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The Role of Phototaxis in the Initial Swim Bladder Inflation of Larval Yellow Perch (*Perca Flavescens*).

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THE ROLE OF PHOTOTAXIS IN THE INITIAL SWIM BLADDER INFLATION OF
LARVAL YELLOW PERCH (PERCA FLAVESCENS)

by

Christopher Ryan Suchocki

A Thesis Submitted in
Partial Fulfillment of the
Requirements for the Degree of

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ABSTRACT

THE IMPLICATIONS OF LOW-LEVEL, NIGHTTIME ILLUMINATION ON THE INITIAL SWIM BLADDER INFLATION OF YELLOW PERCH (*PERCA FLAVESCENS*) LARVAE

by

Christopher Ryan Suchocki

The University of Wisconsin-Milwaukee, 2017
Under the Supervision of Professor Osvaldo Jhonatan Sepulveda Villet

The North Central Regional Aquaculture Center has designated the yellow perch (*Perca flavescens*) as a high priority species for culture. The demand for this species is high and it is estimated that the market could readily consume 50 to 100 million pounds per year. Tank culturing of yellow perch has several advantages over pond culture and this method has been growing in popularity, but is currently held back by problems in larval development. One of these problems, failed swim bladder inflation (SBI), is frequently reported in the literature as a bottleneck in the culture of many fishes. Unsuccessful SBI increases metabolic demands, inhibits prey capture, and increases a fish's overall probability of death. Initial SBI can occur within a finite period during ontogeny, and missing this opportunity results in permanent malformation of the organ. Advances in this problem have been made, but the challenge of increasing SBI success remains. The literature suggests that light cues appear to trigger the response of rising to the surface to gulp air and initially inflate. The yellow perch is photopositive in the larval phase and this phototactic response

correlates with the window of opportunity for SBI to occur. The goal of this research was to examine the role that phototactic behavior plays in initial SBI in yellow perch. The results of this study reveal that low-intensity nighttime light reduces the proportion of perch larvae to initially inflate. It seems that the photopositive response does not contribute to SBI success, and in fact it can significantly hinder the process when light sources exist below the water's surface. This could potentially explain previous research where increased SBI is seen in tanks with less reflective internal surfaces, and suggests that nighttime lighting and below surface light should be reduced as much as possible to increase SBI success.

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Introduction and Background

The Current Global State of Aquaculture

The current world population is over 7 billion, and this number is expected to grow to 9.7 billion by the year 2050 (Gerland et al., 2014). This growing populace has fostered an ever increasing need for expanding current food production. Fish and seafood has been essential in strengthening global food security. So far, the growth in global fish supply has outpaced that of the world population.

Correspondingly, average fish availability and consumption per capita has risen resulting in fish becoming a larger proportion of total protein consumed (FAO, 2016).

73.8 million tonnes of fish with a first-sale value estimated at over US \$160 billion were harvested from aquaculture practices in the year 2014. For decades, aquaculture has been responsible for an increasing percentage of total fish production across the globe. In 2014, aquaculture production accounted for 44.1 percent of the total production of fish by aquaculture and capture fisheries, up from 42.1 percent in 2012 and 31.1 percent in 2004 (FAO, 2016). It is likely that this trend will continue and our reliance on aquaculture as a food source will soon out-supply capture fisheries.

With the expanding amount of aquaculture products being sold, the diversity of cultured fish species has also increased to a staggering 362 different finfish

species when including hybrids (FAO, 2016). Of relatively recent interest as an aquaculture species is the yellow perch *Perca flavescens*, the only North American fish in the genus *Perca*. Two additional species in the genus *Perca* exist in Eurasia, the European perch *P. fluviatilis* and Balkhash perch *P. schrenkii* of which the former is also becoming more commonly seen in the aquaculture setting (Kestemont, Dabrowski, & Summerfelt, 2015).

Yellow Perch Ecology and Status in the Great Lakes

In the wild the yellow perch fills the ecological role of intermediate or top piscivore, and the species has a long history in the Great Lakes region of being a highly valued food, and sport fish. In the early 1990's there was an abundance of yellow perch in Lake Michigan with about 85 percent of fish caught recreationally (by weight) being yellow perch often due to their accessibility by near-shore anglers (Francis, Robillard, & Marsden, 1996). This totaled to over one million kilograms annual between 1985 and 1993 (Clapp & Dettmers, 2004). Yellow perch fillets are a popular food item in the Great Lakes region. Although almost 70 percent of US. perch sales occur within 50 miles of the Great Lakes, there is far more demand than supply (Malison, 1999). Suppliers have indicated that the current market could absorb 50-100 million pounds of yellow perch per year, and suppliers have stopped looking for new sales due to the failure to meet the demand of current customers. This lack of supply has resulted in the illegal

sales of other species such as walleye, and zander as yellow perch. Clearly there is room for the industry to grow, and substantial space for suppliers to fill.

Unfortunately for anglers and commercial fishers of yellow perch, the wild perch populations in Lake Michigan have declined to historical lows. Researchers started seeing the problem emerging in the late 1980's as annual perch surveys were finding little to no age-0 perch even though yellow perch larvae were being observed. This indicates that the problem is not overfishing, but rather that fish were experiencing poor recruitment (making it out of the larval stage and into the juvenile (age-0) stage of life). Although adult populations of perch were still being sustained, a decline in the future was predicted based on this poor recruitment data (Marsden & Robillard, 2004).

Unfortunately, these predictions have been proven true according to many researchers. Commercial harvests of yellow perch on Lake Michigan were reduced in the 1990's and completely closed by 1997 except for a limited harvest in Green Bay (Clapp & Dettmers, 2004). In 1998 the Wisconsin Department of Natural Resources was able to capture over 4,000 yellow perch. In the following years these numbers dropped significantly. By 2013 less than 100 perch were captured and the same was said for 2014 (WDNR, 2015). This decline in yellow perch populations is, in part, responsible for the newfound interest in culturing the

yellow perch since the traditional food supply was acquired through commercial harvests on the Great Lakes.

Yellow Perch Aquaculture

Since the decline of perch populations began to be observed, The Lake Michigan Committee of the Great Lakes Fishery Commission (GLFC) has held several, public, multi-jurisdictional meetings in an effort to explain to the public what research is being done, what results are being found, and as a sounding board for the public to express their thoughts. In these discussions members of the public have almost always encouraged yellow perch stocking of the Milwaukee Harbor for near-shore anglers. It has been shown that for yellow perch stocking to be a viable option for rejuvenating the fishery, we must lower the cost of rearing perch in captivity. Presently, survivorship of cultured yellow perch larvae is too low, and thus the cost to rear a sufficient number of fingerlings is too high (about 8-10 cents per inch) to be effective in a large stocking scenario. During breakout sessions of the 2014 Yellow Perch Summit researchers estimated that for stocking to have a noticeable effect on natural Lake Michigan perch populations, upwards of 100 million fingerlings would need to be stocked. This brings an estimated cost of \$20 million to \$100 million depending on the size, quality, and price of fingerlings (Lake Michigan Committee, 2014). That being said, the cost and numbers of perch fingerlings necessary for stocking to be a viable option are not the only aspects of why stocking the lake is not currently

feasible. For example the lack of yellow perch fingerling production and their unknown fate in the lake demand further research into improving yellow perch culture methods as well as their movement and population dynamics within the lake.

Most of the methods applied to yellow perch aquaculture are modified from previous methods developed for species such as catfish, salmon, and trout which have been in culture longer than any perch species. While these methods do work to a degree, there is much room for improvement. Currently, there is no culture of yellow perch fingerlings on a commercial scale. In order to increase profit margins revised culture methods, feeds, and equipment need to be developed specifically for yellow perch. Because fish are such a diverse group of animals each new species brought into culture brings unique rearing challenges.

First Inflation of the Swim Bladder

The swim bladder is an important gas filled organ in most bony fish which has a varying range of functions, such as buoyancy regulation and communication. Swim bladder morphology of fishes is divided into two distinct classifications based on the organs development and mode of function. These classes of fish are termed physoclistous fish and physostomous fish. Physoclistous fish have swim bladders that are isolated from the digestive tract, and rely on a highly vascular capillary network and gas gland to regulate gas exchange of the swim

bladder. Contrary to physoclistous fish, physostomous fish have a connection from the swim bladder to their digestive tract allowing them to gulp atmospheric air and pass it into their swim bladders via a pneumatic duct as well as expel it through the mouth or anus. This duality confers certain advantages to both physoclistous, and physostomous fish allowing them to exploit a wide variety of ecological niches, and make use of varying survival strategies (Helfman, Collette, Facey, & Bowen, 2009).

Percids, including the yellow perch, are physoclists, and like most physoclistous fish, during larval ontogeny they undergo a period of physostomous swim bladder inflation (SBI) in the first weeks of life. The exact point in time in which this inflation period exists varies between species, but for yellow perch it has been observed to begin between the 7th and 14th day post hatch. This process involves the developing larvae breaking the water surface to gulp air which then passes from the digestive tract into the swim bladder via a pneumatic duct providing the initial inflation. Two events occur that limit the time frame of first SBI. First, the pneumatic duct connecting the digestive tract to the swim bladder in yellow perch and most other physoclistous fish is a temporary feature which atrophies over time leaving a small window of opportunity for this inflation to take place. Second, if the bubbles gulped by the larvae are too large, they are prevented from passing into the swim bladder. Bile has been shown to be used as an internal surfactant that functions to break large, swallowed bubbles into smaller ones that

can successfully pass into the swim bladder. The closing of the pyloric sphincter occurs around 12 days post hatch and prevents bile reaching the swallowed bubbles. Regardless of which condition occurs first, if the opportunity for inflation is missed the larvae is permanently unable to develop a functioning swim bladder (Marty, Hinton, & Summerfelt, 1995; Rieger & Summerfelt, 1998; Rieger, 1995; Summerfelt, 2013).

The lack of a functioning swim bladder results in several developmental and survival problems. Fish that use their swim bladders as a buoyancy mechanism often have trouble with balance, locomotion, and regulating their position within the water column (Jacquemond, 2004; Peruzzi, Westgaard, & Chatain, 2007; Prestinicola, Boglione, & Cataudella, 2014). Affected fish have a higher oxygen demand, more difficulty obtaining prey items, and resulting nutritional and energetic deficiencies not seen by fish who have successfully inflated. Failed SBI regularly leads to malformations of the skeletal system often seen in the lower jaw and spine, but various other defects have been observed (Czesny, Graeb, & Dettmers, 2005; Kurata et al., 2014; Tsuji et al., 2016).

Failed SBI is a problem encountered both in captivity, as well as in the wild, although it is more often seen in culture due to the favorable conditions for uninflated fish to survive. When ample food and a lack of predators is provided it seems that more uninflated fish have the ability to make it to adulthood (Czesny

et al., 2005; Egloff, 1996; Jacquemond, 2004; Woolley & Qin, 2010). In the wild, Egloff (1996) found a Swiss lake in which SBI failure occurred in almost eight percent of wild adult European perch. Czesny et al. (2005) studied the consequences of failed SBI in wild yellow perch residing in Lake Michigan. They found uninflated fish early in the season that were consistently smaller than their conspecifics. Also, the relative occurrence of uninflated fish declined as the sampling season progressed to a point at which only fish with inflated swim bladders were caught pointed to their conclusion fish which did not inflate their swim bladder had an increased chance of mortality. It is likely that SBI success has an effect on the recruitment dynamics of yellow perch in Lake Michigan.

In captive culture, SBI failure has proven to be a significant impediment to the success of rearing yellow perch, (Craig, 2000; Czesny et al., 2005; Hart, Garling, & Malison, 2006; Kestemont et al., 2015) and many other physoclistous species such as, yellowfin tuna *Thunnus albacares* (Partridge et al., 2011), burbot *Lota lota* (Rekecki et al., 2016), and the yellow perch's close relative the European perch *Perca fluviatilis* (Egloff, 1996; Jacquemond, 2004). Yellow perch larvae that have failed to inflate their swim bladders exhibit a constant upward swimming behavior which seems to be attempts to counter their negative buoyancy. Yellow perch larvae without inflated swim bladders have also been proven to capture prey less effectively than conspecifics with inflated swim bladders (Czesny et al.,

2005). Wild inflation rates are estimated to be between 75 and 95 percent in Lake Michigan (Czesny et al., 2005).

The problem of failed inflation is significant enough to have led to the development of gravimetric sorting methods to remove uninflated fish from culture (Friedmann & Shutty, 1999). Although innovations in the processes of removing the uninflated fish is helpful to the aquaculture industry, focus on improving the proportion of successful SBI in the first place would be more cost effective. Methods developed to improve inflation so far are involved mostly with abiotic environmental factors, such as water surface properties, photoperiod modification, flow manipulation, and air injection. Surface films and particles impede the inflation process and there has been significant improvement in inflation rates in tanks which have some method of removing surface substances either by skimming, spraying, or absorbing surface impediments (Clayton & Summerfelt, 2010; Hart et al., 2006; Kestemont et al., 2015; Trotter, Pankhurst, & Battaglione, 2005; Tsuji et al., 2016).

Regardless of the previous improvements made in understanding and improving initial SBI, the factors influencing this process are still poorly understood and there is much work to be done to further improve success in intensive culture. The problem is substantial enough, and the economic advantage of potential

solutions is large enough that initial SBI should be one of the main foci of future research when studying yellow perch for aquaculture or hatchery purposes.

It is likely that broodstock health and nutrition has a direct effect on the health and inflation rate of larval yellow perch although this has not yet been proven. In other species it has been shown a direct link between broodstock nutrition and larval health including SBI success proportions (Czesny et al., 2005). It also seems that the abiotic factors, particularly the air-water interface and photoperiod of the rearing environment play a crucial role in inflation success (Woolley & Qin, 2010). Therefore, in future research it makes sense to further manipulate and examine the role these factors have on the initial SBI of physoclistous fish in aquaculture situations.

Light and Development

Abiotic, environmental factors often play a crucial role in the early life history of fishes, and light is of no exception. The large majority of animals have evolved under the influence of the familiar natural day and night cycling. For many lifeforms, light is an essential part of their existence. The regular variation in photoperiod animals experience throughout time has given rise to a variety of unique patterns, processes, adaptations, and preferences both behavioral and physiological dictated by an internal circadian clock (Vallone et al., 2007). Fish are no exception to this phenomena. A considerable amount of research exists

investigating the role of light in fish biology, but the relationships between light and life forms are complex and there is still much we do not yet understand.

The effects of varying photoperiod on fish are well researched and have been shown to have strong relationships to the growth and survival of larval fish. Partly because fish are such a diverse group of vertebrates, the effects of varying photoperiod have a wide range of responses between different species. For example, an extended photoperiod has been shown to improve survival out of the larval phase in gilt-head bream *Sparus aurata* (Tandler & Helps, 1985), while in the same family *Sparidae*, larval Western Atlantic seabream *Archosargus rhomboidalis* exhibit a decline in survival under an extended photoperiod (Dowd & Houde, 1980). It is not safe to assume a species will perform a certain way based only on our knowledge of related species. This aspect of fish diversity is especially relevant in the aquaculture industry. For the most efficient methods of culture to be developed for a species, we must understand the biology as thoroughly as possible, and this often requires directed research on the species of interest.

Unsurprisingly, certain developments of percids are cued by changes in photoperiod. In the wild, yellow perch spawning occurs in spring, and gonadogenesis is triggered by changes in photoperiod and temperature. For captive culture purposes, yellow perch can be kept from reaching sexual maturity

by keeping the photothermal environment constant. Initial maturation can be triggered by changes in photoperiod regardless of temperature, but late maturation processes require a temperature change similar to natural changes (Shewmon, Godwin, Murashige, Daniels, & Losordo, 2007). In the closely related European perch, photoperiod variation impacts gametogenesis and spawning activity. Seasonal photoperiod changes significantly affect egg quality, spawning rate, and broodstock mortality in this species (Abdulfatah, Fontaine, Kestemont, Gardeur, & Marie, 2011; Migaud, Wang, Gardeur, & Fontaine, 2006). Similarly, in pikeperch it has been shown that a constant photoperiod can be used to suppress sexual maturation regardless of temperature changes (Ben Ammar et al., 2015; Sara Poursaeid, Falahatkar, Takami, & Efatpanah, 2012). While the reaction of these percids to photothermal changes are comparable, there are notable differences in the importance of either light or temperature in sexual development for each species. This again helps to illustrate the species specific research that is necessary in developing the most efficient culture methods.

Additionally, the growth rate of yellow perch varies with photoperiod. Shewmon et al. (2007) showed that adult yellow perch exhibited an increased growth rate under constant photoperiod when compared to a natural photoperiod, but this growth rate was more dependent on temperature than photoperiod. The greatest growth rates were observed under a constant photoperiod and a constant temperature of 23°C. Huh (1976) found that the growth of yellow perch was

higher when exposed to a photoperiod of 16 hours versus a photoperiod of 8 hours over a period of 14 weeks and, in contrast to Shewmon et al., that this growth rate was more dependent on photoperiod rather than temperature. It is thought that this increase in growth is linked to the delay in sexual maturation experienced under an unchanging photothermal regime since growth slows upon gonadogenesis. There is also speculation that the increase in growth under an extended photoperiod has to do with an increased amount of allowed foraging time.

Light and First Swim Bladder Inflation

In many species of fish, the presence of a circadian clock is involved in the development of a properly functioning swim bladder. The rate of successful initial SBI in larval striped bass *Morone saxatilis* increases with a shorter photoperiod which, like yellow perch, have temporary physostomous swim bladders (Martín-Robichaud & Peterson, 1998). The authors also noted that when kept under continuous light exposure, SBI was almost totally inhibited. This is a common trend with similar conditions observed in Yellowfin tuna *Thunnus albacares* (Partridge et al., 2011), striped trumpeter *Latris lineata* (Trotter, Battaglione, & Pankhurst, 2003), Pacific bluefin tuna *Thunnus orientalis* (Kurata, Tamura, Honryo, Ishibashi, & Sawada, 2017), Australian bass *Macquaria novemaculeata* (Battaglione & Talbot, 1990), snapper *Pagrus auratus* (Fielder, Bardsley, Allan, & Pankhurst, 2002), meagre *Argyrosomus regius*, (Vallés & Estévez, 2013) and

others. In contrast, SBI in chub *Leuciscus cephalus* and bleak *Alburnus alburnus* is higher in a continuous photoperiod than in a natural one. In European perch a continuous photoperiod has been shown to decrease the time before larval fish inflate their swim bladders when compared to a more natural photoperiod (Brüning, Hölker, & Wolter, 2011). Data on yellow perch SBI and light was found in only one case. This information was found second-hand in a culture guide (Kestemont et al., 2015), but the details of the experiment were not published in a peer-reviewed journal, and the authors could not be contacted for comment. The only reported incidence of SBI success percentages of larval yellow perch in aquaculture conditions under 24 hour light are significantly lower ($39.6 \pm 8.3\%$) than fish under a 12 hour light, 12 hour dark photoperiod ($60.7 \pm 14.5\%$) (Wojno, Kwasek, Dabrowski, & Wick, 2012).

Phototaxis

The length of the day and night cycle is only one facet of light that can influence an organism's development. Many lifeforms, fish included, exhibit a phototactic response. This response is characterized as being attracted towards (photopositive) or driven away from (photonegative) a light source by recognizing an intensity gradient.

Although it is commonly stated that yellow perch larvae are positively phototactic, the majority of evidence for this behavior seems to be anecdotal or taken from

first-hand observations. In a 1983 paper by Mancini et al. nighttime lights were used to remove larval fish from a pond successfully. Similar techniques are reported frequently in guides and websites. Perch begin life being positively phototactic, and as they age and their visual systems develop they become negatively phototactic. This is estimated to occur after 50 mm TL. This change in phototactic response is correlated with many other physiological and behavioral changes in yellow perch larvae and it is likely that at least some of these changes are directly related (Hart et al., 2006; Mancini, 1983).

There have been studies of the phototactic response of other percids, but since behavior can vary significantly within a family or even genus of fishes it is important to study specific species, especially when they are of interest for intensive aquaculture applications. The same can be said for research on the relationship of photoperiod and initial SBI in yellow perch. There is little more than anecdotal evidence that initial SBI in yellow perch is reduced under an extended photoperiod, but this is often stated as fact. Because of this many sources state that SBI in yellow perch happens at night or requires darkness to cue the development.

Research Objectives and Relevance

The main goal of this work is to further understand the biology, specifically early life development, of the yellow perch. There are significant interests in yellow

perch as a candidate for intensive aquaculture similar to salmon, catfish, and tilapia along with over a decade's worth of previous research on the subject. In order for this venture to succeed, the current low survivorship of perch out of the larval stage must be increased in order to reduce costs (Wetmore, 2011).

Currently, failed swim bladder inflation (SBI) plays a major role in early life stage mortality. While significant increases in SBI has been made by reducing surface obstructions, failed inflation persists, and the processes behind this development are poorly understood. It seems that abiotic factors, particularly the air-water interface and photoperiod, of the rearing environment play a crucial role in inflation success (Woolley & Qin, 2010). Therefore, it makes sense to further manipulate and examine the role these factors have on the initial SBI of physoclistous fish in aquaculture settings.

While many researchers have examined the role of unnatural photoperiods, the intensity of the light during these experiments often goes unreported. In the cases in which nighttime light intensity is reported or specifically looked at, it is far brighter than moonlight or any other nighttime illumination found in nature. This could be a crucial error in understanding the role of darkness in initial SBI, and it also prevents these studies from providing accurate information about the environmental relevance of moonlight on SBI.

The objective of this research is to examine the role of the phototactic response of larval yellow perch and low-level, night time illumination on the larva's initial SBI, growth, and survival. The short, photopositive phase of larval yellow perch ontogeny, and the temporary window of opportunity for initial SBI correlate with each other. Because of this, it is hypothesized that larval yellow perch rely on their phototactic response, and the presence of moonlight to locate the water's surface and inflate their swim bladders during the evening in the wild. If this hypothesis is correct, transferring this knowledge to culture conditions would provide a simple solution to help improve yellow perch survival to adulthood by increasing proportions of successful SBI.

Methods

Egg incubation

Yellow perch egg ribbons were obtained from the laboratory of Fred Binkowski at The University of Wisconsin-Milwaukee's School of Freshwater Sciences. All larvae for this experiment were sourced from the same egg ribbon which was spawned by hand ensuring the gametes were from only one female and one male. Multiple egg ribbons were incubated, and the best looking ribbon was used for the experiment based on the number of opaque eggs observed. The eggs were incubated in 22 L, clear, food-grade, plastic containers holding 20 L of water. The eggs were pinned to a plastic, elevated rack with zip ties to ensure proper water flow and oxygenation around the ribbons. The ribbons were

incubated using flow-through, dechlorinated water which began at a temperature of 10.5°C, and increased to a temperature of 17.9°C over a period of 12 days.

The eggs were treated with a 10% formalin solution (Dot Scientific Inc., Burton, MI) to help prevent fungus from forming. Formalin was dosed once daily, starting on the second day. The volume of formalin used was 6 ml for days two and three, 12 ml for days four through nine, back down to 6 ml on day ten, and omitted in the final two days thereafter before hatching. Because of the flow-through nature of the incubation tanks, the resulting concentration was 300 or 600 ppm, for 6 or 12 ml dosed respectively, at the moment of dosing which decreased rapidly with time. This procedure was done following the protocol used by Mr. Binkowski's lab. By the end of the 11th day numerous fish were seen hatching, and on the morning of the 12th day an acrylic rod was used to help break up the egg ribbon and assist the remaining unhatched fish in hatching.

Larvae Culture

After hatching, fish were removed from the incubation chambers manually by hand using a beaker. Larvae were counted in the beakers and 250 larvae were transferred into one of 12 respective experimental holding containers. The layout of the replicates and their holding containers can be seen in Figure 1. These containers consisted of two-gallon, black, HPDE buckets with an internal standpipe surrounded by a 300 micron screen. Each replicate bucket was

capable of holding 6 L of water. These tanks received flow-through, dechlorinated water at a rate of 8 L/hr from a spray nozzle oriented at 90° to the water's surface. The spraying of influent water was done to assist in breaking up and removing lipids and other floating particulates which can impede air-access and thus hinder swim bladder inflation of the larvae. The water temperature was raised from 17.9 to 20.0°C over the first two days in the experimental tanks and was left at 20.0°C for the 19 days of the experiment.

Feeding

The larvae were hand fed 100 ml of concentrated, saltwater rotifers (*Brachionus plicatilis*) by hand twice daily at 9:00 am and at 3:00 pm for all 19 days of the experiment. These rotifers were cultured in the lab, and fed a diet of RGcomplete algae paste (Reed Mariculture, San Jose, CA). Rotifers were visually confirmed to be in the experimental tanks at multiple points in the day to verify the continuous presence of food for the larvae. The concentration of rotifers in the experimental tank four hours after feeding was about 15 rotifers per ml. After two days, artemia nauplii (Artemac USA LLC, Ogden, UT) were added twice daily along with the rotifers. All nauplii were decapsulated, hatched and under 36 hours old when fed to larvae. A single feeding consisted of approximately 20,000 nauplii.

Lighting

Three low-level, nighttime illumination treatments were kept in separate sub-containers each containing four replicate populations in the previously mentioned two gallon buckets. These four replicates were separated by black, corrugated plastic sheets, and draped in black plastic sheeting to block out all residual light. All lighting was controlled by automated electronic timers. All replicates in each of the three treatments were lit for 14 hours during the day by a shared 50 lumen, 2700K LED driven at 12v. This resulted in a daytime light intensity of 80-100 lx at the water surface. For the nighttime light treatments, nighttime lights were turned immediately after the daytime light shut off and remained on for the remaining 10 hours of night until the daylight led again came on. The nighttime light was created with a 24 lumen, 4000K LED driven at 7.5v resulting in a nighttime light intensity of less than 1.0 lx at the water surface. This 14L:10D photoperiod was chosen due it being similar to the natural photoperiod that exists when yellow perch hatch in the wild during spring. The LEDs for the treatment in which nighttime illumination came from below the surface were encased in a clear resin enabling them to be submerged within the replicate buckets without seeing a change in intensity. This color temperature and intensity of the nighttime light are roughly analogous to that of natural moonlight, although the natural intensity is at most 0.3 lx (Kyba, Mohar, & Posch, 2017).

Mortalities and Recovery

Checks for mortalities occurred twice per day, in the morning and afternoon before feedings. Apparent mortalities were removed from the tank, counted, and preserved in 95% ethanol. A mortality curve was created by dividing the mean cumulative daily mortalities by the initially stocked 250 larvae and plotted against time.

Recovery is defined as the proportion all fish accounted for. This percentage was calculated by dividing the sum of mortalities plus fish which survived to the end of the experiment by the 250 fish initially stocked in the treatments. The mean recovery percentages was then calculated from the three replicates for each treatment before being compared. Recovery was calculated to address any missing fish and to ensure these fish do not impart a bias on the final SBI proportions and other statistical analyses.

Sampling

From one of the four replicates in each treatment, five randomly sampled larvae were removed, and euthanized with an overdose of buffered tricaine methanesulfonate (MS-222) (Western Chemical Inc., Ferndale, WA) at a concentration of 300 mg/L. These fish were then lit from beneath, and imaged with a dissecting microscope as per the recommendations for examining swim bladder inflation by (Barrows, Kindschi, & Zitzow, 1993). After inspection and measurement the carcasses were preserved in 95% ethanol.

Digital images of each fish were taken and their total length (TL) was measured using previously calibrated software (Motic Images Plus 2.0). Swim bladder inflation success was easily visually confirmed by looking for a prominent bubble above and just in front of the gut, in uninflated fish the swim bladder lumen could be observed as well. Only fish in which the SBI success was clearly obvious were counted as successful inflates. Questionable inflation status occurred infrequently, but when seen these fish were treated as uninflated.

Sampling was done to observe the time point at which the fish begin to inflate their swim bladders. These fish were measured to calculate a growth curve and the specific growth rates of each treatment to see if there is an effect of the treatments on the growth of the larvae. It is worth noting that this process was not replicated, and lacks any statistical power, but is used to anecdotally explain other observed effects. Because this one replicate was manipulated differently than the others, it cannot be considered a true replication. The surviving fish, mortalities, and sampled fish in this tank were not used in any other analyses other than in growth calculations and the timing of first observed inflation.

Growth Calculations

An exponential regression was employed to illustrate the differences in growth rates of sampled larval yellow perch total lengths (TL) over time (t) between

treatments. The specific growth ratio (SGR) was calculated for each of the three treatments using the following formula:

$$\text{SGR} = 100 * [(\ln \text{TL}_f - \ln \text{TL}_0) / t]$$

SGR traditionally uses weight to determine a growth rate of larval and juvenile fish since weight increases exponentially in young fish. In this situation, TL was used instead of weight. During our short sampling period TL grew exponentially, this is evidenced by the high coefficients of determination, and thus high degree of fit of an exponential curve.

Survivors

After 19 days the experiment was ended, and all surviving fish left were euthanized as above. The number of fish left at the end of the experiment was divided by the initial 250 larvae stocked, and this proportion was termed survival in all future mentions. Upon euthanasia, the larvae were imaged, TL was measured, and swim bladder inflation was visually confirmed as stated above. This data was recorded before the larvae carcasses were preserved in 95% ethanol.

Statistical Analysis

Separate contingency tables were used to analyze homogeneity of swim bladder inflation success proportions within each treatment to see whether or not the

replicate values could be combined. Due to the significant differences between replicates they were not combined and each replicate was treated as a sample.

An effect of the treatments on the means of recovery, survival, final TL, and the proportion of fish to successfully inflate their swim bladders was examined using a one-way Analysis of Variance (ANOVA). Differences at the 5% level were considered significant. In the single case in which a significant difference was found, pairwise comparisons of percent abundance between study sites were made using Tukey's Honest Significance Test.

In order to transform the proportions of fish with successfully inflated swim bladders into a normal distribution, the values were logit transformed as below (Warton & Hui, 2011):

$$\text{logit}(p) = \ln\left(\frac{p}{1-p}\right)$$

Where 'p' is the mean proportion of larvae with successfully inflated swim bladders within a replicate.

Analysis of covariance (ANCOVA) was used to compare the regressions using TL as the dependent variable, day post hatch (dph) as the covariate, and the different nighttime light treatments as the group variable.

Results

Survival and recovery

The term recovery is used to describe the proportion of fish known to be recovered as a mortality, or larvae which survived to the end of the experiment. The mean percent recovery for each of the treatments in which the low-level nighttime illumination source was above the water surface, below the water surface, and completely absent (from here on referred to as the “above”, “below”, and “control” treatments) was $87.7 \pm 4.3\%$, $78.9 \pm 9.9\%$, and $79.1 \pm 10.0\%$ respectively (Table 1). ANOVA indicates that there was no significant difference in the percent recovery between the treatments ($F_{2,6} = 1.161$, $p = 0.375$).

After 19 dph, yellow perch larvae survival ranged from 17.6% to 65.2%. Survivorship was highest in the “control” treatment ($59.6\% \pm 3.12\%$) followed by the “below” treatment ($54.8\% \pm 7.33\%$), and “above” ($46.4\% \pm 25.33\%$)(Table 1). It is worth noting the large standard deviation (25.33%) of the “above” treatment. This is mostly due to the poor survivorship of one replicate (17.6%) while the other two replicates were much higher (65.2% and 56.4%). There was no significant difference of survivorship between treatments when tested via ANOVA ($F_{2,6} = 0.599$, $p = 0.579$).

Cumulative mortality remained below 5% until the 10th dph in all treatments. On the 8th dph there was a notable increase in mortality seen in the “control” treatment. During this period, mortality remained low in the “above” and “below” treatments. The “above” and “below” treatments experienced a spike in mortality

from the 13th to 15th days post hatch which was not seen in the “control” treatment, although in the “control” mortality grew consistently over this time. Final cumulative mortality was 19.3%, 24.1% and 19.5% for the “above”, “below”, and “control” treatments respectively (Figure 2).

Final length and growth

The mean TL and standard deviation of all surviving fish at the end of the experiment was 8.261 ± 0.727 mm, 8.334 ± 0.838 mm, and 9.068 ± 0.825 mm for the “above”, “below”, and “control” nighttime illumination treatments respectively (Table 1); this difference was found to not be significant ($F_{2,6} = 3.101$, $p = 0.119$). The fish that were sampled daily were measured and the means of these lengths was plotted and can be seen in Figure 3. An exponential regression fit the growth data of sampled fish best with coefficients of determination of 0.86, 0.93 and 0.93 for the “above”, “below”, and “control” treatments respectively. Specific growth rate (SGR) over 19 dph was $1.54\% \text{ day}^{-1}$, $1.78\% \text{ day}^{-1}$, and $2.16\% \text{ day}^{-1}$ for the “above”, “below”, and “control” nighttime illumination treatments respectively (Table 1).

A One-way ANCOVA was used to determine a statistically significant difference between the “above”, “below”, and “control” treatments on length controlling for time. There is no significant effect of the nighttime light treatments on the TL of the larvae after controlling for time ($F_{2,265} = 0.457$, $p = 0.634$). The slope and

intercepts are determined to be indistinguishable, and thus the growth rates between treatments are considered to be the same.

Swim bladder inflation

Separate contingency tables were used for each treatment to examine whether the proportions of successful SBI were significantly different between replicates. The replicates in the nighttime illumination treatments were significantly different, $X^2(df = 2, N = 348) = 22.72, p < 0.05$ and $X^2(df = 2, N = 459) = 32.55, p < 0.05$ for “above” and “below” treatments respectively, and thus the results are considered to be non-homogeneous. Alternatively, the replicates of the “control” treatment were determined to not be significantly different from each other, $X^2(df = 2, N = 452) = 9.19, p > 0.05$, and the results from this treatment are considered to be homogeneous (Table 2).

Inflation was clearly visible as a large bubble just above and in front of the stomach under the dissecting light microscope. An example of an unsuccessfully inflated larvae versus a successfully inflated larvae can be seen in Figure 4. The mean proportion of surviving yellow perch larvae with successful SBI was highest in the “control” treatment where no nighttime illumination was present, $92.84 \pm 4.47\%$. The treatments with low-level, nighttime illumination had SBI success fractions of $79.89 \pm 11.82\%$ and $45.99 \pm 16.85\%$ for the “above” and “below” treatments respectively (Table 1). ANOVA indicated a significant difference ($F_{2,6} =$

11.02, $p < 0.01$) of SBI success proportions between the “control” and the “below” treatments (Figure 5).

Time of first inflation

When sampling fish daily, the first sighting of successful SBI occurred on the 10th dph in the “control” treatment where two of the five sampled fish had inflated their swim bladders. This occurred two days before any SBI was seen in either of the treatments with low-level, nighttime illumination. The “above” treatment showed the first inflation on the 12th dph and the “below” treatment showed the first inflation on the 15th dph (Figure 6).

Discussion

The fragile nature of yellow perch larvae during post hatch development is reflected in the low observed survival which never exceeded 66% and fell as low as 17.6% in one replicate (“above” treatment). This characteristic of the larvae resulted in large variations within treatments for example the standard deviation of mean survivorship in the “above” treatment was 21.5% and the standard deviation of the mean swim bladder inflation (SBI) success were 11.8% and 16.9% for the “above” and “below” treatments respectively. Further replication may have eliminated this problem, but limitations in space and the number of eggs per female prevent further replication from being feasible without introducing other confounding factors.

Clinging behavior was observed in living fish and it may have contributed to early life stage mortality. The transition from endogenous to exogenous feeding is known to be a critical point for larval perch survival, and clinging may prevent this. Early life stage perch have extremely limited energy stores. They cannot survive long without a sufficient amount of exogenous feeding, and death from starvation would occur after the yolk sac has been consumed, and is expected around the first week of life. (Withers, Sesterhenn, Foley, & Troy, 2015).

The amount of larvae unaccounted after collecting survivors, mortalities, and samples had a range of 19 to 79 animals. There are numerous possible causes for missing fish including rapid decay of the carcasses preventing enumeration, human error, and cannibalism. In the past, cannibalism has been observed of larval yellow perch in the wild, as well as in culture (Malison, 1999; Tarby, 1974). Cannibalism requires a large size difference, and the range in the size of survivors (6.33 – 12.04 mm) suggests that cannibalism most likely impacted the number of recovered fish and differences in final total length (TL) (Baras, Kestemont, & Mélard, 2003). It is important to note that the mean percent recovery and mean TL of survivors were not significantly different between treatments.

It is suggested that the photopositive behavior of the larvae was responsible for these results, but it is important to note that phototaxis was not directly observed in this experiment. The significant difference in inflation proportions between the “control” and “below” treatments is thought to be due to the larvae being attracted towards the light at the bottom of the tanks, preventing access to air, leaving them unable to successfully inflate their swim bladders. This idea is corroborated by the lack of a significant difference between inflation proportions in the “above” and “control” treatments. Furthermore, if the 4th replicate in which larvae were sampled daily is included in the final SBI proportions, the difference between the “above” and “below” treatments becomes significant while the lack of a significant difference in the “above” and “control” treatments remains. Regardless of whether this replicate is included or not, it is believed that this study, along with the successful use of light traps to catch larval perch, Mancini et al. (1983), and numerous anecdotal accounts of photopositive behaviors, provides more than enough evidence to confidently state that larval perch are photopositive at least up until 19 dph, and most likely longer.

It does not appear that yellow perch rely on this phototactic response to assist them in initially inflating their swim bladders. If the perch do use their photopositive reaction to help inflate their swim bladder, it would be expected that the proportion of inflation in the “above” group would be significantly higher than in the “control” and “below” groups, which was not the case. Because there was

no significant differences in survivorship between treatments, it can be said that failed SBI does not contribute to mortality in the first 19 days of life. This is consistent with the literature on the performance of yellow perch with failed SBI which suggests that failed SBI doesn't directly lead to mortality, but leads to a decrease in overall performance which, in many environments, may result in mortality (Czesny et al., 2005; Jacquemond, 2004).

Nighttime illumination reduces initial SBI success when compared to the "control" group, although this reduction is not necessarily significant. First SBI has been observed *in vivo* (Rieger, 1995; Rieger & Summerfelt, 1998), and these observations show that there is a period of rest before fish vigorously swim towards the surface in an effort to break the water's surface tension and gasp air. In the "above" treatment it is likely that the photopositive larvae swam towards to the light due to their photopositive response, and were near the top of the water column. This could possibly prevent inflation by exhausting them to the point that they couldn't generate enough burst speed to breach the surface. It could also possibly prevent inflation by having the larvae situated too close to the water's surface, making it impossible for the fish to build up enough velocity to breach by the time they reached the surface.

The role of phototaxis in reducing SBI agrees with previous studies on the colors of tank walls (Cobcroft, Shu-Chien, Kuah, Jaya-Ram, & Battaglione, 2012;

Downing & Litvak, 1999; Jentoft, Øxnevad, Aastveit, & Andersen, 2006; Tamazouzt, Chatain, & Fontaine, 2000). Perch have shown increased rates of inflation in black and gray tanks, as opposed to white tanks. The improvement seen in black and gray tanks has been attributed to the lack of incidental light reflecting off the tank's sides and bottom, as well as better contrast when visually detecting food items. Internal reflections may hinder inflation by attracting the larvae to the tank surfaces in a behavior termed clinging (Craig, 2000). Dark-walled tanks have been shown to increase proportions of successful first feeding, swim bladder inflation, and a reduction in clinging behavior (Hinshaw, 1985). This idea is supported by observations of Nickum (1978), who showed that larval walleye in dark tanks were less attracted to the sides of their tanks than when kept in light tanks. Reiger (1995) then showed that walleye attempting to inflate their swim bladders along the edges of a tank were less successful than larvae that penetrated the surface of open water, due to the meniscus of the water on the tanks edge preventing the larvae from breaching the water.

Because the difference in inflation proportions was significant between the "below" and "control" treatments, and the only difference in lighting was during the evening hours, there is the possibility that a significant amount of initial SBI occurs at night. This would make sense ecologically, since swimming towards the surface to inflate leaves the larvae particularly susceptible to predation. During

the evening, the chance of predation is lowered due to decreased visibility conferring an advantage to nighttime inflation (Milinski, 1993).

There is also an advantage of inflating during the evening since feeding is less frequent without light because perch are highly visual feeders (Hinshaw, 1985). By inflating and feeding at different times, an overlap of these behaviors is prevented, which in turn increases the time available for both activities. This idea is supported by the observations of Trotter et al. (2003), where swim-up behavior of striped trumpeter *Latris lineata* was observed more in the dark than during light periods. Additionally, sand whiting *Sillago ciliata* have been shown to initially inflate their swim bladders at night while feeding only during the day (Battaglione, McBride, & Talbot, 1994), and Australian bass reared under continuous darkness exhibited high instances of successful SBI, but poorer growth and survival (Battaglione & Talbot, 1990). While yellow perch may initially inflate during night, the data cannot prove this without a doubt since there are many possibilities as to the role of darkness in SBI success.

Alternatively, the suppression of SBI success in the “above” and “below” treatments may have to do with a lack of environmental cues for development. For many animals, a regular photoperiod is necessary to develop a normal circadian rhythm, which regulates other early life stage developments. It is possible that darkness is needed to trigger certain behaviors and developments

which enable the larvae to inflate, rather than darkness being necessary because the fish actually inflate at night.

In the closely related European perch *Perca fluviatilis* L., light of an intensity as low as 1 lx inhibits the production of the hormone melatonin, although it could not be shown that this level of light induces a stress response in the fish (Brüning, Hölker, Franke, Preuer, & Kloas, 2015). In the early stages of ontogenesis in gilthead sea bream, melatonin has been suggested to be more than a time-keeping hormone, and may play a role in early life development. The digestive tract was shown to be an important source of melatonin (Kalamariz et al., 2009). This is particularly interesting since development and changes in the digestive tract has an important role in allowing physostomous SBI.

The lag in the time in which first SBI was seen in treatments with nighttime light suggests that this illumination, even when very dim, plays a role in the suppression of first SBI. These results are consistent with previous studies on extended photoperiod and SBI (Migaud et al., 2006; Tandler & Helps, 1985; Trotter et al., 2003). These studies point to a compromise between periods of light and dark being needed for successful SBI to occur (Fielder et al., 2002). Another interesting aspect of this delay in inflation is that Brüning, Hölker, & Wolter (2011) showed that a 24 hour photoperiod, as opposed to a 12 hour light photoperiod, resulted in earlier SBI in the closely related European perch,. This is

the exact opposite of what was observed in this study and further highlights the requirement of species specific research.

The high percentage (93.0%) of fish to inflate in the “control” group is particularly interesting. The way this treatment was executed seems to have created an environment that favors inflation success more than should be expected. A replicate in the “control” treatment had a SBI success percentage of 96.6% (140 of 145 surviving fish) which is considered exceptional, and it is suggested that further research on improving this proportion is not necessary, but rather efforts should be taken to understand why this treatment performed so well. The density of fish and prey items, ensuring complete darkness at nighttime, and the spraying of influent water may have played a role in this success.

Because of the large variation between replicates, and the low number of replications within treatments, the proportions of successful SBI were not homogeneous, and the statistical power of the analyses is low. When comparing the proportions of SBI success, it is not possible to parse out whether the significant effects are due to the treatment variables or other random effects. This high variability in the performance and survival in the first weeks of life is frequently encountered when studying larval fish (Helvik et al., 2009). Because of this problem, effort was made to elucidate these random factors by examining differences in survival, recovery, and growth between the treatments. The lack of

significant difference in survivorship, mortality, recovery, growth rate, and final TL of the three treatments helps strengthen the argument that the significant difference seen in SBI success of the treatments is due to the independent variables rather than other factors, such as differences in feeding or stress responses.

There are significant implications of the knowledge that nighttime illumination suppresses swim bladder inflation. Many culture conditions, both indoor and outdoor may introduce unnatural nighttime illumination. There are accounts of farmers taking advantage of the photopositive response of yellow perch larvae in feed training, enumeration, and observation of larval fish at night without knowing that this practice could be hindering the successful development of their fish (Craig, 2000; Hart et al., 2006; Mancini, 1983).

Indoor culture facilities may have incidental light in the evening hours in the form of equipment screens or indicator lights, safety lighting, and others. While the impacts of these lights may seem negligible, it is shown here that even relatively dim light can result in a decrease in the fraction of fish with successful SBI, especially if incident light is able to be reflected on the interior of holding tanks. There is the possibility of larvae being attracted to such reflections resulting in the reduction of successful SBI fractions. Based on this research, the covering of

any nighttime light, no matter the brightness, is recommended along with the use of tanks with minimally reflective interior surfaces.

Because growth rates increase significantly under an extended photoperiod (Huh, 1976; Shewmon et al., 2007), there could be the temptation for farmers to apply this treatment early in life which, according to these results, could hinder final production. Uninflated fish have been shown to perform significantly worse than inflated conspecifics by many benchmarks, such as oxygen consumption, growth rate, ability to capture prey, and others (Czesny et al., 2005). SBI and proper food availability should be the main concerns in the first two to three weeks of life post hatch, and thus a 14 L: 10 D period is recommended, after which an extended photoperiod may be used in an effort to produce increased growth rates and facilitate feed training. Further research into the optimal photoperiod for larval development may help refine this suggestion.

Because the nighttime lights used were similar to that of natural moonlight during a full moon, these results can be interpreted in an ecological context. In the wild, the moon phase upon hatching and inflation date may account for incidences of failed SBI, but there is lack of significant differences between the “above” and “control” treatments that would suggest this effect, especially since natural waters often have some degree of turbidity that would reduce the penetration of any moonlight. In recent decades, man-made light emission has increased globally

between three and six percent per year (Hölker, Moss, Griefahn, Kloas, & Voigt, 2010). Nearshore, artificial lighting could negatively impact SBI in larval yellow perch, but this is unlikely since yellow perch are distributed exclusively offshore during early life stages (Post & McQueen, 1988).

Conclusion

In conclusion, these results provide experimental evidence that yellow perch larvae are positively phototactic under light of an intensity as low as 1 lux until at least 19 dph, but this behavior was not directly observed. It is possible that the larvae's phototactic behavior negatively affects swim bladder inflation if the light is below the surface, and possibly if a significant amount of light is reflected off of the internal surfaces of culture tanks, but this cannot be said definitively from the results of this experiment. Low intensity nighttime illumination was not shown to improve the successful swim bladder inflation in yellow perch. This type of light seems to delay the point of first inflation, and it may interfere with the development of a natural circadian rhythm. However, this type of nighttime light does not affect survival or growth.

Inflation success in the "control" group was exceptional, and efforts should be made to understand why these fish performed so well. If the results of SBI in the "control" group can be replicated, the majority of the inflation problem can be said to be solved. The lab from which the eggs for this experiment were obtained has

reported high inflation percentages using an oil boom to remove surface oils. It seems that the reduction of surface films largely solves the inflation problems. Regardless, this method is labor intensive, and it may not be feasible on a commercial scale. Thoughts on the future direction of this research and problems encountered when designing and executing this experiment can be found in the appendix.

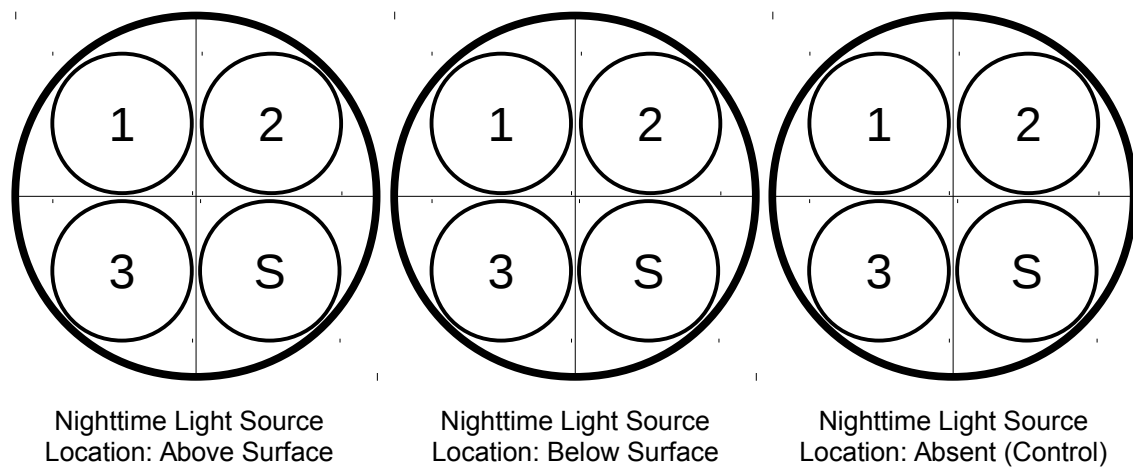


Figure 1: Experimental design. Replicates were placed into three “pods” sorted by treatment. Replicates within these pods were separated by tall pieces of corrugated plastic to prevent night light from bleeding between replicates. The entirety of the pods were draped in black plastic to prevent the influence of any residual outside light. Daytime lights were shared among pod replicates. Of the replicates, one (labeled “S”) had five larvae removed daily to chart growth and SBI over time. This pseudo-replicate was not pooled with any other replicates in any analyses.

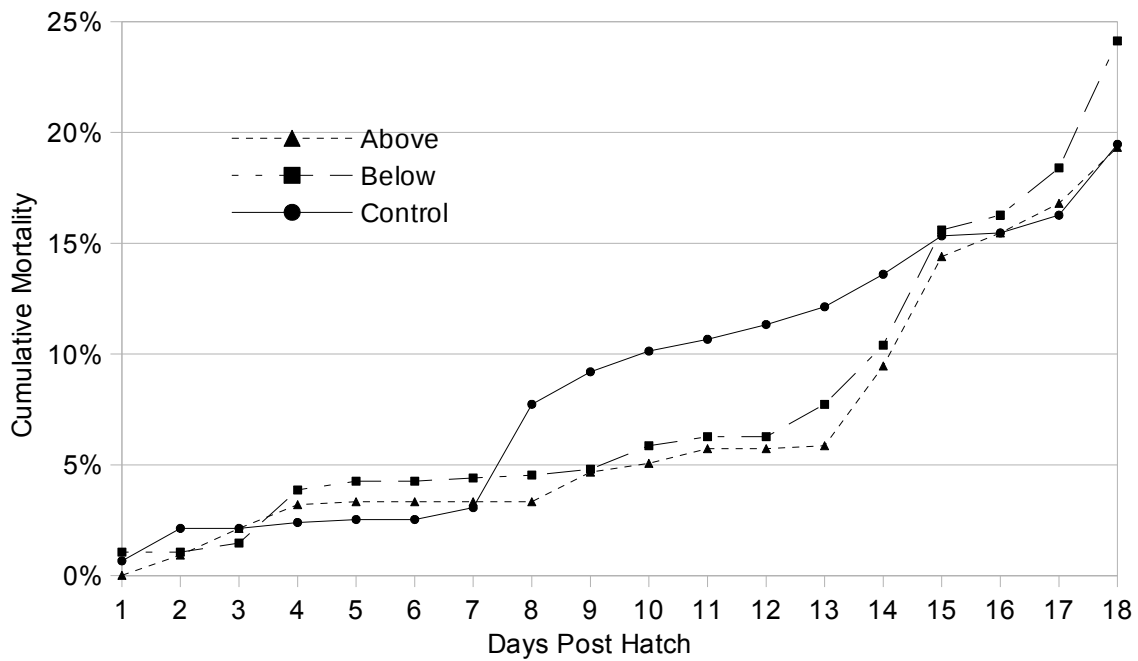


Figure 2: Cumulative mortality of larval yellow perch under different low-level nighttime light treatments of different source locations (above and below the water's surface, the control had no nighttime light) over 18 days after hatching.

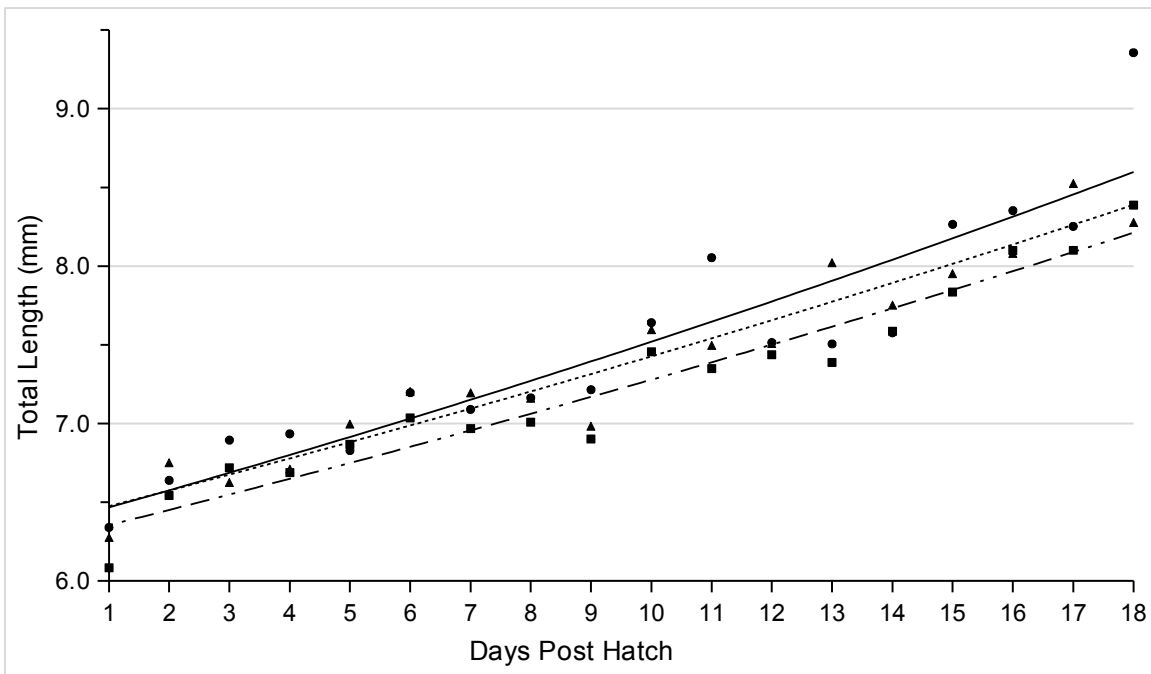


Figure 3: Mean total length of five larval yellow perch (*Perca flavescens*) sampled daily over 18 days post hatching from different low-level nighttime light treatments of different source locations (above and below the water's surface, the control had no nighttime light). Exponential regression lines are fitted to the data. ANCOVA revealed no significant effect of the treatments on the length of larvae after controlling for time, $F_{2,265} = 0.457$, $p = 0.634$.

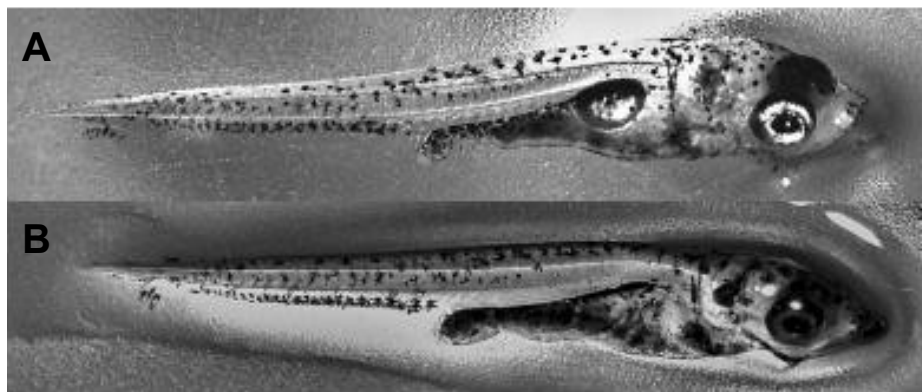


Figure 4: Images of larval yellow perch (*Perca flavescens*) 11dph showing successful (A) and unsuccessful (B) first swim bladder inflation. In 'A' the swim bladder can be seen to be inflated via the presence of a large bubble just above the gut while this feature is absent in 'B'.

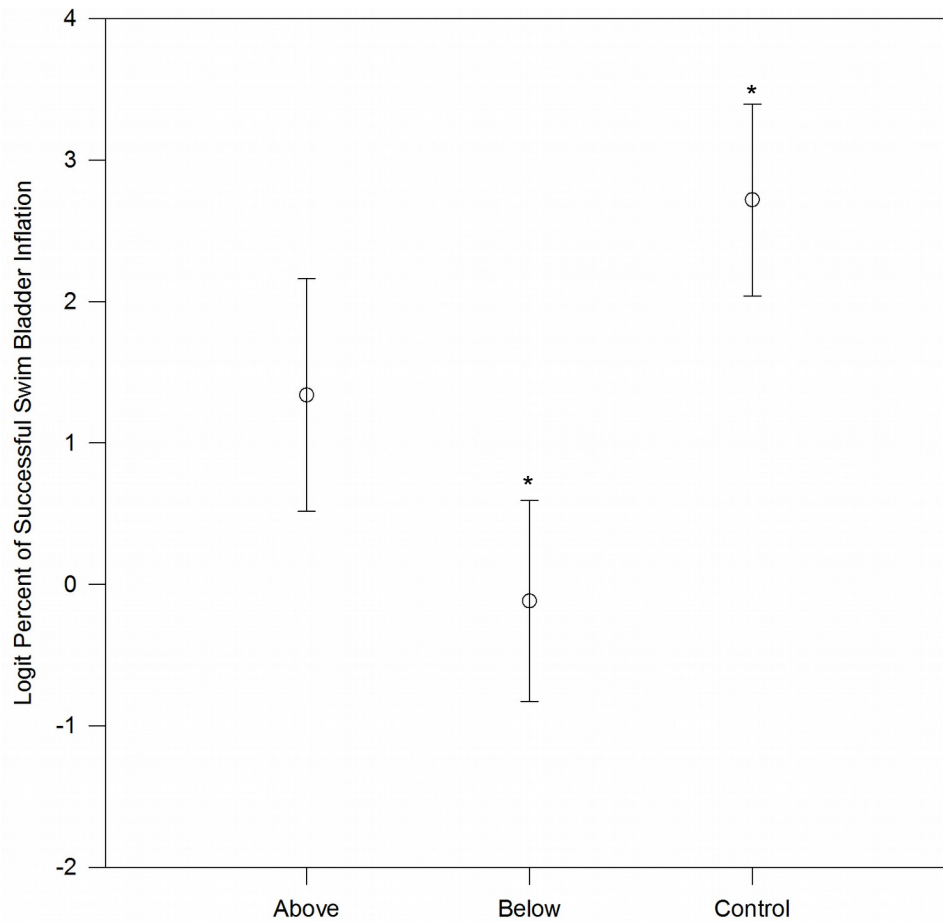


Figure 5: ANOVA of logit transformed mean percentages of successful swim bladder inflation of larval yellow perch (*Perca flavescens*) from different low-level nighttime light treatments of different source locations (above and below the water's surface, the control had no nighttime light). * indicates a significant difference, $F_{2,6} = 11.02$, $p < 0.01$.

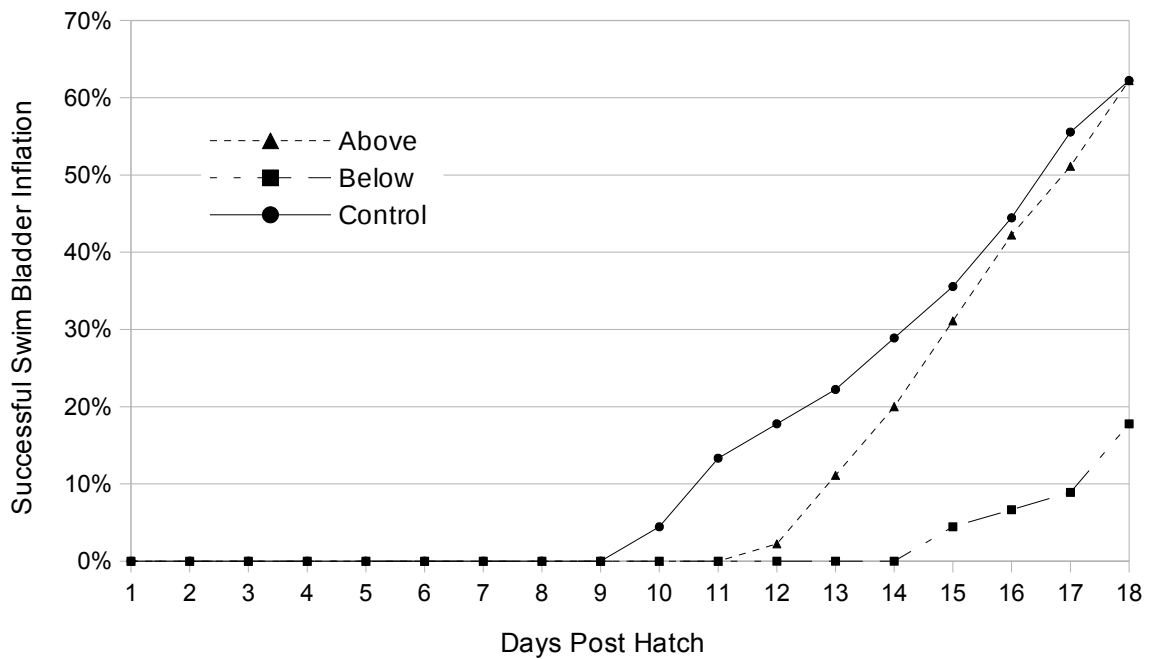


Figure 6: Cumulative percent of daily sampled larval yellow perch (*Perca flavescens*) with successfully inflated swim bladders from different low-level nighttime light treatments of different source locations (above and below the water's surface, the control had no nighttime light) over 18 days post hatching.

Table 1: Effects of low-level nighttime light of different source locations (above and below the water's surface) on survival, recovery, growth, and swim bladder inflation of yellow perch (*Perca flavescens*) until 19 dph. The control group had no nighttime lighting. (mean \pm S.D., n = 3). * indicates significantly different means within columns ($P < 0.05$).

Treatment	Survival (%)	Recovery (%)	Final total length (mm)	Specific Growth Rate (% day ⁻¹)	Swim bladder inflation (%)
"Above"	46.4 \pm 25.3	87.7 \pm 4.3	8.261 \pm 0.727	1.54	79.89 \pm 11.82
"Below"	54.8 \pm 7.3	78.9 \pm 9.9	8.334 \pm 0.838	1.78	45.99 \pm 16.85*
"Control"	59.6 \pm 3.1	79.1 \pm 10.0	9.068 \pm 0.825	2.16	92.84 \pm 4.47*
P-values (F-ratio)	0.579 (0.599)	0.375 (1.161)	0.119 (3.101)	-	0.009 (11.02)

Table 2: Results of contingency table analysis of successful swim bladder inflation proportions within low-level nighttime light treatments of different source locations (above and below the water’s surface, the control had no nighttime light). *indicates a significant difference ($P < 0.05$).

Treatment	X^2 Result
“Above”	$X^2(df = 2, N = 348) = 22.72^*, p < 0.05$
“Below”	$X^2(df = 2, N = 459) = 32.55^*, p < 0.05$
“Control”	$X^2(df = 2, N = 542) = 9.19, p > 0.05$

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Appendix: Future Research Suggestions

Future research on SBI may want to focus on improving and scaling up methods for surface film removal. Although effective methods for surface film removal have been developed, the literature of these methods suggests that they have only been used on small-size, experimental systems, and have not yet be tried on a commercial scale. For effective commercial yellow perch aquaculture to flourish, this development is absolutely necessary.

Outside of the inflation problem, this research suggests that future effort to improve survival through the first two weeks of life should be a priority. Mortality in all treatments exceeded 40% at the end of the experiment. The properties of lights used in culture should be examined further, and specifically light's role in the typical clinging behavior should be focused on as a factor preventing first exogenous feeding, but other factors may play a role in clinging behavior and this early life mortality spike.

If effort is taken to expand phototaxis research, evidence of the phototactic behavior should be gathered. Currently, the only information suggesting that yellow perch are photopositive is anecdotal or assumed from related behaviors, such as using light traps to capture larvae. Direct evidence is needed to positively confirm the existence of this behavior and would expand on its role in SBI or other behaviors. Using cameras to actually observe this photopositive

behavior would help to ground-truth previous studies on phototaxis in yellow perch. If further research is done on the clinging behavior observed in yellow perch, direct observations of phototaxis may also suggest that light plays a role in clinging.

Additional research on photoperiod in relation to SBI success would help further expand the understanding of the role that light plays in SBI. It is possible that the results seen in this experiment may have been due, in part, to the presence of an extended photoperiod. There is a significant amount of research on the role of photoperiod and SBI. Designing an experiment could be done easily by following previous studies such as those by Kurata et al., (2017); Martín-Robichaud & Peterson, (1998); Partridge et al., (2011); and Trotter et al., (2003).

Because this research was the first to examine SBI and phototaxis, there was a considerable amount of problems encountered when attempting to gather reliable data. This experiment took three attempts to run successfully, and anyone trying to expand on this research should take note of the mistakes made here.

At first, feeding was done by an automated dosing pump that delivered a food mixture hourly. This resulted in the tanks becoming dirty very quickly due to uneaten feed and the resulting fungal growth. The most success occurred when feeding was done by hand twice per day as described in the methods sections.

When doing this it was noted that excess food and fungus gathered on anything submerged in the tank. Because of this, it is necessary to ensure that all influent lines, dosing lines, and other materials are kept out of the tanks.

Consistency between treatments and replicates was a problem initially. It was important to ensure that the light from the “above” and “below” treatments were the same distance from the surface for consistency of intensity, this was not done in the first two trials. Additionally, sampled fish were initially taken from all tanks. This was changed in the final trial to only sampling from one replicate to prevent effects caused by this pseudo-random sampling.

Ideally, the statistical power of this experiment could have been improved by randomizing the placement of treatments within pods. That is, an “above”, “below”, and “control” replicate should have been placed together in the same pod. Currently, pods contained replicates from one entire treatment. By mixing replicates of treatments together, one could eliminate location or pod as a factor that may have influenced any statistically significant results. An increase in replicates or skipping the sampling replicate would also help improve the statistical power of this experiment.

Finally, when observing inflation it was necessary to look for an inflated swim bladder immediately after euthanasia when the fish are still transparent.

Originally, fish were preserved in formalin with the goal being to observe inflation by seeing which fish floated and sank as per the results of Barrows et al., (1993). Unfortunately, upon preserving the fish they all sank and became a foggy white color so that the actual SBI could not be seen. It could be possible to see inflation via x-ray or histology, but it seems that visual confirmation immediately after euthanasia is the most practical method.