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Thermal dynamics of ovarian maturation in Atlantic cod (*Gadus morhua*)

Olav Sigurd Kjesbu[1,*], David Righton[2], Maria Krüger-Johnsen[1], Anders Thorsen[1], Kathrine Michalsen[1], Merete Fonn[1] and Peter R. Witthames[2,3]

[1]Institute of Marine Research, P.O. Box 1870, N-5817 Bergen, Norway (e-mail: olav.kjesbu@imr.no; maria.kruger-johnsen@imr.no; anders.thorsen@imr.no; kathrine.michalsen@imr.no; merete.fonn@imr.no)

[2]Centre for Environment Fisheries and Aquaculture Science, Pakefield Road, Lowestoft, Suffolk NR330HT, UK (e-mail: david.righton@cefas.co.uk; peter.witthames@cefas.co.uk)

[3]Present address: Fecund Fish Consultancy, 40 Plumtrees, Lowestoft, Suffolk, NR32 3JH, UK (e-mail: fecund-fish@tiscali.co.uk)

*Corresponding author (tel.: +4755238500, fax: +4755238555)

Abstract: The timing and success of spawning in marine fish is of fundamental importance to population persistence, distribution and, for commercial species, sustainability. Their physiological processes of reproduction are regulated, in part, by water temperature, and therefore changes in marine climate may have dramatic effects upon spawning performance. Using Atlantic cod (*Gadus morhua*) as a case study, we examined the links between water temperature, vitellogenesis and spawning time by conducting extensive laboratory and field studies. Our experiments documented that vitellogenesis generally starts at autumnal equinox, and that oocyte growth and investment is greater in cod held at warmer temperatures. Furthermore, spawning occurred earlier when oocyte growth was more rapid. The experimental results were confirmed by measurements of oocyte growth collected from wild caught cod in northern (Barents Sea) and southern (Irish and North Seas) populations. A model of oocyte maturation was successfully developed to explain the results. This model was consistent with published egg production curves of cod from the Barents Sea, North and Irish Seas, considering *in situ* temperatures recorded by individual data-storage tags on cod in those areas. These findings have considerable relevance for future studies of fish recruitment in relation to climate change.

Keywords: cod, vitellogenesis, temperature, light, spawning

Introduction

Current scenarios of climate change are based on extensive analyses of a suite of environmental variables and have led to the conclusion that many natural systems are being affected by regional climate changes, particularly temperature increases (IPCC 2007). The focus on temperature has resulted in many studies examining the link between species distributions and climate change (Parmesan and Yohe 2003). The marine environment is no exception, with some authors suggesting that the southern limit of species distributions are rapidly moving northwards (Perry et al. 2005).

While species distributions are likely to change, many populations will persist under sub-optimal conditions and therefore it is also necessary to understand how changes in marine climate will affect vital processes (Drinkwater 2005; Pörtner et al. 2008). This can be achieved by undertaking process-oriented and mechanistic studies, and placing the results in the framework of metabolic ecology (Brown et al. 2004; Sousa et al. 2008). For example, Pörtner et al. (2008) concluded that, based on studies on eelpout (*Zoarces viviparous*) and Atlantic cod (*Gadus morhua*), temperatures beyond pejus ('turning worse') are first felt at the whole organism level (due to oxygen-limited thermal tolerance) followed by reductions in growth and reproductive investment. Thus the physiological effects of extreme warming seas are likely to have far-ranging effects on population dynamics (Brander 2007).

Atlantic cod is a species of high commercial and socioeconomic value that occurs throughout the north Atlantic in waters predicted to be amongst those which show the largest temperature rises in the world (Drinkwater 2005; IPCC 2007). Cod grow faster, becomes fatter, mature at an earlier age, and are distributed further north with increasing environmental temperature (Dutil and Brander 2003; Drinkwater 2005; Sundby and Nakken 2008). The reproductive performance of cod is also sensitive to

temperature. Overall fecundity seems to increase with temperature (Kjesbu et al. 1998; Pörtner et al. 2001), but the quality of the eggs may be reduced beyond ~10°C (Pepin et al. 1997; Geffen et al. 2006; van der Meeren and Ivannikov 2006). The physiological links between water temperature and reproductive success are likely traceable to the aerobic demands of their large reproductive organs (up to 20% of body weight) (Pörtner and Farrell 2008; Pörtner et al. 2008). The ovary of a spawning cod is highly active sequestering vitellogenin from the plasma, i.e., during spawning there is a significant increase in the transport of protein from the white muscle via the liver to the high number of developing oocytes (Kjesbu et al. 1991, 1996).

In addition to the number and quality of eggs, the timing of spawning behaviour is also critical to reproductive success (Wright and Trippel 2009). Cod spawn earlier in the year in the warmer (more southerly) areas of the species' distribution (Brander 2005), which appears to be the result of the interaction between three main factors. First, the environmental conditions suitable for larval development occur earlier in the year in warmer seas (Planque and Fredou 1999). Second, the onset of sexual maturation in cod is related to day length (Bromage et al. 2001; Norberg et al. 2004; Davie et al. 2007). Finally, the temperature that cod experience during the vitellogenic period influences the timing of egg release (Kjesbu 1994), with egg release being delayed in colder waters.

Developing a clearer and more precise understanding of the exogenous factors regulating reproductive development, and their interaction, is a necessary prerequisite for predicting the effect of warming seas on reproductive success in marine fish. To do so requires a comprehensive, process-oriented analysis based on existing knowledge combined with new information from experimental and field studies. We took advantage of recent advances in image analysis and ovary sampling techniques

(Kjesbu et al. 1996; Thorsen and Kjesbu 2001; Witthames et al. 2009) to track oocyte growth in cod under experimentally controlled conditions. Our target was to determine the underlying principles regulating the natural maturity cycle of cod under different environmental conditions, and to establish an accurate and precise oocyte growth curve, ideally applicable to all stocks. Gonad growth apparently commences around the time of autumnal equinox (Woodhead and Woodhead 1965; Kjesbu 1991; Davie et al. 2007) but more exact information is required to pin-point the time of vitellogenesis initiation in the year. Likewise, the rate of development of oocytes under different temperature regimes has not yet been adequately shown but modelled using physiological principles (Kjesbu 1994). Secondly, we integrated this understanding with temperature data collected using electronic archival tags on wild cod under natural conditions to enable us to answer the question of how trade-offs between body growth and reproductive performance are influenced in different thermal environments, addressing in particular the effect of temperature on variation in reproductive traits like fecundity and size-specific spawning time. Finally, the overall spawning time model should as far as possible be simple to run, i.e., be based on mechanistic principles rather than new raw data, and properly tested by consulting published egg production curves for cod in different waters.

Materials and methods

Laboratory study

Main protocol

The experiment took place at the Institute of Marine Research (IMR) in Bergen between 1 June 2005 and 26 January 2006 using reared local Norwegian Coastal cod brought to the laboratory for acclimation in December 2004. The fish were produced

semi-extensively at the IMR marine pond facility Parisvatnet, Øygarden, west of Bergen (Blom et al. 1994) in the spring of 2003, subsequently vaccinated against vibrosis, and transported to IMR Austevoll Research Station in March 2004 for on-growth. At arrival in Bergen an equal number of fish were placed into two identical semi-rectangular 30-m^3 outdoor concrete tanks (water depth: 1.8 m). The top of the tank was covered by a net to reduce light intensity (by about 70%). Sea water was continuously supplied from the bottom of the nearby deep fjord, after sand-filtration and degassing, to refresh the water in each tank. In January 2005, all individuals were anaesthetized (60 ppm benzocaine in oxygenated sea water) and sex determined by gonad catheterization ('biopsied') using a Pipelle de Cornier® endometrial suction curette (Witthames et al. 2009), and PIT-tagged. As expected (Svåsand et al. 1996), all these 2-yr-old individuals were sexually mature; both running males and maturing/running females were noted. As for the period at Austevoll, the fish were given dry feed (www. skretting.no: Amber Neptun) consisting of 52% protein, 18% fat and 9.6% nitrogen-free extracts with a total energy content of 21.0 $MJ \bullet kg^{-1}$. In late spring 2005 a special broodstock feed (Vitalis Repro Cod) from the same producer was used to speed up recovery after the completion of the first spawning season.

At the start of the experiment all fish were mixed together and randomly reassigned to the same tanks used previously. In one of the tanks (n = 80) the water was consistently maintained at ambient temperature (AT), i.e., at approximately 9-10 ^{o}C throughout the year, while in the other tank (n = 75) the temperature was gradually reduced overnight to 5 ^{o}C, designated as low temperature (LT). Thus, the guidelines of Schmidt-Nielsen (1983) for further Q_{10} studies (see below) were followed strictly, including that the experimental temperatures should be 'sufficiently far apart' but still fall within the natural temperature range of the fish (Sundby 2000; Brander 2005).

Fish were handfed *ad libitum* three times per week (Kjesbu et al. 1991) using the standard dry feed described above but no food was given 2-5 days before weighing of the fish. Care was taken to stop feeding when appetite dropped markedly. The amount of uneaten pellets (waste feed) was judged from the number of pellets remaining on the tank bottom 1 h after feeding whilst the maximum stocking density was 8 $kg \bullet m^{-3}$. The tank was vacuum-cleaned to remove any waste feed and excreta once a week.

Every month, 1 June (Day 0), 5 July (Day 35), 3 August (Day 64), 6 September (Day 98), 6 October (Day 128), 2 November (Day 155), 6 December (Day 189) and 10 January (Day 224) all specimens were anaesthetized, weighed (W, 1 g) and measured for total length (TL, 0.5 cm below). In total 75 LT and 74 AT individuals (both genders) could be successfully followed over the whole experiment. Biopsies were taken from Day 128 onwards to establish oocyte growth curves (Kjesbu 1994). Initiation of spermiation was tested by hand stripping. Oocyte sizes, measured on Day 224, together with established oocyte growth rates were used to define the time for termination of the experiment, with the aim that the groups would consist of both prespawning ('prespawners') and spawning females ('spawners'). Consequently, all specimens were exposed to a lethal dose of anaesthetic and killed on Day 240 (26 January) by a blow to the head. Total length and whole body weight was measured and the whole gonad, liver and remaining viscera were carefully removed and weighed (0.1 g). Apart from a few exceptions, only data from the females are presented here.

Analysis of ovarian biopsies

About 0.25 g of ovarian tissue (n = 344) was collected from each female on Day 128, 155, 189, 224 and 240, removing a total of ≤ 1 % referring to the final ovary weight measured on Day 240. The samples were fixed in 3.6 % phosphate buffered

formaldehyde (Bancroft and Stevens 1996), stored and analysed automatically for oocyte diameter > 200 µm (Thorsen and Kjesbu 2001). Out of the 200 normal oocytes (follicles) measured per sample the largest 10% was defined as the leading cohort (LC) and the corresponding mean diameter (LC diameter) used to specify the maturity phase (West 1990; Kjesbu 1994; Thorsen and Kjesbu 2001). Similar data on the smallest 10% of oocytes were taken to reflect the smallest cohort (SC diameter), to study hiatus (gap) development between previtellogenic (≤ 250 µm) and vitellogenic oocytes (> 250 µm) (Sivertsen 1935) and thereby termination of oocyte recruitment. Some caution should be expressed for data between 200-250 µm due to contrast problems during the automated procedure. The vitellogenic oocyte distribution of cod should be considered as unimodal and homogeneous throughout the ovary (Witthames et al. 2009). The width of this distribution, reported as standard deviation ($SD_{diam.}$), was included to strengthen the understanding of oocyte growth dynamics and to indicate portion of eggs spawned, seen by a gradual fall in $SD_{diam.}$(Kjesbu et al. 1990). Hydrated or ovulated oocytes, used as spawning markers, were noted but not measured. Individual fecundity was given from oocyte packing density (number of oocytes•g^{-1}), estimated from the mean diameter of all 200 oocytes, multiplied with whole ovary weight, i.e., the Auto-diametric method (Thorsen and Kjesbu 2001). To test for normal oocyte development a limited numbers of samples were processed histologically using conventional protocols, i.e., Technovit® as embedding medium and 2 % toluidine blue and 1 % sodium tetraborate as stain.

Experimental definitions and calculations

Data on body growth were split by gender and tank (LT and AT), while spawning status (prespawners or spawners) was added as a third category when considering reproductive information. Common expressions were applied in all calculations.

196 Growth analyses included: 1) specific growth rate (G, in percentage), $G = 100\times(\ln W_2$
197 $- \ln W_1)/(t_2 - t_1)$, where W_1 is initial weight at time t_1 and W_2 final weight at time t_2,
198 and 2) daily length increment (DLI, in mm•day^{-1}), $DLI = 10\times(TL_2 - TL_1)/(t_2 - t_1)$
199 (Svåsand et al. 1996), with TL_1 corresponding to total length at t_1 and TL_2 to total
200 length at t_2. To be able to compare overall G with previous studies, males were also
201 included in the calculation.
202 Reproductive investment was defined as: 1) fecundity (F, number of vitellogenic
203 oocytes); 2) relative fecundity (RF, number of vitellogenic oocytes•whole body
204 weight^{-1}); or 3) gonadosomatic index (GSI, 100×gonad weight•whole body weight^{-1}).
205 Fish condition was presented either as liver (hepatosomatic) index (HSI, 100×liver
206 weight•whole body weight^{-1}), or Fulton's condition factor (K, 100×whole body
207 weight•total length^{-3}). Occasionally ovary-free weight (somatic weight) replaced
208 whole body weight, marked with subscript $_S$. The influence of experimental
209 temperature on daily growth in LC diameter was established by: 1) common linear
210 regression analysis at either the group (tank) or the individual level and 2) estimation
211 of the Q_{10} value, $Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$ (Schmidt-Nielsen 1983) where estimated slopes
212 (rates) from the previous regressions were labelled as $R_{1,LT}$ and $R_{2,AT}$ and
213 corresponding temperatures as $T_{1,LT}$ and $T_{2,AT}$. Predictions of a new rate (R_{new}) at
214 another temperature (T_{new}) was found after rearrangement of the standard Q_{10} formula:
215 $R_{new} = R_{1,LT}\times Q_{10}^{(T_{new}-T_{1,LT})/10}$ (or $R_{new} = R_{2,AT}\times Q_{10}^{(T_{new}-T_{2,AT})/10}$) (Schmidt-Nielsen
216 1983). Final maturation for the presently fixed oocytes was set to start (eccentric
217 germinal vesicle (GV)) and end (GV breakdown) at a LC diameter of 875 and 1000
218 µm, respectively, found after conversion (Thorsen and Kjesbu 2001) of fresh oocyte
219 data (Kjesbu et al. 1996). The length of the vitellogenic period, i.e., from 250 to 875
220 µm, was given as $625/R_{1,LT}$ and $625/R_{2,AT}$ (days).

Feeding ration (FR) was calculated for each of the eight successive periods between fish measurements, and for the whole experiment as such. In the first situation FR was calculated as the total amount of feed eaten during each period divided by estimated total fish biomass in the tank midway in the period ((ΣW_1 + ΣW_2)/2) and the number of days in question ($t_2 - t_1$). FR for the whole experiment was weighted mean periodic feeding ration.

Experimental water temperature was reported as grand mean weekly temperature based on 3-7, usually 5, measurements per week. Temperatures limited to the vitellogenic period ($T_{vit.}$) were given separately.

Field study

DST-recorded temperatures

Information on temperatures experienced during the length of the maturation cycle, recorded by data storage tags (DSTs), was acquired from previous projects studying free-ranging cod. The temperature data were compiled from the longest DST records available in the English Channel (Channel), southern North Sea and Irish Sea (southern waters) and the Barents Sea (northern waters).

In the case of the northern individuals (n = 6), all appear in Godø and Michalsen (2000), showing the following total length at release (tag number in parenthesis) in March 1996: 64 (246), 74 (117), 72 (131), 65 (204), 73 (206) and 81 (44) cm. Successful recording times were 12 ± 1 month. The last fish mentioned was tagged at the spawning ground in Lofoten, i.e., considered to be sexually mature, while the others were tagged at the Finnmark coast and mostly believed to be sexually immature. However, the majority were likely to be sexually mature at recapture in 1997, seen by consulting the corresponding length-at-age key and maturity-at-age ogive reflecting a probability of 73 – 93% (ICES Advisory Committee 2008). The

246 DST was attached externally and the temperature recorded (precision: ± 0.2 ºC) in
247 weekly cycles of every 2 h for the first 6 days and every 12 h on the 7th day. In
248 southern waters DST data (accuracy: ± 0.1 ºC; precision: 0.03 ºC) were collected from
249 10 specimens tagged between 1999 and 2005 showing recording times comparable to
250 those given above for the Barents Sea cod but using a higher measuring frequency of
251 once per 10 min (Neat and Righton 2007). Data were collected from tags attached
252 either externally or implanted internally but the difference in site was considered
253 unimportant (Righton et al. 2006). Also, any between-year variation recorded was
254 considered negligible in relation to within-year fluctuation (Neat and Righton 2007).
255 Thus, to ease visual comparison, monthly-resolved data, including also for the cod in
256 the north, were plotted within a single year. The tagged cod in southern waters were
257 released in February, except for the largest fish measured, i.e., an 86-cm Channel cod,
258 being released in March. The total length for the other nine specimens ranged 47 – 66
259 cm. The examined Channel and North Sea cod showed 75 – 100 % probability of
260 being sexually mature (R.D.M Nash, IMR, Norway, Final Report, RASER (EU-
261 project Q5RS-2002-01825)) whilst the one examined from the Irish Sea certainly was
262 sexually mature (Armstrong et al. 2004).

263 ***CTD-recorded temperatures***

264 IMR Barents Sea CTD (conductivity, temperature and depth) files were consulted
265 to indicate the effect of annual variation in environmental temperature on gonad
266 maturation. More specifically, the average temperature in August-September (1998-
267 2007) in the Vardø North Transect (72º15'N – 74º15'N, 31ºE, depth: 50-200 m) was
268 compared with development in maturity stage as observed 6-7 months later.
269 Considered temperatures correlated well with bottom temperatures (R. Ingvaldsen,

IMR (personal communication, 2008)) in the centre of the cod distribution (Sundby 2000) just before or at initiation of vitellogenesis (Kjesbu 1991).

Vardø North Transect temperature was in one case contrasted with similar type of information from DSTs. Transect temperature measured in August/September 1996 and January 1997 were averaged and related to average DST temperatures between 1 September 1996 and 1 February 1997 for each of the above-mentioned six Barents Sea cod.

Fish sampling and analyses

Adult cod were collected both from northern and southern waters using very much the same protocols, although in the last situation the sampling was spread over several days, including into the spawning season. Northern fish were worked up just after landing of the catch while this procedure took place onboard for the southern fish.

The sampling in the northern area was part of the regular 'Andenes fecundity time series' (Kjesbu et al. 1998; Thorsen et al. 2006), i.e., examining females (n = 486) captured by commercial vessels over a period of 1-2 days in early-mid March (calendar day 57-74) off the Vesterålen region, Northern Norway (69º19'N 16º09'E). The Andenes study was limited to Barents Sea (Northeast Arctic) cod, excluding 5-30% of the material classified, from the otolith, as Coastal cod. Otoliths were also used for reading of age and spawning zones (Rollefsen 1934). Presently eight years spanning from 1999 to 2008 were included. Thus, only ovarian samples analysed after the introduction of the Auto-diametric method (see above) were considered. Generally, close to 100 % of the fish were prespawners, i.e., only a few spawners were detected based on the presence of hyaline or ovulated oocytes. In 2006 an extra sample was taken in mid-February (calendar day 43-44: 'early 2006') to be compared with the standard sample (calendar day 66-67: 'late 2006'). To further evaluate

representativeness of the adopted sampling scheme, year-specific length-at-age data were contrasted with similar type of data available from the statutory Lofoten-Vesterålen survey in mid-March - late April (Korsbrekke et al. 2001; ICES Advisory Committee 2008). However, as these data were not resolved by sex, the present comparison was limited to 2005-2007, i.e., in years when the Andenes program was extended to include males. Each fish was physically characterized by its total length (1 cm below), weight of the whole body (10 g), ovary, liver and viscera (1 g). Viscera comprised of all organs left in the body cavity after removing the ovary and liver, and as much as possible of the oesophagus. Any stomach content was judged by dominant species. Cases where the stomachs were devoid of contents were noted as a special category.

Fish from the southern area were collected in 2004 in the central North Sea (n = 41) and the eastern Irish Sea (n = 38) from catches made with IBTS gear (North Sea) or a commercial rock hopper trawl (Irish Sea). Due to low catchability, the collection of specimens was stretched over 34 days (22 January – 24 February) and 10 days (10 – 19 February), respectively. Hence special emphasis was placed on the establishment of relevant maturity standardisation techniques, as detailed in the Result Section. Only total lengths (1 cm below) along with measured oocyte data (see below) were considered. Spawners showed either hydrated/ovulated oocytes, or recent post-ovulatory follicles. The latter structures were detected in resin sections specially aimed for this purpose, i.e., using PAS – Mallory's trichrome stain (Witthames et al. 2009). As previously, final oocyte maturation was set to be introduced at 875 µm, as there were no indications of any deviation.

Analysis of ovarian sub-samples

All sampling was carried out from the right ovarian lobe using either a plastic pipette with a wide opening (IMR) or a standardized Wiretroll II pipette (Bohit) (Cefas) (Witthames et al. 2009). The corresponding LC diameter was added to the established fish database and supplemented with mean oocyte diameter for Barents Sea cod to estimate fecundity. Variation in LC diameter across the whole ovary was about ± 10 µm (SE) tested on seven Barents Sea cod (total length: 89-111 cm) in the standard Andenes program (2003), cf. also Fig. 1 in Witthames et al. (2009). Calibration tests performed between institutes showed that the two image analysis programs used were fully compatible (Witthames et al. 2009).

Field-related definitions and calculations

The following relationship was established between weight of viscera with empty stomach (VW_{empty}, g) and total length (TL, cm) for Barents Sea fish:

(1) $VW_{empty} = 1.38\times10^{-5}\times TL^{3.722}$ ($r^2 = 0.940$, df = 1,56, $p < 0.001$,TL: 58-124 cm).

Prior to antilogarithm, the constant had a logarithmic value of -11.19 and an associated SE of 0.57. SE for the exponent was 0.126. No year effect was noted (late 2006 vs. 2008) (slope: $p = 0.074$; intercept: $p = 0.178$) (ANCOVA). Thus, whole body weight (W, g) could be corrected ($W_{corrected}$, g) for varying stomach content:

(2) $W_{corrected} = W – (VW – VW_{empty})$,

where VW is recorded weight of viscera (g). At a given length, based on all standard Andenes samples, expected body weight ($W_{expected}$, g) was:

344 (3) $W_{expected} = 2.76\times10^{-3}\times TL^{3.266}$ ($r^2 = 0.970$, df = 1,445, $p < 0.001$, TL: 54-128 cm).

345

346 The constant showed a logarithmic value of -5.89 with SE equal to 0.12. The

347 exponent SE was 0.027.

348 Based on these approaches, viscera condition, $C_{viscera}$, and fish condition C_{weight},

349 were defined as: $C_{viscera} = VW/VW_{empty}$ and $C_{weight} = W_{corrected}/W_{expected}$. The latter

350 expression was used to handle problems with size-dependency in condition (Scott et

351 al. 2006), simultaneously cancelling out any noise in the data caused by varying

352 stomach content. Specific growth rate was found from the standard formula (see

353 above) defining W_1 and W_2 as $W_{corrected, age\ 8}$ and $W_{corrected, age\ 9}$, respectively, and (t_2-t_1)

354 as 365 days, i.e., studying separate cohorts (Dutil and Brander 2003). Annual total

355 length increment (ALI) was estimated as total length divided by the corresponding

356 age ($cm \bullet year^{-1}$).

357 **Light cycle**

358 The duration of daylight from 1 June 2005 to 26 January 2006 (i.e. the present

359 experimental period) at Guernsey (49º27'N, 02º33'W) (Channel), Isle of Man

360 (54º15'N, 04º30'W) (Irish Sea), Bergen (60º24'N, 05º18'E) (Experiment) and Bear

361 Island (74º27'N, 19º02'E) (Barents Sea) was taken from the Astronomical

362 Applications Department of the U.S. Naval Observatory, USA

363 (http://aa.usno.navy.mil). For plotting purposes, the number of minutes was

364 transformed into decimal fraction of an hour. Total duration refers to when any

365 portion of the sun is above the horizon. This was found for all days corresponding to

366 experimental measurements days (n = 9) but adding summer and winter solstice and

367 autumnal equinox.

368 **Statistics**

All statistical analyses were performed with Systat® 12 and the graphs produced with SigmaPlot® 10. Prior to any statistical test, each subset of data was examined for normal distribution by the Shapiro-Wilk test and the Anderson-Darling test (default options). For proportions normality was in some cases achieved by arcsine transformation (Sokal and Rohlf 1981). Equality of variances was tested with the F-test (incl. the Levene test). Coefficient of variation (CV) was presented as 100×SD/mean (%). Tests between or among groups included both nonparametric (Mann-Whitney test and Kruskal-Wallis test) and parametric methods (Student t-test). For ANCOVA the assumption of homogeneity of slopes was tested prior to any test on intercepts, using ln-transformed data when required. In regression analysis, standard error was attached to each regression coefficient and r^2 replaced with adjusted r^2 at low number of observations. For multiple regressions the entry of an independent variable was based as far as possible on biological relevance consulting experimental findings when establishing field models. Unless specially mentioned, any predictor adopted showed a significant contribution (an absolute value of $t > 2.0$) and 'tolerance' > 0.1, the latter to exclude highly correlated predictors (Systat Software 2007). The Akaike Information Criterion (AIC) was consulted when appropriate, searching for the lowest AIC (Systat Software 2007). In 'tracking studies' on the same experimental individuals across time (i.e. balanced design) observed changes were tested with Linear Mixed Models (LMM) and/or Repeated Measure ANOVA. To clarify the specific influence of a given category at specific points within such time series, Hypothesis Test (Effects) ANOVA was used. For LMM fixed factors were tank, time (month), gender (if relevant) and tank×month, and the random factor set to be fish×tank. The default first-order autoregressive structure was included to adjust for autocorrelation with time. Resulting adjusted *p*-values for

fixed effects were consulted and presented. Rejection of null hypothesis was always set at $p < 0.05$.

Results

Laboratory study

Water temperature and food intake

Grand mean (SD) temperature during the length of the experiment (240 days) was 5.05 (0.49) at LT (low temperature) and 9.33 (0.59) ºC at AT (ambient temperature). Variation in temperature within a week was typically ± 0.5 °C. The smallest between-tank difference in temperature was 2.9 ºC, the largest 5.2 ºC (Fig. 1a). Daily temperature showed evidence of synchrony between tanks (r = 0.474, $p < 0.001$).

Fig 1 near here

Weighted mean feeding ration (FR) for the eight measurement periods at LT and AT was 0.226 and 0.244 dry feed•g wet fish^{-1}•day^{-1}, respectively, i.e., not significantly different ($p = 0.774$) (Student t-test). Periodic FR declined over time in both tanks (Fig. 1b). There were indications that the AT fish took more feed initially (Day 0-64), but later on the appetite in the two tanks was similar. There was a transitory drop in interest in food between Day128 and 155, coinciding with initiation of vitellogenesis (see below).

Fish growth and condition

Individual growth in body weight showed evidence of tank ($p = 0.011$), month ($p < 0.001$), gender ($p = 0.017$) and tank×month ($p < 0.001$) effects (LMM). Despite this overall specific growth rate (G) did not vary statistically between tanks but overall daily length increment (DLI) did vary showing the highest figures for AT (Table 1). Fish at LT and AT were similar in size at the start of the experiment, both in terms of

Table 1 near here

mean length and weight (Table 1). After 224 days their mean lengths were statistically different (Table 1).

More in-depth analyses demonstrated that the specific growth rate varied periodically. Both tanks demonstrated a strong positive relationship between mean periodic G and periodic FR (LT: $r^2_{adj.} = 0.889$, df = 1,6, $p < 0.001$; AT: $r^2_{adj.} = 0.829$, df = 1,6, $p = 0.001$, both genders). The fall in mean periodic G (Fig. 1c) essentially mimicked the one for FR (Fig. 1 b) whilst the pattern of change across time for G differed between tanks ($p = 0.007$) (Repeated Measures ANOVA). There was evidence of a trade-off between initial body weight and subsequent growth rate (LT: r = -0.448, $p = 0.009$; AT: r = -0.382, $p = 0.018$). Around the time of initiation of spawning (see below) G generally became negative (Fig. 1c).

AT fish developed a significantly lower condition factor (K) than those at LT (Fig. 1d). The interaction month×tank was highly significant ($p < 0.001$) (Repeated Measure ANOVA). As a consequence, mean K at LT and AT became increasingly significantly different (Day 0: $p = 0.057$; Day 35-98: $p \leq 0.017$; Day 128-240: $p < 0.001$) (Hypothesis Test ANOVA). Analysis of somatic condition factor (K_S) gave a similar answer for the last measurement point (Day 240) (LT vs. AT prespawners: $p < 0.001$) (Student t-test).

Initiation of spawning

A higher proportion of fish in AT (34%) compared to fish in LT (6%) started to spawn on Day 240 indicated by either running eggs or hydrated oocytes. All ovaries were in a normal state. Freely running (‘spermiating’) males were first noted on Day 224.

GSI, HSI, and fecundity regulation

Prespawners held at AT showed, on average, a significantly higher gonadosomatic index (GSI), fecundity (F) and relative fecundity (RF) than their LT counterparts at Day 240 but a significantly lower hepatosomatic index (HSI) (Table 1). Only the LT regime showed evidence of any influence of maturity status, represented by LC diameter, on somatic relative fecundity (RF_S), but fish in both regimes developed a negative trend in RF_S with increasing LC diameter (LT: r = -0.518, p = 0.003; AT: r = -0.271, p = 0.189) (Fig. 2). Exclusion of a statistical outlier at AT (Fig. 2) associated with an uncertain measurement did not influence the conclusion. AT prespawners demonstrated a significantly higher LC diameter-specific RF_S than LT prespawners (intercept: $p < 0.001$; slope: p = 0.458 (0.604, without outlier)) (ANCOVA).

Fig 2 near here

Testing the temporal influence of body size on fecundity, multiple regression analysis consistently revealed no significant effect of TL ($p >> 0.05$) when W was used as the other independent variable. Hence, this type of analysis did not expose any condition effect as such on fecundity. Use of W as the only independent variable explained up to 36% of the variance (r^2) in F for LT and 40% for AT, referring to W on Day 224 and 240, respectively (Fig. 3). The level of significance was, however, rather similar throughout the experiment for AT ($0.001 < p < 0.004$), but steadily increasing for LT (p falling from 0.054 to 0.001). Inclusion of vitellogenic LC diameter (see below) contributed significantly to the regression for LT ($p \leq 0.046$) but not so for AT ($p \geq 0.077$) (Fig. 3). In the first case r^2 reached 0.681 on Day 240. The relative influence of LC diameter versus W on F, taken as the ratio of the corresponding absolute standard coefficients, increased in the case of LT from about 50% on Day 155 to about 75% on Day 240 but was rather stable around 40% for AT. In all cases W contributed positively to F while LC diameter negatively. The

Fig 3 near here

respective F formulae (millions) based on W (g) and LC diameter (µm), all referring to Day 240, for LT and AT were:

(4) $F = 3.08(SE \pm 0.74) + 9.87\times10^{-4}(SE \pm 1.61\cdot10^{-4})\times W - 4.14\times10^{-3}(SE \pm 0.92\times10^{-3})\times LC$ (LT, $r^2 = 0.681$, df = 1,25, $p < 0.001$)

(5) $F = 2.70(SE \pm 0.76) + 8.46\times10^{-4}(SE \pm 2.16\times10^{-4})\times W - 2.12\times10^{-3}(SE \pm 1.28\times10^{-3})\times LC$ (AT, $r^2 = 0.481$, df = 1,18, $p = 0.004$).

For the sake of comparison LC was withheld in Eq. (5) despite its insignificant statistical contribution: at LC = 500 µm for a standard female of 3500 g the fecundity was 3% higher at AT than at LT but this difference increased to 23% at LC = 800 µm.

Data on oocyte diameter frequency distributions showed differences in oocyte recruitment dynamics both between tanks and between prespawners and spawners. The width of the oocyte distribution ($SD_{diam.}$) increased markedly over time but significantly more in AT prespawners than in LT prespawners (Day 189: $p = 0.163$; Day 224: $p = 0.017$; Day 240: $p < 0.001$) (Student t-test) (Fig. 4a). A comparable situation appeared between AT prespawners and spawners (Day 189: $p = 0.003$; Day 224: $p = 0.013$), but the difference disappeared (Day 240: $p = 0.393$) when the spawners experienced a fall in $SD_{diam.}$ related to initiation of spawning (Student t-test). This analysis was not testable at LT. Indications of differences in $SD_{diam.}$ between AT prespawners and spawners appeared early on (Day 155: $p = 0.051$) but not so between LT and AT prespawners (Day 155: $p = 0.820$) (Mann-Whitney test). When ignoring the subdivision into prespawners and spawners the pooled $SD_{diam.}$ data showed no differences between the two temperature regimes on Day 155 ($p = 0.330$)

Fig 4 near here

(Mann-Whitney test) but diverged by Day 189 ($p = 0.009$) and 224 ($p < 0.001$) (Student t-test). Corresponding data on the smallest cohort of oocytes (SC) revealed that the process of oocyte recruitment had ceased from Day 189, i.e., mean SC was then well above 250 µm (Fig. 4b), though a few individuals apparently recruited oocytes all the way up to Day 224. Any difference in SC development between LT and AT prespawners could not be fully confirmed (Day 155-240, $p > 0.062$) (Student t-test) but examples of such differences existed when contrasting AT prespawners and spawners (Day 155: $p = 0.504$; Day 189: $p = 0.009$; Day 224: $p = 0.054$; Day 240: $p = 0.001$) (Mann-Whitney test). The pooled SC data demonstrated tank differences on Day 155 ($p = 0.009$) but not later on ($p > 0.687$) (Mann-Whitney test).

LC oocyte diameter as maturation criterion

The observation that AT females showed a reasonable mixture of prespawners and spawners on the last day of the experiment made it possible to test the assumption that individuals with larger LC diameter spawn first. This appeared to be generally true: on Day 224 females that subsequently spawned within the next two weeks showed on average a LC diameter of 694 (SE ± 17) µm compared to 585 (SE ± 16) µm for those that did not spawn within that period of time, i.e., a significant difference ($p < 0.001$, Student t-test).

Overall oocyte growth rate

LT and AT females tracked over time showed significantly different oocyte growth trajectories ($p < 0.001$, Repeated Measures ANOVA) resulting in significant differences in mean LC diameter (LC_{group}) at late vitellogenesis (Fig. 5). More explicitly, the two data sets differed statistically from Day 189 onwards (Day 128: $p = 0.521$, Day 155: $p = 0.075$, Day 189: $p = 0.011$, Day 224: $p = 0.005$ and Day 240: $p = 0.003$) (Mann-Whitney test or Student t-test). Dropping spawners from the analysis,

Fig 5 near here

caused the observed differences on Day 189 and 224 to disappear (p = 0.298 and 0.154, respectively) and nearly so on Day 240 (p = 0.048). On Day 128 10% of the LT females and 6% of the AT females contained developing oocytes (LC diameter > 250 µm). This figure increased sharply to 66% and 76% on Day 155, respectively. Hence, in relative terms more AT females entered vitellogenesis between Day 128 and 155. The detailed individual tracking study revealed an example of extreme slow oocyte growth. Exclusion of this LT female did not affect the above statistical conclusions. LC_{group} diameter (µm) increased between Day 128 and 240 (Fig. 5) as:

(6) $LC_{group} = 3.43(SE \pm 0.20) \times ED - 197(SE \pm 39)$ (LT, $r^2 = 0.990$, df = 1,3, $p < 0.001$)

(7) $LC_{group} = 4.10(SE \pm 0.15) \times ED - 281(SE \pm 30)$ (AT, $r^2 = 0.996$, df = 1,3, $p < 0.001$),

where ED is elapsed days since Day 0. The use of a power function in place of a linear function increased r^2 even closer to 1, but had no practical implications. The typical oocyte growth rate (R) of AT females was 19.5% higher than for LT females, i.e., 3.43 at LT vs. 4.10 µm•day^{-1} at AT.

Individual oocyte growth rate

Studies of individual LC diameter data showed that the time of entrance to vitellogenesis in the autumn influenced when each female would likely start to spawn in the subsequent spring. This was most clearly seen for the earliest and latest spawners: females with the largest LC diameter on Day 240 were all vitellogenic on Day 155 (LC diameter > 380 µm) while none of the females with the smallest LC values on Day 240 had yet commenced vitellogenesis on Day 155 (LC diameter ≤ 250 µm). A regression analysis on the complete individual-based data sets indicated that

predicted spawning time for both temperature regimes showed similar dependence on the time of entrance to vitellogenesis (intercept) and subsequent oocyte growth rate (slope) (R), i.e., LC diameter on Day 155 and R influenced final LC diameter (Day 224) by 57-58% and 42-43 %, respectively. A few out of the tracked females deviated from the rest by showing a fall in R past Day 155. Likewise, some apparently had a temporarily increased R, mainly between Day 224 and 240, cf. appearance of spermiating males. Nevertheless, the high individual stability in R preceding the process of egg release was confirmed by separate regression analysis (LT: mean r^2 = 0.979 (SE ±0. 010), AT: mean r^2 = 0.993(SE ±0. 001)), where each fish was represented with four successive LC diameter measurement points. In one single case the r^2 was much lower, i.e., 0.717. Subsequent histology showed a fully normal ovary.

The average individual R per tank was 3.56 (SE ±0.15) and 4.21 (SE ±0.12) $\mu m \bullet day^{-1}$, i.e., about 3-4 % higher compared to the corresponding group-based parameter values given in Eqs. (6) and (7), respectively. The R appeared to be statistically independent (p = 0.928) on when the females entered vitellogenesis in the autumn, being testable for AT (middle vs. late entrance, Mann-Whitney test).

Estimated R values reflected a general vitellogenic Q_{10} value of 1.44 (1.47) and a length of the vitellogenic period of 176 (182) days at LT and 148 (152) days at AT, with the results from Eq. (6) and (7) given in parenthesis. Thus, the respective ovaries matured typically for 6 and 5 months before initiation of spawning, or stated in another way, LT females showed a delay in spawning time of one month compared to AT females. Based on these findings R_{new} ($\mu m \bullet day^{-1}$), i.e., the oocyte growth rate at another environmental temperature (T_{new}) than the present ones can be predicted by the following expression:

566 (8) $R_{new} = 4.21 \times 1.44^{(T_{new} - 9.60)/10}$.

567

568 As noted, input data are from AT and the Q_{10} value refers to vitellogenic females. The
569 alternative use of input data from LT, would, logically, give the same result.

570 ***Size-specific oocyte growth***

571 Only AT females showed a significant relationship between LC diameter on Day
572 224 and body weight and length measurements during the course of the experiment.
573 Of the two explanatory variables, W had, with the exception of Day 64-98, a higher
574 predictive power than TL but both r^2 showed generally falling values with time (TL:
575 Day 0: 0.34, Day 224: 0.19; W: Day 0: 0.37, Day 224: 0.27). All these regressions
576 were significant (TL: $p \leq 0.007$; W: $p \leq 0.001$) but none for LT (TL: $p \geq 0.653$; W: p
577 ≥ 0.082). Exclusion of the above-mentioned LT female with extremely slow oocyte
578 growth had no statistical relevance. All slopes were positive, except for two LT
579 outputs, which were considered irrelevant due to their insignificant nature. In
580 consequence, those AT females that turned out to be spawners before the end of the
581 experiment had on Day 0 a significantly larger body size than their accompanying
582 prespawners (W: $p = 0.023$ (Table 1); TL: $p = 0.026$ (mean: 56.0 vs. 53.6 cm))
583 (Student t-test). These prespawners, however, compared to the other time periods
584 tested ($p \geq 0.677$), showed indications of a higher specific growth rate between Day
585 35 and 98 ($0.171 < p < 0.181$) (Mann-Whitney test). This probably contributed to the
586 finding that AT spawners and prespawners became just insignificantly different in
587 mean body weight on Day 224 (Table 1) ($p = 0.060$) while their corresponding mean
588 lengths (65.0 vs. 63.8 cm) evidently had turned statistically similar ($p = 0.365$)
589 (Student t-test). Too few LT spawners existed for such a test. More comprehensive
590 multivariate analyses did not locate any other additional variables, such as

591 hepatosomatic index or expressions of body growth (G and DLI), which significantly
592 increased the understanding of variation in prespawning LC diameter.
593 The underlying reason for any potential influence of body size on prespawning LC
594 diameter was tested by regressing oocyte growth rate (R, $\mu m \bullet day^{-1}$) on initial length
595 with no relationship in the case of LT ($p >> 0.05$) but noticeably so for AT (Fig. 6):
596
597 (9) $R_{size} = 10.42\times10^{-2}(SE \pm 3.40\times10^{-2})\times TL - 1.50(SE \pm 1.87)$ (AT, $r^2 = 0.232$, df =
598 1,31, $p = 0.005$, 46 < TL (Day 0) < 61 cm).
599
600 For clarity, R was relabelled as R_{size}. Using initial body weight instead of length as the
601 predictor gave similar results. This significant relationship persisted until both Day
602 128 and 224 when studying the same individuals:
603
604 (10) $R_{size} = 8.24\times10^{-2}(SE \pm 2.94\times10^{-2})\times TL - 0.90(SE \pm 1.83)$ (AT, $r^2 = 0.202$, $p =$
605 0.009, 51< TL (Day 128) < 68 cm).
606
607 (11) $R_{size} = 8.02\times10^{-2}(SE \pm 2.85\times10^{-2})\times TL - 0.97(SE \pm 1.85)$ (AT, $r^2 = 0.204$, $p =$
608 0.008, 53 < TL (Day 224) <70 cm).
609
610 These two latter regressions were included due to practical applications in the field
611 study below. Next, the following formula was established including both the above
612 temperature (Eq. (8)) and body size effect (Eq. (9), (10) or (11) depending on the time
613 in the autumn):
614
615 (12) $LC_2 = R_{size}\times1.44^{(T_{new} - 9.60)/10}\times(t_2 - t_1) + LC_1$.

Fig 6 near here

Thus, measured developing LC diameter (LC_1) on any day t_1 can in effect be transferred (standardised) to developing LC diameter (LC_2) on day t_2 in a warm temperature situation resembling the one of the AT regime.

Field study

DST-recorded temperatures in the different waters

Temperature information gathered from DSTs showed that adult cod in the northern and southern waters stayed in highly different temperatures throughout the year but without any evident trend in selected temperature by fish size. Monthly temperature profiles from the Channel, North Sea and Irish Sea clearly differed from those from the Barents Sea (Fig. 7). After the supposed spawning season, the southern category went into significantly warmer water while those belonging to the northern category generally entered cooler water, including temperatures below zero. Both categories showed less variation around expected time of spawning, but particularly the northern one, i.e., between March to May the temperatures experienced by the present individuals of Barents Sea cod were concentrated around 4 ºC, which was far below the introduced threshold value of 9.6 ºC, thought to imply impaired spawning, if met or exceeded (Fig. 7). This was not the case for the three southern stocks showing examples of individuals quite close to this critical line in the months of interest, i.e., January-March. The data indicated that the overall temperature between 1 September and 1 February, assumed to overlap to a large extent with the period of vitellogenesis, was centred on 2 ºC (range: 1 – 3.5 ºC) and 11 ºC (range: 9 – 13 ºC) for cod in the north and south, respectively (Fig. 8). There was no obvious fish size dependency as analysed within the Channel, North Sea and Barents Sea cod stocks (for the Irish Sea only one fish was recorded).

Fig 7 near here

Fig 8 near here

Barents Sea data base

Data from this area included physical descriptors (total length, viscera condition ($C_{viscera}$) and body condition (C_{weight})) (Table 2) and CTD-recorded temperature in August-September (Vardø North Transect, T_{VN}) (Fig. 9). All three physical descriptors demonstrated significant annual variations within the standard time series ($p < 0.001$) (Kruskal-Wallis test), although the length data (54 – 128 cm) should be treated with some caution due to likely examples of non-random sampling. The early and late (standard) 2006 samples also deviated significantly in length ($p = 0.032$) and viscera condition ($p < 0.001$) but not in body condition ($p = 0.172$) (Mann-Whitney test). Viscera condition was included to reflect feeding activity finding two major peaks, 2003 and late 2006 (Fig. 9).

Table 2 near here

Fig 9 near here

To assess the representativeness of the various data, our fish measurement data were first compared with similar survey data but also with present experimental growth data, followed by a study on CTD data in relation to similar DST data, finding some deviations. The comparison with the extensive length-at-age database from the Lofoten-Vesterålen survey showed that the presently sampled cod, focusing on the main age groups 8 and 9 (combined sexes), were consistently 5-10 % larger between 2005 and 2007. Corresponding specific growth rate (females only) varied typically from about 0.05 %·day^{-1} within the period 1999-2005 to about 0.12 %·day^{-1} within the period 2006-2007. Thus, indicated growth in body weight was roughly half, or less, of the above experimental values. Transect temperature during the autumn of 1996, 3.1 ºC, was about 1 ºC higher than the corresponding average DST temperature. The individual range in DST temperature (Fig. 8) showed, however an overlap with this CTD record.

Fecundity regulation in the Barents Sea cod

666 The pooled analysis on standard Andenes samples (i.e., excluding early 2006)
667 showed that the fecundity represented by F (millions) was significantly influenced (p
668 < 0.001) by TL (cm), C_{weight} (without unit), LC diameter (µm) and T_{VN} (ºC):
669
670 (13) $F = 1.95\times10^{-4}\times TL^{3.726}\times C_{weight}^{1.729}\times LC^{-1.141}\times T_{VN}^{0.325}$ ($r^2 = 0.921$, df = 1, 445, $p <$
671 0.001).
672
673 The constant had a logarithm value of -8.54 with SE 0.65. The SE of the exponents
674 was 0.054, 0.098, 0.093 and 0.091, respectively. The corresponding standard
675 coefficient was 0.928, 0.246, -0.166 and 0.050 implying that the absolute relative
676 contribution was 66.8, 17.7, 11.9 and 3.6%, respectively. Thus, TL and T_{VN}
677 contributed clearly the most (t = 69.01) and least (t = 3.59) to F. Tolerance was ≥
678 0.92. Inclusion of ALI as an expression of growth resulted in the same AIC, i.e., its
679 contribution was barely insignificant (t = -1.85, or p = 0.065) and therefore excluded.
680 F for a standard female of 85 cm with LC diameter 750 µm ($F_{85cm\_750\ \mu m}$) ranged from
681 2.3 (1999) to 3.1 millions (2007).
682 The fecundity of each sample could be successfully described (p < 0.001) by the
683 linear combination of body weight ($W_{corrected}$) and LC diameter: r^2 ranged from about
684 0.85 (1999) to about 0.94 (early 2006 and 2008). The higher r^2 in the field compared
685 to the laboratory (Fig. 3) was the result of the longer range in body size of wild
686 specimens. Mean C_{weight} of a sample effectively predicted mean $F_{5500\ g\_750\ \mu m}$ (r^2 =
687 0.911, p < 0.001), 5500 g corresponding to $W_{expected}$ of a 85 cm fish (Eq. 3).
688 ***Overall oocyte growth rate of the Barents Sea cod***
689 Development in mean LC diameter (LC_{group}) over time as observed in the Andenes
690 samples agreed largely with experimental, low-temperature results, although giving

somewhat lower LC values at a given calendar day (Fig. 10). The proper establishment of the field-based equation was complicated by two outliers ('late' 2006 and 2007) and one case of large leverage ('early' 2006). The statistical use of all field data showed an oocyte growth rate (R) of 3.45 (SE ±1.76) µm•day^{-1} and an intercept of -214 (SE ± 196) µm (r^2 = 0.770, p = 0.002). Avoiding the problem of presenting the year 2006 twice by leaving out late 2006 in the test, gave comparable outputs (R: 3.69 (SE ±0.59) µm•day^{-1}; intercept: -278 (SE ± 163) µm; r^2 = 0.866, $p < 0.001$). Based on the data in Fig. 9 the biggest, positive residual in LC_{group} diameter (2007) was associated with non-feeding larger fish, which apparently had experienced the highest vitellogenic temperature. The corresponding most negative residual (late 2006) referred to the most actively feeding fish, and were also among the smallest in size.

Fig 10 near here

Individual oocyte growth rate of the Barents Sea cod

The individual-based study of all standard Andenes samples combined revealed that only about 5-7% of the total variation in LC diameter residuals could be explained by the above defined physical descriptors and transect temperature. These multiple regressions were, however, highly significant ($p < 0.001$), which should be seen in light of the high number of females examined (df = 1,445). Temperature data (Vardø North, T_{VN}) as such did not meet the t value > 2 requirement (t = 1.58), but clearly so when multiplied with length (TL) (t = 3.03) to form an interaction term (t = 3.66). The standard coefficients pointed to that $TL \times T_{VN}$ was about 1.6 more influential than C_{weight} (t = 2.26). The addition of $C_{viscera}$ resulted in another significant, but this time negative contribution (t = -2.96). Dropping out $C_{viscera}$, the comparable contribution by ALI was t = 1.94, i.e., very close to being significant (p = 0.053).

Size-specific spawning time in the different waters

For the Barents Sea cod only three out of eight annual standard samples showed evidence of any relationship between LC diameter and total length of each individual fish (Table 2). More specifically, 9% of the variation in 2004 could be explained and 16-18 % in 2006-2007, otherwise by a few percent at most. The extra sample in early 2006 did not demonstrate such a positive relationship. Year 2000, referring to the latest sample taken in the spring (mid March), showed 12 spawners. Spawners were not significantly different in length from prespawners ($p = 0.536$) (Student t-test). Also, the regression behaved similarly ($p = 0.415$) when spawners were included rounding off their LC diameters to 875 µm to avoid getting false relationships driven by large, swelling oocytes. Similarly, inclusion of five spawners in 2007 did not bias the given regression, i.e., gave $p = 0.003$ instead of $p = 0.002$. Replacement of $W_{corrected}$ with TL throughout the time series gave similar conclusions while age and number of spawning zones did not significantly ($p > 0.05$) influence LC diameter at any point, despite the large spread in observed values (age: 5-13 years (plus one at 18 years); spawning zones: 0-6 (plus one at 7 and 12)).

Both the North Sea and Irish Sea samples showed examples of individuals lagging behind in maturation but in opposite to the Barents Sea samples these were generally more concentrated towards the lower part of the size range. In the North Sea the observed spawners were significantly larger in size than their accompanying prespawners ($p = 0.003$) (Mann-Whitney test), i.e., typically by 18 cm (Table 2). This was much less clear for the Irish Sea ($p = 0.476$) where the spawners were on average only 3 cm larger (Student t-test). However, in this case a meaningful comparison was complicated because prespawners were also very close to spawning, seen by their large LC_{group} diameter (822 µm) (Table 2). Prior to standardisation using Eqs. (11)

and (12), both the North Sea and Irish Sea data showed a significant positive fish size-dependency (North Sea: $r^2 = 0.137$, df = 1,33, $p = 0.029$; Irish Sea: $r^2 = 0.238$, df = 1,35, $p = 0.002$). Following standardisation this significance still prevailed (North Sea: $r^2 = 0.152$, df = 1,39, $p = 0.012$; Irish Sea: $r^2 = 0.155$, df = 1,36, $p = 0.014$), setting, as above, the LC diameter of spawners to 875 µm.

Light cycle in the different waters

The light cycle in the Barents Sea differs markedly from the other areas of interests and is characterised by continuous light during the summer followed by a steep decline in day length to continuous 'darkness' in the winter, i.e., with a much larger amplitude in day length than the Channel, Irish Sea and experimental location (Fig. 11). These latter three show quite similar light cycles, although following, as expected, the general pattern of a larger temporal range in day length northwards.

Fig 11 near here

Conceptual maturation model

Approaches taken

The fact that the oocyte growth curves of the low-temperature experimental cod and the Barents Sea cod were close (Fig. 10) despite the great difference in light cycle suggested that autumnal equinox could be a collective starting point for vitellogenesis, simply because this was the only point between summer and winter time when their day lengths were exactly equal (Fig. 11). The inference of an underlying similar oocyte growth pattern following temperature adjustments was strengthened because the LT curve referred to 5 ºC (Fig. 5) whilst the Barents Sea cod likely stayed in somewhat cooler water (Figs. 8 and 9); the plotted field curve should be located somewhat below the experimental LT one, as noticed in Fig. 10. Detailed examinations of Eq. (6) and (7) showed that these two experimental curves intersected on Day 130 (8 October) corresponding to a LC_{group} diameter of 250 µm (LT: 249 µm;

766 AT: 252 µm) followed in both cases by initiation of vitellogenic oocyte growth. This
767 was seen from the combination of 1) 250 µm is maximum previtellogenic oocyte
768 diameter, 2) oocyte growth is linear and 3) vitellogenesis is well established on Day
769 155, each point being described in full above. Thus, the roughly two-week period
770 from autumnal equinox on Day 114 (22 September) to this intersection point was
771 defined as response time ('latency'), and the two equations rewritten as:
772
773 (6)' $LC_{group} = 3.43(SE \pm 0.20) \times ED_{vit} + 250$ (LT)
774
775 (7)' $LC_{group} = 4.10(SE \pm 0.15) \times ED_{vit} + 250$ (AT),
776
777 where ED_{vit} is number of days after 8 October (ED_{vit} in the following year will
778 therefore be 84 + calendar day). In consequence, the problem of locating the intercept
779 value, known to be important (see above), was assumed solved. A remaining problem
780 was to clarify the actual vitellogenic temperature ($T_{vit.}$) of the present Barents Sea cod
781 bearing in mind that the above data indicated some differences in CTD and DST
782 temperature records. Accordingly, $T_{vit.}$ was considered unknown and indicated by
783 adjusting the oocyte growth rate, R_{new}, in the general equation $LC_{group} =$
784 $R_{new} \times ED_{vit} + 250$ to achieve a fit between this curve and the field-based one. This
785 happened when R_{new} was 3.36 µm•day^{-1}(Fig. 10) reflecting a temperature of 3.4 ºC,
786 found by rearrangements of Eq. (8).Thus, the Andenes cod apparently stayed mostly
787 in the upper part of the relevant DST temperature range (Fig. 8), i.e., consistently in
788 colder water than indicated by the CTD Vardø North Transect (Fig. 9).
789 ***Resulting output***

790 The combined use of Eq. (8) and the expression $ED_{vit} = 625/R_{new}$ made it possible
791 to model the start of spawning (i.e. LC = 875 µm) in the year for an individual female
792 cod in response to a range in $T_{vit.}$ (Fig. 12). As can be seen, Eq. (8) was extrapolated
793 by 3-4ºC on each side of the range to include higher and lower temperatures than used
794 experimentally and adopting the associated level of uncertainty. Any dependency on
795 fish size was tested in a warm water situation (see above) using Eq. (10) followed by
796 the previous standard procedure setting the temperature to 11 °C, i.e., the typical DST
797 $T_{vit.}$ seen for southern waters (Fig. 8). However, the low r^2 of Eq. (10) implied
798 considerable prediction bands (not shown) and thereby gave an uncertain conclusion
799 about the actual levels of response (Fig. 12).

Fig 12 near here

800 ***Realism test***

801 The conceptual model, excluding any body size effects, was tested by consulting
802 published spawning curves (seasonal pelagic egg production curves) of the Barents
803 Sea (Pedersen 1984: Lofoten area) and Irish Sea cod (Armstrong et al. 2001). Thus,
804 start of spawning (egg release) was known meaning that the matching $T_{vit.}$ could be
805 found from Fig. 12 and validated with available oceanographic data (Barents Sea cod)
806 or DST information (Irish Sea cod). Resulting vitellogenic temperatures agreed well
807 with expected environmental water temperatures encountered by the fish, however, in
808 the case of the Barents Sea cod these earliest spawners (calendar day 75) likely
809 originated from local waters. More specifically, $T_{vit.}$ equaled 7.5 °C corresponding
810 with the typical autumnal temperature of 7 to 8 °C seen in Atlantic water masses (50-
811 200 m) off Lofoten in the Gimsøy Transect (68°24'N 14°04E –70°24'N 0812''E) (Dr
812 K.A. Mork (personal communication, 2008). In the case of the Irish Sea start of
813 spawning (calendar day 45) referred to a $T_{vit.}$ of 13°C, which was possible (Fig. 8).
814

Discussion

Our study on reproductively competent Atlantic cod has revealed the interaction between the main factors influencing the maturity (oocyte growth) dynamics of this species. The strong dependence between day length and the initiation of vitellogenesis enabled us to develop temperature-specific maturity formulae based on general physiological principles, i.e., not requiring any new data to run the model in future operations. We were then able to use these formulae to predict convincingly the variation in start of spawning time in cod of several different stocks. Thus, other factors affecting oocyte growth rate such as condition variation and body size did not significantly bias this calculation. These results now make it possible to better understand the variation in cod spawning time as a consequence of past marine climate, and allow us to make forecasts about what may happen in the future as climate changes.

The effect of the autumnal equinox on maturity

Here we demonstrate experimentally that autumnal equinox is the starting point of vitellogenesis in Atlantic cod. This can be deduced from the fact that the fish in two different tanks were maintained at very different temperatures from summer onwards but did not display any sign of different oocyte growth trajectories until 8 October. The models that we fitted to the data show that the growth curves for oocytes in each experimental tank intersected at 250 µm corresponding to the upper previtellogenic oocyte (PVO) diameter described in other studies (Sivertsen 1935; Kjesbu 1991). This result concurs with Woodhead and Woodhead's (1965) conclusion, based on observation of a concurrent sharp increase in thyroid follicle cell height and increase in size and numbers of late PVOs (circumnuclear phase oocytes), that autumnal equinox is the time of 'spawning migration and gonad maturation' for the Barents Sea

840 cod. Previously, Woodhead and Woodhead's work has received little attention,
841 possibly because the spawning migration of the Barents Sea cod starts much later, i.e.,
842 in December-January (Bergstad et al. 1987), and so is easily disassociated from gonad
843 maturation in this stock. However, the regulation of thyroid hormone production was
844 further explored by Comeau et al. (2001) who observed a significant increase in these
845 hormones around the equinox in the southern Gulf of St. Lawrence cod. Their results
846 on estradiol-17β and testosterone show a (minor) pulse around that time. Surprisingly,
847 sentinel catches in the western part of this gulf area consistently peaked on exactly the
848 same date as the present oocyte growth intersection point, 8 October (2-13 October)
849 followed by another catch peak about two weeks later in the eastern part of the gulf
850 when the migrating cod arrived. Comeau et al. (2001) conclude that 'thyroid
851 hormones may facilitate the onset of the autumn migration by enhancing metabolism,
852 sensory biology and swimming capacity'. Although it is new information that
853 vitellogenic oocyte growth in cod typically commences after a latency of about two
854 weeks following autumnal equinox, studies related to cod aquaculture, especially on
855 photoperiod manipulation, agree with our conclusion as these report increased levels
856 of sex steroids and gonad growth from October onwards in the normal day group
857 (Norberg et al. 2004; Skjæraasen et al. 2004; Davie et al. 2007). Such experimental
858 designs also reflect that the oocyte growth rate can show a great level of plasticity
859 (Hansen et al. 2001; Davie et al. 2007), but in a field situation the photoperiodicity
860 should be considered constant between years, ignoring the possible effect of variation
861 in cloud covers. Our study is exceptional in that it finds an almost perfect matching of
862 laboratory and field data on the temperature dependence of oocyte growth, which 1)
863 emphasizes the success of our experimental design and the accuracy of both the

experimental and field datasets and 2) opens the road for similar experiments to take place for other fish species.

Present results showed that the experimental cod lost their appetite and grew less between 6 October and 2 November. Skjæraasen et al. (2004) observed a similar sudden drop in food intake for cod held on natural light and considered the following decline in appetite up to spawning to be a consequence of sexual maturation, although information shows that cod may take food during spawning (Fordham and Trippel 1999; Michalsen et al. 2008). Hence, the time around autumnal equinox is obviously a period where major changes take place in the physiology of adult cod, including a switch in energy allocation patterns to support further gonad growth. The experimentally delayed and compressed seasonal photoperiod data in Norberg et al. (2004) show that testosterone for both female and male cod consistently increases when the day length falls below 12 hours. For females, the corresponding estradiol-17β pattern lags slightly behind, which is as expected; testosterone is 'aromatased' into estradiol-17β (see Norberg et al. 2004). Thus, it is not the calendar as such that determines the onset of maturity, but the time when the duration of darkness first exceeds 12 hrs in the autumn, presumably through the mechanism of melatonin accumulation (Migaud et al. 2007). The present outlined day length threshold value and subsequent vitellogenic response is remarkably similar to mechanisms demonstrated in the marine annelid *Nereis virens* (Olive et al. 1998).

The experimental oocyte growth data do, however, show a variation in initiation of vitellogenesis of about ± 1 month. Thus, a few individuals start to show evidence of oocyte growth in September while others start in November, corresponding to a day length of 14 and 10 h, respectively. Consequently, early spawners (those that require only 10 h of darkness to start oocyte growth) should be considered to be less sensitive

889 to light than late spawners (14 h of darkness). One applied consequence of this
890 discovery is that considerable savings could likely be made in the cod aquaculture
891 industry by replacing the current practise of continuous light (24 h light: 0 h dark) to
892 prevent sexual maturation (Taranger et al. 2006) to obviously somewhat shorter day
893 lengths to reduce the electricity bill.

894 **The effect of temperature on reproductive investment and condition factor**

895 We found ample experimental evidence of an influence of temperature on
896 reproductive investment, temporal variation in body growth and condition factor.
897 Early on in the experiment, females held at the higher, ambient temperature (AT)
898 showed indications of better appetite, compared to females held at the lower, cooled
899 temperature (LT), although the overall feeding ration for the whole length of the
900 experiment was not statistically different. In both treatments, oocyte recruitment
901 ended during the late autumn, with a few exceptions, as is usually observed for
902 determinate spawners such as cod (Kjesbu 2009). However, despite indications of a
903 higher food intake, AT females had a lower condition index (Fulton's K) than LT
904 females. This is in contrast to previous field studies across cod stocks that report
905 higher K at higher temperatures (Rätz and Lloret 2003). In our experiment the main
906 reason appears to be generally more investment in length growth at AT but similar
907 overall weight growth as LT putting the estimated K for AT downwards. This finding
908 was further supported by that 1) tests on somatic K, i.e., following subtraction of
909 ovary weight from the expression, also gave a significant difference and 2) these
910 differences in K were already in place in early October, i.e., before vitellogenesis and
911 thereby gonad growth was well established. Regression analyses standardised for
912 maturity demonstrated that differences in relative fecundity between the two
913 categories of females was established early on (LC diameter ≈ 400 μm) pointing to an

increased production of PVOs at AT compared to at LT. The noted higher fecundity at AT apparently came at the expense of reduced liver size. In contrast, LT females appeared to 'over-recruit' oocytes, and later significantly reduced the number of developing oocytes as vitellogenesis (LC diameter) progressed, a process known as down regulation through vitellogenic atresia (Witthames et al. 2009). These different patterns might suggest that the AT regime is more 'effective' in terms of oocyte recruitment, i.e., assumed to be closer to the upper pejus temperature (Pörtner et al. 2001; 2008): a vitellogenic temperature of 9 °C enabled the AT females to reduce investment in the liver to boost egg production, whereas the LT group retained investment in the liver at a less 'optimal' temperature of 5°C. Taken together these results show that interpretation of K data on cod (for example from field surveys) require a good understanding of feeding conditions as well as thermal experience, and therefore such data should be treated with caution. Conversely, our findings support the conclusion of Skjæraasen et al. (2006) that the period of early vitellogenesis is important for the resulting fecundity of cod but here we clarify that the underlying oocyte regulation pattern depends on temperature and thereby varies with temperature and can be traced back as early as summer time.

The effect of temperature and other factors on oocyte and ovary growth

Unlike previous work (Kjesbu 1989) to estimate Q_{10}, our experiments were conducted during the vitellogenic period rather than the spawning season. The rates of growth of oocytes, and thereby the corresponding Q_{10} values, are therefore lower because the estimates of growth were not made during the period when oocytes swell with water (Kjesbu et al. 1996). As a result, our experiments enabled us for the first time to establish a robust relationship between the rate of oocyte growth and

938 temperature during the full maturity period, paying special attention to the assessment
939 of the Q_{10} value and its underlying rate-specific error terms.
940 In any experiment of this nature, it is not possible or desirable to totally negate
941 uncontrolled effects on oocyte growth created e.g. by variation in fish condition
942 (Kjesbu 2009) or potential trade-off with somatic growth (Yoneda and Wright 2005).
943 However, the modelled specific growth rates (G) of cod (of the size we used in our
944 experimental tanks) are almost constant over the temperature ranges we selected (as
945 per Bjørnsson and Steinarsson 2002) and should cancel out any overall difference in
946 body growth, as we found. More specifically, the present overall G values of LT and
947 AT fell between two earlier published records, one from the laboratory (Bjørnsson
948 and Steinarsson 2002: Icelandic cod), which was 17 and 9% above, and one from the
949 field (Clark et al. 2003: North Sea), which was 15 and 20% below, respectively.
950 Similarly, the applied *ad libitum* feeding protocol should, in theory, remove any
951 general condition effect as such. Thus, the measurements of leading cohort (LC)
952 diameter over time at 5 and 9°C can be considered to very much reflect the actual,
953 typical effect of these two temperature regimes on oocyte growth. Our experiments
954 therefore provide us with overall oocyte growth rates that were, as far as is possible,
955 solely related to temperature.
956 In consequence, we were able to derive accurate models for maturity (expressed as
957 LC diameter growth) at standard (5-9 °C) and extrapolated (2-13°C) temperatures.
958 These did not reveal any deviation from the observed spawning curves in the northern
959 or southern stocks (as derived from field sampling). In addition, the temperature data
960 collected from the electronic tagging experiments showed that the temperatures at the
961 upper limit of this extrapolation can be considered normal for wild cod at the southern
962 end of their distribution. Thus it seems likely that changes in spawning time, based on

963 the maturity curves, can be predicted at these higher temperatures. We are, however,
964 more uncertain about the validity of this model at the other end of the temperature
965 scale (from 2 to 5°C) because the directly measured thermal experience of cod in the
966 southern stocks has rarely fallen this low (Neat and Righton 2007, D. Righton
967 (personal communication, 2009)). Historical data on water temperatures at the
968 southern limits of cod distribution suggest that such circumstances have occurred in
969 the past (Bigg et al. 2008).

970 One potentially important problem not accounted for in the present maturity
971 models is that mature fish in poor condition are known to delay spawning time up to
972 two weeks (see review in Kjesbu 2009). This length of time compares with what was
973 seen in the Andenes time series in terms of total residual variation in LC_{group} diameter
974 (when considering the relevant oocyte growth rate). Only 5-7 % of this variation
975 could, however, be explained by the set of selected predictors in this work including
976 relative condition. This result was markedly different from the corresponding
977 fecundity model where almost all of the variation, 92%, could be explained by the
978 same predictors. In other words, fecundity and spawning time show fundamentally
979 different main regulatory principles, as implied in the above discussion on the
980 dominating role of day light for subsequent spawning time. Other candidates for
981 creating bias when running the present oocyte growth models might be any additional
982 effect of age (Ramsay and Witthames 1996: Dover sole (*Solea solea*) from the
983 English Channel) or spawning experience. These single effects could not be assessed
984 in the experiment because all fish had the same origin. However, the Andenes cod
985 showed large variations in these parametric values but no trace of any relevant
986 implications. Nevertheless, to reduce any uncertainty in the application of the present
987 maturity equations this potential age effect should be further explored, at least in

warmer water. Note here that we did find a temperature-specific effect of body size, dealt with later on. Further tests on the maximum vitellogenic LC diameter in relation to fish size (Ramsay and Witthames 1996), presently set to be 875 µm, should also be considered for fine-tuning of the present model.

The effect of temperature on the timing of spawning

Validations showed that our models of oocyte growth were able to predict convincingly the variation in start of spawning time of northern and southern cod stocks in the north-east Atlantic. Thus, spawning time in the southern stocks always occurred earlier than in the northern stocks and, because the variation in thermal experience during vitellogenesis was greater to the south, spawning time was more variable than in the north. Furthermore, our model can help to explain variation in spawning time for stocks outside of our study region. For example, the observed water temperature and spawning time information presented for cod off Newfoundland (Hutchings and Myers 1994) also agree well with our models despite very low ambient temperatures and a different definition of 'time of spawning'. However, for unknown reasons, our model does not seem suitable for the Eastern Baltic Sea cod, which may also spawn in the early autumn even though the experienced temperatures do not seem to be particularly cold (Wieland et al. 2000). This situation might be explained because the long Baltic spawning season (March-September) is a special adaptation to the extreme fluctuating environmental conditions in this ecosystem (MacKenzie et al. 1996). Also the Baltic tribe is an outlier both physiologically (in terms of egg formation) and genetically (Kjesbu and Witthames 2007) as well as by its more pelagic life style than typical for adult cod (Tomkiewicz et al. 1998).

Understanding the spawning dynamics of cod stocks

In most areas, the progression of the spawning season in cod is observed as a gradual and accelerating increase in egg density to a peak, and a subsequent deceleration (the 'spawning curve'). The experiments we conducted show that the start of vitellogenesis is imprinted (Otterå et al. 2006; Greives et al. 2008; Paul et al. 2008) but the subsequent oocyte growth rate is adjusted by environmental temperature (Olive et al. 1998). Thus, autumnal equinox acts as the oocyte growth trigger and temperature as the main oocyte growth regulator. These results lead to a new perspective of the principles involved in the formation of what appears as this typical spawning curve, as seen for instance in the Lofoten area (Pedersen 1984). The first part of this curve should consist of eggs shed by fish coming from the warmest water followed progressively by fish coming from colder and colder water. In the case of Lofoten this would indicate that local cod (i.e. Coastal cod) eggs are generally spawned first followed later also by eggs from the Barents Sea cod.

Secondly, our data support the contention that the spawning time of larger females in relation to smaller females is advanced in the warmer water but this phenomenon vanishes in the colder water. The underlying reasons for size dependency are, however, unclear (Wright and Trippel 2009). According to Pörter et al. (2008), a larger ectothermic body enhances thermal sensitivity based on allometrical considerations: 'oxygen supply becomes restricted earlier than in a smaller specimen'. For the Barents Sea cod the size dependency seems labile; in a few years larger cod are more developed, in most other years, they are not. For the Irish Sea and North Sea both data sets indicate that the larger cod spawn first, although less convincing for the Irish Sea than the North Sea, possibly due to the less successful sampling program but also truncated age and length distribution following the stock collapse in the Irish Sea stock. Taken together, this suggests that size-specific spawning time apparently has an

underlying physiological reason related to thermal window dynamics, found experimentally to relate back to body size in summer time at AT. In this article, the information from DSTs suggests that the spawning temperature (window) is around 7 °C for cod in southern waters, the Irish and North Seas and the Channel, and around 4 °C for cod in northern waters, the Barents Sea. Thus, the two spawner categories seem adapted to different thermal windows, as expected (Pörtner et al. 2008). More data are, however, needed to stretch this argument any further, e.g. earlier findings suggest that the Barents Sea cod may spawn between 4 and 6 ºC (Ellertsen et al. 1989).

Predicting the effects of climate change on cod reproductive ecology

In sum, the results of our work show that we are now able to explain and predict the maturity (oocyte growth) and likely fecundity and reproductive success of cod in different areas. The tool box of equations that we derived should now make it possible to better understand the variation in cod spawning time as a consequence of future climate change that, in turn, will have great prospects in further recruitment studies. For instance, one could now examine if the effects of climate-mediated changes in the zooplankton community (e.g. Beaugrand et al. 2000), coupled with changes in the time of spawning, could lead to a more frequent mismatch between the critical feeding period for cod larvae and the time of greatest abundance of copepod nauplii. Also, the consequences of warming on the success of ovulation and egg quality in different waters should be further examined paying special attention to the critical threshold value of 9.6 ºC for cod seen in the aquaculture-related work of van der Meeren and Ivannikov (2006).

Conversely, temperatures suitable for optimal vitellogenesis may be actively selected by adult cod, assuming that these temperatures also allow for sufficient food intake to permit sufficient investment in the gonads, and therefore mediate the effect

of any climate change by shifting the relative positions of feeding and spawning habitats. Turning the present series of arguments around, our findings may suggest that observations of spawning curves can be used to indicate the temperatures that adult cod have been experiencing, i.e., the ovaries of cod could be used as a 'biological thermometer'.

Altogether, we show that the underlying oocyte growth and energy allocation patterns of cod is strongly influenced by environmental temperature opening up for a fascinating field of research, and that the issues discussed in this paragraph and the others above will be central research areas for marine fish reproductive physiologists in the years to come.

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Table 1. Summary of experimental results on female Coastal cod held at low temperature (LT) and ambient temperature (AT) during their second maturation cycle, i.e., from the age of about 2½ to 3 years. In one case both genders were analysed for comparison with published results. Prespawner and spawner refer to females that became these categories towards the end of the experiment (Day 224). **Note:** W, whole body weight; TL, total length; G, specific growth rate; DLI, daily length increment; K, Fulton's condition factor; GSI, gonadosomatic index; HSI, hepatosomatic index; F, fecundity; RF, relative fecundity; n, number of fish.

			LT		AT		Between-tank
	Category	No. of days	n	Mean (SD)	n	Mean (SD)	(P-value)
Growth							
W (g)	Female	0	33	1861 (298)	38	1866 (350)	0.955
	Female	224	33	3348 (482)	38	3469 (589)	0.351
TL (cm)	Female	0	33	53.6 (3.4)	38	54.4 (3.2)	0.317
	Female	224	33	61.5 (3.8)	38	64.2 (3.9)	0.004
G (%•day^{-1})	Both genders	224	75	0.252 (0.052)	74	0.253 (0.059)	0.624
	Female	224	33	0.263 (0.036)	38	0.278 (0.045)	0.086
DLI (mm•day^{-1})	Female	224	33	0.35 (0.05)	38	0.44 (0.08)	< 0.001
Reproduction							
W (g)	Female, prespawner	0	31	1855 (306)	25	1774 (304)	0.328
	Female, spawner	0	2	1961 (–)	13	2042 (377)	—
	Female, prespawner	224	31	3354 (496)	25	3340 (535)	0.921
	Female, spawner	224	2	3257 (–)	13	3717 (629)	—
K	Female, prespawner	224	31	1.42 (0.15)	25	1.28 (0.15)	0.001
	Female, spawner	224	2	1.68 (–)	13	1.34 (0.11)	—
GSI (%)	Female, prespawner	240	30	11.8 (3.0)	25	14.1 (3.7)	0.013
	Female, spawner	240	2	15.7 (–)	13	22.2 (4.1)	—
HSI (%)	Female, prespawner	240	31	13.2 (1.5)	25	11.0 (1.2)	< 0.001
	Female, spawner	240	2	12.7 (–)	13	10.1 (1.4)	—
F (millions)	Female, prespawner	240	30	3.72×10^6 (0.70×10^6)	25	4.32×10^6 (1.02×10^6)	0.017
RF (g^{-1})	Female, prespawner	240	30	1117 (167)	25	1307 (242)	0.003

1313

Table 2. Overview of samples, split into prespawners and spawners, used to explore the relationship between leading cohort (LC) oocyte diameter and total length (TL) in the different waters of study. For the Barents Sea cod year-specific regression were established. If significant, slope (a) and intercept (b) values (±SE) are given. In 2006 both an early and late sample was taken. North Sea and Irish Sea LC diameter data were standardised to the last day of sampling at the cruise. Shaded area: a significant result; solid border: an insignificant result (for TL only). n: number of females.

					TL (cm)		LC (µm)		Regression analysis LC vs. TL			
Year	Area	Calendar day	n	Category	Mean	SD	Grand mean	Grand mean SD	r^2	P	a (±SE)	b (±SE)
Northern area												
							Observed values					
1999	Barents Sea	69-70	90	Prespawner	85.2	9.7	752	62	0.001	0.758	–	–
2000	Barents Sea	74	79	Prespawner	80.8	9.4	792	64	0.007	0.456	–	–
2003	Barents Sea	62	48	Prespawner	84.9	14.7	732	64	0.002	0.793	–	–
2004	Barents Sea	57	51	Prespawner	86.1	16.1	722	52	0.086	0.037	0.95 (0.44)	640 (39)
2005	Barents Sea	59	45	Prespawner	89.7	13.4	717	65	0.038	0.199	–	–
2006, early	Barents Sea	43-44	38	Prespawner	90.5	14.8	680	76	0.001	0.825	–	–
2006, late	Barents Sea	66-67	40	Prespawner	82.1	17.9	724	59	0.158	(0.011)	1.31 (0.49)	613 (54)
2007	Barents Sea	67	49	Prespawner	91.3	17.8	785	79	0.180	0.002	1.88 (0.58)	613 (54)
2008	Barents Sea	58-59	46	Prespawner	92.8	14.7	725	64	0.004	0.687	–	–
Southern area												
							Standardised values					
2004	North Sea	22-55	17	Prespawner	60.9	16.1	706	94				
			24	Spawner	78.9	15.3	≥ 875	–				
2004	Irish Sea	41-50	15	Prespawner	62.7	14.6	822	44				
			23	Spawner	65.9	12.4	≥ 875	–				

1314

1315

Figures

Fig. 1. Experimental conditions in low-temperature (LT, filled circles) and ambient-temperature (AT, open circles) tanks as described by water temperature (T) (a), feeding ration (FR) (b), and resulting mean specific growth rate (G) (± SE) (c) and mean Fulton's condition factor (K) (± SE) (d). Vitellogenesis (shaded area) refers to females with developing oocytes. Inserted numbers in (a) are mean vitellogenic temperature at LT (lower position) and AT (upper position) while arrow reflects a transient problem with the temperature cooler. Solid and broken lines in (b) show weighted mean FR at LT and AT, respectively, while overlapping lines in (c) show mean G for the whole experiment in the two tanks. Day 0 = 1 June 2005; Day 240 = 26 January 2006.

Fig. 2. Relative somatic fecundity (RF_S) versus leading cohort (LC) oocyte diameter on Day 240 (experimental end) for prespawners held either at low temperature (LT) or at ambient temperature (AT). LT prespawner is represented by filled circle and AT prespawner by open circle. Separate regression lines are included (LT: solid line; AT: broken line), excluding in one case an outlier (arrow).

Fig. 3. Explanatory power (r^2) of potential fecundity on Day 240 (experimental end) for prespawners at low (LT, solid line) or ambient temperature (AT, broken line) regressed on either body weight (W) or combined with leading cohort (LC) diameter (W + LC) at different measurement points during the experiment. Mean LC diameter is attached to each relevant analysis point. W-based regressions are indicated by filled

circle for LT and open circle for AT while W+LC regressions are indicated by filled and open crosses, respectively. An AT outlier was excluded (see Fig. 2).

Fig. 4. Development in mean values (± SE) of the width of the developing oocyte frequency distribution (SD_{diam}) (a) and the diameter of the smallest cohort of oocytes (SC) (b) in low-temperature prespawners (solid circle) and ambient-temperature prespawners (open circle) and spawners (cross) between Day 155 and 240 (2 November – 26 January). In b) the vertical distance from maximum previtellogenic oocyte diameter (horizontal line) to each measurement point indicates oocyte gap size formation.

Fig. 5. Growth in mean leading cohort (LC) oocyte diameter (± SE) at the group level (LC_{group} diameter) at low (filled circle) and ambient temperature (open circle) between Day 128 and 240 (6 October – 26 January. The same females were tracked over time.

Fig. 6 Individual oocyte growth rate (R) between Day 155 and 224 (2 November – 10 January) at low (LT) (filled circle) and ambient temperature (AT) (open circle) in relation to total length (TL) as measured on Day 0 (1 June). Associated regression lines (LT: solid line; AT: broken line) are included but excluding in the case of LT an outlier (arrow).

Fig. 7 DST-recorded temperatures (T) (monthly mean ± SD) in released-and-recaptured individual cod in southern waters, i.e. the Channel (cross), southern North Sea (square) and Irish Sea (triangle), and Barents Sea (circle). Length of spawning

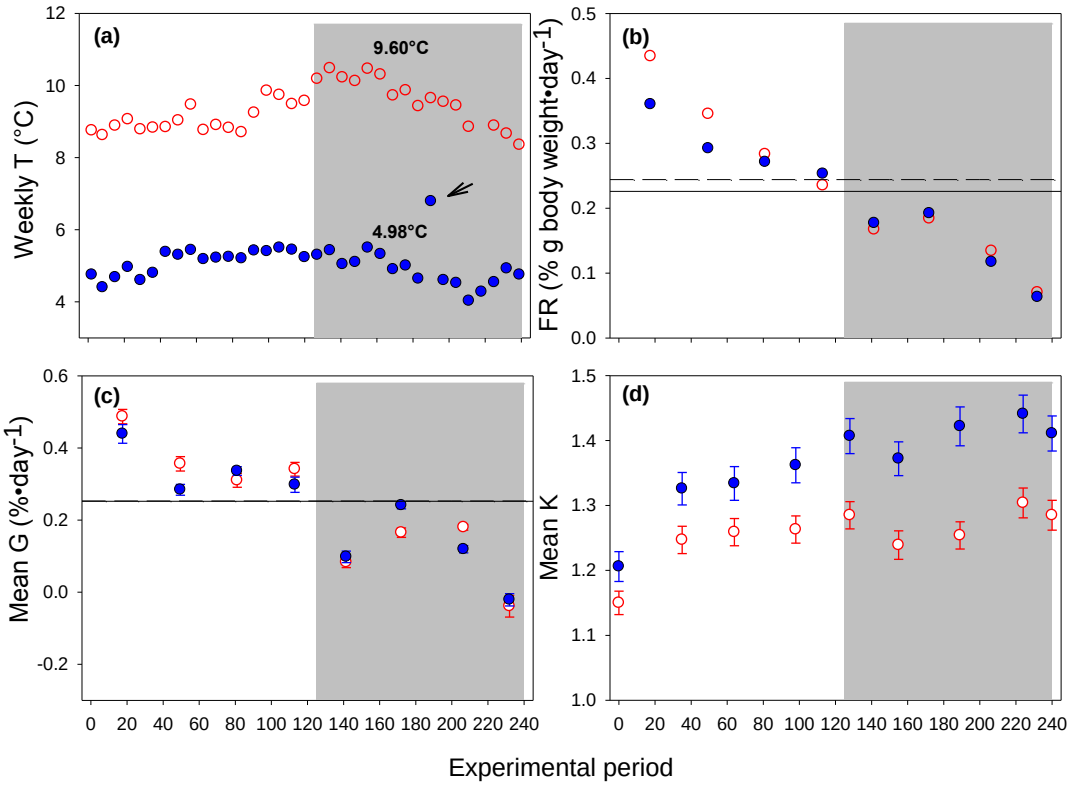
season in southern (left box) and northern waters (right box) is from Brander (1994) and Pedersen (1984), respectively. Temperatures at or above the horizontal line (9.6 ºC) is considered to result in reduced egg fertilization and normal development (van der Meeren and Ivannikov 2006).

Fig. 8 DST-recorded temperatures (mean, min. and max. value) between 1 September and 1 February for cod in northern and southern areas in relation to total length (TL) at release (same material as in Fig. 7). The selected period of time is assumed to encompass the major part of the vitellogenic period and thereby vitellogenic temperature (T_{vit})., see main text. Cross, square, triangle and circle represent the Channel, North Sea, Irish Sea and Barents Sea, respectively.

Fig. 9 Descriptors (mean ± SD) of the Andenes-caught Barents Sea cod females, i.e., total length (TL) (a), viscera condition ($C_{viscera}$) (b) and body condition (C_{weight}) (c) using filled circle for standard sample and open circle for extra sample, and CTD temperature (mean only) in the Vardø North Transect in August-September (T_{VN}) (early vitellogenesis) (d). Extreme values in (b) refer mainly to predation on adult capelin (*Mallotus villosus villosus*) but also adult herring (*Clupea harengus*) and unidentified 'fish species'. Horizontal line in (b) and (c) is the normalised, reference line.

Fig. 10 Grand mean leading cohort oocyte diameter (LC_{group}) as observed in the Andenes sampling program on Barents Sea cod (filled circle with year attached) plotted versus calendar day of sampling (solid line) in comparison with similar experimental data at 5 (short dash, cf. Eq. (6)') and 9.6 ºC (long dash, cf. Eq. (7)')

1390 and conceptually modelled data (3.4 ºC) (dotted line). Shaded area refers to oocyte
1391 final maturation.
1392
1393 **Fig. 11** Duration of daylight at four areas of study (Channel: cross; Irish Sea: triangle;
1394 Experiment: square; Barents Sea: circle) as reported by official US web pages at
1395 present experimental measurement days (Day 0-240: 1 June 2005 – 26 January 2006).
1396 Time of summer and winter solstice and autumnal equinox are added as well as the
1397 time when the sun went below the horizon for the first time after the period of
1398 continuous light in the Barents Sea. Horizontal line is inserted to mark autumnal
1399 equinox.
1400
1401 **Fig. 12** Conceptual modelled relationship (± 2×SE) between start of spawning (i.e.,
1402 leading cohort oocyte diameter equals 875 µm) and vitellogenic temperature ($T_{vit.}$) for
1403 an individual cod female. Spawning time of a large (65 cm, large circle) and small
1404 (55 cm, small circle) warm-water (southern) cod is indicated.
1405



1406

1407 **Fig. 1**

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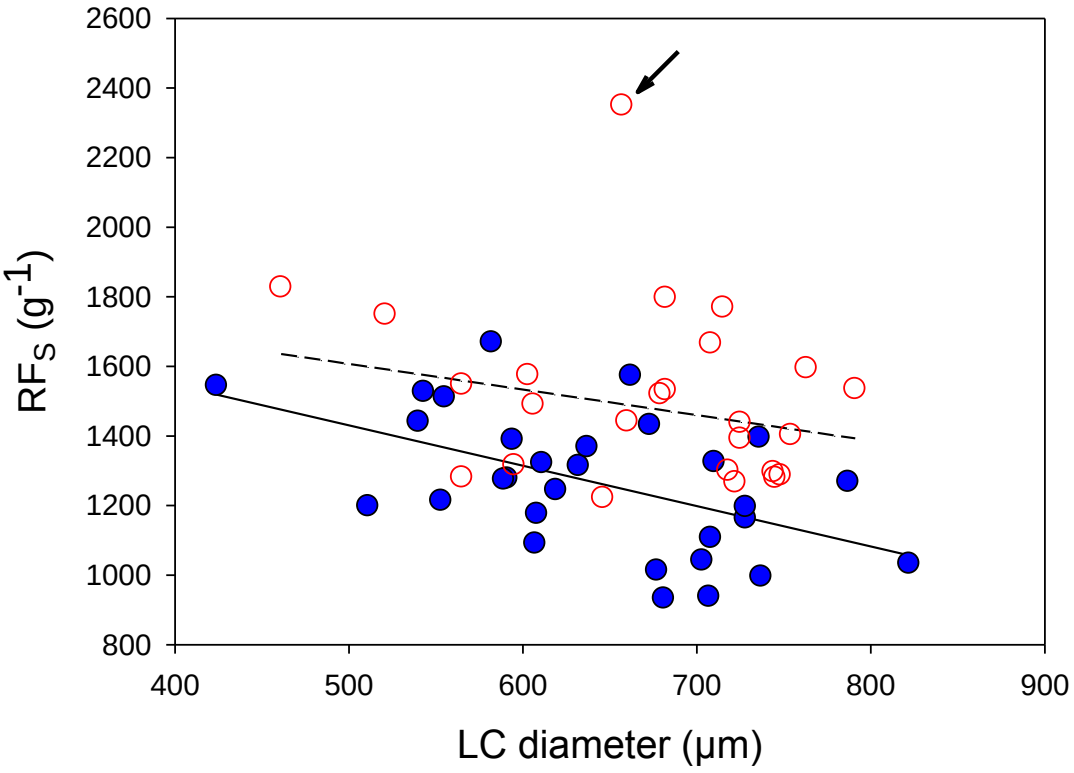
1409


2600
2400
2200
2000
1800
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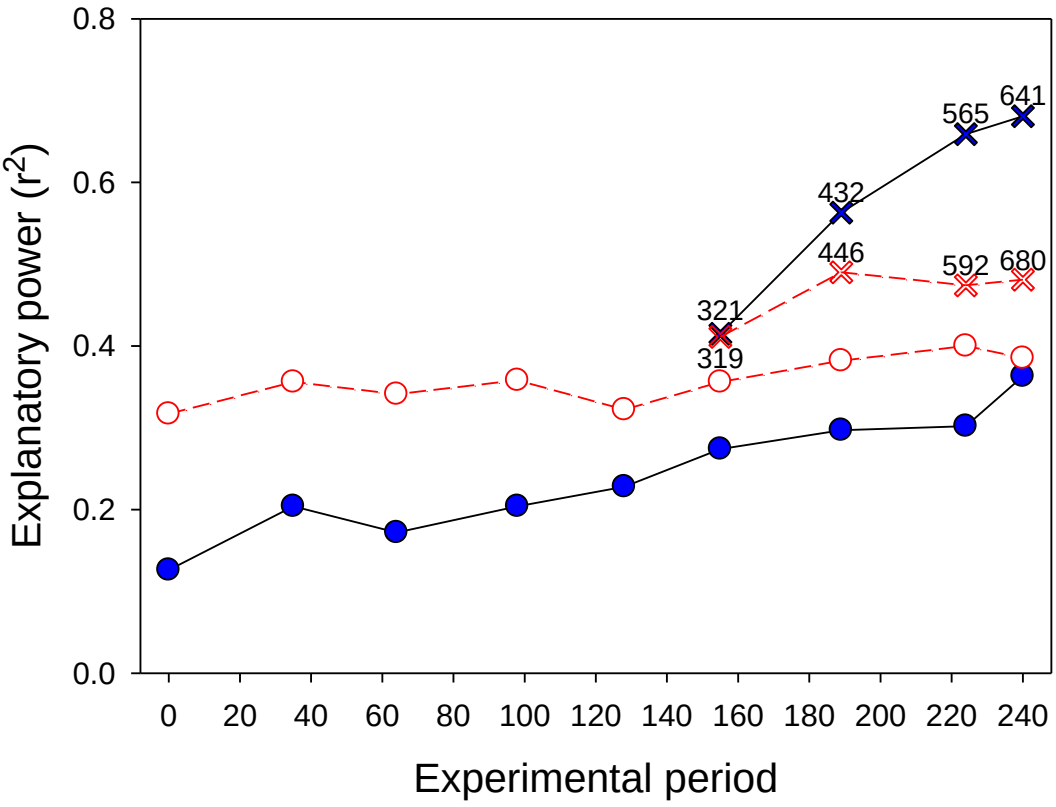
600
700
800
900
RFS (g-1)
LC diameter (µm)

Fig. 2

1414



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1416 **Fig. 3**

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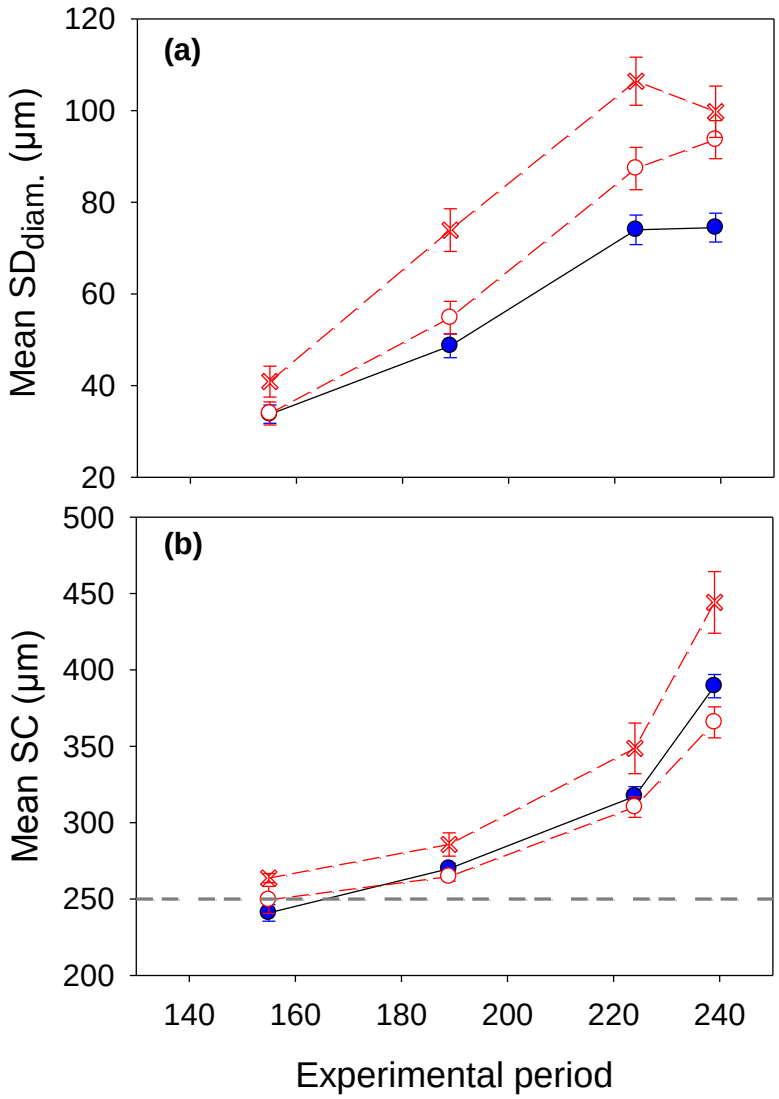
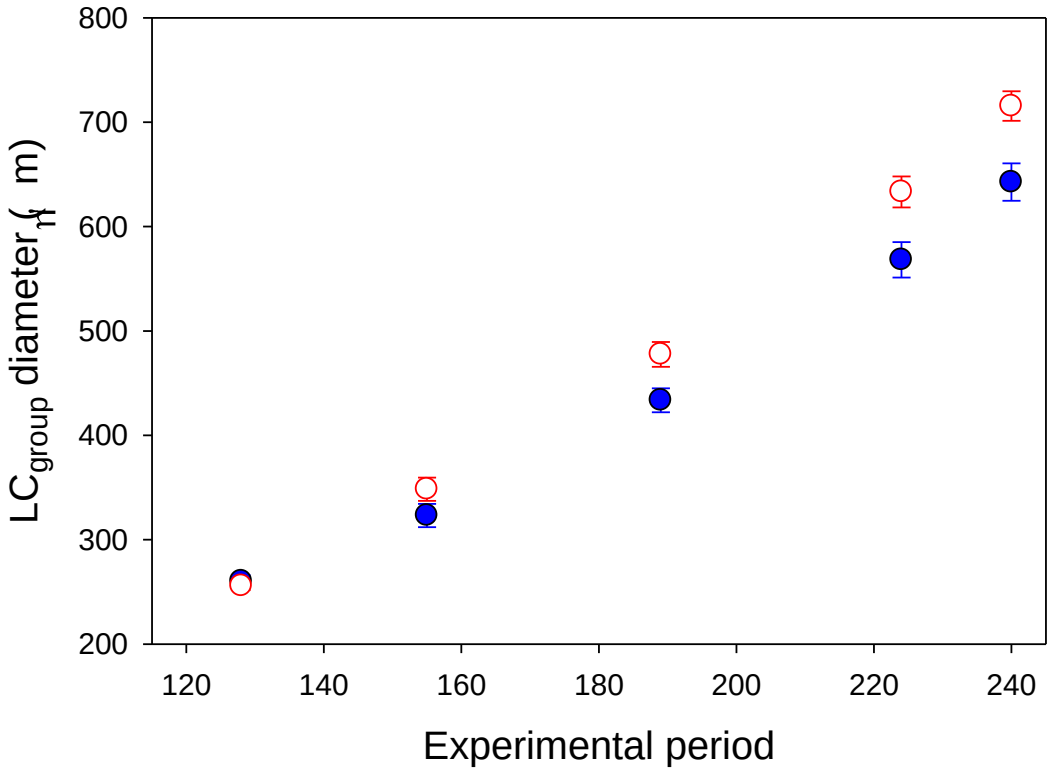


1419

1420 **Fig. 4**

1421


800
700
600
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220
240
Experimental period
LC group diameter (μm)

Fig. 5

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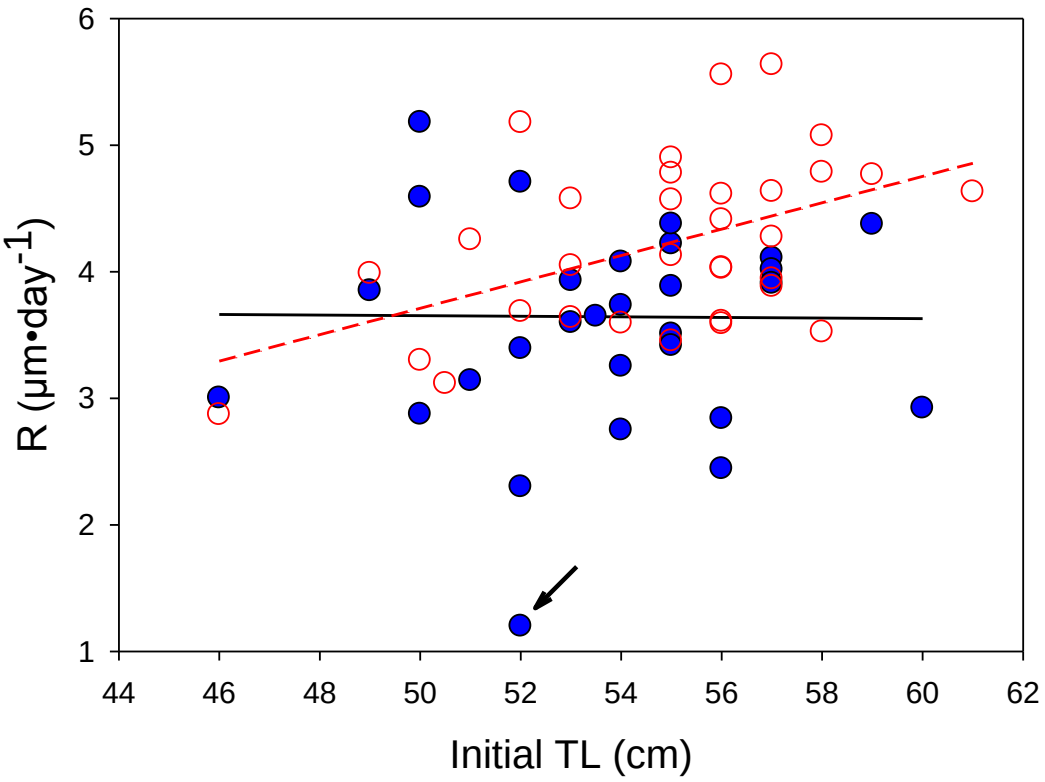

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1429 **Fig. 6**

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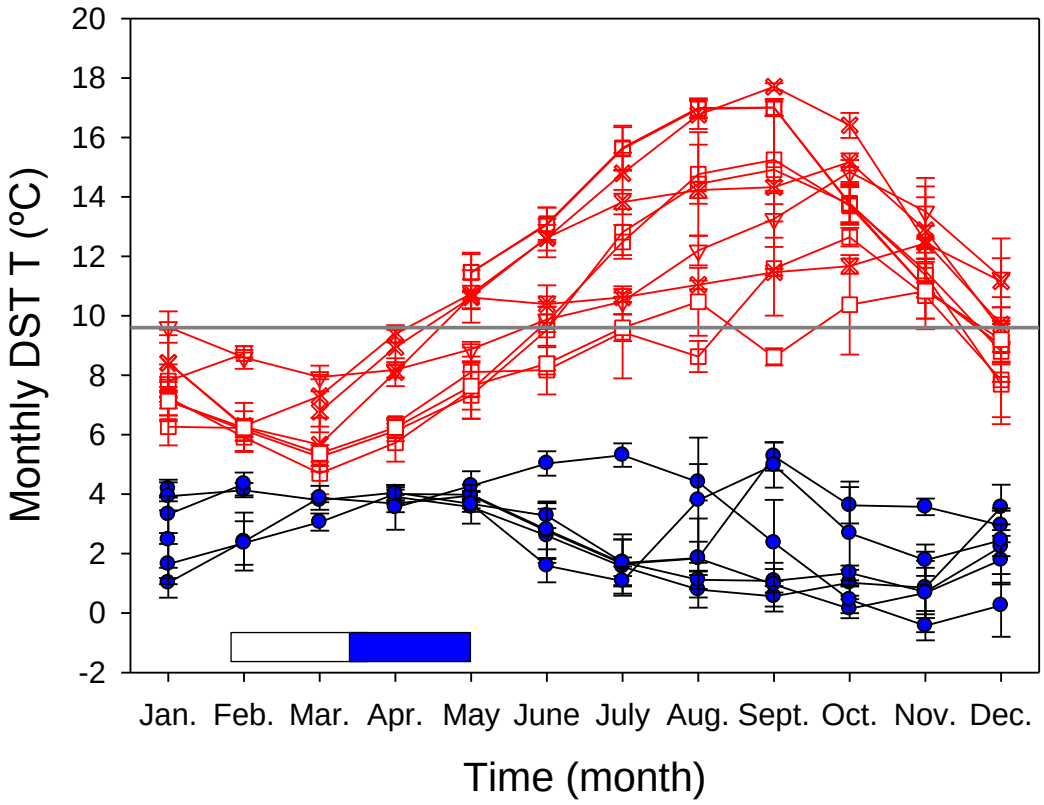

1432

1433 **Fig. 7**

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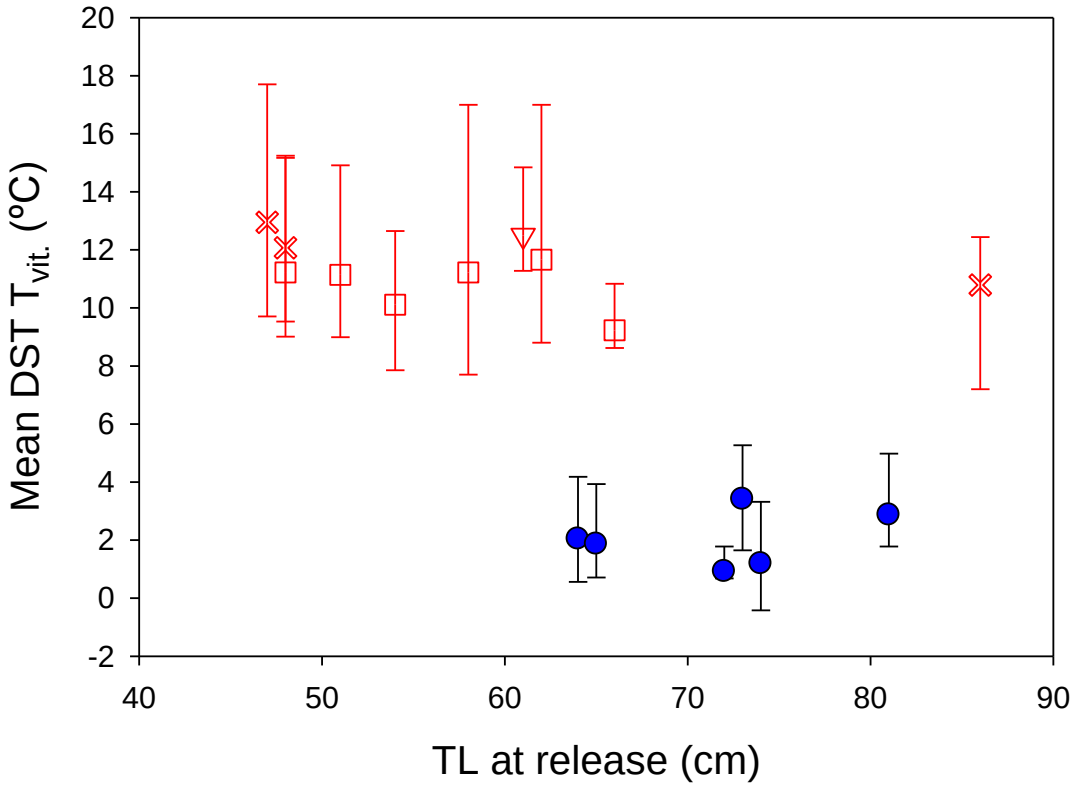

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1437 **Fig. 8**

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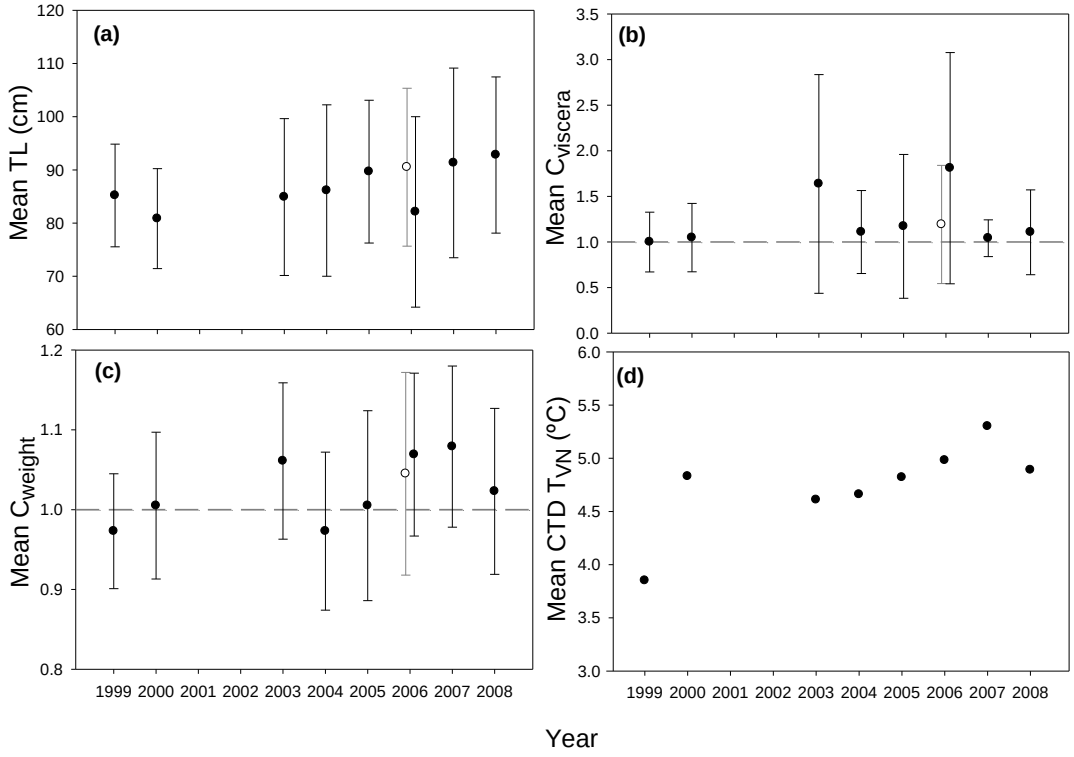

1440

1441 **Fig. 9**

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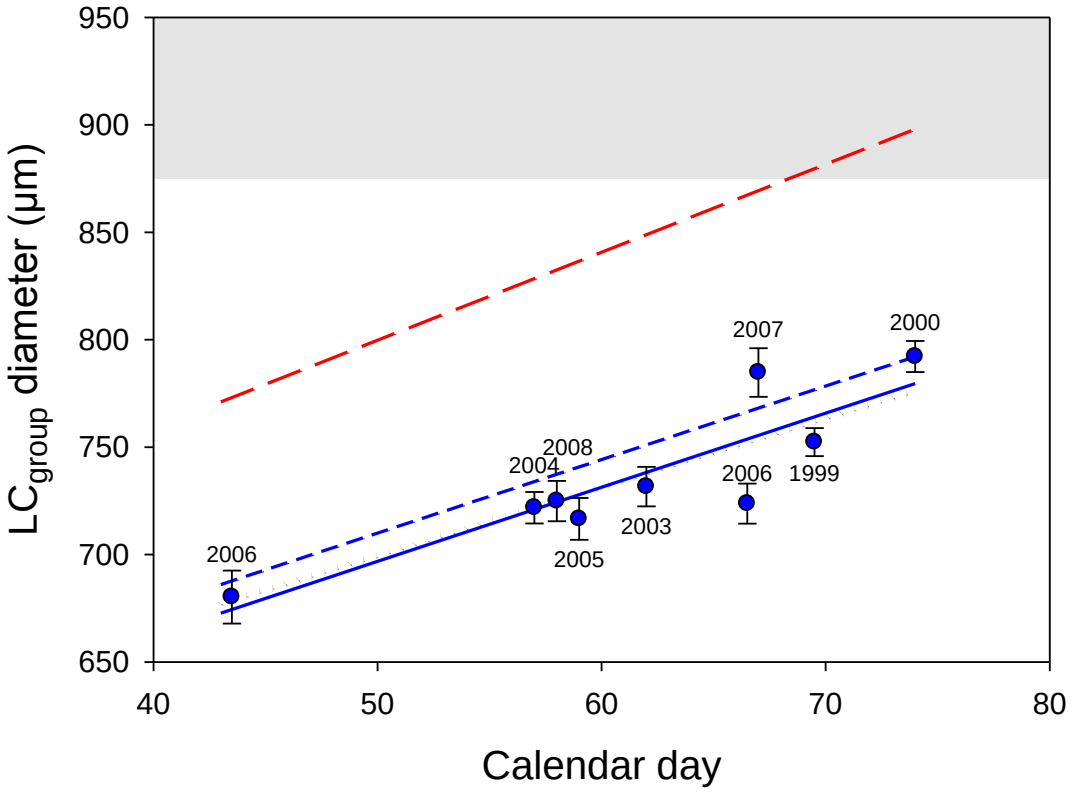

1444

1445 **Fig. 10**

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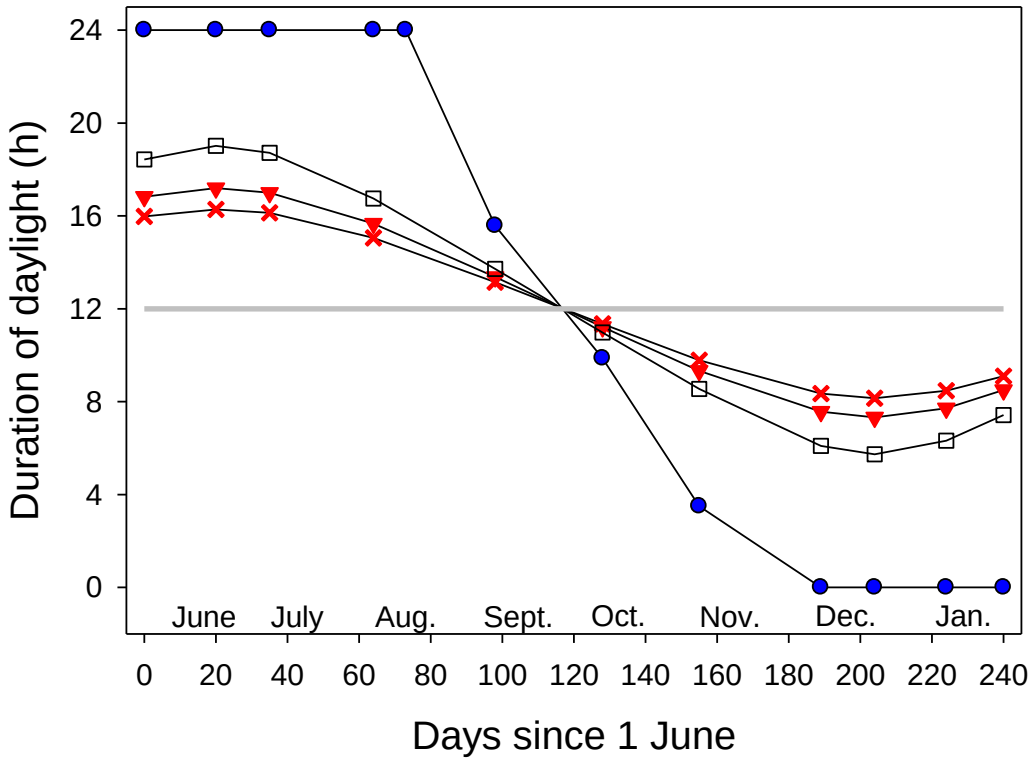

1449

1450 **Fig. 11**

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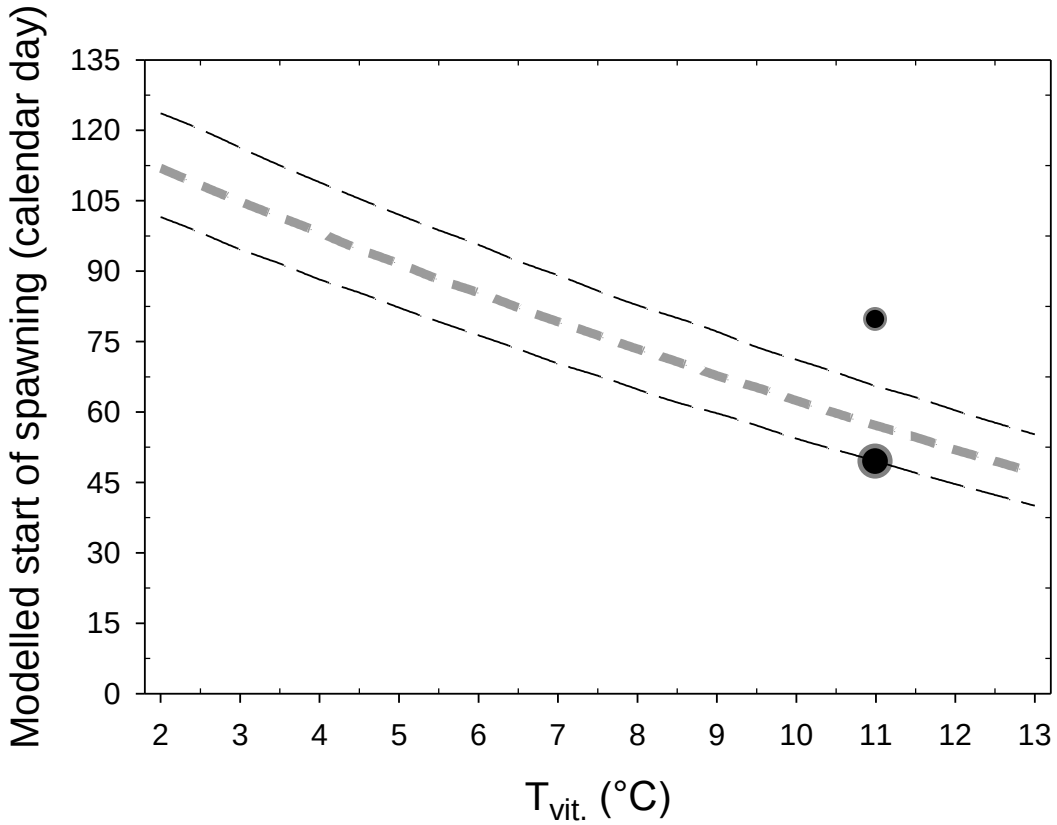
1452



1453

1454 **Fig. 12**