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Reproduction and winter biology of polar cod *Boreogadus saida* from Svalbard waters

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Front picture: Polar cod under the ice (2010).

The picture is used with permission of the photographer: Peter Leopold.

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Content

Abstract

Polar cod (*Boreogadus saida*) is considered to be a key species in the Arctic marine ecosystems. Yet detailed or even basic knowledge regarding its biology and adaptations, especially during the polar night, are in many cases poor. No field data is presently available on the gonad development of polar cod, its reproductive biology and associated bioenergetics. Accordingly, polar cod was sampled at different locations in Svalbard waters in August, September, November and January. Gonadosomatic index (GSI) and hepatosomatic (HSI) indices were calculated as indicators of the physiological state of the fish. Maturity stages were determined by microscopic histological techniques. Energy reserves (total lipids, proteins and carbohydrates) were quantified in liver and gonads as well as cellular respiration through Electron Transport System (ETS) activity. Results indicated that polar cod prepared for spawning and started developing their gonads in August and September. The fish with GSI≥4% were considered mature as they had spawned at least once or were preparing for their first spawning. A general increase in GSI was observed together with a general increase in the total energy content in polar cod gonads from August to January. Males showed fully developed gonads in November, i.e. two months earlier than females. HSI and total energy content in liver tended to be constant over time, except for males where HSI was lower in January compared to the other months. Furthermore, only mature males showed a negative correlation between GSI and HSI that indicated a drain of energy from liver to gonads in males. It was hypothesized that female and male polar cod had different reproductive strategies. Based on histological analysis of the gonads, female polar cod were confirmed to be iteroparous and to be able to spawn more than once in their lifetime. Indeed, the presence of post-ovulatory follicles in the ovaries of mature females in August indicated that the fish had spawned the previous winter, while the progressive oocytes at the stages of cortical alveoli and lipid inclusion formation showed that there would be a new reproductive cycle in the coming spawning season. The histology did not allow concluding a similar unequivocal characterisation of the male reproductive strategy and samples from post-spawning period would be needed. However, results tended to show a higher energy investment and a higher metabolic activity in males than females and a decrease in their abundance compared to females with age. It is therefore hypothesized that males may be semelparous. In the harsh Arctic conditions this gender-specific strategy can be a successful opportunity to maximise the reproduction process and maintain high abundance of the polar cod population. Males should always be ready to spawn, because the timing of female gonad development can be different and dependent on environment.

I. Introduction

Reproduction is a key process in any living organisms that usually involves an element of trade-off and optimization (Fleming, 1996). Different environmental conditions created various reproductive strategies in fish to optimise their reproduction and maximise their fitness (Fleming, 1996; Lassala and Renesto, 2007), resulting in an array of various anatomical, behavior, physiological and energetic adaptations (Moyle and Cech, 2004).

Most marine species are iteroparous, i.e. they reproduce several times during their lifetime, have separate genders without any secondary sexual characteristics and an external fertilization without any parental care (Murua and Saborido-Rey, 2003). However, some fishes such as many of the salmoniformes species are semelparous and die after their first spawning (Altukhov *et al.*, 2000). The North Atlantic redfish, *Sebastes marinus*, is an example of a fish with internal fertilization of eggs (Sorokin, 1967), whereas others like sea basses (e.g. *Centropristis striata*), are hermaphrodites, changing from females to males as they get larger (Lavenda, 1949).

The frequency of reproduction may be dependent on the latitude (Moyle and Cech, 2004; Miller and Kendal, 2009). For instance, the American shad (*Alosa sapidissima*) inhabiting southern rivers are semelparous, while northern species are iteroparous as in the harsh and variable northern conditions the survival rates of their eggs and larvae are very low and energy should be saved for the next spawning season in case there were no survivors in the previous one (Leggett and Carscadden, 1978). Furthermore, the longevity of the reproductive period is getting shorter with increasing latitude, so that tropical species spawn almost continuously (Miller and Kendal, 2009) while arctic fishes spawn during few weeks mainly in winter or early spring to make larvae hatch in summer when the water temperatures are warmer and food is abundant (Fortier *et al.*, 1995; Graham and Hop, 1995). In order to protect the eggs against the risk of freezing, most of the Arctic species (e.g. Greenland cod (*Gadus ogac*) and Arctic flounder (*Liopsetta glacialis*)), produce demersal eggs with large yolk reserves and long incubation periods (Miller and Kendal, 2009). Eggs of polar cod (*Boreogadus saida*), were reported to be buoyant (Graham and Hop, 1995; Ponomarenko, 2000).

The ways of using food reserves in reproduction can be different too (Stearns, 1989; Jonsson, 1997). Some species, such as Atlantic herring (*Clupea harengus*), may be defined as capital breeders (Slotte, 1999; Ganias *et al.*, 2007; Kennedy *et al.*, 2010) and rely on stored energy reserves for developing gonads, while others such as bay anchovy (*Anchoa mitchilli*) and

hake (*Merluccius merluccius*) may be defined as income breeders (Stearns 1993; Murua, 2010,) that depend on new energy for developing gonads.

Polar cod (*Boreogadus saida*) is a true arctic species that belongs to the family Gadidae (Carr *et al.*, 1999). It has a circumpolar distribution, and is common in both open and ice-covered waters of the Arctic shelf seas (Ponomarenko, 1968; Rass, 1968; Craig *et al.*, 1982). It is also a species commonly assumed to play a key role in the Arctic marine food web (Bradstreet *et al.*, 1986; Jensen *et al.*, 1991; Gjøsæter, 2009; Jonsson *et al.*, 2010), being an important link between the lower (e.g. zooplankton) and the higher trophic levels (seabirds and mammals) (Bradstreet and Cross, 1982). However, despite the fact that polar cod is considered a key species, there are still gaps of knowledge concerning its biology, life cycle and adaptations to the Arctic environment, especially during the polar night when light condition is deficient, food availability is low and water temperatures below zero. Polar cod is known to reproduce between January and March (Ponomarenko, 1965; Rass, 1968). Fecundity is high and ranges from 9000 to 12000 eggs (Gjøsæter, 2009), although Hop *et al.* (1995) reported a much higher fecundity (up to 26500) in captive specimens. The eggs of polar cod are buoyant (Graham and Hop, 1995; Ponomarenko, 2000) and are of 1600–1800μm in diameter (Andriyashev, 1954; Graham and Hop, 1995; Hop *et al.*, 1995). Limited access to field data during the harshest month of the year, have hindered the study of biological processes during the polar night. Most of the existing knowledge on polar cod reproductive biology was obtained from studies carried out on captive fish (Graham and Hop, 1995; Sakurai *et al.*, 1998) including experimental data on the spawning energetics (Hop *et al.*, 1995). Hop *et al.* (1995) showed that polar cod invested a lot of energy in the reproduction and lost up to 50% of its total body mass during spawning. Furthermore, liver and somatic tissues (muscle) were providing a substantial portion of energy to reproduction (Hop *et al.*, 1995). Although, captive polar cod survived after spawning in captivity (Graham and Hop, 1995; Hop *et al.*, 1995; Sakurai *et al.*, 1998), little is known about the energy investment in wild populations (Hop *et al.*, 1995) and how polar cod cope with such a substantial energy loss. As polar cod become mature at age 2+ and 3+ (Lear, 1979; Craig *et al.*, 1982) and can grow up to age 7+ (Bradstreet *et al.*, 1986; Hop *et al.*, 1997), iteroparity, the possibility to reproduce several times during the life cycle, is the most accepted reproductive strategy of this fish species.

The overall aim of the project was to investigate the seasonal development of gonads and the associated bioenergetics in wild polar cod, collected in Svalbard waters at different seasons. For this, baseline physiological indicators such as gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated to assess the physiological state of the fish. Gonad maturation was

analysed using microscopic histological methods. Moreover, energy levels in the form of total lipids, proteins and carbohydrates and cellular respiration were measured both in liver and in gonads.

The study presents gender-specific characteristics of the physiology and population dynamics of polar cod that raise questions regarding its reproductive strategy. Based upon this, a new hypothesis concerning a gender-specific reproductive strategy is presented and discussed.

The outcome of the project is highly relevant to the understanding of the polar cod life cycle strategy, its fitness and survival in Arctic. Furthermore, the use of histological tools is a first effort to provide a clear description of ovaries and testes structure and their development over time in this species.

II. Materials and methods

2.1 Sampling

Polar cod were collected by bottom trawling during cruises on RV/Helmer Hanssen in Svalbard waters. Fish were sampled in November 2010 (n=37) and January 2011 (n=30) during the polar night, and in August 2011 (n=10) and September 2011 (n=15) during the twilight period (Figure 1).

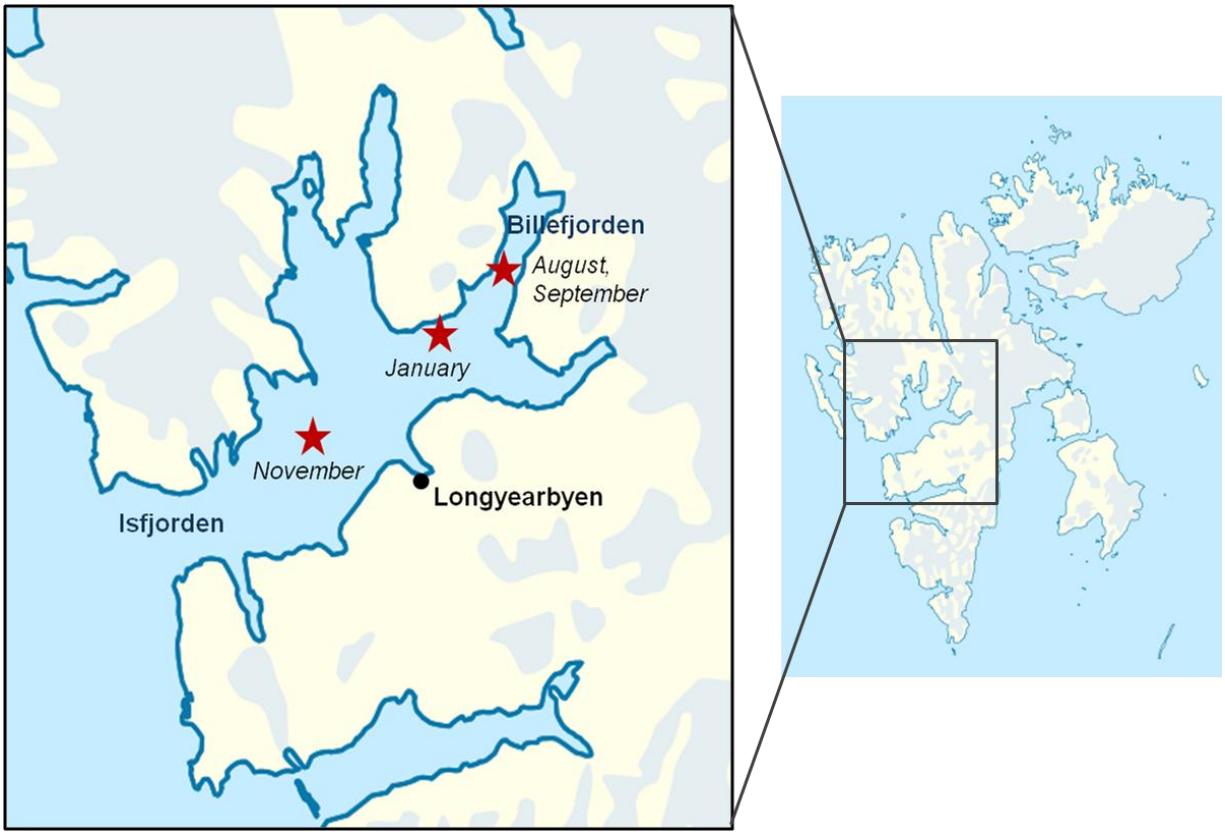

Figure 1. Sampling locations of polar cod in Svalbard waters in November 2010 (n=37), January 2011 (n=30), August 2011 (n=10) and September 2011 (n=15).

Total length (±1mm) and total wet weight of polar cod (±1g wwt) were recorded. Gender and macroscopic maturity stages were determined. Liver and gonads were dissected out from the fresh material and weighted (±0.01g wwt). Tissue samples were snap frozen in liquid nitrogen and stored at -80^{0}C until further biochemical analyses. Sections of gonad tissue (1cm×1cm) were cut from the middle and posterior parts of gonads of each fish specimen and fixed in 4% formaldehyde for histological analysis. Stomach fullness (%) and content were recorded as well as the presence of parasites and observed diseases. Due to possible misinterpretation of the

content of some stomachs, only the stomach fullness is presented. Finally, otoliths were sampled for age estimation.

2.2 Physiological indicators

Two physiological indicators were calculated to access the biological state of the caught polar cod:

1) Gonadosomatic index (GSI) = (gonad wet weight/somatic weight)*100

2) Hepatosomatic index (HSI) = (liver wet weight/somatic weight)*100

The somatic weight was computed as (total wet weight–liver wet weight–gonad wet weight).

2.3 Age estimation

Whole otoliths were used to estimate the age of the sampled polar cod. Otoliths were examined under a microscope Leica M205 C with a Planapo 1.0x objective lens in sub-surface light. The age was estimated by counting the white winter rings (slow growth) (Gjøsæter and Ajiad, 1994). The pictures were taken with a DFC420 camera connected to PC running Leica Application Suite (LAS) v. 3.8.

2.4 Biochemical analyses of energy content and cellular respiration

Quantitative biochemical analyses of energy reserves (lipids, proteins and carbohydrates) and cellular respiration (Electron Transport System (ETS) activity) in liver and gonad of polar cod were conducted according to De Coen and Janssen (1997).

Samples were thawed on ice, weighted (±0.001g wwt), homogenised in 10x volume of a homogenisation buffer pH=7.5 (0.1M Trizma HCl/base buffer, 0.4M $MgSO_4$, 15% polyvinylpyrrolidone and 0.2% (w/v) Triton X-100) using Potter-Elvehjem homogenizer and centrifuged for 10 minutes (3000g, 4^0C). Supernatants were split into three subsamples for ETS, lipids and proteins/carbohydrates analyses.

For determination of the ETS activity (µmol O_2 consumed/hour/g tissue wwt), homogenates of each sample (100µl) were 2x diluted in homogenization buffer. 50µl of the

resulting solution were pipetted as triplicates into microwells. 100µl buffered substrate solution (0.1M Trizma HCl/base buffer, pH=7.5 and 0.3% Triton X-100) and 50µl NADH/NADPH solution (1.17mM/250µM) were added. The reaction was started by 100µl of iodonitrotetrazolium chloride (4 mg/ml INT in distilled water). The increase in absorbance was followed every 15 seconds for 10 minutes at room temperature (20^0C) at 490nm wavelength using Perkin Elmer Victor^3 plate reader spectrophotometer.

For lipid extraction, the homogenates (200µl) were thawed on ice, mixed with 500µl of chloroform, 500µl of methanol and 250µl of distilled water and centrifuged for 5 minutes (10000g, 4^0C). Then, 100µl of the lower chloroform phase was carefully pipetted into a glass reagent tube. At the same time a standard curve (0–0.5 mg/ml) of stock solution of glyceryl tripalmetate (3 mg/ml in chloroform) was prepared. 500µl of sulphuric acid H_2SO_4 (95–97%) was added to the samples and standards. The content was gently mixed using a pipette and charred at 200^0C for 15 minutes. The samples and standards were diluted 1:6 in distilled water. 300µl of the resulting solution were pipetted into microwells and absorbance was measured at 340nm wavelength using the Victor^3 plate reader.

For protein and carbohydrate contents, the homogenates (300µl) were thawed on ice, mixed with 100µl of 15% trichloroacetic acid and centrifuged for 5 minutes (10000g, 4^0C). Supernatants containing carbohydrates were transferred to a new tube and stored on ice. Pellets containing proteins were washed with 100µl of 5% trichloroacetic acid and centrifuged again for 5 minutes (10000g, 4^0C). Supernatants were combined with the last one and stored at -80^0C for the carbohydrate analyses.

Pellets were dissolved in 500µl of 1N NaOH and incubated on 60^0C for 30 minutes. Then 300µl of 1.67N HCl was added to neutralize the solution. Subsequently, samples were diluted in appropriate dilutions: 100x for liver and 500x for gonads. Protein concentration (mg/ml) was determined according to Bradford (1976) using a bovine serum albumin standard curve (0–0.008 mg/ml). 100µl of standard solution or diluted samples were pipetted in four replicates into microwells. 250µl of 5x diluted Bradford reagent was added, and the plate was left for saturation for 2 minutes. Absorbance was measured at 595nm wavelength using the Victor^3 plate reader.

Carbohydrate concentrations (mg/ml) were determined against a glycogen standard curve (0–0.250 mg/ml). 50µl of thawed supernatants or standard solutions were pipetted in four replicates into microwells. 200µl of H_2SO_4 (95-97%) and 50µl of 5% phenol solution were

added. The plate was incubated under the fume cabinet at room temperature for 30 minutes. Absorbance was then measured at 490nm wavelength using the Victor^3 plate reader.

The total content of lipids, proteins and carbohydrates (mg/ml) were converted into Joules per g tissue wet weight (J/g wwt) using their specific enthalpy of combustion i.e.39.5kJ/g for lipids, 24.0kJ/g for proteins and 17.5kJ/g for carbohydrates (Gnaiger, 1983). Total energy content in a tissue (J/tissue) was determined with help of the total tissue wet weight (g).

2.5 Histological analysis of gonads

Gonads of female and male polar cod were analysed histologically to determine and verify maturation stages and seasonal development of oocytes and testis. In average, 30% of all gonad samples of polar cod from the four different seasons were taken for the histological analysis. The samples from August and September were combined in order to increase the amount of individuals for each gender group (Table 1).

Table 1. Number and range of gonadosomatic index (GSI) (%) of female and male polar cod (*Boreogadus saida*) caught at different seasons in Svalbard waters and taken for the histological analysis.

Season	Gender	n	GSI range (%)
August and September	female	2	4.9-6.4
	male	9	3.9-11.8
November	female	5	5.4-8.0
	male	8	20.4-41.6
January	female	10	0.4-25.6
	male	13	0.1-31.9

The samples were analysed in the environmental biochemistry lab of the Institute of Biology of the Karelian Research Centre, Russian Academy of Sciences (Petrozavodsk, Russia) using modern histological equipment. Classical histological methods of sample embedding into paraffin and staining with hematoxylin and eosin (H&E) were applied for this study (Mikodina *et al*., 2009). Gonad tissues were dehydrated through ascendant concentrations of ethanol series (70% 30 min, 80% 30 min, 90% 10 min, 96% 10 min and 2x 100% 30 min), then cleaned in o-xylene (10, 30 and 30 min) and embedded in paraffin (50 and 80 min) (Histomix, BioVitrum, Russia) using a MICROM spin tissue processor STP-120 (Thermo fisher scientific, USA).

In order to make paraffin moulds of dehydrated and paraffin infiltrated tissues, a MICROM paraffin embedding centre EC-350 (Thermo fisher scientific, USA) was used. The paraffin moulds were cut on a sliding microtome HM 450 (Thermo fisher scientific, USA) in transverse serial sections of thickness 7µm and 9µm. The slides were manually processed through the series of o-xylene and ethanol for paraffin elimination (dewaxing) and tissue dehydration and stained with hematoxylin (Mayer's hematoxylin) and eosin (Table 2). The combination of H&E is the most simple and effective method to identify cell structures as nucleus, nuclei and other cytoplasmic inclusions (Pirs, 1962). Finally, the slides were cover slipped and were mounted using synthetic Bio Mount BM500 (Bio Optika, Italy).

Table 2. Order of reagents and immersion time used for paraffin elimination, dehydration of tissue on the microscope slides and hematoxylin and eosin staining (manual procedure).

Order	Reagent	Immersion time, minutes
1	o-Xylene	5
2	o-Xylene	5
3	96% Ethanol	5
4	96% Ethanol	5
5	70% Ethanol	5
6	Water distilled	5
7	Hematoxylin	7-10
8	Tap water	2-3
9	Eosin	5
10	96% Ethanol	5
11	96% Ethanol	5
12	100% Ethanol	5
13	o-Xylene-Acetone (2:1)	5
14	o-Xylene	10
15	o-Xylene	10

Histological sections were studied in the light microscope (50–1000x) Axioskop 40 (Carl Zeiss) with eye lens x10 and objective lenses x5, x10, x20, x40, x100. Sections on the slides were photographed with camera Pixera Pro 150ES connected with the microscope. All photos were analysed with Videotest programme and cell structures and inclusions were measured.

Immature fish were defined as fish that had never spawned. Mature fish were those that had spawned at least once or were preparing for their first spawning.

2.6 Statistical analysis

Microsoft Excel and software package XLSTAT Version 2012.2.01 were used for the statistical analyses. Assumptions of normality and homogeneity of data were checked using normal P-plot and Levene's test, respectively (Berk and Carey, 2009). As both assumptions were not violated for all the parameters, a one-way ANOVA was performed. Statistical significance was considered when $p \leq 0.05$.

Significant differences ($p \leq 0.05$) in the biological parameters (total length, total weight, GSI, HSI, as well as lipid, protein and carbohydrate content and ETS activity in liver and gonads) were tested among seasons (4) using one-way ANOVA. Moreover, a Bonferroni correction factor was performed to make a multiple pair wise comparison and find which pairs of seasons were significantly different ($p \leq 0.05$). Significant differences ($p \leq 0.05$) in the parameters between females and males, between immature and mature fish, and among different age classes were also tested using one-way ANOVA. Pearson correlation matrix was used to check the correlations between GSI and total energy content in gonads, between HSI and total energy content in liver and between GSI and HSI (data were normally distributed and homogenous).

For the histological analysis one-way ANOVA and Bonferoni correction factor were applied to check significant differences ($p \leq 0.05$) in female ovaries (mean square, length, width, diameter and rotundity coefficient) among seasons.

Parameters based on one individual fish were excluded from the statistical analysis.

III. Results

3.1 Description of polar cod

Polar cod of age 1+, 2+ and 3+ were caught at each sampling month except for September where only 2+ and 3+ fishes were caught. 2+ age class was dominant at all sampling seasons (Figure 2). More male individuals (75%) were presented in 1+ age class; however, in 2+ and 3+ age classes females were the most represented (73% and 67%, respectively).

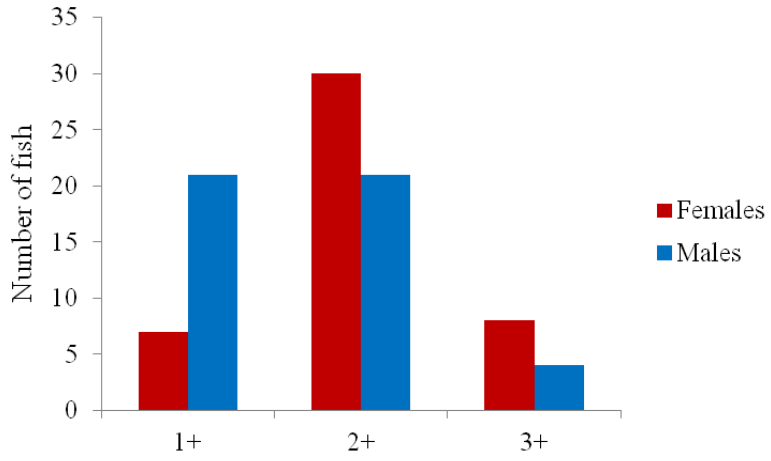

Figure 2. Frequency distribution of female and male polar cod (*Boreogadus saida)* in the different age classes (1+, 2+, 3+) from all sampling seasons.

Maturity was determined by microscopic histological methods. At all sampling months, it was observed that polar cod with GSI≥4% had spawned at least once or were preparing for their first spawning, and were considered mature. It was found that each age class included both mature and immature polar cod except for 1+ age class in August where only immature fish were presented. Furthermore, age estimation of the caught polar cod showed that both females and males of the age 1+ could be mature. However, not all females and males of age 3+ were mature (e.g. Figure 8A represents 3+ immature female caught in January).

No significant gender-specific differences in either total length or total wet weight were observed for any age class in any season (Table 3). Moreover, total length and total weight of polar cod of the same gender and season were not significantly different among age classes.

Due to heterogeneity of sampling locations and small sample size for each age class and month, it was not possible to compare fish size and potential growth of fishes over the seasons. Indeed, 1+ males in November were significantly larger in length and weight than those in August and January ($p=0.039$ and $p=0.014$, respectively) (Table 3). 2+ females in September were significantly larger in length and weight than females in November and January ($p=0.000$ and $p=0.004$). No significant differences in length and weight were found for the 3+ fish among the seasons.

Table 3. Total length (mm) and total weight (g) of polar cod (*Boreogadus saida*) of different age classes (1+, 2+, 3+) and gender (F: female, M: male). Values are mean±SE. Letters (a, b) indicate significant differences ($p \leq 0.05$) among seasons in fish of the same gender and age. No significant differences were found either between females and males of the same age and season or among age classes in fish of the same gender and season.

Season	Age	n		Total length (mm)		Total weight (g)	
		F	M	F	M	F	M
August	1+	2	3	106 ± 8^{a}	117 ± 6^{a}	7 ± 2^{a}	9 ± 1^{a}
	2+	1	0	183	-	42	-
	3+	3	1	169 ± 36^{a}	180	37 ± 22^{a}	44
September	1+	0	0	-	-	-	-
	2+	7	7	158 ± 8^{a}	141 ± 8^{a}	23 ± 4^{a}	17 ± 2^{a}
	3+	0	1	-	192	-	42
November	1+	3	11	125 ± 3^{a}	137 ± 5^{b}	12 ± 1^{a}	18 ± 2^{b}
	2+	9	8	131 ± 3^{b}	136 ± 6^{a}	14 ± 1^{b}	17 ± 2^{a}
	3+	4	1	141 ± 6^{a}	174	15 ± 4^{a}	27
January	1+	2	7	127 ± 7^{a}	122 ± 2^{a}	12 ± 4^{a}	11 ± 1^{a}
	2+	13	6	128 ± 3^{b}	128 ± 4^{a}	13 ± 1^{b}	13 ± 1^{a}
	3+	1	1	135	124	16	12

- Gonadosomatic index

In general, mature females and males had significantly higher GSI than immature fish (all $p<0.0001$) (Figure 3A). There was an increase in GSI for mature fish over time. Mature females showed significantly higher GSI in January than in November ($p<0.0001$). Mature males developed their gonads earlier than females and had significantly higher GSI than females in November ($p<0.0001$). Also, GSI did not show any significant differences among age classes either for females or for males in any season (data not shown).

In January, small mature males of the size range 110–120mm and 120–140mm had a higher percentage of individuals with GSI≥15% (100% (n=6) and 92% (n=11), respectively), than mature females of the same size range (38% (n=3) and 71% (n=10), respectively).

- Hepatosomatic index

Seasonal variations of HSI of immature and mature polar cod were found to be constant (Figure 3B). Only mature males showed significantly higher HSI in November compared to September and January ($p<0.0001$). A significant difference in HSI between females and males was only observed for mature fish in January where females had significantly higher HSI than males ($p=0.000$). Moreover, mature females in November showed significantly higher HSI than immature females ($p=0.033$). No significant differences in HSI were found among age classes except in November where females at age 1+ and 2+ (10.1±0.3% and 9.4±0.3%, respectively) had a significantly higher HSI than 3+ females (4.5±2.4%) ($p=0.011$) (data not shown).

- Stomach fullness

The analysis of polar cod stomach fullness in September showed that the fish had been recently feeding. 40% and 33% of the sampled fish had 25% and 50% full stomachs, respectively (Table 4). In November, 64% of the collected polar cod had empty stomachs, however 45% of them had full guts (data not shown). In January polar cod were found to feed too. Indeed, 63% had at least 25% stomach fullness, of which 23% of caught polar in January had full stomachs.

Gender and maturity differences in stomach fullness could not be analysed statistically due to small sample size.

Table 4. Frequency distribution (%) of polar cod (*Boreogadus saida*) with different stomach fullness (0%, 25%, 50%, 75% and 100%) caught in August, September, November and January. NA= not available data.

Season	n	Frequency distribution (%)				
		0%	25%	50%	75%	100%
August	10	NA	NA	NA	NA	NA
September	15	7	40	33	7	13
November	37	64	11	3	11	11
January	30	27	20	17	13	23

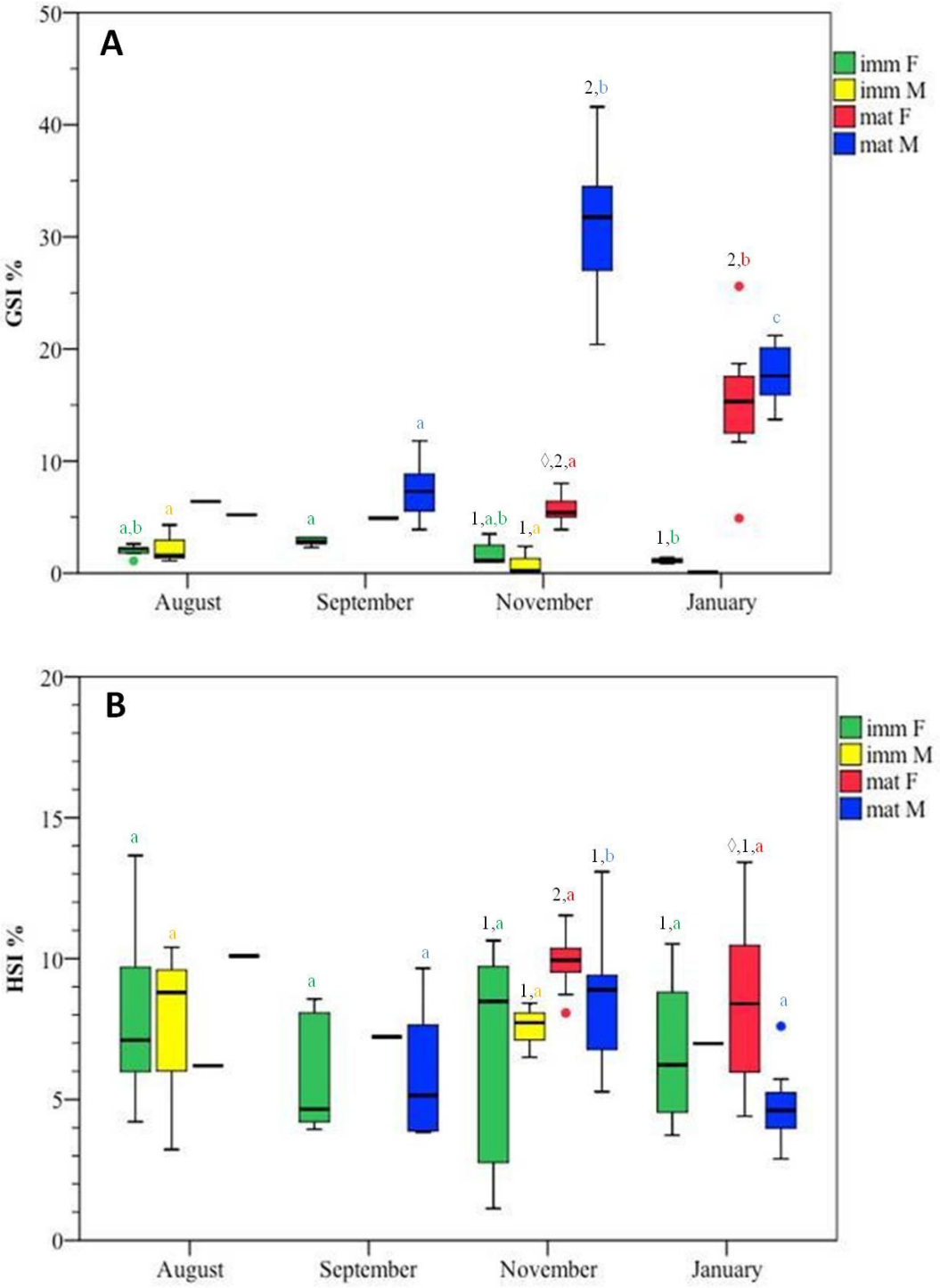

Figure 3. Seasonal variation of (A) gonadosomatic index (GSI, %) and (B) hepatosomatic index (HSI, %) of immature (imm) and mature (mat) female (F) and male (M) polar cod (*Boreogadus saida*). Plots represent the median (line), 25%-75% percentiles (box), min-max range (whisker) and moderate outliers (dots). Symbol (◊) indicates significant difference ($p \leq 0.05$) between females and males of the same maturity and season; numbers (1, 2) indicate significant difference ($p \leq 0.05$) between immature and mature fish of the same gender and season; letters (a, b, c) indicate significant differences ($p \leq 0.05$) in fish of the same gender and maturity among seasons.

3.2 Energy content in gonads

Lipids and proteins were the main sources of energy in polar cod gonads, followed by carbohydrates (Appendix, Table I). Both immature and mature females significantly increased their total energy concentration (sum of lipids, proteins and carbohydrates, J/g gonad wwt) in the gonads from September to January ($p=0.001$ and $p=0.004$, respectively) (Figure 4A). But, no significant changes in total energy concentrations were observed in gonads of mature males. Immature males were not considered in the statistical analysis due to small sample size.

Gender-specific differences were observed in November and January where mature females showed a significantly higher total energy concentration in the gonads than mature males (both $p<0.0001$) (Figure 4A). This was due to higher protein and carbohydrate concentrations in mature females in November ($p<0.0001$ and $p=0.000$, respectively) and higher lipid and protein concentrations in January ($p=0.010$ and $p<0.0001$, respectively) (Appendix, Table I). Moreover, in November, mature females had significantly higher total energy concentration than immature females ($p=0.020$), mainly due to higher protein concentration ($p=0.000$) (Figure 4A and Appendix, Table I). In January, total energy concentration was not significantly different between immature and mature females (Figure 4A). However, protein concentration was also significantly higher in mature females than in immature females ($p=0.021$), and lipid and carbohydrate concentrations were similar (Appendix, Table I).

When considering the total energy content in gonads (J/gonad), there was a significantly strong correlation ($R^2=0.692$, $p<0.0001$) between total energy content in gonads and GSI at all sampling seasons for both males and females. Mature females significantly increased total energy content in the gonads from September to January ($p=0.001$) while immature females showed no significant differences (Figure 4B). Mature males had significantly higher total energy in the gonads in November compared to other months ($p=0.000$).

When comparing levels between gender and maturity stages, mature female polar cod showed higher total energy in the gonads than immature females (in November $p=0.000$ and in January $p=0.002$) (Figure 4B). In November, mature males had accumulated about three times more total energy in their gonads than mature females ($p<0.0001$). In January, this trend changed with mature females showing a significantly higher total energy content per gonad than mature males ($p=0.006$).

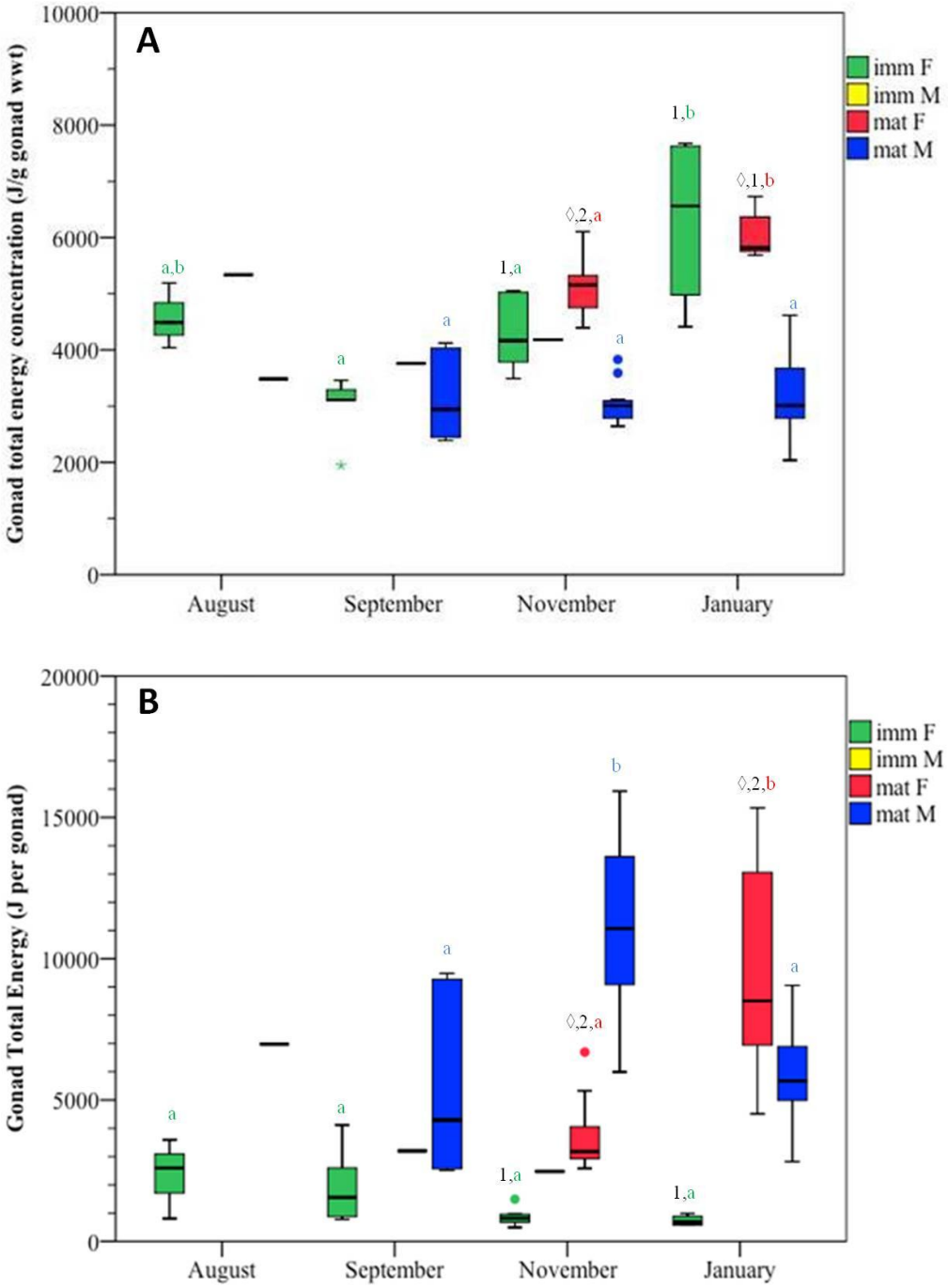

Figure 4. (A) Total energy concentration (sum of lipids, proteins and carbohydrates, J/g gonad wwt) and (B) total energy content (sum of lipids, proteins and carbohydrates, J/gonad) in gonads of immature (imm) and mature (mat) female (F) and male (M) polar cod (*Boreogadus saida*). Plots represent the median (line), 25%-75% percentiles (box), min-max range (whisker), moderate outliers (dots) and extreme outliers (asterisks). Symbol (◊) indicates significant difference ($p \leq 0.05$) between females and males of the same maturity and season; numbers (1, 2) indicate significant difference ($p \leq 0.05$) between immature and mature fish of the same gender and season; letters (a, b) indicate significant differences ($p \leq 0.05$) among seasons in fish of the same maturity and gender.

Only mature polar cod from November and January were taken for comparison of total energy content in gonads among age classes. No significant differences in gonad total energy were observed between 1+ and 2+ fish (data not shown). However, in November 3+ females showed a two-fold higher total energy (6695 J/gonad) in the gonads than 1+ and 2+ females (2752±173 J/gonad and 3696±389 J/gonad, respectively).

3.3 Energy content in liver

Lipids represented the major part of energy in liver of polar cod, followed by proteins and carbohydrates (Appendix, Table II). No significant differences in liver total energy concentrations (sum of lipids, proteins and carbohydrates, J/g liver wwt) were observed between gender, maturity stages and among seasons (Figure 5A). However, mature males showed significantly lower protein and carbohydrate concentrations in liver in November compared to other sampling months ($p=0.036$ and $p<0.0001$, respectively) and mature females showed significantly higher carbohydrate concentration in January compared to November ($p=0.017$) (Appendix, Table II). No significant gender-specific differences were found in lipid and protein concentrations in liver at any season. Carbohydrate concentration in liver of mature fish caught in January was found to be significantly higher in males than in females ($p=0.026$). Moreover, in November, immature females showed significantly higher lipid concentration in liver than mature females ($p=0.048$). In January, carbohydrate concentration in liver of immature females was also significantly higher than in mature females ($p=0.010$).

The total energy content in liver (J/liver) showed a significant correlation with the HSI in each sampling month (August: $R^2=0.580$, $p=0.028$; September: $R^2=0.559$, $p=0.028$; January $R^2=0.773$, $p<0.0001$) except November ($R^2=0.128$, $p=0.052$). Furthermore, no significant differences in total energy content were found in liver of females among the seasons, while mature males showed significantly lower liver total energy in January compared to other sampling months ($p=0.002$) (Figure 5B). The total energy content in liver did not significantly change between females and males and between immature and mature polar cod in any season, except for the mature fish in January where females had significantly higher energy reserves than males ($p=0.005$).

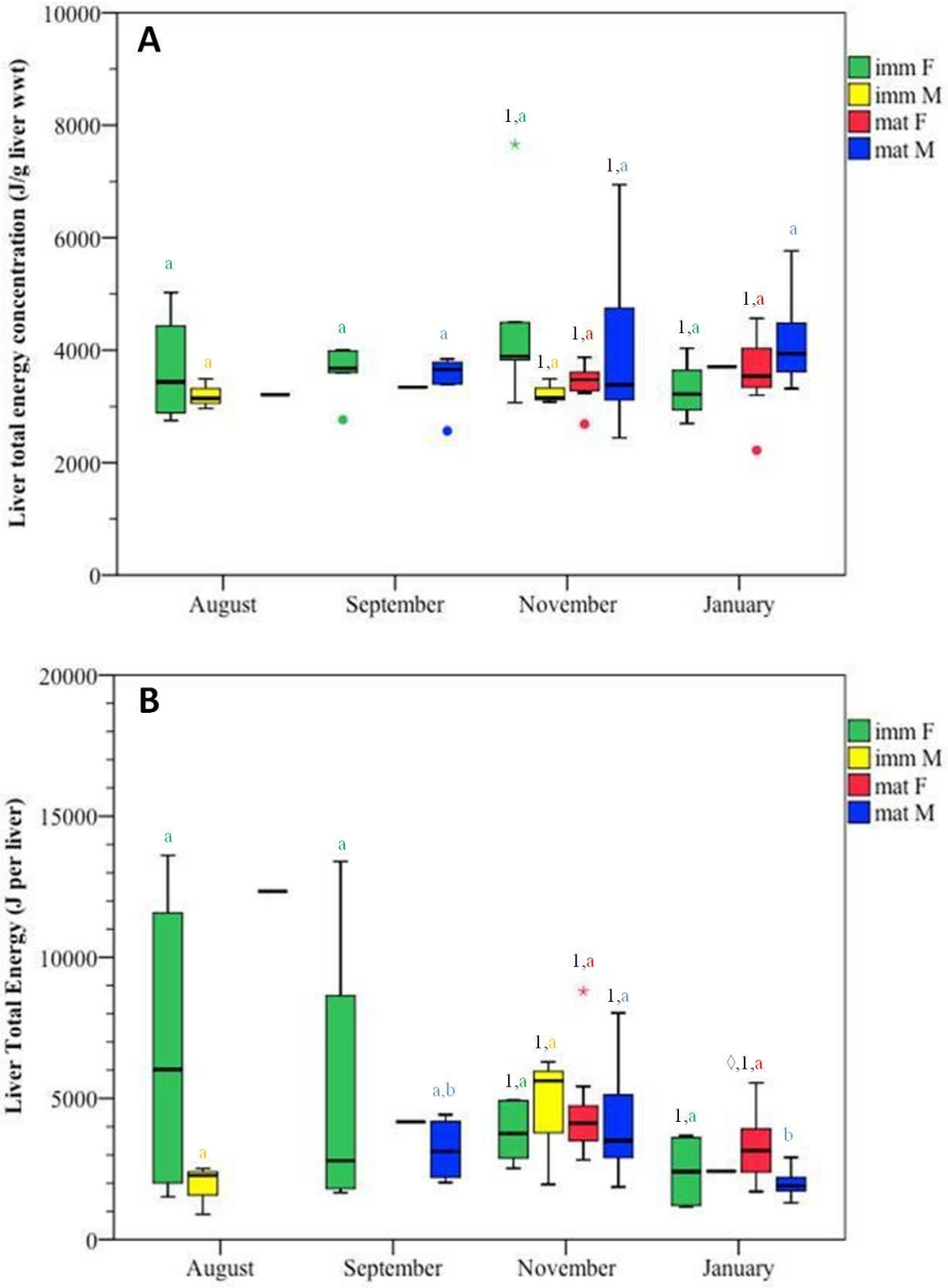

Figure 5. (A) Total energy concentration (sum of lipids, proteins and carbohydrates, J/g liver wwt) and (B) total energy content (sum of lipids, proteins and carbohydrates, J/liver) in liver of immature (imm) and mature (mat) female (F) and male (M) polar cod (*Boreogadus saida*). Plots represent the median (line), 25%-75% percentiles (box), min-max range (whisker), moderate outliers (dots) and extreme outliers (asterisks). Symbol (◊) indicates significant difference ($p \leq 0.05$) between females and males of the same maturity and season; numbers (1, 2) indicate significant difference ($p \leq 0.05$) between immature and mature fish of the same gender and season; letters (a, b) indicate significant differences ($p \leq 0.05$) among seasons in fish of the same maturity and gender.

Only mature polar cod from November and January were considered for comparison of total energy content in liver among age classes. No significant differences in liver total energy were observed between 1+ and 2+ fish (data not shown). However, it seemed that females increased total energy content in liver with age. 3+ females showed a two-fold increase in total energy stored in liver compared to 1+ and 2+ females both in November and in January. On the contrary, males slightly decreased total energy content in liver with age. Increase in total liver energy with age in females and decrease in males was directly dependent on liver wet weight (data not shown).

3.4 Cellular respiration in gonads

Specific ETS activity (µmol O_2/hour/g gonad wwt) in gonads was significantly higher in females than in males in August for immature fish ($p=0.001$), in November for both immature and mature fish ($p=0.002$ and $p<0.0001$, respectively) and in January for mature fish ($p=0.002$) (Figure 6A). Mature females showed a significantly higher specific ETS activity in gonads in November (36±2 µmol O_2/hour/g gonad wwt) than in January (14±1 µmol O_2/hour/g gonad wwt) ($p<0.0001$). Mature males had significantly higher specific ETS activity in gonads in January (20±1 µmol O_2/hour/g gonad wwt) compared to September (13±0 µmol O_2/hour/g gonad wwt) and November (13±1 µmol O_2/hour/g gonad wwt) ($p<0.0001$). No significant changes in specific ETS activity were observed in gonads of immature fish among the seasons. Effect of maturation on specific ETS activity was only found for female gonads. In November and January mature females showed significantly lower specific ETS activity in the gonads than immature females ($p=0.009$ and $p<0.0001$, respectively).

Total ETS activity (µmol O_2/hour/gonad) in gonads was observed to have a significant and strong correlation ($R^2=0.578$, $p<0.0001$) with GSI at all sampling seasons. Mature females in November and January ($p=0.009$ and $p=0.003$, respectively) as well as mature males in November ($p=0.002$) had significantly higher activities than immature fish (Figure 6B). Furthermore, the total ETS activity in gonads in November and January was significantly lower in mature females compared to mature males ($p=0.003$ and $p=0.020$, respectively). When considering the seasonal pattern of the total ETS activity, immature females significantly decreased the total ETS activity in gonads from September to January ($p=0.021$). Mature males in November showed a significant increase in the total ETS activity in gonads compared to other

sampling months ($p<0.0001$). However, mature females and immature males showed no significant differences in total ETS activity in gonads over time.

No significant changes in total ETS activity in gonads of mature males were found among age classes either in November or in January (Table 5). However, in November, mature females showed a significant increase in gonad total ETS activity with age ($p=0.011$). This trend seemed also visible in January although statistical analysis could not be performed due to small sample size.

Table 5. Total Electron Transport System (ETS) activity (µmol O_2/hour/gonad) in gonads of mature polar cod (*Boreogadus saida*) of different gender (F: female, M: male) and age classes (1+, 2+ and 3+). Values are mean±SE. Letters (a, b) indicate significant differences ($p \leq 0.05$) among age classes in fish of the same gender and season.

Season	Age	ETS activity in gonad (µmol O_2/hour/gonad)			
		n	F	n	M
November	1+	2	17±2[a]	6	60±6[a]
	2+	6	26±1[b]	6	45±10[a]
	3+	1	56	0	-
January	1+	1	27	7	31±2[a]
	2+	10	22±3	5	43±7[a]
	3+	1	35	1	28

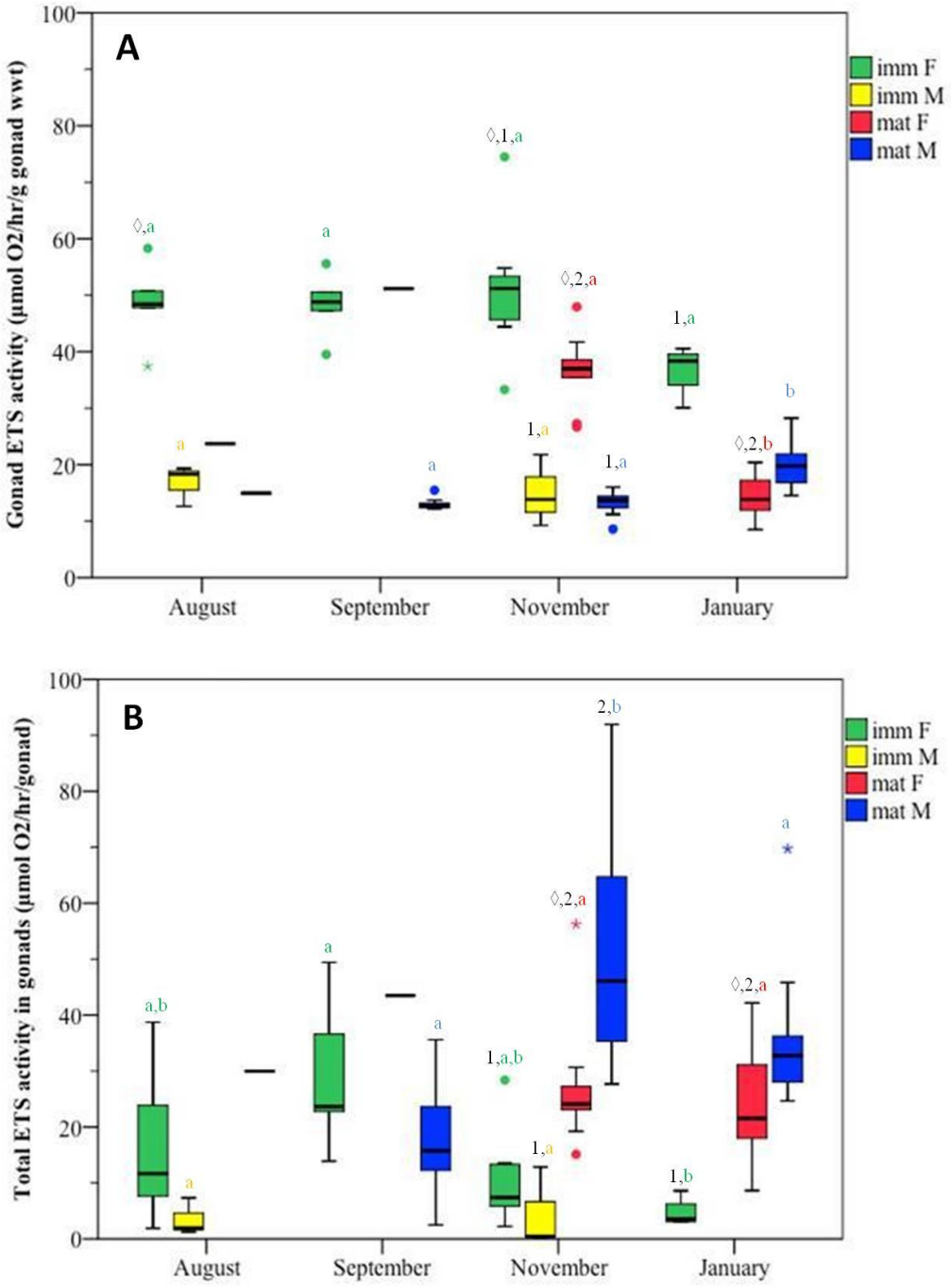

Figure 6. (A) Specific Electron Transport System (ETS) activity (µmol O_2/hour/g gonad wwt) and (B) total ETS activity (µmol O_2/hour/gonad) in gonads of immature (imm) and mature (mat) female (F) and male (M) polar cod (*Boreogadus saida*). Plots represent the median (line), 25%-75% percentiles (box), min-max range (whisker), moderate outliers (dots) and extreme outliers (asterisks). Symbol (◊) indicates significant difference ($p \leq 0.05$) between females and males of the same maturity and season; numbers (1, 2) indicate significant difference ($p \leq 0.05$) between immature and mature fish of the same gender and season; letters (a, b) indicate significant differences ($p \leq 0.05$) among seasons in fish of the same maturity and gender.

3.5 Cellular respiration in liver

There were no significant differences in specific ETS activity (µmol O_2/hour/g liver wwt) in liver among the sampling seasons except for immature males, which showed significantly higher specific ETS activity in August compared to November (p=0.015) (Figure 7A). Gender-specific differences in ETS activities could only be observed in January where mature males showed significantly higher specific ETS activity in liver than mature females (p=0.001) (Figure 7A).

Total ETS activity (µmol O_2/hour/liver) in liver showed a strong and significant correlation with HSI in August (R^2=0.698, p=0.010) and September (R^2=0.657, p=0.000). No significant correlation was found between total ETS activity in liver and HSI in November (R^2=0.048, p=0.227), however, in January, the correlation was weak but significant (R^2=0.143, p=0.043). There were neither gender nor maturity specific differences in total liver ETS activity (Figure 7B). Furthermore, no significant seasonal changes in total ETS activity in liver were observed except for mature males, which showed a lower total ETS activity in January compared to September and November (p=0.033). A high individual variability and a quite low median in total ETS activity in liver of immature females in August were observed probably due to small sample size (n=5).

Finally, no significant differences in total ETS activity in liver of mature female and male polar cod were found among age classes either in November or in January (data not shown).

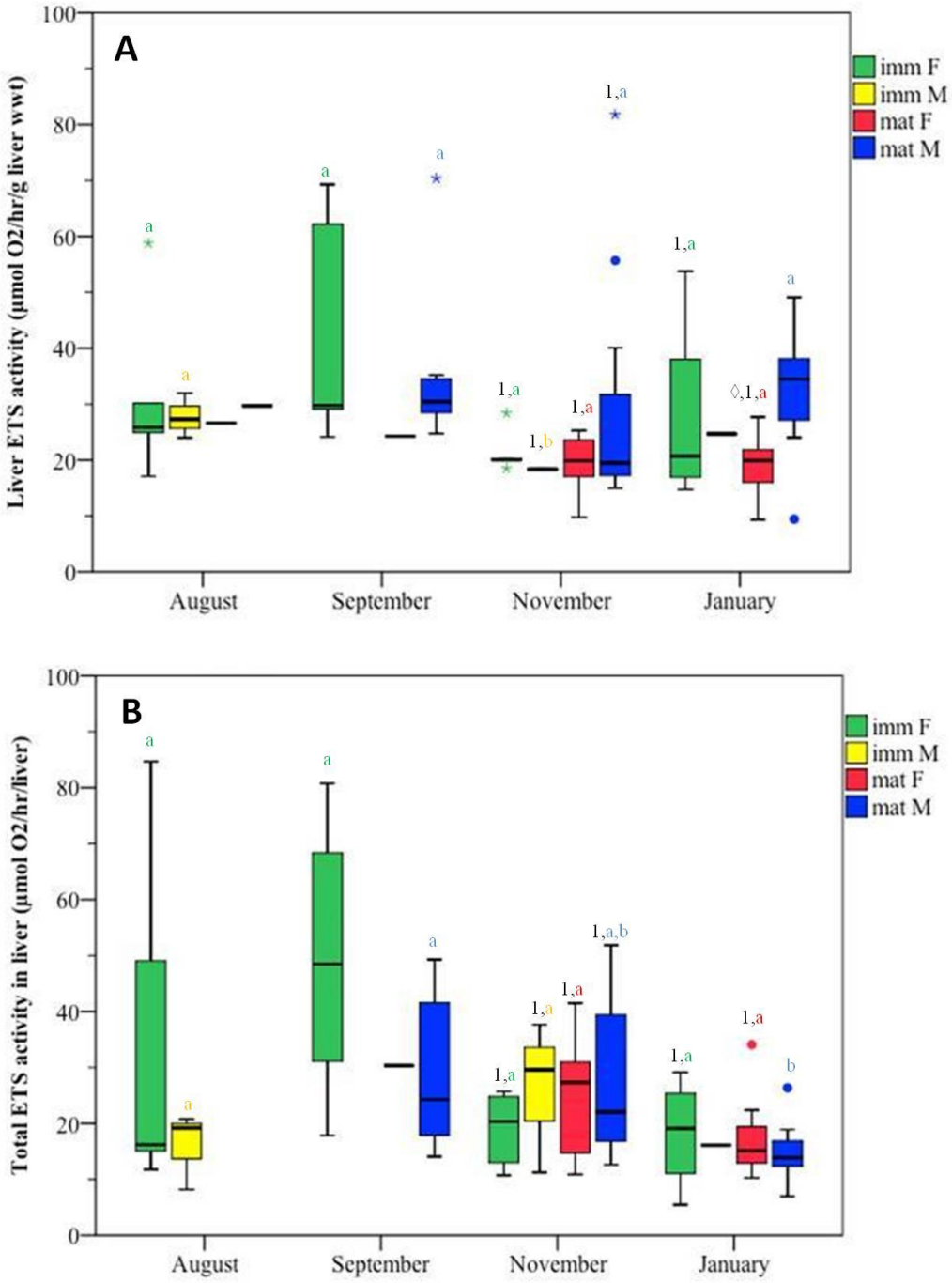

Figure 7. (A) Specific Electron Transport System (ETS) activity (µmol O_2/hour/g liver wwt) and (B) total ETS activity (µmol O_2/hour/liver) in liver of immature (imm) and mature (mat) female (F) and male (M) polar cod (*Boreogadus saida*). Plots represent the median (line), 25%-75% percentiles (box), min-max range (whisker), moderate outliers (dots) and extreme outliers (asterisks). Symbol (◊) indicates significant difference ($p \leq 0.05$) between females and males of the same maturity and season; letters (a, b) indicate significant differences ($p \leq 0.05$) among seasons in fish of the same maturity and gender. The number (1) indicates no significant difference ($p \leq 0.05$) between immature and mature fish of the same gender and season.

3.6 Histological analysis of gonads

The ovarian structure of immature female polar cod at age 2+ and 3+ caught in January was represented by developing oocytes of earlier generations: oogonia (36±6%) and previtellogenic oocytes (64±6%) (Table 6, Figure 8A). The oogonia (36±1μm) had a significant three-fold smaller mean diameter than the previtellogenic oocytes (107±2μm) ($p<0.0001$) (Appendix, Table III).

The difference between immature and mature females was clearly visible. Ovaries of mature polar cod at age 2+ and 3+ caught in August and September contained oocytes at five different maturation stages (Figure 8B and 8C). The dominant cohort was the previtellogenic oocytes (66±14%), followed by oogonia (12±12%), oocytes at the stage of cortical alveoli (7±7%), oocytes at the stage of lipid inclusion formation (7±7%), oocytes at the final vitellogenesis stage under resorption (5±2%), and post-ovulatory follicles (4±4%) (Table 6). The basic size characteristics of these female germ cells (mean oocyte diameter, oocyte length, oocyte width, oocyte square and rotundity coefficient) are presented in the Appendix (Table 3).

Table 6. Occurrence of different oocyte stages (%) based on oocyte counts (n) in the ovaries of immature and mature polar cod (*Boreogadus saida*). Values are presented as mean±SE. Oo: oogonia, PVit: previtellogenesis, CA: cortical alveoli, LIF: lipid inclusions formation, Vit: vitellogenesis, Mat: maturation, POF: post-ovulatory follicles, AO: atretic oocytes.

Season	Maturity	n oocytes	Age (years, min-max)	Oogenesis stages (%)							AO
				Oo	Pvit	CA	LIF	Vit	Mat	POF	
August and September	mature	102	2-3	12±12	66±14	7±7	7±7	5±2	0	4±4	0
November	mature	224	1-3	9±4	66±5	0	0	24±6	0	0	1±1
January	immature	450	2-3	36±6	64±6	0	0	0	0	0	0
	mature	254	1-2	24±4	50±4	0	0	23±4	0	0	2±2

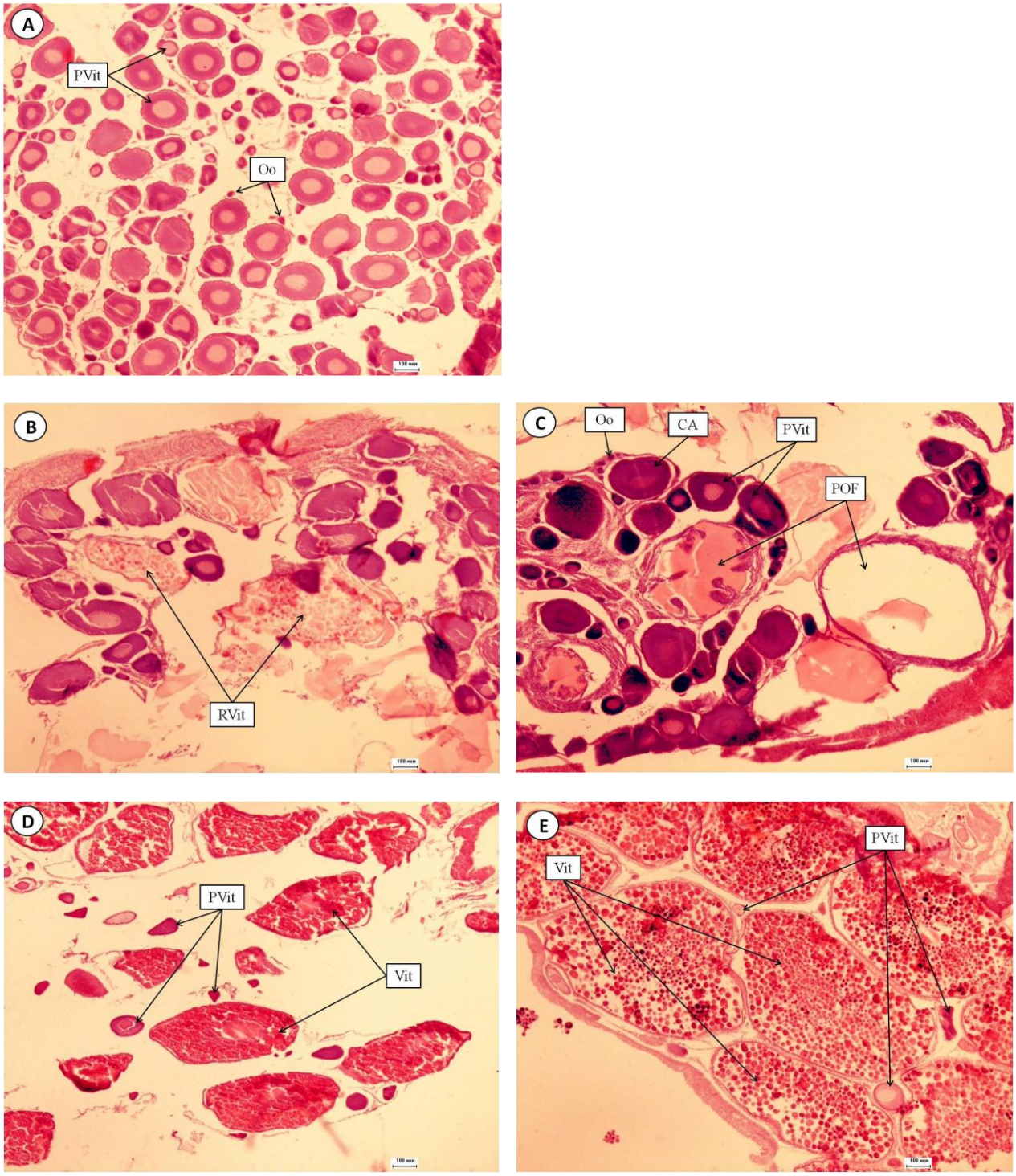

Figure 8. Ovarian structure of immature female polar cod (*Boreogadus saida*) in (A) January and mature female polar cod in (B, C) August, (D) November and (E) January. Oo: oogonia, PVit: previtellogenic oocyte, RVit: vitellogenic oocyte under resorption, CA: oocyte at cortical alveoli stage, POF: post-ovulatory follicle, Vit: vitellogenic oocyte. Magnification: 50x. Bar: 100µm.

Oogonia and previtellogenic oocytes represented oocytes at early stages of development while oocytes at cortical alveoli stage and lipid inclusion formation stage indicated more progressive stages of oocyte growth. The oocytes at the cortical alveoli stage (202±6µm) showed a significantly increased mean diameter compared to the previtellogenic oocytes (79±4µm) ($p<0.0001$) (Appendix, Table III). The cortical alveoli were located close to the membrane (Figure 9). The zona radiata membrane was thin but well visible under the light microscope. The oocytes at the lipid inclusion formation stage (306±19µm) were significantly larger in the mean diameter than the oocytes at the cortical alveoli developmental stage ($p<0.0001$) (Appendix, Table III). The lipid droplets and lipid drops appeared inside the oocyte space. They mixed with the cortical alveoli and lay as alveoli close to the oocyte periphery (Figure 10). The lipid inclusions formation is the transitional stage between the previtellogenesis and vitellogenesis.

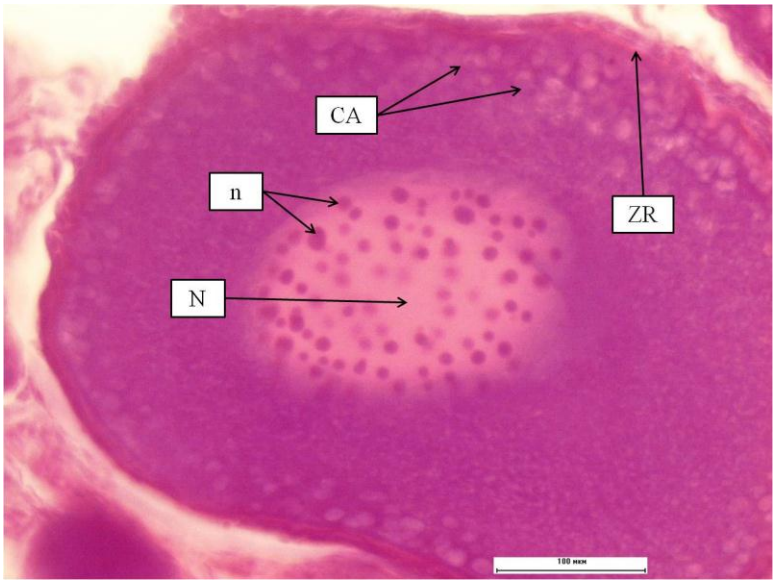

Figure 9. Oocyte at cortical alveoli developmental stage in the ovary of polar cod (*Boreogadus saida*) collected in August and September. CA: cortical alveoli, N: nucleus, n: nucleoli, ZR: zona radiata. Magnification: 400x. Bar: 100µm.

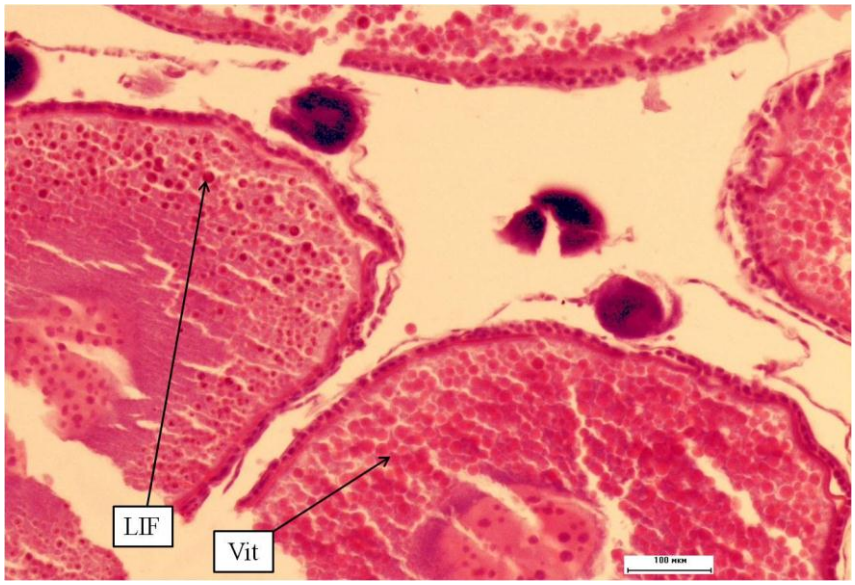

Figure 10. Oocyte at the stage of lipid inclusions formation (LIF) lying beside a vitellogenic oocyte (Vit) in ovary of polar cod (*Boreogadus saida*) collected in September. Magnification: 200x. Bar: 100µm.

Mature females at age 1+, 2+ and 3+ caught in November and January had similar structures of ovaries. Their ovaries consisted of previtellogenic oocytes, as a dominant generation of developing oocytes (66±5% in November and 50±4% in January), vitellogenic (maturing) oocytes (24±6% and 23±4%, respectively) and oogonia (9±4% and 24±4%, respectively) (Table 6). General views of ovarian structure of mature female polar cod from November and January are presented in Figures 8D and 8E. Previtellogenic oocytes made loose groups interspaced between considerably large vitellogenic oocytes. The presence of previtellogenic oocytes indicated the potential for future development of vitellogenic oocytes later that spring or in the next spawning season. The number of previtellogenic oocytes significantly increased from August to November (from n=54 to n=150, respectively). However, previtellogenic oocytes (79±4µm) in August were significantly larger in mean diameter than in November (60±2µm) ($p=0.000$) (Appendix, Table III). This was due to the larger size of the selected female specimens in August compared to November. Furthermore, in January, previtellogenic oocytes became significantly larger in the mean diameter (109±3µm) than in November (60±2µm) ($p<0.0001$) (Appendix, Table III).

In November, vitellogenic oocytes (396±1µm) were at the early vitellogenic stage in the ovaries of mature polar cod. In January, vitellogenic oocytes (606±17µm) were at the middle and final vitellogenesis. Hence, the mean diameter of vitellogenic oocytes, their nucleus and yolk globules were significantly larger in January than in November ($p<0.0001$, $p=0.013$ and $p<0.0001$, respectively) (Appendix, Table III). The entire volume of vitellogenic oocyte was filled with yolk globules (Figure 11). The yolk globules fused together forming bigger yolk globules representing the final stage of egg formation. The vitellogenic oocyte contained wide and plainly visible zona radiata membrane threaded by numerous erect radiate channels. Moreover, the vitellogenic follicle surrounded by the follicle membrane consisted of the theca and the membrane granulose (Figure 11B).

Few atretic oocytes during previtellogenesis were observed in the ovarian structure of mature polar cod collected in November and January (Table 6, Figure 12). No significant differences were observed in the mean diameter of atretic oocytes of the fish from these two seasons (Appendix, Table III).

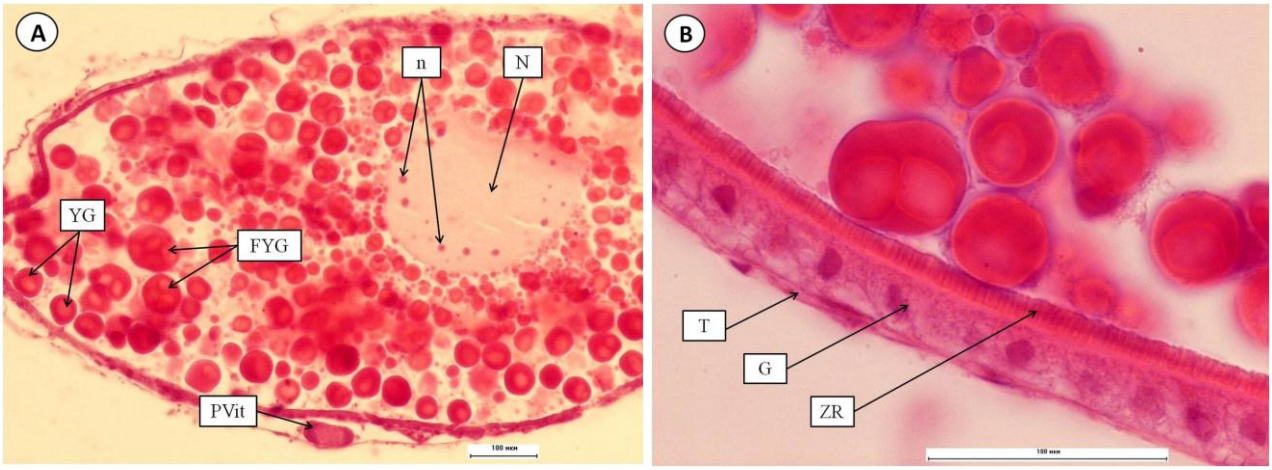

Figure 11. Vitellogenic oocyte in the ovary of mature polar cod (*Boreogadus saida*) collected in January. (A) Oocyte at mid-vitellogenesis, (B) Membrane organisation. N: nucleus, n: nucleoli, YG: yolk globule, FYG: fused yolk globule, PVit: previtellogenic oocyte, T: theca, G: granulose, ZR: zona radiata. Magnification: (A) 200x, and (B) 1000x. Bar: 100µm.

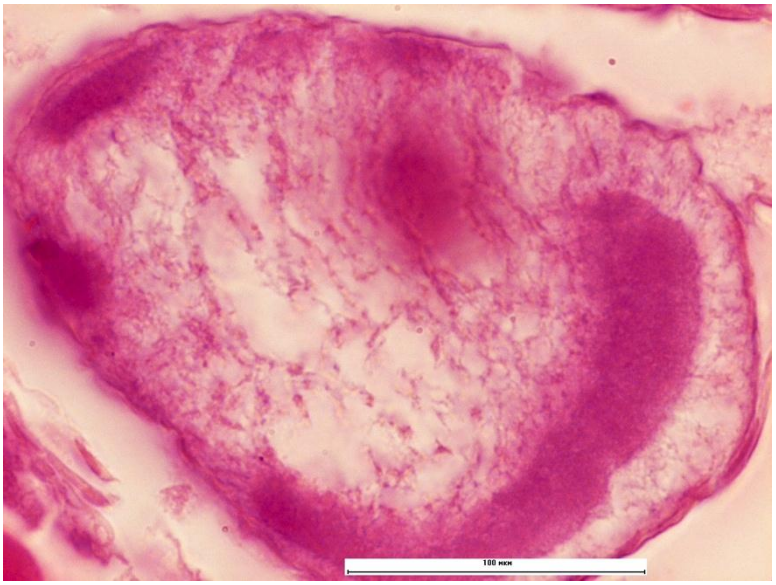

Figure 12. Atretic oocytes of polar cod (*Boreogadus saida*) collected in January. Magnification: 1000x. Bar: 100μm.

Immature males collected in January were characterized by simple and poor developed testes structures containing spermatogonia and spermatocytes (Figure 13A). The similar testes structure was observed in the immature males from August. November samples of immature fish were not taken for the histological analysis.

The testes of mature male polar cod started to develop in August and September. They presented spermatogonia, spermatocytes in different generations (primary and secondary spermatocytes) and partly spermatids (Figure 13B). The testicular structure of polar cod collected in November was more or less orderly in a view of dominance spermatids and presence of spermatozoa groups (Figure 13C). At this developmental stage the sperm ducts were clearly visible. Mature males collected in January had typical lobular structure of testes (Figure 13D). Lobule spaces were full of spermatozoa, which partly were observed in sperm ducts (Figure 14). Many tubules were empty of spermatozoa close to the periphery of the testes (Picture 13E). Thus, the testes of polar cod in January were ready for spawning and observed emptiness of peripheral testes lobules was caused artificially by finger pressing on a ventral part of male body.

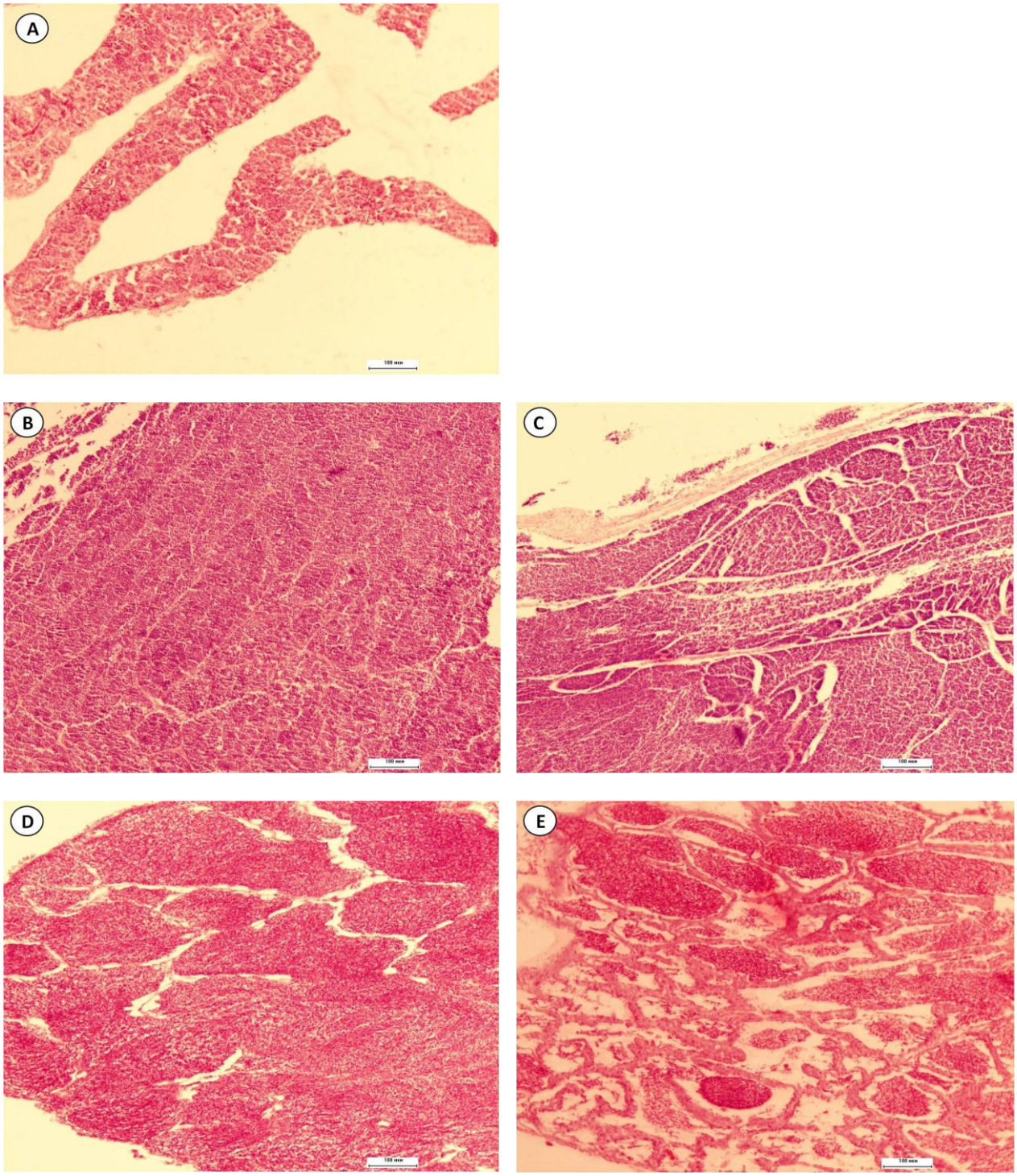

Figure 13. Testes structure of (A) immature male polar cod in January and mature polar cod (*Boreogadus saida*) in (B) September, (C) November and (D and E) January. E. shows the discharge of sperm and emptiness in the tubules. Magnification: 100x. Bar: 100µm.

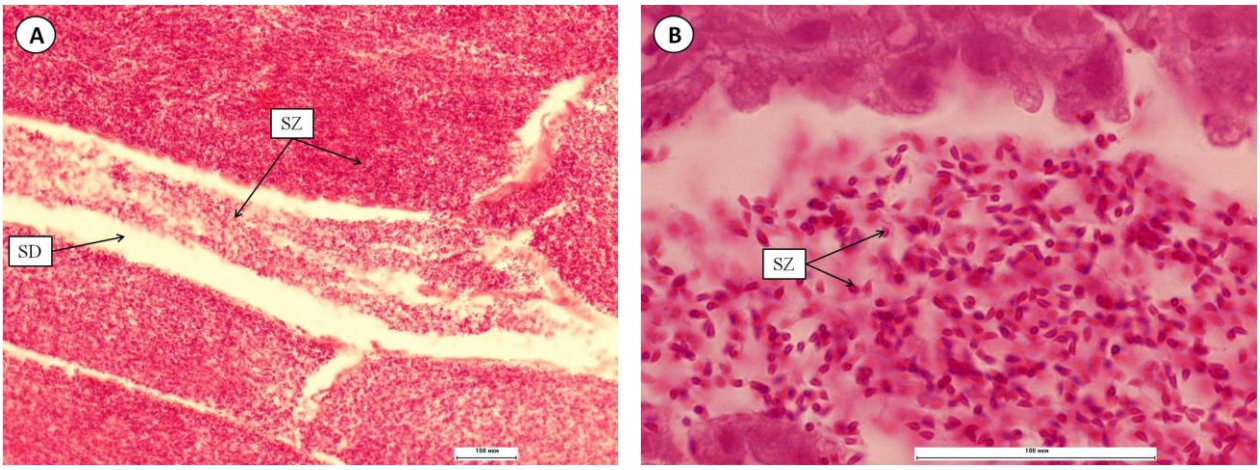

Figure 14. Spermatozoa of mature male polar cod (*Boreogadus saida*) collected in January. SZ: spermatozoa, SD: sperm duct. Magnification: (A) 200x and (B) 1000x. Bar: 100µm.

VI. Discussion

4.1. Seasonal development of polar cod gonads

The active growing and development of polar cod gonads started in the end of summer and early autumn. Due to different sampling locations and respective fish size, the seasonal changes in gonad mass and its energy content were difficult to observe. However, a general increase in GSI was observed together with a general increase in the total energy content in gonads from August to January. Polar cod with GSI≥4% were found to be mature and had spawned at least once or were preparing for their first spawning in the coming winter. Polar cod, especially males, invested a high amount of energy in the reproduction process. The highest GSI were observed to be 26% in females in January and 42% in males in November. In captivity gonad mass was reported to reach even 50% of the total body weight (Hop *et al.*, 1995), possibly because the captive polar cod were regularly fed three to five times per week throughout the year (Hop *et al.*, 1995). Also, the captive fish were larger in size (size range 154–276mm) (Hop *et al.*, 1995) compared to the fish in the present field data (in November and January, size range 111–174mm).

Female gonads showed higher energy concentrations of lipids and proteins (J/g gonad wwt) and higher specific ETS activity (µmol O_2/hour/g liver wwt) than male gonads. Higher protein concentration and higher specific ETS activity in gonads of females compared to those of males suggested active metabolic processes in the previtellogenic oocytes of immature and mature females at all sampling seasons. Almost all previtellogenic oocytes had a Balbiani body around the nucleus. This area consists of the RNA material, mitochondria, the endoplasm reticulum (Zelazowska *et al.*, 2007). The function of the Balbiani body has not been confirmed yet (Kloc *et al.*, 2004). However, numerous nucleoli and wideness of the Balbiani body indicated high rates of protein synthesis in the oocyte nucleus (Abascal and Medina, 2005; Junqueira and Carneiro, 2005). Hence, female polar cod always maintained high protein synthetic activities and therefore high specific ETS activity in the gonads. Furthermore, the observation of the wavy boundaries of previtellogenic oocytes and the enlargement of the size and volume of young oocytes of immature females in January may also indicate active synthetic processes. However, to the best of our knowledge, this feature has never been observed in the other species.

The appearance of progressive oocytes at the stages of cortical alveoli and lipid inclusion formation in the ovaries of mature fish in August indicated that these specimens were at the beginning of the egg production period. Mature ovaries in November were characterised by

development of vitellogenic oocytes and appearance of yolk globules in their cytoplasm. The yolk in the vitellogenic oocytes is considered as an essential energetic reserve of the ripe eggs that supplies necessary components for an embryo to grow and develop (Nefedova, 1988; Rainuzzo *et al.*, 1997; Johnson, 2009). In January, yolk globules started to accumulate and therefore provided an increase in lipid concentration in the ovaries. This would guarantee the survival rate of the hatched larvae in the beginning of its life (Wiegand, 1996). It is also notable that no significant differences in lipid concentration were found between immature and mature females at any season. This, however, may be due to a difference in density (g gonad/cm^3) of the gonads of mature and immature females. Indeed, it can be assumed that one gram of mature gonads represented a smaller volume than one gram of immature gonads (Figures 8A and 8E). Therefore, the expression of energy concentration per gram gonad tissue may be biased and should be presented per volume gonad instead. Unfortunately, the density of gonads at the different maturation stages was not measured during the present study and this hypothesis cannot be tested.

Vitellogenesis is characterized by the appearance of true yolk globules produced in liver and transported by blood vessels to the cytoplasm of the oocyte (Murua and Saborido-Rey, 2003; Moyle and Cech, 2004; Miller and Kendal, 2009). The duration of the vitellogenesis stage is generally dependent on the amount of energy reserves stored in the individuals and on external factors such as food and temperature that control the admission and penetration of lipid inclusions and vitellogenin to the oocytes (Kalaida *et al.*, 2011). Indeed, the vitellogenic oocytes significantly increased in size in January compared to November together with an increase in GSI and energy content in gonads. The mean diameter of the vitellogenic oocytes in January was 606±17µm and would continue to grow and accumulate yolk globules. Indeed, a vitellogenic oocyte becomes an egg at the diameter about 1600–1800µm (Andriyashev, 1954; Graham and Hop, 1995; Hop *et al.*, 1995). Therefore, the spawning would probably take place later, in the middle of February or in the beginning of March.

The occurrence of few atretic oocytes in the ovaries of mature polar cod in November and January was found as a normal physiological process of resorption of developing oocytes (Murua *et al.*, 2003; Agulleiro *et al.*, 2007). Ucuncu and Cakici (2009) confirmed that atresia in fish was regulated by apoptosis, a programmed cell death. As for Atlantic herring, *Clupea harengus*, (Kurita *et al.*, 2003; Kennedy *et al.*, 2011), atresia in polar cod can be assumed to control fecundity and recruitment of oocytes for the next reproductive season.

The spermatogenesis of polar cod followed the same stages of development as described for other teleost fishes (Ratty *et al.*, 1990; Rideout and Burton, 2000; Dziewulska and Domagala, 2003; Fishelson *et al.*, 2006; Bucholtz *et al.*, 2008). The development of male polar cod gonads started late August to early September, at the same time as females started producing eggs. No significant changes in lipid, protein and carbohydrate concentrations (J/g gonad wwt) were observed in male gonads over time. On the contrary, female gonads significantly increased lipid and protein concentrations and significantly decreased carbohydrate concentration from August to January. Male gonads developed faster than female gonads (Hop *et al.*, 1995; this study). Higher GSI and tubules full of spermatozoa indicated that males were ready for spawning in January.

4.2 Energy allocation

Since hepatosomatic index (HSI) of females did not significantly change over time, total lipid and protein contents as well as total ETS activity in female liver also showed no significant differences among the seasons. However, mature males had significantly smaller HSI in January and therefore also showed lower total protein content and total ETS activity in January compared to other sampling months. It is generally known that gonad development of many fish lead to a high drain of energy from other tissues (Love, 1970; Delahunty and de Vlaming, 1980; Neves and Brayton, 1982; Dorucu, 2000), and especially liver lipids are a main source of energy for reproduction (Shulman, 1974; Rinchard and Kestemont, 2003). In the present field data, only mature male polar cod showed a significant negative correlation between GSI and HSI in January (R^2=0.395, p=0.021). This correlation was weak but may show that at least in male fishes the liver was an important energy source to the gonads. In contrast, mature females did not have the same negative trend between GSI and HSI and may have thus most likely drawn the energy for gonad development from other sources than liver. For instance, somatic tissues can also be used to supply fish gonads with energy (Hayesa and Taylor, 1994; Arellano-Martínez and Ceballos-Vázquez, 2001). In general, proteins of somatic tissues are mobilised for gonads prior to spawning (Shulman, 1974; Medford and Mackay, 1978). In the present study, energy in somatic tissues was not available; therefore the importance of somatic tissues as energy source for gonads could not be determined. However, Hop *et al.* (1995) calculated that energy loss in muscle was 16% in females and 23% in males during their gonad development. Moreover, it was also reported that captive fish decreased muscle mass by 5% just before spawning (Hop *et al.*, 1995). Energy for reproduction can also be taken from active feeding, as observed for other

income breeders such as hake, *Merluccius merluccius* (Murua, 2010) and bay anchovy, *Anchoa mitchilli* (Stearns, 1993). Although records of stomach fullness are only snapshots of the feeding activity, and are not necessarily representative of the overall fish's feeding activity, polar cod were observed to have food in their stomachs in winter during gonad maturation. Unfortunately, the sample size in January was too small to test whether there was a difference in stomach fullness between genders. However, it may be assumed that male polar cod potentially received energy for the gonad development from both liver and food while females took most of their energy during the reproduction period from the food and less from liver. Hop *et al.* (1995) who showed that captive polar cod significantly decreased HSI and liver lipids during gonad development, however, did not notice gender differences. As polar cod were regularly fed under this experiment (Hop *et al.*, 1995), males did not spend additional energy to look for prey as they would in the wild, and they had thus no reasons to refuse food and die.

4.3 Reproductive strategy

The presence of vitellogenic oocytes at the final vitellogenesis stage under resorption and few post-ovulatory follicles in the ovaries of mature polar cod in August showed that these females had spawned the previous winter. Gonzalez and Larraneta (1996) reported the possibility to observe post-ovulatory follicles in the ovaries of Atlantic cod (*Gadus morhua*) up to seven months after spawning. Furthermore, development of progressive oocytes at the stages of cortical alveoli and lipid inclusion formation in August indicated that these specimens prepared for the coming spawning season. The occurrence of different generations of oocytes, oogonia and previtellogenic oocytes between the vitellogenic oocytes, in the ovaries of mature fish in January showed a group synchronous ovarian organisation of polar cod (Murua and Saborido-Rey, 2003). The vitellogenic oocytes would ovulate in the coming season while oogonia and previtellogenic oocytes indicated the potential for a new reproduction cycle next winter. All these facts confirm that wild female polar cod are able to spawn more than once in their lifetime. Moreover, it is known that polar cod become mature at a young age (Lear, 1979; Craig *et al.*, 1982), live up to 6+ or 7+ (Bradstreet *et al.*, 1986; Hop *et al.*, 1997) and may therefore reproduce several times. Under laboratory conditions it was also observed that the fish survived after spawning (Graham and Hop, 1995; Sakurai *et al.*, 1998). Hence, female polar cod are iteroparous (Graham and Hop, 1995).

The histological data did not allow a similar unequivocal characterisation of the male reproductive strategy. In order to investigate this question with histological tools, samples of mature males in post-spawning state (in March or April) would be needed to observe the presence or absence of both empty lobules and a new generation of spermatogonia. However, male polar cod generally showed higher GSI than females, and their gonads developed earlier than female gonads (Hop *et al.,* 1995; Hop *et al.,* 1997; this study). Moreover, in November, total energy content in gonads was three times higher in males than in females. Also, as a result of bigger gonads, the metabolic activity (total ETS, J/gonads) in male gonads was found to be higher than in females. Thus, waiting for the females for two–three months requires higher energy investment from males compared to females. Indeed, males may use all energy they have including liver and external food source for the reproduction, while females that have low energy costs over the whole reproductive period may take most of their energy from the food and less from liver (cf. section 4.2). Furthermore, the present field data showed that the number of males in the catches decreased with age, and it therefore indicates a higher male mortality compared to that of females. Nikolskii (1950) reported a high spawning mortality of polar cod in nature, but did not note if this mortality was gender-specific. Lear (1979), Craig *et al.* (1982) and Hop *et al.* (1997) observed that more females were generally presented in the samples of large specimens. For instance, only females of age 7+ were caught in Hinlopen in August 2011 (Berge J., pers.com.). Thus, in wild populations, it seems that female polar cod usually get older than males. Moreover, in the present study, a higher proportion of small males (110–140mm) with large gonads (GSI≥15%) than small females were observed in January. The highest GSI (42%) of the whole dataset was found in 1+ male of 140mm total length from November that indicated a high-energy investment at a very young age, which would most likely lead to post-spawning death. All these data suggest that males have a different reproductive strategy than females. It can be hypothesized that in general, male polar cod are semelparous and exhibit a combination of breeding strategies, i.e. being partly capital breeders.

Such gender-specific reproductive strategy could be an adaptation of polar cod in general, and not merely a specific observation from a limited dataset from Svalbard waters. It has been shown that females are determinate spawners, producing only a finite number of eggs every year (Hop *et al.*, 1995; Sakurai *et al.*, 1998). Hence, it is more beneficial for them to reproduce several times to increase the amount of offspring (Hop *et al.*, 1995; Moyle and Cech, 2004). Moreover, the development of female gonads and timing of spawning process are known to be dependent on environmental conditions such as water temperature, its salinity and light regime

(Graham and Hop, 1995). To maximise reproductive success in a strongly seasonal and variable environment, males may therefore develop their gonads earlier than females in order to be ready to fertilise the eggs from several females (Graham and Hop, 1995). However, this adaptation in males represents a very large energy investment. Males may thus allocate all available energy from both body reserves (capital breeders) and continuous feeding (income breeders) into one single reproductive event, while females rather rely on an income strategy, primarily through continuous feeding activity, for optimizing their probability of several reproductive events throughout their lifetime. A comparable gender-specific reproductive strategy was described for another circumpolar Arctic species, the capelin (*Mallotus villosus*) (Huse, 1998). Huse (1998) suggested that semelparity in males was a trade-off between variable juvenile mortality that supports iteroparity and high adult mortality that forces optimal reproductive strategy towards semelparity. This hypothesis can also be adapted for polar cod. Even if the hatching occurs under the ice (Hognestad, 1968; Drolet *et al.*, 1991) and no predators around (Davis *et al.*, 1980), the predation on adult polar cod by seabirds and mammals during post-spawning season remains high (Welch *et al.*, 1992; Welch *et al.*, 1993).

V. Conclusion

This study showed that gonad development in polar cod started already in late August. The gonads increased in size and accumulated a high amount of lipids and proteins over time both in females and in males until January. Liver was a main source of energy for this development in males, but seemed less important in females. Furthermore, indications of active feeding during the entire sampling period showed that polar cod at least to some degree is an income breeder. Although females showed a higher energy concentration in gonads than males due to their lipid rich eggs, males were believed to invest more energy in reproduction when considering the entire reproductive season as they maintained fully developed gonads over several months before females were ready to spawn.

While it is known that female polar cod are iteroparous, the present study indicated that male polar cod may be semelparous based on histological examination of the gonads, bioenergetics and population dynamics. Indeed, male polar cod seemed to invest all their energy in one reproduction cycle and potentially die after spawning while female polar cod rely on a more cautious and balanced energy use for successful reproductive activity over several years. This hypothesis is, however, based on a quite limited number of samples. Moreover, the present field data was heterogeneous, and it was difficult to see the seasonal trend of gonad development and associated bioenergetics due to different sampling locations. For future research it is highly recommended to sample fish at the same location and include as well fish in a post-spawning state, from March or April.

The knowledge on life cycle strategies of polar cod acquired in this study is very important to understand fitness of the species in the severe Arctic environment and to predict its susceptibility to global warming that may challenge polar cod ability to survive and reproduce.

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Appendix

Table I. Concentration of lipids, proteins and carbohydrates (J/g gonad wwt) in gonads of immature and mature females (F) and males (M) polar cod (*Boreogadus saida*). Values are represented as mean±SE. Symbol (◊) indicates significant difference ($p \leq 0.05$) between females and males of the same maturity and season; numbers (1, 2) indicate significant difference ($p \leq 0.05$) between immature and mature fish of the same gender and season; letters (a, b) indicate significant differences ($p \leq 0.05$) among seasons in fish of the same maturity and gender.

Season	Maturity	n		Lipids, J/g wwt		Proteins, J/g wwt		Carbohydrates, J/g wwt	
		F	M	F	M	F	M	F	M
August	immature	4	2	2375±372^{a,b}	2021±204^{a}	1880±146^{a}	-	50±4^{a}	-
	mature	1	1	3507	1929	1766	1529	65	27
September	immature	6	0	2140±192^{a}	-	803±180^{b}	-	39±3^{a,b}	-
	mature	1	8	2226	2310±173^{a}	1493	786±128^{a}	42	21±1^{a}
November	immature	7	3	2165±203^{1,a}	2512±392^{1,a}	2027±87^{1,a}	1264	32±4^{1,b}	14
	mature	9	17	2314±258^{1,a}	2090±105^{1,a}	2804±112$^{◊,2,a}$	928±41^{a}	32±2$^{◊,1,a}$	22±1^{a}
January	immature	4	0	3821±601^{1,b}	-	2457±293^{1,a}	-	25±1^{1,b}	-
	mature	12	13	3015±136$^{◊,1,b}$	2364±184^{a}	3102±66$^{◊,2,a}$	894±55^{a}	23±1^{1,b}	21±1^{a}

Table II. Concentration of lipids, proteins and carbohydrates (J/g liver wwt) in liver of immature and mature females (F) and males (M) polar cod (*Boreogadus saida*). Values are represented as mean±SE. Symbol (◊) indicates significant difference ($p \leq 0.05$) between females and males of the same maturity and season; numbers (1, 2) indicate significant difference ($p \leq 0.05$) between immature and mature fish of the same gender and season; letters (a, b) indicate significant differences ($p \leq 0.05$) among seasons in fish of the same maturity and gender.

Season	Maturity	n		Lipids, J/g wwt		Proteins, J/g wwt		Carbohydrates, J/g wwt	
		F	M	F	M	F	M	F	M
August	immature	5	3	2695±374[a]	2049±201[a]	879±191[a]	1097±53[a]	50±7[a]	55±9[a]
	mature	1	1	-	2282	938	861	79	63
September	immature	6	0	2196±190[a]	-	1349±84[a]	-	62±11[a]	-
	mature	1	8	1873	2099±96[a]	1428	1250±106[a]	39	64±4[a]
November	immature	7	3	3530±689[1,a]	2265±64[1,a]	1024±128[1,a]	952±116[1,a]	33±2[1,a]	26±4[1,b]
	mature	9	17	2389±111[2,a]	3044±303[1,a]	1022±32[1,a]	893±70[1,b]	28±2[1,a]	31±3[1,b]
January	immature	4	1	2205±205[1,a]	2947	1012±94[1,a]	735	77±17[1,a]	23
	mature	12	13	2523±158[1,a]	3078±236[a]	1020±63[1,a]	1015±89[a,b]	41±4[◊,2,b]	59±6[a]

Table III. Seasonal characteristics and measurements of the oocytes determined in the ovaries of polar cod (*Boreogadus saida*).

Values are represented as $\frac{\text{mean}\pm\text{SE}}{\text{min-max}}$. Letters (a, b, c) in the same column indicate significant difference ($p\leq0.05$) among seasons; numbers (1, 2) in the same column indicate significant difference ($p\leq0.05$) between immature and mature polar cod caught in January.

Season	Maturity	Oocyte	Nucleus / Inclusions	Square (μm^2)	Length (μm)	Width (μm)	Diameter (μm)	Rotundity coefficient
August and September	mature	Oogonia (n=21)		$\underline{381\pm26}^{a}$ 212-726	$\underline{28\pm1}^{a}$ 20-40	$\underline{17\pm1}^{b}$ 11-24	$\underline{22\pm1}^{b}$ 16-30	0.95^{a}
		Previtellogenic oocyte (n=57)		$\underline{5572\pm580}^{a}$ 684-18176	$\underline{96\pm5}^{a}$ 33-209	$\underline{68\pm4}^{a}$ 29-135	$\underline{79\pm4}^{a}$ 30-152	0.91^{a}
			Nucleus of previtellogenic oocyte (n=57)	$\underline{1504\pm120}^{b}$ 251-4704	$\underline{52\pm2}^{a}$ 22-97	$\underline{34\pm1}^{a}$ 15-64	$\underline{42\pm2}^{a}$ 18-77	0.94^{a}
		Oocyte at cortical alveoli stage (n=12)		$\underline{32437\pm1969}$ 22525-43391	$\underline{232\pm9}$ 189-283	$\underline{184\pm8}$ 141-234	$\underline{202\pm6}$ 169-235	0.92
			Nucleus of oocyte at cortical alveoli stage (n=12)	$\underline{4902\pm355}$ 2574-6543	$\underline{95\pm6}$ 63-122	$\underline{67\pm3}$ 56-86	$\underline{78\pm3}$ 57-91	0.94
		Oocyte at stage of lipid inclusions formation (n=2)		$\underline{73946\pm8983}$ 64963-82929	$\underline{391\pm11}$ 380-402	$\underline{259\pm27}$ 233-286	$\underline{306\pm19}$ 288-325	0.85
			Nucleus of oocyte at stage of lipid inclusions formation (n=2)	$\underline{4635\pm1958}$ 2677-6594	$\underline{99\pm22}$ 77-122	$\underline{58\pm14}$ 44-72	$\underline{75\pm17}$ 58-92	0.88
		Vitellogenic oocyte (n=4)		$\underline{89949\pm24620}^{b}$ 20849-137243	$\underline{407\pm73}^{b}$ 194-516	$\underline{275\pm45}^{b}$ 149-353	$\underline{324\pm56}^{b}$ 163-418	0.88^{a}
			Nucleus of vitellogenic oocyte (n=1)	7809^{b}	120^{a}	87^{b}	100^{b}	0.95^{a}

			Yolk globules of vitellogenic oocyte (n=20)	406±35[a] 212-900	23±1[a] 16-39	20±1[a] 15-28	22±1[a] 16-34	1.00[a]
		Post-ovulatory follicles (n=6)		125114±36440 20849-281799	451±78 194-751	324±47 149-486	376±60 163-599	0.90
November	mature	Oogonia (n=21)		407±30[a] 223-717	28±1[a] 17-45	18±1[b] 13-27	22±1[a,b] 17-30	0.96[a]
		Previtellogenic oocyte (n=150)		3324±227[b] 527-14182	80±3[b] 28-192	49±2[b] 18-108	60±2[b] 26-134	0.87[b]
			Nucleus of previtellogenic oocyte (n=150)	1026±65[b] 179-5348	44±1[b] 17-129	27±1[b] 11-58	34±1[b] 15-83	0.93[a]
		Vitellogenic oocyte (n=50)		128298±7361[b] 59068-258026	557±17[b] 333-853	306±11[b] 207-527	396±11[b] 274-573	0.76[b]
			Nucleus of vitellogenic oocyte (n=15)	12197±1386[b] 5754-25908	153±8[a] 119-194	102±8[b] 63-184	122±7[b] 86-182	0.87[a]
			Yolk globules of vitellogenic oocyte (n=280)	125±4[b] 31-415	12±0[b] 6-22	11±0[b] 6-21	12±0[b] 6-23	1.00[a]
		Atretic oocyte (n=3)		9964±2064[a] 6045-13046	162±22[a] 130-205	90±11[a] 68-106	111±12[a] 88-129	0.78[a]
January	immature	Oogonia (n=165)		1157±66[1] 124-6697	44±1[1] 14-108	32±1[1] 11-82	36±1[1] 13-92	0.95[1]
		Previtellogenic oocyte (n=285)		10297±438[1] 1442-28377	126±3[1] 54-247	94±2[1] 32-182	107±2[1] 43-190	0.91[1]
			Nucleus of previtellogenic oocyte (n=285)	2917±89[1] 785-8111	71±1[1] 34-126	51±1[1] 23-93	59±1[1] 32-102	0.93[1]

mature	Oogonia (n=58)		706±110[a,2] 150-6102	34±2[a,2] 16-128	23±1[a,2] 8-62	28±2[a,2] 14-88	0.96[a,1]
	Previtellogenic oocyte (n=133)		10547±619[c,1] 842-27054	136±5[c,1] 40-253	91±3[c,1] 25-174	109±3[c,1] 33-186	0.88[a,b,2]
		Nucleus of previtellogenic oocyte (n=133)	3037±164[a,1] 333-9059	74±2[c,1] 23-141	49±2[c,1] 18-104	59±2[c,1] 21-107	0.90[b,2]
	Vitellogenic oocyte (n=57)		301505±15791[a] 80343-569998	850±23[a] 446-1281	455±15[a] 225-661	606±17[a] 320-852	0.77[b]
		Nucleus of vitellogenic oocyte (n=13)	17835±1584[a] 7888-25654	177±11[a] 103-234	132±6[a] 95-158	149±7[a] 100-181	0.89[a]
		Yolk globules of vitellogenic oocyte (n=430)	489±12[a] 97-1826	25±0[a] 11-49	22±0[a] 9-46	24±0[a] 11-48	1.00[a]
	Atretic oocyte (n=6)		6783±1279[a] 2840-10874	105±13[b] 65-156	77±7[a] 56-96	91±9[a] 60-118	0.95[b]