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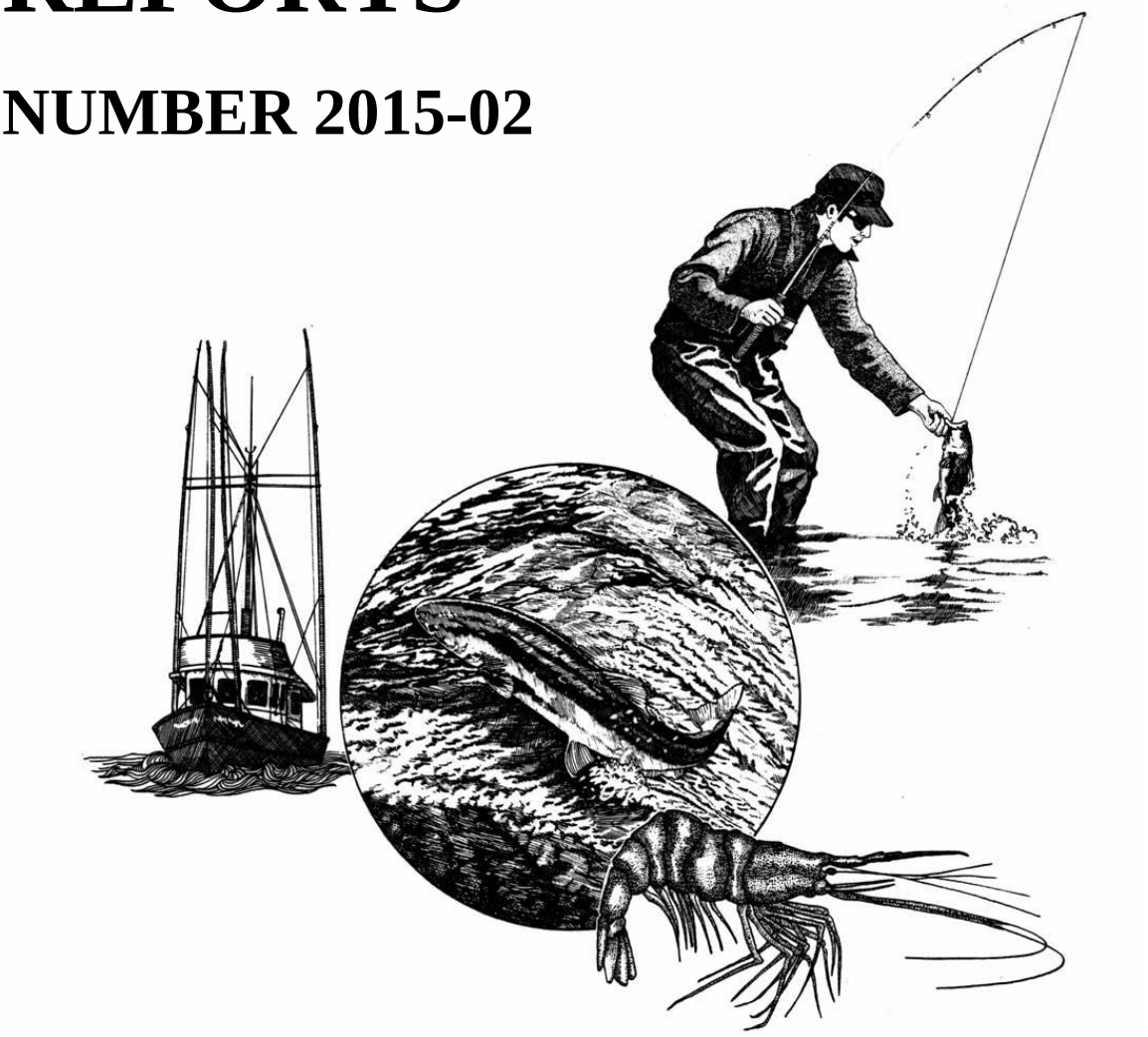

FISH DIVISION
Oregon Department of Fish and Wildlife

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Photo above by: Oregon Coast Aquarium

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Introduction

This is the ninth publication in a series documenting results from a long-term sampling program aimed at developing improved life history data for groundfish species commonly captured in Oregon fisheries. When this study was begun in 2000, the available life history data for many U.S. west coast groundfish species were of questionable quality. Length-weight relationships, age-at-length, and reproductive seasonality were either poorly known or derived from very limited sampling. In addition, the maturity curves then used in stock assessment models were typically based on macroscopic (visual) assessment of maturity status. However, studies have shown that histological evaluation of ovarian thin-sections, especially if combined with optimal seasonal sampling, is much more accurate (Gunderson et al. 1980, Wyllie Echeverria 1987, West 1990, Nichol and Pikitch 1994, Hannah and Parker 2007). In previous publications in this series, improved female maturity data have been developed for Petrale Sole (*Eopsetta jordani*, Hannah et al. 2002), Pacific Ocean Perch (*Sebastes alutus*, Hannah and Parker 2007), Yelloweye Rockfish (*S. rubberimus)* and Cabezon (*Scorpaenichthys marmoratus*, Hannah et al. 2009), Aurora Rockfish (*S. aurora*, Thompson and Hannah 2010), Quillback (*S. maliger*) and China Rockfish (*S. nebulosus*, Hannah and Blume 2011), Vermilion Rockfish (*S. miniatus*, Hannah and Kautzi 2012), Copper Rockfish (*S. caurinus*, Hannah 2014), and Blue-sided (*S. diaconus*) and Blue Rockfish (*S. mystinus*, Hannah et al. 2015). This report describes the development of length-at-weight, age-at-length, spawning seasonality, and histologically-based female length-at-maturity life history parameters for Kelp Greenling (*Hexagrammos decagrammus*).

Methods

Kelp Greenling used for this study were sampled from Oregon's marine waters from Astoria to Brookings. This species is encountered in both commercial and recreational fishery catches. Most fish used in this study were sampled from these fisheries. The 10-inch minimum size limit in the recreational fishery and the 12-inch minimum size limit in the commercial fishery precluded the collection of samples for juvenile and adolescent fish through fishery-dependent sampling. Therefore, various special projects were conducted by the Oregon Department of Fish and Wildlife to sample fish as needed to fill in the size range. These special samples were collected primarily from areas near Gold Beach, Port Orford and Newport.

Data for the length-weight relationship were derived from commercial fishery and special project samples collected from 2000 to 2014. To determine a length-weight relationship for Kelp Greenling, these data were fit in one model using linear regression in the statistical program R (Ver. 3.1.2.) on $\log_{10}$-transformed data in the form:

$y_i = b_0 + b_1 x_i$ where,

y_i is the predicted $\log_{10}$(weight) based on each x_i ($\log_{10}$(length)), and b_0 and b_1 are parameters that define the intercept and slope of the regression line. Parameters were

back-transformed to graphically depict the predicted geometric mean weight based on length. Nonlinear least squares regression was explored in this analysis, but that model over-predicted weight for smaller fish. Therefore, it was determined a log-linear model best fit the data.

To determine age-at-length, samples were utilized from the recreational and commercial fisheries, and from special projects, spanning the years 2003 through 2014. Otoliths were hydrated overnight in a 50% ethanol solution to improve clarity of patterns, and then broken and burned as described in Chilton and Beamish (1982). The standard three parameter von Bertalanffy growth equation was used to fit age-at-length using nonlinear least squares regression in R.

To determine female maturity and spawning seasonality, samples collected from the commercial fishery and from special projects from 2003 through 2014 were used. Fish were measured and ovaries were examined and assigned a macroscopic maturity stage (Table 1). Whenever possible, both female gonads were weighed and a small section of ovary was collected and prepared for histological microscopic evaluation. These samples for microscopy were preserved in 10% buffered formalin and later transferred to 70% ethanol for storage. A subset of ovary weights from size classes containing 100% mature fish, sampled from 2003 through 2010, were summarized by month to identify the season of peak ovary weight as a proxy for the Kelp Greenling spawning season. A gonadosomatic index was not used to determine spawning seasonality because fish weights were not available for many samples.

Table 1. Visual (macroscopic) maturity stages and descriptions for Kelp Greenling ovaries.

Maturity	Stage	Condition	Description
Immature	1	Immature	Small, translucent
	2	Developing	Small, yellow-orange, translucent or opaque
Mature	3	Maturing	Granular, yellow-orange, opaque
	4	Mature	Filled w/ large translucent eggs (1-2 mm) & smaller yellow-orange eggs
	5	Spawning	Large, translucent eggs (2-3 mm) fill ventral portion
	6	Spent	Large, flaccid, thick, w/ opaque white membrane. A few eggs still present
	7	Resting	Small, red to grayish-red, no granular tissue present, rare

The maturity status of individual specimens was determined primarily based upon microscopic examination of stained ovary sections. One difficulty with determining maturity status based solely on the macroscopic evaluation of ovaries is, for many species, “maturing” and "resting" ovaries cannot be reliably separated (Wallace and Selman 1981, McDermott and Lowe 1997). Externally, these stages can appear quite similar but represent different states of maturity. In some fish species, young females undergo abortive maturation, characterized by atresia of a developing class of oocytes,

further complicating the macroscopic assessment of maturity (Hannah and Parker 2007, McDermott et al. 2007, Hannah and Blume 2011).

For microscopic evaluation, ovarian tissue samples were embedded in paraffin, sectioned at 5 μm and stained with Harris's hematoxylin and eosin Y (West 1990), then examined using a binocular microscope at 100x magnification. The stage of the most advanced oocyte was recorded, following McDermott and Lowe (1997). McDermott and Lowe's (1997) Atka Mackerel (*Pleurogrammus monopterygius*) oocyte stages were used as the basis of our oocyte staging due to both the close phylogenetic relationship between Kelp Greenling and Atka Mackerel, two hexagrammids, and because Kelp Greenling display oocyte maturation patterns similar to Atka Mackerel. Maturity status was assigned as mature, immature or unknown. Ovaries with oocytes showing eosin-positive dark-staining vitellogenic yolk gobules (stage 5) that had developed beyond the oil droplet stage (stage 4) were classified as mature (Figure 1), unless ovaries showed clear indications of extensive atresia (Hunter and Macewiz 2001), typified by a complete lack of cell nuclei (Figure 2). Fish with ovaries displaying obvious signs of post-release reorganization such as post-ovulatory follicles (POFs; Figure 3) and/or residual atretic egg remnants (Figure 4) were also classified as mature. Fish with non-vitellogenic oocytes that appeared well-organized were classified as immature (stages 1 through 4; Figure 5). Fish with ovaries with oil droplet oocytes showing some signs of atresia and reorganization, but without egg remnants, POFs or other definitive indicators of maturity were classified as unknown, because it was not possible to determine if the reorganization was a result of abortive maturation in an immature female or the late stages of reorganization following spawning. Females classified as unknown were not used for analysis of length-at-maturity. Fish with ovaries showing abortive maturation were classified as immature, unless these fish were notably larger or older than the length interval in which both immature and mature fish were being encountered (adolescent phase; Hannah and Parker 2007, Thompson and Hannah 2010). For a photographic guide of oocyte maturity stages see Appendix A.

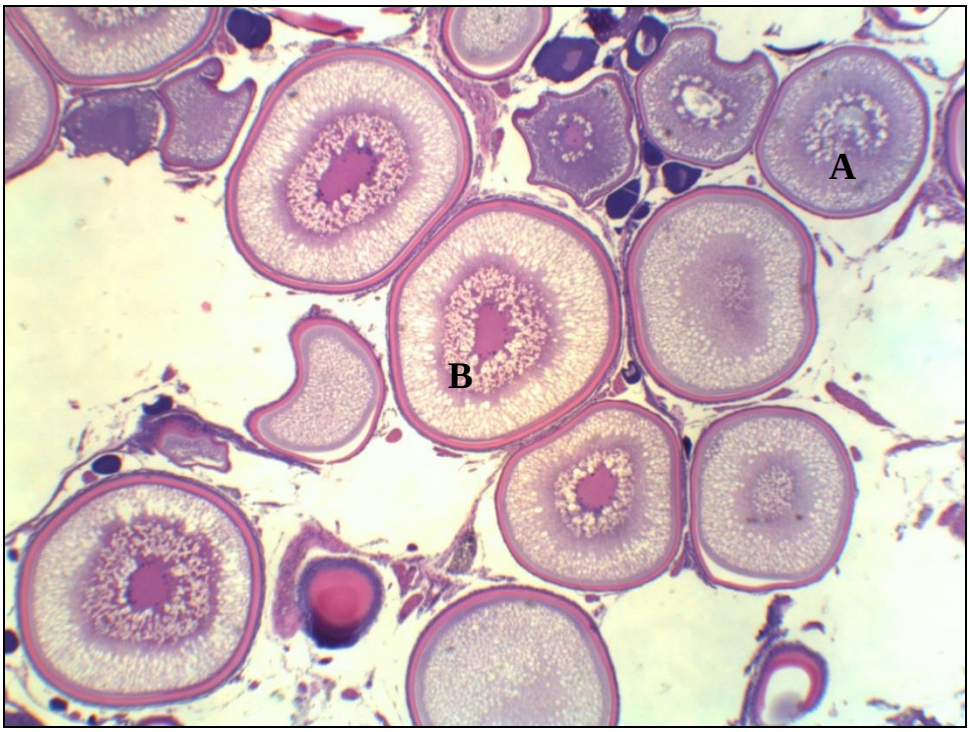

Figure 1. Stained ovary thin-section of a Kelp Greenling showing development of immature oil droplet oocytes (stage 4; A) and mature early magenta-staining vitellogenic yolk globule oocytes (stage 5; B). Largest oocytes are ~730 µm; 40x magnification.

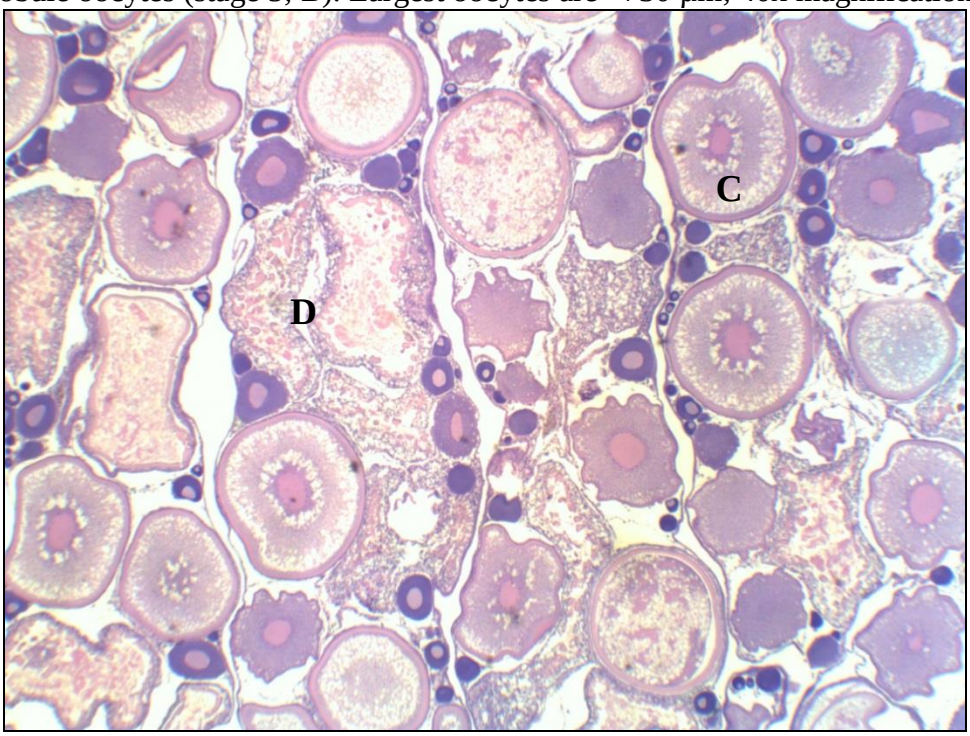

Figure 2. Stained ovary thin-section from a Kelp Greenling showing oil droplet oocytes (stage 4; C), and extensive atresia and abortive maturation of developing oocytes (D). Largest oocytes are ~ 400 µm and magnification is 40x.

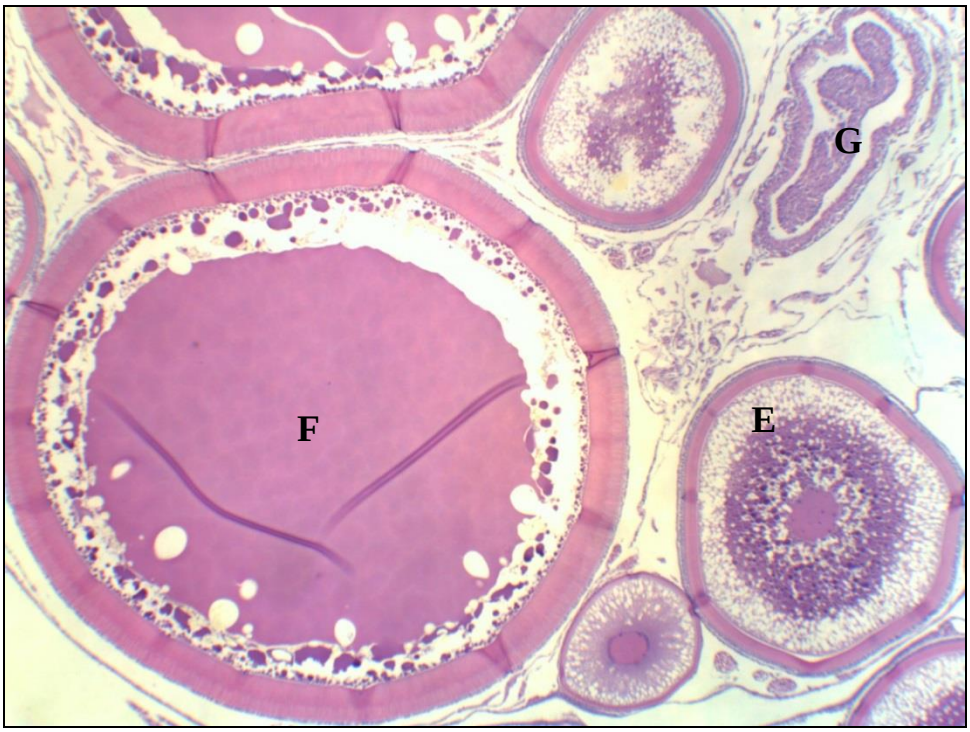

Figure 3. Stained ovary thin-section from a mature Kelp Greenling showing multiple oocyte stages. The late vitellogenic yolk globule oocyte (stage 5; E) is ~750 µm, the early hydration oocyte (stage 7; F) is ~1,500 µm, and the post ovulatory follicle (G) is ~650 µm. Magnification is 40x.

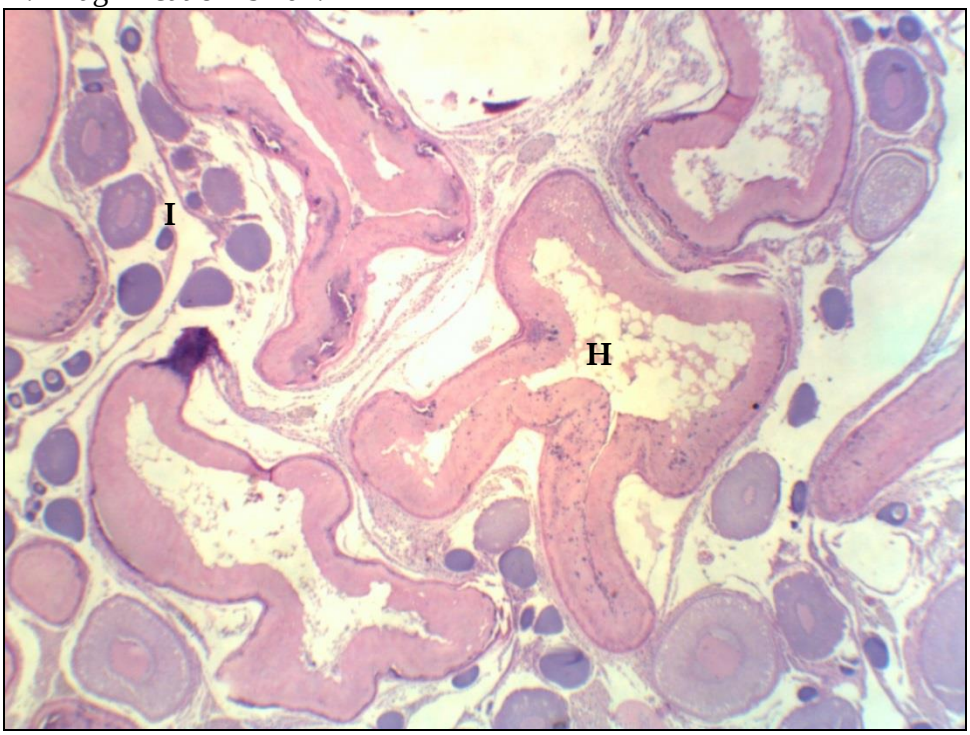

Figure 4. Stained ovary thin-section from a spent Kelp Greenling showing residual atretic egg remnants (H) among reorganizing perinuclear to cortical alveoli oocytes (stages 1 – 3; I). Egg remnants are ~1,200 µm and magnification is 40x.

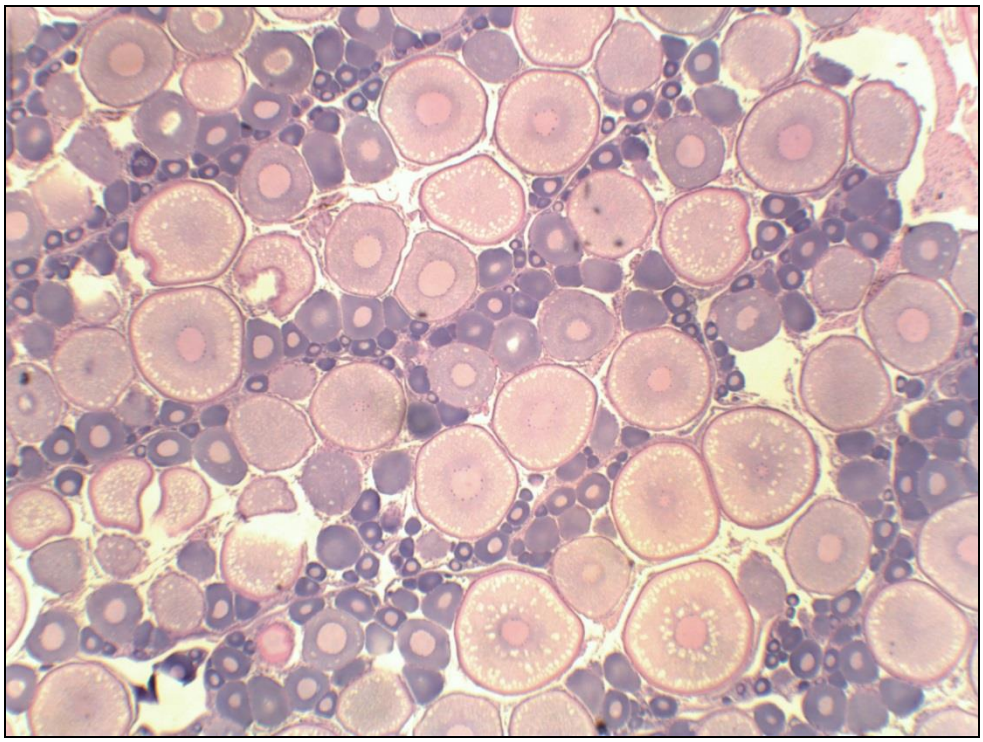

Figure 5. Stained ovary thin-section from a Kelp Greenling showing immature perinuclear through oil droplet oocytes (stages 1 – 4). The largest oocytes pictured are ~330 µm and magnification is 40x.

We evaluated Kelp Greenling maturity in this study only as a function of length because enough reliably aged fish with associated histology samples were not available to produce an age-at-maturity curve. Logistic regression, in R, was used to fit sigmoid curves to the proportion mature by length in the form,

$$p_{x_i} = e^{(b_0+b_1x_i)}/(1+e^{(b_0+b_1x_i)}) \text{ where,}$$

p is the probability that a fish is mature in a given length (cm) interval x_i, and b_0 and b_1 are parameters that define the shape and location of the fitted sigmoid curve. The predicted length at 50% maturity (L_{50}) was calculated as,

$$L_{50} = -b_0/b_1.$$

Results

Kelp Greenling collections resulted in 17,000 weight-at-length samples collected from 2000 through 2014 (Table 2). Length was a significant predictor of weight ($p < 0.001$) explaining 88% of the variation in weight for both sexes combined. Additional variation in weight-at-length for larger Kelp Greenling is likely also influenced by gravidity, seasonality, stomach fullness, spatial location, sample density, and measurement error. The back-transformed geometric mean fitted curves for females, males, and for both

sexes combined are plotted in Figure 6, however, curves for males and females are graphically indistinguishable from the curve for both sexes.

Table 2. Log_{10}-linear length-at-weight model parameters (± standard error) for Kelp Greenling. Length range observed, by sex, is also shown.

Parameter	Both Sexes	Females	Males
b_0	-2.09 (0.01)	-2.17 (0.02)	-2.01 (0.02)
b_1	3.17 (0.01)	3.21 (0.01)	3.12 (0.01)
r^2	0.88	0.91	0.86
p	< 0.001	< 0.001	< 0.001
N	17,000	7,270	9,730
Length range (cm)	7 - 49	7 - 49	7 - 49

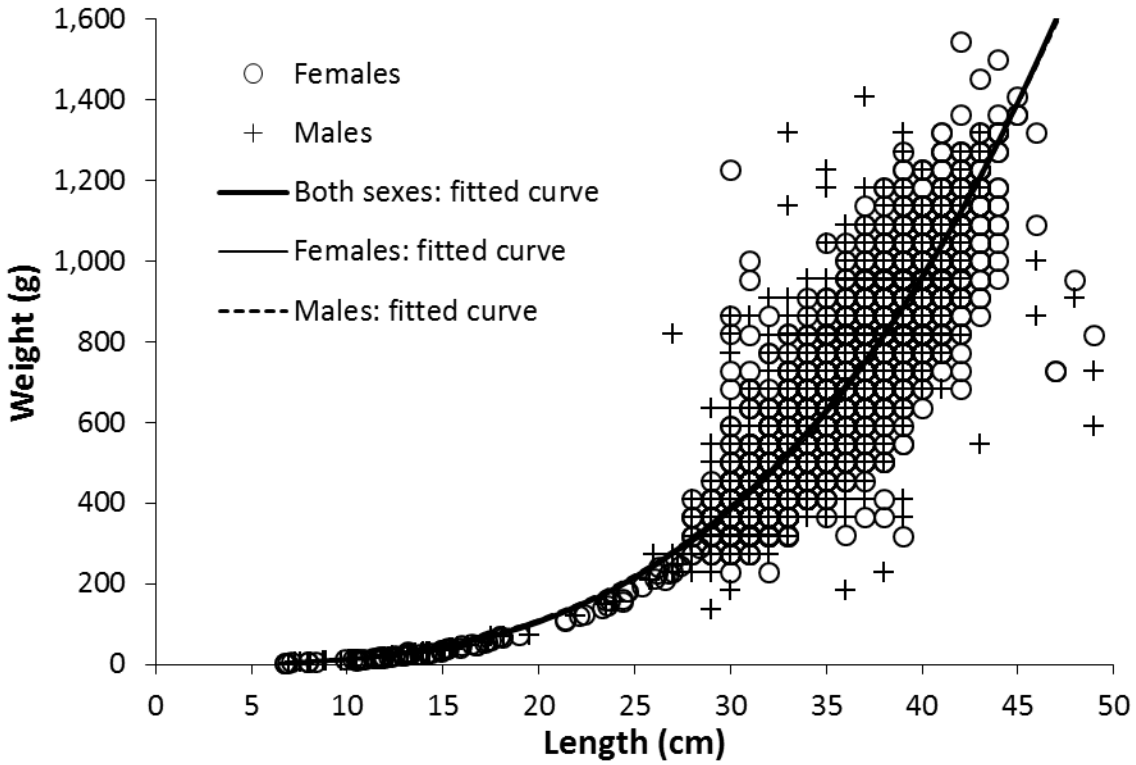

Figure 6. Fork length (cm) versus weight (g) and back-transformed geometric mean curves for Kelp Greenling fork length versus weight for females, males, and both sexes combined.

Ages were generated for 953 female and 1,026 male Kelp Greenling collected from 2003 through 2014 (Table 3). The data fit von Bertalanffy curves well for both sexes combined (R^2 = 0.51; Figure 7) as well as for each sex separately (females: R^2 = 0.54; males: R^2 = 0.56), and showed that females grow larger than males. Asymptotic length estimates (± standard error) were 38.51 (±0.14) and 37.33 (±0.11) for females and males, respectively.

Table 3. Parameter estimates (± standard error) for standard von Bertalanffy growth formulae fitting fork length (cm) against age for Kelp Greenling females, males, and both sexes combined. L_∞ = asymptotic length; k = growth coefficient; t_0 = hypothetical age at length zero; R^2 = coefficient of determination; and N = sample size. Age range observed, by sex, is also shown.

Parameter	Both Sexes	Females	Males
L_∞	37.84 (0.09)	38.51 (0.14)	37.33 (0.11)
k	0.534 (0.009)	0.527 (0.013)	0.527 (0.012)
t_0	-0.69 (0.03)	-0.68 (0.03)	-0.72 (0.04)
R^2	0.51	0.54	0.56
N	1,979	953	1,026
Age range	0 - 17	0 - 15	0 - 17

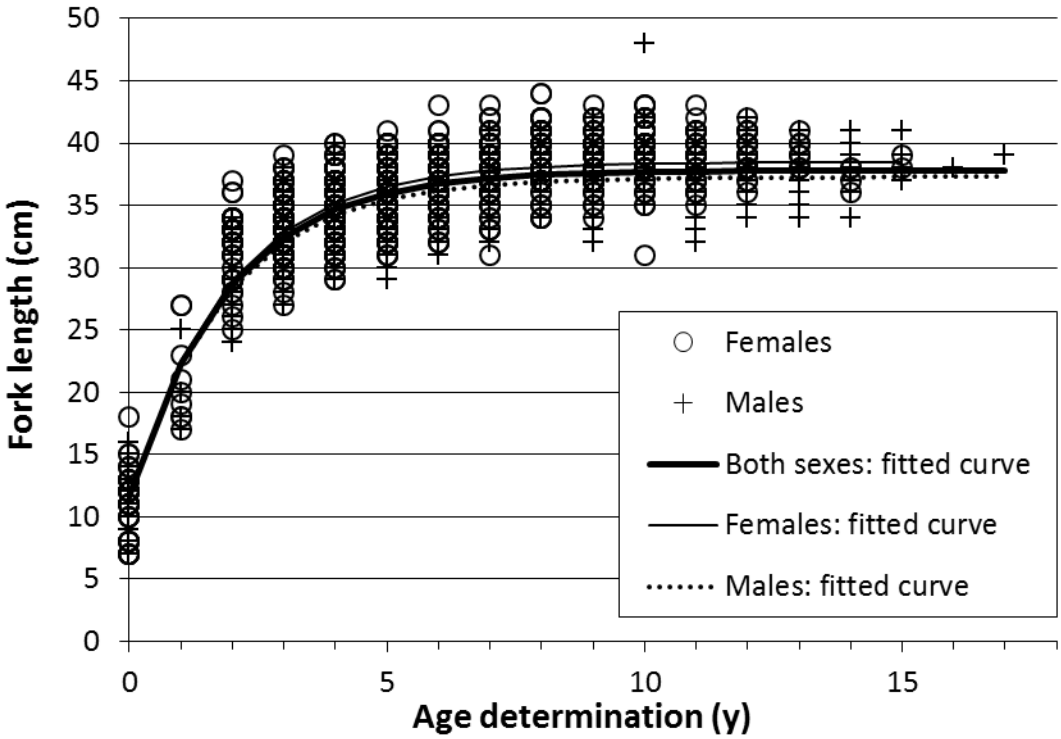

Figure 7. Age (y) versus fork length (cm) for female and male Kelp Greenling, and fitted von Bertalanffy growth curves for females, males, and both sexes combined.

Ovary evaluation resulted in macroscopic maturity stage data for 1,300 Kelp Greenling collected between 2003 and 2014 (Table 4). Histology samples collected and processed for microscopic evaluation of female maturity totaled 675 (Table 4) and ovary weights were collected for 406 specimens.

Based on the macroscopic staging of ovaries, spawning in Kelp Greenling was not highly synchronous, with mature and spawning ovaries (stages 4 and 5) observed at low frequencies from May through January, peaking in frequency in September and October (Figure 8).

Table 4. Numbers of female Kelp Greenling maturity (M) and histology (H) samples collected and processed, by month and macroscopic maturity stage (Table 1), 2003 - 2014.

Maturity stage	Immature		Developing		Maturing		Mature		Spawning		Spent		Resting		Total	
Month	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H
January	0	0	6	6	17	13	1	0	0	0	1	1	1	1	26	21
February	0	0	17	8	21	9	0	0	2	2	16	7	39	21	95	47
March	2	2	0	0	8	8	0	0	0	0	20	18	23	21	53	49
April	2	2	5	2	12	10	0	0	0	0	29	25	36	22	84	61
May	9	9	6	3	26	16	6	2	2	1	32	22	35	23	116	76
June[1]	9	9	8	2	36	16	10	1	7	0	23	4	57	23	150	55
July	7	7	2	1	77	33	8	2	12	0	35	4	56	15	197	62
August[1]	4	4	6	4	139	66	5	1	0	0	0	0	15	4	169	79
September[1]	11	10	3	3	110	65	39	10	23	5	44	4	6	1	236	98
October	18	17	2	2	29	24	25	14	14	10	0	0	5	4	93	71
November	3	3	2	1	26	16	7	4	2	1	0	0	6	3	46	28
December	6	6	2	2	18	15	4	2	1	1	4	2	0	0	35	28
Total	71	69	59	34	519	291	105	36	63	20	204	87	279	138	1,300	675

[1] Note that histology samples were collected from 9 female kelp greenling (1 in June; 1 in August, 7 in September) without valid macroscopic stage assignments

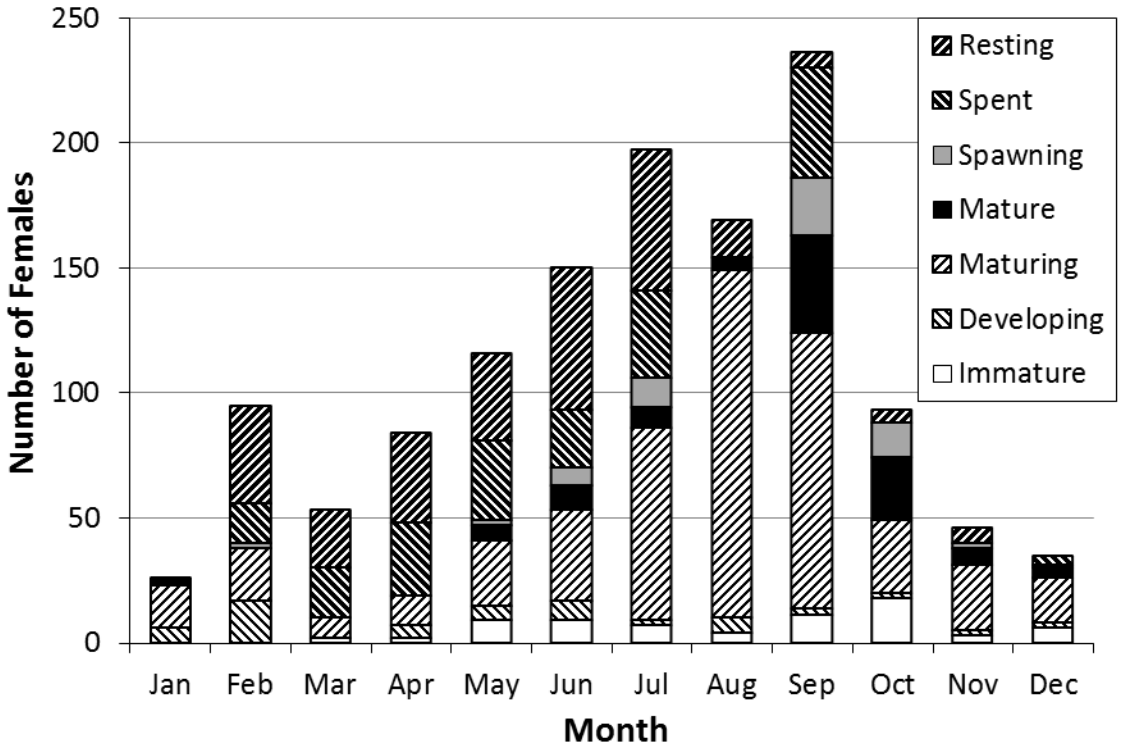

Figure 8. Number of female Kelp Greenling sampled for maturity, by macroscopic (visual) maturity stage and month, 2003 through 2014.

Comparison of macroscopic and microscopic evaluations of female maturity showed that macroscopic staging of Kelp Greenling ovaries was not as accurate as microscopy staging (Table 5). Microscopic evaluations of 675 ovaries resulted in the reclassification of maturity status for 96 specimens (14.2%) and "unknown" status for 52 specimens (7.7%, Table 5). Of the 12 immature fish that were reclassified as mature based on microscopy, all showed clear evidence of prior spawning based on the presence of POFs or residual atretic egg remnants in the ovary. This indicated these fish were actually "resting" instead of "developing", as recorded. Of the 84 fish that were reclassified from mature to immature, all lacked POFs, residual eggs and/or development beyond oil droplet (stage 4) oocytes, and all were of an "adolescent" length (17 to 32 cm). Forty-five of the fish reclassified to immature showed evidence of atresia, six had extensive atresia likely associated with abortive maturation. The remaining fish showed well-organized oocytes with no evidence of vitellogenesis, POFs, or residual eggs. The six females of adolescent length with abortive maturation were treated as immature fish for the fitting of logistic regressions of length at maturity (Hannah and Parker 2007). No examples of skipped spawning were observed in our samples.

Table 5. Comparison of macroscopic and microscopic determinations of maturity in female Kelp Greenling collected from Oregon waters from 2003 through 2014.

	Macroscopic classification		Microscopic classification			
Month	Condition	Number	Confirmed	Reclassified	Unknown	Not used
January	Immature	6	3	2	1	0
	Mature	15	5	9	1	0
February	Immature	8	7	1	0	0
	Mature	39	35	3	1	0
March	Immature	2	1	0	0	1
	Mature	47	34	11	2	0
April	Immature	4	3	1	0	0
	Mature	57	49	5	3	0
May	Immature	12	9	3	0	0
	Mature	64	55	6	2	1
June	Immature	11	9	2	0	0
	Mature	45	40	2	3	0
July	Immature	8	7	0	0	1
	Mature	54	41	2	10	1
August	Immature	8	7	0	1	0
	Mature	71	52	5	14	0
September	Immature	13	11	1	1	0
	Mature	85	62	10	9	4
October	Immature	19	18	0	1	0
	Mature	52	39	11	2	0
November	Immature	4	3	1	0	0
	Mature	24	13	10	1	0
December	Immature	8	7	1	0	0
	Mature	20	9	10	1	0
Total		675	519	96	52	8

The large number of ovaries classified as "unknown" microscopically (7.7%) showed that histology was only moderately effective in resolving maturity status for female Kelp Greenling (Table 5). Ovaries microscopically classified as "unknown" were most often from fish sampled in late summer months, and were classified macroscopically as "maturing" (stage 3). All but 14 of these fish were of adolescent length. Microscopically, these fish displayed developing oil droplet (stage 4) oocytes, but lacked definitive markers of prior spawning (i.e. residual atretic egg remnants or POFs). These fish also lacked signs of extensive atresia, indicative of abortive maturation. Eight slides were not used in evaluation of length-at-maturity because of poor slide quality.

The final data set evaluated female length-at-maturity for 615 specimens ranging in length from 7 to 45 cm, from the months of January through December, for which maturity determinations were considered definitive (Table 6). These data showed that

females mature as small as 26 cm, and are 100% mature at 33 cm (Table 6). A logistic regression of maturity against length fit the data well ($p < 0.0001$, $R^2 = 0.93$) and indicated a length at 50% maturity (L_{50}) of 29.34 cm (Table 7, Figure 9). Ovaries from Kelp Greenling in size classes of 100% mature fish were much heavier in September through December suggesting fall as the primary Kelp Greenling spawning season in Oregon waters (Figure 10).

Table 6. Number of female Kelp Greenling used in determining length-at-maturity and proportion mature, by length (cm).

Length (cm)	Number sampled	Proportion mature
7	5	0.00
8	4	0.00
9	0	--
10	4	0.00
11	6	0.00
12	8	0.00
13	7	0.00
14	3	0.00
15	8	0.00
16	3	0.00
17	6	0.00
18	6	0.00
19	2	0.00
20	1	0.00
21	3	0.00
22	6	0.00
23	15	0.00
24	18	0.00
25	16	0.00
26	13	0.15
27	13	0.08
28	14	0.21
29	14	0.29
30	10	0.70
31	14	0.86
32	14	0.86
33	21	1.00
34	26	1.00
35	41	1.00
36	53	1.00
37	69	1.00
38	78	1.00
39	44	1.00
40	40	1.00
41	17	1.00
42	10	1.00
43	1	1.00
44	0	--
45	2	1.00
Total	615	

Table 7. Results of logistic regression analysis of maturity status of female Kelp Greenling versus length (cm).

Independent variable	Coefficients	Standard error	*p*-value	L_{50}	95% confidence limits	Coefficient of determination (R^2)
Length				29.34 cm	±0.54	
Constant	-29.3183	3.8515	< 0.001			
Length	0.9991	0.1303	< 0.001			
Full model			< 0.001			0.93

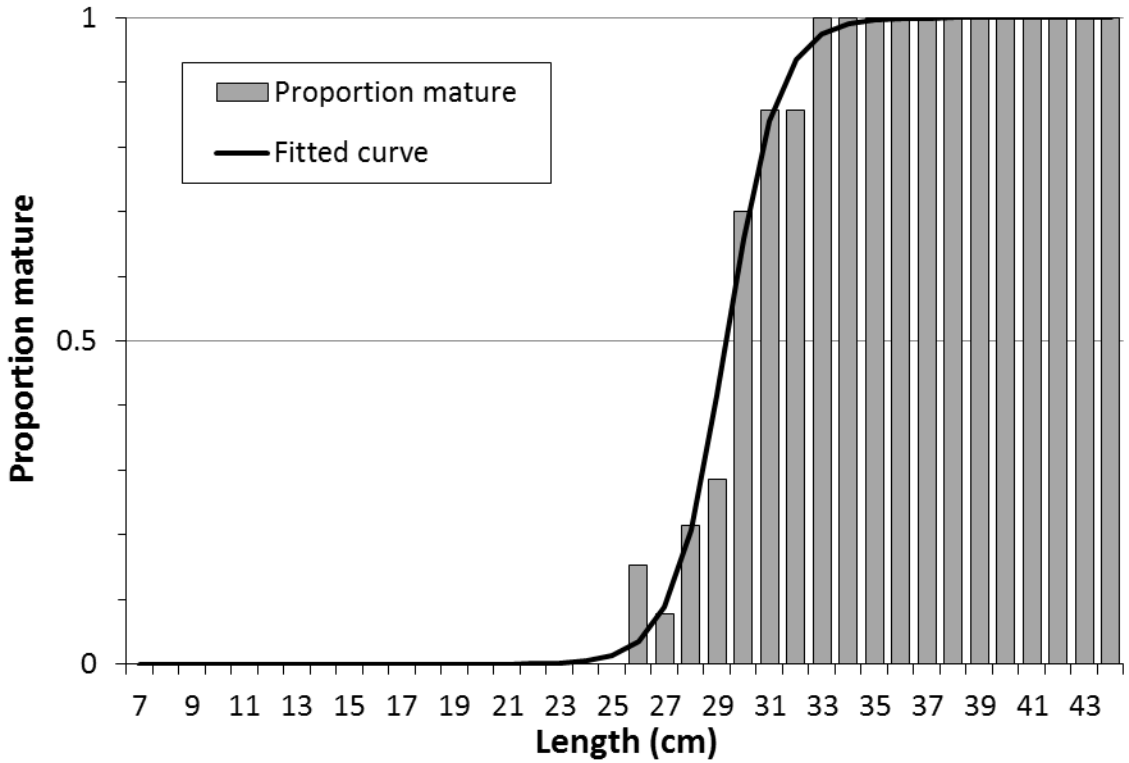

Figure 9. Proportion of mature female Kelp Greenling, as a function of length (cm), showing the fitted logistic curve.

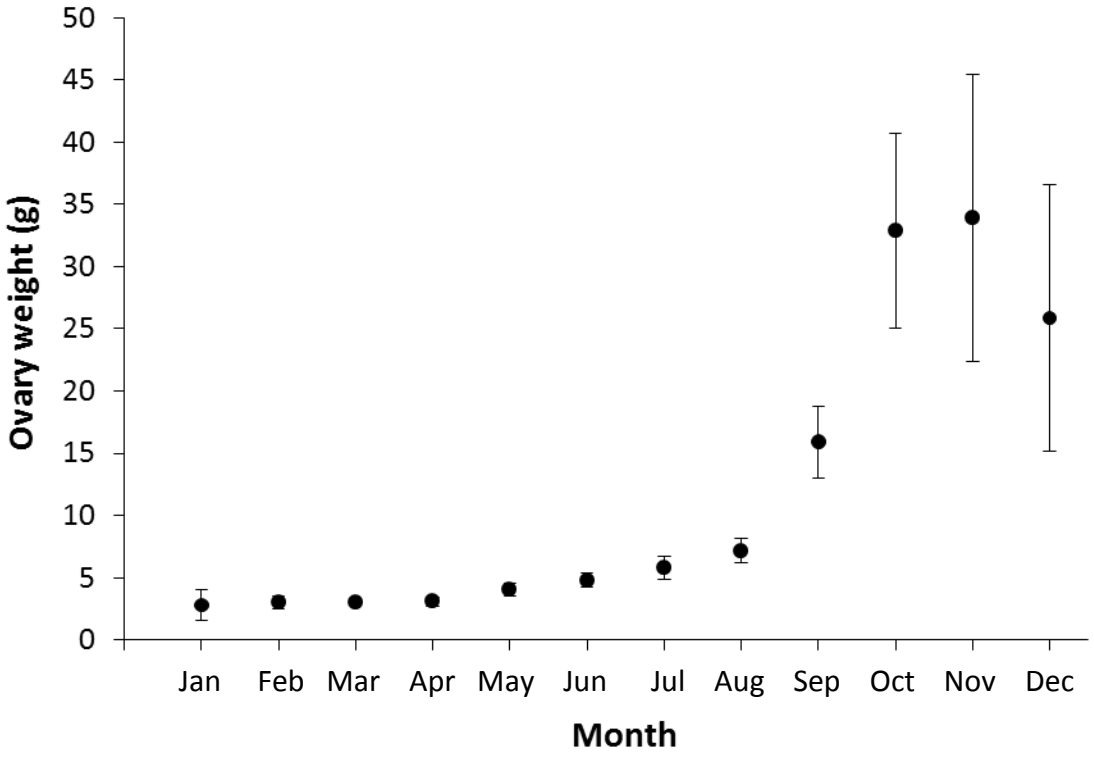

Figure 10. Female mean ovary weight for mature Kelp Greenling only (≥ 33 cm) by calendar month with 95% confidence intervals.

Discussion

To our knowledge, this is the first study to evaluate Kelp Greenling life history parameters based on large sample sizes, including both female macroscopic and histology-based microscopic maturity evaluations, specific to Oregon waters. Rothrock (1983) studied length-weight and age-at-length for Kelp Greenling from central and southern California using standard length for fish measurements. The length-weight slope coefficients determined in our study (Table 2) fell within the range of coefficients identified by Rothrock (2.61 to 3.27) from multiple data sets, however our hypothetical weights at length zero (b_0 coefficients) were larger than values for California (range = -5.31 to -3.66). Additionally, length-weight slope parameters identified in the California study (Table 2) were within the range of values for Oregon for females (3.32) and males (3.14) that were used in the 2005 stock assessment for Kelp Greenling (Cope and MacCall 2005). Rothrock (1983) did not provide von Bertalanffy parameter estimates, but did observe Kelp Greenling that had spawned by the 2+ age class, which generally aligns with the 26 to 32 cm mature fish observed in our study (Figures 7 and 9). Von Bertalanffy parameters for female and male asymptotic length identified in our study were also consistent with values for Oregon used in the 2005 assessment of Kelp Greenling (female = 38.98 cm; male = 37.05 cm, Cope and MacCall 2005). However, our analysis indicated this species grows faster (assessment k = 0.3 to 0.4), has a larger

hypothetical age at length zero (assessment t_0 = -2.46 to -1.21), and does not live as long as in Oregon waters as suggested in the 2005 assessment (25 years). Length-at-50% maturity for females, produced from histological examination of ovaries, (Table 7) was substantially smaller in our study than the value for both sexes combined (35.19 cm) used in the 2005 assessment. Individual ovaries observed in this study showed multiple stages of mature oocytes and POFs (Figure 3) consistent with batch spawning by Kelp Greenling (Kurita et al. 1995 and Crow et al. 1997).

Regarding the female macroscopic maturity staging scale (Table 1), results from this microscopic evaluation of maturity confirm fish with "maturing (stage 3)" and "resting (stage 7)" ovaries were misidentified most often. "Maturing" macroscopic evaluations were incorrect approximately 30% of the time, and occurred in all months of the year. However, misidentifications were more common from September through January, the primary spawning season. Macroscopic staging difficulties in these months are likely related to atresia and abortive maturation in adolescent fish. These patterns of atresia and abortive maturation in developing Kelp Greenling ooctyes are consistent with observations by McDermott et al. (2007) who concluded atresia is used to control reproductive output at the end of the spawning season in Atka Mackerel. Additionally, results from this study suggest fish with macroscopic "maturing" ovaries may (~70%) or may not (~30%) be mature. Maturity designations of fish with "resting" ovaries were incorrect in both spring and fall months, but errors were most common in March (42%) in adolescent length fish (22 to 28 cm). Given this pattern of macroscopic misidentification, our results suggest that "maturing" and "resting" stages should be treated with caution when used for maturity designations, and should be verified with histology samples, whenever possible, to minimize misidentification. Optimal sampling of ovaries for microscopic histology should occur in fall through winter months based on the maturation and spawning seasonality of this species. Unfortunately, this is a seasonal period with low fishing effort in both recreational and commercial fisheries, making samples more difficult to obtain.

Acknowledgements

Field staff from ODFW's Groundfish Monitoring, Oregon Recreational Boat Survey Black Rockfish PIT-tagging projects assisted with field sampling of Kelp Greenling. Amy J. Lindsley collected many juvenile Kelp Greenling samples. Melissa Head double-read a subset of microscopic histology samples and provided valuable document review.

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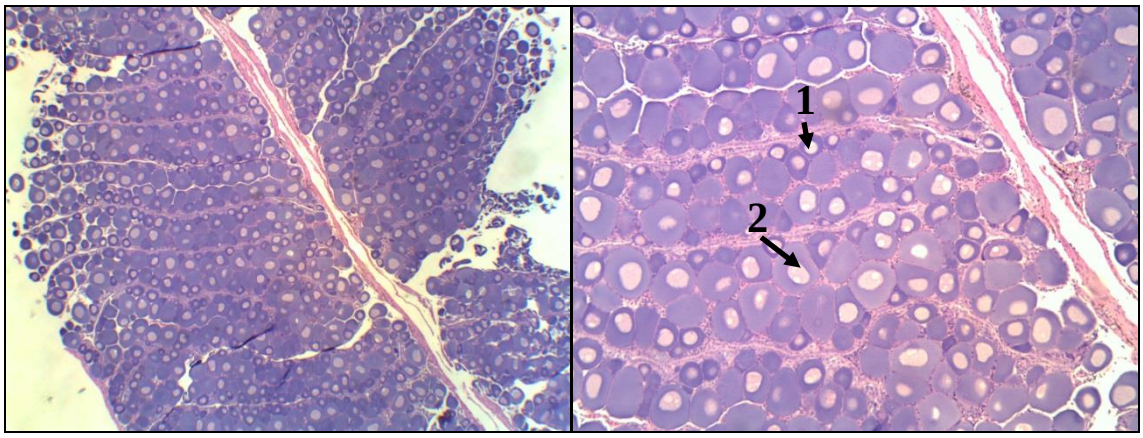
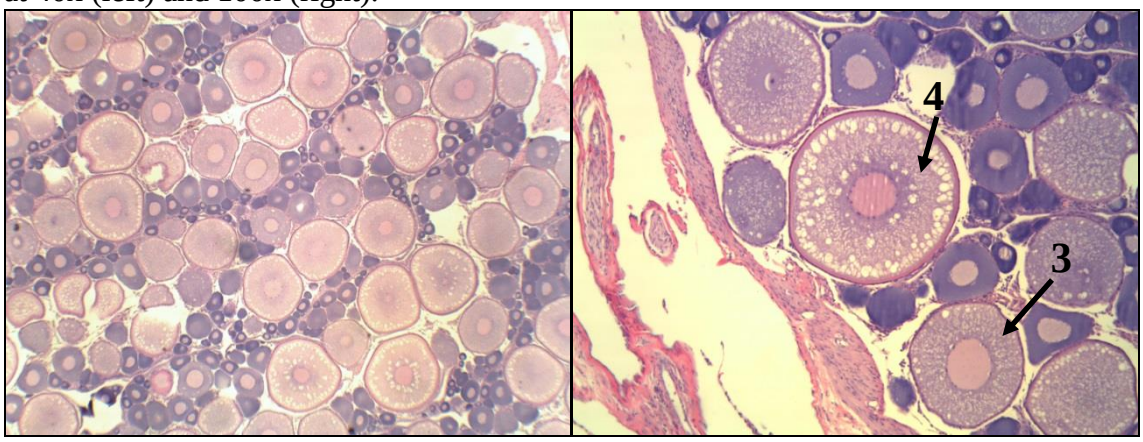
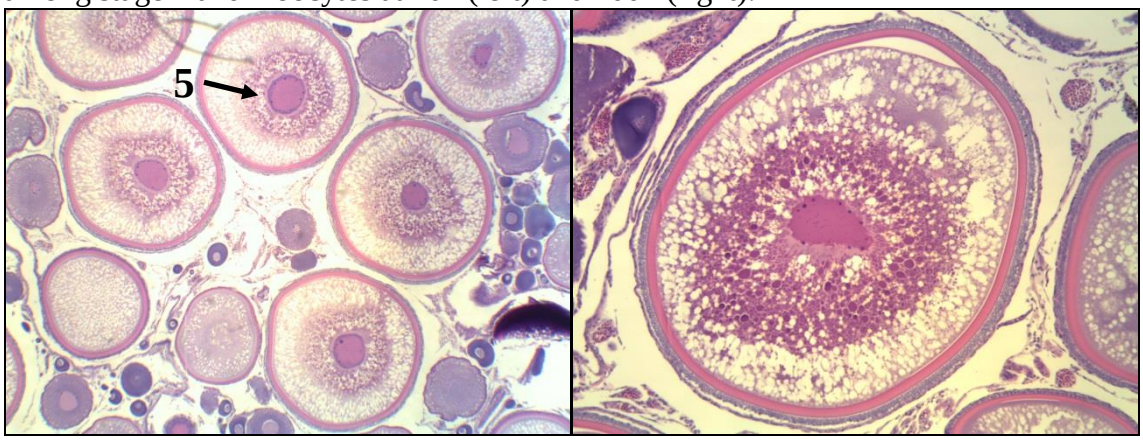
Appendix A. Examples of microscopic oocyte maturity stages 1 through 9 based on McDermott and Lowe (1997).



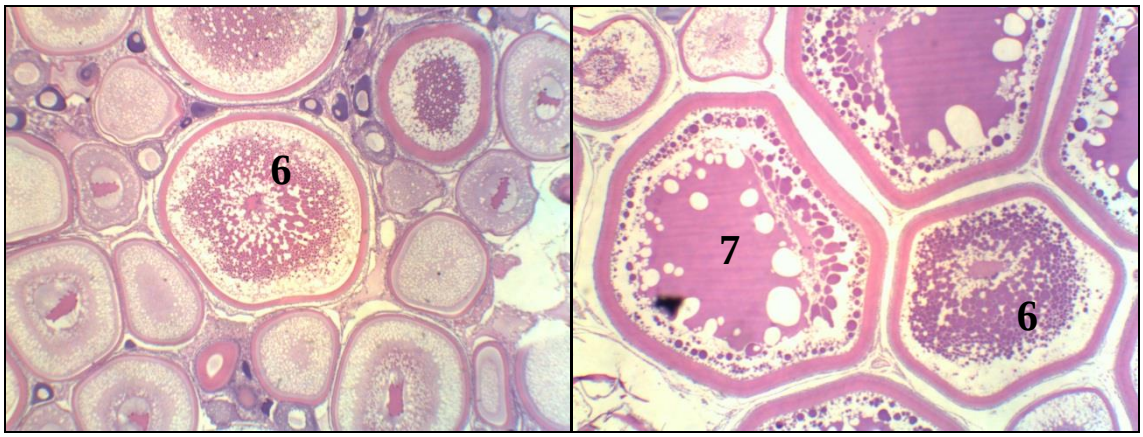
Figures 1A & B. Immature early perinucleus stage 1 and late perinucleus stage 2 oocytes at 40x (left) and 100x (right).



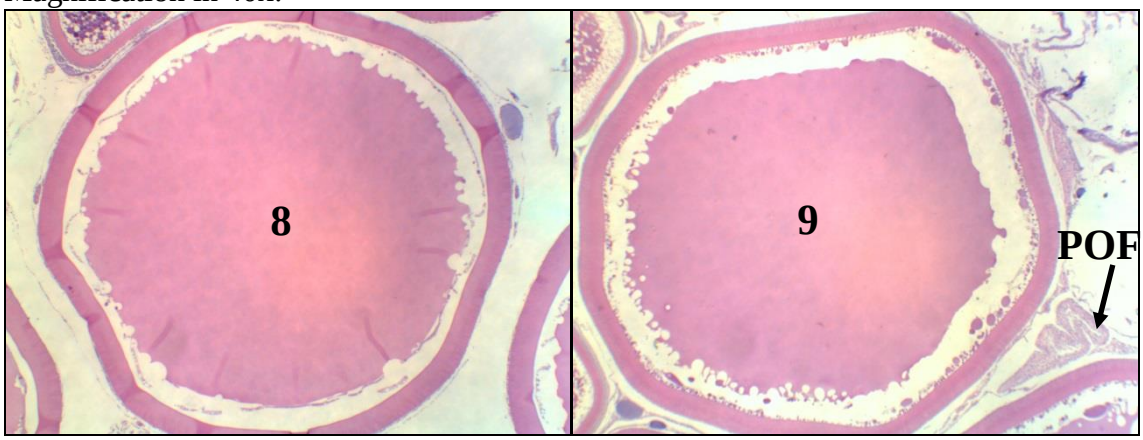
Figures 2A & B. Immature cortical alveoli stage 3 and oil droplet stage 4 oocytes among stage 1 and 2 oocytes at 40x (left) and 100x (right).



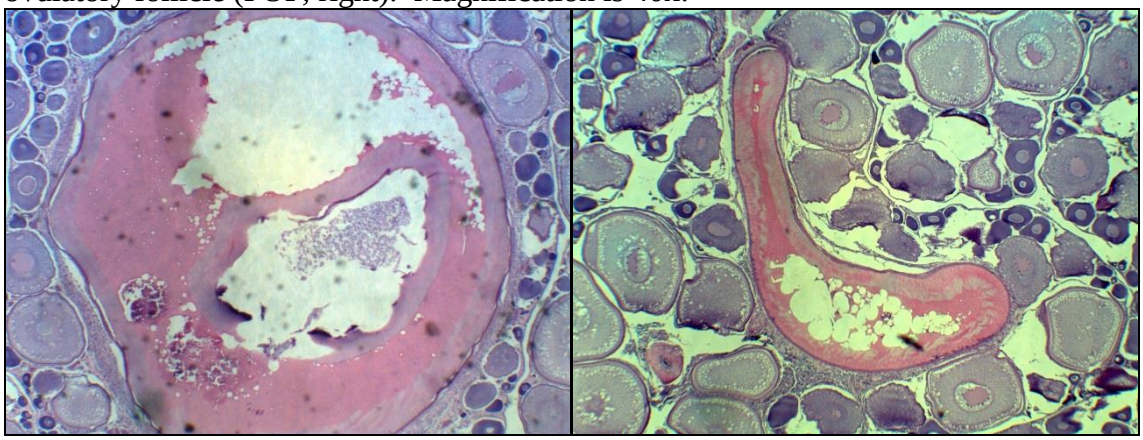
Figures 3A & B. Mature yolk globule vitellogenic stage 5 oocytes 40x (left) and 100x (right).



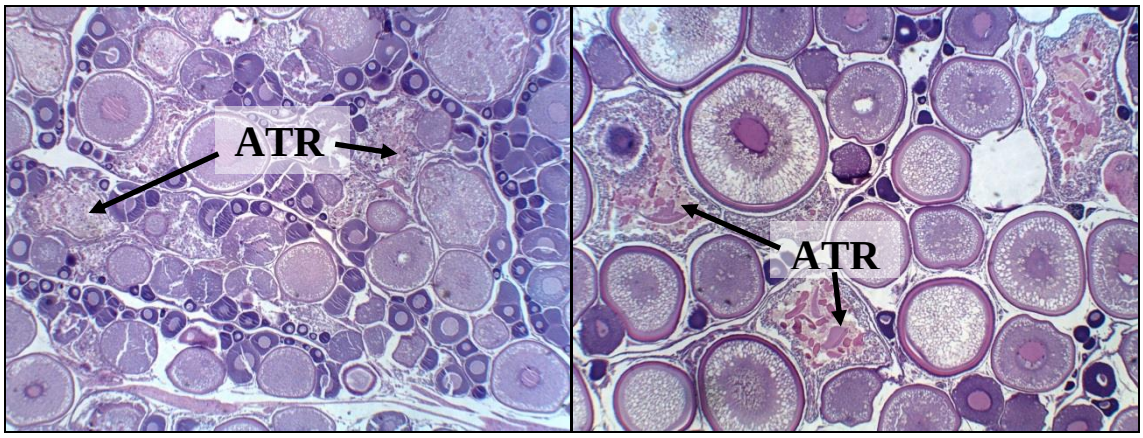
Figures 4A & B. Mature migratory nucleus stage 6 and early hydration stage 7 oocytes. Magnification in 40x.



Figures 5A & B. Mature late hydration stage 8 (left), ovulated stage 9, and post ovulatory follicle (POF, right). Magnification is 40x.



Figures 6A & B. Residual atretic egg remnants. Magnification is 40x.



Figures 7A & B. Atresia (ATR) examples. Magnification is 40x.

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