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

Mathias Danielsen*, Ane Vigdisdatter Nytrø**, Thor Arne Hangstad**, Inger-Britt Falk-Petersen*, Albert Kjartansson. Imsland** & Thor Magne Jonassen**

*The Arctic University of Norway (UiT)
**Akvaplan-niva AS, Tromsø, Norway
Email: tmj@akvaplan.niva.no

## Background

Temperature may affect the development and viability of fish embryos (Geffen et al., 2006), and temperature tolerance in fish eggs during embryonic development is narrower than during later life stages (Rombough 1997). Early embryonic stages may also be more sensitive than the later stages (Kazuyuki et al., 1988). In Atlantic cod (*Gadus morhua*) gradual increment in temperature from 4.5 °C to 9.5 °C in 32 h was suggested as a better choice than constant 4.5 °C and more rapid increments based on embryonic cleavage pattern, hatching success and larval morphology and histology (Puvanendran et al., 2013). This study is testing similar effects of different incubation temperatures in common lumpfish (*Cyclopterus lumpus* L.).
The project was sponsored by The Norwegian Seafood Research Fund (FHF project no. 900977)

## Objective

Study how different incubation temperatures effected; early cell symmetry, egg development, mortality, hatching success and early larvae size, deformities and histomorphology.

## Materials and methods

The experiment was conducted at Akvaplan-niva, Tromsø, Norway 11 March to 30 May 2015. Studies on the hatched larvae was done at the Arctic University of Norway (UiT). Eggs were incubated in 2 L incubators at constant low (4-6 °C, group Cold, C), constant high (10 °C, groups Warm, W) and gradual increasing temperature from approx. 4.2 °C to 10 °C during 4 days (group Gradient, G) (Figure 1).

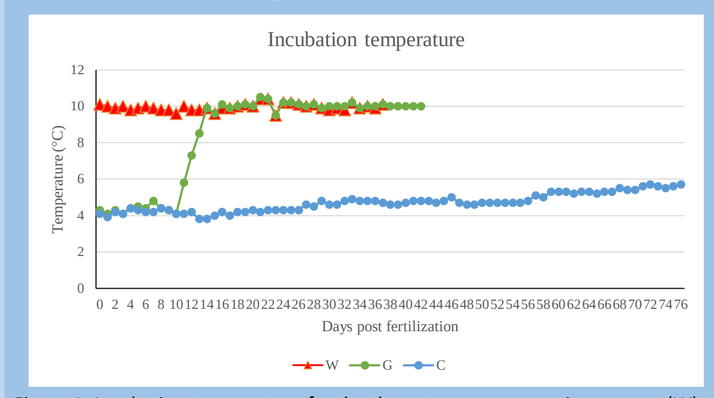


Figure 1: Incubation temperature for the three temperature regimes warm (W), gradient (G) and cold (C).

There were 5 replicates with 200 eggs for each of the 3 temperature regimes and there were two separate test lines with eggs from one separate females for each test line (Batch 1 and 2). All eggs from both females were fertilized with sperm from the same two males.
Time-series samples were taken from 2 incubators in each test line, while the remaining 3 incubators in each line was un-disturbed until the end of the experiment.
Fertilization rate and egg diameter was measured on 100 eggs from both females before incubation.
Egg survival and hatching rate was measured
Standard length, myotom height, wet weight, yolk sac height, spinal deformities and organohistology was measured on 20 newly hatched larvae and 20 two week old larvae from each triplicate incubators from each of the three experimental groups (Figure 2).


Figure 2: Left: Newly hatch lumpsucker larva and measurements done, standard length (1), myotom height (2) and yolk sac height (3). Right: Newly hatched deformed lumpsucker larva.

## Results

- Eggs were similar in color, size (2,33 and 2,28 mm) and fertilization (97,79 and 98,89 %) for batch 1 and 2 respectively.
- Egg development was faster in the warm temperature regime but similar in term of day-degrees for all groups (Table 1 and Figure 3).
- Egg mortality was highest in the cold group and lowest in the warm group (Figure 4).
- Hatching occurred at approximately 280 day-degrees. 278,6 for group W, 279,9 for group G and 285 for group C.
- Hatching success (%) was highest in the group G and lowest in group C (Figure 5).

Table 1: Overview of lowest day degree at which developmental stage of lumpsucker larvae was observed.

| Development | d° | Development | d° | Development | d° |
|---|---|---|---|---|---|
| 2 cells (B) | 0,0 | Embryo (J) | 49,8 | Body pigment (N) | 138,4 |
| 4 cells (C) | 4,1 | Eyes (J) | 49,8 | YS vein spread (O) | 173,8 |
| 8 cells (D) | 4,1 | Lipid compression (K) | 70,3 | Headgrowth (O) | 183,3 |
| 16 cells (E) | 4,1 | Otocyst (L) | 89,4 | Open mouth (O) | 209,8 |
| 64 cells (F) | 8,0 | Eye pigment (M) | 117,3 | Body growth (O) | 209,8 |
| Morula (G) | 10,1 | Otolith (M) | 117,3 | Egg filled (P) | 254,3 |
| Blastula (H) | 20,1 | Heartbeat (N) | 128,9 | Hatch | 278,6 |
| Gastrula (I) | 29,2 | Yolk-sac vein (N) | 128,9 | Dorsal fin | 308,4 |

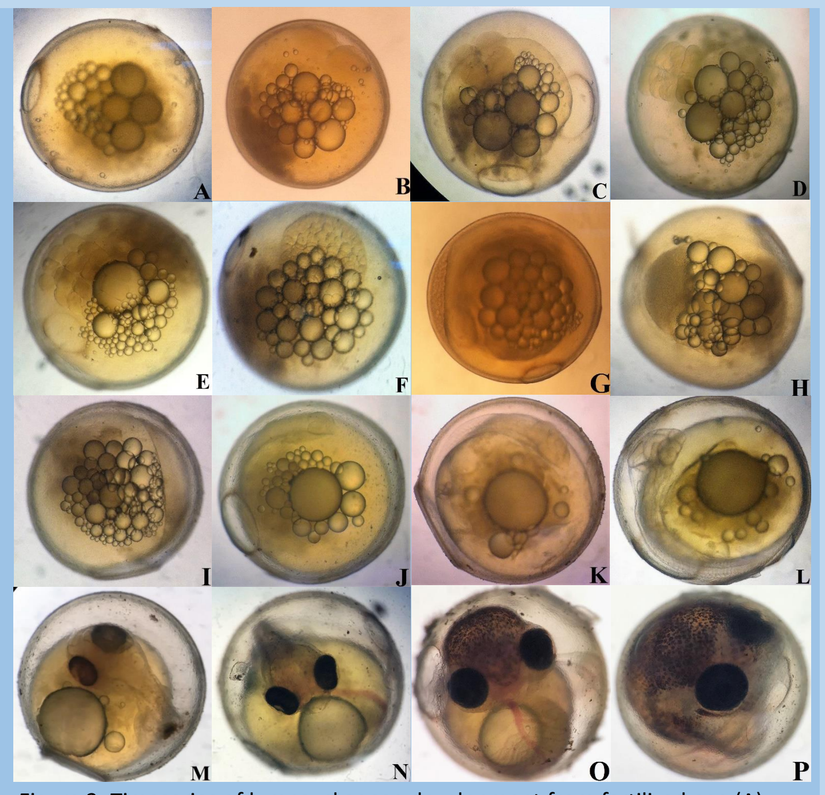


Figure 3: Timeseries of lumpsucker egg development from fertilized egg (A) to just before hatch (P). See table 1 for more info.

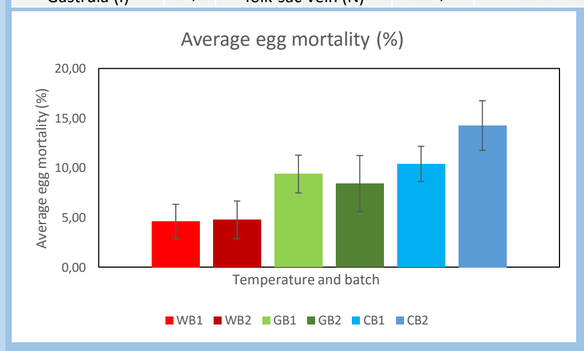


Figure 4: Average egg mortality for lumpsuckers for group warm (W) gradient (G) and cold (C) and batch 1 (B1) and batch 2 (B2).

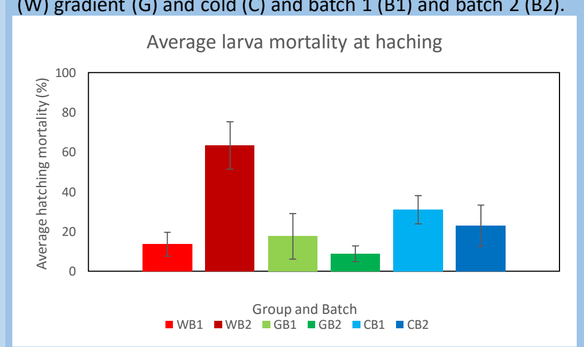


Figure 7: Average lumpsucker larva mortality at hatching for group nwarm (W) gradient (G) and cold (C) and batch 1 (B1) and batch 2 (B2).

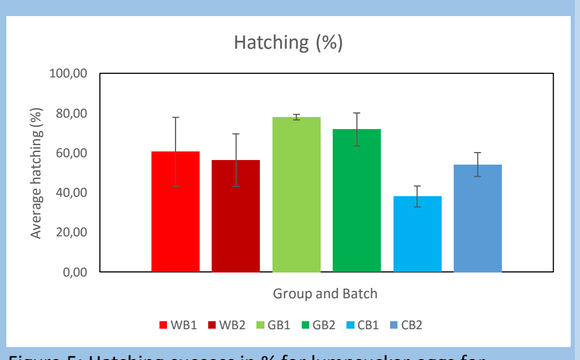


Figure 5: Hatching success in % for lumpsucker eggs for groups warm (W) gradient (G) and cold (C) and batch 1 (B1) and batch 2 (B2).

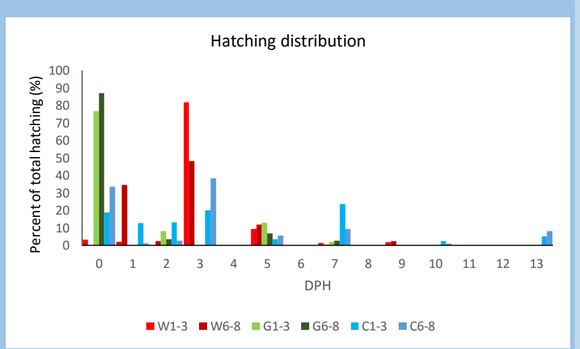


Figure 6: Hatching distribution for lumpsucker larvae from warm (W) gradient (G) and cold (C) and batch 1 (1-3) and batch 2 (6-8).

- Most eggs in the gradient group hatched the first day. Group W and C both had a hatching peak after 3 days post first hatching. The cold group had the longest hatching period of (Figure 6)
- Newly hatched larvae mortality was lowest in the gradient group (Figure 7).
- Larvae mortality after two weeks was similar between groups.
- There was not found any difference in internal development from the histomorphological samples taken between temperature groups, ether from newly hatched or two week old larvae

Table 2: Average standard length, body height, yolk-sac height and wet weight for newly hatched lumpsucker larvae from group warm (W) gradient (G) and cold (C).

| Group | Length (mm) | SD | Height (mm) | SD | Yolk-sac (mm) | SD | Weight (mg) | SD |
|---|---|---|---|---|---|---|---|---|
| Warm | 5,33 | 0,85 | 0,95 | 0,14 | 1,12 | 0,08 | 4,37 | 0,75 |
| Gradient | 5,71 | 0,64 | 1,02 | 0,14 | 1,15 | 0,08 | 4,88 | 0,79 |
| Cold | 6,11 | 0,72 | 1,08 | 0,14 | 1,11 | 0,09 | 5,55 | 0,84 |

Table 3: Average occurrence in percent of dorsal fin development, tail bend, spine damage, body deformities and mechanical damage on newly hatched lumpsucker larvae for warm (W) gradient (G) and cold (C).

| Group | Dorsal fin (%) | Tail bend (%) | Spine damage (%) | Deformed (%) | Damaged (%) |
|---|---|---|---|---|---|
| Warm | 20,09 | 44,86 | 35,05 | 48,13 | 25,23 |
| Gradient | 8,63 | 12,16 | 3,14 | 18,43 | 2,35 |
| Cold | 0,00 | 11,06 | 8,04 | 15,58 | 4,02 |

- Larvae from the cold temperature regime were the larges while larvae from the warm temperature regime were samlest, both for newly hatched and two week old larvae (Table 2).
- Newly hatched larvae from the cold group had not separated the dorsal fin from the larvae finfold.
- Larvae from the warm group had highest presence of larvae deformities (Table 3).
- Two week old larvae had no difference in larvae deformities between temperature groups.

## Discussion

The combination of highest hatching rate, low egg mortality and lowest larval mortality at hatching for the temperature gradient group suggests that this incubation regime, with gradual increasing temperature from approx. 4 °C to 10 °C during 4 days, is favorable compared to constant low (4 °C) or high (10 °C) temperature. Gradient group also showed a more synchronized hatching compared to the two other groups, which may contribute to shorter production cycles, more uniform size distribution and less need for handling and grading in the later juvenile stage.

## Conclusion

| Temperature regime | Pro | Con |
|---|---|---|
| Warm; constant 10°C | High egg survival and good hatching rate, short incubation time. | High occurrence of deformities. |
| Cold; constant ambient water (4-6°C) | Large larvae with little deformities | Low egg survival and hatching rate, long incubation time and unsynchronized hatching |
| Gradient; Ambient water for 10 days, then gradually increased to 10 over 4 days. | Good egg survival, high hatching success, short incubation time. Syncronized hatching and low larva mortality. Little deformities. | |

## References

Geffen A.J., Fox C.J., Nash R.D.M., 2006. Temperaturedependent development rates of cod Gadus morhua eggs. Journal of Fish Biology 69: 1060–1080.

Kazuyuki S., Hisashi K., Shogoro K., 1988. Changes in low temperature tolerance of the eggs of certain marine fish during embryonic development. Comparative Biochemistry and Physiology A 91: 183–187.

Puvanendran V., Falk-Petersen, I.-B., Lysne, H., Tveiten, H., Toften, H., Peruzzi, S. 2013. Effects of different step-wise temperature increment regimes during egg incubation of Atlantic cod (Gadus morhua L.) on egg viability and newly hatched larval quality. Aquaculture Research 46: 226-235

Rombough P.J., 1997. The effects of temperature on embryonic and larval development. In: Global Warming: Implications for Freshwater and Marine Fish (ed. By C.M. Wood, & G.D. McDonald), pp. 177–224. Cambridge University Press, Cambridge, UK.