

Effects of various early life history temperature regimes on development and metamorphosis of Atlantic cod (*Gadus morhua*) larvae



Tolga Bashan

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Supervisors:

Inger-Britt Falk Peterson

inger.falk-petersen@uit.no

Velmurugu Puvanendran

velmurugu.puvanendran@nofima.no

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Abstract

The growth and important structures development in different temperature regime histories were studied during mid-metamorphosis stage of Atlantic cod (*Gadus morhua*) larvae. In order to see the growth and the organ development differentiation due to different temperatures at a given stage, we exposed the larvae into 4 different temperature regimes; L-L (low temperature at incubation, low temperature after hatching), L-H (low temperature at incubation, high temperature after hatching), H-L (high temperature at incubation, low temperature after hatching) and H-H (high temperature at incubation, high temperature after hatching) (low $4\pm 0.2^{\circ}\text{C}$ and high $9\pm 0.2^{\circ}\text{C}$). The sampling was done according to the expected stage at a given sampling stage. Samples were fixed and studied under light microscope. Standard length and myotome height were measured for growth, swim bladder size, alimentary tract and fin differentiation were studied for organ development. The L-H group fish showed the best growth but had the highest variation in myotome heights. There was a lot of variation in organ developments at the given stages among the temperature groups, yet the groups with high temperatures after hatching period showed better organ development, postulating that besides the age is the key factor for fish to reach a given stage, also temperature is an extrinsic factor in organ development.

Key words: *Gadus morhua*, different temperatures, growth, organ development

Contents

ACKNOWLEDGEMENTS.....	3
ABSTRACT.....	4
1.INTRODUCTION.....	8
2.MATERIALS AND METHODS.....	10
2.1. Broodstock.....	10
2.2. Experimental setup.....	10
2.3. Larval rearing.....	12
2.4. Sampling.....	12
2.5. Measurements.....	15
2.6. Data Analysis.....	15
2.7. Detailed studies of samples of larvae.....	15
3.RESULTS.....	18
3.1. Larval Growth	18
3.1.1. Standard length.....	18
3.1.2. Myotome height.....	21
3.2. Organ development.....	26
3.2.1. Swim bladder.....	29
3.2.1.1. L-L Group.....	29
3.2.1.2. L-H Group.....	33
3.2.1.3. H-L Group.....	37
3.2.1.4. H-H Group.....	38
3.2.2. Alimentary tract and liver development	40
3.2.2.1. L-L Group	40
3.2.2.2. L-H Group	41
3.2.2.3. H-L Group	45
3.2.2.4. H-H Group	46
3.2.3. Fin development.....	48
3.2.3.1. L-L Group	48
3.2.3.2. L-H Group	49
3.2.3.3. H-L Group	50
3.2.3.4. H-H Group	50
4.DISCUSSION.....	51
4.1. Larval growth.....	51
4.1.1. Standard length.....	52
4.1.2. Myotome height.....	53
4.2. Organ development.....	54
4.2.1. Swim bladder.....	54
4.2.2. Alimentary tract.....	56
4.2.3. Fin development.....	57

5.CONCLUSION.....	58
6.REFERENCES.....	59
7.APPENDIX.....	63
APPENDIX A.....	63
APPENDIX B.....	71

Content of figures and tables

Figure 1. Experimental setup procedure.....	11
Figure 2. Description and staging of feeding landmarks with respect to Atlantic cod larval developmental stage.	17
Figure 3. Representative drawings of developmental stages of cod larvae.....	17
Figure 4. Standard lengths of cod larvae from each temperature groups at different ages..	18
Figure 5. Least square means of log standard length in L-L group.....	19
Figure 6. Least square means of log standard length in L-H group.....	20
Figure 7. Least square means of log standard length in H-L group.....	20
Figure 8. Least square means of log standard length in H-H group.....	21
Figure 9. Myotome heights of each temperature group at stages 8,11 and 12.....	22
Figure 10. Least square means of myotome heights in L-L group.....	23
Figure 11. Least square means of myotome heights in L-L group.....	23
Figure 12. Least square means of myotome heights in L-H group.....	24
Figure 13. Least square means of myotome heights in L-H group.....	24
Figure 14. Myotome heights of cod larvae in H-L group.....	25
Figure 15. Least square means of myotome heights in H-H group.....	25
Figure 16. Pictures of larvae from stage 8 from the four different groups.....	27
Figure 17. Pictures of larvae from stage 11 from the three different groups.....	28
Figure 18. Pictures of larvae from stage 12 from the three different groups.....	28
Figure 19. Swim bladder size of each temperature group.....	29
Figure 20. Different swim bladder shapes in L-L group stage 8 fish.....	30
Figure 21. Swim bladder shapes in L-L group stage 11.....	31
Figure 22. Random pictures of swim bladder at stage 12 in L-L group.....	31
Figure 23. Least square means of swim bladder lengths in tanks in L-L group.....	32
Figure 24. Least square means of swim bladder lengths in tanks in L-L group.....	32
Figure 25. Random stage 8 pictures from three different replicates of L-H group.....	34
Figure 26. L-H group stage 11 pictures.....	34
Figure 27. L-H group stage 12 pictures from three different replicates.....	35
Figure 28. Least square means of swim bladder lengths in tanks in L-H group.....	36
Figure 29. Least square means of swim bladder lengths in tanks in L-H group.....	36
Figure 30. Different swim bladder shapes from H-L group at stage 11.....	37
Figure 31. Swim bladder lengths range at stage 11 in H-L group.....	38
Figure 32. Stage 11 H-H group swim bladder shapes.....	39
Figure 33. Random stage 12 pictures from three different replicates of H-H group.....	39
Figure 34. Least square means of swim bladder sizes in H-H group.....	40
Figure 35. Alimentary tract differentiations at stage 8 L-L group.....	41
Figure 36. Alimentary tract differentiations at stage 11 L-L group.....	42
Figure 37. Stage 12 L-L group intestine development.....	42

Figure 38. Stage 8 L-H group alimentary tract.....	44
Figure 39. Pictures of L-H group stage 11 intestines.....	44
Figure 40. Random stage 12 pictures from three different replicates of L-H.....	45
Figure 41. Pictures of alimentary tract from stage 11 H-L group.....	46
Figure 42. Pictures of alimentary tract from stage 11 H-H group.....	47
Figure 43. Random pictures of alimentary tract of stage 12 H-H group.....	48
Table 1. Replicate tanks in each temperature group.....	11
Table 2. Landmarks used as a reference for staging cod (<i>Gadus morhua</i>) larvae.....	13
Table 3. Landmarks used as a reference for staging cod (<i>Gadus morhua</i>) larvae.....	14
Table 4. Sampling ages in different temperature treatment groups	14
Table 5. Morphological landmarks and their functional relationships in developing Atlantic cod larvae.....	16
Table 6. Mean standard lengths at the respective sampling stage at each temperature group.....	18
Table 7. Mean lengths of myotome heights at the respective sampling stage at each temperature group.....	21
Table 8. Mean lengths of swim bladder sizes at the respective sampling stage at each temperature group.....	29
Table 9. L-L group fish swim bladder development according to the shape and size at each sampling stage.....	30
Table 10. L-H group fish swim bladder development according to the shape and size at each stage.....	33
Table 11. H-L group fish swim bladder development according to the shape and size at each stage.....	37
Table 12. H-H group fish swim bladder development according to the shape and size at each stage.....	38
Table 13. L-L group intestine development at each sampling stage.....	41
Table 14. L-H group intestine development at each sampling stage.....	43
Table 15. H-L group intestine development at each sampling stage.....	46
Table 16. H-H group intestine development at each sampling stage.....	47
Table 17. L-L group fin development at each sampling stage.....	49
Table 18. L-H group fin development at each sampling stage.....	49
Table 19. H-L group fin development at each sampling stage.....	50
Table 20. H-H group fin development at each sampling stage.....	51

1-Introduction

Fish egg incubation time and hatching depend on different environmental conditions such as temperature, dissolved oxygen concentration or physicochemical composition of water (Bagenal, 1971; Bonislawska et al., 2001). The egg incubation temperature is inversely proportional to incubation time in both haddock (*Melanogrammus aeglefinus*) (Martell et al., 2005), and cod (Thompson & Riley, 1981; Fitzsimmons & Perutz, 2006). In laboratory experiments, cod have been incubated and hatched at temperatures from -1.5 °C to 12 °C. LT₅₀ studies have demonstrated that thermal tolerance range of cod embryos is -1.8 °C to 12.0 °C (Johansen & Krogh, 1914; Valerio et al., 1992) with a midpoint of 6 °C to 6.5 °C (Rombough, 1997). After hatching, the early life of cod can be divided into different main periods such as the yolk sac period, start-feeding and feeding larval period and metamorphosis period. Temperature affects the hatching time, the hatching success, survival at hatching, survival after hatching, growth, organ development and malformations during these stages (Thompson & Riley, 1981; Pryor & Brown, 1998; Otterlei et al., 1999; Peterson, Martin & Harmon, 2004; Fitzsimmons & Perutz, 2006). Yolk consumption is temperature dependent as are all of the physiological functions in most ectotherms (Brett, 1970), hence utilization of yolk should be considered as positively correlated with temperature (e.g. Howell, 1980 ; Johns & Howell, 1980 ; Eldridge et al., 1982). The yolk utilization efficiency may indicate larval growth but on the other hand as the larvae grows, metabolic requirements also increase, so that a change in either processes will be reflected in growth performance (Kuftina & Novikov, 1986 ; Steinarsson & Björnsson, 1999).

After hatching, there are two main development strategies in the early life stages of fish ; directly and indirectly reaching the juvenile stage (Balon 1999). The Atlantic cod (*Gadus morhua*) larvae evolved a strategy of passing through a relatively long metamorphosis period before the juvenile stage is reached. In this intermediate larval period between the hatched embryo and juvenile stage, the rudimentary organs start to develop and differentiate to functional and more sophisticated organs or organ systems which fulfil the needs of juvenile fish during post metamorphosis. The developmental changes are most pronounced with regard to changes in muscle type and mass, fins, sensory, respiratory & osmoregulatory organs and digestive tract differentiation. The word metamorphosis does not only reflect the morphological and anatomical changes in fish but also physiological,

behavioral and biochemical changes taking place in order to adapt the fish to the new habitat where it will spend the juvenile and adult stages.

Since the natural habitat of cod is in the northern temperate waters, their preferences for low temperatures make these fish more sensitive towards small temperature changes; particularly during the early life history (Pedersen & Jobling, 1989; Blaxter, 1992; Kamler, 1992; Björnsson et al., 1999; Hochachka & Somero, 2002). These authors show that the optimal temperature for growth for Atlantic cod decreases with fish growth. This indicates that the early life history of cod is the most manipulative phase in terms of changing the growth and developmental rates by temperature.

The early life stages of fish have an incredible growth rate and Finn et al. (2002) described that at a given temperature, cod larvae can increase their body weight about 2000 times within the first 50 days of their life. Hunt Von Herbing (2001) described that the growth of cod larvae is divided into two main periods; the first period extends from hatching to 35-50 days post-hatch (at 10 C⁰, start of metamorphosis and mid metamorphosis) and the second period extends between 40-90 days post-hatch (end of metamorphosis, transformation into juvenile).

This master thesis project is a part of a national project established to build a knowledge platform for studies of function, mechanisms and processes linked to developmental, nutritional, environmental and management aspects of the early life stages of cod (CODE-Cod Development, subproject CODLIFE). For the master's thesis, larval groups with various temperature histories were sampled from hatching through metamorphosis at similar developmental stages but different ages (days post-hatch). The investigations include comparisons between size and growth as well as observations of potential differences in organogenesis among the various temperature history groups.

In order to study the growth and developmental differentiations caused by various temperature regimes during embryonic and larval stages of Atlantic Cod (*Gadus morhua*) larvae and juveniles, Atlantic cod eggs and larvae were exposed to 4 different temperature regimes.

The aim of this study is to identify the any differences that occur in comparison to external and internal organs development as the fish grow in 4 different temperature groups during mid-metamorphosis stage.

2. Materials and Methods

2.1 Broodstock

Cultured Atlantic cod adults, originating from the 2007 and 2008 year class kept at the National Cod Breeding Centre (NCBC) in Tromsø, north Norway, were used as broodstock in this experiment. They were kept in two 25 m³ circular tanks and the water temperature was maintained at 4 -5 °C. The fish density ranged from 8 to 12 kg m⁻³. The water used in the tanks was untreated ambient water with a rate of 350-450 L min⁻¹. Adult cod started spawning in mid March 2011 and gametes were hand stripped 2 weeks after the initial spawning to ensure good quality eggs and sperm (Kjesbu et al. 1996).

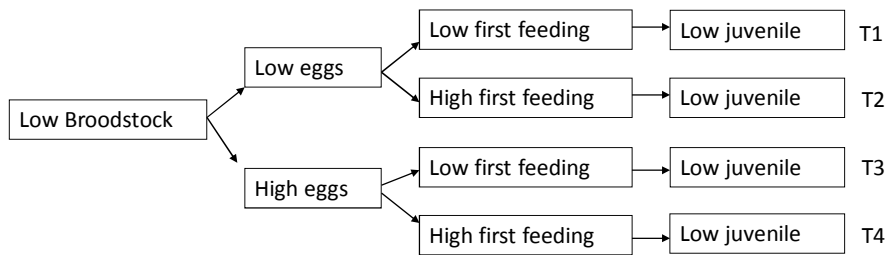
Sperm from 8 males and eggs from 18 females were collected by hand stripping. A total of 6L of eggs were collected. Just before commencing the fertilization process, all the eggs and sperm were pooled and the fertilization was performed using standard dry fertilization method (Hansen & Puvanendran, 2010). Stripped eggs were fertilized by using a standard dry fertilization method. Milt and eggs were mixed together with a 1:25 ratio and 200 mL of ambient seawater was added afterwards in order to activate the sperm and gently stirred. After waiting for 1-2 minutes, the mixture was washed to remove the excess sperm and transferred to incubators.

2.2. Experimental setup

The experimental setup been used in this work was formulated by the CODLIFE project and conducted by Nofima AS, in Tromsø. The setup is constituted of 3 different phases, spawning, incubation-hatching and feeding phases (Fig. 1). Soon after fertilization, equal volumes of eggs (375 mL) were transferred to sixteen 25 L up-welling incubators with a flow rate of 1.5 L min⁻¹. Temperatures in 8 of the incubators were maintained at 4±0.2°C until hatch while temperatures of remaining 8 incubators were gradually increased to 9.5°C in 32 hours (~ 0.5°C every 3 hours) and maintained at 9.5±0.2°C until hatch. Subsequently, the two embryo groups (LE, and HE) were established. Constant aeration ensured even distribution and mixing of eggs in the water column. Dead eggs were removed daily during the first 6 days and every 2nd day until hatch by flushing out the water from the conical tank bottom. At 100% hatch, larvae were transferred to 190 l circular experimental tanks. Larval stocking density was 26,000 larvae L⁻¹. Eggs incubated at low temperature (4°C) were transferred to 8 tanks kept at 4°C while eggs incubated at high temperature (9.5°C) were transferred to another 8 tanks kept at 9.5°C. . Larvae from each incubator were

equally distributed to corresponding tanks. From 5-10 dph, temperature of 4 tanks at 4.5°C was gradually increased to 9.5°C (LH) while other 4 tanks were kept at 4°C (LL).

Similarly, from 5-10 dph, temperature of 4 tanks at 9.5°C was gradually decreased to 4°C (HL) while the other 4 tanks were kept at 9.5°C (HH). Thus, we created 4 different groups in order to study the effects on development and growth during the larval and early juvenile stages (Fig. 1, Table 1).



Low temp groups: 4-5 °C

High temp groups: Eggs: 9-10 °C; Larvae: 10-12 °C

Figure 1. Experimental setup procedure (T1 is referred as low temperature at egg incubating and low temperature at larval rearing, T2 is LH, low temperature at egg incubating and high temperature at larval rearing, T3 is HL, high temperature at egg incubating and low temperature at larval rearing, T4 is HH high temperature at egg incubating and high temperature at larval rearing temperature groups).

Table 1. Replicate tanks in each temperature group, -: not sampled (tanks 2, 3 and 4 were only sampled for CODE LIFE project).

Temperature groups	Tank no	Tank no	Tank no	Tank no
L-L	7	8	9	-
L-H	17	18	19	20
H-L	2	3	4	5
H-H	13	15	16	-

2.3. Larval rearing

After hatching, the following 10 days microalgae (Nannochloropsis®, Reed Mariculture) was applied to the experimental tanks; in addition to that, larvae were fed with Phosphonorse® and Micronorse® enriched rotifers with a density of 4000 individuals per tank. Enriched rotifers were kept in a cooled storage tank for 24 hours at 5°C and were fed to the larvae every fourth hour by a feeding robot. Rotifer feeding ended up at 29 dph. At the last 4 days of rotifer feeding, artemia was added to the diet of larvae and ended up at 46 dph (days post hatching). In the weaning period, larvae were fed with dry feed after 32 dph. Algonorse coldwater® was used as the weaning diet. The size of the pellets was 200-300 µm at the beginning and was gradually increased to 300-500 µm at 64 dph.

2.4 Sampling

Larval samples were taken at 5 pre-determined larval stages from all 4 treatments (T1-T4) by using the methods described by von Herbing et al. (1996) (Table 2, 3). For this master's thesis, sampling included during the metamorphosis period, from stage 8 to 12.

In order to ensure that the fish were in the right stage for sampling, some individuals from each tank were regularly examined. When the right stage was reached, ten larvae from each tank were sampled randomly (see appendix, Table 4) by siphoning fish out from the tank by using a hose. After the fish were collected in a bucket, they were anaesthetized by using MS-222 and transferred into small glass tubes filled with 4% buffered formaldehyde and kept in the fridge until they were analysed in the laboratory.

Table 2. Landmarks used as a reference for staging cod (*Gadus morhua*) larvae (von Herbing et al., 1996) (H head; YS yolk-sac; AT alimentary tract; GC gill cover; G gills; SB swim bladder; FF finfold; F paired fins; P body and eye pigmentation).

Landmark	Stage 5	Stage 6	Stage 7	Stage 8
H	viscerocranial structures enlarge; mouth sub-terminal position; sharp jaw angle	increased girth of cranial elements	jaw cartilage thicker; mouth terminal; jaw angle prominent	upper jaw more pronounced; lower jaw larger
YS	much reduced; 25–50% yolk left	small sac, 10–25% yolk left	no yolk left, only remnants of the sac	sac remnants
AT	mid-gut enlarged; intestine convoluted	intestine is more convoluted	enlarged oesophagus, highly convoluted intestine	one small loop between mid-gut and intestine; liver elongated
GC	epithelial membrane growing postero-laterally from the hyoid	epithelial membrane covers 50% of gill cavity	> 50% of gill cavity covered	> 50% covering gill cavity
G	the epithelial layer on the gill arches has thickened; no filaments	first gill filaments appear on 2nd or 3rd gill arches	gill filaments growing longer	numerous gill filaments on 2nd and 3rd arches
SB	bladder inflated, but inflation is not complete; pigment clusters thicker over dorsal portion	inflated in most larvae; pigment spreading ventrally	enlarged; dorsal portion covered by black/silvery pigment, fully functional	enlarged, pigment spreading over whole bladder
FF	the supracephalic sinus remains elevated	supracephalic sinus still elevated	supracephalic sinus elevated	supracephalic sinus beginning to collapse
F	larger rounded pectoral fins with cartilaginous base	large pectoral fins, cartilaginous base; thickened cleithrum	caudal peduncle thickened	caudal peduncle thickening, base of pectoral fin enlarged
P	more pigmentation generally, none between the post-anal pigment bars	pigments filling two post-anal bands, more over intestine and liver	covering trunk, no pigment bars left, covers also head and dorsal portion of intestine	more numerous and spreading over head, lower jaw, trunk and intestine

Table 3. Landmarks used as a reference for staging cod (*Gadus morhua*) larvae (von Herbing et al., 1996) (H head; YS yolk-sac; AT alimentary tract; GC gill cover; G gills; SB swim bladder; FF finfold; F paired fins; P body and eye pigmentation).

Landmark	Stage 9	Stage 10	Stage 11	Stage 12
H	cranial elements larger, thicker	large cranial structures; branchiostegal rays, small opercular bones; teeth on lower and upper jaws, large pharyngeal teeth	head more elongated; jaws partly mineralized, larger teeth	elongated head, mouth terminal
YS	no remnants visible	no remnants	no remnants	none
AT	mid-gut large, intestine coiled	large oesophagus, highly coiled intestine, large liver	large stomach and intestine lumen, coils of intestine indistinct, as covered by pigment	large and obscured by pigment
GC	gill covered entirely by opercular membrane	dorsal growth of membrane containing opercular bones, seven branchiostegal rays projecting from hyoid arch	well formed operculum with large opercular margin (valve)	well formed operculum, covers whole gill cavity
G	gill filaments numerous, first secondary lamellae	two rows of gill filaments with secondary lamellae	two rows of gill filaments with numerous lamellae	two rows of large gill filaments and large lamellae
SB	very distinct, filled	distinct and filled	more elongated, extending posteriorly over the mid-gut and intestine	elongated and extends posteriorly above the anus, covered entirely by silver pigment
FF	sinus almost collapsed off head	sinus completely collapsed	sinus collapsed and finfold covering the trunk; narrower and scalloped at the extreme caudal end	not present
F	fin rays in pectoral fins, first caudal fin rays	caudal fin rays, thickened pectoral fin	well formed caudal fin rays, precursors to the dorsal and ventral paired fins present	dorsal, anal and pelvic fins are formed but still small, caudal fin is well formed and large
P	plentiful around upper and lower jaws, intestine, head and trunk	over upper and lower jaws, intestine, extending to end of trunk, lateral line pigment streak	chromatophores are larger, thicker and more numerous	covers entire body

Table 4. Sampling ages (dph days post hatching) in different temperature treatment groups (T1 LL; T2 LH; T3; HL; T4 HH), - : no data of the group of fish at that stage, due to over-sampling in T1 group and due to mortality in T3 group (T3 stage 11 was sampled only to be used in a-d and swim bladder sizes in this master thesis project, the age was not recorded).

	Stage 8	Stage 11	Stage 12
T1 group	29 dph	60 dph	-
T2 group	22 dph	43 dph	66 dph
T3 group	23 dph	-	-
T4 group	17 dph	42 dph	57 dph

2.5. Measurements

15 larvae from each tank were sampled, anesthetized using metacaine methanesulfonate (MS-222) and then digitally photographed using a camera fitted to a dissecting microscope. The digital photographs were used for the measurement of larval standard length. The myotome height (above anal opening) and swim bladder size measurements of ca. 10 larva from each temperature regime, stage and tank from my samples were done at the laboratory at the Faculty of Biology, Fishery Science and Economy, University of Tromsø by using a Leica Wild M 10 stereomicroscope under different magnifications.

2.6. Data Analysis

Graphical illustrations and statistical evaluations were carried out with the MySTAT and SYSTAT programs. For the statistical approach, ANOVA analysis was used. For the growth analysis, standard lengths were transformed into log standard lengths. The data included in the statistical analysis of sl (standard length) were obtained from the common samplings in the CODE LIFE project. Of the 480 individuals from CODE LIFE project, 105 were L-L group, 180 were L-H group, 60 were H-L group and 135 were H-H group. The swim bladder and the myotome height data were obtained from the master thesis samplings. Of the 259 samplings from master thesis samplings, 71 individuals were L-L group, 109 were L-H group, 19 were H-L group and 60 were H-H. There was inadequate amount of specimens left in the tanks to be sampled in L-L group replicates at stage 12 and H-L group replicates at stages 11 hence the standard lengths for these stages of L-L and H-L group were not included in the statistical analysis. There was only one tank (tank5) for H-L group fish at stage 11 with a very few individuals left inside to be sampled, hence there is only box plot graphic for this group in myotome heights result.

2.7. Detailed studies of samples of larvae

Fixed samples of cod larvae and early juveniles were analysed at the laboratory at the Faculty of Biology, Fishery Science and Economy, University of Tromsø by using a Leica Wild M 10 stereomicroscope under different magnifications. The standard length, swim bladder size and anal-dorsal muscle height were measured by using a scaled ocular. The landmarks stated in Table 2 and 3 were taken as a basis for staging the individual fish larvae and juveniles. With increasing size the larvae had to be dissected in order to reveal the abdominal organs. After making relevant examinations and stagings of each individual,

pictures were taken with an Olympus digital camera mounted on the stereomicroscope and the larvae were transferred to glass vials with 70% alcohol solution for later histological processing for a further study.

There were very few individuals left in the tanks due to mortality in the H-L group after stage 8 and due to over-sampling in the L-L group replicates at stage 11. The few individuals of the H-L group were sampled at stage 11 and the L-L group at stage 12 and used for only the detailed studies of larvae for the thesis project. These data were not used in the standard length analysis. Three external diagnostic landmarks were studied after observation of the specimens from the four temperature combinations (Table 5). These landmarks are easy to identify with a stereo microscope and when it was necessary, the individuals were dissected in order to see internal organ development when the pigmentation on skin became stronger.

Table 5. Morphological landmarks and their functional relationships in developing Atlantic cod larvae from yolk-sac to transformation stage (von Herbing et. al.1996).

Landmark	Functional relationship
Swim bladder	Locomotion: controls buoyancy and may aid in swimming activity and prey capture efficiency.
Alimentary tract	Feeding: increases in size and complexity, enhances food digestion and assimilation.
Finfold	Locomotion: provides stability in the water and enhances forward thrust due to its large surface area.

The morphological differentiation landmarks in alimentary tract and finfold for each stage are based on Hunt von Herbing (1996) (Figs. 2, 3).






Larval Stage	Intestinal Stage	Description of intestinal structure	
1	1	simple straight gut, no differentiation, no anus	
2-7	2	intestine expanded, three part gut present; oesophagus, mid-gut and hind-gut; peristaltic waves present	
8	3	intestine contains one small lateral loop, posterior to mid-gut	
9	4	mid-gut (stomach) large, intestine has two coils	
10	5	large oesophagus, stomach, highly coiled intestine	

Figure 2. *Gadus morhua*. Description and staging of feeding landmarks with respect to larval developmental stage. (■ liver; □ intestine; ▨ swim bladder)

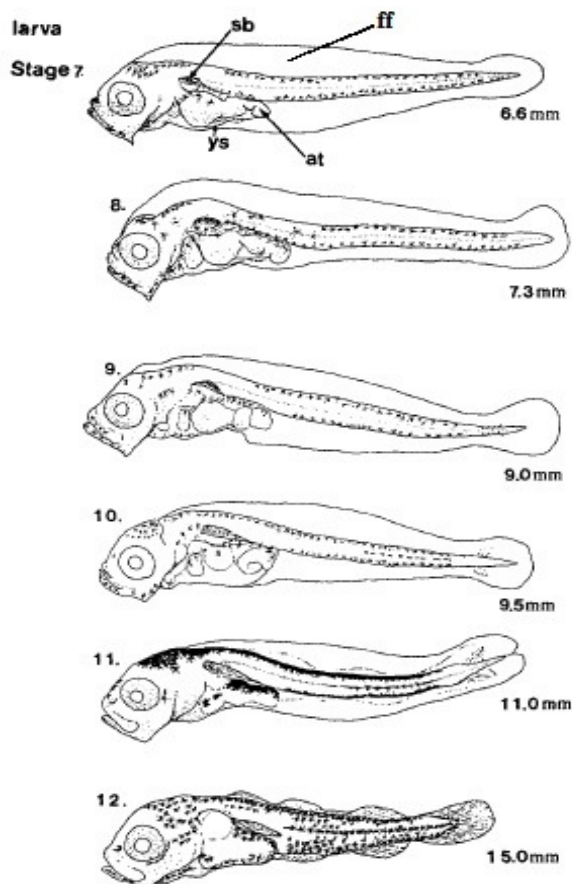


Figure 3. *Gadus morhua*. Representative drawings of developmental stages of cod larvae (sb swim bladder; at alimentary tract; ff finfold; ys yolk sac) (von Herbing et al., 1996).

3. Results

3.1. Larval Growth

3.1.1. Standard length

The standard length of the larvae derived from the CODE LIFE project is presented in the figures and tables below. Larvae at the L-L group reached the start of sampling stage (8) 29 days post hatch whereas the H-H group fish reached the same stage at 17 days (Table 4, Fig. 4). The L-H and the H-L groups reached stage 8 almost at the same time period (22 and 23 days post hatch). The fish in all of the replicates of H-L group started to die after stage 8 and were not available during the next sl sampling stages.

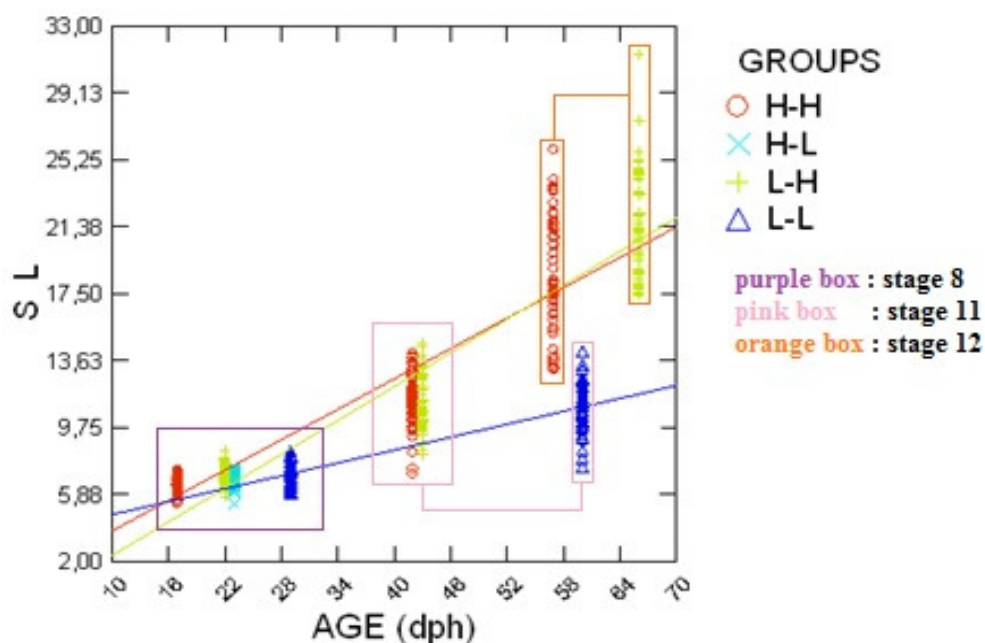


Figure 4. Standard lengths (SL, in mm.) of cod larvae from each temperature groups at different ages (dph: days post hatching) and stages. In stages 8 and 11 larvae from each temperature group show similar standard lengths but at different ages. At stage 12, L-H group larvae show a higher standard length than H-H group.

Table 6. Mean standard lengths (in mm.) at the respective sampling stage at each temperature group (n/a : No data for the corresponding stage).

group/stages	Stage 8	Stage 11	Stage 12
L-L	7,09	10,96	n/a
L-H	7,19	11,37	21,46
H-L	6,73	n/a	n/a
H-H	6,52	11,37	18,73

L-L group fish showed the slowest growth in terms of reaching advanced stages (Fig. 4) and also in terms of standard length measurements at a given time. The mean lengths of L-L group at stage 8 showed a higher value whereas at stage 11 the larvae from the other group had higher standard length means (Table 6). L-H group fish reached stage 8 in between H-H and L-L group fish but they reached stage 11 almost at the same age as the H-H group (Fig. 4). The mean lengths of this group are the highest at stage 8 and 11, at stage 11 the mean lengths of L-H and H-H group are similar (Table 6).

The least square means of L-L group at stage 8 and 11 are shown in figure 5. Among the tanks, tank 9 showed the highest standard length values in both stages whereas tank 7 had the lowest values in both stages. The increase in standard lengths from stage 8 to 11 showed almost the same trend in all the tanks.

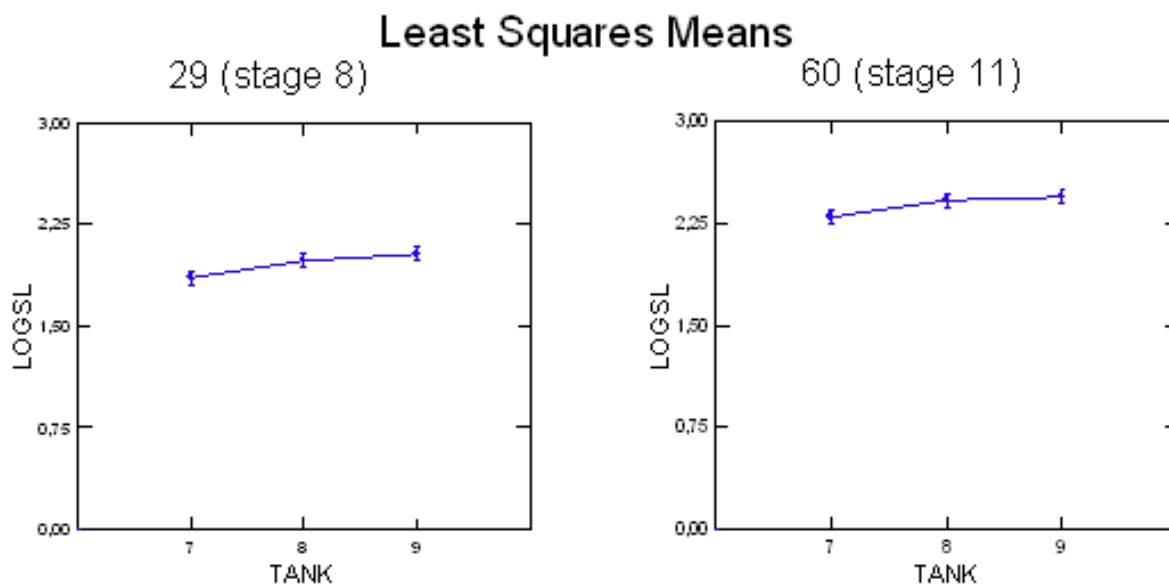


Figure 5. Least square means of log standard length (LOGS_L) of cod larvae with regard to age (days post hatch) and tanks in L-L group ($r^2 = 0,85377$, $p > 0,5$). Tank 9 shows the highest standard length values in both stages.

The least square means of L-H group are shown in figure 6. There was no significant variation in standard lengths of replicates at stages 8 (22 dph) and 11 (43 dph) whereas at stage 12 (66 dph) tank 20 showed higher standard length values (Fig. 6).

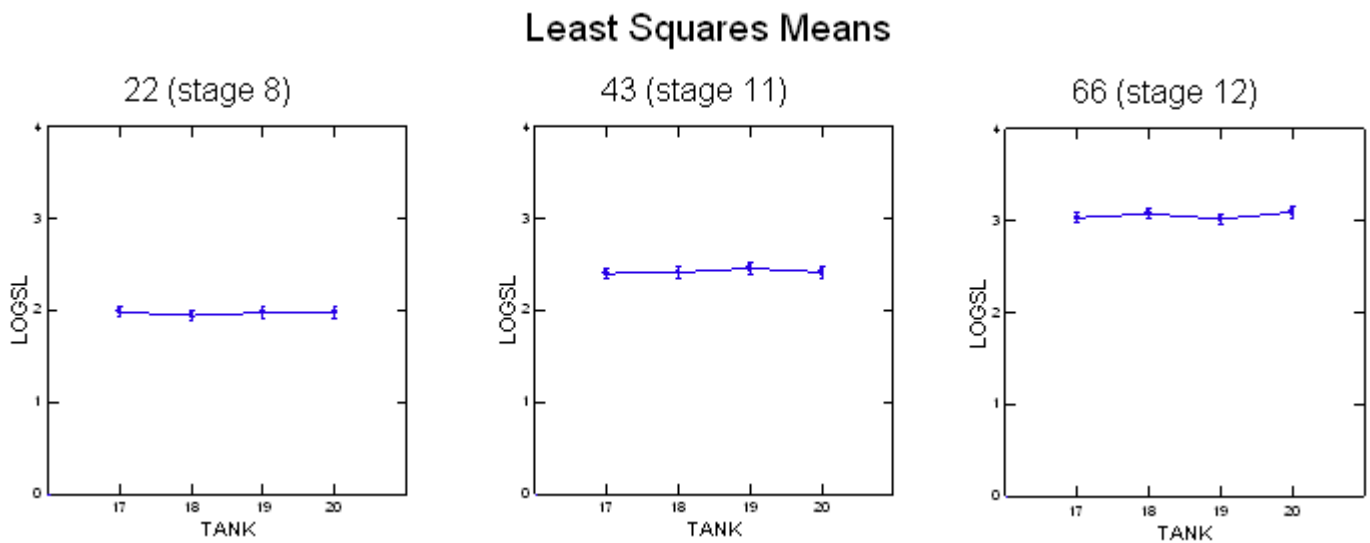


Figure 6. Least square means of log standard length (LOGS_L) of cod larvae with regard to age (days post hatch) and tanks in L-H group ($r^2 = 0,94$, $p > 0,1$). There is no significant larval sl variation among the tanks.

The least square means of standard lengths of H-L group fish are shown in figure 7. There was no significant variation among the tanks at stage 8.

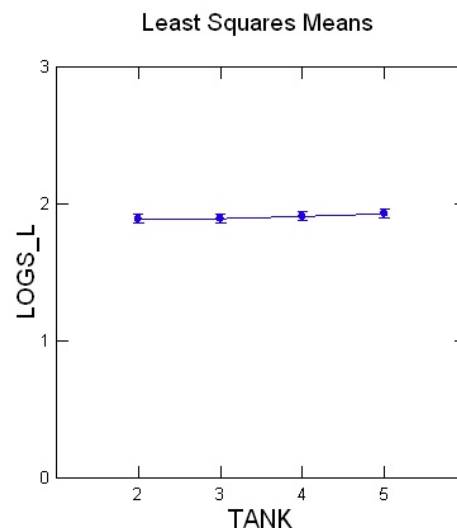


Figure 7. Least square means of log standard length (LOGS_L) of cod larvae at stage 8 with regard to tanks in H-L group ($r^2 = 0,062$, $p > 0,1$). No significant larval sl variation observed among the tanks.

At stage 8 H-H group fish, there was no significant variation in standard length among the tanks (Fig. 8) whereas at stage 11, tank 15 showed the lowest values among the tanks. Tank 16 had slightly higher sl values at stage 11 but at stage 12 very small increase in standard length was found (Fig. 8).

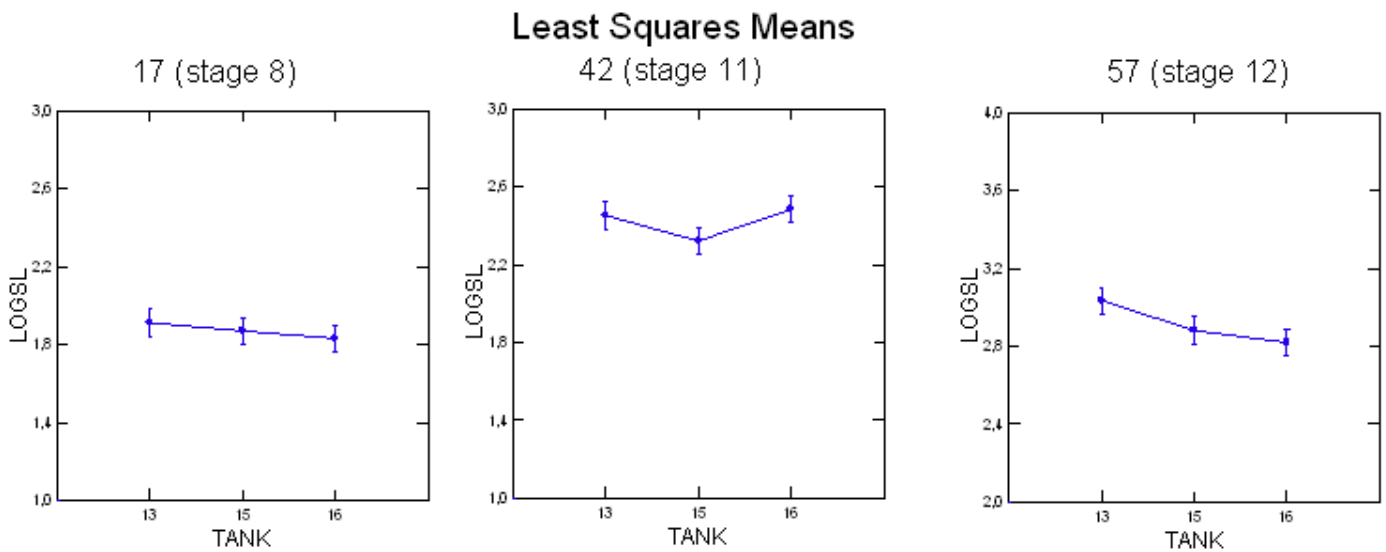


Figure 8. Least square means of log standard length (LOGS_L) of cod larvae with regard to age (days post hatch) and tanks in H-H group ($r^2 = 0,91$, $p < 0,01$). Tank 13 larvae show a better growth compared to those in other tanks.

3.1.2. Myotome height

The myotome heights of L-L and L-H groups at stage 8 were almost similar (Table 7, Fig. 9). At stage 11, the L-L group had the lowest myotome height whereas the rest of the temperature groups values were close to each other (Table 7, Fig. 9). At stage 12, H-H group fish had the highest myotome height followed by L-H and L-L temperature groups respectively (Table 7, Fig. 9).

Table 7. Mean lengths of myotome heights (in mm.) at the respective sampling stage at each temperature group (n/a : No data available for the corresponding stage, H-L and H-H groups stage 8 fish were only sampled for CODE LIFE project). N= 259

Groups/stages	Stage 8	Stage 11	Stage 12
L-L	0,906	1,197	1,674
L-H	0,91	1,475	2,978
H-L	n/a	1,432	n/a
H-H	n/a	1,477	3,144

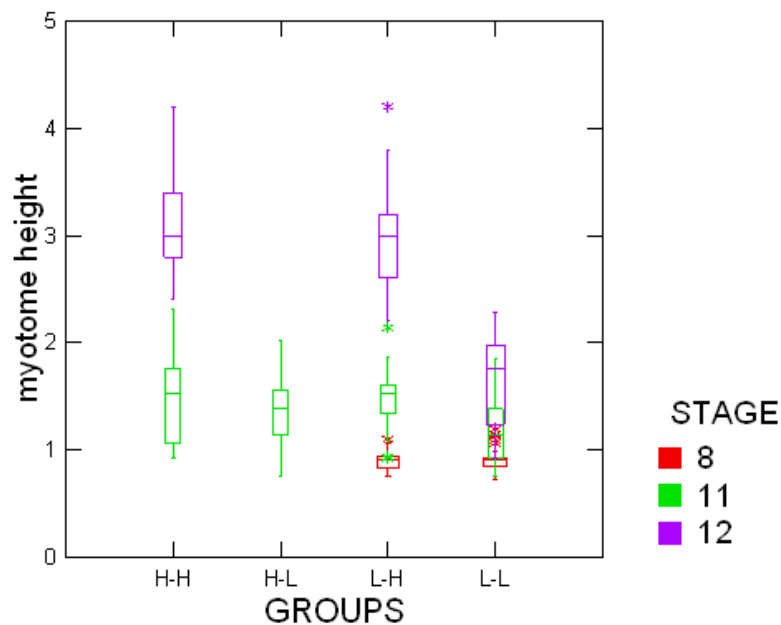


Figure 9. Myotome heights (in mm) of each temperature group at stages 8,11 and 12. The red boxes show stage 8, greens show stage 11 and purples show stage 12 myotome heights of the corresponding temperature groups. H-H group fish has the highest myotome heights. L-L group stage 12 fish shows significantly lower values compared to L-H and H-H stage 12 fish.

L-L temperature group fish had almost no variation among the tanks at stage 8 (Fig. 10). At stage 11, there was a significant increase in myotome heights of tank 8 fish whereas the rest of the replicates still had almost similar myotome heights as they had at stage 8 (Fig. 10). At stage 12, tank 8 showed higher myotome heights than tank 7 fish (Fig. 11).

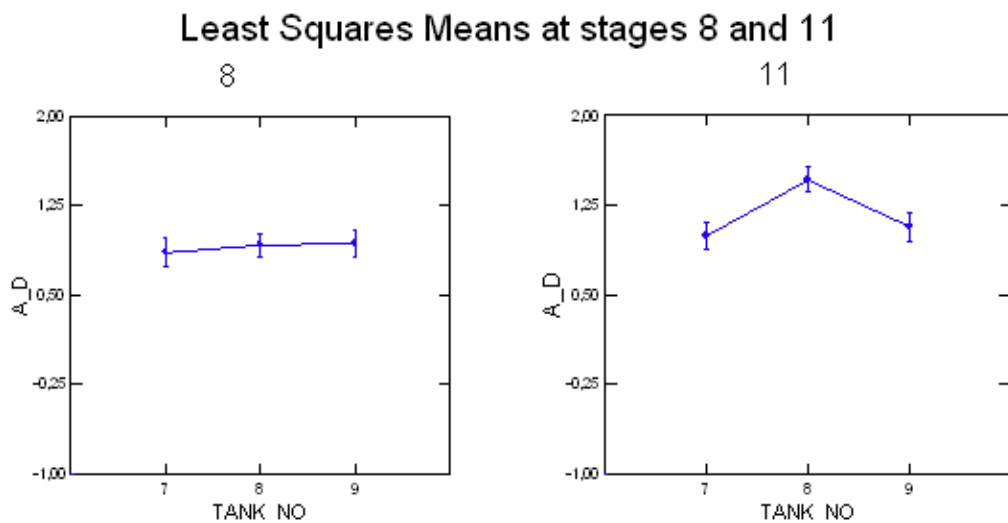


Figure 10. Least square means of myotome heights (a-d) of cod larvae with regard to stages 8 and 11 in L-L temperature group replicates ($r^2 = 0,63$, $p < 0,001$). Among the tanks, tank 8 shows higher myotome values (right) at stage 11. There is no significant increase in myotome height in tanks 7 and 9 from stage 8 to stage 11.

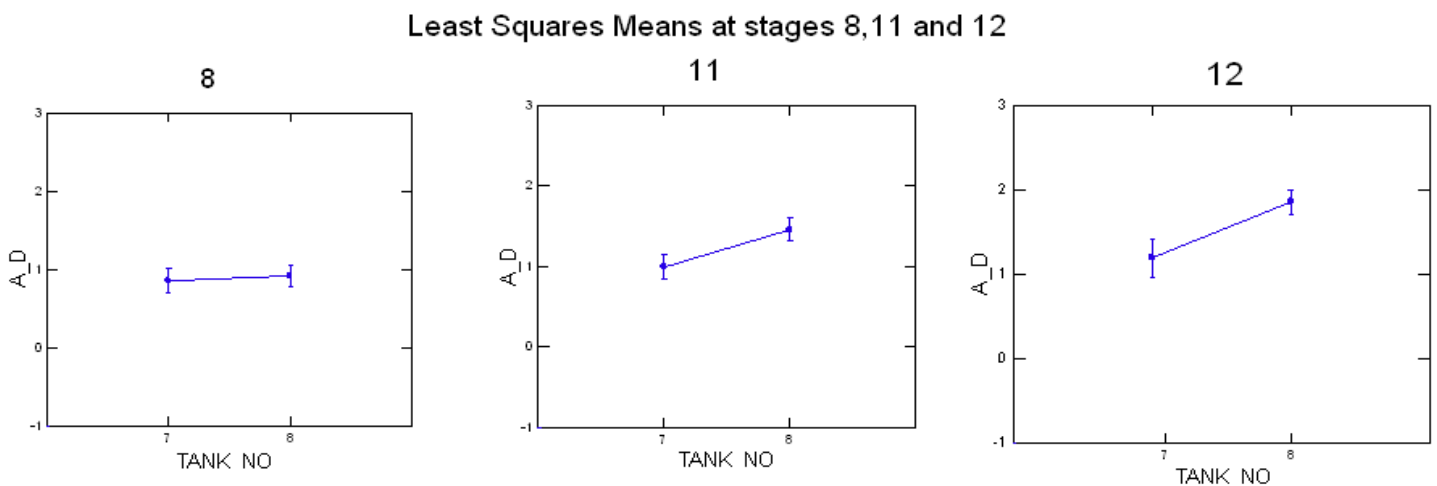


Figure 11. Least square means of myotome heights (a-d) of cod larvae with regard to stages 8,11 and 12 in tanks 7 and 8 of L-L temperature group replicates ($r^2 = 0,75$, $p < 0,01$). Tank 8 shows higher myotome heights at both stage 11 and 12 (middle, right).

Among the temperature groups, L-H group fish had the highest variations of myotome heights in each stage (Fig. 12,13). There was no difference in myotome heights among the tanks at stage 8 whereas at stages 11 and 12, tank 17 showed higher values (Fig. 12, 13).

Least Squares Means at stages 8,11 and 12

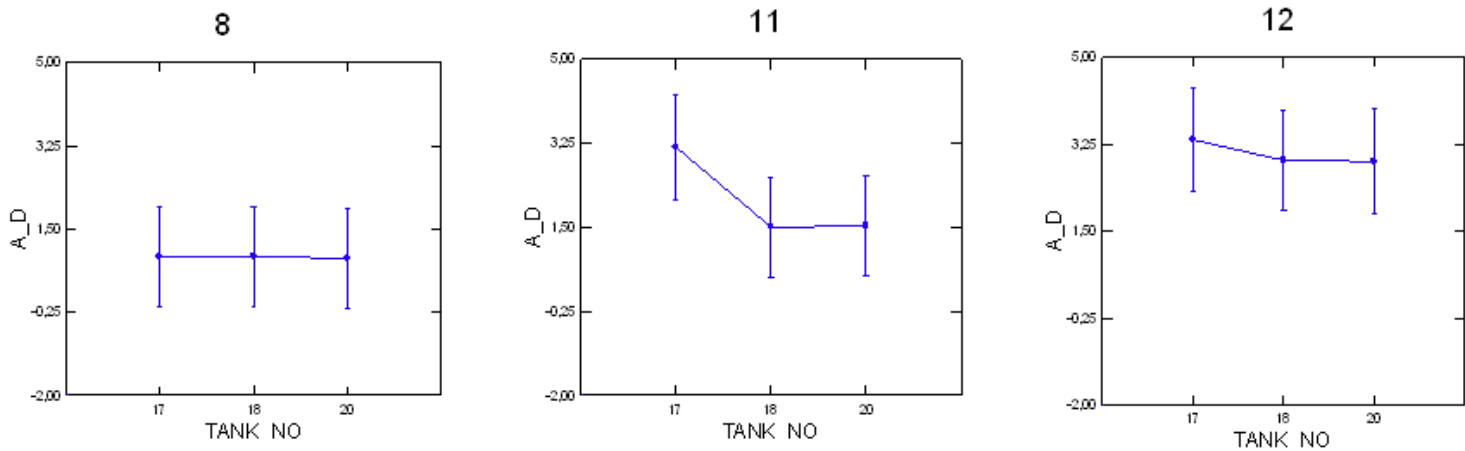


Figure 12. Least square means of myotome heights (a-d) with regard to stages 8,11 and 12 in tanks 17,18 and 20 of L-H temperature group ($r^2 = 0,28219$, $p > 0,5$). Tank 17 shows higher myotome heights at stages 11 and 12 (middle, right). At stage 8 replicates show almost a similar variance (left).

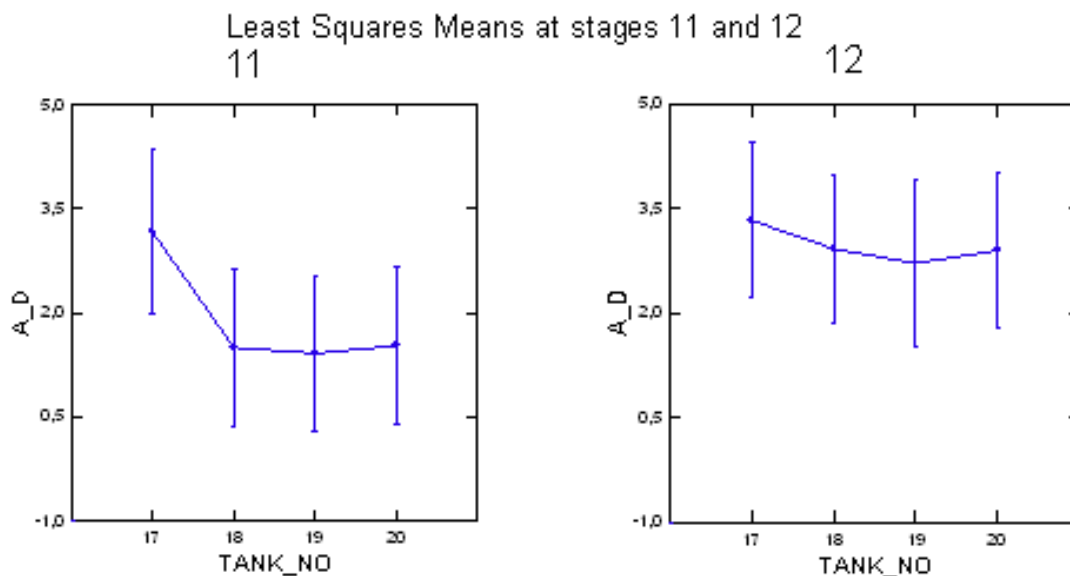


Figure 13. Least square means of myotome heights (a-d) of cod larvae with regard to stages 11 and 12 in tanks 17,18, 19 and 20 of L-H temperature group ($r^2 = 0,16936$, $p > 0,5$). Tank 17 shows higher myotome heights at stage 11 and 12.

H-L group fish had a large variance in myotome heights at stage 11 (Fig.14). Nevertheless, the mean myotome height was still higher than L-L group fish (Table 7.)

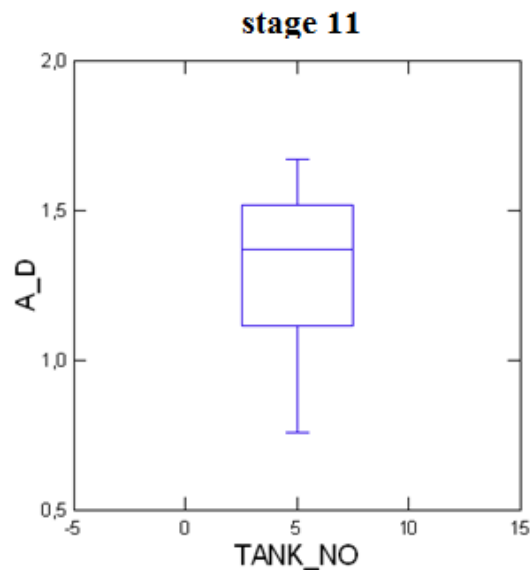


Figure 14. Myotome heights (in mm) of cod larvae in H-L group tanks at stage 11. There is a large variation of myotome height among the individuals.

Stage 11 H-H group fish had no significant variance among the replicates at stage 11 whereas at stage 12 tank 13 fish showed higher myotome heights than the rest of the tanks (Fig. 15).

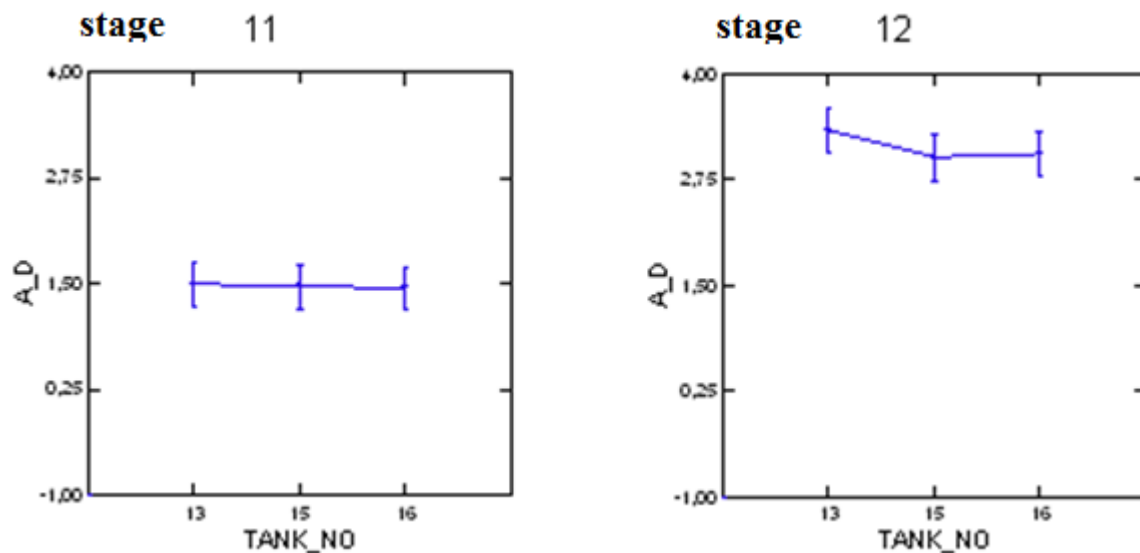


Figure 15. Least square means of myotome heights (a-d) of cod larvae with regard to stages 8,11 and 12 in H-H temperature group replicates ($r^2 = 0,82261$, $p > 0,5$). At stage 11, no significant difference in myotome heights among the tanks was registered (left). Tank 13 shows a higher myotome height (right).

3.2. Organ development

The figures below (Figs. 16, 17,18) show the overall development of selected representatives from the 480 individuals of CODE LIFE project samples in different temperature groups at stages 8,11 and 12 respectively. At stages 8 and 11, not only the morphological development but also the main internal abdominal organ development are seen due to lack of pigmentation whereas at stage 12, only the swim bladder is clearly visible (Figs. 16, 17,18).

Larvae from all temperature groups reached stage 8 whereas only 3 of the temperature groups (LL,LH and HH) could progress through stages 11 and 12 (Figs. 16, 17, 18). Stage 8 fish from all temperature groups are seen in figure 16, with the landmarks of stage 8; small loop between mid-gut and intestine, circular swim bladder, slightly elongated liver and the larval finfold. Stage 11 specimens from three temperature groups (LL, LH and HH) are shown in figure 17, with the landmarks of stage 11; large stomach and indistinct coils in intestine, enlarged liver, elongated and posteriorly extended swim bladder over the mid-gut and intestine and well formed caudal-pectoral fins and precursors of dorsal-ventral fin pairs. Stage 12 specimens of three temperature groups (LL,LH and HH) are shown in figure 18, with the landmarks of stage 12; large stomach and intestine obscured by pigment, more enlarged liver, swim bladder elongated and extended posteriorly above the anus and covered by silver pigment, formed but still small dorsal-ventral fin pairs and well formed caudal fins. Most of the HH group individuals had larger fins than the rest of the temperature groups at this stage (Fig. 18-C).

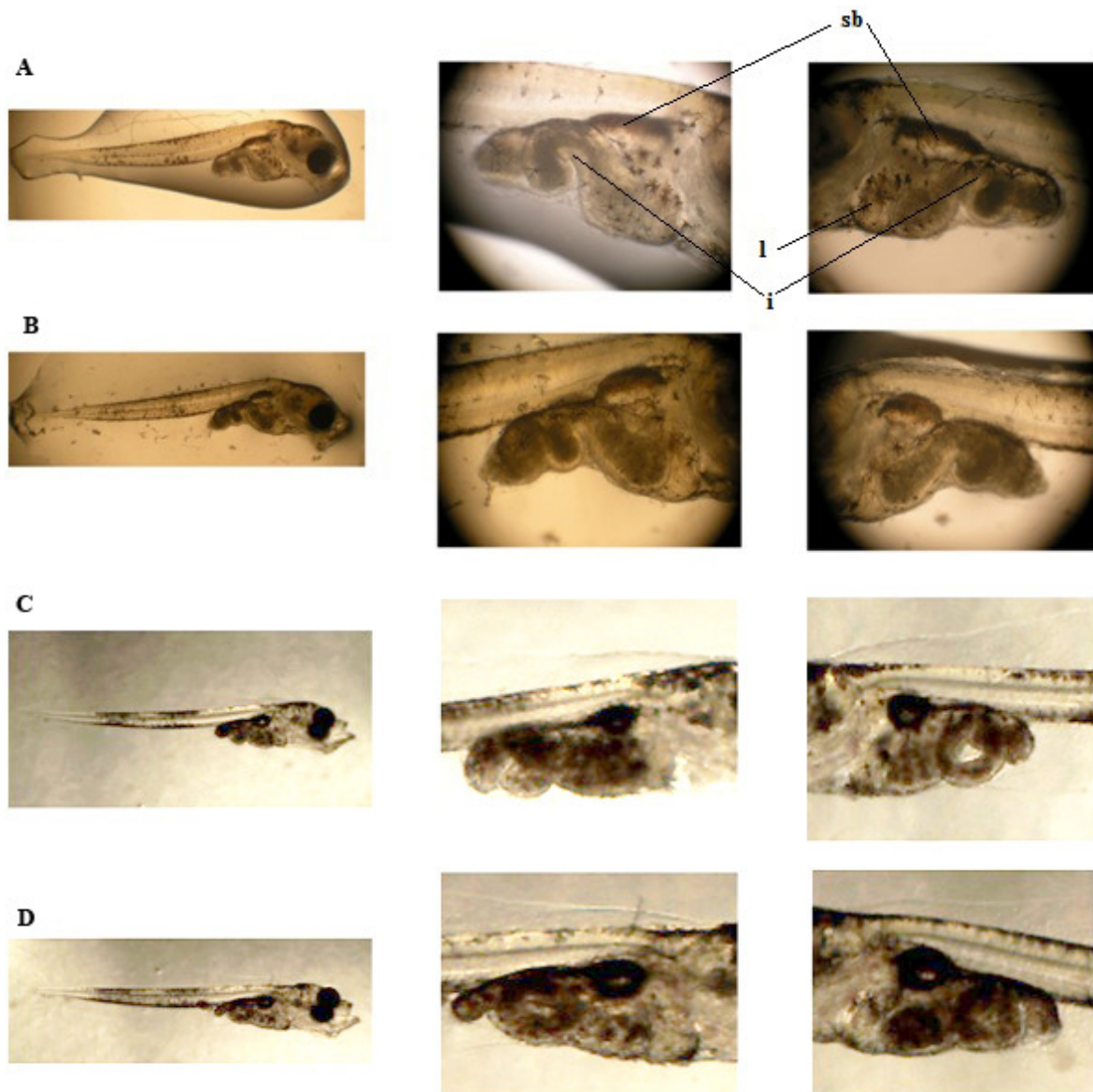


Figure 16. Pictures of larvae from stage 8 from the four different groups : a-LL group, b-LH group, c- HL group, d- HH group (overview, enlarged mid-body seen from right and left sides respectively, sb swim bladder, l liver, i intestine).

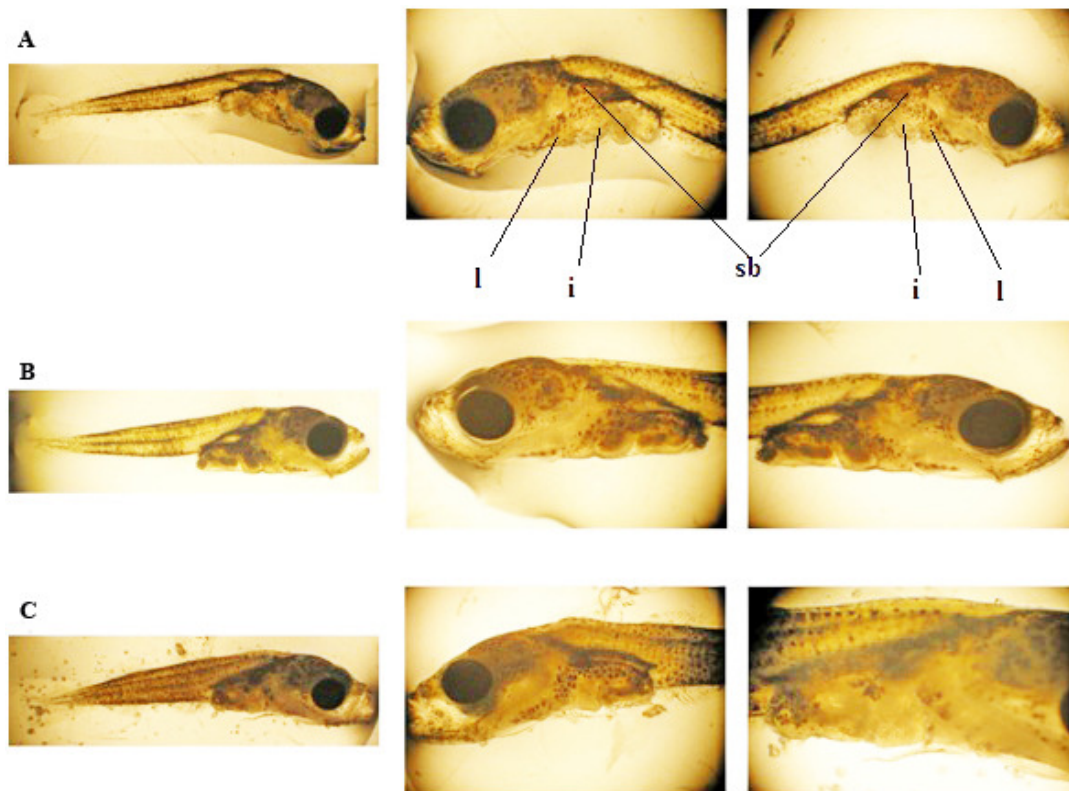


Figure 17. Pictures of larvae from stage 11 from the three different groups : a-LL group, b- LH group, c- HH group (overview, enlarged mid-body seen from right and left sides respectively, sb swim bladder, l liver, i intestine).

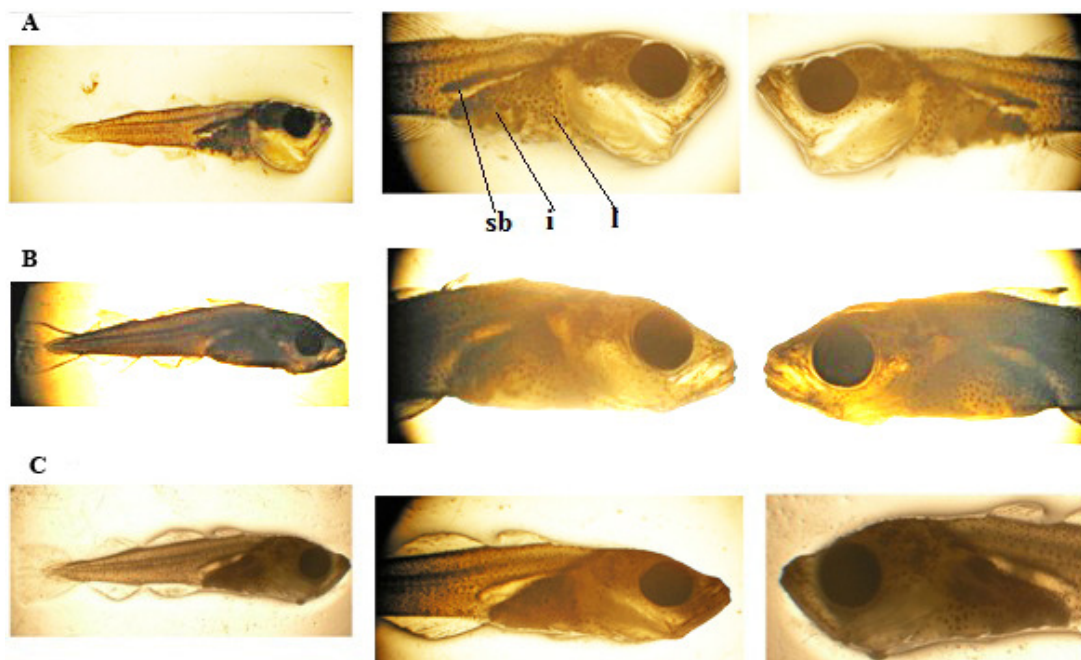


Figure 18. Pictures of larvae from stage 12 from the three different groups : a-LL group, b- LH group, c- HH group (overview, enlarged mid-body seen from right and left sides respectively, sb swim bladder, l liver, i intestine).

3.2.1. Swim bladder

The mean lengths of swim bladders at the respective sampling stage at each temperature group are shown in table 8 and figure 19. Among the temperature groups, L-H group has shown the highest swim bladder sizes at each stage (Fig. 19, Table 8), H-L group had the lowest value at stage 11 followed by L-L and H-H group respectively. At stage 12, L-H and H-H groups had almost similar means of swim bladder sizes (Table 8).

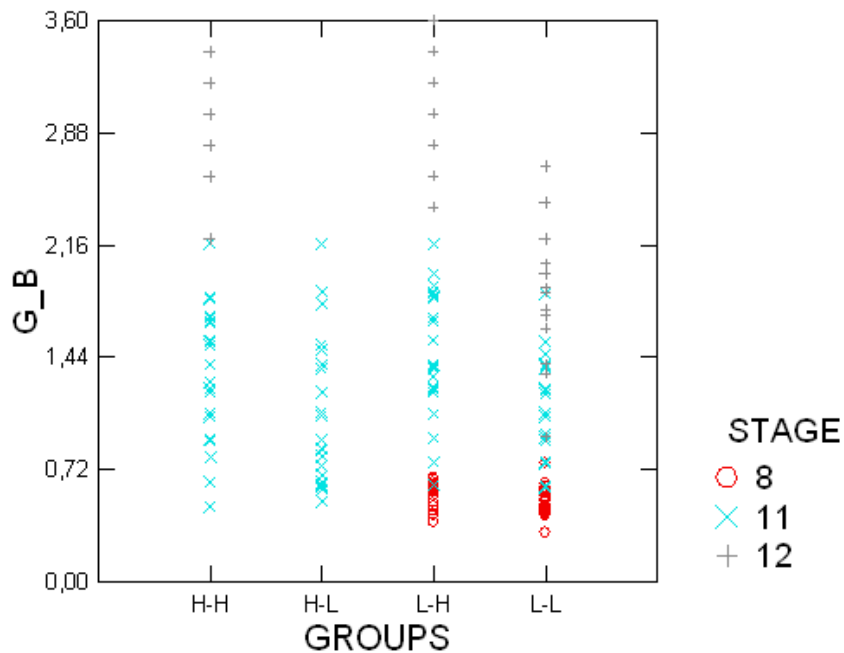


Figure 19. Swim bladder size (in mm.) of each temperature group at stages 8,11 and 12.

Table 8. Mean lengths of swim bladder sizes (in mm.) at the respective sampling stage at each temperature group (- : No data available for the corresponding stage). N= 259

Group/Stage	Stage 8	Stage 11	Stage 12
L-L	0,501	1,14	1,87
L-H	0,56	1,47	2,94
H-L	-	0,95	-
H-H	-	1,35	2,92

3.2.1.1. L-L Group

At stage 8, the dorsal pigmentation of the swim bladder was noted. Some individuals showed more scattered pigmentation on the swim bladder. The shape of the swim bladder

of most of the individuals was still circular whereas many individuals had a more elongated swim bladder referred to as more developed (Fig. 20, Table 9).

At stage 11, all of the individuals had developed pigmentation on the swim bladders covering the whole bladder. Swim bladders of most of the larvae were elongated and enlarged above the mid-gut and intestine but two individuals still had totally circular shaped swim bladder and the rest of the underdeveloped individuals had slightly elongated swim bladders (Fig. 21, Table 9).

At stage 12, all of the individuals showed a silvery pigmentation on the swim bladders. The shape of the swim bladder of each individual was elongated and extended posteriorly above the anus (Fig. 22, Table 9). Only three individuals had underdeveloped swim bladders at this stage.

Table 9. L-L group fish swim bladder development according to the shape and size at each sampling stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal.

Sampled/observed	Stage specific development	Underdeveloped	More developed
Stage 8	19	0	11
Stage 11	17	8	3
Stage 12	11	3	0

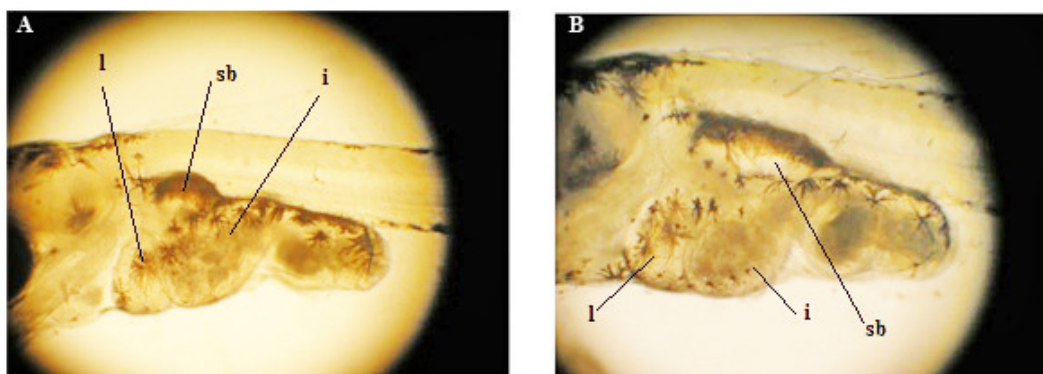


Figure 20. Different swim bladder shapes in L-L group stage 8 fish, a: circular shaped, b: more elongated shape. (sb swim bladder, l liver, i intestine)

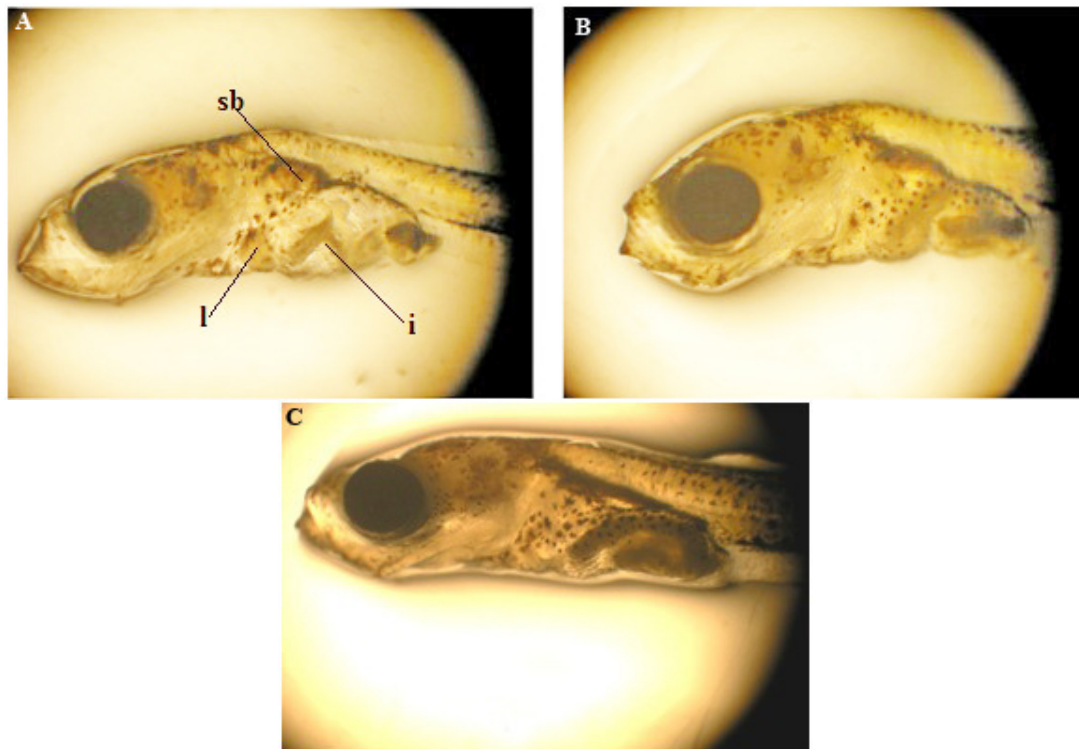


Figure 21. a,b: Circular shaped, c: more elongated swim bladders in L-L group stage 11. (sb swim bladder, l liver, i intestine)

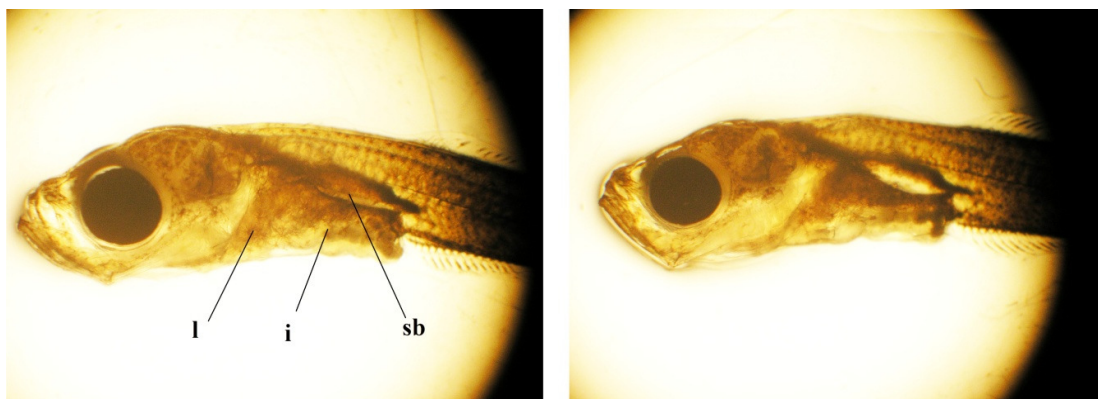


Figure 22. Random pictures of swim bladder at stage 12 in L-L group. (sb swim bladder, l liver, i intestine)

The least square means of L-L group fish swim bladder at stage 8 had no significant difference among the replicates whereas at stages 11 and 12 tank 8 fish had significantly higher values than the rest of the replicates (Fig. 23 and 24). Figure 24 shows that the variance in tank 7 is higher than in tank 8.

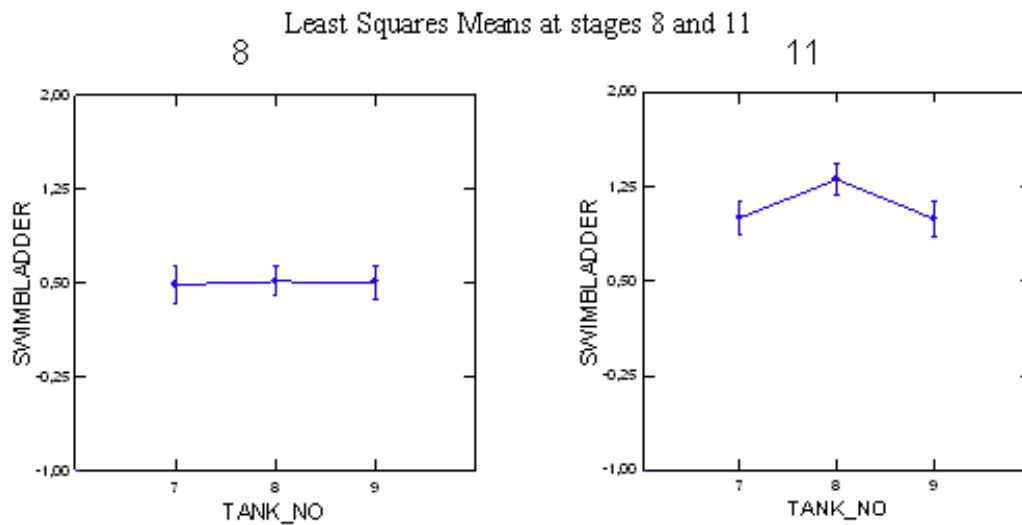


Figure 23. Least square means of swim bladder lengths in tanks 7,8 and 9 at stages 8 and 11 of L-L group ($r^2 = 0,73728$, $p < 0,5$). Tank 8 shows a higher value in swim bladder size at stage 11 than the rest of the replicates (right). At stage 8 there is no significant size difference among the tanks (left).

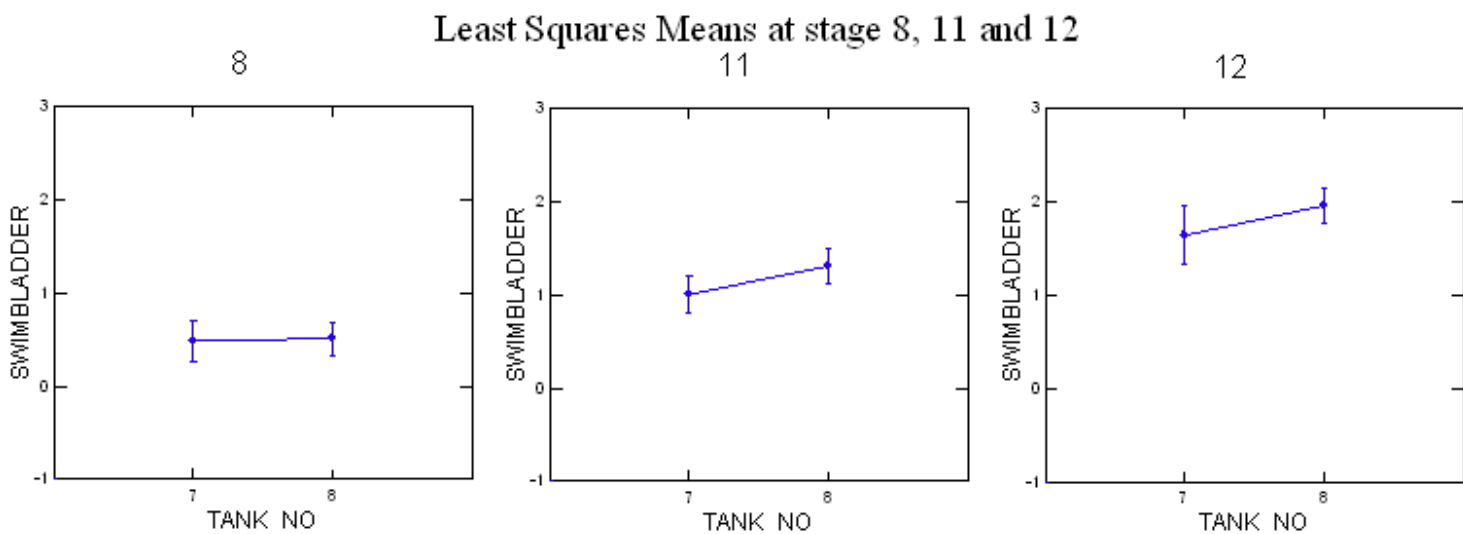


Figure 24. Least square means of swim bladder lengths in tanks 7 and 8 at stages 8, 11 and 12 of L-L group ($r^2 = 0,78921$, $p < 0,5$). At stage 8 there is no significant difference among the tanks (left), at stage 11 and 12 tank 8 shows larger swim bladder size (middle, right). The variance in tank 7 is higher than in tank 8 (left, right).

3.2.1.2. L-H Group

Stage 8 specimens showed almost a similar pigment development, pigmentation starting to cover the swim bladder from the top and slightly scattering abdominally. 15 of the 30 individuals had circular swim bladder shape whereas the rest of the specimens started to develop a more enlarged swim bladder (Fig. 25, Table 10).

Pigmentation of the swim bladders at stage 11 was similar for all individuals; all of them were covered with pigments. 1 of the 30 individuals had a circular shaped swim bladder and the other 2 underdeveloped specimens had slightly elongating swim bladders (Fig. 26, Table 10) whereas the rest of the individuals developed an elongated swim bladder that extended over mid-gut.

At stage 12, all of the L-H group fish developed silver pigmentation covering the swim bladder. The shape of the swim bladder in each individual was elongated and extended over mid-gut and anus. There were no apparent differences in terms of size and pigmentation between the specimens (Fig. 27, Table 10).

Table 10. L-H group fish swim bladder development according to the shape and size at each stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal.

Sampled/observed	Stage specific development	Underdeveloped	More developed
Stage 8	15	0	15
Stage 11	19	3	17
Stage 12	40	0	0

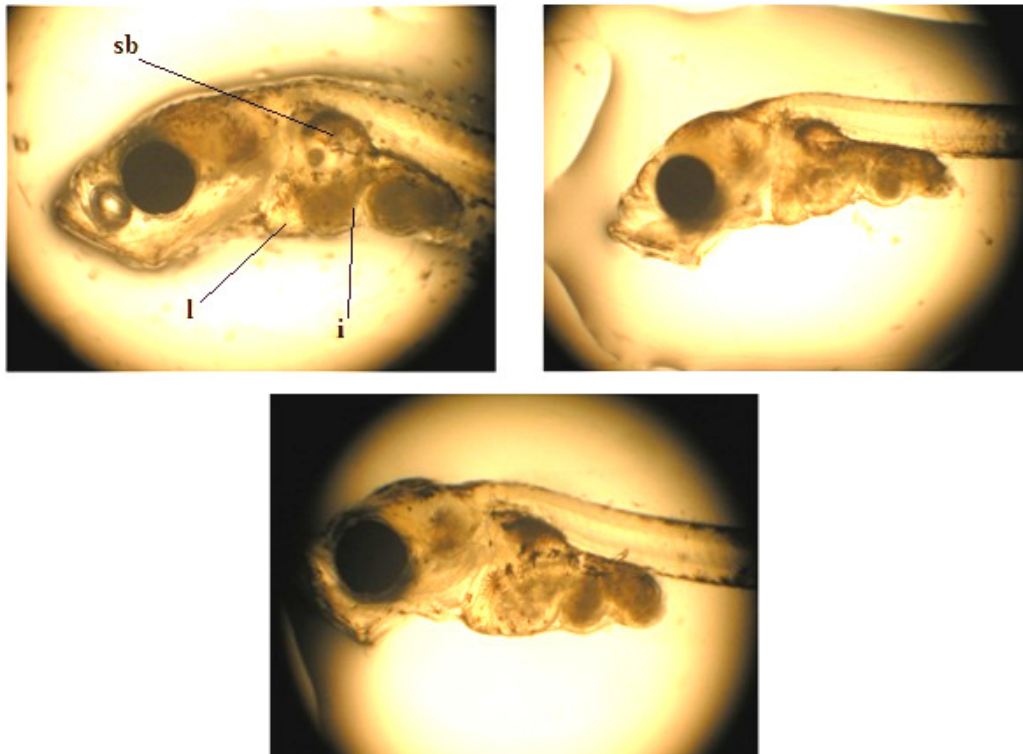


Figure 25. Random stage 8 pictures from three different replicates of L-H group. (sb swim bladder, l liver, i intestine)

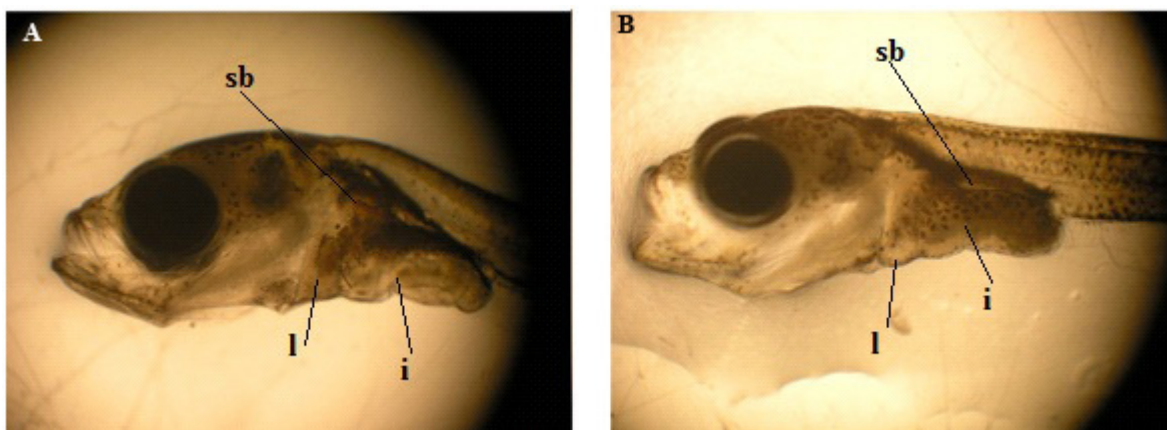


Figure 26. L-H group stage 11 pictures; a: still circular swim bladder, b: elongated swim bladder. (sb swim bladder, l liver, i intestine)

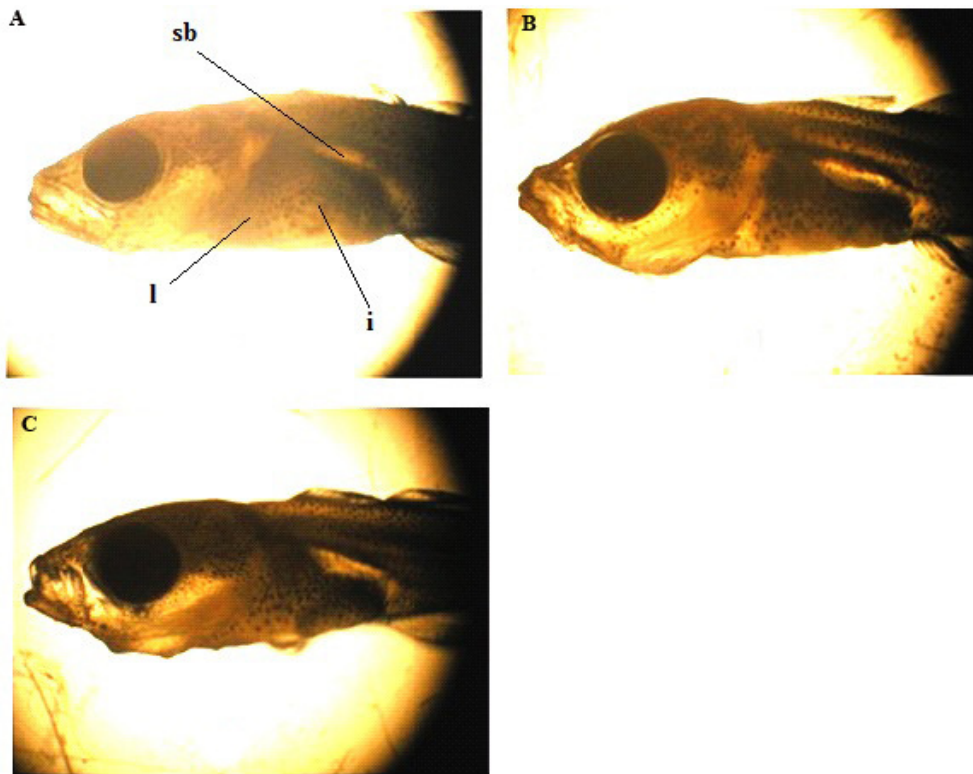


Figure 27. L-H group stage 12 pictures from three different replicates. (sb swim bladder, l liver, i intestine)

The least square means of swim bladder sizes in L-H group replicates are given in figures 28 and 29. At stage 8 there was no significant difference among the tanks however tank 18 has a higher swim bladder size at stage 11 and tank 17 has a higher swim bladder size at stage 12 (Figs. 28, 29). Tank 19 had the lowest swim bladder size at stages 11 and 12 (Fig. 29).

Least Squares Means at stages 8,11 and 12

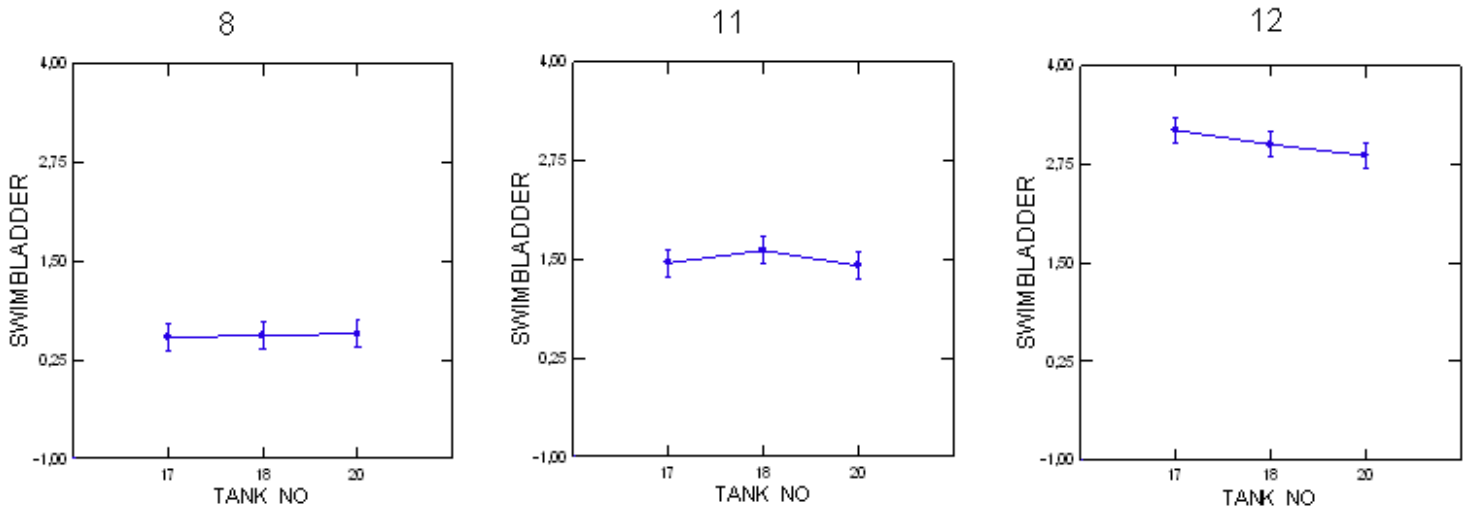


Figure 28. Least square means of swim bladder lengths in tanks 17, 18 and 20 at stages 8, 11 and 12 of L-H group ($r^2 = 0,94265$, $p < 0,5$). At stage 8 there is no significant difference among the tanks (left) whereas at stage 11 tank 18 has higher values (middle right) and at stage 12 tank 17 has the highest values (right). Tank 20 shows the lowest swim bladder size (middle, right). There is a significant increase in swim bladder size after stage 11 (middle).

Least Squares Means at stages 11 and 12

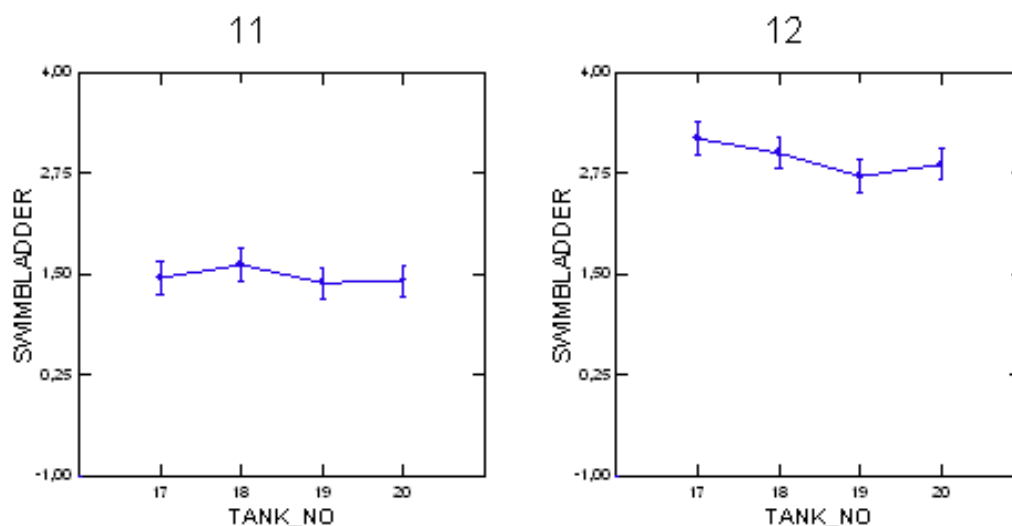


Figure 29. Least square means of swim bladder lengths in tanks 17, 18, 19 and 20 at stages 11 and 12 of L-H group ($r^2 = 0,86591$, $p < 0,5$). Tank 19 shows lowest swim bladder size in both stages.

3.2.1.3. H-L Group

Stage 11 H-L group fish had developed pigmentation spreading all over the swim bladder. 9 individuals still had underdeveloped swim bladders, the rest (13) of the fish developed slightly elongating and elongated over mid-gut swim bladders (Fig. 30, Table 11).

Table 11. H-L group fish swim bladder development according to the shape and size at each stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal (- : not sampled).

Sampled/observed	Stage specific development	Underdeveloped	More developed
Stage 8	-	-	-
Stage 11	8	9	4
Stage 12	-	-	-

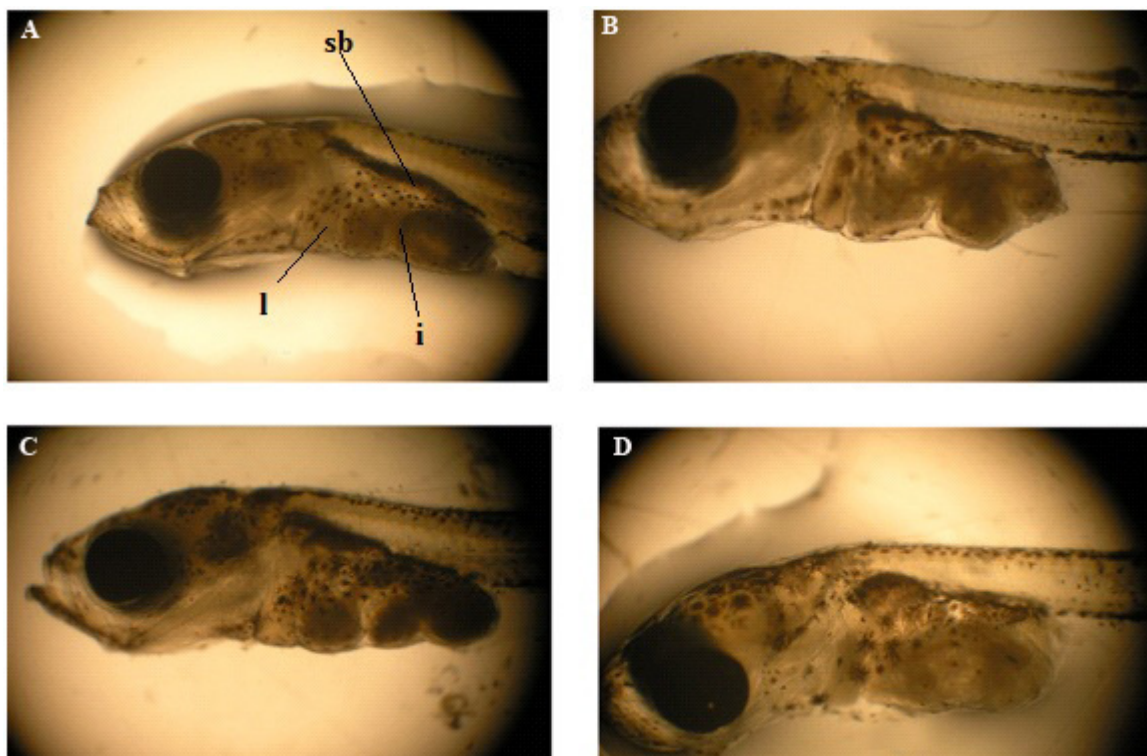


Figure 30. Different swim bladder shapes from H-L group at stage 11; a: Elongated over mid-gut and anus, b: Slightly elongating, c: More elongated, d: Still circular. (sb swim bladder, l liver, i intestine)

Since there is only one tank and one stage at this group, ANOVA analysis was not used for this group. The range of swim bladder lengths of this group is shown in Figure 31.

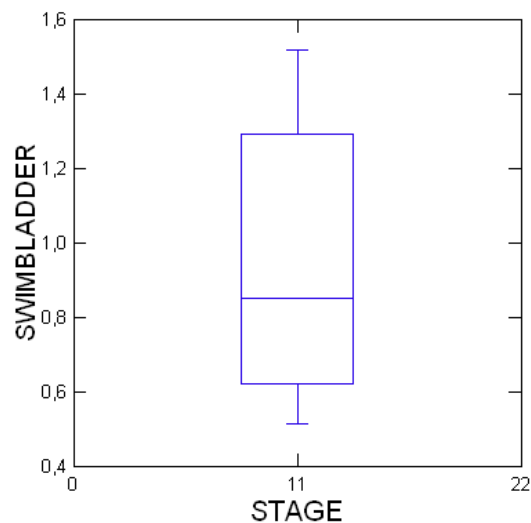


Figure 31. Swim bladder length (in mm) range at stage 11. There is a large variation in swim bladder sizes in this group of fish. The mean swim bladder size is 0,95 mm (see Table 7), the lower limit is 0,5 mm and the higher limit is 1,5 mm. Most of the fish had swim bladder sizes in between 0,6 and 1,3 mm.

3.2.1.4. H-H Group

At stage 11, most of the fish had developed a pigmentation covering the whole bladder. The size of the swim bladder showed a variance at this stage, 12 out of 30 individuals had extremely elongated swim bladders whereas 4 had slightly elongated. And one of the specimens still had a circular shaped swim bladder (Fig. 32, Table 12). Stage 12 H-H group fish developed silver pigments on the swim bladder. All of the individuals developed elongated and extended swim bladders over mid-guts through anus (Fig. 33).

Table 12. H-H group fish swim bladder development according to the shape and size at each stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal (- : not sampled).

Sampled/observed	Stage specific development	Underdeveloped	More developed
Stage 8	-	-	-
Stage 11	13	5	12
Stage 12	2	0	27

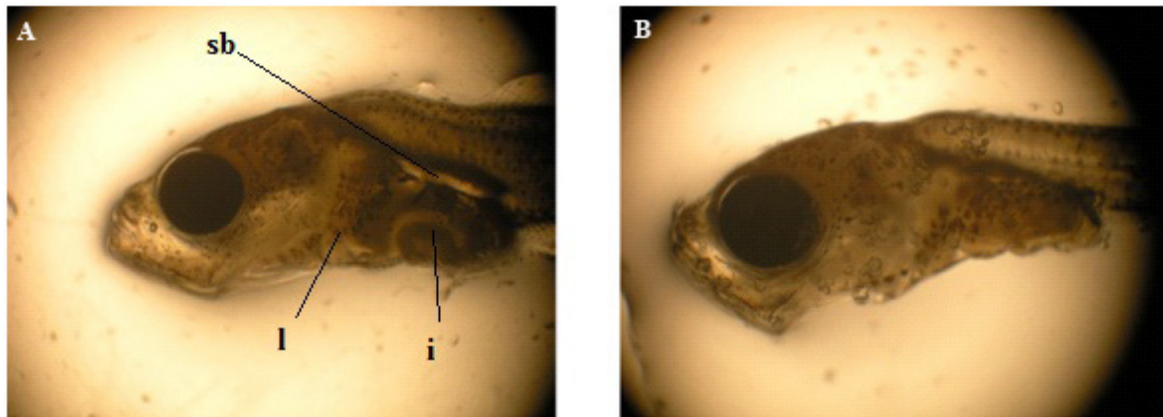


Figure 32. Stage 11 H-H group, a: Elongated swim bladder shape, b: Still circular swim bladder. (sb swim bladder, l liver, i intestine)

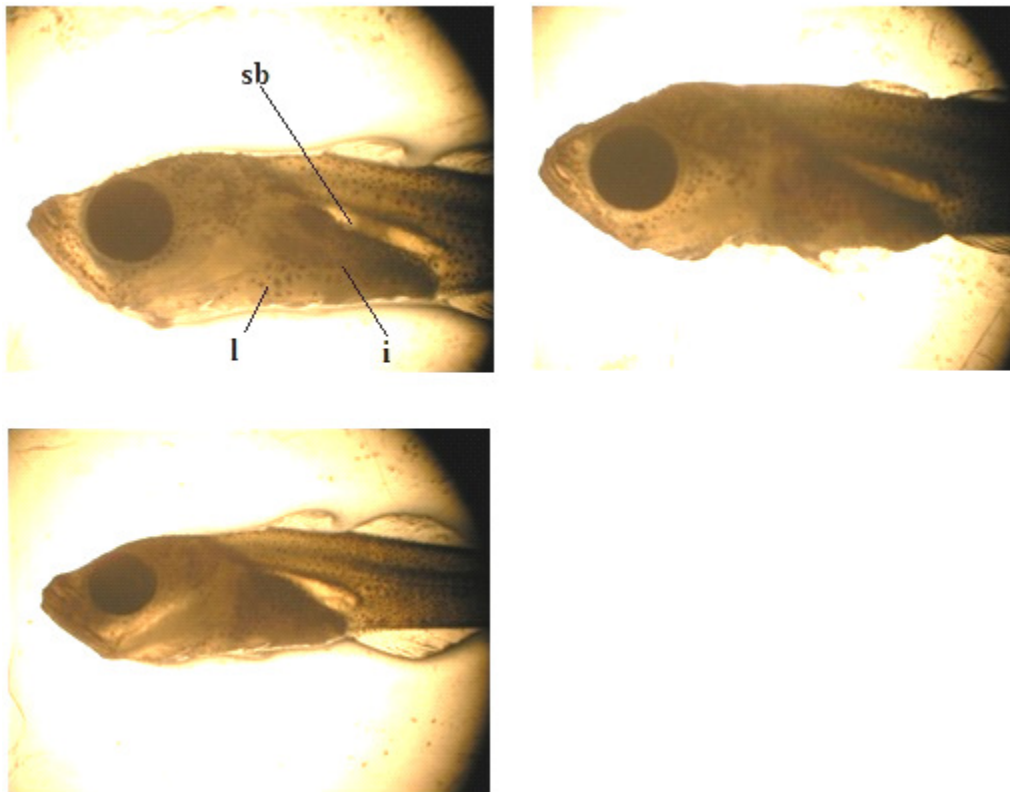


Figure 33. Random stage 12 pictures from three different replicates of H-H group. (sb swim bladder, l liver, i intestine)

The ANOVA results for this group were $r^2 = 0,83$, $p > 0,5$. At stage 11, tank 15 showed slightly lower swim bladder sizes than the other replicates. There was no significant overall length variance among the tanks as they reached stage 12 (Fig. 34).

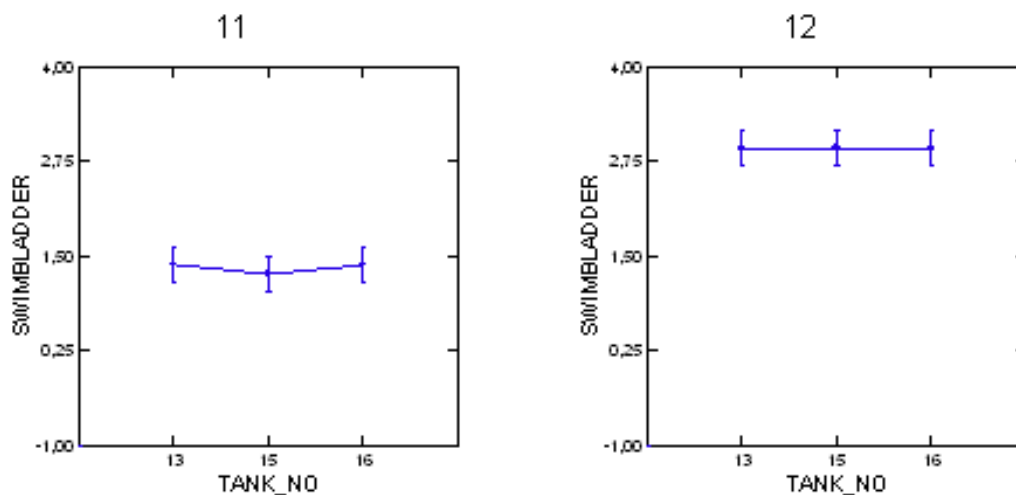


Figure 34. Least square means of swim bladder sizes with regards to stages 11 and 12 in H-H group replicates ($r^2 = 0,83$, $p > 0,5$). There is no significant size differences among the groups.

3.2.2. Alimentary tract and liver development

3.2.2.1. L-L group

Most of the stage 8 L-L group fish had started to develop a small loop between mid-gut and hind-gut, 13 of 40 individuals had developed more enlarged mid-gut and slightly coiling intestine which indicates the developmental stages of stage 9 alimentary tract. 2 of the 40 individuals still had an almost straight alimentary tract without any traces of a loop (Fig. 35). At this stage, there is no yolk or yolk remnants observed in any individuals. The liver of the individuals were still small and round shaped, slightly elongating in some individuals.

At stage 11 ,most of the individuals in each replicate had developed a large stomach and intestine (Fig. 36, Table 13). 5 of the 29 individuals had irregularities in their intestines (shrunken) (Fig. 36, Table 13). The coils of the intestine were indistinct in most of the specimens due to pigmentation. At this stage, the livers were more elongated and enlarged. Stage 12 L-L group had 2 of 14 specimens with irregularities in their alimentary tracts (Fig. 37), the rest of the specimens showed the landmarks of stage 12, highly coiled and large intestine covered with pigments. One of the individuals had a very small and underdeveloped liver even if it had a well formed intestinal development (Fig. 37). The rest of the individuals had developed enlarged and elongated livers.

Table 13. L-L group intestine development at each sampling stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal.

Sampled/Observed	Stage specific development	Underdeveloped	More developed
Stage 8	25	2	13
Stage 11	12	5	12
Stage 12	12	2	0

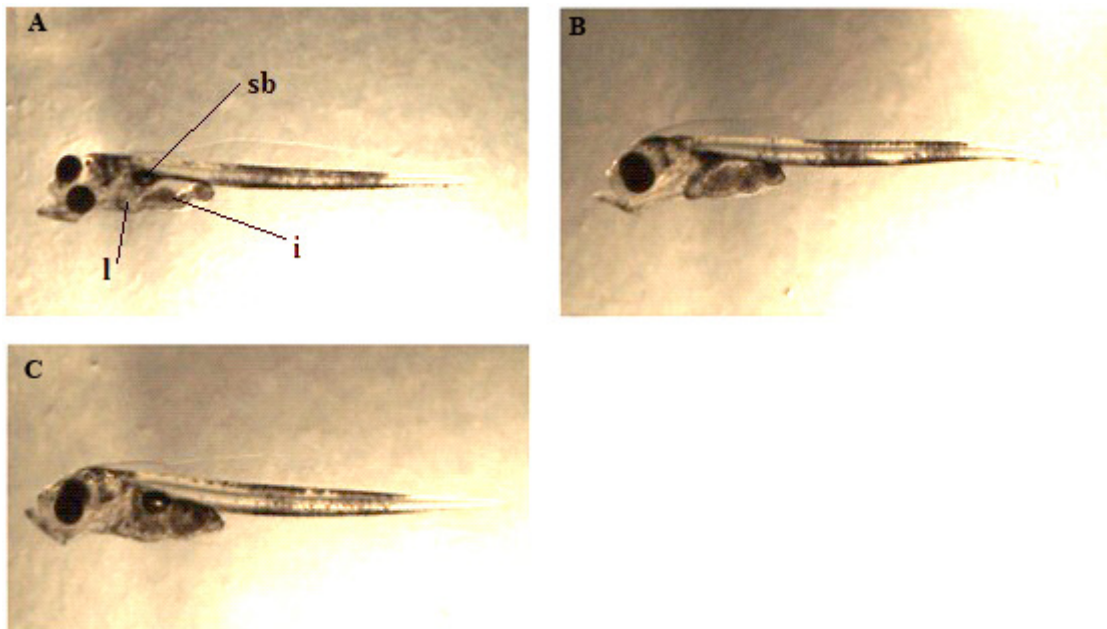


Figure 35. Alimentary tract differentiations at stage 8 L-L group fish; a: Almost straight, b: Small loop between intestine and mid-gut, c: Enlarged mid-gut (sb swim bladder, l liver, i intestine).

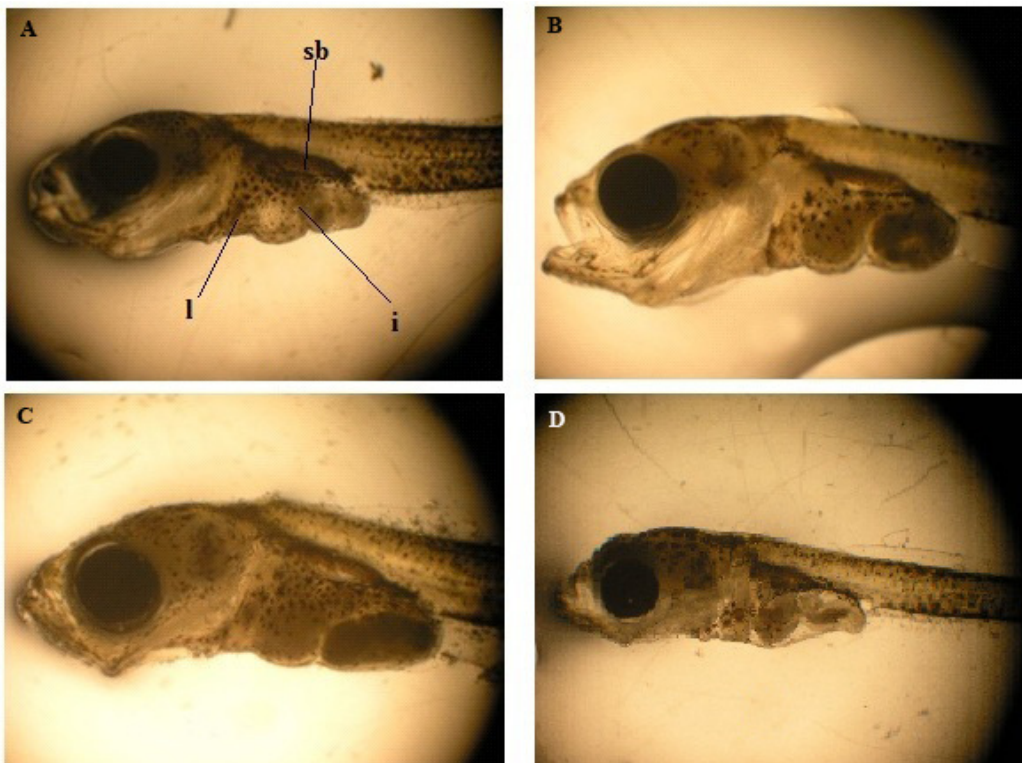


Figure 36. L-L group stage 11 pictures, a,b,c : Normal intestinal development, d: Irregular (shrunken) intestine. (sb swim bladder, l liver, i intestine)



Figure 37. Stage 12 L-L group intestine development; a: Irregularity in intestine (shrunken), b: Normal stage 12 intestine development, c: Underdeveloped liver. (sb swim bladder, l liver, i intestine)

3.2.2.2. L-H group

Most of the larvae at stage 8 had developed a small loop between mid-gut and intestine and some individuals started to develop a larger mid-gut indicating they were closer to stage nine (Fig. 38, Table 14.). Liver sizes of this group are slightly larger than the L-L group with the indications of growth through oesophagus. Only one individual had a slightly smaller liver size than the rest of the group (Fig. 38).

Stage 11 fish had developed highly coiled intestines covered with pigments (Fig. 39, Table 14). One of the 40 individuals had an underdeveloped alimentary tract referring to the landmarks stated at Table 2. The liver at this stage was enlarged and showed a growth through oesophagus.

All of the fish at stage 12 developed more functional, large and fully pigmented intestines and they all reached stage 12 in terms of alimentary tract development (Fig. 40, Table 14). Livers got more elongated and enlarged comparing to stage 11 fish.

Table 14. L-H group intestine development at each sampling stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal.

Sampled/Observed	Stage specific development	Underdeveloped	More developed
Stage 8	16	0	14
Stage 11	12	1	27
Stage 12	28	0	12

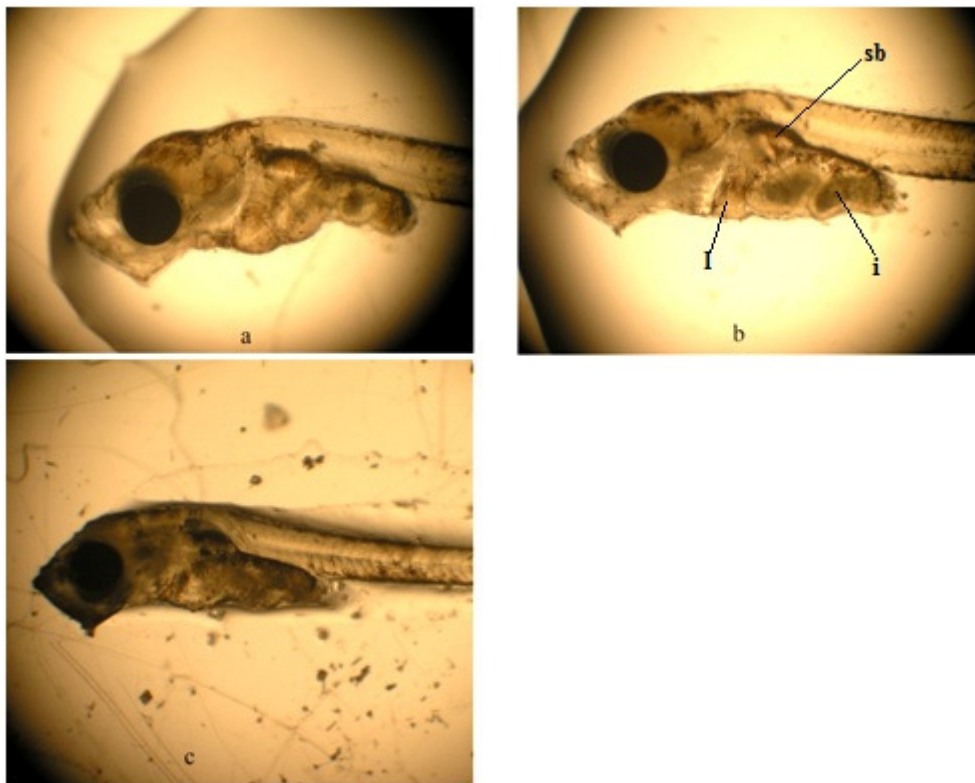


Figure 38. Stage 8 L-H group alimentary tract, a: Small loop between mid-gut and intestine, b: Slightly larger mid-gut, c: Individual with small liver and normal intestinal development. (sb swim bladder, l liver, i intestine)

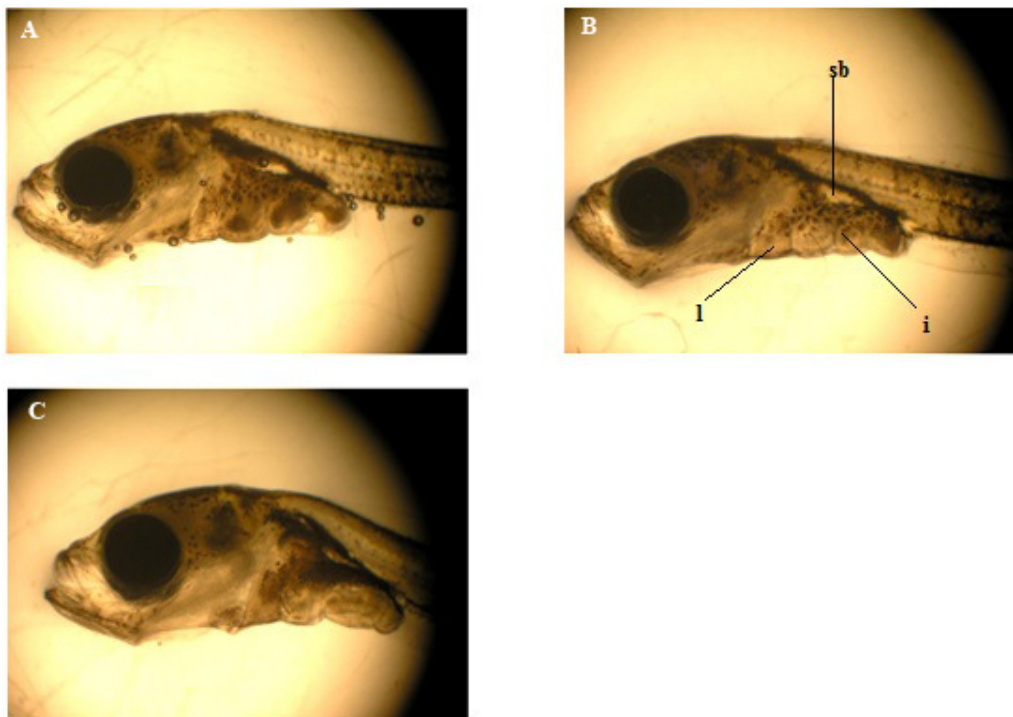


Figure 39. a and b: Random pictures of L-H group stage 11 intestines, c: Underdeveloped intestine with big loop between mid-gut and hind-gut. (sb swim bladder, l liver, i intestine)

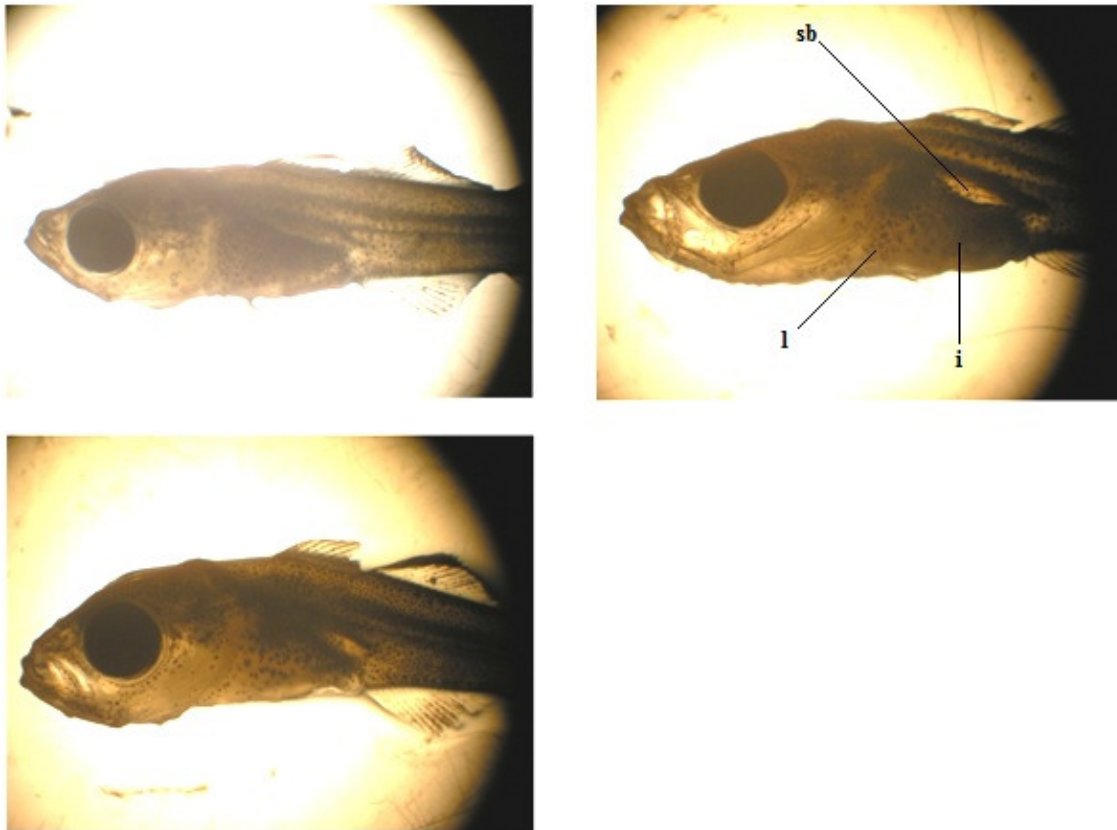


Figure 40. Random stage 12 pictures from three different replicates of L-H group. (sb swim bladder, l liver, i intestine)

3.2.2.3. H-L group

Most of the stage 11 fish had coiled intestines with an indistinct loop at this stage (Fig. 41). 2 of the 29 individuals had underdeveloped intestines with a big distinct loop and 5 of the 20 fish had irregularity in their intestine (Fig. 41, Table 15). Two of the individuals with shrunken intestines had very underdeveloped round shaped small liver (Fig. 41), the rest of the individuals developed elongated and enlarged livers.

Table 15. H-L group intestine development at each sampling stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal (- : not sampled).

Sampled/Observed	Stage specific development	Underdeveloped	More developed
Stage 8	-	-	-
Stage 11	17	7	5
Stage 12	-	-	-

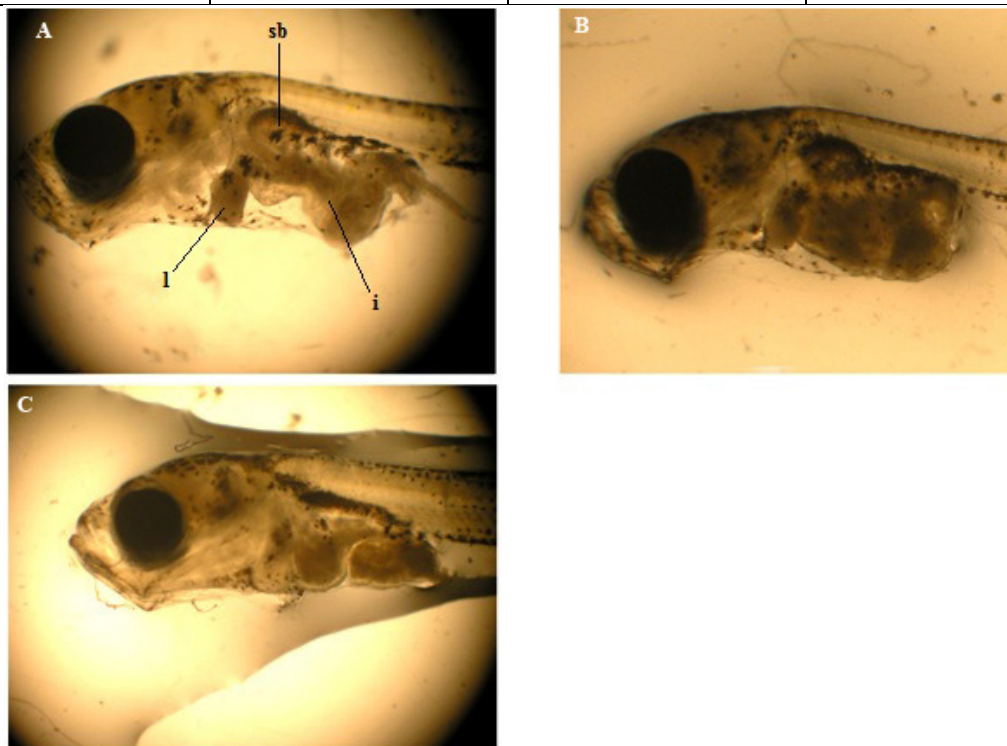


Figure 41. Pictures from stage 11 H-L group, a: Shrunken intestine with small round shaped liver, b: Irregular growth in intestines, c: Normal development in liver and intestine at stage 11. (sb swim bladder, l liver, i intestine)

3.2.2.4. H-H group

Most of the stage 11 fish developed coiled and large intestines. It was observed that some of the individuals had more sophisticated, highly coiled intestines, showing the landmark of stage 12 intestine shape (Fig. 42, Table 16). Stage 11 fish had elongated and enlarged livers. Individuals that are closer to stage 12 had more enlarged livers than the rest of the specimens (Fig. 43).

At stage 12, most of the fish developed advanced intestine that are highly covered with pigments (Fig. 43, Table 16). The shapes of the intestine of most fish were indicating that these fish were about to finish metamorphosing stage. Only 1 of 29 individuals were showing indications of stage 12 intestine development. All the individuals had very enlarged and elongated livers.

Table 16. H-H group intestine development at each sampling stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal (- : not sampled).

Sampled/Observed	Stage specific development	Underdeveloped	More developed
Stage 8	-	-	-
Stage 11	22	0	8
Stage 12	1	0	28

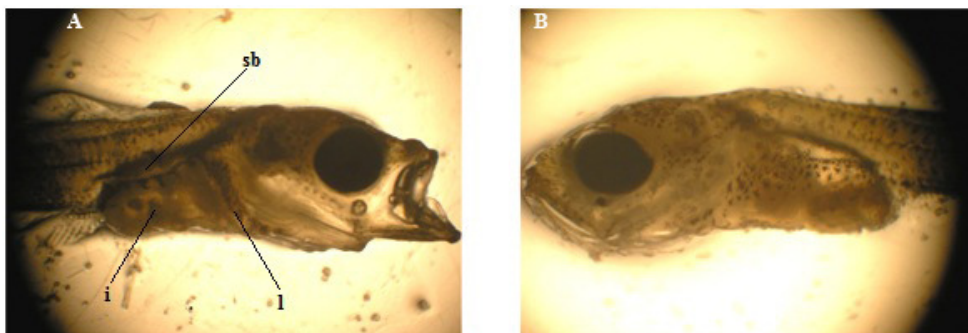


Figure 42. Pictures from stage 11 H-H group; a: Intestine development closer to stage 12, more enlarged liver, b: Stage 11 intestine and liver development (sb swim bladder, l liver, i intestine).

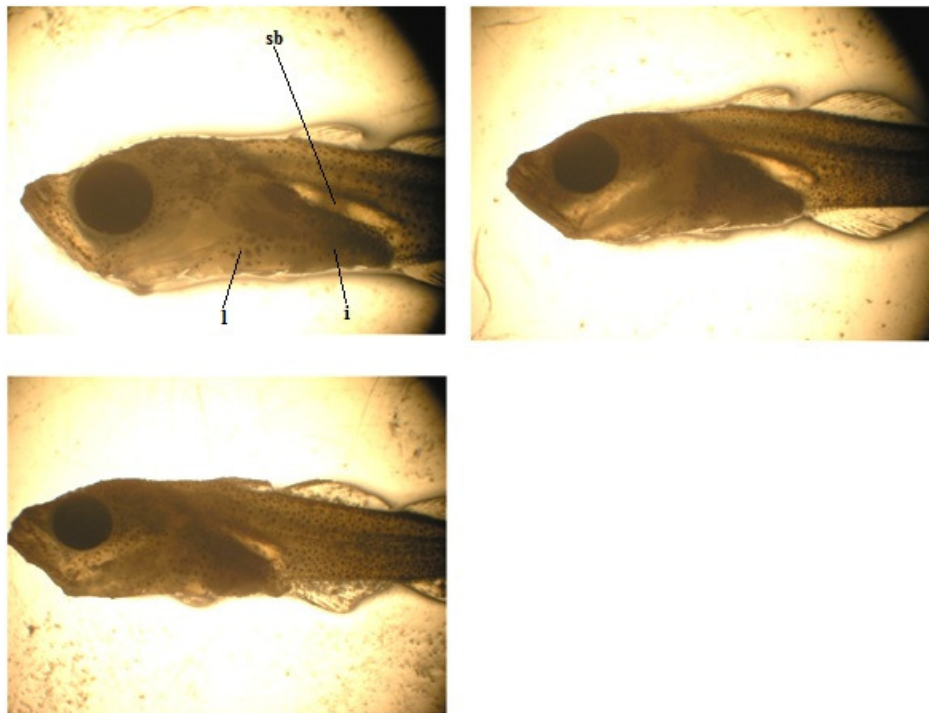


Figure 43. Random pictures of stage 12 samples (sb swim bladder, l liver, i intestine).

3.2.3. Fin Development

3.2.3.1. L-L group

Of the 30 individuals at stage 8, 28 fish had developed large pectoral fins and thickened caudal peduncle which indicates the fin development of stage 8. 2 of the individuals had slightly visible caudal fin rays (Table 17).

15 of the 29 individuals developed fin rays in caudal fin and thickened pectoral fins at stage 11. The rest of the specimens had large pectoral and caudal fins and precursors of dorsal-ventral paired fins (Table 17).

10 of the 14 specimens had developed small dorsal, anal and pelvic fins; well formed and large caudal fin at stage 12. The rest of the specimens still had the precursors of dorsal-ventral fins (Table 17).

Table 17. L-L group fin development at each sampling stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal.

Sampled/Observed	Stage specific development	Underdeveloped	More developed
Stage 8	28	0	2
Stage 11	14	15	0
Stage 12	10	4	0

3.2.3.2. L-H group

Of the 30 individuals, only 1 was showing the landmarks of stage 8 fin development with large pectoral fins and thickened caudal peduncle. 29 individuals developed visible fin rays in caudal and pectoral fins showing the fin development of stage nine (Table 18).

At stage 11, 21 of the 39 specimens had developed precursors of dorsal-ventral paired fins and large caudal and pectoral fins. 10 individuals still had thickened pectoral fins and fin rays on caudal and pectoral fins. 8 individuals developed small dorsal and ventral fins (Table 18).

All of the specimens from the stage 12 sampling had developed larger fins with fully developed dorsal-ventral paired fins, caudal and pectoral fins showing being more differentiated than stage 12 fins according to the landmarks by Hunt von Herbing (Table 18).

Table 18. L-H group fin development at each sampling stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal.

Sampled/Observed	Stage specific development	Underdeveloped	More developed
Stage 8	1	0	29
Stage 11	21	10	8
Stage 12	0	0	40

3.2.3.3.H-L group

At stage 11, 14 of the 29 specimens had developed precursors of dorsal-ventral paired fins and large caudal and pectoral fins. 15 of the 29 specimens still had large pectoral and caudal fins, fin rays on pectoral and caudal fins but no precursors of dorsal-ventral paired fins (Table 19). No more developed fins were observed at H-L group fish.

Table 19. H-L group fin development at each sampling stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal (- : not sampled).

Sampled/Observed	Stage specific development	Underdeveloped	More developed
Stage 8	-	-	-
Stage 11	14	15	0
Stage 12	-	-	-

3.2.3.4. H-H group

14 of the 30 individuals had developed precursors of dorsal-ventral paired fins and large caudal and pectoral fins at stage 11. 7 individuals still had large pectoral and caudal fins, fin rays on pectoral and caudal fins but no precursors of dorsal-ventral paired fins. 9 individuals had developed formed but still small dorsal-ventral paired fins which state stage 12 development according to Hunt von Herbing (see Table 3, Table 20)

At stage 12, only 1 of the 29 individuals had the landmarks of fin development with small dorsal-ventral paired fins at stage 12 as Hunt von Herbing stated (see Table 3). 28 of the 29 specimens had developed large dorsal-ventral paired fins and large caudal and pectoral fins (Table 20).

Table 20. H-H group fin development at each sampling stage. Numbers refer to the amount of individuals at each developmental stage during the sampling period. The expected sampled stages are vertical in the first column and the actual observed stages are horizontal (- : not sampled).

Sampled/Observed	Stage specific development	Underdeveloped	More developed
Stage 8	-	-	-
Stage 11	14	7	9
Stage 12	1	0	28

4. Discussion and conclusions

4.1. Larval growth

Temperature affected larval growth and time of reaching the expected stages till the end of mid-metamorphosis period. The higher the temperature was during the incubation and after hatching period, the faster the fish reached the expected stages (Table 4). The temperature at incubation period takes place at the hatching time, but on the other hand the size of the larvae at hatch and the growth rates during endogenous feeding (Peterson et al., 1977; Chambers, 1997; Otterlei et al., 1999).

The standard lengths of H-H and H-L larval groups at stage 8 were shorter than those of the low incubation temperature groups (Fig. 4, Table 6), which can be correlated to the fact that increased temperatures would result in decreased yolk conversion efficiencies that would reduce the total energy available for growth and consequently produce smaller larvae (Orjanguren & Brana, 2003). Temperature influences biochemical reactions especially on energy supply and demand and hence play an important role on fish growth (Blaxter, 1992; Imsland & Jonassen, 2001). After stage 8, the high temperature groups showed better growth than the low temperature groups. However, the final sizes of H-H group fish were shorter than the L-H group fish at stage 12 (Fig. 4, Table 6). This result correlates with the assumption that the increase in temperature can accelerate developmental rates more than those of growth and can cause different larval sizes at a given developmental stage (Kamler, 1992; Fuiman et al., 1998). At hatching, larval size can be crucial in terms of survival, but after the endogenous feeding period with the start of external feeding, the importance of the morphological development may be decisive for survival of the larvae as the fish shifts into an active predator that has to chase the prey.

Thus, high temperatures give advantage to the growing larvae in terms of mobility that would consequently turn into energy by catching their prey.

4.1.1. Standard length

As the temperature affects the growth by time, the L-L group fish reached the expected stages the latest. Due to the yolk consumption efficiency rates, the L-L group fish have the highest standard length values at stage 8, whereas after stage 8 due to the effects of low temperature, the mean standard length of the L-L group showed lower values compared to the other groups (Fig. 5, Table 6). There was no significant variation among the standard lengths of L-L group replicates, small variations may have been caused by the density of the tanks.

The low temperature exposed to eggs until hatching caused the L-H larvae to reach stage 8 at a number of days in between that of H-H and L-L group fish. The effects of low temperature until hatching and high temperature after hatching are stated above. The effects of high temperature after hatching caused a decent growth in standard length size in this group. Even if the larvae reached stage 11 almost at the same time as the H-H group larvae (Table 4), L-H group fish reached stage 12 after a bit longer time period (66 dph in L-L group, 57 dph in H-H group).

Due to the high temperature during incubation and hatching and low temperature during the larval stage, H-L group fish reached stage 8 almost at the same time as L-H group (23 dph) (Fig. 4, Table 4). The mean standard length at stage 8 was lower than the low temperature incubation and hatching group, but it was slightly higher than H-H group (Fig. 4, Table 6). There was no obvious variance among the tanks at stage 8 (Fig. 5). Right after stage 8, there was a high mortality in the replicates in this group. This collapse in the tanks of H-L group is postulated to be due to the decrease in the temperature after hatching since there was no such mortality in H-H group fish.

The mean standard length of H-H group was the lowest among the experimental groups at stage 8; yet the time to reaching stage 8 was the shortest (Fig. 4, Table 4, 6). The effects of the increased temperature after the hatching period resulted with the significant increase of standard length as they almost had the same standard lengths as the L-H group fish in the advance stages (stage 11 and 12).

There was no significant variation among the tanks at stage 8, even if there was a small difference among the tanks. At stage 11, there was a considerable standard length

difference between tank 15 and the rest (Fig. 8). For all that, at stage 12, the standard length of tank 15 was higher than tank 16. This could be explained by the high number of the sampled individuals and mortality in the tanks at corresponding stages, yet we have no data about the exact larval density in the different tanks.

4.1.2. Myotome height

Galloway et al. (1998) found that at the time of first feeding, cod eggs incubated at low temperatures that are close to their lower temperature tolerance limit (1 °C) produced smaller and less-viable larvae which had fewer functional muscle fibers than the larvae that were incubated at 5 or 8 °C. They postulated that the low somatic growth rates were caused by the larvae putting higher priority into reaching a certain length instead of increasing muscle mass and it is energetically beneficial for cod larvae to increase in length in order to get the advantage of the ratio between inertial and viscous forces acting on swimming larvae (Fuiman and Webb, 1988; Osse and van den Boogaart, 1995).

The means of myotome heights did not show the same growth trend as the means of standard lengths at the corresponding temperature groups and stages. At stage 11 and 12, H-H group fish had slightly higher myotome means than L-H group, and significantly higher values than L-L group (Table 7, Fig. 9) whereas the standard lengths of L-H group fish at stage 11 was equal to those of the H-H group and at stage 12 was higher than the H-H group. This result can be correlated to those of Galloway et al. (1998) as the low temperature groups incubation and hatching had lower muscle growths compared to the high temperature groups.

L-L group stage 8 fish had almost no variation in myotome heights (Fig. 10), whereas as the fish grew into more advanced stages (Fig. 11), tank 8 fish showed a higher muscle height even if there was no significant variation in sl at stage 11 L-L group.

The highest variation of myotome heights at each developmental stage was at L-H group replicates. At stage 8, there was no significant variance among the tanks, but at stage 11 and 12, tank 17 larvae had larger muscle heights (Fig. 12). There was no significant variance among the tanks in sl sizes at each developmental stage, even if some individuals had developed a higher muscle height and some still had lower.

H-L group fish also had a high variance in tank 5 at stage 11 (Fig. 14). Since there is not enough sl data from stage 11 H-L group, it is not possible to compare and correlate the growth in sl and muscle height size.

H-H group fish had no significant myotome height variance among the tanks at stage 11 whereas there was a little variation in each tank (Fig. 15) yet the sl values at stage 11 showed a variance among the tanks. Tank 13 had a higher myotome height at stage 12 than the rest of the replicates, correlating with the sl values (sl higher than the rest of the replicates at stage 12).

The variations either in tanks and among tanks at each temperature group showed that the L-L temperature group had the lowest variation in myotome heights. As the temperature increased in the other groups, the variation in muscle height development increased as well. The myotome heights were postulated to be following an inversely proportional growth with the increasing-decreasing sl values. There was no such correlation determined between sl and myotome heights none of the temperature groups.

4.2. Organ development

As the fish absorb the yolk and proceed through the threshold of exogenous feeding, the early development of either internal or external organs play a key factor in terms of growth and survival. Rather than the differentiation of new structures that occurs before exogenous feeding, the pre-existing structures grow, such as the convolution of intestine which provides more surface area for digestion, the inflation of swim bladder which helps the fish to control the buoyancy as the fish gets heavier and the differentiation of the finfold (Blaxter, 1988; Kjørsvik et al., 1991; Hunt von Herbing, 1994; Hunt von Herbing et al., 1996).

4.2.1. Swim bladder

The swim bladder may partially inflate during endogenous feeding stage yet it is not fully functional until the end of yolk utilization and transition into exogenous feeding stage (Hunt von Herbing, 1994). The inflation of swim bladder plays a key factor on the survival of fish larvae and many studies on Atlantic cod and other species have reported that the failure in inflating the swim bladder can lead to high mortalities (Dannevig & Dannevig, 1950; Riley, 1966; Thompson & Riley, 1981).

As with regard to sl values, the L-H temperature group larvae had the highest swim bladder means at each developmental stage (Fig. 19, Table 8). L-L group fish had no underdeveloped swim bladders and 11 more developed swim bladders at stage 8 whereas they had 8 underdeveloped and 3 more developed at stage 11 and 3 underdeveloped at

stage 12. The variation in the size of the swim bladders among the tanks at the corresponding stage was showing almost the same pattern as in the myotome heights of this group (Fig. 23, 24 in comparison to Fig. 10, 11); no significant difference at stage 8 and larvae in tank 8 showed higher values at stages 11 and 12.

Half of the L-H group fish had more developed swim bladders at stage 8 and at stage 11 they had 3 underdeveloped and 17 more developed swim bladders (Table 8, 10). Stage 12 fish showed the same swim bladder development as stated in table 3. The variation in swim bladder sizes among the tanks were almost showing the same pattern myotome heights variations at stages 8 and 12, whereas at stage 11 tank 17 had lower swim bladder size in contrast to myotome height at that stage (Figs. 28, 29 in comparison to Figs. 12, 13).

The H-L group fish had the lowest swim bladder mean among the tanks at stage 11, a result of having high numbers of larvae with underdeveloped swim bladders (9 of 21) at this stage (Table 8,11).

The swim bladder sizes of the H-H group were very close to those of the L-H group at stages 11 and 12 (Table 8). Almost half (12 of 30) of the stage 11 fish showed more developed swim bladders whereas there were still few some individuals (5 of 30) showing the landmarks of an earlier stage of swim bladder development. At stage 12, most of the fish (27 of 29) had more developed swim bladders (Table 12), however even if the swim bladder means of the H-H group were not higher than L-H group, they developed a more elongated and pigmented swim bladders. There was no significant swim bladder size variance among the tanks at stage 12 but tank 15 showed slightly lower values at stage 11 (Fig. 34). The myotome heights of the H-H group larvae had no significant variation at stage 11 whereas at stage 12, tank 13 larvae had higher myotome heights (Fig. 18).

The graphical comparison of myotome heights and swim bladder sizes showed that there was a correlation between these two structures. The swim bladder development tables put forward that the low temperatures in long term have a negative effect on the development of the swim bladder. The L-L group stage 11 fish had underdeveloped swim bladders in 8 of their 28 individuals, but H-L group fish had underdeveloped swim bladders in 9 of their 21 individuals. As it is stated above about the importance of the swim bladder development for survival, most of the H-L group fish died right after stage 8 and only a few samples could be taken from the tanks and were used only for this master thesis project. The reason for the mortality at this temperature group could be related to the lack of swim bladder development resulting in final starvation.

4.2.2. Alimentary tract

The development of alimentary tract structures is a key factor for survival in terms of providing the needed energy for growth. The development of stomach and larger intestine provides more surface area for digestion and secretion of gastric enzymes from stomach and improves utilization of the nutrients in intestines (Govoni et al., 1986).

The intestine development of L-L group at stage 8 and 11 had a high variation with 2 underdeveloped and 13 more developed intestinal stage among 40 specimens at stage 8 and 5 underdeveloped and 12 more developed intestinal stage among 29 specimens at stage 11 (Table 13). At stage 12, there were only 2 individuals with underdeveloped intestinal stage of the 24 specimens (Table 13). It should be remembered that the sl values of L-L group were lower than L-H group fish at given stages.

The L-H group fish had higher a degree of intestinal complexity in 14 of 40 specimens at stage 8 without any underdeveloped intestinal stage. At stage 11 there was only one individual with an underdeveloped intestinal stage and 27 larvae had a more developed intestinal stage among 40 individuals (Table 14). At stage 12, 12 of the 40 individuals had more developed intestinal stage (Table 14). The sl values of this group fish were the highest at each given stage.

H-L group fish had a high variation of intestinal developmental stage at stage 11 with 7 underdeveloped and 5 more developed intestinal stage among 29 individuals (Table 15). 5 of the 29 specimens had shrunken intestines which could possibly have been caused by starvation even if the sl values at stage 8 and myotome height at stage 11 were not significantly lower compared to the other temperature groups.

The intestine development at stage 11 H-H group had no individuals with underdeveloped intestinal stage. 8 of the 30 specimens had a higher degree of intestinal development. At stage 12, 28 of the 29 individuals had more developed intestines (Table 15). Even if L-H group fish had higher sl values at stage 12, H-H group fish had higher numbers of more developed intestines at this stage.

The developmental stages are assumed to be age-dependent with a little age-independent variations and this concept has been used when describing temperature dependent developmental studies (Thompson & Riley, 1981; Pitman et al., 1989). The results of this study showed that even if the larvae have been raised at the same temperature, there were variations in intestinal development in the same temperature group at a given stage. This

showed that the stage of intestinal development was not age-dependent, but also depended on an extrinsic factor (temperature).

4.2.3. Fin development

As well as the swim bladder development affects on survival, fin development also has a crucial importance on growth. After changing the feeding behaviour (internal to external feeding), larvae need to be an active swimmer in order to feed. The fin development affects on the locomotion of larvae which will consequently give advantage for feeding. The fin development of L-L group had no underdeveloped fins and 2 of the 30 specimens showed an advance stage in terms of fin development at stage 8 (Table 16) whereas at stage 11, 15 of the 29 individuals and at stage 12, 4 of the 14 individuals still had underdeveloped fins (Table 16). Considering that both the sl and the myotome heights of the L-L group were lower than the other groups, this could be the consequence of the lack of locomotor activity caused by underdeveloped fins.

The L-H group at stage 8, 29 of the 30 specimens had more developed fins and there was no underdeveloped fins whereas at stage 11, 10 of the 39 specimens had underdeveloped, 8 of the 39 had more developed fins. All of the individuals showed an advance stage fin development at stage 12 (Table 17). The reason of the fin developmental stage variation in each sampling stage is uncertain, yet the means of sl and the myotome height of this group had high values at each sampled stage.

The H-L group stage 11, 15 of the 29 individuals had underdeveloped fins (Table 18). As it was described in swim bladder and alimentary tract development in this group, the underdevelopment of fins also played a role on the survival of H-L group fish, even the means of sl and myotome height was not significantly lower than the other temperature groups. These comparisons rise the idea that fish kept on using their energy on developing muscle tissue until the time they reached point of no return.

The H-H group fish had 7 underdeveloped and 9 more developed fin stages among 30 specimens at stage 11 (Table 19). At stage 12, the most of the individuals had more developed fins (28 of 29), just as the increase in the more developed fins at stage 12 L-H group fish.

The increase of temperature seemingly affected the fin development in the late stages positively. As the fish had better sl, myotome height, swim bladder development and

alimentary tract development; the fin development showed a parallel development with the other parameters.

In conclusion, the temperature effects on growth showed that the low temperature at hatching causes an extended time period for the fish to reach expected period compared to high temperatures at incubation, whereas it affected the size of the larvae at early mid-metamorphosis stage (stage 8) as the low temperature groups had higher sl values. The effects of high temperature after hatching affected the growth of the larvae at later (stage 11 and 12) stages with an increased sl value. The effects of temperature on myotome heights showed large variations in the temperature groups which were exposed to high temperature at any period whereas at L-L temperature group had the lowest variation. The swim bladder sizes were highly correlated with myotome heights and temperature. The longer time the fish were exposed to low temperatures, the more underdevelopment on the swim bladders occurred. The variations on the alimentary tract development showed that the age is not the only factor on internal organ development; as an extrinsic factor, temperature had a high correlation with the alimentary tract development. The fin development was affected by the increased temperatures in late stages (stage 11 and 12). It is very obvious that the decreased temperatures after incubation and hatching results with low development of fish that would eventually cause huge mortalities and high temperature after incubation and hatching has a positive effect in not only the growth but also the organ development of fish.

5. References

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Appendix

Appendix A

Raw data from CODE LIFE project sampling (stg: larval stage, age: dph, sl : standard length in mm, weight in mm).

group	age	stg	tank	s-l	weight	group	age	stg	tank	s-l	weight
L-L	29	8	7	6,58	0,00022	L-L	29	8	8	7,42	0,00026
L-L	29	8	7	5,97	0,00022	L-L	29	8	8	7,38	0,00026
L-L	29	8	7	6,74	0,00022	L-L	29	8	8	7,23	0,00026
L-L	29	8	7	6,19	0,00022	L-L	29	8	8	7,18	0,00026
L-L	29	8	7	6,47	0,00022	L-L	29	8	8	7,71	0,00026
L-L	29	8	7	6,45	0,00028	L-L	29	8	8	7,74	0,00028
L-L	29	8	7	5,9	0,00028	L-L	29	8	8	7,41	0,00028
L-L	29	8	7	6,67	0,00028	L-L	29	8	8	7,29	0,00028
L-L	29	8	7	6,09	0,00028	L-L	29	8	8	8,18	0,00028
L-L	29	8	7	6,29	0,00028	L-L	29	8	8	7,02	0,00028
L-L	29	8	7	6	0,00028	L-L	29	8	9	7,39	0,00032
L-L	29	8	7	6,72	0,00028	L-L	29	8	9	7,45	0,00032
L-L	29	8	7	6,71	0,00028	L-L	29	8	9	8,05	0,00032
L-L	29	8	7	6,11	0,00028	L-L	29	8	9	7,54	0,00032
L-L	29	8	7	7,41	0,00028	L-L	29	8	9	7,39	0,00032
L-L	29	8	8	6,03	0,00024	L-L	29	8	9	8,14	0,0003
L-L	29	8	8	7,15	0,00024	L-L	29	8	9	7,29	0,0003
L-L	29	8	8	7,73	0,00024	L-L	29	8	9	7,58	0,0003
L-L	29	8	8	6,47	0,00024	L-L	29	8	9	7,48	0,0003
L-L	29	8	8	7,39	0,00024	L-L	29	8	9	7,05	0,0003
L-L	29	8	9	8,39	0,00038	L-L	60	11	7	12,68	0,00486
L-L	29	8	9	7,36	0,00038	L-L	60	11	8	9,14	0,00494
L-L	29	8	9	7,82	0,00038	L-L	60	11	8	9,73	0,00494
L-L	29	8	9	7,59	0,00038	L-L	60	11	8	9,7	0,00494
L-L	29	8	9	8,08	0,00038	L-L	60	11	8	10,41	0,00494
L-L	29	8	10	6,33	0,00024	L-L	60	11	8	10,65	0,00494

L-L	29	8	10	6,51	0,00024	L-L	60	11	8	10,56	0,00402
L-L	29	8	10	7,42	0,00024	L-L	60	11	8	10,68	0,00402
L-L	29	8	10	6,67	0,00024	L-L	60	11	8	11,31	0,00402
L-L	29	8	10	7,16	0,00024	L-L	60	11	8	11,43	0,00402
L-L	29	8	10	7,04	0,00026	L-L	60	11	8	11,62	0,00402
L-L	29	8	10	7,24	0,00026	L-L	60	11	8	12,14	0,00494
L-L	29	8	10	6,4	0,00026	L-L	60	11	8	12,62	0,00494
L-L	29	8	10	6,9	0,00026	L-L	60	11	8	12,41	0,00494
L-L	29	8	10	7,74	0,00026	L-L	60	11	8	13,14	0,00494
L-L	29	8	10	7,15	0,0003	L-L	60	11	8	14,14	0,00494
L-L	29	8	10	7,31	0,0003	L-L	60	11	9	9,87	0,00428
L-L	29	8	10	5,94	0,0003	L-L	60	11	9	9,34	0,00428
L-L	29	8	10	7,78	0,0003	L-L	60	11	9	10,71	0,00428
L-L	29	8	10	8,09	0,0003	L-L	60	11	9	10,99	0,00428
L-L	60	11	7	7,49	0,0069	L-L	60	11	9	11,19	0,00428
L-L	60	11	7	7,97	0,0069	L-L	60	11	9	11,23	0,00386
L-L	60	11	7	9,21	0,0069	L-L	60	11	9	11,81	0,00386
L-L	60	11	7	9,17	0,0069	L-L	60	11	9	12,39	0,00386
L-L	60	11	7	8,47	0,0069	L-L	60	11	9	11,72	0,00386
L-L	60	11	7	9,89	0,00552	L-L	60	11	9	12,51	0,00386
L-L	60	11	7	10,11	0,00552	L-L	60	11	9	11,45	0,0045
L-L	60	11	7	10,06	0,00552	L-L	60	11	9	11,91	0,0045
L-L	60	11	7	10,14	0,00552	L-L	60	11	9	12,52	0,0045
L-L	60	11	7	10,42	0,00552	L-L	60	11	9	12,18	0,0045
L-L	60	11	7	10,96	0,00486	L-L	60	11	9	13,38	0,0045
L-L	60	11	7	11,47	0,00486	L-H	22	8	17	6,68	0,00028
L-L	60	11	7	10,88	0,00486	L-H	22	8	17	7,39	0,00028
L-L	60	11	7	11,31	0,00486	L-H	22	8	17	7,62	0,00028
L-H	22	8	17	7,37	0,00028	L-H	22	8	19	6,66	0,0003
L-H	22	8	17	6,02	0,00034	L-H	22	8	19	7,1	0,0003
L-H	22	8	17	7,86	0,00034	L-H	22	8	19	7,85	0,0003
L-H	22	8	17	7,47	0,00034	L-H	22	8	20	7,38	0,00032

L-H	22	8	17	7,98	0,00034	L-H	22	8	20	6,88	0,00032
L-H	22	8	17	7,19	0,00034	L-H	22	8	20	7,79	0,00032
L-H	22	8	17	6,89	0,00028	L-H	22	8	20	7,19	0,00032
L-H	22	8	17	7,44	0,00028	L-H	22	8	20	6,91	0,00032
L-H	22	8	17	7,5	0,00028	L-H	22	8	20	6,27	0,0003
L-H	22	8	17	7,32	0,00028	L-H	22	8	20	6,5	0,0003
L-H	22	8	17	7,93	0,00028	L-H	22	8	20	5,77	0,0003
L-H	22	8	18	6,57	0,0002	L-H	22	8	20	7,91	0,0003
L-H	22	8	18	6,34	0,0002	L-H	22	8	20	8,39	0,0003
L-H	22	8	18	7,23	0,0002	L-H	22	8	20	7,72	0,00034
L-H	22	8	18	7,16	0,0002	L-H	22	8	20	7,38	0,00034
L-H	22	8	18	6,57	0,0002	L-H	22	8	20	7,73	0,00034
L-H	22	8	18	6,48	0,00024	L-H	22	8	20	7,78	0,00034
L-H	22	8	18	6,37	0,00024	L-H	22	8	20	7,09	0,00034
L-H	22	8	18	6,58	0,00024	L-H	43	11	17	8,17	0,00086
L-H	22	8	18	7,66	0,00024	L-H	43	11	17	8,78	0,00086
L-H	22	8	18	6,7	0,00024	L-H	43	11	17	10,41	0,00086
L-H	22	8	18	7,84	0,00028	L-H	43	11	17	10,48	0,00086
L-H	22	8	18	7,07	0,00028	L-H	43	11	17	11,01	0,00086
L-H	22	8	18	6,94	0,00028	L-H	43	11	17	10,79	0,00136
L-H	22	8	18	8,05	0,00028	L-H	43	11	17	11,68	0,00136
L-H	22	8	18	7,35	0,00028	L-H	43	11	17	10,64	0,00136
L-H	22	8	19	6,8	0,00028	L-H	43	11	17	11,37	0,00136
L-H	22	8	19	7,58	0,00028	L-H	43	11	17	12,06	0,00136
L-H	22	8	19	6,42	0,00028	L-H	43	11	17	11,28	0,00202
L-H	22	8	19	7,21	0,00028	L-H	43	11	17	11,39	0,00202
L-H	22	8	19	6,88	0,00028	L-H	43	11	17	13,19	0,00202
L-H	22	8	19	7	0,00032	L-H	43	11	17	12,09	0,00202
L-H	22	8	19	7,83	0,00032	L-H	43	11	17	13,73	0,00202
L-H	22	8	19	7,46	0,00032	L-H	43	11	18	9,32	0,00088
L-H	22	8	19	7,69	0,00032	L-H	43	11	18	9,55	0,00088
L-H	22	8	19	6,81	0,00032	L-H	43	11	18	10,22	0,00088

L-H	22	8	19	7,35	0,0003	L-H	43	11	18	9,83	0,00088
L-H	22	8	19	7,71	0,0003	L-H	43	11	18	9,69	0,00088
L-H	43	11	18	10,89	0,00144	L-H	66	12	17	17,91	0,00568
L-H	43	11	18	11,17	0,00144	L-H	66	12	17	18,09	0,00568
L-H	43	11	18	11,4	0,00144	L-H	66	12	17	18,42	0,00568
L-H	43	11	18	10,43	0,00144	L-H	66	12	17	17,42	0,00568
L-H	43	11	18	11,95	0,00144	L-H	66	12	17	18,82	0,00568
L-H	43	11	18	12,52	0,0023	L-H	66	12	17	19,6	0,00238
L-H	43	11	18	12,69	0,0023	L-H	66	12	17	19,78	0,00238
L-H	43	11	18	12,55	0,0023	L-H	66	12	17	20,58	0,00238
L-H	43	11	18	12,76	0,0023	L-H	66	12	17	21,71	0,00238
L-H	43	11	18	13,96	0,0023	L-H	66	12	17	22,09	0,00238
L-H	43	11	19	8,86	0,00104	L-H	66	12	17	23,19	0,021617
L-H	43	11	19	10,18	0,00104	L-H	66	12	17	23,29	0,021617
L-H	43	11	19	10,06	0,00104	L-H	66	12	17	23,24	0,021617
L-H	43	11	19	9,95	0,00104	L-H	66	12	17	24,71	0,021617
L-H	43	11	19	10,98	0,00104	L-H	66	12	17	25,22	0,021617
L-H	43	11	19	11,15	0,00146	L-H	66	12	18	18,64	0,00954
L-H	43	11	19	10,17	0,00146	L-H	66	12	18	17,59	0,00954
L-H	43	11	19	12,26	0,00146	L-H	66	12	18	20,63	0,00954
L-H	43	11	19	12,77	0,00146	L-H	66	12	18	20,12	0,00954
L-H	43	11	19	13,28	0,00146	L-H	66	12	18	20,59	0,00954
L-H	43	11	19	13,36	0,00256	L-H	66	12	18	21,13	0,035
L-H	43	11	19	13,32	0,00256	L-H	66	12	18	22,15	0,035
L-H	43	11	19	13,31	0,00256	L-H	66	12	18	22,23	0,035
L-H	43	11	19	12,76	0,00256	L-H	66	12	18	22,05	0,035
L-H	43	11	19	14,4	0,00256	L-H	66	12	18	21,19	0,035
L-H	43	11	20	8,51	0,00106	L-H	66	12	18	23,23	0,030767
L-H	43	11	20	9,78	0,00106	L-H	66	12	18	24,11	0,030767
L-H	43	11	20	10,75	0,00106	L-H	66	12	18	24,8	0,030767
L-H	43	11	20	10,61	0,00106	L-H	66	12	18	24,55	0,030767
L-H	43	11	20	10,68	0,00106	L-H	66	12	18	24,44	0,030767

L-H	43	11	20	11,33	0,00204	L-H	66	12	19	18,95	0,01275
L-H	43	11	20	10,89	0,00204	L-H	66	12	19	18,74	0,01275
L-H	43	11	20	10,75	0,00204	L-H	66	12	19	19,6	0,01275
L-H	43	11	20	10,86	0,00204	L-H	66	12	19	17,48	0,01275
L-H	43	11	20	11,3	0,00204	L-H	66	12	19	17,97	0,01275
L-H	43	11	20	11,21	0,00308	L-H	66	12	19	19,38	0,01331
L-H	43	11	20	12,87	0,00308	L-H	66	12	19	18,69	0,01331
L-H	43	11	20	12,65	0,00308	L-H	66	12	19	18,91	0,01331
L-H	43	11	20	12,5	0,00308	L-H	66	12	19	17,57	0,01331
L-H	43	11	20	14,64	0,00308	L-H	66	12	19	22,18	0,01331
L-H	66	12	19	21,32	0,02438	H-L	23	8	3	7,08	0,00026
L-H	66	12	19	21,11	0,02438	H-L	23	8	3	6,56	0,00022
L-H	66	12	19	24,4	0,02438	H-L	23	8	3	6,42	0,00022
L-H	66	12	19	24,23	0,02438	H-L	23	8	3	6,59	0,00022
L-H	66	12	19	31,35	0,02438	H-L	23	8	3	6,45	0,00022
L-H	66	12	20	20,86	0,00888	H-L	23	8	3	6,62	0,00022
L-H	66	12	20	17,78	0,00888	H-L	23	8	3	6,5	0,00026
L-H	66	12	20	19,87	0,00888	H-L	23	8	3	6,66	0,00026
L-H	66	12	20	21,03	0,00888	H-L	23	8	3	6,31	0,00026
L-H	66	12	20	21,15	0,00888	H-L	23	8	3	7,22	0,00026
L-H	66	12	20	18,23	0,0102	H-L	23	8	3	7,25	0,00026
L-H	66	12	20	20,82	0,0102	H-L	23	8	4	6,98	0,00024
L-H	66	12	20	21,97	0,0102	H-L	23	8	4	6,87	0,00024
L-H	66	12	20	21,54	0,0102	H-L	23	8	4	6,66	0,00024
L-H	66	12	20	24,74	0,0102	H-L	23	8	4	6,06	0,00024
L-H	66	12	20	24,23	0,02226	H-L	23	8	4	6,49	0,00024
L-H	66	12	20	25,12	0,02226	H-L	23	8	4	6,14	0,00028
L-H	66	12	20	23,45	0,02226	H-L	23	8	4	7,09	0,00028
L-H	66	12	20	25,7	0,02226	H-L	23	8	4	7,46	0,00028
L-H	66	12	20	27,5	0,02226	H-L	23	8	4	6,54	0,00028
H-L	23	8	2	6,91	0,00028	H-L	23	8	4	6,61	0,00028
H-L	23	8	2	6,96	0,00028	H-L	23	8	4	6,99	0,00028

H-L	23	8	2	6,37	0,00028	H-L	23	8	4	7,05	0,00028
H-L	23	8	2	5,36	0,00028	H-L	23	8	4	6,84	0,00028
H-L	23	8	2	6,57	0,00028	H-L	23	8	4	7,02	0,00028
H-L	23	8	2	6,51	0,00028	H-L	23	8	4	6,54	0,00028
H-L	23	8	2	6,97	0,00028	H-L	23	8	5	6,45	0,00018
H-L	23	8	2	6,9	0,00028	H-L	23	8	5	5,98	0,00018
H-L	23	8	2	7,28	0,00028	H-L	23	8	5	7,13	0,00018
H-L	23	8	2	6,75	0,00028	H-L	23	8	5	6,55	0,00018
H-L	23	8	2	6,59	0,0003	H-L	23	8	5	7,1	0,00018
H-L	23	8	2	6,4	0,0003	H-L	23	8	5	7,09	0,00016
H-L	23	8	2	6,57	0,0003	H-L	23	8	5	7,16	0,00016
H-L	23	8	2	6,36	0,0003	H-L	23	8	5	6,92	0,00016
H-L	23	8	2	7,01	0,0003	H-L	23	8	5	6,4	0,00016
H-L	23	8	3	6,08	0,00026	H-L	23	8	5	6,87	0,00016
H-L	23	8	3	6,25	0,00026	H-L	23	8	5	7,03	0,0002
H-L	23	8	3	6,41	0,00026	H-L	23	8	5	6,89	0,0002
H-L	23	8	3	7,22	0,00026	H-L	23	8	5	6,81	0,0002
H-L	23	8	5	7,48	0,0002	H-H	17	8	16	6,21	0,0003
H-H	17	8	13	6,84	0,00024	H-H	17	8	16	5,79	0,0003
H-H	17	8	13	5,54	0,00024	H-H	17	8	16	7,01	0,0003
H-H	17	8	13	6,9	0,00024	H-H	42	11	13	10,12	0,002917
H-H	17	8	13	5,9	0,00024	H-H	42	11	13	10,47	0,002917
H-H	17	8	13	7,07	0,00024	H-H	42	11	13	13,15	0,002917
H-H	17	8	13	7,28	0,00028	H-H	42	11	13	11,08	0,002917
H-H	17	8	13	6,51	0,00028	H-H	42	11	13	9,73	0,002917
H-H	17	8	13	7,03	0,00028	H-H	42	11	13	13,5	0,004117
H-H	17	8	13	6,56	0,00028	H-H	42	11	13	13,5	0,004117
H-H	17	8	13	6,96	0,00028	H-H	42	11	13	12,57	0,004117
H-H	17	8	13	7,14	0,00022	H-H	42	11	13	10,19	0,004117
H-H	17	8	13	7,16	0,00022	H-H	42	11	13	13,01	0,004117
H-H	17	8	13	7,06	0,00022	H-H	42	11	13	10,29	0,0034
H-H	17	8	13	6,83	0,00022	H-H	42	11	13	12,04	0,0034

H-H	17	8	13	7,08	0,00022	H-H	42	11	13	9,8	0,0034
H-H	17	8	15	6,62	0,00028	H-H	42	11	13	12,13	0,0034
H-H	17	8	15	6,36	0,00028	H-H	42	11	13	14,04	0,0034
H-H	17	8	15	6,71	0,00028	H-H	42	11	15	8,3	0,00136
H-H	17	8	15	6,28	0,00028	H-H	42	11	15	10,69	0,00136
H-H	17	8	15	6,35	0,00028	H-H	42	11	15	11,22	0,00136
H-H	17	8	15	5,77	0,00024	H-H	42	11	15	9,21	0,00136
H-H	17	8	15	6,22	0,00024	H-H	42	11	15	10,9	0,00136
H-H	17	8	15	6,43	0,00024	H-H	42	11	15	12,07	0,0016
H-H	17	8	15	6,45	0,00024	H-H	42	11	15	11,66	0,0016
H-H	17	8	15	6,96	0,00024	H-H	42	11	15	7,06	0,0016
H-H	17	8	15	7,19	0,00026	H-H	42	11	15	9,72	0,0016
H-H	17	8	15	5,82	0,00026	H-H	42	11	15	11,75	0,0016
H-H	17	8	15	6,78	0,00026	H-H	42	11	15	11,51	0,00172
H-H	17	8	15	6,57	0,00026	H-H	42	11	15	10,82	0,00172
H-H	17	8	15	7,09	0,00026	H-H	42	11	15	7,36	0,00172
H-H	17	8	16	5,39	0,00034	H-H	42	11	15	9,53	0,00172
H-H	17	8	16	6,07	0,00034	H-H	42	11	15	13,43	0,00172
H-H	17	8	16	6,64	0,00034	H-H	42	11	16	12,85	0,00218
H-H	17	8	16	6,86	0,00034	H-H	42	11	16	11,57	0,00218
H-H	17	8	16	6,84	0,00034	H-H	42	11	16	11,96	0,00218
H-H	17	8	16	5,8	0,00034	H-H	42	11	16	11,87	0,00218
H-H	17	8	16	5,89	0,00034	H-H	42	11	16	11,81	0,00218
H-H	17	8	16	7,13	0,00034	H-H	42	11	16	13,85	0,00234
H-H	17	8	16	6,52	0,00034	H-H	42	11	16	12,72	0,00234
H-H	17	8	16	5,36	0,00034	H-H	42	11	16	10,66	0,00234
H-H	17	8	16	6,48	0,0003	H-H	42	11	16	11,76	0,00234
H-H	17	8	16	6,08	0,0003	H-H	42	11	16	13,47	0,00234
H-H	42	11	16	11,47	0,0019	H-H	57	12	15	22,73	0,035817
H-H	42	11	16	10,54	0,0019	H-H	57	12	15	23,44	0,035817
H-H	42	11	16	11,09	0,0019	H-H	57	12	15	23,54	0,035817
H-H	42	11	16	11,33	0,0019	H-H	57	12	16	13,23	0,00704

H-H	42	11	16	13,77	0,0019	H-H	57	12	16	13,82	0,00704
H-H	57	12	13	17,54	0,009	H-H	57	12	16	13,12	0,00704
H-H	57	12	13	17,99	0,009	H-H	57	12	16	13,15	0,00704
H-H	57	12	13	19,83	0,009	H-H	57	12	16	15,2	0,00704
H-H	57	12	13	18,06	0,009	H-H	57	12	16	16,34	0,0034
H-H	57	12	13	20,33	0,009	H-H	57	12	16	16,72	0,0034
H-H	57	12	13	19,44	0,0128	H-H	57	12	16	16,29	0,0034
H-H	57	12	13	20,81	0,0128	H-H	57	12	16	17,03	0,0034
H-H	57	12	13	20,72	0,0128	H-H	57	12	16	17,14	0,0034
H-H	57	12	13	21,3	0,0128	H-H	57	12	16	17,66	0,02665
H-H	57	12	13	21,45	0,0128	H-H	57	12	16	18,28	0,02665
H-H	57	12	13	21,19	0,031617	H-H	57	12	16	22,21	0,02665
H-H	57	12	13	23,69	0,031617	H-H	57	12	16	22,1	0,02665
H-H	57	12	13	21,56	0,031617	H-H	57	12	16	23,42	0,02665
H-H	57	12	13	24,09	0,031617	H-H	57	12	15	15,98	0,0037
H-H	57	12	13	25,85	0,031617	H-H	57	12	15	18,21	0,0037
H-H	57	12	15	13,41	0,00566	H-H	57	12	15	17,37	0,0037
H-H	57	12	15	13,62	0,00566	H-H	57	12	15	17,7	0,0037
H-H	57	12	15	14,27	0,00566	H-H	57	12	15	18,96	0,0037
H-H	57	12	15	15,5	0,00566	H-H	57	12	15	20,4	0,035817
H-H	57	12	15	15,44	0,00566	H-H	57	12	15	21,71	0,035817

Appendix B

Raw data of master thesis project sampling (stg: larval stage, sl : standard length in mm, tl: total length in mm, ad: myotome height in mm, sb swim bladder in mm).

Tank no	group	sl	tl	ad	sb	stg
7	LL	6,08	6,384	0,8816	0,471	8
7	LL	6,697	6,908	0,903	0,481	8
7	LL	6,03	6,3	0,9	0,45	8
7	LL	5,4	5,7	0,72	0,45	8
7	LL	6,732	7,038	0,933	0,581	8
7	LL	6,364	6,609	0,887	0,474	8
7	LL	6,426	6,747	0,841	0,49	8
7	LL	6,273	6,518	0,78	0,474	8
8	LL	7,12	7,48	0,912	0,532	8
8	LL	6,232	6,414	0,76	0,425	8
8	LL	7,6	7,904	1,064	0,76	8
8	LL	6,536	6,81	0,912	0,425	8
8	LL	6,776	7,05	0,942	0,462	8
8	LL	6,622	6,899	0,924	0,446	8
8	LL	6,232	6,505	0,744	0,44	8
8	LL	7,326	7,57	1,14	0,608	8
8	LL	6,384	6,612	0,912	0,593	8
8	LL	7,144	7,448	0,912	0,547	8
8	LL	6,04	6,296	0,906	0,438	8
8	LL	6,065	6,311	0,906	0,453	8
9	LL	7,003	7,45	1,192	0,566	8
9	LL	7,7	8,008	1,093	0,43	8
9	LL	6,75	7,05	0,75	0,315	8
9	LL	7,003	7,301	0,909	0,521	8
9	LL	6,437	6,705	0,819	0,447	8
9	LL	7,08	7,365	0,93	0,63	8
9	LL	7,05	7,35	0,885	0,585	8
9	LL	6,545	6,853	0,924	0,462	8

9	LL	7,6	7,828	0,988	0,532	8
9	LL	6,232	6,384	0,836	0,547	8
7	LL	9,394	9,856	0,924	0,77	11
7	LL	8,162	8,47	0,847	0,77	11
7	LL	8,393	8,778	0,77	0,616	11
7	LL	10,64	11,172	1,216	1,368	11
7	LL	9,576	9,88	0,912	0,76	11
7	LL	8,056	8,36	0,912	0,76	11
7	LL	9,424	9,804	1,064	1,064	11
7	LL	9,086	9,394	1,078	1,54	11
7	LL	10,626	10,934	1,232	1,386	11
8	LL	11,858	12,782	1,848	1,463	11
8	LL	10,164	10,626	1,386	1,078	11
8	LL	9,086	9,548	1,078	0,924	11
8	LL	12,32	13,09	1,694	1,848	11
8	LL	11,78	12,4	1,627	1,395	11
8	LL	11,005	11,47	1,24	1,395	11
8	LL	10,23	10,85	1,55	1,395	11
8	LL	8,835	9,3	1,085	0,95	11
8	LL	11,935	12,4	1,55	1,395	11
8	LL	11,315	11,78	1,55	1,24	11
9	LL	9,664	9,966	0,906	0,906	11
9	LL	9,211	9,815	1,208	1,057	11
9	LL	9,966	10,419	1,359	1,208	11
9	LL	7,399	8,003	0,755	0,604	11
9	LL	9,455	10,075	1,24	0,93	11
9	LL	9,455	10,23	1,085	1,085	11
9	LL	9,61	10,23	0,93	0,93	11
9	LL	9,92	10,54	1,085	1,24	11
7	LL	14,105	14,725	1,705	1,86	12
7	LL	11,78	12,4	1,24	1,705	12
7	LL	10,85	11,16	0,93	1,395	12

7	LL	11,625	11,935	0,93	1,627	12
8	LL	13,345	14,13	2,041	2,041	12
8	LL	12,874	13,659	1,884	1,884	12
8	LL	16,359	17,584	2,198	2,669	12
8	LL	14,13	14,915	1,978	2,198	12
8	LL	12,312	13,072	1,672	1,976	12
8	LL	12,616	13,376	1,824	1,748	12
8	LL	16,262	17,48	2,28	2,432	12
8	LL	14,592	15,504	1,976	2,432	12
8	LL	13,175	13,95	1,705	1,337	12
8	LL	10,23	10,85	1,085	0,93	12
17	LH	6,688	7,022	0,912	0,486	8
17	LH	7,524	7,752	1,064	0,667	8
17	LH	6,992	7,174	1,033	0,608	8
17	LH	6,566	6,84	0,836	0,456	8
17	LH	6,688	6,992	0,912	0,486	8
17	LH	6,353	6,566	0,866	0,486	8
17	LH	7,296	7,6	1,018	0,608	8
17	LH	6,87	7,144	0,912	0,547	8
17	LH	6,644	6,87	0,906	0,604	8
17	LH	5,738	6,07	0,755	0,425	8
18	LH	6,839	6,867	0,964	0,581	8
18	LH	6,915	7,313	0,918	0,612	8
18	LH	7,42	7,741	1,101	0,658	8
18	LH	5,967	6,12	0,78	0,382	8
18	LH	6,779	6,992	0,912	0,608	8
18	LH	6,779	7,144	0,942	0,608	8
18	LH	7,083	7,296	0,912	0,577	8
18	LH	6,232	6,536	0,76	0,456	8
18	LH	6,688	6,992	0,912	0,486	8
18	LH	7,326	7,63	1,064	0,638	8
20	LH	7,296	7,448	0,927	0,623	8

20	LH	6,384	6,536	0,897	0,608	8
20	LH	7,6	7,752	1,064	0,608	8
20	LH	6,536	6,84	0,805	0,608	8
20	LH	6,12	6,303	0,79	0,612	8
20	LH	5,897	6,11	0,765	0,505	8
20	LH	6,426	6,58	0,942	0,612	8
20	LH	6,58	6,732	0,942	0,592	8
20	LH	7,14	7,326	0,942	0,608	8
20	LH	6,384	6,612	0,76	0,456	8
17	LH	11,005	11,625	1,55	1,55	11
17	LH	11,47	12,09	17,05	1,55	11
17	LH	9,3	9,765	1,317	1,317	11
17	LH	8,525	8,835	0,93	0,62	11
17	LH	11,16	11,625	1,55	1,55	11
17	LH	11,935	12,555	1,86	2,17	11
17	LH	11,625	12,245	1,705	1,86	11
17	LH	10,075	10,85	1,55	1,24	11
17	LH	8,736	9,048	0,936	1,248	11
18	LH	9,24	9,856	1,232	1,078	11
18	LH	9,086	9,671	1,232	1,232	11
18	LH	13,86	14,784	1,848	1,848	11
18	LH	12,32	13,09	1,54	1,848	11
18	LH	12,008	12,616	1,52	1,9	11
18	LH	12,312	12,768	1,672	1,976	11
18	LH	9,272	9,728	1,368	1,368	11
18	LH	10,792	11,4	1,52	1,824	11
18	LH	11,704	12,32	1,694	1,694	11
18	LH	10,01	10,78	1,386	1,386	11
19	LH	11,935	12,71	1,86	1,86	11
19	LH	10,075	10,695	1,55	1,395	11
19	LH	11,47	12,09	1,55	1,395	11
19	LH	12,23	10,85	1,55	1,55	11

19	LH	9,92	10,385	1,317	1,24	11
19	LH	10,54	10,85	1,55	1,55	11
19	LH	10,54	11,16	1,395	1,55	11
19	LH	8,99	9,455	1,24	0,924	11
19	LH	9,086	9,548	1,078	1,386	11
19	LH	9,086	9,548	1,078	1,078	11
20	LH	10,251	11,016	1,53	1,377	11
20	LH	10,251	11,016	1,53	1,377	11
20	LH	10,71	11,322	1,53	1,377	11
20	LH	12,24	13,005	2,142	1,836	11
20	LH	11,704	12,464	1,672	1,824	11
20	LH	9,88	10,184	1,368	1,368	11
20	LH	11,704	12,312	1,672	1,672	11
20	LH	10,336	10,792	1,52	1,216	11
20	LH	10,472	10,934	1,386	1,386	11
20	LH	7,084	7,392	0,924	0,77	11
17	LH	21,4	23,2	3,6	3,6	12
17	LH	19,2	21	3,4	3,4	12
17	LH	22,4	24,4	3,6	3,6	12
17	LH	20,2	21,8	3,2	3,2	12
17	LH	18,6	19,8	3,2	3	12
17	LH	19	20,6	3	3	12
17	LH	20,5	22,4	3,6	3	12
17	LH	19	20,4	3,2	2,8	12
17	LH	20,4	22	3,2	3	12
17	LH	19,8	21,4	3,4	3,2	12
18	LH	22,4	24,4	3,8	3,4	12
18	LH	24	26,2	4,2	3,6	12
18	LH	17,2	18,8	3	3,2	12
18	LH	16	17,2	2,6	3,2	12
18	LH	17,5	19	3	3,2	12
18	LH	16,8	18,2	2,6	2,6	12

18	LH	16,2	17,6	2,6	2,6	12
18	LH	17	18,4	2,4	3	12
18	LH	13,8	14,8	2,2	2,4	12
18	LH	15,4	16,8	2,8	2,8	12
18	LH	16,8	18,4	3	3	12
19	LH	15,2	16,2	2,6	2,8	12
19	LH	18,4	20	3,2	3	12
19	LH	16	17,2	2,8	2,4	12
19	LH	15,4	16,6	2,4	2,4	12
19	LH	16,3	17,6	2,5	2,8	12
19	LH	16,4	17,6	2,6	2,4	12
19	LH	16,4	17,64	2,6	2,8	12
19	LH	16,6	18	2,6	2,6	12
19	LH	19,8	21,4	3,2	3,2	12
20	LH	19,2	20,1	3	3,2	12
20	LH	17,4	19	3	3	12
20	LH	17,4	19	3,2	2,8	12
20	LH	17,4	18,8	2,8	2,8	12
20	LH	16,4	18	2,8	2,6	12
20	LH	16,4	17,8	2,6	2,8	12
20	LH	17	18,4	2,8	3	12
20	LH	18,2	20	3,04	2,8	12
20	LH	18,4	20,2	3,4	2,8	12
20	LH	16,2	17,6	2,4	2,8	12
5	HL	10,728	11,175	1,639	1,49	11
5	HL	8,493	8,93	1,192	0,596	11
5	HL	7,301	7,673	0,894	0,596	11
5	HL	9,685	9,983	1,564	0,745	11
5	HL	8,968	9,272	1,216	0,851	11
5	HL	8,664	8,968	1,14	0,608	11
5	HL	9,88	10,153	1,52	0,912	11
5	HL	10,032	10,488	1,52	1,216	11

5	HL	8,968	9,272	1,368	0,805	11
5	HL	8,208	8,512	1,094	0,684	11
5	HL	7,144	7,372	0,76	0,608	11
5	HL	10,488	10,792	1,672	1,368	11
5	HL	7,6	7,904	1,064	0,516	11
5	HL	9,88	10,184	1,368	0,912	11
5	HL	10,032	10,412	1,52	1,064	11
5	HL	8,056	8,36	1,64	0,638	11
5	HL	10,184	10,549	1,52	1,52	11
5	HL	10,184	10,488	1,52	1,368	11
5	HL	7,448	7,752	1,064	1,52	11
13	HH	12,464	13,528	1,824	1,824	11
13	HH	8,36	8,664	1,216	0,912	11
13	HH	10,488	10,944	1,672	1,52	11
13	HH	9,424	9,804	1,216	1,52	11
13	HH	12,684	13,741	1,976	1,661	11
13	HH	8,456	8,758	1,057	0,906	11
13	HH	11,627	12,533	1,812	1,812	11
13	HH	7,852	8,154	1,057	0,906	11
13	HH	12,464	13,376	1,672	1,672	11
13	HH	10,4	10,8	1,52	1,216	11
15	HH	11,552	12,16	1,672	1,824	11
15	HH	8,664	8,968	1,064	0,912	11
15	HH	11,856	12,464	1,824	1,824	11
15	HH	11,552	12,16	1,824	1,672	11
15	HH	12,628	13,399	2,31	1,54	11
15	HH	11,396	11,858	1,694	1,694	11
15	HH	10,4	10,72	1,44	1,28	11
15	HH	9,44	9,76	0,96	0,8	11
15	HH	9,12	9,6	0,96	0,48	11
15	HH	9,76	10,08	0,96	0,64	11
16	HH	11,78	12,632	2,015	1,705	11

16	HH	11,315	11,935	1,705	1,705	11
16	HH	10,23	10,85	1,55	1,395	11
16	HH	10,54	11,16	1,55	1,55	11
16	HH	10,075	10,54	1,395	1,24	11
16	HH	12,555	13,33	2,17	2,17	11
16	HH	9,765	10,23	1,395	1,24	11
16	HH	9,765	10,075	1,24	1,24	11
16	HH	9,513	9,815	1,057	1,057	11
16	HH	9,664	10,117	1,057	0,906	11
16	HH	9,702	10,01	0,924	1,078	11
16	HH	17,4	19	3,2	3,2	12
16	HH	18,4	20	3	3,4	12
16	HH	23,2	24,2	4,2	3,4	12
16	HH	18	19,4	2,8	3,2	12
16	HH	16,6	18,2	2,8	2,6	12
16	HH	16,4	17,8	2,8	2,8	12
16	HH	17,6	19	3,2	2,8	12
16	HH	16,4	17,6	3	2,8	12
16	HH	18,4	20	3,2	2,8	12
16	HH	12,8	14	2,4	2,2	12
13	HH	22,4	24,2	3,6	3,2	12
13	HH	24,4	26,4	4,2	3	12
13	HH	20	21,8	3,8	2,8	12
13	HH	19,6	21	3,4	3,2	12
13	HH	17,6	19,2	3,4	2,6	12
13	HH	16,6	18	2,8	3	12
13	HH	17	18,4	3	2,8	12
13	HH	16,6	18	2,8	3	12
13	HH	19	20,4	3,6	2,8	12
13	HH	16,4	17,4	2,8	2,8	12
15	HH	21,4	23,2	3,4	3,4	12
15	HH	16,4	17,8	3,2	3,2	12

15	HH	17,7	19,4	3	3,2	12
15	HH	17,4	18,8	3	2,8	12
15	HH	16,8	18	3	2,8	12
15	HH	16,8	18	3	2,8	12
15	HH	18,6	20	3,2	2,8	12
15	HH	16,4	17,6	2,8	2,8	12
15	HH	15,4	17,2	2,6	2,6	12