

Aus dem Institut für Tierzucht und Tierhaltung
der Christian-Albrechts-Universität zu Kiel

**The effect of out-of-season production and light regime
manipulation for intensive pike-perch (*Sander lucioperca*)
larvae culture**

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“Kept in a small bowl, they will remain small. With more space, the fish can double, triple or quadruple its size.”

Edward Bloom

TABLE OF CONTENTS

LIST OF TABLES	5
LIST OF FIGURES	6
GENERAL INTRODUCTION	8
Chapter I. Quality of out-of-season produced pike-perch (<i>Sander lucioperca</i>) larvae	16
Chapter II. The effect of light intensity on performance of larval pike-perch (<i>Sander lucioperca</i>)	41
Chapter III. Positive phototaxis in larval pike-perch (<i>Sander lucioperca</i>) triggering self grading mechanism	64
SUMMARY	91
APPENDIX	97
DANKSAGUNG	99
LEBENS LAUF	100

LIST OF TABLES

Table I-1: P-values for larval quality parameters of conducted linear and mixed models. Left column showing the parental properties that were modeled as explanatory variables. Upper row showing the larval quality parameters Hatching rate (Hr), standard length at 0 dph (SL0), Yolk-size at 0 dph (YoS), Lipid-droplet size at 0 dph (LiS), feed consumption average (FCa), natural mortality rate after 15 dph (Mn), salinity induced mortality after stress test at 15 dph (Mn), Swim bladder inflation after 15 dph (SBI), standard length growth (SLG) and dry weight growth (DWG).

Table II-1: Overview of performance indicators sorted according to light treatment. Daily length growth (GRSL), daily weight growth (GRInDW), natural mortality (Mn), day of 50% swim bladder inflation (SBI50), average feed consumption (FCa) and stress mortality (Ms).

Table III-1: Statistical analysis of grading experiment. Mann-Whitney U test testing on inequality between grading groups.

LIST OF FIGURES

Figure I-1: Schematic illustration of four gut fullness intensities on the example of 15 dph old pike-perch larvae. No gut content (I), little gut content (II), average gut content (III) and high gut content (IV). Illustrations redrawn from original photographs.

Figure I-2: Boxplot of larval standard length (SL) growth over 21 dph showing median, quartiles and whiskers. Season 1 (white), season 2 (light grey), season 3 (dark grey) season 4 (black).

Figure I-3: Boxplot of larval growth in dry weight (DW) over 21 dph showing median, quartiles and whiskers. Season 1 (white), season 2 (light grey), season 3 (dark grey) season 4 (black).

Figure I-4: Boxplot of larval standardized RNA-DNA ratio (sRD) over 21 dph showing median, quartiles and whiskers. Season 1 (white), season 2 (light grey), season 3 (dark grey) season 4 (black). Grey and white background marks transition between sampling days. Numbers above a season plot name seasons which are significantly different from the marked group at that day.

Figure I-5: Relative proportion of gut fullness intensities (no gut content (white), little gut content (light grey), average gut content (dark grey) and high gut content (dark)) 2 hours after feeding divided in spawning seasons.

Figure I-6: Mean proportion of larvae with inflated swim bladder divided in different spawning seasons from 6 to 21 dph. Error bars visualize standard deviation of the data. Season 1 (white), season 2 (light grey), season 3 (dark grey) and season 4 (black).

Figure I-7: Boxplots of larval quality parameters divided in different seasons showing median, quartiles and whiskers. Season 1 (white), season 2 (light grey), season 3 (dark grey) season 4 (black).

Figure I-8: Pairsplot of larval quality parameters including dotplots of each pairing and p-values of the associated linear regression. P-values lower than 0.05 are printed bold.

Figure I-9: Dotplots of larval quality parameter pairings that showed significant correlations (Fig. I-8). Trendlines visualizing fitted regressions. Corresponding regression equation and R^2 are given top left of the plots.

Figure II-1: Spectrum of light used during the experiment. Intensity is depicted between 380 and 780 nm wavelength.

Figure II-2: Schematic illustration of four gut fullness intensities on the example of 15 dph old pike-perch larvae. No gut content (I), little gut content (II), average gut content (III) and high gut content (IV). Illustrations redrawn from original photographs.

Figure II-3: Standard length (SL) boxplot of larvae from four different light treatments over the first 21 dph showing median, quartiles and whiskers. 100 lx (black), 500 lx (dark grey), 1000 lx (light grey) and 2500 lx (white).

LIST OF FIGURES

Figure II-4: Weight boxplot of larvae from four different light treatments over the first 21 dph showing median, quartiles and whiskers. 100 lx (black), 500 lx (dark grey), 1000 lx (light grey) and 2500 lx (white). Asterisks indicate p-values lower than 0.05 after performing a multiple contrast test between logarithmic weight data of pictured treatments.

Figure II-5: Boxplot of accumulated mortality from different light treatments during two hours of salinity-stress exposure showing median, quartiles and whiskers. 100 lx (black), 500 lx (dark grey), 1000 lx (light grey), 2500 lx (white). Asterisks indicate p-values lower than 0.05 after performing a multiple contrast test between pictured treatments.

Figure II-6: Boxplot of natural mortality under different light treatments after 15 dph, showing medians, quartiles and whiskers. Asterisks indicate p-values lower than 0.05 after performing multiple contrast tests.

Figure II-7: Mean proportion of larvae with inflated swim bladder divided in different light treatments from 6 to 21 dph. Error bars visualize variability of the data. 100 lx (black), 500 lx (dark grey), 1000 lx (light grey) and 2500 lx (white).

Figure II-8: Relative proportion of gut fullness intensities (no gut content (white), little gut content (light grey), average gut content (dark grey) and high gut content (dark)) 2 hours after feeding divided in treatment groups. 100 lx (a), 500 lx (b), 1000 lx (c) and 2500 lx (d).

Figure II-9: RNA-DNA ratio of larvae during the first 21 dph (top left), length to weight growth (top right), RNA-DNA ratio to weight growth (bottom left) and residuals from the linear regression model of length and logarithmic weight against RNA-DNA ratio (bottom right). Shading of dots and boxplots represents treatments 100 lx (black), 500 lx (dark grey), 1000 lx (light grey) and 2500 lx (white).

Figure III-1: Spectrum of light used during phototaxis and grading experiment. Intensity is depicted between 380 and 780 nm wavelength and underlined with the corresponding color scheme.

Figure III-2: Assemblage of light channel for phototaxis and grading experiment. Diagonal view (a) shows dark i.e. black side (1), removable mesh (2), bright i.e. white side (3) and channel wall with water overflow (4). Side view (b) and top view (c) are shown below.

Figure III-3: Relative distribution of larvae after two hours of phototaxis experiment. Light grey depicts larvae collected at bright side (700 lx), dark grey stands for larvae at dark side (0 lx) of the channel. Scaling of x-axes reflects sampling points. Phototaxis between sampling points is interpolated.

Figure III-4: Absolute distribution of larvae after two hours of grading experiment at 16 dph with 1.5 mm mesh (a), 22 dph with 1.5 mm mesh (b), 28 dph with 1.5 mm mesh (c) and 34 dph with 3 mm mesh (d). Light grey depicts larvae collected at the bright side (700 lx), dark grey stands for larvae at the dark side (0 lx) of the channel.

GENERAL INTRODUCTION

Aquaculture

Global aquaculture is a billion dollar industry that in 2014 produced a total quantity of 73.8 million tons of food fish that was valued 160.2 billion US\$ and represented 44.1% of the world wide fish production. Compared with 13.4% in 1990 the share of aquaculture production tripled over the last 25 years and yet global aquaculture production is still growing (FAO, 2017b). Reason for the steady production increase is the exponentially growing human population that is progressively reliant on aquatic food to cover their protein demands. Hence the fishing pressure increased tremendously over the last decades leading to an overexploitation of global fish populations (Christensen et al., 2014) that has provided a stagnating harvest of about 80 billion tons for the last 20 years. Today a further increase of wild caught fish seems impossible and not wise whereas at the same time people eat roughly twice as much fish per capita than 50 years ago when the world's population was only half as great as today (UN, 2015; FAO, 2017b). In order to still meet the world's increasing demand of fish in the future, further expansion of global aquaculture is imperative and will continue to play a socio-economic key role for global food security.

In many cases intensification of aquaculture is linked with environmental pollution through waste water, uneaten feed, feces and medicinal drugs (Chua Thia et al., 1989; Seymour and Bergheim, 1991). Therefore, to avoid environmental problems, methods for upscaling need to be environmentally friendly and sustainable. Today in practice, sustainable production increase can be attained via a variety of different methods that include genetic improvement, health management, control and development of reproduction, nutritional improvement of feed, technical improvements of aquaculture systems and optimization of resource use (Parker, 2011). During the last decades technical improvements led to an increased utilization and propagation of partially and fully enclosed recirculation aquaculture systems (RAS). With the use of innovative technologies for water purification, RAS operate with low fresh water use and high stocking densities while still maintaining a healthy environment for the fish. Furthermore, technical control of RAS allows the optimal adjustment of rearing conditions and a year-round fish production. Since a lot of the applied rearing protocols are based on unique biological requirements of specific species, methods cannot be transferred directly in the rearing protocols of other aquaculture species. Especially for species that are newly introduced in intensive aquaculture production systems, a lot of basic research has to be conducted to define optimal rearing conditions and to overcome critical phases within the fish's life history until production can be economically viable. However, fish production in a RAS is associated

with relatively high investment and operation costs that limit the cost effective fish production in RAS to species with high market value.

Pike-perch biology

The European pike-perch (*Sander lucioperca*) is a piscivorous ambush-pursuit predator that is indigenous in fresh- and brackish-waters typically reaching sizes up to 70 cm (Hochleithner, 2005). It is most active during twilight and night times relying on excellent eyesight that allows the fish to hunt at very dim light conditions (Ali et al., 1977). Pike-perch is a valuable recreational and commercial fish species (Rundberg, 1977; Overton et al., 2015) and has been intentionally introduced outside of their natural dispersal limits until the late 19th century (Smith et al., 1998; Eschbach et al., 2014). Today pike-perch occurs in lakes and rivers of Middle and Eastern Europe as well as in large areas of Northern Asia (FAO, 2017a). Depending on prevailing water temperatures, growth rates and sex, pike-perch reaches maturity between 2 and 6 years (Petrova and Živkov, 1998; Lappalainen et al., 2003). Male pike-perch mature about one year earlier than females (Lappalainen et al., 2003). Final gamete maturation occurs after a temperature increase of the cold winter water (Kestemont and Henrotte, 2015) leading to natural spawning between April and June. During natural spawning the male inhabit a spawning territory defending it towards conspecifics for 2 to 6 weeks (Jepsen et al., 1999). During that time the male's mating behavior attracts a mature female and both begin to carry out a 30- to 90-minutes long "mating dance" at the end of which the female releases and the male fertilizes the eggs (Horváth et al., 1984). After fertilization the female leaves the spawning site and the male remains with the eggs for 4 to 8 days until hatching (Hochleithner, 2005). As spawning substrate to which the sticky eggs are attached pike-perch prefer plant roots and other plant materials (Schlumberger and Proteau, 1996).

Pike-perch in aquaculture

Pike-perch is traditionally wild caught by inland fishery or farmed in extensive pond aquaculture. Limits of this traditional pike-perch farming are suboptimal somatic growth that mainly depends on the surrounding water temperatures and a restricted reproduction that is limited to a single annual year-class. Nevertheless, from the beginning of systematic pike-perch rearing, methods have constantly been evolved to further increase production.

Historically production of pike-perch in aquaculture started as uncontrolled, natural reproduction and grow-out in earthen ponds. Pike-perch were released into the ponds during spawning season and remained there until natural spawning took place and pike-perch fry had been obtained. For grow out pike-perch fry stayed in the earthen ponds and were reared solitarily in monoculture or in polyculture together with carp (Steffens et al., 1996; Zakęś and Demska-Zakęś, 2009). Later

enhancements to this method created a variety of different rearing approaches that lead to continuous increases in productivity. Most relevant of those methods are the controlled natural reproduction (Zakęś and Demska-Zakęś, 2009), lake cage reproduction (Demska-Zakes and Zakes, 2002), artificial reproduction (Steffens et al., 1996; Zakęś and Demska-Zakęś, 2009) and out-of-season reproduction (Hermelink et al., 2013). Today a combination of artificial out-of-season reproduction and RAS based grow out is the most effective way to produce a high amount of larvae and to quickly grow the fish to marketable size. Within this method pike-perch spawners are held under an artificial temperature- and photo-period controlling the fish's spawning cycle. Eggs are fertilized artificially and reared until hatching. Larvae are reared under optimal environmental conditions up to marketable size within a RAS system. Thus, today in indoor RAS the artificial reproduction and grow out of pike-perch is possible during the whole year (Hermelink et al., 2013). Unfortunately, RAS based out-of-season production is linked to high operating costs for technology and staff. Therefore a further increase in productivity is desirable to counter the high production costs. Problems during reproduction and suboptimal rearing conditions need to be identified and improved. Thereby it is particularly promising to focus on the early life stages of pike-perch since mortality i.e. production loss during that time is highest (Balon, 1984; Summerfelt, 1996; Kestemont et al., 2007) and needs to be improved.

Production improvements

Reproduction success and hence the quality of larval pike-perch depends on breeder selection, breeder's rearing conditions and environmental conditions during rearing of eggs and larvae (Gjedrem and Baranski, 2010; Schaerlinger and Źarski, 2015). By modifying one of those factors, it is possible to increase or to decrease offspring quality. During out-of-season reproduction, photo-thermal manipulation of spawners is used to alter the neuroendocrine pathways that regulate gonad maturation (Hermelink et al., 2011; Hermelink et al., 2013). Thus, it is possible to produce pike-perch offspring independently from seasonal influences and to substantially increase production. However, out-of-season reproduction includes an extensive modification of breeder's rearing conditions and therefore potentially alters larval quality. Thus, it is possible that out-of-season pike-perch production is accompanied by an unnoticed decrease of larval quality. To date such an effect was not observed but cannot be ruled out either.

Further potential for improvements exists in the specific selection of breeders. Research on other commonly farmed fish like Baltic cod (*Gadus morhua*), Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) linked egg and larval quality to a variety of parental properties and rearing conditions like maternal genetics, lipid composition, fatty acid composition, level of domestication and water quality (Brooks et al., 1997; McCormick, 1998; Izquierdo et al.,

2001; Trippel et al., 2005; Bobe and Labbé, 2010; Evans et al., 2010; Gjedrem and Baranski, 2010; Migaud et al., 2013; Valdebenito et al., 2013). With knowledge about the influence of parental properties on larval quality, it is possible to implement successful breeding programs (Teletchea and Fontaine, 2014) and to establish predictive quality factors that allow a profound prognosis on expected larval quality (Kjørsvik et al., 2003; Ottesen and Babiak, 2007). However, species specific knowledge about effects of breeder selection and rearing on larval pike-perch quality is scarce but holds potential for further production increase.

Direct influences from the rearing environment of fish in an aquaculture system are relatively easy to investigate and, thus, today optimal rearing conditions for juvenile and adult pike-perch are widely described (Kestemont et al., 2015). Whereas for the relatively short larval period during pike-perch life history optimal settings of some important environmental factors are not described as well as for the older life stages. Nevertheless, physiological development progresses rapidly during that time and physical requirements change accordingly (Kolkovski et al., 2009; Ott et al., 2012). So far the effect of different light intensities on larval pike-perch development has not been described sufficiently. For juvenile and adult fish, light requirements are studied in detail and fish at that age are described to prefer very dim light conditions (Luchiari et al., 2006; Kozłowski et al., 2010). Larval pike-perch on the other hand is known to undergo a short period of positive phototactic behavior (Jäger et al., 1984; Schlumberger and Proteau, 1996) which shows that fish at that age behave fundamentally different to high light intensities. Thus, it is likely that larval pike-perch is best reared under different light intensities than juvenile and adult specimens. To describe the optimal light intensities for pike-perch during larval age could provide valuable knowledge for intensive larval rearing and further increase the production outcome.

Another way to increase aquaculture production is to find technical solutions for known issues during the rearing period or to improve old methods with new techniques that are more advantageous. For larval pike-perch rearing one prominent obstacle is the cannibalistic behavior that larvae develop starting at a total length of 15 mm about two weeks after hatching (Szkudlarek and Zakes, 2007). With the onset of cannibalistic behavior, mortality can increase noticeably and if no measures are taken to inhibit this behavior, the losses will increase rapidly. Studies showed that reducing size heterogeneity via grading is an effective way to inhibit cannibalism (Szczepkowski et al., 2011; Knaus and Gallandt, 2012). During a mechanical grading process, fish are typically concentrated in a set up including a barrier with a specific mesh size or gap width that can only be passed by certain fish sizes. Unfortunately the widely applicable, mechanical grading processes are typically associated with physical forces that are accompanied by high handling stress for the fish (Dunlop et al., 2004). Furthermore, size sorting of larval fish is very delicate due to the small individual size and often labor

intensive. Developing a grading method for pike-perch larvae that is easier i.e. faster to apply and associated with less stress for the fish could improve larval pike-perch rearing significantly.

Prospects of the study

During the course of this study, reproduction and rearing methods of RAS based out-of-season pike-perch production were investigated for the first 21 days post hatch (dph). Three experiments were conducted with different approaches to settle open questions and find new solutions in larval pike-perch aquaculture.

For the first experiment 13 batches of fertilized pike-perch eggs originating from four separate seasonally shifted broodstocks were hatched and larvae were reared in an indoor aquaculture system. During incubation and rearing, a variety of quality indicators were recorded to assess the effect of out-of-season spawning on the quality of produced offspring. The aim was to (i) give a profound estimation on potentially negative effects of out-of-season spawning on quality of pike-perch offspring. In an additional approach to the experiment individual morphological and life history traits of breeders were compared to larval and egg quality. Furthermore, early larval quality was compared to late larval quality. Thus, it was tried to (ii) locate parental body characteristics and early larval life history traits that could be used to predict and increase larval performance.

In a second experiment larval pike-perch of a single batch were reared under four different light intensities until 21 dph. During the rearing period multiple quality indicators were recorded and compared to the respective light treatments. Within the extend of the experiment it was tried to (iii) give a depiction on the effect of light intensities during larval rearing and to answer which light intensity was most advantageous for larval development.

During the third experiment phototactic behavior of pike-perch larvae from 1 to 50 dph was evaluated in a channel system that provided darkness at one side of the channel and bright light at the other side. Subsequently the observed phototactic behavior was used to evaluate the effectiveness of a light triggered self grading mechanism during 16 and 34 dph. The aim was to (iv) demonstrate if light can be used as an attractant to implement a gentle self grading mechanism during the larvae's positive phototactic phase.

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Chapter I. Quality of out-of-season produced pike-perch (*Sander lucioperca*) larvae

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Abstract

In the present study, 13 different batches of fertilized pike-perch (*Sander lucioperca*) eggs were hatched and larval fish were reared for 21 days post hatch (dph) in an indoor aquaculture system. The fertilized pike-perch eggs originated from four different broodstock groups held under temporarily shifted temperature and light regimes that enabled an out-of-season offspring production with four spawning seasons per year (spring, summer, autumn and winter). Aim of this study was to determine potentially negative effects of out-of-season spawning on the quality of larval offspring. Furthermore, different parental quality properties (female length, spawning experience, domestication history, total egg volume, number of fertilizing males) and larval quality properties (Hatching rate, standard length at 0 dph, Yolk-size at 0 dph, Lipid-droplet size at 0 dph, feed consumption, natural mortality, stress induced mortality, Swim bladder inflation, standard length growth, dry weight growth) were evaluated for their potential to predict larval quality. During the course of the study parental properties were tested on their influence on larval quality and the larval quality parameters were tested on their influence among each other. The results showed one

out of three out-of-season groups to have a noticeably lower standardized RNA-DNA ratio (sRD) and a lower dry weight growth (DWG). Nevertheless, the two other remaining out-of-season spawning groups produced larvae that were of equivalent quality to those that were produced during natural spawning season. Further parental properties were shown to be insufficient predictors for larval quality. Additionally it was shown that the size of the lipid droplet (LiS) at 0 dph substantially affected larval stress resistance and it was proposed to regularly use LiS at 0 dph as larval quality predictor.

1. Introduction

The European pike-perch (*Sander lucioperca*) is a high valued fresh- and brackish-water fish that naturally occurs in Middle and Eastern Europe as well as in large areas of Northern Asia (FAO, 2017). Traditionally pike-perch is wild caught by inland fishery or farmed in extensive pond aquaculture. Over the last two decades, however, the favorable traits like fast growth and good market acceptance has led to focus on pike-perch for intensive production in land based recirculation aquaculture systems (RAS). One major obstacle for intensification of pike-perch production was the dependency on natural spawning for offspring production. This dependency on a single spawning event per year led to short periods with a glut of fish and long periods with shortage of intensively produced pike-perch (Overton et al., 2015). During the last two decades a lot of progress has been made regarding the optimization of pike-perch rearing methods and techniques (Overton et al., 2015). Nowadays, reproduction of pike-perch in RAS can be controlled by manipulation of water-temperature and photo-period (Hermelink et al., 2013). Thus, offspring can be produced out-of-season and year-round. The continuous offspring production is typically based on several broodstock groups that are held at different times of an artificial, annual light and temperature cycle that induces and shifts the maturation process of the broodstock to the farmers' needs. After a cold wintering phase, final maturation is induced by an elevation of water temperatures that simulate spring conditions and fertilized eggs are received by controlled nest spawning or stripping of the fish (Zakęś and Demska-Zakęś, 2009). The implementation of controlled out-of-season spawning ascertains a constant availability of intensively produced pike-perch larvae, which is a prerequisite for economically viable intense RAS production of this species.

Availability, however, is only one criterion that is of particular importance for the viability of production. Another key concern is high quality of reproduced fish in terms of favorable traits like fast growth, low mortality and high resilience against stress. The reproductive success and hence the quality of eggs and larvae depend on breeding selection, their rearing conditions and the rearing environment of eggs and larvae (Gjedrem and Baranski, 2010; Schaerlinger and Źarski, 2015). While direct influences from the rearing environment of larvae and eggs are relatively easy to investigate, the assessment of indirect effects that originate from parental traits and rearing conditions is more

challenging. A lot of work has been done on the rearing environment of pike-perch eggs and larvae whereas knowledge on the effects of breeder selection and rearing is scarce. Nevertheless, research on other commonly farmed species showed a variety of parental effects on quality of gametes, eggs and larvae. By today gamete, egg and larval quality have been attributed to maternal and paternal genetics, domestication, temperature protocols, fish size, use of hormones, nutrition and handling stress (Brooks et al., 1997; McCormick, 1998; Izquierdo et al., 2001; Trippel et al., 2005; Bobe and Labbé, 2010; Evans et al., 2010; Gjedrem and Baranski, 2010; Migaud et al., 2013; Valdebenito et al., 2013). Unfortunately, those effects are highly species specific and have to be characterized for each species individually. To ensure a more effective breeder selection and increasing reproduction success in future pike-perch farming potential, parental and early larval quality indicators need to be assessed on their interaction with offspring quality.

The work of McCormick (1998) showed that quality of the tropical damselfish's (*Pomacentrus amboinensis*) offspring can be negatively affected by disturbance of hormonal balance. An elevated cortisol level of breeders led to significantly smaller larvae with smaller yolk sac. During out-of-season reproduction of pike-perch the environmental factors light and temperature are used to artificially alter the neuroendocrine pathways that regulate this hormonal balance (Hermelink et al., 2011; Hermelink et al., 2013). Thus it is possible that the application of out-of-season reproduction in pike-perch affects hormonal balance of breeders in a manner that not only allows the shift of spawning but also negatively affects quality of eggs and larvae. Therefore it is imperative to investigate the effects of out-of-season reproduction on quality of obtained eggs and larvae. Parameters that can provide information about the quality of larvae are numerous and can be divided into morphological, behavioral and biochemical quality parameters. The morphological quality indicators include size measurements and the monitoring of specific morphological developments that are linked to critical milestones during larval ontogeny (Balon, 1984). Behavioral quality relates to larval behavior that is either desirable or undesirable and biochemical quality parameters potentially include the observation of every metabolic product that is present within the larvae. Furthermore, mortality is an important indicator that integrates the aforementioned quality indicators, reflecting either natural, stress- or otherwise induced factors.

For the experiments of this work, fertilized eggs of 13 different females were hatched and the larvae were reared up until 21 days post hatch (dph). The spawning females originated from four differently shifted broodstocks of a commercial company (AquaPri, Denmark). During the incubation and rearing period a variety of morphological, behavioral, lethal and biochemical quality indicators were used to assess the effect of light and temperature induced out-of-season spawning on the quality of produced offspring. Furthermore, individual morphological and life history traits of reproducing

males and females were used to locate possible paternal and maternal effects on larval quality. Also early larval quality was compared with late larval quality at 15 and 21 dph.

Aim of this study was (i) to give estimation on potentially negative effects of out-of-season spawning on quality of pike-perch offspring and (ii) to locate parental body characteristics and early larval life history traits that could be used to predict and increase larval performance up to 21 dph.

2. Material and Methods

2.1 Stripping and broodstock management

All larvae that were used during the experiments originated from four broodstocks of the commercial pike-perch hatchery. The four different broodstocks were held under the same rearing conditions but temperature and light regime were temporarily shifted in intervals of three months. Thus, four spawning seasons per year could be induced (spring, summer, autumn and winter). Maturation was induced by a wintering phase of four months where temperature was gradually decreased and a subsequent warming phase with a temperature increase up to ~16°C. At time of ovulation the fish were anesthetized with a 20% benzocain solution (Kalmagin, Centroviet, Santiago de Chile) and the eggs were stripped. The stripped eggs were subsequently fertilized with freshly stripped sperm. Afterwards the fertilized eggs were incubated in McDonald-type hatching jars (Pentair Hatching Jar, Apopka, United States) at about 16°C water temperature. Eggs were monitored every 24 hours and fertilization rates i.e. embryo survival was calculated. During the stripping process parental qualities of spawning females and males had been measured and recorded.

2.2 Larval rearing

The fertilized pike-perch eggs of 13 different females were obtained from AquaPri between August 2013 and March 2015 (Summer 2013, n = 3; Autumn 2013, n = 2; Winter 2014, n = 3; Summer 2014, n = 2; Autumn 2014, n = 1; Spring 2015, n = 2) at an egg ripening between 10 and 40 degree days (d°C). Eggs were transported in plastic bags placed in a styrofoam box to maintain the water temperature at 16-17°C. The plastic bags were each filled with about 5 L hatchery water, 5 L ambient air and the fertilized eggs. After a transport of three hours, the eggs were transferred into McDonald-type hatching jars (Pentair Hatching Jar, Apopka, United States) at the experimental facility of the Gesellschaft für Marine Aquakultur mbH (GMA) (Büsum, Germany). Incubation temperature of the hatching jars was 18°C and ambient light intensity was below 50 lx. Hatching was initiated after the eggs reached 72 d°C by stopping the water in-flow. Subsequently, within 2 hours larvae were

transferred into the rearing system with a density of 100 larvae per liter. Rearing took place in three structurally identical aquaculture systems that each contained four 40 L rearing tanks of green coloration placed inside the system with a total water volume of 1700 L. The experimental rearing was performed in triplicates that were evenly distributed on each of the three aquaculture systems. Each rearing tank had a water inflow of about 100 ml/min that evoked a gentle circular current. The outflow was placed in the middle of each tank in shape of a leveled drain that was covered with a mesh (mesh size 1 mm). Inside the rearing tanks a plastic tube was placed to provide gentle aeration and perturbation at a rate of 30-60 bubbles per minute. Water temperature was maintained at 18°C with a heater (Aquamedic TH-500W, Bissendorf, Germany) respectively via a cooling system (Aquamedic Titan 1500, Bissendorf, Germany). Inside the RAS system a UV sterilizer (Aquamedic Helix Max UV 55W, Bissendorf, Germany) was integrated and throughout the whole experiment water of the system was treated with algae concentrate (BlueBioTech Nannochloropsis sp., Büsum, Germany) maintaining green water at 2-5 Nephelometric Turbidity Units (NTU). The water parameters were monitored every day to keep them at $18 \pm 0.5^\circ\text{C}$ water temperature, $\geq 95\%$ oxygen saturation, $\leq 0.1 \text{ mg/dm}^3$ ammonia, $\leq 0.2 \text{ mg/dm}^3$ nitrite and pH 7.8 to 7.6. A 24 h light regime with 500-700 lx light intensity at the water surface was provided by light tubes (Lampen XXL CMI T 36W IP 65, Kronau, Germany). 24 h lightning period was oriented on commercial hatcheries where continuous lightning regimes are commonly used based on the assumption that early feeding success increases (Kestemont et al., 2015). The use of artificial lightning via light tubes was also oriented on common procedure from commercial producers where artificial lightning tubes are widely used.

Larvae were fed with live feed from 4 dph onwards, periodically every 6 h throughout 24 h (6, 12, 18 and 24). The larvae were fed with *Artemia spec.* nauplii (INVE AF Specialty Cysts, Dendermonde, Belgium) that were harvested every day and stored on ice until the fourth feeding. Larvae were fed with a density of 10 nauplii per ml per day until the end of the experiment at 21 dph, which provided sufficient ad libitum prey under those stocking densities.

2.3 Sampling

Sampling of the differently treated larvae started at 0 dph and continued in three-day intervals until the last sampling at 21 dph. At each sampling day, ten larvae per tank were removed from the rearing system and stored in a solution of pH-stabilized tricaine methansulfonat (MS222). The sedated larvae were then photographed under a stereo microscope (Olympus SZ61, Tokio, Japan) for subsequent morphometric measurements. Thereafter larvae were washed in deionized water and individually stored in 1.5 ml eppendorf flasks in which they were immediately snap-frozen at -80°C. Sampling of all tanks took about 2 hours and was conducted between 2 and 4 o'clock pm after the second feeding respectively at the same time of day for sampling days with no feeding.

2.4 Measurements

Characteristics of parental fish that were suspected to have an influence on larval quality were measured at the pike-perch farm. These characteristics included female length, spawning experience (first time spawner or spawner with at least one spawning of a previous cycle) and domestication history (wild caught spawner or captive born spawner) as well as the total egg volume and the number of fertilizing males (one or two). The different spawning seasons to which the parental fish were allocated were oriented on the natural spawning season of pike-perch between April and June. Thus fish from the hatchery that were stripped during natural spawning season i.e. in-season were considered to spawn in a natural rhythm (season 1). However, fish that spawned in an artificial rhythm between July - September (season 2), October - December (season 3) and January – March (season 4) were considered to be off-season spawners.

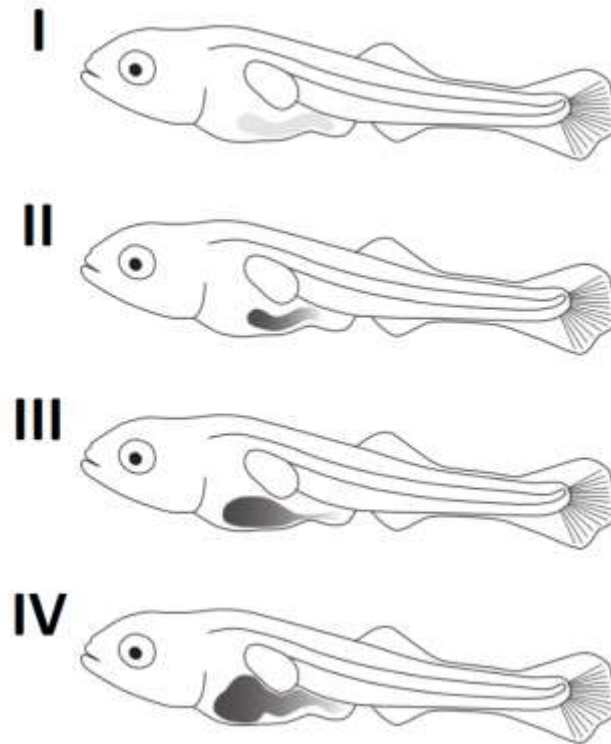


Figure I-1: Schematic illustration of four gut fullness intensities on the example of 15 dph old pike-perch larvae. No gut content (I), little gut content (II), average gut content (III) and high gut content (IV). Illustrations redrawn from original photographs.

Before hatching of fertilized pike-perch eggs, survival of the eggs i.e. embryo survival was measured every 24 hours. After hatching, a hatching rate (Hr) was calculated based on the fertilization rate and the consecutive survival rates. The images that were taken from each larva during the sampling were subsequently analyzed with the graphic program ImageJ 1.45s, standard length (SL) was measured for each fish from the tip of the upper jaw to the posterior end of the last vertebra and the rate of feed consumption (FC) was determined and ranked into four different stages (Fig. I-1). Moreover, an average daily FC (FCa) was calculated by assigning the four gut fullness stages to numbers (I=0, II=1, III=2 and IV=3), adding the numbers and dividing the summed up ranks by the amount of experiment days. The proportion of swim bladder inflation (SBI) was recorded over the whole experiment. Also at 0 dph the yolk sac size (YoS) and lipid droplet size (LiS) were calculated. At dph 15, natural mortality (Mn) and stress induced mortality (Ms) were determined. At the age of 15 dph, it was assumed that the critical phase of swim bladder inflation had ended (Steenfeldt, 2015) and cannibalistic behavior that can appear at a larval SL of 15 mm (Szkudlarek and Zakes, 2007) had no influence on the larvae yet. Thus Mn observations were not biased by cannibalism and Ms was not influenced by an elevated stress level that could have originated from cannibalism or the swim bladder inflation process. Mn was measured by counting the larvae of initially 4000 fish that were introduced per tank. The

difference between the initial stock size and the counted stock size at dph 15 was the basis for mortality calculations. Furthermore Ms was calculated, exposing larvae to an acute salinity change to examine the capability of larvae to perform osmoregulation and to counteract stress (Kestemont et al., 2007; Lund et al., 2012). During the test 120 larvae from each tank were gently transferred to a 1 L glass beaker containing water of 19 PSU and 18°C. Over a period of two hours cumulative mortality i.e. fish lying on the bottom without movement was counted in 5-min intervals as described by Dagar et al. (2010) and registered as Ms.

After the sampling, frozen fish were dried for 24 hours in a (Christ Alpha LD 1-2, Osterode, Germany) freeze dryer which extracted any water from the samples. The dried fish were then weighed on a high-resolution balance (Sartorius Cubis Balance MSA2.7S, Göttingen, Germany) and individual dry weight (DW) was measured. After weighing, larvae were put back into the eppendorf flasks and preserved at -80°C until further usage for RNA-DNA analysis. The RNA-DNA ratio as quality parameter is based on the principle of a fairly constant DNA level and a RNA content that varies with protein synthesis activity, the majority of cellular RNA being tRNA (Bulow, 1970; Meyer et al., 2012). A high RNA-DNA ratio is consequently associated with high protein synthesis and hence somatic growth and, with a time lag of around two days, nutrition as well (Meyer et al., 2012). Individual RNA and DNA content of larvae were measured following Caldarone et al. (2003). Within this measurement whole freeze-dried larvae were used to determine the nucleic acid content by means of the intercalating fluorescent dye ethidium bromide (Clemmesen, 1987; Caldarone et al., 2001) in an untreated homogenate. Afterwards in two consecutive steps, RNase and DNase were added to the homogenate and respectively two more fluorescence readings were performed. The second reading measured the homogenate fluorescence without the degraded RNA and the third without RNA and DNA fluorescence. Based on the difference between the first and the second and between the second and the third reading, the nucleic acids were quantified. The resulting RNA-DNA ratios were standardized (sRD) to a reference slope ratio based on the slopes of the standard curves in Caldarone et al. (2006). All experiments of this study were conducted in agreement with German legal regulations on animal experiments.

2.5 Statistical analysis

For the evaluation of parental influence on larval quality parameters Hr, SLO, YoS, LiS, FCa, Mn, Ms and SBI at 15 dph, an appropriate linear model was defined. Based on graphical residual analysis, data were assumed to be normally distributed and to be homoscedastic. The statistical model included season (1 – 4) and the parental properties female length, female spawning experience, female domestication history, total egg volume and number of fertilizing males. The batch was

regarded as random factor. Based on this model, an analysis of variances (ANOVA) or an analysis of covariances (ANCOVA) was conducted. Because of inter correlations between the parental properties female length, female spawning experience and total egg volume the two properties with highest p-values were excluded from the model and a new model was defined with the remaining explanatory variables. For the second model, an analysis of variances was performed once more to answer if the parental characteristics and the time of spawning i.e. season were influencing larval quality parameters. For the analysis of parental influence on larval quality parameters growth in SL and DW, an appropriate statistical mixed model was defined (Laird and Ware, 1982; Verbeke and Molenberghs, 2009). Based on a graphical residual analysis, data were assumed to be normally distributed and homoscedastic. The statistical model included season 1 – 4 and the parental properties female length, female spawning experience, female domestication history, total egg volume and number of fertilizing males. The batch was regarded as random factor. The subsequent analysis of variances and definition of a second model followed the procedure for the linear models that was described above. For the analysis of season on larval sRD, data were assumed to be normally distributed and to be heteroscedastic based on graphical residual analysis. Statistical analysis of the data started with the definition of an appropriate mixed model that included the factors season (season 1 - 4) and dph as well as their interaction term as fixed factor. The analysis of sRD evaluated the differences between seasons at each sampling day and the factor dph was included as a qualitative parameter. To evaluate the potential of early larval quality parameters as predictors for late larval quality parameters, multiple nested linear regressions of Hr, SLO, YoS, LiS, FCa, SBI, Mn, Ms, DW and SL were calculated. The parameters batch and tank were included in the models as nested variables.

Outlier detection was performed via graphical boxplot examination and subsequent Cook's distance analysis. Observations that exceeded a value of 1 were defined as outlier and excluded. The statistical software R (2015) was used to evaluate the data.

3. Results

Comparing the increase in SL over time between different spawning seasons (Fig. I-2) up to 21 dph, no significant differences were found ($p=0.85$). The increase in SL was well described by a linear model and variability within the individual groups increased over time. However, for season 1 a slightly albeit insignificant higher length variation was present between 9 and 18 dph, but at 21 dph this difference was no longer detectable.

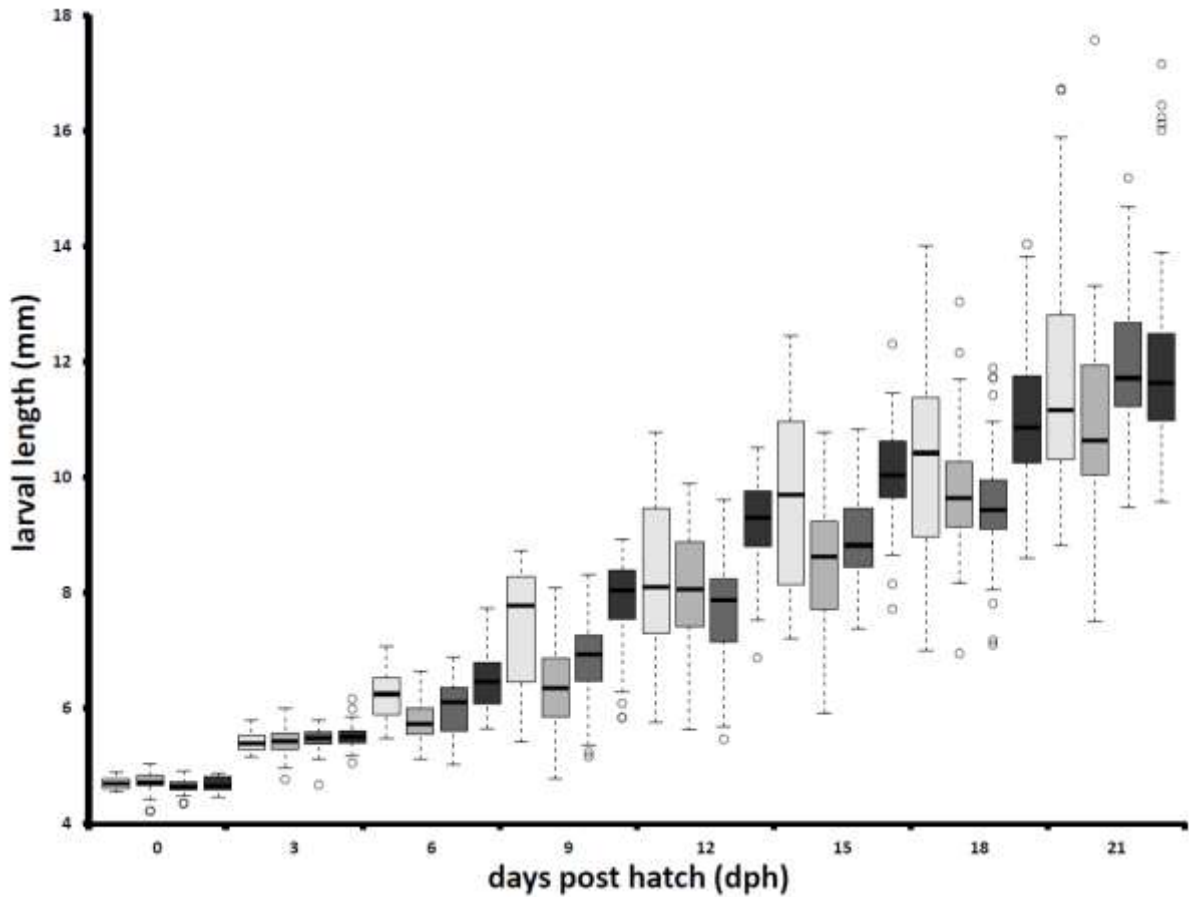


Figure I-2: Boxplot of larval standard length (SL) growth over 21 dph showing median, quartiles and whiskers. Season 1 (white), season 2 (light grey), season 3 (dark grey) season 4 (black).

Evaluating the growth curves of larval DW between spawning seasons (Fig. I-3) up to 21 dph, no significant differences were found ($p=0.72$). Larval DW growth appeared to be approximately exponential or logistic. Distinct differences in mean dry weight were not apparent. Nevertheless, weight increase of season 3 seemed to become lower than those of the other seasons beginning at dph 12. An increase of variance in all four seasons was evident with the upper and lower quartiles while especially the upper quartiles became bigger between 15 and 21 dph.

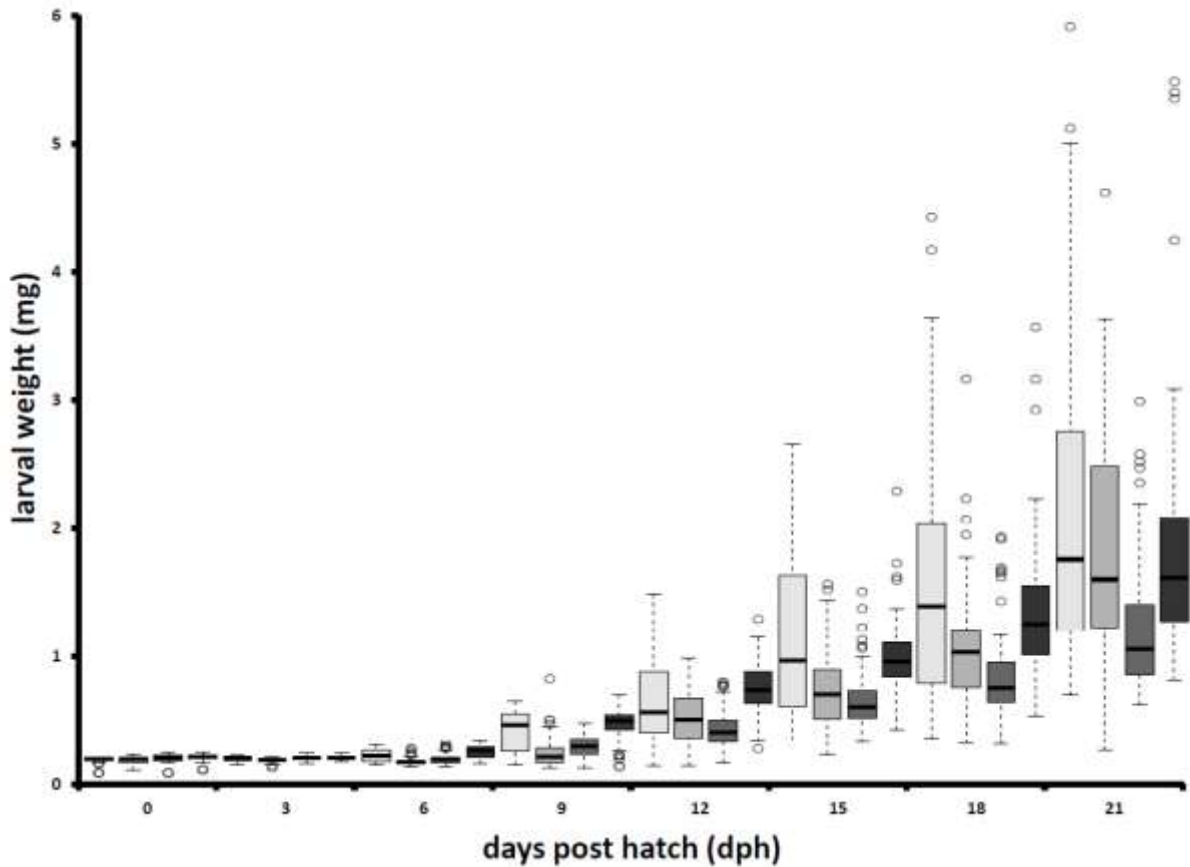


Figure I-3: Boxplot of larval growth in dry weight (DW) over 21 dph showing median, quartiles and whiskers. Season 1 (white), season 2 (light grey), season 3 (dark grey) season 4 (black).

From 0 to 21 dph the average sRD of all seasons remained constant between 3.3 and 2.2 (Fig. I-4). An increase of variance over time in all four seasons was evident equally for the upper and lower quartiles. Particularly prominent was a decrease in minimum sRD beginning at dph 9. Significant differences between seasons were found at sampling days 6, 9, 12, 15 and 18. Lowest average sRD beginning at dph 9 was consistently shown for season 3. Nevertheless, no spawning season showed constant significantly higher sRD over another season and at the last sampling day no significance was shown at all.

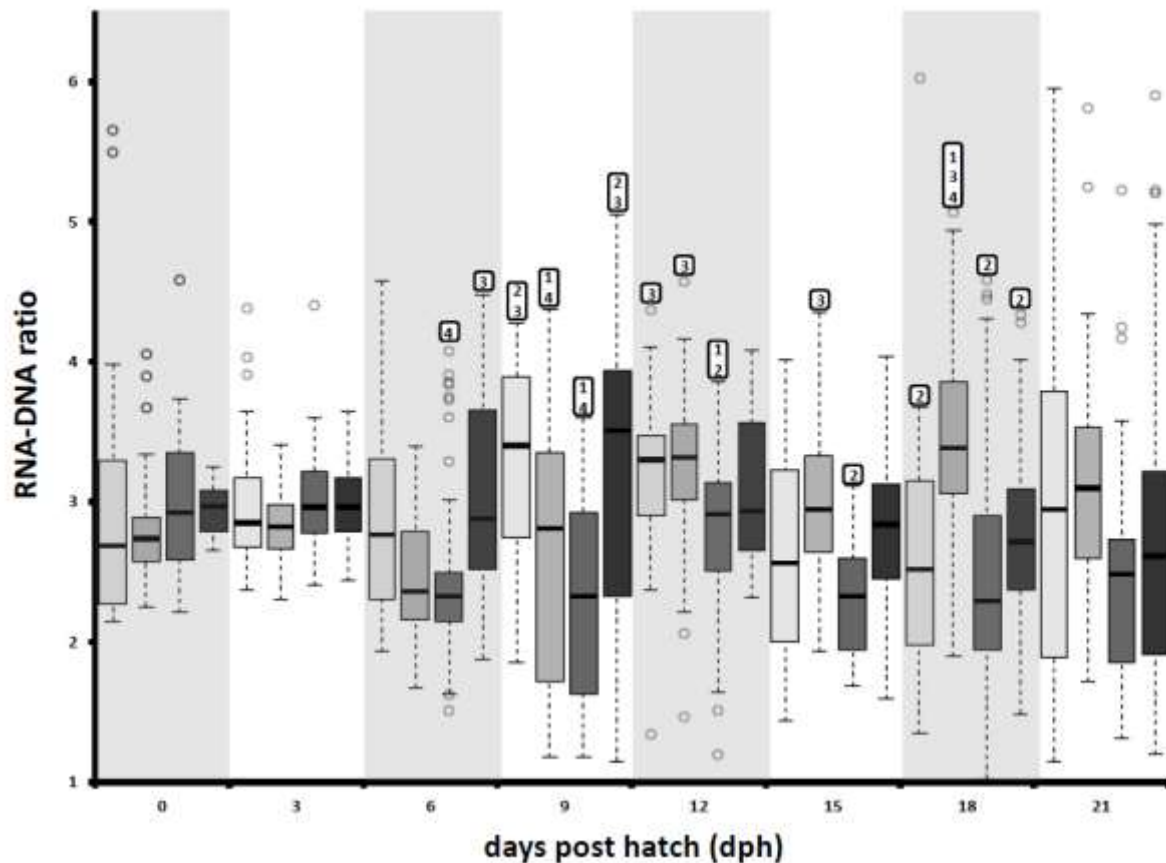


Figure I-4: Boxplot of larval standardized RNA-DNA ratio (sRD) over 21 dph showing median, quartiles and whiskers. Season 1 (white), season 2 (light grey), season 3 (dark grey) season 4 (black). Grey and white background marks transition between sampling days. Numbers above a season plot name seasons which are significantly different from the marked group at that day.

FC was recorded in four intensity categories beginning at 6 dph (Fig. I-5). Continuous differences in FC between different spawning seasons were not evident. While at dph 6 seasons 2 and 3 seemed to have a lower FC than seasons 1 and 4, clear differences at later sampling days were no longer evident. For all seasons, an increase in FC was noticeable between dph 6 and 9. After 9 dph FC for seasons 1, 2 and 3 fluctuated slightly but overall FC remained at an average to high gut content. For season 4, however, the amount of individuals with little gut content seemed to be a slightly higher. A significant difference between FCa and season could not be found ($p=0.75$).

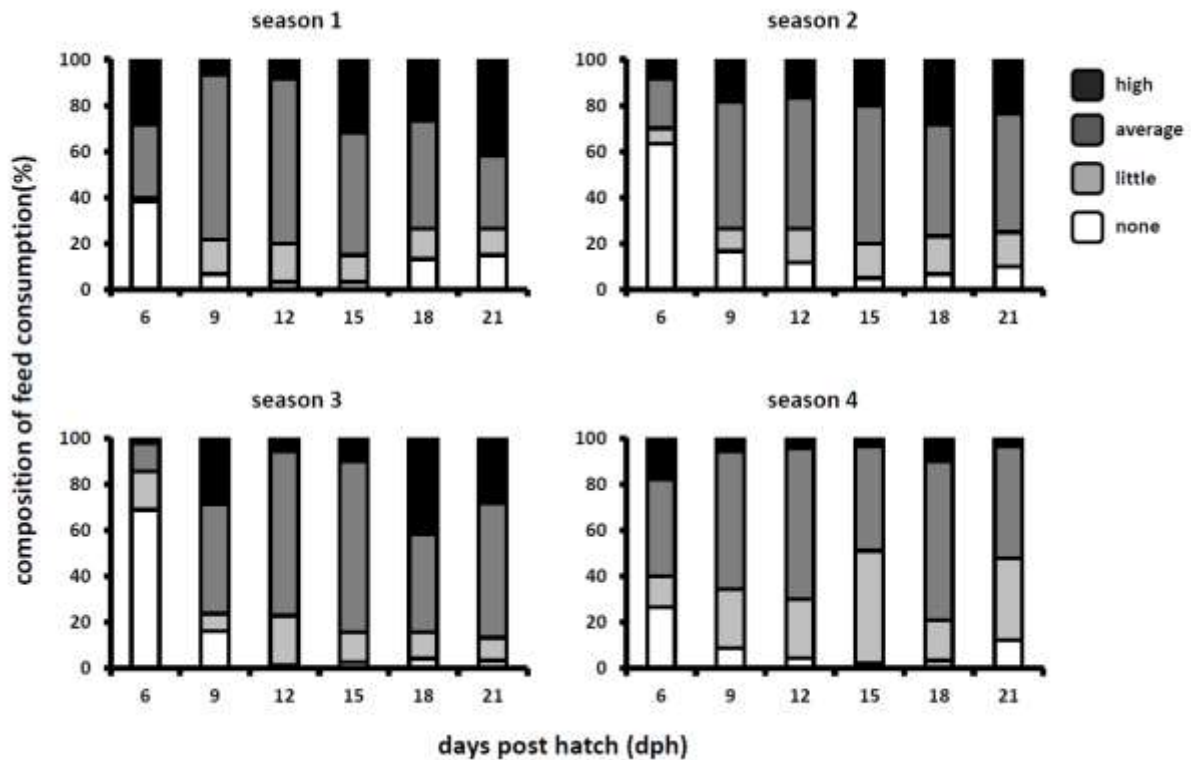


Figure I-5: Relative proportion of gut fullness intensities (no gut content (white), little gut content (light grey), average gut content (dark grey) and high gut content (dark)) 2 hours after feeding divided in spawning seasons.

SBI started between 6 and 9 dph (Fig. I-6) and increased markedly between 9 and 12 dph. After 12 dph noticeable changes in the amount of individuals with inflated swim bladder were not present. Apparently seasons 1 and 2, beginning at 12 dph, showed considerably lower swim bladder inflation than seasons 3 and 4. Nevertheless, a statistically significant influence between season and SBI was not found ($p=0.308$).

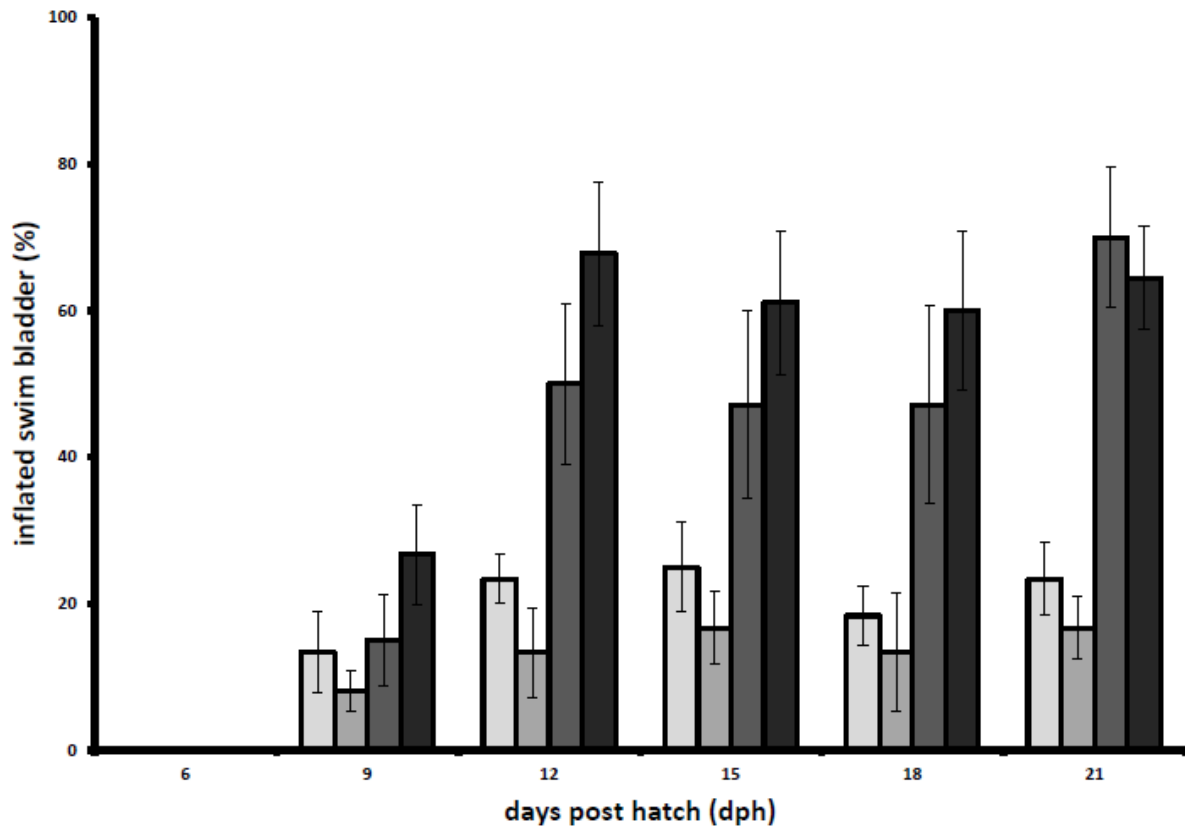


Figure I-6: Mean proportion of larvae with inflated swim bladder divided in different spawning seasons from 6 to 21 dph. Error bars visualize standard deviation of the data. Season 1 (white), season 2 (light grey), season 3 (dark grey) and season 4 (black).

Optical analysis of larval quality parameters Hr, SL0, YoS, LiS, Mn and Ms divided in four spawning seasons (Fig. I-7) revealed a high extent of variability between as well as within seasons. Thus differences in median and inner quartiles between seasons were mostly within the range of their whiskers. While no seasonal differences were apparent for Hr and SL0, the YoS for seasons 1 and 4 seemed to be equally lower compared to seasons 2 and 3. The LiS however showed no clear difference within seasons. A high Mn was visible for season 2 while Mn for season 1, 3 and 4 was considerably lower. Nevertheless, the highest measured Mn in season 1 and 3 lay within the data range of season 2 Mn. Ms after two hours of salinity stress exposure was lowest for season 3 while again a high variability within the different seasons was evident. Hr, SL0, YoS, LiS, Mn and Ms showed no statistically significant difference within seasons (Tab. I-1).

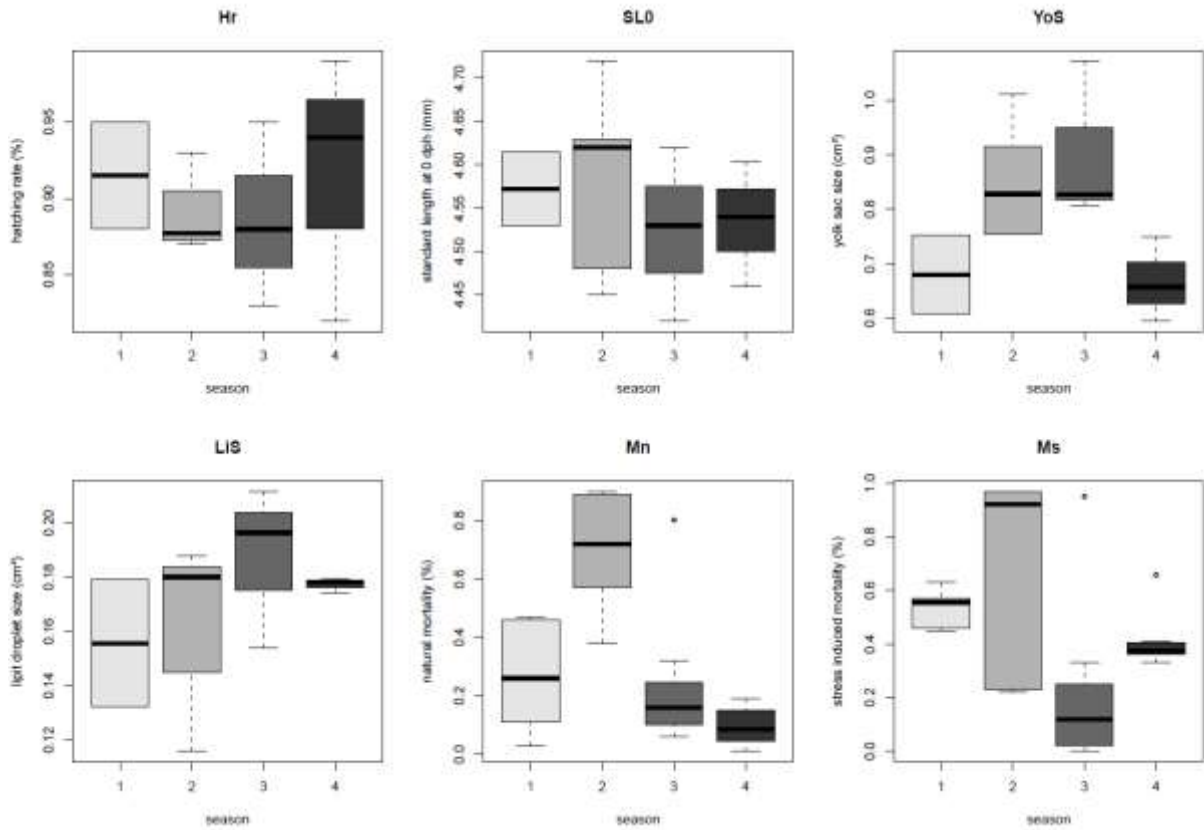


Figure I-7: Boxplots of larval quality parameters divided in different seasons showing median, quartiles and whiskers. Season 1 (white), season 2 (light grey), season 3 (dark grey) season 4 (black).

Table I-1: P-values for larval quality parameters of conducted linear and mixed models. Left column showing the parental properties that were modeled as explanatory variables. Upper row showing the larval quality parameters Hatching rate (Hr), standard length at 0 dph (SL0), Yolk-size at 0 dph (YoS), Lipid-droplet size at 0 dph (LiS), feed consumption average (FCa), natural mortality rate after 15 dph (Mn), salinity induced mortality after stress test at 15 dph (Ms), Swim bladder inflation after 15 dph (SBI), standard length growth (SLG) and dry weight growth (DWG).

parental properties	Hr	SL0	YoS	LiS	FCa	Mn	Ms	SBI	SLG	DWG
experience	0.402									
length female		0.3			0.191			0.268	0.165	0.312
egg volume			0.214	0.078		0.195	0.16			
Domestication	0.909	0.724	0.358	0.856	0.762	0.181	0.7	0.975	0.477	0.542
fertilising males	0.623	0.106	0.558	0.709	0.996	0.878	0.88	0.423	0.333	0.49
season	0.405	0.25	0.365	0.968	0.756	0.283	0.805	0.308	0.846	0.715

Regressions between larval quality parameters (Fig. I-8) showed significant linear relations the following parameters: YoS and LiS were the only early larval quality parameters that showed significant relations with late larval quality parameters (Ms, SLG, DWG). Significant correlations were also identified between the late larval parameters Ms and SLG as well as for SBI and DWG. Furthermore, YoS with LiS and SLG with DWG correlated strongly.

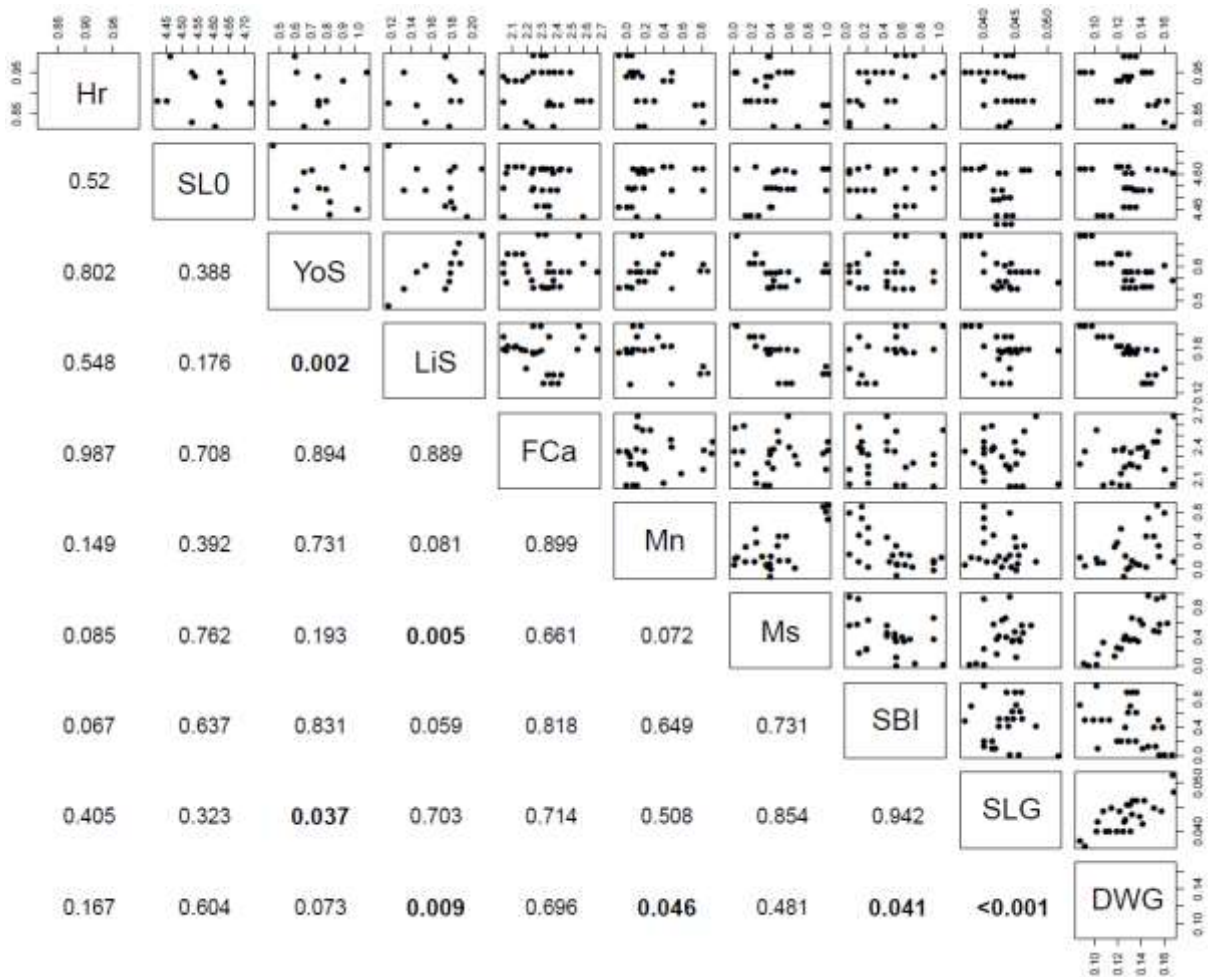


Figure I-8: Pairsplot of larval quality parameters including dotplots of each pairing and p-values of the associated linear regression. P-values lower than 0.05 are printed bold.

Fitted linear regressions of statistically significant quality parameter pairings showed a wide range of R^2 values from 0.1 for YoS and SLG to 0.7 for LiS and Ms. YoS and LiS showed to be positively correlated and a high LiS correlated negatively with Ms. However, DWG seemed to be negatively affected by high LiS and a high YoS was interacting with low SLG. Furthermore, with increasing Mn an increase in DWG was detected and high SBI correlated negatively with DWG.

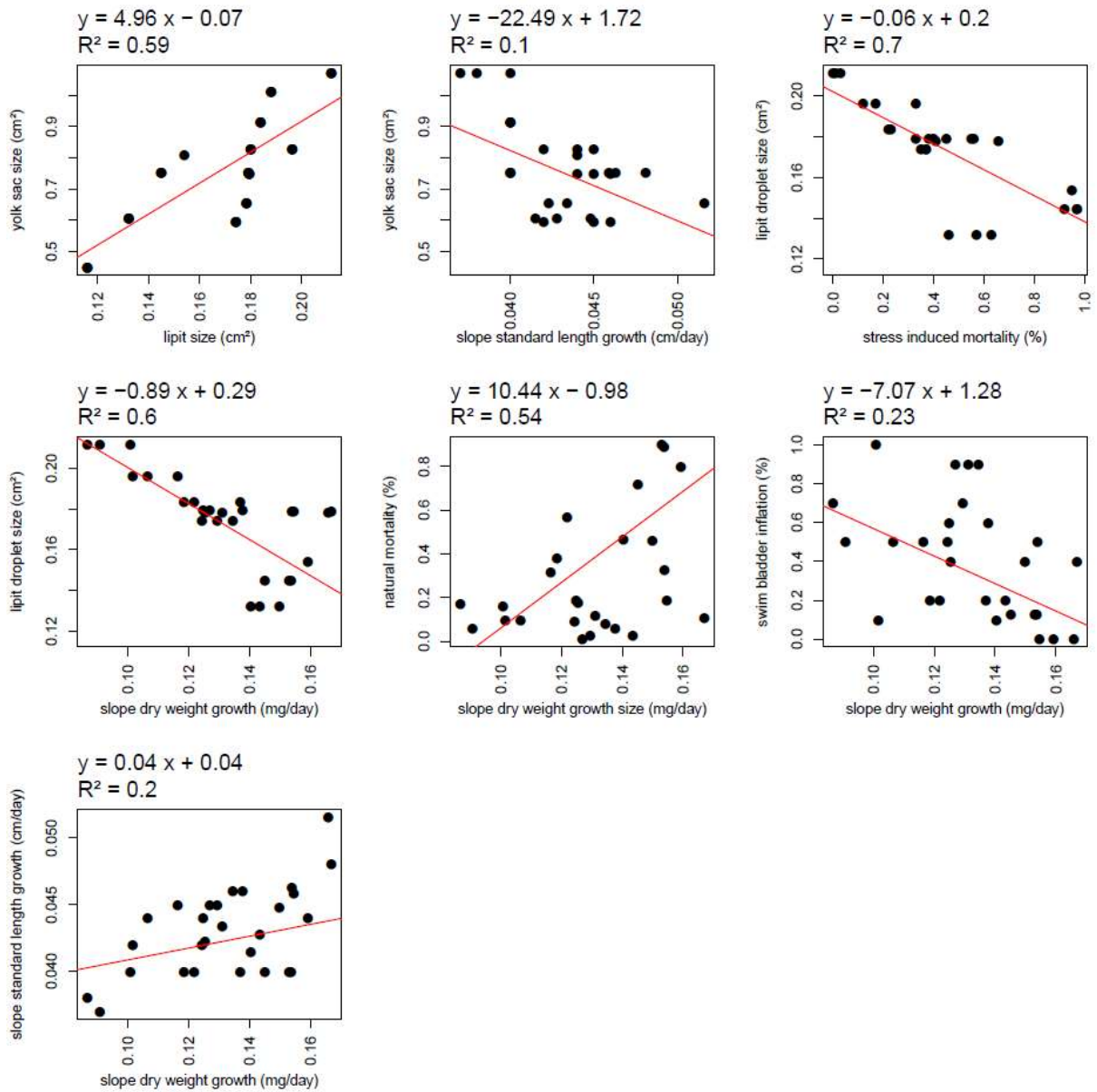


Figure I-9: Dotplots of larval quality parameter pairings that showed significant correlations (Fig. I-8). Trendlines visualizing fitted regressions. Corresponding regression equation and R^2 are given top left of the plots.

4. Discussion

4.1 Season

Except for the sRD content no statistically significant relations could be observed between out-of-season spawning and larval offspring quality. While graphical data presentation displayed distinguishable differences for DWG, Mn and SBI, linear models found neither positive nor negative significant correlations. It was noteworthy that variability of larval quality parameters within seasons was high and that variability for SLG, DWG and sRD increased over time.

It can be stated that in average, larvae of season 3 showed a constantly low sRD between dph 6 and 18 while sRD of seasons 1, 2 and 4 were alternating in its relative proportion. Taking into account that sRD is a condition factor closely related to fish growth (Buckley, 1984), it could be expected that the constant low sRD of season 3 had to be accompanied by low growth performance. While SLG showed no prominent differences between seasons (Fig. I-2), a clear albeit insignificant decrease in DWG was monitored for larvae of season 3 beginning at dph 12 (Fig. I-3). Thus a combination of significantly low sRD and an insignificantly low DWG suggested that larvae of season 3 grew slower because of low feed intake or conversion. Evaluating the FC, it was noteworthy that no clear difference could be found in gut fullness intensity between seasons (Fig. I-5). At most larvae of season 3 showed a slightly lower count of larvae with no FC and a higher count of high FC. Therefore it seemed unlikely that the documented low sRD was caused by low feed intake. Thus another stressing factor had to be the reason for a low RNA level i.e. low biosynthesis. Considering all other documented quality indicators that potentially could have influenced sRD, it was noticeable that performance of season 3 larvae was similar to the other seasons or even better. Early larval properties like Hr, SL0, YoS and LiS were not visibly different while after 15 dph stress and natural mortality was comparatively low and SBI was high. Therefore, an interaction between the low sRD and one of the documented quality indicators seemed unlikely.

Although statistical analysis disclosed a lower sRD in season 3 larvae, which can be considered an effect of out-of-season spawning, the question rises why larvae of season 2 and 4 did not show the same low sRD since they were spawned out-of-season as well. A possible explanation could be that other factors than light and temperature had influenced quality characteristics of produced larvae. Factors that were previously shown to have an influence on the neuroendocrine control of reproduction are factors like nutrition, energy reserves and stress (Cerdá et al., 1995; Schreck et al., 2001; Milla et al., 2009; Taranger et al., 2010). The nutritional feed supply of tested broodstocks was identical and energy reserves of the stripped females differed but were randomized. Differences in stress exposure on the other hand was much more difficult to rule out since the cause of stress

potentially include a wide range of genetic, developmental and environmental stressors (Barton, 2002). Thus, provided that parental stress exposure can influence offspring characteristics (Schreck et al., 2001), a difference in parental stress history could explain the observed lower sRD and DWG for season 3 larvae. Nevertheless, this explanation for observed lower sRD and DWG of season 3 larvae was left to be speculative since observations could not be reproduced for the other out-of-season reproduced treatments (season 2 & 4) and a potential stress factor of the broodstock could not be narrowed down.

Thus the results of this work could not find evidence for a negative effect of out-of-season spawning on quality of pike-perch offspring since a decrease in sRD and DWG could not be found for all out-of-season spawning treatments. Results allowed speculations but gave no prove for an unknown parental stressor which could have evoked the observed low sRD and DWG. Future studies on parental stress exposure related to offspring quality are needed to validate this hypothesis.

4.2 Parental properties

The parental properties spawning experience, female length, total egg volume, history of domestication and amount of fertilizing males were tested for possible significant correlations with larval quality parameters of the produced offspring. It was striking that not one significant correlation could be found for any of the early and late larval quality parameters.

The presented data showed that the tested parental properties did not represent efficient indicators for the prediction of early and late larval quality parameters. Nonetheless, it is important to emphasize that results showed that the effect of parental properties on larval quality is comparatively small and not significantly verifiable since variability in larval quality was very high. However, that parental properties, in fact, do have an effect on offspring quality is well-founded in the fundamental characteristics of genetic inheritance (Gjedrem and Baranski, 2010). In the past, genetic breeding programs showed to be a major opportunity to substantially boost favorable traits in aquaculture species over the course of a view generations (Teletchea and Fontaine, 2014). Therefore, the future implementation of professional breeding programs on pike-perch is highly promising and repeatedly discussed among aquaculturists (Blonk and Komen, 2015). Thus, over a period of several generations of selective breeding, it is likely that larval quality and performance can be increased. Nonetheless, presented data showed that the prediction of early offspring quality using observed parental properties of one previous generation is not possible.

4.3 Early and late larval quality

Statistical analysis of early and late larval quality parameters produced 45 pairings of whom 7 showed a significant linear correlation (Fig. I-8 and I-9). Observed correlations were partially highly significant and comprised low to medial explained variance. Evaluating the 7 observed correlations between larval quality parameters, it is important to notice that the biological origin of those correlations is interpretable but since a lot of the parameters potentially depend on each other, ripple effects and autocorrelations are likely.

To forecast the quality of pike-perch larvae at an early developmental stage is of major interest for offspring producer and grow-out farmer. Therefore correlations between larval quality parameters are of particular interest if the parameters are originated at different times in larval life history. Therefore the two observed correlations between YoS - LiS and SLG - DWG are of limited value for larval quality prediction since parameters are measured during the same time of larval life history. Furthermore, both correlations describe biological interdependencies that are also of limited informative value. Since the lipid droplet is part of the yolk sack, a positive correlation between YoS and LiS was expected. A highly significant correlation between growth in length and weight was also expected and described the basic positive correlation between linear SLG and exponential DWG that resulted in a low R^2 because of their different curve characteristics (McGurk, 1987). Particularly remarkable were the observed correlations between YoS – SLG and LiS – DWG. Both correlations described a negative regression between the larvae's initial maternal feed supply at 0 dph and larvae's body growth over the first 21 dph. While one could think that larvae with more yolk or lipid are set for a better start resulting in higher growth than larvae with smaller YoS or LiS, exactly the opposite seemed to be the case. A possible explanation for this connection could lay in the onset of first feeding. While larvae with low maternal energy reserves rely on exogenous feeding earlier, it is possible that those larvae started to feed on artemia earlier than larvae with a high YoS and LiS (Yúfera and Darias, 2007). Such a shift in time of first feeding could have resulted in a temporarily delayed growth. Another possible reason could be that larvae with more initial energy reserves were potentially fitter and more larvae with low growth potential survived during the first 21 dph while slow growing larvae of batches with low energy reserves died during that time (Garrido et al., 2015). This could have resulted in higher average growth rates for batches with higher mortality due to natural selection. Looking at the correlations YoS – Mn and LiS – Mn, no link between size of yolk and natural mortality at 15 dph was apparent while a tendency of low Mn for larvae with high LiS was visible albeit insignificant. The theory of a higher selection pressure for larvae with low LiS was supported by the highly significant correlation between LiS and Ms. Larvae with high initial LiS showed low mortality during the salinity stress test at 15 dph while Ms for larvae with low LiS was

considerably high. Thus, it can be hypothesized that the higher stress tolerance of larvae with high LiS resulted in lower Mn during the rearing period which caused a lower DWG. Additionally to this hypothesis, the observed positive correlation between Mn and DWG can be interpreted as a high growth rate that is not caused by the correlating Mn but by lower stress tolerance of larvae that causes high Mn and in turn a higher growth rate of fewer larvae. Considering the high correlation between YoS - LiS and the absence of a significant correlation between YoS – Mn, it can even be speculated that the correlation between YoS and SLG is more the result of an autocorrelation between YoS and LiS than a real biological connection. Reason for the prominent correlations between LiS and the presented larval quality data could be that the lipid droplet is likely the major source of mono unsaturated fatty acids (MUFA) in larval, teleost fish (Wiegand, 1996; Schaerlinger and Żarski, 2015). The work of Lund and Steinfeldt (2011) showed that stress tolerance of larvae fed with a diet rich in MUFAs was significantly higher than for larvae that were fed with a MUFA poor diet. Therefore, it is likely that pike-perch larvae with a high LiS have an elevated MUFA level which positively correlates with larval stress tolerance. Additionally to the correlations that directly or indirectly involved LiS, a further correlation was observed between SBI and DWG. Unfortunately the correlation appeared to be negative which means that larvae with high SBI showed lower DWG. For older fish it is well observed that a non inflated swim bladder leads inevitable to higher mortality and a strong decrease in growth since the fish consumes much more energy during swimming than fish with an inflated swim bladder that allows them to float (Czesny et al., 2005). That the presented data are contradictive to those findings is puzzling. A lower DWG for higher SBI could be explained by a higher mortality of non inflated individuals over time. But since the temporal depiction of SBI showed no substantial fluctuation in SBI after 12 dph such a ripple effect can be excluded. Another reason could be that larval fish did not yet depend on the inflation of the swim bladder since they were not that heavy at that age. Nonetheless, an increase in DWG due to non inflated swim bladders cannot be explained in this work. Overall, the analysis of larval quality parameters did suggest that the initial size of larvae's lipid droplet was decisive for their stress tolerance and could be used as a standard measure to predict late larval quality. Furthermore, it remains questionable if the observed decrease in DWG was an artifact caused by lower Mn or an effect that was somehow caused by a high LiS.

4.4 Conclusion

During the course of this work no evidence was found for a negative effect of out-of-season spawning on quality of pike-perch offspring. It was speculated, that differences in parental stress exposure could have lead to observed low sRD and DWG of tested autumn spawned larvae. Nevertheless, a constantly low sRD could exclusively be found in one of three out-of-season spawning groups and all other related larval quality parameters were not affected. Therefore it is postulated, that out-of-season spawning is likely to have no negative influence on larval quality. Based on the results of this work and the obvious benefits of a seasonally independent larvae production a further use of out-of-season technology is advised. Furthermore, it was shown that an increased size of the lipid droplet at 0 dph positively affected larval stress resistance which was probably due to an increased reserve of MUFAs. Based on the findings of this work, it is recommended to generally measure LiS at 0 dph to predict larval quality. However, parental properties were shown to be insufficient for the prediction of produced larval quality.

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Chapter II. The effect of light intensity on performance of larval pike-perch (*Sander lucioperca*)

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Abstract

In this work performance parameters of larval pike-perch (*Sander lucioperca*) reared under four different light intensities (100, 500, 1000 and 2500 lx) until 21 days post hatch (dph) were investigated. As performance parameters change in length and weight, swim bladder inflation, feed consumption, natural mortality, stress induced mortality and RNA-DNA ratio were measured. Aim was to investigate the influence of light intensity on pike-perch performance during the first three weeks of larval rearing. Significant differences were found in natural and stress induced mortality as well as in weight growth. No single light exposure level combined optimal performance of all tested performance parameters. Highest light intensity of 2500 lx showed good weight growth but an increase in stress induced mortality. Bright light of 500 and 1000 lx intensity was found to improve growth and stress mortality whereas dim light conditions of 100 lx showed significantly lower natural mortality. Thus data suggested that most favorable illumination during larval pike-perch rearing comprise a tradeoff between optimal natural mortality under dim light conditions (100 lx) or optimal larval growth and stress resistance under bright light conditions (500 and 1000 lx). It is shown that high light intensities during larval rearing can be beneficial for pike-perch rearing if offspring supply is not limited.

1. Introduction

1.1 Commercial relevance

Pike-perch (*Sander lucioperca*) is a piscivorous fresh- and brackish-water fish that commonly occurs in Middle and Eastern Europe as well as in large areas of Northern Asia (FAO, 2017). It is a high valued food as well as a popular gaming fish that is marketed as full grown fish for the gastronomy and as fingerling for restocking purposes (Kestemont et al., 2015). Due to the good market acceptance and a rapid growth rate (Dil, 2008; Schaefer et al., 2015), pike-perch is considered to be a species with high potential for the diversification of recirculation aquaculture systems (RAS) in Europe (Wang et al., 2009; Dalsgaard et al., 2013). While the majority of consumed pike-perch still arises from wild catches, a proportionally small but increasing amount is produced in RAS (FAO, 2017). Although land-based RAS require a lot of technical knowhow and cost-intensive maintenance, the extensive control of most environmental conditions led to substantial improvements in RAS-based larvae culture and grow out (Steenfeldt, 2015). Over the last decades, researchers were able to describe optimizations of husbandry conditions that led to an increase in growth (Wang et al., 2009), survival (Hilge and Steffens, 1996; Szkudlarek and Zakes, 2007) and to less deformities (Divanach et al., 1997; Kestemont et al., 2007). Thus, nowadays, pike-perch can be produced in RAS on a price and quality competitive level.

1.2 Pike-perch & light

However, environmental factors influencing early larval development are manifold (Robb and Abrahams, 2003; Frisk et al., 2012; Zeytin et al., 2016) and specifically for pike-perch larvae not yet fully understood. High mortality rates and skeletal deformities are common during that time of larval development (Balon, 1984; Summerfelt, 1996; Kestemont et al., 2007) and were previously associated with characteristics of the prevailing light intensity during the rearing of other common aquaculture species like cod (*Gadus morhua*) (Van Der Meeren and Jørstad, 2001), sea bream (*Sparus aurata*) (Saka et al., 2001) and haddock (*Melanogrammus aeglefinus*) (Downing and Litvak, 1999). Still, for pike-perch the effect of light regime on larval development is not sufficiently described yet. While larval pike-perch are known to undergo a short period of positive phototactic behavior (Jäger et al., 1984; Schlumberger and Proteau, 1996), the specific impact of different light qualities, quantities and periodicities during the first days post hatch (dph) are uncertain. For juvenile and adult fish, however, light requirements are studied with more detail and fish at that age are described as very sensitive and best reared at dim light conditions (Luchiari et al., 2006; Kozłowski et al., 2010) with long wavelength (Luchiari et al., 2009). But since many physiological changes occur from the larval towards a juvenile i.e. adult developmental stage (Ott et al., 2012), it cannot generally

be assumed that the optimal rearing conditions are consistent throughout pike-perch's life history. Owing to the lack of knowledge concerning optimal light conditions for larval pike-perch rearing, light intensities in laboratory and practical scale vary widely between different rearing facilities and were reported between 50 and 500 lx (Steenfeldt et al., 2011; Rod, 2014). Therefore further experiments are needed that determine the optimal light intensity for larval pike-perch by evaluating larval performance parameters after i.e. while rearing under different light conditions.

1.3 Performance parameters

Parameters that can provide information about larval performance are numerous and can be divided into morphological, behavioral, lethal and biochemical performance parameters. Morphological behavioral and lethal parameters are typically easy to evaluate but are specific for each species while biochemical parameters lean on general molecular mechanisms that apply across species but require advanced laboratory equipment (Schaerlinger and Źarski, 2015). Morphological performance parameters include among others observations of critical developmental mile stones like first exogenous feeding (Steenfeldt et al., 2011), swim bladder inflation (Ott et al., 2012) and forming of deformities (Kestemont et al., 2007). Furthermore, included in the morphological parameters are size measurements over time. Lethal performance parameters solely comprise the count of mortality that can either be natural or induced by a stress factor. The count of induced mortality is typically performed within the course of challenge experiments that determine the potential of organisms to withstand unfavorable environmental conditions. One of those challenge experiments is the recently introduced salinity stress test that examines the capability of larvae to perform osmoregulation and to counteract stress (Kestemont et al., 2007; Lund et al., 2012). Behavioral performance instead relates to larval behavior that is either desirable, like constant and high feed consumption or undesirable, like the development of cannibalistic behavior (Szczepkowski et al., 2011). Biochemical performance parameters however potentially include every metabolic product that is present within the larvae. Therefore biochemical performance parameters are very divers. One commonly used parameter is the RNA-DNA ratio which is based on the principle of a fairly constant DNA level and a RNA content that varies with nutritional condition (Bulow, 1970; Meyer et al., 2012).

1.4 Objectives

Within extend of this study pike-perch larvae were reared between 0 and 21 dph under four different light intensities between 100 and 2500 lx. During the experiment morphological, behavioral and biochemical performance parameters were assessed encompassing natural mortality, stress induced mortality, length growth, weight growth, feed consumption, swim-bladder inflation and RNA-DNA

ratio. The diverse indicators were used to assess the effect of different light intensities on larval pike-perch performance for the first three weeks of rearing.

2. Material and Methods

2.1 Larval rearing

Fertilized pike-perch eggs from a single male – single female mating were obtained from the commercial pike-perch hatchery AquaPri (Egtved, Denmark). Thus it is important to mention that all presented data resulting from this single male – single female mating and can comprise genetically individual characteristics that are not necessarily reproducible with pike-perch larvae of other origin. To exclude a genetic influence repetitions of the presented experiments need to be performed in future studies. The obtained eggs were transported with 40 degree days (d°C) in two plastic bags that were placed in a styrofoam box to maintain the water temperature at 16-17 °C. The plastic bags were each filled with 5 L hatchery water, 5 L ambient air and 230 ml fertilized eggs. After a transport of three hours, the eggs were transferred into McDonald-type hatching jars (Pentair Hatching Jar, Apopka, United States) at the experimental facility of the Gesellschaft für Marine Aquakultur mbH (GMA) (Büsum, Germany). Incubation temperature of the hatching jars was 18 °C and the ambient light intensity was below 50 lx. Hatching was initiated after 72 d°C by stopping the water in-flow. 90% of larvae hatched within 2 hours after initiation. Newly hatched larvae were transferred into the rearing system with a density of 100 larvae per liter. The rearing system contained 12 glass aquaria with a water volume of 40 L that were integrated in the same water circuit with a total volume of about 1900 L, including two water reservoir tanks, used as pump-sump and head-tank, respectively. In each of the aquaria a plastic tube was placed that provided gentle aeration and perturbation at a rate of 30-60 air bubbles per minute. Each of the tanks was equipped with a mesh (mesh-size 335 µm) covered water overflow and a water inflow that was set at a flow rate of about 150 ml/min, i.e. one full water replacement per 4.4 hours. The aquaria were separated by black, opaque tarpaulin to shut out any light from neighboring light treatments. The same tarpaulin was used to cover the aquaria walls from the outside to prevent larvae from detecting movements outside of the aquaria. 30 cm above the tanks customary, T8, 1200 mm halogen tubes (Lampen XXL CMI T 36W IP 65, Kronau, Germany) were assembled in such a way, that all twelve aquaria were exposed to an even light distribution of 1000 at the edges up to 2500 lx in the middle of the aquaria i.e. directly beneath the installed light tube. Light intensity was measured at the water surface with a digital luxmeter (BEHA-Amprobe Unitest 93560 luxmeter, Glottertal, Germany). To decrease the light quantity in accordance to the individual light treatments, layers of opaque cover sheets were used to cover the surface of the light tubes and decrease the light quantity, which had no influence on the light

spectrum. The light intensity within the different tanks decreased from the center of the tank that was placed directly beneath the light tube towards the edges of the tank. Thus light intensities of the four different light treatments varied between 40 – 100, 250 – 500, 500 – 1000 and 1000 – 2500 lx. For declaration of the four treatments maximum illumination was used. The impact of different light treatment was tested in a triplicate experimental design in which the individual tanks were allocated randomly across the experimental setup. The light periodicity was set at a continuous 24 h/d⁻¹ illumination and the light quality i.e. spectrum was measured with a spectrometer (Ocean Optics USB 2000, Ostfildern, Germany) and consisted of several peaks between yellow and blue light (Fig. II-1). 24 h lightning period was oriented on commercial hatcheries where continuous lightning regimes are commonly used based on the assumption that early feeding success increases (Kestemont et al., 2015). The use of artificial lightning via light tubes was also oriented on common procedure from commercial producers where artificial lightning tubes are widely used.

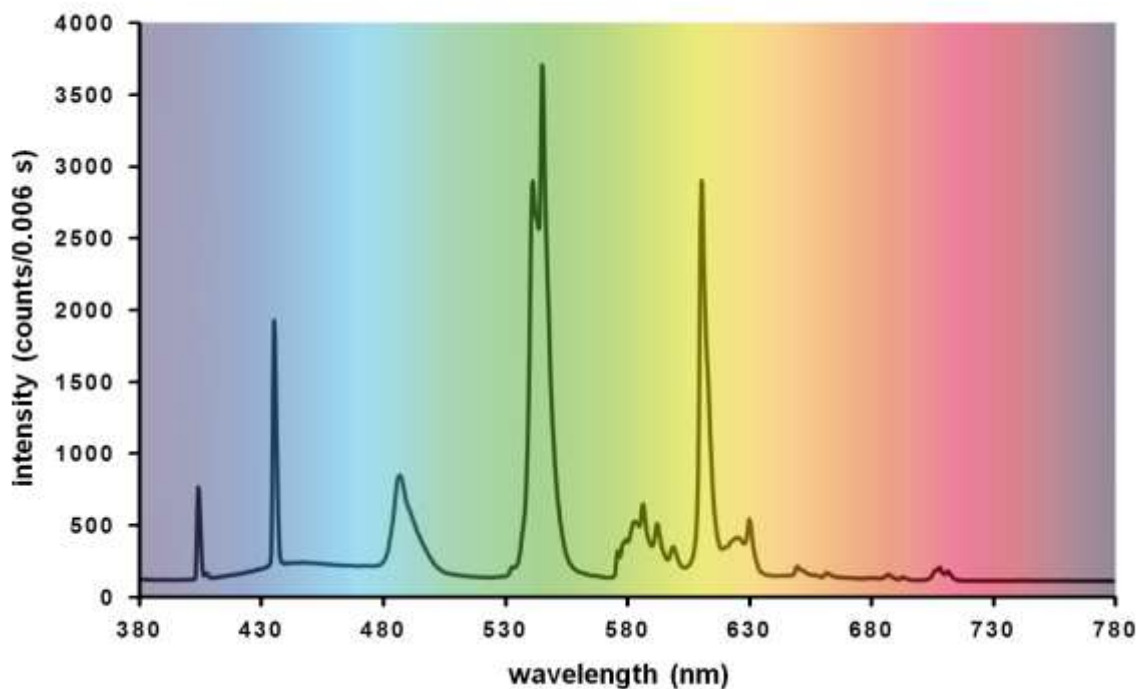


Figure II-1: Spectrum of light used during the experiment. Intensity is depicted between 380 and 780 nm wavelength.

Water temperature was maintained with a heater (Aquamedic TH-500W, Bissendorf, Germany) respectively via a cooling system (Aquamedic Titan 1500, Bissendorf, Germany). Inside the water circuit a UV sterilizer (Aquamedic Helix Max UV 55W, Bissendorf, Germany) was integrated throughout the whole experiment, algae concentrate (BlueBioTech Nannochloropsis sp., Büsum, Germany) was added to maintain green water at 2-5 Nephelometric Turbidity Units (NTU). The water

parameters were monitored every day to keep them at 18 ± 0.5 °C water temperature, $\geq 95\%$ oxygen saturation, ≤ 0.1 mg/dm³ ammonia, ≤ 0.2 mg/dm³ nitrite and 7.8 to 7.6 pH.

Larvae were provided live feed from 4 dph onwards, periodically every 6 h throughout 24h (6, 12, 18 and 24). The larvae were fed with *Artemia spec.* nauplii (INVE AF Specialty Cysts, Dendermonde, Belgium) that were harvested every day and stored on ice until the fourth feeding. Larvae were fed with a density of 10 nauplii per ml per day until the end of the experiment at 21 dph, which provided sufficient ad libitum prey under those stocking densities.

2.2 Sampling

Sampling of the experimental groups started at 0 dph and continued in 3-day intervals until the last sampling at 21 dph. Based on the experiments of Tielmann et al. (2016), where larvae preferred bright light conditions with increasing positive phototactic behavior between 0 and 10 dph, a peak between 10 and 22 dph and a starting decrease in positive phototactic behavior after 22 dph, larvae were assumed to be highly positive phototactic during the first 3 weeks post hatch. After this period an increasing negative effect of high light intensities was anticipated. Therefore, the experiments were exclusively performed during the first 21 dph. At each sampling day, ten larvae per tank were removed and stored in a solution of pH-stabilized tricaine methansulfonat (MS222). The sedated larvae were then photographed under a stereo microscope (Olympus SZ61, Tokio, Japan) for subsequent morphometric measurements. Thereafter larvae were washed in deionized water and individually stored in 1.5 ml capped vials in which they were snap-frozen at -80 °C. Sampling of all tanks took about 2 hours and was conducted between 14 and 16 o'clock after the second feeding respectively at the same time of day for sampling days with no feeding.

2.3 Measurements

For larval performance analysis standard length (SL) as well as dry weight (DW) was measured. Swim bladder inflation (SBI), natural mortality (Mn) after 15 dph and salinity stress induced mortality (Ms) were monitored. Furthermore, feed consumption (FC) and standardized RNA-DNA ratio (sRD) of individual larvae were analyzed.

After each sampling (every 3 days), frozen fish were dried for 24 hours in a freeze dryer (Christ Alpha LD 1-2, Osterode, Germany) which extracted any water from the samples. The dried fish were then weighed to the nearest .1 µg on a high-resolution balance (Sartorius Cubis Balance MSA2.7S, Göttingen, Germany). After weighing, larvae were put back into the capped vial and preserved at -80

°C until further usage for RNA-DNA analysis. Individual RNA and DNA content of larvae was measured following Caldarone et al. (2003). Within this measurement whole freeze-dried larvae were used to determine the Nucleic acid content by means of the intercalating fluorescent dye ethidium bromide (Clemmesen, 1987; Caldarone et al., 2001) in an untreated homogenate. Afterwards in two consecutive steps, RNase and DNase were added to the homogenate and respectively two more fluorescence readings were performed. The second reading measured the homogenate fluorescence without the degraded RNA and the third without RNA and DNA fluorescence. Based on the difference between the first and the second and between the second and the third reading, the nucleic acids were quantified. The resulting RNA-DNA ratios were standardized to a reference slope ratio based on the slopes of the standard curves in Caldarone et al. (2006). The images that were previously taken from each larva were analyzed with the graphic program ImageJ 1.45s, SL was measured from the tip of the upper jaw to the posterior end of the last vertebra, SBI was counted and the rate of FC was determined and ranked into four different stages (Fig. II-2). A linear regression of SL and lnDW was performed and daily growth rates were calculated. Linear regression of SBI between 9 and 12 dph was performed and the time of 50% SBI (SBI50) was determined. Furthermore, an average FC per day was calculated (FCa) by assigning the four gut fullness stages to numbers (I=0, II=1, III=2 and IV=3), adding the numbers treatment specific and dividing the added ranks by the amount of experiment days.

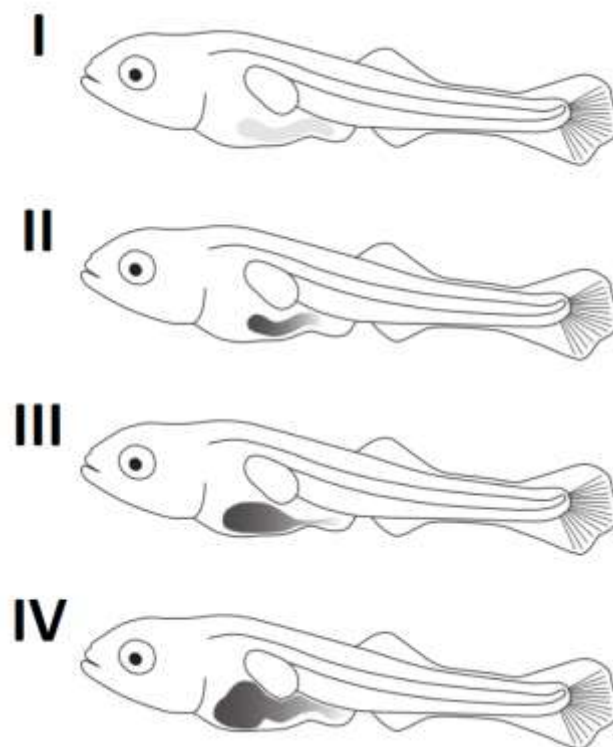


Figure II-2: Schematic illustration of four gut fullness intensities on the example of 15 dph old pike-perch larvae. No gut content (I), little gut content (II), average gut content (III) and high gut content (IV). Illustrations redrawn from original photographs.

At dph 15, Mn and Ms were determined. During that time of development it was certain that the critical phase of swim bladder inflation had ended (Steenfeldt, 2015) but cannibalistic behavior that can appear at a larval SL of 15 mm (Szkudlarek and Zakes, 2007) had no influence on the larvae yet. Thus Mn observations were not biased by cannibalism and Ms was not influenced by an elevated stress level that originated from cannibalism or the swim bladder inflation process. Mn was calculated by counting back the larvae of initially 2000 fish that were introduced per tank. The difference between the initial stock size minus the so far sampled larvae and the counted stock size at dph 15 was the basis for mortality calculations. The salinity stress test at dph 15 was carried out to examine the capability of larvae to perform osmoregulation and to counteract salinity stress (Kestemont et al., 2007; Lund et al., 2012). During the stress test about 120 larvae from each tank were gently transferred to a 1 L glass beaker containing water of 19 PSU and 18 °C. Over a period of two hours larval mortality (i.e. fish lying on the bottom without movement) was counted in 5-min intervals and registered as cumulative mortality as described by Dagar et al. (2010). After performing a linear regression the Ms rate per minute was calculated. All experiments were conducted in agreement with the animal protection law.

2.4 Statistical analysis

Mn at dph 15 was assumed to be binomially distributed and an appropriate generalized model after Dobson (2010) was defined, including the four light treatments as fixed factors. Subsequently an analysis of variances (ANOVA) was conducted, followed by multiple contrast tests (Bretz et al., 2010) comparing the four treatments with each other.

For data analysis of Ms, SBI and FC a binomial distribution of the data was assumed and appropriate generalized mixed models after Schall (1991) were defined. The models included the four light treatments (100, 500, 1000 and 2500 lx) as factors, time in 5-min intervals (5 - 120 min) or days (0 - 21 dph) as covariate and their interaction terms as fixed factors. Tanks of the triplicate design were treated as random factor.

Based on graphical residual analysis of the SL, DW and sRD, data were assumed to be normally distributed and to be heteroscedastic. Statistical analysis of the data started with the definition of appropriate mixed models (Laird and Ware, 1982; Verbeke and Molenberghs, 2009) that included the

factors light treatment (100, 500, 1000 and 2500 lx) and dph as well as their interaction term as fixed factor. For the analysis of SL and DW, the factor dph is included as quantitative factor with a regression from 0 to 21. The analysis of sRD, in contrast, evaluates the differences between treatments at each sampling day and the factor dph is included as a qualitative parameter. Furthermore the exponential DW data were logarithmized (lnDW) to calculate statistics with a linear model. For each analysis tanks of the triplicate design were treated as random factor.

Based on the defined models for Ms, SBI, FC, SL, DW and sRD, analysis of covariance (ANCOVA) was conducted (Cochran, 1957). In case of a significant difference of the response variable ($\alpha = 0.05$), subsequent multiple contrast tests (Bretz et al., 2010) were performed in order to compare the model parameters corresponding to the light treatments. For further analysis linear regressions of SL, lnDW and cumulative Ms were performed to calculate SL and DW growth per day and Ms rate per minute. Furthermore, an average FC was calculated on basis of the individual FC of each sampled larva. For additional graphical analysis residuals from a SL-lnDW were calculated and plotted against their sRD.

Outlier detection was performed via graphical boxplot examination and subsequent Cook's distance analysis. Observations that exceeded a value of 1 were defined as outlier and excluded. All statistical analysis was carried out using the statistical analysis software R 3.2.3.

3. Results

3.1 growth performance

Comparing the increase in SL over time between different light treatments (Fig. II-3) up to 21 dph, no significant differences were found ($p=0.11$). The increase in SL was well described by a linear model and variability within the individual groups increased over time. However, there was a slight albeit insignificant tendency at 21 dph SL of the light treatment 100 lx that appeared to be lower than in the other groups and the curve progressed from a linear to a limited shape.

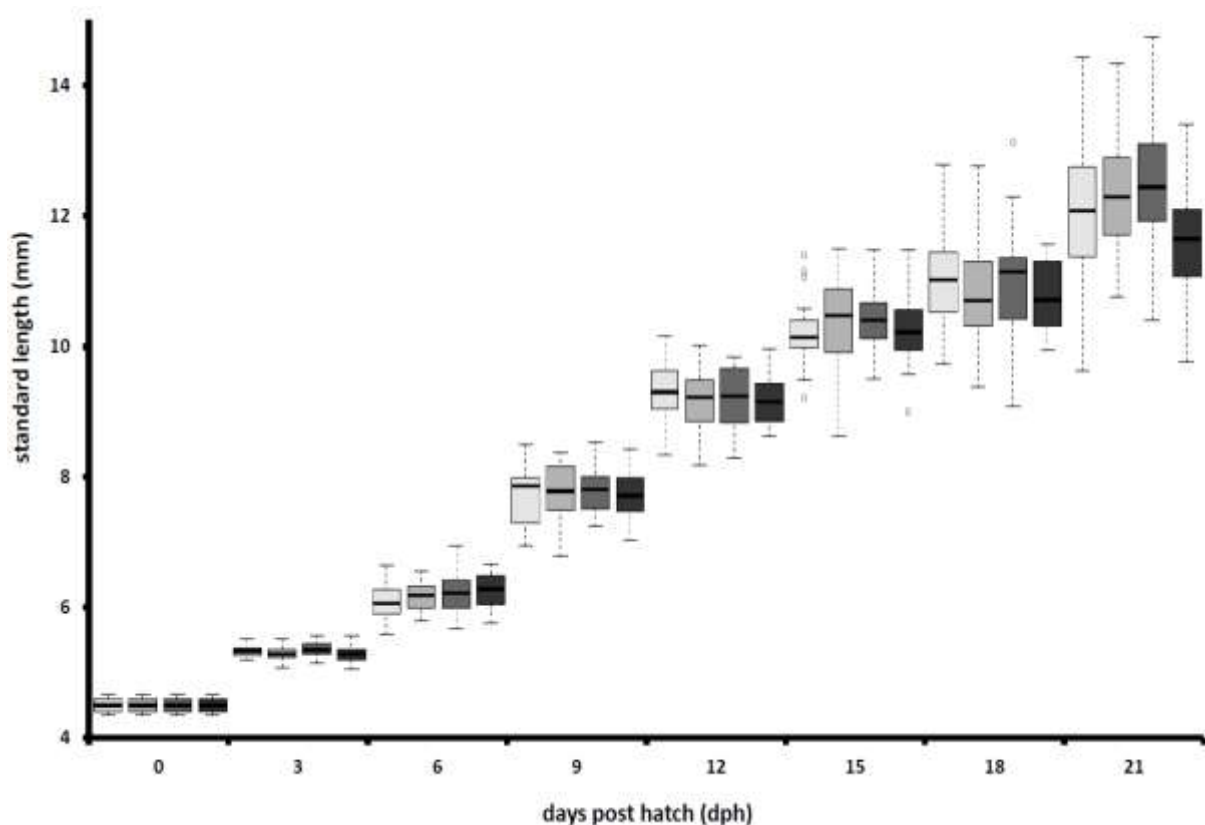


Figure II-3: Boxplot of Standard length (SL) of larvae from four different light treatments over the first 21 dph showing median, quartiles and whiskers. 100 lx (black), 500 lx (dark grey), 1000 lx (light grey) and 2500 lx (white).

Evaluating the growth curves of larval DW between treatments (Fig. II-4), it was apparent that the lowest light treatment showed, starting at 15 dph, lower growth performance than the other three treatments. At 21 dph the difference between the 100 lx treatment and the other groups was most pronounced. Differences between the three groups of higher light intensity, however, were not apparent. Each of the treatments defined an exponential growth curve and similar to SL, variance within the individual treatments increased over time. The statistical analysis of DW showed significant differences between the treatments 100 lx – 2500 lx ($p < 0.001$), 100 lx – 1000 lx ($p =$

0.002) and 100 lx – 500 lx ($p = <0.001$) and with an average logarithmized DW growth rate (GRlnDW) of 14.7 (Tab. II-1) the 100 lx treatment showed the lowest growth performance.

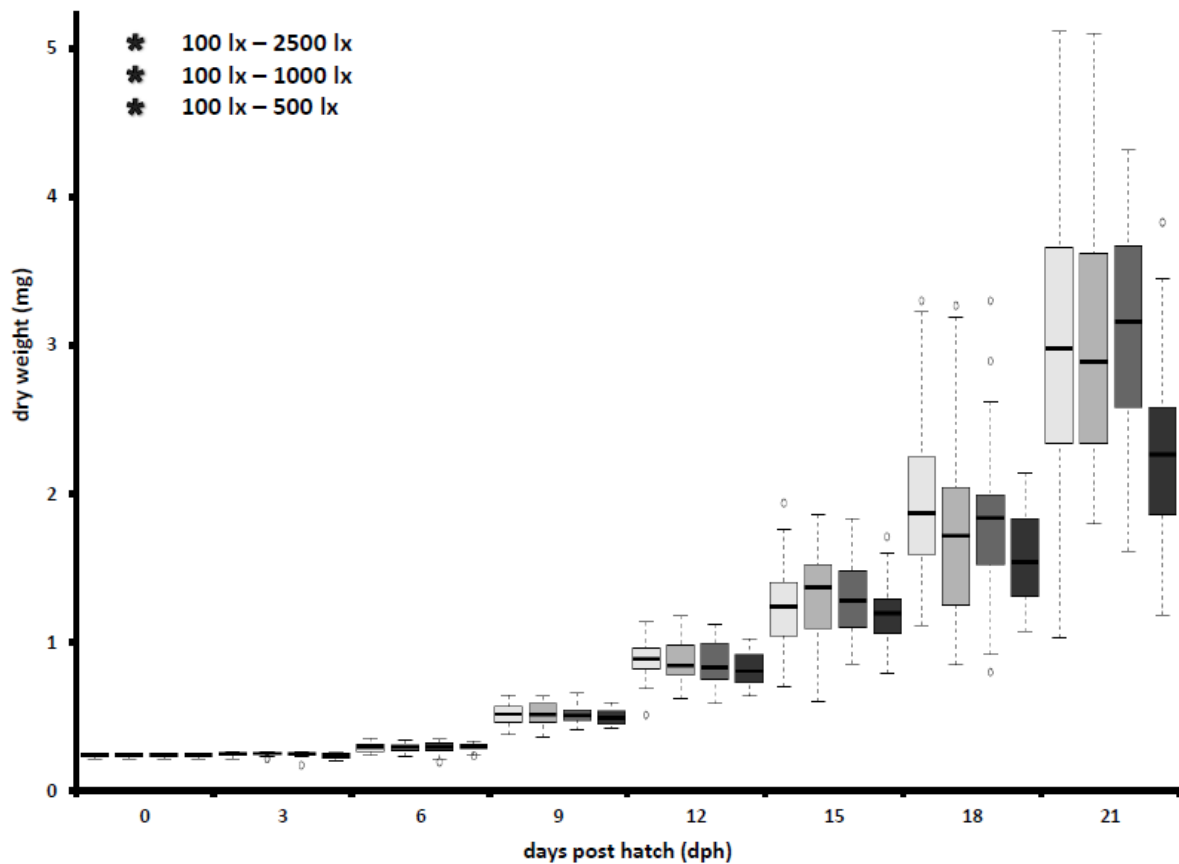


Figure II-4: Boxplot of weight of larvae from four different light treatments over the first 21 dph showing median, quartiles and whiskers. 100 lx (black), 500 lx (dark grey), 1000 lx (light grey) and 2500 lx (white). Asterisks indicate p-values lower than 0.05 after performing a multiple contrast test between logarithmic weight data of pictured treatments.

Table II-1: Overview of performance indicators sorted according to light treatment. Daily length growth (GRSL), daily weight growth (GRInDW), natural mortality (Mn), day of 50% swim bladder inflation (SBI50), average feed consumption (FCa) and stress mortality (Ms).

treatment	GRSL (%/d ⁻¹)	GRInDW (%/d ⁻¹)	Mn (%)	SBI50 (dph)	FCa	Ms (%/min ⁻¹)
100	∅ 34.0 ± 1.5	∅ 14.7 ± 0.7	∅ 12.5 ± 4.1	∅ 10.24 ± 0.45	∅ 2.54 ± 0.28	∅ 0.37 ± 0.07
500	∅ 36.8 ± 1.3	∅ 15.9 ± 0.5	∅ 18.6 ± 2.6	∅ 9.28 ± 0.89	∅ 2.52 ± 0.17	∅ 0.29 ± 0.11
1000	∅ 36.2 ± 2.3	∅ 15.7 ± 0.9	∅ 23.9 ± 12.5	∅ 10.09 ± 0.79	∅ 2.66 ± 0.13	∅ 0.32 ± 0.08
2500	∅ 35.9 ± 1.0	∅ 15.7 ± 0.4	∅ 15.5 ± 4.7	∅ 10.17 ± 0.72	∅ 2.83 ± 0.26	∅ 0.43 ± 0.22

3.2 Mortality

3.2.1 Salinity stress induced mortality

Cumulative Ms of the salinity stress experiment (Fig. II-5) described a linear increase over time and an increase in variability between repeated measurements for all treatment groups.

After 2 hours of experiment highest Ms per minute (Tab. II-1) was measured for larvae of the highest (2500 lx; 0.43%) and the lowest light intensity (100 lx; 0.37%). Lower mortalities were measured for larvae of the light treatment 500 lx (0.29%) and 1000 lx (0.32%). Statistically significant differences could be found for 2500 lx – 500 lx ($p = 0.003$) and 2500 lx – 1000 lx ($p = 0.002$).

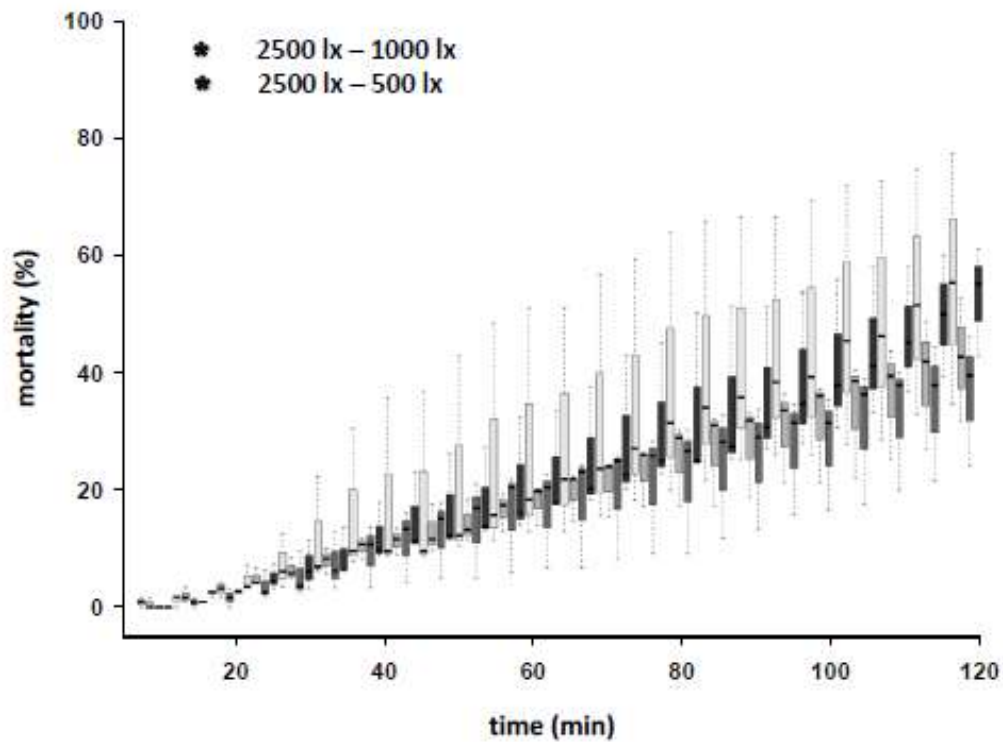


Figure II-5: Boxplot of accumulated mortality from different light treatments during two hours of salinity-stress exposure showing median, quartiles and whiskers. 100 lx (black), 500 lx (dark grey), 1000 lx (light grey), 2500 lx (white). Asterisks indicate p-values lower than 0.05 after performing a multiple contrast test between pictured treatments.

3.2.2 Natural mortality

Larval Mn at 15 dph ranged in average from 12.5% at lowest light intensity (100 lx) to Mn of 18.6% (500 lx) and 23.9% (1000 lx) for the higher intensities (Fig. II-6, Tab. II-1). However, Mn for the highest light intensity of 2500 lx was with 15.5% lower than Mn of the 500 and 1000 lx treatments but higher as Mn of the 100 lx treatment. The performed multiple contrast tests showed significant differences between all treatments.

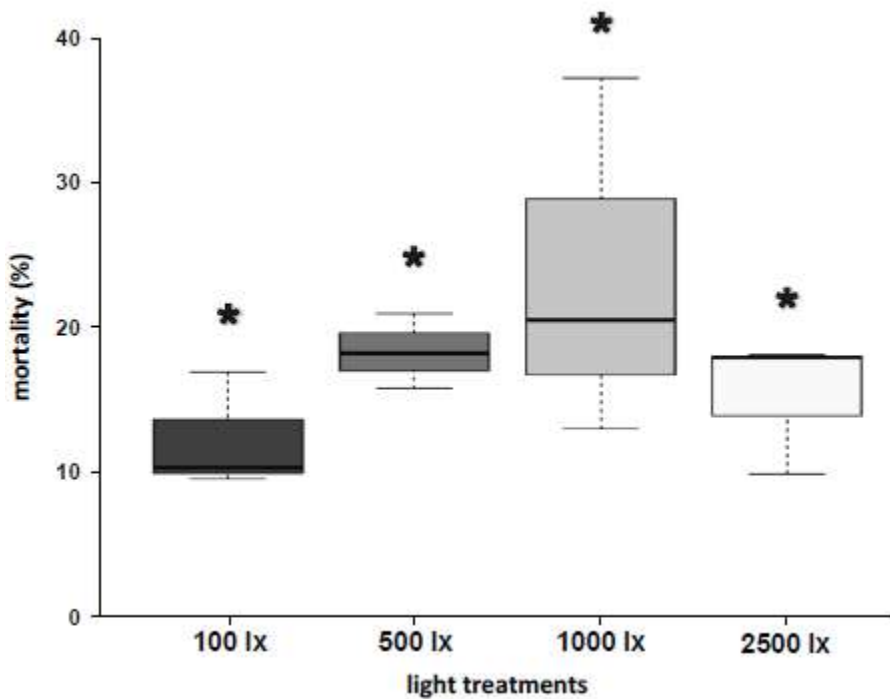


Figure II-6: Boxplot of natural mortality under different light treatments after 15 dph, showing medians, quartiles and whiskers. Asterisks indicate p-values lower than 0.05 after performing multiple contrast tests.

3.3 Swim bladder inflation

SBI started between 6 and 9 dph (Fig. II-7) and increased markedly between 9 and 12 dph. At dph 9 larvae of the lowest light intensity treatment showed a noticeably lower amount of inflated individuals than the other groups. However, at dph 12 strong differences between the four treatments were no longer noticeable. Calculated SBI50 showed the 500 lx treatment to reach the SBI50 first at an average 9.28 dph while the latest SBI50 was calculated for the 100 lx treatment with 10.24 dph (Tab. II-1). The statistical analysis found that no significant variance differences between the treatments were present.

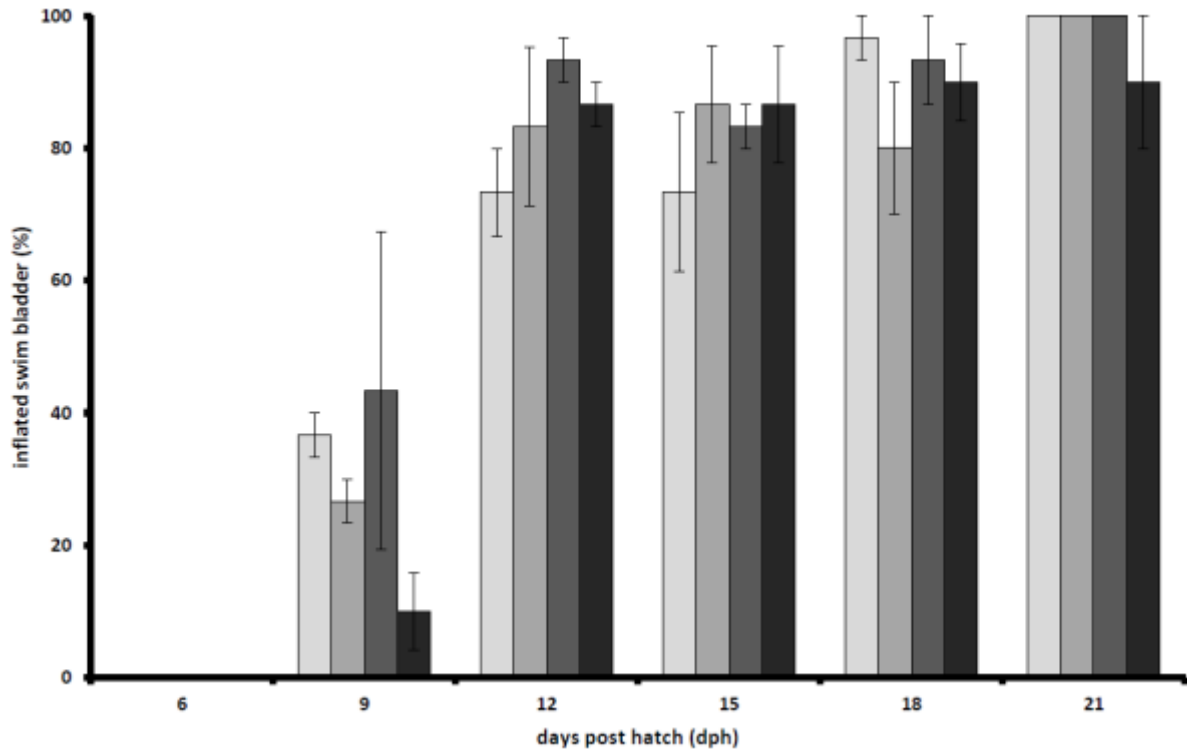


Figure II-7: Mean proportion of larvae with inflated swim bladder divided in different light treatments from 6 to 21 dph. Error bars visualize variability of the data. 100 lx (black), 500 lx (dark grey), 1000 lx (light grey) and 2500 lx (white).

3.4 Feed consumption

FC was recorded in four intensity categories beginning at 6 dph (Fig. II-8). While differences in gut fullness between the treatments were not clearly evident, it was noticeable that larvae with high gut content were in each treatment particularly prominent at 6 dph and again at 21 dph. However, between 12 and 18 dph the amount of larvae with low and no gut content was most prominent. The calculated FCa ranged from 2.54 (100 lx) and 2.52 (500 lx) to slightly higher FCa of 2.66 for the 1000 lx and 2.83 for the 2500 lx treatments (Tab. II-1). The statistical analysis showed no significant differences for high ($p = 0.42$), average ($p = 0.71$), little ($p = 0.84$) or no gut content ($p = 0.35$).

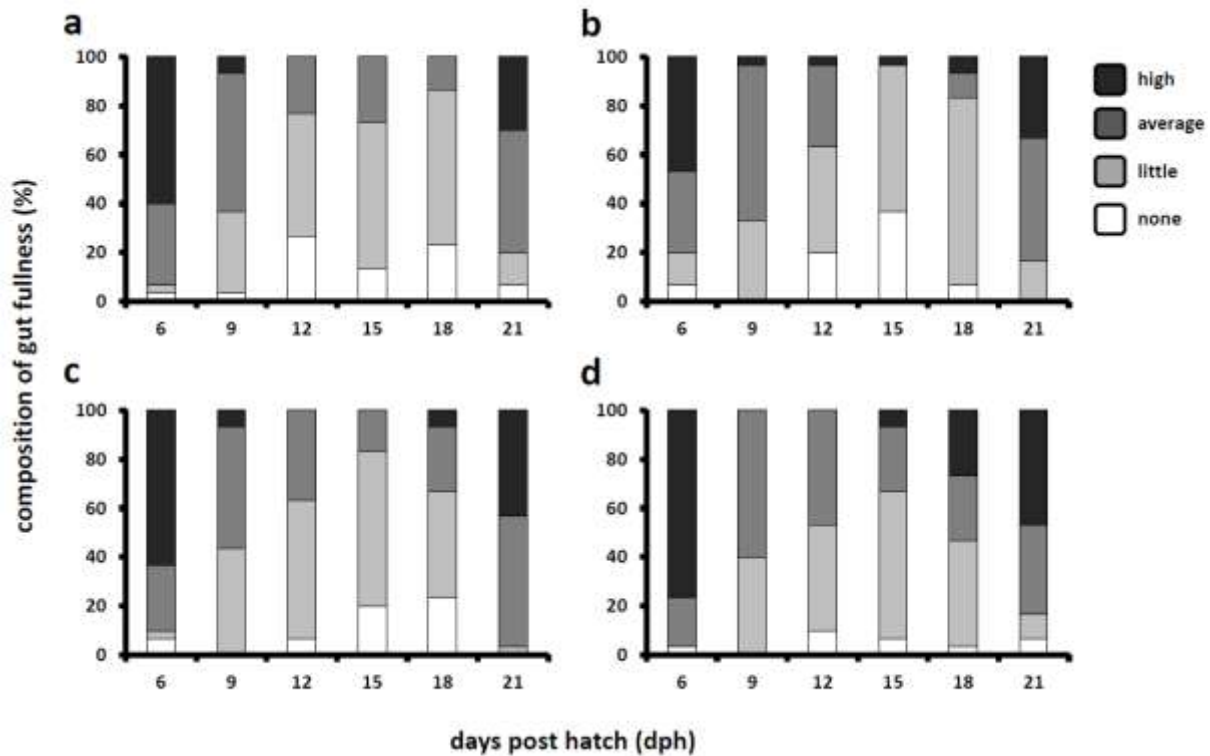


Figure II-8: Relative proportion of gut fullness intensities (no gut content (white), little gut content (light grey), average gut content (dark grey) and high gut content (dark)) 2 hours after feeding divided in treatment groups. 100 lx (a), 500 lx (b), 1000 lx (c) and 2500 lx (d).

3.5 RNA-DNA analysis

From 0 dph up to 9 dph sRD increased slightly and remained constant between 9 and 12 dph (Fig. II-9). From 15 to 18 dph the ratio is decreased again. The only exception was sRD of the 100 lx treatment at 15 dph that was slightly higher than those of the other treatments. At 21 dph a pronounced increase in sRD for all treatments was apparent and with the higher ratio, the variance within the groups increased as well. No significant differences between treatments could be found ($p = 0.88$). The linear increase in SL over time and the exponential increase in DW resulted in an allometric relation between DW and SL, which is given in Figure II-9. No treatment related aggregation was observed in this data presentation. The plot of sRD against DW (Fig. II-9) revealed for larvae up to 3 mg a relatively stable sRD that varies between 1 and 4. For larvae heavier than 3 mg the sRD increased and varied between 2.5 and 7. This observation was consistent with the increase in sRD for older larvae of 21 dph. A visible difference between treatments was again not detectable. Furthermore the residuals of SL and \ln DW, plotted against sRD (Fig. II-9) showed no explicit pattern of high or low sRD at specific residuals. Thus a pattern for a specific light treatment was not visible as well.

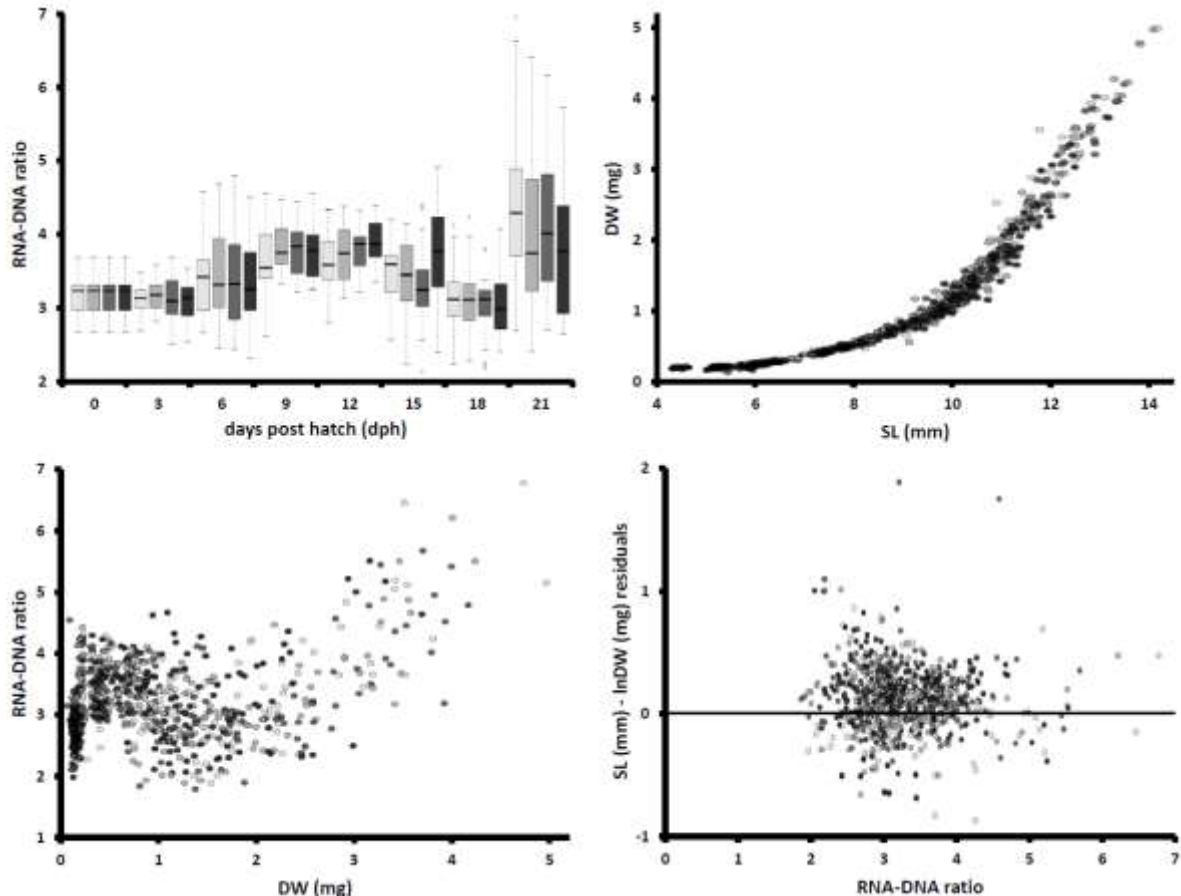


Figure II-9: RNA-DNA ratio of larvae during the first 21 dph (top left), length to weight growth (top right), RNA-DNA ratio to weight growth (bottom left) and residuals from the linear regression model of length and logarithmic weight against RNA-DNA ratio (bottom right). Shading of dots and boxplots represents treatments 100 lx (black), 500 lx (dark grey), 1000 lx (light grey) and 2500 lx (white).

4. Discussion

4.1 Growth performance

The statistical analysis of SL could not find any significant differences between the treatments while visually the growth curve for 100 lx appeared to become less steep than the growth curves of the higher light treatments (Fig. II-3). For DW the same effect, in a more pronounced shaping, was visible (Fig. II-4) and statistically detectable. Therefore, data suggested that weight gain and longitudinal growth was positively affected by light intensities over 100 lx. The differently steep shaping of SL and weight growth was expected and has been shown before by Szkudlarek and Zakes (2007). Size and weight gain of tested pike-perch larvae was moderate but not exceptional when compared to other studies (Szkudlarek and Zakes, 2007; Schaefer et al., 2015). Reasons for the observed light intensity driven growth effect can be premised in either a higher energy intake i.e. higher FC or a more effective, energetic use of the ingested feed.

4.2 Mortality

4.2.2 Salinity stress induced mortality

Results for the salinity stress experiment (Fig. II-5 & Tab. II-1) showed Ms that was highest for larvae of the treatment 100 and 2500 lx with significant difference between high Ms of 2500 lx and low Ms of 500 and 1000 lx treatments. Thus a high light intensity of 2500 lx showed to affect stress resistance of pike-perch larvae negatively. Nevertheless, the low stress resistance did not seem to have a negative impact on SL and DW growth which were shown to be equally high as for the 500 and 1000 lx treatments. For the 100 lx treatment, on the other hand, a slight albeit insignificant tendency of low stress resistance i.e. high Ms was accompanied by low SL and DW growth. It is conceivable that extremely high light intensities like 2500 lx have the potential to directly stress pike-perch larvae without affecting growth performance. However, low light intensities of 100 lx do not stress the larvae directly but possibly create an indirect higher hunting expense which in turn decreases growth and increases stress as a result. For future studies the investigation of Mn and Ms under different light intensities should also target earlier and later developmental stages of larvae. Thus, additional insight on temporal changes in the effect of light intensity during different ontogenetic development could be provided.

4.2.1 Natural mortality

Mn varied between 36.4 and 8.4% which is within the range of other growth studies on larval pike-perch (Szkudlarek and Zakes, 2007). Results of the statistical analysis for Mn at 15 dph (Fig. II-6 & Tab. II-1) showed a positive effect of low light treatment on survival, whereas a linear relationship between light intensity and Mn was not evident. Possible reasons for differences in Mn are SBI success that has been linked to larval mortality by Czesny et al. (2005) or differences in FC. Anyhow, both factors showed no significant differences between treatments and were therefore insufficient explanatory for the observed differences in Mn. Low stress tolerance is another explanatory variable that has been linked by Lund and Steinfeldt (2011) to increased natural mortality in larval pike-perch. Nevertheless, while larvae of treatments 100 and 2500 lx showed the lowest Mn, they simultaneously comprised the highest Ms i.e. lowest stress tolerance. This observation depicted a seemingly opposite interaction between Mn and stress tolerance as described by Lund and Steinfeldt (2011). An explanation for this connection could be an interdependency of factors where a low growth rate could be linked to a slower ontogenetic development and therefore to a delay of mortality intensive ontogenetic mile stones. In that case low Mn of the 100 lx treatment would not so much be an expression of good larval performance but a temporary effect of slow larval

development. Another effect could be that a low Mn lead to a longer survival of week individuals which in turn led to a higher Ms during the salinity experiment while weak larvae of treatments with high Mn were already singled out, leading to a lower Ms.

4.3 Swim bladder inflation

The inflation of swim bladder is a critical mile stone in larval pike-perch development between 7 and 14 dph (Steenfeldt, 2015) and has previously been associated with light treatment as well as posterior growth performance (Demska-Zakêœ et al., 2003). Analysis of SBI during the experiment could not find significant differences between treatments at any of the sampling days although at dph 9 the inflation rate appeared to be considerably lower for larvae of the 100 lx treatment (Fig. II-7). Those observations were backed by the calculated SBI50 that was with 10.24 dph highest i.e. latest for the 100 lx treatment (Tab. II-1). However, a continuity of low SBI for the 100 lx treatment during 12 to 21 dph was not present. Therefore, it appeared unlikely that the late but coughed up SBI solely accounted for constant lower growth of the 100 lx treatment. For future studies comparison of a high light treatment up to 7 dph with a treatment until 14 dph and longer could evaluate the influence of SBI during larval light treatment.

4.4 Feed intake

Looking at the FC between 6 and 21 dph (Fig. II-8) no conspicuous differences between light treatments were found, affirmed by the calculated FCa (Tab. II-1) which showed low variation between treatments. Lowest FCa was calculated for the 500 lx treatment although it showed, together with the 1000 and 2500 lx treatments, best SL and DW growth. Accordingly lower growth performance of the 100 lx treatment seemed to be an effect of energy use rather than an effect of energy intake. Analyzing the FC data it is important to mention the tank wall color has been shown to interact via light reflection with the provided light regime and can significantly influence FC (Blaxter, 1980; Hinshaw, 1985; Malison and Held, 1992). The dark tank wall color of this experiment ensured lowest light reflection but experiments for older pike-perch showed that a green tank wall can be beneficial (Grozea et al., 2016), while larval Eurasian perch (*Perca fluviatilis*) benefits from light grey tank walls (Tamazouzt et al., 2000).

4.5 RNA-DNA ratio

Analysis of sRD (Fig. II-9) did not provide statistically significant differences in larval performance throughout the treatment groups. The characteristics of sRD varied over time but between the treatments no clear differences could be found. Even the comparison of SL–lnDW residuals with the individual sRD showed no dependency between sRD and deviation from the SL/DW relation. Nevertheless, the overall development of sRD can be used to speculate about larvae metabolic and nutritive utilization in context with ontogenetic development. Thus the overall increase in sRD beginning at dph 6 is in accordance with the start of artemia feeding that began at dph 4. The increase of RNA content could mark the period in which pike-perch larvae started to feed on artemia and increased their metabolic processes. The subsequent decrease of sRD between 15 and 18 dph could mark a period in pike-perch life history in which maternal yolk nutrition is absorbed but the larval stomach is not yet fully developed to completely compensate for the lack in yolk nutrition (Ostaszewska et al., 2005; Hamza et al., 2015). However, sRD did not seem to be affected by different light intensities.

5. Conclusions

A holistic evaluation of all results led to the conclusion that a decision of rearing pike-perch larvae until 21 dph under dim or bright light intensities cannot be generalized. This work shows that pike-perch during its first 3 weeks post hatch could benefit from high light intensities between 500 and 1000 lx in terms of improved growth and low Ms i.e. higher stress resistance. In turn rearing larval pike-perch under dim light conditions of 100 lx showed to have a positive effect on Mn. In practice an illumination of 500 – 1000 lx could make the detection of larvae within their rearing environment easier and facilitate the working processes at a hatchery due to a better illumination of the working environment. Accordingly, the decision of illumination intensity during larval rearing should depend on the farmers rearing capacity, availability i.e. amount of larvae and the practical use of an illuminated working environment. For further rearing beyond the first three weeks larvae will become more light sensitive and light intensities of over 25 lx will become disadvantageous for the fish (Luchiari et al., 2006; Lund et al., 2012). Thus a gradual down regulation of light intensity after the third week post hatch is recommended (Tielmann et al., 2016).

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Chapter III. Positive phototaxis in larval pike-perch (*Sander lucioperca*) triggering self grading mechanism

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Abstract

Early size grading of intensively reared pike-perch (*Sander lucioperca*) culture is an effective way to postpone and reduce cannibalistic behavior and hence increase larval survival. The commonly used manual grading methods are associated with intense handling stress and the risk of injuring the small and fragile larval fish. Thus gentle but still accurate methods for grading pike-perch during its early life history are needed to further improve intensive larvae culture. In this work the phototactic behavior of pike-perch larvae from 1 to 50 days post hatch (dph) were evaluated in a channel system with 0 and 700 lx light treatment. In a second experiment the larvae's observed positive phototaxis were then used to evaluate the effectiveness of a light triggered self grading mechanism during 16 and 34 dph. The findings of this work show larval pike-perch to be highly positive phototactic during its larval stage with a peak of positive phototactic behavior between 10 and 22 dph. After 22 dph positive phototaxis decreased and pike-perch increasingly preferred the lower light treatment. The use of larvae's positive phototaxis for a gentle self grading was successful at 22 dph and is recommended to be used in larval rearing protocols. Also it is suggested to implement a light triggered self grading mechanism within future tank designs for larval pike-perch.

1. Introduction

The European pike-perch (*Sander lucioperca*) is one of the upcoming species for intensive production in recirculation aquaculture systems (RAS). Pike-perch in aquaculture is growing fast, reaches relatively high selling prices and the competing wild catches are low since 1994 (FAO, 2017). However the amount of wild caught fish highly influences the market price of pike-perch and to successfully rear pike-perch in RAS the production has to be competitive regarding quality and costs. For that reason a lot of research has been conducted that focused on the improvement of rearing and reproduction methods throughout the life history of pike-perch. During that time great progress has been made and today aquaculture production of pike-perch is more efficient and sophisticated than ever (Dil, 2008; Szczepkowski et al., 2011; Hermelink et al., 2013). Yet there is still potential to further improve rearing methods. Especially high mortality during critical phases in larval life history (Balon, 1984; Summerfelt, 1996; Kestemont et al., 2007) is still a problem. For pike-perch one of the main causes of mortality during larval rearing is the development of cannibalistic behavior that occurs when first larvae reach around 15 mm total length about two weeks after hatching (Szkudlarek and Zakes, 2007). During that time size differences in fish of same age increase and bigger individuals can prey on smaller ones. Recent studies showed that reducing size heterogeneity via grading is an effective way to lower cannibalistic behavior and hence improve the survival rate (Szczepkowski et al., 2011; Knaus and Gallandt, 2012).

Size grading of fish is a common practice in aquaculture which can, depending on the species, reduce cannibalism and/or increase growth rate (Lambert and Dutil, 2001; Szczepkowski et al., 2011). The grading methods are manifold and change with species and age specific requirements. A lot of the various grading methods commonly used are based on a mechanical selection process and range from simple brailers and nets over box graders up to heavy machinery that grades automatically (Coche and Muir, 1998; Ibrahim and Sultana, 2006). During mechanical selection fish are typically concentrated in a set up including a barrier with a specific mesh size or gap width that can only be passed by certain fish. In those systems fish are forced through the gaps by gravity and shaking of the selective barriers. The main advantage of mechanical grading is its wide applicability throughout different species and age classes. Downsides however are the short term removal from tank waters and the applied physical forces that are associated with high handling stress for the fish (Dunlop et al., 2004) as well as injuries to eyes, fins and muscles (Willoughby, 1999). To avoid the rough and stressful handling a variety of grading methods use natural behavior to create a self sorting effect by attracting or repelling the fish. One method for instance uses the instinct of salmonids to swim upstream and grades the fish via selective barriers that are installed within an artificial current (Coche and Muir, 1998). In another approach water volume of the rearing tank gets reduced via

movable walls until the fish are crowded and only small fish escape from the crowded group via a selective net (Summerfelt et al., 2009). Those minimal invasive grading methods hold less stress for the fish but are also very specific since they are based on biological responses that are not necessarily constant between species and throughout life history. Thus to create a self sorting grading method it is crucial to consider the specific characteristics of the targeted fish and find an attractant or repellent it reacts to.

One biological factor that is likely to trigger directed movements in pike-perch is light intensity. Juvenile and adult pike-perch are described as shadow loving fish which is best held at dim light conditions below 25 lx (Luchiari et al., 2006) while larval pike-perch undergo a short period of high phototrophism at the beginning of their ontogenesis (Schlumberger and Proteau, 1996). During that time the positive phototaxis results in high density crowding towards light sources (Proteau et al., 1993) and is strong enough to direct the larvae towards a net with 1.5 mm mesh size (Jäger et al., 1984). The change from positive to negative phototactic behavior is believed to correlate with the development of the *tapetum lucidum* in the pike-perch eye that increases sight (Wunder, 1930; Luchiari et al., 2006). For pike-perch the exact time frame in which the *tapetum* evolves and the phototaxis changes has not been defined yet but experiments conducted on a closely related species, the walleye (*Sander vitreus*), have shown beginning *tapetum lucidum* development during the first month post hatch (Braekvelt et al., 1989; Kozłowski et al., 2010). Thus it can be speculated, that the ontogenetic development of pike-perch is similar and that the shift from positive to negative phototaxis possibly begins during the first 30 days post hatch (dph).

To verify this assumption we conducted an experiment to describe the larvae's phototactic behavior during the first 50 dph. Based on the findings of this experiment we subsequently conducted a second experiment in which we tried to grade larvae between 16 and 34 dph in different length classes using a light source as trigger to create a self sorting effect. Aim of this study was to evaluate the intensity of pike-perch phototaxis throughout the first 50 dph and to evaluate the suitability of light as an attractant in a self grading setup for larval pike-perch.

2. Material and Methods

2.1 Larval rearing

Fertilized pike-perch eggs were obtained from AquaPri in Egtvedt Denmark. The eggs were transported in plastic bags that were placed in a styrofoam box to maintain the water temperature at 16-17°C. At the facility of the Gesellschaft für Marine Aquakultur mbH (GMA) the eggs were transferred into McDonald-type hatching jars (Pentair, Hatching Jar) where the eggs were incubated at 18°C. Hatching was initiated after the eggs reached 72 day degrees by stopping the waterflow. Subsequently the larvae were transferred into the rearing system with a density of 100 larvae per liter. The rearing system contained four 40L rearing tanks of green coloration that were placed in a RAS system with a total water volume of 1700 L. Each rearing tank had a water inflow of about 100 ml min⁻¹ that evoked a gentle circular current. The outflow was placed in the middle of each tank in shape of a leveled drain that was covered with 1 mm mesh sized gauze. Inside the rearing tanks a plastic tube was placed to provide aeration and surface velocity via 30-60 bubbles per minute. Water temperature was maintained at 18°C with a heating rod (Aquamedic, TH-500W) respectively via a cooling system (Aquamedic, Titan 1500). Inside the RAS system a UV sterilizer (Aquamedic, Helix Max UV 55W) was integrated and throughout the first 21 dph water of the system was treated with algae concentrate (BlueBioTech, Nannochloropsis sp.) maintaining green water of 2-5 NTU. The water parameters were monitored every day to keep temperature, oxygen saturation, ammonia, nitrite and pH inside optimal ranges. A 24 h light regime with 500-700 lx light intensity at the water surface was provided by light tubes (CMI, T 36W IP 65). 24 h lightning period was oriented on commercial hatcheries where continuous lightning regimes are commonly used based on the assumption that early feeding success increases (Kestemont et al., 2015). The use of artificial lightning via light tubes was also oriented on common procedure from commercial producers where artificial lightning tubes are widely used.

Feeding of the larvae was started at 4 dph periodically every 6 h. The larvae were fed with *Artemia spec.* nauplie (INVE, AF Specialty Cysts) that were harvested every day and stored on ice until the fourth feeding. Larvae were fed with 10 artemia nauplie per ml per day. Most pike-perch larvae used in the experiments were fed exclusively on *Artemia spec.*, one exception are larvae that reached 50 dph. Those larvae were fed with 200-300 µm dry feed (INVE, O.range START-L) starting at 15 dph continuing with 300-500 µm dry feed (INVE, O.range GROW-S) beginning at 30 dph.

2.2 Experimental design

Phototaxis experiment

The development of phototactic behavior was tested in a 150 cm long channel that was constructed out of a DN 250 plastic pipe that has been opened lengthwise (Fig. III-2). The channel walls of one side of the channel were lined with white plastic foil and the other side with black plastic foil. Except of a small window between the black and the white side of the channel the black side was sealed with tarpaulin, so no light could enter the black side from another place than the connecting window. The adjacent white side of the channel was illuminated with a lightning rod that provided 700 lx light intensity at the water surface. Light spectrum was measured with a spectrometer (Ocean Optics USB 2000, Ostfildern, Germany) and consisted of several peaks between yellow and blue light (Fig. III-1). The water used in the light channel was not in motion and originated from the rearing system that has been described in section 2.1.

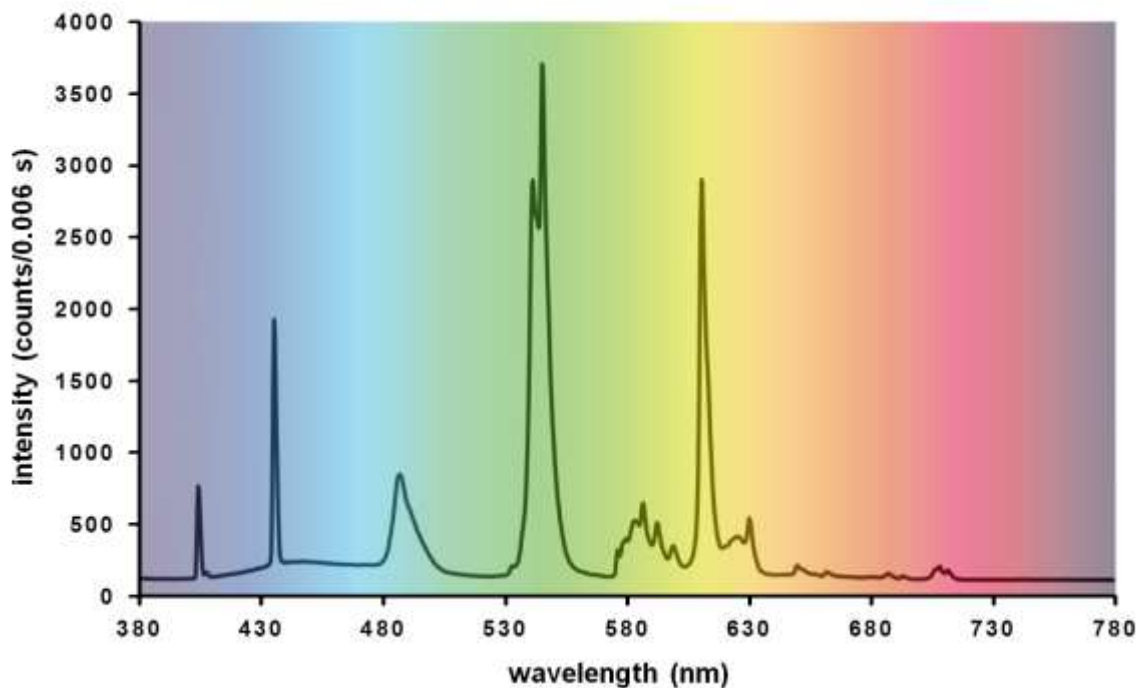


Figure III-1: Spectrum of light used during phototaxis and grading experiment. Intensity is depicted between 380 and 780 nm wavelength and underlined with the corresponding color scheme.

During the phototaxis experiment, pike-perch larvae were evenly distributed within a static water column inside the light channel. The black side of the channel was sealed carefully and light on the white site was turned on. After a period of two hours a wall was inserted between the black i.e. dark

side and the white i.e. bright side of the channel. Afterwards larvae were collected according to their current distribution and dispersed on scale paper to take pictures.

Grading experiment

The grading experiment was conducted in the same light channel that has been described for the phototaxis experiment (Fig. III-2). Experiments were conducted for larvae at the age of 16, 22, 28 and 34 dph.

During the grading experiment the larvae were introduced into the water column at the dark side of the channel while the net was installed between the bright and the dark side of the channel. For a successful grading it is crucial, that the selected mesh size meets the average size of the initial, ungraded group to hold back big larvae and at the same time let pass smaller individuals. Thus the tested mesh sizes of 1.5 and 3 mm were selected based on the findings of Knaus and Gallandt (2012). After introducing the larvae, the dark side was sealed and light on the bright side was turned on. After a period of two hours the separating wall was installed, larvae were collected and photos of the larvae placed on scale paper were taken.

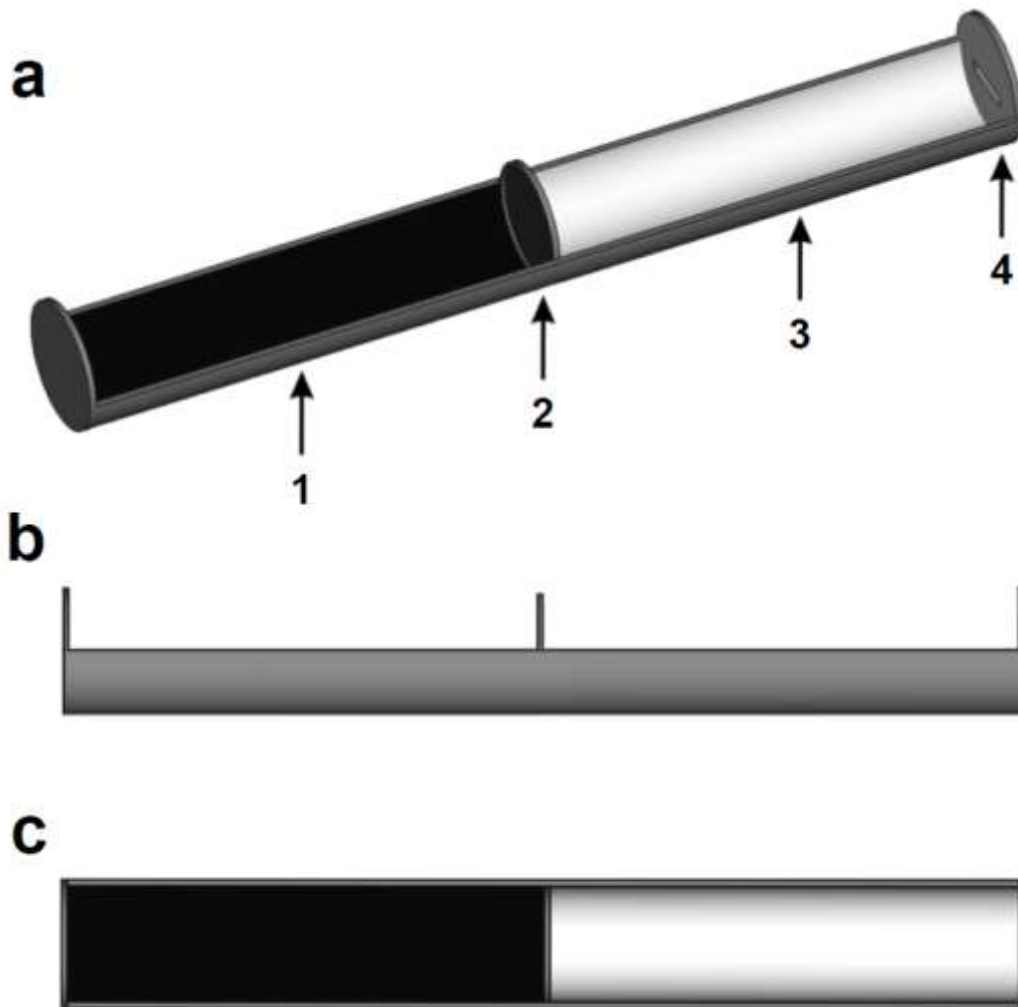


Figure III-2: Assemblage of light channel for phototaxis and grading experiment. Diagonal view (a) shows dark i.e. black side (1), removable mesh (2), bright i.e. white side (3) and channel wall with water overflow (4). Side view (b) and top view (c) are shown below.

2.3 Data collection and analysis

The photos that were taken during the phototactic and grading experiments were evaluated with the graphic analysis program ImageJ 1.45s. During the grading process larvae attempt to pass the net by swimming towards the mesh head first. Thus height and width of larvae are the limiting factor while length has no direct influence on the passing of larvae. Nevertheless length of pike-perch larvae at 29 dph correlates with width (Knaus and Gallandt, 2012) hence we used larval length as size measure. The amount of larvae on each photo as well as the individual length was determined. Differences in distribution of length between bright and dark groups of the phototaxis experiment were analyzed via a Shapiro-Wilk test and a Mann-Whitney U test with the statistical software R 2.13.2. With the Shapiro-Wilk test the dark and bright groups of each grading trial were tested for normality. Based on the results of the Shapiro-Wilk test a Mann-Whitney U test with $\alpha=0.05$ was conducted to test the

length distribution of bright and dark groups on inequality. Together with minimum, maximum and group average the resulting p-values were used to evaluate the grading success of each grading trial.

3. Results

3.1 Phototaxis experiment

During the phototaxis experiment we investigated the phototactic behavior of pike-perch larvae during the first 50 dph. The larvae were exposed to 0 and 700 lx light conditions and were counted with regard to their destination.

Figure III-3 shows the development of phototactic behavior between 1 and 50 dph. The percentage of larvae that preferred bright light conditions ranged between 80.4% at 50 dph and 100% at 22 dph. The amount of larvae distributed at the bright side increased from 82.9% at 1 dph to 97.7% at 2 dph and decreased again over the next two days to 94.9%. At 10 dph 99.0% of the tested larvae swam during the experiment towards the light and at 22 dph every tested fish were originated at the bright side of the setup. From 28 dph to 50 dph positive phototactic behavior started to decrease again with only 80.4% of the larvae orientating towards the light at the end of the recordings.

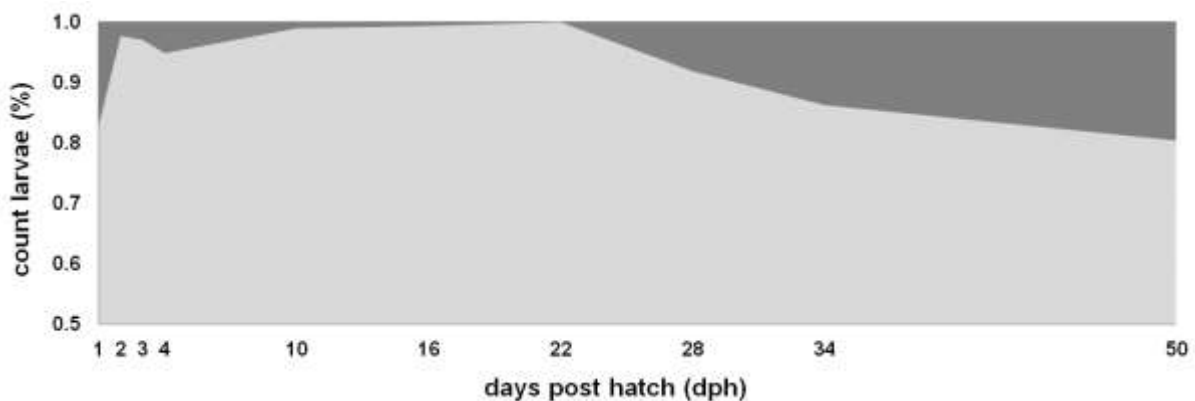


Figure III-3: Relative distribution of larvae after two hours of phototaxis experiment. Light grey depicts larvae collected at bright side (700 lx), dark grey stands for larvae at dark side (0 lx) of the channel. Scaling of x-axes reflects sampling points. Phototaxis between sampling points is interpolated.

3.2 Grading experiment

During the grading experiment trials were executed with larvae of age 16, 22, 28 and 34 dph. For optical analysis, length distribution of the groups was plotted in Figure III-4 and to quantify the grading success i.e. length difference between bright and dark groups a Mann-Whitney U test was conducted (Tab. III-1).

With more than seven times as much individuals in the bright than in the dark group, Figure III-4a shows for 16 dph larvae a clear difference in group size. Also a grading effect in terms of difference in length distribution minimum and maximum is visible. The smallest larvae between 0.4 and 0.5 cm were exclusively destined in the bright group, while the largest larvae between 1.2 and 1.3 cm only occurred within the dark group. Instead the average length with 0.76 cm for the bright and 0.81 cm for the dark group differs little. At 22 dph (Fig. III-4b) the smallest larvae between 0.6 and 0.7 cm again were destined in the bright group while the biggest larvae of 1.3 to 1.5 cm solely appeared in the dark group. The amount of individuals in the bright group was more than three times higher than in the dark group and the difference between averages ranged between 0.95 cm for the bright and 1.17 cm for the dark group. At 28 dph (Fig. III-4c) the smallest larvae with 0.9 cm and the biggest larvae between 2.3 and 1.7 cm were both distributed in the dark group. Size of the bright group was 1.4 times higher than the dark group and average length differed from 1.28 cm for the bright group to 1.5 cm for the dark group. At 34 dph (Fig. III-4d) the smallest larvae of 1.5 cm were present in both groups while the biggest larvae from 2.1 to 2.9 cm were exclusively observed in the dark group. Both groups were of nearly the same individual size and the average length ranged from 1.76 cm for the bright group to 2.14 cm for the dark group.

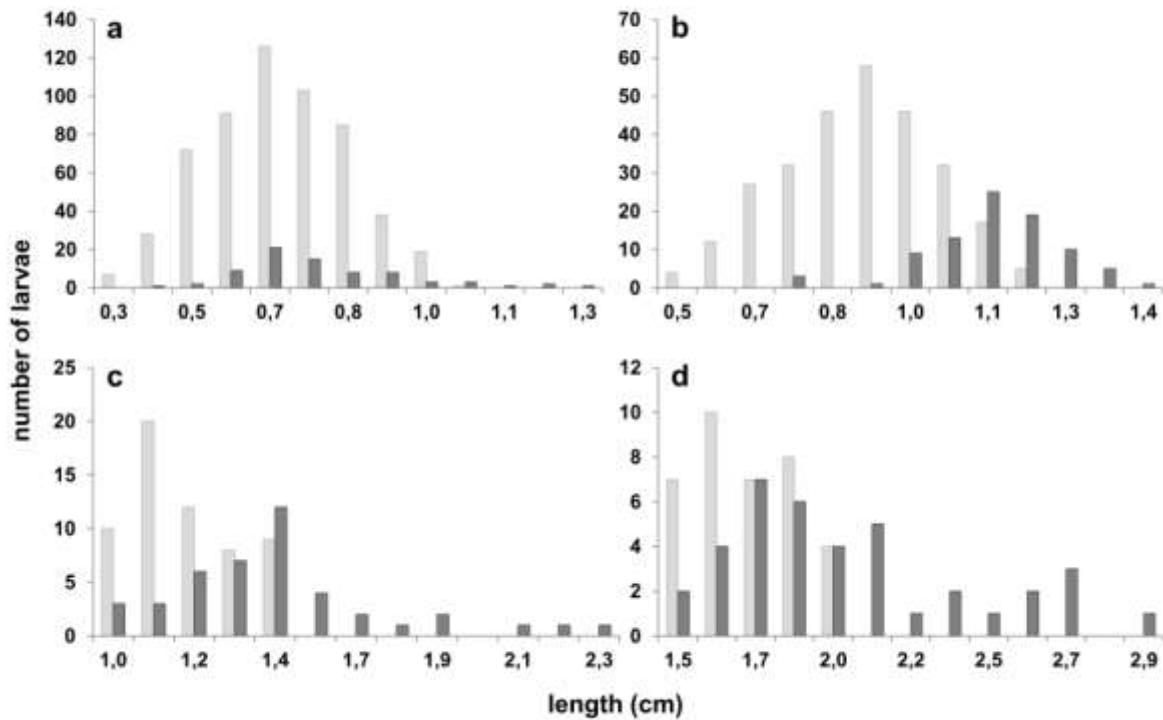


Figure III-4: Absolute distribution of larvae after two hours of grading experiment at 16 dph with 1.5 mm mesh (a), 22 dph with 1.5 mm mesh (b), 28 dph with 1.5 mm mesh (c) and 34 dph with 3 mm mesh (d). Light grey depicts larvae collected at the bright side (700 lx), dark grey stands for larvae at the dark side (0 lx) of the channel.

The statistical analysis being a Mann-Whitney U test for the bright and dark group of every tested age provided a p-value as measure for inequality in length distribution (Tab. III-1). The lowest p-value i.e. the most inequality in length was reached at 22 dph. At the ages of 28 and 34 dph the inequality was still significant but the p-value was much higher. The p-value for larvae at 16 dph in turn was higher than the 0.05 significance level and therefore the bright and dark group were statistically equally distributed.

Table III-1: Statistical analysis of grading experiment. Mann-Whitney U test testing on inequality between grading groups.

group	p-value Mann-Whitney U test ($\alpha=0.05$)
dph16 bright	0.0608
dph16 dark	
dph22 bright	2.20E-16
dph22 dark	
dph28 bright	1.78E-06
dph28 dark	
dph34 bright	4.05E-07
dph34 dark	

4. Discussion

4.1 Phototaxis

With the analysis of phototactic behavior throughout the first days of pike-perch development we were able to describe a very early increase of positive phototaxis and a subsequent decrease within the fourth week of rearing (Fig. III-3). The described behavioral development depicts the change from positive phototactic behavior of larval (Proteau et al., 1993) to negative phototactic behavior of juvenile and adult pike-perch (Marshall, 1977; Luchiari et al., 2006). This behavioral change is based on the gradual eye development that begins during the egg stage (Ott et al., 2012), progresses with cone and rod development (von der Emde et al., 2004; Luchiari et al., 2009) as well as the development of the *tapetum lucidum* (Wunder, 1930; Luchiari et al., 2006). Only the exact temporal appearances of those ontogenetic developments are not known. Nevertheless the beginning decrease in positive phototactic behavior is very similar to the behavioral response of the closely related Walleye (*Sander vitreus*) which is highly attracted by bright light for the first eight weeks of its life. After that time the walleye shifts to negative phototaxis, being attracted by light intensities of 4 lx and below (Bulkowski and Meade, 1983). Simultaneously to the shift the *tapetum* starts to develop, while rods and cones develop later during life history (Braekvelt et al., 1989). Thus the shift from positive to negative phototaxis is likely to be correlated with the walleyes *tapetum lucidum* development. Due to the close relation and the very similar habitat of walleye and pike-perch it can be speculated that during pike-perch ontogenesis the shift to negative phototaxis as well is related to the onset of *tapetum lucidum* development. Nevertheless this connection leaves to be unverified and needs to be confirmed in future work on the subject of eye physiology of larval pike-perch.

Regarding the optimal light intensity for larval pike-perch rearing, the observed phototactic behavior implies that pike-perch larvae during their first weeks post hatch can cope with and even prefer higher light conditions. For fish performance during the first four weeks post hatch neither high nor low light intensities have yet been reported to have a positive or negative effect. This work is also not able to definitely prove if high or low light during larval age is preferable but it provides new evidence. Since it is shown that larvae during their first four weeks post hatch are constantly swimming towards high light intensities (Fig. III-3) it is well possible that they are not negatively affected by light up to 700 lx. Nevertheless as soon as the shift to negative phototaxis begins, light needs to be shifted to lower intensities in order to avoid stress and provide optimal rearing conditions.

4.2 Grading

To verify the effectiveness of a light triggered self grading system we conducted four grading trials at 16, 22, 28 and 34 dph (Fig. III-4). At the ages 16, 22 and 28 dph grading was performed via 1.5 mm mesh size while larvae at 34 dph were graded with 3 mm mesh size.

Evaluating the results minimum and maximum length, the destination of the groups average length and the p-values as mathematical measure for inequality were taken in to account. By examining the length distribution of the 16 dph bright and dark group it is conspicuous, that the statistical analysis found both groups to be statistically equal backed by the average length that only differed about 0.05 cm. The minimum and maximum length on the other hand illustrate a small grading effect with a lower minimum length in the bright and a higher maximum length in the dark group. A reason for those contradicting observations could be that length at 16 dph is not yet a reliable measure for larval width which would lead to a mismatch between the grading criterion i.e. width and the selected size measure i.e. length. A second possibility is that the urge to swim towards light is not yet strong enough in some of the larvae to overcome the mesh size they potentially would fit through. Considering that the phototaxis experiment showed a very strong positive phototaxis at 16 dph already (Fig. III-3) and that Knaus and Gallandt (2012) observed a correlation between larval length and width for older larvae with age 29 dph onwards we perceive the first assumption as more likely. At 22 dph the grading experiment showed a very clear grading effect. The shift between minimum and maximum length was similar to the 16 dph experiment but the average group length differed with 0.22 cm bigger larvae at the dark side more than four times as much as at 16 dph. This change is also reflected by the p value which is the lowest throughout all trials. Based on those findings and the literature which connects low size heterogeneity in larval pike-perch with a decrease in cannibalistic mortality (Baras and Jobling, 2002; Szczepkowski et al., 2011; Knaus and Gallandt, 2012) it is obvious

that the tested self grading mechanism succeeded to grade 22 dph larvae in two more homogenous size groups that are likely to postpone and/or decrease its cannibalistic behavior. The grading trials at later age 28 and 34 dph on the other hand identify the limits of light triggered self grading. At both trials the minimum larval length is nearly the same for the dark and bright groups while the maximum length for the dark group is much higher than maximum length in the bright groups. Also the average length is much smaller for the bright than for the dark groups. The depicted length distributions show smaller heterogeneity only for the bright group demonstrating the suitable mesh size. At the same time the dark group stays highly heterogenic with individuals that stay in the dark although they would fit through the net. The statistical analysis depicts the groups for both ages as similarly unequal, which is based on the higher maximum in the dark groups and the smaller average size in the bright groups. The dark groups size heterogeneity increases from 28 to 34 dph and reflects the decrease in positive phototaxis that was discussed in 4.1. Accordingly for a sufficient self grading it is crucial to grade within the time of larvae's maximum phototactic behavior.

4.3 Applicability

Based on the findings of phototaxis and grading experiments it was possible to distinguish a small time frame for a successful self grading and to describe the behavioral change of pike-perch larvae exposed to high light conditions. Those findings could find practical use in larval pike-perch rearing and tank design. The high positive phototaxis from the first days after hatching and its subsequent decrease after about three weeks could mark a time window in which larvae do not negatively react on high light intensities. Since larvae at that time are very small the handling and assessment of larvae culture is increasingly difficult under dim light conditions. Based on the larvae's behavior this study suggests, that if needed it is possible to rear larval pike-perch for the first three weeks using bright light conditions without harming the fish. After this period the light preferences change to lower light conditions accordingly should the used light treatment be regulated down to less than 25 lx (Luchiari et al., 2006).

The use of larvae phototaxis for self grading was shown to be sufficient at 22 dph and should be implemented in larval rearing protocols to postpone and reduce cannibalistic behavior without stressing or injuring the fish. After this first further gradings would need to be executed every two weeks (Knaus and Gallandt, 2012) until cannibalism naturally decreases at a fish weight of about 10 g (Schmidt and Kühn, 2013). All gradings beyond the positive phototactic period would need to be executed either manually or by differently triggered self grading mechanisms. Conceivably using the observed flight reflex to deeper waters (Müller-Belecke and Kaufhold, 2013) and grade the fish vertically or trying to grade negative phototactic fish from bright light towards darkness. Those

approaches would need to be tested in further studies and might also be applicable to similar aquaculture relevant species like the walleye. In any case should future tank designs try to include grading mechanisms within the rearing tanks to further reduce handling stress during the grading process.

5. References

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GENERAL DISCUSSION

During the course of the present study, common reproduction and larval rearing methods of RAS based, intensive pike-perch aquaculture were evaluated and potential problems as well as improvement opportunities were outlined. Three different chapters were conducted with the aim to reply to the posed objectives:

- (i) Give a profound estimation on potentially negative effects of out-of-season spawning on quality of pike-perch offspring.
- (ii) Locate parental body characteristics and early larval life history traits that could be used to predict and increase larval performance.
- (iii) Give a depiction on the effect of light intensity during larval rearing and predicate which light intensity was most advantageous for larval development.
- (iv) Demonstrate if light can be used as an attractant to implement a gentle self grading mechanism during larvae's positive phototactic phase.

The overall aim of this study was to contribute new knowledge that could lead to an improvement of larval pike-perch rearing and reproduction.

Out-of-season spawning

Out-of-season reproduction of pike-perch is a highly acclaimed method of intensive RAS based pike-perch reproduction that enables a seasonally independent offspring supply over the whole year. Unfortunately, it is uncertain how the involved alteration of breeder's neuroendocrine pathways affects the quality of produced offspring. Thus, chapter one of this study compared 13 different batches of in- and out-of-season produced pike-perch to its egg and larval quality parameters.

During the course of the experiment, no statistically significant effect could be found between out-of-season spawning and the quality parameters Hr, SL0, YoS, LiS, FCa, Mn, Ms, SBI, SLG, DWG (Fig. I-7). However, a strong variability in larval quality parameters was observed and for the growth parameters SLG, DWG and sRD an increase in variability over time was detected. Similar observations were made during the experiments of chapter two where variability in larval quality was high from the beginning and growth parameters increased in heterogeneity over time (Fig. II-3, II-4, II-5 & II-9). Furthermore, larvae of the experiments in chapter three were shown to increase in size heterogeneity over time as well (Fig. III-4). Explanations for the high and increasing variability can be found in the work of Kestemont et al. (2003) who stated that quality of larval fish depends on a wide range of intrinsic and environmental factors, of which the respective influences are largely unknown.

Much of larvae's variability emerges from those factors via interactive effects. Thus, a possible effect of out-of-season spawning would need to be proportionally strong to be detected in data with such high variability. However, statistical analysis of sRD and season showed, beginning at dph 6 a continuously low sRD for larvae that were spawned during autumn. Larvae that were spawned during winter, summer and spring (in-season) did not show a notable, season related variation in sRD. The observed low sRD of autumn spawned larvae was linked to a lower, albeit insignificant DWG which is supported by the work of Buckley (1984) who closely connected sRD as condition factor to fish growth. Furthermore, a positive correlation between sRD and DWG could be observed for larvae of experiment 2 as well (Fig. II-9) However, since feed consumption of all tested larvae were similar and inconspicuous in its characteristics a lower DWG caused by low feed intake was not likely. Instead it was more likely that another factor influenced autumn spawned larvae in a way that larvae's feed conversion was impaired which in turn decreased DWG and sRD. Since the other recorded quality parameters of autumn spawned larvae showed consistently good values an interaction between low sRD and one of the documented quality indicators seemed unlikely. A negative effect of out-of-season spawning was also implausible since low sRD and DWG was only detected for one of the three out-of-season spawning treatments. Instead it was speculated that performance of autumn spawned larvae could have been low because of a higher parental stress exposure. That stress exposure of breeders can be transmitted to offspring characteristics as was shown by Schreck et al. (2001) and the large number of genetic, developmental and environmental stressors that potentially can affect stress exposure (Barton, 2002) indicate that such a connection is possible. However, the exact nature of a relevant stress factor that influenced parental stress exposure could not be uncovered.

(i) A potentially negative effect of out-of-season spawning on quality of pike-perch offspring could not be detected. However, low sRD and DWG of autumn spawned larvae were speculated to be caused by an elevated stress exposure of the specific broodstock. Nevertheless, it remained highly speculative that breeders stress level altered larval quality since two other off-season treatments showed no decrease in quality and direct evidence for a higher stress level of autumn spawners was not measured.

Prediction of larval quality

Early prediction of larval quality is one of the most important challenges in controlled pike-perch reproduction since it allows the farmer to choose only highest quality eggs and larvae for further production. Thus rearing and production effort can be minimized by rearing fewer larvae with higher quality. To identify functional, predictive quality indicators, in chapter one, parental and larval quality parameters of the 13 batches were recorded until 21 dph and analyzed.

During the course of the experiment, significant correlations could be found for larval quality parameters YoS – LiS, YoS – SLG, LiS – Ms, LiS – DWG, Mn – DWG, SBI – DWG and SLG – DWG. Significant correlations for parental quality parameters could not be found. The observed correlations between YoS - LiS and SLG – DWG were of limited value for larval quality prediction since both correlations described the relationship of two factors during the same time of larval life history. Thus, a prediction of larval quality based on one of the factors was not possible. Furthermore, the correlation between YoS – LiS described an expected interdependency since LiS is part of the YoS. Moreover, the correlation of SLG – DWG did also describe a biological interdependency (McGurk, 1987) which was confirmed by the results of chapter two where an allometric growth relation between SLG and DWG was observed (Fig. II-9). Nevertheless, of particular interest were the observed correlations between YoS – SLG, LiS – DWG and SBI – DWG. All three correlations described a connection between an early larval quality parameter and the growth performance during the 21 day rearing period. Therefore an early assessment of larval growth potential based on YoS, LiS or SBI would be possible. Unfortunately the observed correlation between SBI and DWG was negative which excluded SBI as applicable quality predictor since an inflated swim bladder is a prerequisite for long-lasting good growth and development (Czesny et al., 2005), while a short dated increase in larval growth cannot compensate for a high amount of non-inflated swim bladders. Reasons for the negative correlation between SBI and DWG, which was contradictory to the findings for older pike-perch, remained unclear. The significant correlations between YoS – SLG and LiS – DWG described a negative connection between larvae's initial maternal feed supply at 0 dph and larvae's body growth over the first 21 dph. As possible explanation for this negative connection, it was suspected that larvae with high maternal energy reserves were potentially fitter resulting in high survival during the first 21 dph while slow growing i.e. weak larvae of batches with low energy reserves died during that time (Garrido et al., 2015). This could have resulted in higher calculated growth rates for batches with high mortality due to a natural selection i.e. survival of fast growing larvae. Supported was this theory by a tendency of low Mn for larvae with high LiS, a negative correlation between LiS and Ms and a positive correlation between Mn and DWG. Furthermore, by looking at Mn of the 2500 lx treatment of chapter two (Fig. II-4 & II-6) data confirmed that high Mn can be accompanied by high growth rates as it has been shown for larvae of chapter one. However, looking at the 100 lx treatment of the same experiment it becomes obvious that low Mn can also be connected to low growth. Those observations do not necessarily undermine the postulated theory of size selective mortality that leads to a higher calculated growth. However, based on the high quantity of factors and their great potential for ripple effects, it is important to realize that such a theory remains speculative.

(ii) Overall, the analysis of parental body characteristics and early larval life history traits showed that initial size of the lipid droplet could be used as standard measure to predict later stress tolerance and growth of the larvae. Parental body characteristics on the other hand could not be shown to have substantial predictive value for larval quality parameters. For future studies it was suggested to closely investigate the negative correlation between LiS and DWG to determine the exact mechanism of this correlation.

Light intensity

Implementation of specific light conditions within a controlled aquaculture environment is a relatively easy and cost-effective task which can have great influence on fish's growth and developmental performance (Boeuf and Le Bail, 1999; Luchiari et al., 2009). Unfortunately light regime consists of a complex interaction of light-intensity, -periodicity and -quantity which leads to high research expense. For juvenile and adult pike-perch, optimal light requirements are studied in detail (Luchiari et al., 2006; Kozłowski et al., 2010) while research on larval pike-perch is scarce. Thus, in chapter two pike-perch of the same batch of fertilized eggs were reared under four different light intensities (100, 500, 1000 and 2500 lx) until 21 dph to investigate which light intensity was most advantageous for early larval development.

During the course of the study, it was possible to describe significant differences in Mn after 15 dph, Ms after 15 dph and in DW growth over 21 dph. No statistically significant differences were found for FC, SBI, SL growth and sRD. For the highest light intensity of 2500 lx as well as for the lowest of 100 lx Ms was shown to be negatively affected by the light treatment. Furthermore, for the 2500 lx treatment the low stress resistance was accompanied by high growth rates while the 100 lx treatment showed low stress resistance and a low DWG (Fig. II-4 & II-5). Moreover, Mn of the 100 and 2500 lx group was significantly lower than Mn of the 500 and 1000 lx treatments which in turn showed higher stress resistance (Fig. II-5 & II-6). The observed negative connection between Mn and Ms was contradictory to the positive connection that was described by Lund and Steinfeldt (2011) and the visible positive albeit insignificant correlation that was observed during the experiments of chapter one (Fig. I-8). As possible explanations for the negative connection between Mn and Ms, it was postulated that a low Mn could have led to a more heterogenic group of larvae in which weaker individuals could have stayed alive that would have died in groups with higher Mn. In turn these weaker individuals could have led to a higher Ms during the salinity experiment while weak larvae of treatments with high Mn were already singled out, leading to a lower Ms. Another effect could have been that the low growth performance of the 100 lx group led to a slower ontogenetic development. Compared with the higher light treatments, a slowed down ontogenetic development would have led

to a delayed undergoing of critical mile stones in larval life history. The associated increase in Mn that is linked to those mile stones would have been delayed as well. In that case, low Mn of the 100 lx treatment would not so much be an expression of good larval quality but a temporary effect of slow larval development. Those theories are similar to the explanations for negative correlations between YoS – SLG and LiS – DWG in chapter one and try to explain relationships of parameters that seem inexplicable via a cascade of effects that define each other. It is important to emphasise that those theories remain speculative.

For sRD no significant differences could be found between light treatments. Nevertheless, the development of sRD varied over time and it is possible to speculate about connections with metabolic and nutritive utilization in context with ontogenetic development. Thus, the overall increase in sRD beginning at dph 6 is in accordance with the start of artemia feeding that began at dph 4. The subsequent decrease of sRD between 15 and 18 dph could mark a period in pike-perch life history in which larvae traverse ontogenetic developments that prohibit the organism from an effective feed consumption and processing. Possible ontogenetic processes that explain the decreased sRD can be found in stomach and yolk development. While the visible yolk of pike-perch larvae is absorbed at about 6 dph, remaining traces of yolk nutrition can be found up to 15 dph (Ostaszewska et al., 2005). Up until the complete absorption of yolk nutrition, larvae need to be able to synthesize all metabolic products on their own. To do so, a fully developed stomach is required. While yolk nutrition can be fully absorbed at 15 dph, the stomach development in pike-perch occurs not until 15 and is completed at 20 dph (Hamza et al., 2015). The observed decrease in sRD of all light treatments between 15 and 18 dph is presumably ascribed to these developmental processes. This thesis is supported by the sudden increase in sRD and feed consumption at 21 dph, marking the completion of stomach development and onset of self-containing metabolism of larvae. Looking at the sRD data of chapter one (Fig. I-4), the described ontogenetic and metabolic development is not reflected and, thus, do not reinforce those interpretations. Anyhow, reasons for the relatively constant sRD values of chapter one could be that the depicted average sRD development of 13 batches does not depict the developments of specific batches as clearly as the single batch of larvae from chapter two. Another possibility is that larvae of chapter two have a very distinct and unique sRD development which larvae with another genetic background do not have. Especially the strong increase of sRD at dph 21 of larvae in chapter two could be a marker for a comparatively fast stomach completion which was slower for larvae in chapter one. Unfortunately the experiments end with 21 dph. This makes it impossible to determine if a sRD increase i.e. stomach development of chapter one larvae was delayed for a few days. An average lower DW growth of chapter one larvae (Fig. I-3) encourages the hypothesis of slower larval stomach development. Additionally lower DW of

chapter one larvae matches with a slightly lower sRD compared to higher DW growth and sRD of chapter two larvae. Those findings correspond with the positive correlation of RNA-DNA ratio and DW that was shown by Buckley (1984) and confirm that sRD is a suitable factor to measure nutritional value of pike-perch.

(iii) In conclusion the experiments of chapter two showed that larval pike-perch during the first 21 dph of rearing can benefit from high light intensities between 500 and 1000 lx in terms of improved growth and high stress resistance. In turn larval rearing under dim light conditions of 100 lx showed to have a positive effect on larval mortality. Thus light intensity during larval pike-perch rearing should not exceed 1000 lx and the decision to rear under dim or bright light conditions should be based on the farmer's rearing capacity, availability i.e. amount of larvae and the practical use of an illuminated working environment.

Self grading

Early size grading of intensively reared pike-perch (*Sander lucioperca*) culture is an effective way to postpone and reduce cannibalistic behavior and hence increase larval survival. Cannibalistic behavior can appear as soon as first larvae reach a total length of 15 mm about two weeks after hatching (Szkudlarek and Zakes, 2007). Because of the small and fragile organisms during that early age a size sorting is very labor intensive or even impossible. By finding a way to sort larval pike-perch in a cost effective and organism friendly manner, one could substantially improve larval survival and rearing conditions. Thus, in chapter three it was tried to characterize the intensity and duration of a short positive phototactic period during the beginning of pike-perch's life history (Schlumberger and Proteau, 1996). Thereupon it was tried to implement a gentle, light driven self grading mechanism throughout the positive phototactic period.

During the course of the experiments pike-perch larvae were shown to be highly positive phototactic from the day of hatching. Positive phototactic behavior between 0 and 10 dph was already high but still increasing with a peak of up to 100% positive phototaxis between 10 and 22 dph. After 22 dph positive phototaxis was slowly decreasing. Thus, larvae were assumed to be highly positive phototactic during the first 3 weeks post hatch. Those findings match the findings of chapter two that showed larval pike-perch up to 21 dph to benefit from bright light intensities between 500 and 1000 lx in terms of a higher growth rate and higher stress resistance (Chap. 2; Fig 4 & 5). Nevertheless, findings of chapter two also underlined that Mn during the rearing under bright light intensities was increasing (Chap. 2; Fig 6). Evaluating the observed development of phototactic behavior, it is necessary to consider that larvae of the experiment were held under an artificial light regime of 24 h

illumination with 700 lx light intensity. This treatment is likely to have influenced larvae's behavioral development and, thus, phototactic development of larvae held under another light regime could differ. Anyhow, since larvae of chapter one and two were held under similar light regimes like chapter three larvae, it can be expected that phototactic development of chapter one and two larvae was similarly.

Grading experiments were conducted during the time of highest positive phototaxis and during the period of decreasing positive phototactic behavior. Results showed that a light driven self grading mechanism where larval pike-perch is actively swimming through a size selective mesh size of 1.5 mm can be successfully conducted during the time of peak positive phototaxis at 22 dph. Furthermore, results suggested that an even earlier size grading at 16 dph could be successful as well but would need to be conducted with a smaller mesh size. Attempts to conduct this self grading mechanism beyond the time of peak phototaxis were shown to be inefficient. In accordance with the experiments of chapter two, it is plausible to postulate that successful light driven self grading can and should be conducted during the third week of larval rearing and that the exposure of larvae to high light intensities can be beneficial for the organism during that time. Overall the experiments showed larval pike-perch to be highly positive phototactic with a peak between 10 and 22 dph and a slow decrease after 22 dph.

(iv) It was also shown that light can be used as an attractant to implement a gentle self grading mechanism during larvae's peak in positive phototactic behavior.

Applicability of gained results

By considering the presented results for the application in commercial aquaculture, it is important to carefully consider costs and benefits of each method. Especially methods that are complicated in their application and methods that require constant repeating labor hours are cost intensive. The commercial use of sRD, for instance, would not be feasible because the method is cost intensive, labor intensive and not easy to interpret. Nevertheless, RNA-DNA ratio still is a valuable measure for explanatory research that focuses on nutritive and developmental relations (Meyer et al., 2012).

Thus, low performance of out-of-season produced larvae from autumn breeders could be detected due to sRD. Nevertheless, since the exact mechanisms of observed lower sRD for autumn spawners could not be revealed and the possibility of a year round offspring production is highly relevant for commercial success, authors think it is still advisable producing larvae out-of-season.

The measurement of parental parameters for prediction of larval quality is another method that is accompanied by a relatively high labour expense and did not show significant correlations with larval quality parameters. At this it must be differentiated between a direct prediction of larval qualities based on specific properties of the parents or a selective breeding program that is specifically targeting desired properties across many generations. A prediction of larval quality based on its parent's properties showed to be inefficient and not advisable for commercial use. A breeding program across many generations, however, promises great production and revenue increase despite major investment and running costs. Examples for such breeding programs can be found for common carp (*Cyprinus carpio*) (Vandeputte, 2003), rainbow trout (*Oncorhynchus mykiss*) (Janssen et al., 2015), Atlantic salmon (*Salmon salar*) (Gjedrem and Baranski, 2010) and the sea bass (*Dicentrarchus labrax*) (Vandeputte et al., 2009). For pike-perch, however, selective breeding programs are still at their infancy but are strongly promoted to further increase production (Blonk and Komen, 2015).

Labour expense and results for measuring early larval quality parameters as predictors of later development differ greatly and have only LiS shown to be simultaneously practicable and of predictive value. Monitoring of LiS is a relatively easy to use, one-time method that was shown to enable a prediction of future stress tolerance and growth performance at the age of 0 dph. Thus, LiS could contribute to the decision making of whether or not a batch of larvae should be considered for further rearing. However, exact effects of the interdependencies remain unknown.

Adjusting ambient light conditions regarding to specific preferences that change with larval development is a cheap and a straightforward method to improve larval rearing conditions. However, obtaining the knowledge about optimal light conditions during different phases in pike-perch's life history is complex and to date not completed for every light related variable at any time of the fish's life history. Anyhow the present study showed that larval pike-perch from 0 to 21 dph can benefit from high light intensities between 500 and 1000 lx in terms of improved growth and high stress resistance. On the other hand, Mn of fish that were reared under those light intensities was slightly higher. Nevertheless, under the circumstances that a slightly higher Mn can be compensated by sufficient produced larvae, farmers are encouraged to rear their larvae up to 21 dph under high light intensities.

Implementing a light driven self sorting mechanism into the procedure of commercial larval rearing would be related to constructing a light tunnel. Optimally, this light tunnel would be included into the rearing system avoiding unnecessary handling. Once such a system was constructed the present

study showed that it would be possible to grade pike-perch larvae between two to three weeks post hatch into more size homogenous sub groups, avoiding high labour expense and handling stress for the fish. In the long term, such a system would decrease labour expense and cannibalistic mortality. Thus, pike-perch farmers should consider implementing a light driven self grading into their larval rearing protocol.

Further perspective

In order to further increase production output and decrease costs in the future of pike-perch rearing it is very promising to further look at the interactions between LIS at 0 dph and later stress resistance as well as weight growth. To fully understand the negative correlation of LIS and DWG that was observed in the present study, it is important to perform experiments that consider a long term effect and rear larvae up to commercial size. Such a study could demonstrate if a high or a low LIS leads to significant differences in production. If such a long term effect was proven, pike-perch farmers would be provided with an easy to use measurement that could predict future performance of larvae. Another very straight forward approach to further improve rearing conditions is to fully uncover the light requirements of pike-perch over time. At this it is especially necessary to look at the largely unknown light requirements of larval pike-perch and to create continuous light treatment protocols. In doing so it is vital that not only light intensity is part of this research but also periodicity and quantity. It is postulated that an optimal light regime that changes according to the fish's development could substantially increase production. It is also promising that the extension of the presented self grading approach into a specifically designed larval rearing tank that provides a light grading system for larvae between two to three weeks post hatch. It would also be imaginable to integrate a mechanism for the grading of older larvae that uses the observed flight reflex of older pike-perch (Müller-Belecke and Zienert, 2015).

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SUMMARY

Pike-perch (*Sander lucioperca*) is a high valued food and gaming fish that is traditionally wild caught or farmed in extensive pond aquaculture. Naturally pike-perch occurs in Middle and Eastern Europe and in large areas of Northern Asia. Over the last two decades, however, the favorable traits like fast growth and good market acceptance led researchers and aquaculturists to focus on pike-perch for intensive production in land based recirculation aquaculture systems (RAS). Within the surrounding of a RAS system, technical control of environmental factors allow a very close monitoring and adjustment of factors that influence the fish's development. Thus, rearing protocols are developed based on the unique biological requirements of pike-perch during specific developmental stages. To complete such a rearing protocol for all environmental factors at all stages during pike-perch's life history, a lot of basic research has to be conducted to define optimal rearing conditions and to overcome critical phases within the fish's life history. Today husbandry conditions for intensive RAS are advanced enough to produce pike-perch on a price and quality competitive level despite the high running costs. However, pike-perch is a relatively new species for intensive RAS rearing and a lot of unresolved questions still exist regarding the optimization of rearing conditions. Especially for the rapidly changing early life stages, knowledge gaps exist that need to be closed. Thus, in this study rearing methods of pike-perch larvae culture were investigated and in three chapters it was tried to settle open questions and find new solutions for larval pike-perch aquaculture. Aim of each of the experiments was to provide new information that could improve existing rearing methods and lead to an increase in production.

In chapter one, 13 batches of fertilized pike-perch eggs originating from four separate seasonally shifted broodstocks were hatched and larvae were reared in an indoor aquaculture system. During incubation and rearing, different parental, early and late larval quality properties were measured and analyzed. Aim was to give a profound estimation on potentially negative effects of out-of-season spawning on quality of pike-perch offspring. Also it was tried to locate parental body characteristics and early larval life history traits that could be used to predict and increase larval performance. During the course of the study lower standardized RNA-DNA ratio (sRD) and a lower dry weight growth (DWG) was determined for one of the four different spawning seasons. However, larvae of the other three spawning seasons showed no conspicuities and an exact mechanism that led to the observed lower sRD and DWG could not be revealed. Evidence for a negative effect of out-of-season spawning on quality of pike-perch offspring was not apparent since a decrease in sRD and DWG could not be found for all out-of-season spawning treatments. For the early prediction of larval quality parental characteristics were shown to be of no value. Whereas, for the early larval quality

parameter lipid droplet size (LiS) at 0 dph significant correlations with larval stress mortality (Ms) at 15 dph and DWG could be determined.

In chapter two larval pike-perch of one batch of eggs were reared under four different light intensities (100, 500, 1000 and 2500 lx) until 21 days post hatch (dph). Quality parameters were observed during the whole experimental phase. Aim was to investigate the influence of light intensity on pike-perch larvae during their short period of positive phototaxis. Highest light intensity of 2500 lx showed good DWG but an increase in Ms. Bright light of 500 and 1000 lx intensity was found to improve DWG and Ms but also showed slightly higher natural mortality (Mn) at 15 dph. Dim light conditions of 100 lx showed significantly lower Mn but higher Ms and low DWG. The experiments of chapter two showed that larval pike-perch during the first 21 dph of rearing can in fact benefit from high light intensities between 500 and 1000 lx in terms of improved DWG and low Ms. Thus, it was advised to rear larval pike-perch for the first 3 weeks under bright light conditions that not exceed 1000 lx under the circumstance that a slightly higher Mn can be compensated by an initially high larvae production.

In chapter three it was tried to use the positive phototactic behavior of larval pike-perch to introduce a self grading mechanism that could prevent cannibalistic behavior. Thus, it was tried to develop a gentle grading alternative to the commonly used manual grading methods that are associated with high handling stress and risk for injuries. In a first step the phototactic behavior of pike-perch larvae from 1 to 50 days post hatch (dph) were evaluated in a channel system with 0 and 700 lx light treatment. In a second step the larvae's observed positive phototaxis was then used to evaluate the effectiveness of a light triggered self grading mechanism during 16 and 34 dph. Results showed larval pike-perch to be highly positive phototactic from the beginning with a peak between 10 and 22 dph and a slow decrease after 22 dph. The approach of grading larvae via light as an attractant showed to be successful at 22 dph and was assumed to be possible for an earlier phase as well. With the decrease in positive phototactic behavior after 22 dph, it was not possible to successfully grade older larvae. The use of a light driven self grading was recommended and a decrease in labour expense as well as a higher survival rate was postulated.

This study revealed in three different approaches possibilities to improve common rearing and reproduction methods to further optimize larval pike-perch aquaculture and increase its production outcome. Authors recommend implementing a bright intensity light regime during the first three weeks of rearing to improve larval growth and stress resistance. Furthermore, the inclusion of one to two light triggered self gradings is recommended to prevent cannibalistic behavior. LiS at 0 dph

SUMMARY

showed promising results regarding predictability of late larval quality but to be able to draw the right conclusions further work is needed. A negative effect of out-of-season spawning on quality of pike-perch offspring could not be found and it is advised to continue producing larvae out-of-season.

ZUSAMMENFASSUNG

Der Europäische Zander (*Sander lucioperca*) ist ein hochwertiger Speisefisch sowie beliebte Zielart in der Sportfischerei. Traditionell stammt vermarkteter Zander aus Wildfängen oder aus extensiv bewirtschafteten Teichanlagen. Die natürlichen Verbreitungsgrenzen des Zanders erstrecken sich von Mittel- bis Ost-Europa und über weite Teile Nord-Asiens. Gute Eigenschaften wie schnelles Wachstum und hohe Marktakzeptanz führten dazu, dass innerhalb der letzten Jahrzehnte die intensive Zanderproduktion in landbasierten Kreislaufsystemen (RAS) von Wissenschaftlern und Aquakulturisten vorangetrieben wurde. Durch technische Kontrolle ist es innerhalb von RAS möglich, Umwelteinflüsse die die Entwicklung der Fische beeinflussen können zu überwachen und zu steuern. Um diese Kontrollmöglichkeiten optimal zu nutzen, werden spezielle Aufzuchtprotokolle genutzt und weiterentwickelt, die auf die spezifischen biologischen Bedürfnisse des Zanders während unterschiedlicher Entwicklungsphasen eingehen. Dabei muss im Vorfeld viel wissenschaftlicher Aufwand betrieben werden, um die optimalen Einstellungen für jede Einflussgröße zu jeder Entwicklungsphase des Zanders bestimmen zu können. Heutzutage ist das Wissen um die Aufzucht von Zandern in intensiven RAS weit genug entwickelt um Zander zu produzieren, die preislich und qualitativ auf dem Markt bestehen können, obwohl laufende Kosten einer RAS relativ hoch sind. Nichtsdestotrotz ist der Zander eine vergleichsweise neue Art für die Aufzucht in RAS, dessen Haltungsoptima noch nicht in Gänze erforscht sind. Insbesondere während der frühen larvalen Phasen, deren physiologische und morphologische Entwicklung besonders schnell voranschreiten, bestehen Wissenslücken die geschlossen werden müssen. In der vorliegenden Arbeit wurden bestehende Aufzuchtmethoden für larvalen Zander evaluiert und es wurde in drei Kapiteln versucht Wissenslücken zu schließen, sowie Verbesserungsmöglichkeiten zu finden. Ziel jedes der hier vorgestellten Experimente war es neue Informationen zu erschließen die potenziell zu einer Methodenverbesserung sowie Produktionssteigerung führen könnten.

In Kapitel Eins wurden 13 Gelege befruchteten Zanderlaichs in einer geschlossenen Aquakulturanlage bis zum Schlupf inkubiert und anschließend herangezogen. Die Gelege stammten von 13 unterschiedlichen Elterntierpaarungen aus drei saisonal verschifteten und einem unverschifteten Laichtierbestand. Während Inkubation und Aufzucht wurden unterschiedliche Elterntierparameter, frühe larvale Qualitätsparameter und späte larvale Qualitätsparameter gemessen und analysiert. Ziel war es eine fundierte Aussage darüber zu treffen, ob eine verschiftete Laichperiode der Elterntiere negative Auswirkungen auf die Qualität produzierter Nachkommen haben kann. Des Weiteren wurde versucht Elterntier- und larvale Qualitätsparameter zu identifizieren, anhand derer eine frühe Einschätzung späterer Larvenqualität möglich sein könnte. Im Laufe der Arbeit wurde für eine der

vier unterschiedlich verschifteten Laichtierbestände ein niedrigeres standardisiertes RNA-DNA Verhältnis (sRD) gemessen und ein niedrigeres Wachstum in Trockengewicht (DWG). Larven der anderen drei Saisons zeigten hingegen keinerlei Auffälligkeiten. Dies führte dazu, dass eine exakte Wirkungsweise die hinter dem einmalig beobachteten niedrigen sRD und DWG vermutet wurde nicht eindeutig beschrieben werden konnte. Beweise für einen negativen Effekt von verschifteter Laichperiode auf Larvenqualität konnten dementsprechend nicht eindeutig erbracht werden. Für die frühe Einschätzung späterer Larvenqualität konnten keine Elterntierparameter identifiziert werden die von prognostischem Wert waren. Von den frühen larvalen Qualitätsmerkmalen hingegen konnte die Größe des Lipidtropfens (LiS) 0 Tage nach Schlupf (dph) mit stressbezogener Sterblichkeit (Mn) 15 dph und DWG in Verbindung gebracht werden.

In Kapitel Zwei wurde larvaler Zander von einem Gelege mit vier unterschiedlichen Lichtintensitäten (100, 500, 1000 und 2500 lx) bis 21 dph aufgezogen. Dabei wurden über den gesamten Zeitraum des Experiments Qualitätsparameter der Larven gemessen. Ziel war es, während ihrer kurzen positiv phototaktischen Phase, den Einfluss von Lichtintensität auf die Entwicklung von Zanderlarven zu untersuchen. Larven die mit der höchsten Lichtintensität von 2500 lx aufgezogen wurden zeigten dabei gutes DWG aber hohe Ms. Lichtintensitäten 500 lx und 1000 lx zeigten vergleichsweise gutes DWG und niedrige Ms aber auch etwas höhere natürliche Mortalität (Mn) 15 dph. Niedrige Lichtstärke von 100 lx führte zwar zu der niedrigsten Mn aber auch zu hoher Ms und niedrigem DWG. Die Experimente aus Kapitel Zwei zeigten, dass larvaler Zander innerhalb der ersten 21 dph von der Aufzucht unter hohen Lichtintensitäten (zwischen 500 und 1000 lx) durch gutes DWG und niedrige Ms profitieren kann. Es wurde empfohlen, larvale Zander für die ersten 3 Wochen unter hohen Lichtintensitäten, die 1000 lx nicht überschreiten, zu halten so lange die leicht erhöhte Mn durch hohe Larvenproduktion ausgeglichen werden kann.

In Kapitel Drei wurde versucht das positiv phototaktische Verhalten von Zanderlarven für eine lichtgetriebene Selbstsortierung zu nutzen, die kannibalistischem Verhalten vorbeugen könnte. Hierbei wurde versucht eine schonende Alternative zu den mit Stress und Verletzungen verbundenen manuellen Sortiermethoden zu schaffen, die üblicherweise angewendet werden. Zunächst wurde die Entwicklung des phototaktischen Verhaltens in Zanderlarven während 1 bis 50 dph untersucht. Hierbei wurden die Zanderlarven mit einem Rinnensystem, in welchem 0 lx auf einer und 700 lx Lichtstärke auf der anderen Seite auf die Oberfläche des Wasserkörpers strahlten, untersucht. In einem weiteren Experiment wurde dann das beobachtete, positiv phototaktische Verhalten der Zanderlarven genutzt, um die Effizienz eines lichtgetriebenen Selbstsortierungsmechanismus zwischen 16 und 34 dph zu bewerten. Die Ergebnisse zeigten, dass larvaler Zander von 0 dph an stark

positiv phototaktisch ist und sich sein phototaktisches Verhalten bis zwischen 10 und 22 dph zu einem Maximum aufbaut. Danach nimmt das positive phototaktische Verhalten langsam wieder ab. Der Versuch, auf Basis dieses Verhaltens eine lichtgetriebene Selbstsortierung anzuwenden, war erfolgreich an 22 dph und es wurde vermutet das eine Sortierung zu einem früheren Zeitpunkt auch möglich wäre. Übereinstimmend mit der Abnahme in phototaktischem Verhalten zeigten die Ergebnisse, dass eine Sortierung nach 22 dph nicht mehr möglich war. Es wurde empfohlen eine lichtgetriebene Selbstsortierung anzuwenden um Arbeitsstunden und kannibalistische Mortalität zu senken.

Die Vorliegende Arbeit eröffnete in drei unterschiedlichen Ansätzen jeweils Möglichkeiten zur Optimierung larvaler Zanderanzucht und Reproduktion. Die Autoren empfehlen die Aufzucht von Zanderlarven unter hoher Lichtstärke innerhalb der ersten 3 Wochen nach Schlupf, um Wachstum und Stressresistenz zu stärken. Außerdem wird die Anwendung von ein bis zwei lichtgetriebenen Selbstsortierungen innerhalb der ersten 3 Wochen nach Schlupf empfohlen, um Ausfällen durch Kannibalismus vorzubeugen. LIS an 0 dph zeigte vielversprechende Ergebnisse hinsichtlich Vorhersagbarkeit von späterer Larvenqualität, jedoch sind weitere Untersuchungen von Nöten um mit LIS als Prediktor richtige Entscheidungen treffen zu können. Ein negativer Effekt von verschifteter Laichperiode auf die Qualität resultierender Larven konnte nicht eindeutig nachgewiesen werden. Es wird empfohlen auch weiterhin Zanderlarven durch verschiftete Laichperioden ganzjährig zu produzieren.

APPENDIX

General method description

Hatching rate (Hr) was used as measure for early larval pike-perch quality and determined via the rate of successfully fertilized eggs (Fr) and the rate of embryo survival during egg incubation (ESr). Embryo survival was measured after 24, 48 and 72 hours post fertilization.

$$Hr = Fr * ESr_{24} * ESr_{48} * ESr_{72}$$

Yolk sac size (YoS) was measured at 0 days post hatch (dph) as an early larval quality indicator. The Yolk sac is source of initial larval nutrition that is provided and determined by parental fish pairings. During the beginning of larval development larvae are not able to ingest or digest external food sources and heavily rely on yolk sac nutrition. The YoS was measured as surface area via size scaled pictures of larvae and the graphical analysis software ImageJ 1.45s.

Lipid droplet size (LiS) was measured as early larval quality indicator at 0 dph in the same manner as YoS. The lipid droplet is adhered to the Yolk sac and is likely the major source of energy during the development to exogenous feeding.

Feed consumption (FC) in larval fish starts with the onset of mouth opening and the depletion of initial yolk nutrition. First exogenous feed consumption is considered a critical mile stone in larval life history and can be associated with increased mortality. For pike-perch first feeding can occur between 5 and 9 dph. FC was determined as a late larval quality measure and ranked into four different stages (Fig. I-1). Moreover, an average daily FC (FCa) was calculated by assigning the four gut fullness stages to numbers (I=0, II=1, III=2 and IV=3), adding the numbers and dividing the summed up ranks by the amount of experiment days.

Swim bladder inflation (SBI) is an important process in larval development which permanently influences the fish's ability to float i.e. swim energy efficient. For pike-perch SBI occurs during a small time frame between 7 to 14 dph. Non inflated individuals can survive for a long time but will grow much slower than inflated individuals. SBI was measured as a late larval quality indicator and was calculated by counting inflated and non inflated individuals of a sample group under a microscope.

Natural mortality (Mn) of larval pike-perch can be a measure for early or later larval quality depending on the time of measurement. During the present work Mn was calculated as a late larval quality indicator at 15 dph. Thus the critical phase of SBI was elapsed and cannibalistic mortality did not yet occur. Therefore calculated Mn was an aggregated reflection of every factor that influenced

larval performance from 0 to 15 dph. For an exact calculation larvae were individually counted while stocking experimental tanks at 0 dph and subsequently recounted at 15 dph for the calculation of Mn at 15 dph.

Stress induced Mortality (Ms) is measured by exposing larvae to a physical, chemical or perceived stress factor over a specific period of time. Hereby, the amount of individuals that pass during the experiment is used as a performance measure of how tolerant the fish are towards the stressor. During the present work Ms was measured by exposing larvae of 15 dph, for 2 hours to a 19 PSU salinity at 18 °C. Thus the capability of larvae to perform osmoregulation and to counteract salinity stress was observed.

The RNA-DNA ratio (RD) is a popular measurement for estimating size, condition and growth of fish. DNA content is an index of cell number or biomass and remains relatively constant throughout the development of fish. RNA content on the other hand comprises much of the cell's machinery for protein synthesis and fluctuates according to food availability and the demand for protein synthesis. Several techniques exist for measuring RD and methodological differences can affect the estimate of RD. Thus it is important to achieve comparability between different methods by standardizing measured RD to a common slope ratio (slope DNA standard curve/slope of RNA standard curve) receiving a standardized RNA-DNA ratio (sRD). During the course of this Work sRD was measured as continuous larval quality indicator. Whole body samples of freeze dried pike-perch were homogenized and nucleic acid content was determined by means of the intercalating fluorescent dye ethidium bromide. Afterwards in two consecutive steps, RNase and DNase were added to the homogenate and respectively two more fluorescence readings were performed. The second reading measured the homogenate fluorescence without the degraded RNA and the third without RNA and DNA fluorescence. Based on the difference between the first and the second and between the second and the third reading, the nucleic acids were quantified. The resulting RNA-DNA ratios were standardized (sRD) to a reference slope ratio based on the slopes of a standard curve.

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