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Stress and Welfare of African Catfish (*Clarias gariepinus* Burchell, 1822) in a Coupled Aquaponic System

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Abstract: African catfish (*Clarias gariepinus*) were investigated for physiological stress parameters to assess the influence of different cultivation techniques on fish welfare. The fish were reared in two conceptually identical recirculating aquaculture systems (RAS) constructed in a greenhouse. One system was combined with a floating raft hydroponic unit to culture cucumbers (*Cucumis sativus*) only using the process water. The second system remained without the plant cultivation unit and functioned as the control. Water quality was monitored regularly by measuring dissolved nutrients and physical water parameters. During 87 days, a total of 71 fish from each system (n = 142) were studied, with a final sampling of all fish (n = 107) at the end of the experiment. Blood from the caudal vein was analyzed for the stress hormone cortisol and the glucose concentration. In addition, fish were investigated for external injuries on their skin, fins and barbels. The results demonstrate that the system design had no influence on fish mortalities and growth rates. Furthermore, cortisol and blood glucose levels did not differ significantly between the two systems. However, the number of external injuries was significantly lower in the aquaponics, providing evidence that co-cultivation of fish and plants might offer benefits to the welfare of the fish.

Keywords: African catfish; animal welfare; aquaponics; cortisol; glucose; skin lesions

1. Introduction

The rapidly-growing human population [1] with an increasing demand for food supply makes aquaculture the fastest-growing sector in the agricultural industry. To avoid negative impact to the environment, modern integrated aquaculture systems provide a maximum output of fish products with a minimum release of nutrients and waste water. Aquaponics is the combination of aquaculture and hydroponics that uses the process waters from aquaculture to provide valuable nutrients for plant cultivation [2,3]. Fish reared in aquaponic systems provide a number of fertilizing components [4], for example various nitrogen compounds and phosphorus, which usually are discharged with the waste water.

The fish most frequently kept in aquaponics are cyprinids, tilapia and catfish. The global production of African catfish (*Clarias gariepinus* Burchell, 1822) in 2012 reached 181,601 t [5]. Recirculation systems for this species including aquaponics have developed since the 1980s in Europe [6] and also in Mecklenburg-Pomerania, Germany. *C. gariepinus* is easy to cultivate because of its resilience and high tolerance level for suboptimal environmental conditions, e.g., high ammonium and nitrite concentrations [7–9]. Based on their specialized arborescent organ near the branchial arches, they are able to breathe atmospheric oxygen as an adaptation to oxygen-deficiency. They grow quickly

and have an effective feed conversion ratio (FCR). However, it remains uncertain to what extent the aquaponic environment influences the wellbeing of this fish species [10].

Animal welfare principles applied to other farmed animals have not yet deeply been extended to fish, and there remains a general lack of scientific information on fish welfare [11,12]. However, consumers and consequently fish farmers are increasingly aware of the health and 'happiness' of reared animals [13]. Fish welfare depends on various abiotic factors, such as the water quality or temperature, and biotic factors, such as stocking density, parasites and pathogens, generic behavior, intraspecific aggression, body condition, different physiological parameters, food quality and distribution, growth performance, as well as on anthropogenic influences, such as sorting and handling [14–17]. The different fish species, as well as their respective developmental stages differ in their need for specific environmental conditions and husbandry.

Especially physiological parameters such as stress hormones, metabolites together with the external body condition are often considered for welfare assessments. Measures of the stress response, in particular stress hormones, can be used as an indicator of environmental quality and how well an organism is coping in this environment [18,19]. If stress exceeds the homeostatic state, it most likely negatively influences the wellbeing of fish [20]. Once an organism is exposed to a stressor, the hypothalamus produces corticotropin-releasing hormone (CRH) that stimulates the anterior pituitary gland to emit adrenocorticotropic hormone (ACTH). When ACTH is transported to the adrenal cortex (or in the case of fish: the interrenal cells of the head kidney), cortisol is released [18,21]. It is the final product of the hypothalamic-pituitary-interrenal axis (HPI axis) and is also responsible for feedback interactions between the involved endocrine glands. Alongside with adrenaline and norepinephrine, cortisol is one of the most important stress hormones. The stress response prepares the body for a stressful situation; for example, it mobilizes and (re)directs energy reserves so that the organism can perform optimally under conditions that take the animal out of homeostasis [22]. However, there are various factors that alter cortisol levels in addition to stress, including the sex of the fish, maturity level, season, time of day or feeding condition [9,19,23].

In this study, different parameters reflecting the welfare status of *C. gariepinus* from an experimental recirculating aquaculture system (RAS) compared to a RAS coupled with a hydroponic unit were investigated (Figure 1). Cortisol as the primary stress hormone and indicator for long-term stress, as well as blood glucose levels as an important metabolic factor [18,24] were surveyed. In addition, external injuries of body, fins and barbels were assessed since they appear often on scaleless fish in aquaculture [16] and are therefore most likely a useful indication for the stress and welfare of African catfish [25].

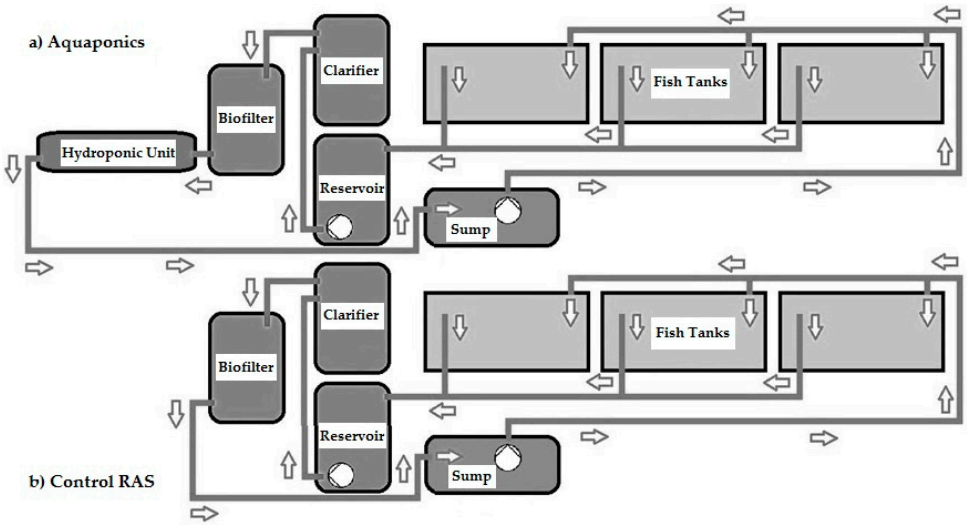


Figure 1. Schematic arrangement and water flow of (**a**) aquaponics and (**b**) control recirculating aquaculture system (RAS).

2. Materials and Methods

2.1. Experimental Systems

Two conceptually-identical RAS were constructed in a greenhouse; one with a floating raft hydroponic unit, the other without. The RAS without the hydroponic unit functioned as the control (Figure 1).

Both systems consisted of three separate 250-L fish-rearing tanks (Length/Width/High = 100 × 50 × 50 cm) organized as triplicate groups without randomization. The tanks were isolated with Styrofoam plates on the sides, the back and the bottom against noise and vibrations. Tanks were covered with acrylic glass plates preventing the fish from escaping. Since the control tanks were installed below the aquaponic tanks, the incoming daylight intensity was adjusted by shading the lids on the aquaponic tanks to provide a similar influence of daylight, which is known to possibly affect welfare [26,27]. Light intensity was measured with a digital luxmeter (Dr. Meter, LX1330B, spectral, range: 0.1–200.000 lux) close above the water surface. The measured surface lux values after shading ranged between 410 and 1060 lux in both RAS with respect to the weather conditions. All tanks were aerated with a single air pump (Aqua Medic Mistral 4000, Bissendorf, Germany). Tower outlets delivered the tank surface water into a reservoir. With an adjustable pump (Eheim compact+ 5000, Deizisau, Germany), the water was transferred into a custom-made clarifier consisting of a rain barrel equipped with a filter pad inside (1 m^2, 3 cm diameter, coarsely structured), bent sickle shaped to slow down water movement. The process water continued flowing by gravity into a moving bed filter with approximately 100 L of floating filter medium (Helix Biocarrier, diameter/height: 17 mm/15 mm, surface: 720 m^2/m^3, protected surface: 589 m^2/m^3), aerated and moved with an air pump (Nitto Kohki LA-120A, Steinenbronn, Germany). The moving bed filter temperature was stabilized with a heater (Tetra Tec HT 300, Melle, Germany).

In the control RAS, the water was collected directly behind biofiltration within a sump and pumped back into the fish rearing tanks using a second adjustable pump (Eheim compact+ 5000). In the experimental coupled aquaponic system, the water continued flowing by gravity into the hydroponic unit, consisting of two floating raft trays with a mud cavern and screen plate (250 × 50 × 30 cm each, AGK Kronawitter, Wallersdorf, Germany). The combined process water from both hydroponic basins was directed into a sump and returned with the second pump (Eheim compact+ 5000) into the fish tanks. Valves were installed in different positions to control water flow. Total water volume was 2120 L in the aquaponic system (including 750 L inside both hydroponic basins) and 1370 L in the control system. Accumulated sludge (approximately 1 L) from the biofilters and clarifiers was flushed once a week.

The experiment was conducted for 87 days (14 June–8 September 2014), with a daily control of system functionality. Algae were removed regularly from the glass panes of the tanks.

2.2. Process Water

Water parameters were measured daily inside the reservoir with a portable two-channel multimeter (Hach-Lange HQ40D, Düsseldorf, Germany), including temperature, pH, electrical conductivity (EC), dissolved oxygen (DO) and oxygen saturation. The pH was buffered with 5 kg oyster shells in each system located inside the reservoir. Other parameters such as total ammonium nitrogen (TAN, NH_4^+N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) were analyzed once a week. Water samples were filtered over glass fiber filter pads (Whatman, GF6, diameter: 47 mm, Maidstone, U.K.), using Hach Permachem Reagents (NitraVer 5 Nitrate Reagent, NitriVer 3 Nitrite Reagent, Ammonia Salicylate Reagent, Düsseldorf, Germany) and analyzed with a spectral photometer (Hach Lange DR 3900, Düsseldorf, Germany) at 500 nm for NO_3, at 507 nm for NO_2 and at 655 nm for NH_4. Water turbidity was measured with the spectral photometer (Hach Lange DR 3900), with an extinction at different wavelengths (320 nm, 450 nm, 600 nm and 750 nm) at the end of the experiment.

There was no water exchange in both systems, and only evaporated water was refilled once a week to the same amount in both RAS.

2.3. Hydroponics

Cucumber plants (*Cucumis sativus*, Kiluna F1) were chosen for the hydroponic unit because of their fast growth and notable nutrient uptake [28]. Ten plants were germinated on rock wool cubes (L:W:H = 7 × 7 × 6 cm, Grodan Delta, Roermond, The Netherlands) and fertilized with a nutrient solution (Scotts/Everris, Universol Orange, Geldermalsen, The Netherlands). Seedlings were transferred after two weeks (with a size of approximately 10 cm and two foliage leafs) onto floating rafts of polystyrene granting direct root contact with the process water. Each plant unit was vented at three points supporting nitrification on the roots and optimal plant growth [4]. The cucumber stems were attached to cords with plant clips. During plant proliferation, side branches were cut regularly. Stem length and leaf area were measured once a week. The leaf area was calculated after Cho et al. [29], with the formula:

$$LA = 0.88\ L{\cdot}W - 4.27.$$

LA: Leaf area; L: Leaf length; W: Leaf width

After the experiment, roots were cut from each stem base, dried in a compartment dryer (Memmert UF750plus, Schwabach, Germany) for 24 h at 60 °C, and the total dry weight of all produced root material was measured (Shimadzu UW6200H, 0.5–620 g, Kyoto, Japan). Fruit biomass production was recorded on a wet weight basis.

2.4. Fish Cultivation

Fish were obtained from a local fish farmer (PAL GmbH, Abtshagen, Germany). Before stocking, the fish were measured and weighed (Shimadzu UW6200H, 0.5–620 g). The average starting size was 31.45 cm (±1.95 cm) of body length and 214.42 g (±6.17 g) of body weight. Each of the three tanks per system was then stocked with 18 *C. gariepinus* (in total 54 fish per system), which equals a stocking density of 15.4 kg/m^3 (per tank). Genders were distributed randomly. Within the daily feeding regime, 25 g of sinking trout feed pellets (Skretting Optiline 3P, Stavanger, Norway) with an adequate nutritional composition for *C. gariepinus* were given by hand at 9:00 a.m. To avoid disturbance regarding the physiological data collection, the feeding regime was not adjusted, even in the case of fish loss. The feed conversion ratio (FCR), the specific growth rate (SGR), as well as the daily growth rate (DGR) were calculated after the end of the experiment.

2.5. Sampling and Data Analyses

Specimens were taken at Days 6, 11, 13, 40, 41, 42, 68, 69, 73 and 74. From each of the three tanks of both systems, small groups of 3–5 fish (n = 9–15) were sampled on those days. The netting was accomplished quickly, but carefully minimizing netting stress of other fish and the increase of cortisol as a consequence (baseline sampling). The fish were caught from 10:00–18:00 well after morning feeding time, to avoid changes to hormone levels caused by adaption to the feeding period [19]. On Days 87–89, the experiment was ended, and all fish (n = 107) were sampled again for the respective parameters. Before blood sampling, the fish were directly placed into a narcotic bath (eugenol: 50 mg L^{-1}) until they were completely anaesthetized (loss of reactivity and all reflexes).

The gender and possible external body injuries were counted. This was conducted by the same two trained persons on every sampling day. All fish were treated independently, and if it was unclear whether a wound should be counted or not, these two persons made a decision. The documentations included only fresh excoriations that were not already in the healing process, i.e., secondary skin lesions (Figure 2, A) or larger fin and barbel damages, but no wounds with renewed mucus or epidermal abrasions (Figure 2, B) or restored skin structures with the regeneration of a new basement membrane cell layer [30]. Furthermore, we did not differentiate between the various lesion sources, e.g., ulcerative skin lesions or those caused by intraspecific competitions and fights (Figure 3).

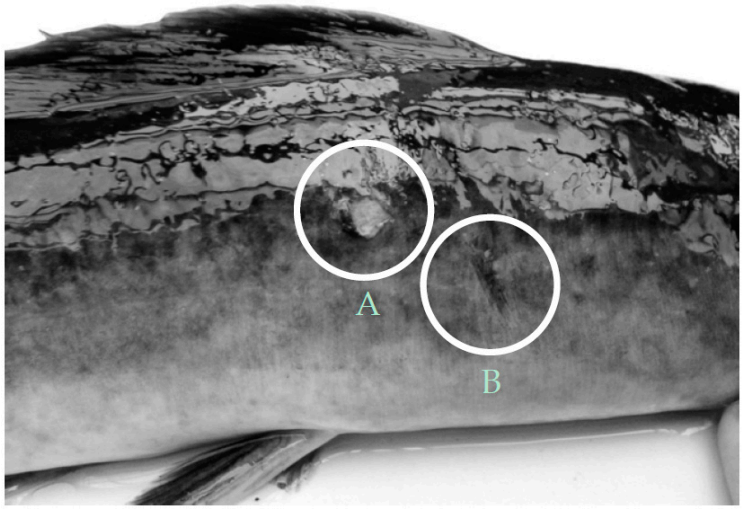
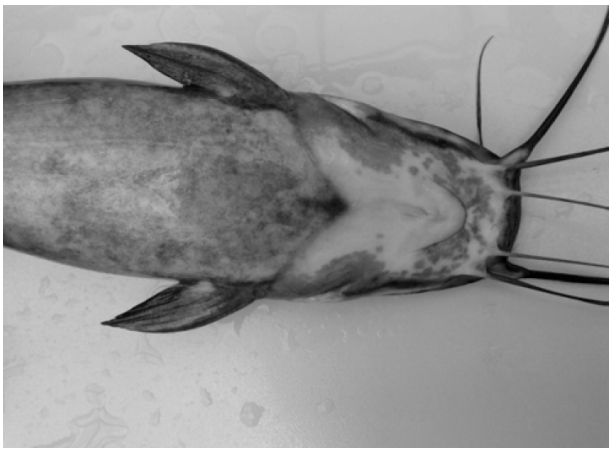


Figure 2. Heteromorphic skin injuries of *C. gariepinus*; A: penetrating wound reaching down to the underlying tissue; B: non-penetrating wound (abrasion). Photo processed with amplified contrast (+20%).

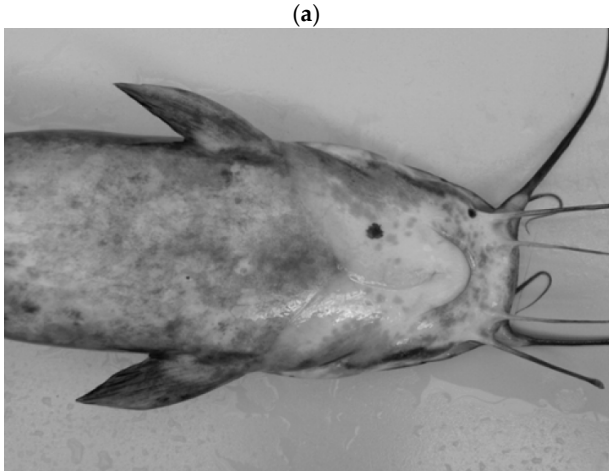
Figure 3. Skin damage with obvious teeth marks and the typical jaw form of *C. gariepinus*, the result of an intraspecific fight. Photo processed with amplified contrast (+20%).

About 0.5 mL blood were taken with a syringe (B Braun 2 mL; B Braun disposable hypodermic needle, Ø 0.45 × 25 mm/26 G × 1″) from the caudal blood vessels. These treatments were carried out in accordance with the EU guidelines for animal experiments and were approved by the responsible ethics committee (Reference 7221.3-1-005/14). Blood glucose was measured immediately with an Accu Check Aviva (Roche Diabetes Care, Mannheim, Germany) in mmol L^{-1}. Remaining blood was transferred to cuvettes prepared with a coagulation inhibitor (BD Vacutainer EDTA 2 mL, PET).

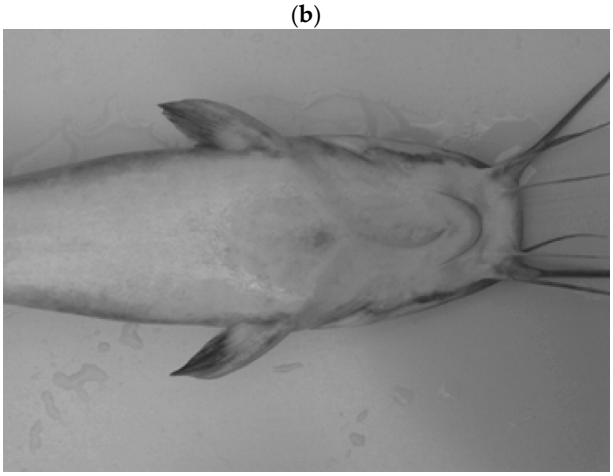
Photos of the ventral side were taken of each sampled fish to distinguish individuals by their distinct skin patterns (Figure 4). This was done in order to avoid repetitive sampling of the same specimens (pseudo-replication) on ensuing sampling days. This noninvasive method guaranteed the meaning of cortisol baseline sampling and can be recommended for *C. gariepinus*.



(**a**)



(**b**)



(**c**)

Figure 4. (**a**–**c**) Examples of individual differences in skin patterns of *C. gariepinus*.

Each fish was placed in a recovery tank, and after regaining all reflexes, they were put back into their respective aquaria from which they were caught. In the meantime, blood samples were stored on ice and subsequently centrifuged (Hettich Universal 320 R, Tuttlingen, Germany) with 1500 *g* at 4 °C for 10 min to separate plasma and cells. The plasma was stored at −80 °C, and cells were discarded. A sandwich-ELISA for fish cortisol (detection minimum: 0.0023 ng mL^{-1}, Cusabio, MD, USA) was conducted with a microplate reader (BioTek Synergy HT, Winooski, VT, USA) at 450 nm to determine cortisol levels.

2.6. Statistical Analyses

IBM SPSS Statistics 22 was used for all statistical analyses. For both the samplings in small groups over the entire experiment, as well as the big sampling at the end of the experiment (cf. Section 2.5), the stress parameters (cortisol, blood glucose and external injuries) were checked individually for a normal distribution using Kolmogorov–Smirnov (significance corrected after Lilliefors). Then, if normally distributed, an unpaired *t*-test and, if not, the non-parametric Whitney-Mann U-test were applied. The fish growth and the water quality parameters were analyzed as just described for the stress parameters except the water turbidity, which was tested through a Kruskal–Wallis-test for independent non-parametric samples.

3. Results

3.1. System Characteristics and Water Quality

The water parameters nitrite-nitrogen and temperature were not significantly different ($p > 0.05$) in both systems throughout the entire experiment (Table 1). Nitrate-nitrogen, TAN, oxygen, pH and EC showed significant differences ($p < 0.05$) between the treatments.

Table 1. Mean values ± SEM of water quality parameters; * significance ($p < 0.05$). EC, electric conductivity; TAN, total ammonium nitrogen.

System	DO (mg L^{-1})	O_2 (%)	pH	Temperature (°C)	EC (µS cm^{-1})	TAN (mg L^{-1})	Nitrite-N (mg L^{-1})	Nitrate-N (mg L^{-1})
Aquaponics	8.04 * ± 0.05	95.87 * ± 0.25	7.61 * ± 0.03	24.21 ± 0.29	620.74 * ± 8.50	0.91 * ± 0.26	0.06 ± 0.01	29.42 * ± 4.15
Control	7.79 * ± 0.05	93.53 * ± 0.31	6.60 * ± 0.07	24.50 ± 0.51	934.84 * ± 26.31	4.96 * ± 1.33	0.09 ± 0.02	56.22 * ± 7.04

Nitrate-N, nitrite-N and TAN were reduced to a greater extent under the aquaponic conditions (Table 1, Figures 5–7).

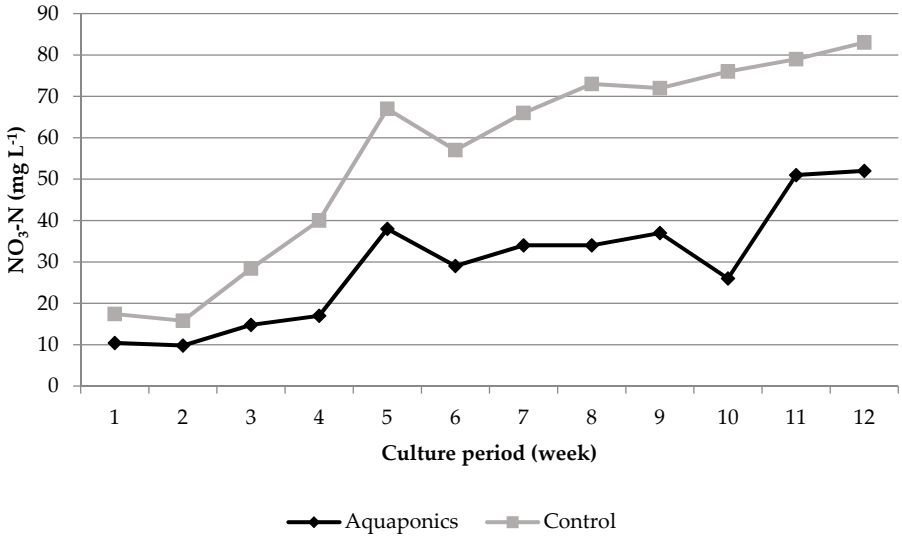


Figure 5. Temporal changes of nitrate (NO_3-N) over the experimental period of 12 weeks.

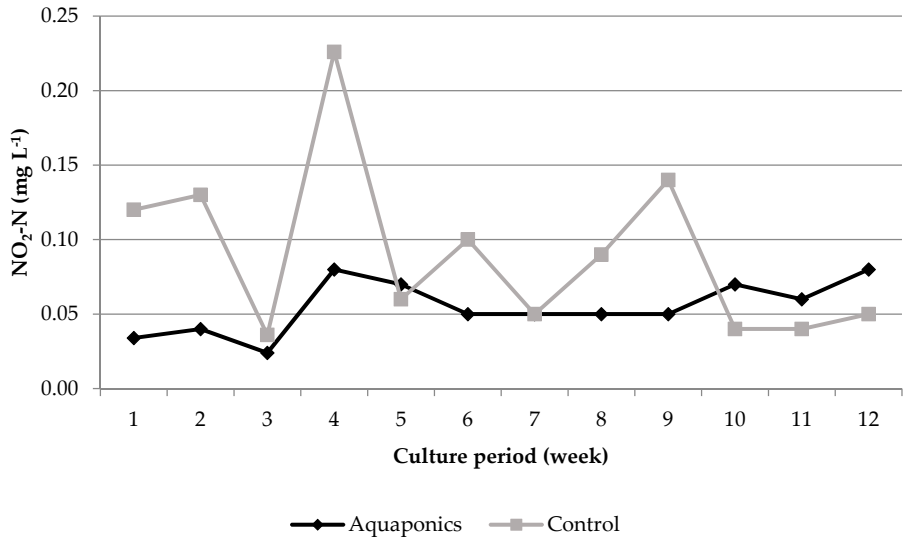


Figure 6. Temporal changes of nitrite (NO_2-N) over the experimental period of 12 weeks.

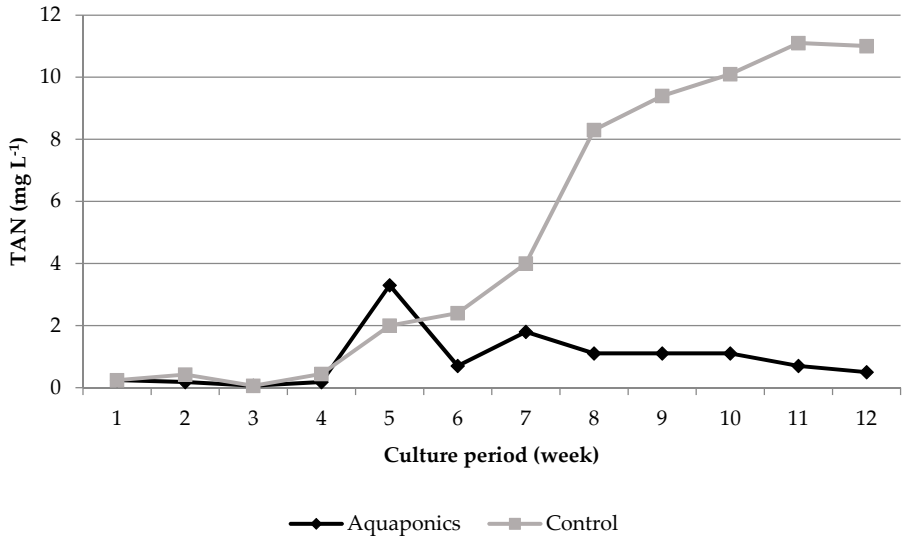


Figure 7. Temporal changes of total ammonium nitrogen over the experimental period of 12 weeks.

Particularly during the last days of the experiment, TAN reached higher levels in the control RAS (Figure 7). The temperature varied over the experiment, but it was mostly between 22 and 27 °C in both RAS (Figure 8). Oxygen was usually approximately 0.2 mg L^{-1} lower in the control system (Figure 9). There, the starting pH-value of 8.3 fell more rapidly than inside the aquaponic system and levelled off in the range from 6 and 6.6, while it fell more slowly and remained at approximately 7.5 in the aquaponic system (Figure 10). The oyster shells could not buffer the pH sufficiently in the control system. EC increased relatively quickly in the control system. It started at about 500 µS cm^{-1} and ended at 768 µS cm^{-1} in the aquaponic system and at 1286 µS cm^{-1} in the control system (Figure 11).

Measurements of water turbidity (extinction) revealed significant differences ($p < 0.05$) between the treatment groups at each tested wavelength (Table 2).

Table 2. Mean values ± SEM of water turbidity extinctions for both systems at different wavelengths; * statistical significance ($p < 0.05$).

System	320 nm	450 nm	600 nm	750 nm
Aquaponics	0.313 * ± 0.007	0.064 * ± 0.003	0.025 * ± 0.002	0.015 * ± 0.002
Control	0.561 * ± 0.001	0.112 * ± 0.002	0.036 * ± 0.002	0.020 * ± 0.002

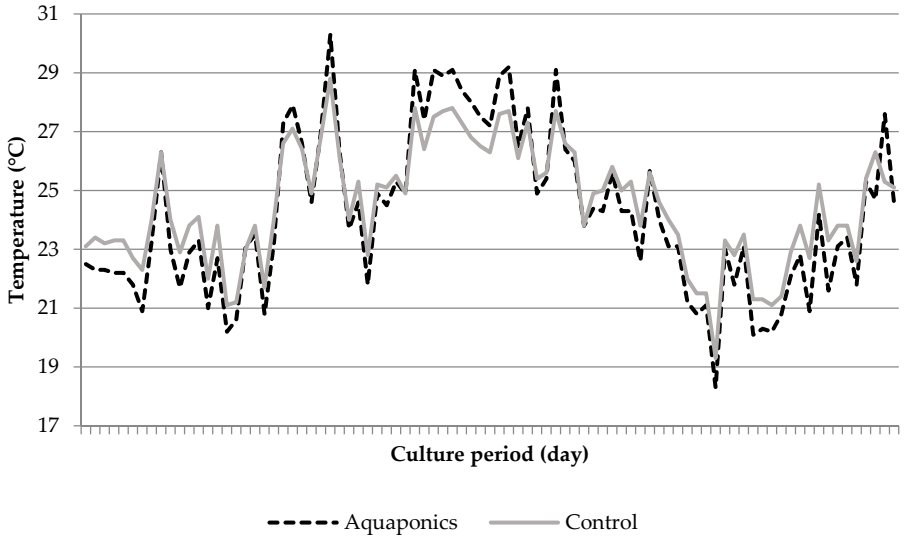


Figure 8. Temporal changes of temperature over the experimental period of 12 weeks.

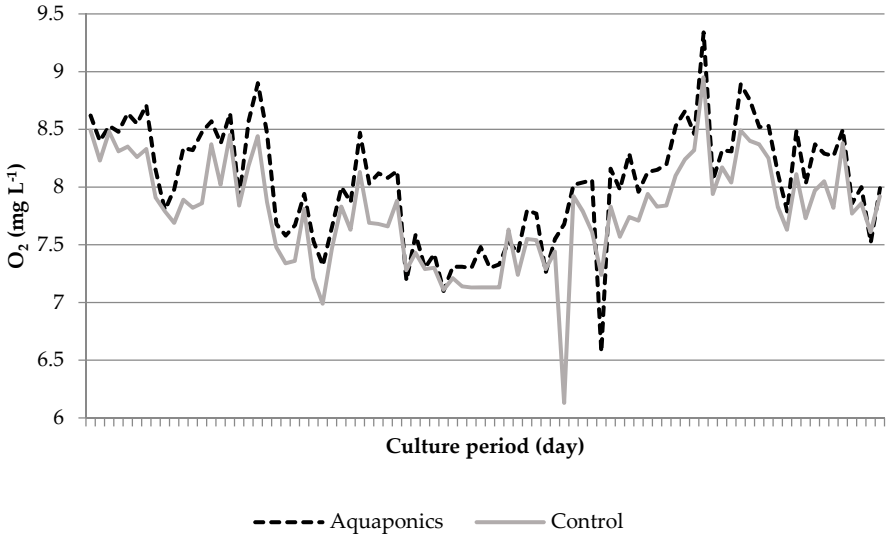


Figure 9. Temporal changes of dissolved oxygen (DO) over the experimental period of 12 weeks.

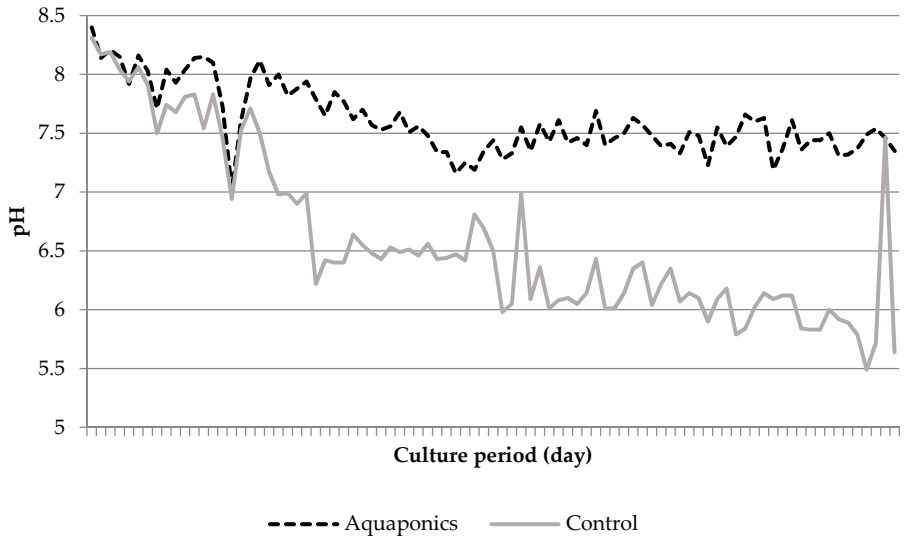


Figure 10. Temporal changes of the pH-value over the experimental period of 12 weeks.

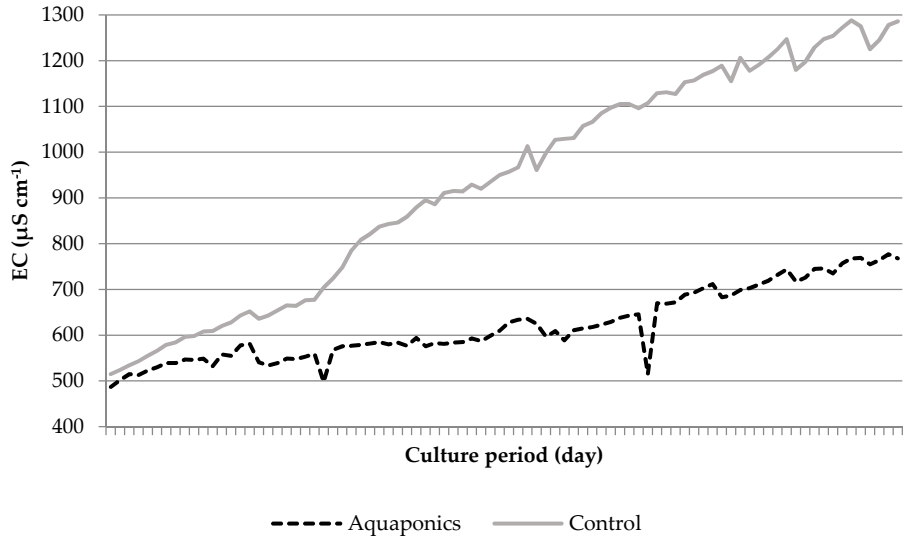


Figure 11. Temporal changes of the electric conductivity over the experimental period of 12 weeks.

3.2. Plant Growth

During the experiment, the cucumber plants sprouted up to an average stem length of 445.6 cm (±39.71 cm), while the leaf area grew to an average of 800.29 cm^2 (±74.82 cm^2). The roots (Figure A1) reached a total dry weight of 137 g. Twenty two-point-zero seven kilograms of fruits (fresh weights) were harvested in 87 days. Slightly yellowish brown laminas at some leaf margins with a darker venation were observed.

3.3. Fish Growth

Mortality during the experiment was low. Within the aquaponics, no fish died, and in the control RAS, only one single loss was observed after six weeks; the cause of death was unclear. Both treatment groups grew to comparable sizes. There were no significant differences ($p > 0.05$) between both treatment groups. The growth data and the calculated FCR, SGR and DGR are shown in Table 3.

Table 3. Performance (±SEM) of the African catfish in the experimental culture systems.

System	Initial Weight (g/Fish) [a]	Final Weight (g/Fish) [a]	Initial Length (cm/Fish) [a]	Final Length (cm/Fish) [a]	FCR [b]	SGR [c]	DGR (g/Fish/Day) [d]
Aquaponics	214.42 ± 5.95	333.33 ± 8.61	31.45 ± 1.39	35.17 ± 1.78	1.02	0.48	1.37
Control		327.58 ± 9.10		34.91 ± 1.71	1.09	0.41	1.30

Note: [a] Initial weight/length as the average of 33 fish before stocking; final weight/length as the average of 33 fish per system; [b] feed conversion rate calculated as feed conversion ratio (FCR) = total weight of feed given/total wet weight gain; [c] specific growth rate calculated as specific growth rate (SGR) = $(\ln W_f - \ln W_i \times 100)/t$; [d] daily growth rate calculated as daily growth rate (DGR) = weight gained by fish/culture days.

3.4. Stress Responses

The chronic stress response based on the rearing conditions showed no significant differences between the treatment groups. Cortisol levels were almost the same, even between the different genders in the same group. Most fish had cortisol levels between 10 and 12 ng mL^{-1} (Figure 12). The mean cortisol levels at the end of the experiment were slightly increased in the RAS with 14.7 ng mL^{-1} and in the aquaponics with 12.7 ng mL^{-1}. Larger temporal changes of the cortisol response were not detected (cf. Table A1).

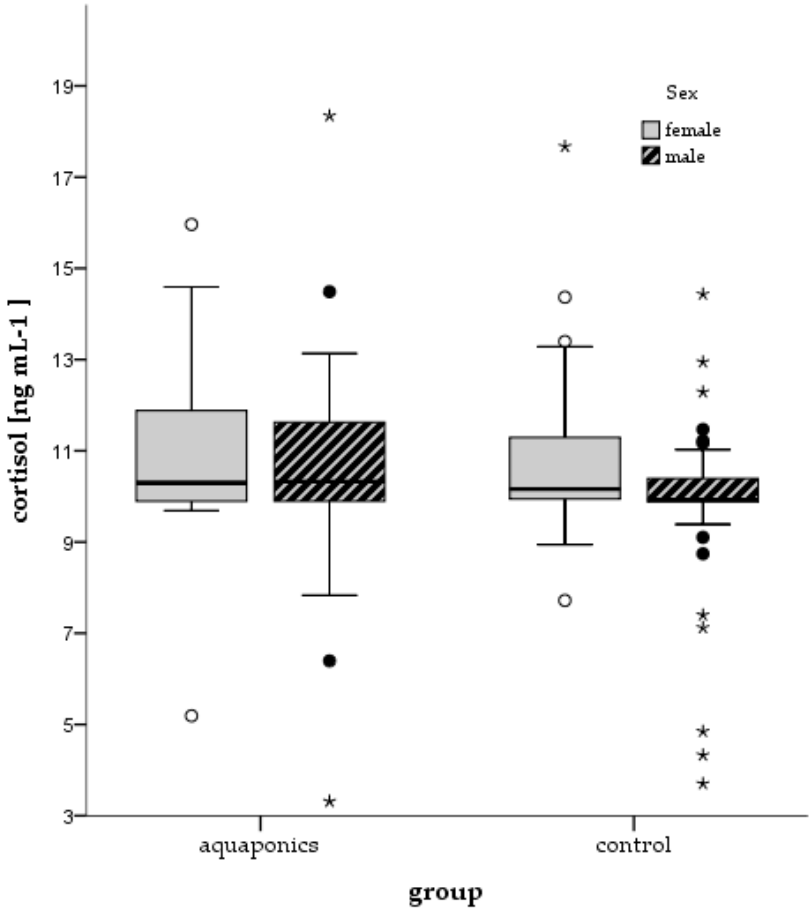


Figure 12. Cortisol levels of African catfish (*C. gariepinus*) in different cultivation groups, subdivided by genders. Illustrated is the sampling in small groups over the entire experiment. Outliers: ○/●; extrem values: *.

The metabolic responses showed no significant differences between the treatment groups. Most fish had a blood glucose level between 2.0 and 3.0 mmol L^{-1}. The males tended to have a slightly higher

blood glucose level than the females in each group. A few bigger males had blood glucose values up to 8.5 mmol L^{-1} (Figure 13). The mean glucose level at the end of the experiment was 3.2 mmol L^{-1} in both systems. There were only minor temporal increases over the experiment (cf. Table A1).

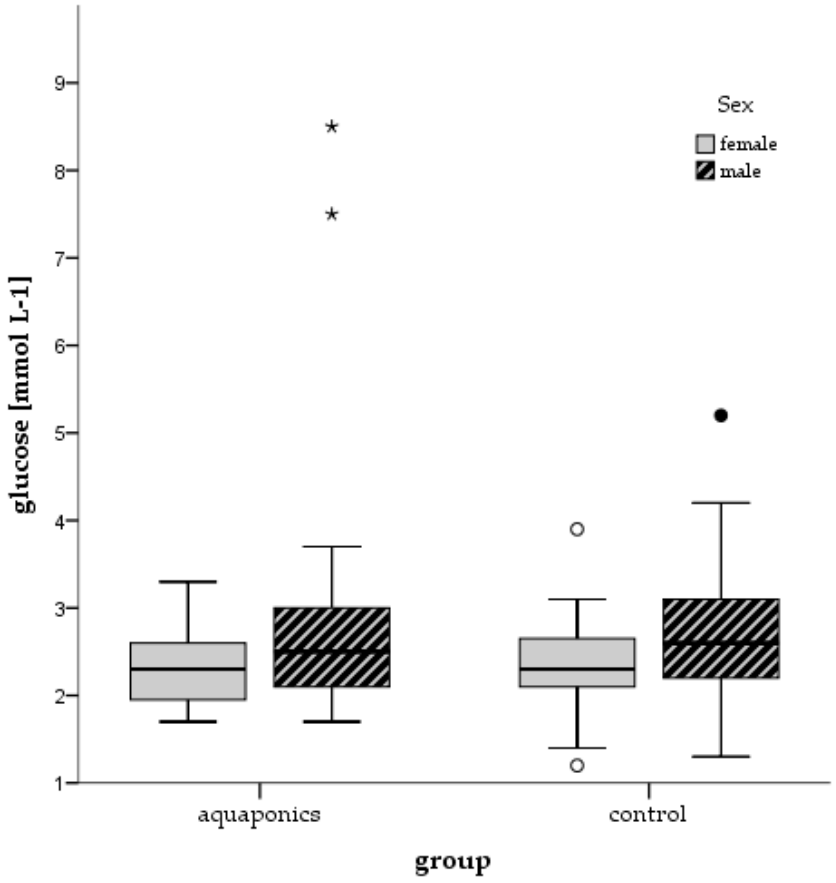


Figure 13. Blood glucose levels of African catfish (*C. gariepinus*) in different cultivation groups, subdivided by genders. Illustrated is the sampling in small groups over the entire experiment. Outliers: ○/●; extrem values: *.

The number of external injuries per individual fish differed significantly between the two treatment groups in both sampling methods (cf. Section 2.5). The aquaponic group showed more fish with an entirely healthy skin with no injuries and flawless fins and barbels, whereas the control group included more fish with one or two skin lesions or flawed fins and barbels within the sampling of small groups over the entire experiment (Figure 14). The average number of skin injuries was 1.3 (RAS) and 0.8 (aquaponics). Within the sampling at the end of the experiment, the number of fish with 0, 1, 2, 3 and 4 injuries was 26, 9, 7, 7 and 2 (control RAS) and 43, 10, 1, 0 and 0 (aquaponics), respectively. Therefore, in the final sampling, no fish in either the control RAS or the aquaponics had more than four injuries, with a significant difference $p < 0.05$ (average 1.0 for control RAS and 0.2 for aquaponics). In both sampling methods, more than 95% of the wounds seemed to be the result of intraspecific behavior in both RAS. Variations between the tanks were determined: the sampling of smaller groups over the entire experiment revealed average injury numbers of: 1.04, 0.64 and 0.67 for the three aquaponic tanks, respectively; in the control tanks: 0.72, 1.44 and 1.71, respectively. While the final sampling the aquaponic system revealed 0.22, 0.17 and 0.28 injuries, respectively, in the control system: 0.94, 1.44 and 0.53 injuries were observed, respectively (also, cf. Table A1).

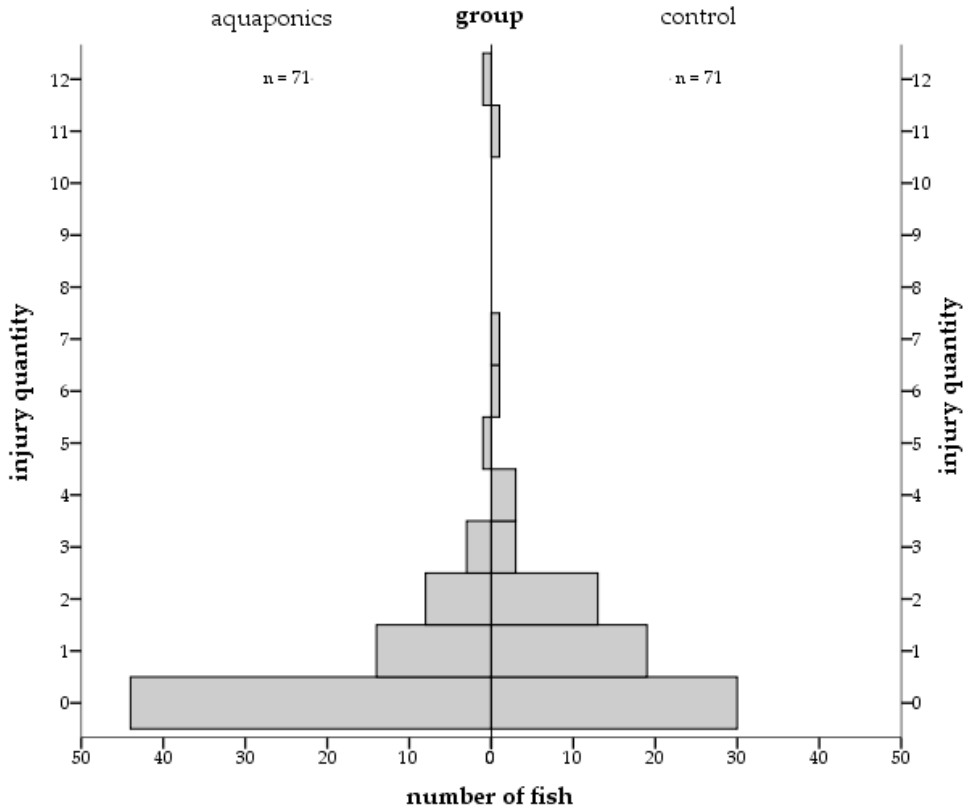


Figure 14. Quantity of external injuries on African catfish (*C. gariepinus*) in different cultivation groups. Illustrated is the sampling in small groups over the entire experiment.

In the first two weeks after stocking, African catfish normally shows a boosted agonistic behavior, which can lead to more external injuries. After that phase, it is normal to see another behavioral pattern: usually intraspecific competitions decrease. In the first two weeks of this study, an average of 1.5 wounds in the aquaponic RAS and 1.1 wounds in the control RAS were determined (based on the first three measurements), while after a month, 1.1 wounds in the aquaponic RAS and 2.4 wounds in the control RAS were found (based on the three continuing measurements). Since this fish species regenerates wounds very quickly (usually within one week), the wounds that occurred in the respective phases will not change the overall results of both treatment groups.

4. Discussion

Comparison of the physiological stress parameters plasma cortisol and blood glucose of African catfish kept in an experimental RAS and a coupled aquaponic system showed no significant differences. On the other hand, the external injuries of the bodies, fins and barbels were significantly lower in the aquaponics, providing evidence for the benefits of combined fish and plant cultivation for fish welfare.

4.1. Water Quality

The water parameters in both systems were within the range found suitable for the cultivation of African catfish during the entire experiment. This is supported by the death of only one fish in the control RAS. *C. gariepinus* is able to withstand adverse water conditions such as nitrite-N between 1 and 3 mg L^{-1}, NH_3-N up to 6 mg L^{-1} or nitrate-N above 200 mg L^{-1} [31], in addition to fairly low DO levels down to 2 mg L^{-1} [32]. According to Eding and Kamstra [33], African catfish even withstand NH_3-N levels up to 8.8 mg L^{-1} and a pH of 6.5–8. The average DO level of about 8 mg L^{-1} during our experiments reflected in fact optimal conditions, supplemented by the air breathing ability of *C. gariepinus*. The relatively high mean TAN-level of almost 5 mg L^{-1} in our control RAS was still in

the tolerance. pH mean values of 6.6 (control RAS) and 7.6 (aquaponics) and an average temperature of >24 °C in both systems were also in the proper range [32]. When comparing the dissociation equilibrium of TAN in relation to the average temperature and pH values, only minor amounts of NH_3 could be detected in the water of both RAS. For the control RAS, we calculated 0.025 and 0.019 mg L^{-1} for the aquaponic RAS, respectively [34]. It can be assumed that these amounts of un-ionized ammonia are relatively harmless to fish [35].

Comparison of the control RAS and the coupled aquaponic system revealed significant differences, resulting in higher EC, ammonium, nitrite and nitrate levels and lower pH and DO in the former. This is a direct consequence of the hydroponic unit within the aquaponics. Though a deep water hydroponic unit always increases the total water volume (here by an additional 750 L), thereby diluting solutes, the plants help to remove harmful substances emanating from the fish. Therefore, the hydroponic unit helped to maintain system stability for a longer time, particularly under higher TAN levels caused by the small size of the attached biofilters. All nitrogen-compounds were lower inside the aquaponics; the pH was more stable; and also the EC remained at a lower level (up to 786 µS cm^{-1}) compared with the control RAS (1286 µS cm^{-1}). Due to the higher ammonium level, the pH-value in the control RAS was lowered to a higher extent. The amount of oyster shells we used could not sufficiently slow down this development. As can be seen in the good growth of the plants including fruit harvesting (see below), the plants must have absorbed the respective nutrients [36]. Furthermore, minerals were absorbed, so they slow the accumulation and were responsible for the lower EC inside the aquaponics. Because we did not observe larger anaerobic sections in either system, degassing through denitrification was not responsible for the reduced nitrate levels inside the aquaponic system.

4.2. Plant Production

The oxygen saturation in the hydroponic unit was more than sufficient for supporting the plant growth [37,38]. The plants performed well considering that no extra fertilizer was provided throughout the experiment. The nutrient supply from the fish husbandry units was enough to produce 22.07 kg fruits. However, the total nutrients provided from the aquaculture unit were rather suboptimal [36]. There was a minor lack of certain nutrients due to no extra fertilization [2]. A yellowish brown lamina, especially on older leaves, might have been caused by this minor nutrient deficiency, or more likely by the high radiation intensity due to the weather conditions in summer with high temperatures, especially inside the greenhouse. This was, however, limited, and the cucumber plants grew to full size and produced immaculate fruits.

In addition to an increased water volume of the aquaponic system and an enhanced nutrient consumption by the plants, also the growth of roots might provide positive effects for the fish in the aquaponics system. Roots have the ability to filtrate water and retain suspended sediment load. The absence of roots leads most likely to the murky water conditions in the control system as shown by measurements of the water turbidity (Table 2). Other effects, such as the release of substances from the roots into the process water, were not a matter of this study, but cannot be excluded [10]. As discussed in Section 4.1, the plants inside the hydroponic units helped to maintain system stability, reducing water replacement due to their nutrient uptake. Thus, emissions from aquaculture units were minimized. Consequently, the applied experimental aquaponic unit performed as expected in order to test the effects of this cultivation method on African catfish welfare, in comparison to the RAS alone.

4.3. Fish Production

The used stocking density of 54 fish per system (15.4 kg/m^3) was comparable to the extensive holding conditions chosen by Palm et al. [39]. A pre-study using the same two systems at a higher stocking density (e.g., 105 fish per system ≅ 30.0 kg/m^3) and the double feed load revealed distinctly high ammonium and nitrite values, caused by the limited biofilter dimension.

The chosen feeding regime of 25 g/d allowed stable system performance. Based on the estimated FCR (aquaponics vs. control RAS: 1.02/1.09), SGR (0.48/0.41) and DGR (1.37/1.30), *C. gariepinus* had

good growth in both systems despite the reduced feed intake (cf. Table 3). Growth performance in both of our systems was not significantly different, demonstrating that the aquaponics, as well as the RAS provided suitable conditions for the African catfish. Palm et al. [39] reported a comparable FCR (1.0) with a better SGR (0.65) using a larger ebb-flood substrate aquaponic system (3.7 m^3) and a stocking density of 12.2 kg/m^3. Due to the observed nutrient consumption of *C. sativus* (Figure 5), a coupled aquaponic system have the ability to maintain more fish than a comparable RAS without plants: in this case, the control system.

4.4. Stress and Welfare Aspects

The magnitude of corticosteroid responses among different teleosts or within a species can vary considerably [21] between 30 and 300 ng mL^{-1} [19]. Such high corticosteroid magnitudes were caused by induced stressors during experiments (e.g., caused by netting). This contrasts unstressed wild teleosts, showing basal cortisol values of typically <10 ng mL^{-1}. However, some species, e.g., Chub, *Leuciscus cephalus*, reach higher cortisol values of 50–100 ng mL^{-1} while resting and up to 1500 ng mL^{-1} during stress [19].

Van de Nieuwegiessen et al. [16,17] investigated different welfare parameters in African catfish in the context of varying stocking density, age or chronic stressors. The authors recorded no significant effect on SGR, FCR or cortisol responses, even after netting stress. In these experiments juveniles of *C. gariepinus*, with body weights between 7.5 and 90.0 g, showed a cortisol baseline level of about 20 ng mL^{-1}, depending on the stocking density, and a glucose baseline level lower than 4 mmol L^{-1} [16]. Larger African catfish with a final body weight of max. 313.1 g had cortisol baseline levels between 64 and 135 ng mL^{-1} and glucose baseline levels between 3.0 and 3.8 mmol L^{-1} [17]. In the present study, most fish in both treatment groups had relatively low cortisol baseline levels between approximately 10 and 12 ng mL^{-1} over all sampling days, without any cortisol peak during the start-up phase on Days 6, 11 or 13. The glucose levels found between approximately 2 and 3 mmol L^{-1} had a normal magnitude, related to the low feed intake of 25 g/d. Though the environment can lead to physiological or psychological changes, which often are accompanied by behavior adaptions, in animals in diverse ways [40], neither cortisol nor blood glucose levels were altered as adaptive responses in the aquaponic system.

Schram et al. [9] demonstrated that high NH_3-N levels can reduce feed intake and growth, but in their experiments, *C. gariepinus* did not show any major physiological disturbances in the observed blood parameters when chronically exposed to higher NH_3-N concentrations. In our study, following the definition of stress in terms of cortisol and blood glucose levels, African catfish were not affected by the water quality, either. Towards the end of the experiment, when water parameters were degraded, there were neither cortisol, nor blood glucose level increases, demonstrating the very high tolerance of African catfish to changing water conditions. This partially matches the results from Schram et al. [9].

Comparison of the body condition between the two groups showed significantly increased skin, fin and barbel damages to the fish from the RAS. In both cases, these external injuries did not seem to be related to degrading water parameters because we could not observe any increasing damage rates towards the end of the experiment. Instead of that, the number of external injuries was even more decreased inside all aquaponic tanks within the final sampling. In contrast, there was only one tank in the control with decreased external wounds (cf. Section 3.4). In addition to the altering water quality, increased water turbidity in the control RAS was the major difference between the two rearing systems. African catfish is able to cope with diverse habitat conditions. In some of their natural habitats, the volume of lentic waters shrinks during the annual dry season, and the remaining fish often concentrate in muddy pools to survive. It may be assumed that African catfish adapts to these conditions, changes behavior and/or fights for preferred places. This would either imply that water turbidity increases the aggression capability of this species, encountering each other through bites and leading to more wounds through intraspecific competition and fights under murky or suboptimal conditions. Although the sources for the observed lesions were not stated, most wounds appeared to

be the result of aggressive behavior between the fish, as illustrated in Figure 3. Several publications have reported on the behavioral adaption of fish in the face of environmental changes including water turbidity [41].

Another possible explanation for the differences in external injuries might be the influence of light intensity on the fish. The light intensities in both RAS were comparable during the experiment (Section 2.1), but the turbidity could have influenced the light intensity inside the water body by absorption. There are indications that swimming behavior and aggression are affected by light intensity, which results in more wounds [42], likely caused by higher stress under an intense light regime. African catfish is not a primarily visual creature, but surely reacts on visual interference [43]. However, it is interesting to note that in this case, fish from the less turbid waters with higher light intensities of the aquaponics system had less wounds compared with the RAS system. In this case, additional explanations, such as the possible effects of plants in the hydroponic system on reared fish, must be taken into consideration.

It can be assumed that increased external injuries also promote bacterial, fungal or protozoan infections [44], applying pressure on the immune system. This immune stress also diminishes welfare [21,25] and has the effect of weakened fish. Several publications affirm this [18,45–47]. Analyzing immune parameters such as lymphocytes, neutrophil granulocytes, etc., would help to complete the picture of immune stress and welfare in this context.

5. Conclusions

African catfish kept under aquaponic conditions, as well as in the control system did not show any differences in physiological stress parameters. The levels of blood glucose and plasma cortisol were low compared with earlier studies, demonstrating that the physiological stress response in the experimental units was also low. On the other hand, we could observe significantly fewer external injuries in the aquaponic fish, possibly indicating a better wellbeing of these fish. Significant differences in the number of bite wounds might be related to an adapted intraspecific behavior due to the differences in turbidity and/or light intensity between both systems. While increased turbidity inside the RAS system also decreases the visual sense and might increase territorial fights, the filtering capacity of the plant roots inside the hydroponics decreased the suspended matter and reduced nutrient load. Consequently, aquaponic systems might support African catfish rearing through increased filtering of the process water. On the other hand, other possible effects of the plants and plant-originating substances on the behavior of the fish were not studied. This needs to be performed in future approaches.

Compared with RAS, aquaponics offers many benefits such as maintenance of water parameter stability, reduction of waste water, reduced emissions, thereby leading to a smaller impact onto ground water or the surrounding ecosystems. This study suggests a better welfare status of fish in a coupled aquaponic system even under low levels of the stress hormone cortisol and the metabolic factor blood glucose. This might be considered in the development of modern aquaculture technologies, also considering fish welfare, in the future.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. Supplementary Data

Table A1. The average (±SEM) temporal changes for the different stress and welfare parameters, in different phases of the experiment (Days 6–13, Days 40–42, Days 68–74 and the final sampling on Day 87).

System	Parameter	Sampling Days 6–13	Sampling Days 40–42	Sampling Days 68–74	Final Sampling on Day 87
Control	Cortisol	10.04 ± 0.05	12.17 ± 0.43	9.33 ± 0.30	14.77 ± 0.60
Aquaponics	(ng mL^{-1})	10.17 ± 0.10	12.51 ± 0.31	10.12 ± 0.38	12.69 ± 0.42
Control	Glucose	2.00 ± 0.14	2.51 ± 0.18	2.78 ± 0.09	3.16 ± 0.10
Aquaponics	(mmol L^{-1})	2.27 ± 0.12	2.49 ± 0.13	2.75 ± 0.23	3.16 ± 0.14
Control	External	1.07 ± 0.32	2.35 ± 0.63	0.75 ± 0.17	0.98 ± 0.17
Aquaponics	injuries	1.53 ± 0.79	1.00 ± 0.23	0.36 ± 0.16	0.22 ± 0.06

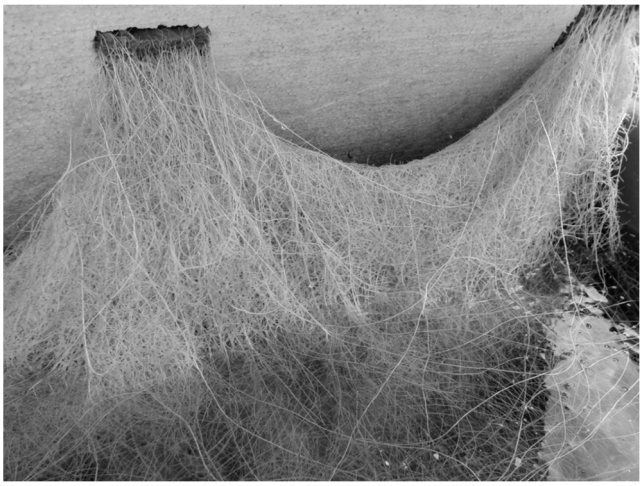

Figure A1. Root meshes of cucumber plants (*C. sativus*) in the final phase of the experiment.

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