ZURICH UNIVERSITY OF APPLIED SCIENCES
DEPARTMENT LIFE SCIENCES AND FACILITY MANAGEMENT
INSTITUTE OF NATURAL RESOURCE SCIENCES

Effect of feeding regime and light intensity on size heterogeneity, fin damage and growth performance of juvenile European perch (*Perca fluviatilis*) in aquaculture

Master Thesis

of
Seitz Andreas
Master programme Environment and Natural Resources 2017
Specialisation in Ecological Engineering
Submission date 01.07.2021

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Imprint

Keywords:	growth, *Perca fluviatilis*, size heterogeneity, fin damage, feeding behaviour, self-feeders, sexual growth dimorphism, welfare
Suggested citation:	Seitz, A. (2021). *Effect of feeding regime and light intensity on size heterogeneity, fin damage and growth performance of juvenile European perch (Perca fluviatilis) in aquaculture*. Master thesis. Wädenswil, Zurich University of Applied Sciences. Unpublished.
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Abstract

Light and feeding regime are among the key factors that determine growth of farmed fish. Light is known to affect behaviour, which then, in turn can be matched with an adequate feeding regime to obtain best growth performance. A better understanding of the interplay between these two factors is of great interest to commercial perch farms due to the lack of information and the need to improve production efficiency. Here, we evaluated for the first time the combined effects of the factors light intensity and feeding regime (compromise between lower and upper levels currently applied in perch farms) on size heterogeneity, fin damage and growth performance of European perch, *Perca fluviatilis*. Two light intensities (15 lux and 100 lux) and three feeding regimes (5 and 24 feeding events per day and self-feeders) were applied in a factorial design with a four-fold replication of groups of 80 fish (initial body weight was 8.6 ± 1.7 g) that were reared for 42 days in a recirculating system. A light intensity of 15 lux improved growth and feed conversion when fish were fed with self-feeders. Size heterogeneity (CV) in all groups increased during the study with a tendency for lower CV in groups with 24 feeding events. No sexual growth dimorphism occurred, which, thus, did not influence size heterogeneity. Mortality and fin damage were low and were not affected by light intensity or feeding regime. Our results indicate that already marginal changes in light intensity alter the behaviour of perch when environmental factors permit fish to express their feeding preferences. The results contribute to the improvement of rearing conditions and production efficiency of commercial perch farms.

Zusammenfassung

Licht und Fütterungsregime gehören zu den Hauptfaktoren, die über das Wachstum von Zuchtfischen bestimmen. Es ist bekannt, dass Licht das Verhalten beeinflusst, welches dann mit einem angemessenen Fütterungsregime abgestimmt werden kann, um die beste Wachstumsleistung zu erzielen. Ein besseres Verständnis über das Zusammenspiel dieser beiden Faktoren ist für kommerzielle Barschfarmen von grossem Interesse, da es derzeit keine Informationen dazu gibt und die Notwendigkeit besteht, die Produktionseffizienz zu verbessern. Hier haben wir zum ersten Mal die kombinierten Auswirkungen der Faktoren Lichtintensität und Fütterungsregime (die Levels stellen ein Kompromiss zwischen dem unteren und oberen Niveau dar, das derzeit in Barschfarmen angewendet wird) auf die Grössenheterogenität, Flossenschäden und Wachstumsleistung des Flussbarschs, *Perca fluviatilis*, untersucht. In einem faktoriellen Design wurden zwei Lichtintensitäten (15 Lux und 100 Lux) und drei Fütterungsregime (5 und 24 Fütterungen pro Tag und Self-Feeder) angewendet. Die Gruppen, bestehend aus 80 Fischen (das anfängliche Körpergewicht betrug 8.6 ± 1.7 g), die während 42 Tagen in einem Kreislaufsystem gehalten wurden, wurden vierfach repliziert. Eine Lichtintensität von 15 Lux verbesserte das Wachstum und die Futterverwertung von Fischen, die mit Self-Feedern gefüttert wurden. Während der Studie nahm die Grössenheterogenität (CV) in allen Gruppen zu. Es zeigte sich aber eine Tendenz zu einem geringeren CV in den Gruppen, die 24 Fütterungen erhielten. Da kein sexueller Wachstumsdimorphismus auftrat, wurde die Grössenheterogenität dadurch nicht beeinflusst. Die Mortalität und die Flossenschäden waren gering und beide wurden weder durch die Lichtintensität noch durch das Fütterungsregime beeinflusst. Unsere Ergebnisse deuten darauf hin, dass bereits marginale Änderungen in der Lichtintensität das Verhalten von Barschen verändert, wenn es die Umweltfaktoren zulassen, dass die Fische ihre Fresspräferenzen auszudrücken können. Die Ergebnisse tragen zur Verbesserung der Haltungsbedingungen und der Produktionseffizienz von kommerziellen Barschzuchten bei.

Acknowledgements

First and foremost, I would like to express my deepest gratitude to the entire team of the research group for aquaculture systems at the Zurich University of Applied Sciences, which gave me the unique possibility to develop my own project and to perform the study in all its extent. Furthermore, special thanks go to Dominik Refardt for his excellent advice and support during the statistical analyses. His constructive inputs and corrections were always helpful in the preparation of this thesis and contributed significantly to its success. Vlastimil Stejskal and Timo Stadtlander were very helpful in the project development for which I am very grateful. I would especially like to thank Vlastimil Stejskal for providing me with the self-feeding systems and for taking on the position of second corrector. I would also like to thank Jan Matoušek for teaching me how to handle the self-feeders and for taking the long journey to bring them to Switzerland. Further thanks go to Mathias Sigrist and Fridolin Tschudi for their assistance and advice in setting up the experiment. I would also like to thank the staff of Valperca SA, succursale Percitech, especially Beat von Siebenthal and Guirec Dewavrin, for the pleasant collaboration and for providing feed and experimental fish. My sincerest gratitude goes to Linda Tschirren for her tremendous support during the experiment. Last but not least, I would like to thank all the people who were involved in data acquisition and fish sampling, Linda Tschirren, Mathias Sigrist, Luca Regazzoni, Jan Schellenberg, Ali Cem Güler, Jonas Windisch, Sophia Egloff and Fridolin Tschudi, without whom it would not have been possible to carry out this study.

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1 Introduction

The European perch, *Perca fluviatilis*, has been identified as a promising species for production in aquaculture and a conversion from extensive pond farming to intensive production in recirculating aquaculture systems (RAS) has been implemented to increase production efficiency (Overton et al., 2015). Switzerland remains the most important market for perch in Europe in which the biggest perch farms are exclusively RAS based (Toner, 2015). According to FAO (2021) data, the amount of perch produced in Swiss aquaculture facilities nearly tripled between 2015 and 2019, making Switzerland the largest aquaculture producer of perch in Europe in 2019 (468 tonnes live weight, 51.6% total share). Despite the substantial progress made in recent years, there are still open issues in intensive perch production. These concern the improvement of production efficiency in general as well as a moderate growth performance and size heterogeneity of fish in particular (Policar et al., 2019).

The feeding regime is a main factor for optimal fish growth (Geay & Kestemont, 2015). Adequate regimes can reduce size heterogeneity (Sun et al., 2016; Wang et al., 1998), whereas restricted regimes led to increased stomach volumes (Ruohonen & Grove, 1996) and hyperphagia (Jobling, 1983). Thus, the adequacy of feeding regimes affect fish welfare but also farm profitability (Alanärä & Strand, 2015), since aquafeeds usually account for the biggest costs in aquaculture production (Rana et al., 2009). Feeding regimes with more frequent feeding events affected the growth of many fish species positively (Lee et al., 2000; Sun et al., 2016; Wang et al., 1998). However, a recent study with juvenile perch did not find differences in growth performance when fish were fed three times a day or continuously, although a slight tendency for better growth was observed in tanks with continuous feed supply (Wysujack & Drahotta, 2017). As only few studies have been conducted to investigate the effects of different feeding regimes in juvenile perch, there is still limited information about optimal feeding schedules (Geay & Kestemont, 2015; Valperca SA, personal communication; Wysujack & Drahotta, 2017).

Light intensity has been repeatedly demonstrated to be a factor that affects many behavioural and biological processes of fish, such as the foraging activity and success (Czarnecka et al., 2019) as well as growth (Trippel & Neil, 2003). Light intensity significantly modulated the growth of juvenile pikeperch (Kozłowski et al., 2010) and fish showed a preference for the lowest light intensity when different levels between 1 lux and 50 lux or 25 lux and 300 lux were applied (Luchiari et al., 2006). On the other hand, Strand et al. (2007a) could not confirm these results for juvenile perch when they were reared at 200 lux and 1100 lux and no main effect of light intensity on growth performance was observed. However, another study could show that high light intensity affected the activity level of perch during daytime, which seemed to indicate elevated stress (Staffan, 2004). Therefore, general specifications for optimal light intensities in perch range from 200 lux to 1100 lux (Policar et al., 2015), but it should be emphasized that most of the light intensities tested so far, as well as those which are specified as optimal, are well above the intensities currently applied in commercial perch farms (Valperca SA, personal communication).

Size heterogeneity in intensively reared perch is high, with body weight of seven-month-old perch ranging from 7 to 89 g, while the average was 25.9 g (Mélard et al., 1996). The sexual growth dimorphism that perch may exhibit under intensive culture conditions is another factor that might add to size variation in stocks (Fontaine et al., 1996; Juell & Lekang, 2001), however, it is suggested that the origin of the huge size heterogeneity is not exclusively due to genetic characteristics (Fontaine et al., 1997; Melard et al., 1995).

In intensive perch farming, already small differences in size can provoke aggression and even in its absence, dominance hierarchies may be manifested (Magnhagen, 2015). Aggressive behaviour among fish can also promote the occurrence of fin damage (Latremouille, 2003). Therefore, a common practice to reduce size heterogeneity is size-sorting (Policar et al., 2015), which is performed routinely (at least biweekly) in larval and early on-growing stages (Fontaine & Teletchea, 2019). The advantage of size sorting was evident in perch post-larvae and resulted in higher biomass gain due to higher survival (Król et al., 2019). However, the advantage of size sorting in juvenile perch can be partially offset by the emergence of fast-growing fish in each sorted group, which induced a further increase of heterogeneity during the culture phase and did not increase the overall productivity (Mélard et al., 1996). Furthermore, size-sorting is an additional stressor (Policar et al., 2015) and repeated stressful events reduce feed intake and increase energy expenditure (Strand et al., 2007b), resulting in reduced growth performance of perch (Jentoft et al., 2005; Strandet al., 2007b). Generally, there is a need to reduce the frequency of sorting events (Policar et al., 2015) and other regular disturbances to increase the welfare of farmed fish (Strand et al., 2007b), yet alternative methods to keep the level of size heterogeneity low, are limited.

Both factors light intensity and feeding regime need to be considered for optimizing fish growth (Geay & Kestemont, 2015; Strand et al., 2007a), whereas the latter might even reduce size heterogeneity (Sun et al., 2016; Wang et al., 1998). However, earlier studies conducted with juvenile perch were limited in several aspects. Only one factor (either light intensity or feeding regime) was observed and higher light intensities than in commercial perch production were used (Strand et al., 2007a; Wysujack & Drahotta, 2017), or the light intensity applied was not mentioned at all (Wysujack & Drahotta, 2017). Moreover, fish in both previously mentioned studies were fed with automatic feeders and therefore, the feeding regimes applied were extrinsically controlled. Self-feeding systems, however, allow the fish to intrinsically control feeding time, frequency and quantity, and may even increase the welfare status of farmed fish, while serving as a suitable tool to investigate the effect of several factors regarding the feeding behaviour (Attia et al., 2012). To our knowledge, no study was conducted that focused on both factors (feeding regime and light intensity) simultaneously. As earlier studies have documented that both factors affect growth and other biological processes in fish, this might be necessary to better understand both the individual effects and their interaction to improve the rearing conditions and production efficiency in commercial perch farms. However, this requires that the levels of light intensity and feeding regime are within the range currently applied in perch farms.

Hence, the aim of this study was to assess the effects of light intensity and feeding regime in a factorial design on size heterogeneity, fin damage and growth performance of juvenile European perch cultured in RAS. The experiment should explicitly correspond to commercial culture conditions. Thus, the study design represents a compromise between focusing on lower and upper levels of both the light intensity (15 lux and 100 lux) and the feeding regime (5 and 24 feeding events per day) while considering physiological preferences of the fish (extrinsically and intrinsically controlled feeding regimes, i.e., automatic feeders and self-feeders, respectively). Light intensity and tank wall colour together create specific light conditions in rearing tanks which might modulate feed visibility and feed intake, thus influencing growth of perch (Strand et al., 2007a), whereas increasing the feeding frequency could improve growth performance an reduce size heterogeneity of fish (Sun et al., 2016; Wang et al., 1998). Therefore we hypothesized that a moderate increase in light intensity (15 lux to 100 lux) combined with more frequent feeding events (i.e., 24 feeding events or self-feeders) can increase growth performance while reducing size heterogeneity and aggression (i.e., fin damage) in juvenile perch.

2 Materials and methods

2.1 RAS setup and culture conditions

The experiment was carried out in a recirculating aquaculture system (RAS; total volume 2200 L) that consisted of 24 square plastic tanks. The RAS was equipped with a biofilter (500 L, Kunststoff-Spranger GmbH, Plauen, Germany), aerated with an air blower (MEDO BLOWER LA-120A, Nitto Kohki Europe GmbH, Steinenbronn, Germany), a protein skimmer (Turboflotor 5000 baby ECO, AB Aqua Medic GmbH, Bissendorf, Germany) and an UV disinfection unit (Pro Pond Advantage UV110, Tropical Marine Centre Ltd, Hertfordshire, UK). Solids were removed using three different types of filters; a fleece filter (Smartpond GmbH, Friedrichsfehn, Germany), a sand filter (Ultima II 60,000, Aqua Ultraviolet, Temecula, USA) and the water flowed back into the biofilter chamber cleaned by a filter mat. The water was aerated using an oxygen concentrator (Woodland® Oximaxx, Koi Andreas GmbH, Hammersbach, Germany) before being returned to the fish tanks. Average flow rate (± SD) in the tanks was 5.0 ± 0.1 Lmin^{-1}. Approximately 250–375 L of fresh water were exchanged daily.

Each tank (55 × 55 × 33 cm, water volume 69 L) had black walls and a glass front. Tanks were arranged on four shelves (six tanks each) in a climate chamber (2.7 × 5.0 m; Kälte 3000 AG, Landquart, Switzerland), which allowed control of water temperature, room temperature and humidity. All tanks were covered with a transparent plastic sheet. To protect the fish against external disturbances, the glass front and the space between the top of the tanks and the top of the shelf were covered with a PVC foil (black outside, white inside). Additionally, all tanks were separated by thin metal plates to ensure that the specified light intensities were not affected by the illumination of adjacent tanks.

Illumination was provided separately for each tank by mounting a 30 cm LED strip (eco+ Day 5500K, LEDaquaristik GmbH, Hövelhof, Germany) centrally above the tanks. The light regime used throughout this study was 9L:15D, with daytime between 07:00 and 16:00 CET. At the changes between day and night, light was dimmed gradually for 30 min. Specific light intensities during daytime are indicated in the text.

Two types of feeders were used in this study. Automatic point source feeders (PFLANZER - Fütterungssysteme, Simmozheim, Germany) mounted above 16 tanks or a 24-hr self-feeding system with a string sensor for fish feed demand (IMETRONIC, Marcheprime, France), mounted on the tank cover of the remaining eight tanks.

Water temperature, pH, oxygen saturation and electrical conductivity were constantly monitored with probes (oxygen: LDO sc; pH: 1200-S sc; electrical conductivity: 3798-S sc; water temperature: all probes, Hach Lange) connected to a display module (SC1000, Hach Lange) and values were stored online. Additional measurements of the same parameters were carried out weekly with a multimeter (HQ40D, Hach Lange). Ammonium, nitrite and nitrate concentrations were analysed with spectrophotometric test kits (LCK 304, LCK 341, LCK 339, Hach Lange) (Table 1).

Table 1: Water parameters during the acclimatisation period and the experiment.

Parameter	Unit	Acc. period	Experiment
Water temperature	°C	20.7 ± 0.3	20.8 ± 0.5
pH		7.2 ± 0.1	7.2 ± 0.1
Oxygen saturation	%	122.5 ± 10.0	117.3 ± 7.2
Electrical conductivity	$\mu S\ cm^{-1}$	3838.9 ± 294.1	3971.7 ± 199.3
NH_4-N	$mg\ L^{-1}$	0.43 ± 0.07	0.69 ± 0.33
NO_2-N	$mg\ L^{-1}$	0.24 ± 0.06	0.29 ± 0.12
NO_3-N	$mg\ L^{-1}$	45.98 ± 15.12	72.57 ± 5.79

Note: Data are expressed as mean ± standard deviation.

2.2 Fish acclimatisation and training

2760 juvenile European perch (*Perca fluviatilis*), with an average body weight (± SD) of 2.9 ± 0.1 g (own measurements), were brought from a fish hatchery (Valperca SA, succursale Percitech, Switzerland) to the research facility of the university (ZHAW Wädenswil, Switzerland) and were distributed into the tanks of the RAS (115 fish per tank). Fish were then acclimatised for three weeks and fed commercial feed (pellet size 1.1 mm, INICO Plus, BioMar). According to the producer, the feed contains 56% crude protein, 18% crude fat, 8.9% nitrogen-free extracts, 10.8% crude ash, 1.6% total phosphorous and 22.0 $MJ\ kg^{-1}$ gross energy. During the acclimatisation period, fish in the eight tanks with self-feeders were trained to operate the sensors by feeding them multiple times per day by hand near the sensor. In the remaining 16 tanks with automatic feeders, feed was provided nine times per day at regular intervals between 7:20 and 15:40 CET. The daily feed ration was set at 3.5% at the beginning and reduced to 3% body weight until the end of the acclimatisation period. Light intensity was set to 25 lux at the water surface (measured with PG100N, UPRtek Corp., Taiwan).

2.3 Experimental setup

Acclimatised and trained fish were size sorted before initiating the trial. A total of 1920 fish, with an initial average body weight (± SD) of 8.6 ± 1.7 g were distributed into 24 square plastic tanks of the same system that was used for acclimatisation and training. Each tank contained 80 fish. The experiment ran from 5 January 2021 until 16 February 2021, a total of 42 days.

Two environmental factors (light intensity and feeding regime) at two (15 and 100 lux light intensity) and three levels (5 and 24 feeding events per day, self-feeding system), respectively, were applied in a fully crossed and balanced design, with each combination replicated four times. The surface light intensity was 15.2 ± 1.1 lux and 99.1 ± 2.7 lux (mean ± SD) for the 15 and 100 lux level, respectively. Fish were provided commercial feed of pellet size 1.1 mm (INICO Plus) for the first two weeks and then of pellet size 1.5 mm (START PREMIUM, Alltech Coppens) for the rest of the experiment. According to the producer, the latter feed contains 54% crude protein, 15% crude fat, 10.4% crude ash, 1.6% total phosphorous and 21.1 MJ kg^{-1} gross energy. Feed was delivered using the same feeders as during the acclimatisation period. In tanks with 5 and 24 feeding events (extrinsically controlled regimes), feed was delivered at regular intervals between 7:20 and 15:40 CET. The daily feed ration was set to 2% body weight. Self-feeders (intrinsically controlled regime) were adjusted to release 2 g of feed per demand and demands were limited to one every three min. to avoid hedonic behaviour. The number of demands was automatically recorded using POLY Files software (IMETRONIC, Marcheprime, France). Feed was weighed and the amount for one week was filled into the feeders. Feed leftovers and faeces were continuously removed through a drainpipe (0.5 cm above bottom, in the centre of the tank) and through skimmer slots at the surface. The number and weight of pellets flushed from each tank was not collected.

2.4 Sampling procedure

At the beginning of the experiment and every two weeks until the end, biomass (B) and average body weight (avg BW) were determined by individual weighing (beginning and end of the experiment) and bulk weighing (after two and four weeks). Fish were starved for 16 h prior to weighing and fed by hand after weighing. The regular feeding regime was initiated the next day at 7:20 CET. Mortalities were recorded, and dead fish removed daily, but tanks were only cleaned when fish were already outside for weighing. After each weighing, the amount of feed was adjusted. At the end of the experiment, individual measurements were carried out on all fish. Fish were immediately killed with an overdose of anaesthetic (2-phenoxyethanol; at least 3 ml L^{-1}). Dead fish were weighed and stored in a freezer in labelled zip-lock bags. Standard length (SL), sex and fin damage were recorded on thawed fish. Visual assessment of fin condition was done for each fish and fin (dorsal first, dorsal second, caudal, anal, ventral left, ventral right, pectoral left and pectoral right fins) and total fin score (TFS) was calculated by summing score points for each fin per fish in a given tank (see Stejskal et al. (2011) for details). Sexes were determined by dissection.

2.5 Calculations

The following variables were calculated per tank:

$$Specific\ growth\ rate\ (\%\ d^{-1}), SGR = \frac{lnB_t - lnB_i}{\Delta t} \times 100$$

$$Coefficient\ of\ weight\ variation\ (\%), CV = \frac{SD}{avg\ BW} \times 100$$

$$Apparent\ feed\ conversion\ ratio, AFCR = \frac{F}{B_t - B_i}$$

$$Mortality\ (\%)\ = \frac{D_t}{N_i} \times 100$$

where B_i is the initial fish biomass, B_t is the fish biomass at the end of the experiment and Δt is the duration of the experiment (Ricker, 1979). Coefficient of weight variation (CV) was calculated at the beginning and at the end of the experiment, where SD is the standard deviation of body weight (individual weighing) and avg BW is the average body weight of fish in a given tank. During the experiment, uneaten food was not collected. Hence, exact feed conversion calculations were not possible. Therefore, values were calculated as apparent feed conversion ratio (AFCR), where F is the amount of feed supplied. Mortality was calculated throughout the whole experiment, where N_i is the initial number of fish and D_t is the number of dead fish at the end of the experiment.

2.6 Statistics

Statistical analyses were performed in R v4.0.4 (R Core Team, 2021) based on average values from each tank. Assumptions of parametric tests (normality of residuals, homoscedasticity) were verified using Shapiro-Wilk's and Levene's test, respectively, and non-parametric tests were used if necessary. At the beginning of the experiment, avg BW and CV were compared across tanks with a Kruskal-Wallis test to ensure comparable weight distributions among experimental groups. Variables that were collected at the end of the experiment, or within a two-week interval, were tested with separate two-way analyses of variance (ANOVA) using the fixed factors light intensity (two levels: 15 lux and 100 lux) and feeding regime (three levels: 5 feeding events, 24 feeding events and self-feeders). A light intensity × feeding regime interaction was always considered. Differences regarding sexes of the fish were tested with separate two-way ANOVAs using the fixed factors gender (two levels: male and female) and experimental group (six levels: see Table A1) considering the gender × experimental group interaction. Where factors had a significant effect ($p < 0.05$), Tukey's test was applied. To exclude carry-over effects from differences in avg BW or CV that were already present at the beginning of the experiment, correlations between variables obtained at the beginning and at the end of the experiment were tested with a Spearman's rank correlation.

3 Results

3.1 Growth parameters

At the beginning of the experiment, neither avg BW (8.3 ± 0.2 to 8.9 ± 0.6 g, mean ± SD, Table A1) nor CV (18.2 ± 1.7 to 20.3 ± 0.3%, mean ± SD, Table A1) differed significantly between experimental groups (Kruskal-Wallis test, avg BW: $H_5 = 7.656$, $p = 0.176$; CV: $H_5 = 8.960$, $p = 0.1107$). However, fish in tanks with self-feeders had difficulty operating the sensors during the first two weeks of the experiment (own observation), this delayed growth and caused an artifactual effect on growth parameters. Therefore, comparisons between tanks with self-feeders and the other two feeding regimes cannot be made and results are presented separately where necessary.

Growth (measured as SGR) was not affected by light intensity as a main effect. However, a main effect of feeding regime and an interaction between both factors were observed during the whole experiment (Table 2). Light intensity influenced growth when self-feeders were installed, where SGR was higher when fish were reared at 15 lux than at 100 lux (Tukey's test, $p = 0.029$). This suggests that light can have an effect on feeding if the behaviour is allowed to be controlled intrinsically. The difference between self-feeding regimes is unlikely to be an artifact, as fish in both treatments had the same initial avg BW (Table A1). This supports the conclusion that the observed effect arose during the experiment. Fish from tanks with self-feeders grew less (most likely due to the initial delay, as explained above) and significantly higher SGR in tanks with 5 and 24 feeding events were found (both Tukey's tests, $p < 0.001$). At the same time, the extrinsically controlled regimes did not differ significantly (Tukey's test, $p = 0.972$) (Figure 1).

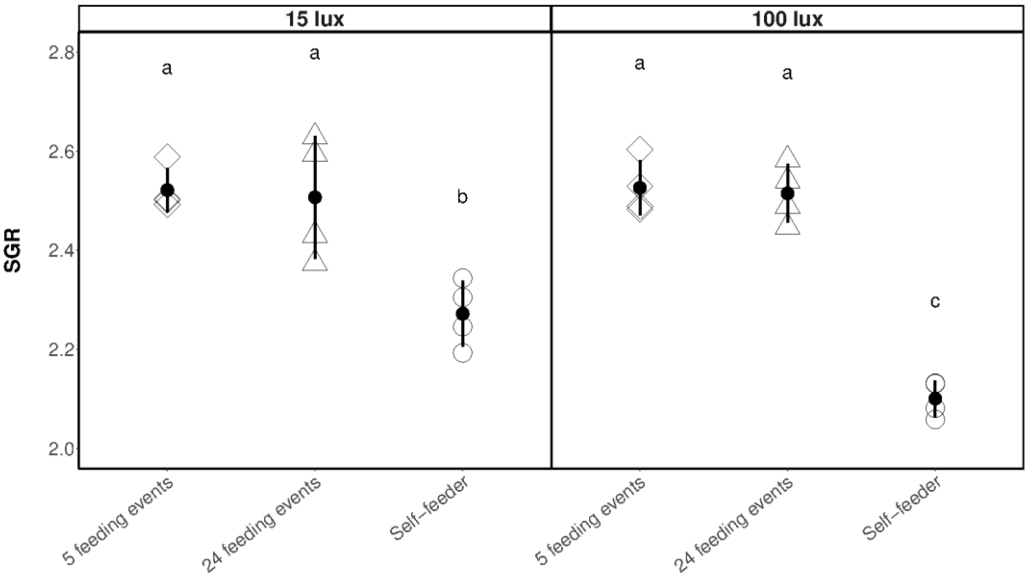

Figure 1: Effect of light intensity (15 lux, 100 lux) and feeding regime (5 feeding events, 24 feeding events, Self-feeder) on SGR [% d^{-1}]. Data are expressed as single values per tank including mean ± standard deviation (n = 4). Different letters indicate significant differences (p < 0.05).

Size heterogeneity (measured as CV) was not affected by light intensity, yet a marginally significant main effect of feeding regime was observed (Table 2). This was mainly driven by the feeding frequency of extrinsically controlled regimes. A posthoc comparison showed that tanks with 24 feeding events tended to have a lower CV than tanks with 5 feeding events but did not differ significantly (Tukey's test, *p* = 0.255) (Figure 2). No correlation was found between CV at the beginning and at the end of the experiment (Spearman's ρ = -0.047, *p* = 0.828) and between avg BW at the beginning and CV at the end of the experiment (Spearman's ρ = -0.204, *p* = 0.339), which supports the conclusion that the slight tendency for extrinsically controlled regimes arose during the experiment.

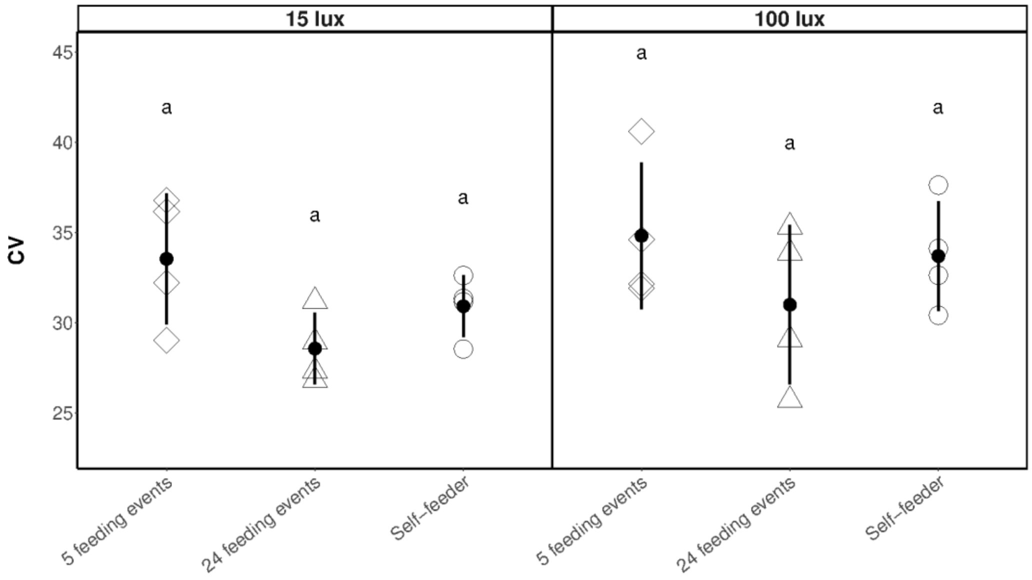

Figure 2: Effect of light intensity (15 lux, 100 lux) and feeding regime (5 feeding events, 24 feeding events, Self-feeder) on CV [%]. Data are expressed as single values per tank including mean ± standard deviation (n = 4). Different letters indicate significant differences (p < 0.05).

Table 2: Analysis with two-way ANOVA for the effect of light intensity (15 lux and 100 lux) and feeding regime (5 feeding events, 24 feeding events and self-feeder) on calculated variables during the whole experiment (specific growth rate, SGR; apparent feed conversion ratio, AFCR) and at the end of the experiment (coefficient of weight variation, CV). The light intensity × feeding regime interaction was always considered.

	df	SGR			CV			AFCR		
		MS	*F*	*p*	*MS*	*F*	*p*	*MS*	*F*	*p*
Light intensity	1	0.017	3.402	0.082	28.050	2.583	0.125	0.006	7.919	0.011
Feeding regime	2	0.292	59.177	<0.001	38.950	3.588	0.049	0.116	149.652	<0.001
Light intensity × feeding regime	2	0.021	4.254	0.031	1.240	0.114	0.893	0.005	6.984	0.006
Within groups	18	0.005			10.860			0.001		

Note: *df* = degrees of freedom, *MS* = mean squares, *F* = variance ratio.

The feed conversion ratio (measured as AFCR) was affected by light intensity and feeding regime as a main effect during the whole experiment and an interaction between both factors was observed (Table 2). Light intensity, however, appeared to influence AFCR only in the tanks with an intrinsically controlled feeding regime and there, values were significantly lower at 15 lux compared to 100 lux (Tukey's test, *p* = 0.003). This repeats the observation made with the SGR and is not independent from it, as both AFCR and SGR are calculated using fish biomass. Due to the reason explained above, the effect of the feeding regime is at least partially artifactual, which is also the likely explanation for the significantly higher AFCR in tanks with self-feeders compared to tanks with 5 and 24 feeding events (both Tukey's tests, *p* < 0.001) at either intensities. At the same time, tanks with an extrinsically controlled regime did not differ significantly (Tukey's test, *p* = 0.389) (Figure 3).

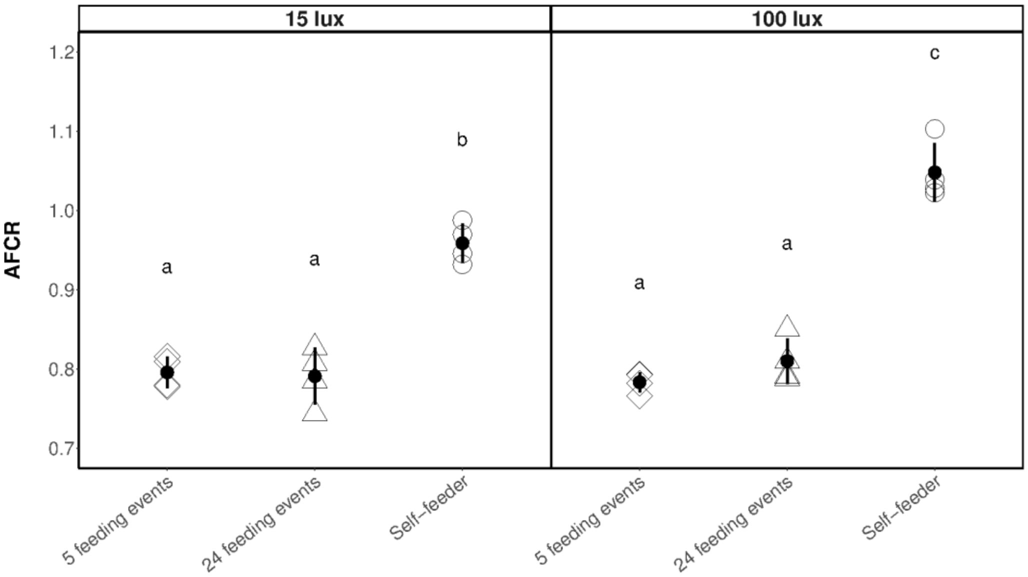

Figure 3: Effect of light intensity (15 lux, 100 lux) and feeding regime (5 feeding events, 24 feeding events, Self-feeder) on AFCR. Data are expressed as single values per tank including mean ± standard deviation (n = 4). Different letters indicate significant differences (p < 0.05).

The effect of light intensity on growth and feed conversion ratio in experimental groups with self-feeders is an intriguing result, as it suggests that light intensity can influence fish behaviour (i.e., feeding) in a permissive setting (i.e., self-feeders). However, as issues with self-feeders were observed, these observations must be carefully scrutinized to exclude an artifact. In the following section, we present supporting evidence that the effect of light intensity indeed arose during the experiment. We do this by considering experimental periods (two-week intervals) individually.

In terms of SGR, a significant interaction between light intensity and feeding regime was measurable towards the end of the experiment (weeks 4–6, Table 3), which then also influenced the data for the whole experiment (Table 2). The interaction effect was due to a significantly higher SGR in tanks with self-feeders at 15 lux than at 100 lux (Tukey's test, *p* = 0.031), while no other significant differences were found. On the other hand, the only main effect of feeding regime on SGR was observed during weeks 0–2 (Table 3), with significantly lower values in tanks with self-feeders compared to tanks with 5 (Tukey's test, *p* = 0.014) and 24 feeding events (Tukey's test, *p* = 0.003). At the same time, tanks with an intrinsically controlled regime did not differ significantly (Tukey's test, *p* = 1.000). This corroborates the observation that tanks with self-feeders at either light intensity had difficulty operating the sensors at the beginning.

Table 3: Analysis with two-way ANOVA for the effect of light intensity (15 lux and 100 lux) and feeding regime (5 feeding events, 24 feeding events and self-feeder) on specific growth rate (SGR) of all experimental periods (two-week intervals). The light intensity × feeding regime interaction was always considered.

	df	Weeks 0–2			Weeks 2–4			Weeks 4–6		
		MS	*F*	*p*	*MS*	*F*	*p*	*MS*	*F*	*p*
Light intensity	1	0.197	1.061	0.317	0.188	1.125	0.303	0.159	2.278	0.149
Feeding regime	2	2.682	14.441	< 0.001	0.042	0.248	0.783	0.003	0.038	0.963
Light intensity × feeding regime	2	0.027	0.143	0.868	0.065	0.389	0.683	0.353	5.061	0.018
Within groups	18	0.186			0.167			0.070		

Note: *df* = degrees of freedom, *MS* = mean squares, *F* = variance ratio.

The same pattern was observed for AFCR, where a significant interaction between light intensity and feeding regime arose during the last two weeks of the experiment (Table 4). Again, this suggests that this is not an artifact but an effect that reflects an alteration in feeding behaviour in response to different light intensities. More specifically, a posthoc comparison revealed a tendency for AFCR in tanks with self-feeders being higher at 100 lux than at 15 lux (Tukey's test, *p* = 0.073).

Table 4: Analysis with two-way ANOVA for the effect of light intensity (15 lux and 100 lux) and feeding regime (5 feeding events, 24 feeding events and self-feeder) on apparent feed conversion ratio (AFCR) of all experimental periods (two-week intervals). The light intensity × feeding regime interaction was always considered.

	df	Weeks 0–2			Weeks 2–4			Weeks 4–6		
		MS	*F*	*p*	*MS*	*F*	*p*	*MS*	*F*	*p*
Light intensity	1	0.200	1.058	0.317	0.004	0.110	0.743	0.026	1.936	0.181
Feeding regime	2	0.350	1.855	0.185	0.048	1.521	0.245	0.237	17.412	< 0.001
Light intensity × feeding regime	2	0.012	0.063	0.939	0.032	1.016	0.382	0.054	3.952	0.038
Within groups	18	0.189			0.032			0.014		

Note: *df* = degrees of freedom, *MS* = mean squares, *F* = variance ratio.

Considering these observations in addition to those for the entire experimental duration, this supports the conclusion that the effect of light intensity on intrinsically controlled regimes occurred during the experiment (after week four).

3.2 Mortality, fin damage and sexes

Mortality in all tanks was low and reached an average value of 1.5 ± 1.5% (mean ± SD, n = 24) at the end of the experiment (Table A1). The total fin score (TFS), as a measure of fin damage, was also low and an average value of 5.2 ± 0.7 (mean ± SD, n = 24) was found among all experimental groups (Table A1). Neither main effects of nor interactions between light intensity and feeding regime on either mortality or TFS were significant (Table 5). Thus, the data do not support any evidence that light intensity or feeding regime influenced mortality or the occurrence of fin damage substantially.

Table 5: Analysis with two-way ANOVA for the effect of light intensity (15 lux and 100 lux) and feeding regime (5 feeding events, 24 feeding events and self-feeder) on calculated variables at the end of the experiment (mortality and total fin score, TFS). The light intensity × feeding regime interaction was always considered.

	df	Mortality			TFS		
		MS	*F*	*p*	*MS*	*F*	*p*
Light intensity	1	4.167	1.574	0.226	0.337	0.684	0.419
Feeding regime	2	1.628	0.615	0.552	0.291	0.591	0.564
Light intensity × feeding regime	2	0.065	0.025	0.976	0.537	1.090	0.357
Within groups	18	2.648			0.492		

Note: *df* = Degrees of freedom, *MS* = Mean squares, *F* = Variance ratio.

The amount of male and female perch was not affected by sex (two-way ANOVA, $F_{1,36}$ = 0.228, *MS* = 7.520, *p* = 0.636) or experimental group (see groups in Table A1) (two-way ANOVA, $F_{5,36}$ = 0.034, *MS* = 1.140, *p* = 0.999) and no interaction between both factors was observed (two-way ANOVA, $F_{5,36}$ = 2.418, *MS* = 79.870, *p* = 0.055). Thus, sexes were equally distributed, and the experiment was conducted with 40 ± 6 male and 39 ± 6 female perch (mean ± SD, n = 24) per tank (Table A1). Neither avg BW (two-way ANOVA, $F_{1,36}$ = 2.081, *MS* = 4.960, *p* = 0.158) nor SL (two-way ANOVA, $F_{1,36}$ = 1.910, *MS* = 0.075, *p* = 0.176) were influenced by sex, which suggests that sex had no influence on the calculated parameters. Furthermore, the data do not support the occurrence of a sexual growth dimorphism between sexes.

4 Discussion

The results show an effect of light intensity and feeding regime on growth performance of juvenile perch. Light intensity had a measurable effect when the feeding behaviour was allowed to be controlled intrinsically, thus affecting growth rate and feed conversion ratio (which are interdependent) of fish. There, a lower light intensity of 15 lux appeared to be favourable when compared to 100 lux. The same effect has also been observed in another percid species, where the lower light intensity (45.1 lux and 1.2 lux, respectively) of two consecutive experiments enhanced the growth rate of juvenile pikeperch while it decreased the feed conversion ratio (Kozłowski et al., 2010). In contrast, light intensity did not affect growth of juvenile perch when they were reared at 200 or 1100 lux for three weeks (Strand et al., 2007a). However, the previously mentioned study was conducted with large perch of 59.6 g, whereas we used small perch of 8.6 g, which were reared twice as long. Since growth of fish decreases with increasing size, it appears that a three-weeks period was not long enough for light intensity to affect physiological responses in large juvenile perch (Geay & Kestemont, 2015). This is corroborated by our finding, as the effect of light intensity in self-feeding regimes was significant only after the fourth week of the experiment.

Since 15 lux substantially increased the growth performance of perch with self-feeding regimes, our results do not correspond to the common assumption that the optimal light intensity for perch is between 200 and 1100 lux (Policar et al., 2015). This leads to the conclusion that light intensity is indeed an important factor in perch farming and it should be considered to rear fish at rather dim light conditions. That perch were able to feed efficiently at low light intensity is also reflected in reasonable average growth rates (ranging from 2.10 to 2.53% d^{-1}, Table A1) and feed conversion ratios (ranging from 0.78 to 1.05, Table A1) which even appear to be better than those of Fontaine et al. (1997), Jourdan et al. (2000) and Wysujack & Drahotta (2017), which used perch of similar size.

The light intensities applied could have altered the activity level of perch, which might explain the lower growth rate and higher feed conversion ratio of fish in tanks with self-feeders at 100 lux compared to those at 15 lux, as an increase in light intensity resulted in higher swimming activity of perch (Staffan, 2004). However, Strand et al. (2007a) did not observe differences in energy expenditure when juvenile perch were kept at 200 and 1100 lux. This makes it questionable whether the differences in growth performance in self-feeding fish arose due to higher swimming activity at 100 lux, especially since both extrinsically controlled regimes did not differ significantly.

A possible explanation for the effect of light intensity on growth performance under self-feeding regimes could be found in the activity patterns of wild perch, since self-feeding systems allow fish to feed according to their biological rhythm (López-Olmeda et al., 2012). In wild perch, it is generally assumed that their activity is directly linked to foraging (Jacobsen et al., 2015; Kerr, 1982). Perch exhibited crepuscular activity peaks in a clear lake, whereas under turbid conditions, they were active throughout the entire diel cycle (Jacobsen et al., 2015). In our study, the low light intensity of 15 lux may represent turbid conditions, while the high light intensity of 100 lux could be comparable to clear lakes. This would imply that perch in tanks with self-feeders at low light intensity may have triggered the sensor throughout the photoperiod between 7:00 and 16:00 CET and even continued during the night, while fish in tanks with self-feeders at 100 lux tended to limit their triggering activities to twilight conditions. If so, self-feeding fish at 15 lux might have had an advantage compared to self-feeding fish at 100 lux, which would explain the better growth rate and feed conversion ratio at 15 lux. However, to support this hypothesis, the recorded number of demands from tanks with self-feeders need to be considered and should be included in further analysis. Nevertheless, our results indicate that the observed effects of light intensity arose by changes in feeding behaviour of fish in a permissive setting (i.e., self-feeders).

The effect of feeding regime on growth was most likely due to the initial delay where fish had difficulty in operating the sensors, as it has been shown that growth of fish is positively correlated with feed consumption at optimal rearing conditions (Condrey, 1982). Moreover, the same issue concerning initial difficulty in activating the triggering mechanism of self-feeders has been observed in another study with juvenile perch, where the authors suggested that this delay may have contributed substantially to the reduction in growth performance (Jourdan et al., 2000). Taking into account the fact that fish in tanks with self-feeders grew worse than fish with an extrinsically controlled regime only in the first two weeks of the experiment, we conclude that it was not the self-feeding regime in general that was the detrimental factor, but rather the fish size, the duration of the acclimatisation period, or the sensitivity of the sensors.

Several studies have documented that an increase in feeding frequency could enhance growth performance and lead to reduced size heterogeneity in fish (Sun et al., 2016; Wang et al., 1998). In terms of growth performance, this was obviously not the case in our experiment, however, we observed a slight non-significant tendency of lower size heterogeneity in tanks with 24 feeding events at either light intensity. This at least partially in contrast to an earlier study with juvenile perch where no effect of feeding frequency on growth performance or size heterogeneity was found, even when the feed was continuously administered (Wysujack & Drahotta, 2017). The sexual growth dimorphism in perch may add to size variation (Fontaine et al., 1996; Juell & Lekang, 2001), whereas an increase in size heterogeneity was proposed to indicate the establishment of dominance hierarchies in stocks (Brett, 1979). We did not observe the occurrence a sexual growth dimorphism, however, average size heterogeneity of all experimental groups increased from 19.3 to 32.1% (Table A1) during the course of the study. Therefore, it is likely that dominance hierarchies have been established, but the slightly lower size heterogeneity was due to the effect of increased feeding frequency of the extrinsically controlled regime with 24 feeding events. Our study lasted 42 days, so we suspect that the effect could even become significant if such a feeding regime is applied over a longer period. Nevertheless, our results show the importance of an adequate feeding regime under intensive rearing conditions and it should be considered to generally increase the feeding frequency.

Aggressive behaviour is one of the main causes for fin damage in aquaculture (Latremouille, 2003) and also occurred in intensively reared perch in RAS (Stejskal et al., 2020). Furthermore, fin condition as well as mortality can be used as welfare indicators in farmed fish (Ellis et al., 2002). In our experiment, both fin damage, expressed as total fin score, and mortality were lower than in other studies with juvenile perch of similar size (Fontaine et al., 1997; Jourdan et al., 2000; Stejskal et al., 2011). Neither total fin score nor mortality was influenced by light intensity or feeding regime and no differences were observed between experimental groups. This indicates that the levels of both environmental factors did not favour intracohort aggression and did not adversely affect the welfare status of fish in the present study.

In conclusion, this is the first study conducted with juvenile European perch to investigate the effects of light intensity and feeding regime (intrinsically and extrinsically controlled) in a factorial design under commercial farming conditions. We could not confirm our hypothesis where we assumed that increasing the light intensity to 100 lux in combination with more frequent feeding events or self-feeders would increase growth performance while reducing size heterogeneity and fin damage in intensively reared juvenile perch. However, our results showed that already marginal changes in light intensity influence the behaviour of perch when environmental factors (i.e., self-feeders) permit fish to express their feeding preferences, where the lower light intensity (15 lux) in such a setting substantially increased the growth performance. Furthermore, when feed was administered at fixed intervals, an increase in feeding frequency (24 feeding events per day) tended to reduce size heterogeneity. Thus, our results highlight the importance of adequate levels of both the light intensity and feeding regime in farming environments and show that it may be possible to further improve the rearing conditions and production efficiency of commercial perch farms by adjusting these factors to optimal levels.

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Appendix

Appendix 1: Supplementary data

Table A1: Parameters of perch at the beginning (initial average body weight, avg BWi; initial coefficient of weight variation, CVi), at the end (final average body weight, avg BWf; final coefficient of weight variation, CVf; mortality; total fin score, TFS; amount of males; amount of females) and during the whole experiment (specific growth rate, SGR; apparent feed conversion ratio, AFCR) of all experimental groups.

	Experimental group					
Parameter	24 feeding events, 100 lux	24 feeding events, 15 lux	5 feeding events, 100 lux	5 feeding events, 15 lux	Self-feeder, 100 lux	Self-feeder, 15 lux
avg BWi [g]	8.3 ± 0.2	8.9 ± 0.6	8.4 ± 0.6	8.9 ± 0.4	8.4 ± 0.4	8.4 ± 0.2
avg BWf [g]	24.0 ± 1.3	26.1 ± 1.1	24.8 ± 2.5	26.1 ± 0.5	20.3 ± 1.1	21.9 ± 0.8
CVi [%]	19.5 ± 1.3	19.8 ± 0.9	20.3 ± 0.3	18.9 ± 0.7	18.2 ± 1.7	19.1 ± 0.9
CVf [%]	31.0 ± 4.4	28.6 ± 2.0	34.8 ± 4.1	33.5 ± 3.6	33.7 ± 3.0	30.9 ± 1.7
SGR [% d^{-1}]	2.51 ± 0.06	2.51 ± 0.12	2.53 ± 0.06	2.52 ± 0.04	2.10 ± 0.04	2.27 ± 0.07
AFCR	0.81 ± 0.03	0.79 ± 0.04	0.78 ± 0.01	0.80 ± 0.02	1.05 ± 0.04	0.96 ± 0.02
Mortality	1.3 ± 1.0	2.2 ± 1.9	1.3 ± 1.0	2.2 ± 3.0	0.6 ± 0.7	1.3 ± 1.0
TFS	5.2 ± 0.7	5.5 ± 0.4	5.3 ± 0.7	4.6 ± 1.0	5.4 ± 0.5	5.1 ± 0.6
Amount males	39 ± 6	34 ± 5	43 ± 2	40 ± 10	41 ± 4	43 ± 4
Amount females	41 ± 7	44 ± 6	36 ± 2	39 ± 9	39 ± 3	37 ± 5

Note: Data are expressed as mean ± standard deviation (n = 4).

Appendix 2:

Erklärung betreffend das selbständige Verfassen einer Masterarbeit im Departement Life Sciences und Facility Management

Mit der Abgabe dieser Masterarbeit versichert der/die Studierende, dass er/sie die Arbeit selbständig und ohne fremde Hilfe verfasst hat.

Der/die unterzeichnende Studierende erklärt, dass alle verwendeten Quellen (auch Internetseiten) im Text oder Anhang korrekt ausgewiesen sind, d.h. dass die Masterarbeit keine Plagiate enthält, also keine Teile, die teilweise oder vollständig aus einem fremden Text oder einer fremden Arbeit unter Vorgabe der eigenen Urheberschaft bzw. ohne Quellenangabe übernommen worden sind.

Bei Verfehlungen aller Art treten Paragraph 39 und Paragraph 40 der Rahmenprüfungsordnung für die Bachelor- und Masterstudiengänge an der Zürcher Hochschule für Angewandte Wissenschaften vom 29. Januar 2008 sowie die Bestimmungen der Disziplinarmassnahmen der Hochschulordnung in Kraft.

Ort, Datum:	Unterschrift:
Buchs SG, 01.07.2021	[redacted]