5.6 Initial description of oocyte development in summer and winter caught female Pacific halibut

Josep Planas, Tracee Geernaert, and Kirsten MacTavish

Abstract

Current maturity estimates in female Pacific halibut are derived from macroscopic visual criteria of the ovaries collected in the field. In order to improve maturity estimates and to provide updated estimates of maturity-at-age, the International Pacific Halibut Commission (IPHC) is conducting studies destined to improve our knowledge on reproductive development in female Pacific halibut. In this pilot study, Pacific halibut females from three geographical locations throughout the distribution range of the species were collected during the summer, or non-spawning season, and during the winter, or spawning season. Histological examination of ovaries of winter and summer caught females evidenced differences in oocyte size distribution and predominant oocyte stages that appeared to be relatively consistent with females sexually maturing in the winter and with females undergoing vitellogenesis in the summer, as a prerequisite for spawning in the winter.

Introduction

Each year, the fishery-independent setline survey collects biological data on the maturity of female Pacific halibut that are used in the stock assessment. In particular, female maturity schedule is used to estimate spawning stock biomass. Currently used estimates of maturity-at-age indicate that the age at which 50% of female Pacific halibut are sexually mature is 11.6 years in average. However, not only maturity is estimated with the use of macroscopic visual criteria, implying a relative level of uncertainty associated with the employed semi-quantitative assessment, but the estimates of maturity-at-age have not been revised in recent years and may be outdated. For this reason, efforts need to be put in place to further understand reproductive maturity in female Pacific halibut. Unfortunately, relatively little is known regarding the actual changes that take place in the ovary during reproductive development leading to spawning in this species. This study aimed at describing oocyte development in female Pacific halibut by comparing oocyte stages and characteristics between the non-spawning season (summer) and the spawning season (winter).

Materials and Methods

Sampling locations and timing

Ovarian sample collection has been described previously (Geernaert, 2005; Loher, 2005). In brief, ovaries were collected from females captured in three geographical regions (Fig. 1), two in the central and south Gulf of Alaska (Portlock and Queen Charlotte, respectively) and one in the southeast Bering Sea (Misty Moon). The Queen Charlotte region, encompassing the Queen Charlotte Islands in British Columbia, was chosen to represent the southernmost known major spawning location in the Gulf (St. Pierre 1984). The Portlock region, east of Kodiak Island, Alaska, represented the center of the Gulf. The Misty Moon region, encompassing the Pribilof Canyon in the southeast Bering Sea, was chosen to represent the northernmost known major spawning location (St. Pierre 1984). During the winter sampling, females were captured between January 7

and February 21 of 2004, corresponding to the peak in known spawning activity (St. Pierre 1984), by three vessels: *F/V Free to Wander* (Queen Charlotte region), *F/V Kema Sue* (Misty Moon region) and *F/V Nopsa* (Portlock region). During the summer sampling, females were captured between June and July of 2004 during the summer SSA survey by three vessels: *F/V Pender Isle* (Queen Charlotte region), *F/V Heritage* (Misty Moon region) and *F/V Predator* (Portlock region).

Ovarian histological analyses

In total, 126 females were processed for histology from the summer sampling (40 in the eastern Bering Sea, 43 in the central Gulf of Alaska and 43 in the southern Gulf of Alaska) and 133 from the winter sampling (43 in the eastern Bering Sea, 50 in the central Gulf of Alaska and 40 in the southern Gulf of Alaska). Ovaries were fixed in buffered formalin and processed for routine histological examination at Histology Consultation Services Inc.[1]. Ovarian fragments were embedded in paraffin and sections were mounted on glass slides. Two slides for each ovary were stained with Hematoxylin and Eosin. From each slide, the diameters of 10 randomly selected oocytes were measured, yielding a total of 20 measured oocytes per ovary analyzed. Measures were conducted using the Image-Pro Premier 9.1 software[2]. Although with this method a reasonable number of oocytes per ovary were measured, it was not optimal for staging. Given that accurate ovarian staging must be based on the largest and, therefore, most developed oocytes, some of the sets of oocyte diameters measured did not include the largest oocytes present in the section because those were not randomly selected. Therefore, for staging purposes, each slide had to be visually inspected for the largest present oocytes and classification of the stage of that ovary (i.e. female) was performed according to the developmental stage of the largest oocyte. The measurements of oocyte diameters were used instead to describe the distribution of oocyte sizes in ovaries from females caught in a particular location in the summer or the winter.

Results

Oocyte size distribution in ovaries from Pacific halibut females collected in summer and winter

In Pacific halibut ovaries collected from the summer, non-spawning season, similar oocyte size distribution patterns were observed in females from the three geographical locations: eastern Bering Sea, and central and southern Gulf of Alaska. Mainly a single major population of oocytes was observed, with the highest number of oocytes corresponding to sizes between 0.04 and 0.4 mm in diameter and with a maximum size of approximately 1 mm in diameter. A similar degree of variation was observed regarding the most abundant oocyte size category, with oocyte diameter values around 0.1-0.2 mm in females from the eastern Bering Sea (Fig. 2A), 0.16-0.28 mm in females from southern Gulf of Alaska and 0.16-0.28 mm in females from central Gulf of Alaska.

In Pacific halibut ovaries collected in the winter, spawning season, a population of oocytes with diameters between 0.1 and 0.4 mm were most abundant in ovaries from females from all three geographical areas (Fig. 3), similar to the population of oocytes found in ovaries collected in the summer. However, a second population of larger oocytes could be clearly observed only in ovaries from females captured in southern and central Gulf of Alaska (Figs. 3B, 3C), but not in ovaries from females captured in the eastern Bering Sea (Fig. 3A). Specifically, in ovaries from females from

[1]Histology Consultation Services, Inc. PO Box 770, Everson, WA 98247.
[2]Manufacturer's address: Media Cybernetics, Inc. 401 N. Washington Street, Suite 350, Rockville, MD 20850

the southern and central Gulf of Alaska a group of oocytes of sizes between 1 and 1.8 mm was observed with approximate median values between 1.3-1.54 mm (Figs. 3B, 3C).

When oocyte diameters of all females captured in each season irrespectively of their geographical location of capture are plotted, the above-stated patterns in oocyte size distribution between summer and winter appear clear (Fig. 4). Both summer and winter caught females have a smaller population of oocytes of 0.04-0.4 mm in diameter but only winter caught females have an additional larger population of oocytes of 1.12-1.78 mm in diameter.

Pacific halibut oocyte developmental stages

As in many other teleost species that have been described to date (Lubzens et al., 2009), oocyte stages in ovaries from female Pacific halibut ranged from early developmental to maturing stages depending on the maturational status of the female. The oocyte stages identified were, in growing order of development: early perinucleolar (ePN), late perinucleolar (lPN), cortical alveoli (CA), early yolk granule (eYGr), mid yolk granule (mYGr), late yolk granule (lYGr), primary yolk globule (pYGl), secondary yolk globule (sYGl), tertiary yolk globule (tYGl) and migrating germinal vesicle (GV) (Fig. 5A). Pre-vitellogenic stages include the ePN, lPN and CA stages; early vitellogenic stages include the eYGr and mYGr stages; mid vitellogenic stages include the lYGr and pYGl stages; late vitellogenic stages include the sYGl and tYGl stages, and the maturing stage includes the migrating GV stage, that is characteristic of oocytes undergoing hormone-induced maturation. In parallel to the increased degree of development, Pacific halibut oocytes increase in diameter from approximately 0.2 mm in ePN oocytes to approximately 1.4 mm in mature oocytes (Fig. 5B). The actual range, as well as the means and SD, of oocyte diameters for each of the oocyte stages are indicated in Table 1.

Although the proportion of oocyte developmental stages in each female could not be assessed due to the random selection of only 20 oocytes per ovary, the proportion of oocyte developmental stages throughout the entire sample of summer- or winter-caught females was estimated (Fig. 6). In the ovaries collected in the summer, the most abundant oocyte developmental stage was lPN (slightly over 30% of the total number of oocytes measured) followed by eVTG and mVTG oocytes (approximately 23% and 25% of the total number of oocytes measured, respectively) with no maturing or mature oocytes observed (Fig. 6A). In the ovaries collected in the winter, lPN oocytes were again the most abundant oocyte stage (over 30% of the total number of oocytes measured) followed by CA and eVTG oocytes at approximately 20% and 17% of the total. Interestingly, winter-collected ovaries contained approximately 10% of maturing oocytes and a small percentage of mature follicles, in contrast to summer-collected ovaries (Fig. 6B).

Relationship between microscopic oocyte developmental stages and macroscopic stages

Pacific halibut maturity status is assessed through the classification of gross macroscopic attributes of the dissected ovaries into four categories: F1, Immature; F2, Maturing; F3, Spawning; F4, Resting. Given that macroscopic stages were available for all females evaluated by histological examination, the distribution of oocyte developmental stages according to each gross morphological stage was examined (Table 2). In the summer, ovaries classified as immature (F1) contained a predominance of lPN oocytes (48.5%) followed by eVTG oocytes (21.2%) and mVTG oocytes (10.6%). In contrast, ovaries classified as mature (F2) in the summer contained only mVTG oocytes (66.7%) and lVTG oocytes (33.3%). In this regard, recent morphological evaluation of ovaries classified under the maturing (F2) category that were collected in the summer

of 2016 (Goose Banks area, BC) evidenced marked differences in size, vascularization and oocyte size (Fig. 7). Resting (F4) ovaries in the summer contained a majority of oocytes at the eVTG stage (77.8%) and a smaller percentage of lPN (11.1%) and mVTG oocytes (11.1%). In the winter, ovaries classified as immature (F1) contained a predominance of lPN oocytes (50.8%), followed by CA and eVTG but a small percentage of maturing and mature oocytes. As expected, winter ovaries classified as mature (F2) contained a predominance of maturing oocytes followed by less developed oocytes at the lPN (21.4%) and CA (14.3%) stages. Along the same lines, winter ovaries classified as spawning (F3) contained equal percentages of lVTG (50%) and maturing oocytes (50%), although only one female at each stage was caught. Finally, winter ovaries classified as resting (F4) contained predominantly ooctyes at the eVTG (41.9%) and CA (32.3%) stages, followed by lPN (12.9%) and mVTG (9.7%) oocytes.

Discussion

This study represents the first attempt at describing ovarian development in female Pacific halibut. By following a histological approach, Pacific halibut oocyte stages were identified, measured and oocyte stages compared among females collected during the summer and winter seasons, corresponding to non-spawning and spawning periods, respectively. Furthermore, a comparison of oocyte development between these two seasons was performed at three regions of the distribution range of the species that may correspond to actual spawning areas: eastern Bering Sea, central and southern Gulf of Alaska.

Analysis of the oocyte size distribution shows that all females, irrespective of season and geographic location, contain in their ovaries a population of small oocytes that, according to their size, represent oocytes at the ePN, lPN and CA stages. Thus, it appears that the ovary of Pacific halibut, at least during two windows of their reproductive cycle (e.g. summer/non-spawning and winter/spawning), contains a predominant population of early vitellogenic oocytes. It is likely that between the summer and the fall, batches of early vitellogenic oocytes will be recruited to grow into full vitellogenic oocytes and subsequently contribute to the spawning season (i.e. November – March), as occurs in other batch spawners (Lubzens et al., 2009). Partial evidence for this hypothesis is found by examining the oocyte size distribution of ovaries from females caught in the spawning season (winter) and, specifically, at the presence of a second population of larger oocytes. These larger oocytes, according to their size and features (e.g. migrating or disintegrated GV, hydration, etc.), likely represent maturing or mature oocytes. Maturing oocytes are oocytes that have already started oocyte maturation, that is, the hormone-dependent process that capacitates an immature oocyte for fertilization and that is characterized by the migration of the nucleus or GV from the center of the oocyte towards its periphery (Nagahama and Yamashita, 2008). In contrast, mature oocytes are oocytes that have already undergone oocyte maturation and are ready to be ovulated (i.e. also referred to as preovulatory). It is interesting to point out that females caught in the eastern Bering Sea in the winter did not show the second population of larger oocytes, resembling more the pattern observed in the ovary of females caught in the summer. This could be an indication that female Pacific halibut in the Bering Sea were not as advanced reproductively as females from the Gulf of Alaska. Whether this observation reflects differences in the timing of spawning throughout the distribution range of the species is a question that cannot be answered with the current data, but that deserves further investigation.

The proportion of oocyte stages in females caught in the summer and winter survey can also provide some information as to the degree of development according to season. In particular, the

presence of a relative high proportion of early and mid VTG oocytes and a smaller proportion of late VTG oocytes in females caught in the summer strongly suggests that a large proportion of oocytes were already invested in vitellogenic growth, as supported by the low proportion of CA follicles, and that would likely be moving along towards maturation as the fall progressed. Therefore, females caught in the summer were not yet maturing but their advanced vitellogenic state would suggest that they would be likely maturing in the winter. In contrast, the lower presence of mid and late VTG oocytes and the higher proportion of CA oocytes in winter caught females in comparison to summer caught females, suggests that these females were likely initiating vitellogenesis at the same time when the ovary contained maturing and mature oocytes that would be spawned during the winter season. At this point, it is not known if the presence of early vitellogenic oocytes in winter caught females is indicative of these oocytes contributing to spawning later during the spawning season or whether they will be reserved for the subsequent annual reproductive cycle. The presence of a large proportion of pre-vitellogenic oocytes (lPN) suggests that these may represent the cohort of oocytes that will be recruited for the following annual reproductive cycle and that more advanced oocyte stages may represent oocytes recruited later during the actual reproductive season.

Oocyte staging has also evidenced the inaccuracy of the macroscopic ovarian classification in maturity estimates. This is clearly observed in the immature (F1) stage in the summer, when these ovaries contain more than 10% of mid vitellogenic oocytes that may be capable of being recruited successfully to maturing oocytes in the winter. Another intriguing observation is the high predominance of early vitellogenic oocytes in summer caught ovaries classified as resting (F4). Whereas females with resting ovaries are truly resting or are skipping spawning is an important question that needs to be addressed. Clearly, more intense efforts to sample fish throughout the entire annual reproductive cycle and study in detail morphological, histological, endocrine and functional changes will lead to a better understanding of the temporal and spatial progression of sexual maturation in Pacific halibut females, and to a better estimate of maturity for stock assessment purposes.

References

Geernaert, T. 2005. Analysis of halibut gonad staging. Int. Pac. Halibut Comm. Report of Assessment and Research Activities 2004:319-320.

Loher, T. 2005. Cruise report for 2004 IPHC winter charters. Int. Pac. Halibut Comm. Report of Assessment and Research Activities 2004:287-292.

Lubzens, E., Young, G., Bobe, J., and Cerda, J. 2009. Oogenesis in teleosts: how fish eggs are formed. Gen. Comp. Endocrinol. 165:367-389.

Nagahama, Y., and Yamashita, M. 2008. Regulation of oocyte maturation in fish. Dev. Growth Diff. 50:S195-S219.

St.-Pierre, G. 1984. Spawning locations and season for Pacific halibut. Int. Pac. Halibut Comm. Sci. Rep. 70.

Table 1. Oocyte sizes according to stage

Oocyte stage	Mean ± SD	Range (min - max)
Early PN	0.258 ± 0.04	0.178-0.311
Late PN	0.331 ± 0.08	0.221-0.482
CA	0.506 ± 0.10	0.329-0.743
Early VTG	0.776 ± 0.13	0.392-1.023
Mid VTG	0.869 ± 0.12	0.639-1.621
Late VTG	0.850 ± 0.29	0.639-1.621
Maturing	1.263 ± 0.30	0.692-1.599
Mature	1.406 ± 0.05	

Table 2. Microscopic ovarian stages present in ovaries classified by macroscopic criteria. The presence of oocytes at the various stages is expressed as the percentage of the total number of fish with oocytes at that particular stage for each particular macroscopic stage. The number of fish classified at each of the various oocyte stages in indicated in parentheses.

		Oocyte stages[1]							
	Macroscopic stages	ePN	lPN	CA	eVTG	mVTG	lVTG	Maturing	Mature
Summer									
	F1	9.09 (6)	48.48 (32)	10.61 (7)	21.21 (14)	10.61 (7)	-	-	-
	F2	-	-	-	-	66.67 (16)	33.33 (8)	-	-
	F3	-	-	-	-	-	-	-	-
	F4	-	11.11 (1)	-	77.78 (7)	11.11 (1)	-	-	-
Winter									
	F1	10.77 (7)	50.77 (33)	16.92 (11)	12.31 (8)	4.62 (3)	1.54 (1)	1.54 (1)	1.54 (1)
	F2	-	21.43 (3)	14.29 (2)	-	-	-	57.14 (8)	-
	F3	-	-	-	-	-	50.00 (1)	50.00 (1)	-
	F4	-	12.90 (4)	32.26 (10)	41.94 (13)	9.68 (3)	-	-	-

[1] early perinucleolar (ePN), late perinucleolar (lPN), cortical alveoli (CA), early vitellogenic (eVTG), mid vitellogenic (mVTG), late vitellogenic (lVTG)

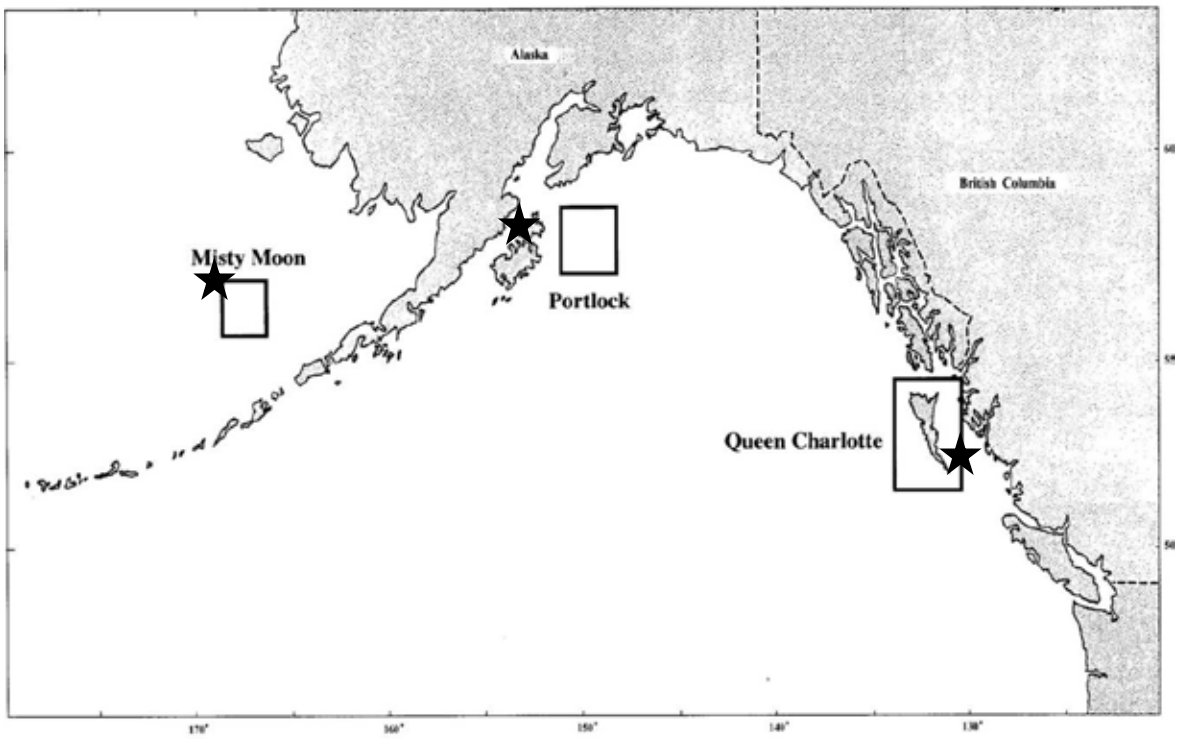

Figure 1. Geographic location of sample collection sites. Summer collection sites (non-spawning season) are indicated by a star and winter collection sites (spawning season) are indicated by a black box.

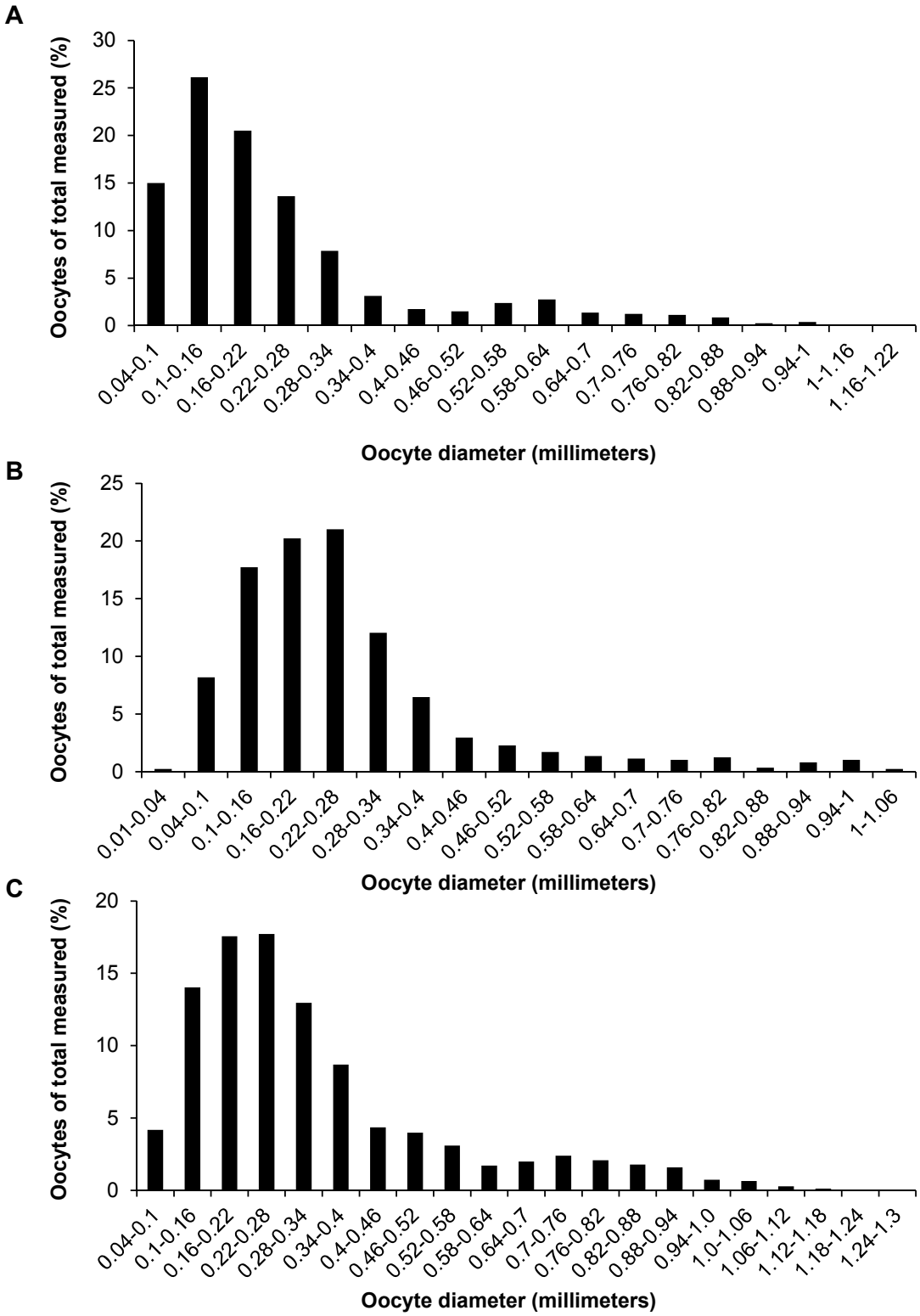

Figure 2. Pacific halibut oocyte size distribution in females caught in the summer. A) Misty Moon region in SE Bering Sea; B) Queen Charlotte region in southern Gulf of Alaska; C) Portlok region in the central Gulf of Alaska

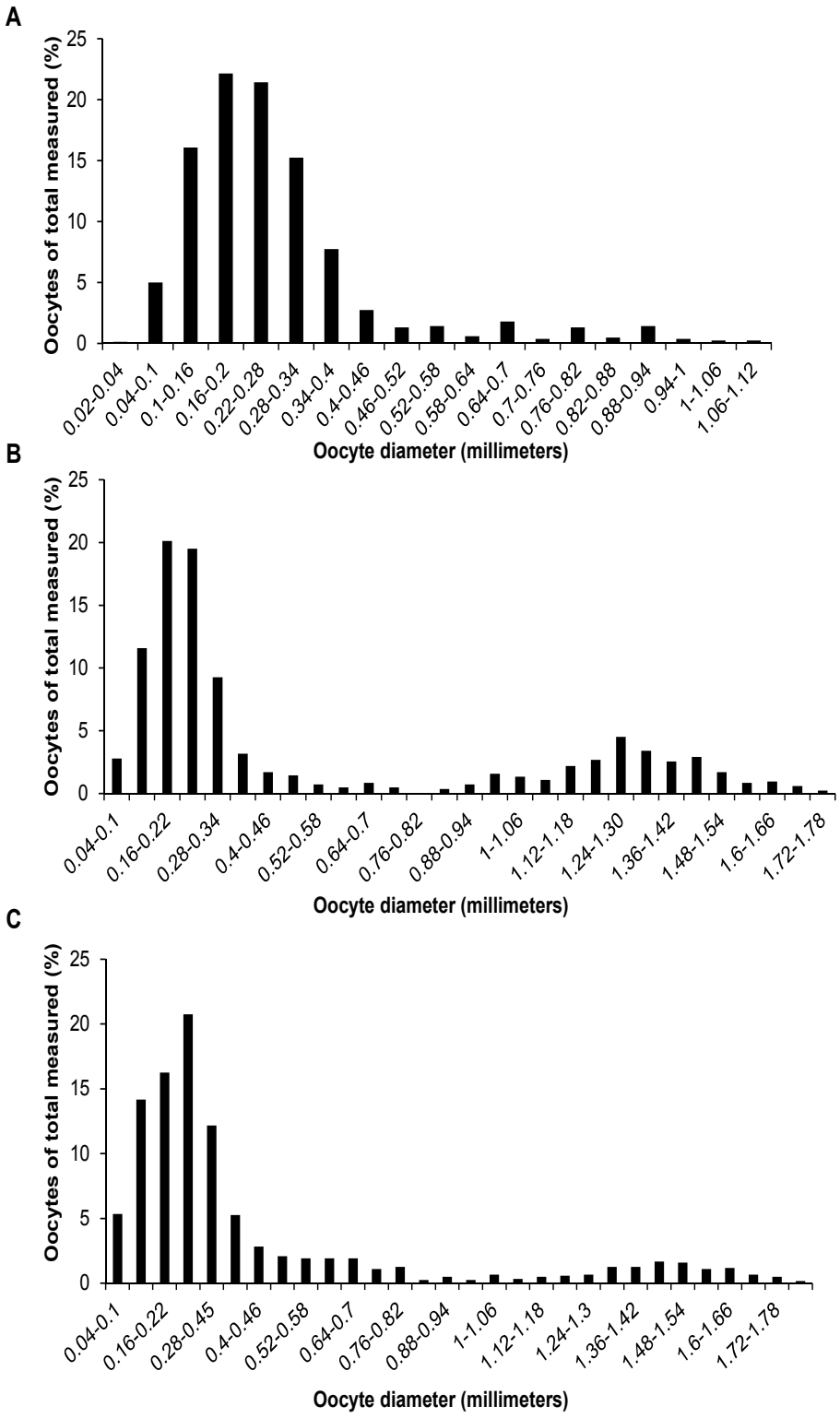

Figure 3. Pacific halibut oocyte size distribution in females caught in the winter. A) Misty Moon region in SE Bering Sea; B) Queen Charlotte region in southern Gulf of Alaska; C) Portlok region in the central Gulf of Alaska

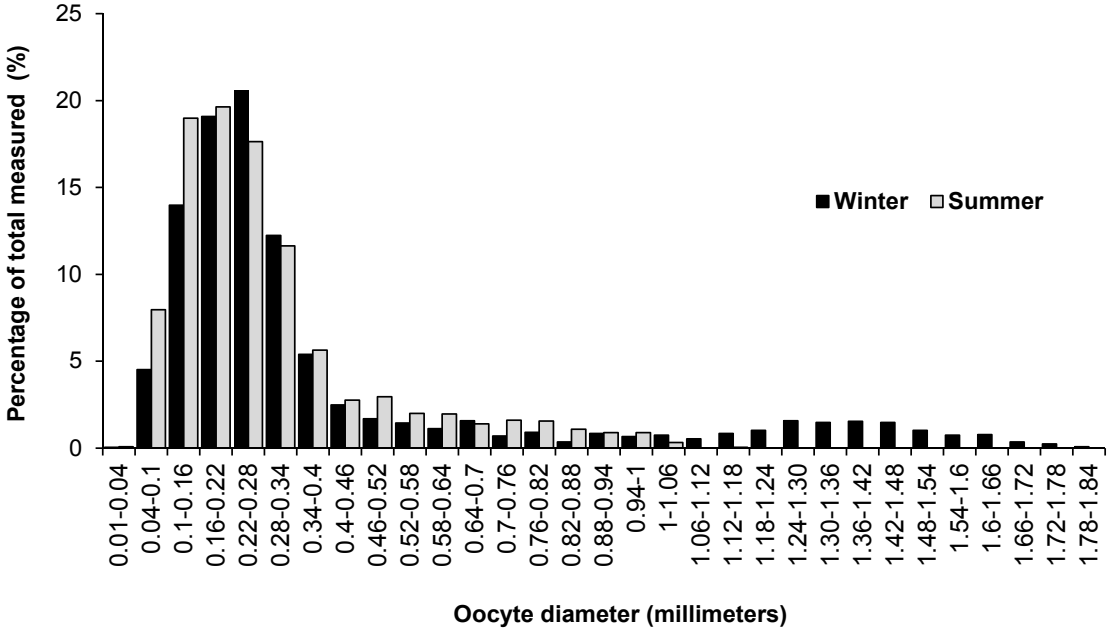

Figure 4. Pacific halibut oocyte size distribution in females caught in summer and winter.

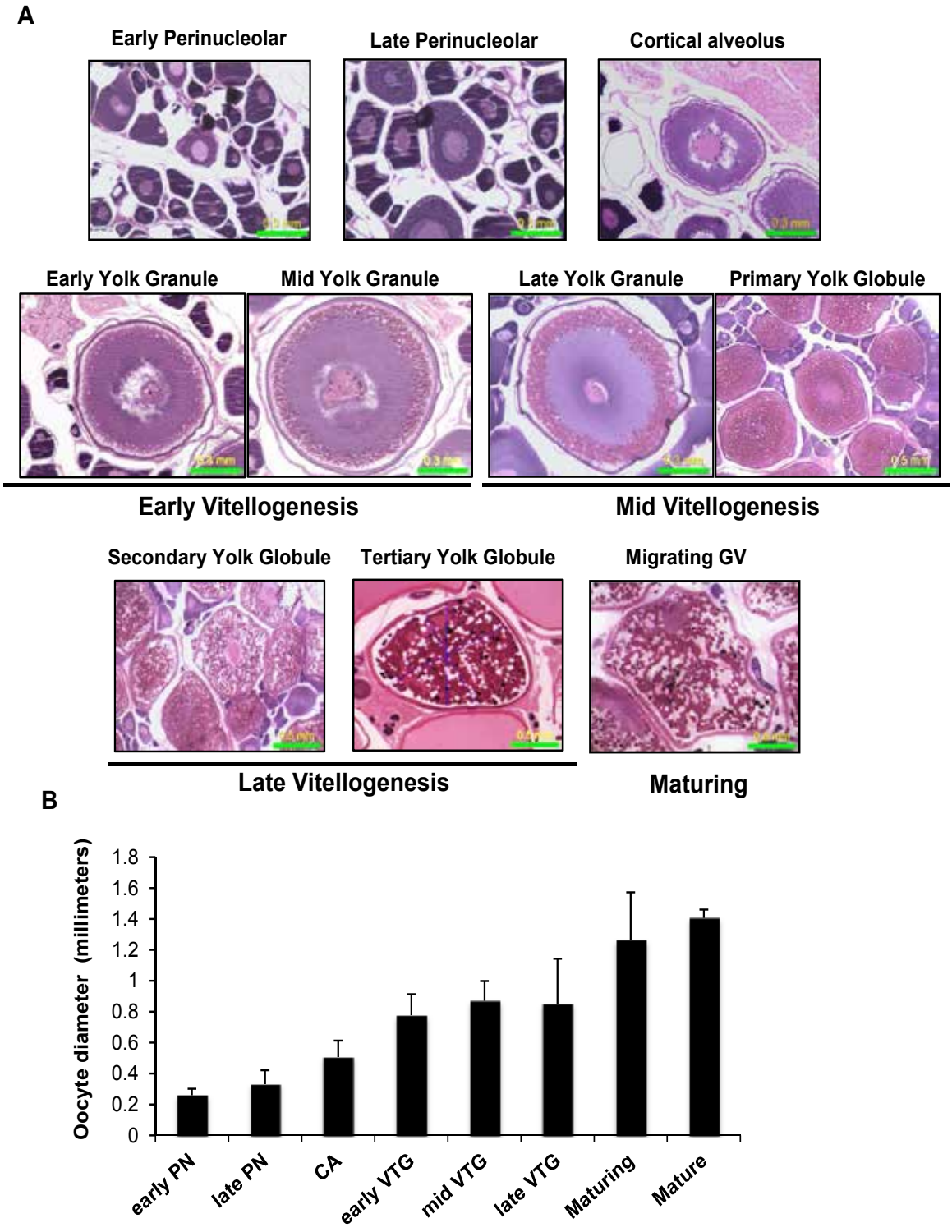

Figure 5. Pacific halibut oocyte stages and diameters. A) Pictures of representative oocytes at the various stages during oocyte development. B) Oocyte diameters (in millimiters) at different stages in oocyte development. Oocyte stage classification included oocytes at the early and late perinucleolar (PN), cortical alveoli (CA), mid and late vitellogenesis (VTG), maturing and mature stages.

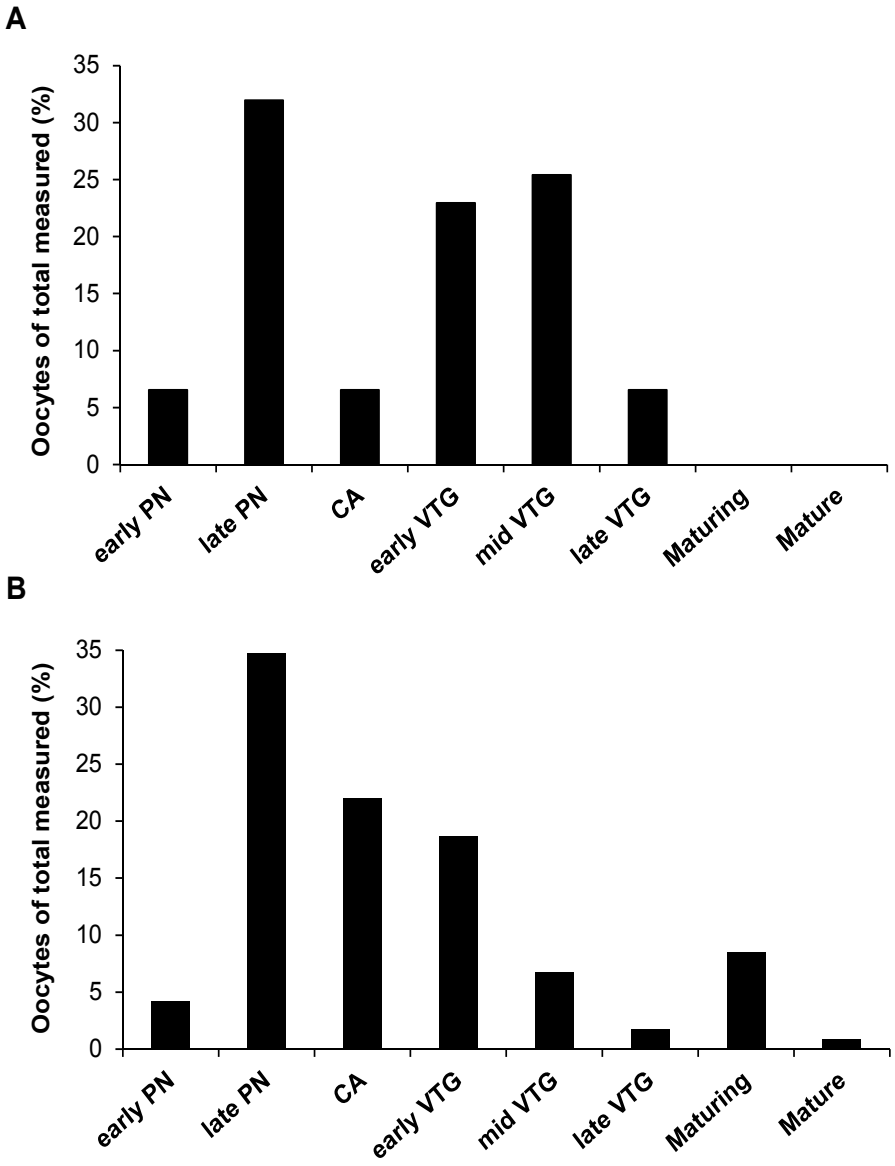

Figure 6. Pacific halibut oocyte stages in females caught in the summer (A) and winter (B). Oocyte stage classification included oocytes at the early and late perinucleolar (PN), cortical alveoli (CA), mid and late vitellogenesis (VTG), maturing and mature stages.

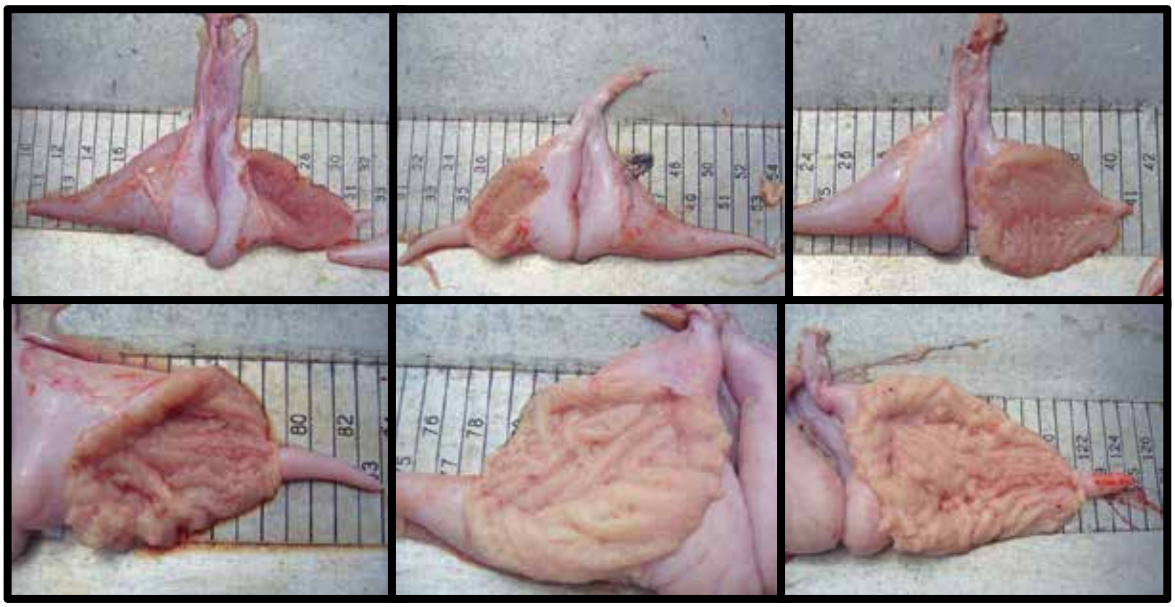

Figure 7. Pacific halibut ovaries classified as maturing (F2). The three examples in the top row show less developed ovaries with no visible oocytes, whereas the three examples in the bottom row show more developed ovaries with visible oocytes.