

## **Assessment of length- and age-at-maturity for California halibut (*Paralichthys californicus*), including a histologically-based description of the reproductive cycle**

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Estimates of length- and age-at-maturity for California halibut (*Paralichthys californicus*) have been reported for southern California, but not central California. To provide new estimates of length- and age-at-maturity for central California halibut, we macroscopically examined gonads from 635 fish caught between 2012 and 2014 and additionally examined ovaries histologically. We developed a detailed description of the reproductive phases and spawning states for California halibut, and assigned sex-specific length- and age-at-maturity to each individual. Males (n=333) ranged from 19.1 to 95.9 cm fork length (FL) and 1 to 16 yr of age and females (n=302) ranged from 18.6 to 111.0 cm FL and 1 to 19 yr of age. Males matured at younger ages and shorter lengths than females. The smallest mature male was measured at 25.7 cm (1 yr), 50% of males were mature by 27.0 cm (1.1 yr), and 100% were mature by 29.0 cm (3 yr). The smallest mature female was measured at 46.6 cm (2 yr), 50% of females were mature by 47.3 cm (2.6 yr), and 100% were mature by 51.3 cm (4 yr), according to histological criteria. Therefore, all California halibut examined were mature before reaching the commercial and recreational minimum legal size limit of 55.9 cm (22 in). When comparing central California maturity data with information from southern California, we found that central California halibut matured at larger sizes (both sexes) and older ages (females only) than southern California halibut, according to macroscopic criteria.

**Key words:** California halibut, *Paralichthys californicus*, length-at-maturity, age-at-maturity, reproductive cycle, minimum legal size limit, histology, spawning state, batch spawner, flatfish

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California halibut (*Paralichthys californicus*) are most commonly encountered from Bodega Bay, central California to Bahía de San Quintín, northern Baja California (Rosales-Casián 1996), although their geographic range extends from the Quillayute River in the state of Washington, USA, to Cabo Falsa in southern Baja California, Mexico (Fitch and Lavenberg 1971, Feder et al. 1974, Allen 1990, Martínez-Muñoz and Ramírez-Cruz 1992). This large predatory flatfish has supported important commercial and recreational fisheries in California since the early 1900s (Frey 1971, Allen 1990, Kramer et al. 2001). Because of its great economic and ecological importance (Allen 1990), California halibut is considered a high priority species for life history research by the California Department of Fish and Wildlife (CDFW), which manages these fisheries for long-term sustainability.

California halibut are oviparous, broadcast spawners that exhibit external fertilization (Allen 1990). This reproductive strategy involves the release of gametes (i.e., eggs and sperm) directly into the water column (Cailliet et al. 1986) where, in the case of California halibut, fertilized eggs develop into pelagic larvae (Frey 1971, Allen 1988). California halibut are batch spawners (Caddell et al. 1990) that release hydrated (i.e., fully developed) ova during reproductive events, while less developed oocytes remain in the ovary and mature for release at a later date (Cailliet et al. 1986, Murua et al. 2003). Thus, ovaries of mature California halibut always contain oocytes in various developmental stages, even after a spawning event has occurred. Although data to evaluate spawning seasonality off the central California coast are limited, a peak in reproductive activity has been observed within the summer months between Monterey and San Luis Obispo (Barnes et al. 2015).

Biological data, including reproductive strategy and timing of maturation, aid fisheries managers in evaluating the effectiveness of minimum legal size limits (Reed and MacCall 1988, Maunder et al. 2011). Since the 1970s, take of California halibut measuring less than 55.9 cm (22 inches) has been prohibited in all California fisheries. One of the intended purposes of this minimum legal size limit was to allow at least 50% of the California halibut population to reach maturity before becoming susceptible to take. Although available estimates of California halibut length-at-maturity suggest that the minimum legal size limit effectively protects immature individuals from take by the fishery along southern California (Love and Brooks 1990), no maturity studies have been conducted for the central California region. Because spatially varying environmental conditions and different degrees of fishing pressure can result in different rates of maturation (e.g., Packer et al. 1999, Yoneda et al. 2007), it is important to construct region-specific estimates of length- and age-at-maturity throughout a species' range.

Here, we estimate length- and age-at-maturity for central California halibut and provide a preliminary assessment of regional differences in maturation by comparing data from central California with those previously collected along the Southern California Bight (Love and Brooks 1990). We also present the first detailed description of the reproductive cycle for California halibut based on histology and include a macroscopic guide for use in assigning spawning states during field-based research.

## MATERIALS AND METHODS

*Sample collections.*—California halibut were collected from recreational, commercial, and research fishing activities off central California (i.e., north of Point Conception). Almost all fish were collected inside San Francisco Bay and from nearshore waters adjacent to Santa Cruz, Moss Landing, Monterey, Morro Bay, and Port San Luis (Figure 1).

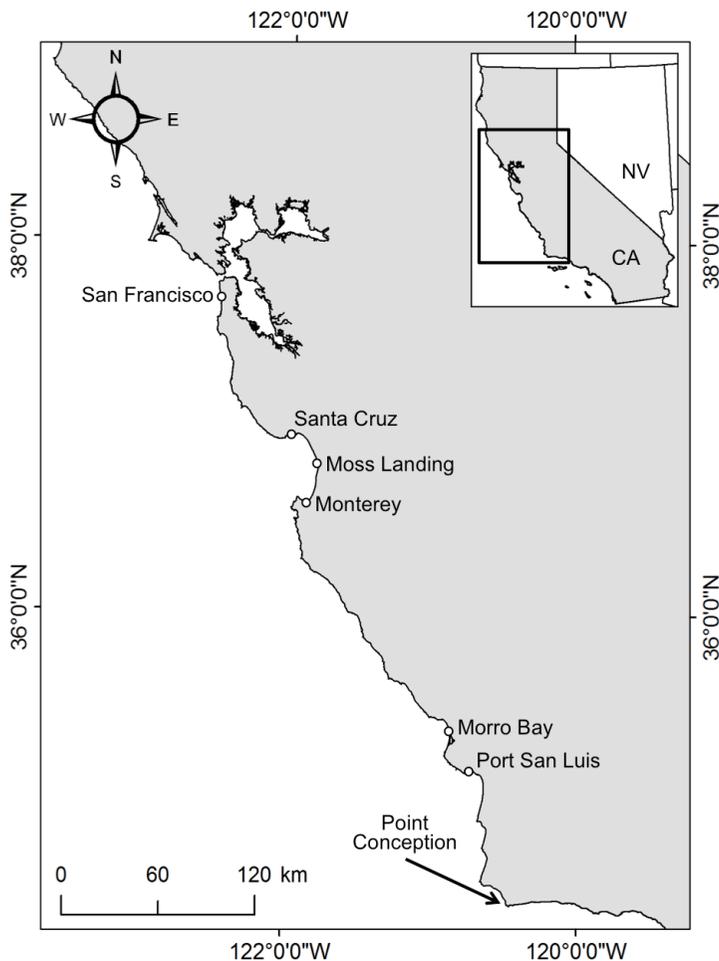


FIGURE 1.—Primary locations used to collect California halibut in nearshore waters off central California. Point Conception denotes the boundary between central and southern California.

In San Francisco Bay, California halibut were collected opportunistically by CDFW staff from July 2012 to November 2014. California halibut were not collected during the months of January and February, and only one sample was collected in December 2012. This was due, in part, to a lack of fishing effort during winter. Fish were obtained from two research trawl vessels, a commercial bay shrimp trawler, and a Commercial Passenger Fishing Vessel (CPFV) using hook-and-line gear, and fishery-independent researchers using hook-and-line gear.

From Santa Cruz, Moss Landing, Monterey, Morro Bay, and Port San Luis, California halibut were collected opportunistically between June 2012 and November 2013; these methods are further described in Barnes (2015). The majority of fish from these areas were caught during the summer months (i.e., June to August), when

California halibut are known to be reproductively active in these areas (Barnes et al. 2015). Samples from fish of legal size were collected from recreational fisheries that used hook-and-line and spear, and from the commercial hook-and-line fishery.

*Laboratory processing.*—California halibut were examined as fresh dead specimens. Fork length (FL, cm), body weight (kg), and eyed-side were recorded. Pre-filleted fork lengths for fish received as filleted carcasses were calculated using the equation:  $0.137 + (0.99 * \text{post-filleted FL})$ ;  $R^2 = 0.999$ ,  $P < 0.001$  (Barnes 2015). Fulton's condition factor (K) was calculated, whenever possible:  $100,000 (\text{body weight} / \text{fork length}^3)$  (Fulton 1902). Sagittal otoliths were processed into thin sections, a technique that has previously been used to age California halibut (MacNair et al. 2001) and is considered a reliable method for ageing longer-lived fishes (Christensen 1964, Power 1978, Beamish and McFarlane 1987). The methodology for thin sectioning was derived from the Committee of Age Reading Experts (CARE 2006) and the formation of one annulus per year was previously validated for California halibut using chemical marking (Pattison and McAllister 1990). California halibut were aged by two or three independent readers until agreement was reached, as described by Barnes 2015.

For both males and females, sex was initially assigned based upon macroscopic characteristics and, if necessary, histology was used to confirm sex. Gonads and livers were removed and weighed (g) for calculations of gonadosomatic index (GSI):  $100 (\text{gonad weight} / \text{gonad free body weight [g]})$  and hepatosomatic index (HSI):  $100 (\text{liver weight} / \text{liver free body weight [g]})$  (Le Cren 1951, Delahunty and de Vlaming 1980, de Vlaming et al. 1982). Ovaries were then preserved in 10% buffered formalin before transfer to 70% ethanol for storage.

Transverse sections of the preserved ovary were sent to an independent laboratory for histological preparation, where they were dehydrated, embedded in paraffin wax, thin-sectioned, mounted on a microscope slide, stained with hematoxylin and eosin (H&E), and returned for analyses. An initial batch of ovaries ( $n=18$ ) was processed to determine if there was a difference in the most advanced oocyte stage among anterior, middle, and posterior sections of both blind- and eyed-side lobes. Because preliminary analyses demonstrated no difference by section or lobe, a single sample (i.e., the mid-anterior transverse section of the blind-side lobe) was analyzed for remaining females.

*Reproductive phase, spawning state, and maturity assignments.*—Prior to preservation, ovaries were macroscopically examined and described according to presence or absence of individual oocytes visible to the naked eye, color, and blood vessel configuration (e.g., color and amount of branching). Each ovary was also histologically examined to identify the most advanced stage of oocyte development. In order of least developed to fully developed, the oocyte developmental stages identified were chromatin nuclear (CN), perinucleolar (PN), cortical alveolar (CA), yolk granule (YG), final maturation (FM), and hydrated (HD), (Murua et al. 2003). CN and PN stages were considered primary growth oocytes and all others were considered secondary growth oocytes (Wallace and Selman 1981). Additionally, histological slides were examined for the presence of postovulatory follicles (i.e., evacuated follicles that collapse when a hydrated oocyte is released [POFs]) and atresia (i.e., resorption of oocytes that are not released). POFs were estimated as new or old and atresia was recorded as alpha atresia (i.e., fresh [*aAT*]) or beta atresia (i.e., old [*bAT*]) based upon the level of degradation (Hunter and Macewicz 1985).

Histological criteria and corresponding macroscopic characteristics were used to assign females to one of six reproductive phases: immature, developing, spawning capable, actively spawning, spent, and resting (Table 1; terminology modified from

Brown-Peterson et al. 2011). Considerations for macroscopically assigning reproductive phases were also outlined. Females histologically assigned to developing, spawning capable, actively spawning, spent, and resting reproductive phases were grouped as mature.

TABLE 1.—Histological characteristics used to describe reproductive phases for female California halibut. Oocyte developmental stages (CN = chromatin nuclear, PN = perinucleolar, CA = cortical alveolar, YG = yolk granule, FM = final maturation, HD = hydrated), other histological characteristics (aAT = alpha atresia, bAT = beta atresia, POFs = postovulatory follicles), and maturity assignments (0 = immature, 1 = mature) are indicated. Corresponding macroscopic characteristics and considerations for assigning reproductive phases based upon macroscopic assessments alone are also described.

Reproductive Phase	Histological Characteristics	Macroscopic Characteristics	Considerations for Macroscopic Assessments
Immature <sup>0</sup>	The most advanced oocytes are in CN or PN stages of development.	Individual oocytes are not visible to the naked eye. Ovaries are light pink to pale orange in color.	Separating this phase from the initial onset of maturity is difficult.
Developing <sup>1</sup>	The most advanced oocytes are in the CA stage.	Individual oocytes are not visible to the naked eye. Ovaries are bright orange in color. Red blood vessels are present.	This phase can be confused with the resting phase.
Spawning Capable <sup>1</sup>	The most advanced oocytes are in the YG or FM developmental stage. Old POFs may be present.	Individual oocytes are visible to the naked eye. Ovaries are yellowish-orange in color.	This phase can be confused with actively spawning and spent phases.
Actively Spawning <sup>1</sup>	HD oocytes and/or new POFs are present. Old POFs may also be present.	HD oocytes may be visible to the naked eye, are interspersed throughout the ovary, and can be accumulated near the oviduct. Ovaries are yellowish-orange in color.	This phase can be confused with spawning capable and spent phases if HD oocytes are not accumulated in the oviduct.
Spent <sup>1</sup>	The most advanced oocytes are in the YG stage. However, greater than 50% of YG stage oocytes are undergoing aAT. No POFs are present.	Individual oocytes may be visible to the naked eye. Ovaries are orange, bright orange, or purple in color.	This phase can be confused with spawning capable and actively spawning phases.
Resting <sup>1</sup>	The most advanced oocytes are in CA or PN stages. However, greater than 50% of all CA oocytes (if present) are undergoing aAT or bAT.	Individual oocytes are not visible to the naked eye. Ovaries are orange to bright orange in color. White (i.e., empty) blood vessels are present.	This phase can be confused with the developing phase.

Histological depictions of female reproductive phases were then used to illustrate the directionality of the California halibut reproductive cycle (Figure 2). In the earliest immature phase of the reproductive cycle, all oocytes can be classified as primary growth oocytes in the chromatin nuclear stage. This is a stage that the ovary never returns to or resembles again. In the next and final phase of immaturity, the most advanced oocytes remain in primary growth, but develop into the perinucleolar stage. The initial progression from the perinucleolar to cortical alveoli stage represents a transition into secondary growth oocytes, progression into the developing reproductive phase, and the initial onset of maturity. Once in the developing phase, the ovary never returns to an immature status. However, the final phase of immaturity can closely resemble the resting phase, which represents an unknown duration of reproductive inactivity that is identified by a return to primary growth stage oocytes. Ovaries were identified as 'early resting' if more than half of secondary growth-size oocytes were undergoing beta atresia, a possible sign of past reproductive activity (Hunter et al. 1992). Relatively large, old females that did not possess any histological signs of past reproductive activity were identified as 'late resting' and mature, even though ovaries resembled the final phase of immaturity. This was due to the assumption that they had previously spawned, based upon their relative size and age. In between the immature and resting phases, females release multiple

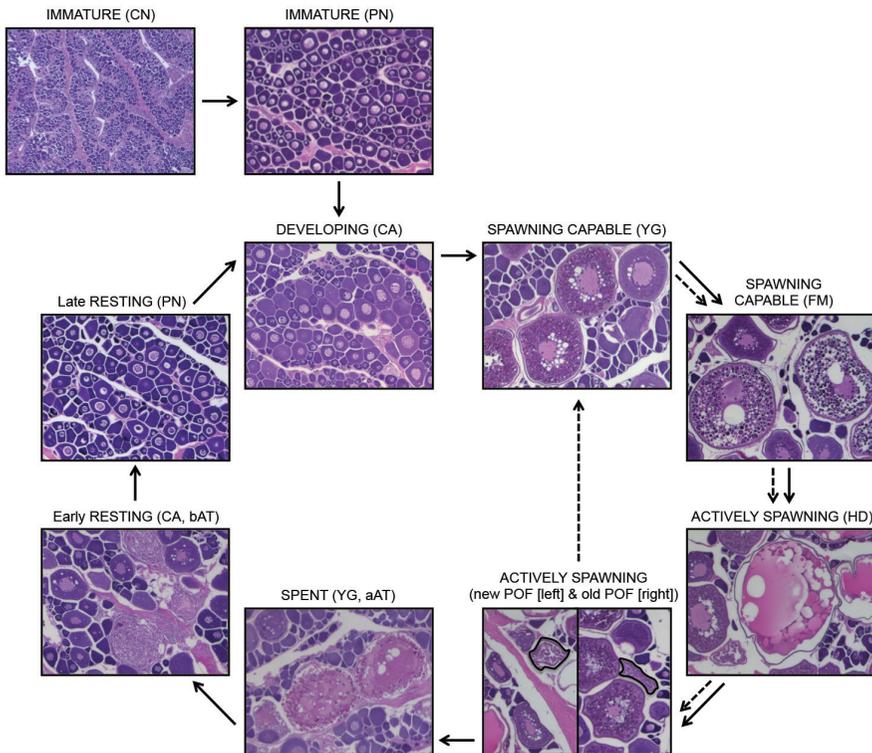


FIGURE 2.—Images of histological slides (50x, ImagePro Plus v7) depicting the most advanced oocyte stage (CN = chromatin nuclear, PN = perinucleolar, CA = cortical alveoli, YG = yolk granule, FM = final maturation, HD = hydrated) and other characteristics (aAT = alpha atresia, bAT = beta atresia, new and old POFs = postovulatory follicles) that were used to determine the reproductive phase for female California halibut. Solid arrows indicate the direction of the reproductive cycle. Dashed arrows indicate that females spawn multiple times by transitioning between spawning capable and actively spawning phases.

batches of eggs through transitions between spawning capable and actively spawning phases, before entering a relatively short spent phase evidenced by mass atresia. The reproductive cycle repeats when the individual leaves the resting phase and enters the developing phase.

Histological analyses were not conducted for male California halibut. Testes were macroscopically examined and described according to lobe shape and incidence of milt in the sperm duct. Males were assigned into one of three reproductive phases: immature, spawning capable, and actively spawning. Immature males had developing (i.e., oval-shaped) testes and no milt in the sperm duct. Spawning capable males had fully developed (i.e., triangular-shaped) testes, but no milt in the sperm duct. Actively spawning males had both fully developed testes and milt in the sperm duct at the time of capture. Males assigned to spawning capable and actively spawning reproductive phases were subsequently grouped as mature.

The incidence of milt in the sperm duct was used to assign an inactive or active spawning state for males. Females were macroscopically categorized into inactive and active spawning states based upon the incidence of individual oocytes visible to the naked eye. A female in an active spawning state was further identified as ‘fully hydrated’ if hydrated oocytes were accumulated in the oviduct at the time of capture. This macroscopic information was used to construct a guide for assigning spawning states during field-based assessments (Figure 3).

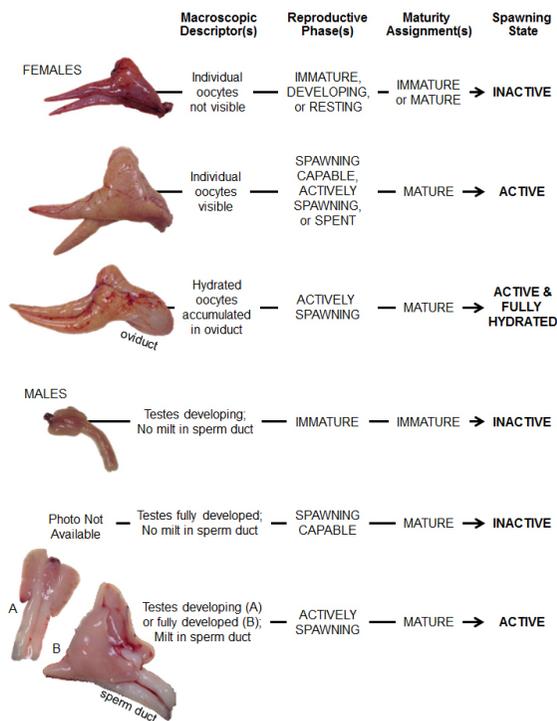


FIGURE 3.—Macroscopic guide to assigning sex-specific spawning states (i.e., inactive or active) to California halibut during field-based assessments. For each spawning state, possible corresponding photo(s), macroscopic descriptor(s), reproductive phase(s), and maturity assignment(s) are listed. The ephemeral ‘fully hydrated’ condition within the active spawning state for female California halibut confirms an actively spawning reproductive phase at the time of capture. Maturity cannot be macroscopically assigned to inactive females. The presence of milt in the sperm duct confirms an active spawning state at the time of capture for males. A photo was unavailable for mature males collected in an inactive spawning state due to the rare occurrence of this condition.

*Maturity curves.*—A histologically-based female maturity ogive and a macroscopically-based male maturity ogive were constructed for central California halibut. Data provided by Love and Brooks (1990) were used to represent southern California halibut in constructing maturity ogives for regional comparisons. Additionally, maturity classifications for central California halibut females were reassigned to be consistent with the macroscopic maturity criteria used for southern California females (i.e., based solely upon the incidence of individual oocytes visible to the naked eye). Therefore, developing and resting phase females from the histologically-based central California ogive (i.e., those with individual oocytes not visible to the naked eye) were reclassified as immature for regional comparisons. Methods used to construct male maturity curves for central California did not change because they were categorized macroscopically for both regions. Finally, unpublished data provided by the CDFW Bay Study were used to develop a conversion from total length (TL, cm), to FL for California halibut. The conversion to FL:  $(0.97 * TL) + 0.60$  ( $R^2 = 0.999$ ;  $P < 0.01$ ) was only applied to southern California halibut because FL was initially recorded for all central California halibut.

For all maturity curves described above, relationships between the proportion of mature individuals and length (cm) or age (yr) were established using a generalized linear model (GLM) with a binomial distribution and logit link function (stats, R v3.2.2). Parameters  $a$  (slope) and  $b$  (intercept) from the equation  $P_x = \frac{1}{1 + e^{ax+b}}$  (where  $P_x$  is the proportion of mature individuals at a given age or length  $x$ ) were calculated using sex- and region-specific models (Gunderson et al. 1980). Lengths and ages at 50% maturity were calculated from fitted models using the dose.p function and a proportion of mature individuals ( $p$ ) set to 0.5 (MASS, R v3.2.2).

*Reproductive phase, spawning state, and maturity comparisons.*—A one-way ANOVA was used to test for differences in age (yr), length (cm), weight (kg), Fulton's K, GSI, and HSI among female reproductive phases, female spawning states, and male maturity assignments. The Tukey HSD post-hoc multiple comparisons test was used to evaluate relationships among significant ( $P < 0.10$ ) factors (SPSS v23). Finally, oocytes from a subsample of whole mount (i.e., preserved eggs) and histologically processed females were measured to evaluate potential differences in oocyte size by developmental stage (Appendix I).

## RESULTS

*Sample collections.*—A total of 635 California halibut (302 females and 333 males) were collected off of central California (Figure 4). The majority of fish collected from San Francisco Bay (92.5%) were shorter than the minimum legal size limit, whereas the majority of fish caught in Monterey Bay (i.e., Santa Cruz, Moss Landing, Monterey) and Morro Bay/Port San Luis (96.7%) were of legal size. As a result, the vast majority of immature fish were collected from San Francisco Bay, whereas mature fish were largely collected along the outer coast of central California. One additional female (82.0 cm; not shown in Figure 4) was collected from Half Moon Bay. Females ranged from 18.6 to 111.0 cm (1 to 19 yr) and males ranged from 19.1 to 95.9 cm (1 to 16 yr). The majority of fish were collected during the summer months (June through August,  $n=468$ ), followed by fall (September to November,  $n=107$ ) and spring (March to May,  $n=59$ ). One fish was collected during winter (December through February).

*Reproductive phase, spawning state, and maturity assignments.*—Based on histological examinations, female California halibut were classified into six reproductive phases: immature ( $n=66$ ), developing ( $n=27$ ), spawning capable ( $n=109$ ), actively spawning ( $n=77$ ), spent ( $n=7$ ), and resting ( $n=16$ ). Histological assignments of female

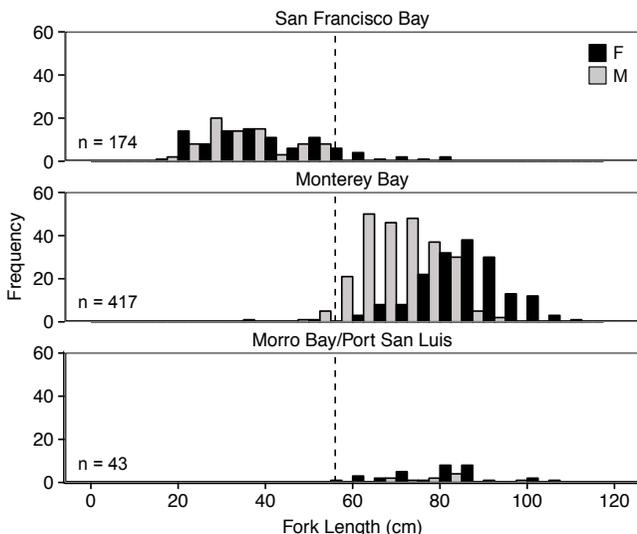


FIGURE 4.—Length frequency distributions for California halibut collected off central California, by sex and location. Black bars denote numbers of females and gray bars denote numbers of males. The dashed line indicates the minimum legal size limit (55.9 cm).

maturity resulted in 66 immature and 236 mature individuals. When reassigning maturity based solely upon macroscopic characteristics, we observed 109 immature females in an inactive spawning state and 193 mature females in an active spawning state.

Macroscopic evaluations of testes yielded three classifications for males: immature (n=19), spawning capable (n=6), and actively spawning (n=305). Malformed males (n=3) possessed testes that were oddly-shaped, more solidly textured, and without milt in the sperm duct. These individuals were excluded from analyses. Of those sampled, 98% of mature males were captured in an actively spawning state. Only a few mature males (2%) were classified as inactive because they possessed fully developed testes, but no milt in the sperm duct.

*Maturity curves.*— Female California halibut matured at greater lengths (cm) and ages (yr) than male conspecifics in central California, based on histological examination for females and macroscopic examination for males (Figure 5). All females had reached maturity by 51.3 cm (4 yr) and all males had reached maturity by 29.0 cm (3 yr). The fork length ( $\pm$  standard deviation [SD]) at which 50% of the samples collected were considered mature was  $47.3 \pm 0.88$  cm for females and  $27.0 \pm 0.43$  cm for males. The ages ( $\pm$ SD) at which 50% of the samples collected were considered mature was  $2.6 \pm 0.10$  yr for females and  $1.1 \pm 0.10$  yr for males. The smallest mature female was measured at 46.6 cm (2 yr) and the smallest mature male was measured at 25.7 cm (1 yr).

Using histological criteria to determine maturity for central California halibut females resulted in lower estimates of length and age at 50% maturity than those made using macroscopic criteria alone (Table 2). Comparisons of macroscopically-based maturity curves for central and southern California halibut showed that males and females matured at longer lengths off of central California (Figure 6). Females from central California were older at 50% maturity, but age at maturity did not differ for males between the two regions.

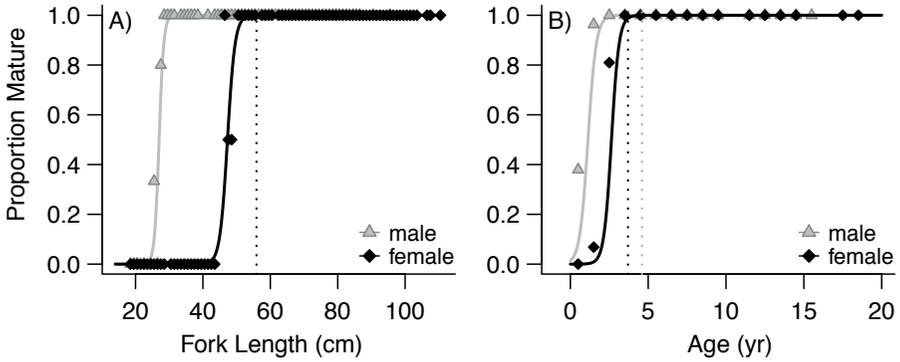


FIGURE 5.—Proportions of mature California halibut collected from central California, by fork length (cm; A) and age (yr; B). Males (classified macroscopically) are shown as gray triangles and females (classified using histological techniques) are shown as black diamonds. Dotted lines represent the single length (as determined by the minimum legal size limit of 55.9 cm) or sex-specific age (estimated by Barnes et al. 2015) at which California halibut become available to the fishery.

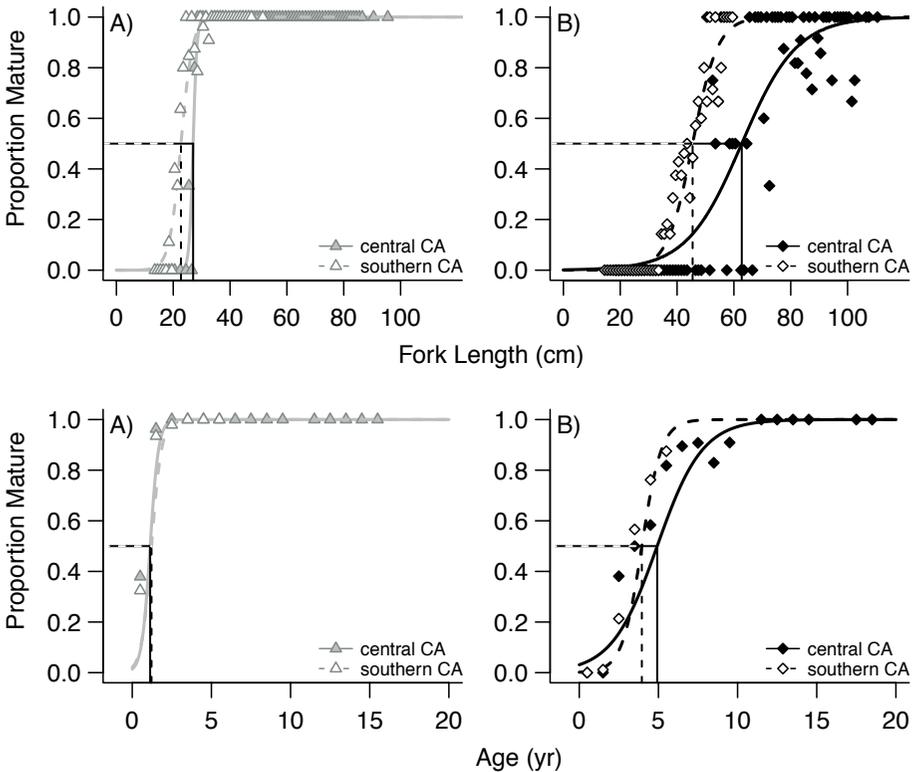


FIGURE 6.—Proportions of mature California halibut collected from central California (this study, shaded shapes and solid curves) and southern California (Love and Brooks 1990, open shapes and dashed curves), by fork length (cm; top) and age (yr; bottom). These curves are based on macroscopic examination only. Males are shown as gray triangles (A) and females are shown as black diamonds (B). Perpendicular lines illustrate region-specific estimates for length and age at 50% maturity.

TABLE 2.—Maximum likelihood estimates (and 95% confidence intervals) for parameters a (slope) and b (intercept) from generalized linear models relating sex- and region-specific proportions of mature California halibut to length (cm) and age (yr). Estimates based upon histological staging criteria developed as part of this study are denoted by asterisks. All other estimates resulted from macroscopic maturity criteria comparable to Love and Brooks (1990). Data for southern California halibut were provided by Love and Brooks (1990). Predicted lengths ( $L_{0.50}$ ) and ages ( $A_{0.50}$ ) at 50% maturity are shown in bold.

	Female		Male	
	central CA	southern CA	central CA	southern CA
<b>Length (cm)</b>				
<i>a</i>	* - 0.9 (- 1.6 to - 0.2)			
	- 0.1 (- 0.1 to - 0.1)	- 0.2 (- 0.3 to 0.1)	- 1.5 (- 2.5 to - 0.5)	- 0.5 (- 0.6 to - 0.4)
<i>b</i>	* 42.1 (8.8 to 74.4)			
	6.5 (5.0 to 8.1)	10.3 (7.9 to 12.7)	39.3 (11.8 to 66.8)	11.1 (8.0 to 14.2)
$L_{0.50}$	* <b>47.3 (45.6 to 49.0)</b>			
	<b>62.8 (59.1 to 66.5)</b>	<b>45.5 (44.8 to 47.2)</b>	<b>27.0 (26.2 to 27.8)</b>	<b>22.7 (21.8 to 23.6)</b>
<b>Age (yr)</b>				
<i>a</i>	* - 4.1 (- 5.6 to - 2.6)			
	- 0.7 (- 0.8 to - 0.6)	- 1.6 (- 2.0 to - 1.2)	- 3.8 (- 5.9 to - 1.7)	- 3.1 (- 3.9 to - 2.3)
<i>b</i>	* 10.9 (6.9 to 14.9)			
	3.4 (2.6 to 6.0)	6.4 (4.9 to 7.9)	4.3 (1.8 to 6.8)	3.8 (2.7 to 4.9)
$A_{0.50}$	* <b>2.6 (2.4 to 2.8)</b>			
	<b>4.9 (4.4 to 5.4)</b>	<b>4.0 (3.75 to 4.25)</b>	<b>1.1 (0.9 to 1.3)</b>	<b>1.2 (1.1 to 1.3)</b>

*Reproductive phase, spawning state, and maturity comparisons.*—Immature females were younger and smaller (both in length and weight  $p < 0.001$ ) than all other reproductive phases (Table 3). There were no significant differences in mean Fulton's K among reproductive phases. Estimates of mean GSI for spawning capable and actively spawning

TABLE 3.—Summary statistics for California halibut, categorized by reproductive phase and spawning state for females and maturity assignment for males. Female maturity assignments (0 = immature, 1 = mature) are also indicated for each reproductive phase. Mean, standard deviation, and sample size (parentheses) are indicated for each physical descriptor (age, length, weight, Fulton's K, GSI, and HSI). Different symbols represent statistically significant groups within a particular physical descriptor and reproductive category.

Female Reproductive Phase	Age (yr)	Length (cm)	Weight (kg)	Fulton's K	GSI	HSI
Immature <sup>0</sup>	1.7±0.6 (66) <sup>*</sup>	32.8±8.0 (66) <sup>*</sup>	0.47±0.30 (66) <sup>*</sup>	1.13±0.12 (66)	0.32±0.18 (65)	1.07±0.42 (60) <sup>‡</sup>
Developing <sup>1</sup>	6.2±2.6 (27)	72.0±16.7 (26)	4.95±3.75 (18)	1.15±0.13 (18)	1.09±0.29 (17) <sup>∞</sup>	1.48±0.39 (18)
Spawning Capable <sup>1</sup>	8.3±2.7 (105)	84.7±12.2 (109)	7.73±3.24 (60)	1.17±0.12 (60)	4.05±1.34 (57) <sup>*</sup>	1.68±0.35 (54) <sup>‡</sup>
Actively Spawning <sup>1</sup>	7.8±1.9 (77)	83.0±10.8 (77)	6.92±2.98 (41)	1.18±0.09 (41)	5.21±1.84 (39) <sup>^</sup>	1.71±0.36 (40) <sup>‡</sup>
Spent <sup>1</sup>	7.4±2.1 (7)	79.2±16.1 (7)	6.51±4.27 (5)	1.18±0.03 (5)	1.91±0.53 (5) <sup>∞</sup>	1.65±0.51 (5) <sup>‡</sup>
Resting <sup>1</sup>	4.9±2.2 (16)	66.0±14.4 (16)	3.23±2.45 (12)	1.14±0.09 (12)	1.23±0.24 (11) <sup>∞</sup>	1.12±0.30 (11) <sup>‡</sup>
<b>Female Spawning State</b>						
Inactive	3.3±2.5 (109)	47.2±21.5 (108)	1.65±2.57 (96)	1.13±0.12 (96)	0.57±0.44 (93)	1.16±0.43 (89)
Active	8.0±2.4 (189) <sup>*</sup>	83.9±11.8 (193) <sup>*</sup>	7.36±3.19 (106) <sup>*</sup>	1.17±0.10 (106)	0.57±0.44 (93) <sup>*</sup>	1.16±0.43 (89) <sup>*</sup>
<b>Male Maturity Assignment</b>						
Immature	1.1±0.2 (19)	23.3±3.0 (19)	0.15±0.05 (19)	1.11±0.09 (19)	0.14±0.11 (19)	0.97±0.35 (9)
Mature	6.7±3.0 (232) <sup>*</sup>	63.8±15.3 (311) <sup>*</sup>	3.08±2.23 (167) <sup>*</sup>	1.09±0.12 (129)	2.06±1.45 (127) <sup>*</sup>	1.02±0.36 (122)

females were different from one another and from all other reproductive phases ( $p < 0.001$ ). Results from the Tukey HSD post hoc test indicated that spawning capable and actively spawning females represented a relatively similar subset of individuals ( $p = 0.06$ ;  $n = 14.7$ ), distinct from all other reproductive phases. Mean GSI for females in the spent phase was different from immature ( $p = 0.03$ ), spawning capable ( $p = 0.001$ ), and actively spawning ( $p < 0.001$ ) phases, but not developing ( $p = 0.71$ ) or resting ( $p = 0.87$ ) phases. Statistical comparisons of HSI yielded various results across reproductive phases for female California halibut. However, mean HSI values for immature and resting females were most similar to one another, whereas mean HSI for spawning capable, actively spawning, and spent females were most closely estimated. HSI for developing females was not distinct from either grouping. When aggregating females into spawning states, active females were older, longer, heavier, and exhibited greater values of GSI and HSI ( $p < 0.01$ ) than those in an inactive state. Fulton's K was also greater for reproductively active females ( $p = 0.08$ ). Mature males were older, longer, heavier, and exhibited greater GSI ( $p < 0.001$ ), but there were no differences in mean Fulton's K ( $p = 0.72$ ) or HSI ( $p = 0.88$ ) from immature males.

## DISCUSSION

*Male reproductive biology.*—Because maturity for male California halibut can be assigned macroscopically, maturity curves may be constructed without the use of histology. In addition, the incidence of milt in the sperm duct can be used to determine the spawning state of male California halibut examined macroscopically in the field. Assigning a spawning state can lead to a better understanding about the proportion of males that spawn in a particular area over a given time period and should be documented.

*Female reproductive biology.*—Our histological analyses, which illustrated a co-occurrence of secondary growth oocytes (i.e., evidence that the ovary is preparing for another spawning event) and postovulatory follicles (i.e., evidence for recent spawning activity), support the idea that California halibut are batch spawners (Caddell et al. 1990). California halibut also demonstrate asynchronous ovarian development, as evidenced by the fact that the most advanced oocyte stage co-occurred with all preceding stages of oocyte development regardless of reproductive phase (Murua et al. 2003).

*Assigning maturity based on histological examination for females.*—We found that histological analyses were more accurate in determining maturity for California halibut than depending upon macroscopic characteristics alone. This is because histology reveals characteristics that are not identifiable to the naked eye (i.e., specific oocyte developmental stages, postovulatory follicles, and atresia). However, it remained difficult to histologically discern between immature and resting individuals (Hunter and Macewicz 2003), complicating the assignment of maturity for individuals in these phases.

Because our sampling design provided only a snapshot of reproductive activity, we cannot be certain that fish grouped as mature but collected in a phase other than actively spawning would have spawned within the cycle of capture. We also were unable to ascertain the amount of time it takes an individual found in the developing phase to become capable of spawning. The transition between developing and spawning capable reproductive phases can have substantial effects on estimates of length- or age-at-maturity, given that it may take up to a year to complete (Junquera et al. 2003). However, the time it takes for cortical alveoli oocytes (i.e., developing phase) to develop into yolk granule form (i.e.,

spawning capable phase) remains unknown for California halibut. Additionally, adverse environmental and/or ecological factors may cause a mature individual to forego spawning, undergo atresia, and redirect finite energy reserves toward maintenance (Rideout et al. 2005). There may also be an increased probability of failed reproduction during the first year of maturity (e.g., Jørgensen et al. 2006), which is not known for California halibut.

*Assigning spawning state and maturity without histology for females.*—Although histological processing is necessary to categorize female California halibut into specific reproductive phases, it is both expensive and time consuming. Therefore, macroscopic examination of the ovaries is highly preferred, especially during field-based assessments. There were some macroscopic similarities in ovary color and the prevalence of blood vessels; however, these characteristics alone were not enough to accurately assign an ovary to a specific reproductive phase. We found that the incidence of macroscopic oocytes [i.e., YG, FM, and HD stages; (Hunter et al. 1992)] was the most straightforward and accurate characteristic to use for field-based assessments of spawning state and maturity. Oocytes in earlier stages of development (i.e., CN, PN, and CA stages) are not individually visible to the naked eye and instead appear as a single mass.

Using this criterion, females can be placed into one of two spawning states: inactive or active. On a finer scale within the active category, a ‘fully hydrated’ state can be assigned if hydrated oocytes are accumulated in the oviduct and are released when pressure is applied to the organ cavity. This ephemeral state (Hunter and Macewicz 1985) suggests that the female was spawning at the location and time of capture. Although the resolution of these macroscopic assignments would not be as fine as those provided by histological analyses, it can lead to a better understanding about the proportion of females that spawn in a particular area over a given time period and should be documented. We assert, however, that macroscopic assignments should only be used to identify spawning state for females and not to assess maturity. Using macroscopic criteria alone could result in underestimations of proportional maturity, as demonstrated in this study when comparing assignments based on macroscopic and histological characteristics for central California females. If female maturity were to be assessed histologically in southern California, it would be expected that the curves would shift further to the left displaying that females mature at younger ages and smaller sizes than previously reported, provided that there were no temporal changes in maturation between studies. This is because resting females may be mistakenly classified as immature. Developing females, which are assumed to spawn soon after capture, may also be mistakenly categorized as immature individuals. Although assessing maturity macroscopically increases the chance for misclassification, we found that GSI can help guide categorizations of females. Thus, we highly recommend the collection of body and ovary weights, as long as they can be accurately measured.

*Management considerations.*—Although the first comprehensive stock assessment for California halibut separated the species into two distinct stocks, estimates of maturity were only available from fish collected south of Point Conception (Maunder et al. 2011). Our study is the first to estimate length- and age-at-maturity for the central California halibut stock, thereby providing region-specific data to inform upcoming assessments. We found that the lengths at 50% maturity for central California halibut (27.0 cm [1.1 yr] for males and 47.3 cm [2.6 yr] for histologically-assigned females), were well under the minimum legal size limit of 55.9 cm. This suggests that the minimum legal size limit likely meets the management objective of protecting immature individuals from removal by the fishery north of Point Conception, given current conditions.

When combining results from our maturity ogives with growth rate information from Barnes et al. (2015), we conclude that half of central California halibut males have the opportunity to spawn for 3.5 years before reaching the minimum legal size limit, whereas half of central California halibut females are capable of reproduction 1.1 yr before becoming susceptible to fishery take. For California halibut, reproductive success is related to optimal temperature conditions (i.e., warmer water is associated with better egg and larval survival within tolerance limits [Gadomski and Caddell 1991]) and may be affected by other ecological variables (e.g., prey availability). Varying environmental and ecological conditions can affect the number of successful spawning years available to California halibut prior to becoming susceptible to take by the fishery and should be considered when assessing productivity during different regimes.

Substantial effort and many different gear types (i.e., hook-and-line, spear, beach seine, trawl) were employed in an attempt to collect immature fish along the central California coast. However, the vast majority of immature California halibut were obtained within San Francisco Bay, an area that comprises the greatest proportion of estuarine habitat north of Point Conception. Additionally, California halibut utilize estuaries as nursery habitats (Haaker 1975, Allen and Herbinson 1990, Kramer 1990), and are known to benefit from occupying these environments during early life via increased growth and decreased mortality (Valle et al. 1999). Although the extent of California halibut migration out of this estuary to other portions of the central California coast is unknown, we think it is possible that some of the mature individuals collected along the central California coast were once juveniles inside San Francisco Bay. We do not believe that the sample locations of immature fish substantially affect our length- and age-at-maturity estimates at the regional level, although estimates may differ on a finer spatial scale.

*Regional comparisons of length- and age-at-maturity.*—There is an energetic trade-off between growth and reproduction in marine fishes (Jones and Johnston 1977, Rijnsdorp 1990). Differences in growth between central and southern California halibut have been demonstrated, with central California halibut growing at faster rates than southern California conspecifics (MacNair et al. 2001, Barnes et al. 2015), which could be due to relatively early energy allocations toward reproduction over somatic growth and/or maintenance. With consideration that a substantial amount of time had passed between our study and that conducted by Love and Brooks (1990) off of southern California, we found differences in timing of maturation between central and southern California halibut. Our results showed a greater length and age at 50% maturity for central California females. Males from central California also exhibited a slightly greater length at 50% maturity, but there was no regional difference in age. However, similar ages at 50% maturity for central and southern California males could have resulted from a combination of relatively early maturation (between 1 and 3 yr) and rounding age estimates to the nearest year.

There are several potential explanations for the observed differences in length- and age-at-maturity by region. Biogeographic variation in environmental conditions (e.g., temperature, irradiance) and/or ecological interactions (e.g., prey availability, predation rates) have been attributed to intraspecific differences in the life history traits of many flatfish species (e.g., Witthames et al. 1995, Abookire and Macewicz 2003, Spencer 2008, Nissling and Dahlman 2010). Regional variation in fishing pressure can also lead to differences in timing of maturation (e.g., size-selective fishing can cause a shift to maturation at younger ages and/or smaller sizes) and has been documented in exploited fish populations (Rijnsdorp 1989, Bowering and Brodie 1991, Trippel 1995, Grift et al. 2003). Faster rates

of maturation may be problematic to fish populations in the long run because of negative impacts to reproductive potential (e.g., fecundity is known to increase with body size [Bagenal 1966, Wootton 1979]). In comparison with the earliest preliminary study on southern California halibut maturation (Higgins 1919), the data collected by Love and Brooks (1990) suggest that a population-level shift towards earlier maturation may have occurred.

*Future studies.*—A comparable study using the reproductive phase, spawning state, and maturity assignment criteria described herein should be conducted to re-assess sex-specific length- and age-at-maturity for southern California. Histological analyses would provide more precise estimates of female maturity for construction and comparison of maturity ogives. Relying on macroscopic characteristics alone is an inaccurate way to assess maturity because it only accounts for individuals in a reproductively active state. Because environmental conditions (e.g., PDO, [Chavez et al. 2003]) and relative fishing pressure (Maunder et al. 2011) have changed over the past three decades, it is also necessary to formulate contemporary estimates of length- and age-at-maturity (Rijnsdorp 1989). A study conducted in southern California that is similar to ours would enhance regional comparisons of length- and age-at-maturity, increase accuracy in evaluating the effectiveness of the minimum legal size limit south of Point Conception, and provide an opportunity to compare temporal variation in maturation of southern California halibut.

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## APPENDIX I: OOCYTE MEASUREMENTS

Oocyte circumference ( $\mu\text{m}$ ) and area ( $\mu\text{m}^2$ ) were measured from two types of ovarian tissue: preserved (i.e., whole mounts fixed in formalin and stored in ethanol; Table A) and histologically processed (Table B). Because California halibut oocytes are not perfectly spherical, estimates of diameter ( $\mu\text{m}$ ) were calculated by dividing circumference ( $\mu\text{m}$ ) by pi ( $\pi$ ).

TABLE A.—Whole mount measurements (8x magnification, ImagePro Plus v7) obtained from actively spawning California halibut females (83.6±29.0 cm TL; n=5). Mean, standard deviation, and number of individual oocytes measured (parentheses) are listed by aggregated oocyte developmental stage. Because developmental stages are difficult to differentiate from whole mounts, oocytes in PN and CA stages were aggregated, as were those in YG and FM stages.

Developmental Stage	Diameter ( $\mu\text{m}$ )	Circumference ( $\mu\text{m}$ )	Area ( $\mu\text{m}^2$ )
perinucleolar (PN) or cortical alveoli (CA)	271±43 (51)	851±136 (51)	52,390±16,101 (51)
yolk granule (YG) or final maturation (FM)	491±73 (59)	1,543±229 (59)	169,971±46,923 (59)
hydrated (HD)	902±71 (68)	2,834±222 (68)	564,366±73,911 (68)

TABLE B.—Histological measurements (50x magnification, ImagePro Plus v7) obtained from spawning capable and actively spawning California halibut females (82.7±12.9 cm TL; n=12). Mean, standard deviation, and number of individual oocytes measured (parentheses) are listed by oocyte developmental stage. Damage caused by histological processing prevented the measurement of HD stage oocytes from histological samples.

Developmental Stage	Diameter ( $\mu\text{m}$ )	Circumference ( $\mu\text{m}$ )	Area ( $\mu\text{m}^2$ )
perinucleolar (PN)	89±20 (271)	279±61 (271)	5,056±2,217 (271)
cortical alveoli (CA)	200±57 (167)	628±180 (167)	26,245±16,025 (167)
yolk granule (YG)	365±62 (85)	1,147±196 (85)	80,661±29,418 (85)
final maturation (FM)	489±80 (42)	1,537±252 (42)	149,642±39,018 (42)