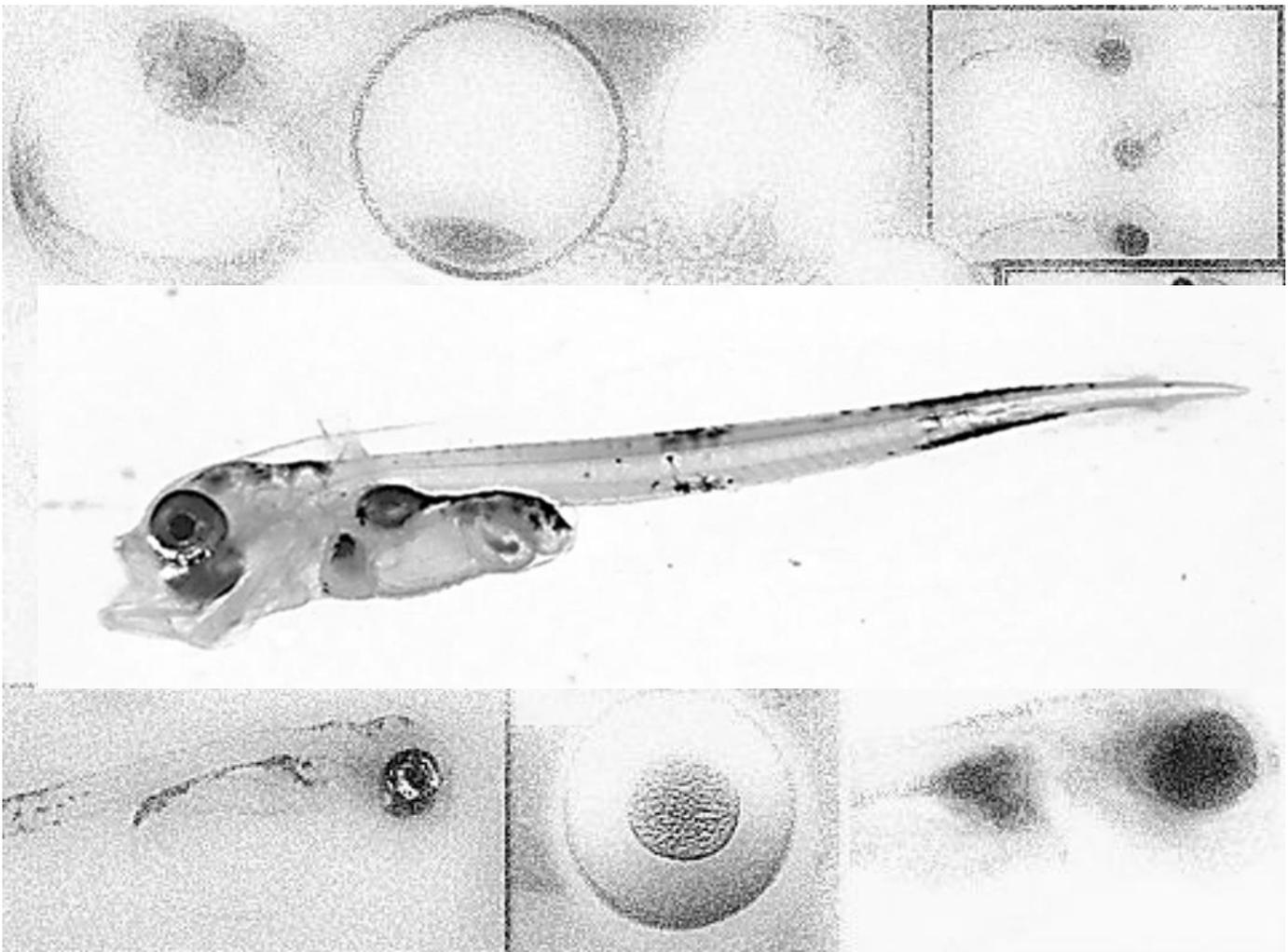




Comparative effects of two temperatures (0 & 3 °C) on Polar cod embryonic and larval development

CONNIE JONES

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Abstract

The Arctic environment is projected to be the most affected by rising seawater temperature. Polar cod (*Boreogadus saida*) a key stone species are particularly vulnerable to climate change with their restricted thermal tolerance and a slow embryogenesis. Increasing our knowledge of polar cod development will help improve our understanding of the impact of climate change and a warming Arctic in the future and the impacts this might have on the population. This study aimed at characterizing the development of Polar cod from fertilisation to 86 days post fertilisation (dpf) at two temperatures within their biological tolerance (0 & 3 °C) in order to determine the thermal response during early life histories. Adult polar cod were wild caught from Svalbard waters, stripped and artificially fertilised. The embryos were distributed among 8 incubators, 4 incubators per temperature (0 & 3 °C). The present study found that the larvae incubated at 0 °C experienced less mortality, greater hatching success and a larger size at hatch and during early feeding than 3 °C larvae. However the larvae had a slower feeding initiation, a lower feeding success and less yolk sac at the corresponding developmental ages. Comparatively the larvae incubated at 3 °C experienced faster development, greater feeding success and larger yolk reserves at hatch and first feeding. However these larvae experienced higher mortality and lower hatching success, a smaller hatching size and were less developed at hatch. In regards to the results of this study 0 °C treatment provided the ideal conditions for survival. However polar cod early life development might benefit from a slightly warmer Arctic from faster development leading to less time spent as vulnerable larvae and a better predatory avoidance and thus better juvenile recruitment. However under accelerated temperatures the temperature optimum may be exceeded and larvae will experience significantly negative biological and environmental consequences.

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1. Introduction

1.1. Climate change and the expected effects of elevated seawater temperature

The Arctic and sub-arctic seas are projected to be the most affected by rising seawater temperature and CO₂ concentrations (Fransson et al, 2009). The climate is already changing rapidly in areas such as the Norwegian Sea, Barents Sea, Iceland and East Greenland Sea. Arctic water has a high solubility of CO₂ which further enhances impacts of climate change. Atlantic waters are increasing in temperature which flow in to the Arctic Ocean accelerating the decline in sea ice cover, decreasing buffer capacities and aggravating ocean acidification (Igor et al 2010). Ocean warming and acidification threatens fish populations through narrowing of embryonic thermal ranges and reducing suitable spawning habitats (Dahlke et al, 2018). Average sea surface temperature will potentially rise 3 °C or more by the year 2100 having profound effects on early life stages of fish inhabiting those surface waters (Llopiz et al, 2014). The sea ice cover in the Arctic Ocean previously behaved in a more stable and predictable way. During the first half of the 20th century, arctic sea ice would reach 15 million km² each March retreating 7 million km² each September. The multi-year ice (MYI) was thick enough to survive the melt season and in 1970s MYI occupied more than two thirds of surface area of the Arctic basin. The remaining one third of the Arctic basin was occupied by first year ice (FYI). Since 1979 the seemingly stable Arctic sea ice cover started to change considerably (Kwok and N. Untersteiner 2011). Comiso (2002) studied multiyear ice cover (MYI) using satellite passive microwave data from 1978 to 2000. Their findings found that MYI cover is shown to be declining at a relatively fast rate of 8.9 % per decade. If this decline persists, MYI cover will likely disappear within this century. Wang and Overland (2012) used climate models to address the question of the extent of sea ice loss. The results suggest a nearly Sea ice free Arctic by the 2030s. Laxon et al (2013) using estimates of ice volume from cryo sat-2 satellite radar altimeter measurements of sea ice thickness found 2012 summer ice extent to be the lowest on satellite record. Perovich and Richter-Menge (2015) collected data from various sources on 41 sites within the Arctic region, compiled to investigate the amount of surface and bottom ice melt from 1957 to 2014. They found between the periods 2000 to 2014 near the edge of summer ice cover there was enough melting to completely melt first year ice and on two occasions completely melt MYI cover in the Beaufort Sea. Comiso (2002) concludes that a reduction in ice cover will result in a large influx of solar radiation into the Arctic Ocean which will change the characteristics of the

water column. There will be an increase wind strength over the water surface reducing stratification and encouraging mixing of nutrient rich waters and nutrient replenishment from upwelling's. In turn this will change the productivity and biota of the region increasing primary production and consequently increasing zooplankton and fishery production (Arrigo, et al, 2008,. Bouchard and Fortier, 2008). The increasing CO₂ concentrations and temperature of surface waters may increase phytoplankton growth rates however potentially resulting in a negative physiological response of the breakdown of phytoplankton enzymatic activity (Sobrino et al 2008). A reduction in sea ice will also make the Arctic oceans more accessible to human activity such as oil exploration (Comiso 2002). Climate warming and ecosystem change will cause range expansion of boreal species into the Arctic regions. This could result in an overlap in diet between Arctic native species such as polar cod and advancing competing species resulting in potential habitat displacement (Renaud et al 2012).

It is widely considered that the early ontogeny of fish species is the most temperature sensitive stage in development (Laurel et al, 2018). Arctic species such as polar cod have adapted to low temperatures and low thermal fluctuations within the natural environment through evolution to maintain performance and endurance. This specialisation of a species keeps them bound to a strict range of environmental conditions (Speers-Roesch et al, 2018). These life history traits are bottlenecks for some species resulting in a reduced ability to adapt to rapid environmental changes such as future predictions of climate change (Dahlke et al, 2018). A slight change or fluctuation of abiotic conditions in the previously stable Arctic environment can have great impacts on such species metabolic demands, resulting in high energy costs potentially negatively affecting general fitness performance such as growth, reproduction, swimming capacity and feeding success (Butler 1992). Temperature is known to have significant effects on the rate of development in larvae incubation period and size at hatch (Perrichon, et al 2018). This alone can have adverse effects on populations, considering the timing of spawning and subsequent hatching has likely evolved to provide a favourable feeding environment for larvae. Such as the early spring plankton bloom in the marginal ice-zone (Graham 1995). Higher ocean temperatures result in faster growth and earlier hatching and a faster absorption of the vitelline reserves (Perrichon, et al 2018), until the optimal temperature is exceeded. A warmer climate may lead to changes in polar cod phenotypic traits including earlier maturation, smaller size, increased investment in reproduction at early age, and in sum a reduced fecundity (Nahrgang 2014).

1.2. Polar cod

Polar cod (*Boreogadus saida*) is an abundant Arctic fish species, found in diverse arctic and subarctic habitats ranging from brackish to fully marine waters (Bouchard, et al 2011) coastal and offshore waters (Nahrgang 2014), both pelagic and benthic habitats during the juvenile and adult stages (Gradinger and Bluhm 2004). Polar cod are an important element of a strong bottom up controlled ice-associated food web (Gradinger and Bluhm 2004), they are considered an important energy rich resource for many marine birds and mammals in the Arctic ecosystem (Christiansen 2012). Welch et al (1992) found that Polar cod channel up to 75 % of energy flow from zooplankton to higher trophic levels. The early life stages of Polar cod have low thermal tolerance with upper thermal limits of 3 to 3.5 °C and a declining hatching success above 2 °C (Laurel et al 2018). Young polar cod are often observed within cracks and brine channels of sea ice (Gradinger and Bluhm 2004) which is a successful way of avoiding predation from below and above the ice (Lønne and Gulliksen 1989). Peak hatching occurs in April and May but extends from January to July (Bouchard et al, 2017). They spawn large transparent pelagic eggs (Graham 1995) which develop under the ice (Bouchard 2015). Age 0 ice associated juvenile polar cod develop in the top 100 m surface layer over the spring and summer (Lønne and Gulliksen 1989). By early fall they descend to intermediate depths to join the deep adult population at temperatures below 0 °C (Bouchard et al, 2017). Age 2+ polar cod are mostly mesopelagic forming dense aggregations within a deep embayment and along the shelf slope (Bouchard et al, 2017). Understanding the thermal sensitivity of eggs and larval stages is one way of assessing their likelihood of survival with a changing climate. Polar cod have large eggs with larger lipid reserves which give large larvae at hatch. This corresponds with the longer development time of the embryos in order to ensure survival to onset of feeding (Laurel et al 2018). A larger pre-winter size is an important survival advantage; higher temperatures will improve growth and thus survival of larvae during winter (Bouchard et al 2017). Summer hatching experience higher temperature, have more food and grow faster which will help them reach adult size in a shorter period of time. This reduces the time spent most vulnerable to predation compared to winter hatching who have a longer growing season (Fortier et al 2006). Polar cod live closely to the surface sea ice during their most vulnerable early life stages, their lives are largely connected to and affected by the condition of the ice which is receding and thinning with increasing temperatures. Declining sea-ice extent and thickness may alter community dynamics and structure, a change in abundance of Polar cod may have damaging effects on the Arctic marine ecosystem (Bouchard 2017). Polar cod abundance has been found reduced in the

southern ranges of its distribution, the Barents Sea, due to the rising water temperatures (Eriksen et al, 2015).

To better understand how further temperature changes may affect Polar cod chances of survival we must first acquire the in-depth knowledge of their early life development. These findings will act as a baseline study and will be used as a direct comparison for future temperature dependent experiments (as well as toxicology) and help us to determine what climate change may mean for this species.

1.2. Objectives

This study characterizes the embryogenesis of Polar cod (*Boreogadus saida*) while comparing two temperatures 0 & 3 °C. The larvae were compared both over chronological time and over corresponding development stage. The reference temperature 0 °C represents the preferred temperature (Dahlke et al. 2018) of polar cod embryos and 3 °C represents a thermal stress temperature relative to a climate change scenario (Dahlke et al. 2018, Lind et al. 2018).

We hypothesized that increased sea water temperatures will affect the development and survival of polar cod early life stages.

We predict that larvae of 3 °C will experience a higher mortality induced by thermal stress and develop faster resulting in an earlier hatch with a smaller hatching size compared to 0 °C larvae. It is expected to find correlations between particular developmental stages and peaks in mortality expressing particular vulnerable developmental stages over time.

This study will help improve our understanding of the impact of a changing Arctic and surrounding oceans related to future climate change predictions and what this might mean for Polar cod and how this might alter the Arctic ecosystem.

2. Method

2.1. Ethical statement

All work was performed according to and within the regulations enforced by the Norwegian Animal welfare authorities and no specific permissions were required. The R/V Helmer Hanssen is owned by UiT, The Arctic University of Norway, which has all the necessary authorization from the Norwegian Fisheries Directorate to use a bottom trawl to collect fish for scientific purposes. The experiment was approved by the Norwegian Animal welfare authorities (ID14192).

2.2. Sampling Area and broodstock maintenance

The sampling area was focused on the Arctic and Atlantic waters from North and Southeast of Hinlopen and trawls were taken at two stations; N1 (80° 19'N, 15° 37'E) and B1 (78° 55'N, 23° 40'E) on November 20th 2017. Bottom trawls (6 total) were taken. The Campelen 1800 shrimp trawl was fitted with a live fish lift to insure a more fish friendly trawling experience. Trawls were 20 minutes in duration and taken between 170-200m depth (at bottom). The B1 station where all polar cod used for experimentation were collected can be classified as Arctic water with reduced salinity and cold temperatures (-1 °C at bottom and at surface). When received on deck, trawls were roughly sorted for polar cod which were immediately placed into four flow through seawater tanks (ca. 2500 L) strapped on the starboard side of the trawl deck. These tanks were secured and fitted with lids to prevent losing fish during ship movement. Seawater temperature was ambient and varied from -1 °C at the capture location to 7 °C as the ship neared Tromsø. Roughly 1500 live polar cod were transferred to the biological station in Kårvika, Norway from the pier using an aerated trailer with 2 °C seawater. Upon arrival fish were transferred to a 3000 L holding tank at 2.3 °C and natural light at 69°N, feed daily with Calanus (Calanus AS) and treated daily with Halamid solution.

2.3. Experimental set up

Firstly 8 (4 per temperature of 0 & 3 °C) conical shaped incubators (25 L) were filled with natural UV treated and filtered (60 µm) sea water 33 PSU from the fjord. The water was brought to the correct temperatures, 0 and 3 °C, and checked regularly with thermometers. Hobo loggers were placed in randomly selected tanks and recorded light and temperature every 30 minutes, starting logging the day before the fertilisation occurred. The water flow

was controlled and maintained at 25L/hr. Air stones ensured a 90% oxygen saturation at minimum.

A preliminary test was carried out the day before stripping and fertilisation of the eggs and milt in order to confirm if the brood stock were ready to spawn. In this preliminary test 4 females were selected, the fish were anaesthetised in a finquel bath. 3 of the 4 individuals spawned within one minute of being placed in the solution, 1 of which without any pressure applied to the abdomen. This confirmed that the brood stock were ready for stripping.

2.3.1. Rearing system, Stripping and *in vitro* fertilization

On 28/01/2018 49 mature females (18.8 ± 0.2 cm and 45.9 ± 2.0 g) and 21 mature males (16.7 ± 0.5 cm and 34.9 ± 4.0 g) were sedated with MS222 (50 mg.L⁻¹) and gently stripped between the hours of 10:30-13:00. The eggs of each female (20ml) were placed in two separate 10ml sterile and dry tubes, which were labelled and placed on ice. The fecundities of the spawning females varied depending on fish size, it was observed the larger fish had a larger swollen abdomen and greater spawning capacity. Age of the sample fish ranged from 2-4 years. The egg quality was carefully checked during and after the stripping process to ensure the samples quality and health, the samples were discarded if in too little quantity, discoloured or with blood. All care was taken to avoid the samples coming in contact with water. The eggs seemed homogenous in size within and between females. 21 males were stripped of the milt using the same process but not of the same quantity. The eggs of the 49 females were mixed at a ratio 1:1 and split for *in vitro* fertilisation at 0 and 3 °C respectively. Milt from the 21 males were mixed at a ratio 1:1 similarly to the females and split equally between the two fertilisation temperatures. A total of 490 mL of eggs per temperature were distributed to two glass bowls maintained in an ice water bath (0 °C group) and in water equilibrated to 3 °C respectively. Milt (7mL) was added simultaneously over both bowls under time control at 14:30 and gently mixed for 10 minutes. Activation of the sperm and fertilization started with the addition of 2L of seawater stabilized to 0 and 3 °C respectively. After the 10 minutes of mixing, the excess of sperm was rinsed with seawater adjusted to the respective temperature groups. Potentially fertilised eggs (28 mL) were evenly distributed in each of the 8 incubators using 2 ml spoons, yielding approximately 8000 eggs per incubator. The extra eggs and milt were frozen at 20 °C. Polar cod embryos were raised under a 0:24h light/dark photoperiod cycle during four weeks and then the number of hours of light per day increases gradually to simulate polar light cycle at 80°N.

2.4. Mortality protocol

Egg mortality was recorded on a 24hour basis, until all individuals within an incubator had either died or hatched. Eggs were assumed dead or dying if they were no longer buoyant and had sunk to the bottom of the incubator. First the aeration in the incubators were removed and left for 10 minutes to allow dead eggs to sink to the bottom. The valves on the bottom of the incubators were opened slowly releasing 1L of water collected in to a labelled beaker. All samples were taken within an hour. The aeration was replaced straight after the samples were collected. The samples were stored on ice. A sieve was used to rinse the eggs with seawater; they were then transferred to a petri dish with a pipette where they could be counted accurately using a counter. Eggs were also photographed using the same camera settings for all. The same protocol was done for mortality of larvae.

2.5. Total hatching success protocol

The hatching success rate was evaluated daily from first hatch to 80 % hatch. To determine the proportion of hatched larvae to eggs duplicate water samples were taken of each incubator corresponding to about 1% of the well mixed water column using a plastic pipe which has approximately the same length as the incubator, 5cm in diameter and holds a volume of ca. 200 mL. The pipe was inserted in the water column straight down and all the way to the bottom, suction was applied to pull the sample out the incubator and in to a labelled beaker with the volume of 200 mL. Good aeration within the incubators was important to ensure the larvae and eggs were evenly distributed in the water column so each sample collected contained a representative proportion of embryos and larvae. The eggs and larvae were rinsed on to a sieve and transferred in to Bogorov counting chambers, using counters the eggs and larvae were counted separately and recorded to estimate the percentage of hatching per day. After counting the eggs and larvae were placed back in to the correct incubators. The total hatching success was calculated from the total embryo mortality counts by end hatch per incubator. The total amount of dead eggs was subtracted from the assumed total amount of fertilised eggs (8000) per incubator. This was divided by the total mortality and multiplied by 100 to get the percentage of hatched larvae. Equation = $8000 - \text{total dead eggs} \% \text{ total dead eggs} \times 100$. The assumed start number of 8000 was based on the count of a subsample.

2.6. Feeding protocol

Larvae from both treatments were fed 3 million rotifers (provided by NOFIMA) twice a day at densities 7.5 rotifer per mL, mixed with Nannochloropsis (Nanno 3600, Reed Mari culture). *Artemia spp. Nauplii* (Micro *Artemia* Cysts, Ocean nutrition) was given at densities

of 2 prey per mL. Rotifer introduction to larvae of 3 °C treatment started at 35 dpf, *Artemia spp. nauplii* were first introduced to the larvae at 47 dpf and both rotifers and *Artemia* were fed simultaneously for 11 days. From 69 dpf a mix of enriched (INVE Selco S.presso) and newly hatched *Artemia nauplii* were given, *Artemia nauplii* were enriched to ensure high nutritional value. Rotifer introduction to larvae of 0 °C treatment started 57 dpf (while hatching continued), *Artemia nauplii* were introduced 60 dpf and both rotifers and *Artemia* were fed simultaneously until 68 dpf then only *Artemia* was continued.

2.7. Biological sampling

2.7.1. Larval feeding success

To determine and follow feeding success both temperatures were sampled two times at different ages 3°C – days 52 and 68 and 0°C – days 76 and 86. The percentage increase of feeding success was calculated using these selected days which represent corresponding development age of both temperatures in order to cross analyse the difference between feeding success percentage increase depending on temperature. The samples were taken 1 hour after feeding to give the larvae time to ingest the prey using the same method as size at hatch. Feeding success using the images was determined through observation based on absence or presence of food in the larvae's gastrointestinal tract (Figure 1).

2.7.2. Developmental imaging techniques

Post fertilisation images were taken once every 24 hours in order to highlight key stages in development with comparisons between the two temperatures (3 & 0 °C). The frequency of photographing 3 °C embryos decreased around 50 dpf when yolk sac utilisation is 100% and events within development occur less often. Between 4-8 Specimens were collected from the incubators via pipette, placed on a slide or in a petri dish, images of specimens were captured using a 5MP microscope camera, leica MC 170HD and leica application suite software (LasV46) under a leica M205 C stereo microscope. The specimens were then euthanized with an overdose of buffered tricaine methanesulfonate.

Some challenges were encountered during the photographing of the early life history of Polar cod. The translucent nature of the egg made it difficult at times to photograph the development of the embryo due to light reflection and shine. The formation of the blastodermal cap becomes heavy and faces downwards, showing the yolk side up, making it very difficult to study and photograph detail of the development stage. Water droplets or small air bubbles compromise the image quality. If there was too much water the larvae

would move too rapidly for sufficient photography and the eggs may move due to buoyancy. Larvae being fragile can become damaged easily by potential mechanic damage from pipette tips and from the heat of the microscope lighting. Water was removed to avoid larvae movement but then the larvae were more likely to deform and die.

2.7.3. Larval length and yolk sac measurements and image analysis

To determine larval length increase over time as a comparison between temperature at the same development age four days were selected per temperature 0 °C – days 50, 66, 76 and 86 and 3°C – days 28, 39, 52 and 68. Using the same method as for the hatching rate protocol using the plastic pipe to sample the water column larvae were collected from all incubators. Once euthanized with MS-222, 58 larvae were selected at random over the 4 incubators for both temperatures for each of the four selected days and photographed using the 5MP microscope camera, leica MC 170 HD and leica application suite software (Las V 4.6) under a leica M 205 C stereo microscope. From the images the larvae length (mm) and yolk sac size was measured using the open source software image J. Yolk sac size was measured from two days per temperature, 0 °C – days 50 and 66 and 3 °C – days 28 and 39. The scale in image J was set using the analyse option and the straight line to measure the scale bar on the opened image, the scale bar was changed depending on magnification used on the image. Body length measurements were made to the nearest 0.1 mm (Figure 1). The standard length of the larvae was measured from the back of the head and the start of the neck (excluding the nose and the skull) to the tip of the tail (excluding the fin folds) using the segmented line in image J (Figure 1). Yolk sac area was measured using the oblong circle in image J. Also observations of feeding success were recorded.

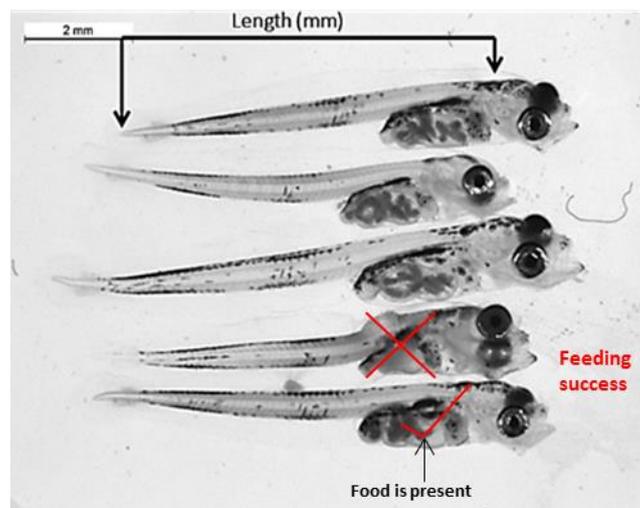


Figure 1 Larvae length measurement and feeding success analyses.

2.8. Statistical analysis

All experimental data was analysed in R studio. Graphs were generated with the free package ggplot. All data is presented as mean value with standard error of the mean. Normality of residuals for all data was tested using the aid of visual assessments such as qqplots, box plots and kurtosis, as well as the Shapiro-Wilk test. For normally distributed variables a T-test was performed to compare the temperate treatments. If assumptions of normality and homoscedasticity were not met a Mann-Whitney U test was performed.

Larvae from both temperatures were compared based on similar developmental stages, newly hatched larvae corresponded to 50 dpf (0°C) and 28 dpf (3 °C), yolk sac larvae corresponded to 66 dpf (0 °C) and 39 dpf (3 °C), feeding stage larvae corresponded to 76 dpf (0 °C) and 52 dpf (3 °C) and pre-flexion stage larvae corresponded to 86 dpf (0 °C) and 68 dpf (3 °C).

3. Results

External developmental features and effects of temperature were identified over the course of the 86 day experiment at two temperatures (0 and 3 °C) observations of the embryo and larval stages are represented in figures 2, 3 & 4.

3.1 Embryo development and morphological landmarks

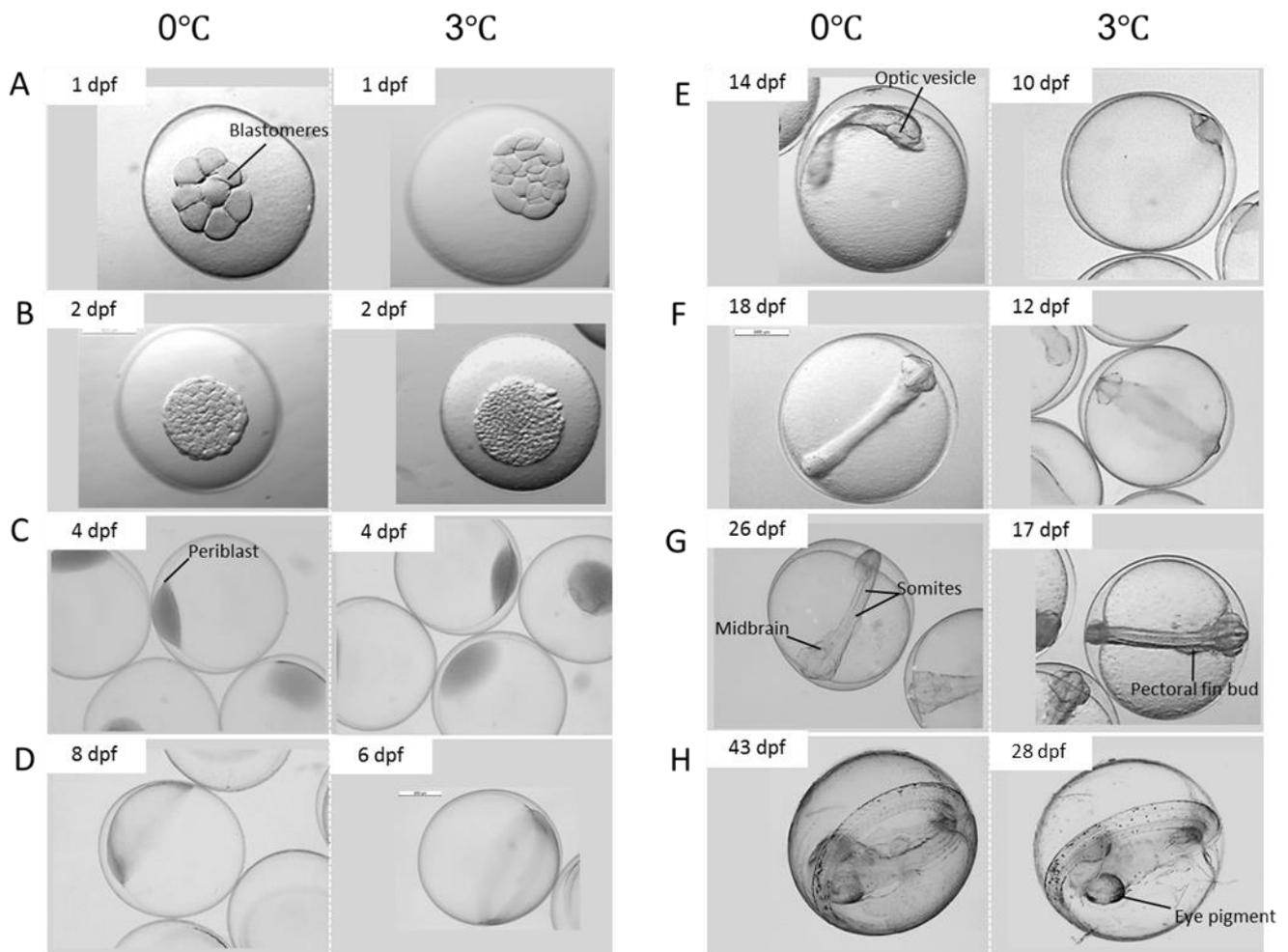


Figure 2 (A-H). Sampled polar cod embryos, photographed using 5MP microscope camera. Embryonic development incubated at 0 & 3 °C. The development is given in degree days; (dpf) days post fertilisation. Points of interest are illustrated in the images. A: 0 °C shows early cleavage 24 hour after fertilisation, 8 cell stage; 3 °C shows early cleavage 24 hour after fertilisation, 16 cell stage with 4x4 array of blastomeres; B: 0 °C 64 cell division with 3 regular tiers of blastomeres; 3 °C 128 cell division; C: 0 °C early morula; 3 °C mid morula stage; D: 0 °C start of Gastrulation stage; 3 °C start of gastrulation stage; E: 0 & 3 °C shield stage; F: 0 & 3 °C bud stage; G: 0 & 3 °C segmentation stage; H: 0 & 3 °C first hatch.

Polar cod embryos reared at the reference temperature (0 °C) began early cleavage right after *in vitro* fertilization and reached the 8 cell stage within 24 hours (Fig 2. A, 0 °C) and the 64 cell stage was reached 48 hours post fertilisation (Fig 2. B, 0 °C). In the early morula stage 9-10 cleavages had occurred and the blastodisc consists of a solid ball of 500 cells which was reached at 4 dpf (Fig 2. C, 0 & 3 °C). Gastrulation began 8 dpf, this is when the cell ball spreads laterally and the cells of the blastodisc began a migration over and around the yolk towards the vegetal pole (Fig 2. D, 0 °C). The shield stage was reached 14 dpf (Fig 2. E, 0 °C) during this time epiboly tissues began to differentiate within the shield and the migration of cells completed the envelopment of the yolk. Shortly after the shield stage 100 % epiboly is reached along with the closure of the blastopore, this marks the Bud stage which was reached at 18 dpf (Fig 2. F, 0 °C). At the segmentation stage (26 dpf) the embryos body length had grown just beyond the yolk sac, the eyes had some pigmentation and slight body movements were observed (Fig 2. G, 0 °C) and a few days later the heartbeat could be seen. By 34 dpf the embryos fin fold and pectoral fin buds were visible, the eyes had strong pigmentation and the embryos were active within the egg. At the time of first hatch (43 dpf) the tail of the embryos had grown one and a half times the egg circumference (Fig 2. H, 0 °C). Hatching success reached 50 % at 52 dpf and 80 % at 60 dpf, in total the hatching event took 17 days.

Embryos incubated at 3 °C exhibited significantly faster development and very short hatching event duration compared to 0 °C embryos. During the early cleavage stage 3 °C embryo blastomere divisions occurred faster than that of the 0 °C embryos (Fig 2. A-B, 3 °C). Gastrulation was reached two days earlier (Fig 2. D, 3 °C), the shield stage occurred 4 days before (Fig 2. E, 3 °C) and the bud stage was reached 6 days before 0 °C embryos (Fig 2. F, 3 °C). Soon after the segmentation stage the embryos first movements could be seen (19 dpf), which was 6 days faster than 0 °C embryos, visible body segmentation and fin development could also be seen at this time for embryos of 3 °C treatment (Fig 2. G, 3 °C). The embryos (3 °C) heart beat could be seen at 22 dpf, with strong pigmentation around the eyes. First hatch occurred 28 dpf, 15 days earlier than 0 °C embryos (Fig 2. H, 3 °C), the total hatching event duration of embryos in 3 °C treatment lasted 3 days.

3.1.1. Larvae development

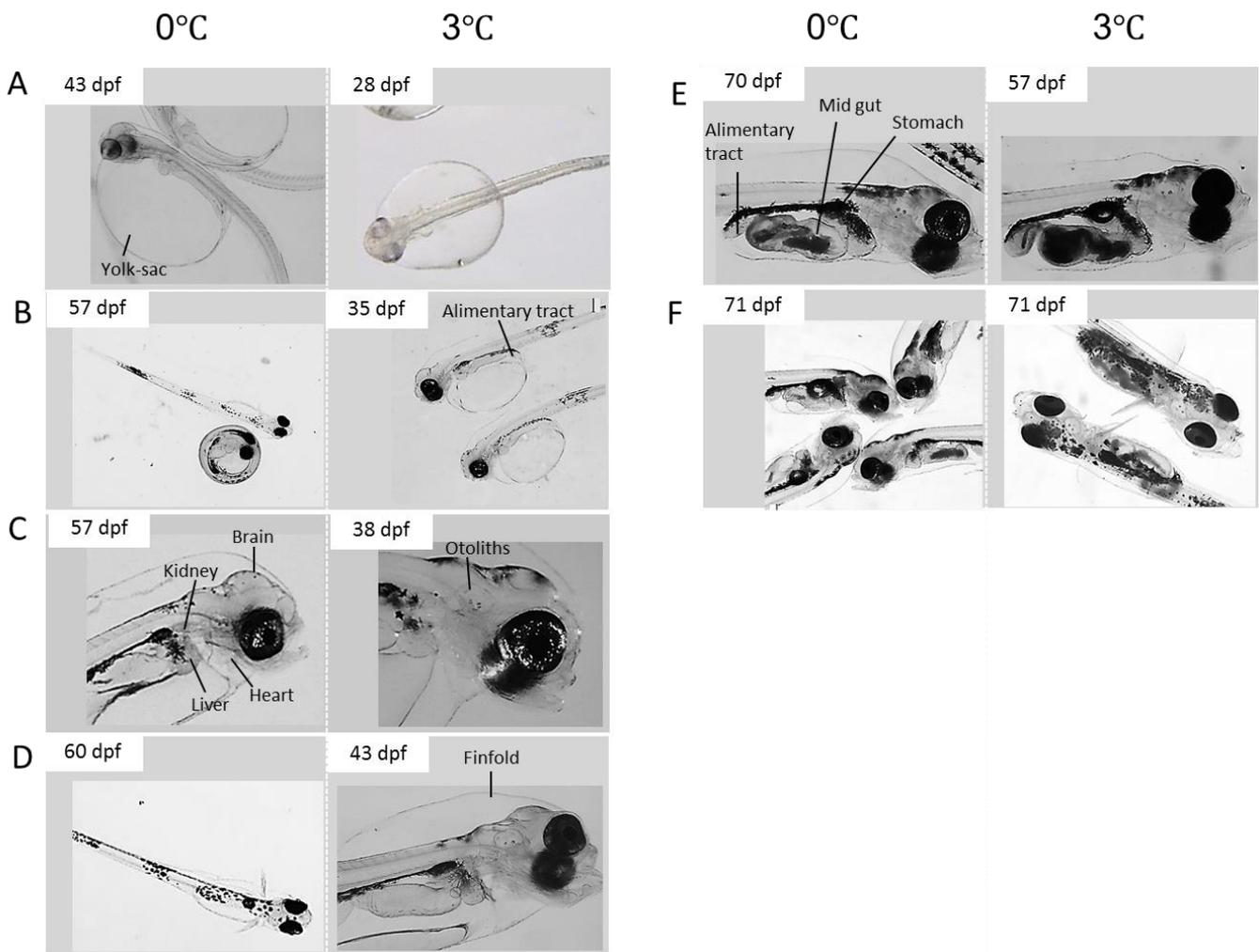


Figure 3 (A-F) Sampled polar cod larvae, photographed using 5MP microscope camera. Larvae development of *Boreogadus saida* incubated at 0 & 3 °C. The images show the development stages in degree day's dpf (days post fertilisation). Points of interest are illustrated in the images. A: 0 °C 43 dpf first hatching seen, shows a newly hatched larvae, 3 °C 28 dpf first hatching seen, shows newly hatched larvae; B: 0 & 3 °C first introduction of rotifers, 0°C hatching is still not complete; C: 0 & 3 °C open mouth stage; D: 0 & 3 °C first introduction of artemia; E: 0 & 3 °C segmented gut stage; F: represents a direct comparison between the two temperatures towards the end of the experiment at 71 dpf.

Polar cod reared at the reference temperature (0 °C) hatched with a straight digestive tract underdeveloped and non-functioning with no differentiation between the sections of the gut (Fig 3. A 0 °C). First introduction of rotifers occurred 57 dpf (Fig 3. B 0 °C), the larvae that hatched during this time, towards the end of the hatching event, did so with an open mouth and with some yolk remaining (Fig 3. C, 0 °C). Many developing features could be seen such as the liver, body pigmentation, otoliths, lower jaw formation, golden eye stage, fins, gill

slits, intestine and a steady heartbeat. During this time observations of the unhatched embryos indicated that there was a restriction of space impacting on healthy growth resulting in spine curvature and deformation. First introduction of Artemia occurred 60 dpf, same time as 80 % hatch and first observed feeding (Fig 3. D 0 °C). The segmented gut stage was reached 70 dpf (Fig 3. E 0 °C) and by this time exogenous feeding had been commenced for 10 days. The larvae were relatively homogenous in size and still had large fin folds at 71 dpf (Fig 3. F 0 °C).

The larvae of 3 °C treatment hatched (28 dpf) with underdeveloped organs, a large yolk sac, simple intestinal tract, and no gills and were less developed compared to 0 °C larvae at hatch (Fig 3. A 0 & 3 °C) Embryos reared at 3 °C had a smaller average larval length at first hatch of 4.5 mm compared to 5.1 mm (0 °C) and a larger average yolk size of 1.6 m² compared to 1.0 m² (0 °C). Larvae first introduction of rotifers was 35 dpf (Fig 3. B 3 °C), the larvae had jaw movements and open mouth at 38 dpf (Fig 3 C 3 °C). First introduction of Artemia and first exogenous feeding was 43 dpf, at this time the larvae have developed eye pigmentation, an extended jaw, and gill formation and there was little yolk remaining (Fig 3. D 3 °C). The differentiation of the digestive tract can be observed 35 dpf (3 °C) and 48pdf (0 °C) 5 days after 80% hatch for both temperatures. It is possible to see the fore gut, the mid gut and the hind gut 37 dpf (3 °C) and 51 dpf (0 °C) similarly for both temperatures. Larvae from both temperatures initiate first feeding 4-5 days after the mouth first opened. Segmented gut stage is reached 57 dpf in 3 °C larvae (Fig 3. E 3 °C) and exogenous feeding had been commenced for 14 days which is 4 days longer than 0 °C larvae. As a comparison of chronological time the larvae of both temperatures are represented at 71 dpf in Figure 3 (Fig 3. F 0 & 3 °C). During this time the larvae of 3 °C appeared more developed than 0 °C and are more active aided with long pectoral fins and the fin rudiments could be clearly seen at the tip of the tail. Also defining sections of the brain started to become apparent, the stomach was positioned above the intestine and the gills were protruding from the side of the neck (3 °C). The larvae (3 °C) had almost no fin folds at this time whereas 0 °C larvae have large fin folds around the body (Figure 3, F 0 & 3 °C).

3.2. Time line of early life history events

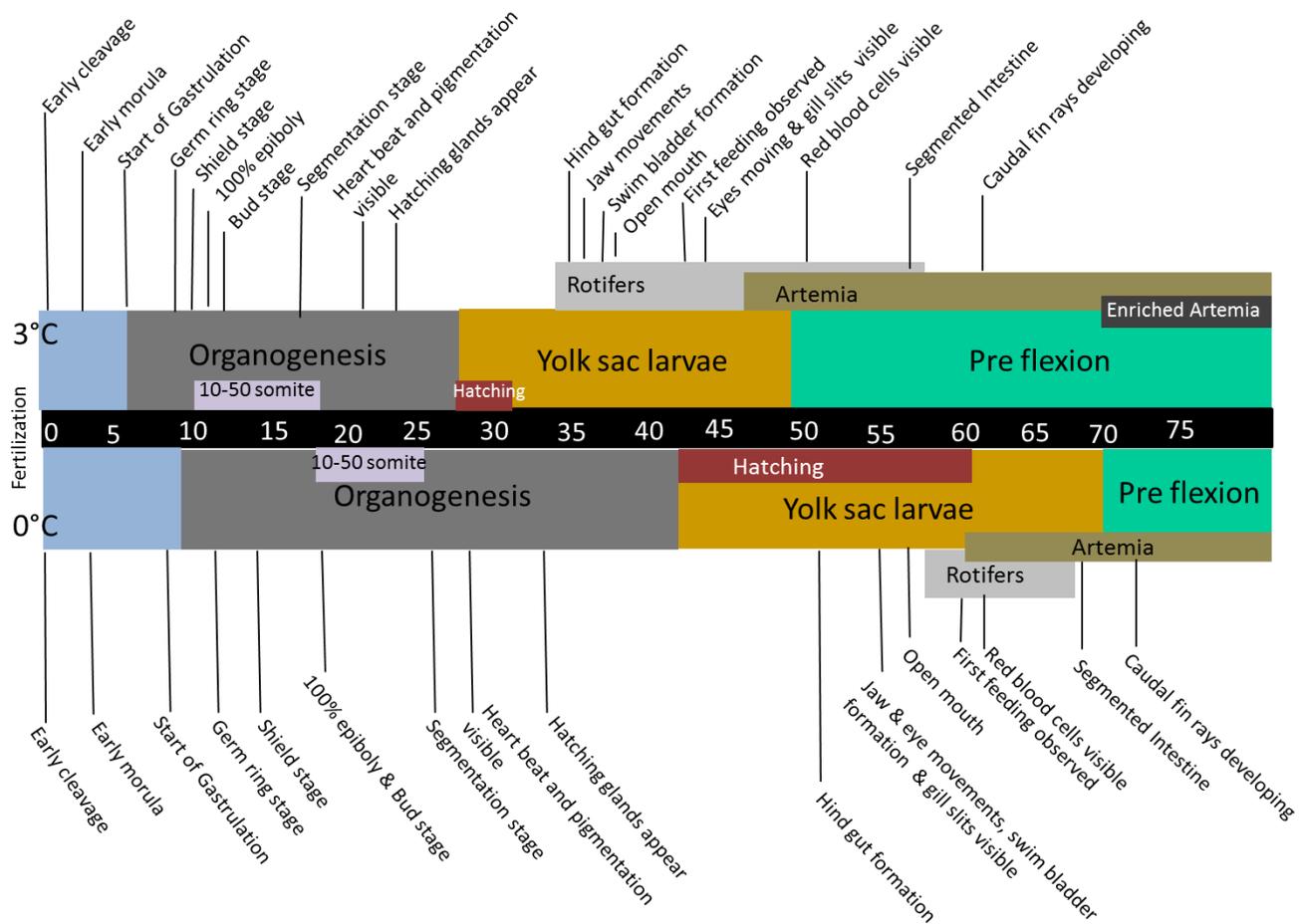


Figure 4 shows a time line of the embryo and larval development of polar cod from fertilization to 86 dpf as a comparison in chronological time between 3 & 0 °C. The figure shows the development stages in degree day's dpf (days post fertilisation).

Visible morphological events were rapid during early development at both temperatures. Development occurred faster at 3 °C for all stages and the intervals between morphological events were shorter compared to 0 °C. There was a pause in the visible morphological development between the hatching gland stage and the hind gut formation stage for both temperatures, during this time the hatching event occurred and the yolk sac larvae stage begins. Throughout the entire experiment 0 °C larvae were behind 3 °C larvae in development, the largest difference was at 80 % hatch when 0 °C larvae were 30 days slower in development however these larvae were able to catch up in development towards the end of the experiment leaving a smaller difference of 10 days at 73 dpf (Figure 4).

3.3. Mortality

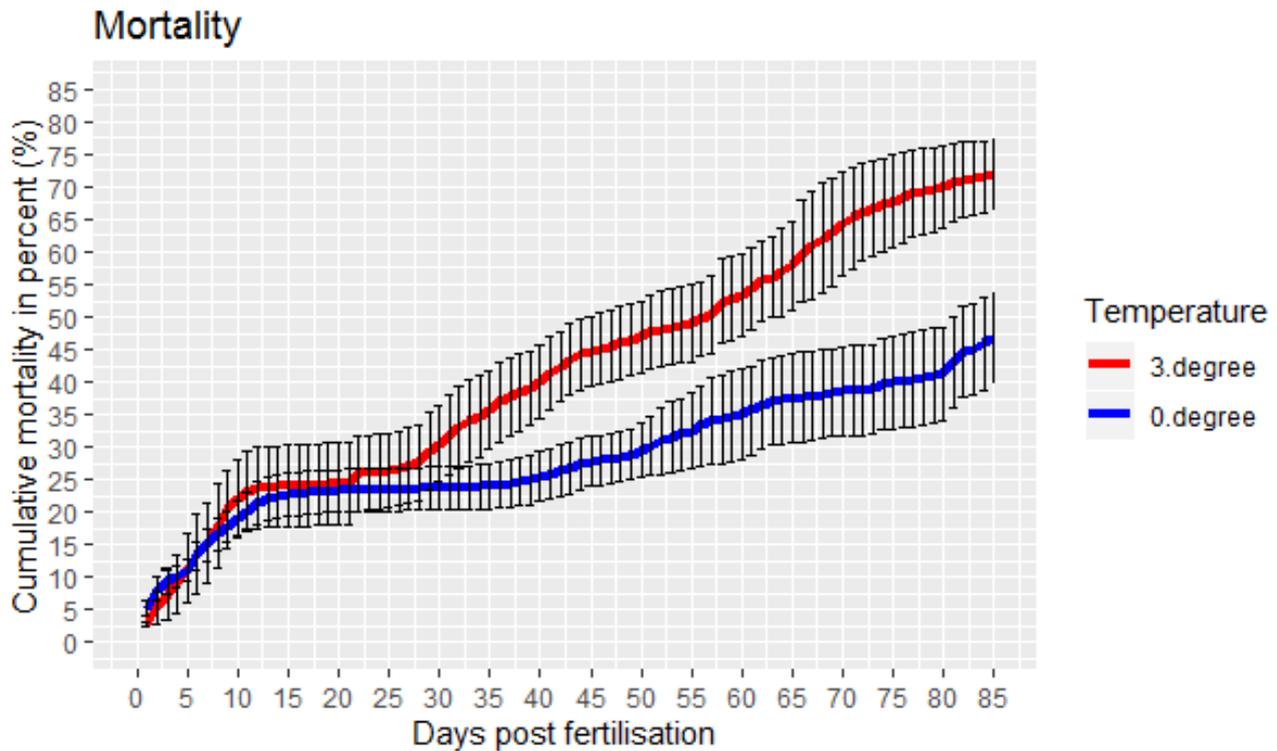


Figure 5 Cumulative mortality (mean percentage with standard error bars) of polar cod embryos and larvae from four replicate incubators per temperature (3 & 0 °C) (A.C.P.M) over 86 days. The two linear regressions were tested for coefficients and then a paired sample t-test was applied to establish a pair wise difference. The two regressions were significantly different with p -value <.0001.

Both temperatures experienced a high mortality increase directly after fertilisation in the first 11 days of development from early cleavage to shield stage, then the mortality stabilises at ± 25 %. At around 22 dpf the larvae of 3 °C treatment experienced a spike in mortality this marks the heart beat and pigmentation stage. Both treatments after the start of the hatching event experienced a steady increase of mortality until the end of the experiment. Larvae of both treatments experienced a slight increase in mortality at the onset of feeding although not significant (Figure 5). The cumulative mortality at 86 dpf was 47.0 % (0 °C) and 71.9 % (3 °C) representing a difference of 24.9 % between the two temperatures with a p -value <.0001. Larvae of 3 °C experienced a significant higher mortality rate compared to 0 °C larvae.

3.4. Total hatching success

The mean hatching success in percentage with standard deviation was higher in 0 °C (69.3 ± 4.0 %) compared to 3 °C (68.2 ± 6.2 %). First hatch occurred 43 dpf (0 °C) and lasted 17 days and 28dpf (3 °C) and lasted 3 days. Mann whitney U test was used to test the hatching

success data for significance between the temperature treatments, a P-value of 0.001 concludes that there is a significant difference in hatching success between the two temperatures.

3.5. Larval length measurements

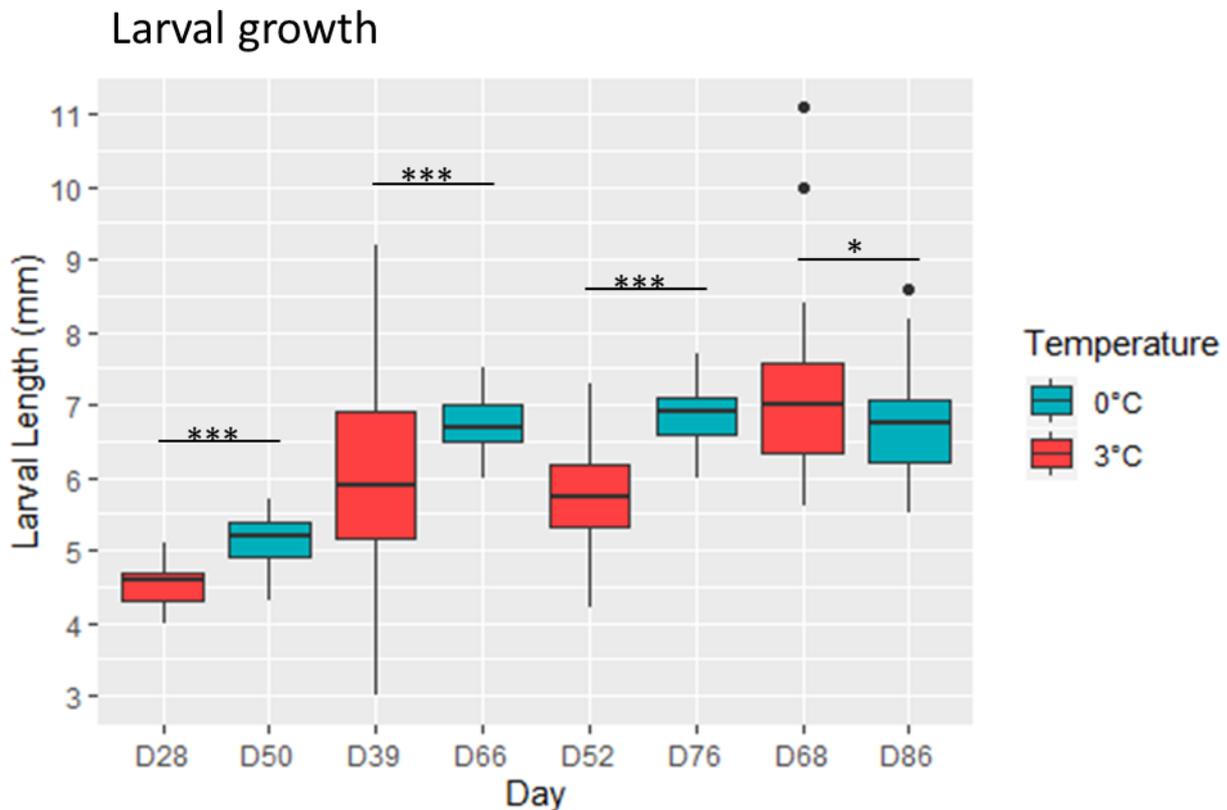


Figure 6 shows larval growth over time with larval length (mm) and measurements of 58 randomly selected larvae from four selected days for each temperature (3 & 0 °C). 28 dpf (3 °C) and 50 dpf (0 °C) represent newly hatched larvae. 39 dpf (3 °C) and 66 dpf (0 °C) represent yolk sac larvae. 52 dpf (3 °C) and 76 dpf (0 °C) represent feeding stage larvae. 68 dpf (3 °C) and 86 dpf (0 °C) represent pre-flexion stage larvae. Plot represent the median (Bar), 25–75 % percentiles (box), non-outlier range (whisker), outliers (circle), the symbol * (asterisk) indicate significant differences, (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) between treatments.

There are significant differences in larval body length induced by rearing temperature at all stages. During newly hatched larvae, yolk sac larvae and feeding stage larvae the average body length was longer in 0 °C larvae compared to 3 °C larvae ($p < 0.001$). Interestingly at pre-flexion stage there was a slightly larger average body length in 3 °C larvae compared to 0 °C larvae ($p < 0.05$). From the newly hatched stage to pre-flexion stage the total larval length grew 31.3 % (0 °C) and 57.7 % (3 °C), therefore larvae incubated at 3 °C had a greater growth compared to the larvae incubated at 0 °C. Newly hatched larvae average length

measurements were 5.3 mm (0 °C) and 4.7 mm (3 °C). At the yolk sac stage 0 °C larvae average length ranged from 6 mm to 7.5 mm whereas 3 °C larvae had a much larger variation in length from 3 mm to 9.2 mm. Larvae growth (0 °C) slowed between the yolk sac stage (66 dpf) and feeding stage (76 dpf) during the time of 100 % yolk sac utilisation (70 dpf) and the average length reduced slightly at pre-flexion stage (86 dpf). The average length for 3 °C larvae reduced between yolk sac stage (39 dpf) and feeding stage (52 dpf) during the time of 100 % yolk sac utilisation (50 dpf) and the length increased again by pre-flexion stage (68 dpf) and the larvae had a slightly larger length compared to 0 °C. During all stages (from newly hatched larvae to pre-flexion stage) larvae incubated at 3 °C treatment had a leaner body when compared to the corresponding development ages of 0 °C larvae (Figure 6). When comparing chronological time between temperatures 3 °C larvae were slightly greater in average length than 0 °C larvae. Newly hatched larvae 50 dpf (0 °C) average larval length was 5.3 mm and the corresponding feeding stage larvae 52 dpf (3 °C) average larval length was 5.8 mm. Yolk sac stage 66 dpf (0 °C) average larval length was 6.7 mm and the corresponding Pre-flexion stage 68 dpf (3 °C) average larval length was 7 mm.

3.6. Feeding success

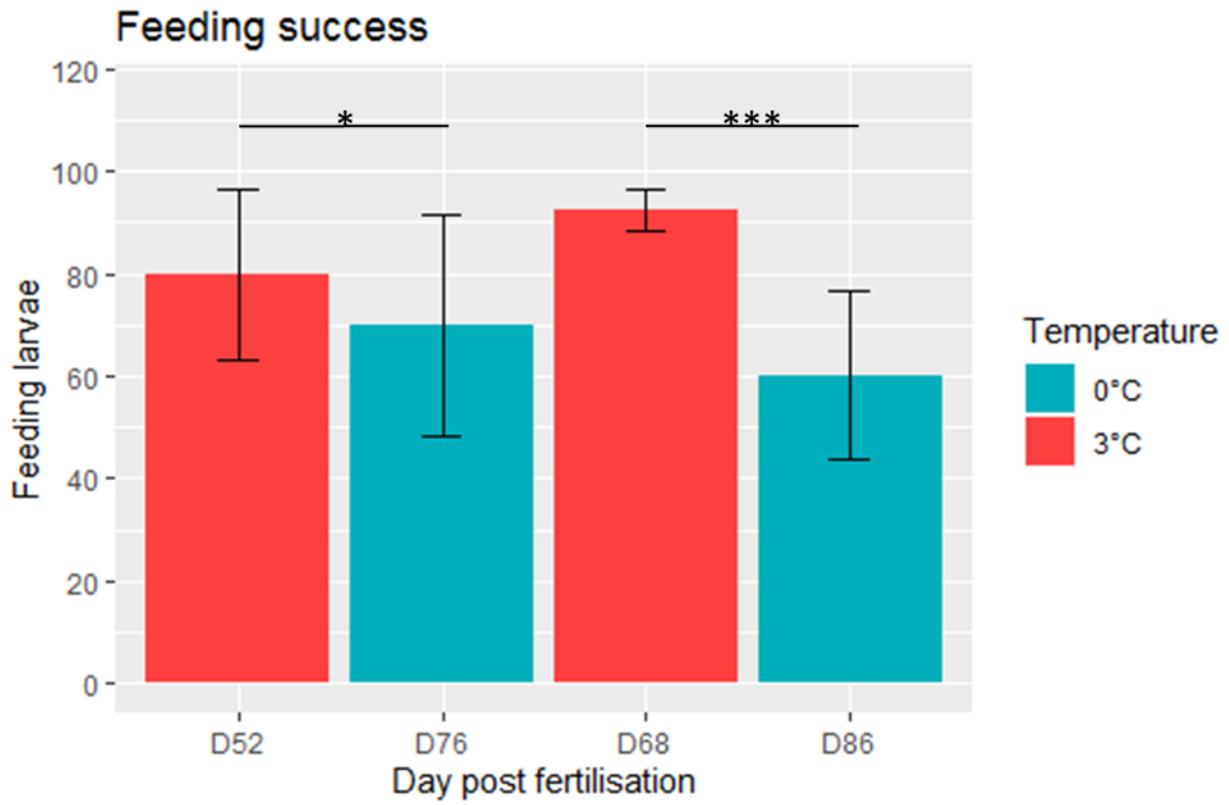


Figure 7. Feeding success of 58 randomly selected larvae of two days for each temperature (3 & 0 °C). 52 dpf (3 °C) and 76 dpf (0 °C) represent feeding stage larvae and 68 dpf (3 °C) and 86 dpf (0 °C) represent pre-flexion stage larvae. Histogram represents the feeding larvae percentage of the mean of four incubators, standard deviation (error bars). The symbol * (asterisk) indicate significant differences, (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) between treatments.

Larvae incubated at 0 °C showed a lower feeding success compared to 3 °C in both stages and had a feeding success percentage decrease of 25 % from feeding stage to pre-flexion stage. Larvae incubated at 3 °C experienced a 50 % feeding success increase between feeding stage and pre-flexion stage. We can conclude that there is a significant difference between feeding success of both stages, feeding stage larvae had a p -value 0.02 and pre-flexion stage larvae had a p -value < 0.001 (Figure 7).

3.7. Yolk sac size

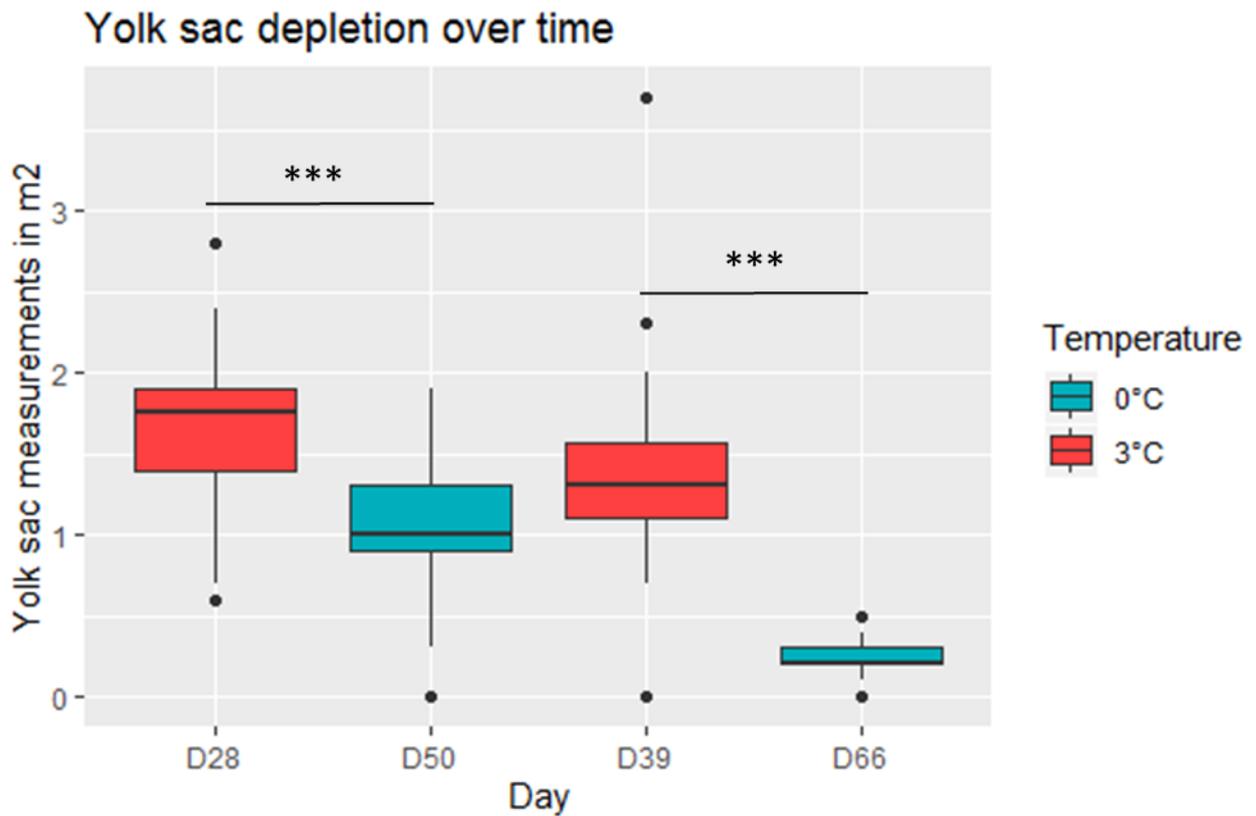


Figure 8 Yolk sac depletion over time, yolk sac area measured in (m2). 58 randomly selected larvae were measured over two days for each temperature (3 & 0 °C). 28 dpf (3 °C) and 50 dpf (0 °C) represent newly hatched larvae and 39 dpf (3 °C) and 66 dpf (0 °C) represent yolk sac larvae. The plot represents the median (Bar), 25–75% percentiles (box), non-outlier range (whisker), outliers (circle). The symbol * (asterisk) indicate significant differences ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$) between treatments.

Larvae incubated at 0 °C had less yolk sac than 3 °C larvae at both stages. From newly hatched larvae to yolk sac larvae stage, larvae incubated at 0 °C showed 75 % decrease in yolk sac size, whereas larvae incubated at 3 °C showed a 12.5 % decrease in yolk sac size from newly hatched larvae to yolk sac larvae stage. There are significant differences in yolk sac depletion induced by rearing temperature. Newly hatched larvae and yolk sac larvae stage both had a p -value < 0.001 (Figure 8).

4. Discussion

The present study investigated the effects of two temperatures (0 & 3 °C) within the thermal tolerance of polar cod during early life. Significant differences in survival, hatching success, feeding success and yolk sac size were observed. Sakurai et al (1998) identified the ideal temperature range of 0.5 to 3 °C of which had the highest polar cod embryonic survival rates of 65 %. Dahlke, et al (2018) found that when Polar cod embryos were placed under 4.5 °C they showed increased mortality from severe heat stress. In the present study, mortality was significantly lower for 0 °C treatment (42 % mortality) compared to 3 °C treatment (72 % mortality) (Figure 4). Larvae of both temperatures experienced a similar trend in mortality although larvae of 3 °C treatment had more peaks in mortality compared to larvae of 0 °C treatment. Post fertilisation both temperatures showed a rapid increase in mortality in the first 11-12 days of development from early cleavage to shield stage, similar results were found by Thompson (1981) and Rombough (1996). Suggesting that regardless of temperature this is a highly vulnerable and sensitive period in early development. From the start of the hatching event until the end of the experiment, both treatments experienced an increase in mortality, although larvae of 3 °C treatment experience a more intense increase and larvae of 0 °C treatment experience a more steady increase in mortality. A study by Bonnett (1939) also found a sharp increase in Atlantic cod egg mortality as hatching approached at all temperatures used in the experiment with higher mortality at higher temperatures. In this study there was also a slight increase in mortality for both treatments during the initiation of early feeding although not significant (Figure 4). Other studies have conflicting results regarding the effects of temperature on mortality. Increasing temperatures within the species thermal tolerance had been found to have no direct influence on survival (Gracia-Lóópez, et al 2004., Rankin and Sponaugle 2011). However on the contrary some studies have found the relationship between temperature and daily mortality rates of fish eggs and larvae to be highly correlated (Houde 1989., Fossum 1988) and survival prior to hatching has been found significantly compromised at higher temperatures (Gagliano et al 2007). In spite of this, mortality rates of fish eggs and larvae regardless of temperature are generally high (Fossum 1988), conclusively a study by Dann (1981) found that only 2 % of spawned North Sea Cod eggs survived to produce larvae. Despite the importance of understanding the causes of mortality during the egg phase to studies of recruitment of egg production very little is known about the processes of which may influence it (Bunn et al 2000).

In the present study hatching success was higher for larvae in the 0 °C treatment (69.3 ± 4.0 %) compared to larvae of 3 °C treatment (68.2 ± 6.2 %). A study by Laurell (2018) found that the hatch success of Polar cod embryos quickly declined above 2 °C. Larvae of both temperatures hatched as transparent larvae with larval fin folds, simple intestinal tract, and with eye pigmentation. However larvae of 3 °C hatched earlier, with a larger yolk sac, no gills and more underdeveloped compared to 0 °C larvae leaving the larvae unable to swim and thus highly vulnerable to predation and more exposed to external pollutants. The hatching period duration was shorter at the higher temperature which was also found by Gracia-Lo´pez et al (2004). Towards the end of the (0 °C) hatching event some embryos were clearly restricted for space and consequently started to develop a deformed spine and even if hatching did occur at this stage it seemed unlikely the larvae would survive. Low temperature might partially inhibit the action of the hatching enzyme (Tay and Garside 1975) which might explain why the embryos of 0 °C treatment had a longer hatching event and why some did not hatch at all. In the present study 50 % hatch occurred at 52 dpf (0 °C) and at 29 dpf (3 °C), similar results have been found by Sakurai et al (1998) where 50 % hatch occurred at 75 dpf at -1.0 °C, 44 dpf at 1.5 °C and 35 dpf at 3 °C. In this study 0 °C treatment can be identified as the optimal temperature for best performance of larvae regarding lower mortality and a higher hatching success.

It is well known that temperature influences larval size at hatch, although size can be influenced by yolk conversion efficiency (Gracia-Lo´pez, et al 2004), and yolk utilization efficiency can be influenced by temperature. Larvae incubated at 0 °C showed on average a longer length at newly hatched larvae, yolk sac larvae and feeding larvae stage when compared to 3 °C larvae of the same development age. Larvae of 0 °C treatment were also observed to be more active at hatch compared to 3 °C larvae at hatch. Thus larvae incubated at 0 °C would potentially have an advantage at first hatch which is a highly vulnerable stage (Fortier et al 2006) from a stronger swimming capacity enabling larvae better able to avoid predation, as well as an advantage at early feeding stage to establish a better prey capture from a less lean and a longer body length (Ninness et al 2006). However over chronological time the average length was slightly greater for larvae incubated at 3 °C (Figure 6), suggesting that temperature had relatively little effect on larval average length between 0 & 3 °C but rather higher temperatures resulted in a greater development speed and larval stage duration declined rapidly as temperature increased. Larvae reared at 3 °C reached the onset of preflexion stage after 50 dpf compared to 70 dpf for 0 °C larvae. Growing faster is an

advantage as to reduce the time spent as vulnerable larvae and mortality has been found to decrease with increased larval size and development age (Mc Gurk 1984). Although studies have found that larval length can reduce with increasing temperatures (Tay and Garside 1975), larvae kept at higher temperatures have a higher metabolic demand which requires more energy for sustaining its necessary functions and growth can be affected if food is limited (Buckley 2004, Barrionuevo and Burggren 1999, and Schirone and Gross 1968). Additionally, Kunz (2018) found that under temperatures above 0 °C polar cod experienced an elevated metabolic rate, with a reduced maximum swimming capacity, which under future predictions of warming ocean temperatures may directly impair polar cod foraging success and escape response to predators and reducing the species competitive strength. Therefore, temperature has many trade-offs, a faster development can reduce the time spent as vulnerable larvae, larger size can aid in feeding success and predatory avoidance however if the temperature optimum is exceeded the larvae will experience a higher metabolism and energy deficiency if unable to consume sufficient amounts of food.

The onset of feeding is a critical and sensitive time point. Larvae incubated at 0 °C showed a lower feeding success compared to 3 °C larvae. Higher temperature has been found to decrease the time to reach the point at which feeding is initiated (Jordaan and Kling, 2003). Bouchard (2011) found that polar cod embryos developing in colder strictly marine environments hatched later and experienced a poor first-feeding success. Michaud et al (1996) also found that in the Northeast Water, the swimming activity and the feeding success of recently hatched polar cod larvae decreased with decreasing temperature in the range +4 to -1.8 °C. Aronovich et al (1975) noted that Polar cod incubated at 1.5 °C can survive without food 14-16 days after hatching without affecting the percentage of larvae capable of establishing feeding, however a starvation period of 20 days after hatching proved critical, and feeding should not start later than 17-20 days post hatch at 3-4 °C. Feeding success of larvae might also be influenced by the amount of time between the hatching event and the introduction to an external food source from establishing feeding behaviour (Wallace and Aasjord 1984). Larvae incubated at 0 °C were exposed to exogenous food source 3 days before total hatch and before first feeding was observed. There was a total of 17 days from first hatch (43 dpf, first hatching) to first observed feeding (60 dpf, last hatching). Larvae incubated at 3 °C were exposed to exogenous food source 5 days after total hatch and for 8 days before first feeding was observed. This is a total of 15 days from first hatch to first feeding. This earlier contact with food at the 3 °C treatment may have influenced feeding

behaviour and thereafter improved food consumption resulting in a better feeding success (Wallace and Aasjord 1984). The lower feeding success of 0 °C larvae might be a consequence from a shorter introduction to an exogenous food source allowing little time for the larvae to develop the necessary skills for prey capture and consumption before the point of no return (Wallace and Aasjord 1984).

Polar cod rely on an endogenous energy source for a prolonged duration. In the present study complete yolk utilisation of larvae incubated at 0 °C occurred at 70 dpf, 10 days after 80 % hatch and first exogenous feeding. Larvae incubated at 3 °C completely utilised the yolk reserves 50 dpf, 20 days after 80 % hatch and 7 days after first exogenous feeding (Figure 8). Ojanguren et al (1991) found that increasing temperature accelerated developmental rates and increase metabolic demand in Atlantic salmon which may be the cause of a faster yolk absorption (Blaxter, 1992), however higher temperature also increase larval activity which can reduce the yolk utilisation efficiency as more of the yolk energy is spent on the additional activity rather than growth (Peterson and Martin-Robichaud,1995). The growth of the larval length slowed down in 0 °C treatment between the yolk sac larvae stage (66dpf) and feeding stage (76dpf) during this time 100 % yolk sac utilisation occurred (70 dpf) and the average larval length reduced slightly at the pre-flexion stage (86dpf). The average length of larvae incubated at 3 °C reduced between yolk sac larvae stage (39dpf) and feeding stage (52dpf) during the time of 100 % yolk sac utilisation (50 dpf). It then increases again by pre-flexion stage (68dpf) (Figure 6). A study by Heming (1988) helps to explain these results, they found that towards the end of the yolk sac stage, yolk absorption efficiency reduces and then becomes negative resulting in the reabsorption of larval tissue mass which supports the findings in the present study. Larger yolk reserves during the early larval period is advantageous and larvae with larger yolk reserves tend to exhibit diurnal feeding pattern which reduces the chance of predation (Blaxter and Hempel 1963). Yolk re-absorption occurred more slowly in 0 °C larvae and there was a slightly longer duration from first feeding and complete yolk utilisation of 3 days compared to 3 °C. This cold water species rate of digestion is slow especially in the first days of feeding, which means food density needed is low (Aronovich et al 1975). However in a warming arctic in suboptimal temperatures metabolic demands will change and larvae will have to search and capture more prey, thus exposing themselves to a higher potential for predation (Skiftesvik 1992). Therefore larvae of lower temperatures have an advantage and it could be concluded that the 0 °C treatment was the preferred temperature regarding yolk utilisation in the present study.

Ecological consequences of sea ice decline

If the sea ice continues to decline polar cod will experience a decrease in suitable spawning habitats, nursery grounds (Dahlke et al 2018) and feeding habitat for early life stages and there will be a loss of protection by Sea ice from predation for spawning adults (Christiansen 2017). The use of model projections and simulations of climate change have shown that by 2100 unrestricted ocean warming and acidification will cause a substantial decline in polar cod egg survival from decreasing embryonic tolerance (Dahlke et al 2018). Although an earlier ice break up and more frequent winter polynyas could improve juvenile recruitment by increasing survival of earlier hatchers from faster development and growth and improved feeding success from the light necessary for prey perception and capture (Fortier et al 1996), in order to reach larger pre-winter size before the fall migration (Bouchard and Fortier 2008., Bouchard et al 2017). However species recruitment success depends on a combination of complex environmental factors, of which will alter due to climate change and future predictions of polar cod disturbance in connectivity and increase risk of adjective losses, and recruitment failure (Dahlke et al 2018). Bouchard (2015) explains the theory that an earlier hatching season of polar cod is desired through selection pressures dictated by the need to reach a maximum pre-winter size to reduce chances of predation and increase survival to adulthood. Not giving importance to the mismatch theory for first feeding larvae to coincide with the maximum abundance of their prey. Climate change can affect biological production and food web structure in the Arctic Ocean through the warming of the upper water layer and increased stratification due to surface sea ice melt (Carmack 2011). Stratification constrains the flux of nutrients in to the euphotic zone which can reduce primary production in a nitrate limited system (Carmack 2004., Lee et al 2012) which in turn selects for smaller less energy satisfying pelagic algae (Li et al 2009). A reduction in sea ice will cause a loss of habitat for sea ice algae and sub ice phytoplankton which together were found to account for 57 % of the annual primary production in the central arctic ocean from July to August in 1994 (Gosselin et al 1997). Alternatively sea ice retreat beyond the shelf break increases the potential for upwelling which brings high nutrients in to the Beaufort shelf (Carmack and chapman 2003). Also there may be an increase in primary production due to increased solar radiation from less ice covered waters (Falk-petersen et al 2006., Wassmann et al 2011). The timing of the phytoplankton bloom is critical for copepod survival which in turn provides food for polar cod. It is difficult to predict if primary production will increase or decrease under ice retreat conditions, and will depend on regional conditions (Carmack 2011). Furthermore prolonged ice free seasons in the arctic may displace the larger lipid rich copepods such as *C.glacialis*

and *C. hyperboreus* by the smaller less lipid rich copepods *C. finmarchicus* and *Pseudocalanus* spp and create a shift in the current zooplankton availability (Falardeau et al 2014). A study by Falardeau et al (2014) found that Polar cod strongly selected larger calanus spp, with a shift in zooplankton availability Polar cod will acquire less energy which will affect their growth efficiency for a large pre-winter size. In a warmer Arctic polar cod is threatened with the advancement of boreal species with potential dietary competition predation and habitat displacement. Atlantic Cod and haddock have expanded distributions northwards all year round in the Barents Sea (Johansen et al 2013), this implies increased predation pressures on small arctic fish species such as polar cod (Fossheim 2015, Renaud et al 2012). Current research has found an extensive dietary overlap between polar cod and capelin in the Barents Sea where both rely primarily on copepods such as *C. hyperboreus* and *C. glacialis*, this supports the potential for interspecific competition between these species (McNicholl et al 2016). Little dietary overlap was seen in Atlantic sand lance which was found to focus on selecting smaller prey (Falardeau et al 2014) and a study by Renaud et al (2012) found little dietary overlap between Atlantic cod and Haddock with Polar cod during the larval stages. However Capelin, Atlantic sand lance, Atlantic cod and Haddock during their early life stages may have higher foraging capability should zooplankton assemblages shift towards smaller prey taxa under a warming climate. However Polar cod are well adapted to low temperatures and are able to convert food to energy more efficiently which could give an additional advantage over advancing Atlantic species (Kunz 2018). Arctic warming and sea-ice loss will also facilitate invasions by new pathogens and disease it may also influence ecological dynamics indirectly through effects on movement, population mixing, and pathogen transmission (Post et al 2013).

Warmer Sea temperatures may benefit polar cod in the near future until temperatures exceed optimal thermal limits and cause adverse effects on the development and survival of polar cod during early life stages. It is important to identify vulnerable life stages and habitat needs of highly specialised and sensitive species such as polar cod and to look at polar cod response to environmental stress and resilience to climate change as this is crucial for predicting the future abundance and distribution of a species. The response time to a changing environment is unlikely equal to the speed of current and future predictions of climate change, increasing the risk of extinction (Kristensen et al 2018). Other major challenges in the near future include addressing pressures arising from anthropogenic use of arctic coastal and near-shore

areas as Sea ice diminishes (Wassmann et al 2011), such as commercial extraction of fish species having an impact on top/down processes (parson 1992).

5. Conclusion

This experiment was designed to investigate temperature effects on early life history of polar cod by using two temperature treatments, 0 °C represents reference temperature and 3 °C represents predictions of future ocean warming and climate change to investigate how this will affect polar cod survival and population recruitment.

Results of this study have shown that temperature had a significant effect on the speed of development, aiding polar cod larvae of 3 °C treatment to reach a less vulnerable larval stage more quickly. There was also a greater feeding success for 3 °C larvae compared to the larvae of 0 °C treatment. However the results of this study showed that higher temperature led to higher mortality and lower hatching success. Larvae of 3 °C had an earlier temperature induced hatching event, hatching smaller and less developed than 0 °C. In the field the early hatching larvae would become exposed to environmental pollutants and ambient changes at a more underdeveloped and vulnerable stage no longer protected in the chorion. This study identified vulnerable developmental stages of mortality regardless of temperature although this was not as pronounced in the developmental stages as previously expected. Considering the larvae had similar length during the same larval sampling days it could be concluded that temperature has little effect on larval length over chronological time. With all of this in consideration it remains difficult to determine whether elevated temperatures will or will not benefit polar cod larvae.

This study gave insights into polar cod sensitivity during early life development, the results highlighted that polar cod larvae within their biological temperature tolerance are capable of withstanding and surviving slightly increasing temperatures and that there are both pros and cons during early life stages within both temperatures. It also emphasises that our study was only a small look into the effect of temperature on polar cod. Therefore it would be beneficial to follow polar cod development to adulthood and reproduction potential to observe and analyse long term effects of elevated temperatures. In regards to a warmer ice free arctic and consequently a greater degree of light penetration to surface waters it would be of interest and importance for future research to assess polar cod larvae under elevated temperatures

combined with the effects of light stimuli on behaviour and activity looking closely at feeding behaviour and predatory avoidance.

These findings may suggest that the relationship between temperature and growth rate, mortality, hatching success, feeding success and yolk sac utilisation is complex. Regarding the fact that there is no simple answer to how temperature increase will impact the development and survival of larval early life stages in the natural environment. Additional investigation is needed in this field of study to gain more insight of the direct and indirect consequences of temperature on embryo and larval development which as a whole is a complex system of many contributing factors.

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