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Effects of temperature on early life stages of Atlantic cod *Gadus morhua: A descriptive study*

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ABSTRACT

Temperature is an important parameter during the early life stages of fish and has been a topic of interest for marine species for a long time. Atlantic cod *Gadus morhua* is an economically important species and a great candidate for aquaculture diversification despite the present bottlenecks of high production costs and low market prices. The aim of this study was to describe the effects of temperature on early life stages of Atlantic cod, *G. morhua*. Secondary data was obtained from laboratory studies done on various Atlantic cod stocks and inductive reasoning research strategy was used applying the systematic literature review approach. Numerous studies showed that temperature has a large effect on the timing of important developmental stages like time to hatching. A synthesis of five case studies showed that time to hatching in days (T_{days}) was inversely related to temperature in an exponential way ($T_{days} = 28.904e^{-0.125x}$). Development rates were faster for higher temperatures than lower temperatures although structural changes slowed and accelerated at different developmental stages. Results also confirmed that temperature has significant effects on fertilization success and egg quality whereas effects on egg mortality show a high degree of variability. Temperature altered the number and size of embryonic muscle fibres in different ways for different stocks, while respiration rate and otolith growth generally increased with temperature. Prevalence of particular vertebral malformations was also found to be temperature dependent. Finally, a 32 hour stepwise increase from ambient temperature (4.5˚C) to optimal temperature (9.5 ˚C) was found as the best option of increasing temperatures whilst avoiding a reduction in the quality and quantity of viable larvae. Temperature changes may cause a mismatch between the occurrence of first feeding cod larvae and their prey organisms, and in a changing climate, variable and changing environmental conditions may affect growth and mortality and generate recruitment variability. In conclusion, temperature is an important factor in both the aquaculture of cod and for recruitment of wild cod stocks.

Keywords: *Atlantic cod, Gadus morhua, temperature, development, cod eggs, cod larvae, organogenesis, embryogenesis, morphology and malformations*

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1. INTRODUCTION

Several developmental stages are temperature sensitive during the early life of fish. Water temperature may affect larval viability, rate of development and growth (Blaxter, 1992; Johnston *et al*., 1997; Galloway *et al*., 1998), meristic counts (Brander, 1979), yolk absorption, behavior and swimming speed. A great deal of work has focused on the timing and level of morphological development of key early life-history stages, from egg fertilization to the beginning of the larval period. This considerable deal of literature shows that somatic cell growth and differentiation, morphological landmarks, the ontogeny of tissues, vertebral deformations, body movement and muscle development are all affected by egg incubation or larval rearing temperatures. The eggs and larvae of fishes are of particular interest because it is generally held that younger stages are more susceptible to potentially harmful environmental effects than older individuals (Blaxter, 1992) and are so important for the recruitment of new fish into the population. This thesis aims at providing a systematic review of the information available on the temperature effects on early life stages of Atlantic cod, *Gadus morhua* (Linnaeus, 1758). Information on cultured cod is complemented by information on wild stocks throughout the text.

1.1. Geographical distribution of Atlantic cod; case studies

The Atlantic cod, (*G. morhua*), is an economically important (Puvanendran *et al*. 2013) cold water marine fish species (Tveiten, 2008) of the northern hemisphere with a natural distribution that covers Cape Hatteras to Ugava Bay along the North American Coast, the East and West coasts of Greenland, around Iceland, and throughout coastal Europe from the Bay of Biscay to the Barents Sea, (Cannon, 1997 cited in Hall *et al*.,2004, p. 255) which is their most important feeding area (figure. 1). Like many other gadoids, Atlantic cod experience a wide range of environmental conditions, including temperature and light conditions (Brander, 1995; Suthers and Sundby,1999), in the different parts of their distribution area (Imsland *et al.*, 2007). The northeast Arctic or Arcto-Norwegian stock, sometimes referred to as '*skrei'* (Norwegian word for 'the wanderer'), as it is the migratory cod, is at present the world's largest population of Atlantic cod.

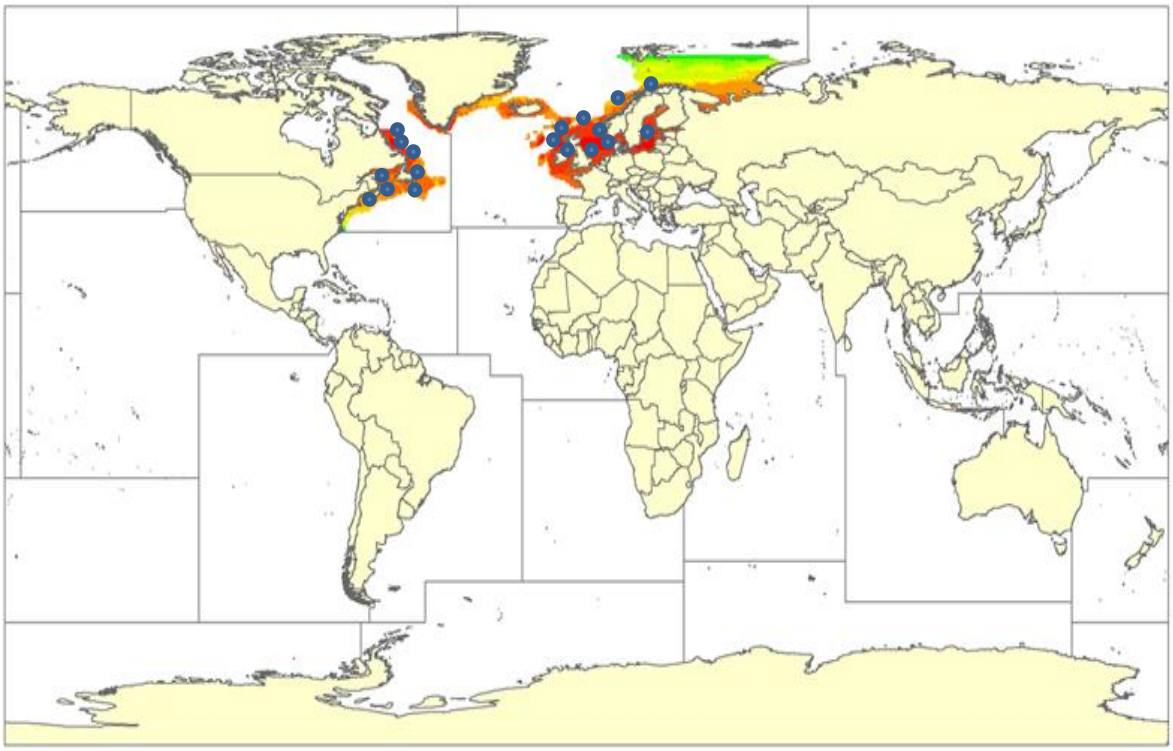


Figure 1. The global distribution of the Atlantic cod, *G. morhua* with populations included in this review marked (blue dots). (Source: seaaroundus.org)

The several cod stocks throughout the extensive area over which they are distributed across the Atlantic, are exposed to a variety of environmental conditions which generates systematic differences in ambient temperature between geographical areas (Otterlei *et al*., 2002). There is no question today that growth of these fish is significantly influenced by environmental temperature. Stock specific differences in weight at age for wild stocks are documented (Brander 1995; Otterlei *et al*., 1999) as well as differences in morphological or structural development of cultured stocks (Von Herbing *et al.*, 1996; Otterlei *et al*., 2002; Johnston and Andersen, 2003). Thus it was important to distinguish results of one stock from another in case of any genetic or brood stock differences which may still affect results on egg or larval development in laboratory grown Atlantic cod. Concerning the North- East Atlantic stocks, a genetic differentiation between North-East Arctic (NEA) and Norwegian coastal (NC) populations of Atlantic cod is possible using specific molecular markers (Fevolden and Pogson, 1997; Pogson and Fevolden, 2003).

1.2. Research purpose and questions to address

Studies with early life history stages of cod have been done as early as the 1970's, with a comprehensive review on the influence of temperature on the embryonic development of cod being published by Rosenthal and

Alderdice (1976). Page and Frank (1989) also undertook a review on this topic focusing on North Atlantic stocks. Additional work has been done on early life stages and their response to different environmental conditions like salinity oxygen, light and temperature with more recent work on some stocks in print today (Puvanendran *et al*., 2013).

The purpose of this study is to review the effects of incubation and rearing temperature on the rate or timing of developmental processes throughout embryogenesis and larval morphological growth of Atlantic cod, *G. morhua*. Interest in cod is not only due to its long importance as a commercially exploited fish species and its development as an aquaculture species, but also more recently, it is due to its central position in the study of the effects of climate. Literature shows that temperature is very important for egg and larval viability (Zhao *et al*., 2001) and disruption of normal early developmental patterns occur at high and low temperature conditions in this and other fish species. The purpose of this thesis is to review the state of knowledge on the possible effects of temperature; both negative and positive; on survival, early development and growth of the Atlantic cod. This work is not only relevant but important for predicting population dynamics and recruitment in fishery management and for improving hatchery rearing conditions in aquaculture operations.

The chief aim of this study is to determine the possible effects of temperature (if any) on:

- embryonic development
- Egg and larval mortalities
- Organogenesis / morphological development
- Timing of important developmental stages from fertilization to early larval period
- Larval development

The following research questions are addressed by this study:

- Primary question

What are the effects of environmental temperature on early life stages of Atlantic cod, *Gadus morhua*?

- Secondary questions
  - How does temperature affect developmental rates of cod eggs and larvae?
  - How does temperature affect organogenesis in Atlantic cod's early life stages?

1.3. Research strategy, approach and constraints

The strategy used in this study was that of inductive reasoning (bottom-up logic) as conclusions were reached not from general statements, but from specific examples or case studies of Atlantic cod cultured from different populations. The study was an analysis of both quantitative and qualitative secondary data. In order to achieve the objectives of this paper a summary map of results from the relevant studies and their experimental temperatures was created (APPENDIX IV). The specific methodology used to identify and screen literature is given in the methodology section (chapter 3).

Due to the existence of numerous and different staging schemes and the breadth of the subject, a meta-analysis proved to be a challenge. This study is therefore mainly a descriptive study with some narrative meta-analyses where staging and data was comparable. It focuses on cultured Atlantic cod development from the period of fertilization to early larval period 50%-100% yolk absorption. The approach used in this work was that of a systematic literature review as explained in Gough *et al*. (2012) which adopted the participants, interventions, comparators, outcomes and study design elements (PICOS) as stipulated by the Centre of Reviews and Dissemination (2009) as inclusion and exclusion criteria bases for literature to include in the review after searching various databases (refer to APPENDIX 1). Some literature search results yielded studies written in languages other than English (e.g. French and German). However, although inclusion of only English published articles may introduce language bias, this was unavoidable due to time and resource constraints and the non-English sources were merely mentioned when found in other English articles.

1.4. The structure of the paper

Chapter 2 describes the biological background of Atlantic cod and the environmental clues necessary or significant for its development. Some background of Atlantic cod fisheries and the historical development of cod culture are described. Developmental stages for cod early life stages are identified and described. The flow of literature for this review and the methods used are explained in *chapter 3*. *Chapter 4* firstly gives a description of the process of events in embryogenesis and early larval development for Atlantic cod from the most recent of a few baseline studies on the subject. Then an exposition of temperature effects on body tissues, developmental rates, metabolism, egg and larval sizes, and other diverse effects like morphological deformities and genetic expression are given. Some quantitative synthesis of secondary data, where comparability was possible, is also presented in this chapter. Finally, the final chapter, *chapter 5*, addresses the implications of findings to aquaculture wild stock populations, and in the event of climate change.

2. BACKGROUND ON SPECIES

2.1. The economic significance of Atlantic cod

Atlantic cod is one of the most important commercial fish species in Northern Europe and North America's eastern coast (FAO, 2013). Historical records show that there has been a commercial cod fishery in the North Atlantic since the 17th century. Traditional fisheries however date back many centuries and have been an important basis for many coastal communities, and important merchandise. Divided into several more or less separate stocks with different population size and harvest regimes, the largest fishery for Atlantic cod is on the Northeast Arctic and Icelandic cod stocks. Approximately 73 percent of the total worldwide catch in 2008 (~765 000 tons) was taken by just Norway, Iceland and the Russian Federation.

Cod stocks in North America were heavily reduced during the 1980s with the collapse of stocks in Newfoundland (Pryor and Brown, 1998) creating a necessity for new avenues of producing cod. In addition, the socioeconomic climate since the early 1980s saw retail prices of cod rise high enough to make cod farming commercial attractive (Hall *et al*. 2004). Fuelled by the successes of the Atlantic salmon industry, the interest for cod farming in The Northern hemisphere grew in the 20th century. Atlantic cod was a promising species for aquaculture with potential for natural stock enhancement procedures as well. Early attempts of cod aquaculture were merely using extensive rearing conditions in coastal ponds. Despite major constraints primarily related to mass rearing of fry (Pryor and Brown, 1998) limiting commercialization and initial challenges like the difficulty of larval feeding (Hall *et al*., 2004; Knutsen and Tilseth, 1985; Kjørsvik *et al*., 1991; Pedersen and Falk-Petersen, 1992) and cannibalism (Folkvord, 1991), attempts to raise Atlantic cod were first successful economically in lagoon systems in the Norwegian fjords in the 1980s (Van der Meeren and Næss, 1993).

Significant advances in larval feeding strategies since 1995 (Hall *et al.*, 2004) improved the viability of intensive culture, to the point where it became a commercial reality. Together with new advances in light manipulation of brood stock to achieve year-round egg production, as well as reducing the problem of early sexual maturation, the biological basis for cod aquaculture was achieved (FAO, 2013). This breakthrough began the acceleration in investments towards cod aquaculture enterprises, both hatcheries and on-growing farms. Within a few years an annual production capacity in the order of 60 million juveniles and around 400 on growing sites, corresponding to a theoretical annual production capacity of about 180 000 tons was built up in Norway alone. Modern industrial cod farming thus started in northern Europe. Therefore, in the past decade, the aquaculture potential for Atlantic cod has been mostly due to the efforts initiated by Norway, Scotland and later by Canada and the United States fuelled by government and private investments (Puvanendran *et al*., 2009). A 2009 report stated that Norway accounted for around 80% of the world's farmed cod production, and had

increased its national production by 59% from 10,375 tons in 2007 to 16,523 tons in 2008. Finally, production reached a peak of around 20 000 tons in Norway in 2010 (Directorate of Fisheries, 2013).

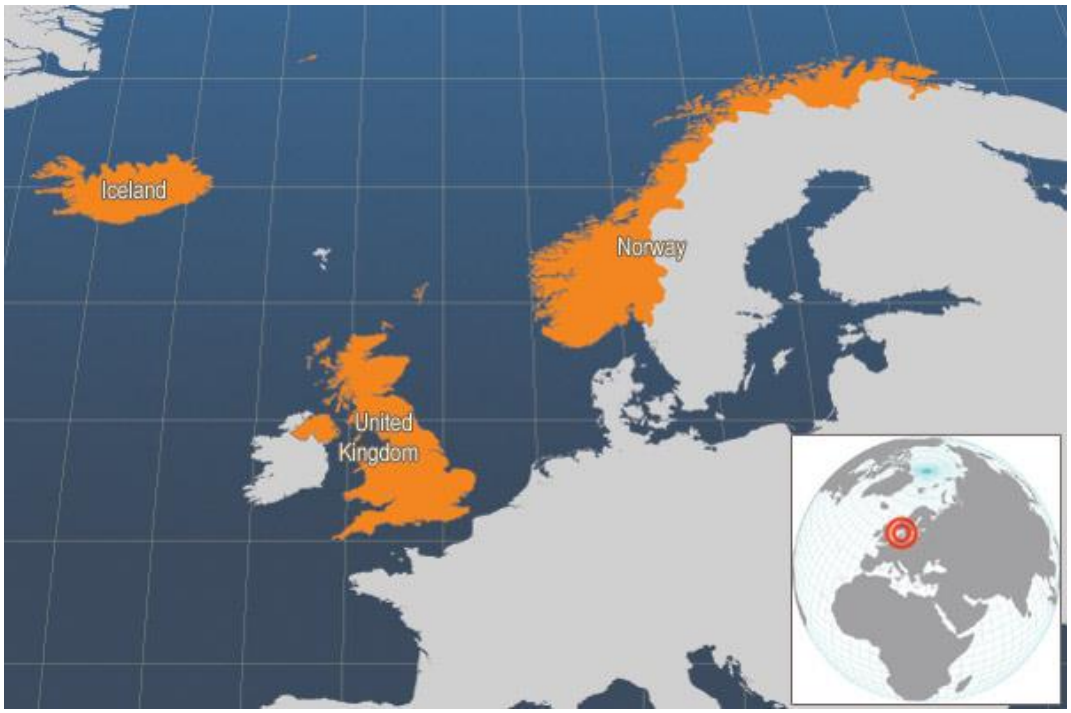


Figure 2. Main producer countries of Atlantic cod, *Gadus morhua* in 2006
(Source: FAO fisheries statistics)

In the beginning of the last decade, some analysts had optimistic predictions of 400,000 tons per annum in farmed production of cod by 2020, (Solsletten, 2001). However, the financial market crisis in 2008, together with much more challenging biological problems than were expected has caused most of these enterprises to close down (FAO, 2013) Present bottlenecks to cod farming are that of high production costs and the low market prices.

Concerning wild cod stocks, FAO (2013) reports that today the cod fishery in North America is very low compared to historical levels after the stock collapsed. A similar situation exists for several of the European stocks. Cod is highly appreciated as food and is sold as fresh whole or filleted product, salted or as stock-fish, supporting many coastal communities and industries thus making it a species of great socio-economic importance (Hall *et al.*, 2004). Despite the challenges in aquaculture, cod remains a promising candidate for the diversification of the aquaculture industry in Norway and other Northern countries.

2.2. Species Biology and diet

Atlantic cod (G. *morhua*) is major fish species in certain systems like the Baltic Sea where it has great ecological significance as the dominant piscivore species. Cod preys on sprat and herring and the latter prey on cod eggs and early larvae. Trophic interactions link these stocks thus recruitment success of cod is important, thus the interest to know whether temperature plays a role in cod recruitment. The larvae prey on natural zooplankton (calanoid copepods) at first and as juveniles they start schooling, feeding on a variety of both benthic and pelagic organisms including crustaceans, fish and some conspecifics (cannibalism). Some cod stocks perform large migrations between feed areas in the open ocean and spawning areas along the coast while other stocks are very stationary during their whole life. Growth and age/size at maturity vary widely between different cod stocks. Coastal cod in the southern regions may reach sexual maturity when they are 2-4 years old (40 cm) while some oceanic stocks, such as North East Arctic cod may be 6-9 years and 60 cm when reaching their first spawning (FAO, 2013).

2.3. Spawning habitat and egg characteristics

Cod inhabit waters down to 600min depth (Geffen *et al*., 2006) and because of their wide distribution, they are subdivided into different stocks or subpopulations that often exhibit differences in growth and reproductive characteristics (Brander, 2005). Geffen *et al*. (2006) record that cod eggs are found over a wide range of temperatures from 1.5° C in the north-west Atlantic to 9° C in the Celtic Sea (North-east Atlantic).

Cod is a highly fecund batch spawner (Kjesbu, 1989), spawning 10 to 20 batches during a 2 to 3 month period in December – June (dependent on stock) (FAO, 2013). This is in agreement with the findings of Hall *et al.*, (2004) who records that the spawning season varies geographically between January and May and usually lasts for 60 to 90 days. Egg size is around 1.4 mm and fecundity accordingly high (500 000 to 1 000 000 eggs per kg female weight for farmed cod). The eggs are planktonic and hatch after approximately two weeks (5 °C, 70 day-degrees). Under captive experimental conditions cod eggs may develop and hatch within temperatures of -1.5 to 12 ˚C (Galloway *et al*., 1998; Laurence and Rogers, 1976; Thompson and Riley, 1981). This is because various cod stocks of the North Atlantic spawn within a wide range of temperatures. North Atlantic cod has been found to spawn within a range of -1, 5 to 12ºC (Brander 1979 in Galloway *et al*., 1998). Fitzsimmons and Perutz (2006) state that the natural temperature experienced by the Shetland Isles cod stock during the spawning season range from 6 to 11°C. On the other hand Schmidt (1909) states that cod spawns between 3 and 9.5°C with the peak cod spawning season extending from December to April depending on location (Laurence and Rogers 1976). In the Baltic, cod spawns in the deep basins at strongly variable hydrographical

conditions with eggs occurring in and below the halocline (vertical salinity gradient). Valerio *et al*., (1992) found that cod of the northern cod stock (NAFO Divisions 2G, 2J, 3K, L) spawns near the seabed at depths greater than 250m, with temperatures around 2 to 4°C. Due to buoyancy, cod eggs can therefore be found at depths above 60m, with the majority occurring in the upper 30m of the water column in the Newfoundland and Labrador region. Survival during the egg and early larval stage regarded as a major bottleneck (Nissling, 2004).

2.4. Ambient environmental temperature conditions for Atlantic cod eggs

Historical temperature data from the Georges Bank area showed that cod experience extreme temperature ranges of 1.4 to 12.7°C for spawning seasons. In laboratory studies, 8°C is the temperature most commonly used for rearing cod (Peck *et al.,* 2006). This is also the temperature that is mostly experienced by larvae in the wild and included in most studies dealing with temperature effects on Atlantic cod. Temperatures ranging from 5 to 7 degrees have been suggested to be where Atlantic cod spawns and embryonic development (Bjørnsson et *al.*, 2012).

Studies by Thompson and Riley (1981) found out that death occurs in the early stages of cleavage at temperatures below 1.5°C in cod from the southern North Sea. Pepin *et al.* (1997) demonstrated that cod eggs from the Northeast Newfoundland shelf stock can develop normally at ocean temperatures as low as -1°C although high mortality occurs during this early development. Mortality for that population increased with decreasing temperatures. Furthermore, in comparison to this increasing mortality for decreasing temperature, other studies (Laurence and Rogers, 1976; Iversen and Danielssen, 1984) suggested that the cumulative mortality during development reaches a minimum in the 5 to 10°C range but increases at higher and lower temperatures. This suggests that when considering mortality rates, the best temperatures to rear Atlantic cod would be between 5 and 10 ºC.

2.5. Environmental clues for development

Falk-Petersen (2005) records that the developmental mechanisms are similar in all teleosts with differences related to genetic and environmental clues being only with respect to the timing of such developmental events. There have been numerous studies with early life history stages of marine fish species, which indicate that abiotic factors have significant effects on survival and early life development. Although it has been said that water temperature represents one of the most critical factors to egg survival and quality and larval viability, this is not the sole abiotic factor important during Atlantic cod and other fish's early life. In their experiments, Puvanendran *et al*. (2009) found that egg incubation densities had a significant effect on the standard length of the larvae. Temperature, salinity and oxygen are stated by Wieland *et al*. (1994) as some of the most important

factors for growth and development too. Pepin *et al*. (1997) found that only 6% of variation in larval size can be attributed to egg size, and that temperature had a much more significant effect on the size at hatch.

Egg survival and viability has been mostly related to the environmental conditions in several studies but other factors have been shown to play an important role too. Zhao *et al*. (2001) showed that other parameters which are maternally inherited like egg or larval size can influence survival and growth in this species. Large eggs of Atlantic cod reared in the laboratory produced large larvae. Under culture conditions egg survival is also affected by the water used to incubate the eggs (Van der Meeren and Ivannikov, 2006). Survival can be increased by using green water achieved by continuously adding *Isochrysis galbana* (IWAKI Tokyo, japan) (9.0% - 46.6%) from a lower 29.3% survival for eggs reared in clear water. Not only temperature, but also food availability, affects egg and larval performances like developmental rate, growth and mortality (Nissling, 2004) due to variability in zooplankton production as explained by Cushing's (1982) 'match and mismatch' hypothesis. In a later study Van der Meeren and Ivannikov (2006) stated that egg survival has also been shown to be correlated with both initial and average feeding conditions (Zhao *et al*., 2001) and found that exogenous feeding tends to give faster growth than endogenous feeding (yolk absorption). Other studies also show that artificial photomanipulation to which spawners are exposed can affect larval survival and consequent growth (Van der Meeren & Ivannikov, 2006; Hansen *et al* 2013). A study where continuous winter light, as a result of light regime manipulations by Van der Meeren and Ivannikov (2006), improved larval survival in a range between 9.0% and 46.6% from the 3.1% and 7.7% range obtained with spawners kept under natural light conditions. The percentage increase in weight per day (SGR) of larvae also was also higher falling between 8.7% and 13.6% in daily weight increase (from a previous 8.3% and 9.5% daily increase). Finally, water quality as connected to pollution and stocking density has a consequential effect on survival, growth and development of cod and other fish species. A review by Jezierska *et al*. (2009) concludes that metal intoxication of embryos results in disturbances of developmental processes and causes embryonic and larval malformation and mortality. They suggest that the initial period of embryonic development, just after fertilization, and probably the period of hatching are the most sensitive to metal intoxication.

2.6. Development stages of Atlantic cod eggs and larvae

Cod egg development was first described in the late 1800s and the earliest publication of temperature effects on time to hatch was for a Western Atlantic stock by Earll, (1878) and for an Eastern Atlantic stock by McIntosh and Prince (1888) (Geffen *et al*., 2006). Numerous publications on the topic have been made since then on

development stages, rates of early life development abnormalities and organogenesis as affected by different parameters like temperature.

Although different staging methods for early life cod development exist (see table 2 of authors with their studies listed in appendix), there are distinguishable, clear stages in early life development of Atlantic cod which some authors have described and named or numbered. Vollset *et al.* (2009) record three developmental stages for Atlantic cod initially held at 6 °C until 20 days post hatch (dph) and then moved to 8°C namely the period after yolk absorption (10 days post hatch, dph), the established feeding stage (26 dph) and the stage for metamorphosing larvae (47 dph).

Morphological landmarks important to feeding, respiration and locomotion can be chosen to define major developmental stages from hatching to the juvenile stage (Hall *et al.*, 2004; Von Herbing *et al*., 1996). However, these landmarks may vary with age and size. Named developmental stages allow for future additions and can be grouped into larger time intervals or periods. Geffen *et al.*, (2006) synchronized the development stages used by various authors in their independent staging schemes by assigning the stages to broad, common embryonic periods, namely:

- cleavage (fertilization to blastodisc formation, c. 64 cells),
- blastula (blastula to germ or signet ring formation),
- gastrula (start of gastrulation to closure of blastopore),
- embryo (tailbud formation to hatching) and
- hatching (start of hatching to 100% hatch).

Although various authors have used stages that subdivided these broad periods in different ways (Bonnet, 1939; Von Westernhagen, 1970; Laurence and Rogers 1976; Frigeirsson, 1978; Thompson and Riley, 1981; Makhotin *et al*., 1984; Markle and Frost, 1985; Page and Frank, 1989; Hall *et al*., 2004), the events marking the start and end of these periods are specifically defined in the schemes of each author (APPENDIX V)

2.6.1. Egg staging

Laurence and Rogers (1976) recorded four stages of development from fertilization to hatching. This is a similar total number of stages as identified by Markle and Frost (1985) However, substantial differences exist when it comes to events marking the beginning of a stage or the end of it (table 1).

Table 1. Description of egg stages as defined by Laurence and Rogers (1976) and Markle and Frost (1985)

Stage	Laurence and Rogers 1976	Markle and Frost 1985
Stage I	From fertilization to the formation of a complete blastodermal cap	From fertilization until the visible formation of an embryonic axis around the midgastrula stage
Stage II	From the formation of a completed blastodermal cap through the development of the segmentation cavity to the first appearance of the germinal ring and embryonic axis	From the formation of the embryonic axis until the embryo is halfway around the yolk, approximately at the time of blastophore closure
Stage III	From the first appearance of the germinal ring and embryonic axis to the closure of the blastopore	From the end of stage II until the tip of the tail reaches or could reach the snout
Stage IV	From the closure of the blastopore to hatching.	From the end of stage III until hatching

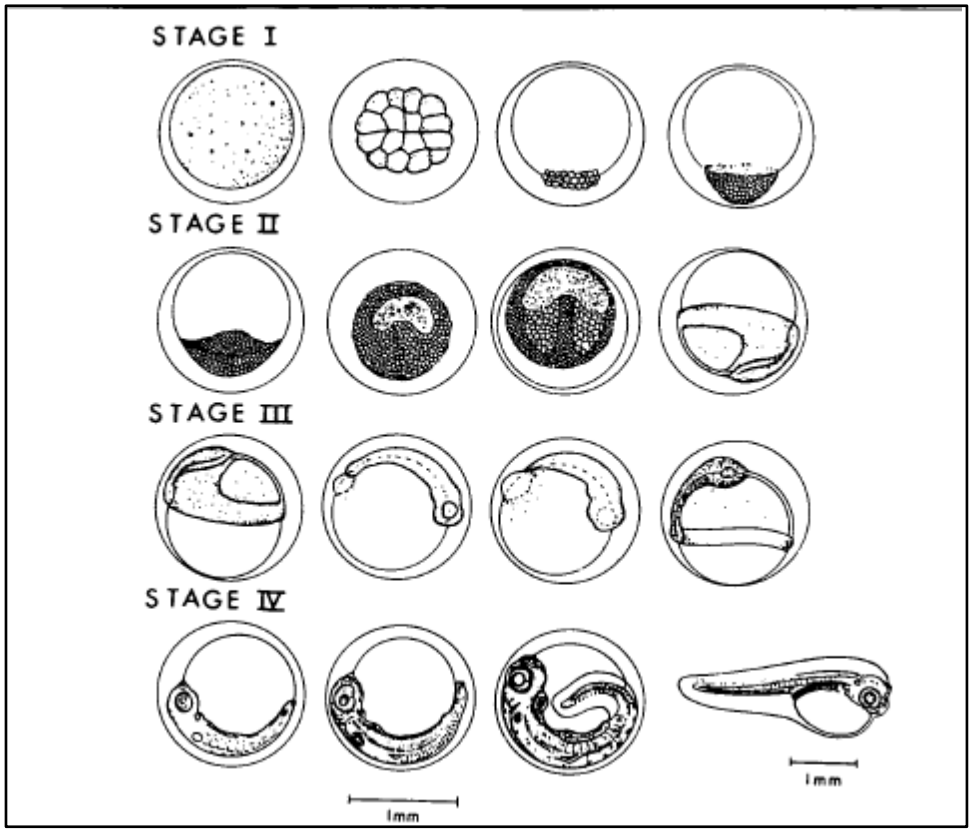


Figure 3. Staging classification for Atlantic cod and haddock embryos from fertilization to hatching (Source: Laurence and Rogers, 1976)

In a later study, Thompson and Riley identified five stages for Atlantic cod egg development (table 2)

Table 2. Criteria for egg staging following Thompson & Riley (1981)

Stage	Criteria
I	**A** blastula stage lasting from fertilization until successive cleavages produce a cellular mass in which individual cells are not visible **B** The continuing development of the blastodisc, which becomes visible as a signet ring, up to the first indication of the primitive streak
II	**A** gastrulation stage lasting from the first sign of the primitive streak until the closure of the blastopore
III	Growth of the tail occurs until the embryo spreads around three-quarters of the circumference of the egg. There is development of the eye structure and pigment spots
IV	Growth of the tail occurs until the embryo fills the whole egg with the tail touching the head
V	Growth of the tail past the head. Pigmentation of the eyes begins. At the end of this stage the larva hatches

Some modifications to this by other authors split or divide some stages. Galloway *et al.,* (1998) divided the gastrula stage (II) into an early phase (IIA) and a late phase (IIB). Laurence and Rogers (1976) have a large period from closure of the blastophore to hatching (stage IV in table 1.) whereas Thompson and Riley (1981) subdivide this period into three distinct stages (II, IV and V in table 2).

2.6.2. Larval staging

In his review of early organogenesis of various cultured fish species, Petersen (2005) described early life in temperate cultured species in much broader developmental stages from fertilization namely the egg / embryo stage, the yolk-sac stage (eleutheroembryonic stage), the larval stage and the metamorphosis stage of maturation of larval morphology and anatomy into juvenile structure and function.

Larval staging specifically for Atlantic cod has been carried out as early as the late 1970s (Frigeirsson, 1978). Thompson and Riley (1981) defined four developmental stages from the yolk sac stage after hatch to the time

of first feeding of early cod larvae. A more recent study, among others, by Von Herbing *et al*. (1996) describes nine external diagnostic structures as morphological landmarks for a total of 12 larval stages from hatch (0 days post hatch, dph) to stages of complete exhaustion of the yolk-sac (70 dph). Jordaan *et al*. (2007), with the help of digital images, have staged Atlantic cod larvae by applying three staging characters, believed to reflect the majority of the changes in gross morphology. These included (1) head morphology, (2) yolk-sac morphology and (3) position of head relative to body coupled with gut morphology.

Table 3. Criteria for larval staging following Thompson & Riley (1981)

Developmental stage	**Main Characteristics**
1a	Large yolk sac; profile extends beyond fin margin. Simple and straight gut
1b	Yolk sac profile almost in line with fin margin. Gut differentiation
1c	Yolk sac profile in line with fin margin Gut differentiated and slightly convoluted. Mouth slightly open
1d	Small amount of yolk left. Mouth open and functional. Some food in gut.

2.7. Temperature; an important factor in fisheries and aquaculture

Numerous studies with early life-history stages of marine fish species indicate that abiotic factors such as temperature and salinity and oxygen content may have significant effects on their survival (Wieland and Schnack 1994). Temperature has been a topic of interest for marine species for a long time and an early study (Hempel and Blaster, 1961) concluded that different temperature conditions on fish eggs influence a range of meristic characteristics such as vertebral number in the adult stage. Following Fry (1947), temperature effects on the early life stages of fish may be divided into five categories namely lethal (i.e. destroying organisms, including sub-lethal, less drastic effects), controlling (i.e. pacing metabolism, behavior and other physiological responses), directing (i.e. stimulating orientation responses), limiting (i.e. restricting activity and distribution) and masking (i.e. interacting with other environmental factors) even though nothing is known of limiting or masking effects of temperature on young fish stages (Blaxter, 1992). Jordaan *et al*. (2006) states that temperature is an important parameter not only during the early life stages of fish, but also continues to play a major role after metamorphosis and into late life stages in terms of growth, survival and feed efficiency. Other

studies focusing on muscle growth and development have proven, for example, that embryonic temperature can set the final number of fast muscle fibres per myotomal cross-section (FFN) and alter the fibre size distribution and the myonuclear content of individual muscle fibres in adult fish (Johnston *et al*. 2003; Macqueen *et al*. 2008). Different temperature regimes interact with size of the fish to give significant effects on growth and feed conversion efficiency (Imsland *et al*., 2005). Fish otoliths, which are bony structures in used as gravity, balance, movement and directional indicators with a secondary function in sound detection have growth that is dependent on temperature and size of fish (Otterlei *et al*., 2002).

The early stages of embryonic development is a period critical to developmental temperatures as genetic expression can be affected under thermally stressed conditions (Skjærven *et al.*, 2011). Since cellular defense mechanisms are activated even in the earliest stages of embryonic development, and many other metabolic processes important for growth and organ development in larvae occur during early life stages, it is important to monitor the thermal environment in a period so critical to development and future viability of individuals. Therefore temperature is an important parameter to consider in successfully culturing Atlantic cod (Skjærven *et al.*, 2011).

However, temperature during the egg and early larval stages is not only an essential factor in farmed cod but also for wild populations. It has been stated as one of the most important parameters related to cod recruitment, like in the case of Northeast Arctic cod **(**Galloway *et al*., 1998)**.** Vikebø *et al*. (2005) calls it the most influential physical parameter on growth and recruitment on cod. This is similar to what Laurence and Roger (1976) state about Atlantic cod and its gadoid relative haddock *Melanogrammus aeglefinus* (L). As temperature determines the match or mismatch between the occurrence of first feeding cod larvae and their prey organisms, recruitment success in wild cod populations is well dependent on the temperature (Ellertsen *et al*., 1989). Not only does it have direct effects on growth and development of the wild stocks, but also indirect effects through lower trophic levels. In addition to that, behavioral responses to gradients of temperature and light in the pelagic can potentially regulate the distribution and survival of early life stages of fish (Vollset *et al.*, 2009).

Van der Meeren and Ivannikov (2006) state that variable temperature conditions have been shown to have significant effects on other gadoids and fish species. Higher temperatures have been shown to reduce the egg production in Atlantic halibut (*Hippoglossus hippoglossus*), give lower fertilization and hatching rates which are even more dramatic for temperatures above 8°C (Brown et al. 1995). Abnormal cell cleavage patterns are also observed at these high temperatures. Malfunctions like endocrine dysfunction in Atlantic salmon (*Salmo salar*) (King and Pankhurst, 2004a; 2004b), alevin deformities and low egg viability or survival in brook trout (*Salvelinus fontinalis*) (Hokanson *et al*., 1973 and common wolfish (*Anarhichas lupus*) (Tveiten *et al*., 2001).

Extreme cases of no egg survival can occur at temperature far above the survival limit, for example in rainbow trout (*Oncorhynchus mykiss*) (Pankhust *et al*., 1996).

Since temperature explains most of the variance in planktonic egg development times, such relationships can be used to derive estimates of larval production from egg surveys (Geffen *et al.*, 2006). In addition, temperature-dependent egg development relationships are necessary for spawning stock biomass estimation using egg production methods. Environmental temperature has been also suggested as a proxy for other climatic parameters. In view of all the above and in the wake of a changing climate and warming oceans, it is important to understand, even in the smallest way, the possible impacts of these temperature changes on cod early life stages and recruitment (Ellersten et al., 1989). Malformations or poor development in cultured cod, would have a significant impact upon commercial aquaculture (Fitzsimmons and Perutz, 2006) therefore a further understanding of rearing temperature protocols is necessary to reduce the effect of malformations on larval quality and maximize survival rate, growth and development. One source of variation in early life-history traits is the maternal effect described by Chambers & Leggett (1996). Jordaan *et al.*, (2006) emphasize that it is important to recognize the presence of parental influence on traits exhibited by eggs and larvae. External abiotic factors have a great influence growth and development of early life stage. They also interact with the internal engineering of each individual to gene level to ultimately influence egg and larval development.

2.8. Temperature profiles for Atlantic cod

Although cod occurs over a fairly wide range of temperatures, the species has an upper and lower lethal temperature limit for embryonic and post-embryonic stages which varies in relation to geographic location or latitude (Blaxter, 1992). Dannevig (1894) made the first comprehensive attempt to find the temperature range for cod. Experiments by Johansen and Keogh (1914) led to their establishment of a an upper temperature limit for cod of 10.2 ºC as eggs did not hatch for their experimental temperatures higher than this of 12 ºC, 13 ºC, 16.5 ºC and 20 ºC. Bonnet (1939) was able to hatch cod eggs from Ipswich Bay, Massachusetts from temperatures of 0 ºC to 14 ºC. However Bonnet (1939) concluded that 12 ºC was very near the upper limit of temperature for the development of cod egg as greater temperatures of 13 ºC and 14 ºC failed showing no survival after 24 hours. Despite the eggs hatching in Bonnet (1939), the result of no or reduced viability at temperatures higher than 12˚C is the similar to that observed in the previous work by Dannevig (1894) and Johansen and Keogh (1914).

Laurence and Rogers (1976) concluded through an examination of the Georges Bank area temperature data that cod tolerates extreme temperature ranges of 1.4°C to 12.7°C in spawning seasons. Galloway *et al*., (1998) concluded that a temperature of 1ºC is close to, or below, the lower thermal tolerance limit for normal

functional development of Northeast Arctic cod (NEA). However, NEA cod eggs could develop and hatch within a fairly wide range of -1.5 to 12ºC. Valerio *et al.* (1992) established that Newfoundland cod eggs did not freeze at -1.8°C (the freezing point of seawater) but only froze at temperatures between -4.1 and -17°C exhibiting considerable freeze resistance.

Numerous laboratory studies (Laurence and Rogers, 1976; Iversen and Danielsen, 1984; Galloway *et al*., 1998; Von Herbing et al., 1996; Nissling, 2004; Hall *et al*., 2004; Puvanendran *et al.*, 2013) in this review have indicated that the maximum optimal incubation temperature at which Atlantic cod hatch successfully is below 10°. A study by Peterson *et al*. (2004) on cod from the Bay of Fundy stocks, concluded that optimal temperature for incubation of Atlantic cod eggs falls between 2- 4°C as these lower incubation temperatures resulted in larger lengths of hatched cod larvae in comparison to those from higher incubation temperatures. In a more recent study Puvanendran *et al.*, (2013) confirmed 9.5°C as the optimal incubation temperature. Puvanendran *et al.*, (2013) further explain that that the egg incubation temperature of Atlantic cod eggs can be elevated the from ambient temperature 4.5°C to the maximum optimal incubation temperature 9.5°C using gradual increment periods 8-96 hours (32h yielding significantly better results) without arresting or altering the normal embryonic and organ development and hatching or reducing the quality or quantity of viable larvae.

Higher incubation temperatures have been shown to have long-lasting adverses effects on fish. Veterbral column deformities (Fitzsimmons & Perutz, 2006) or a phenomenon Lucas (1998) called ‘epigenetic developmental programming’ (Puvanendran *et al*., 2013) could have large effects on viability and egg or larval quality which could transcend into later life stages. Environmental temperature can become a stressor and alter metabolic pathways, organ and tissue structures which in turn become apparent at later developmental stages even after the stress stimulant is no longer there (Lucas 1988).

3. METHODOLOGY

A total of 15 scientific databases were searched for relevant literature using key words in search fields to obtain relevant studies (refer to APPENDIX II and III). The entire relevant search results were exported to ENDNOTE X7 where all duplicates (86) was deleted leaving a total of 305 studies/articles. Abstract of these were obtained and screened thoroughly for relevance. Microsoft Excel Spreadsheets and Microsoft Word processor were the software used to manage data for this review.

3.1. Reviews flow of references

A brief summary of the flow of references throughout this review process:

- 3215 references were found by searching and available for title and abstract screening.
- 305 references were found to be potentially relevant from titles and previews and required retrieval
- 101 reports were obtained and available for keyword screening after abstract review
- After keywording, 50 studies were relevant to the focused review question and thus were screened full text for inclusion in data extraction.
- 24 of these reports met the review's inclusion and exclusion criteria and were described in the summary map (appendix table 4) after screening of the full texts for use in an in-depth review.
    - An additional total of 13 other relevant studies discovered through hand searching journals or as literature referred to in other studies (figure 4).

The list of keywords used in screening the full texts of different studies were *Atlantic cod, Gadus morhua, temperature, development,* cod *eggs, cod larvae, organogenesis, embryogenesis, morphology* and *malformations*.

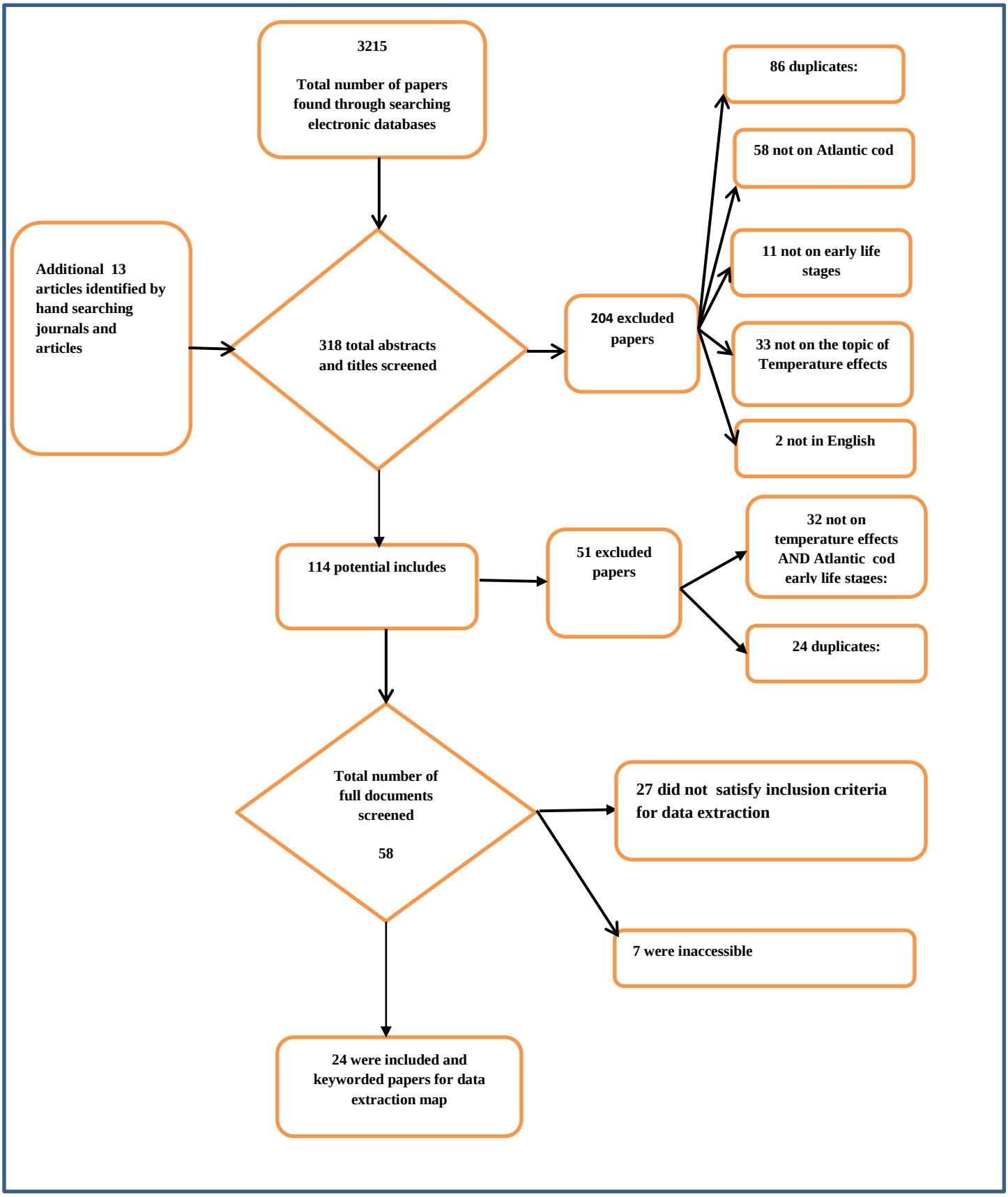


Figure 4. The flow of studies as represented by a PRISMA diagram.

4. RESULTS

4.1. Baseline study references

In a baseline study for multiple families if Atlantic cod reared at 7°C, Hall *et al.*, (2004) described numerous stages from fertilization to first feeding in embryonic development. An earlier study by Von Herbing *et al*., (1996) had determined morphological landmarks for cod larvae at 5˚C and gone further to check the influence of temperature on the variation in developmental rates of the described external diagnostic structures by comparing two temperature regimes (5 ˚C and 10 ˚C).

4.1.1. Egg development staging timeline (7˚C)

Early development of Atlantic cod eggs goes through various periods namely the zygote period (300–335 335 minutes post fertilization, mpf), cleavage period (335 mpf – 22 hours post fertilization, hpf), blastula period (22–56 hpf), gastrula period (56–113 hpf), and the segmentation period (113–256 hpf) wherein organogenesis commences (Hall *et al*., 2004)

At a temperature of 7˚C, a broad time window of the zygote stage period occurs after fertilization is complete (Hall *et al*., 2004). A cleavage period occurs synchronously between embryos with a cell cycle time of 135 between cleavages up until the 32-cell stage. Thereafter cleavage events become more irregular after which the embryo enters the blastula period (22-56hpf). The blastodisc consists of a solid ball (blastula) of 500 cells approximately 9-10 cleavages by 36hpf. Between 56-113 hpf, gastrulation takes place (gastrula period). The embryo at this stage attains dorsoventral and anteroposterior axes and closure of the blastopore occurs as the free floating embryo orientates shield-side up. Epiboly begins and following 100% epiboly, cells in the epiblast give rise to the ectoderm, which eventually becomes the epidermis and neural tissue. The hypoblast gives rise to the mesoderm, which forms such tissues as muscle and bone, and the endoderm, which forms such tissues as the liver and gut. Following completion of epiboly, a transient structure known as Kupffer's vesicle can often be seen within the cells of the tail bud during this period. Segmentation period follows at 113-256dpf. Because the first processes of organogenesis begin at 50% epiboly, gastrulation and segmentation overlap. Tissues and organs begin to differentiate within the shield as epiboly completes its envelopment of the yolk. The number of somites (*s*) during segmentation period appear linearly with respect to time. The regression equation is:

$s = 0.29t - 18.14$, R^2 (adjusted) 0.89 and residual degrees of freedom, df, 208.

There is no distinct pharyngula period in cod and staging by the primordium of the lateral line is unnecessary according to Hall *et al*., (2004) as new somites are added throughout until just prior to hatch, unlike in other

teleost species. The first somite furrow appears at 82 hours (h) soon after the notorchord rudiment appears at 45% epiboly. More somite formation occurs at 162 minute intervals and all somites are formed by 220 hpf, 36h prior to hatching. A neural plate (neurulation) is apparent by 50% epiboly. By 75% epiboly most differentiation is apparent (neurons, optic placodes. Complete epiboly (100%) occurs at the 10-somite stage. By the 40-somite stage the first indications of retinal lamination are discernible.

Hall *et al*., 2004 found the mean cell cycle time for the first six cleavages to be 135 minutes and that the segmentation period began 220hpf. Furthermore, the addition of new somites continued throughout the majority of embryonic development until just prior to hatching; something that does not happen in other teleosts (Hall *et al*., 2004). Hatching at this temperature of 7˚C occurred at 256 hpf and a functional jaw and hindgut was developed enough for first feeding by the end of the 3rd dph.

4.1.2. Embryogenesis

The notochord is the first visible structure within the embryo shield. First somite furrow appears at 82h a few minutes after the notochord rudiment at 45% epiboly (Hall *et al*., 2004). The neural plate is apparent by 50% epiboly and the floorplate may be discerned in transverse section above the vacuolating notochord by 10-somite stage. Towards brain development, the first three neuromeres were distinguishable as swellings in the head at 70 % epiboly (5-somite stage) (Hall *et al*., 2004). By the 20-somite stage, neuromeres strongly affect head shape. A further 7 neuromeres develop at 25-somite stage. Patterning of brain areas is virtually complete by the 30-somite stage (Hall *et al*. 2004). According to the same author the optic placodes are fully differentiated from shield as solid masses of large losely packed cells by 75% epiboly in the initial stages of eye development. At 100% epiboly (10-somite stage) a faint infolding becomes visible rostrocaudically in the centre of the eye. The first indication of retinal lamination is discernible at 40-somite stage and pigment disposition occurs after the completion of somitogenesis. Octic placodes become visible as solid masses of ectorderm on either side of hind-brain at 17-somite stage (Hall *et al*., 2004)

During ear development, the first indication of lumen development is at 20-somite stage, seen as an invagination in the centre of each placode. The invagination enlarges and tiny otoliths can be seen. However further development of inner ear is within the larval period.

The heart field is not yet a true pericardial cavity but visible by the 20-somite stage as a seemingly empty space. Two mesodermal tubular primordial are visible on either side of the ventral midline by 25-somite stage. Tubular primordial fuse into a cone by the 30-somite stage. Additional somites are added and sporadic heart beats occur after 40-somite stage. After the 50-somite stage, the two minor chambers (sinous venosus and

bulbous arteriosus) delineate at the extremities. The heart however is not viewable beyond this as chambers are in different focal planes.

Kidney primordial is apparent, as yet without lumina, in transverse section at the 30-somite stage. The glomerulus visible in longitudinal section and in post-hatch fish, the pronephric ducts adjoin the urinary bladder.

The first visible protrusion of unpaired embryonic finfold is just visible at 17-somite stage. The finfold expands to surround the entire body (except the head) by the 25-somite stage. Pectoral fin development is comparatively late, with first sight of these structures at 50-somite stage. However, their expansion is rapid and an apical fin-fold surrounds the periphery of the proximal mesenchyme by the hatching gland stage. Appendages have a vertical orientation (with reference to the anterior-posterior body axis) but rotate, by time of first feeding, to a nearly horizontal position. An intestinal lumen is visible early (10-somite stage) but the internal epithelium of gut is only highly villiarized by 50-somite stage. A cloaca only develops during the early larval period.

In a study using different incubation temperatures of 4, 7 and 10ºC, Hall and Johnston (2003) concluded that the timing of appearance of the otic placode, unpaired median fin fold, gut lumen, otic vesicle, lens of the eye, otoliths, first muscular contractions, swim bladder and hindgut were all unaffected by developmental temperatures. However, epiboly was relatively delayed at higher temperature such that at closure of the blastopore, a 4˚C embryo possessed 3 somites; a 7 ºC embryo, 10 somites; and a 10 ºC embryo, 12 somites. On the other hand, the first regular heartbeat occurred earlier at higher temperatures.

4.1.3. Larval morphological development timeline

Von Herbing *et al*. (1996) selected nine external diagnostic structures as landmarks for larval development namely the head, yolk-sac, alimentary tract, gills, gill cover, swim bladder, fin fold, pigmentation and paired fins. A total of 12 stages for each landmark comprehensively explain the morphology of these structures and their progression in development pre-metamorphosis. This paper focuses on stage 1 (0-1dph) to stage 3 (3-4 dph) (refer to Von Herbing *et al*. (1996) staging table in APPENDIX VII) as this represents the early larval period in comparison with the work done by Hall *et al*., (2004) who describe 274hpf (hatch) to 346hpf (3,75 days post hatch), the time of recorded first feeding.

At 7 ºC cod from Mallaig Scotland hatched at 256 hpf (10.66dph), a urinary bladder was present in newly hatched floating larvae (Hall *et al*., 2004). A swim bladder could be seen for the first time dorsal to the liver in the whole-mount embryo. Similarly, Von Herbing *et al*., (1992) identified a small distinct sac dorsal to the intestine and covered in pigment 2-3 dph.

The Yolk sac for newly hatched larvae was relatively large after completion of last somites and pigmentation of eye (Hall *et al*., 2004). However yolk size diminished and embryos re-orientate to the adult position and underwent negative photo axis away from the water's surface. The yolk sac for the 5˚C reared eggs was also large a hatch. Spherical yolk-sac full of yolk, approximately 95% was observed by Von Herbing *et al.* (1996). An elliptical reduction in yolk size was also observed post hatch from 70-100% yolk left at 2-3dph to 50-70% yolk left at 3-4dph. Hall *et al*. (2004) observed seven pairs of neuromasts barely visible on the epidermis arranged symmetrically on both sides of the body within the early larval period (0-4dph).

Actinotrichia were present in the caudal embryonic fin fold .Von Herbing *et al.,* (1996) observe the supracephalic sinus of the dorsal finfold. It reached its maximum elevation at stage 3 (3-4dph) from a small unelevated state at stage 1 (0-1dph).

Two days post hatch (304 hpf) three areas of the gut (the foregut, midgut and hindgut) were recognizable (Hall *et al,* 2004) and first feeding stage occurred 346 hpf (90 hph). At 5˚C, Von Herbing *et al*. (1996) identified the alimentary tract as a simple tube with no differentiation 1dph. At stage 2 (2-3dph) the intestine was expanded with a constriction formed between the mid-gut and hind-gut (the rectal valve which separates food in foregut and hindgut), results very similar to the findings by Hall *et al.,* (2004) at a different temperature of 7˚C.

4.2. Effect of temperature on rate or timing of important developmental stages

4.2.1. Time (absolute and relative) spent in each developmental stage

Von Herbing *et al* (1996) checked morphological progression at 5˚C for two populations, Newfoundland (NF) and Scotian Shelf (NS) cod. Both populations progressed through the stages at the same rate, with intervals between stages becoming longer later in larval development. Comparing the results to those of Hall *et al.,* (2004) for cod from Mallaig Scotland population, the larvae progressed through the early larval period (Stage 1-3 according to Von Herbing *et al.,* (1996) at similar rates when comparing similar landmarks of the yolk-sac, alimentary tract or gut, swim bladder and the fin fold. Similarly, Hall and Johnston (2003) recorded that newly hatched and first feeding larvae from temperature groups of 4, 7 and 10°C were indistinguishable in terms of

their gross morphology. This suggests that the stages in development are consistent for Atlantic cod despite of temperature for early larval periods.

However, Von Herbing *et al*., (1996) state that developmental rates were faster at 10˚C than at 5˚C for all the stages. They found that the structural change is very rapid at both 5 ˚C and 10 ˚C during the early larval period from early to mid-yolk-sac stages, (stage 1-5; 0-9dph in APPENDIX VII). Structural change is then slowed for both temperatures after the early stages. However late in development, after stage 9 (>35dph), the rate of development accelerated in the 5 ˚C larvae, while it was maintained at the slowed pace for the 10 ˚C larvae, consequently taking larvae at 5 ˚C almost twice as long (58days) to reach Stage 11 than it did for larvae kept at 10 ˚C which took just 29days (Von Herbing *et al*., 1996).

Developmental rate had also been found to be faster at higher temperatures than lower ones in an earlier study by Bonnet (1939). The study showed that the time in days to move through different stages from first cleavage to newly hatched larvae decreased significantly as temperature was increased. Time to development stage at hatch for eggs incubated at 6 ºC took almost twice as long as it did at 12 ºC (Bonnet, 1939). However, the difference between development at 10 º and 12 ºC was not the same as that between 6 ºC and 8 ºC even though the temperature change (2 º C unit changes) was the same for both.

Pryor and Brown (1998) recorded development times 40% longer at their low temperature regime of 0-1°C in comparison to the medium and high temperature regimes of 3-4°C and 7-8°C respectively. Larval development was slowest at 0.0°C -1.0°C with yolk-sac absorption taking nearly twice as long as in the other treatments. In a study with 3 incubation temperature groups of 3, 5 and 8°C, Galloway *et al*., (1998) found out that the highest temperature (8°C) gave the most rapid developmental rate, thus the shortest absolute time spent in each stage. However the relative time spent in stage II (refer to table 2) was longer for the eggs incubated at 5°C and shorter in the corresponding stage III than other temperature groups of 1˚C and 8˚C. Even after 5dph the larvae from the 8°C temperature group was at a more advanced developmental stage in comparison to the other groups.

Hall and Johnston (2003) found that the absolute development time during embryogenesis was inversely correlated with temperature across the different embryo rearing temperatures of 4°C, 7°C and 10°C. Embryos raised at higher temperatures hatched and reached the first feeding stage in a shorter time. With the time of first feeding defined as 100% development time, figure 5, it took embryos reared at 4, 7 and 10°C 23, 14 and 11 days respectively to reach '100% development' after development and opening of the jaw (Hall and Johnston, 2003).

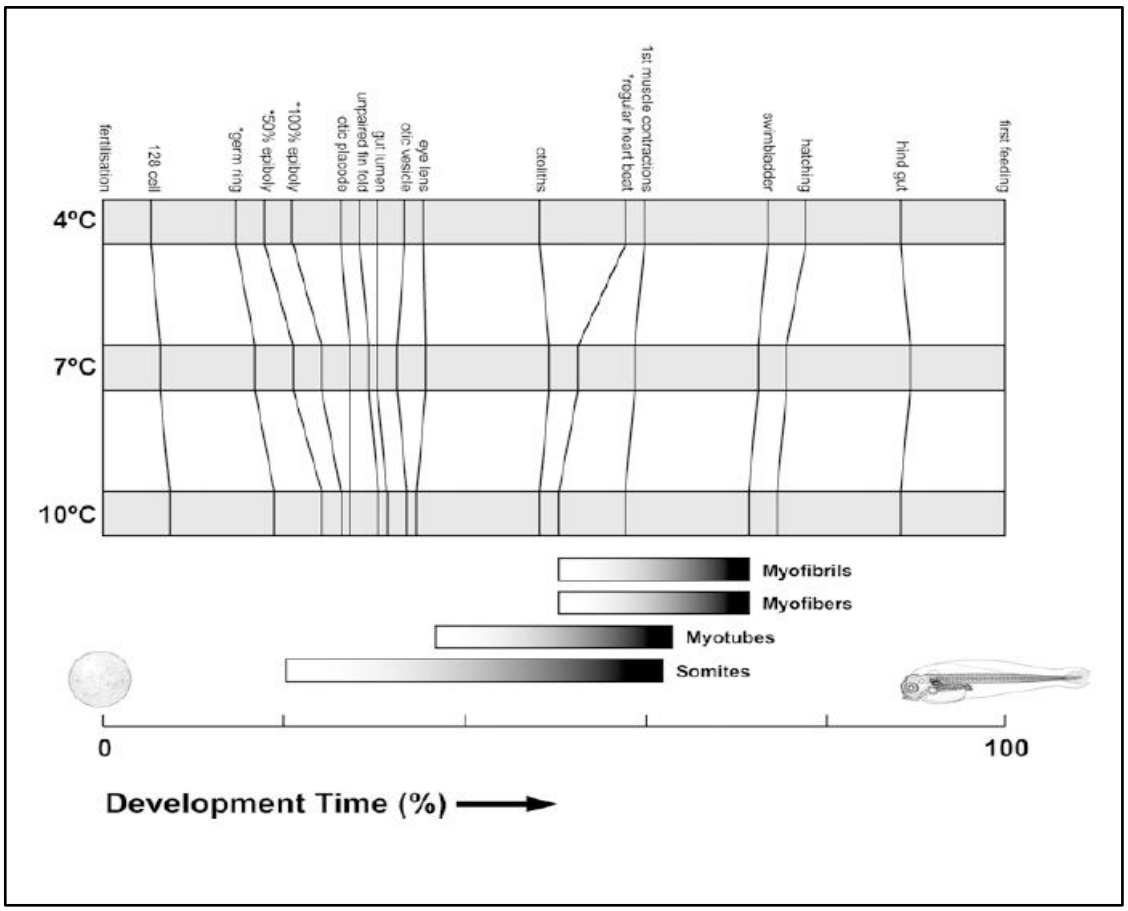


Figure 5. Relative timing of developmental events during embryogenesis at different temperatures. differences of greater than 5% development time are indicated by an *asterisk* (*) (Source: Hall and Johnston, 2003).

Nissling (2004) found that the time from start of feeding until exhausted yolk was considerably shorter at high temperatures than at lower temperatures. This is consistent with results from Von Herbing *et al.*, (1996) of temperature caused differences in developmental rates after the early larval period. Nissling (2004) recorded varied averages in time from functional jaw to yolk sac absorption for different temperature with the shortest time being for the highest temperature (~3 days at 11°C; 3.5 days at 9°C; 4 days at 7°C; 8.5 days at 3°C and 13 days at 1°C). This implies a higher demand for food availability for early larvae kept at high temperatures.

4.2.2. Hatching rate

There is evidence that temperature during embryonic stages has a large role to play in determining the hatch success and incubation time.

Bonnet (1939) records the mean percentage hatching success for different temperatures. Higher temperatures reduced the mean percentage hatched out of the total number of eggs. Eggs were kept at 6 ºC, 8 ºC, 10 ºC and

12 ºC and the average percentage hatched eggs was 11.2%, 3.3%, 1.9%, 0.04% respectively. However, the batches kept at 6 ºC had very low initial numbers of eggs and due to the factor of overcrowding, they not comparable with the other temperature groups. For a experiments ranging from 2°C to 12°C by Laurence and Rogers (1976), the mean times to 50% hatching of viable prolarvae (i.e. larvae which have hatched but still retain a yolk sac) over all combinations of temperature and salinity (26 – 36 $^{\circ}/_{oo}$) were 12.86 days. As temperature increased, the time to 50% hatch decreased and concurrently the duration in days of the hatching period decreased from an average of 10 days at 2°C to 2.5 days at 12°C (Laurence and Rogers, 1976). Various studies concur with this general trend that there is a significant inverse relationship of 50% hatching time to temperature for cod species. Pryor and Brown (1998) record a shorter incubation period at high (7.0 – 8.0°C) and medium (3.0-4.0°C) temperatures than observed for low temperatures (0.0 to 1.0°C). Galloway *et al.*, (1998) record an inverse correlation between 50% hatching and incubation temperature; the opposite being true for time to hatching measure in degree days (1 degree day = mean temperature x number of days). Jordan *et al.*, (2006) ascertain a consistent trend of decreasing time to 50 and 100% hatch with increasing temperature.In this present study, the extraction of data on time to hatching from four case studies (Galloway *et al*., 1998; Jordaan, 2002; Laurence and Rogers, 1976; Bonnet, 1939), and fitting it to a trendline showed that time to hatching in days decreased exponentially according to the model

Time to hatching (days) = $28.904e^{-0.125x}$, for sample size (N) of 65.

80% of the total variation of outcomes explained by the model, (R^2 of 0.80) (figure 6).

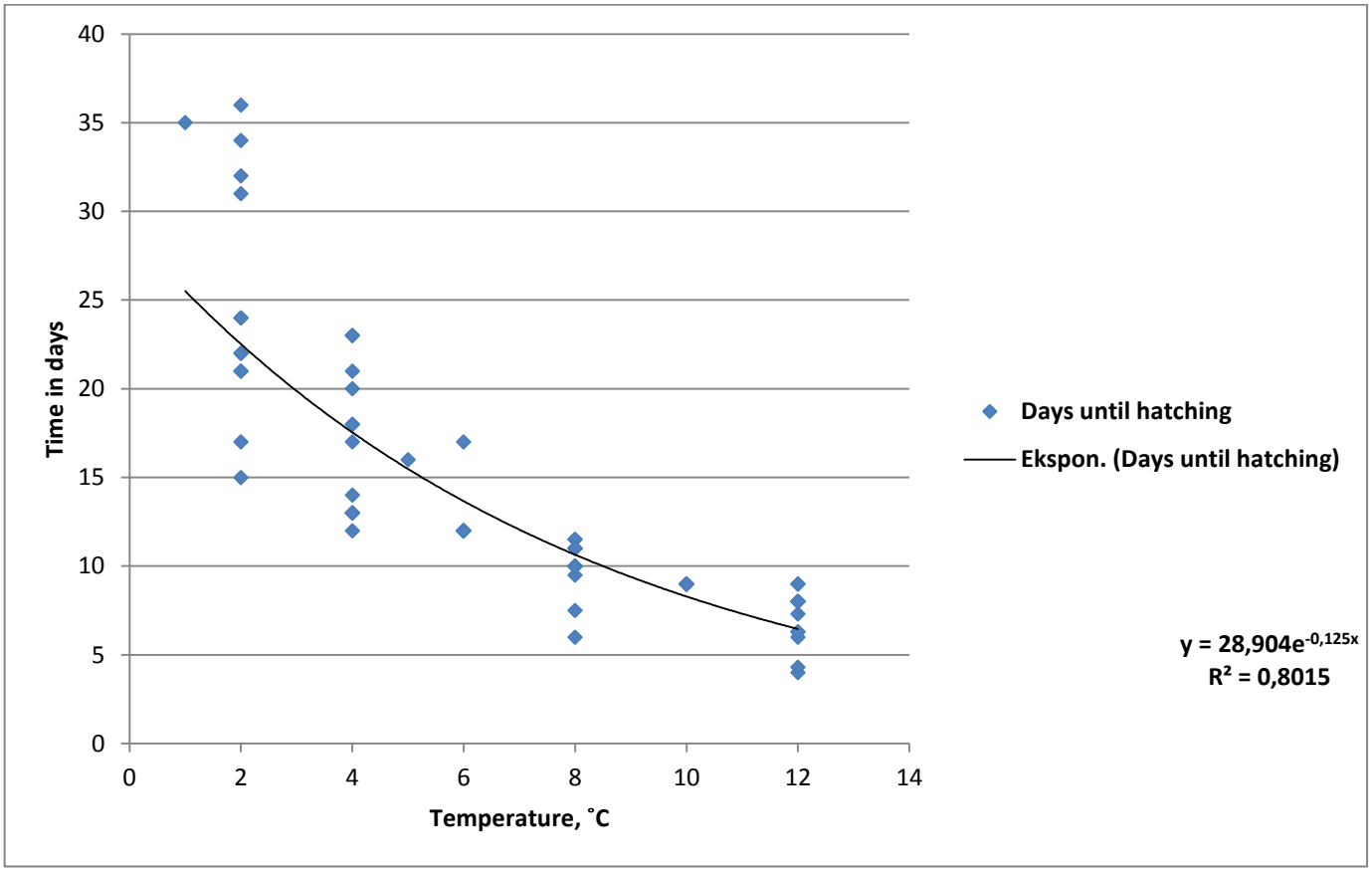


Figure 6. Time to hatching (50% and 100%) for cod from Northeast Arctic, Newfoundland and Narragansett Bay, Massachusetts.

4.3. Effect on fertilization success and egg quality

In an experiment where ambient temperature was reduced after reaching 13.7 °C during the first half of the spawning period to monitor egg quality and viability by Van der Meeren and Ivannikov (2006), it was found that temperatures above 9.6°C resulted in significant reductions in fertilization and normal egg development. Simultaneously, the incidence of unfertilized or dead eggs mortality increased with elevated temperature. However, for lowered temperatures below 9.6°C, the egg characteristics improved. Tveiten (2008) also records a decrease in the proportion of eggs showing normal cell cleavage by exposing brood stock fish to elevated temperatures (4°C, 96%, 8°C, 85%, 12°C, 12%). This asserts how critical the environmental temperature prior to any fertilization is for future egg development. Furthermore exposing Atlantic cod brood stock to elevated temperatures significantly reduces the egg quality (Tveiten, 2008) and can reduce the fertilization percentage by more than 50% when brood stock are exposed to elevated temperatures during ovarian growth. His results showed 60% fertilization at 4°C, 27% at 8°C, and 13% at 12 °C.

4.4. Egg and larval mortality

Results on percent mortality by Laurence and Rogers (1976) showed that mortality was independent of temperature for cod at 36 different combinations of temperature (2 to 12 ºC) and salinity (26 to 36 $^{o}/_{oo}$). However, the stage from closure of the blastopore to hatching (Stage IV in table 1) was statistically the most significant period of mortality for cod and a factor analysis of variance demonstrated that the effects of temperature significantly accounted for the greatest variability in cod mortalities. High embryo mortality similar to that mentioned by Laurence and Rogers (1976) for cod had been previously noted by Bonnet (1939) who demonstrated increasing embryo mortality of cod as hatching approached.

In his studies on Baltic cod, egg survival was shown to be unaffected in the temperature range 3°C-9°C but significantly lower survival was observed at higher temperatures (11°C). In another study on cod from Shetland Isles, Fitzsimmons and Perutz (2006) found out that even if there were significant differences in survival between groups of larvae, there were no significant differences in survival between different incubation temperatures. Mean survival at hatch was 34%, 24%, 35%. 26%, 21% and 24% for temperatures of 6 ºC, 6.5 ºC, 8 ºC, 9 ºC, 10 ºC and 11ºC respectively. Similar conclusions were arrived at Puvanendran *et al.*, (2009) who found that egg incubation temperatures ranging between (10 ºC and 11˚C) had no effect on larval survival (8% and 10% respectively). Galloway *et al.,* (1998) also found that mortality until 50% hatching was approximately 75% for Northeast Arctic cod eggs regardless of the temperature. Otterlei *et al.,* (2002) report that larval mortality may vary with respect to temperature, year, and stock without showing any distinctive trends. They conclude that temperature differences were the cause of a large variation in larval mortalities in their studies. Similar conclusions have been made for eggs and larvae in wild stocks and Blaxter (1992) reports that it is not common to hear of cases of temperature-induced mortality in large bodies of water.

4.5. Temperature effects on growth and development of tissues and organs

4.5.1. Muscle growth

Wheater *et al.,* (1987) state that multicellular organisms possess specialized cells to enable movement of tissues or organs. Three types are namely skeletal muscle (i.e. voluntary muscle responsible for the movement of the skeleton and organs such as the eye globe), visceral muscle (i.e. involuntary muscle which forms the muscular component of visceral structures such as blood vessels, the gastrointestinal tract and urinary bladder) and cardiac muscle which has many structural and functional characteristics which provide for the continuous, rhythmic contractility of the heart. Many studies have been done on a wide range of species to discover the

developmental plasticity of muscle growth at different temperatures (Johnston, 2006).

In teleost fish, the myotomal muscles form the main edible part, thus the central goal of finfish aquaculture is the production of myotomal muscle (Johnson *et al*., 2008). Teleost muscle is unique in many aspects of its structure compared to other vertebrates and temperature is known to have major effects on early muscle development in teleosts (Hall *et al*., 2003). Johnston and Anderson (2008) found that the temperature prior to hatching has been shown to affect the timing and intensity of muscle hyperplasia as well as fibre sizes in larval stages in Atlantic cod (figure 7). This has also been the case in a number of marine fishes including Atlantic herring *Clupea harengus* (Johnston *et al*., 1998), plaice *Pleuronectes platessa* (Brooks and Johnston, 1993), sea bass *Dicentrarchus labrax* (López-Albors *et al*., 2003), and haddock *Melanogrammus aeglefinus* (Martell and Kieffer, 2007). Stickland *et al.* (1988) discovered that egg incubation temperature affected the number and size distribution of myotomal muscle fibres in Atlantic salmon alevins at hatching. Another similar study by Johnson *et al.* (1995) found that temperature alters the timing of microfibril assembly with respect to somite stage as in studies with Atlantic herring.

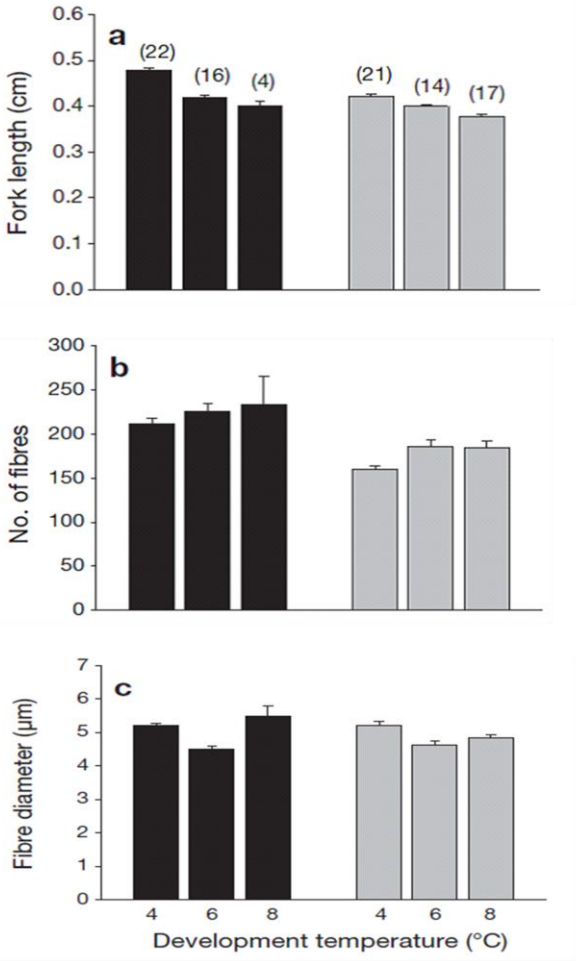


Figure 7. Source: Johnston and Andersen (2008)

The relationship between embryonic development temperatures and **(a)** larval fork length, **(b)** number of fast muscle fibres per trunk cross-section and **(c)** average diameter of fast muscle fibres.
Black bars: Northeast Arctic cod;
Grey bars: Norwegian coastal cod.
All results are mean ± SE. Number of fish sampled (*Gadus morhua*) is shown in brackets in panel (a).

However, it has been suggested that the reaction norm of larval fibre number with respect to the embryonic temperature regime differs in different populations. A study involving farmed cod of western Scottish origin found no significant differences in number of fibres at hatching for incubation temperatures of 4 and 7°C (Hall & Johnston 2003). Only for the 10°C treatment were there more muscle fibres (~14% more). The same author

reports similar findings for Northeast Arctic cod eggs reared at different temperature groups by Galloway *et al.* (1998). The total cross-sectional area of axial muscle (refer to figure 8) 1dph was not affected by incubation temperature. There were no significant differences between temperature groups and the mean number and size of superficial fibres per myotome 1dph.

In addition, no differences in number and size of muscle fibres 5dph were observed although the deep fibres of the 1ºC-group contained less myofibrils than the higher temperature groups of 5 or 8ºC (Galloway *et al.*, 1998). Furthermore, a significantly high number of deep fibres and a significantly smaller mean diameter of deep fibres in the 1°C temperature group compared to the 8°C group. No significant differences in axial muscle development were found between the 1°C and the 5°C temperature groups. These differences also exist in other species like Atlantic herring (Johnston *et al*. 1998) and Atlantic salmon (Johnston *et al*. 2000)

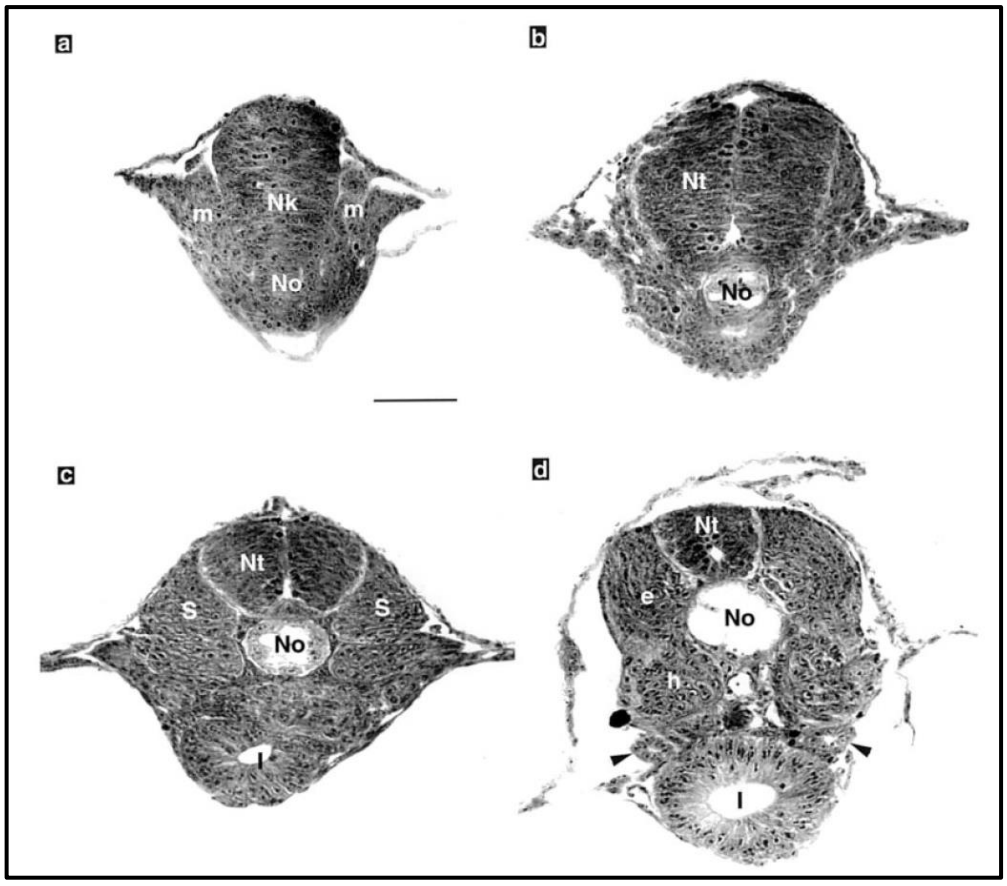


Figure 8. **Development of axial structures during embryogenesis in the Atlantic cod, *Gadus morhua*.** Transverse sections taken immediately posterior to the position of the liver (H&E, bar=100µm). **a**: 75% epiboly. Nk, neural keel; m, mesoderm; No, notochord. **b**: 10-somite stage. Nt, neural tube; No, notochord. **c:** 30-somite stage. Nt, neural tube; S, somites; No, notochord; I, intestine. **d:** Golden eye stage. Nt, neural tube; No, notochord; e, epaxial muscle mass; h, hypaxial muscle mass; I, intestine; arrowheads, pronephric ducts. (**Source: Hall *et al*., 2004**).

4.5.2. Otoliths

Temperature has been found to significantly affect the growth of lapillus in Atlantic cod larvae and juvenile. Otterlei *et al*. (2002) record that the somatic growth and otolith growth rates of increased with increasing temperature from 4°C to 14°C for cod fed in excess. Higher temperatures of 14°C gave approximately 6 to 8 times bigger lapilli compared to those reared at the much lower 4°C at the age of 56 days. Differences in average otolith growth between the Norwegian coastal (NC) cod and the Northeast Arctic (NA) cod stock for different temperature groups were also observed. On average, the otolith radius of larval NC cod stock was found to be 5.7% larger at given lengths, than that of larval NA cod. Juvenile NC was found to be 4.5% larger, at given lengths, than that of the juvenile NA stock (Otterlei *et al*., 2002). However, the response of otolith growth rate to temperature has been found to be different from the optimum curve of somatic growth rate for different fish species (Mosegaard *et al*., 1988; Maillet and Checkley, 1990; Sorgard, 1991) including herring, *Clupea harengus*, (Folkvord *et al*., 1996). Thus the results on development of these bony structures may not necessarily be indicative of somatic growth rates for the fish. Even though the response in otoliths growth was measured at juvenile stage by Otterlei *et al*. (2002), results are included in this review as the temperatures used for incubating eggs during early life affected the size differences at different temperatures. It is important to note also that larger cod had larger otoliths than smaller ones reared under identical condition thus size differences also affect the growth rate of these important structures.

4.6 Temperature effects on metabolic processes

4.6.1. Feed Conversion Efficiency (FCE)

In general, high temperatures lead to a faster evacuation rate in part due to the effect of temperature on the reaction rate of the digestive enzymes (Govoni *et al*., 1986). Blaxter 1992 suggests that rates of passage of food are very dependent on the rate of feeding since earlier larval stages may have simple uncompartmentalised guts. Thus the results from Imsland *et al.* (2005) of different optimal temperatures for growth and feed conversion efficiency are reasonable. They report that the optimum temperature for growth of juvenile cod in relation to size drops from 14.7°C for 5-10g juvenile to 12.4°C for 40-50g juveniles.

4.6.2. Respiration rate as reflected by the Q_{10} factor

Experimentation makes it possible to relate temperature to certain life processes by the use of statistical concepts like the $Q_{10.}$ This is a factor by which rates change over certain temperature ranges. However, Q_{10}

values do not remain constant over wide ranges of temperature so that extrapolations cannot be made (Blaxter, 1992). These values can be useful over narrower ranges of temperature for predictive purposes. Q_{10} is given by the formula:

$$\mathbf{Q}_{10} = (\mathbf{K}_1/\mathbf{K}_2)^{10/ t_1 - t_2}$$

OR

$$\mathbf{Q}_{10} = \mathbf{K}_{(t+10)} / \mathbf{K}_{(t)}$$

K1 and K2 are the reciprocals of times or rates at temperatures t_1 and t_2 respectively (Blaxter, 1992; Peterson *et al*., 2004).

Peck and Buckley (2007) describe an exponential increase in cod larvae respiration rate (RR) with temperature which is evident in their experimental study by a Q_{10} of 3.00. However, although they recorded an increase in respiration rate (RR) with increasing temperature for groups of cod that had a mean dry mass of 71 to 77µg, respiration rate increased not solely because of temperature but other parameters like larval age and absence of food (Davenport and Lonning, 1980; Solberg and Tilseth, 1984; Serigstad 1987; Finn *et al*, 1995) for newly hatched larvae. Their Q_{10} estimates suggest a linear decrease in the effect of temperature on the respiration rate of cod with increasing ln(dry mass) for sizes encompassing larvae to large juveniles (Peck and Buckley, 2007). This could be the reason behind observations of cod juveniles exploiting a wider range of in situ temperatures than larvae. It is important to note that the data collected in their study was ln-transformed (transformed to natural logarithm) when needed for linear regression analyses.

4.6.3. Effects on swimming speed

Mackenzie and Kiørboe (1995) recorded values of 2.6-2.7bls^{-1} swimming speed for cod raised at 6-8°C. Temperature has an effect on these as a comparable study by Skiftesvik (1992) observed swimming speeds of 0.8bls-1 during larval active periods at 5°C. Studies have shown that larval cod do not swim continuously but forage using burst movements and pauses (MacKenzie and Kiørboe, 1995; Hunt von Herbing and Gallager, 2000). Peck *et al.* (2009) provide estimates of swimming (foraging) speed for larval cod reared at 8°C.They found out that the mean routine swimming speed estimates (both bursts and pause phases) equaled an average of ~ 2.4bls^{-1} at 8°C. This is similar to the mean result from Von Herbing and Gallager (2000) of 2.8bls-1 at the same temperature of 8°C. Therefore of the above mentioned studies, no apparent differences exist across populations in swimming speed at similar temperatures ranging from 6˚C to 8˚C. A measure of the 'scope for activity' which is the maximum factor by which routine metabolic rate can rise during activity usually lies

between 1.4 and 3.5 for larval fishes even though it may be as high as 10 (Rombough,1988 in Blaxter, 1992). It is a potential criterion of the potential for movement However; more work has shown that temperature has no detectable effect on the scope factor.

4.7. Temperature effects towards malformations and deformities

4.7.1. Egg and vertebral deformities

In their studies on temperature and salinity effects on cod, Laurence and Rogers, (1976) record checking for any abnormal conditions including shortened and thickened bodies, spinal curvatures and enlarged yolk sacs. These were observed for Atlantic cod but it was found that such deformities were independent to temperature. Galloway *et al*. (1998) observed no abnormal development for cod eggs from the Northeast Atlantic incubated at 1°C, 5°C and 8°C temperature groups. A combination of the percentage sum of vertebral malformations (Fitzsimmons and Perutz, 2006) and the mean total percentage abnormalities in cod (Laurence and Rogers, 1976) in a graph (figure 9)- shows variations in sum total or mean total deformations with temperature increase or decrease.

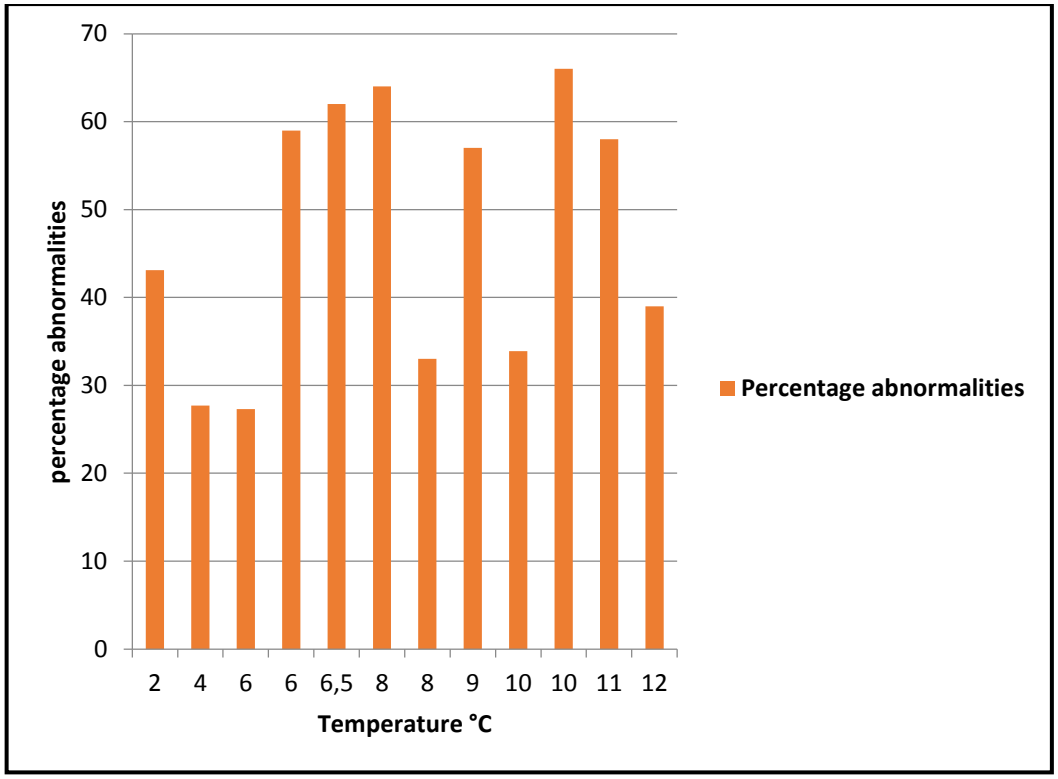


Figure 9. Percentage (mean) total abnormalities in cod

A Plot made from data extracted from Laurence and Rogers (1976); Fitzsimmons and Perutz, (2006)

However, Fitzsimmons and Perutz (2006) found out that temperature can affect the extent of deformations when it came to vertebral malformations in cod larvae. Vertebral malformations in newly hatched larvae reared under 6 temperature groups from 6º to 11ºC ranged from 57% to 66%, numbers slightly higher than the 27.3% to 43.1% range observed by Laurence and Rogers (1976). Types of malformations recorded for all temperature treatments included kyphosis (V shape), lordosis (Λ shape), scoliosis (lateral malformation), kyphosis / lordosis complex, and larvae with severe vertebral curvature (including larvae with shortened vertebral columns as a result of severe vertebral curvature) (see figure 10).

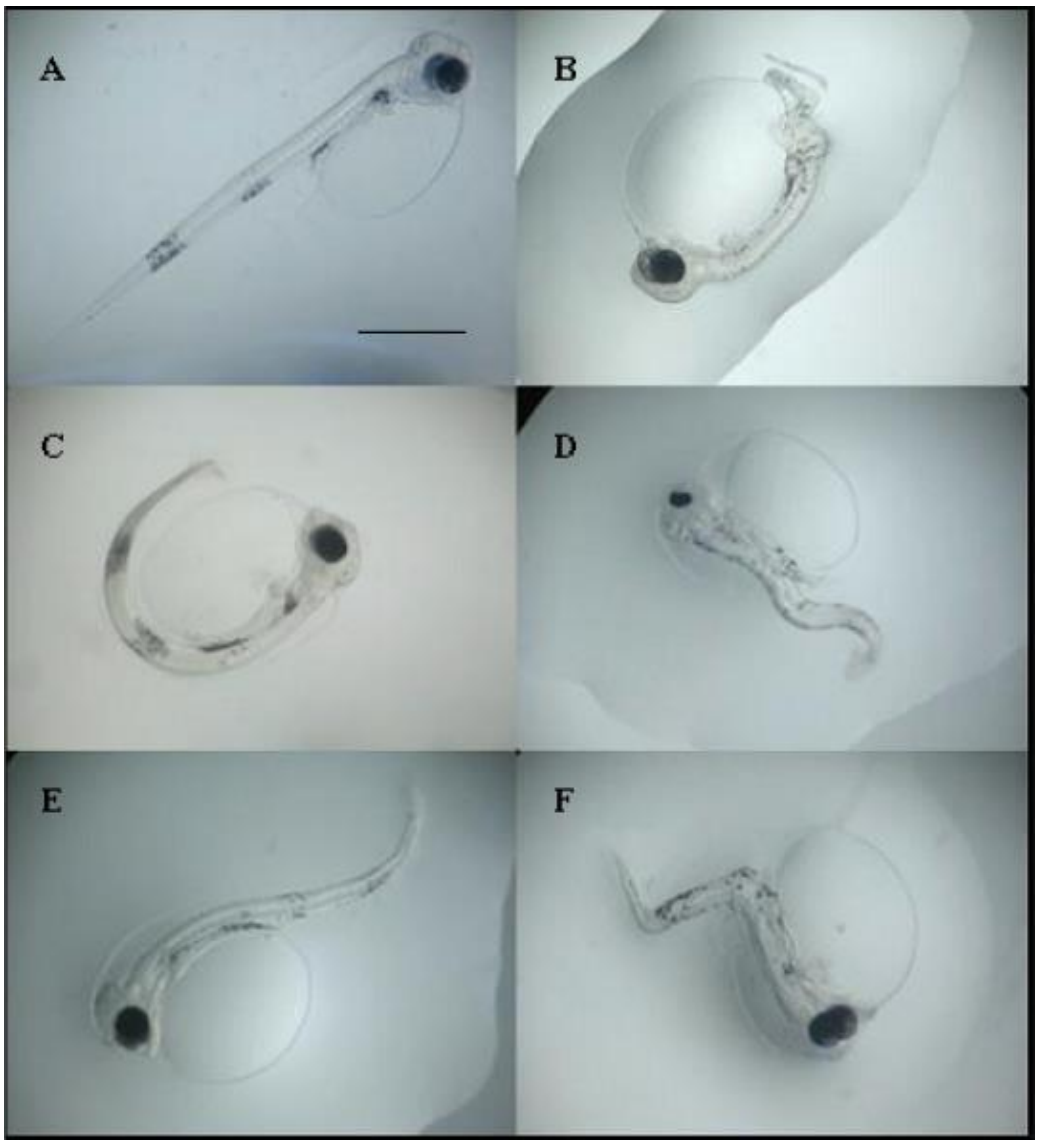


Figure 10: normal larvae without signs of vertebral column malformation; B – severe vertebral curvature; C – kyphosis; D – scoliosis; E – lordosis; F – lordosis / kyphosis complex. Scale bar 1mm shown in A. (Photographs of one day post-hatch larvae taken at camera Magnification of 3.2)

(Source: Fitzsimmons and Perutz, 2006).

Despite observing v-notched and compressed notochord vertebrae deformities in their study, Puvanendran *et al.*, (2009) concluded that egg incubation temperatures were not the cause for the differences in vertebral deformity levels in hatched cod at a rearing temperature ranging between 10 ˚C and 11˚C. This could have been because they were just two temperature treatments and the absence of different groups to compare, which was the case for Fitzsimmons and Perutz (2006) who tested 6 different temperature groups.

The prevalence of larvae with severe vertebral curvature with shortened tails increased significantly at higher temperatures even though the other deformities did not did not differ significantly between incubation temperatures (figure 11).

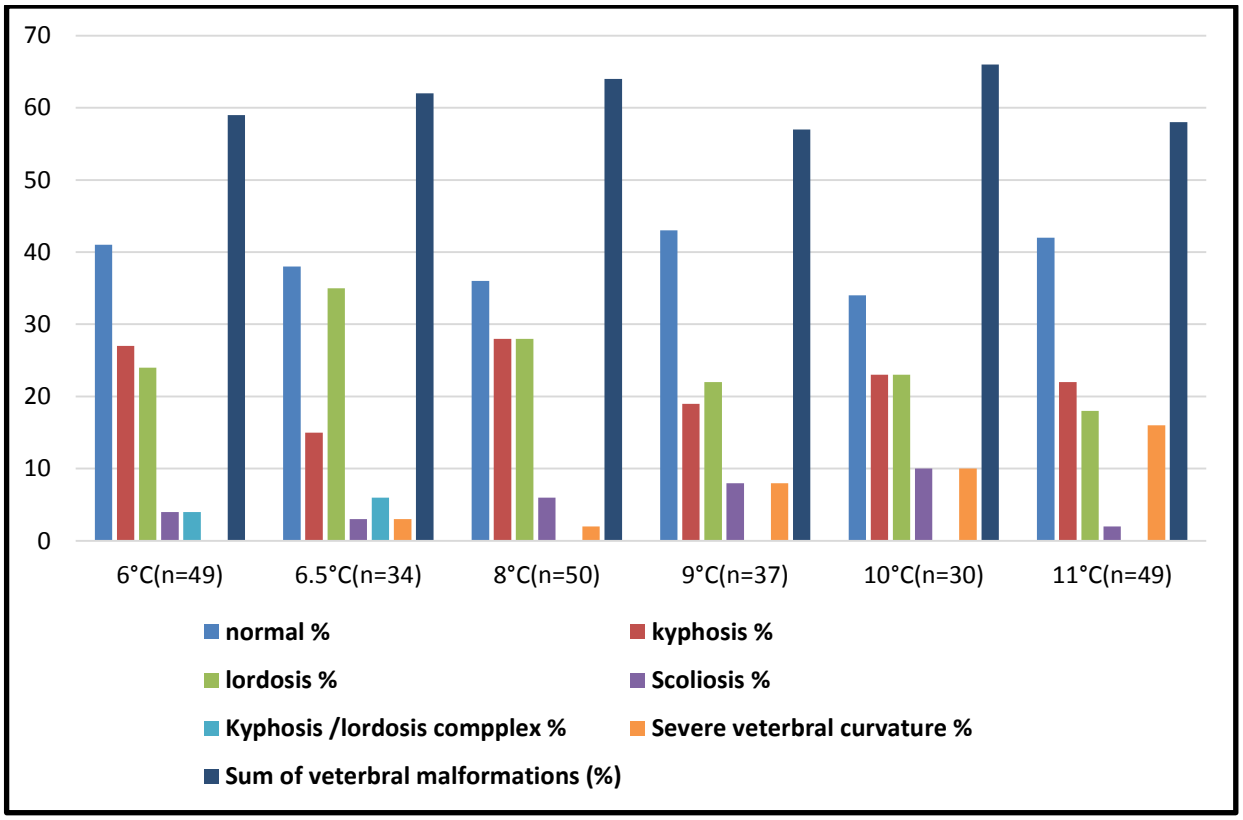


Figure 11. Prevalence and types of vertebral malformations (%) in larval Atlantic cod at hatch (Data extracted for plot from Fitzsimmons and Perutz, 2006)

4.8. Temperature effects on size

4.8.1. Dry Mass,

Pryor and Brown (1998) found no significant differences between the dry weights of eggs incubated at low (0-1°C), medium (3-4°C) and high (7-8°C) temperatures. Von Herbing *et al*., (1996) states that growth, measured as an increase of dry weight, *wt*, over time, *t*, was significantly correlated with dph at 10 º. Growth rates were significantly higher in Newfoundland (NF) larvae than in the Scotian Shelf (NS) population at 5ºC and 10 ºC. This suggests that growth as a measure of dry weight over time varies significantly among populations than with different temperature regimes. That may be a possible explanation for Atlantic cod mean larval dry mass (at 50% hatch and at the end of yolk-sac stage) in µg increasing with larval age in Peck and Buckley (2008). However, this excluded the youngest individuals measured at the highest temperature which was 10°C. This was the case for larval dry weights

4.8.2. Temperature effects on egg (diameter) and larval (length at hatch) sizes

Field studies on cod ascertain that there is strong negative relationship between the average size of fish eggs and the temperature of the environment in which the eggs were caught. In addition to that, gained knowledge is that there is also a strong positive relationship between the size of cod larvae and the egg from which each individual hatches. The conclusion from egg-larval size studies (Ware and lambert, (1985); Miller *et al.,* 1995) has been that large eggs are produced in colder waters and yield larger larvae. This implies a strong negative relationship (in the field) between cod larvae size and temperature where the environment in which the eggs were found**.**

In laboratory experiments, Pryor and Brown (1998) found significant differences in egg diameters and larval growth among different temperature treatment regimens ranging from low to medium to high. Diameters increased proportionately with temperature. The same authors found that the total length of larvae was greatest at the lowest temperature both at 50% hatch and at the end of the yolk-sac stage. Furthermore, yolk-sac width at 50 % hatch was significantly greater at 3-4°C (Pryor and Brown, 1998). Larvae from eggs incubated at 1°C were found to have significantly larger yolk sac volume (YSV) at hatching than larvae form eggs incubated at the higher temperatures of 5°C , but only slightly larger than larvae whose eggs were incubated at and 8°C (Galloway *et al.*, 1998). Larval size at consumed yolk sac differed significantly among temperatures ranging from 1 to 11°C and no differences were observed between 7°C and 9°C (Nissling, 2004).

In terms of standard length at hatch, Laurence and Rogers (1976) state that although temperature accounted for the greatest variability in mean standard hatching lengths for cod, hatching lengths were not associated with temperature for cod eggs incubated in a range of temperatures between 2°C and 12°C. Galloway *et al*. (1998)

found that the larval size at hatch from cod eggs kept at 1°C was significantly shorter than larvae from eggs incubated at higher temperatures of 5°C and 8°C and this significant difference was present 5dph even though all hatched larvae had been moved to a similar temperature of 5°C. The standard length between larvae from groups incubated in at 5 and 8°C was found to have no differences Galloway *et al*. (1998). In addition, Hall and Johnston (2003) on the other hand, state that no significant differences in standard length for cod embryos reared at 4, 7 and 10°C were apparent both at the hatching or first feeding stages. In contrast, Jordan (2002) recorded that larvae in the warmer temperature treatments of 2, 4, 8 and 12 °C had a smaller standard length at hatch although one of three replicated groups (Batch 2) showed no trend in either size at hatch across the temperature treatments. This is consistent with results from Nissling (2004) who recorded significantly lower larval sizes at the high temperature of 11°C. Larval size at hatching was found to be smaller and significantly lower at high temperatures (11 °C) and both larger and significantly higher at lower temperatures (1°C and 3°C). In addition to that, Peterson *et al*. (2004) found that the lengths of hatched Atlantic cod larvae reared at a series of constant temperatures from 4-10 °C from fertilization to hatch are greater at lower incubation temperatures, with the temperature range 2-4°C being suggested as the optimal temperature with regard to larval length at hatch.

On the other hand, Pepin *et al.,* (1997) through a study with a restricted number of experimental trials did not find strong negative relationships between size at hatch and incubation temperature but the inverse. This inverse relationship observed by Pepin *et al*., (1997) may be explained by the overall metabolic load placed on the embryo as temperature was decreased.

4.9. Other diverse effects of temperature

4.9.1. Effects of stepwise increments in temperature on growth and development

A more recent study by Puvanendran *et al*., (2013) shows how rapidly the early incubation temperature of Atlantic cod eggs can be increased without affecting hatching or viability of cod larvae. A control group kept at 4°C incubation temperature yielded significantly longer larvae, with smaller yolk reserves at hatch than with stepwise increments of 8h, 32h, 64h and 96 h (T1, T2, T3 and T4 respectively) Puvanendran *et al*., (2013). Although Cell asymmetries and embryo mortalities were not significantly different between control and T2-T4 groups, the T1 group of (8h-stepwise increments) yielded higher egg mortalities and lower hatching success. Figure 12 shows a horizontal section of the control and larvae whose temperature environment was changed from 4.5˚C to 9.5˚C in 8h steps. Puvanendran *et al.* (2013) found that the egg incubation temperature of

Atlantic cod eggs can be elevated the from ambient temperature 4.5°C to the maximum optimal incubation temperature 9.5°C using gradual increment periods 8h - 96h without arresting or altering the normal embryonic and organ development and hatching or reducing the quality or quantity of viable larvae. However, Of the four treatment groups, 32h yielded significantly better results. T2 larvae had largest yolk reserves in comparison with the other groups (figure 13) , making 32h steps (T2) the best choice of gradual temperature increments

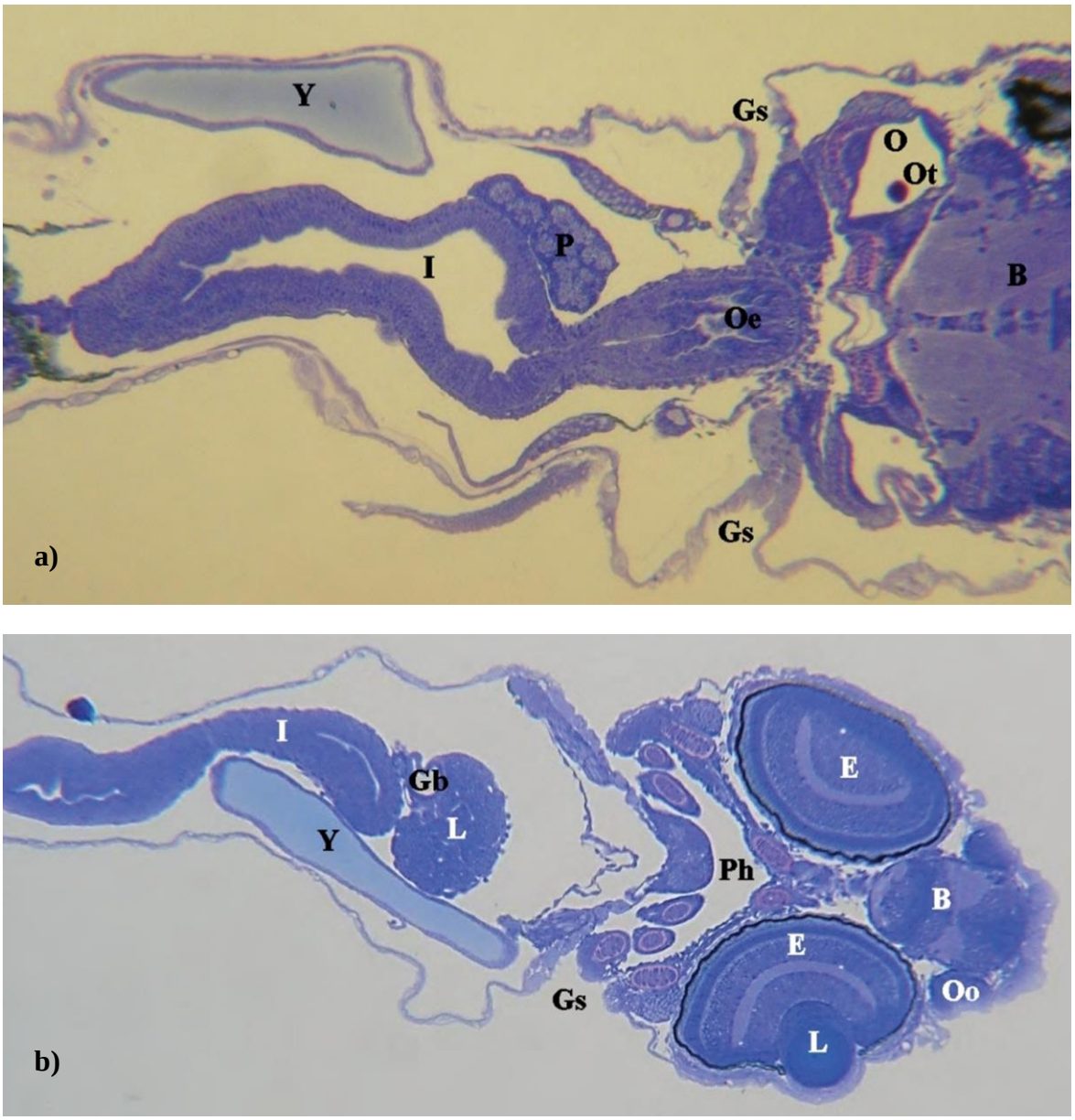
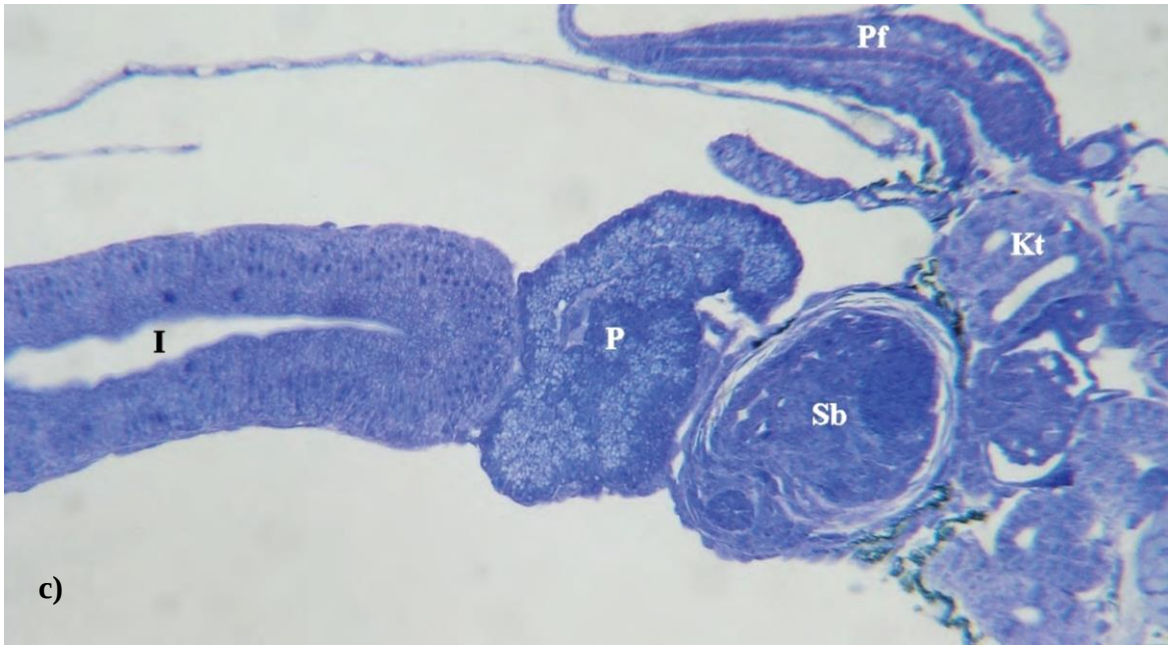


Figure 12. caption:

(a). Horizontal section through newly hatched cod larva from control group showing brain (B), otocysts (O), otolith (Ot), gill slits (Gs) oesophagus (Oe), pancreas (P), intestine (I) and yolk sac (Y)

(b) Horizontal section through newly hatched cod larva from T1 group (8h steps) showing olfactory organs (Oo), brain (B), eyes (E) with lens (L), pharynx (Ph), gills (G), liver (L), yolk sac (Y) and intestine (I). **(Source: Puvanendran *et al* ., 2013)**



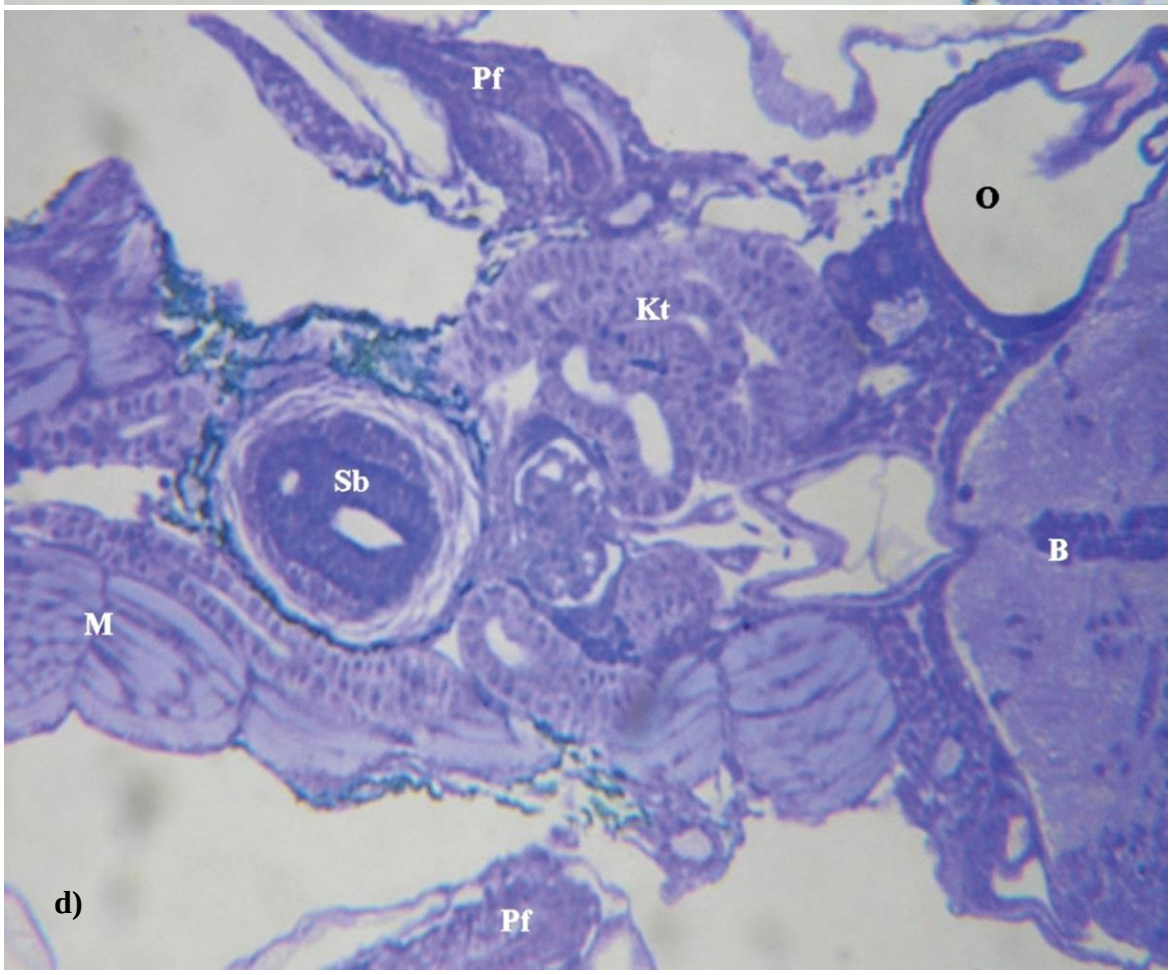


Figure 13 caption

(c). Horizontal section through newly hatched cod larva from T2 (32h steps) group showing pectoral fin (Pf), kidney tubuli (Kt), swim bladder (Sb), pancreas (P) and intestine (I)

(d). Horizontal section through newly hatched cod larva from T3 (64h steps) group showing brain (B), otocyst (O), kidney tubuli (Kt), swimbladder (Sb), musculature (M) and pectoral fins (Pf). **(Source: Puvanendran *et al*., 2013)**

for future developmental programming of cod when considering embryonic cleavage pattern, hatching success, larval histology and larval morphology.Figure 14 shows the larvae from the last group T4 (96h).

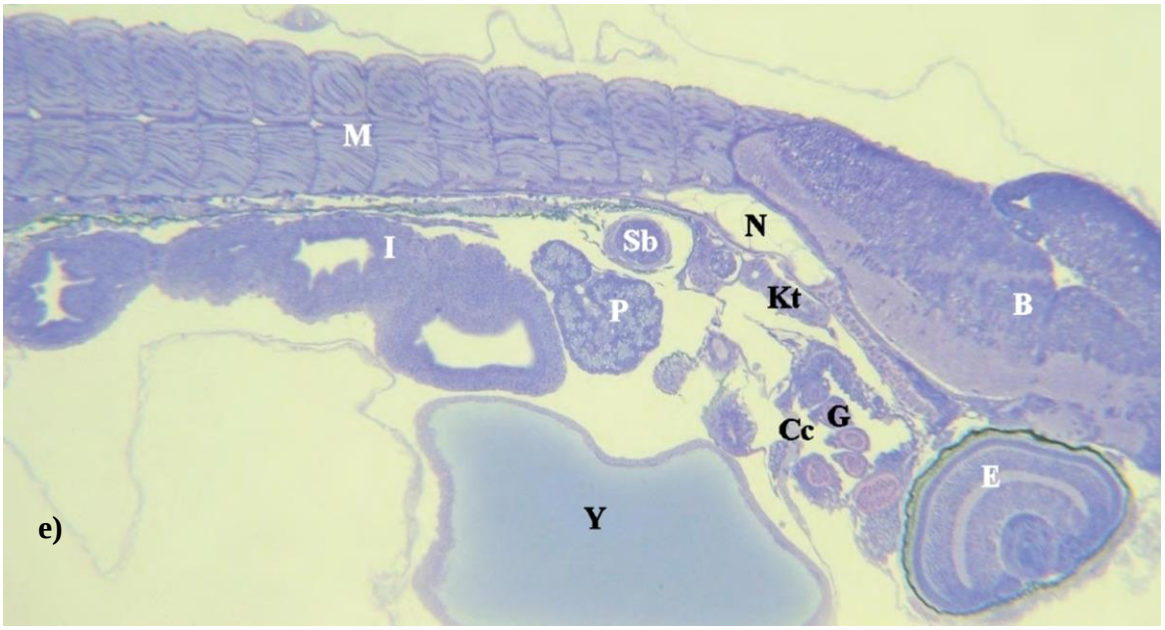


Figure 14. caption
e). Longitudinal section through newly hatched cod larva from T4 (96h steps) group showing brain (B), eye (E), gill (G) cavity, chloride cells (Cc), kidney tubuli (Kt), notochord (N), swim bladder (Sb), pancreas (P), intestine (I) and musculature (M) **(Source: Puvanendran *et al.*, 2013)**

4.9.2. Effect on body movements

A study by Peterson *et al.* (2004) shows that the frequency of cod embryo body movements can be affected by the temperature they are reared under. Cod embryo body movements increased from zero (at 42 degree-days post fertilization) to a maximal at 73- 32 degree days with the most active embryos (having a mean of 5.5 movements over 10 min) being those kept at 2°C. They conclude that embryo body activity declines with an increase in temperature (to less than 1 per 10min at 8°C-10°C).

4.9.3. Effect on genetic expression

comparison with the other groups (figure 13) , making 32h steps (T2) the best gradual increment choice of temperature for future developmental programming of cod when considering embryonic cleavage pattern, hatching success, larval histology and larval morphology.

Skjærven *et al.* (2011) found out that embryonic stages of Atlantic cod, *G. morhua* are especially sensitive to incubation temperature as ontogenetic expression of maternal genes can be reduced and zygotic and reference genes showed increased expression under continuous long term (6 °C) and acute short term heat exposure in Atlantic cod embryos.

4.9.4. Effect of ice and low (sub-zero) temperatures

A number of studies have established that post-metamorphic teleost fish freeze and die when they come into contact with ice at temperatures below the freezing point of their blood plasma (Valerio *et al*., 1992). In their laboratory studies with Atlantic cod, Valerio *et al*.(1992) found out that despite the absence of antifreeze proteins, egg homogenates, did not freeze at -1.8°C (the freezing point of seawater) in icy aerated seawater and could be undercooled to -4°C in ice. Cod eggs only froze at temperatures between -4.1 and -17°C with freeze resistance depending on the integrity of the chorion and larvae could be cooled to -1.8°C if there was no direct contact with ice crystals (Valerio *et al.*, 1992). Ice increased the freezing point to -1,36 if directly touched with ice. Cod's freeze resistance during early life stages maybe due to the nature of cods external epithelium and delayed development of sensitive gill structures below 0°C (Valerio *et al.*, 1992).

Newfoundland and Labrador waters are often covered by ice and temperatures in the top 30m can approach the freezing point of water. The majority occurrence of cod eggs after spawning is in this upper region of the water column. It is apparent therefore that early cod development may occur at subzero temperatures and in intimate association with ice in the field.. This environment however, is lethal for most fish.

5.1. Summary

Summary tables of temperature high temperature effects and low temperature effects are provided in APPENDIX VIII and APPENDIX IX respectively. Water temperature is important in the early development of Atlantic cod. It has been found to significantly affect egg diameters among different temperature treatment regimens (Pryor and Brown (1998). Elevated temperatures significantly reduce the egg quality (Tveiten, 2008) and can reduce the fertilization percentage by more than 50% but also to increase respiration rate (Peck and Buckley, 2007) and shorten the time to hatching. However, egg survival was proven to be unaffected by temperature. Even though egg and larval mortality was independent of temperature increasing embryo mortality of cod as hatching approached was characteristic in most case studies. Temperature also has a greater influence on development rates of cod eggs and yolk larvae than egg size (Pepin *et al*., 1997), with relatively little impact on development rates prior to hatch caused by variation in embryo sizes and having a significant effect on the characteristics of yolk sac larvae. Low temperatures yield a larger yolk sac volume (YSV) at hatching (Galloway *et al*., 1998; Nissling, 2004). However there are contrasting conclusions for standard larval length at hatch among some case studies in relation to temperature. Observations of hatching lengths not being associated with temperature for cod eggs incubated in a range of temperatures (Laurence and Rogers, 1976; Galloway *et al*., 1998; Hall and Johnston, 2003) and of significantly larger standard lengths at hatch for lower incubation temperatures (Pryor and Brown 1998) or of significantly shorter larvae from eggs incubated at low temperatures than at higher temperatures (Galloway *et al*., 1998) have been reported in this review. However, one common thing among the contrasting studies is that temperature found to account for the greatest variability in the mean standard hatching lengths for cod. Some average hatching rates differed among different cases for the same temperature level. Time to hatching in Laurence and Rogers (1976) was lower than that recorded by Bonnet (1939) and Jordaan and Kling (2006) for the same temperatures. These differences may be attributed to differences in racial stocks and differences in experimental methods used in each case study. Temperature also has significant effect on development of structures and tissue in early stages. Otolith growth has been shown to increase with temperature (Otterlei *et al*., 2002). Several authors (Galloway *et al.*, 1998; Hall and Johnston 2003; Hall *et al.*, 2003) establish that temperature alters the number and size of embryonic muscle fibres in numerous species including Atlantic cod, *G. morhua*. Although abnormalities in cell cleavages and have been found to be independent to temperature, the prevalence of vertebral malformations has been shown to affect in cod larvae to be affected by water temperature. Puvanendran *et al*., 2013 conclude that the best option to increase incubation temperature of cod embryos from their ambient temperature (4.5°C) to the maximum optimal incubation temperature of 9.5°C without reducing the quality and quantity of viable larvae is by using 32h steps.

All of the above information is of great relevance to future Atlantic cod farmers or fingerling producers in hatcheries. Muscle growth and development, larval sizes, egg viability and larval mortalities are some of the things critical in aquaculture production of Atlantic cod. Therefore, hatchery operators and on grower of cod have knowledge on the optimal temperature ranges for their particular stock of cod that can give less variable mortality, avoid lowered fertilization success, avoid unwanted reduced egg quality and viability but operate grow out cod with faster times to hatching , yielding good sizes at hatch and getting fast body muscle and tissue growth of fish. Malformations in fish, however, are prevalent in many species and are particularly important in species cultivated for commercial aquaculture (Fitzsimmons and Perutz, 2006). Increased temperature increase the prevalence of vertebral malformations in Atlantic cod and this can result in serious economic implications for both hatchery operators and on-growers of the fish (Fitzsimmons and Perutz, 2006). However, even though prevalence and types of malformations are specific to hatchery rearing conditions like temperature, there exist also general malformations associated with rearing protocols in many hatcheries (Boglione *et al*., 2001).

5.2. Discussion

Underlying the normal, presumably genetically based, lethal temperature limits of a species there is an optimum range, which may change seasonally or geograpically and within which the species normally resides (Blaxter, 1992). The studies reviewed in this paper on temperature effects reveal that Atlantic cod has temperatures optimal for growth or development. However these vary with populations and thus generalizations cannot be made. However some stocks respond in similar ways to environmental temperature. For example, Von Herbing *et al*. (1996) discovered that growth rates and structural complexity differs between larvae from different populations and were the case between Newfoundland and Scotian shelf larvae. However, Geffen *et al.* (2006) found that there were no consistent trends that differentiated Eastern and Western, or Northern and Southern populations cod populations of the Irish Sea cod. Therefore it is important that individual stock relationships should be used where possible as the case may differ for another stock or population, particularly in models.

Reviewed data on the timing to 50% hatching from Irish Sea cod, Northwest Atlantic cod (Newfoundland Shelf, Georges Bank and Bay of Fundy) and northeast Atlantic cod (North Sea, Baltic Sea and Norwegian Sea) showed that temperature significantly affected time to 50% hatching in an inverse manner. A synthesis of five cases (Narragansett Bay Massachusetts cod, Ipswich Bay, Massachusetts, Northeast Arctic cod and Newfoundland cod) for a range of temperatures from 1 to 12 ˚C, showed an inverse exponential relationship between time to hatching (in days) and temperature as explained by the model:

$T_{days} = 28.904e^{-0.125x}$

The model is derived from a sample size, N, of 65 (refer to APPENDIX X) and explains the total variation of outcomes by 80%.

A closely related species in the same family as cod, *Gadidae*, and occurring on both sides of the Atlantic ocean, haddock (*Melanogrammus aeglefinus*, L.) is influenced by temperature in a similar way to cod with regard to the time to hatching. Haddock hatching times were found by Martell *et al*. (2005) to be inversely proportional to incubation temperature and ranged from 20.3 days at 2°C to 9.1 days at 10 °C. Pryor and Brown, 1998) also establish that increasing temperature generally decreases incubation time for other species as well like Atlantic halibut (*Hippoglossus hippoglossus*) and yellowtail (*Limanda ferruginea*) larvae.

Temperature only affects variability in cod mortalities but mortalities are independent of temperature. However, irrespective of temperature, highest mortalities for cod are reported to be just prior to hatching for cod (Bonnet, 1939; Laurence and Rogers, 1976). The susceptibility of the egg to harmful factors in the environment is least during this stage just before hatch. Cod embryos can be said to be more eurothermal than haddock (Laurence and Rogers, 1976) whose mortalities are affected by temperature.

Bonnet (1939) stated that development rates are much less for the same unit change in temperature for comparisons between 'higher than the optimal temperature groups' (e.g 10 ºC and 12 ºC) and 'lower but still above the optimal temperature groups' (6 ºC and 8 ºC). Bonnet (1939) suggests that the retardation in development is due to the fact that the temperature maximum approaches. As the maximum temperature limit is approached there is retardation of development. Similar observations have been made for mackerel too (Worley, 1933).

5.3.Temperature, wild stock recruitment and climate change

Amongst others, temperature is one of the parameters most important for cod recruitment. In the Northeast Arctic lower sea water temperatures produce smaller year classes while higher temperature give year classes of all strengths (Ellersten *et al.*, 1989). Since pelagic marine embryos are subjected to currents which may displace them into regions of different oceanographic conditions, and in the light of a warmer climate and warmer seas, the adaptability of these embryos to changes in temperature may affect the viability of wild cod eggs and ultimately year class strength. Furthermore, Stock development of cod is linked by trophic interactions. Temperature changes may cause a mismatch between the occurrence of first feeding cod larvae and their prey organisms resulting in poor recruitment, the match and mismatch hypothesis, of Cushing (1969; 1982; 1990). A

lot of literature ascertains that variable and changing environmental conditions may affect growth and mortality and generate recruitment variability (Cushing, 1982; Nissling, 2004) Imsland *et al*., 2007). An example of potential effects on wild stocks is Baltic cod. A shift in peak spawning of cod from spring to summer during the 1990s has been observed in the Baltic Sea (Nissling, 2004). Depending on recruitment success, the Baltic system may be pushed towards a cod or a clupeid dominated system (Nissling 2004). Delayed spawning which may involve egg development at too high temperatures has been suggested as the reason behind the poor recruitment of cod observed therein. Therefore Atlantic cod is vulnerable in the event of a warming climate and warmer seas, which would have an effect on stock recruitment. Already, correlations between increasing temperature and gadoid recruitment are known to be found in the northern waters whereas negative relationships are observed in the warmer waters and none found at intermediate temperatures (Imsland *et al*. 2007). An increase of temperature, as would occur with global warming will change the timing of ecological events and influence the match or mismatch of larvae and their prey and their predators in nature. Therefore temperature could offset the trophic interactions and upset the ecological balance in nature. Blaxter (1992) states that aquatic poikilothermal animals; cod being one of them; are buffered against short term and even seasonal changes in temperature and are consequently more stenothermal- and therefore potentially more vulnerable- than their terrestrial counterparts to a rise of temperature of a few degrees celcius (Blaxter, 1992).

5.4 Conclusion

Several parameters are temperature sensitive in the early life stages of Atlantic cod and it is arguably the most important environmental influence driving development, growth, and survival of marine fish during their early life history. Studies have shown significantly shorter duration of the development period at high temperatures, faster respiration rates, low egg viabilities, lowered fertilization success, varied muscle growth and standard length at hatch, smaller egg sizes and temperature dependent prevalence of vertebral malformations at high temperatures. Therefore water temperature can affect metabolism, other physiological processes, structural changes and developmental rates in early stages of Atlantic cod. How it affects body sizes, growth, differentiation of muscle and meristic characters varies among different populations of cod due to genetic differences among stocks. Temperature is an important topic in aquaculture as it can result in large economic costs in hatcheries or grow out farms through abnormal cell cleavages during embryogenesis and deformities in early larval morphology. Temperature changes may cause a mismatch between the occurrence of first feeding cod larvae and their prey organisms resulting in poor recruitment by affecting larval survival in wild stocks

indirectly in terms of food availability. In the event of climate change, variable and changing environmental conditions may affect growth and mortality and generate recruitment variability.

REFERENCES

BLAXTER, J. H. S. 1992. The effect of temperature on larval fishes. *Netherlands Journal of Zoology*, **42** (2-3): 336-357

BJØRNSSON B., STEINARSSON A. & ODDGEIRSSON M. 2001 Optimal temperature for growth and feed conversion of immature cod (*Gadus morhua* L.). *ICES Journal of Marine Science*, **58**: 29 –38

BOGLIONE C, GAGLIARDI F, SCARDI M & CATAUDELLA S (2001). Skeletal descriptors and quality assessment in larvae and post-larvae of wild-caught and hatchery-reared gilthead sea bream (*Sparus aurata* L. 1758). *Aquaculture*, **192**: 1-22.

BRANDER, K. M. 1995. The effect of temperature on growth of Atlantic cod (*Gadus morhua.* L). *ICES Journal of Marine Science*, **52**: 1-10

BRANDER, K. 1979. The relationship between vertebral number and water temperature on cod. *Journal du Conseil / Conseil Permanent International pour l'Exploration de la Mer*, **38**: 286-292

BROOKS, S. AND JOHNSTON, I. A. (1993). Influence of development and rearing temperature on the distribution, ultrastructure and myosin subunit composition of myotomal muscle fiber types in the plaice *Pleuronectesplatessa*. *Marine Biology*, **117**: 501-513.

BROWN N.P., BROMAGE N.R. & SHIELDS R.J. 1995. The effect of spawning temperature on egg viability in the Atlantic halibut (*Hippoglossus hippoglossus*). In: *Proceedings of the 5th International Symposium on the Reproductive Physiology of Fish*. 2-8 July 1995. The University of Texas at Austin.

CANNON J. 1997. Northeast Atlantic. In: Review of the state of world fishery resources: marine fisheries. Rome: FAO. p 15–23.

CENTRE FOR REVIEWS AND DISSEMINATION. 2009. *Systematic Reviews*: *CRD's guidelines for undertaking reviews in health care.*University of York:CRD

CHAMBERS, R. C. 1997. *Environmental inflluences egg and propagule sizes in Marine fishes. In Early Life History and Recruitment in fish population.* London: Chapman & Hall

CHAMBERS, R.C., LEGGETT, W. C. (1996) Maternal influences on variation in egg sizes in temperate marine fishes. *American Zoologist*, **36**:180–196

CUSHING, D. H. 1969. The regularity of the spawning season of some fishes. *Journal du Conseil / Conseil Permanent International pour l'Exploration de la Mer*, **33**: 81-97

CUSHING, D. H. 1982. *Climate and fisheries*. London: Academic press

CUSHING, D. H. 1990. Plankton production and year-class strength in fish population: an update of the match/mismatch hypothesis. *Advances in Marine Biology*, **26**: 249-293

BONNET, D. D. 1939. Mortality of the Cod Egg in Relation to Temperature. *Biological Bulletin*, **76**, 428-441.

DANNEVIG, A. 1894. The influence of temperature on the development of the eggs of fishes. *Thirteenth Annual Report Fish. Bd., Scotland*, pp. 147-152

DAVENPORT, J., LONNING, S., 1980: Oxygen uptake in developing eggs and larvae of the cod, *Gadus morhua* L. *Journal of Fish Biology*. 16, 249–256.

EARLL, R. E. (1878). A report on the history and present condition of the shore cod fisheries of Cape Ann, Mass, together with notes on the natural history and artificial propagation of the species. *US Commission for Fisheries Report*, 6:685–740.

ELLERTSEN B, FOSSUM P, SOLENDALP, SUNDBY S. 1989. Relation between temperature and survival of eggs and first feeding larvae of the Northeast Arctic cod (Gadus *morhua* L.). *Rapports et procès-verbaux des réunions / Conseil permanent international pour l'exploration de la mer.* **191**: 209-219

DEPARTMENT OF FISHERIES AND AQUACULTURE. 2013. Atlantic Cod, *Gadus morhua*: Rome: FAO. Available at: http://www.fao.org/fishery/culturedspecies/Gadus_morhua/en [Accessed 13 August 2013]

FALK-PETERSEN, I. B. 2005. Comparative organ differentiation during early life stages of marine fish. *Fish & Shellfish Immunology,* **19**: 397-412

FEVOLDEN, S. E & POGSON, G. H. 1997. Genetic divergence at the synaptophysin (SypI) locus among Norwegian coastal and north-east Arctic populations of Atlantic cod. *Journal of Fish Biology***, 51**: 895–908.

FINN, R. N.; FYHN, H. J.; EVJEN, M. S., 1995: Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*).L. Respiration and nitrogen metabolism. *Marine Biology*, **124**:355–369.

FITZSIMMONS, S. D. AND PERUTZ, M. 2006. Effects of egg incubation temperature on survival, prevalence and types of malformations in vertebral column of Atlantic Cod (*Gadus morhua*) larvae. *Bulletin of the European Association of Fish Pathologists*, **26**: 80-86

FOLKVORD, A. 1991. Growth, survival and cannibalism of cod juveniles (*Gadus morhua*): effects of feed type, starvation and fish size. *Aquaculture,* **97**(1): 41–59

FOLKVORD, A., YSTANES, L., JOHANNESSEN, A., AND MOKSNESS, E.1996. RNA: DNA ratios and growth of herring (*Clupeaharengus*) larvae reared in mesocosms. *Marine Biology*, **126**:591–602.

FRIGEIRSSON, E. 1978. Embryonic development of five species of gadoid fishes in Icelandic waters. *Rit Fiskideildar,* **5**: 1–20.

FRY, F. E. J., 1947. Effects of the environment on animal activity. Univ. Toronto Stud. Biol. Ser, **55**: 1-61

GALLOWAY, T. F., KJØRSVIK, E. AND KRYVI, H. 1998. Effect of temperatur on viability and axial muscle development in embryos and yolk sac larvae of teh Northeast Arctic cod (*Gadus morhua*). *Marine Biology,* **132**: 559-567.

GOUGH, D., OLIVER, S. AND THOMAS, J. 2012. *An introduction to systematic reviews*. Los Angeles: Sage

GOVONI, J. J., BOEHLERT, G. W. AND WATANABE, Y. 1986. The physiology of digestion in fish larvae, *Environmental Biology of Fishes*, **16**: 59-77

GEFFEN, A.J., FOX, C, J. AND NASH, D. M. 2006. Temperature-dependent development rates of cod *Gadus morhua* eggs. *Journal of Fish Biology*, **69**: 1060–1080

HALL, T. E., COLE, N. J. AND JOHNSTON, I. A. 2003. Temperature and the expression of seven muscle-specific protein genes during embryogenesis in the Atlantic cod *Gadus morhua* L. *The Journal of experimental biology*, **206**: 3187-3200.

HALL, T. E., AND JOHNSON, I. A. 2003. Temperature and developmental plasticity during embryogenesis in the Atlantic cod *Gadus morhua* L. *Marine Biology*, **142**: 833–840

HALL, T. E., SMITH, P. AND JOHNSTON, I. A. 2004. Stages of Embryonic Development in the Atlantic cod, *Gadus morhua*. *Journal of morphology,* **259**: 255-270

HANSEN, T., KARLSEN, Ø.,TARANGER, G. L., HEMRE, G. I., HOLM, J. C. AND KJESBU, O.S. 2001.Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. *Aquaculture*, **203**:51-67.

HEMPEL, G. AND J. H. S. BLAXTER, 1963. On the condition of Herring larvae. *Rapp. Cons. Explor. Mer*, **154**: 3540

HOKANSON K.E.F., MCCROMICK J.H., JONES B.R. AND TUCKER J.H. (1973) Thermal requirements for maturation, spawning, and embryo survival of the brook trout,*Salvelinus fontinalis. Journal of Fisheries Research Board of Canada,* **30**: 975-984.

IMSLAND, A. K., FOSS, A., FOLKVORD, A., STEFANSSON, S. O. AND JONASSEN, T. M. 2005. The interrelation between temperature regimes and fish size in juvenile Atlantic cod (Gadus *morhua*): effects on growth and feed conversion efficiency. *Fish Physiology and Biochemistry*, **31**: 347-361.

IMSLAND, A. K., FOSS, A., KOEDIJK, R., FOLKVORD, A., STEFANSSON, S. O. AND JONASSEN, T. M. 2007. Persistent growth effects of temperature and photoperiod in Atlantic cod *Gadus morhua*. *Journal of Fish Biology,* **71**: 1371–1382

IVERSEN, S. A. & DANIELSSEN, D. S.1984. Development and mortality of cod (*Gadus morhua* L.) eggs and larvae in different temperatures. *Flødevigen Rapportser*, **1**: 49–65.

JEZIERSKA, B., ŁUGOWSKA, K. AND WITESKA, M. 2009. The effects of heavy metals on embryonic development of fish (a review). *Fish Physiology and Biochemistry* , **35**:625-640

JOHANSEN, A. C., & A. KEOGH, 1914. The influence of temperature and certain other factors upon the rate of development of the eggs of fishes. *Conseil perm. internal, pour l'esplor. de la mer, Publ. de Circonstances,* No. 68.

JOHNSTON, I. A., FERNANDEZ, D., CALVO, J., VIERIA, V. L. A., NORTH, T. W., ABERCROMBY, M. AND GARLAND, T., JR (2003). Reduction in muscle fibre number during the adaptive radiation of Notothenioid fishes: a phylogenetic perspective. *Journal of Experimental Biology*, **206**:2595 -2609.

JOHNSTON, I. A. 2006. Environment and plasticity of myogenesis in teleost fish. *Journal of Experimental Biology*, **209**:2249–2264

JOHNSTON, I. A., COLE, N.J., VIEIRA, V.L.V., DAVIDSON, I. 1997. Temperature and developmental plasticity of muscle phenotype in herring larvae. *Journal of Experimental Biology*, **200**:849–868

JOHNSTON, I. A. AND ANDERSEN, Ø. 2008. Number of muscle fibres in adult Atlantic cod varies with temperature during embryonic development and pantophysin (PanI) genotype, *Aquatic Biology*, **4**: 167–173

JOHNSTON, I. A., ALDERSON, R., SANDHAM, C., DINGWALL, A., MITCHELL, D., SELKIRK, C., NICKELL, D., BAKER, R., ROBERTSON, B., WHYTE, D., SPRINGATE, J. 2000 Muscle fiber density in relation to the colour and texture of smoked Atlantic salmon (*Salmo salar* L.). *Aquaculture*, **189**:335–349

JOHNSTON, I. A., VIEIRA VLV, ABERCROMBY M (1995) Temperature and myogenesis in embryos of the Atlantic herring *Clupea harengus*. *Journal of Experimental Biology*,**198**:1389–1403

JORDAAN, A. 2002. The Effect of Temperature on the Development,Growth and Survival of Atlantic Cod (*Gadus morhua*) During Early Life-Histories. [Electronic Theses and Dissertations] The University of Maine: Fogler Library: Available at http://digitalcommons.library.umaine.edu/etd

JORDAAN A., HAYHURST S.E. AND KLING L.J. (2006) The influence of temperature on the stage at hatch of laboratory reared *Gadus morhua* and implications for comparisons of length and morphology. Journal of Fish Biology 68, 7–24.

KING H. R. AND PANKHURST N.W. 2004a. Effect of maintenance at elevated temperatures on ovulation and luteinizing hormone releasing hormone analogue responsiveness of female Atlantic salmon (*Salmo salar*) in tasmania.Aqua culture, **233**:583-597.

KING H.R. AND PANKHURST N.W. 2004b. Effect of short-term temperature reduction on ovulation and LHRHa respon- siveness in female Atlantic salmon (*Salmo salar*) main- tained at elevated water temperatures. *Aquaculture*, **238**: 421-436.

KJESBU, O.S. 1989. the spawning activity of cod, *Gadus morhua* L. *Journal of Fish Biology*, **34**: 195–206.

KJØRSVIK E. AND LØNNING S. 1983. Effects of egg quality on normal fertilization and early development of the cod, *Gadus morhua* L. *Journal of Fish Biology* **23**, 1–12.

KNUTSEN, G. M. AND TILSETH, S. 1985. Growth, development, and feeding success of Atlantic cod larvae *Gadus morhua* related to egg size. *Transactions of the AmericanFisheries Society*, **114:** 507–511

LAURENCE, G. C. AND ROGERS, C. A. 1976. Effects of temperature and salinity on comparative embryo development and mortality of Atlantic cod (*Gadus-morhua-L*) and haddock (*Melanogrammus-aeglefinus L*). *Journal Du Conseil*, **36**: 220-228.

LINNAEUS. C. 1758. Tomus I. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata. Holmiae. *Laurentii Salvii*, (1-4): 1-824

LÓPEZ-ALBORS, O., AYALA, M.D., GARCÍA-ALCÁZAR, A., ABELLÁN, E., LATORRE, R., RAMÍREZ-ZARZOSA, G., VÁZQUEZ, J.M. 2003. Early temperature effects on muscle growth dynamics and histochemical profile of muscle fibres of sea bass Dicentrarchus *labrax* L. during larval and juvenile stages. *Aquaculture*,**220**:385–406

LUCAS A. 1998. Programming by early nutrition: an experimental approach. *Journal of Nutrition* **128**,401–406.

MACQUEEN D.J., ROBB D.H.F., OLSEN T., MELSTVEIT L.,PAXTON C.G.M. & JOHNSTON I.A. 2008 Temperatureuntil the 'eyed stage' of embryogenesis programmes the growth trajectory and muscle phenotype of adult Atlantic salmon. *Biological Letters*, **4**: 294–298

MACKENZIE, B.R. AND T. KIØRBOE. 1995. Encounter rates and swimming behavior of pause-travel and cruise larval fish predators in calm and turbulent laboratory environments. *Limnol. Oceanogr*. 40: 1278–1289

MAILLET, G. L., AND CHECKLEY, D. M. 1990. Effects of starvation on the frequency of formation and width of growth increments in sagittae of laboratory-reared Atlantic menhaden *Brevoortia tyrannus* larvae. *Fishery Bulletin*, 88: 155–165.

MAKHOTIN, V. V., NOVIKOV, G. G., SOIN, S. G. & TIMEIKO, V. N. 1984. The peculiarity of the development of White Sea cod. *Flødevigen Rapportser*, **1**: 105–120.

MARKLE, D. F. AND FROST, L. A. 1985. Comparative morphology, seasonality, and a key to planktonic fish eggs from the Nova Scotian Shelf. *Canadian Journal of Zoology*, **63**: 246–257.

MARTELL D.J., KIEFFER J.D. AND TRIPPEL E.A. 2005. Effects of temperature during early life history on embryonic and larval development and growth in haddock. *Journal of Fish Biology*, **66**: 1558–1575.

MARTELL, D. J., KIEFFER, J. D. AND TRIPPEL, E. A. 2007. Effects of temperature during early life history on embryonic and larval development and growth in haddock. *Journal of Fish Biology*, **66**:1558–1575

MCINTOSH, W. C. AND PRINCE, E. E. 1888. On the development and life history of the teleostean food- and other fishes. *Transactions of the Royal Society of Edinburgh* 25 (part 3, no. 19), 665–944.

MILLER, T.J., HERRA, T., AND LEGGETT, W.C. 1995. An individual-based analysis of the variability of eggs and their newly-hatched larvae of Atlantic cod (*Gadus morhua*) on the Scotian Shelf. *Canadian Journal of Fisheries and Aquatic Sciences*. **52**: 1083–1093

MOSEGAARD, H., SVEDANG, H., AND TABERMAN, K. 1988. Uncoupling of somatic and otoliths growth rates in Arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**: 1514–1524.

NORWEGIAN FISHERIES DIRECTORATE 2013.*Atlantic cod: About the fishery*. Available at: http://www.fisheries.no/ecosystemsandstocks/marine_stocks/fish_stocks/cod/#.UjZdssZmgyo [Accessed 25 July 2013]

NISSLING, A. 2004. Effects of temperature on egg and larval survival of cod (*Gadus morhua*) and sprat (*Sprattus sprattus*) in the Baltic Sea - implications for stock development. *Hydrobiologia*, **514**: 115-123.

OTTERLEI, E., NYHAMMER, G., FOLKVORD, A. AND STEFANSSON, S. O. 1999. Temperature and size dependent growth of larval and early juvenile Atlantic cod (*Gadus morhua*): a cooperative study of Norwegian coastal cod and Arctic cod. *Canadian Journal of Fisheries and Aquatic Science.* **56**: 2099- 2011

OTTERLEI, E., FOLKVORD, A. AND NYHAMMER, G. 2002. Temperature dependent otolith growth of larval and early juvenile Atlantic cod (*Gadus morhua*). *ICES Journal of Marine Science*, **59**: 401-410.

PANKHURST N.W., PURSER G.J., VAN DER KRAAK G.,THOMAS P.M. AND FORTEATH G.N.R. 1996. Effect of holding temperature on ovulation, egg fertility, plasma levels of reproductive hormones and in vitro ovarian steroidogenesis in the rainbow trout *Oncorhynchus mykiss*. Aquaculture, **146**: 277-290

PAGE, F. H. AND FRANK, K. T. 1989. Spawning time and egg stage duration in Northwest Atlantic haddock (*Melanogrammus aeglefinus*) stocks with emphasis on Georges and Browns Bank. *Canadian Journal of Fisheries and Aquatic Sciences,* **46** (Suppl. 1), 68–81.

PECK, M. A. AND BUCKLEY, L. J. 2008. Measurements of larval Atlantic cod (*Gadus morhua*) routine metabolism: temperature effects, diel differences and individual-based modeling. *Journal of Applied Ichthyology*, **24**: 144-149.

PECK, M. A., LAWRENCE, D., BUCKLEYB, J. AND. BENGTSONC , D. A. Effects of temperature and body size on the swimming speed of larval and juvenile Atlantic cod (*Gadus morhua*): implications for individual-based modeling. *Environmental Biology of Fishes*, **75**:419–429

PEDERSEN T, FALK-PETERSEN IB (1992) Morphological changes during metamorphosis in cod (*Gadus morhua* L.) with particular reference to the development of the stomach and pyloric cecae. *Journal of Fisheries Biology* , **41**:449–461

PEPIN, P., ORR, D. C. AND ANDERSON, J. T. 1997. Time to hatch and larval size in relation to temperature and egg size in Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2-10.

PETERSEN, I. B., 2005. Comparative organ differentiation during early life stages of marine fish. *Fish & Shellfish Immunology*, **19**:397- 412

PETERSON, R. H., MARTIN-ROBICHAUD, D. J. & HARMON, P. 2004. Influence of incubation temperature on body movements of Atlantic cod (*Gadus morhua* L.) embryos and on size at hatch. *Aquaculture Research*, **35**: 453-457.

POGSON, G. H. AND FEVOLDEN, S.E. 2003. Natural selection and the genetic differentiation of coastal and Arctic populations of the Atlantic cod in northern Norway: a test involving nucleotide sequence variation at the pantophysin (PanI) locus. *Molecular Ecology*, **12**:63–74

PRYOR, M. AND BROWN, J. A. 1998. Effect of temperature on incubation time and development of Atlantic cod (*Gadus morhua*) eggs and larvae. *Bulletin of Aquacuture Association Canada, **98***(2)

PUVANENDRAN, V., CALDER-CREWE, C. AND BROWN, J.A. 2009. Vertebral deformity in cultured Atlantic cod larvae: ontogeny and effects on mortality. *Aquaculture Research,* **40**:1653-1660

PUVANENDRAN, V., FALK-PETERSEN, I.-B., LYSNE, H., TVEITEN, H., TOFTEN, H. AND PERUZZI, S. 2013. Effects of different step-wise temperature increment regimes during egg incubation of Atlantic cod (*Gadus morhua* L.) on egg. *Aquaculture Research*, 1–10

ROMBOUGH,P. J., 1988. Respiratory gas exchange, aerobic metabolism and effects of hypoxia during early life. In: HOAR, W. S. AND RANDALL, D. J. (Eds): *Fish Physiology* Vol. XIA: 59-61. Academic Press, San Diego

ROSENTHAL, F.C. AND ALDERCDICE, D. F. 1976. Effects of salinity and temperatur eon embryonic development of Atlantic cod (*Gadus morhua*). *Journal of Fisheries Research Board Canada*, **23**: 319-340

SERIGSTAD, B., 1987: Oxygen uptake of developing fish eggs and larvae. *Sarsia*, **72**: 369–371.

SKIFTESVIK, A.B. 1992. Changes in behaviour of onset of exogenous feeding in marine fish larvae. *Canadian Journal of Fisheries and Aquatic Sciences*. 49: 1570–1572.

SKJÆRVEN, K. H., OLSVIK, P. A., FINN, R. N., HOLEN, E. AND HAMRE, K. 2011. Ontogenetic expression of maternal and zygotic genes in Atlantic cod embryos under ambient and thermally stressed conditions. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 159, 196-205.

SOLBERG, T.; TILSETH, S., 1984: Growth, energy consumption and prey density requirements in first feeding larvae of cod (*Gadus morhua* L). In: The propagation of cod *Gadus morhua*, L. E. Dahl, D. S. Danielssen, E. Moksness, P. Solemdal (Eds), Institute of Marine Research Flødevigen Biological Station, Arendal, Norway, pp. 145–166.

SOGARD, S. M. 1991. Interpretation of otolith microstructure in juvenile winter flounder (*Pseudopleuronectes americanus*): ontogenetic development, daily increment validation, and somatic growth relationships. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**: 1862–1871.

STICKLAND, N. C., WHITE, R. N., MESCALL, P. E., CROOK, A.R., THORPE, J. E. 1988) The effect of temperature on myogenesis in embryonic development of the Atlantic salmon (*Salmo salar* L). *Anat Embryol (Berl)* **178**:253–257

SUTHERS, I. M. AND SUNDBY, S. 1999. Role of the midnight sun: comparative growth of pelagic juvenile cod (Gadus morhua) from the Arcto-Norwegian and a Nova Scotian stock. ICES Journal of Marine Science 53, 827–836.

THOMPSON, B. M. ANDRILEY, J. D. 1981. Egg and larval development studies in the North Sea cod (*Gadus morhua* L.). *Rapports et procès-verbaux des réunions / Conseil permanent international pour l'exploration de la mer*.,**178** : 553–559.

TVEITEN, H. 2008. Temperature influence on reproductive development and gamete quality in Atlantic cod (*Gadus morhua*). *Cybium*, **32**: 195-195.

Tveiten H., Solevåg, S. E. AND Johnsen, H.K. 2001. Holding temperature during the breeding season influences final maturation and egg quality in common wolfish. *Journal of Fish Biology*, **58**:3743-85.

VALERIO, P. F., GODDARD, S.V., KAO, M. H. AND FLETCHER, G. L. 1992. Survival of Northern Atlantic cod (*Gadus morhua*) Eggs and Larvae when Exposed to Ice and Low Temperature. *Canadian Journal of Fisheries and Aquatic Sciences*, **49**: 2588-2595

VAN DER MEEREN, T. AND IVANNIKOV, V. P. 2006. Seasonal shift in spawning of Atlantic cod (*Gadus morhua* L.) by photoperiod manipulation: egg quality in relation to temperature and intensive larval rearing. *Aquaculture Research*, **37**: 898-913.

VAN DER MEEREN, T. AND NÆSS, T. 1993. How does Atlantic cod (Gadus *morhua* cope with variability in feeding conditions during early larval stages? *Marine Biology*, **116**: 637-647

VIKEBØ, F., SUNDBY, S., ADLANDSVIK, B. AND FIKSEN, O. 2005. The combined effect of transport and temperature on distribution and growth of larvae and pelagic juveniles of Arcto-Norwegian cod. *Ices Journal of Marine Science*, **62**: 1375-1386.

VOLLSET, K. W., FIKSEN, O. & FOLKVORD, A. 2009. Vertical Distribution of larval Cod in Experimental Temperature Gradients. *Journal of Experimental and Marine Biology.*

VON HERBING, H.I., BOUTILIER, R. G., MIYAKE, T. AND HALL, B. K. 1996. Effects of temperature on morphological landmarks critical to growth and survival in larval Atlantic cod (*Gadus morhua*). *Marine Biology*, **124**: 593-606.

VON HERBING, H. I. AND S.M. GALLAGER. 2000. Foraging behavior in early Atlantic cod larvae (*Gadus morhua*) feeding on a protozoan (*Balanion sp*.) and a copepod nauplius (*Pseudodiaptomus sp*.). *Marine Biol*ogy, 136: 591–602.

VON WESTERNHAGEN, H. 1970. Erbrutung der Eier von Dorsch (*Gadus morhua* L.), Flunder (*Pleuronectes flesus* L.) und Scholle (*Pleuronectes platessa* L.) unter kombinierton Temperatur – und Salzgehaltsbedingungen. *Helgolander wissenschaftliche Meeresuntersuchungen*, **21**, 21–102.

WARE, D.M., AND LAMBERT, T.C. 1985. Early life history of Atlantic mackerel (*Scomber scombrus*) in the southern Gulf of St.Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**: 577–592.

WHEATER, P. R., BURKITT. H.G., DANIELS, V. G. 1987.*Functional Histology; A Text and Colour Atlas* 2nd ed. London:Longman group (FE) Ltd

WIELAND, K., WALLER, U. & SCHNACK, D. 1994. Development of Baltic cod eggs at different levels of temperature and oxygen content. *Dana*, **10**: 163-177.

WORLEY, L. G., 1933. Development of the eggs of the mackerel at different tem peratures. *Journal of General Physiology*, **16** (5): 841-857.

ZHAO, Y. CHEN, Y. & BROWN , J. A. 2001. Impacts of egg and larval size on survival and growth of Atlantic cod under different feeding conditions. *Journal of Fish Biology*, **59:** 569–581

APPENDICES

APPENDIX 1. Inclusion criteria based on PICOS (CRD, 2008)

Review Objective The objective of this review is to assess the effects of temperature on the early life stages of Atlantic Cod, *Gadhus morhua*	
P- Participants	Studies where the population studies was Atlantic cod, *Gadus morhua* Studies which included early life of Atlantic cod, *Gadus morhua (eggs, larvae or both).*
I- Interventions	Studies in which temperature or a combination of temperatures was one of more or the only factor in the experiments with Atlantic cod, *Gadus morhua*.
C- Comparators	Comparators included are any studies on early-life (embryo or larval) development within the same family as *G. morhua* with the same criteria for participants, interventions, outcomes and study designs stated in this table.
O- Outcomes	Any morphological or rate of development (timing) differences observed. These include (but not restricted to) survival, somite development, segmentation period in hours post-fertilization (hpf), hatching time (hpf), first feeding in days post hatch (dph), functional jaw and hind gut development (dph), vertebral malformations or other deformities.
S- Study design	Studies with a clear methodology using Randomised Controlled Trials (TCTs) and controlled trails and duplicates were preferred.

APPENDIX 11. Databases searched for literature to include in review

Name of database	Number of search hits	Number of relevant search results from title screening title
Aquatic Sciences and Fisheries Abstracts(ASFA)	323	31
CoS Conference papers Index (ProQuest)	341	24
CoS conference Index	33	31
FishBase	1	1
IOS Premetapress	nil	nil
JSTOR-History of Science and Technology	842	11
JSTOR-History of Science and Technology	110	13
NRC Canada Journals (research press)	931	57
Science Citation Index (ISI)	150	92
Web of Knowledge	19	17
Web of Science (ISI)	2	1
ScienceDirect(Elsevier)	48	14
ScienceDirect(Elsevier)	5	5
SCIRUS (Elsevier)	45	42
Zoological record Plus	42	23
Zoological Record Plus	23	15
Total	**3215**	**377**

APPENDIX III. Search fields used in literature search

- **su**(temperature) AND **su**((fish larvae OR embryogenesis)) OR **su**((effect of rearing temperature on cod developmnent))
- atlantic cod
- rearing temperature AND atlantic cod AND egg OR larvae AND development
- rearing OR (incubation AND temperature AND cod AND eggs)) AND **Abstract**: ((temperature effects AND atlantic cod AND eggs) OR larvae)
- Topic=(temperature effects) AND Topic=(atlantic cod) AND Topic=(embryology OR larvae OR development)
- Timespan=All years. Databases=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH.
- Topic=(temperature effects) AND Topic=(atlantic cod larvae) AND Topic=(egg development)

- Timespan=All years. Databases=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH.
- TS=(Temperature effects AND Atlantic cod AND embryogenesis SAME larval development)
- Databases=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH Timespan=All years

- 48 articles found for: TITLE-ABSTR-KEY(temperature OR embryology OR larvae development) and TITLE-ABSTR-KEY(atlantic cod)
- 5 articles found for: TITLE-ABSTR-KEY(temperature AND embryology OR larvae development) and TITLE-ABSTR-KEY(atlantic cod)
- title:temperature AND title:atlantic AND title:cod (embryology OR larvae OR development OR morphology OR organogenesis)
- temperature AND atlantic AND cod AND larvae AND growth
- temperature AND effects AND atlantic AND cod AND eggs AND development

APPENDIX IV. Summary map of studies included in this review

Paper ID.	Temperature (˚C)	Experiment duration	Response variables	Results	Cod Stock/ Population	Comments
1. Johnston and Øyvind. 2008	4 6 8	Incubation until adults	Muscle fibres at hatching	At hatching Number of fast myotomal muscle fibres (determined at 0.7 standard length) were greater at higher rearing temperature (10.4% greater at 8°C than at 4°C in NEA cod) At hatching number of fast myotomal muscle fibres was greater for NC cod than for NEA populations (32.7% greater at 4°C)	NC (Norwegian Coastal cod) and NEA (North East Arctic cod)	Effects of pantophysin (Pan1) genotype also checked simultaneously. Muscle fiber development differs with genotype differences between NC and NEA cod.
2. Von Herbing *et al.* (1996)	5°C ± 0.5 10°C± 0.5	Spawning to larval transformation to pelagic juveniles	9 functional morphological landmarks important to feeding respiration, locomotion:	Faster development at 10°C than at 5°C for all stages. Rapid structural change during early to mid yolk-sac stages (1-5) at both temperatures. Slower at later stages for both temperatures. Then late in development (after stage 9) rate accelerated for 5°C larvae but kept maintained or lower for the 10 °C larvae.	NF (Newfoundland) and NS (Scotian Shelf)	Eggs produced from two genetically discrete populations. NF eggs were between the 8 and 16- cell stages when they were acclimated to the experimental temperatures. NS batches of eggs between the 2- to 16-cell stages were acclimatized to experimental tempertures over a period of hours.

3. Pepin et al. (1997)	Fertilization : 1 2 3 Incubation : -1 1 3 5 7			Increased temperature = faster transition times Temperature had significant effect on development rates of cod eggs and yolk sac larvae than egg size	Broodstock of 30-50 adult cod bottom trawled and kept at University of Newfoundland (North East Newfoundland)	-Ranges of temperature chosen reflect the those that occur during spawning of cod on northeast NF shelf -Entire distributions of event times (not merely (mean and median) was used to depict schedule of transitions from one stage to the next.
4. Imsland et al. (2005)	7 10 13 16	Juvenile-Adult				Long term temperature effects: Not for data extraction as focus is on eggs and larvae
5.Van der Meeren & Ivannikov 2006	<9.6					No data extracted
6. Wieland et al. (1994)	2 7	Spawning to Pelagic juveniles	Study identified 5 development stages as in Westernhagen (1970) -**developmental time** was the incubation time from fertilization to the end of different developmental stages (5 equations for each stage) -mortality mainly due to oxygen..	-Higher temp (7˚C) from 2 ˚C, reduces the incubation period (time from fertilization to 50% hatching to 13.0 days from 27.5days -highest mortality rates throughout incubation period are at gastrulation period & imeediately b4 hatching -larvae always hatched prior to stage 5	Baltic Cod	Temperature and oxygen control were both factors tested -oxygen is a limiting factor for reproductive success of Baltic cod
7. Tveiten (2008)	4 and 12 when approaching spawning 4; 8; ;12 during ovarian growth			at elevated temperature : -Egg quality significantly reduced -reduced fertilization -reduced normal cell cleavages		
8. Puvanendran et al. (2013)	4.5 ± 0.5 throughout was control Control increased to 9, 5±0.5 in steps: 8hours=T1; 32hours=T2: 64hours= T3, 96hours= T4		Embryonic cell symmetry -embryonic mortality -hatching success -larval skeletal abnormalities Length and yolk sac volume	Fast increments in temperature (T1 8h step from 4.5 to 9.5°C) yielded higher egg mortalities and lower hatching success Larvae: -control larvae (4°C) were significantly longer, had smaller yolk reserves at hatch than T1-T4 T2 larvae had largest yolk reserves Embryonic Cleavage pattern hatching success and larval morphology and histology shows that T2 is the best gradual increment choice of temperature for future developmental programming of cod	Norwegian Cod Breeding Centre NCBC trosmsø	Cell asymmetries and embryo mortalities not significantly different between control and T2-T4 groups
9.Peterson et al. 2004	5.4° 0-10°C		-length at hatch -embryonic movements	Body movements not slow body flexes as in salmon but rapid movements ranging from predominat rapid twitchingto fictive swimming movementswith	Bay of Fundy Cod stock	

				the embryo revolving within the egg. No cod embryos active until 42 degree days post fertilization Number of active embryos increased to a Max of 9-10 for temp :0-6°C at 73.3-82.1degree days Increasing temperature decrease activity to 5-6 at 8°C And only an activity of 2 at 10°C		
10.Hall and Johnston (2003)	4 7 10		% time to first feeding and somite age Time of hatching	Time of first feeding equivalent across temperatures therefore % time to first feeding was used No differences in time of hatching and timing of appearanceof the otic placode, unpaired median fin, gut lumen, otic vesicle, lens of the eye otoliths.etc BUT Closure of blastophore delayed with respect to segmentation for high temperatures at 3-somite (at 4°C), 10-somite (at 7°C) and 12 somite (at 10°C)stages Muscle cellularity markedly altered in 10°C group compared to 4 and 7°C groups. 10°C larvae had significantly more deep white fibres at hatch (+14%) but numbers of superficial red fibres remained unchanged	Ardtoe Bay Scotland broodstock	
11. Peck and Buckley. (2008)	5.0 7.5 10.0		M_D; larval dry mass R_R routine respiration rate	Linear relationship between respiration and body size (ln R_R= .16.LnM_D – 6.57. Exponential increase in RR with temperature; Q_{10}`=3.00	Coastal Massachusetts, USA, cod in the vicinity of the Great South Channel	I hourly measurements of R_R
12.Otterlei et al, (2002)	(SD = 0.2) 4.1 ± sd 8.0± 0.1 for 1995 batch 6.1±0,1 10.0±0,2 12.0±0,3 14,1±0,2		Lapillus radius (whole and mean)	AT HATCH: Mean radius range of lapillus for NC = 10.8-11.3µm, and NA = 9.3-9.6µm for embryos incubated at 8°C However Note that pronounced temperature and size specific effects on lapillus growth were observed in experiments. Mean daily growth of otoliths (lapillus radius) increased with temperature from 4 to 14°C with a maximum observed otolith growth at 14°C being -10.5µmd^{-1} (mean 4.6),-NC -6.9 µm/d (mean 4.2) Quite the opposite, average otolith growth was -<1 (mean= 0.4-0.7) µ/d for both NC and NA stocks	Northeast Arctic cod (NA) Norwegian coastal cod (NC)	50% hatching time aka day 0 of larval stage (occurred 12dpf) at temperatures (7.3 - 7.9°C) for both seasons of natural spawning (April '95 and March'96) .NB. Larger cod had developed larger otoliths than the smaller ones of the same age under identical conditions, suggesting that temperature is not the only factor. Standard length and lapillus radius follows a common third order polynomial; RL = 1.871 + 2.043SL + o.239SL^2- 0.0033SL^3
13. Peck *et al*.(2006)	8±0.2°C		Two dimensional	The mean routine swimming speed estimates (both bursts and	Great South Channel fidh,	Three Larval cod swimming trials

			Swimming speed	pause phases) in equaled an average of ~ 2.4bls^{-1} at 8°C.	Massachusetts, USA	conducted: Computer image analysis of timed videos
14. Hall *et al* (2004)	7	Fertilization until first-feeding.	-Cell number -Somite number -Mean cell cycle time for the first 6 cleavages -Timing of segmentation period -Time of hatching -First feeding stage	-mean cell cycle time for the first six cleavages was 135min -somite development eqn: 0.29t – 18.14 -segmentation period began 220h post fertilization (hpf) -somite addition continued throughout most of embryonic development(unlike other teleosts) -hatching at 256 hpf -first feeding at end of 3rd dph -fiunctional jaw and hindgut developed by end of 3rd dph. -	Mallaig Scotland	Used as the baseline study for egg development stage descriptions.
15. Geffen et al. (2006)	6 8 10 12	Fertilization-50% hatch	4 camparable stage-endpoints: -end of blastula -end of gastrula -point of growth of embryo completely surrounding yolk -point when 50% eggs are hatched	Hatching rates: 16.4 days after fertilization (DAF) at 6° C, -10.3 DAF at 8° C - 9.4 DAF at 10° C -7.4 DAF at 12° C. Egg mortality (instantaneous mortality rate, z, Nt ¼ N_0e^{zt} whereN0 is total number of eggs sampled over the incubation period, Nt is the number of live eggs remaining on each sampling occasion after subtracting the number of dead eggs at each sampling occasion and t is stage duration in days) increased at the higher temperatures, but survival was >80%.	Irish Sea cod Gadus morhua	No consistent tends to differentiate Western or Northern vs. Southern populations
16. Fitzsimmons and Perutz (2006)	6 7 8 9 10 11	Fertilization – 1dph	survival/mortality malformations in veterbral column	-no significant differences in mortality between eggs incubated at diff temperatures. -61% (152 out of 249) hatched larvae showed at least one veterbral deformities: -kyphosis -lordosis -scoliosis -severe vertebral curvature -significant increase in prevalence of malformed larvae with severe veterbral curvature as egg incubation temperature increased. No significant differences in sum of prevalence of malformations between different groups (fish) and temperatures BUT significant differences netween types of malformations and different temperatures.	Shetland Isles	Malformations assessed immediately at hatch only for larvae with a heartbeat (living) nmmn
17. Galloway et al. (1998)	1 5 8 (transferred to 5 after hatch)	1dpf to hatching	At hatch axial musculature 5dph axial musculature		Northeast Arctic cod	Time of hatching was 50% eggs hatched, Larvae kept at 5°C after hatching
18. Jordan (2002) unpublished thesis	2 4 8 12	50% hatch to first feeding (samples taken every 4 degree days	standard length, development stage, yolk-sac area and whether or not	a consistent trend of decreasing time to 50 and 100% hatch with increasing temperature (trend not seen when time is expressed in degree days)	Broodstock; Logy Bay, Newfoundland, Canada	A thesis

		for the 4, 8, 12 temperature groups and everyday for the 2°C treatment)	the larvae were feeding.	There was no relationship between size at hatch and the incidence of first-feeding larvae, found a dome-shaped relationship between temperature and the incidence of first-feeding.		
19. Jordan et al. (2007)	2 4 8 12	50% hatch to first feeding	Standard length Yolk-sac area Feeding Development stage	there was a consistent trend of decreasing time to 50 and 100% hatch with increasing temperature.	Trinity Bay (48–00 N; 53–30 W)	Samples were taken until larvae was >45% of sample. Thereafter it was considered 50% hatch and hatching was initiated
20. Blaxter		Larval fish	Biochemical reaction rates -diffusion -complex biological processes: - development -muscle contraction -nerve conduction -locomotor performance	Lethal effects -controlling effects -directing effects -limiting effects -masking effects		Generalizations for larval fishes not Atlantic cod in particular
21. Laurence and Rogers (1976)	2 4 6 8 10 12	-embryo mortality -total mortality and viable prolarvae(larvae which have hatched but still retain a yolk sac) -time to 50% hatch -mean standard length at hatching -percentage of abnormalities		-highest percentage of variable hatch observed for 2-10°C -variable mean length at hatch across all temperatures -Time to 50 % hatch inversely related to temperature -Abnormalities appeared during development and were independent of temperature or salinity. -	Broodstock captured by demersla trawl off the mouth of Narragansett Bay, Rhode Island, 18km South of Chatham, Massachusetts at depth of 40m.	A factor experiment of temperature and salinity for cod and haddock
21. Bonnet , (1939)	6 8 10 12 14	Fertilization to hatch	Time to hatching	Time to hatch was 17 dpf	Ipswich Bay, Massachusetts	
22. Nissling, (2004)	Incubation temperatures: 1,0 2,9 7,3 9,0 10,8	-time to50% hatching -	Fertilzation to yolk absorption	-larval size significantly lower at 11°C and higher at 1°C -50% hatching to yolk absorption periods: 11°C (6-8dph) 9°C (7-9 dph) 7°C (8-10dph) 3°C (15-18dph) 1°C (22-26dph)	Baltic Sea	Hatch to 50% was in darkness
23. Valerio et al, (1992)	Sub zero temperatures	Egg Freezing point Larval homogenate freezing point	-freeze resistance of eggs -	Egg and larval homogenates did not appear to have antifreeze proteins (froze at meanf.p. -0.78 and – 0.88°C respectively) -But cod eggs dodnt freeze at -1.8°C in icy aerated seawater -Cod eggs could be undercooled to -4°C in ice Cod froze at temperatures between -4.1 and -17°C with	Off Newfoundland	Larvae could be cooled to -1.8°C withstanding freezing provided they were not brought into direct contact with ice crystalsLarvae froze at -1.36°C (Feeding stage) or -1.34°C (yolk-sac stage) if directly touched with ice. Approx. 0.5 lower than than expected

				freeze resistance depending on the integrity of the chorion		from the freezing temperatures of their body fluids -Freeze resistance maybe due to the nature of cods external epithelium and delayed development of sensitive gill structures below 0°C.
24. Pryor and Brown (1998)	3 temperature regimes Low (0-1°C) Medium (3-4) High 7-8	Fertilization to end of larval yolk stage	Incubation time Developmental time Dry weight Egg diameters Total length Yolk-sac width		Broodstock from Ocean Sciences Centre	Abstract only

TABLE II. Development stage schemes for cod eggs, synchronized to show comparable stage transitions or end points. Stage names and definitions in each column are based on the authors' own terminology

Period	Descriptor of start and end of stage	Laurence & Rogers (1976)	Thompson & Riley (1981)	Page & Frank (1989)	Bonnet (1939)	von Westernhagen (1970)	Markle & Frost (1985)	Makhotin *et al.* (1984)	Friðgeirsson (1978)	Hall *et al.* (2004)
Cleavage	First cleavage							1(I) Zygote formation	1: First cleavage	Zygote: first cleavage
	Two cells				First cleavage: two cells			2(II) Cleavage	2: Cleavage of blastodisc (two cells)	Cleavage: two cells
	Four cells								2: (four cells)	Cleavage: four cells
	Eight cells								2: (eight cells)	Cleavage: eight cells
	16 cells								2: (16 cells)	Cleavage: 16 cells
	64 cells					Iaα: 64 cells		3(II) Cleavage		
Blastula	128 cells (blastula)							4(III) Blastulation	2: Cleavage of blastodisc (big cell morula)	Cleavage: 128 cells (blastula)
	Blastodisc	I: Blastodisc				Iaβ: blastodisc		5(III) Blastulation	2: Cleavage of blastodisc (midcell morula)	Blastula: blastodisc
	Blastodisc				Late blastula: blastula	Iaγ: blastula		6(III) Late blastula	2: Cleavage of blastodisc (small cell morula)	Blastula: sphere stage
	Blastula							7(IV) Gastrulation	2: End of blastula	
	Germ ring		IA: Blastodisc	I			I: Germ ring	8(IV) Signet ring	2: Germ ring	Blastula: germ ring

TABLE II. Continued

Period	Descriptor of start and end of stage	Laurence & Rogers (1976)	Thompson & Riley (1981)	Page & Frank (1989)	Bonnet (1939)	von Westernhagen (1970)	Markle & Frost (1985)	Makhotin *et al.* (1984)	Friðgeirsson (1978)	Hall *et al.* (2004)
Gastrula	Start of gastrulation				Quarter gastrula: start of gastrulation	Ibα: Gastrula early			3: Gastrulation	Gastrula: germ ring
	Embryonic axis	II: Blastula to embryonic axis	IB: Signet ring to embryonic axis		Half gastrula: embryonic axis	Ibβ: Gastrula middle		9(V) Organogenesis	3: Embryonic axis	Gastrula: embryonic axis
	Epiboly					Ibγ: Gastrula late		10(V) Organogenesis	3: Epiboly	Gastrula: 25% epiboly
						IIα: Embryo primitive			4: Midgastrula	
						IIβ: Head, eye visible, blastopore still open		11(V) Organogenesis	4: Tailbud formation	
						IIγ: Pigmentation, myomeres			4: Optic bulbs	
	Closure of blastopore	III: Embryonic axis to closure of blastopore	II: Embryonic axis to closure of blastopore	II	18 somites: closure of blastopore	IIδ: Eyelense formed blastopore closed	II: Embryonic axis to closure of blastopore		4: Closure of blastopore	Gastrula: closure of blastopore
Embryo	Tailbud					IIIα: Embryo 180–270° around yolk eyelense complete pectoral buds visible		12 (VI) Embryo mobile state		Segmentation period: heart visible
						IIIβ: heart visible				

TABLE II. Continued

Period	Descriptor of start and end of stage	Laurence & Rogers (1976)	Thompson & Riley (1981)	Page & Frank (1989)	Bonnet (1939)	von Westernhagen (1970)	Markle & Frost (1985)	Makhotin *et al.* (1984)	Friðgeirsson (1978)	Hall *et al.* (2004)
	Tail at 90° angle to body		III: Embryo 180° to 270° around yolk		35 somites: tail at 90° angle to body	IIIγ: Slow heartbeats			5: Tailbud formation to first heartbeat (embryo 180–270° around yolk)	Segmentation period: regular heartbeat
	Embryo 270–360° around yolk		IV: Embryo 270–360° around yolk	III	Three quarters circle: embryo 270–360° around yolk	IVα: Embryo 270–360° around yolk, heart movement, eye pigment, embryo movement	III: Closure of blastopore to embryo 360° around yolk	13(VI) Embryo mobile state		Segmentation period: golden eye stage (embryo 270° around yolk)
						IVβ: Regular heartbeat, pectoral fins		14(VII) Embryo about to hatch		
	Embryo 360° around yolk to hatching	IV: Closure of blastopore to hatching	V: Embryo 360° around yolk to hatching	IV	Whole circle: embryo 360° around yolk to hatching		IV: Embryo 360° around yolk to hatching	15(VII) Embryo about to hatch	6: Embryo 270° around yolk to hatching	Segmentation period: 50% epiboly to hatching
Hatching	Hatching starts		Hatching starts		Hatching starts	Vα: Hatching starts				Segmentation period: hatching
	50% hatching	50% hatching		50% hatching		Vβ: 50% hatch				
	100% hatched		100% hatched			Vγ: 100% hatch				

APPENDIX VI. Temperature-dependent development rate equations on different cod stocks (Geffen et al., 2006)

TABLE III. Data sources for temperature-dependent development rates of cod from different stocks

Authors	Source for stage scheme	Stock	Temperature equation
Pepin *et al.* (1997)	Laurence & Rogers (1976)	North-west Atlantic, Newfoundland Shelf	Various
Markle & Frost (1985)	Markle & Frost (1985)	North-west Atlantic, Nova Scotian Shelf	None
Bonnet (1939)	Bonnet (1939)	North-west Atlantic, Georges Bank	Extracted data
Laurence & Rogers (1976)	Based on Markle & Frost (1985)	North-west Atlantic, Georges Bank	$D = 21{\cdot}96 - 1{\cdot}307$
Peterson *et al.* (2004)	Laurence & Rogers (1976)	North-west Atlantic, Bay of Fundy	$D = 42{\cdot}24e^{-0{\cdot}17T}$
Makhotin *et al.* (1984)	Makhotin *et al.* (1984)	North-east Atlantic, White Sea	None
Friðgeirsson (1978)	Friðgeirsson (1978)	North-east Atlantic, Icelandic	None
Iversen & Danielssen (1984)	von Westernhagen (1970)	North-east Atlantic, Norwegian Sea	Extracted data
Thompson & Riley (1981)	Based on Ryland *et al.* (1975)	North-east Atlantic, North Sea	$\mathrm{Ln}(D) = B + AT$
Dannevig (1895)		North-east Atlantic, North Sea	Extracted data (time to hatch)
Hall *et al.* (2004)	Hall *et al.* (2004)	North-east Atlantic, West of Scotland	None
von Westernhagen (1970)	von Westernhagen (1970)	North-east Atlantic, Baltic	Extracted data
Wieland *et al.* (1994)	von Westernhagen (1970) and Thompson & Riley (1981)	North-east Atlantic, Baltic	$D = ae^{-b*Tx}$
Nissling (2004)	Thompson & Riley (1981)	North-east Atlantic, Baltic	Extracted data (time to hatch)
Page & Frank (1989)	Based on Meek (1924)	Based on data from different cod stocks	$D = a\,(T + 2)^b$
This study	Thompson & Riley (1981)	North-east Atlantic, Irish Sea	$D = \alpha + \beta\,\ln(T)^{-1}$

D, time (days); *T*, temperature (° C).

APPENDIX VII. Morphological landmarks for Atlantic cod larvae as described by Von Herbing *et al*. (1996)

Landmark	Stage 1: 0–1 dph	Stage 2: 2–3 dph	Stage 3: 3–4 dph	Stage 4: 6–7 dph
H	rounded, deflected downward; mouth not open, oropharyngeal membrane intact; hatching gland cells on top of head	square shaped, detached ventrally from yolk-sac membrane; upper jaw cushion and lower jaw well defined, oropharyngeal membrane may be perforated	mouth open, subventral: jaw angle distinct	jaw angle of the head is distinct formed by posterior growth of the retroarticular cartilage; mouth is sub-ventral
YS	large, spherical, full of yolk; > 95% yolk remains	elliptical and reduced; 70–100% yolk left	yolk-sac reduced; 50–70% yolk left	still contains yolk; 25–50% yolk left
AT	straight simple tube, no differentiation; anus not formed	intestine has expanded; constriction formed between mid-gut and hind-gut (rectal valve, separates food in foregut from hind gut); anus formed	liver enlarged; rectal valve present	foregut (oesophagus) is elongated and the mid-gut has expanded; intestine convoluted
GC	rounded vent or gill cavity at junction of head and yolk-sac membrane	gill cavity has grown ventrally	gill cavity has extended ventrally forming a gill slit, separating the head from the abdominal region	thin epithelial border growing from the hyoid
G	four gill arches; no gill filaments or secondary lamellae	four gill arches; no filaments or lamellae	gill arches covered by epithelial layer, which will later give rise to gill filaments; arteries with pigmented blood cells present in each arch	four gill arches with epithelial covering
SB	connected to the intestine (Morrison 1993), future position can be seen antero-dorsally to intestine under a dense pigment cluster	small distinct sac, dorsal to intestine and covered with pigment	a distinct sac covered dorsally by chromatophores	enlarged rounded sac, dorsal pigmentation is dense; SB not yet functional
FF	surrounds the whole body including yolk-sac; supracephalic sinus is small, not elevated	supracephalic sinus is elevated	supracephalic sinus of the dorsal finfold has reached its maximum elevation	supracephalic sinus maximally elevated
F	pectoral fins are small, round and well formed	simple, rounded pectoral fins	rounded pectoral fins	large cartilaginous base to pectoral fin
P	transparent with a few pigment spots spread along the trunk; eyes are large in proportion to the head, but only partially pigmented	2 post-anal bands on the trunk, some cover the hindgut; eye is heavily pigmented	pigment bars extend further along the body, additional pigment covering the abdominal region; eyes are fully pigmented displaying deposits of guanine which make them appear golden in reflected light	body pigmentation extended anteriorly covers most of the anterior trunk area and some of the intestine

Landmark	Stage 9: 35 dph	Stage 10: 50 dph	Stage 11: 60 dph	Stage 12: 70 dph
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

Landmark	Stage 5: 8–9 dph	Stage 6: 12–13 dph	Stage 7: 17–18 dph	Stage 8: 25–26 dph
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 posterio-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

APPENDIX VIII. Summary of results for high temperature effects on Atlantic cod early life

Effect of high temperature on Egg/Embryo development	**References**
Reduced incubation period	Wieland *et al*., 1994 Pryor and Brown, 1998
Reduced egg quality; reduction in fertilization percentage; High occurrence of abnormal cell cleavage	Tveiten, 2008
High proportion of normally cleaving eggs for faster temperature increase (stepwise); Higher mortality of embryos at T1 of an 8h-stepwise increase from 4.5 to 9.5°C	Puvanendran *et al*., 2013
embryo body activity	Peterson *et al*. 2004
Significantly low or smaller larval size (11°C) ; Shorter time from start of feeding to completely exhausted yolk implying a high demand for food availability;	Nissling, 2004; Pryor and Brown,1998; Peterson *et al*., 2004
Faster developmental rates (short developmental time	Galloway *et al.*, 1998; Pryor and Brown, 1998
Higher egg mortalities, lower hatching success for fast temperature increments (8hourly) from zygote stage to hatching.	Puvanendran *et al.*, 2013
Increased prevalence of severe vertebral curvature	Fitzsimmons and Perutz, 2006
Lower time to hatch and much shorter hatching period	Laurence and Rogers, 1976

APPENDIX IX. Summary of results for low temperature effects on Atlantic cod early life

Effect of low temperature on Egg/Embryo development	**References**
Significantly longer larvae, with smaller yolk reserves at hatch Long incubation period	Puvenandran *et al*., 2013
High embryo body movement; most active embryos (a mean of 5.5 movements per 10 min) being those kept at 2°C	Peterson *et al*.,2004
Longer developmental time (approximately by half)	Pryor and Brown , 1998; Von Herbing *et al*. 1996, Hall and Johnston, 2003
Shorter larvae with larger yolk sac and more small deep fibres at hatching (at 1°C); less myofibrils 5dph.	Galloway et al, 1998
Significantly greater sized larvae (1°C); Longer periods from 50% hatching to yolk absorption	Nissling, 2004

APPENDIX X. Time to hatching for different Atlantic cod stocks in days and degree days.

Reference	Hatch definition	Stock	Incubation Temperature°C	Days until hatching	Degree days (D°) until hatching
Galloway et al., 1998	50 % hatch	Northeast Arctic cod	1 5 8	35 16 11	37 79 89
Jordan 2002	50 % hatch	Newfoundland cod batch 1	2 4 8 12	31 21 11,5 6,3	59 84 93 79
Jordan 2002	50 % hatch	Newfoundland cod batch 2	2 4 8 12	15 12 6 4	32 47 48 50
Jordan 2002	50 % hatch	Newfoundland cod batch3	2 4 8 12	34 20 9,5 6	32 70 77 74
Jordan 2002	100 % hatch	Newfoundland cod	2 4 8 12	32 23 11,5 7,3	61 92 93 91
Jordan 2002	100 % hatch	Newfoundland cod	2 4 8 12	17 14 7,5 4,3	36 55 66 54
Jordan 2002	100 % hatch	Newfoundland cod	2 4 8 12	36 23 10 6,3	33 81 81 79
Laurence and Rogers, 1976	50 % hatch	Narragansett Bay, Massachusetts	2 2 2 2 2 2	24 22 21 22 21 24	-
Laurence and Rogers, 1976	50 % hatch	Narragansett bay, Massachusetts	4 4 4 4 4	18 18 17 13 13 13	-
Laurence and Rogers, 1976	50 % hatch	Narragansett bay, Massachusetts	6 6	12 12	

			6 6 6 6	12 12 12 12	
Laurence and Rogers, 1976	50 % hatch	Narragansett bay, Massachusetts	8 8 8 8 8 8	10 10 11 10 11 10	-
Laurence and Rogers, 1976	50 % hatch	Narragansett bay, Massachusetts	10 10 10 10 10 10	9 9 9 9 9 9	--
Laurence and Rogers, 1976	50 % hatch	Narragansett bay, Massachusetts	12 12 12 12 12 12	8 8 8 9 9 8	-
Bonnet, 1939	Unknown	Ipswich Bay, Massachusetts	6	17	-
Otterlei et al	50% hatch	Northeast arctic and Norwegian coastal cod	7.6	12	-