UiT
THE ARCTIC
UNIVERSITY
OF NORWAY

FACULTY OF BIOSCIENCES, FISHERIES AND ECONOMICS

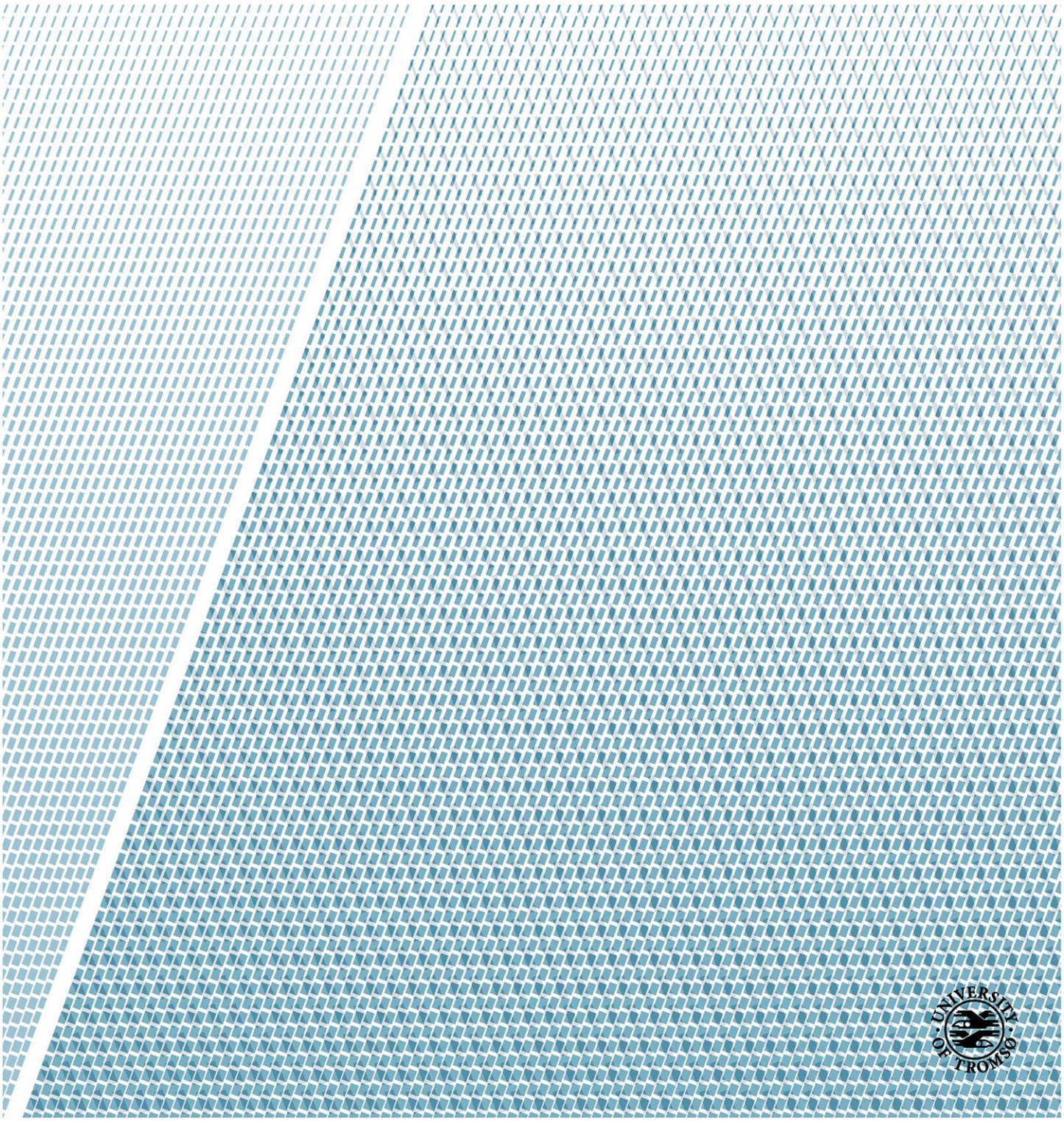
DEPARTMENT OF ARCTIC AND MARINE BIOLOGY

Effect of incubation temperature on eggs and larvae of lumpsucker (*Cyclopterus lumpus* L.)

—

Mathias Danielsen

Master thesis in biology August 2016



Acknowledgements

I wish to thank my supervisors Inger-Britt Falk-Petersen, Thor Magne Jonassen and Albert K. Imsland for their support, feedback, guidance, comments, input and patience, and for including me in this project. I also want to thank Ane Vigdisdatter Nytrø and Thor Arne Hangstad from Akvaplan Niva for help and input before, during and after the experiment. Everyone working at TMY also did an amazing job in helping with the experiment and also gave me a lift back and forth from Tromsø to Kraknes several times. Thanks to Akvaplan Trondheim for lending me an office spot in April 2016, it was most helpful. Fride Tonning at UiT did a splendid job with the histology, thank you very much.
Finally I wish to thank my mom, dad and girlfriend for a tremendous support and motivation.

Thank you very much everyone, I could not have done this without you!

Mathias Danielsen

Table of content

Abstract

In this study the aim was to investigate how incubation temperature effected lumpsucker eggs and larvae; by comparing early cell symmetry, egg/embryo development, mortality, hatching success and early larvae size weight and histomorphology.
Two batches of eggs were incubated at 3 temperature regimes; 1- Ambient seawater 4-6°C (cold), 2-Ambient seawater for 10 days then gradually increased to 10°C (gradient), 3-constant 10°C seawater (warm).
Early cell symmetry, development and hatching time was similar with regard to day degrees between all temperature groups, although faster (in days) with warmer temperature. The eggs incubated in cold water had the highest egg mortality and lowest hatching success. The warm group had lowest egg mortality while the gradient group had highest hatching success. The gradient group also had the most synchronized hatching; most of the eggs hatched during the first day. The warm and cold group both had a hatching peak 3 days post first hatching. The larvae mortality was highest in the warm group and lowest in the gradient group. The cold group had the longest, heaviest and thickest larvae followed by the gradient and warm group respectively. Newly hatched larvae from the warm group had most body deformities. Larvae from all groups kept fed at 10°C for two weeks showed a difference in body size; larvae from the cold regime being largest. No difference between groups were found in mortality or body deformeties. Studies of organ and tissue histomorphology of hatched and two week old larvae did not reveal differences between the temperature groups.

This study demonstrated that incubation temperature will effect: mortality, hatching success and early larvae conditions of lumpsucker. A gradual rise in incubation temperature at an early embryo stage seemed to be most beneficial.

Keywords: Lumpsucker, incubation temperature, early development.

Introduction

Since the aquaculture production of Atlantic salmon in Norway (*Salmo salar*) started in the 1970´s the industry has grown to become the biggest producer of Atlantic salmon in the world. In 2009 the worldwide production of Atlantic salmon in aquaculture reached 1,5 million tons, with Norway producing 944600 tons and the United Kingdom and Chile producing 141800 and 129500 tons respectively (Torrisen et al, 2011). According to the Norwegian Ministry of Trade, Industry and Fisheries, the full-time equivalent (FTE) in 2010 in Norway was over 9500 directly from aquaculture, in addition there many are FTE´s connected indirectly to aquaculture in form of transport, equipment, development and fish-food production (NFD, 2015).

There are some issues that can be connected to aquaculture, and the Norwegian government produced a strategy for an environmentally sustainable aquaculture industry in 2009 and presented the 5 following areas where the aquaculture affects the environment (FKD, 2009).

- Genetic influence and escape
- Pollution and emissions
- Disease
- Area use
- Feed resources

The growth of the Atlantic salmon production has made an artificially high density of Atlantic salmon, which in turn has made the spreading of Salmon lice very high and independent from the wild Atlantic salmon population. The Salmon lice population is unnaturally high in some fjords and along the cost of Norway (Skilbrei et al, 2015).

The Salmon lice (*Lepeophteirus salmonis*) is a highly modified parasitic copepod. They reach a length of 10-30 mm, females have a long egg sacs attached on their back. Salmon lice live externally attached on salmonid fish, feeding on: dermal tissue, blood and body fluids (Moen & Svensen, 2004).

Wild salmonids have also been effected by the salmon lice. Infection on wild fish close to sea pens have been shown to be higher than fish far away (Bjørn et al, 2001; Costello 2009) and salmon lice has been blamed for sea trout (*Salmo trutta*) stock collapses. (Heuch et al, 2005).

Fish infected with Salmon lice are effected in several ways; heavy infections can cause large wounds which in turn increase the risk of lethal bacterial and/or fungal infection (Moen & Svensen, 2004; Skilbrei et al, 2015). In addition, infected salmonids can suffer from osmotic imbalance, physiological stress, anaemia, lower appetite, reduced growth, delayed sexual maturity and higher risk of predation (Skilbrei et al, 2015).

The salmon lice also have an economic impact because of lost production and preventive actions, the salmon aquaculture industry was predicted in 2012 to spend 200 million $ on actions against the Salmon lice, mainly chemical treatment (Bergheim, 2012).

The use of chemicals have been common in several countries over the years to reduce the occurrence of Salmon lice including: organophosphates, pyrethroids, avermectins, chitin synthesis inhibitors, formaldehyde and hydrogen peroxide (Denholm et al, 2002). Although use of chemicals has helped reduce the Salmon lice levels, they are expensive (Costello, 2009; Bergheim, 2012). Another problem that has been noticed is that they have caused the development of resistance (Denholm et al, 2002; Jimenez et al, 2012) and also have possible risks of effecting the environment (Burridge et al, 2010).

Cleaner-fish

Since the 1989 wrasses (Labridae) have been used commercially by stocking them together with Atlantic salmon. Wrasses turned out to be a good biological control for reducing the Salmon lice in sea pens (Treasurer, 2002). Species of wrasses have different depth preferences, however, a mixture of species will give best results. A 1-50 ratio of wrasse per salmon is considered effective density (Moen & Svensen, 2004). Wrasses are caught from the wild in traps and transferred to the sea pens. Most of the cleaner-fish used in aquaculture in Norway are wrasse, and in 2014 approximately 21 million fish were distributed (Skiftesvik & Nedreaas, 2015). The Wrasses are distributed in the wild from the coast of Morocco, along the Mediterranean, to the British Isles, the North-Sea, the West Baltic and up to mid-Norway (Skiftesvik & Nedreaas, 2015; Moen & Svensen, 2004).

Due to the wrasses southern distribution, use of wrasses in the northern parts of Norway may be a challenge (Durif, 2015). Furthermore, it was also suggested that the appetite of wrasses is reduced in low temperature water (Lein et al, 2013). This provided the need for a cleaner-fish adapted to colder waters.

The lumpsucker (*Cyclopterus lumpus* L.) has a more widespread natural distribution, further north than the northernmost species of wrasses, reaching Novaya Zemlya, Svalbard, Iceland, South Greenland, Hudson Bay Newfoundland, New Jersey, coast of Portugal, British Isles, North Sea, Baltic Sea and Norwegian coast (Andriyashev, 1964; Cox & Anderson, 1922; Davenport, 1985; Moen & Svensen, 2004; Durif, 2015).

Imsland et al (2014a) studied the use of Llumpsuckers as cleaner fish in sea pens with Atlantic salmon, and found clear signs of grazing on Salmon lice. This indicated that the lumpsucker can be a suitable cold-water cleaner-fish alternative. Lumpsuckers and Atlantic salmon did not show any antagonistic behaviour between each other in another study by Imsland et al. (2014b). It is important for the welfare of lumpsuckers that they have access to attach themselves to a substrate for resting, however they seem to adapt to and prefer artificial substrate like smooth plastic (Imsland et al, 2015). Small lumpsuckers (50g) are preferred because they have showed higher grazing of salmon lice than larger lumpsuckers (>350g). Larger lumpsuckers has also shown a negative effect on overall growth and food conversion in Atlantic salmon (Imsland et al, 2014c).

Lumpsucker (Cyclopterus lumpus L.)

The lumpsucker has a very characteristic appearance, and should not be confused with other species. It has a slightly oval shape with seven dorsolateral rows of bony projections running along its body. Its skin is thick and scale-free. A large suction disc is located on its belly between the pectoral fins. The first dorsal fin is overgrown and forms a dorsal crest. Females grow to 30-40 cm, maximum 60 cm and males to 25-30 cm, maximum 50 cm. They can weigh up to 5.5 kg. The skin colour is blackish-grey or bluish-grey, but during the spawning season the male skin changes colour; usually red, orange or purple (Andriyashev, 1964; Davenport, 1985; Moen & Svensen, 2004).

As mentioned earlier, it is a widely spread species in the North Atlantic Ocean.

Juveniles are usually located close to the surface, attached to seaweed and kelp which provides cover and food availability, they can also be found in intertidal pools. After a year they migrate out to deeper oceans becoming semi pelagic like the adults (Davenport, 1985; Durif, 2015; Moen & Svensen, 2004; Moring & Moring, 1991). Some juveniles have however been found in the in open sea areas (Daborn & Gregory, 1982). Juveniles feed on different zooplankton; mainly amphipods, copepods and isopods, located near the surface (Daborn & Gregory, 1982; Moring ,1989). Previously it was thought that adult lumpsucker were benthic living, however Blacker (1983) suggests that the adults in fact spend most of their lives in the upper 50 meters of open oceans. Adults feed on pelagic crustaceans and jellyfish (Blacker, 1983; Moen & Svensen, 2004). From February and onwards in the spring, sexually mature fish return for spawning in shallow water in coastal areas (Andriyashev, 1964; Davenport, 1985; Durif, 2015; Moen & Svensen, 2004). Females spawn in several batches and have high fecundity laying between 100-400.000 eggs in total (Brown, 1992; Moen & Svensen2004). The eggs are 1,8-2,6 mm in diameter and can have a variety of colours; pink, orange, yellow, green, brown and red. They also stick to each other after exposure to saltwater (Andriyashev 1964; Collins, 1976; Cox & Anderson, 1922; Davenport, 1985; Davenport & Thorsteinsson 1989; Moen & Svensen, 2004). Males guard the eggs, which can be from several females, until they hatch after approximately 2 months (Durif, 2015; Moen & Svensen, 2004;).

In some places, like in Norway, lumpsuckers have traditionally been fished only for their roe, considering the fish itself as trash fish. In other countries however, it is considered a delicacy (Davenport, 1985; Moen & Svensen, 2004). In 2008 the lumpsucker roe catch was 675 tons which had a value of 18,9 million NOK (Sunnanå, 2009). The total quota of roe catch recommended by the Norwegian institute of Marine Research in 2015 is set to 400 tons (Durif, 2015).

The commercial production and use of lumpsuckers as cleaner fish in fish pens is fairly new. Challenges and new experiences are most likely to occur in the future. This call for new research on the lumpsucker to help improve the welfare of lumpcukers and salmonids used in the aquaculture, and increase efficiency of lumpsucker production.

Temperature

The environmental conditions during early life can affect; fish growth, adult body size, sex ratio, egg sizes, lifespan and migration, and temperature is considered to an important factor (Jonsson & Jonsson, 2014). Several studies that show temperature as a physiological factor have an effect on development and survival of fish egg and larvae. During early life, fish from temperate zones appear to be more sensitive to temperature changes, than juveniles and adults (Rombough, 1997)

Greffen et al (2006) showed mortality in cod eggs (*Gadus morhua*) increased with higher constant incubation temperature. Puvanendran et al (2015) exposed cod eggs to incubation temperature of 4,5°C increasing at different rates to 9,5°C and found that it did not affect mortality or cell asymmetries significantly, but an increase over 32h was recommended over 8h, 64h and 96h.

Time from fertilization to 50% hatching was reduced with increased temperature for fat greenling (*Hexagrammos otakii*) and hatching percentage was higher at 12°C and 16°C compared to 8°C and 20°C (Hu et al., 2015). Temperature also increase length of incubation for lumpsuckers (Cox & Anderson, 1922).

Mueller et al. (2015) showed that temperature effected hatch timing, size at hatching, survival and energy use of embryos from lake whitefish (*Coregonus clupeaformis*), higher incubation temperatures produced fewer and smaller larvae. It was also suggested that end of gastrulation and hatching were critical windows for the effect of temperature on survival.

Kazuyuki et al. (1988) suggested that marine fish embryos have four periods sensitive to low temperature during development; cleavage, early gastrula, embryo appearance and blastopore closure.

Collins (1978) found that lumpsucker eggs incubated at average temperatures of 6,4°C and 9,8°C hatched after 31 and 25 days respectively, and eggs incubated at an average temperature of 3,8°C did not hatch at all.

Objective

Currently there is no in depth study done on the effect of incubation temperature on the lumpsucker eggs and early larvae. Since the lumpsucker is now produced commercially as a

cleaner-fish, information about incubation temperature is important in order to optimize rearing conditions.

The objective of this study was to see how different incubation temperatures effected; early cell symmetry, egg development, mortality, hatching success and early larvae size, deformities and histomorphology.

Incubation temperatures used in this study was constant 10°C, ambient water 4-6°C and ambient water 4-6°C for 10 days and gradually increased over 4 days to 10°C.

The study was a part of the Akvaplan-niva project "Stamfiskhold av rognkjeks", project number: 900977, and FHF (Fiskeri- og havbruksnæringens forskningsfond) as project assigner.

Materials and methods

Experimental location and design

The experiment was carried out at Tromsø Marin Yngel (Akvaplan NIVA), Kraknes Troms Norway, between 11th of March and 30th of May 2015.
Larval measurements and histological preparations and analyses were carried out between August 2015 and April 2016 in the laboratory at the Department of Arctic and Marine Biology at the University of Tromsø.

Two batches of eggs were incubated at 3 temperatures, in 5 replicates:

- 1: Ambient seawater temperature 4-6°C (Cold, C).
- 2: Ambient seawater temperature for the first 10 days and then gradually increased to 10°C over 4 days (Gradient, G).
- 3: Constant 10°C seawater (Warm, W).

Lumpsucker roe was collected from two females and put in two separate plastic containers. Milt from two males was then added to both roe samples, and distributed evenly into it. All four lumpsuckers were caught from the wild at Hekkingen, Malangen, Norway. One mL of eggs was subtracted from each bowl using a syringe and placed on two petri-dishes, the eggs were then counted to estimate the number of eggs/mL. For every incubator, two ml of eggs were then put on a petri-dish and carefully separated from each other, using tweezers. Saltwater was slowly added into these petri-dishes using a pipette, after a few minutes the eggs were lowered into the incubators, trying to avoid the eggs to stick together.

Eggs from each batch were placed in a total of 30 incubators, 5 replicates from each batch, at all 3 temperature regimes. Two incubators from each batch were sampled for eggs during the incubation period; while two triplicates from each regime and batch were left undisturbed until hatching.
Approximately 200 eggs (2mL) were put into all 30 incubators. From the triplicate unsampled incubators, 50 larvae were kept alive and fed with 0,1-0,2mm pellets (AgloNorse Extra) for 2 weeks after hatching to study possible late effects.

A summary of the incubators, temperature groups and egg batches is shown in Table 1, and a close up photo of an incubator can be seen in Figure 1.

Table 1: Overview of incubators, incubation temperature regimes, male and female lumpsuckers used, batch number and which incubators were sampled during the egg stage.

Temperature	Cold	Gradient	Warm				
Incubator				Female	Male	Batch	Sample incubator
1	C1	G1	W1	1	1 & 2	1	No
2	C2	G2	W2	1	1 & 2	1	No
3	C3	G3	W3	1	1 & 2	1	No
4	C4	G4	W4	1	1 & 2	1	Yes
5	C5	G5	W5	1	1 & 2	1	Yes
6	C6	G6	W6	2	1 & 2	2	No
7	C7	G7	W7	2	1 & 2	2	No
8	C8	G8	W8	2	1 & 2	2	No
9	C9	G9	W9	2	1 & 2	2	Yes
10	C10	G10	W10	2	1 & 2	2	Yes

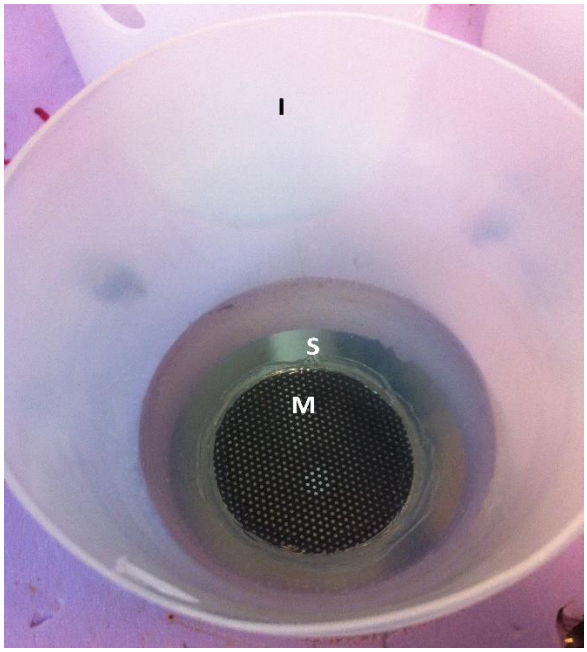


Figure 1: Close-up photo of incubator (I), bottom mesh (M) glued in place with a rim of silicone (S).

The experimental rig consisted of 30 incubators (Fig. 2). Each incubator was made using a 2-liter plastic bottle turned upside down and stuck in a styrofoam plate. The bottom of the bottles was removed and a hole drilled in the bottle-cap. Inside the bottle a plastic plate with 1,5 mm mesh holes was glued with silicone to make the bottom of the incubator where the eggs would lie. The incubators were set up in three rows of 10, with each row representing a different temperature exposure groups. Seawater was supplied with PVC pipes running under each row with an outlet under each incubator, a silicone hose connected from the outlets of the pipe to the incubators through the hole in the bottle-cap. On the top of the incubators a hole was drilled and a silicone and rubber hose was used to make a water outlet.

The first row of incubators was supplied with ambient seawater from the sea. The third row was supplied with 10 degree heated seawater. The second row was supplied with both water types, making it possible to run on either one or the other, or a mixture of both. The amount of water flowing through the experimental rig was controlled by vents in the water supply pipes, this was set to approximately 2L/min, but was adjusted, sometimes more than once per day, to keep the water level even in all incubators. The incubator position in the Styrofoam plate could also be adjusted to even out water levels.

Both the heated water and the ambient temperature water had passed through a 60-µm mesh filter, a UV filter and been saturated with oxygen before entering the experimental rig. The larvae were expected to hatch around 280 day degrees; at 260 day degree a cap with a 0,5 mm mesh was put on the water outlet of the incubators to hinder any larvae from going down the drain.

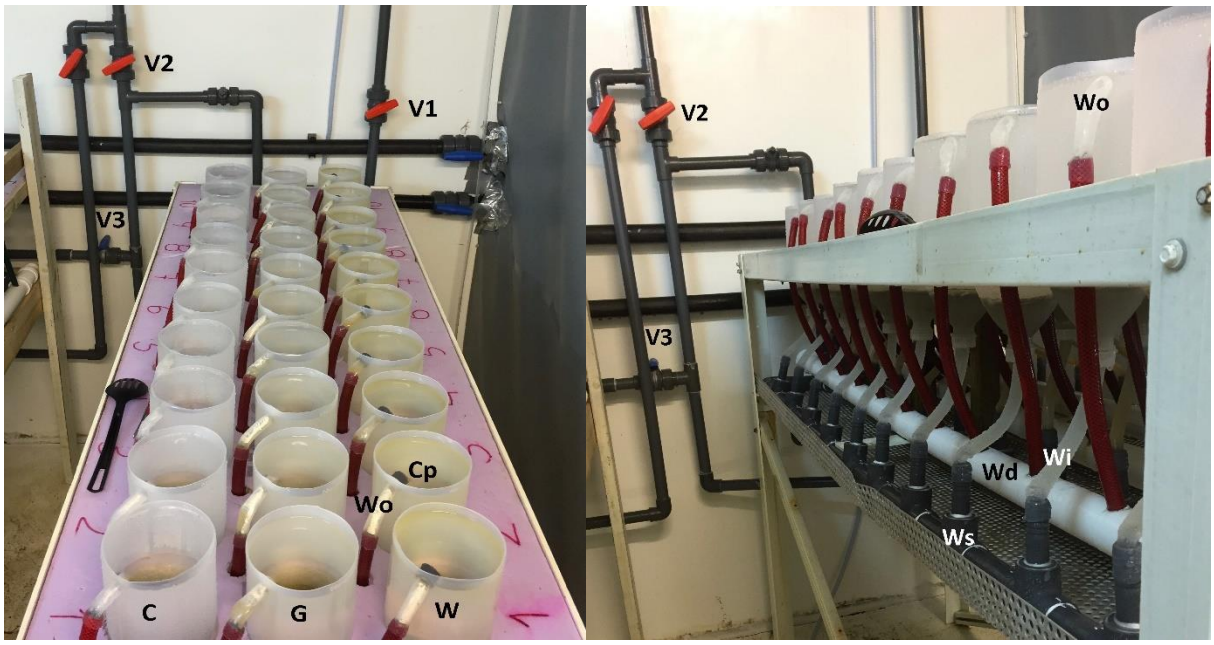


Figure 2: Overview of experimental rig with all 30 incubators, left row is cold (C), the middle gradient (G) and right warm (W). Water outlet from incubator (Wo), outlet cap (Cp), warm-water supply vent 1 (V1), warm-water supply vent 2 (V2) and cold-water supply vent (V3). Water supply to incubators (Ws) water intake (Wi) and water drainage (Wd).

The hatched larvae were kept in containers similar to the incubators, however the water inlet and outlet were switched with each other (Fig. 3). In addition, a 0,5 mm mesh was glued with silicone over the plastic plate inside to keep larvae from being sucked out of the container. The water outlet hose was longer and was raised up to the height of the water level inside the container and had an air vent on the top. This air vent was used to create a vacuum that would rapidly suck out the water from the container and thus help to keep excess food and bacterial growth at a minimum. The water temperature in the larval containers was 10°C.

Temperature and oxygen saturation levels were recorded daily using an Oxyguard Handy Alpha (Sterner Aquatech, Ski, Norway). The temperature was measured in one incubator from every temperature treatment. If adjustments were done to the water input flow, the water level of all the incubators on that row were checked to be correct.

Light was on during working hours, from 08:00 to 16:00 every day, and during samplings that took place out of working hours.

Cleaning of the incubators was done if the accumulation of debris inside the incubators became too visible. The eggs were then removed using a plastic spoon and a plastic pipette and put in a bucket with seawater at the respective temperature regime while the incubator was rinsed.

Sampling of eggs

Fertilization percentage and average egg diameters were calculated by taking 15 eggs from each sampling-incubator and studying them under a stereomicroscope (Leica WILD M10).

To study the development, abnormalities and mortality of the eggs incubated at different temperatures, egg samples were taken throughout the incubation period from the sample incubators. The first two days, egg samples were taken twice a day. From day three and onwards, sampling was done every second or third day until hatching occurred. During sampling, a minimum of 5 eggs were taken from each sampling incubator (i.e. 10 eggs from each batch, and 20 from each temperature regime).
The egg samples were taken using a plastic spoon, lifting the eggs to the surface and then carefully separating them, if they stuck together, and put into glass vials with water from the incubator until they were studied under the stereomicroscope. The eggs were photographed through the ocular of the stereomicroscope using a mobile phone camera (iphone 4 and iphone 6) and then stored on 4 % buffered formaldehyde in case additional examinations were needed. Number of abnormal and dead embryos were estimated from each sample.

Sampling of larvae

When the larvae started to hatch in incubators from one temperature regime, up to a maximum of 50 larvae from the each of the triplicate (non-sampled) incubators of both batches were moved to other containers to be kept alive for 2 weeks after the hatching peak. The larvae were caught using a plastic pipette and transferred either to a container to be kept alive for 2 more weeks or a glass vial. Only larvae that appeared to be in good condition were transferred. Larvae stuck on the water outlet or swimming in consecutive circles were excluded. All other larvae, beside the 50 transferred to the containers, were killed with an overdose of anesthetics (FINQUEL) and stored on 4 % buffered formaldehyde to be examined later.

Figure 3: Container for hatched larvae. Water supply (Wi), water intake (Wi), Water outlet (Wo), air vent (Av) and water drainage (Wd). Food remains (F) and bacterial growth (B) can be seen on the right.

Feeding was done by hand during working hours. The larvae were fed 4 times a day at around 08.00, 10.00, 13.00 and 15.00h. They were given approximately 1 cl of pellets (AgloNorse Extra) each time. Around half an hour after the last feeding, the excess feed accumulated on the bottom and bacterial growth was rinsed away.

Two weeks after the peak of the hatching, the larvae kept in the containers were taken out using a plastic pipette, killed with an overdose of anesthetics (FINQUEL) and stored on 4 % buffered formaldehyde for later examination.

Examination of larvae

A subsample of up to 20 larvae from each day of hatching from all triplicates of both groups, as well as a subsample of 20 two week old larvae, were studied under the stereomicroscope. Several measurements and notes were done: body length, body height above anal opening, yolk-sac height (Fig. 4), weight, dorsal fin development, tail bend, spine damage, deformed body and mechanical damage (for example, missing bodyparts, bursted yolk-sac, degradation and so on). Yolk-sac height could not be measured on the two week old larvae due to them having used most of the yolk-sac and being less transparent.

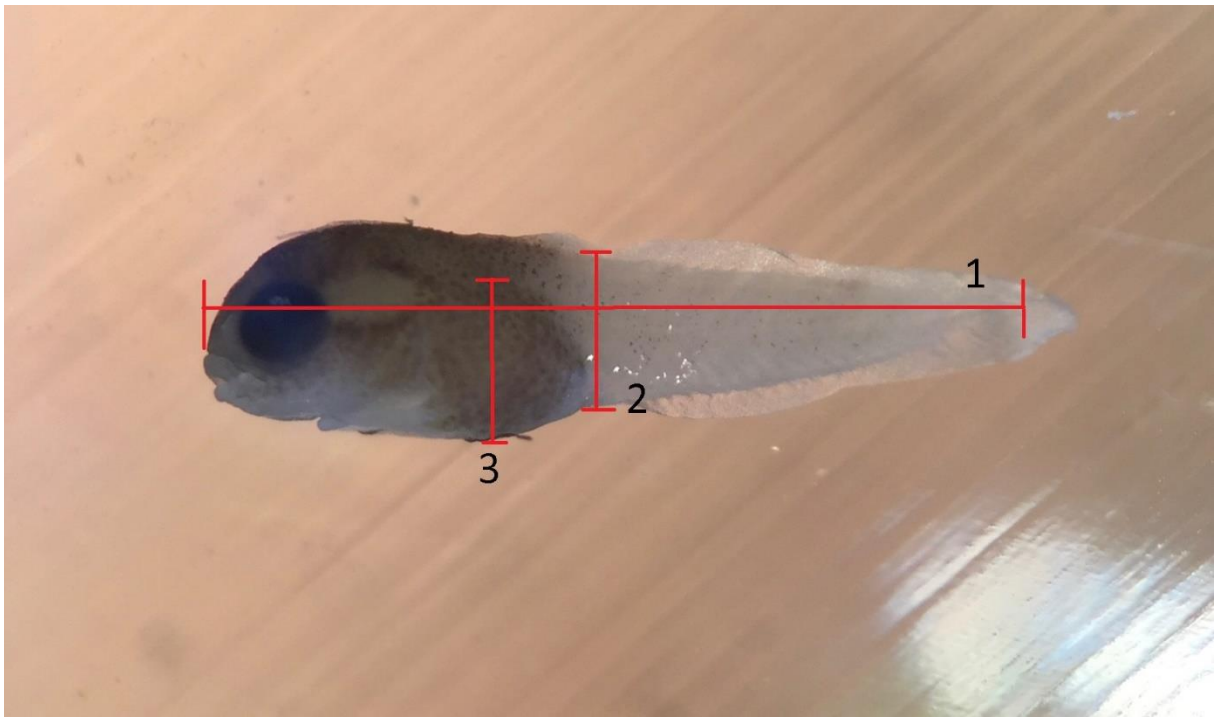


Figure 4: Newly hatched lumpsucker larva illustrating measurements for length (1), body height (2) and yolk-sac height (3).

The bodyweight of the larvae was measured using a Mettler MX5 weight (Figure 5). Using tweezers, the larvae were carefully picked up and rolled on a piece of paper towel to dry of excess moisture, then put on a small disc made of aluminum foil and then weighed. The larvae were mostly measured 10 at the time to account for an unstable number on the weight due to evaporation.

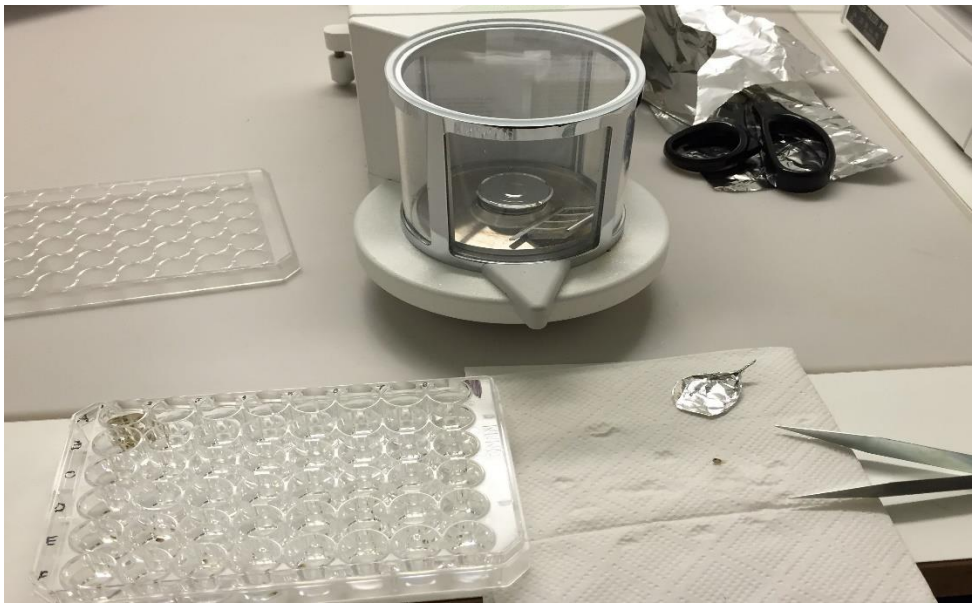


Figure 5: Mettler MX5 used for measuring the weight of the larvae.

Histology

Histological studies were carried out on larvae to reveal possible developmental differences and organ or tissue abnormalities in individuals from different temperature regimes. Hatched and two week old larvae (3 from each of the replicates) were fixed in buffered formaldehyde (4%), embedded in wax (Paraplast) and sectioned longitudinally at 5 µm with a rotation microtome. Sections were stained with eosin and haemotoxylin and examined and photographed under a microscope (LEICA DM2000 LED). Photos were taken of these slides using a LEICA DFC295 camera and computer software (LEICA APPLICATIONSUITE V4.7).

Statistics

A t-test was carried out to check for significant difference in egg size between the batches. Egg mortality was calculated from the number of dead eggs found in the samples taken during incubation. A Two-Way ANOVA was then carried out to check for statistical significant differences between groups from the three temperature regimes.
Hatching percentage was calculated in all replicate incubators based on the number of larvae hatched compared to the estimated number of eggs put into them. A Two-Way ANOVA was carried out on the hatching percentage of all replicates to see if there was a statistical significant difference between the temperature regimes.
Larvae mortality was calculated based on the number of dead larvae present among hatched larvae during the hatching period, and when the 2 week old larvae were removed. A Two-Way ANOVA was conducted to check for statistically significant differences.
To check for statistical significant differences in length, body height, yolk-sac height, and weight of larvae, a three-way nested ANOVA and a Newman-Keuls test were carried out. Differences in dorsal fin development, tail bend, spine damage, deformities and mechanical damage were recorded as present or not present and were analyzed using a Kruskal-Wallis test. For the newly hatched larvae these statistical tests were done when hatching occurred in a minimum of two of the temperature regimes at the same days post hatching (DPH), with the first day of hatching being 0 DPH.
All statistical analyses, figures and data plotting were done in Microsoft Excel 2013 and Statistica.

Results

Incubation

Egg colour

Eggs from both batches had an orange colour (Fig. 6), and batch 2 eggs were slightly darker.

Figure 6: Eggs from batch 1 with an orange colour.

Egg sizes and numbers

Both egg batches had eggs of similar size. The average egg diameter was 2,23 mm (SE ±0,0049) in batch 1 and 2,28 mm (SE ±0,0045) in batch 2 (Fig. 7). Although the average egg size was larger in batch 2, the difference was not statistically significant (T.test P= 8,8998*10^-13).

The number of eggs per ml was higher in batch 1 (109) than in batch 2 (94). Thus, an estimate of 218 eggs from batch 1 and 188 from batch 2 were distributed into each incubator, as shown in Table 2.

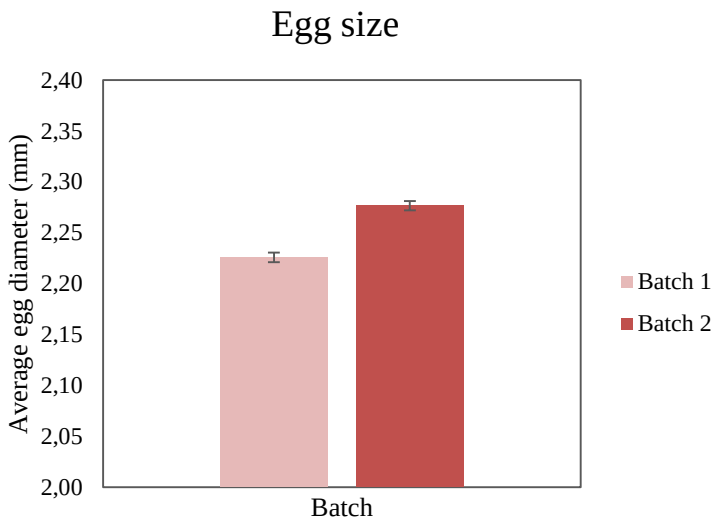


Figure 7: Average egg diameter (mm) of lumpsucker eggs from batch 1: 2,23 mm (±SE 0,0049) and 2: 2,28 mm (±SE 0,0045).

Table 2: Number of eggs per ml and number of eggs incubated in each replicate incubator from batch 1 and 2.

Batch	Eggs/ml	Incubated
1	109	218
2	94	188

Temperature and oxygen

Oxygen saturation was stable both during incubation and after hatching. During incubation the average oxygen saturation was 109,18 % for the warm group 108,30 %for the gradient group and 103,12 % for the cold group. After hatching it was 108,76 %, 110,13 % and 105,50 % for group warm, gradient and cold, respectively. A summary of oxygen saturation can be found in Table 4 for incubation, and Table 6 for hatched larvae.

Water temperature in the cold group was on average 4,7°C throughout the incubation period starting at around 4°C and gradually rising to approximately 6°C. The warm group was stable at around 10°C with an average of 9,95 °C. The gradient group was similar to the cold group until 10 DPF, after the temperature rise it was stable at around 10°C like the warm group. Incubation temperature over time can be seen in Figure 8, averages are displayed in Figure 9 and a summary can be found in Table 3.

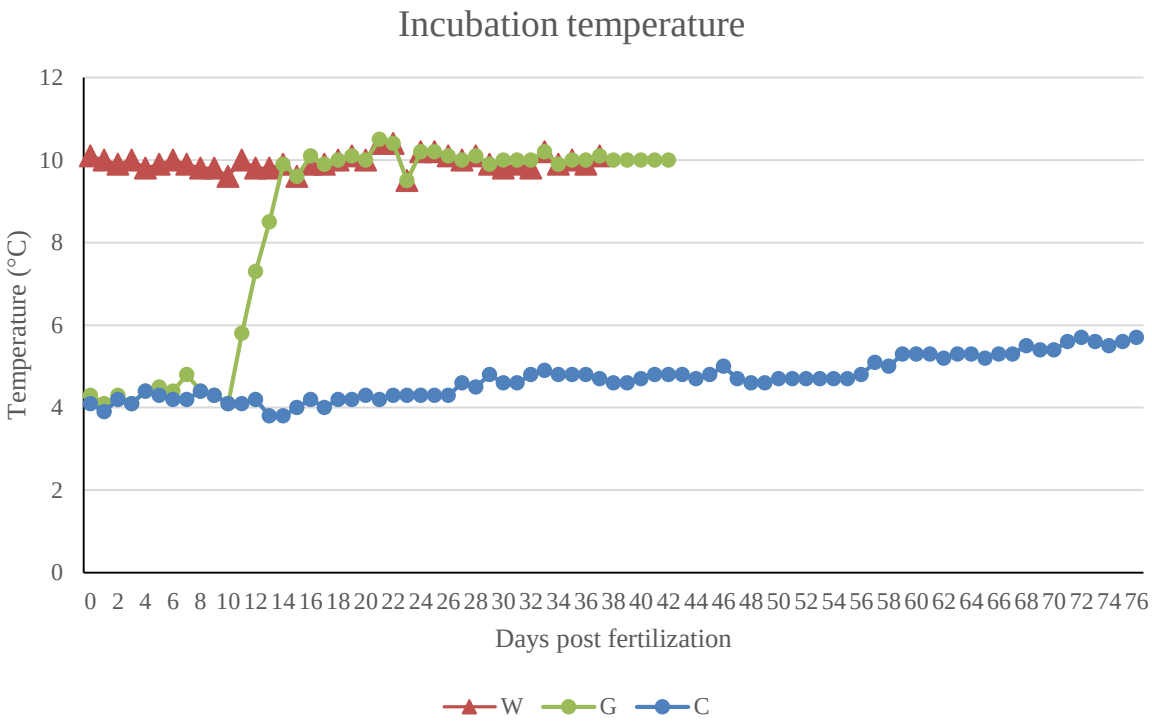


Figure 8: Incubation temperature regimes for the lumpsucker egg groups W=warm, G=gradient and C=cold.

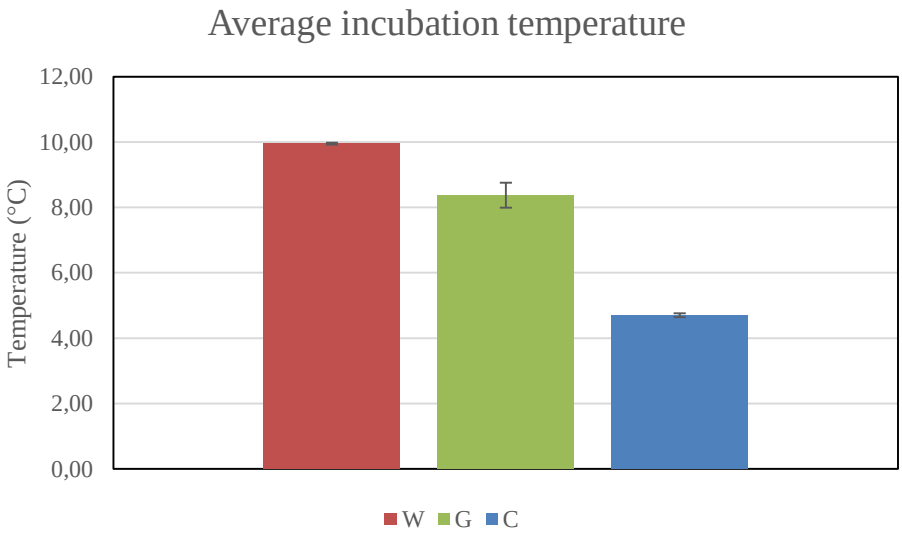


Figure 9: Average incubation temperature for each temperature regime W=warm, G=gradient and C=cold.

Table 3: Overview of temperature during egg incubation of lumpsucker.

Incubation temperature (°C)

	Warm	Cold	Gradient	G (0-10 DPF)	G (10-14 DPF)	G (14-42 DPF)
Average	9,95	4,70	8,37	4,34	10,02	7,12
Min	9,50	3,80	4,10	4,10	9,50	4,10
Max	10,40	5,70	10,50	4,80	10,50	9,90
SD	0,19	0,49	2,52	0,21	0,19	2,27
SE	0,03	0,06	0,38	0,06	0,04	1,01
Variance	0,04	0,24	6,37	0,04	0,04	5,13

Table 4: Overview of oxygen saturation during egg incubation of lumpsucker W=warm, G=gradient and C=cold.

Incubation oxygen saturation (%)

	W	G	C
Average	109,18	108,30	103,12
Min	95,00	89,00	95,00
Max	120,00	115,00	111,00
SD	6,43	6,37	3,20
SE	1,10	1,01	0,37
Variance	41,30	40,63	10,27

Table 5: Overviev of temperature for lumpsucker larvae.

Larvae

Temperature, hatched larvae (°C)

	W	G	C
Average	9,97	9,99	9,70
Min	9,80	9,90	9,50
Max	10,20	10,10	10,20
SD	0,11	0,07	0,20
SE	0,03	0,02	0,05
Variance	0,01	0,00	0,04

Table 6: Overview of oxygen saturation for lumpsucker larvae W=warm, G=gradient and C=cold.

Oxygen, hatched larvae (%)

	W	G	C
Average	108,76	110,13	105,50
Min	99,00	100,00	102,00
Max	115,00	115,00	108,00
SD	4,44	3,31	1,69
SE	1,08	0,86	0,40
Variance	19,69	10,98	2,85

Fertilization and development

Fertilization was high in both batches, with 97,79 % in batch 1 and 98,89 % in batch 2.

Early cell symmetry (2-16 cell stage) appeared normal in all temperature regimes and in both batches.

Eggs in the warm temperature regime sampled 7 hours post fertilization (HPF) had reached 2 cells. The next sample was taken 20 HPF and the eggs had then reached 64 cells. This is 5 cell divisions over 13 hours which equals 2,6 cell divisions per hour.
Samples from the cold and gradient groups taken 22 HPF, which at the time both were running on ambient water, revealed that some eggs had reached 4 cells and others 8 cells. At 29 HPF they were at 8 and 16 cells, and at 47 HPF they were at 64 cells. This means 0,14 divisions per hour between the first two samples, and from 0,22 to 0,17 cell divisions per hour between the second two samples. Number of cell divisions related to HPF can be seen in Figure 10.

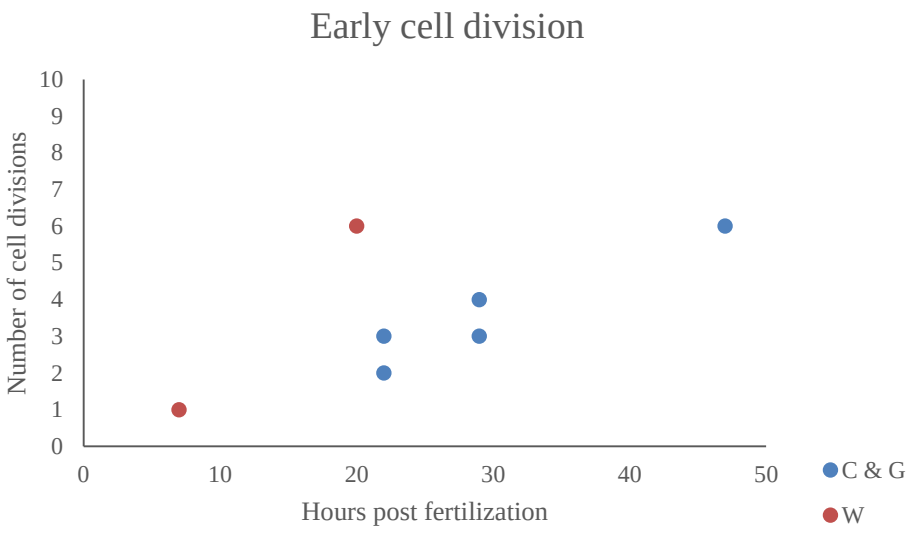


Figure 10: Early cell division of lumpsucker eggs as number of cell divisions related to time after fertilization W=warm, G=gradient and C=cold.

Development was as expected faster with increasing temperature, but in relation to day degrees it was fairly similar between all groups. The lowest day degrees a certain developmental stage was observed is summarized in Table 7. Photos of *C. lumpus* development is presented in Appendix I.

Table 7: Overview of lowest day degree (d°) at which a developmental stage of lumpsucker was observed.

Egg development

Development	d°	Development	d°	Development	d°
2 cells	0,0	Embryo	49,8	Body pigment	138,4
4 cells	4,1	Eyes	49,8	YS vein spread	173,8
8 cells	4,1	Lipid compression	70,3	Headgrowth	183,3
16 cells	4,1	Otocyst	89,4	Open mouth	209,8
64 cells	8,0	Eye pigment	117,3	Body growth	209,8
Morula	10,1	Otolith	117,3	Egg filled	254,3
Blastula	20,1	Heartbeat	128,9	Hatch	278,6
Gastrula	29,2	Yolk-sac vein	128,9	Dorsal fin	308,4

Egg Mortality

All experimental groups had low egg mortality early in the incubation period and the mortality gradually increased as shown in Figure 11. The average egg mortality for each batch in all groups is displayed in Figure 12. Egg mortality was lowest in the warm group and highest in the cold group, as shown in Figure 12. A Two-Way ANOVA showed a statistically significant difference in egg mortality between groups, displayed in Table 8.

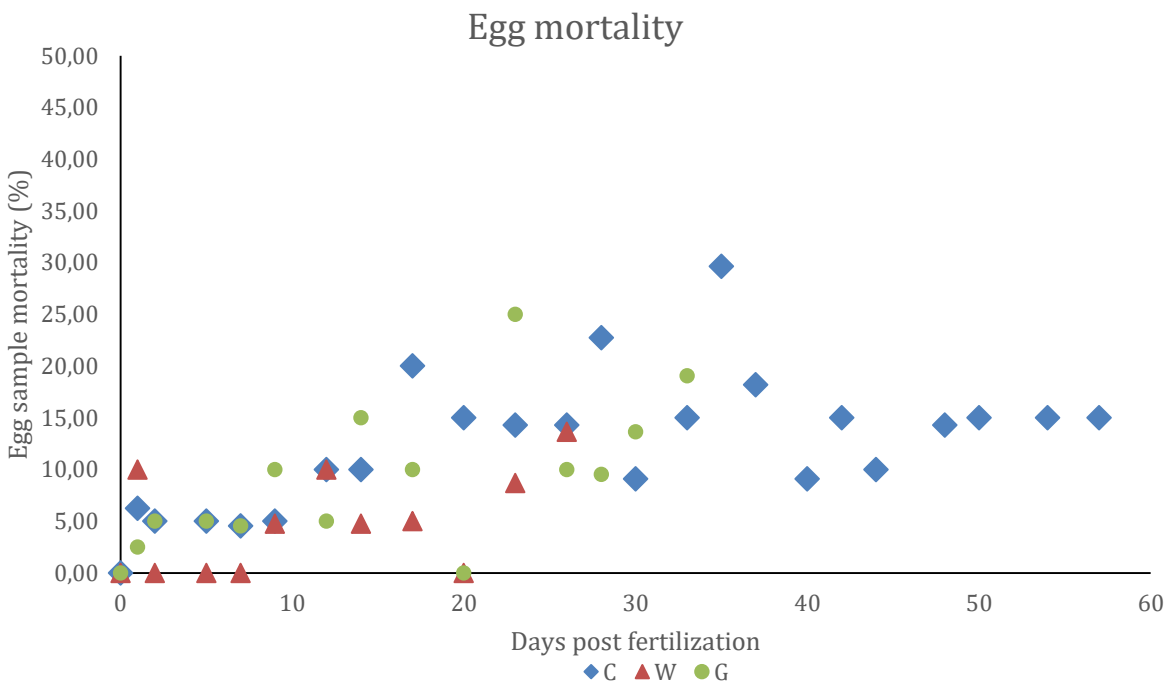


Figure 11: Lumpsucker egg mortality over time W=warm, G=gradient and C=cold.

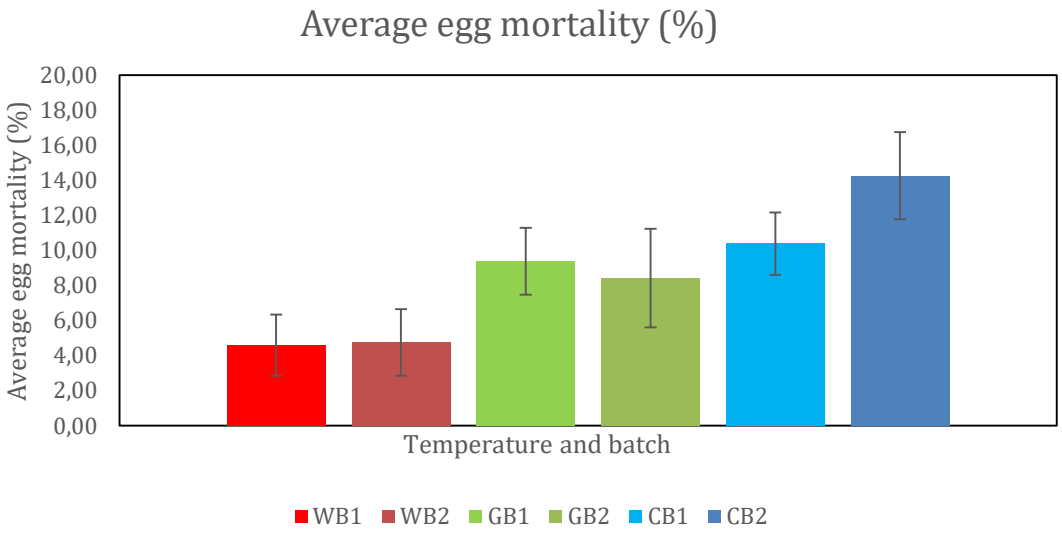


Figure 12: Average egg mortality for both lumpsucker egg batches and all temperature groups W=warm, G=gradient and C=cold B1= batch 1 B2= batch 2.

Table 8: Two-Way ANOVA, Egg mortality

	SS	*df*	*MS*	*F*	*p*
Intercept	7008,154	1	7008,154	79,54893	0,000000
Group	954,009	2	477,005	5,41444	0,005915
Batch	24,805	1	24,805	0,28156	0,596908
Group*Batch	124,197	2	62,099	0,70487	0,496710
Error	8457,472	96	88,099		

Hatching

Larvae colour

Compared to the eggs, the colouration of the larvae was more distinguished. Larvae from batch 2 had stronger pigmentation compared to larvae from batch 1 which resulted in a blackish colour, batch 1 larvae appeared light brown in colour (Figure 13). This pigmentation difference was observed at hatch as well as after two weeks.

Figure 13: Two week old lumpsucker larvae, left batch 1 and right batch 2.

Table 9: Overview of d°, days post fertilization (DPF) and days post hatching (DPH) for start, peak and end of hatching for all temperature regimes.

	Start		Peak (50 %)		End	
	d°	DPF	d°	DPH	d°	DPH
Warm	278,6	28	308,4	3	368,1	9
Gradient	279,9	35	279,9	0	350	7
Cold	285	63	301,3	3	356,2	13

Hatching started at 278,6 d° 28 DPF in the warm water group, which reached a hatching peak (50 % of total hatching) at 3 DPH, and ended at 9 DPH. The gradient group started hatching at 279,9 d° at 35 DPF and reached the hatching peak the same day with almost 80 % of all larvae hatching. Hatching ended after 7 days at 350 d° in the gradient group. At 63 DPF hatching started in the cold group at 285 d°. The cold group reached the hatching peak at 3 DPF and the hatching lasted until 13 DPH (Table 9).

The distribution of hatching differed between groups, in the gradient group most larvae hatched the first day. Although both the warm and cold group had a hatching peak at 3 DPH, it was less pronounced in the cold group which also had the longest hatching period. The

hatching distribution is displayed in Figure 15.

The average hatching percent was highest in the gradient group and lowest in the cold group (Figure 14). A Two-Way ANOVA showed that there was a statistical significant difference in hatching percentage between the temperature groups (Table 10).

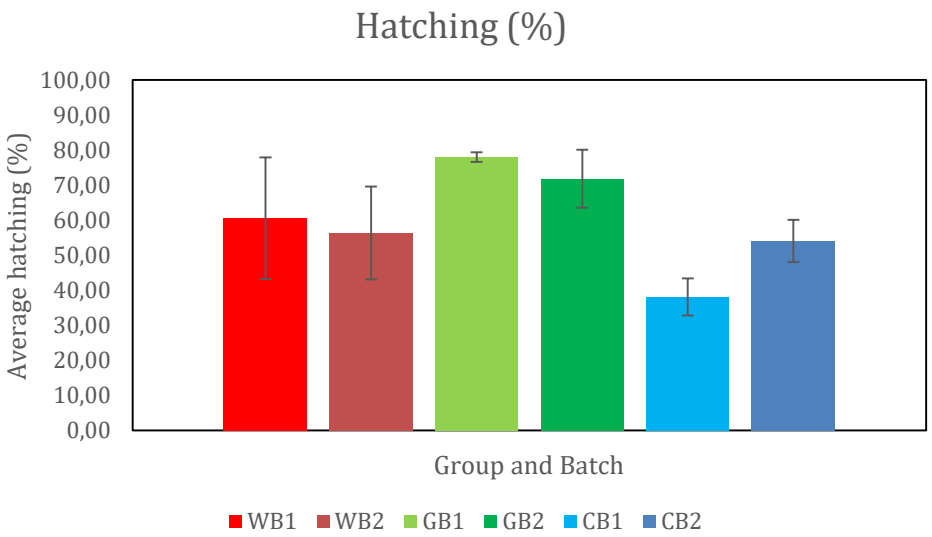


Figure 14: Average hatching percent for both lumpsucker batches and all temperature regimes W=warm, G=gradient and C=cold B1= batch 1 B2= batch 2.

Table 10: Two-Way ANOVA, Hatching %

	SS	*df*	*MS*	*F*	*p*
Intercept	64397,43	1	64397,43	211,0263	0,000000
Batch	16,02	1	16,02	0,0525	0,822645
Group	2507,52	2	1253,76	4,1085	0,043731
Batch*Group	451,41	2	225,71	0,7396	0,497845
Error	3661,96	12	305,16		

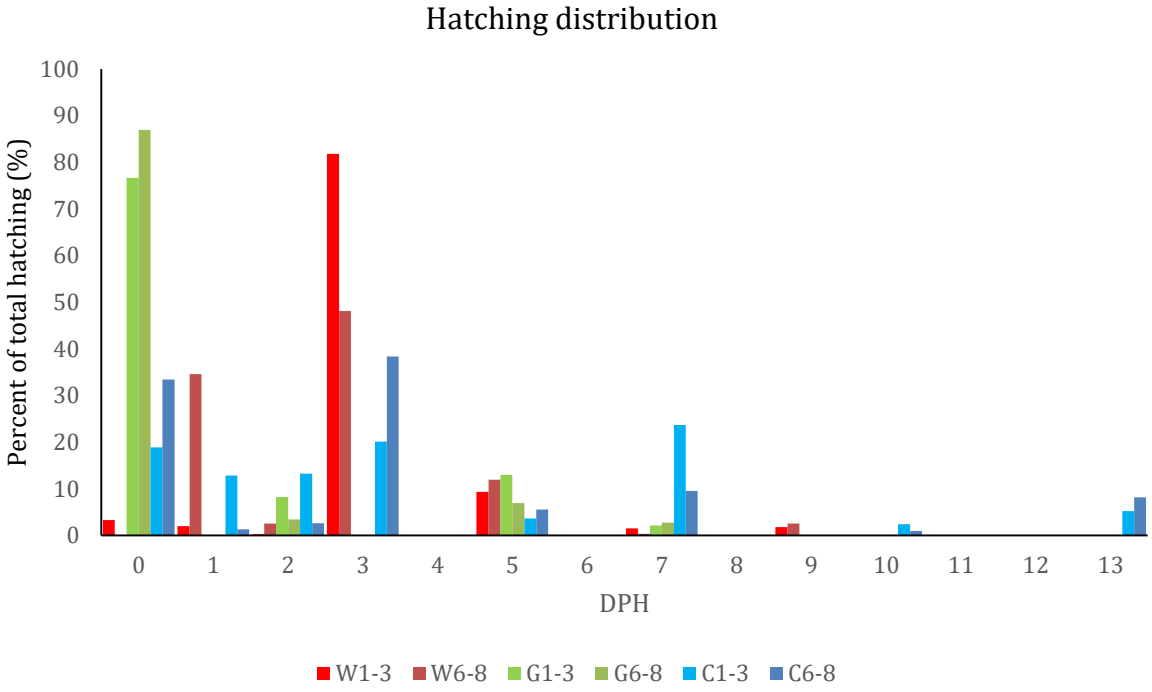


Figure15: Percent of total hatching for each batch of lumpsucker larvae from all temperature regimes, distributed over days post hatching W=warm, G=gradient and C=cold 1-3= batch 1, 6-8= batch 2.

Larvae mortality

The larvae mortality was lower during the peak of hatching for all groups. The gradient group had low mortality the first day, during peak hatching, and higher later in the hatching period. Both the warm and cold groups had higher mortality before and after the hatching peak (Figure 16).

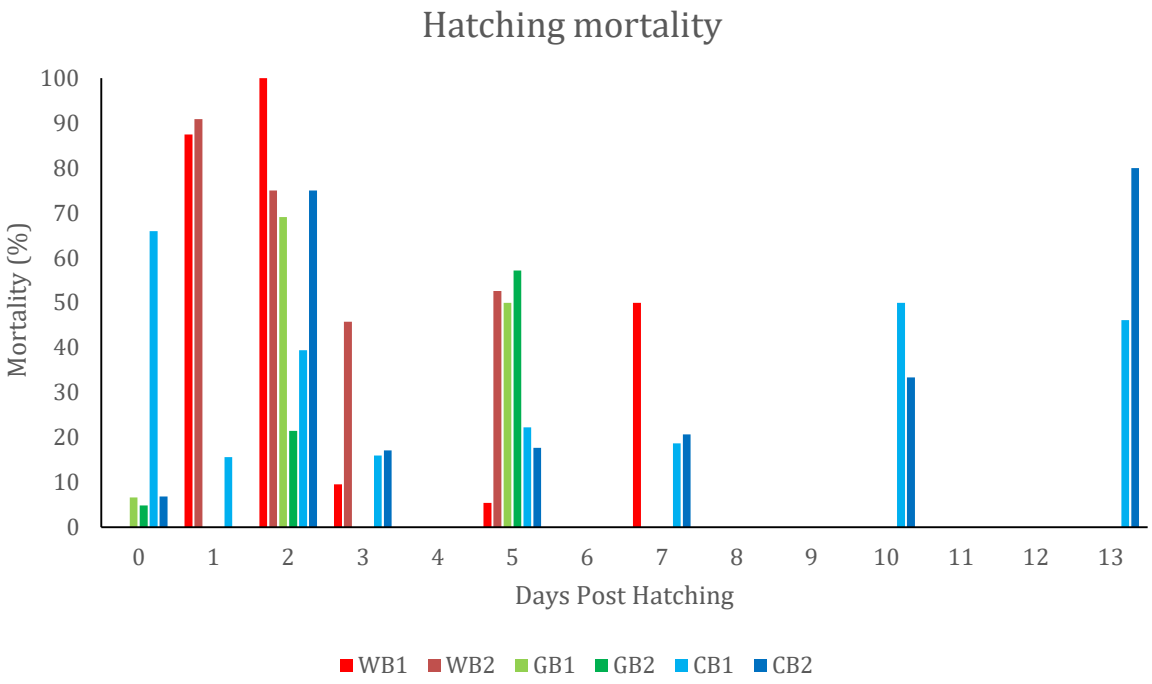


Figure 16: Larvae mortality in percent, of lumpsucker at hatching for each day post hatching W=warm, G=gradient and C=cold B1= batch 1 B2= batch 2.

The average hatching mortality was highest in batch 2 from the warm group and lowest in batch 2 from the gradient group (Figure 17). There was a statistical significant difference in mortality at hatching between groups (Table 11), for the two weeks old larvae however, there was not (Table 12).

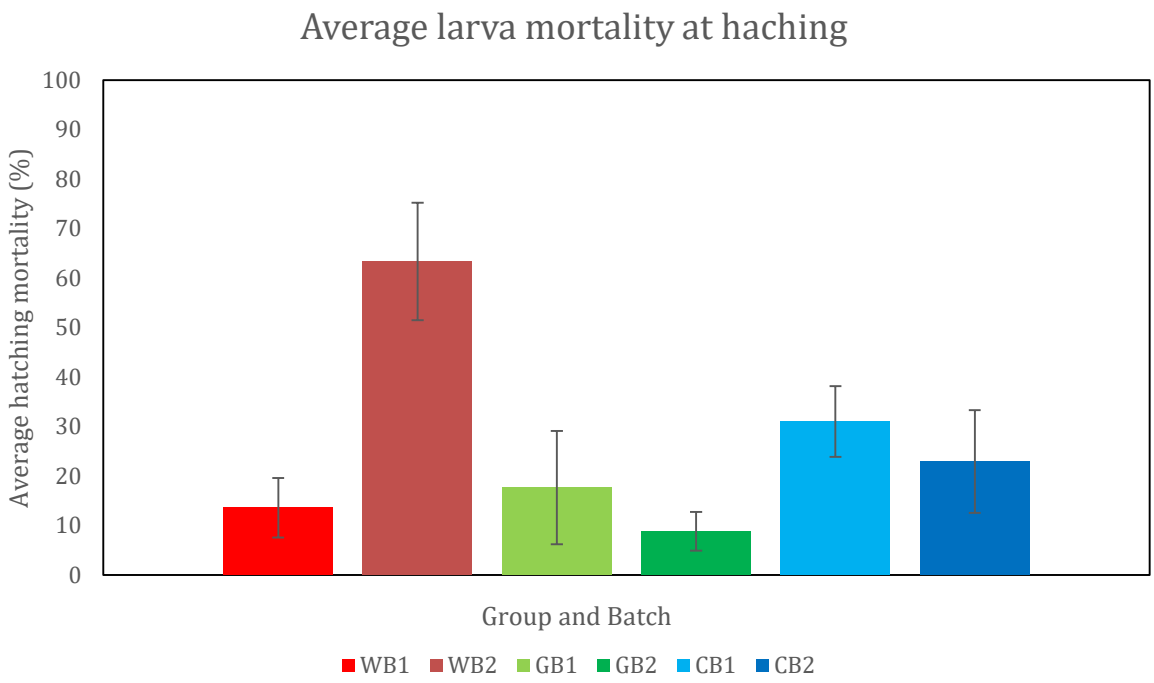


Figure 17: Average lumpsucker larvae mortality at haching W=warm, G=gradient and C=cold B1= batch 1 B2= batch 2.

Table 11: Two-Way ANOVA, Larvae mortality (hatching)

	SS	*df*	*MS*	*F*	*p*
Intercept	12379,10	1	12379,10	51,24428	0,000012
Batch	540,91	1	540,91	2,23915	0,160386
Group	1914,56	2	957,28	3,96274	0,047713
Batch*Group	3390,15	2	1695,07	7,01689	0,009591
Error	2898,84	12	241,57		

Table 12: Two-Way ANOVA, Larvae mortality (two weeks old)

Effect	*SS*	*df*	*MS*	*F*	*p*
Intercept	1351,473	1	1351,473	7,876081	0,015854
Group	389,293	2	194,646	1,134356	0,353817
Batch	13,277	1	13,277	0,077373	0,785624
Group*Batch	241,613	2	120,807	0,704033	0,513914
Error	2059,105	12	171,592		

Larvae measurements

During hatching, larvae from the cold temperature regime were longest, had highest bodies and were heaviest, while larvae from the warm regime were shortest lowest and lightest. The gradient group larvae had the largest yolk-sac, while the cold group larvae had the smallest. There was statistical significant difference in length, body height, yolk-sac size and weight between the temperature groups, and the replicates. Body height and weight was statistically different between batches as well. Table 13 summarises averages in measurements of the larvae at hatching and figure 18-21 displays the development over time.
The development of the dorsal fin was only noted in the warm and gradient groups. The warm group had statistically significantly higher occurrence of bended tail, spine damage, deformities and other body damages compared to the gradient and cold group. A summary can be found in Table 14.

Table 13: Summary of average; length, height and weight for batch and temperature regime in newly hatched lumpsucker larvae W=warm, G=gradient and C=cold B1= batch 1 B2= batch 2.

Batch			Height		Yolk-sac		Weight	
Group	Length (mm)	SD	(mm)	SD	(mm)	SD	(mg)	SD
WB1	5,70	0,66	1,02	0,14	1,14	0,08	4,70	0,71
WB2	5,11	0,88	0,91	0,14	1,10	0,08	4,17	0,73
W	5,33	0,85	0,95	0,14	1,12	0,08	4,37	0,75
GB1	5,67	0,71	1,00	0,14	1,14	0,09	4,74	0,76
GB2	5,76	0,52	1,03	0,14	1,16	0,09	5,07	0,78
G	5,71	0,64	1,02	0,14	1,15	0,08	4,88	0,79
CB1	5,91	0,83	1,02	0,14	1,09	0,08	5,27	0,81
CB2	6,32	0,51	1,14	0,14	1,13	0,09	5,84	0,82
C	6,11	0,72	1,08	0,14	1,11	0,09	5,55	0,84

Table 14: Summary of average occurrence of; dorsal fin, tail bend, spine damage, deformities, body and body damage for batch and temperature regime in newly larvae.

Group/Batch	Dorsal fin (%)	Tail bend (%)	Spine damage (%)	Deformed (%)	Damaged (%)
WB1	39,51	30,86	20,99	14,81	4,94
WB2	8,27	53,38	43,61	68,42	37,59
W	20,09	44,86	35,05	48,13	25,23
GB1	13,51	12,16	4,73	24,32	3,38
GB2	1,87	12,15	0,93	10,28	0,93
G	8,63	12,16	3,14	18,43	2,35
CB1	0,00	17,00	12,00	23,00	7,00
CB2	0,00	5,05	4,04	8,08	1,01
C	0,00	11,06	8,04	15,58	4,02

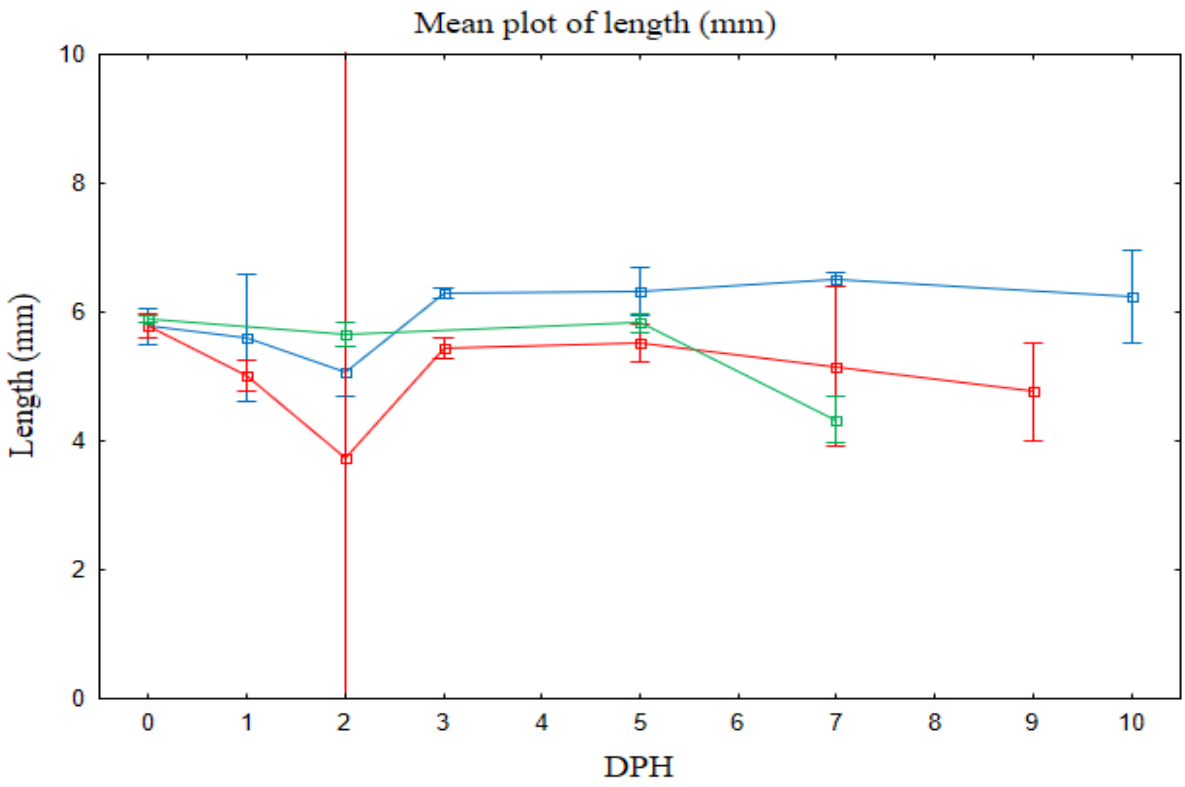


Figure 18: Mean plot of length for lumpsucker larvae during hatching, cold (blue) gradient (green) and warm (red) temperature regime.

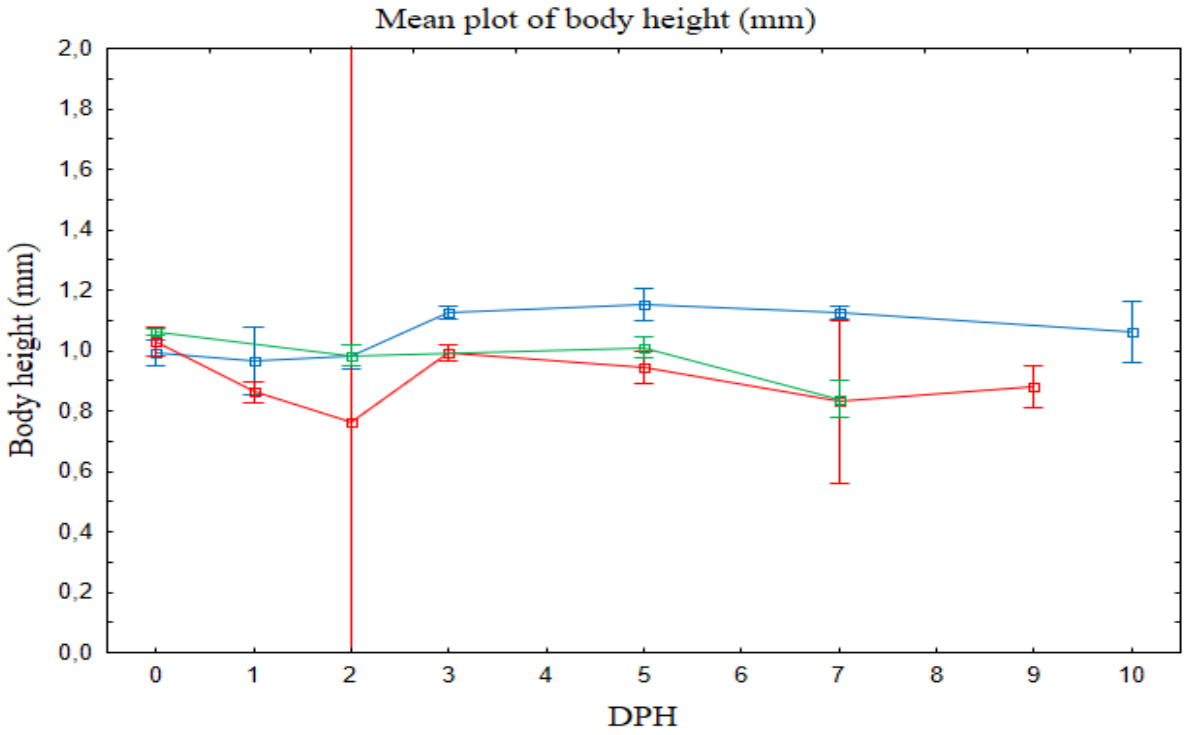


Figure 19: Mean plot of body height for lumpsucker larvae during hatching, cold (blue) gradient (green) and warm (red) temperature regime.

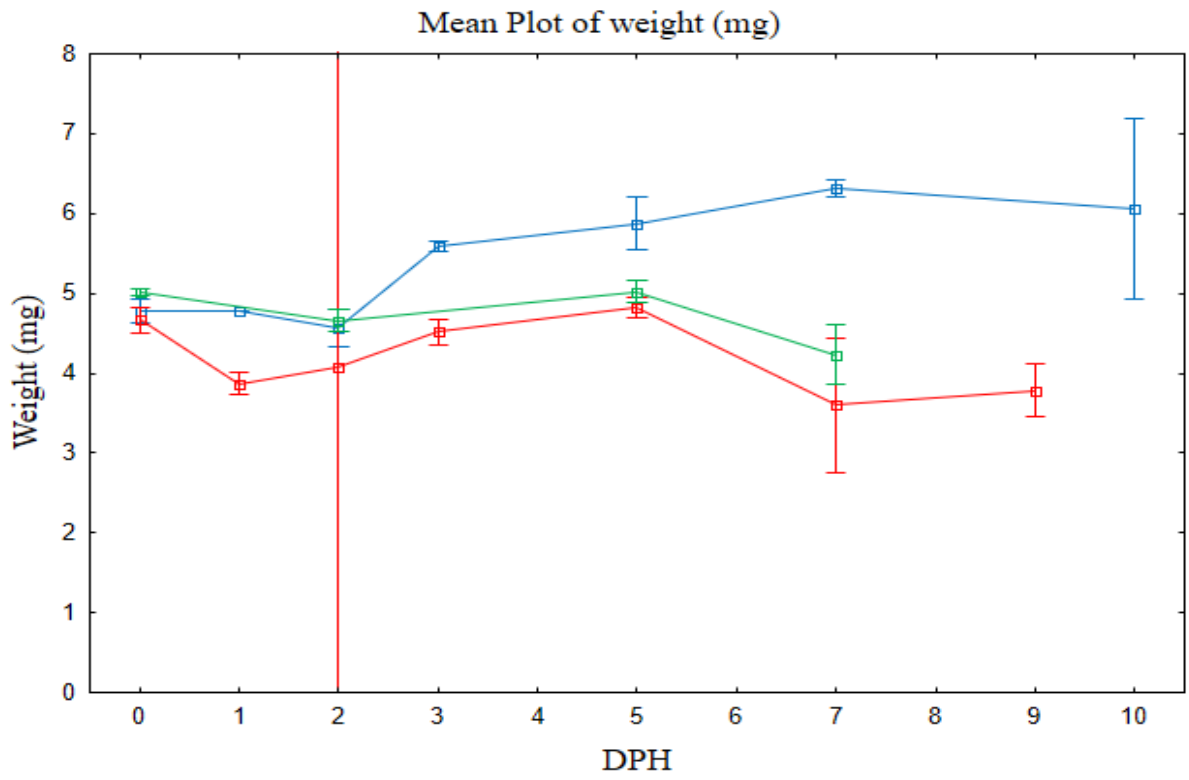


Figure 20: Mean plot of weight for lumpsucker larvae during hatching, cold (blue) gradient (green) and warm (red) temperature regime.

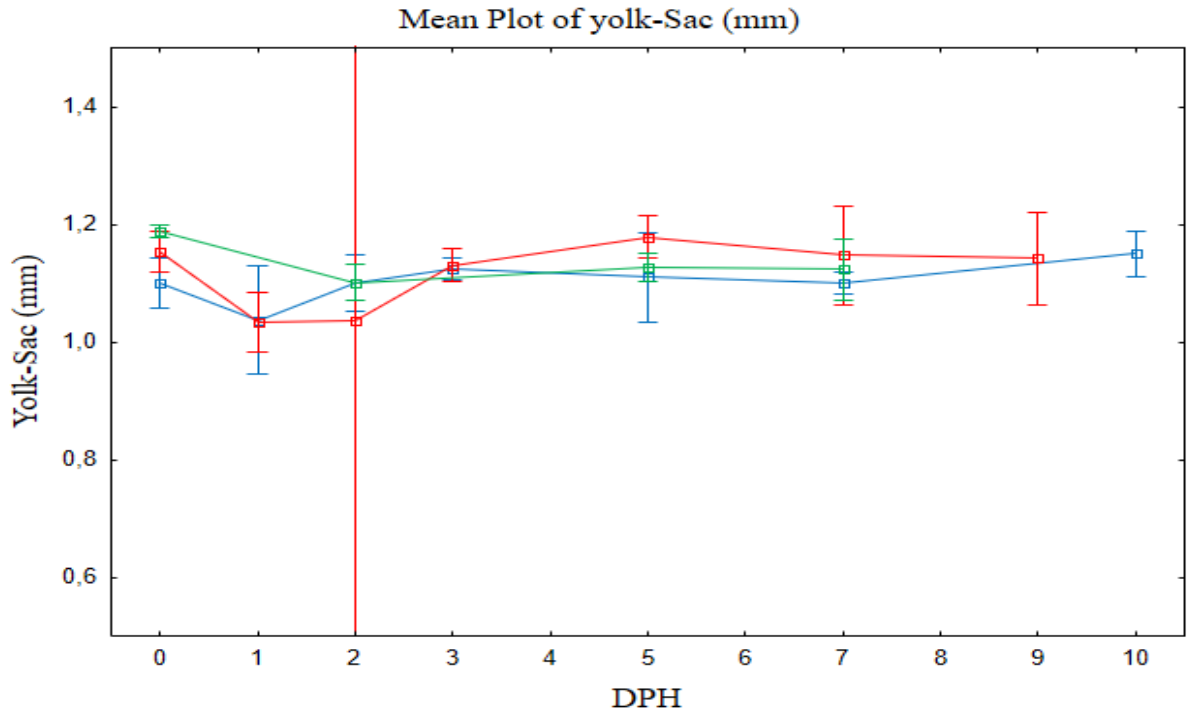


Figure 21: Mean plot of yolk-sac size for lumpsucker larvae during hatching, cold (blue) gradient (green) and warm (red) temperature regime.

There were statistical significant differences in length, body height and weight between temperature groups and batches for the 2 weeks old larvae. Between replicates there was also a difference in weight and height.
There was not found a statistical difference in tail bend, spine damage, deformities nor body damage between the groups in the two weeks old larvae.

Results from the statistical tests that were carried out on the larvae measurements data can be found in appendix II.

Table 15: Summary of average; length, height and weight of two weeks old lumpsucker larvae from the two batches and various temperature regimes W=warm, G=gradient and C=cold B1= batch 1 B2= batch 2.

Group/Batch	Length (mm)	SD	Height (mm)	SD	Weight (mg)	SD
WB1	6,39	0,56	1,14	0,10	6,74	0,79
WB2	6,49	0,66	1,17	0,09	7,40	0,72
W	6,44	0,61	1,16	0,08	7,07	0,56
GB1	6,35	0,28	1,11	0,08	6,39	0,60
GB2	6,49	0,12	1,19	0,08	7,04	0,61
G	6,42	0,23	1,15	0,08	6,71	0,62
CB1	6,62	0,18	1,08	0,08	7,34	0,63
CB2	6,73	0,28	1,17	0,08	8,31	0,64
C	6,67	0,24	1,12	0,08	7,82	0,65

Table 16: Summary of average occurrence of; tail bend, spine damage, deformities, body and body damage of two weeks old lumpsucker larvae from each batch and temperature regime W=warm, G=gradient and C=cold B1= batch 1 B2= batch 2.

Group/Batch	Tail bend (%)	Spine damage (%)	Deformed (%)	Damaged (%)
WB1	9,68	1,61	1,61	3,23
WB2	3,28	0,00	6,56	4,92
W	6,50	0,81	4,07	4,07
GB1	1,61	0,00	0,00	3,23
GB2	1,67	0,00	0,00	0,00
G	1,64	0,00	0,00	1,64
CB1	8,33	1,67	0,00	0,00
CB2	15,00	1,67	3,33	0,00
C	11,67	1,67	1,67	0,00

Histology

The organ- and tissue histomorphology of *C. lumpus* is relatively mature. A yolk rest is still present, though. The eyes are heavily pigmented and appear functional, mouth and total digestive system well developed with folded and differentiated mucosa. The liver is large with vacuolated hepatic cells, pancreatic tissue and kidneys present and gill development has been initiated. Numerous mucous cells characterize the skin of *C. lumpus* and the ventral sucker is well developed.

No difference between the histology of larvae from the various temperature regimes were registered, neither in the newly hatched larvae nor the two weeks old. In the two weeks old larvae; there was little or no yolk left, the intestine appeared slightly more expanded and the gill filaments appeared a bit longer. Otherwise the histomorphology did not deviate much from the newly hatched larvae. Food particles were noted in the intestines of larvae from all temperature groups for both newly hatched and two weeks old larvae.

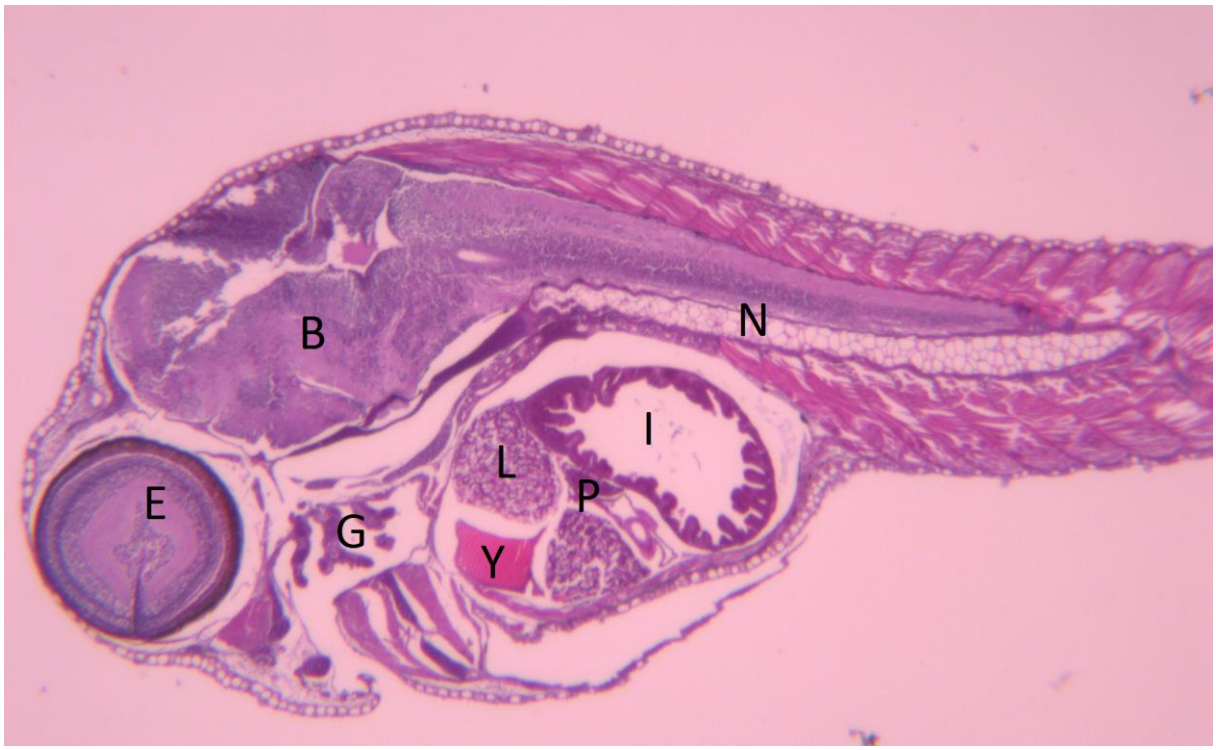


Figure 22: Longitudinal section of a newly hatched lumpsucker larva from gradient regime with; brain (B), eyes (E), gills (G), intestine (I), liver (L), notochord (N), pancreas (P) and yolk-sac (Y).

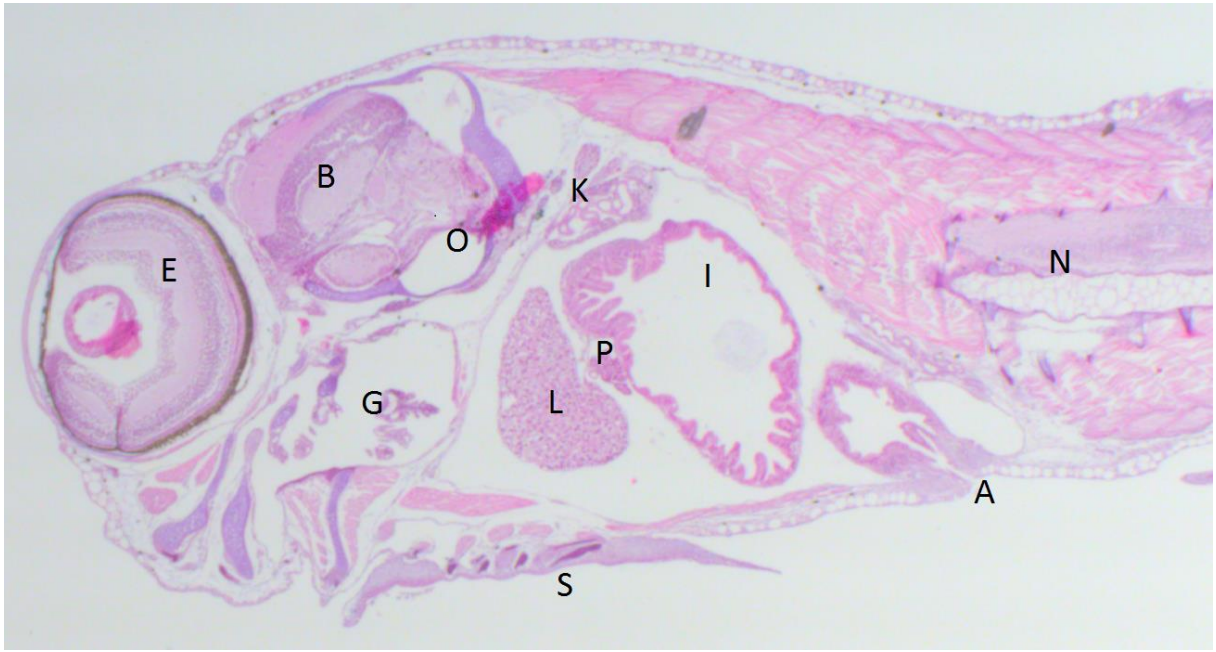


Figure 23: Longitudinal section of a two weeks old lumpsucker larva from gradient regime, anus (A) brain (B), eyes (E), gills (G), intestine (I), liver (L), kidney (K), notochord (N), Otocyst (O), pancreas (P) and suction-disc (S).

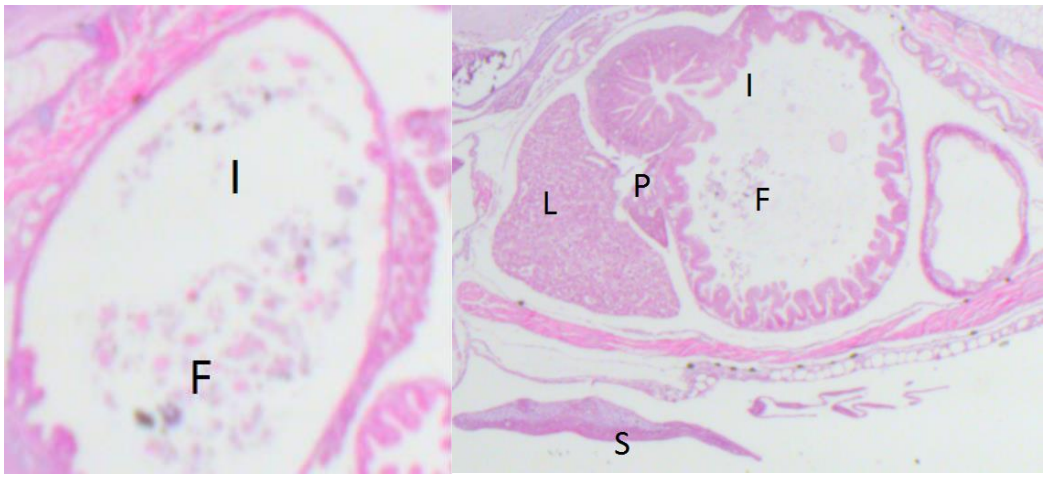


Figure 24: Left: Intestine (I) with food particles (F), from a 2 weeks old lumpsucker larvae from the warm regime. Right: Longitudinal section of a two week old larvae from the cold regime with; intestine (I), food particles (F), liver (L) pancreas (P) and sucktion-disc (S).

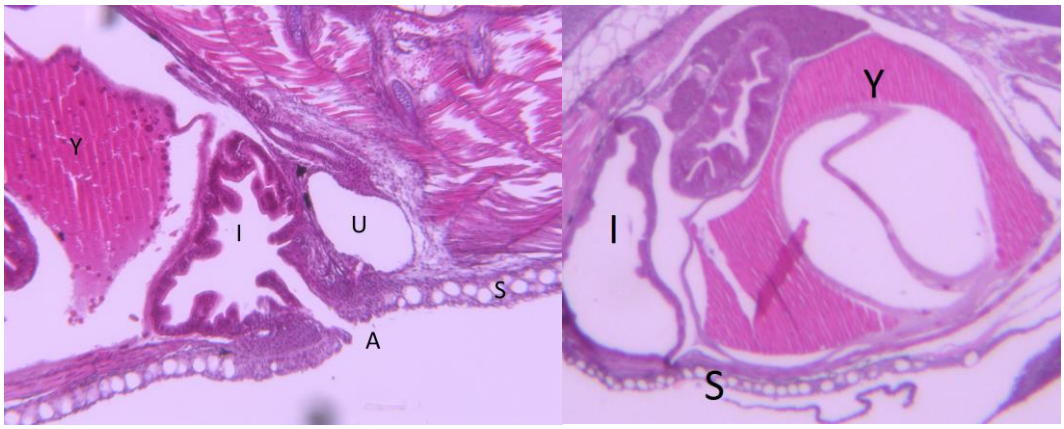


Figure 25: Left: Anus (A), Intestine (I), skin with mucous cells (S) urine bladder (U) and yolk-sac (Y) from newly hatched lumpsucker larvae from the gradient regime. Right: Intestine (I) skin with mucous cells (S) and yolk-sac (Y) from newly hatched larvae from the cold regime.

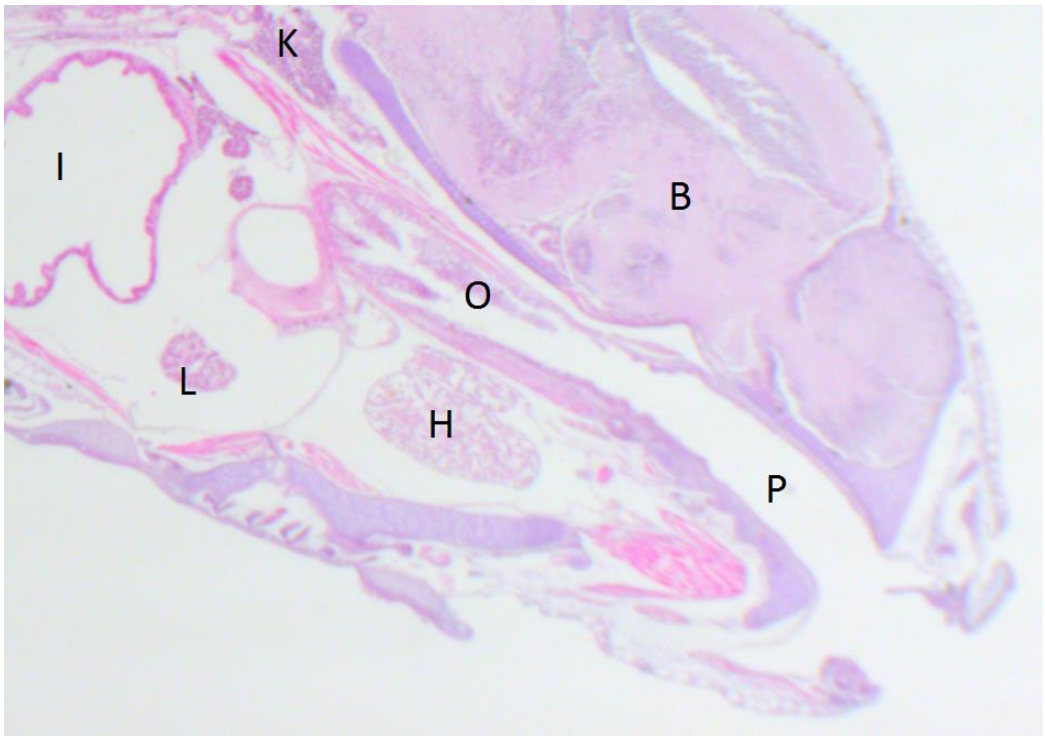


Figure 26: Longitudinal section of a two weeks old lumpsucker larva from the warm regime with: brain (B), heart (H), Intestine (I), kidney (K), liver (L) oesophagus (O), and pharynx (P).

Discussion

Egg colour and larval pigmentation

The colour of the eggs both from batch 1 and batch 2 was orange with batch 2 been slightly darker. The lumpsucker has as mentioned earlier a wide variety in egg coloration and orange is a common colour (Andriyashev 1964; Collins, Cox & Anderson, 1922; 1976; Davenport, 1985; Davenport & Thorsteinsson, 1989; Moen & Svensen, 2004). There is to my knowledge no scientific documentation that shows a correlation between egg color and egg quality. There was not a statistically significant difference in egg mortality or hatching percentage between the two batches in this experiment.

It was very interesting to see that there was a much clearer difference in coloration of the larvae of the two batches. The larvae from batch 2 were much more pigmented and therefore darker than the larvae from batch 1. This could be an indication that egg and larva colour is not linked, however there was no analysis done with regards to the color of the eggs or larvae in this experiment. The temperature did not seem to effect the colour of the larvae, as larvae from all temperature groups had similar colour as the rest of the larvae from each batch.

Egg size and numbers

The size of the eggs was within the expected normal range, with has been reported to between 1,8-2,6 mm (Andriyashev 1964; Collins, Cox & Anderson, 1922; 1976; Davenport, 1985; Davenport & Thorsteinsson, 1989; Moen & Svensen, 2004). The average egg diameter was 2,23 mm (±SE 0,0049) for batch 1 and 2,28 mm (±SE 0,0045) for batch 2. The difference in egg size resulted in an unequal number of incubated eggs from the two batches. It would have been ideal to count exactly the same number of eggs in each incubator, however this proved to be much too time-consuming when the experiment started, and could have have resulted in a difference in incubation time between the incubators of up to several hours.

Even if the number of eggs incubated in batch 2 was slightly higher than in batch 1, I thought it was a fair comparison. This is because the number of eggs or larvae were not directly compared but rather a ratio, for example hatching percent. In addition, the number of eggs in each incubator is high and thus provides a large sample size which provides more certainty.

If in a future experiment of this kind, the exact number of eggs are to be counted, one should be prepared with sufficient manpower to do the job efficiently.
The attempt to separate the eggs from one another was not successful. Keeping 200 eggs separated from each other in a petri-dish while slowly applying saltwater proved to be a difficult task. The separation would have made removing individual eggs easier, but it made cleaning the incubators much more difficult. Making a single layer disc of all eggs together would have been better for cleaning and more consistent between the incubators, as how many eggs that were stuck together or were individual varied somewhat between the incubators.

Temperature

The temperature regulation throughout the experiment seems to have been successful. The seawater used in the warm group had an average temperature of 9,95°C with a minimum of 9,5°C and a maximum of 10,40°C. The cold group started around 4°C and rose to just under 6°C at the end of the incubation. The gradient group had similar temperature to the cold and warm groups when it was running on the same water supply. The increase of approximately 1,5°C each day from 4°C to 10°C was also successful. After the desired temperature was found by using a mixture of cold and warm water the temperature was stable after a few minutes.
Throughout the experiment the temperature in general was very stable at the desired level. The temperature in replicate incubators showed only minor differences when measured. However, if there was a stop in the water-flow through an incubator, the temperature of the still water would rise because of the warmer room temperature, in particular the cold water as the temperature difference was greater. There were some water-flow failures during the experiment, however, the water flow was checked several times daily and corrected if needed. This was no a major problem, and the results from the temperature measurements indicate that the temperature of the water during incubation and post hatching was overall stable.

Oxygen saturation

The oxygen measurements that were carried out throughout the experiment shows that the water was properly saturated with oxygen from start to finish. There is a slight variation in the saturation, but that is true for all temperature groups. More importantly, oxygen saturation was never observed to be critically low.

Egg development

Eggs in the warm incubation group was the fastest to reach hatching; 28 DPF; and the cold group was the slowest at 63 DPF. This was expected as it's known that egg incubation time is slowed down with lower temperature (Hu et al, 2015; Mueller et al, 2015). However, the development rate of the eggs was very similar in all temperature groups in relation to number of day degrees, as no substantial differences were registered in the samples taken during incubation at similar day degrees. It should be noted that egg samples were only taken every second or third day. Because of the difference in temperature regimes between groups, exact comparisons could only be made at similar number of day degrees.

Egg mortality

There was a statistical significant difference in egg mortality between the temperature regimes, with the warm group having the lowest and the cold group the highest total mortality. There was no statistically significant difference in egg mortality between the egg batches. It was surprising that the egg mortality was so high in the cold group, as ambient water was used and the experiment took place in a period when the lumpsucker spawn naturally (Andriyashev 1964; Davenport, 1985; Moen & Svensen 2004; Durif, 2015). One possible explanation for the high egg mortality in the cold group could be that the water has been too cold at some times, as the lowest temperature recorded was 3,8°C. Collins (1978) reported that lumpsucker eggs incubated at an average temperature of 3,8 °C degrees failed to hatch at all. The lumpsucker lays its eggs in shallow water (Andriyashev 1964; Davenport, 1985; Moen & Svensen 2004; Durif, 2015), where temperature stratification can take place if conditions are right. Another possibility is that incubation time may be a crucial factor for

survivability. The longer the incubation time is the greater the chance for infection or attack by fungi, and bacteria. Although the cold group had much less problems with filth and fouling compared to the groups running on warm water, towards the end of the experiment the accumulation was starting to be noticeable.

Hatching

Although the time of hatching varied much in days' post fertilization among the three temperature regimes, 28, 35 and 63 for the groups warm, gradient and cold respectively, hatching started at approximately 280-day degree in all groups. However, there was a large variation in how synchronized the eggs hatched. The warm and cold groups both had a slow start, and reached 50% hatching at 3 DPH. The cold group also had the longest hatching period of all the temperature groups ending at 13 DPH compared to 9 DPH for warm and 7 DPH for the gradient group. The gradient group differed from the other groups as most of the eggs hatched during the first day of hatching, and this happened in all incubators of both batches. It is possible that the change in incubation temperature the gradient group was exposed to influenced the synchronization of egg hatching.

Larvae mortality

There was a statistically significant difference in larvae mortality at hatching between the temperature regimes, the highest mortality was registered in the warm group and the lowest in the gradient group. Mueller et al (2015) also found increased mortality with increased temperature on lake whitefish (*Coregonus clupeaformis*) and Greffen et al (2006) on cod (Gadus morhua). There was some variation between the batches, particularly in the warm group, however they were not significantly different.
The larvae mortality in the two weeks old larvae was not statistically significantly different between temperature groups or between batches. The mortality was on average lower in the warm group and higher in cold and gradient groups. This could have been due to a higher load of microorganisms, because the larvae that were found dead in these containers were stuck in threads of bacteria or fungi.

Larva measurements

Measurements of the lumpsucker larvae showed variation in length, weight, body height and yolk-sac size. There was statistically significant difference in all these measurements between replicates, batches and temperature groups but not at every DPH (appendix II). Yolk-sac size was only statistically significantly different at 0 DPH, with the cold group having significantly smaller size. The overall impression of these data is that larvae from the cold temperature regime is longest, heaviest and thickest, while those from the warm regime are shortest, lightest and thinnest. The analyzes done on the overall data, not considering hatching time, is consistent with this. Smaller larvae as a result of higher temperature has been found in lake whitefish (Mueller et al, 2015)

One difference was observed in larvae development between the incubation regimes; the separation of the dorsal fin from the larval-finfold was only observed on newly hatched larvae from the warm and gradient groups.

There was some variation in tail shape, spine damage, deformities and body damage within the batches and replicates, however, larvae from the warm regime had overall statistically significantly higher occurrence of everyone. Prevalence of malformed larvae with severe vertebral curvature was found by Fitzimmons & Perutz (2006) to significantly increase with egg incubation temperature on cod (*Gadus morhua*).
This suggests that incubation temperature has an important influence on the quality of the lumpsucker larvae that hatch, and that the low temperature of 4-5°C early in the incubation period, compared to a high of 10°C, is more beneficial to the final quality of the larvae. The higher presence of malformed larvae in the warm group is probably the explanation of why the mortality of the larvae from the warm group was higher.

The larvae that was fed for two weeks also had statistically significantly differences in length, body height and weight. Again it was the cold group that stood out from the other two being heaviest and longest, but was now thinnest of the three groups. It seems that larvae from the cold group increased more in length and less in body height compared to those from the warm and gradient group.

Histology

No organ, tissue defects or developmental differences could be revealed between larvae from the three temperature regimes from the histological slides prepared from newly hatched larvae or the two weeks old larvae. Lumpsucker larvae are relatively well developed at hatch (Davenport, 1985; Timeyko, 1986). From newly hatched to two weeks old larvae a slightly more expanded and folded intestine was observed, otherwise changes appeared to be more related to size and shape of the larvae.

The egg samples taken showed a relatively similar development between all temperature groups during incubation, and this seems to be true also for the internal development judging by the results found in this experiment. It should be noted, however, that it is difficult to make similar, comparable histological sections of all samples and this can result in a restricted number of comparable slides.

Rig issues

During the experiment there were some issues with the experimental-rig that occurred. One issue that had to be kept an eye on was the water flow through the incubators. Air bubbles were sometimes stuck in the tubes or pipes of the rig and could influence the water flow. Possibility for water flow regulation at each incubator should be considered for future experiments of this kind.
Some larvae were caught on the outlet not able to get away. However, these larvae were likely deformed in some way as there was higher occurrence of larvae stuck on the water outlet in the warm group incubators. During the experiment, larvae that seemed to be in good condition were observed caught on the water outlet, and then able to swim away from it.

Conclusion

The incubation temperature seems to play an important role with regards to egg mortality and general quality of the lumpsucker eggs and larvae.

The lumpsucker larvae from the cold water regime were biggest, with very few abnormalities, both at hatch and after 2 weeks. However, the egg mortality and hatching success was lowest in the cold group, and had long incubation time. Mortality at hatching was also relatively high.

The eggs from the warm water regime had the lowest egg mortality and a reasonable hatching percent, however the larvae mortality was a bit high, at least in one of the batches. In addition, the warm water larvae showed the highest amount of abnormalities.

In conclusion low ambient seawater temperature 4-5 °C during early incubation period and then increasing to 10°C seem to be preferable as it had relatively low egg mortality, high hatching success, early and high hatching peak with low mortality, medium size and little abnormal body features (tail bend, spine damage, deformities and body damage). From a production perspective it might be best to produce lumpsucker larvae with this kind of incubation temperature, as it has good quality larvae and only takes a few days longer compared to the warm water.

Future research could be done to more exactly pinpoint when an increase in incubation temperature ideally should be implemented and how fast the temperature increase should be.

References

Andriyashev A.P., (1964) Fishes of the Northern Seas of the U.S.S.R. Israel Program for scientific Translations. :471-476.

Bergheim A., (2012) Recent growth trends and challenges in the Norwegian aquaculture industry. Latin American Journal of Aquatic Research, 50 (3):800-807.

Bjørn P.A., Finstad B. & Kristoffersen R., (2001) Salmon lice infection of wild sea trout and Arctic char in marine and freshwaters: the effects of salmon farms. Aquaculture Research, 32: 947-962.

Blacker R.W., (1983) Pelagic records of the lumpsucker, *Cyclopterus lumpus L*. J. Fish biol, 23: 405-417.

Brown J.A., Somerton D.C. & Methven D.A., (1992) Recent Advances in Lumpfish *Cyclopterus lumpus* and Ocean Pout *Macrozoarces americanus* Larviculture. Journal of the world aquaculture society, 23 (4): 271-276.

Burridge L., Weis J.S, Cabello F., Pizarro J. & Bostick K., (2010) Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. Aquaculture, 300: 7-23.

Collins M.A.J., (1976) The lumpfish (*Cyclopterus lumpus L.*) in Newfoundland waters. The Canadian Field-Naturalist, 90: 64-67.

Collins M.A.J., (1978) Experiments on the hatching period of the eggs of the lumpsfish *Cyclopterus lumpus L*. in Newfoundland waters. Le naturauste canadien, 105: 169-171.

Costello M.J., (2009) How sea lice from salmon farms may cause wild salmonid declines in Europe and North America and be a treat to fishes elsewere. Prodceedings of the Royal Society B, 276: 3385-3394.

Cox P. & Anderson M., (1922) A study of the lumpfish (*Cyclopterus lumpus* L.) Contributions to Canadian Biology and Fisheries, 1 (1): 1-20.

Daborn G.R, & Gregory R.S., (1982) Occurrence, distribution, and feeding habits of juvenile lumpfish, *cyclopterus lumpus L.* in the Bay of Foundy. Can. J. Zool., 61: 797-801.

Durif C., (2015) Rognkjeks/-kall. Havforskningsraporten, Fisken og havet, sarnummer 1-2015. Institute of Marine Research, 183.

Davenport J., (1985) Synopsis of biological data on the lumpsucker *Cyclopterus lumpus* (Linnaeus, 1758). FAO Fisheries Synopsis, 147.

Davenport J., & Thorsteinsson V., (1989) Observations on the colours of lumpsuckers, *Cyclopterus lumpus* L., J. Fish Biol. 35: 829-838.

Denholm I., Devine G.J., Horsberg T.E., Sevatdal S., Falland A., Nolan D.V. & Powell R, (2002) Analysis and management of resistance to chemotherapeutants in salmon lice, *Lepeophtheirus salmonis* (Copepoda: Caligidae). Pest Management Science, 58: 528-536.

Fitzsimmons S.D. & Perutz M., (2006) Efects of egg incubation temperature on survival, prevalence and types of malformations in vertebral column of Atlantic Cod (*Gadus morhua*) larvae. Bull. Eur. Ass. Fish Pathol, 26 (2): 80-86.

FKD, Fiskeri- og Kystdepartementet (2009) Strategi for en miljømessig bærekraftig havbruksnæring.

Greffen A.J., Fox C.J. & Nash R.D.M, (2006) temperature-dependent development rates of cod *Gadus morhua* eggs. Journal of Fish Biology, 69 :1060-1080.

Heuch P.A., Bjørn P.A., Finstad B., Holst J.C., Asplin L. & Nilsen F., (2005) A review of the Norwegian "National Action Plan Against Salmon Lice on Salmonids": The effect on wild salmonids. Aquaculture, 246: 79-92.

Hu F., Pan L., Gao F., Jian Y., Wang X., Li L., Zhang S. & Guo W., (2015) Effect of temperature on incubation period and hatching success of fat greenling (*Hexagrammos otakii* Jordan & Starks) eggs. Aquaculture Research, 2015: 1-5.

Imsland A.K., Reynolds P., Eliassen G., Hangstad T.A., Foss A., Vikingstad E. & Elvegård T.A., (2014a) The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). Aquaculture, 424-425: 18-23.

Imsland A.K., Reynolds P., Eliassen G., Hangstad T.A., Nytrø A.V., Foss A., Vikingstad E. & Elvegård T.A., (2014b) Notes on the behaviour of lumpfish in sea pens with and without Atlantic salmon present. J ethol, 32: 117-122.

Imsland A.K., Reynolds P., Eliassen G., Hangstad T.A., Nytrø A.V., Foss A., Vikingstad E. & Elvegård T.A., (2014c) Assessment of growth and sea lice infection levels in Atlantic salmon stocked in small-scale cages with lumpfish. Aquaculture, 433: 137-142.

Imsland A.K., Reynolds P., Eliassen G., Hangstad T.A., Nytrø A.V., Foss A., Vikingstad E. & Elvegård T.A., (2015) Assessment of suitable substrates for lumpfish in sea pens. Aquacult Int, 23: 639-645.

Jimenez D.F., Heuch P.A., Revie C.W. & Gettinby G., (2012) Confidence in assessing the effectiveness of bath treatments for the control of sea lice on Norwegian Salmon farms. Aquaculture, 344-349: 58-65.

Jonsson B. & Jonsson N., (2014) Early environment influences later performance in fishes. Journal of Fish Biology, 85: 151-188.

Kazuyuki S., Kurokura H. & Kasahara S., (1988) Changes in low temperature tolerance of the egg of certain marine fish during embryonic development. Comp. Biochem. Physiol, 91A (1): 183-187.

Lein I., Noble., Nergaard S. & Helland S., (2013) Evaluering av beiteeffektivitet hos berggylt i merd med stor laks. Nofima.

Moen F.E. & Svensen E., (2004), Marine fish & invertebrates of Northern Europe. KOM: 203, 490-492, 529-530, 536-541.

Moring J.R., (1989) Food habits and algal associations of juvenile lumpfish, *Cyclopterus lumpus* L., in intertidal waters. Fishery Bulletin, 87(1): 233-237.

Moring J.R. & Moring S.W., (1991) Short-term movements of larval and juvenile lumpfish, *Cyclopterus lumpus* L., in tidepools. Journal of Fish Biology, 38: 845-850.

Mueller C.A., Eme J., Manzon R.G., Somers C.M., Boreham D.R & Wilson J.Y., (2015) Emryonic critical windows: changes in incubation temperature alter survival, hatching phenotype, and cost of development in lake whitefish (*Coregonus clupeaformis*). J Comp Physiol B, 185: 315-331.

NFD, Det Kongelige Nærings-og Fiskeridepartement, (2015) Forutsigbar og miljømessig bærekraftig vekst i norsk lakse- og ørretoppdrett. Meld.St. 16.

Puvanendran V., Falk-Petersen I., Lysne H., Tveiten H., Toften H. & Peruzzi S., (2015) Effects of different step-wise temperature increment regimes during egg incubation of Atlantic cod (*Gadus morhua* L.) on egg viability and newly larval quality. Aquaculture Research, 46: 226-235.

Rombough P.J., (1996) The effects of temperature on embryonic and larval development. Global warming: Implications for freshwater and marine fish, ed. C.M Wood & D.G. McDonald, Cambridge University Press 1996: 177-223.

Skilbrei O.T., Bjørn P.A. & Vollset K.W., (2015) Hva gjør lakselus med laksefisk. Havforskningsraporten, Fisken og havet, særnummer 1-2015. Institute of Marine Research :24-25.

Skiftesvik A.B. & Nedreaas K., (2015) Leppefisk. Havforskningsraporten, Fisken og havet, særnummer 1-2015. Institute of Marine Research: 169-170.

Sunnanå K., (2009) Rognkjeks og rognkall. Kyst og havbruk 2.4 kystressurser. Institute of Marine Research: 95-96.

Timeyko V.N., (1986) The digestive systems of white sea cod, *Gadus morhua marisalbi* and lumpfish *Cyclopterus lumpus*, at different stages on ontogeny. Voprosy Ikhtiologii, 1: 103-112.

Torrissen O., Olsen R.E., Toresen R., Hemre G.I., Tacon A.G.J., Hardy R.W. & Lall S., (2011). Atlantic Salmon (*Salmo salar*): The "Super-Chicken" of the Sea?. Reviews in Fisheries science, 19(3): 27-278.

Treasurer J.W., (2002) A review of potential pathogens of sea lice and the application of cleaner fish in biological control. Pest Management science, 58: 546-558.

Appendix I

Lumpsucker egg development photographs.

A: Fertilized egg with perivitelline space (1 HPF, 0 d°).

B: 2-cell stage (7 HPF, 0 d°).

C: 4-cell stage (4,1 d°).

D: 8-cell stage (4,1 d°).

E: 16-cell stage (4,1 d°).

F: 64-cell stage (8 d°).

G: Morula (10,1 d°).

H: Blastula (20,1 d°).

I: Gastrula (29,2 d°).

J: Embryo with optic vesicle 49,8 d°).

K: Embryo with segmentation and compression of lipids (70,3 d°).

L: Embryo with otocysts and slightly more developed eye (89,4 d°).

M: Eye pigmentation and otoliths (117,3 d°).

N: Heartbeat, visible vein in yolk-sac (128,9 d°) and weak body pigmentation. (138,4 d°).

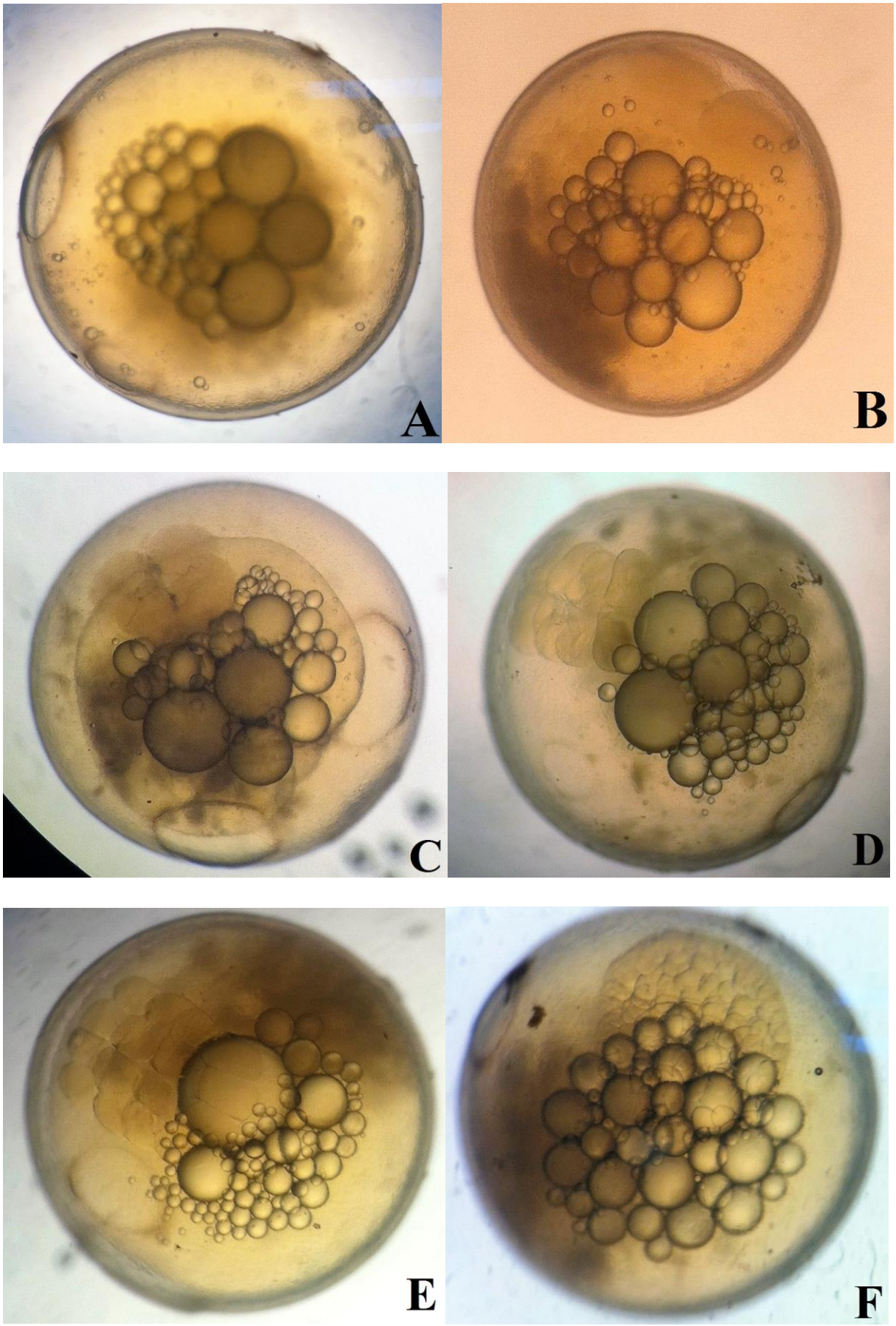
O: Spread in the yolk-sac vein (173,8 d°) growth in head (183,3 d°), mouth open and body growth (209,8 d°)

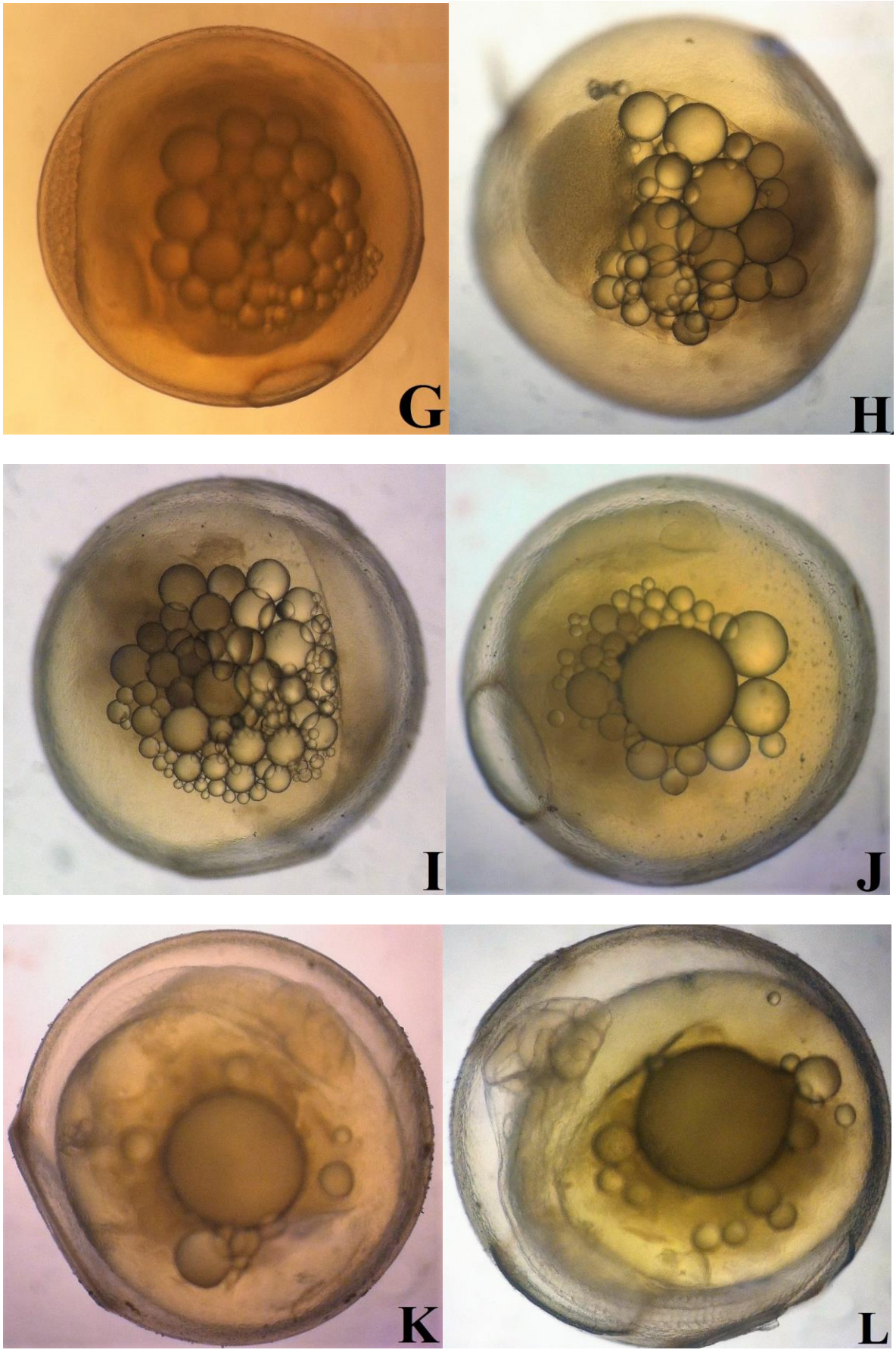
P: Large embryo, ready to hatch (278,6 d°).

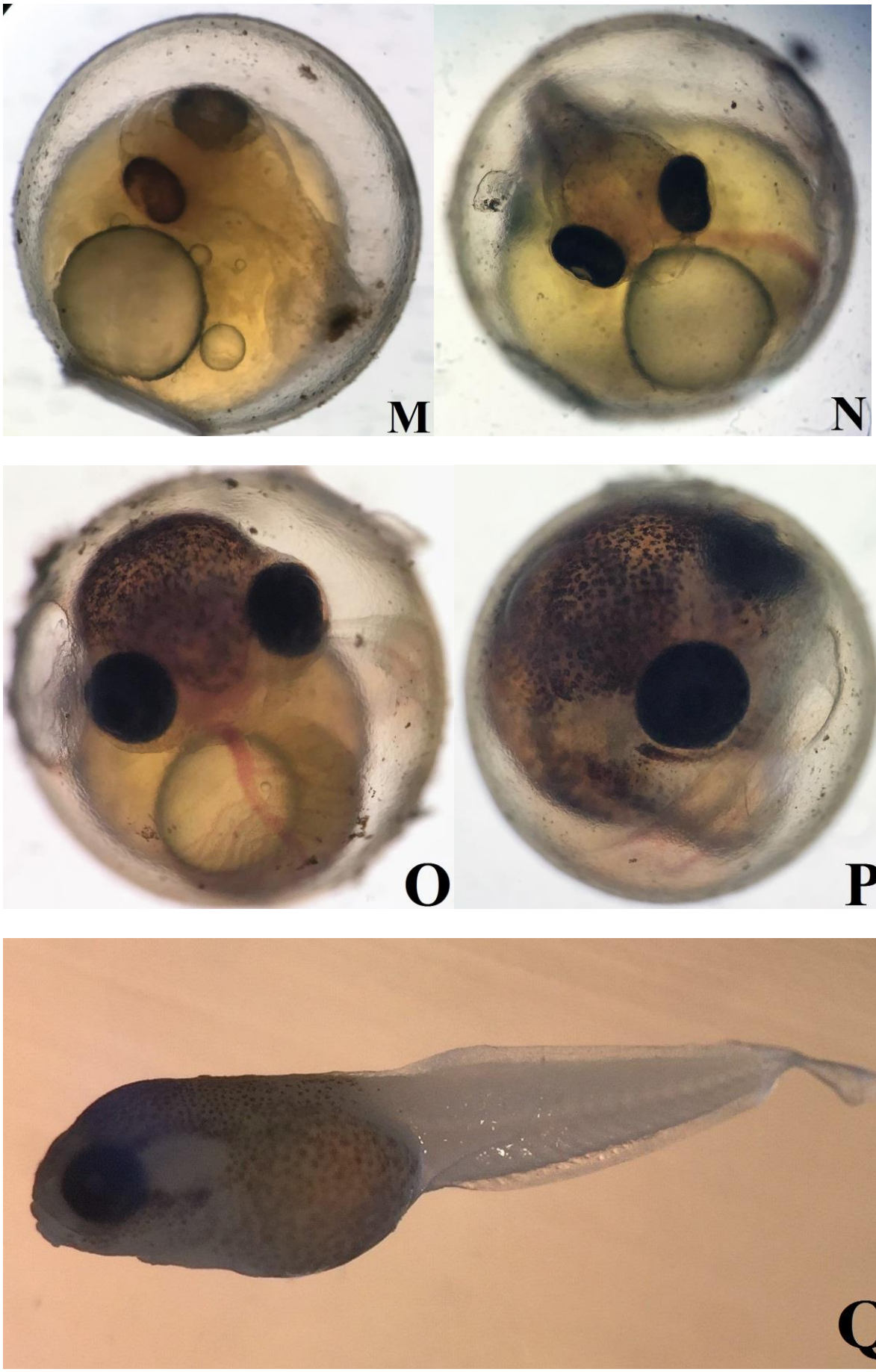
Q: Newly hatched larvae with larvae-finfold and clearly visible yolk-sac (278,6 d°).

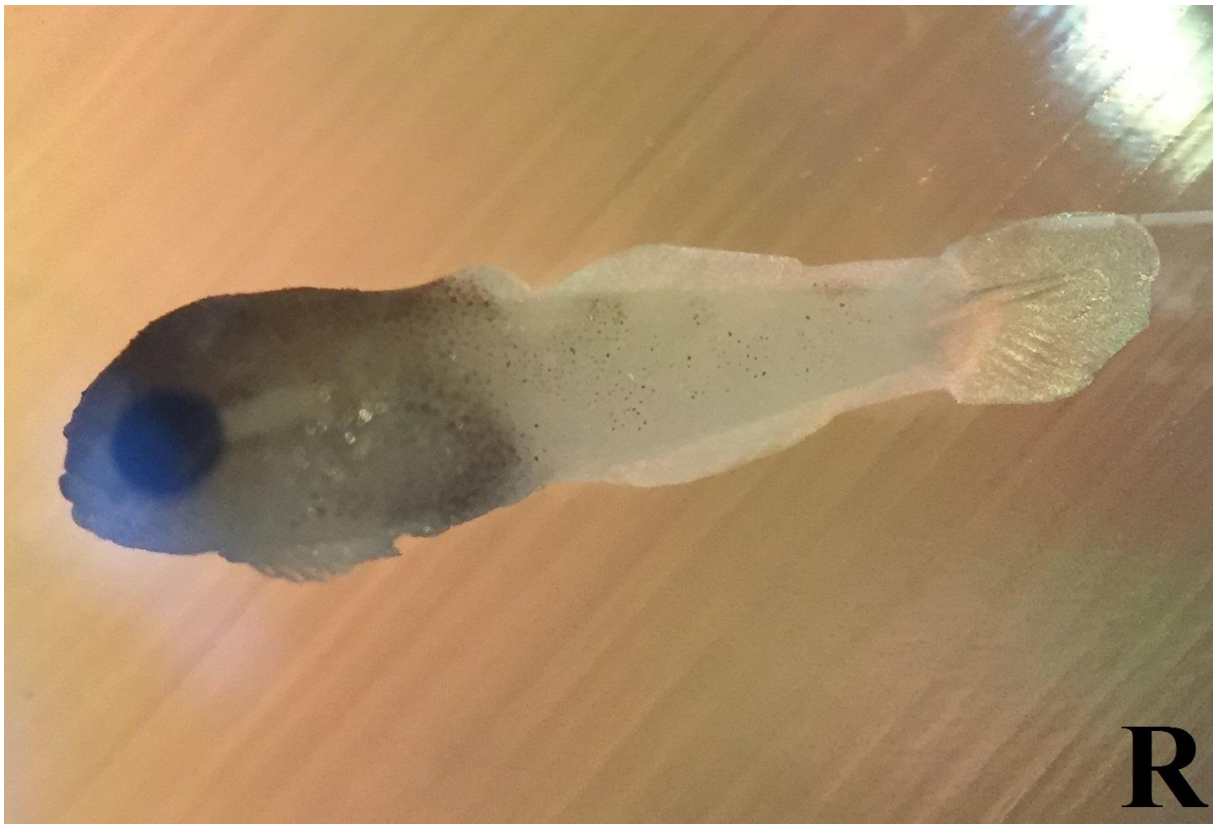
R: Slightly larger newly hatched larvae, with dorsal fin separation from larvae-finfold, less visible yolk-sac (308 d°).

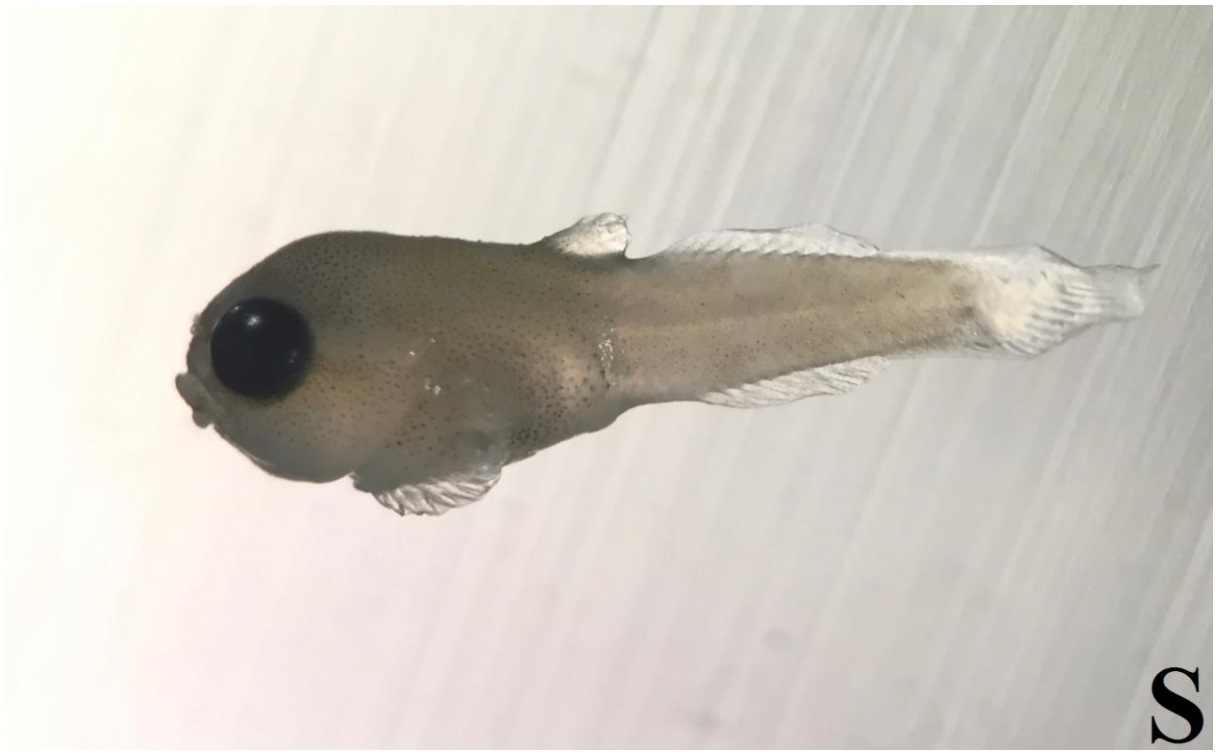
S: Two weeks old larvae, longer body shape and more distinct fins (420 d°).


A
B
C
D
E
F


G
H
I
J
K
L


M
N
O
P
Q


R


S

Appendix II

Height at hatching:

DPH: 0

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00501, df = 157,00
Include condition: DAY=0

Cell No.	GROUP	{1} (1,0319)	{2} (1,0619)	{3} (,99249)
1	WARM		0,132148	0,047755
2	GRADIENT	0,132148		0,001437
3	COLD	0,047755	0,001437	

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00501, df = 157,00
Include condition: DAY=0

Cell No.	BATCH	{1} (1,0191)	{2} (1,0834)
1	A		0,000009
2	B	0,000009	

Univariate Tests of Significance for Height (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0708
Include condition: DAY=0

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	59,54846	1	59,54846	11876,66	0,000000
GROUP	0,04275	2	0,02137	4,26	0,015743
REPLICATE(GROUP*BATCH)	0,28893	8	0,03612	7,20	0,000000
BATCH	0,12307	1	0,12307	24,55	0,000002
Error	0,78718	157	0,00501		

DPH: 1

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00862, df = 42,000
Include condition: DAY=1

Cell No.	GROUP	{1} (,86251)	{2} (,96380)
1	WARM		0,026038
2	COLD	0,026038	

Newman-Keuls test; variable Height (mm)
(Larvedata Klekket)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00862, df = 42,000
Include condition: DAY=1

Cell No.	BATCH	{1} (,96662)	{2} (,83831)
1	A		0,000226
2	B	0,000226	

Univariate Tests of Significance for Height (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0928
Include condition: DAY=1

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	21,18371	1	21,18371	2457,954	0,000000
GROUP	0,00073	1	0,00073	0,085	0,771926
REPLICATE(GROUP*BATCH)	0,13054	3	0,04351	5,049	0,004462
BATCH	0,05645	1	0,05645	6,550	0,014181
Error	0,36197	42	0,00862		

DPH: 2

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00875, df = 61,000
Include condition: DAY=2

Cell No.	GROUP	{1} (,76250)	{2} (,98332)	{3} (,97890)
1	WARM		0,000961	0,000485
2	GRADIENT	0,000961		0,939177
3	COLD	0,000485	0,939177	

Newman-Keuls test; variable Height (mm)
(Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00875, df = 61,000
Include condition: DAY=2

Cell No.	BATCH	{1} (,95648)	{2} (1,0184)
1	A		0,010945
2	B	0,010945	

Univariate Tests of Significance for Height (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0935
Include condition: DAY=2

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	16,40638	1	16,40638	1874,825	0,000000
GROUP	0,15043	2	0,07521	8,595	0,000515
REPLICATE(GROUP*BATCH)	0,38585	8	0,04823	5,512	0,000030
BATCH	0,00016	1	0,00016	0,019	0,891354
Error	0,53380	61	0,00875		

DPH: 3

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00802, df = 153,00
Include condition: DAY=3

Cell No.	GROUP	{1} (,99199)	{2} (1,1250)
1	WARM		0,000009
2	COLD	0,000009	

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00802, df = 153,00
Include condition: DAY=3

Cell No.	BATCH	{1} (1,0412)	{2} (1,0416)
1	A		0,978370
2	B	0,978370	

Univariate Tests of Significance for Height (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0896
Include condition: DAY=3

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	100,7705	1	100,7705	12564,77	0,000000
GROUP	0,1659	1	0,1659	20,69	0,000011
REPLICATE(GROUP*BATCH)	1,0896	8	0,1362	16,98	0,000000
BATCH	0,0302	1	0,0302	3,76	0,054215
Error	1,2271	153	0,0080		

DPH: 5

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01519, df = 94,000
Include condition: DAY=5

Cell No.	GROUP	{1} (,94550)	{2} (1,0107)	{3} (1,1529)
1	WARM		0,091950	0,000106
2	GRADIENT	0,091950		0,000450
3	COLD	0,000106	0,000450	

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01519, df = 94,000
Include condition: DAY=5

Cell No.	BATCH	{1} (1,0294)	{2} (,98300)
1	A		0,054227
2	B	0,054227	

Univariate Tests of Significance for Height (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,1233
Include condition: DAY=5

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	33,84790	1	33,84790	2228,134	0,000000
GROUP	0,18224	2	0,09112	5,998	0,003535
REPLICATE(GROUP*BATCH)	0,56072	11	0,05097	3,356	0,000618
BATCH	0,04738	1	0,04738	3,119	0,080635
Error	1,42797	94	0,01519		

DPH: 7

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00975, df = 72,000
Include condition: DAY=7

Cell No.	GROUP	{1} (,82960)	{2} (,83875)	{3} (1,1264)
1	WARM		0,826927	0,000111
2	GRADIENT	0,826927		0,000115
3	COLD	0,000111	0,000115	

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00975, df = 72,000
Include condition: DAY=7

Cell No.	BATCH	{1} (1,0226)	{2} (1,0656)
1	A		0,057774
2	B	0,057774	

Univariate Tests of Significance for Height (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0987
Include condition: DAY=7

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	27,00118	1	27,00118	2770,640	0,000000
GROUP	1,31036	2	0,65518	67,229	0,000000
REPLICATE(GROUP*BATCH)	0,14803	7	0,02115	2,170	0,046944
BATCH	0,07373	1	0,07373	7,565	0,007521
Error	0,70167	72	0,00975		

Length at hatching:

DPH: 0

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,16908, df = 157,00
Include condition: DAY=0

Cell No.	GROUP	{1} (5,7797)	{2} (5,8885)	{3} (5,7802)
1	WARM		0,614636	0,997159
2	GRADIENT	0,614636		0,348810
3	COLD	0,997159	0,348810	

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,16908, df = 157,00
Include condition: DAY=0

Cell No.	BATCH	{1} (5,7908)	{2} (5,9580)
1	A		0,009749
2	B	0,009749	

Univariate Tests of Significance for Length (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,4112
Include condition: DAY=0

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	1866,835	1	1866,835	11040,96	0,000000
GROUP	0,383	2	0,192	1,13	0,324733
REPLICATE(GROUP*BATCH)	6,528	8	0,816	4,83	0,000025
BATCH	2,134	1	2,134	12,62	0,000504
Error	26,546	157	0,169		

DPH: 1

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,53552, df = 47,000
Include condition: DAY=1

Cell No.	GROUP	{1} (5,0084)	{2} (5,5998)
1	WARM		0,092170
2	COLD	0,092170	

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,53552, df = 47,000
Include condition: DAY=1

Cell No.	BATCH	{1} (5,5182)	{2} (4,9166)
1	A		0,013354
2	B	0,013354	

Univariate Tests of Significance for Length (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,7318
Include condition: DAY=1

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	719,0296	1	719,0296	1342,664	0,000000
GROUP	0,2637	1	0,2637	0,492	0,486346
REPLICATE(GROUP*BATCH)	5,7827	3	1,9276	3,599	0,020153
BATCH	0,6754	1	0,6754	1,261	0,267142
Error	25,1697	47	0,5355		

DPH: 2

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,44381, df = 61,000
Include condition: DAY=2

Cell No.	GROUP	{1} (3,7210)	{2} (5,6559)	{3} (5,0775)
1	WARM		0,000153	0,001683
2	GRADIENT	0,000153		0,163321
3	COLD	0,001683	0,163321	

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,44381, df = 61,000
Include condition: DAY=2

Cell No.	BATCH	{1} (5,3338)	{2} (5,6597)
1	A		0,056912
2	B	0,056912	

Univariate Tests of Significance for Length (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,6662
Include condition: DAY=2

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	468,5505	1	468,5505	1055,735	0,000000
GROUP	11,1823	2	5,5912	12,598	0,000026
REPLICATE(GROUP*BATCH)	6,6647	8	0,8331	1,877	0,080099
BATCH	0,0980	1	0,0980	0,221	0,640158
Error	27,0727	61	0,4438		

DPH:3

Newman-Keuls test; variable Length (mm)
(Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,29987, df = 154,00
Include condition: DAY=3

Cell No.	GROUP	{1} (5,4431)	{2} (6,2920)
1	WARM		0,000009
2	COLD	0,000009	

Newman-Keuls test; variable Length (mm)
(Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,29987, df = 154,00
Include condition: DAY=3

Cell No.	BATCH	{1} (5,8549)	{2} (5,7038)
1	A		0,090468
2	B	0,090468	

Univariate Tests of Significance for Length (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,5476
Include condition: DAY=3

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	3265,730	1	3265,730	10890,52	0,000000
GROUP	14,391	1	14,391	47,99	0,000000
REPLICATE(GROUP*BATCH)	23,809	8	2,976	9,92	0,000000
BATCH	0,789	1	0,789	2,63	0,106920
Error	46,180	154	0,300		

DPH: 5

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,33087, df = 94,000
Include condition: DAY=5

Cell No.	GROUP	{1} (5,5259)	{2} (5,8297)	{3} (6,3196)
1	WARM		0,092533	0,000171
2	GRADIENT	0,092533		0,007447
3	COLD	0,000171	0,007447	

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,33087, df = 94,000
Include condition: DAY=5

Cell No.	BATCH	{1} (5,9246)	{2} (5,6660)
1	A		0,022030
2	B	0,022030	

Univariate Tests of Significance for Length (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,5752
Include condition: DAY=5

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	1063,895	1	1063,895	3215,416	0,000000
GROUP	8,427	2	4,214	12,735	0,000013
REPLICATE(GROUP*BATCH)	13,569	11	1,234	3,728	0,000198
BATCH	0,091	1	0,091	0,276	0,600262
Error	31,102	94	0,331		

DPH: 7

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,24504, df = 72,000
Include condition: DAY=7

Cell No.	GROUP	{1} (5,1606)	{2} (4,3279)	{3} (6,5028)
1	WARM		0,000268	0,000115
2	GRADIENT	0,000268		0,000111
3	COLD	0,000115	0,000111	

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,24504, df = 72,000
Include condition: DAY=7

Cell No.	BATCH	{1} (5,8715)	{2} (5,9399)
1	A		0,542549
2	B	0,542549	

Univariate Tests of Significance for Length (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,4950
Include condition: DAY=7

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	889,9303	1	889,9303	3631,838	0,000000
GROUP	58,5205	2	29,2602	119,412	0,000000
REPLICATE(GROUP*BATCH)	4,9601	7	0,7086	2,892	0,010140
BATCH	0,9829	1	0,9829	4,011	0,048968
Error	17,6426	72	0,2450		

Weight at hatching:

DPH: 0

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00582, df = 157,00
Include condition: DAY=0

Cell No.	GROUP	{1} (4,6644)	{2} (5,0101)	{3} (4,7762)
1	WARM		0,000022	0,000009
2	GRADIENT	0,000022		0,000009
3	COLD	0,000009	0,000009	

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00582, df = 157,00
Include condition: DAY=0

Cell No.	BATCH	{1} (4,7466)	{2} (5,2201)
1	A		0,000009
2	B	0,000009	

Univariate Tests of Significance for Weight (mg) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0763
Include condition: DAY=0

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	1309,927	1	1309,927	224977,3	0,00
GROUP	0,861	2	0,430	73,9	0,00
REPLICATE(GROUP*BATCH)	5,052	8	0,631	108,5	0,00
BATCH	7,163	1	7,163	1230,2	0,00
Error	0,914	157	0,006		

DPH: 1

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,05220, df = 47,000
Include condition: DAY=1

Cell No.	GROUP	{1} (3,8710)	{2} (4,7880)
1	WARM		0,000118
2	COLD	0,000118	

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,05220, df = 47,000
Include condition: DAY=1

Cell No.	BATCH	{1} (4,4703)	{2} (3,7909)
1	A		0,000118
2	B	0,000118	

Univariate Tests of Significance for Weight (mg) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,2285
Include condition: DAY=1

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	468,9709	1	468,9709	8983,646	0,000000
GROUP	0,9668	1	0,9668	18,520	0,000085
REPLICATE(GROUP*BATCH)	7,2517	3	2,4172	46,305	0,000000
BATCH	0,7736	1	0,7736	14,819	0,000356
Error	2,4535	47	0,0522		

DPH: 2

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00021, df = 61,000
Include condition: DAY=2

Cell No.	GROUP	{1} (4,0760)	{2} (4,6588)	{3} (4,5724)
1	WARM		0,000117	0,000109
2	GRADIENT	0,000117		0,000109
3	COLD	0,000109	0,000109	

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00021, df = 61,000
Include condition: DAY=2

Cell No.	BATCH	{1} (4,4948)	{2} (4,8858)
1	A		0,000109
2	B	0,000109	

Univariate Tests of Significance for Weight (mg) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0146
Include condition: DAY=2

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	386,5691	1	386,5691	1813204	0,00
GROUP	3,5513	2	1,7757	8329	0,00
REPLICATE(GROUP*BATCH)	13,7119	8	1,7140	8039	0,00
BATCH	0,0728	1	0,0728	342	0,00
Error	0,0130	61	0,0002		

DPH: 3

Newman-Keuls test; variable Weight (mg)
(Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,03514, df = 154,00
Include condition: DAY=3

Cell No.	GROUP	{1} (4,5128)	{2} (5,5870)
1	WARM		0,000009
2	COLD	0,000009	

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,03514, df = 154,00
Include condition: DAY=3

Cell No.	BATCH	{1} (5,0298)	{2} (4,8450)
1	A		0,000009
2	B	0,000009	

Univariate Tests of Significance for Weight (mg) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,1875
Include condition: DAY=3

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	2360,321	1	2360,321	67162,55	0,000000
GROUP	22,194	1	22,194	631,51	0,000000
REPLICATE(GROUP*BATCH)	54,524	8	6,815	193,93	0,000000
BATCH	0,634	1	0,634	18,04	0,000037
Error	5,412	154	0,035		

DPH: 5

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,02103, df = 94,000
Include condition: DAY=5

Cell No.	GROUP	{1} (4,8195)	{2} (5,0198)	{3} (5,8760)
1	WARM		0,000134	0,000106
2	GRADIENT	0,000134		0,000112
3	COLD	0,000106	0,000112	

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,02103, df = 94,000
Include condition: DAY=5

Cell No.	BATCH	{1} (5,1121)	{2} (4,9759)
1	A		0,000116
2	B	0,000116	

Univariate Tests of Significance for Weight (mg) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,1450
Include condition: DAY=5

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	830,0508	1	830,0508	39462,21	0,000000
GROUP	9,2824	2	4,6412	220,65	0,000000
REPLICATE(GROUP*BATCH)	24,4770	11	2,2252	105,79	0,000000
BATCH	0,9731	1	0,9731	46,26	0,000000
Error	1,9772	94	0,0210		

DPH: 7

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01304, df = 72,000
Include condition: DAY=7

Cell No.	GROUP	{1} (3,6011)	{2} (4,2280)	{3} (6,3183)
1	WARM		0,000115	0,000111
2	GRADIENT	0,000115		0,000115
3	COLD	0,000111	0,000115	

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01304, df = 72,000
Include condition: DAY=7

Cell No.	BATCH	{1} (5,4032)	{2} (6,0457)
1	A		0,000115
2	B	0,000115	

Univariate Tests of Significance for Weight (mg) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,1142
Include condition: DAY=7

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	726,6037	1	726,6037	55738,06	0,00
GROUP	76,5948	2	38,2974	2937,81	0,00
REPLICATE(GROUP*BATCH)	9,3261	7	1,3323	102,20	0,00
BATCH	10,7029	1	10,7029	821,02	0,00
Error	0,9386	72	0,0130		

Yolk-sac at hatching:

DPH: 0

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00523, df = 153,00
Include condition: DAY=0

Cell No.	GROUP	{1} (1,1539)	{2} (1,1880)	{3} (1,0998)
1	WARM		0,098518	0,008734
2	GRADIENT	0,098518		0,000076
3	COLD	0,008734	0,000076	

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00523, df = 153,00
Include condition: DAY=0

Cell No.	BATCH	{1} (1,1565)	{2} (1,1849)
1	A		0,013627
2	B	0,013627	

Univariate Tests of Significance for Yolk-Sac (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0723
Include condition: DAY=0

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	72,35838	1	72,35838	13845,23	0,000000
GROUP	0,04284	2	0,02142	4,10	0,018453
REPLICATE(GROUP*BATCH)	0,14180	8	0,01773	3,39	0,001299
BATCH	0,00698	1	0,00698	1,34	0,249521
Error	0,79961	153	0,00523		

DPH: 1

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01936, df = 37,000
Include condition: DAY=1

Cell No.	GROUP	{1} (1,0338)	{2} (1,0370)
1	WARM		0,961691
2	COLD	0,961691	

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01936, df = 37,000
Include condition: DAY=1

Cell No.	BATCH	{1} (1,0698)	{2} (1,0187)
1	A		0,275577
2	B	0,275577	

Univariate Tests of Significance for Yolk-Sac (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,1391
Include condition: DAY=1

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	27,05965	1	27,05965	1397,547	0,000000
GROUP	0,00936	1	0,00936	0,483	0,491316
REPLICATE(GROUP*BATCH)	0,14380	3	0,04793	2,476	0,076590
BATCH	0,03565	1	0,03565	1,841	0,183044
Error	0,71640	37	0,01936		

DPH:2

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01035, df = 59,000
Include condition: DAY=2

Cell No.	GROUP	{1} (1,0370)	{2} (1,1005)	{3} (1,1009)
1	WARM		0,314541	0,567122
2	GRADIENT	0,314541		0,995483
3	COLD	0,567122	0,995483	

Newman-Keuls test; variable Yolk-Sac (mm)
(Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01035, df = 59,000
Include condition: DAY=2

Cell No.	BATCH	{1} (1,0853)	{2} (1,1272)
1	A		0,109943
2	B	0,109943	

Univariate Tests of Significance for Yolk-Sac (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,1017
Include condition: DAY=2

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	22,28505	1	22,28505	2153,327	0,000000
GROUP	0,01029	2	0,00515	0,497	0,610750
REPLICATE(GROUP*BATCH)	0,12968	8	0,01621	1,566	0,154674
BATCH	0,01379	1	0,01379	1,332	0,253071
Error	0,61060	59	0,01035		

DPH: 3

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00998, df = 133,00
Include condition: DAY=3

Cell No.	GROUP	{1} (1,1307)	{2} (1,1244)
1	WARM		0,711559
2	COLD	0,711559	

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00998, df = 133,00
Include condition: DAY=3

Cell No.	BATCH	{1} (1,1590)	{2} (1,1084)
1	A		0,003053
2	B	0,003053	

Univariate Tests of Significance for Yolk-Sac (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0999
Include condition: DAY=3

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	108,8191	1	108,8191	10900,02	0,000000
GROUP	0,0016	1	0,0016	0,16	0,688006
REPLICATE(GROUP*BATCH)	0,4017	8	0,0502	5,03	0,000018
BATCH	0,0611	1	0,0611	6,12	0,014648
Error	1,3278	133	0,0100		

DPH:5

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00730, df = 92,000
Include condition: DAY=5

Cell No.	GROUP	{1} (1,1781)	{2} (1,1271)	{3} (1,1102)
1	WARM		0,059554	0,033824
2	GRADIENT	0,059554		0,528754
3	COLD	0,033824	0,528754	

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00730, df = 92,000
Include condition: DAY=5

Cell No.	BATCH	{1} (1,1005)	{2} (1,1735)
1	A		0,000140
2	B	0,000140	

Univariate Tests of Significance for Yolk-Sac (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0854
Include condition: DAY=5

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	38,80198	1	38,80198	5317,877	0,000000
GROUP	0,00156	2	0,00078	0,107	0,898544
REPLICATE(GROUP*BATCH)	0,20047	11	0,01822	2,498	0,008584
BATCH	0,01741	1	0,01741	2,386	0,125893
Error	0,67128	92	0,00730		

DPH: 7

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00633, df = 71,000
Include condition: DAY=7

Cell No.	GROUP	{1} (1,1468)	{2} (1,1237)	{3} (1,1001)
1	WARM		0,495720	0,354877
2	GRADIENT	0,495720		0,487110
3	COLD	0,354877	0,487110	

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00633, df = 71,000
Include condition: DAY=7

Cell No.	BATCH	{1} (1,1017)	{2} (1,1190)
1	A		0,339947
2	B	0,339947	

Univariate Tests of Significance for Yolk-Sac (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0796
Include condition: DAY=7

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	37,67023	1	37,67023	5951,224	0,000000
GROUP	0,01950	2	0,00975	1,540	0,221466
REPLICATE(GROUP*BATCH)	0,07200	7	0,01029	1,625	0,142451
BATCH	0,00191	1	0,00191	0,301	0,584929
Error	0,44942	71	0,00633		

Height at hatching, total.

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01173, df = 642,00

Cell No.	GROUP	{1} (,94965)	{2} (1,0159)	{3} (1,0805)
1	WARM		0,000009	0,000022
2	GRADIENT	0,000009		0,000009
3	COLD	0,000022	0,000009	

Newman-Keuls test; variable Height (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01173, df = 642,00

Cell No.	BATCH	{1} (1,0122)	{2} (1,0172)
1	A		0,551063
2	B	0,551063	

Univariate Tests of Significance for Height (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,1083

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	588,1370	1	588,1370	50144,86	0,000000
GROUP	1,2542	2	0,6271	53,47	0,000000
REPLICATE(GROUP*BATCH)	3,2492	14	0,2321	19,79	0,000000
BATCH	0,0957	1	0,0957	8,16	0,004415
Error	7,5299	642	0,0117		

Length at haching, total:

Newman-Keuls test; variable Length (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,45064, df = 649,00

Cell No.	GROUP	{1} (5,3291)	{2} (5,7055)	{3} (6,1123)
1	WARM		0,000009	0,000022
2	GRADIENT	0,000009		0,000009
3	COLD	0,000022	0,000009	

Newman-Keuls test; variable Length (mm) (Haching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,45064, df = 649,00

Cell No.	BATCH	{1} (5,7474)	{2} (5,6672)
1	A		0,123197
2	B	0,123197	

Univariate Tests of Significance for Length (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,6713

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	18745,04	1	18745,04	41596,36	0,000000
GROUP	47,41	2	23,70	52,60	0,000000
REPLICATE(GROUP*BATCH)	68,42	14	4,89	10,84	0,000000
BATCH	0,01	1	0,01	0,02	0,881851
Error	292,47	649	0,45		

Weight at haching, total.

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,29378, df = 651,00

Cell No.	GROUP	{1} (4,3674)	{2} (4,8824)	{3} (5,5534)
1	WARM		0,000009	0,000022
2	GRADIENT	0,000009		0,000009
3	COLD	0,000022	0,000009	

Newman-Keuls test; variable Weight (mg) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,29378, df = 651,00

Cell No.	BATCH	{1} (4,8909)	{2} (4,9413)
1	A		0,229207
2	B	0,229207	

Univariate Tests of Significance for Weight (mg) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,5420

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	14153,99	1	14153,99	48178,69	0,000000
GROUP	112,21	2	56,10	190,97	0,000000
REPLICATE(GROUP*BATCH)	105,64	14	7,55	25,68	0,000000
BATCH	2,88	1	2,88	9,82	0,001807
Error	191,25	651	0,29		

Yolk-sac at haching, total:

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01066, df = 608,00

Cell No.	GROUP	{1} (1,1200)	{2} (1,1508)	{3} (1,1084)
1	WARM		0,002615	0,253924
2	GRADIENT	0,002615		0,000116
3	COLD	0,253924	0,000116	

Newman-Keuls test; variable Yolk-Sac (mm) (Hatching)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,01066, df = 608,00

Cell No.	BATCH	{1} (1,1257)	{2} (1,1320)
1	A		0,448943
2	B	0,448943	

Univariate Tests of Significance for Yolk-Sac (mm) (Hatching)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,1032

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	672,9256	1	672,9256	63135,42	0,000000
GROUP	0,2193	2	0,1097	10,29	0,000040
REPLICATE(GROUP*BATCH)	0,3228	14	0,0231	2,16	0,008024
BATCH	0,0087	1	0,0087	0,82	0,366313
Error	6,4803	608	0,0107		

Body damage at hatching, total:

Kruskal-Wallis ANOVA by Ranks; Damaged (Haching)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 669) =77,78911 p =,0000

	Code	Valid (N)	Sum of (Ranks)	Mean (Rank)
Depend.: Damaged				
WARM	1	215	82778,00	385,0140
GRADIENT	2	255	78762,00	308,8706
COLD	3	199	62575,00	314,4472

Multiple Comparisons p values (2-tailed); Damaged (Hatching)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 669) =77,78911 p =,0000

Depend.: Damaged	WARM (R:385,01)	GRADIENT (R:308,87)	COLD (R:314,45)
WARM		0,000063	0,000617
GRADIENT	0,000063		1,000000
COLD	0,000617	1,000000	

Deformities at hatching, total:

Kruskal-Wallis ANOVA by Ranks; Deformed (Hatching)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 669) =70,79516 p =,0000

Depend.: Deformed	Code	Valid (N)	Sum of (Ranks)	Mean (Rank)
WARM	1	215	87077,00	405,0093
GRADIENT	2	255	77929,00	305,6039
COLD	3	199	59109,00	297,0302

Multiple Comparisons p values (2-tailed); Deformed (Hatching)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 669) =70,79516 p =,0000

Depend.: Deformed	WARM (R:405,01)	GRADIENT (R:305,60)	COLD (R:297,03)
WARM		0,000000	0,000000
GRADIENT	0,000000		1,000000
COLD	0,000000	1,000000	

Development of dorsal fin at hatching, total:

Kruskal-Wallis ANOVA by Ranks; Developed (Hatching)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 669) =47,61054 p =,0000

Depend.: Developed	Code	Valid (N)	Sum of (Ranks)	Mean (Rank)
WARM	1	215	79421,00	369,4000
GRADIENT	2	255	84496,50	331,3588
COLD	3	199	60197,50	302,5000

Multiple Comparisons p values (2-tailed); Developed (Hatching)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 669) =47,61054 p =,0000

Depend.: Developed	WARM (R:369,40)	GRADIENT (R:331,36)	COLD (R:302,50)
WARM		0,100545	0,001300
GRADIENT	0,100545		0,343245
COLD	0,001300	0,343245	

Spine damage at hatching, total:

Kruskal-Wallis ANOVA by Ranks; Spine damage (Hatching)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 669) =103,3481 p =0,000

Depend.: Spine damage	Code	Valid (N)	Sum of (Ranks)	Mean (Rank)
WARM	1	215	86470,00	402,1860
GRADIENT	2	255	75478,50	295,9941
COLD	3	199	62166,50	312,3945

Multiple Comparisons p values (2-tailed); Spine damage (Hatching)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 669) =103,3481 p =0,000

Depend.: Spine damage	WARM (R:402,19)	GRADIENT (R:295,99)	COLD (R:312,39)
WARM		0,000000	0,000007
GRADIENT	0,000000		1,000000
COLD	0,000007	1,000000	

Tailbend at hatching, total:

Kruskal-Wallis ANOVA by Ranks; Taildbend (Hatching)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 669) =91,59644 p =0,000

Depend.: Taildbend	Code	Valid (N)	Sum of (Ranks)	Mean (Rank)
WARM	1	215	88119,50	409,8581
GRADIENT	2	255	76797,00	301,1647
COLD	3	199	59198,50	297,4799

Multiple Comparisons p values (2-tailed); Taildbend (Hatching)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 669) =91,59644 p =0,000

Depend.: Taildbend	WARM (R:409,86)	GRADIENT (R:301,16)	COLD (R:297,48)
WARM		0,000000	0,000000
GRADIENT	0,000000		1,000000
COLD	0,000000	1,000000	

Height at two weeks:

Newman-Keuls test; variable Height (mm) (Two weeks)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00500, df = 349,00

Cell No.	GROUP	{1} (1,1566)	{2} (1,1499)	{3} (1,1244)
1	WARM		0,461947	0,001115
2	GRADIENT	0,461947		0,004842
3	COLD	0,001115	0,004842	

Newman-Keuls test; variable Height (mm) (Two weeks)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,00500, df = 349,00

Cell No.	BATCH	{1} (1,1103)	{2} (1,1772)
1	A		0,000009
2	B	0,000009	

Univariate Tests of Significance for Height (mm) (Two weeks)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,0707

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	478,7933	1	478,7933	95729,82	0,000000
GROUP	0,0727	2	0,0364	7,27	0,000805
REPLICATE(GROUP*BATCH)	0,2399	14	0,0171	3,43	0,000031
BATCH	0,4107	1	0,4107	82,11	0,000000
Error	1,7455	349	0,0050		

Length at two weeks:

Newman-Keuls test; variable Length (mm) (Two weeks)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,16303, df = 349,00

Cell No.	GROUP	{1} (6,4389)	{2} (6,4226)	{3} (6,6729)
1	WARM		0,753094	0,000014
2	GRADIENT	0,753094		0,000025
3	COLD	0,000014	0,000025	

Newman-Keuls test; variable Length (mm) (Two weeks)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,16303, df = 349,00

Cell No.	BATCH	{1} (6,4530)	{2} (6,5668)
1	A		0,006964
2	B	0,006964	

Univariate Tests of Significance for Length (mm) (Two weeks)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,4038

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	15522,43	1	15522,43	95212,44	0,000000
GROUP	4,63	2	2,31	14,19	0,000001
REPLICATE(GROUP*BATCH)	2,00	14	0,14	0,88	0,586464
BATCH	1,23	1	1,23	7,57	0,006251
Error	56,90	349	0,16		

Weight at two weeks:

Newman-Keuls test; variable Weight (mg) (Two weeks)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,25530, df = 349,00

Cell No.	GROUP	{1} (7,0741)	{2} (6,7120)	{3} (7,8224)
1	WARM		0,000009	0,000009
2	GRADIENT	0,000009		0,000022
3	COLD	0,000009	0,000022	

Newman-Keuls test; variable Weight (mg) (Two weeks)
Approximate Probabilities for Post Hoc Tests
Error: Between MSE = ,25530, df = 349,00

Cell No.	BATCH	{1} (6,8172)	{2} (7,5796)
1	A		0,000009
2	B	0,000009	

Univariate Tests of Significance for Weight (mg) (Two weeks)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 0,5053

Effect	SS	Degr. of (Freedom)	MS	F	p
Intercept	19001,21	1	19001,21	74425,54	0,000000
GROUP	76,60	2	38,30	150,02	0,000000
REPLICATE(GROUP*BATCH)	28,59	14	2,04	8,00	0,000000
BATCH	52,35	1	52,35	205,07	0,000000
Error	89,10	349	0,26		

Body damage at two weeks:

Kruskal-Wallis ANOVA by Ranks; Damaged (Two weeks)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 368) =5,240163 p =,0728

Depend.: Damaged	Code	Valid (N)	Sum of (Ranks)	Mean (Rank)
WARM	1	126	23726,00	188,3016
GRADIENT	2	122	22450,00	184,0164
COLD	3	120	21720,00	181,0000

Multiple Comparisons p values (2-tailed); Damaged (Two weeks)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 368) =5,240163 p =,0728

Depend.: Damaged	WARM (R:188,30)	GRADIENT (R:184,02)	COLD (R:181,00)
WARM		1,000000	1,000000
GRADIENT	1,000000		1,000000
COLD	1,000000	1,000000	

Deformities at two weeks:

Kruskal-Wallis ANOVA by Ranks; Deformed (Two weeks)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 368) =5,269368 p =,0717

Depend.: Deformed	Code	Valid (N)	Sum of (Ranks)	Mean (Rank)
WARM	1	126	23726,00	188,3016
GRADIENT	2	122	22082,00	181,0000
COLD	3	120	22088,00	184,0667

Multiple Comparisons p values (2-tailed); Deformed (Two weeks)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 368) =5,269368 p =,0717

Depend.: Deformed	WARM (R:188,30)	GRADIENT (R:181,00)	COLD (R:184,07)
WARM		1,000000	1,000000
GRADIENT	1,000000		1,000000
COLD	1,000000	1,000000	

Spine damage at two weeks:

Kruskal-Wallis ANOVA by Ranks; Spine damage (Two weeks)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 368) =2,073735 p =,3546

Depend.: Spine damage	Code	Valid (N)	Sum of (Ranks)	Mean (Rank)
WARM	1	126	23242,00	184,4603
GRADIENT	2	122	22326,00	183,0000
COLD	3	120	22328,00	186,0667

Multiple Comparisons p values (2-tailed); Spine damage (Two weeks)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 368) =2,073735 p =,3546

Depend.: Spine damage	WARM (R:184,46)	GRADIENT (R:183,00)	COLD (R:186,07)
WARM		1,000000	1,000000
GRADIENT	1,000000		1,000000
COLD	1,000000	1,000000	

Tailbend at two weeks:

Kruskal-Wallis ANOVA by Ranks; Taildbend (Two weeks)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 368) =9,959715 p =,0069

Depend.: Taildbend	Code	Valid (N)	Sum of (Ranks)	Mean (Rank)
WARM	1	126	23207,00	184,1825
GRADIENT	2	122	21413,00	175,5164
COLD	3	120	23276,00	193,9667

Multiple Comparisons p values (2-tailed); Taildbend (Two weeks)
Independent (grouping) variable: GROUP
Kruskal-Wallis test: H (2, N= 368) =9,959715 p =,0069

Depend.: Taildbend	WARM (R:184,18)	GRADIENT (R:175,52)	COLD (R:193,97)
WARM		1,000000	1,000000
GRADIENT	1,000000		0,531989
COLD	1,000000	0,531989	