THIAMINE DEFICIENCY IN BALTIC PIKE?

An experimental pilot study to assess effects of thiamine status on reproductive success, development and behavioural traits in northern pike

Niels Wehrens

HAS Green Academy
&
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Research Report

Applied Biology Research Internship

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In front of you is the finished product of half a year of challenging and exciting research with northern pike. It goes without saying that I would never have been able to finish this project properly without the help of a group of people around me. First of all, I'd like to start off with majorly thanking the lovely researchers from the Swedish University of Agricultural Sciences' Institute of Freshwater Research, first of which being my supervisor, Dr. Elin Dahlgren, for always trusting me to be able to finish this project and for her endless enthusiasm and supportive words. In addition to Elin, our research group consisted of Dr. Joacim Näslund and Dr. Caroline Ek, both of whom have been crucial to this project. Joacim's experience with practical fishkeeping and behavioural studies on fish, and Caroline's ability to always ask the right questions regarding planning and ethics that more often than once caused us to crucially evaluate our plans were all essential to this project. Additionally, my supervisor from HAS Green Academy, Dr. Thijs Frenken, also indubitably deserves a spot in this list of benefactors, as he was always available for comments and feedback on the process expected from my home university.

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With these, for me final, yet for you initial, words I would like to wish you a lot of reading pleasure with my internship report and, for whoever reads this, to invite them openly to contact me if they have any questions or comments about this study.

Niels Wehrens

Maastricht, July 2023

ABSTRACT

Northern pike (*Esox lucius)* is a keystone predator that is important to the Baltic Sea ecosystem. Due to this ecological position, it is crucial that recruitment of new individuals to a population remains at optimal levels. However, it is known from previous studies that Baltic pike appear to have issues with fry survivability and hatch rates, the latter of which has been reported by hatcheries in the Swedish Sankt Anna archipelago. Due to its ecological niche and trophic level, it was hypothesized that Baltic pike could be suffering from thiamine (vitamin B1) deficiency, similar to the M74 syndrome known to affect Baltic salmon (*Salmo salar)* and brown trout (*Salmo trutta)*. In order to quantify data regarding thiamine status in Baltic pike, 17 females were caught from a population near Stora Rimmö in the Sankt Anna archipelago, measured and weighed and had their egg's thiamine values measured in order to get an idea of thiamine levels. From these 17 females, six were subsampled and from these females, eggs were fertilized and hatched in a hatchery from which hatch rates were derived. Hatched fry were subdivided into four groups, a low control group (B1 < 5,0 nmol/g), high control group (B1 > 5,0 nmol/g), low treatment group (B1 <5,0 nmol/g; treated with 2,0 g/l B1) and high treatment group (B1 <5,0 nmol/g; treated with 4,0 g/l B1). Fry were, dependent on group, treated with thiamine hydrochloride and reared for 14 days, after which they were exposed to an open-field behavioural test in order to quantify energy levels in total distance traveled and meandering. Thirty days after hatching, all fry were euthanized and their length and weight were measured to quantify differences in morphology. Additionally, cannibalism was measured as a percentual value per treatment. No significant effects of treatment on open-field behaviour were found. Cannibalistic behaviour was however significantly higher in the low control group when compared to the high control group. Body length and weight were slightly higher in the low control group compared to the high control group. The obtained results did not support the hypothesis that thiamine deficiency and subsequent treatment were associated with low hatch and fry survival rates in this population of Baltic pike. As an alternative explanation, it is hypothesized that either the mothers collected in our relatively small sample were not suffering from thiamine deficiency, or Baltic pike do not suffer from deficiency in general. Additionally, it cannot be excluded that other environmental effects are the leading cause of low recruitment in Baltic Northern Pike. Follow up studies should thus focus on mapping the thiamine status of Baltic pike in the form of a widespread assay or monitoring program and look into different possible causes of low recruitment, such as food availability and ecotoxicological factors.

CONTENTS

1 INTRODUCTION

The Baltic Sea is a large brackish body of water, located in northern Europe, which has a unique mix of salty water from the Atlantic Ocean and fresh water from rivers draining into it. Water salinity ranges from 2-4 PSU in the north, up to 20 PSU in the south, which has led to an ecosystem with low species diversity as most species are either freshwater- or saltwater species, adapted to either one of the salinity extremes (Elmgren, 2001; Ojaveer et al., 2010). This ecosystem is under constant human and natural threat, due to environmental contaminants, eutrophication caused by human activity and its great biological variability like temperature and aforementioned salinity gradients (Elmgren, 1989). Due to these factors the trophic functioning of the Baltic Sea ecosystem is crucial, such as the controlling effect of high trophic level predators (Prato et al., 2013).

One of these high-level predators is the northern pike, *Esox lucius (*hereafter: pike), an anadromous piscivore living in cool-water habitats (Craig, 2008). It is known that pike can influence abundance and distribution of other species, and thereby influence species composition in the ecosystem community, making it a keystone species (Craig, 2008). Additionally, pike has a local population structure with limited movement, around a maximum of 70 km (Wennerström et al., 2017). Because of its ecosystem relevance and low dispersal rate, it is important that local populations of pike remain in good health and numbers. However, a decline in commercial and recreational catches over time, which is indicative of a decreasing population size, has been seen all over the Baltic Sea, ranging from 12% to 100% (Olsson et al., 2023). Research on adult survival rate has found that 84% of tagged adult pike survive over the course of around 10 months which suggests that mortality from fisheries and predators can be low, at least locally (Flink et al., 2023). This leads to the proposition that the reason for population decline might be on the opposite side of the pike's life cycle, mainly relating to its recruitment success. Personal communication with pike hatcheries from the area around Sankt Anna Archipelago (N° 58 03.45'; E° 16 07.05') supports this idea anecdotally, as they have had lower survival rates in fry raised by them compared to the 1990's (Karlsson, 2023).

Reproductive symptoms observed by these pike farmers include: egg brittleness, low fertilization percentage and low general hatch rate, fry with abnormal morphology described as "question-mark-shaped", and random mortality in yolk-sac feeding stages. Whilst outside the scope of this study, males have reportedly very low milt production, often only producing millilitres of milt. Additionally fry hatched in hatching setups that were originally seemingly healthy appeared to have trouble with consuming live zooplanktonic food and occasionally had low growth rates compared to fry of the same age. Looking at other Baltic species, these symptoms have an overlap with those of a reproductive disorder in another keystone species, the Baltic salmon (*Salmo salar*; Vuori et al., 2008). This disorder, often referred to as M74 syndrome, is characterized by low hatch rates, loss of movement coordination in young fry, apathy and lethargy with occasional outbursts of energy (Bengtsson, Hill, Bergman, et al., 1999). After these initial symptoms, fry growth slows down and fry exhibit anorexia, developmental stagnation and eventually death (Börjeson, 2023). Studies have shown that M74 syndrome can be induced by injecting yolk-feeding fry with pyrithiamine, a vitamin B1 (also known as thiamine) antagonist (Amcoff et al., 2002). Additionally, it is known that bathing yolk-feeding fry in a thiamine hydrochloride solution greatly reduces the occurrence of M74 syndrome in salmon fry hatched in hatcheries, from nearly 100% fatality rates down to normal mortality rates (Koski et al., 1999). Due to the overlap in symptoms between salmon and pike and their similar ecological position it can be hypothesized that northern pike in the Baltic could be suffering from a M74 syndrome-like reproductive disorder, possibly caused by low thiamine levels in female egg clutches.

Due to lack of previous M74-related data in pikes the aim of this study was mainly to get an overview on the status of thiamine levels in a single Baltic pike population and to evaluate whether thiamine bathing has an effect on quantifiable parameters regarding activity and developmental success in fry. The study hypothesis was that pike from the Sankt Anna Archipelago suffered from thiamine deficiency and that thiamin baths would mitigate deficiency symptoms in pike fry originating from females with the lowest egg thiamine levels. Specific predictions were that fry from low-thiamine females would be i) smaller in body size and ii) less active and meander more in their swimming path due to low body control. Additionally, a lower hatch rate of eggs from mothers lower in thiamine would be expected. Given effects of low thiamine levels, the thiamine baths were predicted to rescue any deficiency symptoms to normal levels. Two different thiamine concentrations were used for the baths, as no previous studies on such procedures in pike could be found to guide the experimental setup.

2 METHODOLOGY

2.1 PARENTAL PIKE SOURCING, HUSBANDRY AND MEASUREMENTS

Adult northern pike were caught using fyke nets from a Baltic population in the area of the Swedish Sankt Anna Archipelago (N° 58 41.5495'; E° 16 83.013'), hereafter St. Anna, in late April, after which they were separated by sex and kept in holding nets near Stora Rimmö (Figure 1). All fish were inspected daily for their roe or milt development by squeezing the belly near the gonadal region, wherein dispersion of eggs or milt the animal meant that the animal was deemed as spawning-ready. Once ready for spawning, female pike were taken from the water and dried around their urogenital opening after which eggs were stripped by pressing from the middle of the belly towards the tail of the animal. Eggs were squeezed into 500-ml plastic containers and subsequently covered with plastic film in order to keep them from drying out. The adult fish was weighed to the nearest 100 g and measured from snout to folded caudal fin tips in cm (Figure 2). Milt from males was harvested in the same way and collected in 50-ml glass tubes.

Figure 1 - Map showing collection location of adult pike used in this study and Sötvattenslaboratoriet, where collected eggs were hatched and reared.

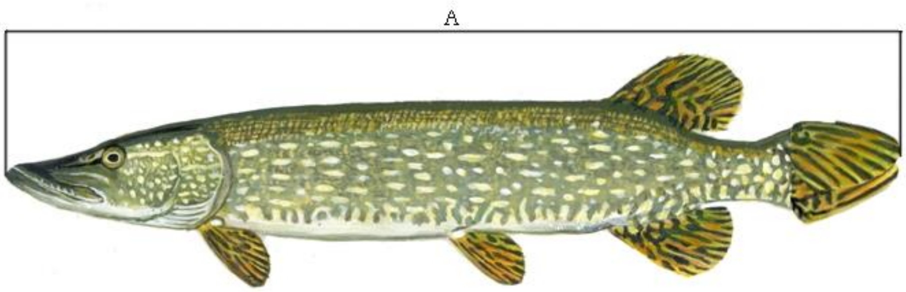

Figure 2 – Length measurement locations (snout; folded caudal fin tips) of adult pike (A). Amended illustration originally created by Beck (2008)

2.2 Egg Fertilisation, Handling and Hatching Process

Gametes collected from the adults were combined directly after collection and stirred gently over the course of a few minutes, using a soft feather, in order to fertilise the eggs. After fertilisation the eggs were moved into upside-down bottomless 1,5 litre PET-bottles, through which water flows upwards, where they were stirred with a feather every 5 minutes during an hour to negate clumping during their water-hardening process (Figure 3). Fertilised eggs were kept in these hatching bottles at St. Anna at a temperature of around 8 °C until they reached 72 degree days (degree days is a measurement used for hatching determined by the cumulative average temperatures over a number of days; a day with average temperature of 10 °C adds 10 degree days to this count). At this point eggs from 8 parents were transported to the hatching- and rearing location at the Institute of Freshwater Research using plastic bag transportation methods such as described by Fey and Greszkiewicz (2023), i.e. eggs being placed in a water filled plastic bag which is inflated using oxygen, keeping into account a density of less than 20% eggs per total water volume. At the Institute of Freshwater Research the eggs were placed into a hatching setup similar to that at St. Anna, with a water temperature of 10 °C and salinity of ca. 4,0 PSU (Figure 4). Circa 75% of the water was replaced daily with fresh water from the lake Mälaren. During this procedure, the eggs were also cleaned, by stirring the eggs with a feather and siphoning away dead or clumped eggs.

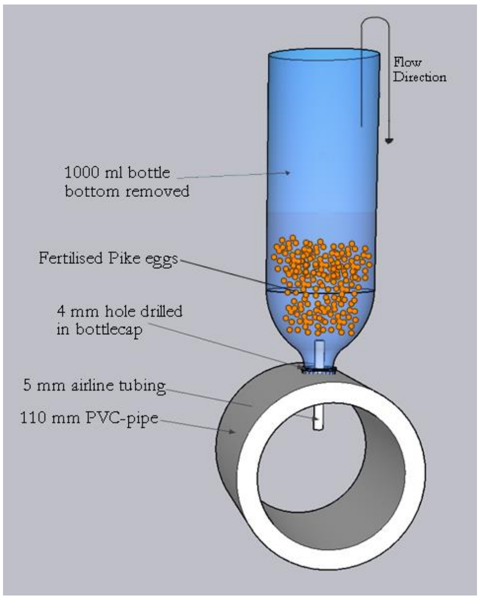

Figure 3 - Diagram of bottle in which eggs were hatched, with water direction flow and relevant measurements indicated.

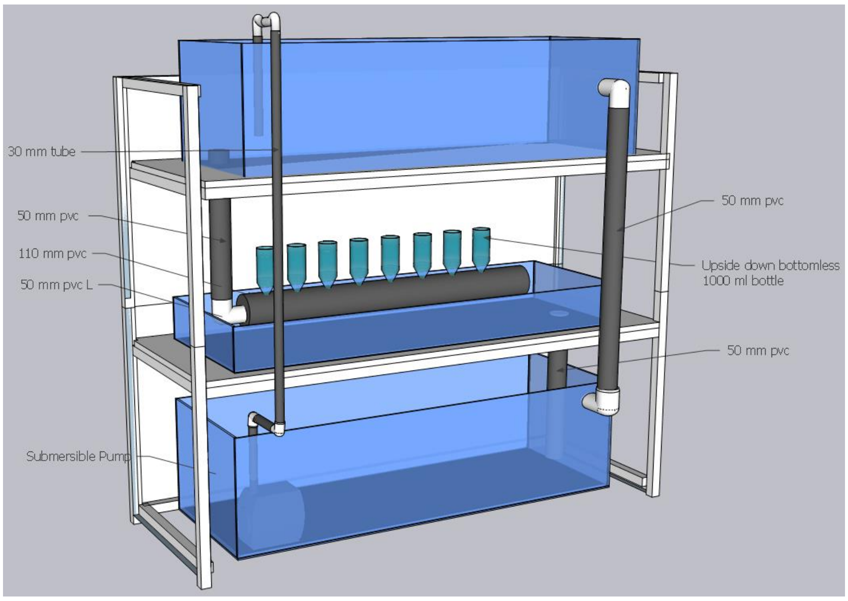

Figure 4 - Diagram showing hatchery setup that was used to hatch fertilised pike eggs. Water was pumped up from lower tank into uppermost tank from where it flowed through hatching bottles back into lower tank.

2.3 HATCHING AND HATCH RATES

Once eggs in the hatchery had reached ~110 degree days and larvae started hatching from the eggs, they were transferred to 24-l holding tanks, in each of which three low density polyethylene plastic stem plants were placed for the fry to attach to and absorb their yolk-sacs. Water circulation was provided using an air stone about halfway down in the water column (Figure 5). Water changes of 70% were performed daily and the tanks bottoms were cleaned of egg residue and dead fry.
A sample of fertilised eggs from all adult pike ($N = 17$) was kept at St. Anna and nurtured in the same way as the transported eggs to get hatch rates without transportation effects. From these samples a percentual hatch rate was derived by comparing the known amount of fertilised eggs to the eventual hatched fry.

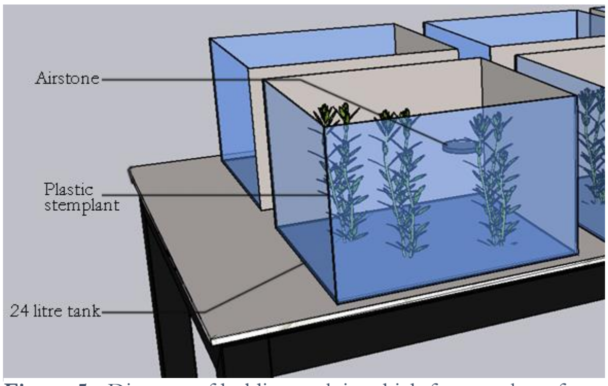

Figure 5 - Diagram of holding tank in which fry were kept for about 8 days to absorb their yolk sacs, with relevant elements indicated

2.4 REARING TANK SETUP

Eight days after the larvae were transferred to the holding tanks (once most fish in a holding tank were free-swimming) fry were culled to 60 similarly-sized individuals per tank. Due to a hatch rate of <1%, fry from two females were excluded in the study, leaving a total of 360 individuals from a total of six females. From this point until euthanization these fish were fed a surplus of artemia nauplii once daily. The artemia remained alive in the water column for a couple of hours post-introduction. The thiamine treatment was conducted by dividing the fry into four treatment groups treated with varying amounts of thiamine (see paragraph 2.5 below).

After treatment, fish were moved into rearing tanks placed in sets of four in each of three climate chambers (temperature: 15 ± 1,0 °C). The tanks were filled with 64 litres of lake water from the lake Mälaren. Tank water was filtered using sponge filters primed with water from Mälaren and water quality parameters (temperature, pH, ammonia, nitrite and nitrate concentration) were checked semi-weekly. Once weekly, a 30% water change was performed, using water from the lake Mälaren. A two cm deep substrate of sand with pebbles (<15 mm) was placed on the bottom of each tank, and 8 stems of artificial LDPE plastic plants were planted in the substrate to provide the pike fry with a structurally enriched environment to provide shelter, promote cognitive development, and reduce aggression (Näslund and Johnsson, 2016). All tanks were illuminated using 20 watt, 5000K, lightbulb fixtures on an automated 14:10 day-night schedule. Tanks were covered using lids made of fine netting, allowing for gas exchange (Figure 6).

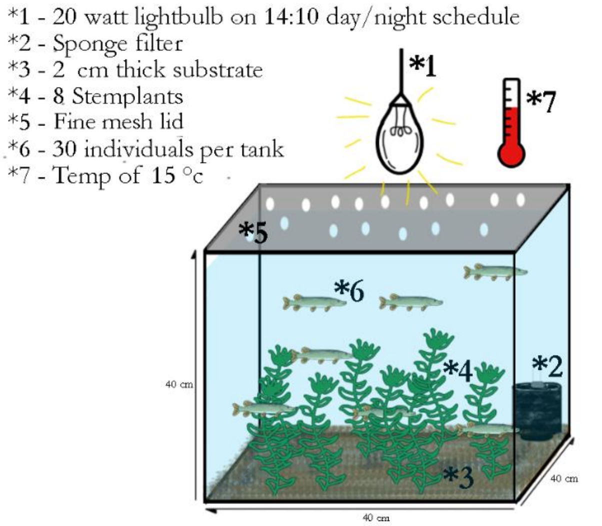

Figure 6 - Diagram of a rearing tank used in this study with relevant aspects indicated. Amount of drawn fry in the diagram is only for illustration and does not indicate actual amounts.

2.5 THIAMINE MEASUREMENTS AND TREATMENTS

Unfertilised egg samples from all 17 female pike caught at St. Anna were immediately frozen at -18 °C after collection. The samples were subsequently analysed at Stockholm University, for free thiamine (hereafter: thiamine) concentration (nmol/gram of eggs) using spectrophotometry methods developed at Stockholm University.

Fry from the fish hatched at the SLU lab were divided into a "low" or "high" subgroup based on natural thiamine level, where eggs below 5,0 nmol/g made up the lower group and eggs above 5,0 nmol/g made up the higher group. From each of these subgroups, 90 fry were taken to form "low control" (C_L) and "high control" (C_H) groups. Additionally, 90 of the fry from the naturally "low" group were treated with a thiamine hydrochloride solution of 2 g/l (LT) and another 90 were treated with a thiamine hydrochloride solution of 4 g/l (HT). The thiamine treatment was carried out eight days after hatching, by moving the fry according to treatment group into an aquarium with two litres of the treatment solution, where the animals were left to absorb the thiamine hydrochloride solution for an hour. During this process oxygen saturation of the water was ascertained using an air pump, and fish health and stress levels were closely monitored. (See 4.1 for details on which group (C_L, C_H, LT, HT) originated from which mothers).

2.6 BEHAVIOUR

An open-field test of general locomotive behaviour was performed 14 days after introduction to rearing tanks, 30 fish from each treatment group (C_L, C_H, LT, HT) were placed separately in 8 aquaria (11 cm × 11 cm × 11 cm, one opaque side), filled with 200 ml of water from the lake Mälaren. Tanks were illuminated from above using a 20 watt diffused LED lightbar and filmed from a height of ca. 30 cm using a Logitech C270 webcam (Figure 7). Fish were collected from their rearing tanks using fine-mesh nets, transported in 50 ml of water in a 100 ml beaker and introduced to an aquarium following a randomly generated schedule, after which they were given five minutes to acclimate to the new environment without the operational researcher in the room. Five minutes after introduction the researcher entered the room and initiated recording the video feed showing exploration and movement behaviour of the fry, using the webcams and Open Broadcaster Software (version 29.1.2; Open Broadcaster Software | OBS, 2023). Fish were then filmed for ten minutes after which they were removed from the aquariums and moved to a temporary holding tank in order to avoid repeated recordings of the same individuals. Video files were analysed using AnimalTA (Chiara et al., 2023), from which total distance travelled and meander were obtained. Fish length was measured using ImageJ (version 1.54d; Schneider et al., 2012), to test for effects of size on behaviour. For specifics on workflow and processing, see appendix 1.

Additionally, cannibalistic behaviour in the rearing tanks was quantified by subtracting the final number of living fish from the starting number of fish corrected for natural deaths. This gave a total percentage of cannibalised fish, however held no information on how many cannibalistic individuals were present per treatment.

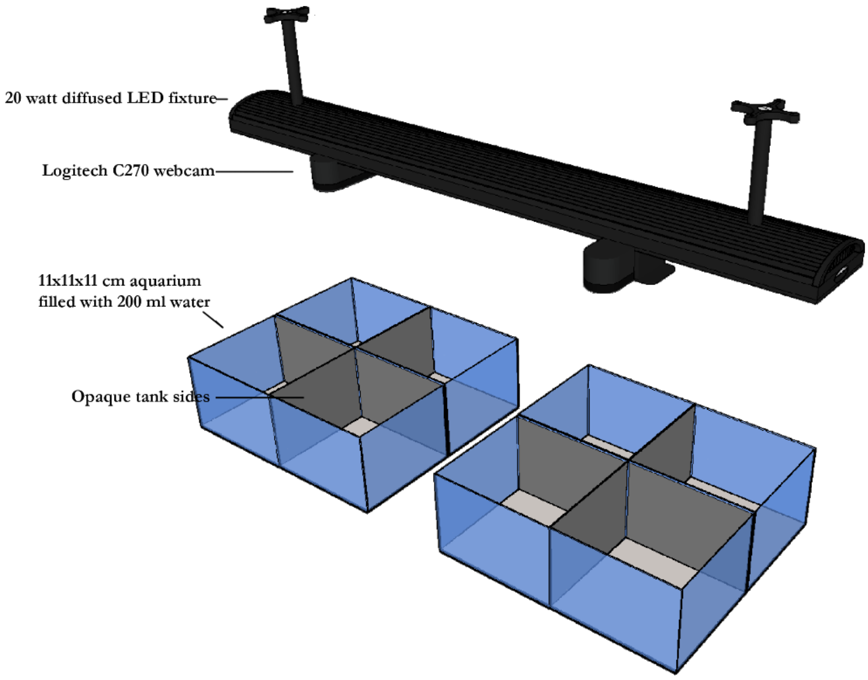

Figure 7 - Diagram showing behavioural recording setup with relevant aspects annotated.

2.7 MORPHOLOGY

On the 21st day after introduction into the rearing tanks all fish were removed from the aquariums using a nylon net and sedated using a 0,15 g/l MS222 (Tricaine Methanesulfonate) solution, after which they were euthanised using a blow to the head. The fry were subsequently individually blotted dry and placed onto mm-paper where a picture was taken from above using a Canon EOS 1100D camera (lens: 55 mm; aperture: *f*/8; ISO: 400) mounted 40 cm above the subject (Figure 8). After photographing, the fish was weighed to the nearest 0,0001g and frozen in a 5 ml microcentrifuge tube at -80 °C. Afterwards all fry were measured digitally, using the photographs taken, to the nearest 0,1 mm, from mouth to fork of caudal fin, using ImageJ (Figure 9).

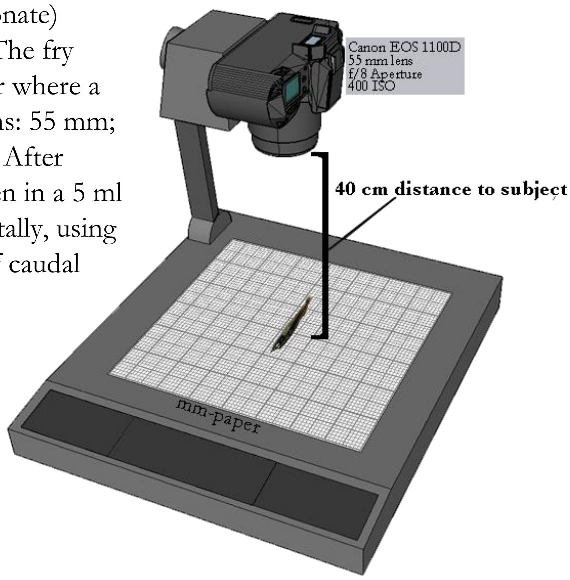

Figure 8 - Diagram showing measurement setup used to measure post-euthanisation fry length.

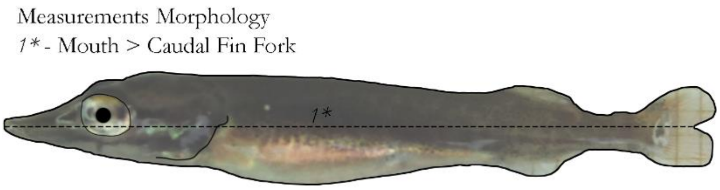

Figure 9 - Fry measurement landmark points (1*, mouth; caudal fin fork)

2.8 DATA PROCESSING AND STATISTICS

2.8.1 Hatch rate

Hatch rate was analysed using a linear model on which a type-III ANOVA was used to test for effects of egg thiamine value on hatching success.

2.8.2 Behaviour

The raw data from the behavioural study, distance travelled and meander, was first analysed visually using AnimalTA's built in tracking path visualiser. If any sections of a movement track were missing, then they were manually interpolated using AnimalTA's interpolation tool, assuming that the animal moved at a steady speed between the missing points. Total distance travelled and meander was also taken from a non-moving animal and this value was subtracted from all other runs, to account for noise in the tracking paths, which adds to the track lengths. After fixing the data, a visual analysis of data normality was performed on a histogram, from which was derived that data was following a leptokurtic distribution. An appropriate data transformation formula was derived using the R-package MASS's Box-Cox function, which indicated that an inverse square-root transformation would be optimal. This transformation was therefore applied to the data. Initial linear models including body length as a covariate were initially used to test for body size effects on behavioural variables (since larger fish sometimes move more than smaller fish in early developmental stages; see e.g. Näslund & Johnsson (2016b)). For distance travelled, body length was found to have a non-significant effect and was therefore excluded from the model. Hence, a type-III ANOVA was used to test for treatment effects on distance travelled. Length affected meander significantly, so it was incorporated into the linear model on meandering.

Cannibalism percentages were recalculated into integers by dividing the percentual data with 100 and then modelled using a beta-regression, after which a type-III ANOVA was used to test for treatment effects on the amount of cannibalism.

2.8.3 Morphology

Data on length and weight was analysed visually for normality and $\log_{10}$ transformed to account for a non-normal distribution, after which a type-III ANOVA was used to test for treatment effects on length and weight.

2.9 ETHICS

Experiments were conducted in accordance with national guidelines for animal care and used procedures were reviewed and approved by the regional ethical review board in Uppsala (Dnr 5.8.18-03489/2023). For all experiments the minimal number of animals was used whilst still guaranteeing viable and statistically powerful results. Animals were euthanized using methods that ensure the least amount of suffering and unwellness, after which they were frozen and kept in freezers for any follow-up studies. If any behaviour was observed that indicates bad general wellbeing, such as gasping for air, excessive cannibalism, unnatural swimming etc. that was not correctable on a day-to-day scale the choice was made to euthanize and exclude those animals from the study (Appendix 2).

3 RESULTS

3.1 FEMALE MORPHOLOGY AND EGG THIAMINE VALUES

The lowest egg thiamine value found in the sample of maternal female pikes collected in St. Anna was 3,6 nmol/g, the highest 8,9 nmol/g. (Table 1). The largest female was 5,0 kg and 80,0 cm, the smallest female was 0,70 kg and 45,0 cm.

Table 1 - Female length and weight measured during egg collection. Egg thiamine value per mother in nmol/g. Mothers from which hatched fry were used in this study denoted under identifier code.

Identifier Code	Female Weight (kg)	Female Length (cm)	Eggs Thiamine Level (nmol/g)
01	3,30	73,5	4,7
02	0,90	47,5	5,1
03	1,50	61,0	5,7
04	1,40	58,0	8,0
05	5,00	80,0	5,4
06 C_H	**2,00**	**63,0**	**6,7**
07	1,10	49,0	6,6
08 $C_{L; LT; HT}$	**1,00**	**54,0**	**4,9**
09 $C_{L; LT; HT}$	**2,80**	**72,0**	**3,6**
10 $C_{L; LT; HT}$	**3,40**	**75,0**	**4,3**
11 $C_{L; LT; HT}$	**1,10**	**56,0**	**4,6**
12 C_H	**0,90**	**50,0**	**7,9**
13	0,85	49,0	5,7
14	1,60	65,0	8,9
15	1,00	55,0	5,3
16	0,70	45,0	5,0
17	1,70	60,0	7,3

Bold: Females from which eggs were used in study

C_L: Low control group; **C_H**: High control group; LT: Low treated group (2g/l); HT: High treated group (4g/l)

3.2 HATCH RATES

Hatch rates (%) varied between 2 and 80 % and were slightly lower on average if female egg thiamine values (nmol/g) were higher (Figure 10). However, the observed effect was very small and not significant (P = 0.746; R^2 = -0,06)

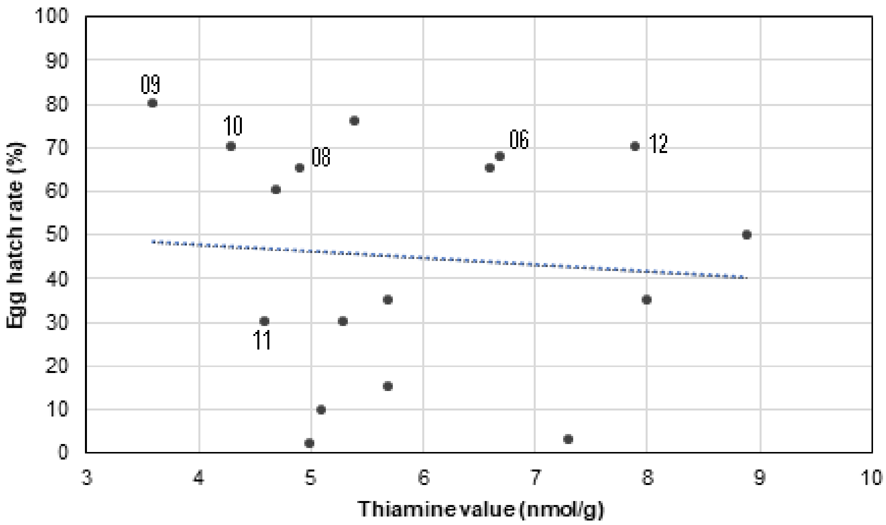

Figure 10 - Egg hatch rate (%) as a factor of thiamine value (nmol/g). Dotted line indicates linear trend. Females from which fry was used in this study are numbered with their identifier code.

3.3 Fry Morphology

The mean length of fry differed significantly between the low and high control groups (F = 3,67, P = 0,013)) with fry from the lower control group (27,8 ± 0,572 mm) being longer than those from the high control (25,6 ± 0,452 mm; Figure 11a). The thiamine treated groups (LT: 27,0 ± 0,495 mm; HT: 26,2 ± 0,441 mm) did not differ significantly, neither reciprocally nor with the control groups (C_L-LT: t = 1,16, P = 0,653; C_L-HT: t = 2,35, P = 0,093; C_H-LT: t = -2,04, P = 0,178; C_H-HT: t = -0,89, P = 0,812; LT-HT: t = 1,21, P = 0,6209). Mean weight also differed significantly between the low and high control (F = 3,07, P = 0,029) as fry from the low control group (0,150 ± 0,0102 g) were heavier than those from the high control group (0,113 ± 0,009 g; Figure 11b). The thiamine treated groups did not significantly differ with either the other treated group or the control groups (C_L-LT: t = 1,16, P = 0,653; C_L-HT: t = 2,35, P = 0,093; C_H-LT: t = -2,04, P = 0,178; C_H-HT: t = -0,89, P = 0,812; LT-HT: t = 1,21, P = 0,621).

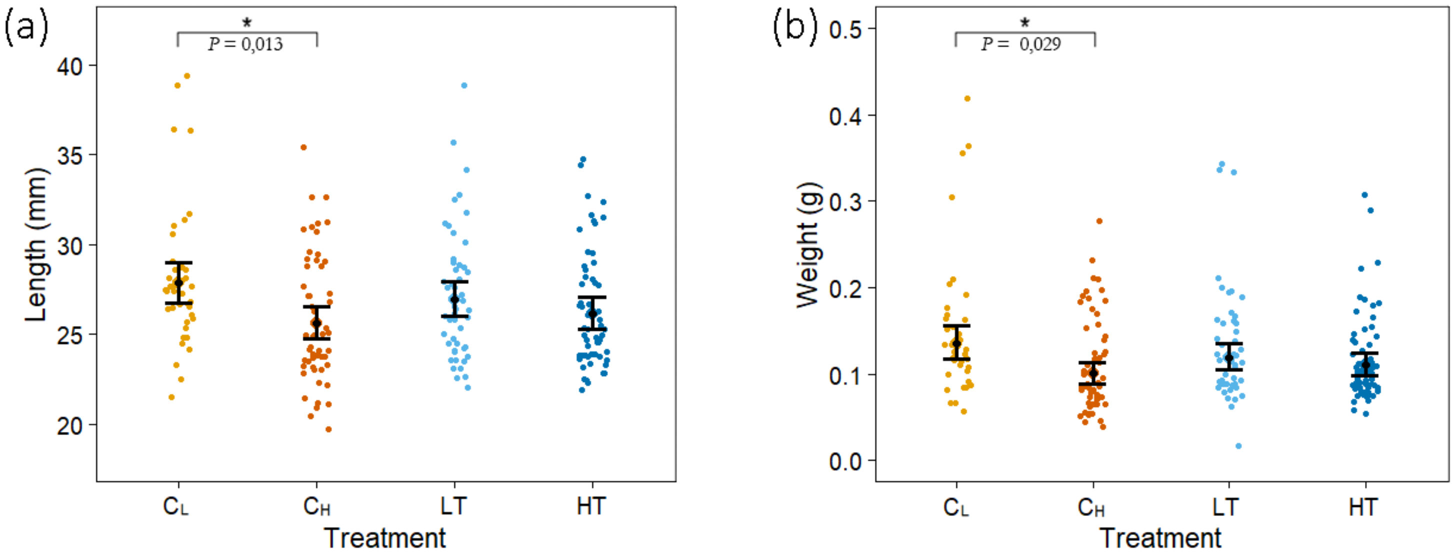

Figure 11 - Fry morphology with (a) as length in mm and (b) as weight in grams. C_L, low control group; C_H, high control group; LT, low treatment group (2g B1/l); HT, high treatment group (4g B1/l). Asterisk denotes significant difference compared to other groups (P value denoted). Error bars show 95% confidence interval with the mean denoted as a black dot.

3.4 Fry Behaviour

Distance moved of fry in an open field behavioural test did not differ significantly between control groups, nor did thiamine treatment have any significant effect on distance moved (F = 0,58, P = 0,628; Figure 12). Meandering was significantly affected by fish length (t = 5,53, P = < 0,001) however there were no significant differences between control groups and treated groups (F = 0,92, P = 0,432; Figure 13).

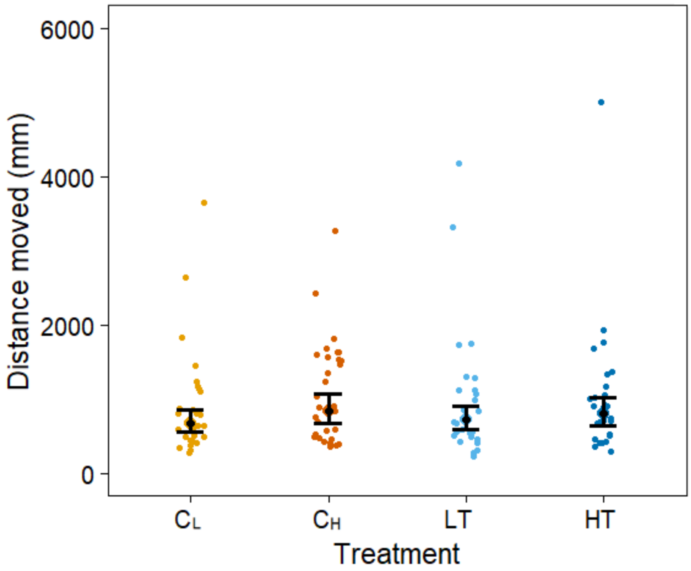

Figure 12- Distance moved of fry in an open field behavioural test C_L, low control group; C_H, high control group; LT, low treatment group (2g B1/l); HT, high treatment group (4g B1/l). Error bars show 95% confidence interval with mean denoted as black dot.

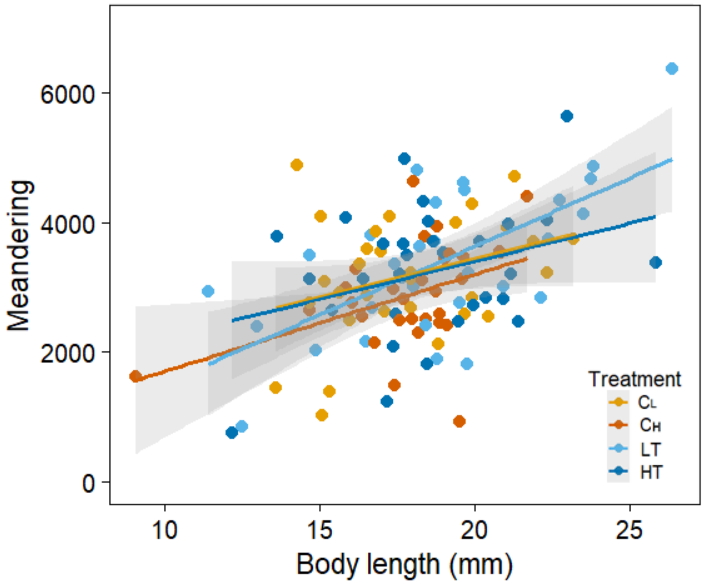

Figure 13 - Linear model plotting meandering during an open field behavioural test as a factor of body length (mm). C_L, low control group; C_H, high control group; LT, low treatment group (2g B1/l); HT, high treatment group (4g B1/l). Gray highlight around slopes shows 95% confidence interval.

3.5 Cannibalism

Percentage of cannibalism was significantly different between the low control group (55,1 ± 0, 710 %) and high control group (27,6 ± 0,630 %), with more perceived cannibalism in the low control group (*z-ratio* = 2,919, P = 0,016). Other groups did not have any significant differences in cannibalism percentages (Figure 14).

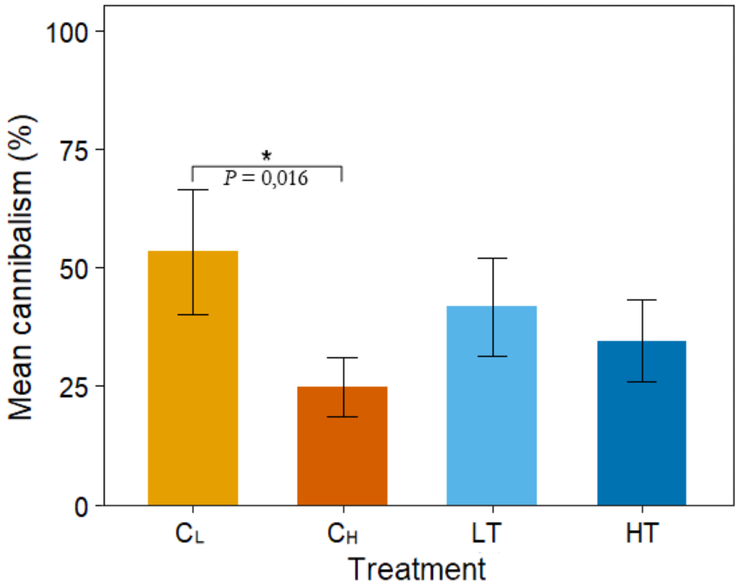

Figure 14 – Mean cannibalism in percentages. C_L, low control group; C_H, high control group; LT, low treatment group (2g B1/l); HT, high treatment group (4g B1/l). Asterisk denotes significant difference compared to other groups (P value denoted). Error bars indicate 95% confidence interval.

4 DISCUSSION & CONCLUSION

4.1 DISCUSSION

In this study, effects of natural thiamine content and thiamine dosing during yolk-sac stage on northern pike fry were investigated. These fry were hatched from eggs obtained from six mothers which were randomly selected from a subsample of 17 females. The lowest found thiamine value in the unfertilized eggs used in this study was 3,6 nmol/g, the highest being 7,9; a subsample representing the whole group relatively well. It is however important to note that it is currently unknown whether the 3,6 nmol/g of thiamine found is actually indicative of acute thiamine deficiency in pike, as no previous studies have been done on thiamine content in pike eggs. When compared to other species of which this information is known, the values found most likely do not correspond with actual deficiency, just lower levels that could be expected in a population (Appendix 3). In the Great Lakes (which are comparable to the Baltic Sea in terms of geographic size, low species diversity, and ecological problems; (Regier et al., 1988), the threshold value for deficiency in salmonids is for example 1,0 nmol/g (Bengtsson, Hill, & Nellbring, 1999; Fisher et al., 1995, 1996). If deficiency levels are comparable among species, this could either mean that Baltic pike do not suffer from thiamine deficiency in the Baltic Sea, or that the relatively small sample taken from the selected population does not contain any deficient females. Further thiamine sampling and monitoring from a wider range of Baltic populations would be recommended to be able to properly chart the thiamine levels expected from northern pike and screen for any females that can be undoubtedly classified as deficient. It is also relevant to mention that thiamine deficiency in salmonids has been shown to follow an interannual trend, where some years have higher counts of deficiency than others, meaning that any samplings should be repeated over multiple years (Amosova et al., 2021; Wolgamood et al., 2011).

Only slight significant differences regarding morphology (length and weight) and cannibalism were found, with fry from the low control group being slightly longer than those from the higher control group. Cannibalism was higher in the low control group compared to the higher control group which is the most likely explanation for the morphological differences, as cannibalistic pike fry tend to grow more rapidly than those feeding on *Artemia nauplii* (Giles et al., 1986). Differences in cannibalism between the low control and high control might be explicable by uneven growth caused by nutrient deficiency in the low control group, early in the growing phase. This would lead to runts being consumed by some of their faster growing peers, a well-known occurrence in pike fry (Bry et al., 1992). If this were to be studied further, a size measurement every few days would be recommended and fish that are in the cannibalistic window (> 1,5 x larger than peers) should not be removed methodically like in this case.

No significant differences in an open-field behavioural test were observed, meaning that fry moved in similar ways in all experimental groups. Expected differences in vitality post-treatment were not directly perceived in the behavioural tests, which can either be because treating with thiamine does not affect pike fry in the same way as it does salmonids, or because any expected post-treatment increase in energy levels is shorter lived (Nicole, 2022). Follow up studies could include an open-field test in an earlier part of the growing stage shortly after treatment with thiamine, to see whether there are any short-term effects on behaviour.

Even though no direct effects of thiamine treatment have been observed that could support the hypothesis that there is widespread thiamine deficiency in Baltic pike, this does not take away from the low recruitment rates and hatchery success in regions of the Baltic Sea (Nilsson et al., 2004). Hypotheses regarding these issues range from egg predation and post-hatch food deficiency to environmental toxins affecting fry health and gamete production (Nikolić et al., 2021; Nilsson, 2006; Nilsson et al., 2004). Whilst wild pike eggs might be predated predominantly by sticklebacks, another known issue in the Baltic Sea, this does not explain low survival rates and small sizes of females in a hatchery environment (Bergström et al., 2015). Although environmental toxins are very broad, one of the issues affecting adult pike might be caused by algal toxins, as harmful algal blooms have been on the rise globally due to warming (Luckas et al., 2005). Previous experiments, such as those done by Nilsson et al. (2004) looked at local effects of algae when hatching pike fry, and found that, whilst minor, presence of diatom algae appears to have a negative effect on fry hatch rates. These experiments did, however, not include a high variety of algae, nor did they include stressed or sub-optimally growing algae. Lack of knowledge on basic functioning of different Baltic Sea algal systems means that it is uncertain whether Baltic algae, during an algae bloom, are in a stressed state and could thus be producing harmful agents (Jamers et al., 2009; Luckas et al., 2005). Future studies could include an assay for algal toxins in eggs, including naturally produced polybrominated dibenzo-*p*-dioxins, as effects of *dioxins* on fish health and gamete production have been described in previous studies, with trout fry being sensitive to as low as 0,034 μg/kg (Haglund et al., 2007; Loonen et al., 1996).

Additionally, it is known that older, more mature, females in other species such as Atlantic cod (*Gadus morhua*), are responsible for high amounts of relatively healthier fry output (Hixon et al., 2014). As northern pike is also a species that grows relatively large and old, it could be hypothesised that big mothers also contribute to higher amounts of recruitments. If, like in other species, bigger females spawn earlier it is possible that low recruitment is caused by temporal mismatch of zooplankton for the young fry, given that the seasonality of Baltic phyto- and zooplankton is known to be changing (Alheit et al., 2005; Kahru et al., 2016). Follow up studies could look into zooplankton diversity and quantity assays in pike spawning grounds, like wetlands, where a higher number of pike gather and thus more sustenance for fry is needed (Tibblin et al., 2023).

4.2 CONCLUSION

No clear correlation between higher natural egg thiamine content and hatch rate could be found, nor did treatment with thiamine in yolk-feeding fry influence measured morphological traits (length, weight) or measured behaviour (distance travelled, meander), which is contradictory to the original hypothesis that Baltic pike from the Sankt Anna Archipelago are suffering from a thiamine deficiency. The sample size from this study was however not very large in terms of e.g. females used, experimental fish originating from only one population, and no larger scale screening for obviously thiamine deficient females was performed. However, this does not take away from observations of female northern pike in the Baltic Sea not being in optimal health. Further monitoring and charting of thiamine status in Baltic northern pike will give a better idea of what values are to be expected from this species. Additionally, ecotoxicological effects such as those from algal blooms should be investigated, as they might also influence reproductive success in the adult fish.

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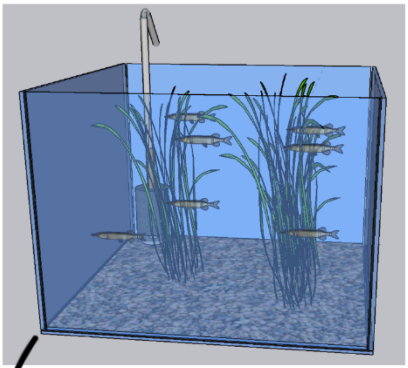
APPENDIX 1 – BEHAVIOURAL WORKFLOW

Collection

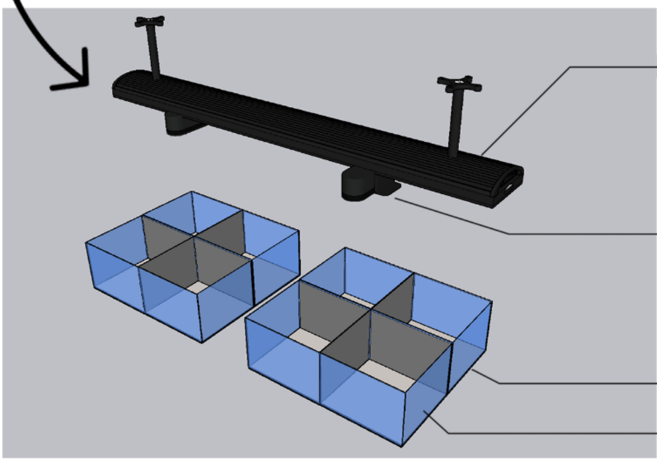


Per round: 4 treatments > 2 fish from each.
Fish were randomly caught using soft net

"Used" fish were temporarily moved to holding tanks afterwards in order to have no replicates

5 minutes acclimation without researcher in room

Open Field Test

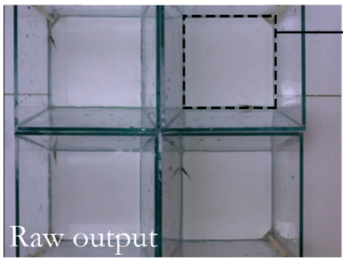


15 repetitions

10 minutes of recording without researcher in room
Recording using OBS version 29.1.2

Fish moved to temporary holding tank

Video Processing



Arena definition

Video's analysed using AnimalTA
Auto-background subtraction
Version 2.3.1

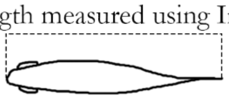
Settings

Correct brightness: No
Correct flickering: No
Threshold: 50
Erosion : 0
Dilation: 0
Area: 0,0 - 5000
Distance threshold: 216,2
Individuals per arena: 1

Path cleanup

Analyses
- Time moving
- Average speed
- Distance
- Meander

Fish length measured using ImageJ



APPENDIX 2 – FISH HEALTH SCORING FORM

Parameter	Score	Value	Observations, explanation
General status		0.0	Alert, active, reacts
		0.1	Lays still, but moves at capture attempts. Abnormal behaviour for the species for max 3 days. Moves to the bottom irregularly
		0.4	Abnormal state for the species; e.g. immobility, does not readily move, does not evade escape at capture attempts. Repeated escape behaviour, hyperactivity or stereotypies (i.e. repeated, ritualistic behaviour). Malformations of skeleton or jaws. Extensive oedema.
Gills		0.0	No discolouration, spots, damages (alt. wounds) or bleeding at the gills
		0.1	Lesser colour changes of the gills
		0.4	Marked colour changes of the gills, for the species abnormal changes, bleedings or visible damages (alt. wounds)
Eyes		0.0	Clear eyes, eyes as usual located in the head
		0.1	Weak clouding of the eyeball. Somewhat protruding eyes if not normal for the species
		0.4	Cloudiness, bleedings, oedema or miscoloured eyes. Strongly protruding eyes if not normal for the species
Scales		0.0	Scales smooth and shining, nothing adheres to the fish
		0.1	Some lack of scales can be seen, small areas with bulging or erect scales or minor mucous veils hanging from the fish
		0.4	Numerous scales are missing/the fish does not look shiny, large fields with erect scales or abnormal amounts of mucous hanging from the fish
Movements and body position		0.0	Normal, calm
		0.1	Hyperactive or hypoactive, changed way of swimming for the species or anxious behaviour.
		0.4	Marked lack of balance, has the belly upwards, cannot coordinate its position in the water/does not keep horizontal position. Abnormal fin movements for the species to keep proper position in the water column.
Skin and fins		0.0	The skin is fully covered with scales. No wounds or other signs of damages
		0.1	Small wounds in the skin < 1% of the body surface, small damages on fins that do not affect swimming, somewhat darker or lighter compared with other fish in the aquarium or compared to what is normal for the species
		0.4	Larger wounds, strongly damaged/missing fin, red skin/bleedings under the scales, markedly changed in colour from other fish in the shoal. Abscesses or abnormal growths

Weight or changes in shape		0.0	Normal shape of body, normal growth for the species
		0.1	Small weight reduction, slimmer shape of body than normal for the species
		0.4	Strong weight reduction, bony, angled back, concave belly or abnormal body shape for the species
Intestinal/ gonadal function		0.0	Intestine in normal condition.
		0.1	Faeces softer/harder than usual but the anus looks normal
		0.4	No signs of faeces/excrements are fluid. The anus seems irritated or infected.
Appetite		0.0	Normal for the species, e.g. feeds from surface at once after feeding until no food is left. Strike times for pike appear normal and precise
		0.1	Some feeding on Artemia or other food designed for the species but do not eat dry food or feed in normal amounts, do not eat all food given. The state must not last longer than 3 days for species normally eating daily. (Is there proper response to the introduction of Artemia)
		0.4	Not interested in Artemia or other food even after more than a day without food. Disinterest in food when pike ought to be hungry and interested. (During feeding times).
Breathing		0.0	Normal breathing, one can hardly see that fish is breathing
		0.1	Compared with what is normal for the species, markedly changed breathing with gill covers and mouth.
		0.4	Almost constant gulping of air at the surface. Clear objective of animal to stay in the stream of oxygen from the bubble filter
Cannibalism		0.4	If a fish visually measures about 1,5x the size of its peers it is to be euthanised and its removal to be noted down in order to avoid excessive cannibalism.
Other observations			
Total score	(>0.4 means fish should be removed, euthanised according to protocol and its removal be written down.)		

APPENDIX 3 – KNOWN EGG FREE THIAMINE VALUES IN FISH, ONLY KNOWN VALUES ADDED.

Fish species	Normal total thiamine (nmol/g)	Thiamine deficiency levels (if known) (nmol/g	Source
lake sturgeon *Acipenser fulvescens*	2,4	< 0,73	(Larson et al., 2021)
lake trout *Salvelinus namaycus*	19,6	< 1,5	(Brown et al., 1998)
alewife *Alosa pseudoharengus*	15,5	-	(Tillitt et al., 2005)
yellow perch Perca flavescens	26,4	-	(Futia et al., 2017)
nine-spine stickleback *Pungitius pungitius*	25,7	-	(Futia et al., 2017)
round goby *Neogobius melanostomus*	14,6	-	(Futia et al., 2017)
rainbow trout (smelt) *Oncorhynchus mykiss*	8,8	-	(Futia et al., 2017)
rainbow trout (adult) *Oncorhynchus mykiss*	20,4 (± 11,3)	< 1,7	(Brown et al., 1998)
chinook salmon *Oncorhynchus tshawytscha*	6,5	< 5,0	(Lindley & Cranford, 2021)
Atlantic salmon *Salmo salar*	> 2,0	< 0,71	(Vuorinen et al., 2021)