with similar-sized fish. The slightly higher FCRs and SGRs in the present study, when compared with the results by Strauch et al. (2019), may be caused by differently applied feeding rates and feed.

The behavioral welfare indicators air-breathing rates, stereotypic behavior, and aggregation behavior did not differ between the groups in the present study. Van de Nieuwegiessen et al. (2008) observed a greater variation of air-breathing rates (mean values 21 – 109 breaths $fish^{-1}$ h^{-1}) depending on stocking density (max. 50 – 300 kg m^{-3}). Compared to these frequencies, the breathing rates in the present study were rather low in relation to much lower stocking densities (present study: max. 17 kg m^{-3}). Obviously, the much higher biomass in the study by (Van de Nieuwegiessen et al., 2008) resulted in much lower DO levels, which resulted in higher air-breathing frequencies when compared with the present study.

It was conspicuous that the fish in K0 had the highest occurrence of agonistic behavior (individual) and fight events (group), while group K600 had the highest number of skin lesions and were least active at the same time. Neither agonistic behavior nor fighting was observed in K600. The lower activity in K600 was also reflected in a significantly elevated feeding time of ≥ 3.7-fold higher than in the other groups.

It seems as if there is a positive influence at K200 and K400 and a negative influence on the fish at highly elevated water K^+ concentrations under K600.

As discussed previously (Strauch et al., 2019), growth is the assimilation of energy in the form of feed-derived fat and protein. To be available for growth, the feed must be digestible, metabolizable, and not be required for maintenance metabolism. The costs for swimming and maintenance are significant, accounting for 15 – 30 % per gross energy intake (Kaushik and Médale, 1994). In freshwater fish, the internal ion concentration is higher than the external, resulting in the passive uptake of water and the loss of internal ions due to diffusion. The fish control internal homeostasis by producing dilute urine and the active uptake of ions from the surrounding water via the gills (Mc Cormick et al., 2013). Located in the mitochondria-rich cells of the gills, Na^+/K^+ ATPase (Mc Cormick et al., 2013), renal outer medullary K^+ channel (ROMKa) (Furukawa et al., 2014; Horng et al., 2017), and NKCC1a ($Na^+/K^+/Cl^-$ cotransporter) (Horng et al., 2017) are involved in the K^+ regulation mechanisms. Furukawa et al. (2014) and Horng et al. (2017) showed an upregulation of ROMKa and NKCC1a mRNA's at elevated water K^+ concentrations (10 mM KCl (Furukawa et al., 2014) and 4.32 mM KCl (Horng et al., 2017)), resulting in higher energy consumption to maintain homeostasis.

It appears possible that in K200 and K400, the ion concentrations in the surrounding water were close to the physiological concentrations in the fish, hence close to a physiological-environmental equilibrium. Consequently, the energetic costs for maintaining osmotic homeostasis were lower, resulting in a better energy balance. Although not significantly, this was also reflected in the apparently slightly better FCRs and SGRs of these groups. It seems as if the fish in K600 use more energy for osmoregulation, struggling with the high K^+ concentrations. Consequently, less energy would be available for feed intake, swimming, agonistic behavior, and growth.

4.3.3 Body Composition

The proximate body composition of the fish did not reveal significant differences between the treatment groups, indicating efficient osmoregulation under reduced (K0) and highly elevated (K600) K^+ concentrations. The body composition data in the present study were comparable with literature references for catfish (Ersoy and Özeren, 2009; Gbadamosi and Osungbemiro, 2016; Toko et al., 2008). The potassium, magnesium, sodium, and phosphorus concentrations in the fish were also comparable with those reported by Strauch et al. (2019). In contrast to their study, where the phosphorus concentration inside the process water of *C. gariepinus* was elevated, the protein content in the fish of the present study was lower, while the fat and calcium content in the fish were higher. The ANNU for phosphorous and potassium were around 10 % higher when compared with the results in their study (Strauch et al., 2019), but at the same time, for protein around 10 % lower. These differences could be a result of different feed and feeding ratios that result in different nutrient uptake and storage. Though we could not detect any significant differences between the sampled treatment groups, there was a tendency that the ANNU of protein and potassium was slightly better in K200 and K400 and for phosphorous in the K400 group.

4.3.4 Potential KOH Application

K^+ is a vital macronutrient in commercial plant production. For hydroponic solutions, recommended K^+ concentrations lie between 156 and 300 mg L^{-1} (Trejo-Téllez and Gómez-Merino, 2012). African catfish aquaculture results in much lower levels of dissolved K^+, ranging between 9 and 122 mg L^{-1} (Palm et al., 2018b; Strauch et al., 2018), much less than required to achieve optimal plant growth, especially in K^+-demanding plants, such as tomatoes. Consequently, to achieve better plant performance in African catfish aquaponics, K^+ water concentrations should be adjusted to reach optimal plant requirements. This would allow improved plant growth, increased harvest, and thus profitability of the system. Our results suggest that it is possible to increase K^+ concentrations in African catfish process water up to 400 mg L^{-1}, high enough to reach suggested optimal plant growth conditions (see above). Consequently, for a better performance of coupled and decoupled African catfish aquaponics, pH adjustment with KOH might be an optimal solution to reach both optimal pH adjustment and K^+ concentrations that optimize plant growth and aquaponics yield.

4.4 Materials and Methods

4.4.1 Experimental Design

The experiment was carried out in the aquaculture research facilities at the University of Rostock (Justus-von-Liebig-Weg 2, 18059 Rostock). The experimental setup consisted of four identical recirculating aquaculture systems (total water volume: 500 L RAS^{-1}), each containing three aquaria (water volume per aquarium: 135 L), one sump with a pump, a heater, an integrated biofilter and a solid separation unit (further specifications see: Strauch et al. (2019)). Each system represented one experimental unit, only differing in potassium concentrations (for details, see Section 4.4.3). The total flow-through in each system was set to 54 L min^{-1} and adjusted to a flow-through of 18 L min^{-1} in each aquarium.

4.4.2 Fish and Feeding

The aquaria of each RAS and one additional aquarium were stocked with 16 juveniles of African catfish (*Clarias gariepinus*) (Fischzucht Abtshagen, Abtshagen, Germany), with a mean weight of 29.5 g $fish^{-1}$. To allow the fish to acclimate to the experimental conditions, they were stocked seven days before the experiment started. After this acclimation period, the fish from the extra aquarium were used as reference (baseline) for proximate whole fish composition.

All fish were hand-fed with a commercial diet (Skretting Meerval ME-2, 2.0 mm) at 2 % body weight per day during acclimation (7 days) and 3 % of body weight during the experimental period (42 days) (calculations for fish biomass and daily feeding ration see [4.1] and [4.2]. The fish were fed once a day between 9:00 – 10:00 a.m. after recording the water parameters (Section 4.4.3). After offering feed to the fish for 30 min, the uneaten feed was removed, counted, and the dry mass of uneaten feed was determined by multiplying the number of uneaten pellets with the mean weight of one dry pellet (0.12 g $pellet^{-1}$, mean value out of 75 pellets). The values were recorded and adjusted in the feeding table for further feeding calculations. Dead fish were removed, and the feeding table was adjusted to the current number of fish in the aquaria. During the experiment's run, two fish died in total, one in K0 and one in K600. Additionally, on 17 days (day 19, days 21 – 31, and days 36 – 40) of the experiment, the feeding time of fish for each aquarium was determined. This was done by measuring the time between the start (the first pellet touching the water surface) and the end (the last pellet was eaten, or the 30 min are over) of feeding.

Fish biomass per aquarium:

$$m_t = m_0 \times e^{SGR \times t} \quad [4.1]$$

Feed ration per aquarium and day:

$$m_F = m_t \times \frac{FR}{100\%} \quad [4.2]$$

where m_t = fish biomass in one aquarium at day t in g, m_0 = fish biomass in one aquarium at stocking in g, SGR = specific growth rate (assumed value = 0.03), t = time since stocking in days, m_F = feeding ration of one day in g aquarium^{-1}, FR = feeding ratio (2 or 3 %).

According to our analysis (see Section 4.4.4), feed dry matter content was 926.8 g kg^{-1} wet weight (ww) containing 514.8 g kg^{-1} ww crude protein, 85.9 g kg^{-1} ww crude fat, 110.2 g kg^{-1} ww crude ash, 8.3 g kg^{-1} ww K^+, and 14.3 g kg^{-1} ww P, complemented by manufacturer's specifications resulted in 14 g kg^{-1} ww fiber, 4 g kg^{-1} ww sodium, 25 g kg^{-1} ww Ca^+, 42 mg kg^{-1} ww Fe, 2.1 mg kg^{-1} ww iodine, 5 mg kg^{-1} ww Cu, 16 mg kg^{-1} ww Mn, and 110 mg kg^{-1} ww Zn. Before each sampling of the fish (baseline and final body composition), the animals were held without feeding for 2 days.

4.4.3 Experimental Units and Water Quality

During the acclimation period, the four RAS were treated equally. With the start of the experiment, the K^+ concentrations were targeted at four levels, with group 1 (control: K0, without addition of KCl): K^+ < 15 mg L^{-1}, group 2 (K200): K^+ = 200 mg L^{-1}, group 3 (K400): K^+ = 400 mg L^{-1} and group 4 (K600): K^+ = 600 mg L^{-1}. The final K^+ concentration in control resulted from feed digestion and excretion of the fish inside the tanks. The adjustment of the treatment concentrations was performed by solving KCl in pre-tempered tap water in separate "conditioning tanks" (HD-polyethylene, one per RAS). To adjust the same conductivity between groups (2400 µS cm^{-1}) in each RAS, NaCl was added to the water in the conditioning tanks from groups K0 to K400. The pretreated water was brought into the RAS as exchange water, which replaced approximately 30 % per total RAS volume three times a week, on Mondays, Wednesdays, and Fridays.

The water quality parameters pH-value, water temperature (T), dissolved oxygen concentration (DO), and electric conductivity (EC), describing the general experimental conditions, were measured in triplicates with a multimeter (HACH® Multimeter HQ40d) daily, between 8:00 – 9:00 a.m. (before feeding), in each aquarium.

To determine the water concentrations of total ammonia nitrogen (TAN), total oxidized nitrogen (TON) (NO_2^-–N + NO_3^-–N), NO_2^-–N, NO_3^-–N, total dissolved nitrogen (TDN), ortho-phosphate phosphorus (PO_4^{3-}–P), Mg^{2+} and K^+, water samples were taken from each sump, one before, and a second two hours after water exchange on Mondays, Wednesdays, and Fridays. The samples were stored in 100 mL plastic centrifuge tubes at −18 °C. The analysis were performed with an automated discrete analyzer (Thermo Fisher Scientific ™ Gallery™), according to the manufacturer's protocols. With the aid of the measured results (NH_4^+ (analytical method includes $NH_4^+ + NH_3$), NO_3^- (analytical method includes $NO_3^- + NO_2^-$), NO_2^-, PO_4^{3-}, Mg^{2+}, K^+), TAN [4.3], TON [4.4], NO_2^-–N [4.5], NO_3^-–N [4.6], TDN [4.7] and PO_4^{3-}–P [4.8] concentrations were calculated.

Total ammonia nitrogen (TAN):

$$c_i(TAN) = \frac{c_i(NH_4^+)}{M(NH_4^+)} \times M(N) \quad [4.3]$$

Total oxidized nitrogen (TON):

$$c_i(TON) = \frac{c_i(NO_3^-)}{M(NO_3^-)} \times M(N) \quad [4.4]$$

Nitrite nitrogen (NO_2^-–N):

$$c_i(NO_2^- - N) = \frac{c_i(NO_2^-)}{M(NO_2^-)} \times M(N) \quad [4.5]$$

Nitrate nitrogen (NO_3^-–N)

$$c_i(NO_3^- - N) = c_i(TON) - c_i(NO_2^- - N) \quad [4.6]$$

Total dissolved nitrogen (TDN):

$$c_i(TDN) = c_i(TON) + c_i(TAN) \quad [4.7]$$

Ortho-phosphate phosphorus (PO_4^{3-}–P):

$$c_i(PO_4^{3-} - P) = \frac{c_i(PO_4^{3-})}{M(PO_4^{3-})} \times M(P) \quad [4.8]$$

where c_i = concentration, M = molar mass, N = nitrogen, TAN = total ammonia nitrogen, NH_4^+ = ammonia nitrogen, NO_3^- = nitrate, TON = total oxidized nitrogen, NO_2^- = nitrite, TDN = total dissolved nitrogen, P = phosphorus, PO_4^{3-} = orthophosphate.

4.4.4 Analysis of Feed and Fish

At the end of the experiment, all fish were counted, weighed, and measured (total length and standard length). Skin lesions were counted. Whole fish samples for chemical analysis were taken and pooled from 8 fish in each group. The fish were weighed and stunned by a

percussion onto the head; afterwards, they were immediately decapitated and frozen at −20 °C. The frozen fish samples (baseline and final sampling) were then homogenized with a meat mincing machine (Bosch ProPower MFW67440) before they were again stored at −20 °C until further analysis. Above that, a composite feed sample was taken by collecting three times 150 g (day 1, day 21, and day 42). The feed was mixed and homogenized by a knife mill (Retsch Grindomix GM 300) and stored at −18 °C until further analysis. Feed, and fish samples were then analyzed for dry matter, ash, protein, fat, and the elements Ca, P, Na, Mg, and K content at LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt der LMS Agrarberatung GmbH, Rostock, Germany) according to standard methods (VDLUFA lll 3.1, VDLUFA lll 8.1, VDLUFA lll 4.1.1, VDLUFA lll 5.1.1 (B), VDLUFA lll 10.8.2).

4.4.5 Performance Calculations

The total feed intake (TFI) [4.9], growth [4.10], feed conversion ratio (FCR) [4.11], specific growth rate (SGR) [4.12], mortality [4.13], and apparent net nutrient utilization (ANNU) [4.14] were calculated.

Total feed intake (TFI):

$$TFI = feed\ eaten\ by\ fish\ over\ the\ experimental\ period\ in\ g\ fish^{-1} \quad [4.9]$$

Growth (G) in g:

$$G = W_t - W_0 \quad [4.10]$$

Feed conversion ratio (FCR):

$$FCR = \frac{TFI}{W_t - W_0} \quad [4.11]$$

Specific growth rate (SGR) in % BW d^{-1}:

$$SGR = \frac{lnW_t - lnW_0}{d} \times 100\% \quad [4.12]$$

Mortality (Mo) in %:

$$Mo = \frac{number\ of\ dead\ fish}{initial\ number\ of\ fish} \times 100\% \quad [4.13]$$

Apparent net nutrient utilization (ANNU) in %:

$$ANNU = \frac{W_t \times X_t - W_0 \times X_0}{TFI \times X_F} \times 100\% \quad [4.14]$$

where W_t = final fish weight, W_0 = initial fish weight, BW = body weight, d = time in days, X_0 = initial nutrient concentration of the fish, X_t = final nutrient concentration of the fish, X_F = nutrient concentration of the feed.

4.4.6 Ethology

To evaluate fish ethology, the fish of each aquarium were observed for five minutes, once in the middle of the experiment (day 23) and once at the end of the experiment (day 42). At the beginning of the experiment, no observations were made to avoid the assessment of stocking-influenced data. All observations were made at 02:30 p.m. and on days where no water exchange was conducted to exclude postprandial somnolence or influence of stress. During observations, the fish behavior was analyzed according to the ethogram in Table 4.6 (adapted from Van de Nieuwegiessen et al. (2008)). To evaluate individual behavior, one randomly chosen fish was observed for five minutes in each aquarium. If the sight of this fish was lost (e.g., due to murky water or fish swimming in front of the observed fish), it was replaced by the fish closest to where it disappeared. To assess group behavior, all visible fish in one aquarium were considered for evaluation.

Table 4.6: *Ethogram – behavioral patterns and their definitions, adapted from van de Nieuwegiessen et al. (2008).*

	Behavior	**Definition**
Individual	Swimming	Active displacement of the body while browsing, moving, and eating.
	Resting	Moving passively through the water or lying still at the bottom of the tank.
	Agonistic behavior	Chasing or biting a fish or being chased upon or bitten by another.
	Air-breathing	The animal moves to the water surface and takes a gulp of air. Air from the gills of the fish escapes when it swims back to the bottom of the tank.
	Stereotypic behavior	Continuous and compulsive swimming under a fixed, repetitive pattern for at least 10 s.
Group	Stock resting	More than 60 % of the fishes in the stock show the behavior pattern “resting”.
	Fight event	Fight events between fishes that are not being individually observed.
	Aggregation behavior	Gathering of more than 30 % of the fishes of the stock in a small area, generally touching each other.

Therefore, the recordings (except for air-breathing) were divided into 10 s sections, and it was counted with “1” if the fish showed the respective behavior in a certain time frame (“0” if not). Afterward, the percentage proportion of the five minutes in which the fish showed the

respective behavior was calculated [4.15]. For air-breathing, the breaths of air within the 5 min were counted and multiplied by 12 to obtain the breathing rate in n fish^{-1} h^{-1}.

Percentage proportion of the shown behavior within the 5 min (= 300 s) of observation:

$$Behavior = x \times \frac{10s}{300s} \times 100\% \quad [4.15]$$

where x = sum of counted "1".

4.4.7 Statistics

The physicochemical water parameters, the growth and feed efficiency parameters, the fish composition, the apparent net nutrient utilization (ANNU), and the welfare indicators were tested for significant differences between the treatment groups ($p < 0.05$). All statistical analyses were conducted with "IBM SPSS Statistics 25". All data were first tested for normal distribution (Shapiro–Wilk test). If the data were normally distributed, the data were tested for homogeneity of variance (Levene's test). In the case of homogeneity of variance, the mean values were compared with ANOVA and Tukey-HSD as post hoc test (FCR; temperature; total length; standard length; whole fish composition: moisture, ash, fat and phosphorous; ANNU: P; fish ethology: resting (individual), aggregation behavior (group)). If the data showed no homogeneity of variance, the mean values were compared with the Welch's test and Dunnett's T3 test as post hoc test (growth; NO_2^-–N). In the case of not normally distributed data, the Kruskal–Wallis test was performed using a Bonferroni-correction (SGR; TFI; DO; pH; conductivity; TAN; TON; NO_3^-–N; TDN; K^+; PO_4^{3-}–P; Mg^{2+}; total weight; whole fish composition: Na, Mg, K; ANNU: protein, potassium; feeding time; skin lesions; fish ethology: swimming (individual), agonistic behavior (individual), air-breathing (individual), fighting events (group), stock resting (group), stereotypic behavior (individual)).

4.5 Conclusion

Modern African catfish recirculating aquaculture production systems and integrated aquaponics have high investment and running costs. To be profitable, stock-, feed-, and water management, and aspects of animal welfare, must be optimized. Many plants in aquaponics require adjusted nutrient solutions to achieve optimal growth and quality. This study demonstrates that K^+ can be added to the rearing water of African catfish juveniles up to 400 mg L^{-1} without impairing the welfare status, productivity, or product quality. This suggests

the safe use of potassium-containing alkalinity agents, such as KOH, to control the pH of the rearing water, with positive effects on nitrifying-biofilter activity, fish growth, and welfare, but also on plant growth and quality in aquaponics.

5 Growth and Welfare of African catfish (*Clarias gariepinus* Burchell, 1822) under Dietary Supplementation with Mixed-Layer Clay Mineral Montmorillonite–illlite/Muscovite (1g557) in Commercial Aquaculture

Abstract

Juvenile African catfish (*Clarias gariepinus* Burchell, 1822) were reared within two experiments (a research facility and a local catfish farm, E1 and E2, respectively) for 102 d each under commercial recirculating aquaculture conditions. The mixed-layer clay mineral montmorillonite–illite/muscovite (1g557) was applied as a feed additive at concentrations of 0.5 % and 2.0 %, which were compared with an unsupplemented control (0.0 %) over 70 d. For E1, feeding was automatic at night, while E2 was fed manually during the day. The growth and physiological welfare parameters of the fish were monitored, including mortality, skin lesions, stress responses after confinement (plasma cortisol and glucose), and additional blood parameters. Tendentially, the most efficient growth in both experiments was observed in the 0.5 % groups, which performed slightly better than the controls (E1: 0.8 % and E2: 3.2 %) despite a lower nutrient content ($p > 0.05$). In E1, the negative skewness of the leptokurtic distribution also revealed the highest number of larger-sized fish per batch. Mortality was low in all the treatment groups (E1 control/0.5 %/2.0 %: 3.6 %/4.9 %/2.9 %; E2 control/0.5 %: 2.6 %/5.5 %). After only 29 d in E1, the number of skin lesions per fish decreased significantly ($p < 0.05$) between each of the 0.5 % and 2.0 % groups, compared to the control (E1 control/0.5 %/2.0 %: 1.2/0.8/0.8). In both E1 and E2, the number of lesions per fish decreased even further after 70 d, significantly between the treatment groups and the control (E1 control/0.5 %/2.0 %: 0.9/0.4/0.5 and E2 control/0.5 %: 0.6/0.3). In E1, the cortisol and glucose concentrations increased strongly in all the groups due to the induced stress, whereas this was not evident in E2 based on the different sampling procedures. The additional blood parameters (aspartate aminotransferase, glutamate dehydrogenase, urea, calcium, phosphate, total protein, leucocytes, erythrocytes, hematocrit, cholesterol, triglycerides, sodium, potassium, and chloride) revealed no significant difference between the treatment groups in either experiment, indicating no negative effects of 1g557 on the organs or metabolism of the fish. Supplementation with 0.5 % 1g557 in the common commercial feeds for African catfish increases growth performance ($p > 0.05$), reduces size variance, and supports fish welfare under different commercial aquaculture conditions in the present study.

Keywords

1g557; feed additive; feed supplementation; fish well-being

5.1 Introduction

The African catfish (*Clarias gariepinus* Burchell, 1822) is a warm-water fish with increasing commercial importance worldwide. For instance, the production of this species in recirculating aquaculture systems (RAS) increased in Germany between 2019 (1,193,137 kg year^{-1}) and 2011 (318,575 kg year^{-1}) (Destatis, 2012, 2020). Due to its high tolerance with regard to adverse water conditions, including low oxygen (Belão et al., 2011) and high ammonium, nitrite, and nitrate concentrations (Păpuc et al., 2019; Roques et al., 2015; Schram et al., 2014), the African catfish can be reared under high stocking densities (up to 500 kg m^{-3}) (Van de Nieuwegiessen et al., 2009). However, rearing fish under very high stocking densities might negatively affect the welfare and survival of the fish. This issue can possibly be overcome through the use of clay minerals, which can be used as feed additives and contribute to the increased fitness and survival of the fish. The welfare of African catfish has previously been assessed by analyzing the behavior, external injuries (i.e., skin lesions), cortisol, glucose, lactate, growth, and mortality of the fish in several studies covering different stocking densities, group compositions, ages, individual differences, and different rearing systems (Baßmann et al., 2017, 2020; Martins et al., 2006a, 2006b; Van de Nieuwegiessen et al., 2008, 2009). All of these may also pertain to the present study.

In general, clay minerals are considered to exert positive effects in relation to aquaculture, ranging from enhanced water conditioning and detoxification to enhanced growth, health, or well-being in farmed aquatic animals. These positive effects have been proven for some clay minerals by scientific research; for many others, this has not yet been confirmed. It is evident that the physical and chemical properties of clay minerals are determined by their chemical composition and spatial crystal structure, which results in such materials exhibiting different abilities to exchange ions or easily hydrate the layered structure. Consequently, some clay minerals have been shown to be able to adsorb different ions, such as nitrogen compounds and phosphates (Eturki et al., 2012; Seger et al., 2015), as well as fatty acids, nucleic acids, or proteins (Edzwald et al., 1976; Heimann, 2010).

According to Attramadal et al. (2012), the addition of clay minerals (mainly illite) resulted in improvements in the water quality during the breeding of Atlantic cod larvae (*Gadus*

morhua L., 1758). In this case, the dissolved organic material was bound, which reduced the bacterial load and, generally speaking, decreased the rate of larval mortality. Seger and Hallegraeff (2022) described a reduction in the ichthyotoxicity in algal blooms, especially following the addition of bentonites. The montmorillonite found in feeds has been determined to adsorb mycotoxins (toxic metabolites of fungi) (Desheng et al., 2005; Hassan et al., 2010; De Mil et al., 2015; Pasha et al., 2008) and the herbicide glyphosate (Khoury et al., 2010), which both pose a growing threat in relation to animal farming due to causing neurotoxic or carcinogenic effects, developmental disorders, decreased weight gain, impaired immunity, and increased mortality (Gill et al., 2018; Koletsi et al., 2021; Marijani et al., 2019; Oliveira and Vasconcelos, 2020). Moreover, some mycotoxins might accumulate in tissues and, therefore, reach end consumers (Anater et al., 2016; Deng et al., 2010; Oliveira and Vasconcelos, 2020). Palm et al. (2015, 2021) reported increased survival, higher final weights, more efficient feed conversion, and reduced size variance in post-larval whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) following the application of feeds containing 2 % montmorillonite-illite/muscovite or a combination of 2 % of this clay mineral and 2 % of the microalgae *Chlorella vulgaris* (Beij). A positive influence on growth performance and feed digestibility has also been described with regard to Nile tilapia (*Oreochromis niloticus* L., 1758) fed with supplemented (Cu^{2+}- exchanged) montmorillonite (Hu et al., 2007, 2008). In addition, Eya et al. (2008) tested feeds supplemented with 0 %, 2.5 %, 5 %, and 10 % bentonite in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). After 90 d, the 5 % and 10 % bentonite supplementation significantly improved the growth parameters of the fish, including the percentage weight gain, specific growth rates (SGR), and feed efficiency. Jawahar et al. (2018) reported immunostimulatory effects after adding sodium bentonite to the diet of stinging catfish (*Heteropneustes fossilis* Müller, 1840).

Mixed-layer clay mineral montmorillonite–illite has been approved as a technological feed additive by the European Food Safety Authority (EFSA, 2014) and, subsequently, the European Union (EU) in Regulation (EU) 2016/1964 under the abbreviation 1g557 (European Comission, 2016). Furthermore, as mentioned above, Palm et al. (2015) demonstrated its positive effects on the survival and growth performance of whiteleg shrimp. However, the potential effects of this clay mineral on fish, including their well-being, remain unknown.

The present study analyzed the external injuries, growth, mortality, and blood parameters of African catfish under dietary supplementation with 1g557. The aim of the

supplementation was to promote the growth and welfare of this species under commercial production conditions at two different aquaculture facilities (a research facility and a local catfish farm) and when applying different feeding regimes (automatic night feeding and hand feeding during the day).

5.2 Materials and Methods

5.2.1 Production Systems and Maintenance

Two experiments (E1 and E2) were conducted in this study. E1 was conducted at the "FishGlassHouse" aquaculture research facility within the University of Rostock, while E2 was performed at a local catfish farm (Fischzucht Abtshagen GmbH & Co. KG, Mecklenburg-Western Pomerania, NE Germany). Both experiments used RAS for catfish production on a commercial scale. The production capacity of the first facility differed according to the research project being carried out (1000 – 5000 kg $year^{-1}$), whereas only stocking fish for third parties and fish for reproduction were produced at the second facility (production capacity unknown). The tank sizes at both facilities were highly comparable, as will be described further below.

The RAS used in E1 has previously been described by Palm et al. (2018b). Briefly put, it comprises nine identical rearing tanks, each measuring (L x W x H) 1.8 m x 1.0 m x 0.7 m (1.26 m^3). The process water is cleaned through a settling tank (1.3 m^3, equipped with lamella inserts, specific surface area of 105 m^2 m^{-3}) and a trickling filter (5.9 m^3, specific surface area of 125 m^2 m^{-3}), collected in a sump (2.7 m^3) and then returned to the fish tanks. When used in E1, the RAS contained a total of 15.1 m^3 water. Regular water exchange was performed with tap water (approximately 624 L d^{-1} = 4.1 % of the total volume). The settling tank was cleaned on a weekly basis. The temperature was set to 27 °C. The pH was adjusted by adding calcium hydroxide as soon as it dropped below 5.5.

In E2, six identical rearing tanks (L x W x H: 1.37 m x 0.94 m x 0.9 m, 1.16 m^3) that formed part of a larger RAS were used. The RAS was equipped with two settling tanks (each 0.95 m^3, equipped with lamella inserts, specific surface area of 125 m^2 m^{-3}) and two biofilters (one trickling filter, approximately 14.1 m^3, specific surface area of 125 m^2 m^{-3}, one moving bed filter, 5.1 m^3, biocarrier volume of approximately 2.75 m^3 with a total surface area > 750 m^2 m^{-3}). The water was collected in a sump (2.5 m^3). This RAS contained a total of

18.8 m^3 of water. Water exchange was performed twice a week when the settling tanks were cleaned (1.9 m^3 each time).

Table 5.1 summarizes the water quality parameters in E1 and E2. In both experiments, the temperature, oxygen concentration and saturation, pH, electric conductivity (EC), salinity, and redox potential were recorded daily (each in triplicate) using a portable multimeter (Hach-Lange HQ40D, Düsseldorf, Germany) at the settling tanks' influx and efflux as well as behind the trickling filter. Twice a week, the water samples were analyzed in triplicate using an automatic photo analyzer (Gallery™, Thermo Fisher Scientific, Waltham, MA, USA) to test for ammonium/ammonia (NH_4^+/NH_3), nitrite (NO_2^-), nitrate (NO_3^-), and ortho-phosphate (PO_4^{3-}). The process water in both facilities was disinfected by means of ultraviolet (UV) radiation. Both experiments were conducted under low-light conditions, as is common in catfish aquaculture.

Table 5.1: *Water parameter (mean ± standard deviation) in E1 and E2, as measured daily in the settling tank influx (SI), the settling tank efflux (SE), and the trickling filter (TF).*

Water Parameters	E1						E2					
	SI	SE	TF	SI	SE	TF
T in °C	27.0 ± 0.2	27.1 ± 0.2	27.3 ± 0.2	28.7 ± 1.0	28.8 ± 1.0	28.8 ± 1.0
O_2 in mg L^{-1}	6.5 ± 0.5	6.0 ± 0.7	7.5 ± 0.2	6.1 ± 0.7	5.0 ± 1.0	6.8 ± 0.5
O_2 in %	81.7 ± 5.9	75.3 ± 9.4	94.2 ± 2.7	78.8 ± 8.9	64.6 ± 11.9	87.7 ± 5.3
pH	6.6 ± 1.1	6.7 ± 1.1	6.8 ± 1.4	7.0 ± 0.7	7.0 ± 0.7	7.1 ± 0.8
EC in µS cm^{-1}	1254.5 ± 260.3	1256.5 ± 259.5	1263.1 ± 263.1	881.2 ± 80.5	881.4 ± 80.8	881.9 ± 81.0
RedOx in mV	153.8 ± 42.6	157.8 ± 42.0	163.0 ± 47.4	160.4 ± 36.7	156.4 ± 34.0	155.8 ± 32.6
NH_4–N* in mg L^{-1}	0.6 ± 0.9	0.6 ± 1.0	0.5 ± 0.9	0.7 ± 2.0	0.7 ± 2.0	0.7 ± 1.9
NO_2–N* in mg L^{-1}	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.3	0.2 ± 0.1
NO_3–N* in mg L^{-1}	71.1 ± 36.4	71.8 ± 36.1	72.5 ± 36.1	196.4 ± 50.3	199.9 ± 52.9	195.5 ± 51.5
PO_4–P* in mg L^{-1}	3.8 ± 1.8	3.9 ± 1.9	3.8 ± 1.9	17.2 ± 9.8	16.6 ± 9.8	16.6 ± 9.8

Note: T = temperature, O_2 = oxygen, EC = electric conductivity, RedOx = redox potential, NH_4–N = ammonium-nitrogen, NO_2–N = nitrite-nitrogen, NO_3–N = nitrate-nitrogen, PO_4–P = ortho-phosphate. Parameters marked by asterisks (*) were measured twice weekly.

5.2.2 Experimental Feeds

This study examined the results of three experimental diets, namely a diet supplemented with 0.5 % or 2.0 % 1g557 and an un-supplemented control diet. Otherwise, all the experimental diets involved standard catfish feed (manufactured by Spezialfuttermittelwerk Beeskow GmbH, Beeskow, Germany). The basic ingredients of the experimental feeds are set out in Table 5.2.

Table 5.2: *Basic ingredients of the experimental feeds.*

Ingredient	**Percentage**
Wheat	27.96
Fish meal 70 M	17.77
Poultry meal	11.22
High protein (HP)-soya extract grist	9.35
Hemoglobin powder	5.61
Hydrolyzed feather meal	5.61
Pea protein	5.61
Monocalcium phosphate	1.03
Additional vitamins	**In IU kg^{-1}**
A; C; D; E	12,000; 160; 1,600; 160

Further information about the proportions of the individual ingredients is available at the manufacturer's discretion. After mixing the respective amount of 1g557 (Palm et al., 2021) to the basic feed ingredients, fish oil (8.88 %) and water (6.5 %) were added according to the manufacturer's recommendation. Next, the feed was pelleted (using a Pelleting Press Model 14 – 175, Amandus Kahl GmbH & Co., Reinbek, Germany), separately packed, and directly deep frozen at –20 °C until feeding in order to prevent any contamination. The feed processing was repeated five times during the experiments so as to obtain fresh feeds. The pellets' stability in water was tested prior to the experiments and found to be sufficient. Information concerning the nutritional values of the specific diets is presented in Table 5.3.

Table 5.3: *Nutritional values of the feeds (dietary values for the 0.5 % and 2.0 % groups were calculated but not practically verified).*

Nutrient	Coppens Special Pro EF 3 – 4.5	Experimental Diets (from Beeskow Feed Mill)		
	Adaptation period	Control group	0.5 % Group	2.0 % Group
Crude protein in %	42.00	45.20	44.97	44.30
Crude fat in %	13.00	15.00	15.00	15.00
Carbohydrates in %	Not specified	19.60	19.50	19.21
Crude ash in %	7.80	5.10	5.08	5.00
Crude fiber in %	1.50	1.40	1.39	1.37
Phosphorus in %	1.14	1.00	1.00	0.98
Digestible energy in MJ kg^{-1}	17.1	20.10	20.00	19.70
1g557 in %	0	0	0.5	2.0

The mixed-layer clay mineral 1g557 originated from an open-cast mine near Friedland in Mecklenburg-Western Pomerania, Northern Germany, which is why it is also known as "Friedland clay." However, in the present study, it will be referred to as 1g557, as that is the official abbreviation used in Regulation (EU) 2016/1964. It is a mixture of different minerals, dominated by 35 – 53 % swellable montmorillonite/illite, around 30 % non-swellable illite/muscovite, and < 20 % kaolinite and quartz. Siderite, pyrite, and other minor constituents (< 1 %) are also present in 1g557 (EFSA, 2014; FIM Biotech, 2017; Henning and Kasbohm, 1998). The empirical formula is $Na_{0.03}Ca_{0.04}K_{0.16}(Al_{1.87}Fe_{0.16}Mg_{0.16})(Si_{3.31}Al_{0.69})O_{10}(OH)_2^-$ (EFSA, 2014). By definition, 1g557 cannot be considered a true bentonite, although its physical properties are determined by montmorillonite, which is the main component of bentonites. When compared with other bentonites, 1g557 has both a lower swelling capacity and a lower specific surface area (Henning and Kasbohm, 1998).

5.2.3 Fish Stocking and Feeding

In E1, 926 presorted juvenile African catfish (with an average weight of 30.9 g) were obtained from Fischzucht Abtshagen GmbH & Co. KG on 1 February 2019. The fish were randomly stocked into the nine tanks so that there were 103 fish tank^{-1} (one tank with 102 fish, approximately 2.5 kg m^{-3}). Respectively, three tanks were allocated to each of the three treatment groups: 0.5 %, 2.0 %, and control.

In E2, 618 juvenile African catfish were bred directly at Fischzucht Abtshagen. The fish were presorted and stocked (with an average weight of 29.8 g) into six tanks (103 fish tank^{-1})

on 24 April 2020. Three tanks were allocated to each of the two treatment groups: 0.5 % and control.

In both experiments, a randomized block design in triplicate was used. During an adaptation period of 31 d, all the fish were fed a regular commercial catfish diet (Coppens Special Pro EF 3 – 4.5 mm, see Table 5.3) with floating pellets, which was the same diet used by the fish farmer. After the adaptation period and the first sampling (see below), the diet was changed by switching to the experimental diets (Table 5.3). The unsupplemented African catfish feed mixture (from Beeskow feed mill) was used as a control. In the supplemented feeds, the replacement of 0.5 % or 2.0 % of the regular feed with 1g557 reduced the nutrient content by a maximum 0.5 % or 2.0 % and the digestible energy by 0.1 – 0.4 %. The experimental diets involved sinking pellets. The amount of feed given per day was based on an existing commercial feeding protocol (between 3.9 % and 1.5 % of the body weight of the fish, depending on the growth stage, Fischzucht Abtshagen). In E1, feeding was performed every two hours between 07:00 p.m. and 05:00 a.m. using automatic feeders (PR5A, Linn Aqua Technology, Lennestadt, Germany). In E2, hand feeding was performed twice daily at approximately 07:30 a.m. and 02:30 p.m. Any remaining feed, if present, was collected by the settling tank and removed routinely.

5.2.4 Sampling

After the adaptation period, sampling was performed every four weeks in both experiments. The first sampling (T0) in E1 involved measuring the body weights, body lengths, initial concentrations of plasma cortisol and blood glucose, and external injuries (skin lesions and fin erosions due to aggressive behavior) of a sub-sample of fish (11 fish per tank = 33 fish per treatment group). After 28 d, the next sub-sample (15 fish per tank = 45 fish per treatment group) was taken over a period of three days (T1), with the growth and welfare parameters of the fish being recorded again. After 58 d, a further sub-sample (15 fish per tank = 45 fish per treatment group) was taken (T2), and the same parameters were recorded. After 70 d, the final sampling (T3) was performed by taking the body weights, body lengths, number of external injuries and mortality of all the remaining fish.

The first sampling (at T0) in E2 involved measuring the same parameters as measured in E1, albeit using three unstressed fish and three stressed fish per tank (18 fish per treatment group). The next sub-sample was taken after 28 d (T1), with the same parameters being

measured in an equal sample size as before. A further sub-sample was taken after 58 d (T2). The final sampling was performed after 70 d (T3) by taking the weights, lengths, number of external injuries, and additional blood parameters (see above) from all the remaining fish.

As some fish were removed from the experiment after each sampling, the feed conversion ratios (FCR) [5.1] were calculated for each sampling date using the following equation. All the remaining fish in the tanks were considered.

$$FCR = \frac{TFI}{W_t - W_0} \quad [5.1]$$

where TFI is the total feed intake in g, W_0 is the initial fish weight in g, and W_t is the final fish weight in g.

The condition index (CI) [5.2] was determined at the time of stocking, T0, and T3. At the time of stocking and T3, all the fish were considered (E1: at stocking: 309, 308, 309; T3: 166, 164, 171; E2: at stocking: 309 each; T3: 172, 163). Moreover, at T0, sub-samples of 33 fish from each group were taken in both E1 and E2. The following equation was used to determine the CI:

$$CI = \frac{fish\ mass\ in\ g}{fish\ length\ in\ cm^3} \times 100 \quad [5.2]$$

5.2.5 Blood Parameter and External Injuries

To compare the welfare, mortality, growth performance, number of external injuries, and blood parameters of the fish, the plasma cortisol and blood glucose concentrations were analyzed. In E1, the aim was to analyze the cortisol and glucose after the fish encountered stressors commonly found in aquaculture production. For this purpose, the water level of a rearing tank was reduced to approximately 20 cm, and all the fish from the tank were quickly removed using nets and placed in 100 L sorting tubs for a short period of time. This process was applied for the weight measurements of the fish stocking per tank, although it also resulted in a confinement stressor for the fish that induced stress responses (i.e., cortisol). Normal or attenuated elevations of cortisol levels, or even the absence of a cortisol response, can contribute to inferences regarding chronic stress conditions (Wendelaar Bonga, 1997). Thus, 15 randomly chosen fish per tank (45 per group) were stunned via brain percussion and

then killed via cutting the gills before blood samples were obtained from their caudal vessels. In Germany, it is legally permitted to kill fish after effective stunning in order to use their organs or tissues for scientific purposes (Deutscher Bundestag, 2020) (according to § 4 (1), § 7 (2) sentence 3, TierSchG [German Animal Protection Act]).

The blood glucose of the fish was measured in situ using test stripes (Accu-Chek Aviva, Roche, Mannheim, Germany). Approximately 0.5 mL of blood was transferred to reaction tubes with a coated coagulation inhibitor (5.4 mg potassium–ethylenediaminetetraacetate, K-EDTA) and then stored on ice. The blood samples were centrifuged (1250 rpm at 4 °C for 10 min; Hettich Universal 320 R, Tuttlingen, Germany), and the plasma phase was used in a cortisol enzyme-linked immunosorbent assay (ELISA) (Cusabio, fish cortisol, sensitivity: 0.0023 ng mL^{-1}) according to the manufacturer's instructions. The plasma samples were analyzed using a micro-plate reader at 450 nm (iMark, Bio-Rad, Feldkirchen, Germany).

In E2, three fish per tank (nine per group) were directly caught, stunned, killed, and blood samples were obtained. This procedure was conducted within 10 min in order to obtain a proper indication of the cortisol baseline (reflecting unstressed fish). Cortisol starts to rise within a few minutes of acute stress being induced (Wendelaar Bonga, 1997). Afterwards, all the remaining fish were treated the same as in E1, that is, stress was induced by means of water level reduction to approximately 20 cm, followed by the catching process and confinement. Then, three fish per tank (nine per group) were stunned and killed, with blood samples being obtained in the same way as in E1 (reflecting stressed fish without specifically considering the temporal influence or intensity stress).

Additional blood samples (approximately 3.0 mL in total) were taken to analyze hematocrit, leucocytes, erythrocytes, aspartate aminotransferase (AST)/glutamic oxaloacetic transaminase (GOT), glutamate dehydrogenase (GLDH), cholesterol, triglycerides, urea, sodium, potassium, calcium, chloride, phosphate, and total protein levels of the fish. The sodium, potassium, and chloride concentrations were measured using an ion-selective electrode. All the other chemical blood parameters were quantified by means of photometry/ flow cytometry. In E1, three fish were sampled per treatment group (one fish per tank) at T0 and T3, while in E2, six fish were sampled per group (two fish per tank) at T0 and T3.

The number of skin injuries on the body and fins of the fish (not on the heads due to the utilized stunning method) was recorded by the same two people throughout the experiments and independently from the treatment groups, which served to exclude bias. Injuries to the

skin occur regularly in the scaleless African catfish, particularly directly after stocking. However, only fresh biting wounds that penetrated the epidermal layer or reached down to the underlying tissue were counted in this study. Multiple skin lesions that were clearly related to a single biting attack were counted as one injury, regardless of their individual size. By contrast, skin lesions that could not be assigned to a single attack were counted as multiple wounds. Injury marks (scars) were not counted if they had already begun to heal (as indicated by a regenerated epidermal layer or mucus), as they would cause no or only minor pressure to the immune system and, therefore, no longer impair the welfare of the fish. Sex, weight, and length were recorded from all the sampled fish. All the remaining fish were weighed as a group, counted, and allocated to the respective rearing tanks.

5.2.6 Statistics

The data gathered in this study were first tested with regard to the distribution. For the normally distributed data and three experimental groups, one-way analysis of variance (ANOVA) and post-hoc multiple range tests were used, whereas Tukey's HSD test was used for variance homogeneity, and Dunnett's T3 test was used for variance inhomogeneity. For the not normally distributed data and unequal numbers, the nonparametric Kruskal–Wallis test was applied. The parameters of the two experimental groups (E2) were analyzed using a t-test if the data were normally distributed; otherwise, the Mann–Whitney test was used to assess the significance. All the tests were performed with a significance level of $p < 0.05$. The significances were only compared between the treatment groups in a single sampling, not between different samplings. In addition, a frequency distribution test was performed, including the range, symmetry, kurtosis, and skewness of the masses and lengths of the fish (Bhujel, 2008). These statistical evaluations were conducted using Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp., 2017, Armonk, NY, USA) software. The tests performed are specified in the Results section with the respective data.

5.3 Results

5.3.1 Fish Growth Performance

The mean weights, lengths, differences in weight (%), and condition indices of the fish at the time of stocking, T0, and T3 are given for E1 and E2 in Table 5.4. At the time of stocking in

E1, the fish in the control group were almost ($p > 0.05$) the same size as those in the 0.5 % and 2.0 % groups.

Table 5.4: *Growth performance (mean ± standard deviation) of the African catfish in E1 and E2 under different diets supplemented with 1g557. Different superscript letters indicate statistical differences.*

	n	Group	Weight in g		Length in cm		Δ [%]	CI in g cm^{-3}	
					E1				
Before stocking and adaptation phase	309	C	30.7 ^{a,b}	± 5.8	16.7 ^{a,b}	± 1.1	0	0.652^{a}	± 0.1
	308	0.5 %	31.6 ^{a}	± 6.0	16.9 ^{a}	± 1.1	+ 2.9	0.652^{a}	± 0.0
	309	2.0 %	30.3 ^{b}	± 6.1	16.6 ^{b}	± 1.1	– 1.3	0.661^{b}	± 0.0
T0 (start of experiment)	33	C	107.8 ^{a}	± 17.2	24.8 ^{a}	± 1.5	n.g.	0.7^{a}	± 0.1
	33	0.5 %	112.6 ^{a}	± 16.2	25.2 ^{a}	± 1.3	n.g.	0.7^{a}	± 0.0
	33	2.0 %	107.9 ^{a}	± 17.1	24.5 ^{a}	± 1.4	n.g.	0.7^{a}	± 0.0
T3 (after 70 d)	166	C	480.5^{a}	± 89.2	39.2^{a}	± 3.0	0	0.8^{a}	± 0.1
	164	0.5 %	484.2^{a}	± 86.7	39.4^{a}	± 2.8	+ 0.8	0.8^{a}	± 0.1
	171	2.0 %	469.5^{a}	± 93.3	39.0^{a}	± 2.9	– 2.3	0.8^{a}	± 0.1
					E2				
Before stocking and adaptation phase	309	C	29.8 ^{a}	± 3.6	17.0 ^{a}	± 0.8	0	0.6^{a}	± 0.1
	309	0.5 %	29.7 ^{a}	± 3.3	17.0 ^{a}	± 0.8	– 0.3	0.6^{a}	± 0.0
T0 (start of experiment)	33	C	115.6 ^{a}	± 20.8	25.3 ^{a}	± 1.6	n.g.	0.7^{a}	± 0.0
	33	0.5 %	115.2 ^{a}	± 16.4	25.3 ^{a}	± 1.4	n.g.	0.7^{a}	± 0.1
T3 (after 70 d)	172	C	409.2^{a}	± 73.1	37.5^{a}	± 2.4	0	0.8^{a}	± 0.2
	163	0.5 %	422.2^{a}	± 76.1	37.9^{a}	± 2.5	+ 3.2	0.8^{a}	± 0.2

Note: C = control group. Δ [%] as the difference in weight relative to C. CI = condition index. At T0, a sub-sample of 33 fish was weighed and measured in terms of the length; at T3, all the remaining fish were weighed and measured in terms of the length; n.g. = not given since sub-sample; $p < 0.05$.

After changing to the test feed at the beginning of E1 (T0), the sub-samples of 33 fish per group weighed 107.8 – 112.6 g and had lengths of 24.5 – 25.2 cm ($p > 0.05$). After 70 d (T3), the 0.5 % group showed the highest weight (with 484.2 g), which was 0.8 % above the weight of the control group (480.5 g). The 2.0 % group was 2.3 % below the weight of the control group (469.5 g). This difference was insignificant, although the 0.5 % group tended to exhibit the best average growth performance, followed by the control group and the 2.0 % group. The differences in the weight and length of the fish in the different groups were insignificant, with a negative trend of 2.3 % being seen in the 2.0 % group.

During E2, the fish in the control and 0.5 % groups showed no significant difference in their size at the time of stocking. At T0, the sub-samples of 33 fish weighed 115.2 – 115.6 g and had an average length of 25.3 cm ($p > 0.05$). After 70 d (T3), the 0.5 % group again showed

the highest weight when compared with the control group (422.2 g vs. 409.2 g; $p > 0.05$). Overall, in E2, the 0.5 % group was found to be 3.2 % above the weight of the control group (with the difference being insignificant).

In E1, under an automatic night-feeding regimen, the FCR ranged from 0.66 to 0.97, whereas in E2 and under a regimen of hand feeding during the day, it ranged from 0.76 to 1.71 (Table 5.5). In E2, although there were no significant differences, there was a tendency toward more efficient FCR between T2 and T3 in the 0.5 % group. A leptokurtic distribution (above 4) (Bhujel, 2008) with negative skewness represents the best batch growth, revealing the highest number of larger-sized fish per batch. In E1, at T3, the highest kurtosis (13.8 fish length, 5.5 weight) with a skewness of – 2.4 (length) and – 1.2 (weight) was observed in the 0.5 % group. The 2.0 % and control groups showed a leptokurtic distribution in terms of the length (7.6, – 1.6; 7.4, – 1.1) and a mesokurtic distribution in terms of the weight (2.2, – 0.8; 3.1, – 1.2). In E2, at T3, the 0.5 % group and the control grew similarly, that is, slightly platykurtic, with a kurtosis and a skewness around 0 (length 0.0, – 0.1; 0.5, – 0.6 and weight – 0.2, 0.4; 0.8, – 0.1).

Table 5.5: *Feed conversion ratio (mean ± standard deviation) in the treatment groups in E1 (on top) and E2 (below). Significance = p < 0.05 (E1: normal distributed, one-way ANOVA, variance homogeneity with Tukey HSD; E2: normal distributed, t-test).*

	Group	Stocking – T 0	T0 – T1	T1 – T2	T2 – T3
E1	C	0.66 ± 0.01[a]	0.78 ± 0.02[a]	0.86 ± 0.03[a]	0.96 ± 0.08[a]
	0.5 %	0.66 ± 0.00[a]	0.76 ± 0.02[a]	0.89 ± 0.02[a]	0.97 ± 0.05[a]
	2.0 %	0.65 ± 0.00[a]	0.77 ± 0.02[a]	0.92 ± 0.03[a]	0.94 ± 0.10[a]
E2	C	0.76 ± 0.01[a]	0.95 ± 0.04[a]	1.06 ± 0.04[a]	2.09 ± 0.52[a]
	0.5 %	0.76 ± 0.01[a]	0.94 ± 0.02[a]	1.04 ± 0.03[a]	1.45 ± 0.19[a]

Note: As a few fish were removed from the experiment during each sampling, the ratio cannot be indicated from the initial stocking to T3. It is, therefore, given for each sampling date based on the respective previous sampling weights. C = control group. Equal superscript letters indicate that there is no statistical significance.

5.3.2 Fish Welfare

The mortality rates in both experiments are given in Table 5.6. No significant differences were found. During E1, the mortality rates (total *n*) in the control, 0.5 %, and 2.0 % groups amounted to 11, 15, and 9 fish. This result indicates a percentage mortality rate of 3.6 %, 4.9 %, and 2.9 %, respectively (Ø 3.8). During E2, the mortality rates (total n) in the control and 0.5 % groups amounted to 8 and 17 fish, indicating percentage mortality rates of 2.6 % and 5.5 %, respectively (Ø 3.7).

Table 5.6: *Mortality rates within the treatment groups in E1 and E2 (both at T3). SD = standard deviation. Significance = p < 0.05 (E1: normal distributed, one-way ANOVA, variance homogeneity with Tukey HSD; E2: normal distributed, t-test).*

Mortality	Groups at T3		
	Control (E1/E2)	0.5 % (E1/R2)	2.0 % (E1)
mean ± SD	3.67 ± 1.53[a] / 2.67 ± 2.52[a]	5.00 ± 0.00[a] / 5.67 ± 2.89[a]	3.00 ± 1.00[a]
total n	11 / 8	15 / 17	9
%	3.6 / 2.6	4.9 / 5.5	2.9

Note: Equal superscript letters indicate that there is no statistical significance.

The number of external injuries decreased in all the treatment groups over the course of both experiments. In E1, however, a significant decrease was observed in the number of external injuries in both the 0.5 % and 2.0 % groups when compared with the control group (Figure 5.1a). At T0, the 0.5 % group had a relatively high mean value of 1.9 (± 1.8) lesions per individual when compared with both the other groups (control group: 1.5 ± 1.4; 2.0 % group: 1.5 ± 1.5). Due to the high standard deviations, there was no significant difference found between the groups. At T1, the number of lesions in the 0.5 % and 2.0 % groups decreased to 0.8 (± 1.3) and 0.8 (± 1.5), respectively, which revealed a significant reduction when compared with the control group (1.2 ± 1.3) ($p < 0.05$). At T2, the number of lesions continued to decrease in all the groups, with the difference between them being insignificant. At T3, the numbers of lesions in the 0.5 % and 2.0 % groups decreased to 0.4 ± 0.8 and 0.5 ± 0.8, indicating a significant difference ($p < 0.05$) when compared with the control group (0.9 ± 1.0).

A similar pattern was observed in E2 (Figure 5.1b). The numbers of external injuries in the control and 0.5 % groups were insignificant at T0 (2.1 ± 1.5 and 1.8 ± 1.8 lesions per fish, respectively, $p > 0.05$). At T1 and T2, the differences between the groups remained insignificant ($p > 0.05$), albeit the 0.5 % group tending to have fewer lesions than the control group. At T3, there was a significant difference ($p < 0.05$) in the number of lesions per fish between the control group (0.6 ± 1.2) and the 0.5 % group (0.3 ± 0.8).

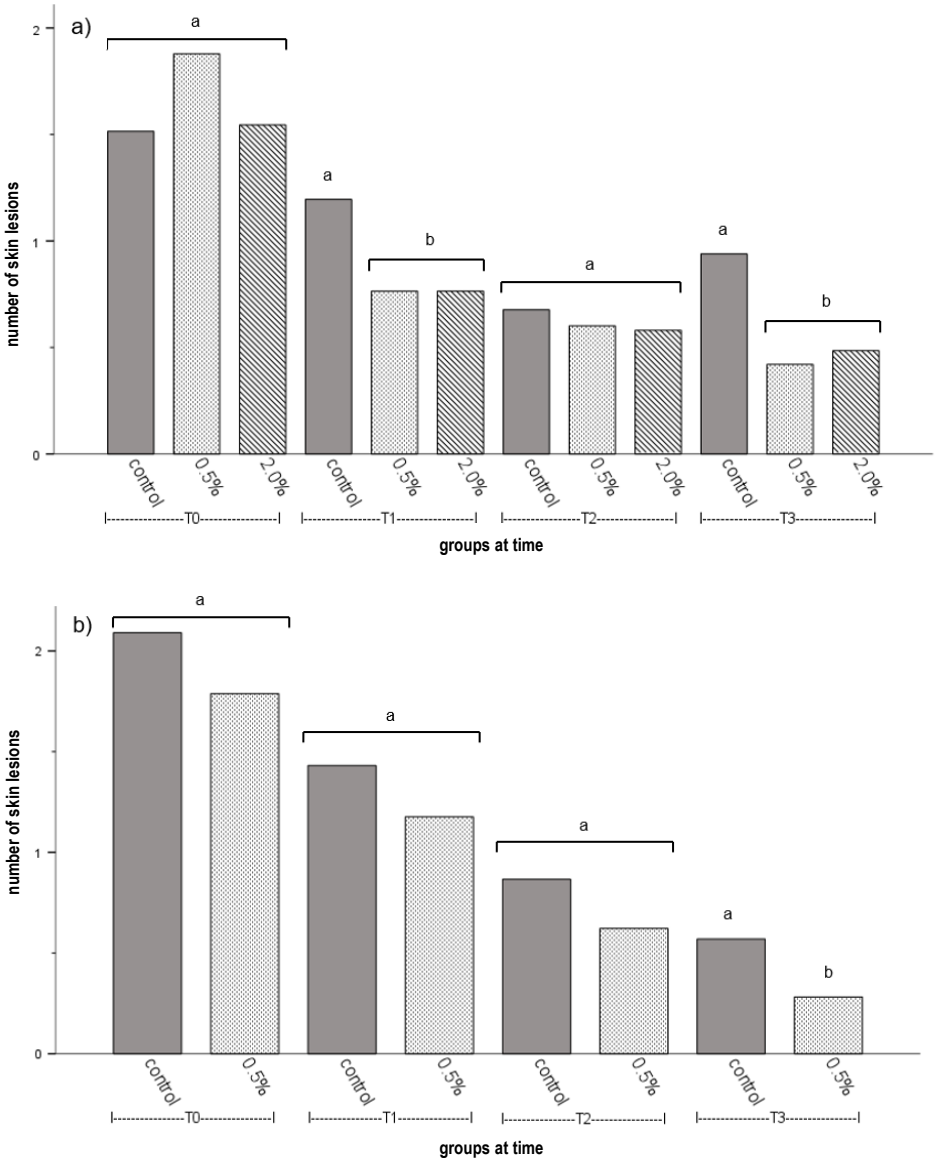

Figure 5.1: *Average number of external injuries in a) the three treatment groups (control, 0.5 %, and 2.0 %) in E1, and b) two treatment groups (control and 0.5 %) in E2, each at T0 (baseline prior to feed supplementation), at T1 (after 28 d of feed supplementation), at T2 (after 58 d), and at T3 (after 70 d), respectively. Significance = $p < 0.05$, Kruskal-Wallis-ANOVA in a), Mann-Whitney test in b). Letters above the bars indicate significant differences between the groups of a sampling.*

In E1, the mean plasma cortisol concentrations prior to the feed supplementation (at T0) in the control, 0.5 %, and 2.0 % groups were 22.7 (± 14.4) ng mL^{-1}, 28.8 (± 15.2) ng mL^{-1}, and 24.7 (± 13.1) ng mL^{-1} ($p > 0.05$), respectively. After the dietary change (at T1), the plasma cortisol concentrations in the 0.5 % and 2.0 % groups increased by approximately 249 % and 190 % (to 100.5 ± 65.5 ng mL^{-1} and 71.5 ± 68.4 ng mL^{-1}, respectively). Yet, an increase of approximately 344 % was also noted in the control group (100.8 ± 68.1 ng mL^{-1}). Significant differences were observed between the 2.0 % group and both the other groups. At T2, the

plasma cortisol concentrations in the control, 0.5 %, and 2.0 % groups were 82.7 ± 43.6 ng mL^{-1}, 140.8 ± 53.2 ng mL^{-1}, and 99.2 ± 76.7 ng mL^{-1}, which means that they were approximately 264 %, 389 %, and 302 % higher when compared with their respective baseline values. Here, the 0.5 % group had a significantly higher concentration than both the other groups (Figure 5.2a).

In E2, the mean plasma cortisol baseline concentrations (at T0) in the control and 0.5 % groups were 19.2 (± 9.0) ng mL^{-1} and 20.8 (± 7.2) ng mL^{-1} in the unstressed fish, respectively, while they were slightly increased in the stressed fish (33.3 ± 17.8 ng mL^{-1} and 32.3 ± 15.3 ng mL^{-1}, respectively). After the dietary change (at T1), the mean concentrations remained nearly identical at 19.7 (± 9.4) ng mL^{-1} and 19.9 (± 3.8) ng mL^{-1} in the unstressed fish and 25.2 (± 8.5) ng mL^{-1} and 24.4 (± 8.1) ng mL^{-1} in the stressed fish. At T2, a minor elevation occurred in all the treatment groups. The unstressed fish in the control and 0.5 % groups had concentrations of 32.7 (± 13.5) ng mL^{-1} and 32.3 (± 15.3) ng mL^{-1}, while the stressed fish had concentrations of 29.5 (± 12.1) ng mL^{-1} and 33.2 (± 11.8) ng mL^{-1} (Figure 5.2b). No significant differences were found between the treatment groups.

In E1, the mean glucose baseline concentrations (at T0) were 2.4 (± 0.4) mmol L^{-1} in the control group and 2.5 (± 0.4) mmol L^{-1} in both the 0.5 % and 2.0 % groups, which indicated there to be no significant differences between the groups. After switching to the supplemented diets (at T1), the mean glucose concentration in the control group increased to 3.8 (± 0.9) mmol L^{-1}, while it increased to 3.6 (± 0.8) mmol L^{-1} in the 0.5 % group and 3.4 (± 0.7) mmol L^{-1} in the 2.0 % group, with the concentration in the 2.0 % group being significantly lower than that in the control group. At T2, the glucose concentration in the control group averaged 4.0 (± 1.0) mmol L^{-1}, while it was 4.4 (± 1.0) mmol L^{-1} in the 0.5 % group and 4.3 (± 0.9) mmol L^{-1} in the 2.0 % group, which indicated there to be no significant differences between the groups (Figure 5.3a).

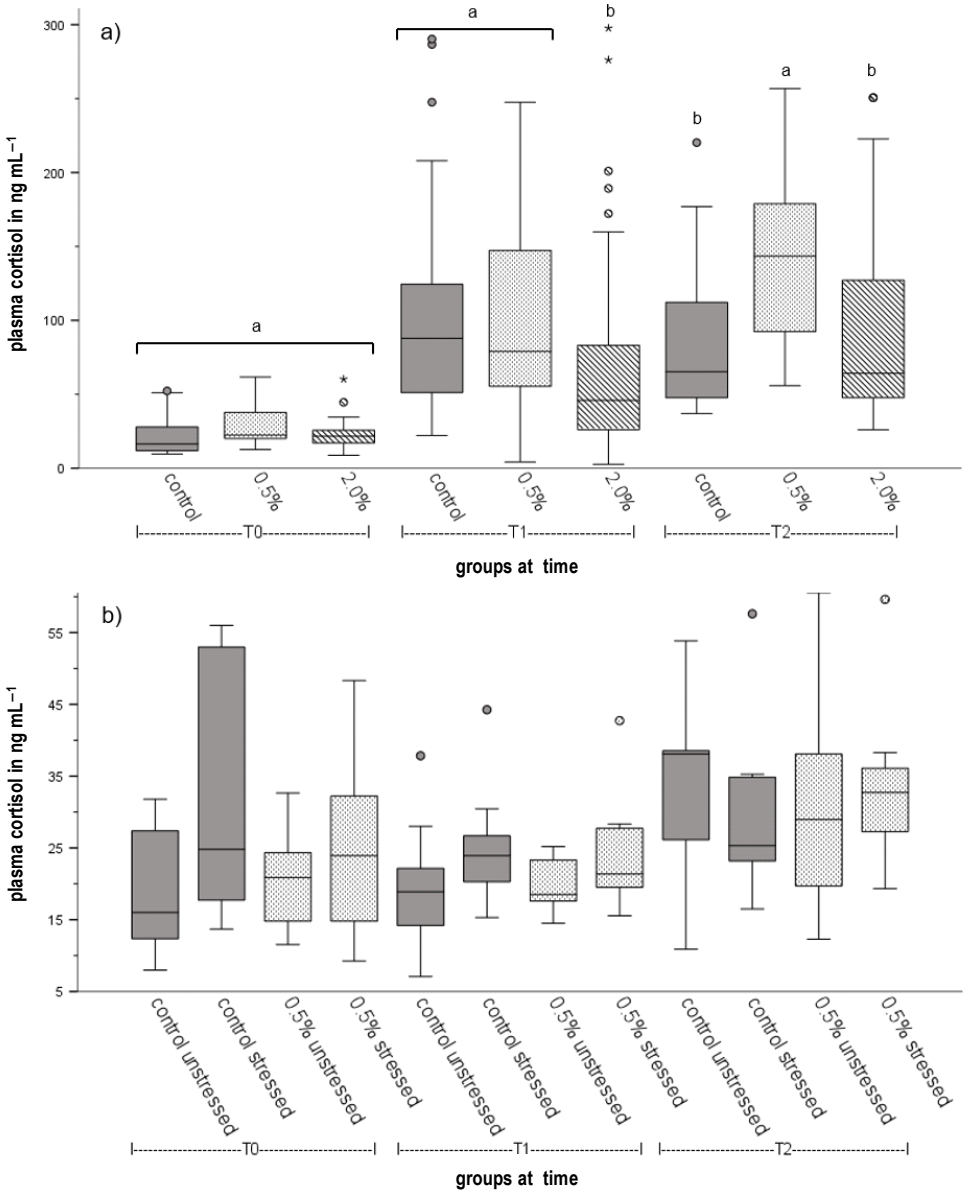

Figure 5.2: *Plasma cortisol concentrations a) in the three treatment groups (control, 0.5 %, and 2.0 %) in E1 after stress, and b) in unstressed and stressed fish in the two treatment groups control and 0.5 %) in E2, each at T0 (baseline prior to feed supplementation), at T1 (after 28 d of feed supplementation), and at T2 (after 58 d), respectively. Asterisk (*) = extreme values; circlets (^{o}) = outliers. In a), at T2, an extreme value of 387.7 ng mL^{-1} in the 2.0 % group is not illustrated. Significance = p < 0.05, Kruskal–Wallis–ANOVA in a). In b), all the data were insignificant (t-test or Mann–Whitney test). Letters above the boxplots indicate significant differences between the groups of a sampling.*

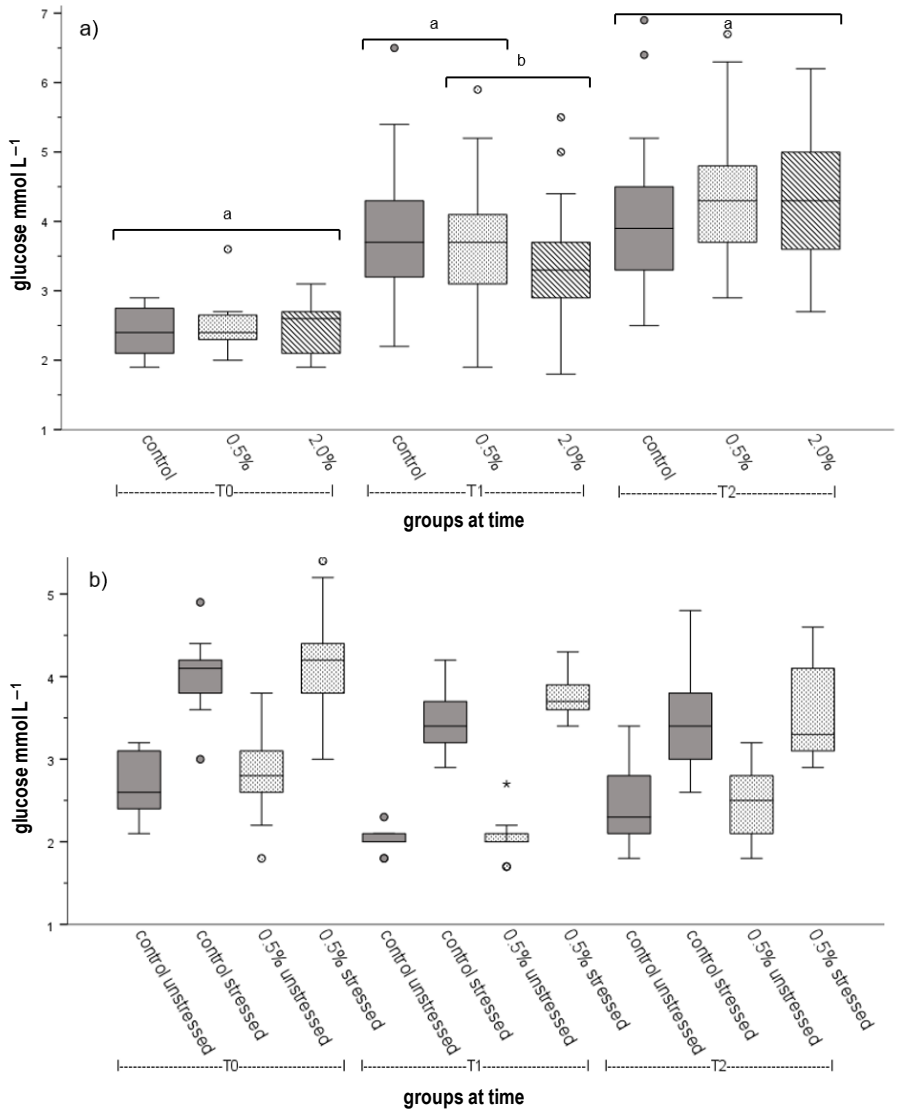

Figure 5.3*: Blood glucose levels in a) the three treatment groups (control, 0.5 %, and 2.0 %) in E1 after stress, and b) in the unstressed and stressed fish in the two treatment groups (control and 0.5 %) in E2, each at T0 (baseline prior to feed supplementation), at T1 (after 28 d of feed supplementation), and at T2 (after 58 d), respectively. Asterisk (*) = extreme values; circlets (^{o}) = outliers. In a), at T2, an extreme value of 8.3 mmol L^{-1} in the 0.5 % group is not illustrated. In b), at T1, an extreme value of 5.6 mmol L^{-1} in the stressed 0.5 % group is not illustrated. Significance = $p < 0.05$, ANOVA, Tukey HSD, Kruskal–Wallis–ANOVA in a). In b), all the data were insignificant (t-test or Mann–Whitney test). Letters above the boxplots indicate significant differences between the groups of a sampling.*

In E2, the mean glucose baseline concentrations (at T0) in the control and 0.5 % groups were 2.7 (± 0.4) mmol L^{-1} and 2.8 (± 0.6) mmol L^{-1} in the unstressed fish, respectively, while they were elevated to 4.0 (± 0.5) mmol L^{-1} and 4.2 (± 0.8) mmol L^{-1} in the stressed fish, respectively. At T1, the glucose concentrations decreased slightly in both groups, with mean levels of 2.0 (± 0.2) mmol L^{-1} and 2.1 (± 0.3) mmol L^{-1} being observed in the unstressed fish in the control and 0.5 % groups, respectively, and 3.5 (± 0.4) mmol L^{-1} and 3.9 (± 0.7) mmol L^{-1}

in the stressed fish, respectively. At T2, the glucose concentrations in the control and 0.5 % groups averaged 2.4 (± 0.5) mmol L^{-1} and 2.5 (± 0.5) mmol L^{-1} in the unstressed fish, respectively, while they were elevated to 3.5 (± 0.7) mmol L^{-1} and 3.6 (± 0.7) mmol L^{-1} in the stressed fish, respectively (Figure 5.3b). There were no significant differences found between the two groups within the different sampling events. The blood parameters reflecting the liver and kidney function, AST (GOT), GLDH, total protein, urea, calcium, and phosphate levels of the fish were found to be insignificant in both experiments (Table 5.7). The leucocytes, erythrocytes, hematocrit, and biochemical blood parameters (cholesterol, triglycerides, sodium, and potassium) were also determined to be insignificant in both experiments (Table 5.8).

Table 5.7: *Liver and kidney function of the African catfish at T0 (baseline) and T3 (end of the experiment) in E1 and E2.*

Sampling Time	n	Group	AST (GOT) in U L^{-1}	GLDH in U L^{-1}	Urea in mmol L^{-1}	Calcium in mmol L^{-1}	Phosphate in mmol L^{-1}	Total protein in g L^{-1}
					E1			
T0	9	Base line	333.7 ± 79.7	29.4 ± 3.7	1.1 ± 0.1	2.8 ± 0.1	3.9 ± 0.2	29.9 ± 1.4
T3	3	C	208.7 ± 38.2	28.2 ± 3.7	1.1 ± 0.1	3.3 ± 0.2	3.7 ± 0.6	39.3 ± 1.2
	3	0.5 %	167.3 ± 12.2	28.1 ± 4.7	1.0 ± 0.1	3.3 ± 0.3	3.3 ± 0.1	38.0 ± 4.1
	3	2.0 %	179.0 ± 5.0	22.7 ± 2.1	1.0 ± 0.1	3.2 ± 0.4	3.2 ± 0.4	37.0 ± 2.2
					E2			
T0	12	Base line	150.8 ± 28.7	14.7 ± 2.5	1.3 ± 0.2	3.0 ± 0.1	3.0 ± 0.2	33.7 ± 1.2
T3	6	C	109.0 ± 18.8	18.4 ± 2.2	1.1 ± 0.1	3.4 ± 0.1	2.5 ± 0.2	39.5 ± 2.4
	6	0.5 %	142.7 ± 48.8	26.5 ± 9.2	1.3 ± 0.1	3.5 ± 0.2	2.8 ± 0.2	41.1 ± 2.2

Table 5.8: *Cellular blood components and biochemical blood parameters of the African catfish at T0 (baseline) and T3 (end of the experiment) in E1 and E2.*

Sampling Time	Group	Leucocytes in G L^{-1}	Erythrocytes in T L^{-1}	Hematocrit in %	Cholesterol in mmol L^{-1}	Triglycerides in mmol/L^{-1}	Sodium in mmol/L^{-1}	Potassium in mmol/L^{-1}	Chloride in mmol/L^{-1}
					E1				
T0	Baseline	3.3 ± 2.7	1.1 ± 0.6	21.5 ± 7.3	2.9 ± 0.2	1.8 ± 0.1	124.6 ± 2.6	13.0 ± 1.7	112.0 ± 2.0
T3	C	0.9 ± 0.3	2.0 ± 0.5	26.2 ± 1.3	3.5 ± 0.1	2.0 ± 0.1	128.3 ± 1.2	9.3 ± 1.6	107.0 ± 1.4
	0.5 %	1.0 ± 0.2	1.7 ± 0.3	25.8 ± 0.2	3.4 ± 0.3	2.0 ± 0.4	129.7 ± 0.9	8.4 ± 0.5	107.7 ± 0.5
	2.0 %	1.0 ± 0.0	1.3 ± 0.4	24.5 ± 0.7	3.1 ± 0.3	1.7 ± 0.2	128.7 ± 1.2	9.5 ± 2.3	110.7 ± 2.1
					E2				
T0	Baseline	3.7 ± 0.8	1.9 ± 0.4	26.7 ± 2.0	2.8 ± 0.3	1.8 ± 0.1	130.0 ± 1.9	7.6 ± 1.3	111.5 ± 1.5
T3	C	13.0 ± 7.5	2.1 ± 0.2	36.3 ± 3.0	3.4 ± 0.2	2.0 ± 0.1	133.5 ± 1.7	7.3 ± 0.6	110.5 ± 1.6
	0.5 %	7.0 ± 3.3	1.7 ± 0.6	34.7 ± 7.1	3.4 ± 0.4	2.0 ± 0.1	133.7 ± 2.0	7.4 ± 0.3	111.0 ± 1.6

5.4 Discussion

5.4.1 Aquaculture Conditions

Despite the known high tolerance of the African catfish (Păpuc et al., 2019), it can be assumed that suboptimal or poor water quality could potentially affect the welfare and growth of this species. Thus, it was considered crucial to provide optimal aquaculture conditions in both experiments.

The water temperature in E1 was close to 27 °C, with a maximum 1.2 °C variation in the entire RAS, while the temperature variations at the individual sampling sites were even closer and deviated by only 0.6 °C. In E2, the mean temperature was slightly higher at 28.8 °C; however, both temperature ranges corresponded to the optimum for African catfish (Păpuc et al., 2019). In E1, the dissolved oxygen was > 90 % saturation or > 7 mg L^{-1} (after the trickling filter). In E2, it was slightly lower with > 70 % saturation or > 5.3 mg L^{-1}. Masser et al. (1999) recommended maintaining dissolved oxygen levels of > 60 % saturation or > 5 mg L^{-1} to ensure optimal growth in most warm-water species. Thus, the oxygen conditions were indeed optimal during both experiments. A consistently high redox potential was also found, which again indicated a good oxygen supply.

In E1, the pH dropped to the slightly acidic range but was kept mostly above 6.5 by water changes and regular liming. In E2, the pH was similar. Ndubuisi et al. (2015) reported the adequate pH range for the growth and survival of African catfish to be between 5 and 9. Therefore, the pH in this study was in the adequate range. An NH_4^+ concentration between 0.1 mg L^{-1} and 0.2 mg L^{-1}, as mainly measured in the present study, can be tolerated very well by African catfish (Păpuc et al., 2019). Under the given temperature and pH ranges, only very minor concentrations of toxic unionized ammonia (NH_3) were present (Losordo et al., 1998; Masser et al., 1999). The unionized form of NH_3 was calculated to reach a maximum of < 0.01 mg L^{-1} to < 0.02 mg L^{-1} at pH 8.2 and 27 °C, with it being distinctly lower with a decreasing pH.

The fluctuations observed in the NO_2^- concentrations up to 0.4 mg L^{-1} in E1 (0.6 mg L^{-1} in E2) were either below or at the recommended maximum (up to 0.6 mg L^{-1}) for African catfish aquaculture, meaning that they did not affect the growth, well-being, or health of the fish (Roques et al., 2015). In E1, the average NO_3^- concentration (after the trickling filter) was 72.5 (± 36.1) mg L^{-1}, although it tended to increase over the course of the experiment to reach a maximum of 170.9 mg L^{-1}. In E2, the mean NO_3^- concentration (after the trickling filter) was

higher, with an average of 190.8 (± 56.9) mg L^{-1} and a maximum of 284.0 mg L^{-1}. Schram et al. (2014) recommended that a NO_3^- concentration of 140 mg L^{-1} should not be exceeded. In E1, the NO_3^- concentration was mostly below this threshold, although it did exceed it for 5 d and reach 170.9 mg L^{-1}, which was still considered to be fairly harmless. In E2, the threshold of 140 mg L^{-1} was exceeded for longer periods; however, the NO_3^- concentration was still relatively low when compared with the concentrations in other studies that assessed African catfish in RAS. For instance, Palm et al. (2018b) reported survival rates of 81.4 % and 88.6 % at NO_3^-–N concentrations of 185.5 mg L^{-1} and 125.6 mg L^{-1}, respectively. Dai et al. (2011) suggested NO_3^- concentrations below 1000 mg L^{-1} and 100 % daily water exchange to be safe. The survival rates reached 95.1 – 97.5 % in the present study. Thus, the slightly elevated NO_3^- concentrations observed during E2 might have had no major effect on the welfare or growth of the African catfish.

In summary, the water quality in both experiments and RAS were considered appropriate to ensure optimal aquaculture conditions for African catfish. As the physicochemical water parameters were within the range reported by Palm et al. (2018b) and the survival rates were sufficiently high, it can be concluded that the use of 1g557 within the test feeds did not negatively affect the functionality of both RAS.

5.4.2 Fish Growth

At T0, the 0.5 % group showed the highest mean weight, although the differences with regard to the other two groups were insignificant. After 70 d of feeding with the supplemented diets (T3), the differences between the control, 0.5 %, and 2.0 % groups were still insignificant, with the mean weights being 480.5 g, 484.2 g, and 469.5 g, respectively. However, a trend was seen in that the 2 % lower nutrient content in the 2.0 % group resulted in a slightly lower weight (2.3 %) when compared with the control group. Thus, the fact that 2 % of the feed was replaced with 2 % 1g557 seemed to have a negative effect on the growth of the fish.

A different picture was seen when comparing the 0.5 % group with the control group. In E1, there was a slight increase in the weight gain in the 0.5 % group (of 0.8 %), despite the fact that 0.5 % of the feed was replaced with 0.5 % 1g557. This trend was verified in E2, where the 0.5 % group had an even higher weight gain (3.2 %) than the control group ($p > 0.05$). In addition, less size variance in the African catfish was observed in E1. This result is consistent with earlier studies in which the addition of montmorillonite or 1g557 to the diets resulted in

good growth performance and lower size divergence, even when other species were involved (Hu et al., 2007, 2008; Palm et al., 2015). This suggests that African catfish, similar to whiteleg shrimp (Palm et al., 2015), can grow more homogeneously when exposed to 1g557 as a feed additive.

In E1, the FCR ranged from 0.65 before and 0.76 – 0.97 after the application of 1g557, constantly increasing with increasing the size from 30 – 32 g (2.5 kg m^{-3}) to 470 – 484 g (21 kg m^{-3}). Palm et al. (2018b) determined an FCR between 0.89 and 1.01 for African catfish in the same RAS within five different production stages, ranging from an initial weight of 40 – 275 g (2.8 – 19.3 kg m^{-3}) to a final weight of 1496 – 1780 g (95.5 – 112 kg m^{-3}). Consequently, the growth performance was as expected during E1, with a slightly better FCR being seen due to the smaller-sized fish and more extensive production conditions (Palm et al., 2018b) throughout the entire experiment. In E2, the FCR was higher and not directly comparable due to the use of a different RAS, different feeding regimens (automatic night vs. regular day feeding by hand), and slightly different cultivation conditions. The FCR was already higher during the adaptation phase, indicating that the difference was not caused by the application of the feed additive but was rather due to the different cultivation methods.

5.4.3 Fish Welfare

In the present study, 2.9 – 5.5 % of the fish fed with 0.5 % or 2.0 % 1g557 did not survive. To a certain extent, the mortality rates were slightly lower in the control groups, although ultimately, they were in a very similar range. Hence, no negative influence on the part of feed supplementation with 1g557 could be detected between the treatment groups. Prior studies that used regular fish feeds showed similar mortality rates, such as 2.5 % (Van de Nieuwegiessen et al., 2008) or 6 % (Baßmann et al., 2020). Palm et al. (2018b) reported survival rates of up to 90.2 % under intensive stocking densities (199.2 kg m^{-3}) and increasing survival rates under more extensive conditions. This may represent the best comparison for the present study, as the same RAS was used with a very similar stocking density and the same system maintenance, albeit with common commercial feeds. Therefore, the mortality rates in the present study can be considered low.

An initial increase in agonistic behavior and, subsequently, a higher number of skin lesions after stocking juvenile African catfish can be considered normal. Based on previous experience, after a few weeks, this tends to decrease. Other studies reported 1 – 8 skin lesions

per fish (Manuel et al., 2014; Van de Nieuwegiessen et al., 2008, 2009). For instance, in a three-week experiment involving African catfish fingerlings, the use of feed containing montmorillonite was found to improve their skin quality and have no adverse effect on their growth (Ismaila et al., 2011). In both the presented experiments, the highest injury rate was recorded at T0. At T1, a reduction was observed in the number of external injuries. More specifically, in E1, the two experimental groups fed with 1g557 showed significantly decreased numbers of external injuries than the controls. In E2, a similar trend was observed. Finally, significantly different numbers of external injuries were recorded independently in both experiments at T3, with all the groups fed diets supplemented with 1g577 having approximately half as many injuries as the control groups. This is considered to indicate the supplementation to have a positive influence on the welfare of the fish.

The cortisol responses were generally highly diverse in the present study, particularly in E1. The mean values ranged between 20 ng mL^{-1} and 140 ng mL^{-1}. Therefore, fewer fish were used for the cortisol analyses in E2. In addition, unstressed fish (baseline cortisol) and fish in which acute stress had been induced were used. The plasma cortisol concentrations of the fish in E2 were comparable to those at T0 in E1, although apart from that, they were lower. The plasma cortisol concentrations of the stressed fish in E2 were mostly elevated when compared with those of the unstressed fish. Solely at T2, the cortisol responses of the stressed control fish were below those of the unstressed control fish, which cannot be explained, although it was probably not due to cortisol suppression. The data were widely scattered in the unstressed 0.5 % group, whereas they were closer together in the stressed fish. For comparison, Martins et al. (2006a) reported the cortisol concentrations of unstressed and stressed African catfish to be mostly between 20 ng mL^{-1} and 100 ng mL^{-1}, with the cortisol concentrations of the unstressed fish being significantly or at least trending toward lower than those of the stressed fish. In general, this is consistent with the present data. It is likely that the sampling method had a strong influence on the experiments in this study, as all the fish were caught and removed from the tanks. Thus, temporal differences, as well as changing stressor intensities in relation to individual fish, could easily have occurred and may have led to the different cortisol responses. The glucose concentrations ranged from 2 mmol L^{-1} to 5 mmol L^{-1} in both experiments, with the stressed fish tending to exhibit higher values than the unstressed fish in E2. This result is also consistent with other studies (Martins et al., 2006a). However, no major differences were found between the experimental groups in this study

despite the significant differences observed in E1 (at T1), which indicates that the 1g557 had no adverse effect in this regard.

The additional blood parameters (AST [GOT], GLDH, total protein, urea, calcium, and phosphate) did not differ significantly in either experiment, suggesting regular liver and kidney function in the fish. In addition, the differences in the cellular blood components (leucocytes and erythrocytes), hematocrit, and chemical blood parameters (cholesterol, triglycerides, sodium, and potassium) were also not significant, indicating that there were no negative effects on the part of the tested feed additive on the organs and metabolism of the African catfish. The present results can serve as reference values for future studies, as data concerning these blood parameters in fish, especially in African catfish, remain very scarce in the literature.

5.5 Conclusions

The application of mixed-layer clay mineral montmorillonite–illite/muscovite (1g557) in the RAS for African catfish has the potential to improve the welfare of the fish without negatively affecting their blood parameters, stress responses, or the RAS itself. After 70 d of cultivation in each of the two independent experiments, the number of external injuries in both the experimental groups under dietary supplementation of 1g557 was significantly ($p < 0.05$) reduced by approximately half when compared with the unsupplemented control fish. Dietary supplementation with 1g557 showed this beneficial effect for the tested fish sizes between 100 g and 500 g. Further studies are required to address why the incidence of external injuries was drastically reduced under supplementation with the tested mixed-layer clay mineral. The findings will support our attempts to improve fish welfare through the application of entirely natural products under recirculation aquaculture conditions in the future.

6 Aquaponics (*s.l.*) Production of Spearmint (*Mentha spicata*) with African Catfish (*Clarias gariepinus*) in Northern Germany

Abstract

Aquaponics production of spearmint (*Mentha spicata*) was evaluated under commercial grow-out conditions of African catfish (*Clarias gariepinus*) in Northern Germany (Mecklenburg-Western Pomerania). Fish batch production under different stocking densities in an extensive aquacultural unit (EAU) and an intensive aquacultural unit (IAU) was connected to conventional plant cultivation on ebb-and-flood planting tables and compared to a liquid fertilizer control. The best growth parameters of *M. spicata* were found under the intensive stocking density of *C. gariepinus* (IAU), resulting in a plant leaf area of 10.9 ± 2.5 cm^2, leaf length of 8.6 ± 1.6 cm, and a cut fresh biomass from aboveground of 31.8 ± 13.8 g $plant^{-1}$, compared to the EAU (5.6 ± 2.1 cm^2; 5.4 ± 1.4 cm; 17.4 ± 4.7 g $plant^{-1}$) and the control (5.7 ± 2.2 cm^2; 5.5 ± 1.4 cm; 11.2 ± 5.3 g $plant^{-1}$). The fresh biomass of the whole plants was not significantly different between the EAU (165.5 ± 71.7 g $plant^{-1}$) and the IAU (190.7 ± 105.6 g $plant^{-1}$), though the latter gained more weight. The initial fish number ratio between the EAU and the IAU of 1/4 increased the *M. spicata* leaf area by twofold in the IAU. Our results demonstrate that aquaponics (*s.l.*) production of *M. spicata* is possible under the direct use of effluent waters from intensive African catfish cultivation without the addition of any liquid fertilizer.

Keywords

Aquaponics; *Mentha spicata*; leaf area; African catfish; FishGlassHouse

6.1 Introduction

Plant cultivation in aquaponics comprises a wide variety of species, from vegetables and herbs to medicinal and ornamental plants (Somerville et al., 2014). In recent years, the production of fast-growing culinary herbs has been emphasized, and mint (*Mentha* spp.) was described as a plant with a high economic potential (Rakocy et al., 2006; Rakocy, 2012). The economic effectiveness of plant cultivation under coupled aquaponics conditions is still under dispute, and feasibility studies with low nutrient crops, including herbs and medicinal plants, are still needed.

Species of the genus *Mentha* (Lamiaceae) are, in general, popular due to their high content of essential oils for medical and culinary purposes. Spearmint's (*Mentha spicata*)

essential oil is rich in carvone (60 – 70 %, monoterpene), which is responsible for the typical spearmint odor and, to a lesser extent, the fragrance limonene (about 10 %) from the same biochemical group (Chauhan et al., 2009). Other ingredients of mint's essential oils possess interesting characteristics, such as pulegone, with a strong insecticidal activity, and menthone, with mutagenic and/or genotoxic activity (Franzios et al., 1997); in general, oils from *M. spicata* are known to be antimicrobial (Sivropoulou et al., 1995).

The essential oils of *Mentha* spp. are used for a wide range of industrial and pharmaceutical products, such as chewing gum, toothpaste, cosmetics, and tea (Lawrence, 2006). The proportion of global production of *Mentha* spp. in 2016 was dominated by Morocco (92.7 %, 98.45 k ton), followed by Argentina (6.7 %), Mexico (0.3 %), Bulgaria (0.2 %), and Spain (0.3 %) (Tridge, 2020). In the United States, the mean value of spearmint (*M. spicata*) oil production was relatively stable at 51,459 k $ (±5372 k $) between 2015 and 2017 (Ross, 2018). In Europe, the demand for mint as a fresh herb or for use as tea is increasing, especially in the United Kingdom, the Netherlands, Sweden, and Germany (CBI, 2020). In *M. spicata*, a significant amount of essential oil substances is formed on the leaf surfaces (Gershenzon et al., 1989). For this reason, the leaf number and the leaf surface area are the most important factors to evaluate the performance and economic potential of *Mentha* spp. (Tiwari, 2008). The combination of *M. spicata* cultivation with a high plant growth performance and the production of *Clarias gariepinus* under aquaponic conditions has great potential for industrial production in the context of the widespread use of *M. spicata* as a culinary and medicinal herb.

Investigations of *Mentha* spp. in aquaponics are limited, though mint has been characterized as a plant with relatively low nutrient requirements and good growth potential (Somerville et al., 2014). Low-nutrient-level aquaponics systems with extensive fish stocking seemed to be ideal for its cultivation. The yield of peppermint (*Mentha piperita*) was 1.8-fold better when using Nile tilapia (*Oreochromis niloticus*), compared with common carp (*Cyprinus carpio*) under summer – autumn conditions in northern Germany in a low nutrient backyard aquaponics system (Knaus et al., 2018). The growth parameters for *Mentha piperita* and *M. sativa* were better in hydroponics, but the essential oil content was higher in *Mentha piperita* under aquaponics (Roosta and Ghorbani, 2011). Good mint yields, with a 26.7 % higher growth rate of field mint (*Mentha arvensis*), were described in combination with *O. niloticus* under aquaponics conditions compared to a system without fish (Wahap et al., 2010). *Mentha arvensis* was successfully cultivated in a gravel substrate aquaponics (Shete et al., 2016) with

common carp (*Cyprinus carpio*) and a mean plant yield of 1.146 kg at two m^2 (three replicates) over an experimental duration of two months, as well as in crushed stone media beds with a mean harvest of 1.076 kg at about 2 m^2 surface area with a volume of 1000 L (Shete et al., 2017). Excellent growth of *Mentha* species was described in brackish water aquaponics, with only slight chlorosis under freshwater aquaponics in the desert (Kotzen and Appelbaum, 2010). Herbs can also be used for biological filters (hydroponics subsystem). *Mentha piperita* and *M. spicata* were successfully tested in *O. niloticus* intensive aquaponics, where herbs removed significant concentrations of nitrogen and phosphate (Espinosa Moya et al., 2016). These studies demonstrate that *Mentha* spp. has already been successfully cultivated in smaller-scale aquaponics; however, so far, there has been no information in the context of large-scale commercial fish production.

African catfish (*Clarias gariepinus*) is a new species to be produced in aquaponics with a very good growth potential (Baßmann et al., 2017; Knaus and Palm, 2017; Palm et al., 2014; Palm et al., 2018b). Due to its well-known flesh quality and uncomplicated fish-rearing management requirements, global aquacultural production of *C. gariepinus* increased, from 1992 to 2015, by 243,195 t (FAO, 2022a). In aquaponics, good growth of *C. gariepinus* was described in combination with water spinach (*Ipomoea aquatica*, (Endut et al., 2010)), basil (*Ocimum basilicum*, (Palm et al., 2014; Pantanella, 2012)), lettuce ((*Lactuca sativa*), cucumber (*Cucumis sativus*), and tomato (*Solanum lycopersicum*) (Palm et al., 2014)). However, the growth of basil and parsley (*Petroselinum crispum*) was reduced in combination with *C. gariepinus* compared with Nile tilapia (*O. niloticus*) (Knaus and Palm, 2017). Thus, the choice of fish species influences plant growth. The combination of spearmint (*M. spicata*) and African catfish (*C. gariepinus*) seems especially promising under commercial aquaponics conditions.

We compared the growth of *M. spicata* with process waters from extensive and intensive batch cultivation of *C. gariepinus* under semi-coupled aquaponics conditions. The spearmint was cultivated in triplicates on ebb-and-flood tables in standard starter fertilized commercial pots and using nutrient-enriched water with a fertilizer from a gardening company (Grönfingers Rostocks Gartenfachmarkt GmbH, Germany) as a control. The production principle followed aquaponics farming (sensu lato: *s.l.*) with garden plants (Palm et al., 2018a). The study was conducted in the “FishGlassHouse,” a modern experimental aquaponics facility in northern Germany (Mecklenburg-Western Pomerania), and mint was cultivated during a short period of the entire fish production cycle. The main purpose of this study was to

demonstrate commercial aquaponics (*s.l.*) production of *M. spicata* by using standard plant cultivation methods and extensive and intensive process waters from *C. gariepinus* aquaculture without conventional liquid fertilizer. The most relevant water parameters for successful decoupled spearmint aquaponics gardening are discussed.

6.2 Materials and Methods

6.2.1 Experimental Facility and System Design

The experiment was carried out in late summer to autumn from 27 August 2015 to 4 November 2015 (70 days) in the FishGlassHouse, Faculty of Agricultural and Environmental Sciences, University of Rostock (UoR, Mecklenburg-Western Pomerania, Germany). The experiment was integrated into the commercial batch production of *C. gariepinus* with a mean grow-out phase of 156.3 days (total 257 days between stocking the first batch and harvesting the final batch).

The FishGlassHouse has a total production area of 1000 m^2 (Palm et al., 2016). The aquaculture unit, with a total area of 300 m^2, consists of three separate recirculating aquaculture systems (RAS, each 100 m^2) with different stocking densities (extensive, semi-intensive, and intensive) of African catfish (*C. gariepinus*), constructed in the same manner as commercial production systems in Mecklenburg-Western Pomerania, northern Germany (PAL Anlagenbau GmbH, Germany). A water management system (100 m^2) enabled the transfer of the aquaponics process water between the fish and plant units. The hydroponics unit (600 m^2 total production area), a VENLO greenhouse (GTW Gewächshaustechnik Werder GmbH, Germany), was equipped with automatic climate control (Hempel & Rülcker, Gesellschaft für elektronische Klimaregelsysteme GmbH, Dresden, Germany). This production system is classified as aquaponics of “intermediate/large-scale commercial for business and industry” (Palm et al., 2018a).

The aquaculture effluents from the extensive (EAU) and intensive (IAU) aquaculture units (Figure 6.1) were connected to the 100 m^2 hydroponics system for cultivation of spearmint (*M. spicata*). The two aquaponics test groups were established as garden plants in peat pots with a 50 % reduced amount of conventional dried fertilizer on ebb-and-flood tables (n = 9) in triplicates (Palm et al., 2018a) and compared with a liquid fertilizer control group. The fish process water was semi-continuously pumped via the water management system into temporary water storage tanks (EAU: WT-E-1; IAU: WT-I-1) and subsequently into the

hydroponics cabin with corresponding aquaculture effluent water storage tanks without the addition of fertilizer (EAU: AET-E; IAU: AET-I). The hydroponics control group was autonomous (NT-Control) and filled repeatedly with a mineral fertilizer solution. Each water storage tank (AET-E; AET-I; NT-Control) was connected to one hydroponics water recirculating system that pumped aquaculture effluents and nutrient-enriched water (NT-Control) to the plant cultivation tables with the mint pots and back into the tanks (Fackler Gewächshaustechnik, Munningen-Laub, Germany).

6.2.2 Fish Production and Feeding

The schematic view of the FishGlassHouse operating extensive (EAU, total volume 13.9 m^3) and intensive (IAU, total volume 16.9 m^3) aquaculture units are given in Figure 6.1. Both units consisted of nine fish tanks of 1.26 m^3 (F 1 – 9, volume 1 m^3), one solids separation unit (EAU: Se-E with 1.1 × 1.2 × 0.9 m and 1.2 m^3; IAU: Se-I with 1.5 × 1.3 × 0.9 m and 1.7 m^3) with a filter material (FAP 627, tube size 54 mm) and total filter volumes of 0.66 m^3 (EAU) and 1.03 m^3 (IAU), and a nitrifying trickling filter (EAU: TF-E with 2.9 m^3 = 368.55 m^2; IAU: TF-I with 11.8 m^3 = 1474.20 m^2), and corresponding sumps (EAU: S-E with 1.6 m^3; IAU: S-I with 4.0 m^3). The process water was transferred from the EAU and IAU solids separation units to a hydroponics cabin, purified from coarse particulate substances.

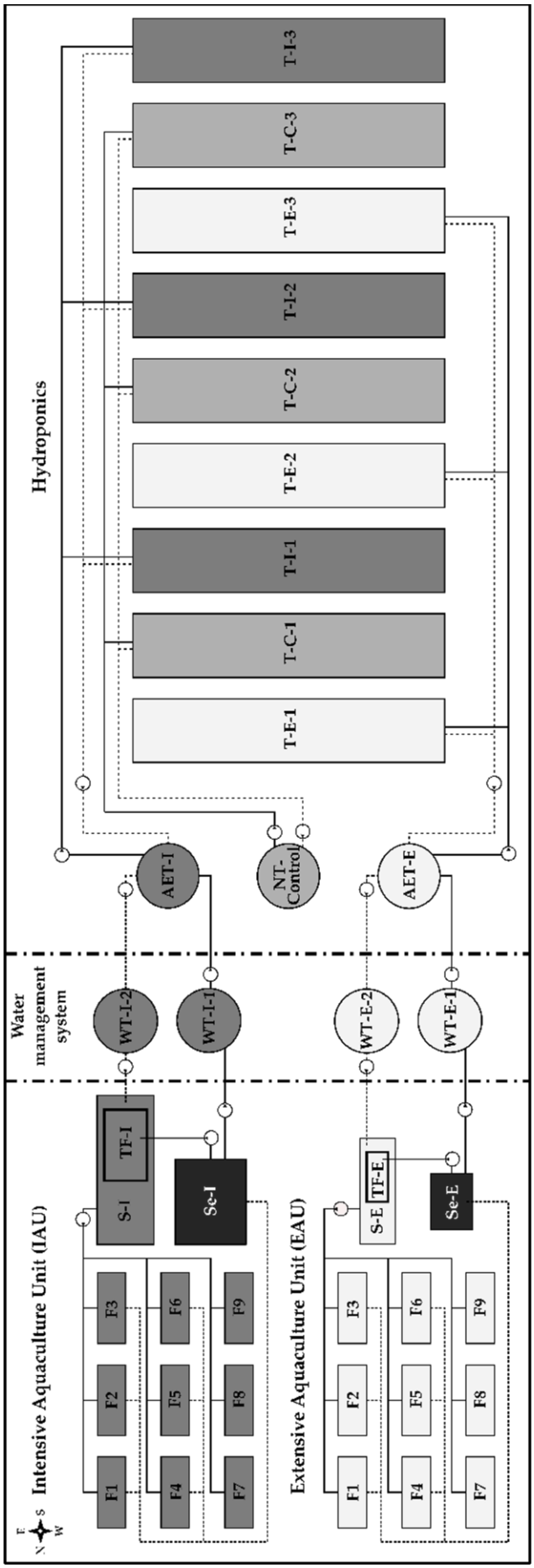

Figure 6.1: *Schematic view of the FishGlassHouse operating units. Intensive Aquaculture Unit (IAU) with fish tank 1 – 9 (F1 – F9), solid separation unit, sedimenter (SE-I), and sump (S-I) with trickling filter (TF-I); Extensive Aquaculture Unit (EAU) with fish tanks 1 – 9 (F1 – F9), solids separation unit, sedimenter (Se-E), and sump (S-E) with trickling filter (TF-E). The aquaculture units were connected to hydroponics via the water management system tanks, with the inflow from the IAU and EAU into the water tanks (WT-I-1/WT-E-1) and the backflow tanks (WT-I-2/WT-E-2). Process water from aquaculture was stored in the aquaculture effluent tanks (AET) of the IAU (AET-I) and EAU (AET-E) and an autonomous fertilizer control tank (NT-Control) and distributed to triplicate groups of planting tables with intensive (T-I-1/3) and extensive (T-E-1/3) fish production water, and the liquid fertilizer control group (T-C-1/3). Continuous lines mark inflows and dotted lines return flows.*

The fish (*C. gariepinus*) were supplied by Fischgut Nord eG (Wittenhagen OT Abtshagen, Germany) with an initial mean weight of 275 g, and each fish tank was stocked with 35 (EAU) and 140 (IAU) fish (total fish growth phase of 257 days, (Figure 6.2). Three tanks were stocked consecutively in the EAU and IAU on 17 July 2015 for fish tanks 3, 6, and 9; on 17 August 2015 for fish tanks 2, 5, and 8; and on 28 September 2015 for fish tanks 1, 4, and 7 (tanks 1, 4, and 7 remained unstocked for 32 days during the run of the experiment). Fish were fed by automated feeding and protocol based on actual *C. gariepinus* feed conversion ratios (PAL Anlagenbau GmbH, Abtshagen, Germany) six times per day (from 06:00 p.m. to 06:00 a.m.) with a single feeding amount in EAU per tank of 41.9 ± 15.8 g, and in IAU per tank of 153.7 ± 55.2 g. A high-protein diet ME-4.5 44-14 Meerval (Skretting, France) was used with 44 % crude protein, 14 % crude lipid, 22.3 % NFE, 10.5 % ash, 1.2 % crude fiber, 1.6 % phosphorus, 0.3 % copper sulfate, and 18.9 MJ digestible energy.

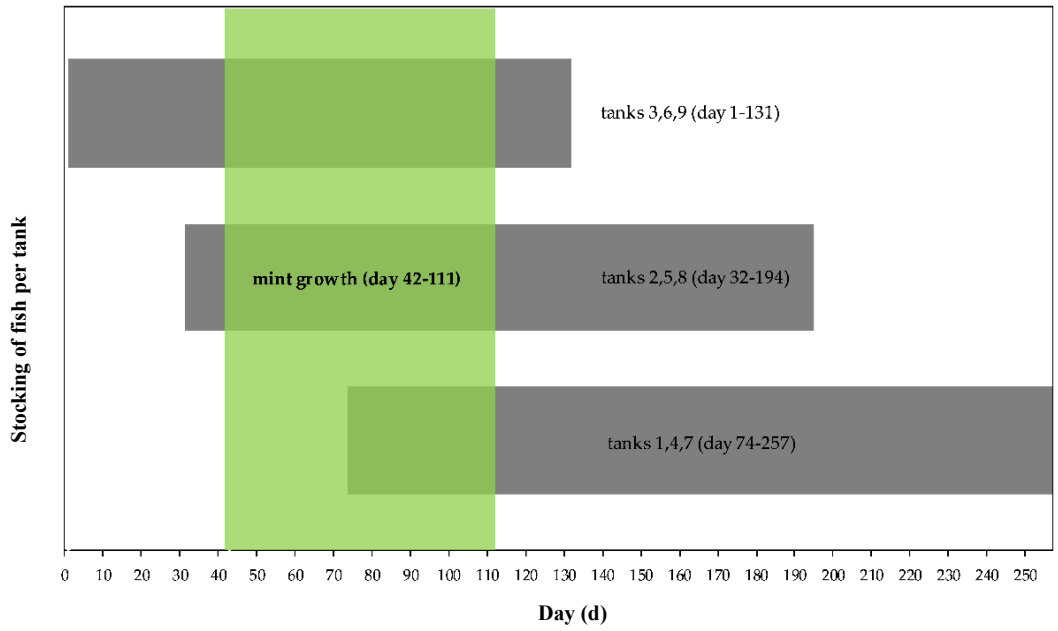

Figure 6.2: *Experimental overview of the fish stocking principle (grey), total time (257 days), and the mint growth phase (green, days 42 – 111) with fish stocked in tanks 3, 6, 9 from days 1 to 131, in tanks 2, 5 and 8 from days 32 to 194, and in tanks 1, 4, and 7 from days 74 to 257.*

Fish growth parameters were taken at the beginning and the end of the grow-out phase using a scale (Kern SFB 100K10HIP, Kern & Sohn GmbH, Balingen-Frommern, Germany). Fish

in tanks 3, 6, and 9 were measured on 24 November 2015; in tanks 2, 5, and 8 on 26 January 2016; and in tanks 1, 4, and 7 on 29 March 2016 (Figure 6.2).

6.2.3 Plant Production

A total of 1260 spearmint plants (*M. spicata*) were supplied by the local company Grönfingers GmbH (Germany) in conventional garden pots (white peat 80 %: 0 – 10 mm and clay 20 %, pH 5.5 – 6.5, EE-Typ 0 "Nullerde", Einheitserdewerk, Werner Tantau GmbH & Co. KG, Uetersen, Germany, with 50 % commercial plant starter fertilizer) as seedlings and distributed on nine (3 × 3) hydroponics ebb-and-flood tables (3.05 × 1.01 m table^{-1}, Otte Metallbau GmbH & Co. KG, Germany, slope: 1.68 ± 0.89 %, n = 3) for the EAU (Table T-E-1/3), the IAU (Table T-I-1/3), and the control (T-C-1/3; Figure 6.1). First, 140 mint pots were placed at a distance of about 10 cm on each ebb-and-flood table. The tables were arranged from north (T-E-1) to south (T-I-3) inside the greenhouse with windows towards the west (natural light) and were connected to aquaculture effluent tanks (EAU: AET-E; IAU: AET-I) and the fertilizer nutrient tank (control: NT-Control), each approximately 1000 L in volume (Fackler Gewächshaustechnik, Germany). The control nutrient tank (NT-Control) operated independently and was filled with a commercial hydroponics fertilizer solution with a nutrient composition of 1.12 mg L^{-1} NH_4^+–N, 9.61 mg L^{-1} NO_3^-–N, and 5.54 mg L^{-1} PO_4^{3-}–P originating from a local commercial plant producer (Grönfingers GmbH, Germany). The plant tables were flooded four times a day, controlled by an automatic clock timer in one direction (4 min flooding, 4 min outflow by gravity, 8 min total irrigation time). The process water from the extensive (AET-E) and the intensive (AET-I) aquaculture effluent tanks was refilled three times a week (Monday, Wednesday, Friday) and returned from the tanks to the specific aquaculture units in a semi-coupled aquaponics system design.

Plant biomass and growth parameters were taken at the beginning of the experiment (n = 9), after 42 days (day 1), and at the end of the experiment (day 111, plant growth duration 70 days), randomly with n = 11 plants per table (n = 33 per treatment group) and measured with a hand ruler (Robbins and Pharr, 1987; Cho *et al.*, 2007; Vazquez-Cruz *et al.*, 2012); leaf area (cm^2) was analyzed and calculated by picture (Easlon and Bloom, 2014). The dry mass of the mint plants was determined using a drying oven (Memmert UN750, Memmert GmbH & Co. KG, Schwabach, Germany) after drying for 3 days at 60 °C and 2 h at 100 °C.

6.2.4 Physical and Chemical Water Parameter

The physical water parameters of temperature (°C), dissolved oxygen (DO, mg L^{-1}), oxygen saturation (%), conductivity (EC, µS cm^{-1}), pH, and redox potential (mV) were measured daily (except on weekends) in triplicates at approximately 09:00 a.m. inside the aquaculture units and the hydroponics tanks (AET-E, AET-I, NT-Control) with an HQ40D multimeter (Hach Lange GmbH, Düsseldorf, Germany). Daylight was measured automatically by a digital lux-meter probe on the top of the FishGlassHouse.

The chemical water parameters were taken twice a week (Monday and Wednesday) in the process water tanks of the hydroponics cabin (AET-E, AET-I, NT-Control) before and after the replacement of the fish effluent waters; means were created and analyzed. Data of NH_4^+-N (mg L^{-1}), NO_2^-–N (mg L^{-1}), and PO_4^{3-}–P (mg L^{-1}) were analyzed three times a week with a Gallery™ Automated Photometric Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and standard protocol NH_4^+: ISO 7150–1 (DIN 38406–5:1983–10), NO_2^-: ISO 6777:1984 (DIN EN 26777:1993–04), PO_4^{3-}: EN ISO 6878–1–1986 (DIN 38405 D11–4). TON (total oxidized nitrogen) as N and nitrate by calculation (TON-nitrite) was analyzed by colorimetric hydrazine method (Template: D08896_01© 2020 Thermo Fisher Scientific, Waltham, MA, USA). Nitrate was reduced to nitrite by hydrazine under alkaline conditions. The total nitrite ions were reacted with sulphanilamide and N-1-naphthylethylenediamine dihydrochloride under acidic conditions to form a pink azo-dye. The absorbance was measured at 540 nm and was elated to the TON concentration by means of a calibration curve. Nitrate (as N) value was obtained by calculating TON (as N) − nitrite (as N).

6.2.5 Mathematical and Statistical Analysis

Fish growth parameters (specific growth rate, Equation [6.1]; feed conversion ratio, Equation [6.2]) were calculated from the biomass of the fish production units (tanks 1, 4, 7; tanks 2, 5, 8; tanks 3, 6, 9) in the EAU and IAU for 257 days. The fish biomass per unit (m_U) at the beginning of the experiment, before and after stocking (tank 1, 4, 7) on day 74 (all tanks stocked), and at the end of the experiment, was calculated according to Equation [6.3].

$$SGR = \frac{lnW_t - lnW_0}{t} \times 100\% \quad [6.1]$$

with W_t = final biomass, W_0 = initial biomass, t = time in days.

$$FCR = \frac{fish\ feed\ quantity}{weight\ gain} \quad [6.2]$$

$$m_u = m_{stock3,6,9} + \frac{m_{feed3,6,9}}{FCR} + m_{stock2,5,8} + \frac{m_{feed2,5,8}}{FCR} + m_{stock1,4,7} + \frac{m_{feed1,4,7}}{FCR} \quad [6.3]$$

with m_U: fish biomass per unit at a specific time; m_{stock}: total fish biomass at stocking in tanks 3, 6, 9, tanks 2, 5, 8, and tanks 1, 4, 7; m_{feed}: feed input into the tanks 3, 6, 9, tanks 2, 5, 8, and tanks 1, 4, 7 until a specific time since stocking.

Significant differences ($p < 0.05$) were calculated from the means of fish and plant growth and water parameters of the hydroponic aquaculture effluent and nutrient tanks. For normally distributed data, a One-Way Analysis of Variance (ANOVA) was used to determine significant differences between the three subgroups (control, EAU, IAU), and a post hoc Tukey-HSD-test was used at variance homogeneity. Otherwise, the Dunnett-T3 test for variance inhomogeneity was used. Non-normalized data were analyzed after Kruskal-Wallis (non-parametric ANOVA) with a Bonferroni adjustment. For two experimental groups, a t-test and the Mann-Whitney test were used. All data were calculated using Microsoft Excel (2010) and SPSS 25 statistical software package (IBM).

6.3 Results

6.3.1 Fish and Plant Production

The duration of grow-out was identical in both aquacultural units (156.3 days, Table 6.1). The individual initial (275 g) and final fish weight were comparable (EAU: 1526.6 ± 284.0 g; IAU: 1458.8 ± 222.5 g), as were the feed conversion and specific growth rates (EAU: 0.94 ± 0.04, 1.09 ± 0.06 % d^{-1}; IAU: 0.96 ± 0.04, 1.07 ± 0.08 % d^{-1}), and the fish reached commercial marketing sizes. Under extensive fish rearing, survival was higher (96.2 ± 6.4 %) than in the intensive production (89.8 ± 5.3 %). In accordance with the final stocking density, total weight per tank (at harvest) and feed input were 3.6, 3.5, and 3.7 times higher, respectively, in the IAU (146.8 ± 20.6 kg m^{-3}, 146.4 ± 25.9 kg, 141.2 ± 27.1 kg) than in the EAU (40.6 ± 6.6 kg m^{-3}, 41.5 ± 8.3 kg, 38.6 ± 6.9 kg). The calculated fish biomasses at the beginning of the experiment, before and after stocking the tanks 1, 4, and 7 on day 74 and at the end of the experiment are given in Table 6.2.

In late summer to autumn conditions, the spearmint growth was relatively good (Figure 6.3). The best results were achieved in the intensive subgroup with the highest total fresh biomass (80.1 kg), plant height (51.0 ± 16.9 cm), shoot number (2.3 ± 1.2), leaf area (10.9 ± 2.5 cm^2), leaf length (8.6 ± 1.6 cm), fresh biomass (190.7 ± 105.6 g $plant^{-1}$), and cut fresh biomass (31.8 ± 13.8 g $plant^{-1}$; Table 6.3).

Table 6.1: *Clarias gariepinus commercial production tank parameters (in mean ± SD) in the FishGlassHouse (Palm et al., 2018b) during grow-out phase (257 days) in the extensive (EAU) and intensive (IAU) aquacultural units*.

Parameter	Extensive (EAU)			Intensive (IAU)			*p*
Initial Parameters							
Tanks	1, 4, 7	2, 5, 8	3, 6, 9	1, 4, 7	2, 5, 8	3, 6, 9	-
Date of stocking	28 Sept 2015	17 Aug 2015	17 July 2015	28 Sept 2015	17 Aug 2015	17 July 2015	-
Day of Grow Out (d)[1]	74	32	1	74	32	1	-
Individual Weight in g	275.0	275.0	275.0	275.0	275.0	275.0	-
Fish Number (no)	105	105	105	420	420	420	-
Biomass Weight in kg	28.9	28.9	28.9	115.5	115.5	115.5	-
Stocking Density kg m^{-3}	7.6	7.6	7.6	30.6	30.6	30.6	-
Final Parameters							
Date of Harvest	29 March 2016	26 Jan 2016	24 Nov 2015	29 March 2016	26 Jan 2016	24 Nov 2015	-
Day of Grow Out (d)[2]	257	194	131	257	194	131	-
Duration Grow Out in d	156.3 ± 23.0[a]			156.3 ± 23.0[a]			1.000
Individual Weight in g	1526.6 ± 284.0[a]			1458.8 ± 222.5[a]			0.436
Mean Fish Number (no)	33.7 ± 2.2[b]			127.2 ± 8.8[a]			0.001
Survival in %	96.2 ± 6.4[a]			89.8 ± 5.3[b]			0.024
Stocking Density in kg m^{-3}	40.6 ± 6.6[b]			146.8 ± 20.6[a]			0.001
Total Weight in kg $tank^{-1}$	41.5 ± 8.3[b]			146.4 ± 25.9[a]			0.001
Feed Input in kg $tank^{-1}$	38.6 ± 6.9[b]			141.2 ± 27.1[a]			0.001
FCR	0.94 ± 0.04[a]			0.96 ± 0.04[a]			0.164
SGR in % d^{-1}	1.09 ± 0.06[a]			1.07 ± 0.08[a]			0.545

[1] Start day of fish grow out between day 1 and 257; [2] End day of fish grow out between day 1 and 257; Different letters ([a], [b]) show statistically significant differences ($p < 0.05$).

Table 6.2: *Calculated values of the fish biomasses in the different fish tanks of the extensive (EAU) and intensive (IAU) aquaculture units in the FishGlassHouse during the experiment, showing the biomasses at the beginning of the experiment and at day 74 (all tanks stocked) before and after stocking tanks 1, 4, and 7.*

	Extensive (EAU)			Intensive (IAU)		
Tanks	**1, 4, 7**	**2, 5, 8**	**3, 6, 9**	**1, 4, 7**	**2, 5, 8**	**3, 6, 9**
	Total Fish Biomass in kg					
Beginning of the Experiment	0	37	43	0	146	174
Day 74 before Stocking Tanks 1, 4, 7	0	68	75	0	242	270
Day 74 after Stocking Tanks 1, 4, 7	29	68	75	116	242	270

Table 6.3: Mentha spicata growth parameter (mean ± SD, total values) of the control, extensive (EAU), and intensive (IAU) aquaculture units.

Parameters	Fertilizer (Control)	Extensive (EAU)	Intensive (IAU)	*p*-I[1]	*p*-II[1]	*p*-III[1]
Initial Parameters						
Initial Biomass in g plant^{-1}	2.7 ± 1.0^{a}	2.3 ± 0.6^{a}	2.8 ± 0.8^{a}	0.831	0.979	0.725
Plant Height in cm	8.5 ± 1.7^{a}	9.7 ± 2.0^{a}	11.2 ± 2.4^{a}	0.558	0.558	0.558
Leaf Length in cm	3.4 ± 0.6^{a}	4.0 ± 0.5^{a}	3.7 ± 1.5^{a}	0.773	0.956	0.912
Leaf Width	2.2 ± 0.3^{a}	2.3 ± 0.7^{a}	2.4 ± 0.7^{a}	0.961	0.812	0.933
Final Parameters						
Plant Height in cm	38.4 ± 8.2^{b}	45.2 ± 11.5^{a}	51.0 ± 16.9^{a}	0.009	0.001	0.109
Shoot Number (no)	1.6 ± 0.7^{b}	1.6 ± 0.6^{b}	2.3 ± 1.2^{a}	0.848	0.004	0.002
Leaf Area in cm²	5.7 ± 2.2^{b}	5.6 ± 2.1^{b}	10.9 ± 2.5^{a}	0.977	0.001	0.001
Leaf Length in cm	5.5 ± 1.4^{b}	5.4 ± 1.4^{b}	8.6 ± 1.6^{a}	0.997	0.001	0.001
Fresh Biomass in g plant^{-1}	120.6 ± 51.8^{b}	165.5 ± 71.7^{a}	190.7 ± 105.6^{a}	0.034	0.003	0.621
Total Fresh Biomass in kg*	50.7	69.5	80.1	-	-	-
Fresh Cut Biomass in g plant^{-1}**	11.2 ± 5.3^{c}	17.4 ± 4.7^{b}	31.8 ± 13.8^{a}	0.006	0.001	0.005
Total Fresh Cut Biomass in kg*	4.7	7.3	13.4	-	-	-
Dry Biomass in g plant^{-1}	18.3 ± 7.4^{a}	23.6 ± 12.4^{a}	21.3 ± 14.2^{a}	0.375	0.375	0.375

*calculated with total number of cultured plants (420) per experimental group and mean of (g plant^{-1}); **represents the aboveground. Biomass section (cut-green-biomass) of the plants; Different letters (^{a}, ^{b}, ^{c}) show statistically significant differences ($p < 0.05$), [1] Significance (p) with: p-I = control/extensive, p II = control/intensive, p-III = extensive/intensive.

Figure 6.3: *Mint plant garden pots of different experimental groups in the hydroponics cabin of the FishGlassHouse.*

Compared to the EAU subgroup, leaf area in the IAU was 1.9 times higher, leaf length 1.6×, and cut fresh biomass 1.8×. The lowest plant growth parameters were found in the fertilizer control group with 50.7 kg total fresh biomass; however, some plant growth parameters were comparable in both the fertilizer control group and the EAU in shoot number (control: 1.6 ± 0.7; EAU: 1.6 ± 0.6), leaf area (control: 5.7 ± 2.2 cm^2; EAU: 5.6 ± 2.1 cm^2), and leaf length (control: 5.5 ± 1.4 cm; EAU: 5.4 ± 1.4 cm); mint dry biomasses were not significantly different between all groups (control: 18.3 ± 7.4 g $plant^{-1}$; EAU: 23.6 ± 12.4 g $plant^{-1}$; IAU: 21.3 ± 14.2 g $plant^{-1}$).

6.3.2 Physical and Chemical Water Parameter

The analysis of the physicochemical water parameters in the aquaculture units during mint cultivation (70 days) showed differences between the EAU and IAU (Table 6.4). Extensive African catfish production showed higher levels of dissolved oxygen (7.1 ± 0.6 mg L^{-1}), saturation level (88.6 ± 7.9 %), and pH (7.2 ± 0.5). With intensive fish production, higher values

were found in salinity (0.6 ± 0.1 ‰), conductivity (1247.7 ± 128.9 µS cm^{-1}), NH_4^+–N (16.69 ± 5.11 mg L^{-1}), TON (76.50 ± 12.53 mg L^{-1}), NO_3^-–N (76.02 ± 12.37 mg L^{-1}), TDN (93.19 ± 14.04 mg L^{-1}), and PO_4^{3-}–P (7.06 ± 1.11 mg L^{-1}). No significant differences between the EAU and IAU were found in redox potential (122.6 ± 32.4 mV; 131.0 ± 26.7 mV) and NO_2^--N (0.29 ± 0.17 mg L^{-1}; 0.48 ± 0.54 mg L^{-1}).

Table 6.4: *Physicochemical water parameters of the extensive (EAU) and intensive (IAU) aquaculture units with Clarias gariepinus production in means (± SD) during mint cultivation (70 days).*

Parameters	Extensive (EAU)	Intensive (IAU)	*p*-Value
O2 in mg L^{-1}	7.1 ± 0.6[a]	4.9 ± 1.7[b]	0.001
O2 in %	88.6 ± 7.9[a]	60.1 ± 20.4[b]	0.001
Temperature in °C	26.7 ± 0.2[a]	25.8 ± 0.8[b]	0.001
pH	7.2 ± 0.5[a]	6.6 ± 0.2[b]	0.001
Salinity in ‰	0.5 ± 0.1[b]	0.6 ± 0.1[a]	0.001
Conductivity in µS cm^{-1}	952.3 ± 87.2[b]	1247.7 ± 128.9[a]	0.001
Redox Potential in mV	122.6 ± 32.4[a]	131.0 ± 26.7[a]	0.241
NH_4^+–N in mg L^{-1}	0.38 ± 0.23[b]	16.69 ± 5.11[a]	0.001
NO_2^-–N in mg L^{-1}	0.29 ± 0.17[a]	0.48 ± 0.54[a]	0.842
TON in mg L^{-1}	44.49 ± 8.73[b]	76.50 ± 12.53[a]	0.001
NO_3^-–N in mg L^{-1}	44.20 ± 8.69[b]	76.02 ± 12.37[a]	0.001
TDN in mg L^{-1}	44.87 ± 8.82[b]	93.19 ± 14.04[a]	0.001
PO_4^{3-}–P in mg L^{-1}	3.06 ± 1.20[b]	7.06 ± 1.11[a]	0.001

Different letters ([a],[b]) show statistically significant differences ($p < 0.05$)

Water parameters in the hydroponics aquaculture effluent and nutrient tanks (AET-E, AET-I, NT-Control) showed differences (Table 6.5). Between all groups, differences in dissolved oxygen concentration (DO) and oxygen saturation were significant, with the lowest values in the intensive group (AET-I) with 2.8 mg L^{-1} (± 1.7) and 31.2 % (± 18.6), respectively. The highest DO and saturation levels were found in the fertilizer control group with 8.3 mg L^{-1} (± 0.8) and 87.4 % (± 2.1). Temperature was relatively stable between the groups; the extensive and intensive subgroups showed slightly higher values at 20 °C. The process water in the fertilizer group was more alkaline (pH 8.1 ± 0.2) than in the EAU group (pH 7.3 ± 0.3) and the IAU group (pH 7.4 ± 0.3). Salinity was higher in the aquaponics groups and significant (IAU: 0.6 ± 0.1 ‰; EAU: 0.5 ± 0.1 ‰) compared with the control (0.4 ± 0.0 ‰). Conductivity was highest in the IAU (1095.2 ± 122.4 µS cm^{-1}), followed by the EAU (821.0 ± 149.3 µS cm^{-1}) and control group (673.7 ± 120.1 µS cm^{-1}). In contrast, the redox potential was lowest in the IAU (100.6 ± 46.1 mV), followed by the control (117.1 ± 102.4 mV) and EAU (142.1 ± 136.9 mV).

Light intensity was highly variable and dropped from 70,300 lx (day 7) to 10,900 lx (day 70) during the experiment (y = − 274.32x + 45,157, R2 = 0.07) under late summer to autumn conditions with a mean of 35,418.6 lx and a minimum of 3,200.0 lx (Figure 6.4), as well as an air temperature (outside) from 30.2 °C (day 5 = maximum) to 5.1 °C (day 70 = minimum) with a mean of 17.0 °C (y = − 0.2039x + 24.202, R2 = 0.51, Figure 6.4).

Table 6.5: *Physicochemical nutrient water parameter (mean ± SD), nutrient ratios (N/P; leaf area/N; leaf area/P), and biomass relations (fresh biomass/leaf area) of experimental aquaculture effluent and nutrient tanks (Fertilizer control: NT-Control; extensive EAU: AET-E; intensive IAU: AET-I) with nutrients for Mentha spicata cultivation in hydroponics (70 days).*

Parameters	Fertilizer (Control)	Extensive (EAU)	Intensive (IAU)	*p*-I*	*p*-II*	*p*-III*
O2 in mg L^{-1})	8.3 ± 0.8[a]	5.1 ± 1.2[b]	2.8 ± 1.7[c]	0.001	0.001	0.001
O2 in %	87.4 ± 12.1[a]	56.1 ± 13.0[b]	31.2 ± 18.6[c]	0.001	0.001	0.001
Temperature in °C	18.9 ± 2.0[b]	20.9 ± 2.6[a]	20.8 ± 2.7[a]	0.001	0.001	0.900
pH	8.1 ± 0.2[a]	7.3 ± 0.3[b]	7.4 ± 0.3[b]	0.001	0.001	0.622
Salinity in ‰	0.4 ± 0.0[c]	0.5 ± 0.1[b]	0.6 ± 0.1[a]	0.001	0.001	0.001
Conductivity in µS cm^{-1}	673.7 ± 120.1[c]	821.0 ± 149.3[b]	1095.2 ± 122.4[a]	0.001	0.001	0.001
Redox Potential in mV	117.1 ± 102.4[a,b]	142.1 ± 136.9[a]	100.6 ± 46.1[b]	0.130	1.000	0.042
NH_4^+–N in mg L^{-1}	0.24 ± 0.31[c]	2.11 ± 3.44[b]	20.46 ± 6.53[a]	0.001	0.001	0.001
NO_2^-–N in mg L^{-1}	0.52 ± 1.11[b]	0.55 ± 0.30[a]	0.60 ± 0.49[a]	0.001	0.001	0.843
TON in mg L^{-1}	7.55 ± 2.12[b]	40.59 ± 12.40[a]	43.99 ± 17.94[a]	0.001	0.001	0.469
NO_3^-–N in mg L^{-1}	7.03 ± 1.95[b]	40.04 ± 12.38[a]	43.38 ± 17.85[a]	0.001	0.001	0.444
TDN in mg L^{-1}	7.79 ± 2.14[c]	42.70 ± 13.92[b]	64.45 ± 20.55[a]	0.001	0.001	0.001
PO_4^{3-}–P in mg L^{-1}	3.44 ± 1.05[b]	3.30 ± 1.16[b]	7.98 ± 1.69[a]	0.392	0.001	0.001
N^1/P^2	2.4 ± 0.7[c]	13.4 ± 3.1[a]	8.2 ± 2.3[b]	0.001	0.001	0.001
Leaf Area/N^1	0.7 ± 0.3[a]	0.2 ± 0.1[b]	0.2 ± 0.1[b]	0.001	0.001	0.356
Leaf Area/P^2	1.6 ± 0.8[a]	2.2 ± 1.5[a]	1.6 ± 0.7[a]	0.148	0.148	0.148
Fresh Biomass/ Leaf Area	23.0 ± 10.5[a,b]	29.2 ± 19.8[a]	16.9 ± 11.0[b]	0.313	0.069	0.009

[1] N = TDN in mg L^{-1}, [2] P = PO_4^{3-}–P in mg L^{-1}; Different letters ([a], [b], [c]) show statistically significant differences ($p < 0.05$), * Significance (p) with: *p*-I = control/extensive, *p*-II = control/intensive, *p*-III = extensive/intensive.

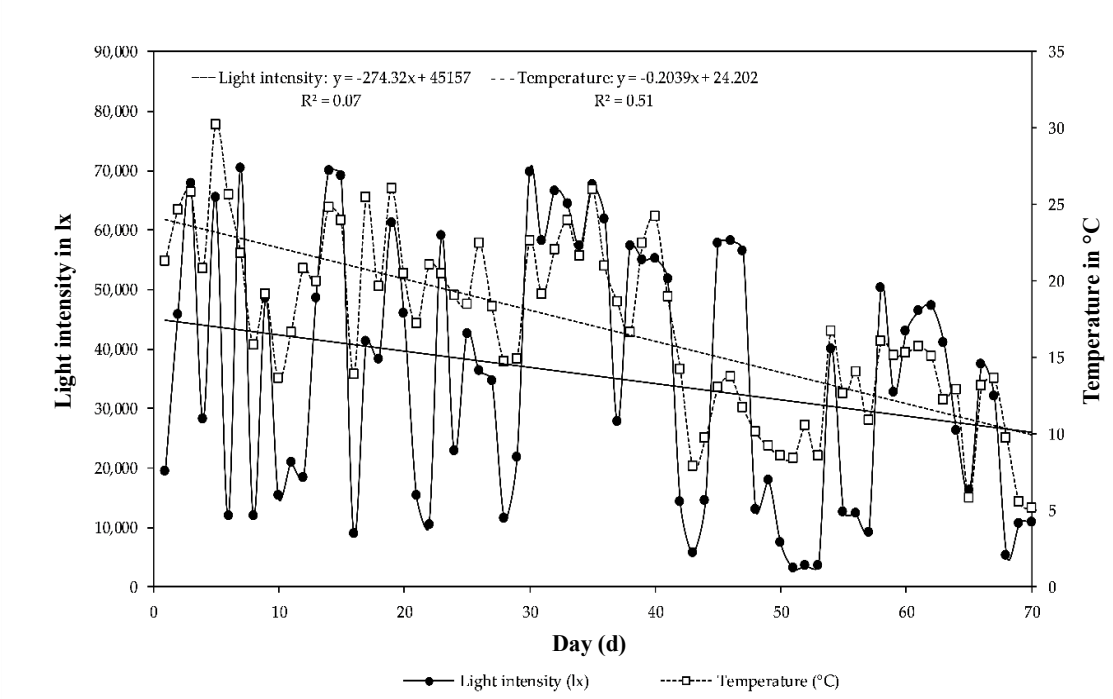

Figure 6.4: *Light intensity in lx and air temperature in °C during the experiment outside the FishGlassHouse at 12:06 for 70 consecutive days.*

Chemical water parameters of the aquaculture effluent and nutrient tanks (Table 6.5) were highest in the IAU for NH_4^+–N (20.46 ± 6.53 mg L^{-1}) by 85-fold compared to the control group and 9.7-fold compared to the EAU. Levels of TON were approximately 5 – 6 times greater in the EAU (40.59 ± 12.40 mg L^{-1}) and also in the IAU (43.99 ± 17.94 mg L^{-1}) compared to the control (7.55 ± 2.12 mg L^{-1}); nearly the same was found for NO_3^-–N values. The TDN value was highest in the IAU (64.45 ± 20.55 mg L^{-1}) by 1.5-fold compared to the EAU (42.70 ± 13.92 mg L^{-1}) and 8.3-fold compared to the control (7.79 ± 2.14 mg L^{-1}). Phosphorus (PO_4^{3-}–P) was generally low in all groups and not significantly different between the control (3.44 ± 1.05 mg L^{-1}) and the EAU (3.30 ± 1.16 mg L^{-1}), with the highest level in the IAU (7.98 ± 1.69 mg L^{-1}).

The levels of NO_3^-–N were variable with comparable developments in both IAU and EAU aquaponic subgroups (Figure 6.5), with the highest values in the IAU (y = 0.5163x + 28.191, R^2 = 0.29) followed by the EAU (y = 0.4829x + 24.158, R^2 = 0.60). In contrast, NO_3^-–N levels in the fertilizer group (control) were lower; they never reached the values of the aquaponics systems and decreased over the duration of the experiment (y = − 0.0281x + 7.8937, R^2 = 0.08).

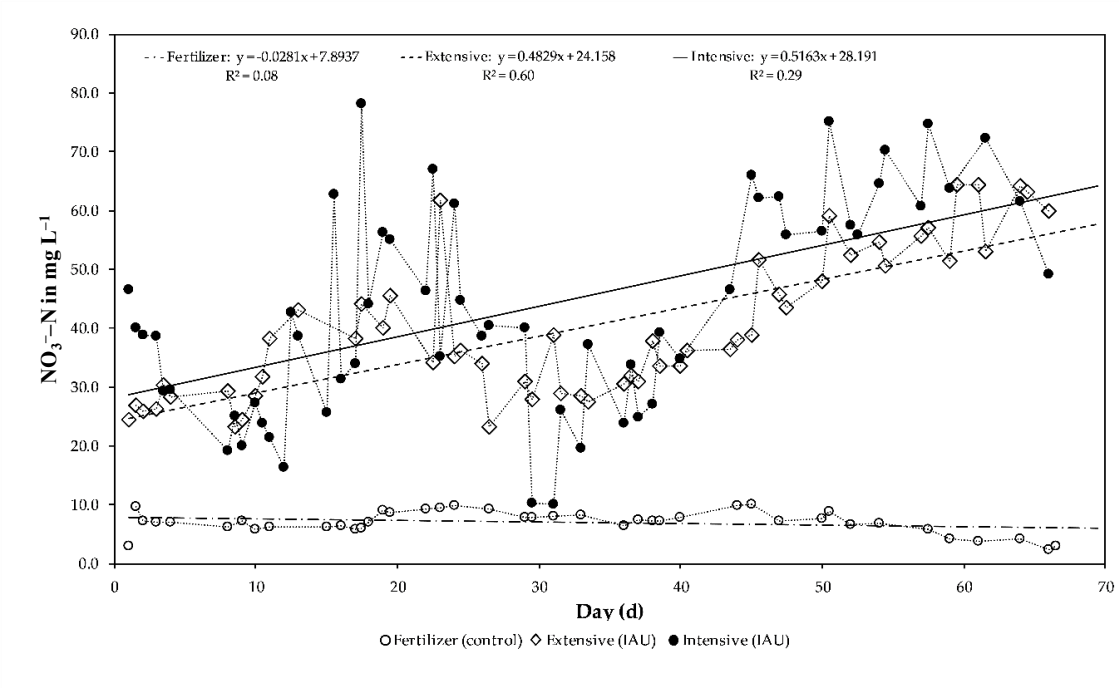

Figure 6.5: *NO_3^-–N in mg L^{-1} values of the fertilizer (control), extensive (EAU) and intensive (IAU) groups on 70 consecutive days during mint cultivation.*

The phosphorus levels showed a strong difference between the IAU (y = 0.0299x + 7.0957, R^2 = 0.11, Figure 6.6) and both other groups. P levels in the EAU nearly reached the intensive p values (days 13, 23) but were generally at lower levels (y = 0.0273x + 2.4013, R^2 = 0.21), nearly parallel to the control. In contrast, the PO_4^{3-}–P levels of the fertilizer group (control) were in the range of the EAU and decreased during the experiment (y = − 0.0193x + 4.017, R^2 = 0.13).

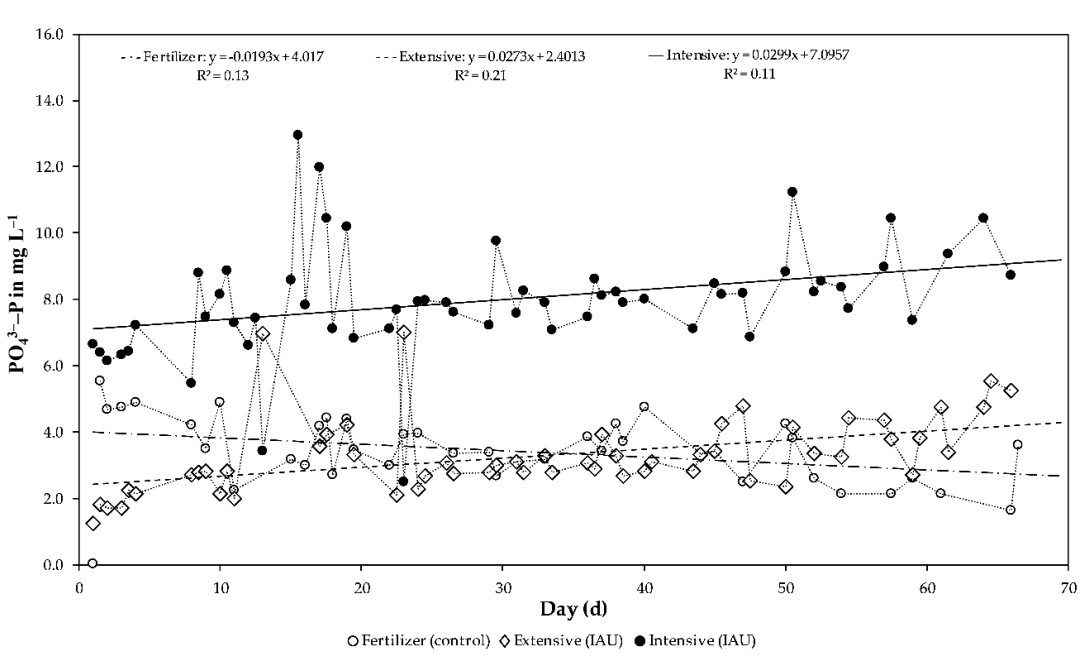

Figure 6.6: *PO_4^{3-}–P in mg L^{-1} values of the fertilizer (control), extensive (EAU), and intensive (IAU) groups on 70 consecutive days during mint cultivation.*

6.3.3 Nutrient Ratios and Biomass Relations

Between the subgroups, the N/P ratio was highest in the extensive group (EAU: 13.4 ± 3.1, Table 6.5), followed by the intensive (IAU: 8.2 ± 2.3) and fertilizer group (control: 2.4 ± 0.7). The ratio of leaf area to N was 3.5 times larger in the control than in the other groups. In contrast, differences in the leaf area to P ratio were generally insignificant between all groups. The relation of fresh biomass (uncut) to leaf area was highest and significant between the EAU (29.2 ± 19.8) and IAU (16.9 ± 11.0); however, there were no significant differences from the control group (23.0 ± 10.5, Table 6.5).

TDN and leaf area values were positively correlated in the experimental groups with the aquaponics process water from *C. gariepinus* effluents (EAU, IAU; Figure 6.7). The highest correlation was found in the EAU group, with the highest m value of 0.98 (y = 30.49 + 0.98x, R^2 = 0.033), followed by the IAU (y = 48.22 + 0.82x, R^2 = 0.013) and the control group with a very low negative correlation (y = 8.81–0.02x, R^2 = 0.002). In contrast, PO_4^{3-}–P and leaf area values were not correlated in the fertilizer and EAU groups (Figure 6.8). Only the IAU group showed a slightly positive correlation (y= 6.79 + 0.08x, R^2 = 0.009).

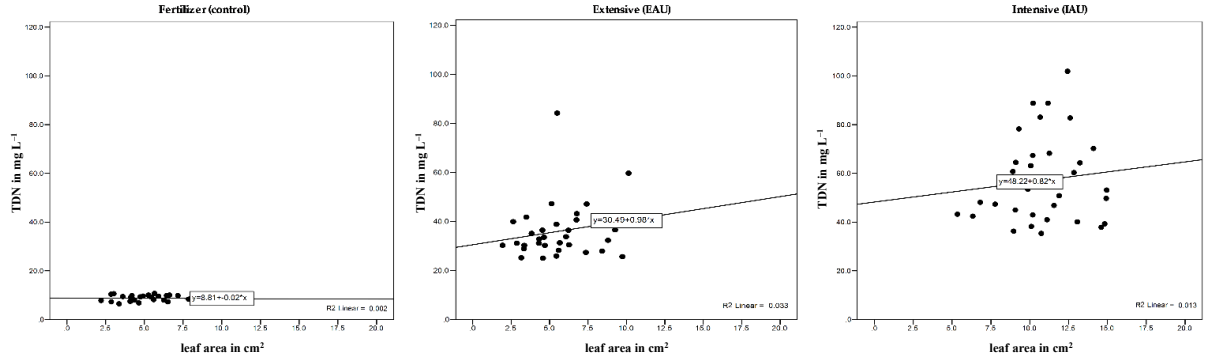

Figure 6.7: *Correlations between TDN values in mg L^{-1} and leaf area in cm^2 of the fertilizer (control), extensive (EAU), and intensive (IAU) groups.*

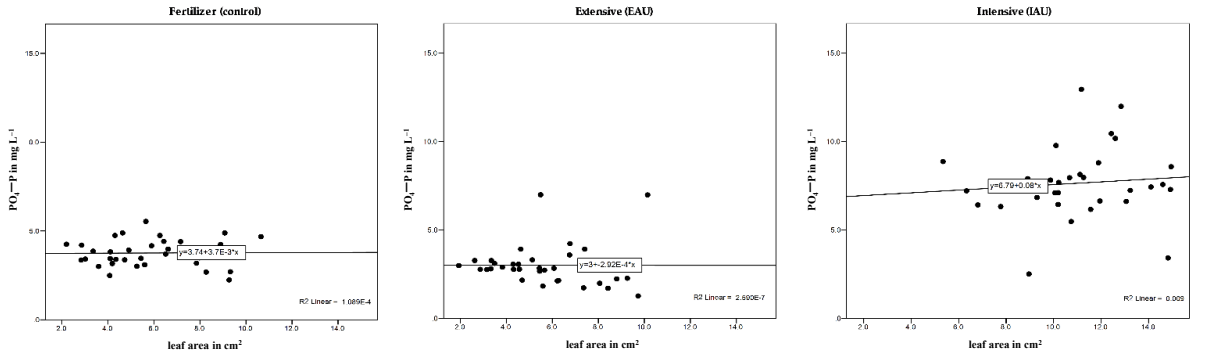

Figure 6.8: *Correlations between PO_4^{3-}–P values in mg L^{-1} and leaf area in cm^2 of the fertilizer (control), extensive (EAU), and intensive (IAU) groups.*

6.4 Discussion

6.4.1 Fish Production

Fish production of *C. gariepinus* followed the “run-in” and “batch production” phases in the FishGlassHouse (Palm et al., 2018b). Even though the final stocking density was 3.6 times higher in the intensive aquaculture unit, no differences in final individual weight, feed conversion, or specific growth were observed, and the fish growth was generally good in both aquacultural units. This is not in accordance with Van de Nieuwegiessen et al. (2009), who recorded a positive influence of an increasing stocking density for 67 – 133 fish m^{-3}.

Commercial African catfish indoor production with a slightly higher stocking density of 166 fish m^{-3} showed a significantly lower feed conversion and final weight (FCR: 1.373 ± 0.01; final weight: 787 ± 6.31 g), starting with juveniles of 12 g and a shorter cultivation period of

154 days (OFF-Farm, Nigeria, (Akinwole and Faturoti, 2007)). Fish in the present study were much older (275 g initial weight), had a lower stocking density (140 fish m^{-3}), and a longer cultivation period (40 % more total time), suggesting highly beneficial cultivation conditions under semi-coupled aquaponics. Feed conversion was more comparable to younger fish (e.g., 190.9 g and 193.0 g), and FCRs were between 0.90 and 0.99 (Leenhouwers et al., 2007). Moreover, 2.7 times younger fish (growth phase from 102.1 ± 3.49 g to 275.12 g) showed a better FCR of 0.81 with stocking of 133 fish m^{-3} than older fish (Van de Nieuwegiessen et al., 2009). With nearly the same initial fish weight of 254.7 g in a nearly closed RAS, a comparable FCR of 0.97 was reported (Mota et al., 2015), indicating that our feed conversion and *C. gariepinus* production was generally similar to earlier experiments under intensive production conditions.

The specific growth of *C. gariepinus* of 1.07 – 1.09 % d^{-1} was relatively low due to the higher fish size. Better growth of 1.4 % d^{-1} was reported with slightly younger African catfish of 200 g at slightly higher temperatures from 27 – 28 °C (Hogendoorn et al., 1983), with 2.05 % d^{-1} from 102.1 – 275.12 g production (Van de Nieuwegiessen et al., 2009) and 2.73 % d^{-1} with fish meal protein substitution by winged bean (*Psophocarpus tetragonolobus*) protein in fish feed (Fagbenro, 1999). Different specific growth rates have also been reported under aquaponics conditions. With an initial body weight of 480.23 g, a specific growth rate of 0.65 % d^{-1} was reported with the production of lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), tomatoes (*Solanum lycopersicum*), and basil (*Ocimum basilicum*) (Palm et al., 2014). In contrast, younger fish (30 – 40 g) grew better with a specific growth of 1.68 – 1.83 % d^{-1} in a gravel substrate aquaponics system (Endut et al., 2010). The reduced specific growth during the present study might be explained by the restrictive feeding protocol (based on actual FCRs and did not correspond to feeding “to saturation”) that was used to prevent nutrient overload inside the catfish RAS.

The physical and chemical water parameters for *C. gariepinus* production were mainly close to or in their optimal range. The mean dissolved oxygen concentration under intensive production was above 3 mg L^{-1} (and 40 %) when young *C. gariepinus* can change to atmospheric air-breathing (Oellermann, 1995). The temperatures were below the optimum (≈ 25 – 26 °C), where the optimal temperature range for *C. gariepinus* juveniles (1.1 ± 0.4 g) was suggested to be 28 – 30 °C (Oellermann, 1995). *C. gariepinus* is tolerant to poor water quality (Britz, 1988), though the data are still contradictory. Reports of the high maximum

NH_4^+–N levels of 16.69 mg L^{-1} in the present study are scarce, and recommended NH_3–N levels reached only 0.34 mg L^{-1} (Schram et al., 2010). For juvenile *C. gariepinus* (5 g), a 96 h LC_{50} level of 2.3 mg L^{-1} NH_3 was found (Britz, 1988). However, toxic levels for African catfish are much higher under real production conditions, where the systems run at a very low pH, suggesting no negative effects of the observed NH_4^+–N levels for our aquaponics fish. Nitrite levels were at the maximum in the IAU at 0.48 mg L^{-1}, below the toxic level of 0.6 mg L^{-1} NO_2^- (Roques et al., 2015), and nitrate was below 140 mg L^{-1} without a negative impact on fish growth (Schram et al., 2014). Interestingly, the NO_2^-–N parameters were comparable between the extensive and intensive catfish production, indicating good functionality of the used biofilters, including their dimension and material.

Under intensive production, the maximum stocking level for African catfish was not reached. This species is tolerant to very high stocking densities (180.7 kg per 900 L), particularly with their air-breathing physiology and lethargic behavior, which results in lower maintenance requirements of recirculating systems (Huisman and Richter, 1987). The highest density of up to 1067 fish m^{-3} (growth range 102.1 – 295.20 g) with good specific growth (2.14 % d^{-1}) and feed conversion (0.81) was reported by Van de Nieuwegiessen et al. (2009). Under commercial production, a final stocking density of 500 kg m^{-3} seems to be possible (Van de Nieuwegiessen et al., 2009), 3.4-fold of the final stocking density in our study.

6.4.2 Mint Production

The best growth of mint was observed in combination with the effluents of intensive *C. gariepinus* aquaponics production. Compared with the extensive rearing of African catfish and the fertilizer control group, the use of intensive aquaponics process water resulted in more fresh plant biomasses of 13.21 % compared to the EAU and 36.76 % in the control, and in higher plant heights of 11.37 % (EAU) and 24.7 % (control). The leaf area, an important parameter for commercial mint production, was highest under intensive catfish production and nearly twofold better compared with the extensive *C. gariepinus* effluents and fertilizer control group. Our results of the leaf area under intensive rearing of African catfish are in agreement with *M. spicata* cultivation in garden soil (leaf area = 9.6 ± 0.5 cm^2 (Patel and Patra, 2015)), demonstrating that culture conditions inside the FishGlassHouse were adequate and the observed differences in mint growth between the three treatment groups were mainly influenced by the increased nutrient supply from the intensive fish cultivation in the IAU.

The dry biomass of *M. spicata* showed no differences between the aquaponics experimental groups and the fertilizer control. This demonstrates the generally good adaptation of mint to wet habitats, low nutrient concentrations, and good cultivation conditions in aquaponics. Earlier investigations showed the advantages of aquaponics compared to the use of commercial fertilizers. Plants grown in aquaponics consumed nutrients faster than in hydroponics, with a higher accumulation of biomass (Savidov, 2005). Lettuce started flowering one week earlier, and cucumber and tomatoes 5 – 10 days earlier compared to plant cultivation in a hydroponics system. Obviously, there is a yet unknown component in the aquaponics solution that increases plant growth versus the exclusive use of conventional hydroponics fertilizer. It was suggested that certain bacteria can promote plant growth in aquaponics, known as plant-growth-promoting rhizobacteria (PGPR) (Savidov, 2005). A better root fresh weight, leaf area, and leaf color were described in Batavian lettuce (*Lactuca sativa* var. *capitata*) and also in iceberg lettuce (*Lactuca sativa* spp.) with the use of aquaponics process water from *C. gariepinus* production combined with a fertilizer in contrast to the use of the fertilizer alone and separate aquaponics (Lehmonen and Sireeni, 2017). In our study, a better growth was observed in the intensive group with a significant 1.4 times higher shoot number, 1.9 × leaf area, and 1.6 × leaf length.

The growth parameters of *M. spicata* showed relatively good results under commercial cultivation conditions (height 38.4 – 51.0 cm). Peppermint production (*Mentha piperita*) was only slightly better in a coupled gravel backyard aquaponics system in combination with common carp (*Cyprinus carpio*, 59.2 cm) and Nile tilapia (*Oreochromis niloticus*, 63.2 cm) under the same late summer to autumn conditions and an experimental time of 70 days (Knaus et al., 2018). In brackish and freshwater desert aquaponics, a slightly higher plant height was achieved for *Mentha* sp. (70 cm) combined with the Nile tilapia red strain (*Oreochromis niloticus* x blue tilapia *O. aureus* hybrids) (Kotzen and Appelbaum, 2010). The mint garden pot production in the present study, applying aquaponics gardening (Palm et al., 2018a), generally also corresponded to earlier experiments in hydroponics (Chrysargyris et al., 2017) or performed even better in the EAU and IAU. Consequently, in terms of fresh plant biomasses, plant heights, and leaf area, the commercial production of spearmint (*M. spicata*) in peat pots with only starter fertilizer by using aquaponics gardening, especially under intensive African catfish stocking has no disadvantages compared with other production methods.

Physicochemical water parameters had an influence on the *Mentha spicata* production depending on the fish stocking density. The general environmental parameters (light, temperature) were optimal for the mint growth, as described for peppermint (*M. piperita*), with sunny, half-shaded, and damp locations and temperatures between 15.5 and 21.1 °C (Vocke, 2002; Wahap et al., 2010). However, the significant and markedly lower pH value in the aquaponics groups might have impacted the nutrient uptake of *M. spicata*. The pH levels in the present study were higher than reported for *Mentha arvensis*, with good growth under slightly acidic conditions with a pH of about 6.5 (Wahap et al., 2010). Good growth of *M. spicata* was also reported under higher pH levels between 7.9 and 8.0 and plant lengths from 59.2 – 63.2 cm in coupled backyard aquaponics combined with common carp (*C. carpio*) and Nile tilapia (*O. niloticus*) (Knaus et al., 2018). Thus, the pH was not decisive for the observed variations in *M. spicata* growth. Conductivity (EC) was highest in the IAU group (1.3 × EAU; 1.6 × fertilizer control group) because the process water from the intensive rearing of *C. gariepinus* received a significantly higher feed input per day. In conventional hydroponics, the EC level strongly influences plant growth and was reported to be optimal for, e.g., peppermint (*Mentha piperita*) at 1.4 dS m^{-1} (Tabatabaie et al., 2006); however, conductivity in aquaponics can be lower at levels from 300 – 600 µS cm^{-1} (Rakocy et al., 2006). In the high-conductivity process water of coupled aquaponics, suitable growth results of *Mentha sp*. were reported at levels of up to 4500 µS cm^{-1} in brackish water and, similar to the present study, at 1060 µS cm^{-1} in freshwater (Kotzen and Appelbaum, 2010). The highest EC level was observed with 1095.2 µS cm^{-1} in the intensive rearing of *C. gariepinus*, which also had the best growth results of *M. spicata*. This demonstrates the importance of the EC level also under aquaponics for mint growth and that even a relatively low EC of about 1000 achieves a good growth performance under aquaponics gardening conditions.

6.4.3 Nutrient Ratios and Biomass Relations

Chemical water parameters were strongly affected by the stocking density. With a fourfold increase in catfish biomass under intensive production, the NH_4^+ level increased markedly. The highest concentration of NH_4^+–N was found in the intensive aquaculture effluent tank, 9.7-fold higher (2.1 vs. 20.5 mg L^{-1}) than in the extensive and 85-fold higher than in the control (0.24 mg L^{-1}). The original process water had an NH_4^+ ratio of 43.9:1, much higher than earlier observed for extensive and intensive African catfish staggered 1 production and

dissolved oxygen below 6 mg L^{-1} (Palm et al., 2018b). Nitrate was comparable between both aquaponics (40.0 vs. 43.4 mg L^{-1}) and 5.9-fold higher than in the control. Nitrogen-derived plant nutrients are considered major growth-limiting factors for plants. Consequently, our plants grew best under an intensive stocking density, resulting in a duplication of the IAU plant leaf area (Table 6.3, Table 6.5). However, *Mentha* species can also perform well under low nitrogen concentrations. In *M. arvensis*, good growth was observed under very low levels of ammonia nitrogen (0.81 mg L^{-1}) and nitrate (0.22 mg L^{-1}) combined with *Cyprinus carpio* (Shete et al., 2017). Obviously, even under the relatively low levels of nitrate from the aquaculture unit combined with relatively high NH_4^+–N in the plant nutrient tanks, the intensive production of *C. gariepinus* provided adequate nutrients for *M. spicata* cultivation during the present study.

The phosphorous content was also low in both plant units run with extensive (3.3 mg L^{-1}) and intensive (8.0 mg L^{-1}) effluent waters of African catfish production. The original process water had a P ratio of 2.3:1 between the intensive (3.1 mg L^{-1}) and extensive (7.1 mg L^{-1}) production units, corresponding to Palm et al. (2018b), who also reported a comparable P ratio between extensive (5.7 mg L^{-1}) and intensive (13.0 mg L^{-1}) African catfish staggered 1 production and dissolved oxygen values below 6 mg L^{-1}. Phosphorus levels in aquaponics are generally low at approximately 6.6 mg L^{-1} (Bittsanszky et al., 2016), similar to the intensive African catfish production in the present study. In backyard aquaponics production of low-level nutrient plants, e.g., basil (*Ocimum basilicum*), parsley (*Petroselinum crispum*), and marjoram (*Origanum majorana*), P levels reached 11.00 mg L^{-1} and 16.86 mg L^{-1} with the production of African catfish (*C. gariepinus*) and tilapia (*O. niloticus*), respectively (Knaus and Palm, 2017). Much lower P values between 1.55 mg L^{-1} and 1.71 mg L^{-1} were reported under the cultivation of Nile tilapia (*O. niloticus*) and common carp (*C. carpio*) in co-cultivation with cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), and lettuce (*Lactuca sativa*) (Knaus and Palm, 2017). Similarly, peppermint (*Mentha piperita*) combined with Nile tilapia (*Oreochromis niloticus*) grew best with a low PO_4–P water content of 1.9 mg L^{-1} and weekly supplements of micronutrients and Fe (Nozzi et al., 2018). Consequently, the observed phosphorus concentration during the present study was also adequate for the aquaponics gardening experiments with *M. spicata* and in accordance with earlier results.

The general growth parameters of *M. spicata* showed good results, especially by using process water from intensive catfish farming with low oxygen (< 6 mg L^{-1}) conditions. The N

and P concentrations, as well as the TDN and P to leaf area correlations, were best in the IAU (Figure 6.7, Figure 6.8), with an N/P ratio of 8.2 (Table 6.5), mainly caused by the higher proportion of phosphorus to TDN (12.38 % P) compared to EAU (7.72 % P). In contrast, the nutrient concentrations and composition of the control were suboptimal, resulting in reduced plant growth (height). It must be taken into account that the fertilizer utilized for the control group maintains plant metabolism for local markets rather than promoting flowering or growth. This might explain the lower performance of the control compared with both aquaponics groups. If the phosphorus content reaches at least a minimal 1:8 P/TDN ratio, the successful cultivation of *M. spicata* in aquaponics under the described conditions is possible.

6.5 Conclusions

The aquaponics (*s.l.*) cultivation of spearmint (*M. spicata*) without the use of additional liquid fertilizer was evaluated inside the "FishGlassHouse", a semi-coupled commercial aquaponics facility in Northern Germany. Effluents from the intensive aquaculture production of African catfish (*C. gariepinus*) achieved the best plant growth parameters in shoot number, leaf area, leaf length, and cut fresh biomass. The 4-fold higher fish biomass under intensive stocking (IAU) resulted in a 1.2-fold higher mint biomass and a 2-fold increase of the leaf area compared to extensive stocking. Aquaponics gardening of *M. spicata* with *C. gariepinus* effluents reveals good growth results and provides marketable plants. The underlying water parameters of oxygen values below 6 mg L^{-1}, ammonium of 2 – 20 mg L^{-1}, nitrate of 40 mg L^{-1}, phosphorous of 8 mg $L^{-1,}$ and an N/P ratio of 8 : 1 are commonly reached in African catfish RAS production, suggesting that *C. gariepinus* effluent waters could be used for aquaponics cultivation of spearmint.

7 Discussion and Conclusions

This work aimed to improve the production conditions and processes of African catfish RASs in Northern Germany (N.G.) to define ideal conditions for optimized production.

For this purpose, first, the influence of different stocking densities on the growth and welfare of catfish during a grow-out period was monitored and evaluated. Second, the effect of stocking density and grading on the behavior, survival rate, and growth of catfish fry was assessed. Third, the influence of different potassium water concentrations on the growth and welfare of juvenile African catfish was investigated. Since potassium is a limiting macronutrient in the fish process water concerning aquaponic use, the goal was to identify whether potassium fertilization is already possible in the fish system, as this could then be combined with pH adjustment. Fourth, it was tested whether the feed additive Montmorillonite-illite/Muscovite (1g557), a mixed clay mineral, positively affects catfish growth and welfare. Last and fifth, it was tested if the process waters from an extensively and intensively stocked African catfish RAS could be used for commercial mint (*Mentha spicata*) production.

This chapter discusses the results of the studies mentioned above and concludes best practices in commercial production systems. Additionally, optimal and poor production conditions are compared concerning survival rate and growth to assess potential differences in yield.

7.1 Status quo and Prospects

According to Pasch and Palm (2021), an African catfish farm in N.G. with a production volume of 300 m^3 should be able to cover its costs. However, according to the farm operators, this is usually not the case. Reasons may be unreliable fingerling availability, suboptimal production, or poor marketing. There are two possibilities to manage RASs profitably due to their expensive operating costs. If the market allows, one possibility is to produce a high-priced product that covers the production costs. The other possibility is to make the yield over the quantity. In that case, the consumer is unwilling to pay a higher price for the offered product. Related to African catfish production in Germany, the second is the case. On average, the producer is paid only 2.20 € kg^{-1} for whole fish, from which hardly anything is left if the costs for feed, labor, energy, water, and repayments are substracted (Pasch and Palm, 2021). It is essential that these farms not only optimize their production but also receive a higher return

for their fish in the future. The prerequisite to achieve this would be that the farmers start to work together and thereby prevent the buyers from being able to depress prices. The current financial situation of the farms shows why it is essential to run them as intensively as possible while optimizing fish growth.

This dissertation has focused exclusively on opportunities for improvements in fish production. Since all farm operators come from an agricultural background but had nothing to do with fish before, they often lack the relevant know-how. Even though catfish is a very tolerant and robust species, there is a difference between simply raising the fish and producing it in large quantities as quickly as possible under highest stocking densities, as it is necessary in commercial farms. That requires personnel with requisite knowledge and skills. In this regard, however, the farm operators often have the problem of finding appropriate personnel who want to work for the paid salary in the usually rural locations over a longer period. Furthermore, there are almost no protocols in the companies so far, so new employees first have to learn all the work steps by themselves, which prevents a smooth transition without restrictions in production. In addition, everything in the existing catfish farms has largely been built as cheaply as possible, which makes the operation very labor-intensive. This high workload and the lack of personnel often lead to the fact that controls, such as monitoring of the water parameters, are only carried out when problems arise. Since practical work, in general, can only be done quickly with routines, high staff turnover also has a negative effect. However, since the change of employees can only be influenced to a very limited extent, the operation must be improved so that optimum production can be reliably achieved under the existing conditions.

7.2 Growth and Improvement Opportunities in African catfish RAS

The African catfish has enormous growth potential and an excellent feed conversion with a mean value of 0.78 during the grow out (**Baßmann et al., 2023**). The FCR is important because feed costs account for 61.4 % of the total variable costs (Pasch and Palm, 2021). From a biological point of view, growth can mainly be influenced by the composition of the feed (Winfree and Stickney, 1981), the way the feed is offered (Rad et al., 2004), genetics of the fish (De-Santis and Jerry, 2007), water quality (Schram et al., 2014), and stocking density (**Baßman et al., 2023**; Irwin et al., 1999).

Concerning feed, it makes a difference not only whether it has a higher or lower energy content (Bromley, 1980) but also how well digestible it is, which in turn is related to the origin (e.g., of animal or plant origin) of the ingredients (Daniel, 2018), but also their ratios (Skalli et al., 2004). Henken et al. (1986) showed that depending on crude protein content and metabolizable energy, the ideal amount of crude protein intake per unit of metabolizable energy considering growth is 25.4 mg kJ^{-1} at 24 °C and 34.7 mg kJ^{-1} at 29 °C in fish with a mean initial weight of around 40 g (experimental period: 12 weeks). Goda et al. (2007) observed specific growth rates of African catfish between 1.52 and 1.93 depending on the protein source in the feed.

With regard to feeding quantity, Marimuthu et al. (2011) found the best feed conversion ratio (initial fish weight: 1.63 g) with a value of 1 at a feeding level of 8 % of body weight per day and the best specific growth rate with a value of 6.59 % d^{-1} at a feeding level of 12 %. The worst FCR and specific growth rate were observed at feeding levels of 5 and 2 %, with values of 1.35 and 1.42 % d^{-1}. While feeding frequencies influence growth, the optimum intervals seem to be age-dependent. Pantazis and Neofitou (2003) observed an improvement in the specific growth rate of more than 0.4 % d^{-1} when fish with a mean initial weight of 102 g were fed twice a day ad libitum compared to 3 times a day. On the other side, Jamabo et al. (2015) observed in fish with a mean initial weight of 4.12 g best growth when fish were fed four times a day compared to once, twice, or three times.

Additionally, some fish always eat slower or more restrained than the rest. These fish are extremely disadvantaged when food is offered only at one point (Rad et al., 2004). That leads to a less homogeneous growth, which makes the slaughter cumbersome if the fish are not graded before. Both factors increase costs.

Concerning the genetic influence on growth, farmers are mostly dependent on fingerling suppliers, and even if they have their own broodstock, they are limited in genetic diversity. In addition, there is no information about a suitable broodstock selection in limited breeding strains of African catfish. However, experiments have shown that there is always a part of slow-growing and fast-growing fish, regardless of whether they were separated from each other or not (Martins et al., 2005b). Additionally, experiments conducted at the University of Rostock have shown that even if fast-growing fish are separated as a group, after a certain time, they will grow apart again (Hummel, 2022, personal communication). Opiyo et al. (2017)

observed differences in fecundity, survival rates, and growth depending on the genetic background. Differences in specific growth rates made up to 3.36 % BW d^{-1}.

Since catfish are kept in RASs, water treatment plays an important role. Despite their high tolerance towards various water parameters, as explained in chapter 1.4.1, deviations from the optimum values can lead to reduced growth and increased mortality rates (Ogunji and Awoke, 2017; Roques et al., 2015; Schram et al., 2014). Moreover, water temperature greatly influences growth as it affects the reaction rate of biological processes (Dell et al., 2011). Between 26 and 32 °C, the growth of African catfish improves with increasing temperature (Kasihmuddin et al., 2021). However, a commercial catfish farm in Germany should include heating costs when determining the most economical temperature.

Regarding water quality, Ajiboye et al. (2015) proved a specific growth rate of more than 0.2 % BW d^{-1} when water was changed every or every second day compared to every fourth day. Schram et al. (2014) observed a reduction in specific growth rates of more than 1 % BW d^{-1} at water nitrate concentrations of 27.04 mM. Nitrite concentrations of 928 µM could reduce the specific growth rate by 0.55 % BW d^{-1} and the survival rate by 8.3 % (Roques et al., 2015). Ammonia concentrations of 1084 µM reduced the specific growth rate by more than 1.5 % (Schram et al., 2010).

Stocking densities can also affect fish growth but also welfare. In commercial RAS in N.G., the stocking densities can be very high, up to 500 kg m^{-3} per fish tank. Thereby production can be increased, but at the same time, the risk of complications increases. Since problems usually appear very quickly and countermeasures are often initiated too late, this can result in high losses. Based on various research studies, optimal stocking densities vary between different age/size classes of fish (Kaiser et al., 1995b; Van de Nieuwegiessen et al., 2008, 2009; Wei et al., 2011). Hossain et al. (1998) observed in catfish with a mean initial weight of approximately 0.8 g better growth when stocked at 5 fish L^{-1} compared to 10 fish L^{-1}. In 0.96 g fish, held in a biofloc-based system, Fauji et al. (2018) found the best specific growth rate with a value of 7.07 % day^{-1} at a stocking density of 6 fish L^{-1} and the best FCR with a value of 0.72 at 8 fish L^{-1} (tested stocking densities: 4, 6, and 8 fish L^{-1}).

In contrast, Van de Nieuwegiessen et al. (2008) found no differences in growth for 10 g fish that were stocked between 500 and 3000 fish m^{-3}, while they found indications of impaired welfare at the highest and lowest stocking density. In fish between 100 and 200 g, they observed an improvement of welfare with increasing stocking density but no influence

on growth (tested stocking densities: 17 – 315 kg m^{-3}). No effect of stocking density on welfare and growth was observed in fish between 1000 and 1500 g (Van de Nieuwegiessen et al., 2009). However, this dissertation demonstrates that these observations were possibly based on experimental conditions and might differ in commercial aquaculture.

In chapter 2 (**Baßman et al., 2023**) of this dissertation, fish were stocked at final stocking densities of around 100, 200, and 400 kg m^{-3} tank volume. The best growth was found at the lowest and the least at the highest stocking density, while the young fish showed improved welfare at higher stocking densities and the old at lower stocking densities. The mean difference in weight gain for the whole grow-out phase between semi-intensively (100 kg m^{-3}) and super-intensively stocked fish (400 kg m^{-3}) at harvest was around 13 %. At the same time, up to an approximate weight of 1300 g, the fish showed no significant differences in weight gain between the groups. The result of Van de Nieuwegiessen et al. (2008, 2009) that younger fish (100 – 200 g) perform better at higher stocking densities matches our results in chapter 2. In contrast, Van de Nieuwegiessen et al. (2008, 2009) finding that the stocking density has no influence at higher weights contradicts the results in chapter 2 of this dissertation. Pasch (2022) also observed poorer growth at higher stocking densities, whereby it must be mentioned that the comparison of different stocking densities was conducted in two different systems.

Chapter 3 of this dissertation investigated the early life stages (approximately three weeks old) of African catfish. It showed they could be held at stocking densities of up to 30 fish L^{-1} without suffering. Thereby stocking densities of 10, 20, and 30 fish L^{-1} were compared.

In summary, if only considering a sustainable use of resources and fish welfare, super-intensive stocking densities (279 fish m^{-3}) are recommended for juveniles up to about 1300 g and semi-intensive stocking densities for older fish. In practice, this would mean that catfish should be size graded and divided at around 1300 g if they are slaughtered with 1500 g. Nonetheless, due to a farm's high investment and running costs, it is not profitable to produce African catfish in RASs at stocking densities below 450 kg m^{-3} (Pasch and Palm, 2021). Therefore it is advisable to slaughter the fish already with 1300 g as the growth reduction in larger fish would be too high. However, because high stocking densities can often abruptly lead to poor water quality if management is not adjusted, it would be necessary to regularly check water parameters and establish protocols for the frequency of water changes and

cleaning operations. That would significantly reduce the risk of sudden failures and counteract production losses due to staff changes. Juveniles (0.06 – 0.32 g) should generally be maintained at a stocking density of 30 fish L^{-1} to maximize production. In addition, regular monitoring should be conducted, and protocols should be established as these are very sensitive age stages, and unexpected losses may occur if countermeasures are not initiated at an early stage.

7.3 Reproduction, Seed Selection, and Grading Regimes

During the last few years, there has been a change of thinking among catfish farmers in N.G. Their goal is now to produce their own fingerlings to make their production independent of suppliers. The disadvantage is that a hatchery is only financially viable if 300 % of its seedling requirements are produced, and additional income is generated through the sale of the remainder (Pasch and Palm, 2021). In addition, separate spaces are needed to isolate the grow-out section from the reproduction and rearing section. That will prevent potential contamination and ensure that the broodstock, larvae, and juveniles have sufficient rest. Another problem is that there is no record of how broodstock selection should be best realized to avoid inbreeding and maintain desirable characteristics. The biggest problem is that if one does not want to buy expensive males from abroad, the own offspring has to be used, which sooner or later results in a certain degree of inbreeding. Furthermore, there are hardly any records on selecting and grading larvae and fry. The goal here is to eliminate as early as possible individuals with deformities, premature gonads, and poor growth. Larval weaning and switching to dry food present another special challenge. Normally, larvae are fed with artemia as live food (or their decapsulated cysts) for the first few days, and after that, they are gradually switched to dry food. However, starting external feeding directly with dry food is also possible. The first feed greatly influences development, growth (Onura et al., 2018), and survival rate (Verreth et al., 1987). Thereby it should be mentioned that feeding live Artemia leads to substantial additional work since these must be set daily to hatch.

Another important point is size grading or removal of shooters and potential cannibals to guarantee an ideal growth and a high survival rate. Up to now, commercial catfish farms have always worked with hand-grading sieves or, in the case of larger fish, by visual examination and manual division on a grading table. These work processes are very time and labor-intensive and require a certain amount of preparation and post-processing in addition

to the grading process itself. For example, the fish should not be fed directly before grading. Otherwise, they will regurgitate the food and cause poor water quality, which can lead to increased mortalities during a stressful situation such as grading.

Furthermore, first feeding after grading must be observed, as they often do not eat at all or only very tentatively at first. Besides the advantage of lower mortality rates due to the removal of cannibals, dividing fish into different size groups has the benefit that the pellet size can be better adjusted. However, the problem is that there is no information on how often and in what quantity splitting should ideally occur in catfish RAS during the various age stages. The fact is that the greatest mortality in African catfish's juvenile stages is normally due to cannibalism (Al-Hafedh and Ali, 2004; Marimuthu et al., 2011). Regular grading can elevate the survival rate 41 times compared to no grading in fish with an initial weight of 0.38 g (Abdelhamid et al., 2010). Moreover, the feeding rate influences survival, resulting in significantly less cannibalism when fish (0.64 – 65.4 g $fish^{-1}$) were fed 6 – 10 % of body weight per day instead of 2 – 4 % (Al-Hafedh and Ali, 2004). Adewolu et al. (2008) observed a reduction in mortality with decreasing light, resulting in a survival rate of 94 % under constant darkness and 81 % under constant illumination. At the same time, African catfish larvae (age: 4 – 25 days) exposed to salinities between 4 – 6 ppt showed less cannibalism compared to 0 – 3 and 7 ppt (Kawamura et al., 2017). Kaiser et al. (1995b) observed that stocking densities of 30 fish L^{-1} showed no differences in growth but different mortalities depending on the available bottom surface area, resulting in 66 % at 0.62 fish cm^{-2}, 54 % at 1.24 fish cm^{-2}, and 49 % at 0.3 fish cm^{-2}. On the other hand, Hossain et al. (1998) compared two stocking densities (5 and 10 fish L^{-1}, initial weight: 0.79 g $fish^{-1}$) under high/low light and sheltered/unsheltered conditions, resulting in a better specific growth of fish held at low stocking densities (5 fish L^{-1}), and low light and sheltered conditions (SGR = 8.1 % BW d^{-1}) compared to fish held at high stocking densities (10 fish L^{-1}), light and no shelter (5.9 % BW d^{-1}), while the survival rate was not affected. Regarding grading, Martins et al. (2005a, 2006b) showed that group composition when grading and dividing siblings does not influence growth but the welfare of African catfish. Separated groups with small fish showed higher aggression than heterogenous groups, while separated groups with big-sized fish showed the least aggression. At the same time, small fish did not grow faster when they were separated from the bigger ones. That indicates that the differences in growth are inherent and not due to social hierarchies (Martins et al., 2005b). Chapter 3 of this dissertation showed that

neither cannibalism nor mortality or growth of juvenile African catfish was influenced by stocking densities of 10, 20, and 30 fish L^{-1}, while grading reduced the mean mortality rate from 25 % to less than 5 % within ten days in fish with an initial weight between 0.06 – 0.08 g. The elevation of survival rates by grading is in accordance with the findings of Abdelhamid et al. (2010), Mollah et al. (1999), and Mwangi et al. (2020), who could reduce mortality rates by grading. In chapter 3, the grading strategy did not influence growth performance parameters but final stocking densities. The highest stocking density could be reached at the lowest handling stress (self-grading), while the lowest stocking density was reached in the group with the highest handling stress (manual-grading every second day). Haylor (1992) also observed a negative effect of handling stress resulting in higher mortality. The results from chapter 3, combined with the literature findings, confirm that cannibals must be removed regularly to ensure maximum production with minimum labor input.

For the best survival rates of 0.06 – 0.3 g fish, grading should be done every five days **(Wenzel et al., 2022**). Since it has been shown that self-grading is possible, a self-grading system should be developed, that is suitable for existing commercial systems. That would save working time and thus personnel. After the fish are brought into the grow-out systems with around 10 g, in commercial farms, they are only size graded once more with approximately 100 – 200 g to counteract a growing apart. Here, it would also be conceivable to work with self-grading systems. If it turns out that these methods can also be used for larger age stages, it makes sense to sort the fish before slaughter once more to remove individuals that are much too small in advance. This is normally not done by now as it is too much work to grade fish with this weight and size manually.

7.4 Water Quality and its Impact on Fish Performance

The fish, as an aquatic animal, is dependent on water. It is constantly exposed to it and cannot survive without it. That is also the reason why water properties play such an important role in successful aquaculture, especially in RAS, where the water exchange rate is very low. Substances in the water are in direct contact with the fish. Therefore, the skin (Glover et al., 2013) and gills (Hwang and Chou, 2013) as barriers have to regulate the homeostasis and the uptake and excretion of specific substances. In RAS, the water exchange is very low, and an adequate filter system primarily ensures the water quality. Thereby fish are usually kept at high stocking densities with a high feed input, which poses the risk of abrupt deterioration of

the water quality. For example, there may be a sharp increase in ammonium if the biofilter fails, or if the aeration is not working properly, a sudden drop in oxygen concentration may occur. Likewise, the pH can drop into the acidic range if the feed input is very high and no pH adjustment is carried out. However, a poorly adjusted pH leading to alkaline pH conditions combined with a poorly functioning biofilter can also be damaging.

Even if all RAS components are functioning well, a high accumulation of waste products in the process water has to be expected. That leads to high nutrient water concentrations, a great number of solids (**Strauch et al., 2018**), and the risk of excessive bacterial growth (Rojas-Tirado et al., 2018). As all of these points can harm the fish, which would result in a decrease in production, optimal water management is necessary to guarantee the best possible production. This water management should include daily control (and measures if necessary) of the fish, the water quality, and the aquaculture system. Thereby the function of the technical equipment for solids removal, nitrification, aeration, degassing, disinfection, and pH adjustment should be checked. Regarding the process water, typically high concentrations of dissolved nitrogen compounds excreted by the fish negatively influence feed uptake and growth (Roques et al., 2015; Schram et al., 2010, 2014), while high pH values, as mentioned before, lead to a high ratio of fish toxic NH_3 to the less toxic NH_4^+ (Lekang, 2019). At the same time, too high or too low water pH values and low oxygen concentrations impede proper biofilter functioning (Chen et al., 2006). In addition, the hatching rate and survival of young African catfish can be negatively affected if the pH is not appropriate (Marimuthu et al., 2019).

Regarding the larval stages of African catfish, the ideal pH value for hatching is between 6.7 and 7.6, and for post-hatch survival, between 6.5 and 7.5 (Marimuthu et al., 2019). At pH values > 10 and < 3.1, hatching is prevented, and at pH values > 9 and < 4.5, 100 % mortality has to be expected after 72 h at the latest. When the freshwater pH value is max. 8.5, a minimum hatching rate of 53 %, and a survival rate of 45 % can be expected without pH adjustment. In the worst case, this could lead to a total loss of 76 % if we assume that only 53 % of the eggs hatch and only 45 % of these eggs survive. In grow-out farms of African catfish, a drop in pH is a larger problem than an increase since both the feeding and breathing of the fish and the nitrification of the biofilter (Chen et al., 2006) result in an acidification of the process water. In the larval stage, however, this is usually not yet relevant since the systems for incubation and first feeding of the larvae have no biofilter, and water exchanges control the water quality. In addition, only very little feed is introduced in relation to the water

volume of the system. If the fish system is included in an aquaponic plant production system, not only the needs of the fish but also the requirements of the plants have to be considered. In coupled systems, it is usually impossible to create ideal conditions for both organisms, but a compromise can be found so that fish and plants grow well. For example, potassium and iron are usually highly deficient in African catfish RAS process waters in relation to plant requirements **(Strauch et al., 2018)**. Moreover, phosphate may be deficient and can be added to the process water of the fish without harming them up to a concentration of 80 mg L^{-1} (Strauch et al., 2019). However, higher concentrations have negative effects on the welfare of the fish.

Chapter 4 of this dissertation showed that very high water potassium concentrations (600 mg L^{-1}) could also negatively affect catfish. Medium concentrations between 200 and 400 mg L^{-1} K^+ positively affect fish welfare, which is expressed in a lower number of biting wounds and fighting events. Even if there was no significant difference in growth performance and final body composition, slightly better growth performance parameters were observed at medium concentrations. Since K^+ concentrations between 200 and 400 mg L^{-1} cover the recommended range for hydroponic nutrient solutions (Al Meselmani, 2022), it is advisable to adjust the pH with KOH in a commercial African catfish RAS if the water is used for aquaponic plant production. Hereby water K^+ concentrations above 400 mg L^{-1} have to be avoided. This results in several advantages. First, the potassium concentration can be optimized regarding aggression and biting wounds of the fish, resulting in better health and welfare status. Second, the pH can be adjusted to ensure the proper functioning of the biofilter and fish health without introducing additional possibly harmful nutrients for fishes or plants, and third, the potassium concentration in the process water can be optimized for plant production. Hereby not only a sustainable use of resources and an additional income for the companies can be achieved, but also an improvement of the welfare of the fish.

7.5 Feed Additives in African catfish Aquaculture

In RAS, feed is the main route of entry besides the water. It introduces nutrients but also pollutants and solid waste. Its quality determines not only fish growth but also water quality. Important parameters for the pellets (besides nutritive properties) are water stability, solidity, uniform size, and high digestibility, which results in low fecal matter production (Alltech Coppens, 2020). Therefore, feed manufacturers are constantly working to improve suitable

RAS feeds. The development of new feed is expensive, but it can result in less pollution of the water, which can lead to higher and more reliable production. Besides plain nutritional ingredients, feed additives are often added. Feed additives are not only used to improve the performance and health of the animals but also to manipulate feed quality, improve nutrient availability, or break down antinutrients (Encarnação, 2016). Increasing the production volume is one of the most relevant parameters for a company, so much effort has been made to find feed additives that support growth. For example, Thangapandiyan and Monika (2020) could improve the feed conversion ratio of *Labeo rohita* by more than 50 % and improve growth by adding zinc oxide nanoparticles to the feed. There are also different commercially available ready-mixed feed additives that can be added to the basic feed and positively influence fish growth (Dada, 2015a). Tawfik et al. (2017) could even quadruplicate the specific growth rate in *Oreochromis niloticus* by adding nano zinc oxide to the fish feed. *Quillaja saponaria* and linseed oil can also be used as feed additives, improving not only the growth but also the welfare profile of *Oreochromis niloticus* (Elkaradawy et al., 2022). Essential oil from *Aloysia triphylla* improves the growth of silver catfish, *Rhamdia quelen* (Zeppenfeld et al., 2016), as well as butyrate in *Sparus aurata*, which is probably due to better availability of nucleotide derivates and essential amino acids (Robles et al., 2013). Many feed additives that positively influence growth affect digestive function by improving the gut microbiota (Giannenas et al., 2012; Rimoldi et al., 2020; Zhou et al., 2013).

In African catfish, medicinal plants can be used as feed additives. *Allium sativum* addition, for example, can improve the specific growth rate by 0.04 % BW d^{-1}, and *Chromolaena odorata* addition can increase the survival rate of the African catfish after a *Pseudomonas aeruginosa* infection by 60 % (Tiamiyu et al., 2021). Adding *Telfairia occidentalis* to the feed can improve growth and feed utilization (Dada, 2015b). Dietary supplementation with L-carnitine suggests lowering the amino acid combustion for energy (Ozorio et al., 2002).

Clay minerals can also be used as feed additives and are typically known for their high surface area, great adsorption capacity, and not being toxic to animals (Nadziakiewicza et al., 2019; Venkateswerlu and Stotzky, 1992). Due to these properties, they can be used to detoxify the body and relieve gastrointestinal infections (Slamova et al., 2011). As feed additives, they bind unpleasant ingredients like mycotoxins and promote animal health and growth (Slamova et al., 2011; Vekiru et al., 2007). In the production of livestock, these toxins not only harm the animals themselves but can also lead to unwanted substances in the animal product, such as

milk, eggs, or meat (Fink-Gremmels and Van der Merwe, 2019; Yiannikouris and Jouany, 2002). In African catfish, the clay mineral bentonite can improve water quality, digestibility, and growth when added as a feed additive (Ayoola, 2016). To evaluate the effect of the regionally mined mixed clay mineral Montmorillonite-illite/Muscovite (1g557), the experiment presented in chapter 5 of this dissertation was conducted. This clay mineral has already been tested as a feed additive for shrimps by Palm et al. (2015), who observed a positive effect on the survival, total weight gain, and size distribution of *Litopenaeus vannamei*. The results of chapter 5 of this dissertation showed that adding 0,5 % of Montmorillonite-illite/Muscovite (1g557) to the basic food can positively influence the growth ($p > 0.05$) and welfare ($p < 0.05$) of African catfish. The study demonstrated a 2 % higher final weight (mean) and fewer biting wounds compared to the control group. Assuming an annual production of 300 tons, an improvement of 2 % would result in 6 tons more production volume per year. Farmers should use Montmorillonite-illite/Muscovite (1g557) as a feed additive in African catfish RAS in N.G. to improve welfare and production.

However, since only a limited number of feed manufacturers offer special food for African catfish RAS, catfish farmers are very dependent on these companies. Therefore, to implement this feed additive, feed manufacturers must first be convinced that this substance has a real added value. To accomplish this, future research regarding the use of it in RAS should focus on its influence on water quality.

7.6 African catfish in Aquaponics Systems

In aquaponic production systems, it is possible to save resources by using the nutrients that accumulate in the process water of the fish instead of commercial artificial fertilizers and using the water itself instead of fresh water. Additionally, aquaponic systems can lower environmental pollution in terms of nutrient discharge, CO_2 emission, and energy consumption through regional plant production and distribution. **Strauch et al. (2018)** showed that the process water of intensive African catfish RAS is nutrient rich. However, according to the summary of Trejo-Téllez and Gómez-Merino (2012), the nutrient profile is not ideal for hydroponic plant production. Knaus et al. (2020a) and Pasch et al. (2021a, 2021b) showed that basil can be cultivated with process water from intensive African catfish RAS. Moreover, Baßmann *et al*. (2017, 2020) showed that cultivating fish and plants in a coupled aquaponic system positively affects fish welfare.

There are several problems in building an economically successful aquaponic farm. First, knowledge of both production sectors requires different skills, which operators often do not have. Second, building such a system is associated with high investment costs, and third the uncertainty of starting in a new business sector and the associated uncertainty of profit (Pasch and Palm, 2021). Despite all these obstacles, combining the production of fish and plants is an ecologically desirable industry once initial obstacles are overcome for future-oriented, long-term sustainable food production. Depending on the plant, the success of the plant production can differ a lot. Therefore, it is important to choose the right species. Plants like tomatoes or cucumbers are heavy feeders. They need more nutrients than herbs, such as mint or basil, which are light feeders (Thurston County, Washington State Department of Ecology, 2010). Light feeders are relatively easy to grow because no additional fertilization or only a small amount is needed in aquaponic production systems, where some nutrients are rather low compared to plant requirements (**Strauch et al., 2018**). A constant monitoring of the nutrient profile of the process water is essential if demanding plants with higher nutrient requirements are chosen for production. The goal is to optimize the process water to be able to produce plants that are competitive with plants produced in traditional production systems. This procedure requires excellent know-how in plant production as well as in fish biology.

Chapter 6 of this dissertation showed that spearmint (*Mentha spicata*), a relatively undemanding plant, can be produced successfully with the process water from an intensively stocked African catfish RAS, even without nutrient supplementation. As the experiment was conducted in a decoupled system with no contact between fishes and plants, fish growth and survival were not influenced and comparable with values from sole fish production (Palm et al., 2018b; **Strauch et al., 2018**). Aquaponic systems allow RAS operators to create a second regional source of income, benefiting from more sustainable overall production and an improved environmental footprint.

In the future, the sustainable production of food will become increasingly important. Due to the large amount of nutrient-rich water that is produced in RAS fish farming, aquaponic plant production is a promising method. Plants can be produced as an additional source of revenue while reducing nutrient discharge to the environment without restricting fish growth. In the longer term, practical methods for combining fish care (e.g., pH adjustment) and the optimization of the process water for the cultivation of special plants should be developed. Hereby, the processes must be developed as simply as possible so they can be implemented

reliably in commercial facilities. With the growing world population, ever-scarcer resources, and eutrophication, aquaponic seems to be a promising production branch. Overall, it is desirable to integrate and expand this sustainable branch of production in African catfish RAS in N.G., whereby appropriate marketing will be necessary to market the plants economically.

7.7 Model Calculations for Production Success under Ideal and Poor Production Conditions

The parameters discussed above show that the yield of a commercial African catfish farm strongly depends on the production conditions of the fish. The following calculations were made to determine the survival rate and growth during a production cycle under ideal and poor husbandry conditions to illustrate the influence of the different parameters. Both literature values and values from the results presented in this dissertation were used for the calculations. No worst-case situations were considered for poor conditions, as this would lead to 100 % mortality, representing a state of emergency and not permanently poor husbandry conditions. The model calculations covered the period from hatching (in case of the survival rate) / first feed (in case of growth) to a slaughter size of approximately 1400 g, while the hatching rate is not considered in the survival rates. Alltech Coppens is the most fed catfish feed in commercial RAS in N.G. Their feeding tables (including growth) for the grow-out could be confirmed by studies at the University of Rostock under commercial conditions **(Baßmann et al., 2023**). For this reason, and as we do not have exact values for all age stages, the grow-out values in Coppens' feeding tables were equated with ideal growth in all following calculations.

7.7.1 Survival Rate

Production can be divided into two phases in commercial African catfish RAS in N.G. Firstly, into the production of juveniles to a size of about 10 g in the hatcheries and secondly, the fattening to about 1200 – 1500 g in grow-out facilities. Since the data that was used to calculate survival rates overlap these two production phases, they are not considered separately in the following but divided into the periods a (hatch – first feed), b (first feed – approximately 0.7 g), c (0.7 g – stocking size), and d (stocking size – slaughter size) (for the calculations see Table 7.1). The time data of the feeding table from Alltech Coppens (2021) were used to determine the duration of growth in the different time periods in Table 7.1. It is assumed that the fish remain in the hatchery for 56 days from first feeding until reaching 10 g,

followed by another 144 days in the grow-out facility until they reach 1400 g. That corresponds to the procedures at commercial farms.

Table 7.1: *Assumed natural mortalities at different time periods during the grow-out of African catfish.*

	Average natural mortality at the different stages			
Production		Hatchery		Grow-out
Time period (t)	**a**	**b**	**c**	**d**
References	Nguyen and Janssen, 2002	**Wenzel et al., 2022** (chapter 3)	Al-Hafedh and Ali, 2004; Mwangi et al., 2020	Palm et al., 2018b **Baßmann et al., 2023**
Assumed age range	From hatch until the first feed	First feed until approximately 0.7 g	0.7 g until stocking size	During grow out to 1.4 kg
Natural mortality (M_n) in %	7.94*	9.09**	9.00***	9.70****
Natural survival rate (SR_n)	***0.92***[b]	***0.91***[b]	***0.91***[b]	***0.90***[b]
Duration of growth (d)[a]:	3	21	35	144

SR_n =(100%–M_n)/100 where SR_n = natural survival rate and M_n = natural mortality in %, exact calculations for M_n: see Tables 15.1-15.4.

* Mean value from the five control values in Nguyen and Janssen (2002). ** Assuming Experiment 2 in Chapter 3 (**Wenzel et al., 2023**)(daily grading) shows the natural mortality within 10 days (= 4.13 %). That 10-day mortality was then extrapolated for 22 days (Table 15.2a). *** Mean value from the non-cannibalistic mortality at 6, 8 and 10 % feeding rates in Al-Hafedh and Ali (2004) and non-cannibalistic mortality rates in Mwangi et al. (2020). **** Mean value from the values of the different stocking densities in **Baßmann et al. (2023)** and staggered production 1 – 3 in Palm et al. (2018b).

[a] Alltech Coppens, 2021.

[b] ***Values used for survival rate calculations under ideal and poor husbandry conditions in Table*** 7.7***.***

The following parameters were taken into account when calculating the survival rates under ideal and poor production conditions (Table 7.7): 1. The naturally occurring mortalities in the time periods a – d (Table 7.1), 2. the type of first feed (Table 7.2), 3. the water pH value at different age stages (Table 7.3), 4. the temperature (Table 7.5), 5. the amount of feed at young age stages (Table 7.4), and 6. size grading of young fish (Table 7.6). Most parameters refer to young age stages of fish as they are much more susceptible and because there are only a few studies on threshold values in older fish. In addition to the parameters mentioned above, survival rates can differ by over 40 % depending on genetics (Opiyo et al., 2017). Moreover, salinities above 7 ‰ can increase the mortality in larvae (Gbulubo and Erondu, 1998). However, this is not considered in the calculations since the parents determine the

genetic prerequisites and can usually not be influenced during grow-out. In addition, salinities of 7 ‰ are normally not reached in fresh water.

Parameter 1, which describes the natural mortalities that also occur under ideal husbandry conditions (for example, due to genetics), is the only parameter that leads to mortalities in the calculation for ideal production conditions. For parameters 2, 3, 4, and 5, the effective survival rates (= survival rates only influenced by one parameter, excluding natural mortalities and mortalities due to general husbandry conditions) were calculated. For calculating the effective survival rates, it is assumed that within a study, the highest survival rate that depends on a parameter represents the ideal parameter value. That means that this parameter value does not cause mortalities and that those that do occur are natural mortalities or mortalities caused by general husbandry conditions to which all individuals in a study are exposed. It is assumed that these parameter-independent mortalities have the same influence on all survival rates within a study. Therefore, these parameter-independent mortality rates are subtracted from the measured survival rates to determine the effective survival rates. For example, for calculating the survival rate in dependence on the pH-value (parameter 3; Table 7.3), this means that for 500 g fish, a pH-value of 8.01 is considered ideal, as the survival rate is highest at 98 %. It is assumed that the 2 % mortality is not related to the pH but to natural mortality or mortalities caused by general husbandry conditions that occur regardless of the investigated parameter. Therefore, the 98 % is normalized to a 100 % survival rate (by the sole influence of the pH) in the calculation. The other survival rates were increased by 2 % in each case, as it is assumed that 2 % of the mortality is unrelated to the pH but is due to mortalities caused by other factors. Against this background, a proportional effective survival rate (K) that was only influenced by a certain parameter (2, 3, 4, or 5) was determined and used for further calculations. K was calculated according to the formula [7.1]. The calculated values are listed in Table 7.2 – 7.6.

$$K_x = \frac{100\% - SR_{x\,max} + SR_x}{100} \qquad [7.1]$$

With x: index for one of the parameters 2 (type of first feed), 3 (pH), 4 (temperature), or 5 (amount of feed at young age stages). K: proportional effective survival rate depending exclusively on a parameter x and excluding mortalities caused by other factors, $SR_{x\,max}$: maximum survival rate in relation to the investigated parameter x, SR_x: the survival rate for a tested value of a parameter x.

For a better understanding, K_{pH} is calculated in [7.2] and [7.3] as an example of the parameter pH. In [7.2], it is shown how K_{pH} is determined for the survival rate at an ideal pH (= 8.01) value and in [7.3] for the survival rate (SR) at pH 6 (in 500 g fish). These values as well as the other calculated K_{pH} values, can be taken from Table 7.3.

$$K_{pH(8.01)} = \frac{100\% - SR_{x\,max} + SR_x}{100} = \frac{100\% - 98\% + 98\%}{100} = 1 \qquad [7.2]$$

With SR_x = survival rate at ideal pH of 8.01 = $SR_{x\,max}$ = 98 %.

$$K_{pH(6)} = \frac{100\% - SR_{x\,max} + SR_x}{100} = \frac{100\% - 98\% + 96\%}{100} = 0.98 \qquad [7.3]$$

With SR_x = survival rate at pH 6 = 96 % and $SR_{x\,max}$ = SR at ideal pH of 8.01 = 98 %.

Grading of fish is done to prevent cannibalism. In order to calculate the proportional effective survival rate that is only influenced by size grading (K_G) excluding natural mortality (Table 7.6), the naturally occurring mortalities (Table 7.1) at the different ages were subtracted from the literature values for survival rates during grading. This was done because not every grading study graded in intervals where all cannibalism could be excluded (meaning that the best values do not necessarily mean cannibalism can be excluded), and mortality rates did not distinguish between death from cannibalism and from other causes. For a better understanding K_G for 0.06 – 0.16 g fish (Table 7.6) is calculated in [7.4] for the grading interval "Every 2nd day".

$$K_G = \frac{SR_G + M_n}{100\%} = \frac{88.34\% + 9.09\%}{100\%} = 0.97\% \qquad [7.4]$$

With SR_G: survival rate for a certain grading interval (here "Every 2nd day") and M_n: natural mortality (see Table 7.1).

Table 7.2: *Survival rate in dependence of the type of first feed (SR_{FF}).*

Duration of feeding in days:	**2d**	**3d**	**5d**	**6d**	**7d**	**14d**	**mean**
	Survival rate (SR_{FF}) in %						
Type of first feed (FF)							
Hatched Artemia Nauplii				91[c]	90[a]		90.5
Decapsulated Artemia Cysts (Survival Rate after 10d)	95.6[e]	90.7[e]	95.6[e]	98[c]	84.3**	88.4[f]	92.1
Decapsulated Artemia Cysts + Commercial Starter Diet						77.5[b]	77.5
Commercial Starter Diet					65.45*	66.2[b]	65.8
Live Daphnia					77.2[d]		77.2
Moina Dubia					92[a]		92.0
Moina + Commercial Starter Diet						69.6[b]	69.6
Mixed Zooplankton					89[a]		89.0
Type of first feed	**Calculated mean proportional effective SR only influenced by the type of first feed (K_{FF}) [+]**						
Hatched Artemia Nauplii	0.98						
Decapsulated Artemia Cysts (Survival Rate after 10d)	***1.00*** [g]						
Decapsulated Artemia Cysts + Commercial Starter Diet	0.85						
Commercial Starter Diet	***0.74*** [g]						
Live Daphnia	0.85						
Moina Dubia	1.00						
Moina + Commercial Starter Diet	0.78						
Mixed Zooplankton	0.97						

References: [a] Adeyemo et al. (1994), [b] Mean values from the single values of Aruho et al. (2017),[c] Bardocz et al. (1999), [d] Olurin and Oluwo (2010), [e] Verreth and Van Tongeren (1989), [f] Mean value from the single values from Verreth and Bieman (1987), [g] ***Values used for the survival rate calculations under ideal and poor husbandry conditions in*** **Table** 7.7***.***

* Mean value from the single values of the references [a] and [d]. ** Mean value from the single values of the references [d] and [e]. [+] For exact calculations of $K_{FF,}$ see Appendix Table 15.5.

Table 7.3: *Survival rate in dependence of the water pH at different life stages (SR_{pH}).*

Initial fish weight in g:	**3.6**	**100**	**500**
Tested pH-value	**Survival rate* (SR_{pH}) in %**		
3	0	0	0
4	20	38	64
5	70	74	88
6	88	90	96
8.01	90	94	98
	Calculated proportional effective survival rate only influenced by the water pH (K_{pH})⁺		
Tested pH-value			
3**	0.00	0.00	0.00
4	0.30	0.44	***0.66[a]***
5	0.80	0.80	0.90
6	0.98	0.96	0.98
8.01	1.00	1.00	***1.00[a]***

***[a]: Values used for the survival rate calculations under ideal and poor husbandry conditions in* Table** 7.7**.**
*: Mustapha and Zainab (2018) as reference for all survival rates, **: assuming that if the SR=0, the result of a lethal pH is independent of natural mortality, ⁺: exact calculations of K_{pH} see Appendix Table 15.6,

Table 7.4: *Survival rate in dependence on the percentage of feed ($SR_{\%F}$) input.*

	Fish size: 0.64 – 65.4 g	
Feed amount in % of body weight per day	**Survival rate* ($SR_{\%F}$) in %**	**Calculated proportional effective survival rate only influenced by the feed amount ($K_{\%F}$)⁺**
0	0	0.00**
2	30	0.52
4	32	0.54
6	78	***1.00[a]***
8	69	0.91
10	67	***0.89[a]***

***[a] Values used for the survival rate calculations under ideal and poor husbandry conditions in* Table** 7.7**.**
* Al-Hafedh and Ali (2004) as reference for all survival rates, ** assuming that if the SR = 0, the result of a lethal temperature is independent of natural mortality, ⁺ exact calculations of $K_{\%F}$ see Appendix Table 15.8

Table 7.5: *Survival rate in dependence of the water temperature and age (SR_T).*

Age / Size:	**Embryo * [a]**	**Larvae ** [a]**	**3 – 20 g [b]**	**Embryo**	**Larvae**	**3 – 20 g**
Temperature in °C	**Survival rate (SR_T) in %**			**Calculated proportional effective survival rate only influenced by water temperature (K_T)[+]**		
17.4	0	0		0.00***	0.00***	
18.9	29	0		0.35	0.00***	
20.6	57.5	15		0.64	0.59	
21.5	92			0.98		
22 (24L : 0D)			40			0.57
22 (12L : 12D)			53			0.70
22 (0L : 24D)			58			***0.75*** [c]
23	72.5	54		0.79	0.98	
23.2		39			0.83	
24	94			1.00		
25.2		56			1.00	
25.4		38			0.82	
25.5	57			0.63		
27.2	39.5	42.5		0.46	0.87	
27.3	90	35		0.96	0.79	
28 (24L : 0D)			57			0.74
28 (12L : 12D)			72			0.89
28 (0L : 24D)			83			***1.00*** [c]
28.6	37.5			0.44		
29.3	35	35		0.41	0.79	
30.3	55.5	52.5		0.62	0.97	
31.6	16.75			0.23		
33.2	14.5	5.75		0.21	0.50	
33.5	8			0.14		
35.2	0			0.00***		
35.6	0	0		0.00***	0.00***	

[a] Prokešová et al. (2015) as reference for the survival rates for embryos and larvae, [b] Orina et al. (2016) as reference for the survival rates for 3 – 20 g fish, [c] ***Values used for the survival rate calculations under ideal and poor husbandry conditions in Table 7.7.*** 0L, 12L, 24L = 0h light, 12h light, 24h light; 0D, 12D, 24D = 0h darkness, 12h darkness, 24h darkness.

* embryo = from fertilization until 50 % of the batch are hatched, ** larvae = from 50 % hatch until 50 % have absorbed their yolk sac, *** assuming that if the SR = 0, the result of a lethal temperature is independent of natural mortality, [+] exact calculations of K_T see Appendix Table 15.7.

Table 7.6: *Survival rate in dependence of grading and fish weight (SR_G).*

	Fish weight in g (experimental period in days)					
	0.06–0.16 (10)	**0.08–0.32 (10)**	**0.38–0.9/3* (28)**	**0.69–36.3 (60)**	**0.89–42.5 (60)**	**0.92–51.7 (60)**
Time space (t):	**b**		**c**			
Time range used for calculations:	First 22 hatchery days after the start of exogenous feeding		Assumed as last 35 days in the hatchery			
Grading intervals	**Survival rate (SR_G) in %**					
No grading		45.00** [a]	1.2 [b]	43.2 [c]		
Every day	90.98** [a]					
Every 2nd day	88.34** [a]					
Every 3rd day			49.17 [b]			
Every 5th day	90.54 ** [a]					
Every 7th day			32.33 [b]			
Every 2 weeks						81.6 [c]
Every 4 weeks					64.4 [c]	
	Calculated proportional effective SR (K_G) only influenced by grading ($K_G = (SR_G + M_n)/100$) [+]					
No grading		***0.54*** [d]	0.10	0.52		
Every day	***1.00*** [d]					
Every 2nd day	0.97					
Every 3rd day			0.58			
Every 5th day	1.00					
Every 7th day			0.41			
Every 2 weeks						0.91
Every 4 weeks					0.73	

References: [a] **Wenzel et al. (2022)**, [b] Abdelhamid et al. (2010), [c] Mwangi et al. (2020), ***[d]: Values used for the survival rate calculations under ideal and poor husbandry conditions in* Table 7.7.** M_n: natural mortality,
* ungraded fish showed the higher weight, ** extrapolated for 22d (Appendix Table 15.2b), [+] exact calculations of K_G see Appendix Table 15.9.

Final survival rates after a whole production cycle were calculated based on the proportional effective survival rates. This was done for ideal and poor production conditions by using the formula [7.4]. Regarding the first feed, in the best case, artemia cysts were fed and, under poor conditions, dry feed (Table 7.2). In terms of pH, it was assumed that at best, it was 8.01 for all ages (ammonium concentrations were not considered in this assessment), and at worst, it dropped to 4 for 500 g fish (Table 7.3), which can happen quickly in a farm with a high feed input. Regarding temperature, for fish between 3 and 20 g, 28 °C was assumed for ideal production conditions and 22 °C and darkness for poor conditions (Table 7.5). Regarding the daily feed amount, it was assumed that ideally, 6 % of body weight was fed per day and, under poor conditions, 10 % (Table 7.4), as overfeeding possibly occurs in grow-out farms

while underfeeding is unlikely. In terms of grading, it was assumed that in the best case, grading was performed at least every 5 days and under poor conditions not at all (Table 7.6).

$$SR_{final} = SR_n(a) \times SR_n(b) \times SR_n(c) \times SR_n(d) \times K_{FF} \times K_{pH} \times K_T \times K_{\%F} \times K_G \quad [7.4]$$

With SR_{final}: Survival rate until slaughter size, SR_n(a), (b), (c), (d): Natural survival rates at the time spaces a, b, c, and d (Table 7.1), K_x: Calculated theoretical survival rates only influenced by a special parameter x (see Table 7.2 – Table 7.6), possible parameters for x: FF = first feed, pH = pH-value, T = Temperature, %F = feed amount in % of body weight per day, G = grading

Table 7.7 shows the single values and the results of the calculations for the survival rates under ideal and poor production conditions. Under ideal conditions, only naturally occurring mortalities reduced the survival rate, resulting in a 69 % survival rate from hatch to slaughter size. In contrast, with poor management, survival rates can drop to 12 %, representing a further loss of 57 % of the original number of fish. Assuming a slaughter weight of 1.4 kg per fish and that a company can produce 300 000 hatchlings per year, under ideal conditions, it can produce 289.8 tons of fish per year, and under poor production conditions, only 50.4 tons per year (calculations see Table 7.8). These values show the extent of the influence of RAS management on the survival rate and, thus, on yield and profit.

Table 7.7: *Survival rate calculations under ideal and poor production conditions.*

Husbandry conditions	SR_n(a)	SR_n(b)	SR_n(c)	SR_n(d)	K_{FF}	K_{pH}	K_T	$K_{\%F}$	K_G	SR_{final}
"Ideal"	***0.92***	***0.91***	***0.91***	***0.90***	***1.00***	***1.00***	***1.00***	***1.00***	***1.00***	0.69
"Poor"	***0.92***	***0.91***	***0.91***	***0.90***	***0.74***	***0.66***	***0.75***	***0.89***	***0.54***	0.12

SR: survival rate; a, b, c, d: time spaces a, b, c and d as defined in Table 7.1; n, FF, pH, T, %F, G: indexes for natural mortality, first feed, water pH, temperature, percentage of given feed and grading; SR_{final}: survival rates until slaughter size in case of ideal and poor production conditions. ***The values used for the calculations under ideal and poor husbandry conditions are highlighted in italics and bold in Table 7.1 – 7.7***.

Table 7.8: *Example calculations for the survival rates under ideal and poor production conditions based on 300 000 fish larvae.*

	Ideal production conditions	**Poor production conditions**
Initial number of larvae (n_{Ini})	300 000	300 000
Survival rate (SR)	0.69	0.12
Final fish weight in kg (W_{fin})	1.4	1.4
Final fish biomass in kg ($m_{fin}=n_{Ini}\times SR\times W_{fin}$)	289 800	50 400
Final fish biomass in tons	289.8	50.4

7.7.2 Growth Rate

In order to assess the growth under ideal and poor production conditions in African catfish RAS in N.G., the parameters first feed (Table 7.9), stocking density (Table 7.10), water temperature (Table 7.11), ammonium (Table 7.12) and nitrite (Table 7.13) concentrations were considered. Since catfish feed from Alltech Coppens is commonly fed in the farms of N.G., their growth data (Alltech Coppens, 2021) was equated with ideal growth in the following calculations. Using the data published by Alltech Coppens, the "ideal" specific growth rates (SGR) were calculated for each production day from the first feed to a slaughter size of 1400 g (daily calculations, see Appendix Table 15.10). To calculate the daily final fish weight (W_{final}) as a function of the SGR (growth under ideal or poor production conditions), formula [7.6] was solved for the final weight (W_{final}) and converted to the formula [7.7]. Thus, growth can be determined by calculating the daily final weight for any given SGR. This was done because growth in poor holding conditions was determined under a negative influence of the above-mentioned parameters by adjusting the SGRs and inserting them into the formula [7.7]. For the calculations under poor holding conditions, it was further assumed that the maximum possible daily SGR was age-dependent and not size dependent.

$$SGR=\frac{lnW_{final}-lnW_{initial}}{t}\times 100\% \quad [7.6]$$

$$W_{final}=e^{\frac{SGR}{100\%}\times t+lnW_{initial}} \quad [7.7]$$

With W_{final}: final fish weight, SGR: specific growth rate, t: time as days of feeding, and $W_{initial}$: initial fish weight.

In order to determine growth under poor husbandry conditions, SGR values from literature and own studies were selected (see Table 7.9 –7.13). These SGR values were not directly used in the formula [7.7] because different feed, the feed quantity, and the type of system also strongly influence growth, independent of the studied parameters. Therefore, an attempt was made to develop a measure to determine the influence of one parameter. That was done by calculating the proportional decline of an SGR, depending on one parameter within a study. For this purpose, a factor Y was calculated (see Tables 7.9 – 7.13), which, when multiplied by the maximum SGR of a study, allows the calculation of the SGR influenced by a certain parameter x (x = first feed, stocking density, water temperature, ammonium, or nitrite concentration). Formula [7.8] shows the relationship between the SGRs and factor Y, and formula [7.9] shows how Y is calculated.

$$SGR_x = SGR_{max} \times Y \quad [7.8]$$

$$Y = \frac{SGR_x}{SGR_{max}} \quad [7.9]$$

With SGR_x: the SGR under the influence of a certain parameter x (first feed, stocking density, water temperature, ammonium, or nitrite concentration) within a study, SGR_{max}: the ideal SGR value within the study, assuming that it reflects an ideal growth under the given circumstances (such as feed, feed quantity, system design), Y: factor to calculate the deterioration of an SGR depending on one of the parameters.

That means, for example, that the literature values in Table 7.9 for SGRs depending on the first feed for days 3 to 8 show an SGR of 33 when feeding artemia and 32 when feeding dry feed. Here it is assumed that 33 is the ideal value under the given circumstances in this study and 32 is due to dry feed administration. This results in a factor Y = 32/33 = 0.97 (more example calculations for the parameter first feed can be found in Appendix Table 15.11). This factor can now be multiplied by the ideal value of Alltech Coppens in this period (days 3 to 8 of feeding) to obtain a corrected SGR value for calculating growth when giving dry feed on days 3 to 8 under the conditions in an African catfish RAS in N.G.

For evaluating growth in relation to holding conditions, the duration of a whole growth cycle from the first feeding to an approximate slaughter weight of 1400 g under ideal Alltech Coppens conditions (= ideal SGR values) and poor production conditions was assessed. According to Alltech Coppens, a weight of 1400 g is reached within 200 days after the onset of

feeding (exact calculations for daily growth according to formula (D) see Appendix Table 15.10). For daily growth calculations under poor husbandry conditions, the given ideal SGRs were corrected by multiplying them with the influencing Y factors (for exact daily calculations, see Appendix Table 15.12).

Under poor production conditions, it was assumed that the first feeding (day 1 to 7) of the fish was conducted with dry starter feed while no artemia were given. From day 8 onwards, it was supposed that the feed was fed according to the specifications of the feeding table (Alltech Coppens, 2021), and therefore, no further growth deficits caused by the parameter "feed" were considered. Moreover, it was assumed that commercial producers always use the highest stocking densities, which is why the SGRs were adjusted for the weight ranges in Table 7.10, if necessary. In case of overlapping periods, the mean value of both Y-factors was used to adjust the SGR. In addition, a two-day drop in temperature to 22 °C (days 73 – 74) was considered, as well as a one-day ammonia increase up to 3 mg L^{-1} (day 101) and a 3-day nitrite increase up to 12.88 mg L^{-1} (days 111 to 113). For exact daily calculations and the use of the factors from Table 7.9 to Table 7.13, see Appendix 15.12. The values in Table 7.9 to Table 7.13 used for the calculations in Table 15.12 are highlighted in italics and bold.

Table 7.9: *Specific growth rates depending on the first weeks of feeding.*

	SGRs in % BW d^{-1}						
	Feeding days						
Feed	1–7[a]	3–8[b]	9–11[b]	12–14[b]	15–17[b]	18–21[b]	22–24[b]
Artemia Nauplii	2.9	33	27	20	15	6	4
Starter dry feed	2.6	32	13	32	30	15	16
	$Y = SGR_x / SGR_{max}$						
	Feeding days						
Feed	1–7[a]	3–8[b]	9–11[b]	12–14[b]	15–17[b]	18–21[b]	22–24[b]
Artemia Nauplii	***1.00***[c]	***1.00***[c]	1.00	0.63	0.50	0.40	0.25
Starter dry feed	***0.90***[c]	***0.97***[c]	0.48	1.00	1.00	1.00	1.00

References for the respective SGRs: [a] Adeyemo et al. (1994), [b] Appelbaum and Mc Geer (1998).

[c] ***Factors used for the specific growth rate calculations under ideal and poor holding conditions.*** SGR: specific growth rate. SGR_{max}: maximum SGR within a study. SGR_x: SGR for a special feed within a study. Y: Factor to calculate the deviation from the optimal SGR.

Table 7.10: *Specific growth rates depending on the stocking density.*

	SGR in % BW per day					
Approx. weight range in g:	0.06–0.17[a]	12–23[b]	20–270[b]	250–700[b]	640–1400[b]	1100–1900[b]
Final stocking densities in kg m^{-3}:						
1.6	10.2					
2		7				
3.3	10.3					
4		7.8				
5.0	10.4					
7		6.8				
24			6			
44			5.8			
61				2.2		
88			6			
119				2.4		
123					1.7	
168						0.9
190					1.4	
225				2.3		
330						1.2
446					1.6	
570						0.7
	Y = SGR_x / SGR_{max}					
Appr. weight range in g:	0.06–0.17	12–23	20–270	250–700	640–1400	1100–1900
Final stocking densities in kg m^{-3}:						
1.6	0.98					
2		0.90				
3.3	0.99					
4		***1.00***[c]				
5.0	***1.00***[c]					
7		***0.87***[c]				
24			1.00			
44			0.97			
61				0.92		
88			***1.00***[c]			
119				***1.00***[c]		
123					***1.00***[c]	
168						0.75
190					0.82	
225				***0.96***[c]		
330						***1.00***[c]
446					***0.94***[c]	
570						***0.58***[c]

References for the respective SGRs: [a] **Wenzel et al. (2022)** (chapter 3), [b] **Baßmann et al., 2023** (chapter 2).
[c]***: Factors used for the specific growth rate calculations under ideal and poor holding conditions.*** SGR: specific growth rate, SGR_{max}: maximum SGR within a study, SGR_x: SGR for a special stocking density within a study. Y: Factor to calculate the deviation from the optimal SGR.

Table 7.11: *Specific growth rates depending on temperature and illumination.*

	SGR in % BW per day[a] (fish size: approx. 3 – 30 g)	
Temperature: Illumination	22°C	28°C
24L: 0D	4.57	5.14
12L: 12D	5.51	6.23
0L: 24D	6.56	7.56
	$Y = SGR_x / SGR_{max}$	
Temperature: Illumination	22°C	28°C
24L: 0D	0.60	0.68
12L: 12D	0.73	0.82
0L: 24D	***0.87*** [b]	***1.00*** [b]

[a] Orina et al. (2016), as reference for all SGRs. [b]***: Factors used for the specific growth rate calculations under ideal and poor holding conditions.*** SGR: specific growth rate. SGR_{max}: maximum SGR within a study, SGR_x: SGR for a special water temperature. Y: Factor to calculate the deviation from the optimal SGR.

Table 7.12: *Specific growth rates depending on ammonia water concentrations.*

NH_3 in mg L^{-1}	**SGR in % BW per day[a] (initial fish weight: 141 g + 34 days experimental period)**	**$Y = SGR_x / SGR_{max}$**
0.07	3.25	0.89
0.24	3.64	***1.00*** [b]
0.65	3.61	0.99
3.00	2.92	***0.80*** [b]
18.46	1.40	0.38

[a] Schram et al. (2010), as reference for all SGRs. [b]***: Factors used for the specific growth rate calculations under ideal and poor holding conditions.*** SGR: specific growth rate, SGR_{max}: maximum SGR within a study, SGR_x: SGR at a special ammonia concentration. Y: Factor to calculate the deviation from the optimal SGR.

Table 7.13: *Specific growth rates depending on nitrite water concentrations.*

NO_2 in mg L^{-1}	**SGR in % BW per day[a] (approx. initial fish weight: 200 g + 28 days experimental period)**	**$Y = SGR_x / SGR_{max}$**
0.28	2.32	***1.00*** [b]
5.11	2.11	0.91
12.88	1.99	***0.86*** [b]
21.12	1.79	0.77
42.69	1.77	0.76

[a]: Roques et al., 2015, as reference for all SGRs. [b]***: Factors used for the specific growth rate calculations under ideal and poor holding conditions.*** SGR: specific growth rate. SGR_{max}: maximum SGR within a study. SGR_x: SGR at a special water nitrite concentration. Y: Factor to calculate the deviation from the optimal SGR.

After 200 days of growth, the growth calculations for African catfish in RAS in N.G. resulted in a final weight of around 900 g under poor production conditions and 1400 g under ideal conditions (for exact daily calculations, see Table 15.12). According to the calculations, under poor husbandry conditions, the fish only reach a slaughter weight of approximately

1400 g after 254 days of feeding. This means that fish under poor production conditions must be kept in the RAS facility for more than 1.5 months longer than fish under ideal conditions. It would reduce the production volume and increases expenses, as more operating costs are incurred per kg of fish produced. This shows the great influence of apparently small growth reductions on the income of a farm.

7.7.3 Conclusions Regarding Ideal and Poor Production Conditions

The preceding calculations show the strong influence of seemingly minor changes in husbandry conditions on production success. For these calculations, chapters 2 **(Baßmann et al., 2023**) and 3 (**Wenzel et al., 2022**) made major contributions by determining the survival rates and growth in African catfish RAS. The study in chapter 2, in which specific growth rates at different stocking densities were calculated, is particularly important as in warm water grow-out RAS, stocking density is one of the most important parameters to increase the yield. Although other publications investigated the effect of stocking densities on the growth of African catfish, none of these studies has investigated a full production cycle in an experimental system that is equivalent to a commercial RAS. The present study, for the first time, allows the establishment of realistic growth values depending on different stocking densities for commercial RAS in N.G. This enables farm operators to stock their systems more wisely and to calculate and plan their production in a more targeted way.

Chapter 3 investigated the mortality rates and growth of African catfish juveniles depending on different stocking densities and grading regimes. In African catfish fingerling production, achieving a high survival rate is one of the greatest challenges to success. As mentioned earlier, mortality at this age stage is largely caused by cannibalism. The study in chapter 3 is important because it not only shows the effects of the different grading regimes to remove cannibals on production but also considers the economic aspects by including the factor of labor.

When considering both the calculations for mortality (see chapter 7.7.1) and growth rates (see chapter 7.7.2), the loss of yield is even higher because, under poor conditions, survival rates decrease, and the growth of the surviving fish is restricted. Thus, if poor production conditions prevail in terms of both survival rate and growth over a complete production cycle of 200 days, starting with 300 000 larvae, 294.7 tons of fish can be produced under good conditions but only 32.9 tons under poor production conditions (for calculations see

Table 7.14). In other words, the yield can differ by more than eight times depending on the management of a farm. In addition to the financial hardship that results from unstable management, it is also difficult to find continuous buyers when production numbers fluctuate since no reliable predictions can be made about the production volumes. This makes it difficult to plan and make longer-term arrangements with potential buyers.

Table 7.14: *Calculations of the production volume under best and poor production conditions considering survival rate and growth.*

	Ideal production conditions	Poor production conditions
Initial number of larvae (n_{Ini})	300 000	300 000
Survival rate (SR)*	0.69	0.12
Final fish weight after 200 days (W_{fin})**	1423.91	912.99
Final fish number ($n_{fin}=n_{Ini}\times SR$)	207 000	36 000
Final biomass in g ($m_{fin}=n_{fin}\times W_{fin}$)	294 749 370	32 867 640
Final biomass in kg	294 749	32 867
Final biomass in tons	295	33

*: see Table 7.7, **: see Table 15.12.

The model described gives a good overview of the most important parameters and their effects on the production success of African catfish RAS. However, due to a lack of data, interactions between the various parameters could not be included. For example, a pH of 8.01 may be optimal for fish under ideal water conditions, but at high ammonia levels, it has a negative effect on fish growth (Schram et al., 2010). Since high nutrient levels can be assumed in African catfish RAS, a pH of 7 should not be exceeded. In addition, a slightly acidic pH may be helpful to reduce bacterial growth and, thus, infections of the fish. This is only one example that shows how difficult it is to operate a system reliably under ideal conditions despite theoretically ideal values. Every RAS is a dynamic system, and farmers must react adequately to the smallest changes at an early stage to be able to guarantee ideal conditions continuously.

In terms of growth, during the last weeks of grow-out to slaughter weight, the best stocking densities are around 100 – 200 kg m^{-3} (**Baßmann et al., 2023**). However, if the financial side is also taken into account, Pasch and Palm (2021) showed that in a farm with a production volume of 300 m^3, a stocking density of at least 450 kg m^{-3} must be maintained in order to be able to cover costs. Thereby, variable costs of 1.51 € kg^{-1}, fixed costs of 0.69 € kg^{-1}, and a selling price of 2.20 € kg^{-1} produced fish were assumed. This shows that under the

current conditions, at the expense of optimal growth, stocking densities in commercial RAS must be at least 450 kg m^{-3} to be able to guarantee a cost-covering economy.

8 Summary

Many factors influence the productivity of intensive African catfish RAS in N.G. This thesis investigates the stocking density, grading regime, water quality, feed ingredients, and the use of process water for aquaponic plant production. Even if it is often assumed that the culture of African catfish in RAS is simple because of its resilience towards harsh environmental conditions, this thesis demonstrates how much the yield depends on certain procedures and external factors that the farmers can influence. Consequently, a certain expertise is required to receive the best results and to be able to cover costs. It is, however, very difficult to define ideal values for all parameters throughout the entire production period, as they also depend on each other and can differ if not only biology but also profitability is considered. This may explain the problems catfish farmers have in running their production in a stable and profitable way.

Within this thesis, a variety of experiments were performed to optimize the production of African catfish in N.G. Considering only growth and welfare, it was found that the fish should best be kept in grow-out systems from 10 g to approximately 1300 g under super-intensive stocking densities (400 kg m^{-3}) and from 1300 g to about 1800 g under semi-intensive (100 kg m^{-3}) stocking densities (**Baßmann et al., 2023**). Hereby the growth can be improved by around 13 %, depending on the chosen stocking density. However, since Pasch and Palm (2021) showed that stocking densities of at least 450 kg m^{-3} are necessary to cover the costs of a farm, it is advisable to lower the slaughter weight to 1300 g as growth reduction at stocking densities above this weight is very high and should be prevented. Moreover, in females above 1300 g, the proportional gonad weight is reported to be considerably higher (Von Merkatz, 2023, personal communication), which should be avoided.

To optimize the commercial production of African catfish fry between 0.06 and 0.32 g, they must be kept at stocking densities of 30 fish L^{-1}. Cannibals must be removed every 5 days, ideally by self-grading to reduce labor costs. This significantly increases the production numbers by reducing mean mortality rates from 25 % to less than 5 % and ensures more stable production. By now, there are no commercial devices for self-grading. Therefore, the staff must do the grading at 5-day intervals manually. The results of chapter 3 (**Wenzel et al., 2022**) provide the framework conditions for reliable and constant fingerling production.

Regarding the sustainable use of the process water for aquaponic plant production and best practices in fish culture, the pH adjustment in the fish systems should be conducted with

KOH to optimize the nutrient profile for the plants and improve the welfare and growth of the fish. Chapter 4 (**Wenzel et al., 2021**) showed that K^+ concentrations between 200 and 400 mg L^{-1} could reduce fighting events and, thus, disturbances in the tanks by more than 50 %. However, it must be ensured that the pH does not increase too much to guarantee that ammonia concentrations will not reach damaging levels, as ammonium levels in the water of grow-out systems can sometimes get very high (> 20 mg L^{-1}, own observations). Therefore, running the pH in a grow-out system below 7 (normally around 6) is advisable to prevent a pH increase above 7 during water exchange, as the freshwater pH is often around 8. In this regard, Palm et al. (2018b) showed that African catfish could grow quite well even at pH levels between 5 and 6 without growth retardation or higher mortality.

Considering feed additives under the aspect of regional resource utilization, it is advisable to integrate Montmorillonit-illite/Muscovite in a concentration of 0.5 % as a fixed component in the feed. In chapter 5 (**Palm et al., 2022**), it could be shown that 0.5 % Montmorillonit-illite/Muscovite leads to fewer skin lesions and a slightly higher final weight after 70 days compared with the control group.

The study in chapter 6 (**Knaus et al., 2020b**) showed that it is possible to directly use the process water of an intensively stocked African catfish RAS to produce an undemanding plant like mint without negative effects on the fish. Therefore, with regard to sustainability and reuse of resources, it is recommended to use process water for aquaponic plant production. This can generate additional income and reduce wastewater discharge. Nevertheless, the decision should be well-considered, as high investment costs and additional know-how are required, and one has to deal with a new, unknown market that requires customers that are willing to pay higher prices for a sustainably produced product (Pasch and Palm, 2021).

Based on the findings in this thesis, new recommendations can be made regarding optimal production conditions for African catfish RAS in N.G. It is now possible to clearly define the advantages and disadvantages of different stocking densities during the grow-out phase. Furthermore, the optimal stocking density of juvenile fish (0.06 – 0.32 g) was found, and an optimal grading system for reducing losses and labor was described. It has been shown that catfish tolerate water potassium concentrations up to 400 mg L^{-1} without negative effects, and therefore a pH adjustment using KOH is recommended if the water is subsequently used for aquaponic purposes. The regionally mined clay mineral Montmorillonite-illite/Muscovite

has been shown to affect fish welfare and growth positively. Its use as a feed additive is, therefore, recommended. Finally, it has been shown that an undemanding plant such as mint can be produced easily without additional fertilization with the water from an intensive catfish RAS. The optimal production conditions for African catfish in RAS are summarized in Table 8.1. There the newly acquired knowledge from this work was supplemented with the help of literature values to obtain a complete overview.

Table 8.1: *Optimal production conditions for recirculating aquaculture systems with African catfish.*

Parameter	Optimum	Reference
First feed	Exclusively *Artemia* for at least 2 days	Adeyemo et al., 1994; Alltech Coppens, 2021; Bardocz et al., 1999; Verreth and Van Tongeren, 1989
pH-value	Hatchery: 7 Grow-out: 6 – 7	Mustapha and Zainab, 2018 Marimuthu et al., 2019; Ndubuisi et al., 2015
Temperature	Larvae/post-larve: 28 – 30 °C Grow-out: 25 – 30 °C	Britz and Hecht, 1987; Verreth and Bieman, 1987 Degani et al., 1989; Hogendoorn et al., 1983; Ogunji and Awoke, 2017; Uys, 1989
Grading	0.06 – 0.17 g: every 5th day 0.38 – 0.9 g: every 3rd day 0.9 – 50 g: at least every 2 weeks	**Wenzel et al., 2022** Abdelhamid et al., 2010 Mwangi et al., 2020
Stocking density	0.06 – 0.32 g: 30 fish L^{-1} *Grow-out: 450 kg m^{-3}	**Wenzel et al., 2022** **Baßmann et al., 2023**; Pasch and Palm, 2021
Ammonia	max. 0.34 mg L^{-1} NH_3–N	Schram et al., 2010
Nitrite	max. 0.6 mg L^{-1} NO_2^-–N	Roques et al., 2015
Nitrate	max. 140 mg L^{-1} NO_3^-–N	Schram et al., 2014
Phosphate	max. 80 mg L^{-1} PO_4^{3-}–P	Strauch et al., 2019
Potassium	max. 400 mg L^{-1} K^+	**Wenzel et al., 2021**
Feed	Addition of 0.5 % Montmorillonite-Illite/Muscovite	**Palm et al., 2022**
Slaughter Size	*1200 – 1300 g	**Baßmann et al., 2023**; Pasch and Palm, 2021
Aquaponic** production	- Production of mint if no additional fertilization is conducted. - pH adjustment in the RAS via KOH if demanding plants shall be produced.	**Knaus et al., 2020b** **Wenzel et al., 2021**

Bold references show results that were collected within the framework of the dissertation.
* Recommended optimal slaughter size and stocking density are the results of a compromise between growth and profitability. ** Recommendations if additional aquaponic plants shall be produced.

9 Outlook

This dissertation aimed to improve the production conditions and processes in commercial African catfish RAS and to give recommendations to enable a continuously stable production. In addition, it was tested if aquaponic mint production is possible without additional fertilization and, thus, has the potential as an additional source of income.

It has been shown that ideal stocking densities of the fish have to be exceeded to be able to cover the costs of a company (at 300 m^3 production volume) (**Baßmann et al., 2023**; Pasch and Palm, 2021). That leads to the first important aspect that should be addressed. African catfish production in N.G. will only have a chance to exist in the future if a reasonable price for the fish can be established. It is, therefore, important to develop strategies to make African catfish more attractive to customers.

It was found that self-grading of juvenile fish is preferable to manual grading, as it reduces handling stress and labor. So far, this is technically only possible on an experimental scale. Therefore, future studies are required to develop commercial-size self-grading systems that can be used in existing companies. The goal should be that such systems can be purchased commercially and easily acquired and used in companies. This would reduce the workload and ensure proper grading also in case of a lack of personnel.

PH adjustment using KOH as an integrated fertilizer for aquaponic use of the process water has proven to be useful. In order to establish this method in commercial farms in the future while ensuring human and animal safety, an automated dosing device especially adapted to catfish RAS should be constructed. This system must be able to adjust the pH to the desired level but also to control the potassium concentrations in the water to ensure that maximum threshold levels are not exceeded. At the same time, the system must be able to handle the high load of suspended solids in the process water, which is a challenge for most probes. The last two suggestions represent technological improvements that should be addressed in the future to make work easier and production more stable. By now, most farmers have helped themselves with self-assembled equipment as there is hardly anything available on the market that is specifically suitable for their farms.

It was found that the regionally mined clay mineral Montmorillonite-illite/Muscovite has a positive effect on the welfare of the fish when added to the feed at 0.5 %. Further research should be done to assess if it also can positively influence water quality, as clay minerals are known to have a high binding capacity. Especially in commercial farms, high amounts of

dissolved nutrients and suspended solids emerge, which can unnecessarily negatively influence the water and, thus, the health status of the fish. If the fish are not in the best condition, this could easily lead to infections and, thereby, growth retardation and production losses. Therefore, it would be helpful to know if Montmorillonite-illite/Muscovite could also be used to bind irritating substances and, by this, reduce potential stress on the fish.

Finally, it has been shown that it is possible to produce mint as an undemanding plant with catfish water without additional fertilization or treatment. However, as it will not be possible to attract customers for aquaponically produced plants at every location and against the background of ever stricter environmental regulations, increasing global pollution, restricted resources, and increasing prices for raw materials, further studies should also focus on the development towards other sustainable possibilities to use “waste-/by-products”. This represents the third important aspect that should be addressed in future research, namely options for the use of by-products. In this context, not only the process water should be considered, but also solid matter and slaughter remains. Particularly regarding slaughter remains, future work should concentrate on developing new higher-valued products like caviar or fish leather or in the animal feed sector. Parallel to the development of new products, large-scale processing and marketing opportunities must be established for the industry. Concerning solid waste, first of all, a proper removal and thickening device should be developed, as conventional methods have not been satisfactory on a commercial scale to date. Only when it is possible to collect the solids in large quantities in a practical way will commercial use be possible at all. Once this has been done, research should be carried out to determine which use of solids is possible and makes the most sense.

All these measures are intended to provide companies with the tools to establish stable production and generate a reasonable profit, which is necessary for their long-term existence.

10 References

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11 Eidesstattliche Erklärung

Hiermit erkläre ich durch eigenhändige Unterschrift, die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben. Die aus den Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Dissertation ist in dieser Form noch keiner anderen Prüfungsbehörde vorgelegt worden.

_____ _____

Ort, Datum Lisa Carolina Wenzel

12 Hypotheses of the Dissertation

Aus der Professur für Aquakultur und Sea-Ranching
der Agrar- und Umweltwissenschaftlichen Fakultät

Cultivation of African catfish (*Clarias gariepinus* Burchell, 1822) in Recirculating Aquaculture Systems (RAS) in Northern Germany

Kumulative Dissertation

zur Erlangung des akademischen Grades
Doktor der Agrarwissenschaften (doctor agriculturae)

an der Agrar- und Umweltwissenschaftlichen Fakultät
der Universität Rostock

vorgelegt von M.Sc. Lisa Carolina Wenzel
Südring 72C, 18059 Rostock

Rostock, den 25.12.2023

12.1 Hypotheses

H1: Stocking density influences growth and performance in African catfish RAS.

H2: Grading regimes influence growth and survival in African catfish RAS.

H3: Selected macronutrients inside the process water and feed ingredients positively affect African catfish's growth performance and welfare in RAS.

H4: Regular African catfish growth in RAS enables successful plant production using the resulting process waters.

H5: Optimized cultivation conditions determine successful African catfish RAS production in Northern German localities.

12.2 Results of the Dissertation

H1: Depending on stocking density and age, African catfish showed differences in growth, skin lesions, and behavior in RAS. After a grow-out period of 23 weeks, from 12 to 1500 – 2000 g, semi-intensively (100 kg m^{-3}) stocked fish grew significantly bigger than super-intensively (400 kg m^{-3}) stocked fish. While after 18 weeks of grow-out, not yet significant differences were seen. At younger age stages (one week after stocking), the fish had fewer skin lesions at higher stocking densities, and vice versa when the fish grew bigger (after 23 weeks). Fish between 0.06 and 0.32 g showed no differences in growth at stocking densities between 10 and 30 fish L^{-1}. At the same time, higher stock activity and less aggressive behavior occurred at higher stocking densities.

H2: Via regular grading every 5 days, the average mortality rate of fish between 0.06 and 0.32 g could be reduced from 25 % to less than 5 % in RAS. Due to cannibalism, the fish grew bigger on average when no grading was conducted compared to regular grading. At the same time, different grading methods (self-grading compared to manual grading) did not influence growth but final stocking densities. Hereby the stocking densities increased with decreasing handling stress.

H3: Both K^+ inside the process water and Montmorillonite-illite/Muscovite (1g577) as an additional feed ingredient could positively affect African catfish's welfare in RAS. The addition of 200 – 400 mg L^{-1} KOH to the process water did not influence growth performance but reduced agonistic behavior and fighting events. Thereby concentrations above 400 mg L^{-1} KOH

led to reduced feed intake and longer feeding times. Adding 0.5 and 2.0 % of 1g577 to the African catfish feed in RAS reduced skin lesions, while the 0.5 % addition also led to slightly better growth.

H4: Aquaponics production of spearmint (*Mentha spicata)* was possible under the direct use of the process water from intensively stocked African catfish RAS without the addition of any liquid fertilizer. Additionally, the aquaponically produced plants grew better than the control group that was cultivated in a commercial fertilizer solution.

H5: It could be shown that the cultivation parameters type of first feed, water pH, water temperature, amount of feed, grading of young fish, stocking density, ammonium and nitrite concentrations in the water strongly influence the survival and growth of African catfish in Northern German RAS. Moreover, it was shown that the annual yield could be increased by more than eight times by optimizing the mentioned cultivation parameters. Showing a successful African catfish RAS production is possible under optimal cultivation conditions.

13 Curriculum vitae

Personal data

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Date and place of birth:	01.08.1987 in Schwabach, Germany
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Work experience

Aug 2023 – today	Employee at Bioenergie Lüchow GmbH & Co. KG
Jan 2021 – Jun 2023	Research fellow at the University of Rostock, Professorship for Aquaculture and Sea-Ranching, Rostock
Feb 2020 – Mar 2021	Executive employee at the Fischzucht Abtshagen GmbH & Co. KG, Abtshagen
Nov 2017 – Jan 2020	Research fellow at the University of Rostock, Professorship for Aquaculture and Sea-Ranching, Rostock
Nov 2011 – Aug 2012	Employee at ANIC Physiotherapy, Altdorf

Secondary employment

Feb 2023 – Jul 2023	Employee at Bioenergie Lüchow GmbH & Co. KG
Jul 2021 – Nov 2021	Employee at Tessiner Edelfisch GmbH
Mar 2017 – Apr 2017	Student assistant at the University of Rostock, Professorship for Aquaculture and Sea-Ranching
Jun 2016 – Dec 2016	Student assistant at the University of Rostock, Professorship for Aquaculture and Sea-Ranching
Oct 2015 – Sep 2017	Employee at Atrium Hotel Krüger, Rostock Sievershagen

Practical experiences during the study

Jan 2017 – Feb 2017	8-week internship at „ECF-Farmsystems GmbH“, Berlin
Mar 2016	4-week internship at „Water-proved GmbH Wassermesstechnik & Aquakultur“, Fürth

Education

	Study
Oct 2015 – Oct 2017	University of Rostock, Study programme: Aquaculture and Sea-Ranching, Degree: Master of Science (1,9); Thesis topic: „Partikuläre Nährstofffrachten bei der Zucht von Afrikanischen Raubwelsen unter verschiedenen Besatzdichten.“
Oct 2012 – Sep 2015	Julius-Maximilians-University of Würzburg, Study programme: Biology, Degree: Bachelor of Science (1,7); Thesis topic: „Die Steuerung der Aktivität durch Licht bei der dunklen Erdhummel.“
	Professional Training
Sep 2008 – Sep 2011	Physiotherapist (Private Berufsfachschule für Physiotherapie in Erlangen)
	Shool
Sep 2006 – Jun 2008	Allgemeine Hochschulreife (Heinrich-Schliemann-Gymnasium, Fürth)
Stay Abroad	
Jan 2006 – Jun 2006	Ontario, Canada (Work on an Icelandic horse farm in exchange for food and accommodation)

Personal Skills and Competences

Language skills:	English, Swedish, basic knowledge of French
Computer skills:	MS-Office, Corel Draw, Image J, R, SPSS
Driving license:	Class BE

Interests and Hobbies

Traveling, hiking, painting

International Presentations

WAS/EAS AQUA 2018, 25 – 29 August 2018, Montpellier, France: Lisa Carolina Wenzel, Sebastian Markus Strauch, Adrian Bischoff-Lang, Olaf Dellwig, Jan Klein, Andrea Schüch, Berit Wasenitz, 2018, Commercial African Catfish (*Clarias gariepinus*) Recirculating Aquaculture Systems: Assessment of Element and Energy Pathways with Special Focus on the Phosphorus Cycle

Publications

- Baßmann, B., Wenzel, L. C., Hahn, L., Palm, H. W. (2023) Effects of Stocking Density, Size, and External Stress on Growth and Welfare of African Catfish (*Clarias gariepinus* Burchell, 1822) in a Commercial RAS. *Fishes*, 8(2), 74.
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15 Appendix

15.1 Calculations of Chapter 7.7.1

15.1.1 Calculations for Natural Mortalities in Table 7.1

Table 15.1: *Calculation for natural mortality during the time-space a (3 days: from hatch – first feed)*

	Survival Rate under ideal water quality %	**References**
	100	Nguyen and Janssen, 2002
	100	Nguyen and Janssen, 2002
	78.8	Nguyen and Janssen, 2002
	95.2	Nguyen and Janssen, 2002
	86.3	Nguyen and Janssen, 2002
Mean:	92.06	
Mean natural mortality in %: (= 100 – mean survival rate)	**7.94**	

Table 15.2: a) *Calculation for natural mortality during the time-space b (22 days from: first feed – 0.7 g),* **b)** *Calculations for the survival rates during the time-space b (22 days from: first feed – 0.7 g) in dependence of different grading intervals*

a)	**Single Values**			**mean**
Natural mortality within 10 days (%)*	4.7	3.6	4.1	4.13
Assumed natural mortality within 22 days (%) (= Natural mortality within 10 days × 2.2)	10.34	7.92	9.02	**9.09**

b) Grading intervals	**Mortality in % in 10 days (M_{10})**	**Mortality in % in 22 days (M_{22}) = M_{10} x 2.2**	**Survival in % after 22 days (SR_{22} = 100% - M_{22})**
no grading	25.00*	55.00	45.00
every day	4.13*	9.09	90.91
every 2nd day	5.30*	11.66	88.34
every 5th day	4.30**	9.46	90.54

*: Values from Table 3.6 (Wenzel et al., 2022).**: Mean value from 5dH and 5dS in Table 3.6.

Table 15.3: *Calculation for natural mortality (M_n) during the time-space c (35 days: from 0.7 g – stocking size)*

Survival rate in % (including cannibalism): SR	Mortality due to cannibalism in %: C	Natural mortality in % (other causes than cannibalism): M_n	Reference
78	16	6*	Al-Hafedh and Ali, 2004
69	25	6*	Al-Hafedh and Ali, 2004
67	27	6*	Al-Hafedh and Ali, 2004
	42.4	11.5	Mwangi et al., 2020
	7.5	10.8	Mwangi et al., 2020
	18.9	13.7	Mwangi et al., 2020
Mean: 67.2	22.8	**9.0**	

*: Calculated values using the formula: $M_n = 100 – SR – C$

Table 15.4: *Calculations for natural mortality during the time-space d (150 days: from stocking – slaughter size)*

	Survival Rates in %	References
	92.88	Chapter 2 (**Baßmann et al. 2023**)
	96.21	Chapter 2 (**Baßmann et al. 2023**)
	90.76	Chapter 2 (**Baßmann et al. 2023**)
	78.1	Palm et al., 2018b
	90	Palm et al., 2018b
	86.4	Palm et al., 2018b
	96.2	Palm et al., 2018b
	96.7	Palm et al., 2018b
	90.2	Palm et al., 2018b
	96.2	Palm et al., 2018b
	88.6	Palm et al., 2018b
	81.4	Palm et al., 2018b
Mean survival rate in %:	90.30	
Mean natural mortality in %: (= 100 – mean survival rate)	**9.70**	

15.1.2 Calculations for the Survival Rates in Dependence on the different Parameters in Chapter 7.7.1

Table 15.5: *Calculations for the proportional effective survival rates (K) only influenced by the type of first feed (FF)*

Type of first feed	Mean K_{FF} = (100 – mean maximum SR_{FF}* + mean SR_{FF}*) / 100
Hatched Artemia Nauplii	(100 – 92.1 + 90.5) / 100 = 0.98
Decapsulated Artemia Cysts (Survival Rate after 10d)	(100 – 92.1 + 92.1) / 100 = 1.00
Decapsulated Artemia Cysts + Commercial Starter Diet	(100 – 92.1 + 77.5) / 100 = 0.86
Commercial Starter Diet	(100 – 92.1 + 65.8) / 100 = 0.74
Live Daphnia	(100 – 92.1 + 77.2) / 100 = 0.85
Moina Dubia	(100 – 92.1 + 92.0) / 100 = 1.00
Moina + Commercial Starter Diet	(100 – 92.1 + 69.6) / 100 = 0.78
Mixed Zooplankton	(100 – 92.1 + 89.0) / 100 = 0.97

With K: proportional effective survival rate = survival rate that only depends on a special parameter (excluding natural mortalities or mortalities due to general husbandry conditions), SR: survival rate and FF: parameter first feed

*: The values and their references are shown in Table 7.2.

Table 15.6: *Calculations for the proportional effective survival rates (K) only influenced by different water pH levels*

	K_{pH} = (100 – maximum SR_{pH}* + SR_{pH}*) / 100		
Tested pH-value	**3.6 g fish^{-1}**	**100 g fish^{-1}**	**500 g fish^{-1}**
3	0.00**	0.00**	0.00**
4	(100 – 90 + 20) / 100 = 0.30	(100 – 94 + 38) / 100 = 0.44	(100 – 98 + 64) / 100 = 0.44
5	(100 – 90 + 70) / 100 = 0.80	(100 – 94 + 74) / 100 = 0.80	(100 – 98 + 88) / 100 = 0.90
6	(100 – 90 + 88) / 100 = 0.98	(100 – 94 + 90) / 100 = 0.96	(100 – 98 + 96) / 100 = 0.98
8.01	(100 – 90 + 90) / 100 = 1.00	(100 – 94 + 94) / 100 = 1.00	(100 – 98 + 98) / 100 = 1.00

With K: proportional effective survival rate = survival rate that only depends on a special parameter (excluding natural mortalities or mortalities due to general husbandry conditions), SR: survival rate and pH: parameter pH-value, *: The values and their references are shown in Table 7.3, **: Here the survival rate does not increase, as it is assumed that this pH is lethal for all fish.

Table 15.7: *Calculations for the proportional effective survival rates (K) only influenced by water temperature (T)*

Age / Size:	Embryo	Larvae	3 – 20 g
Temperature in °C	**K_T = (100 – maximum SR_T* + SR_T*) / 100**		
17.4	0.00**	0.00**	
18.9	(100 – 94 + 29) / 100 = 0.35	0.00**	
20.6	(100 – 94 + 57.5) / 100 = 0.64	(100 – 56 + 15) / 100 = 0.59	
21.5	(100 – 94 + 92) / 100 = 0.98		
22 (24L: 0D)			(100 – 83 + 40) / 100 = 0.57
22 (12L: 12D)			(100 – 83 + 53) / 100 = 0.70
22 (0L: 24D)			(100 – 83 + 58) / 100 = 0.75
23	(100 – 94 + 72.5) / 100 = 0.79	(100 – 56 + 54) / 100 = 0.98	
23.2		(100 – 56 + 39) / 100 = 0.83	
24	(100 – 94 + 94) / 100 = 1.00		
25.2		(100 – 56 + 56) / 100 = 1.00	
25.4		(100 – 56 + 38) / 100 = 0.82	
25.5	(100 – 94 + 57) / 100 = 0.63		
27.2	(100 – 94 + 39.5) / 100 = 0.46	(100 – 56 + 42.5) / 100 = 0.87	
27.3	(100 – 94 + 90) / 100 = 0.96	(100 – 56 + 35) / 100 = 0.79	
28 (24L: 0D)			(100 – 83 + 57) / 100 = 0.74
28 (12L: 12D)			(100 – 83 + 72) / 100 = 0.89
28 (0L: 24D)			(100 – 83 + 83) / 100 = 1.00
28.6	(100 – 94 + 37.5) / 100 = 0.44		
29.3	(100 – 94 + 35) / 100 = 0.41	(100 – 56 + 35) / 100 = 0.79	
30.3	(100 – 94 + 55.5) / 100 = 0.62	(100 – 56 + 52.5) / 100 = 0.97	
31.6	(100 – 94 + 16.75) / 100 = 0.23		
33.2	(100 – 94 + 14.5) / 100 = 0.21	(100 – 56 + 5.75) / 100 = 0.50	
33.5	(100 – 94 + 8) / 100 = 0.14		
35.2	0.00**		
35.6	0.00**	0.00**	

With K: proportional effective survival rate = survival rate that only depends on a special parameter (excluding natural mortalities or mortalities due to general husbandry conditions), SR: survival rate, and T: parameter temperature

*: The values and their references are shown in Table 7.5. **: Here, the survival rate does not increase, as it is assumed that this pH is lethal for all fish.

Table 15.8: *Calculations for the proportional effective survival rates (K) only influenced by the percentage of feeding*

	Fish size: 0.64 – 65.4 g
Feed amount in % of body weight per day	**Calculated theoretic survival rates only influenced by the feed amount $K_{\%F}$ = (100 – maximum $SR_{\%F}$* + $SR_{\%F}$*) / 100**
0	0.00**
2	(100 – 78 + 30) / 100 = 0.52
4	(100 – 78 + 32) / 100 = 0.54
6	(100 – 78 + 78) / 100 = 1.00
8	(100 – 78 + 69) / 100 = 0.91
10	(100 – 78 + 67) / 100 = 0.89

With K: proportional effective survival rate = survival rate that only depends on a special parameter (excluding natural mortalities or mortalities due to general husbandry conditions), SR: survival rate and %F: parameter percentage of feed input, *: The values and their references are shown in Table 7.4. **: Here, the survival rate does not increase, as it is assumed that this pH is lethal for all fish.

Table 15.9: *Calculations for the proportional effective survival rates (K) only influenced by the removal of shooters or potential cannibals by grading (G)*

	Calculated theoretical survival rates only influenced by grading $K_G = (SR_G* + M_n)/100$**		
	Fish weight in g (experimental period in days)		
	0.06–0.16 (10)	**0.08 – 0.32 (10)**	**0.38 – 0.9/3* (28)**
Time space (t):	**b**		**c**
Grading intervals			
No grading		(45 + 9.09) / 100 = 0.54	(1.2 + 9.00) / 100 = 0.10
Every day	(90.91 + 9.09) / 100 = 1.00		
Every 2nd day	(88.34 + 9.09) / 100 = 0.97		
Every 3rd day			(49.17 + 9.00) / 100 = 0.58
Every 5th day	(90.54 + 9.09) / 100 = 1.00		
Every 7th day			(32.33 + 9.00) / 100 = 0.41
Every 2 weeks			
Every 4 weeks			

	Fish weight in g (experimental period in days)		
	0.69 – 36.3 (60)	**0.89 – 42.5 (60)**	**0.92 – 51.7 (60)**
Time space (t):		**c**	
Grading intervals			
No grading	(43.2 + 9.00) / 100 = 0.52		
Every day			
Every 2nd day			
Every 3rd day			
Every 5th day			
Every 7th day			
Every 2 weeks			(81.6 + 9.00) / 100 = 0.91
Every 4 weeks		(64.4 + 9.00) / 100 = 0.73	

With K: proportional effective survival rate = survival rate that only depends on a special parameter (excluding natural mortalities or mortalities due to general husbandry conditions), SR: survival rate, and M_n: natural mortalities, for example, due to genetics.

* The values and their references are shown in Table 7.6). ** The value and their references are shown in Table 7.1. b: Time-space from first feeding until approximately 0.7 g, c: Time-space from 0.7 g until stocking size.

15.2 Calculations of Chapter 7.7.2

15.2.1 Calculations for the Ideal Specific Growth Rates

Table 15.10: *Data for ideal African catfish growth*

Summarized from Alltech Coppens (2021)			Calculated using the data from Alltech Coppens (2021)
Feeding days	**Initial fish weight in g (W_i)**	**Final fish weight in g (W_f)**	**SGR in %BW d^{-1} = $(\ln(W_f) - \ln(W_i)) / t \times 100$**
1	0.0025	0.005	69.31
2	0.005	0.009	58.78
3	0.009	0.015	51.08
4	0.015	0.022	38.30
5	0.022	0.032	37.47
6	0.032	0.044	31.85
7	0.044	0.059	29.33
8	0.059	0.076	25.32
9	0.076	0.098	25.42
10	0.098	0.122	21.91
11	0.122	0.151	21.33
12	0.151	0.184	19.77
13	0.184	0.221	18.32
14	0.221	0.260	16.25
15	0.26	0.310	17.59
16	0.31	0.360	14.95
17	0.36	0.420	15.42
18	0.42	0.480	13.35
19	0.48	0.550	13.61
20	0.55	0.630	13.58
21	0.63	0.710	11.95
22	0.71	0.800	11.93
23	0.8	0.900	11.78
24	0.9	1.000	10.54
25	1	1.100	9.53
26	1.1	1.200	8.70
27	1.2	1.400	15.42
28	1.4	1.500	6.90
29	1.5	1.600	6.45
30	1.6	1.800	11.78
31	1.8	2.000	10.54
32	2	2.100	4.88
33	2.1	2.300	9.10
34	2.3	2.500	8.34
35	2.5	2.700	7.70
36	2.7	2.900	7.15
37	2.9	3.200	9.84
38	3.2	3.400	6.06

Summarized from Alltech Coppens (2021)			Calculated using the data from Alltech Coppens (2021)
Feeding days	**Initial fish weight in g (W_i)**	**Final fish weight in g (W_f)**	**SGR in %BW d^{-1} = $(\ln(W_f) - \ln(W_i)) / t \times 100$**
39	3.4	3.700	8.46
40	3.7	3.900	5.26
41	3.9	4.200	7.41
42	4.2	4.500	6.90
43	4.5	4.800	6.45
44	4.8	5.100	6.06
45	5.1	5.400	5.72
46	5.4	5.800	7.15
47	5.8	6.100	5.04
48	6.1	6.500	6.35
49	6.5	6.900	5.97
50	6.9	7.300	5.64
51	7.3	7.700	5.33
52	7.7	8.100	5.06
53	8.1	8.600	5.99
54	8.6	9.000	4.55
55	9	9.500	5.41
56	9.5	10.000	5.13
57	10	11.000	9.53
58	11	12.000	8.70
59	12	13.000	8.00
60	13	15.000	14.31
61	15	16.000	6.45
62	16	18.000	11.78
63	18	19.000	5.41
64	19	20.733	8.73
65	20.733	22.623	8.73
66	22.623	24.687	8.73
67	24.687	26.938	8.73
68	26.938	29.394	8.73
69	29.394	32.075	8.73
70	32.075	35.000	8.73
71	35	37.619	7.22
72	37.619	40.434	7.22
73	40.434	43.459	7.22
74	43.459	46.711	7.22
75	46.711	50.206	7.22
76	50.206	53.962	7.22
77	53.962	58.000	7.22
78	58	61.757	6.28
79	61.757	65.758	6.28
80	65.758	70.017	6.28
81	70.017	74.553	6.28

Summarized from Alltech Coppens (2021)			Calculated using the data from Alltech Coppens (2021)
Feeding days	**Initial fish weight in g (W_i)**	**Final fish weight in g (W_f)**	**SGR in %BW d^{-1} = $(\ln(W_f) - \ln(W_i)) / t \times 100$**
82	74.553	79.382	6.28
83	79.382	84.525	6.28
84	84.525	90.000	6.28
85	90	95.061	5.47
86	95.061	100.407	5.47
87	100.407	106.054	5.47
88	106.054	112.018	5.47
89	112.018	118.318	5.47
90	118.318	124.972	5.47
91	124.972	132.000	5.47
92	132	138.414	4.74
93	138.414	145.140	4.74
94	145.140	152.192	4.74
95	152.192	159.588	4.74
96	159.588	167.342	4.74
97	167.342	175.474	4.74
98	175.474	184.000	4.74
99	184	191.345	3.91
100	191.345	198.984	3.91
101	198.984	206.927	3.91
102	206.927	215.187	3.91
103	215.187	223.777	3.91
104	223.777	232.710	3.91
105	232.710	242.000	3.91
106	242	250.133	3.31
107	250.133	258.538	3.31
108	258.538	267.227	3.31
109	267.227	276.207	3.31
110	276.207	285.489	3.31
111	285.489	295.083	3.31
112	295.083	305.000	3.31
113	305	313.776	2.84
114	313.776	322.805	2.84
115	322.805	332.094	2.84
116	332.094	341.650	2.84
117	341.650	351.481	2.84
118	351.481	361.595	2.84
119	361.595	372.000	2.84
120	372	381.153	2.43
121	381.153	390.531	2.43
122	390.531	400.140	2.43
123	400.140	409.986	2.43
124	409.986	420.074	2.43

Summarized from Alltech Coppens (2021)			Calculated using the data from Alltech Coppens (2021)
Feeding days	**Initial fish weight in g (W_i)**	**Final fish weight in g (W_f)**	**SGR in %BW d^{-1} = $(\ln(W_f) - \ln(W_i)) / t \times 100$**
125	420.074	430.410	2.43
126	430.410	441.000	2.43
127	441	450.757	2.19
128	450.757	460.729	2.19
129	460.729	470.922	2.19
130	470.922	481.341	2.19
131	481.341	491.990	2.19
132	491.990	502.875	2.19
133	502.875	514.000	2.19
134	514	524.099	1.95
135	524.099	534.397	1.95
136	534.397	544.897	1.95
137	544.897	555.603	1.95
138	555.603	566.519	1.95
139	566.519	577.650	1.95
140	577.650	589.000	1.95
141	589	599.814	1.82
142	599.814	610.827	1.82
143	610.827	622.042	1.82
144	622.042	633.463	1.82
145	633.463	645.094	1.82
146	645.094	656.938	1.82
147	656.938	669.000	1.82
148	669	680.529	1.71
149	680.529	692.257	1.71
150	692.257	704.188	1.71
151	704.188	716.323	1.71
152	716.323	728.668	1.71
153	728.668	741.226	1.71
154	741.226	754.000	1.71
155	754	766.374	1.63
156	766.374	778.951	1.63
157	778.951	791.734	1.63
158	791.734	804.727	1.63
159	804.727	817.934	1.63
160	817.934	831.357	1.63
161	831.357	845.000	1.63
162	845	857.960	1.52
163	857.960	871.118	1.52
164	871.118	884.478	1.52
165	884.478	898.043	1.52
166	898.043	911.817	1.52
167	911.817	925.801	1.52

Summarized from Alltech Coppens (2021)			Calculated using the data from Alltech Coppens (2021)
Feeding days	**Initial fish weight in g (W_i)**	**Final fish weight in g (W_f)**	**SGR in %BW d^{-1} = $(\ln(W_f) - \ln(W_i)) / t \times 100$**
168	925.801	940.000	1.52
169	940	953.674	1.44
170	953.674	967.547	1.44
171	967.547	981.622	1.44
172	981.622	995.902	1.44
173	995.902	1010.390	1.44
174	1010.390	1025.088	1.44
175	1025.088	1040.000	1.44
176	1040	1054.257	1.36
177	1054.257	1068.710	1.36
178	1068.710	1083.361	1.36
179	1083.361	1098.212	1.36
180	1098.212	1113.268	1.36
181	1113.268	1128.529	1.36
182	1128.529	1144.000	1.36
183	1144	1251.000	1.28
184	1158.706	1173.602	1.28
185	1173.602	1188.688	1.28
186	1188.688	1203.969	1.28
187	1203.969	1219.446	1.28
188	1219.446	1235.122	1.28
189	1235.122	1251.000	1.28
190	1251	1361.000	1.20
191	1266.152	1281.488	1.20
192	1281.488	1297.010	1.20
193	1297.010	1312.720	1.20
194	1312.720	1328.620	1.20
195	1328.620	1344.712	1.20
196	1344.712	1361.000	1.20
197	1361	1376.463	1.13
198	1376.463	1392.101	1.13
199	1392.101	1407.918	1.13
200	1407.918	1423.914	1.13
201	1423.914	1440.091	1.13
202	1440.091	1456.453	1.13
203	1456.453	1473.000	1.13
204	1473	1489.038	1.08
205	1489.038	1505.251	1.08
206	1505.251	1521.640	1.08
207	1521.640	1538.207	1.08
208	1538.207	1554.955	1.08
209	1554.955	1571.885	1.08
210	1571.885	1589.000	1.08

Summarized from Alltech Coppens (2021)			Calculated using the data from Alltech Coppens (2021)
Feeding days	**Initial fish weight in g (W_i)**	**Final fish weight in g (W_f)**	**SGR in %BW d^{-1} = $(\ln(W_f) - \ln(W_i)) / t \times 100$**
211	1589	1605.210	1.01
212	1605.210	1621.585	1.01
213	1621.585	1638.127	1.01
214	1638.127	1654.838	1.01
215	1654.838	1671.719	1.01
216	1671.719	1688.773	1.01
217	1688.773	1706.000	1.01
218	1706	1722.648	0.97
219	1722.648	1739.457	0.97
220	1739.457	1756.431	0.97
221	1756.431	1773.571	0.97
222	1773.571	1790.878	0.97
223	1790.878	1808.354	0.97
224	1808.354	1826.000	0.97
225	1826	1842.949	0.92
226	1842.949	1860.056	0.92
227	1860.056	1877.321	0.92
228	1877.321	1894.747	0.92
229	1894.747	1912.334	0.92
230	1912.334	1930.085	0.92
231	1930.085	1948.000	0.92
232	1948	1965.181	0.88
233	1965.181	1982.514	0.88
234	1982.514	2000.000	0.88
235	2000		

SGR: Specific growth rate, W_f: Final weight, W_i: initial weight, t: time in days

Table 15.11: *Factor Y calculations for SGR corrections in correlation to first feeds (example calculation for Y in the case of first feed)*

	SGRs in %BW d^{-1}						
				Feeding days			
Feed	1–7[a]	3–8[b]	9–11[b]	12–14[b]	15–17[b]	18–21[b]	22–24[b]
Artemia Nauplii	2.9	33	27	20	15	6	4
Starter dry feed	2.6	32	13	32	30	15	16
	Factor to calculate deviation from optimal SGR = SGR_x / SGR_{max}						
				Feeding days			
Feed	1–7[a]	3–8[b]	9–11[b]	12–14[b]	15–17[b]	18–21[b]	22–24[b]
Artemia	2.9/2.9=	33/33=	27/27=	20/32=	15/30=	6/15=	4/16=
Nauplii	1.00	1.00	1.00	0.63	0.50	0.40	0.25
Starter dry	2.6/2.9=	32/33=	13/27=	32/32=	30/30=	15/15=	16/16=
feed	0.90	0.97	0.48	1.00	1.00	1.00	1.00

References: [a] Adeyemo et al. (1994), [b] Appelbaum and Mc Geer (1998), SGR: specific growth rate, SGR_{max}: maximum SGR within a study, SGR_x: SGR for a special feed x within a study .

Table 15.12: *Daily growth calculations under best-case (Alltech Coppens) and poor production conditions for African catfish in RAS in Northern Germany*

	Best-case (bc)		Poor production conditions (ppc)			
Days of feeding	SGR_{bc}	$W_{final} = e^{(SGRbc/\ 100\ x\ t\ +\ ln(Winitial))}$	$SGR_{ppc} = SGR_{bc} \times Y$	$W_{final} = e^{(SGRppc\ /\ 100\ x\ t\ +\ ln(Winitial))}$	Y-factor	Comment to Y-factor
1	69.31	0.005	62.38	0.005	0.9	Dry feed instead of Artemia
2	58.78	0.009	52.90	0.008	0.9	Dry feed instead of Artemia
3	51.08	0.015	47.76	0.013	0.935	*Dry feed instead of Artemia
4	38.30	0.022	35.81	0.018	0.935	*Dry feed instead of Artemia
5	37.47	0.032	35.03	0.026	0.935	*Dry feed instead of Artemia
6	31.85	0.044	29.78	0.035	0.935	*Dry feed instead of Artemia
7	29.33	0.059	27.43	0.046	0.935	*Dry feed instead of Artemia
8	25.32	0.076	24.56	0.059	0.97	Dry feed instead of Artemia
9	25.42	0.10	25.42	0.076	1	No negative influence on growth
10	21.91	0.12	21.91	0.094	1	No negative influence on growth
11	21.33	0.15	21.33	0.12	1	No negative influence on growth
12	19.77	0.18	19.77	0.14	1	No negative influence on growth
13	18.32	0.22	18.32	0.17	1	No negative influence on growth
14	16.25	0.26	16.25	0.20	1	No negative influence on growth
15	17.59	0.31	17.59	0.24	1	No negative influence on growth
16	14.95	0.36	14.95	0.28	1	No negative influence on growth
17	15.42	0.42	15.42	0.32	1	No negative influence on growth
18	13.35	0.48	13.35	0.37	1	No negative influence on growth
19	13.61	0.55	13.61	0.42	1	No negative influence on growth
20	13.58	0.63	13.58	0.49	1	No negative influence on growth
21	11.95	0.71	11.95	0.55	1	No negative influence on growth
22	11.93	0.80	11.93	0.62	1	No negative influence on growth
23	11.78	0.90	11.78	0.70	1	No negative influence on growth
24	10.54	1.00	10.54	0.77	1	No negative influence on growth
25	9.53	1.10	9.53	0.85	1	No negative influence on growth

Days of feeding	Best-case (bc) SGR_{bc}	Best-case (bc) $W_{final} = e^{(SGRbc/\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) $SGR_{ppc} = SGR_{bc} \times Y$	Poor production conditions (ppc) $W_{final} = e^{(SGRppc\ /\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) Y-factor	Poor production conditions (ppc) Comment to Y-factor
26	8.70	1.20	8.70	0.93	1	No negative influence on growth
27	15.42	1.40	15.42	1.08	1	No negative influence on growth
28	6.90	1.50	6.90	1.16	1	No negative influence on growth
29	6.45	1.60	6.45	1.24	1	No negative influence on growth
30	11.78	1.80	11.78	1.39	1	No negative influence on growth
31	10.54	2.00	10.54	1.55	1	No negative influence on growth
32	4.88	2.10	4.88	1.62	1	No negative influence on growth
33	9.10	2.30	9.10	1.78	1	No negative influence on growth
34	8.34	2.50	8.34	1.93	1	No negative influence on growth
35	7.70	2.70	7.70	2.09	1	No negative influence on growth
36	7.15	2.90	7.15	2.24	1	No negative influence on growth
37	9.84	3.20	9.84	2.47	1	No negative influence on growth
38	6.06	3.40	6.06	2.63	1	No negative influence on growth
39	8.46	3.70	8.46	2.86	1	No negative influence on growth
40	5.26	3.90	5.26	3.01	1	No negative influence on growth
41	7.41	4.20	7.41	3.25	1	No negative influence on growth
42	6.90	4.50	6.90	3.48	1	No negative influence on growth
43	6.45	4.80	6.45	3.71	1	No negative influence on growth
44	6.06	5.10	6.06	3.94	1	No negative influence on growth
45	5.72	5.40	5.72	4.17	1	No negative influence on growth
46	7.15	5.80	7.15	4.48	1	No negative influence on growth
47	5.04	6.10	5.04	4.71	1	No negative influence on growth
48	6.35	6.50	6.35	5.02	1	No negative influence on growth
49	5.97	6.90	5.97	5.33	1	No negative influence on growth
50	5.64	7.30	5.64	5.64	1	No negative influence on growth
51	5.33	7.70	5.33	5.95	1	No negative influence on growth
52	5.06	8.10	5.06	6.26	1	No negative influence on growth
53	5.99	8.60	5.99	6.64	1	No negative influence on growth

Days of feeding	Best-case (bc) SGR_{bc}	Best-case (bc) $W_{final} = e^{(SGRbc/\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) $SGR_{ppc} = SGR_{bc} \times Y$	Poor production conditions (ppc) $W_{final} = e^{(SGRppc\ /\ 100\ x\ t\ +\ ln(Winitial))}$	Y-factor	Comment to Y-factor
54	4.55	9.00	4.55	6.95	1	No negative influence on growth
55	5.41	9.50	5.41	7.34	1	No negative influence on growth
56	5.13	10.00	5.13	7.73	1	No negative influence on growth
57	9.53	11.00	9.53	8.50	1	No negative influence on growth
58	8.70	12.00	8.70	9.27	1	No negative influence on growth
59	8.00	13.00	8.00	10.04	1	No negative influence on growth
60	14.31	15.00	14.31	11.59	1	No negative influence on growth
61	6.45	16.00	5.61	12.26	0.87	Highest stocking density
62	11.78	18.00	10.25	13.58	0.87	Highest stocking density
63	5.41	19.00	4.70	14.24	0.87	Highest stocking density
64	8.73	20.73	7.59	15.36	0.87	Highest stocking density
65	8.73	22.62	7.59	16.57	0.87	Highest stocking density
66	8.73	24.69	7.59	17.88	0.87	Highest stocking density
67	8.73	26.94	7.59	19.29	0.87	Highest stocking density
68	8.73	29.39	8.16	20.93	0.935	*Highest stocking density
69	8.73	32.07	8.16	22.71	0.935	*Highest stocking density
70	8.73	35.00	8.73	24.78	1	No negative influence on growth
71	7.22	37.62	7.22	26.63	1	No negative influence on growth
72	7.22	40.43	7.22	28.63	1	No negative influence on growth
73	7.22	43.46	7.22	30.77	1	No negative influence on growth
74	7.22	46.71	6.28	32.76	0.87	Assuming 22 °C for 2 days, constant darkness
75	7.22	50.21	6.28	34.88	0.87	Assuming 22 °C for 2 days, constant darkness
76	7.22	53.96	7.22	37.49	1	No negative influence on growth
77	7.22	58.00	7.22	40.30	1	No negative influence on growth
78	6.28	61.76	6.28	42.91	1	No negative influence on growth
79	6.28	65.76	6.28	45.69	1	No negative influence on growth

Days of feeding	Best-case (bc) SGR_{bc}	Best-case (bc) $W_{final} = e^{(SGRbc/\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) $SGR_{ppc} = SGR_{bc} \times Y$	Poor production conditions (ppc) $W_{final} = e^{(SGRppc\ /\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) Y-factor	Poor production conditions (ppc) Comment to Y-factor
80	6.28	70.02	6.28	48.65	1	No negative influence on growth
81	6.28	74.55	6.28	51.80	1	No negative influence on growth
82	6.28	79.38	6.28	55.16	1	No negative influence on growth
83	6.28	84.52	6.28	58.73	1	No negative influence on growth
84	6.28	90.00	6.28	62.53	1	No negative influence on growth
85	5.47	95.06	5.47	66.05	1	No negative influence on growth
86	5.47	100.41	5.47	69.76	1	No negative influence on growth
87	5.47	106.05	5.47	73.69	1	No negative influence on growth
88	5.47	112.02	5.47	77.83	1	No negative influence on growth
89	5.47	118.32	5.47	82.21	1	No negative influence on growth
90	5.47	124.97	5.47	86.83	1	No negative influence on growth
91	5.47	132.00	5.47	91.71	1	No negative influence on growth
92	4.74	138.41	4.74	96.17	1	No negative influence on growth
93	4.74	145.14	4.74	100.84	1	No negative influence on growth
94	4.74	152.19	4.74	105.74	1	No negative influence on growth
95	4.74	159.59	4.74	110.88	1	No negative influence on growth
96	4.74	167.34	4.74	116.27	1	No negative influence on growth
97	4.74	175.47	4.74	121.92	1	No negative influence on growth
98	4.74	184.00	4.74	127.84	1	No negative influence on growth
99	3.91	191.35	3.91	132.95	1	No negative influence on growth
100	3.91	198.98	3.91	138.25	1	No negative influence on growth
101	3.91	206.93	3.13	142.65	0.8	Assuming 1 day of 3 mg/L ammonia
102	3.91	215.19	3.91	148.35	1	No negative influence on growth
103	3.91	223.78	3.91	154.27	1	No negative influence on growth
104	3.91	232.71	3.91	160.43	1	No negative influence on growth
105	3.91	242.00	3.91	166.83	1	No negative influence on growth
106	3.31	250.13	3.31	172.44	1	No negative influence on growth
107	3.31	258.54	3.31	178.23	1	No negative influence on growth

Days of feeding	Best-case (bc) SGR_{bc}	Best-case (bc) $W_{final} = e^{(SGRbc/\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) $SGR_{ppc} = SGR_{bc} \times Y$	Poor production conditions (ppc) $W_{final} = e^{(SGRppc\ /\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) Y-factor	Poor production conditions (ppc) Comment to Y-factor
108	3.31	267.23	3.31	184.22	1	No negative influence on growth
109	3.31	276.21	3.31	190.41	1	No negative influence on growth
110	3.31	285.49	3.31	196.81	1	No negative influence on growth
111	3.31	295.08	2.84	202.49	0.86	Assuming 3 days of 12.88 mg L^{-1} nitrite
112	3.31	305.00	2.84	208.33	0.86	Assuming 3 days of 12.88 mg L^{-1} nitrite
113	2.84	313.78	2.44	213.47	0.86	Assuming 3 days of 12.88 mg L^{-1} nitrite
114	2.84	322.81	2.84	219.61	1	No negative influence on growth
115	2.84	332.09	2.84	225.93	1	No negative influence on growth
116	2.84	341.65	2.84	232.43	1	No negative influence on growth
117	2.84	351.48	2.84	239.12	1	No negative influence on growth
118	2.84	361.60	2.84	246.00	1	No negative influence on growth
119	2.84	372.00	2.78	252.94	0.98	*Highest stocking density
120	2.43	381.15	2.38	259.04	0.98	*Highest stocking density
121	2.43	390.53	2.38	265.28	0.98	*Highest stocking density
122	2.43	400.14	2.38	271.68	0.98	*Highest stocking density
123	2.43	409.99	2.33	278.09	0.96	Highest stocking density
124	2.43	420.07	2.33	284.65	0.96	Highest stocking density
125	2.43	430.41	2.33	291.38	0.96	Highest stocking density
126	2.43	441.00	2.33	298.25	0.96	Highest stocking density
127	2.19	450.76	2.10	304.59	0.96	Highest stocking density
128	2.19	460.73	2.10	311.05	0.96	Highest stocking density
129	2.19	470.92	2.10	317.66	0.96	Highest stocking density
130	2.19	481.34	2.10	324.40	0.96	Highest stocking density
131	2.19	491.99	2.10	331.29	0.96	Highest stocking density
132	2.19	502.87	2.10	338.32	0.96	Highest stocking density
133	2.19	514.00	2.10	345.50	0.96	Highest stocking density
134	1.95	524.10	1.87	352.02	0.96	Highest stocking density
135	1.95	534.40	1.87	358.65	0.96	Highest stocking density

Days of feeding	Best-case (bc) SGR_{bc}	Best-case (bc) $W_{final} = e^{(SGRbc/\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) $SGR_{ppc} = SGR_{bc} \times Y$	Poor production conditions (ppc) $W_{final} = e^{(SGRppc\ /\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) Y-factor	Poor production conditions (ppc) Comment to Y-factor
136	1.95	544.90	1.87	365.42	0.96	Highest stocking density
137	1.95	555.60	1.87	372.31	0.96	Highest stocking density
138	1.95	566.52	1.87	379.33	0.96	Highest stocking density
139	1.95	577.65	1.87	386.48	0.96	Highest stocking density
140	1.95	589.00	1.87	393.76	0.96	Highest stocking density
141	1.82	599.81	1.75	400.70	0.96	Highest stocking density
142	1.82	610.83	1.75	407.76	0.96	Highest stocking density
143	1.82	622.04	1.75	414.95	0.96	Highest stocking density
144	1.82	633.46	1.75	422.26	0.96	Highest stocking density
145	1.82	645.09	1.75	429.70	0.96	Highest stocking density
146	1.82	656.94	1.75	437.27	0.96	Highest stocking density
147	1.82	669.00	1.75	444.97	0.96	Highest stocking density
148	1.71	680.53	1.64	452.33	0.96	Highest stocking density
149	1.71	692.26	1.64	459.81	0.96	Highest stocking density
150	1.71	704.19	1.64	467.42	0.96	Highest stocking density
151	1.71	716.32	1.64	475.15	0.96	Highest stocking density
152	1.71	728.67	1.64	483.01	0.96	Highest stocking density
153	1.71	741.23	1.64	491.00	0.96	Highest stocking density
154	1.71	754.00	1.64	499.12	0.96	Highest stocking density
155	1.63	766.37	1.56	506.98	0.96	Highest stocking density
156	1.63	778.95	1.56	514.96	0.96	Highest stocking density
157	1.63	791.73	1.56	523.07	0.96	Highest stocking density
158	1.63	804.73	1.56	531.31	0.96	Highest stocking density
159	1.63	817.93	1.56	539.68	0.96	Highest stocking density
160	1.63	831.36	1.56	548.18	0.96	Highest stocking density
161	1.63	845.00	1.56	556.81	0.96	Highest stocking density
162	1.52	857.96	1.46	565.01	0.96	Highest stocking density
163	1.52	871.12	1.46	573.32	0.96	Highest stocking density

Days of feeding	Best-case (bc) SGR_{bc}	Best-case (bc) $W_{final} = e^{(SGRbc/\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) $SGR_{ppc} = SGR_{bc} \times Y$	Poor production conditions (ppc) $W_{final} = e^{(SGRppc\ /\ 100\ x\ t\ +\ ln(Winitial))}$	Y-factor	Comment to Y-factor
164	1.52	884.48	1.46	581.76	0.96	Highest stocking density
165	1.52	898.04	1.46	590.33	0.96	Highest stocking density
166	1.52	911.82	1.46	599.01	0.96	Highest stocking density
167	1.52	925.80	1.46	607.83	0.96	Highest stocking density
168	1.52	940.00	1.46	616.78	0.96	Highest stocking density
169	1.44	953.67	1.39	625.39	0.96	Highest stocking density
170	1.44	967.55	1.39	634.12	0.96	Highest stocking density
171	1.44	981.62	1.39	642.97	0.96	Highest stocking density
172	1.44	995.90	1.37	651.86	0.95	*Highest stocking density
173	1.44	1010.39	1.37	660.86	0.95	*Highest stocking density
174	1.44	1025.09	1.37	669.99	0.95	*Highest stocking density
175	1.44	1040.00	1.37	679.25	0.95	*Highest stocking density
176	1.36	1054.26	1.29	688.09	0.95	*Highest stocking density
177	1.36	1068.71	1.29	697.05	0.95	*Highest stocking density
178	1.36	1083.36	1.29	706.12	0.95	*Highest stocking density
179	1.36	1098.21	1.28	715.22	0.94	Highest stocking density
180	1.36	1113.27	1.28	724.43	0.94	Highest stocking density
181	1.36	1128.53	1.28	733.76	0.94	Highest stocking density
182	1.36	1144.00	1.28	743.21	0.94	Highest stocking density
183	1.28	1158.71	1.20	752.19	0.94	Highest stocking density
184	1.28	1173.60	1.20	761.28	0.94	Highest stocking density
185	1.28	1188.69	1.20	770.47	0.94	Highest stocking density
186	1.28	1203.97	1.20	779.78	0.94	Highest stocking density
187	1.28	1219.45	1.20	789.20	0.94	Highest stocking density
188	1.28	1235.12	1.20	798.73	0.94	Highest stocking density
189	1.28	1251.00	1.20	808.38	0.94	Highest stocking density
190	1.20	1266.15	1.13	817.58	0.94	Highest stocking density
191	1.20	1281.49	1.13	826.88	0.94	Highest stocking density

Days of feeding	Best-case (bc) SGR_{bc}	Best-case (bc) $W_{final} = e^{(SGRbc/\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) $SGR_{ppc} = SGR_{bc} \times Y$	Poor production conditions (ppc) $W_{final} = e^{(SGRppc\ /\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) Y-factor	Poor production conditions (ppc) Comment to Y-factor
192	1.20	1297.01	1.13	836.30	0.94	Highest stocking density
193	1.20	1312.72	1.13	845.81	0.94	Highest stocking density
194	1.20	1328.62	1.13	855.44	0.94	Highest stocking density
195	1.20	1344.71	1.13	865.18	0.94	Highest stocking density
196	1.20	1361.00	1.13	875.02	0.94	Highest stocking density
197	1.13	1376.46	1.06	884.36	0.94	Highest stocking density
198	1.13	1392.10	1.06	893.81	0.94	Highest stocking density
199	1.13	1407.92	1.06	903.35	0.94	Highest stocking density
200	1.13	1423.91	1.06	912.99	0.94	Highest stocking density
201	1.13		1.06	922.74	0.94	Highest stocking density
202	1.13		1.06	932.59	0.94	Highest stocking density
203	1.13		1.06	942.55	0.94	Highest stocking density
204	1.08		1.02	952.19	0.94	Highest stocking density
205	1.08		1.02	961.93	0.94	Highest stocking density
206	1.08		1.02	971.78	0.94	Highest stocking density
207	1.08		1.02	981.72	0.94	Highest stocking density
208	1.08		1.02	991.76	0.94	Highest stocking density
209	1.08		1.02	1001.91	0.94	Highest stocking density
210	1.08		1.02	1012.16	0.94	Highest stocking density
211	1.01		0.95	1021.86	0.94	Highest stocking density
212	1.01		0.95	1031.66	0.94	Highest stocking density
213	1.01		0.95	1041.55	0.94	Highest stocking density
214	1.01		0.95	1051.53	0.94	Highest stocking density
215	1.01		0.95	1061.61	0.94	Highest stocking density
216	1.01		0.95	1071.79	0.94	Highest stocking density
217	1.01		0.95	1082.06	0.94	Highest stocking density
218	0.97		0.91	1091.99	0.94	Highest stocking density
219	0.97		0.91	1102.00	0.94	Highest stocking density

Days of feeding	Best-case (bc) SGR_{bc}	Best-case (bc) $W_{final} = e^{(SGRbc/\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) $SGR_{ppc} = SGR_{bc} \times Y$	Poor production conditions (ppc) $W_{final} = e^{(SGRppc\ /\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) Y-factor	Poor production conditions (ppc) Comment to Y-factor
220	0.97		0.74	1110.16	0.76	*Highest stocking density
221	0.97		0.74	1118.39	0.76	*Highest stocking density
222	0.97		0.74	1126.67	0.76	*Highest stocking density
223	0.97		0.74	1135.02	0.76	*Highest stocking density
224	0.97		0.74	1143.43	0.76	*Highest stocking density
225	0.92		0.70	1151.48	0.76	*Highest stocking density
226	0.92		0.70	1159.60	0.76	*Highest stocking density
227	0.92		0.70	1167.77	0.76	*Highest stocking density
228	0.92		0.70	1176.00	0.76	*Highest stocking density
229	0.92		0.70	1184.28	0.76	*Highest stocking density
230	0.92		0.70	1192.63	0.76	*Highest stocking density
231	0.92		0.70	1201.03	0.76	*Highest stocking density
232	0.88		0.67	1209.08	0.76	*Highest stocking density
233	0.88		0.67	1217.17	0.76	*Highest stocking density
234	0.88		0.67	1225.32	0.76	*Highest stocking density
235	0.88		0.67	1233.53	0.76	*Highest stocking density
236	0.88		0.67	1241.79	0.76	*Highest stocking density
237	0.88		0.67	1250.10	0.76	*Highest stocking density
238	0.88		0.67	1258.47	0.76	*Highest stocking density
239	0.88		0.67	1266.90	0.76	*Highest stocking density
240	0.88		0.67	1275.38	0.76	*Highest stocking density
241	0.88		0.67	1283.92	0.76	*Highest stocking density
242	0.88		0.67	1292.52	0.76	*Highest stocking density
243	0.88		0.67	1301.18	0.76	*Highest stocking density
244	0.88		0.67	1309.89	0.76	*Highest stocking density
245	0.88		0.67	1318.66	0.76	*Highest stocking density
246	0.88		0.67	1327.49	0.76	*Highest stocking density
247	0.88		0.67	1336.38	0.76	*Highest stocking density

Days of feeding	Best-case (bc) SGR_{bc}	Best-case (bc) $W_{final} = e^{(SGRbc/\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) $SGR_{ppc} = SGR_{bc} \times Y$	Poor production conditions (ppc) $W_{final} = e^{(SGRppc\ /\ 100\ x\ t\ +\ ln(Winitial))}$	Poor production conditions (ppc) Y-factor	Poor production conditions (ppc) Comment to Y-factor
248	0.88		0.67	1345.33	0.76	*Highest stocking density
249	0.88		0.67	1354.34	0.76	*Highest stocking density
250	0.88		0.67	1363.40	0.76	*Highest stocking density
251	0.88		0.67	1372.53	0.76	*Highest stocking density
252	0.88		0.67	1381.72	0.76	*Highest stocking density
253	0.88		0.67	1390.98	0.76	*Highest stocking density
254	0.88		0.67	1400.29	0.76	*Highest stocking density
255	0.88		0.67	1409.67	0.76	*Highest stocking density
256	0.88		0.67	1419.11	0.76	*Highest stocking density
257	0.88		0.67	1428.61	0.76	*Highest stocking density
258	0.88		0.67	1438.17	0.76	*Highest stocking density
259	0.88		0.67	1447.81	0.76	*Highest stocking density
260	0.88		0.67	1457.50	0.76	*Highest stocking density
261	0.88		0.67	1467.26	0.76	*Highest stocking density

With an initial fish weight of 0.0025 g on day 1 of feeding, SGR_{bc}: ideal specific growth rate under best-case conditions, SGR_{ppc}: specific growth rate under poor production conditions, W_{final}: final weight after a certain day of feeding, $W_{initial}$: initial weight on certain day of feeding = final weight of the day before, Y-factor: factor with which the SGR_{bc} can be adjusted to SGR_{ppc} influenced by a certain parameter (first feed, stocking density, water temperature, ammonium, or nitrite concentration), *: Y factor calculated via the mean value of 2 factors in overlapping size classes.