Timecourse of oocyte development in saithe *Pollachius virens*

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Running headline: OOCYTE DEVELOPMENT IN *P. VIRENS*

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Abstract

Given that information on basic biology such as reproductive physiology, is essentially lacking for saithe the *Pollachius virens*, wild caught North Sea *P. Virens* were monitored for growth, sex steroid profiles and oocyte development pre-spawning and measured for egg size and group fecundity during the spawning season in the laboratory. Vitellogenesis commenced in late October/early November, at a leading cohort size (LC) of ~250 μm whereafter oocytes grew rapidly in size until spawning started in February. Notably, a distinct cortical alveoli stage was virtually absent with yolk granules observed in developing oocytes at the very beginning of vitellogenesis. Little atresia was observed pre-spawning, but atretic reabsorption of remnant oocytes containing yolk granules was found in all females immediately post-spawning. As expected, concentrations of sex steroids, estradiol-17β (females), testosterone (both sexes) and 11-ketotestosterone (both sexes), increased pre-spawning before dropping post-spawning. The present experiment provides the first validation of sex steroid levels in *P. virens*. Post-ovulatory follicles were visible in histological sections from female gonads 9 - 11 months post-spawning, but then disappeared. Spawning commenced around a LC of ~750 μm (700 – 800 μm). Hydrated oocytes (eggs) measured between 1.04 – 1.31 mm (mean = 1.18 mm) with decreasing sizes towards the end of spawning. The average estimated realized fecundity was about 0.84 million eggs (median female length = 60 cm). Spawning lasted from February 13 to March 29.

Key Words; Timing of gonad maturation, oocyte development, atresia, post –ovulatory follicles, fecundity, egg size

INTRODUCTION

Saithe *Pollachius virens* L. 1758 is a gadoid residing in the Northern Atlantic. Despite being a species of considerable cultural and commercial importance, surprisingly little is known about its basic biology. The latter is especially true when compared to other gadoids like the Atlantic cod *Gadus morhua* L. 1758 and to a lesser extent the Atlantic haddock *Melanogrammus aeglefinus* L. 1758.

Traditionally, seven different *P. virens* populations or, perhaps more appropriately named, units of management have been considered; one in the western Atlantic and six populations in the east Atlantic (Olsen *et al.*, 2010). However, the connectivity between these units and their genetic underpinning is still not clear (Reiss *et al.*, 2009). Saha *et al.* (2015) recently published a broadscale account of the genetic structure of *P. virens* throughout its range using seascape genetics. They found that *P. virens* could be distinguished into four broad genetic clusters; the Barents Sea, the Central Northeast Atlantic, Rockall and Canada. This contrasts somewhat to other studies finding less differentiation (Behrmann *et al.*, 2015).

North Sea *P. virens* is the common name given to *P. virens* spawning in north-western areas of the North Sea in February and March. The North Sea *P. virens* stock has fluctuated in size peaking in the early 1970s, but is now at a low level (ICES, 2015). Few studies have reported on individual fecundity in *P. virens*. One notable exception is the report by Storozhuk *et al.* (1974), where they gave information on potential fecundity vs size for wild caught North Sea *P. virens*. van der Meeren & Lønøy (1998) reported a total realized fecundity of 2.21 million eggs in a group containing six females. Studies looking at the timing (onset) of gametogenesis or the sex - steroid levels associated with this process are scarce. Female gametogenesis (oogenesis) can broadly be classified into six stages; oogonial proliferation, primary growth stage, cortical alveolus stage, vitellogenesis, maturation and

58 spawning (cf. Tyler & Sumpter, 1996; Lubzens *et al.*, 2010) with the initial two phases
59 considered previtellogenic.

60 A laboratory experiment was therefore conducted to, primarily, track female oocyte
61 development, and male and female sex steroids concentrations pre- and post- spawning and,
62 secondly, measure resulting egg size and group fecundity during spawning. In addition the
63 formation and duration of post-ovulatory follicles (POFs) was examined. POFs represent an
64 unequivocal marker of past-spawning activity (Witthames *et al.*, 2009). For *G. morhua* and
65 *M. aeglefinus*, POFs have a duration of > 1 year post - spawning and have e.g. been used to
66 identify the phenomenon of skipped spawning in these species (Skjæraasen *et al.*, 2012a;
67 Skjæraasen *et al.*, 2015).

68

69 **MATERIALS AND METHODS**

70 HISTORY OF FISH

71 All *P. virens* were caught by hook and line off the west-coast of Norway (Fig. 1) on Aug 1
72 2013. Captured fish were kept in aerated tanks aboard the research vessel until transport to the
73 Institute of Marine Research (IMR) facility at Parisvannet (Fig. 1) the same day. All fish were
74 pit-tagged for individual identification. Fish were kept in a 3 m d (7 m^3) outdoor tank under
75 an ambient, natural photoperiod and fed on a pellet diet. The tank was supplied with water
76 pumped from 20 m depth with temperature reflecting the ambient temperature at this depth.
77 Little mortality occurred in the tanks in the initial months (~10 %) and fish starting feeding on
78 the pellets immediately. In mid February 2014 fertilised eggs were detected in the tank for the
79 first time with spawning continuing until early April 2014. During the subsequent
80 experimental phase water temperature at Parisvannet varied from approximately 6˚ C in
81 April/May increasing to a peak around 14˚ C in August before decreasing to 12 ˚ C in
82 October.

THE EXPERIMENT

The first part of the experiment evaluated whether POFs could be used as a long-term marker of past spawning in *P. virens* in the same way as has been done for *G. morhua* (Skjæraasen *et al.*, 2009; Witthames *et al.*, 2009; Skjæraasen *et al.*, 2012a) and *M. aeglefinus* (Skjæraasen *et al.*, 2015). On April 2 2014, at the very end of spawning, gonadal biopsies were successfully secured from five of the females. The biopsy sampling was performed on sedated fish. An ovarian sample of about 0.2 ml was withdrawn from the ovary by inserting a thin plastic tube (Pipelle de Cornier®; www.mpmmedicalsupply.com) through the genital pore. The samples were subsequently fixed in 3.6 % buffered formaldehyde for at least 2 weeks prior to analysis.

These females were then subsequently sampled for biopsies ~monthly until October (Table I). From October until the end of spawning, in order to evaluate gamete maturation, biopsy samples was obtained not only for these females, but from all females as long as biopsies could be obtained easily and without any undue physical force. In addition, all fish of both sexes were measured for length (cm), mass (g) and a blood sample was obtained for individual profiles of the sex steroids 11-ketotestosterone (11-KT), testosterone (T) and 17β estradiol (E2) during gametogenesis. In November 2014 the IMR Parisvannet research facility was shut down and all *P. virens* were therefore moved to the IMR Austevoll research facility (Fig. 1) on October 27. Fish were initially kept in two replicate 3 m diameter (7 m^3) outdoor tanks under a natural light and photoperiod until January 10 2015 when all fish were moved to one common outdoor spawning tank (3 m d, 7 m^3) fitted with an egg collector and again kept under a natural light and photoperiod. The tanks at Austevoll were supplied with water pumped from 165 m depth and temperature thus reflects the ambient temperature at this depth (Fig. 2).

Eggs were detected in the egg collectors from February 13 to March 29 2015. During this period, except February 11, 13 and 14 and March 7 and 8, the percentage of fertilized eggs was measured on all days. The latter was determined by examining ~ 200 randomly selected eggs under a microscope (16X) and identifying the number of fertilised and unfertilised eggs. For all days except March 7, 8 and 29 the volume of eggs found in the egg collectors was also noted. From March 11 pictures of the eggs were also taken daily at 16 X magnification allowing for the determination of egg size.

During the course of the experiment some *P. virens* were removed from the tanks due to injury. A total of 13 females and 9 males remained after the completion of the spawning season in 2015 and we have only included data for these fish in the present study. This reduced the amount of females for which biopsy results are shown pre-October 2014 to 3 (but see Table III). No fish were removed during the spawning season. The average temperature experienced by the *P. virens* at Austevoll, i.e. during the vitellogenic phase, was 8.2˚ C. All fish were sedated by 20 g l^{-1} 0.1 M NH_4HCO_3 (metacaine/MS222) at all samplings.

STEROID ANALYSES

Steroids were extracted from blood plasma by a method modified from Pankhurst & Carragher (1992). Briefly, plasma samples (100 µl) were mixed with 1 ml ethyl acetate, vortexed for 20 sec and centrifuged for 3 minutes at 1800 rpm and 4 ˚C. The organic phase was collected by a Pasteur pipette and the hydrophilic phase was extracted once more with 1 ml of ethyl acetate. The extracts were evaporated in a Speed Vac centrifuge (Savant 1000, USA), and dissolved in 1 ml phosphate-buffered saline, pH 7.4 (PBS; 0.1 M KH_2 PO_4, 0.4 M NaCl, 1 mM EDTA) by heating at 60 ˚C for 10 min. The extracted and dissolved steroids were stored at -20 ˚C until analysis by an enzyme-linked immunosorbent assay (Cuisset *et al.*, 1994), previously validated for Atlantic *G. morhua* (Dahle *et al.*, 2003) and further validated

for *P. virens* as described below: Extraction efficiency was determined by addition of a known amount of ^{3}H - labelled steroid to pooled plasma samples (n = 8) and measuring the amount of radioactivity that remained in the extracted and dissolved sample. Recovery after extraction was 65% for E2 and 83-85% for androgens. Steroid concentrations are corrected in relation to percent recovery. Logit-log binding curves of serial dilutions of steroid standards and plasma samples were parallel showing that extracted plasma samples were suited to the assay conditions. ED80 and ED20 were 0.004 ng ml^{-1} and 0.08 ng ml^{-1} for T, 0.04 ng ml^{-1} and 1.00 ng ml^{-1} for 11-KT, and 0.006 ng ml^{-1} and 0.6 ng ml^{-1} for E2. Detection limits of the assays were 0.008 ng ml^{-1} for T, 0.005 ng ml^{-1} for 11-KT and 0.015 ng ml^{-1} for E_2. Internal standards were prepared from mature male (11-KT) and female (E2, T) Atlantic *G. morhua* plasma extracted as described above. Interassay variation was 8.2% for E2 (n = 6), 6.4% for T (n = 9) and 6.2% for 11-KT (n = 11). The intra-assay coefficient of variation was 16.2% for E2 (n = 9), 14.8% for T (n = 14) and 4.6% for 11-KT (n = 9). E2 and T antisera, acetylcholine esterase-labelled tracers and microplates precoated with monoclonal mouse antirabbit IgG were supplied by Cayman Chemicals (USA). Anti-11-KT was a kind gift from dr. David E. Kime, Sheffield University, UK. Standard steroids were purchased from Sigma Aldrich (Sigma reference standards). Cross-reactivities for E2 and T antisera are described by the manufacturer, while details on crossreactivity for the 11-KT antibody are given by Cuisset *et al.* (1994).

IMAGE ANALYSES OF OOCYTE SIZE

All biopsy samples, except the samples obtained in April 2014, were first subjected to digital image analyses to measure the size of vitellogenic oocytes present in the samples. This method uses the contrast between previtellogenic oocytes and vitellogenic oocytes in relation to the set background to specifically select and measure the diameter of the latter oocyte

category (Thorsen & Kjesbu, 2001). Typically 150-250 oocytes are measured in each sample. These measurements were subsequently used to calculate the average oocyte and LC size with the latter defined as the 95 % median of all the measured oocytes. If only pre-vitellogenic (primary growth) stages were present, or alternatively, oocytes were at the very earliest phases of vitellogenesis this method would not produce any size measurements.

HISTOLOGY

Post image - analyses all female biopsy samples were processed for histology using standard protocols for resin embedding (Technovit 7100), producing 4 μm sections stained with 2% toluidine blue and 1% sodium tetraborate. In each section the presence or absence of POFs was noted. Oocytes were then classified into various previtellogenic stages (PVO), cortical alveoli (CAO) and vitellogenic yolk granule (YG) oocytes. For each sample the LC size was also calculated as the average of the five largest oocytes found in the histological section. Finally, atresia was estimated for each oocyte stage following the method introduced by Hunter & Macewicz (1985), i.e. dividing the intensity of atresia into four different categories 0-5, 5-25, 25-50 and > 50 (100) %. Further details on this method can be found in Skjæraasen *et al.* (2009) and Skjæraasen *et al.* (2010a). This 'rough' estimation of atresia was considered adequate for the present study.

EGG SIZE MEASUREMENTS

The egg diameter was measured by analysing images of the eggs, using the Interactive Data Language (IDL, Exelis Visual Information Solutions; www.harrisgeospatial.com) software. The measurements were done on binary versions of the original images to increase the contrast and enhance the edge of the eggs. The binary images were constructed by using a low-pass Fourier filter to reduce the noise, and an intensity scaling (keeping pixels with more

than 1.3 times the mean pixel intensity) to enhance the features in the image. For each egg completely within the field of view, the egg diameter was manually defined on four different axes (with respect to the image axis), horizontally, vertically and two diagonal axes approximately at an angle of 45° with respect to the horizontal and vertical axes. The average of the four measured diameters was used as the egg diameter in further analyses. The measurements were converted from pixels to mm with a calibration image of a ruler taken at the same resolution and magnification. The mm per pixel was calculated by manually defining four points on the ruler 1 mm apart allowing for three measurements of the number of pixels corresponding to 1 mm. The average of these measurements was used for the calibration.

DATA ANALYSES

All mixed - effect analyses were done with the use of R (R Development Core Team 2016) and the *nlme* library (Pinheiro *et al.*, 2016). It was *a priori* expected that body mass would increase prior to spawning, but significantly reduced or even negative during the spawning season. Both experimental day, coded as the number of days past 22/10/2014 (the first sampling date (Table I)) in this and all subsequent tests described below, and its squared term were therefore included as continuous variables in the initial model. In addition, the categorical variable sex and its interaction with sampling date and sampling date squared was included in the initial model. Repeated measures for individual fish were treated as a random effect in this and the mixed - effect analyses described below. To find the most parsimonious model the *Aikaike Information Criterion* corrected for small simple sizes (*AICc*) (Hurvich & Tsai, 1989) was used to remove terms that did not improve overall model fit. Before final model acceptance, residual plots were also checked for patterns in the errors to evaluate if it was necessary to correct for heteroschedastity and it was also tested whether a random effect

in both intercept and the slope for individual fish improved model fit. This general way of model simplification and selection was used for all tests.

For the sex steroid concentrations linear mixed effect ANOVAs were first employed. The different sampling days were treated as a categorical variable and steroid concentrations (ng ml^{-1}), log^e-transformed, as the dependent variable. To quantify the overall temporal patterns sex steroid concentrations were also analysed by employing an initial third order polynomial mixed effect regression with experimental sampling day as a continuous independent variable and steroid concentration (ng ml^{-1}), log^e-transformed, as the dependent variable.

To estimate oocyte growth a linear mixed effect model was used. Leading cohort size (LC; 95 % median egg size obtained from the image analyses) was treated as the dependent variable and regressed against experimental day.

Based on the days when images of the eggs where obtained, the diameter of all eggs in the field of view was measured. In addition, based on visual determination, eggs were classified according to three categories, vital fertilised, dead fertilised or unfertilised eggs. It was tested if there was a significant correlation (Pearson) between the average daily measured diameter of the fertilized eggs and day, hence if the egg size where increasing or decreasing towards the end of the spawning season.

The total average realized fecundity for the 13 females was also calculated. For days where direct measurements of the egg size existed from the images of the eggs, the day specific fecundity was obtained using two conversion formulations from volume to egg number;

$$Fec = \frac{1222}{d^{2.71}} \times vol(ml) \qquad \text{(eq. 1)}$$

231 $$Fec = \frac{1000 * \sqrt{2}}{d^3} \times vol(ml)$$ (eq. 2)

232

233 Eq. 1 is based on an empirical estimation of *G. morhua* eggs in relation to volume (Kjesbu,
234 1989), whereas eq. 2 denotes the maximum packing of spheres in a volume, i.e. it produces a
235 higher fecundity for the same volume and egg diameter (*d* in eq. 1 and 2 (in mm)). Due to
236 technical problems, the egg volumes were not measured on three days in the spawning season.
237 To compensate for this likely bias, a linear interpolation of the volume of the spawning
238 products on these days was made. For days where an egg image was not available the value
239 1.18 mm, i.e. the volume - weighted average diameter of the eggs, was used. Three
240 calculations of 'group fecundity' was then produced; i) a minimum estimate based on eq. 1
241 only including days where volume measurements was available, ii) a median estimate also
242 using eq. 1, but including the estimated volumes for the missing days and iii) a maximum
243 estimate also including the missing days, but using eq. 2.

244

245 **RESULTS**

246 BODY GROWTH

247 No difference in growth, i.e. patterns of changes in mass between males and females, was
248 found. Pre - spawning fish increased in mass while growth ceased or even tended to decrease
249 during the spawning season (Fig. 3, Table II).

250

251 SEX STEROID PROFILES

252 In general sex steroid profiles for both males (T and 11-KT) and females (E2 and T) showed
253 the same temporal pattern; first steroid concentrations increased as the spawning season
254 approached, after which they generally dropped to very low levels in April 2015 (Fig. 4,
255 Table II). However, while the latter was true for all females, some males still displayed high

levels at this last measurement, suggesting they were still in spawning condition. The highest measured concentrations of both T and E2 in females were detected in January and February, at around 3 ng ml^{-1} (T) and 11 ng ml^{-1} (E2), respectively (Fig. 4) 11-KT patterns for females remained low during the entire study (< 1 ng ml^{-1}), although there was a slight increase from the October to the November measurement and the lme-model did yield significant temporal effects (Fig. 4, Table II). The highest measured androgen levels in males were found in February, at around 35 ng ml^{-1} (11-KT) and 16 ng ml^{-1} (T)

OOCYTE DEVELOPMENT, ATRESIA AND POF DURATION

The initial histology samples taken in April 2014 showed that 2 females were still in a spawning state indicated by the presence of both hydrated and vitellogenic oocytes, whereas one had just finished (Table III). Only previtellogenic stages were then found in all biopsy samples until the October 22 sample (Table III, Fig. 5 and 6), when 3 of the sampled females showed an early phase of the yolk granule stage (Table III, Fig. 5 and 6). The transition from the previtellogenic to the vitellogenic stage occurred around an oocyte size of 250 μm, when yolk granules were observed in the oocytes; the smallest LC of vitellogenic occytes was 233, 251 and 253 μm, while the largest LC containing only previtellogenic stages measured 243 μm (Table III, Fig. 5). By November 28, all sampled females had reached the yolk granule stage and the oocytes from all but one female could be measured by automated image analysis. From this point on vitellogenic oocytes grew rapidly in size (4.6 μm day^{-1}; Table II, Fig. 5) until spawning commenced around a *LC* size of 750 μm (range 700 - 800 μm). Females exhibiting the cortical alveolus stage as the most advanced stage were not observed (Table III).

Very little atresia was observed pre-spawning, but reabsoprtion of remnant yolk granule oocytes was found in all sampled females immediately post-spawning. POFs

appeared to last 9 – 11 months post-spawning, but then fully disintegrate and disappear. Immediately post-spawning in April 2014, large POFs could be seen (Table III). Generally, POFs were then found in all histological sections until November, after which they started to disappear. In April 2015, immediately after spawning, no old POFs were seen in any of the histological sections, but large newly formed POFs were once again visible (Table III, Fig. 6).

SPAWNING; EGG SIZES AND FECUNDITY

Eggs were first found on February 13th. Only minute quantities were collected in the first week but, after this, large weekly amounts of eggs, albeit with substantial day to day variation, were collected until March 27th, when volumes dropped significantly (Fig. 7). No eggs were detected after March 29th (Fig. 7). The median daily fertilization percentage was 92.5 %, while fertilized eggs made up 87 % of the total egg volume (Fig. 7).

The average diameter of all measured fertilized eggs was 1.17 mm (range 1.04 - 1.31) and the volume weighted average diameter was 1.18 mm. The day-specific distribution of egg sizes and stages tended to yield a multi - peaked distribution indicative of several females having contributed eggs (see upper panels in Fig. 8). There was a consistent decrease in egg size over the period where egg diameter measurements were obtained (Fig. 8 lower left panel, Pearson correlation coefficient R = - 0.538, P = 0.026). The average fish fecundity was estimated to be 0.84 million eggs, with a lower estimate of 0.77 million eggs and an upper estimate of 0.93 million eggs (Fig. 8).

DISCUSSION

GAMETOGENESIS IN *POLLACHIUS VIRENS*; ONSET, DURATION, VITELLOGENIC STAGES AND STEROID PROFILES

As expected gametogenesis in *P. virens* shows many similarities with other studied gadoids, but there are also distinct and intriguing differences. This applies in particular to the onset and duration of vitellogenesis and the development of the different vitellogenic stages.

Vitellogenesis started around the end of October/early November with 'the group starting date' estimated to be November 7 (Fig. 5). On October 22 oocytes in early vitellogenesis were observed in a few of the sampled female ovaries and by late November all sampled females showed vitellogenic oocytes with yolk granules occupying large proportions of the oocytes (Fig. 6). No vitellogenic oocytes were found before October (Table III, Fig. 6). The presence of a distinct cortical alveoli stage (CA) is thought to be near ubiquitous in teleosts (Tyler and Sumpter, 1996; Lubzens *et al.*, 2010). In *G. morhua*, the appearance of yolk granule oocytes is always preceded by a CA stage (Skjæraasen *et al.*, 2009; Skjæraasen *et al.*, 2010a), and yolk granule oocytes only really become prevalent at LC sizes > 400 μm (Skjæraasen *et al.*, 2010a). The presence of distinct CA follicles has also been shown for another gadoid, the pollack *Pollachius pollachius* L. 1758 (Alonso-Fernández *et al.* 2013). This is clearly not the case for *P. virens* in the present experiment; females never possessed large amounts of CA oocytes at the most advanced stage. This indicates that, if present, this stage is of such a short duration in *P. virens* that it was not captured by the sampling or, perhaps more likely, that yolk granules and cortical alveoli appear simultaneously.

The onset of vitellogenesis appears to be substantially later in *P. virens* than in *G. morhua*, where vitellogenesis is proposed to start around the autumn equinox, i.e. September 22 - 23 (Kjesbu *et al.*, 2010), and where gonad biopsy samples typically show the presence of vitellogenic oocytes in early October (Kjesbu, 1994; Skjæraasen *et al.*, 2009). Naturally, it would be beneficial if the timing of vitellogenesis found in this study were confirmed by future studies. In *G. morhua* (Kjesbu *et al.*, 2010) and *M. aeglefinus* (Martin - Robichaud & Berlinsky, 2004) the main *zeitgeber* is daylength or more specifically, photoperiod, and

experimental studies have shown that artificial manipulation of photoperiod can cause substantial changes in the timing of maturation and spawning in both these species (Hansen *et al.*, 2001; Norberg *et al.* 2004; Skjæraasen *et al.*, 2004; Davie *et al.*, 2007a; Almeida *et al.*, 2009; Karlsen *et al.*, 2014). If photoperiod is the main *zeitgeber* for gametogenesis in *P. virens*, timing of onset of vitellogenesis under natural conditions would be expected to be relatively stable between years and/or studies and the present results would reflect real species differences between these gadoids. Another potential caveat is that the laboratory setting differs from the natural environment in such a way that extrapolation of results becomes uncertain. Generally *P. virens* along the Norwegian coast south of 62˚ N are believed to recruit to the North Sea *P. virens* spawning grounds (Jakobsen 1987, Homrum *et al.* 2013). Juveniles are typically found in Norwegian fjords until ~ 3 years of age when they leave (Heino *et al.* 2012) and migrate to feeding areas in the North Sea (Jakobsen 1987) before joining the spawning population at an age of 5-6 years (ICES 2015). The *P. virens* in the present study were thus caught presumably en route to the North Sea feeding areas (Fig 1). They were thus probably exposed to a light and temperature regime of a slightly more eastern and northern location than their natural wild habitat. However, this slight difference should not have caused any major changes in the timing and onset of gameteogenesis and spawning; e.g. the spawning season reported here closely matches the peak spawning season reported for wild North Sea *P. virens* (ICES 2015).

Interestingly the onset of the actual spawning season in the present study closely matches that of coastal *G. morhua* in the same area, i.e. the vitellogenic phase appears to be of shorter duration in *P. virens*. The transition from pre - vitellogenic to vitellogenic stages occurred around an oocyte size of 250 μm (Fig. 5, Table III). This closely matches previously published values for *G. morhua* (Kjesbu *et al.*, 2010). In contrast hydration and subsequent spawning occurred at an oocyte size of 750 μm (Fig. 5), which is considerably smaller than

355 the 875 μm reported for *G. morhua* (Kjesbu *et al.*, 2010). This difference largely explains the
356 shorter vitellogenic phase found in this study; using the estimated daily oocyte growth rate of
357 4.6 μm it indicates that the vitellogenic period lasts 27 days less in *P. virens*. However, there
358 also are some indications that oocyte development occurred at a faster rate than what would
359 have been expected for *G. morhua* under a similar temperature. Using eq. 8 from Kjesbu *et al.*
360 (2010), the expected oocyte developmental rate for a *G. morhua* at 7.9 C, ambient
361 temperature during vitellogenesis in the present study (Fig. 3), is 4.0 μm day^{-1}. This is
362 somewhat less than the 4.6 μm estimated presently.

363 Plasma profiles and concentrations of E2 and T in female *P. virens* were similar to
364 those previously described in female Atlantic *G. morhua* (e.g. Dahle *et al.*, 2003; Norberg *et*
365 *al.*, 2004; Skjæraasen *et al.*, 2004; Davie *et al.*, 2007b; Karlsen *et al.*, 2014) and female *M.*
366 *aeglefinus* (Martin - Robichaud & Berlinsky, 2004; Davie *et al.*, 2007a). The function of E2 in
367 induction of synthesis of egg shell proteins and vitellogenin is well established in teleosts (cf.
368 Tyler & Sumpter, 1996; Lubzens *et al.*, 2010), including *G. morhua* (Oppen - Berntsen *et al.*,
369 1992; Silversand *et al.*, 1993). In maturing female *G. morhua*, both T and E2 increases in fish
370 in the cortical alveolus stage compared to the previtellogenic stage (Karlsen *et al.*, 2014), and
371 T increases until early vitellogenesis, then decreases during spawning. E2, on the other hand
372 remains elevated until the end of spawning. A similar pattern seems evident in the female *P.*
373 *virens*. Available information suggests that androgens, in particular non-aromatizable ones
374 such as 11-KT, may stimulate primary oocyte growth in some species, such as eel (*Anguilla*
375 *spp*; e.g. Matsubara et al., 2001; Lokman *et al.*, 2007, 2015; Kazeto *et al.*, 2011), coho salmon
376 *Oncorhynhus kisutch* Walbaum 1792 (Forsgren & Young, 2012) and hapuku *Polyprion*
377 *oxygeneios* Schneider and Forster 1801 (Kohn *et al.*, 2013), and elevated circulating levels of
378 11-KT have been documented in previtellogenic and early vitellogenic females of eel
379 (Lokman *et al.*, 1998; Sbaihi *et al.*, 2001), and Waigieu seaperch *Psammoperca waigiensis*

Cuvier 1828 (Hung Quoc *et al.*, 2012). A small, but significant increase in 11-KT was seen in *P. virens* during the transition from previtellogenic to early vitellogenic oocytes between October and November. In Atlantic *G. morhua*, high doses of 11-KT stimulate primary oocyte growth and expression of ovarian genes associated with steroidogenesis and growth (Kortner *et al.*, 2008, 2009). However, the effect of 11-KT at physiologically relevant concentrations in *G. morhua* is still unclear. The current data may support the hypothesis of a function of 11-KT during early oocyte growth in gadoid fish, as has been documented in *Anguillidae* and *O. kisutch*, but further experimental work is needed to verify or reject such a hypothesis.

Plasma concentrations and seasonal variations of T and 11-KT in male *P. virens* followed the same pattern and were similar to what has been described in *G. morhua* (Dahle *et al.*, 2003; Skjæraasen *et al.*, 2004; Almeida *et al.*, 2009). 11-KT was first identified in sockeye salmon *Oncorhynchus nerka* Walbaum 1792 by Idler *et al.* (1961) and is considered the main androgen in teleost males (Borg, 1994), and can be associated with male reproductive behaviour and dominance hierarchies (Borg 1994; Rudolfsen *et al.,* 2006; Skjæraasen *et al.*, 2010b). Distinct male reproductive behaviors have been observed in many gadoids, but apparently have yet to be studied in *P. virens* (Skjæraasen *et al.*, 2012b). If the variation in male 11-KT profiles is also associated with individual variation in behaviour and/or reproductive success is an interesting topic for future studies. As for the other male hormones available data suggest not only a role for T, but also that E2 is active at early stages of spermatogenesis and that the maturation - inducing steroid (MIS) acts during spermiation (cf Schulz *et al.*, 2010). The high plasma levels observed in April, when females had completed spawning, suggest that spermiating males were still present at this time. High steroid levels may be necessary to ensure that sperm can be available during the whole female spawning season.

POF DURATION IN *POLLACHIUS VIRENS*

POFs appear to last between 9 – 11 months post spawning (Table III). In terms of their usefulness as a long-term marker of past spawning activity, it would then appear that they can be relied upon to identify females that spawned in the spring the following autumn. However, it is doubtful that they can be used to separate fish that are skipping a spawning season from immature fish, amongst fish not developing oocytes around the onset of the normal spawning season in the manner done for *G. morhua* (Skjæraasen *et al.*, 2012a) and *M. aeglefinus* (Skjæraasen *et al.*, 2015).

SPAWNING IN *POLLACHIUS VIRENS*; DURATION, EGG SIZE AND FECUNDITY

All *P. virens* were kept in one common tank during spawning due to practical limitations. No information on batch fecundity or the number of batches spawned for individual females is therefore available. There are however, some insights that can be gleaned from the data gathered during the spawning season. Firstly, there were indications of the same temporal patterns as has been documented in *G. morhua* (Kjesbu, 1989; Kjesbu *et al.*, 1996) with egg sizes decreasing towards the end of spawning (Fig. 8). This is thought to occur as females become nutritionally depleted through the spawning season (Kjesbu *et al.*, 1996). Secondly, although the mean egg size (1.18 mm) calculated corresponds quite well with previously published values (Russel, 1976) (1.13 mm), there was substantial variation around this value (range 1.04 – 1.31 mm). Finally, considering the size of the females in the present study, the estimated average realized fecundity corresponds well with the potential fecundity vs length relationship published by Storozhuk *et al.* (1974) for wild - caught North Sea *P. virens*. This is even more true if taking into account that the latter relationship was based on assessing fecundity pre-spawning (potential fecundity) whereas the present values reflect realized fecundity after atretic loss and thus would be expected to be somewhat lower. Considering

that fish were somewhat larger in the present study the fecundity results (~0.84 million eggs female^{-1}) also looks similar to those of van der Meeren and Lønøy (1998) (~0.38 million eggs female^{-1}). The duration of the spawning season for the entire group appears quite short (Fig. 7) in comparison with *G. morhua* (Kjesbu, 1989; Kjesbu *et al.*, 1996) and *M. aeglefinus* (Martin - Robichaud *&* Berlinsky, 2004; Davie *et al.*, 2007a). This may be indicative of *P. virens* spawning fewer batches. However, further studies focusing on individual females are needed to assess if this is the case.

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