INFORMATION REPORTS

NUMBER 2014-04

FISH DIVISION
Oregon Department of Fish and Wildlife

Length and age at maturity of female copper rockfish (*Sebastes caurinus*) from Oregon waters based on histological evaluation of ovaries

Length and age at maturity of female copper rockfish (*Sebastes caurinus*) from Oregon waters based on histological evaluation of ovaries

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April 2014

Introduction

This is the seventh publication in a series documenting results from a long-term sampling program aimed at developing improved female maturity data for a variety of groundfish species commonly captured in Oregon fisheries. When this study was initiated in 2000, the available data on female age and length at maturity for many U.S. west coast groundfish species was of questionable quality. The maturity curves then used in stock assessment models were typically based on macroscopic (visual) assessment of ovary condition. However, there is a large amount of evidence showing 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, new 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) and vermilion rockfish (*S. miniatus*, Hannah and Kautzi 2012). This report describes the development of histologically-based length- and age-at-maturity data for female copper rockfish (*Sebastes caurinus*).

Methods

Copper rockfish used for this study were sampled from Oregon's recreational fishery landings at the ports of Newport and Depoe Bay. Copper rockfish are a minor component of the recreational fishery catch, requiring many seasons of sampling to accumulate enough female fish from a wide range of sizes, ages and months of capture to be able to adequately describe maturity. All fish sampled were measured (cm, fork length) and sexed and otoliths were collected for age determination. Ovaries were examined and assigned a maturity stage (Table 1) following the criteria of Westrheim (1975). Whenever possible, a small section of ovary from fish in stages 1, 2, 3, 6 and 7 (Table 1) was collected for histological preparation and microscopic evaluation. These samples were preserved in 10% buffered formalin and later transferred to 70% ethanol for storage.

Table 1. Visual (macroscopic) maturity stages and descriptions for rockfish ovaries from Westrheim (1975).

Stage	Condition	Description
1	Immature	Small, translucent
2	Maturing	Small, yellow, translucent or opaque
3	Mature	Large, yellow, opaque
4	Fertilized	Large, orange-yellow, translucent
5	Ripe	Large, translucent yellow or gray, with black dots (contain embryos or larvae)
6	Spent	Large, flaccid, red. A few larvae may be present

7	Resting	Moderate size, firm, red-gray, some with black blotches

Female maturity was evaluated using a multi-step process. First, a seasonal "window" for evaluating maturity was chosen based on the prevalence of females with ovaries in stages 4-6 (Table 1), indicative of incipient or recently-completed parturition. In selecting the months to include in this "window" I also considered the need for adequate numbers of small or young fish in the final sample to help define a maturity curve. Then, for samples collected during the months within this "window", the maturity status of individual specimens was determined using a combination of macroscopic maturity stages and microscopic examination of stained ovary sections. Female rockfish with ovaries in stages 4-5 were considered unambiguously mature. One difficulty with determining maturity status based solely on the macroscopic evaluation of ovaries is that, for many species, "maturing" and "resting" ovaries cannot be reliably separated (Wallace and Selman 1981, Wyllie Echeverria 1987). Externally, these stages appear quite similar but represent different states of maturity. In some rockfish species, young females have also been shown to undergo abortive maturation, characterized by mass atresia of the developing class of oocytes, further complicating the macroscopic assessment of maturity (Hannah and Parker 2007, Hannah and Blume 2011). To attain the most accurate maturity classification, I microscopically evaluated all stage 1, 2, 3 and 7 ovaries from the selected seasonal "window". A number of stage 6 ovaries were also evaluated microscopically to verify the accuracy of classifying this stage as unambiguously mature and also to examine the typical structure of post-ovulatory follicles to inform the microscopic evaluation of stage 7 ovaries.

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 Bowers (1992). Maturity status was assigned as either mature, immature or unknown. Ovaries with large oocytes showing dark-staining vitellogenin were classified as mature (Figures 1 and 2), unless they showed clear indications of mass atresia, typified by a complete lack of cell nuclei (Figures 3 and 4). Fish with ovaries showing obvious signs of post-release reorganization, such as post-ovulatory follicles (Wyllie Echeverria 1987) or residual larvae or larval eye pigment were also classified as mature (Figure 5). Fish with non-vitellogenic oocytes that appeared well organized were classified as immature (Figure 6). Fish with ovaries showing some signs of reorganization but without post-ovulatory follicles 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 parturition. Females classified as unknown were not used for analysis of age or length at maturity. Evidence of abortive maturation, characterized by mass atresia of the developing class of oocytes from a vitellogenic stage, was also noted at this time (Hannah and Parker 2007). Fish with ovaries showing abortive maturation were classified as immature, unless they were notably larger or older than the length or age interval in which both immature and mature fish were being encountered (adolescent phase; Hannah and Parker 2007, Thompson and Hannah 2010). Fish with abortive maturation that were older or larger than adolescence were noted, but treated as "mature" for the purpose of fitting curves of length and age at maturity. The accuracy of macroscopic staging of ovaries for fish not in unambiguous macroscopic stages (stages 4-5), was evaluated by comparing the maturity status determined from the macroscopic and microscopic evaluations, within the seasonal “window” for best assessing maturity.

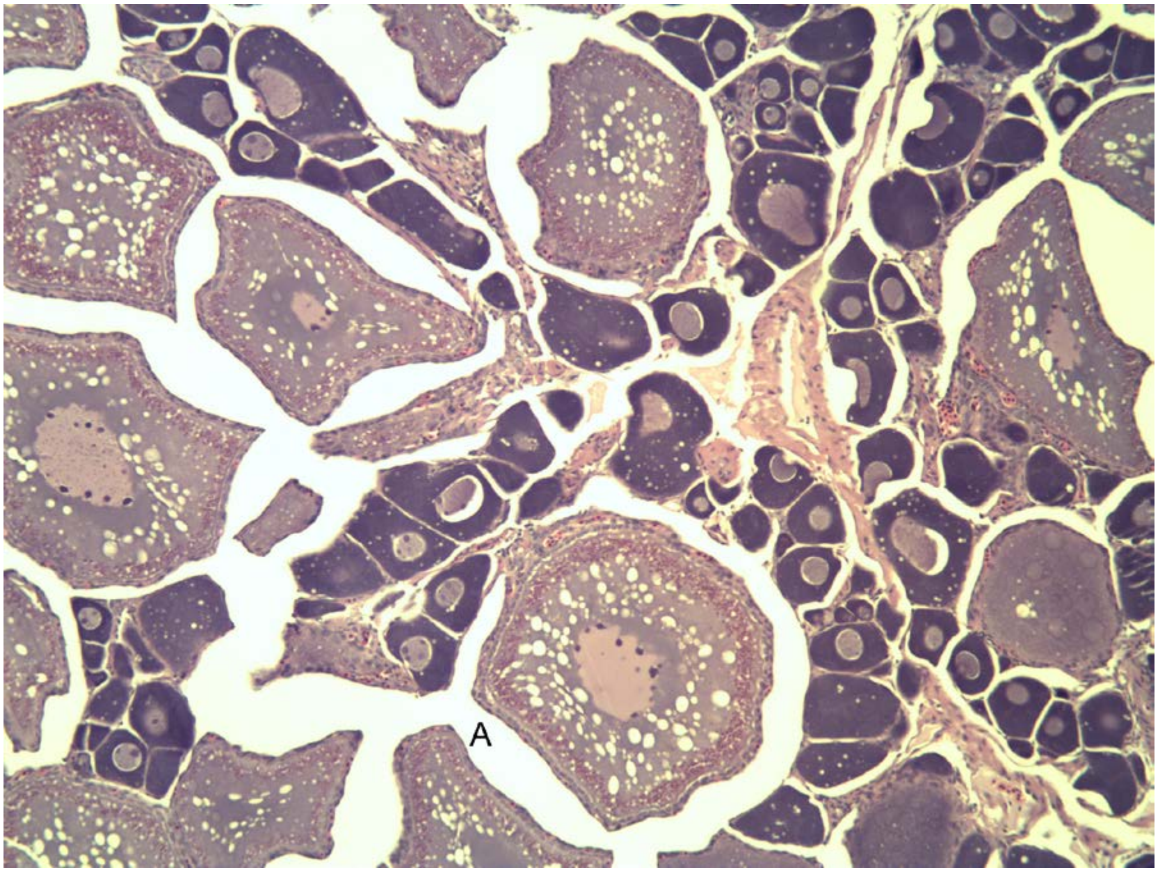

Figure 1. Stained ovary thin-section of a copper rockfish showing the development of magenta-staining vitellogenin (A). Magnification is 100x.

I evaluated copper rockfish maturity in this study as a function of both length and age. Ages of copper rockfish were determined using either the break and burn technique applied to sagittal otoliths (Chilton and Beamish 1982) or a variation of the technique in which sagittal otoliths were broken and “baked” for several minutes prior to age determination.

Logistic regression was used to fit sigmoid curves to the proportion mature by length and age, in the form,

$p_{x_1} = e^{(b_0+b_1x_1)}/(1+e^{(b_0+b_1x_1)})$ where, p is the probability that a fish is mature in a given length (cm) or age inteval x_1, and b_0 and b_1 are parameters that define the shape and location of the fitted sigmoid curve. The predicted length or age at 50% maturity was calculated as,

$L \text{ (or } A)_{50} = -b_0/b_1$.

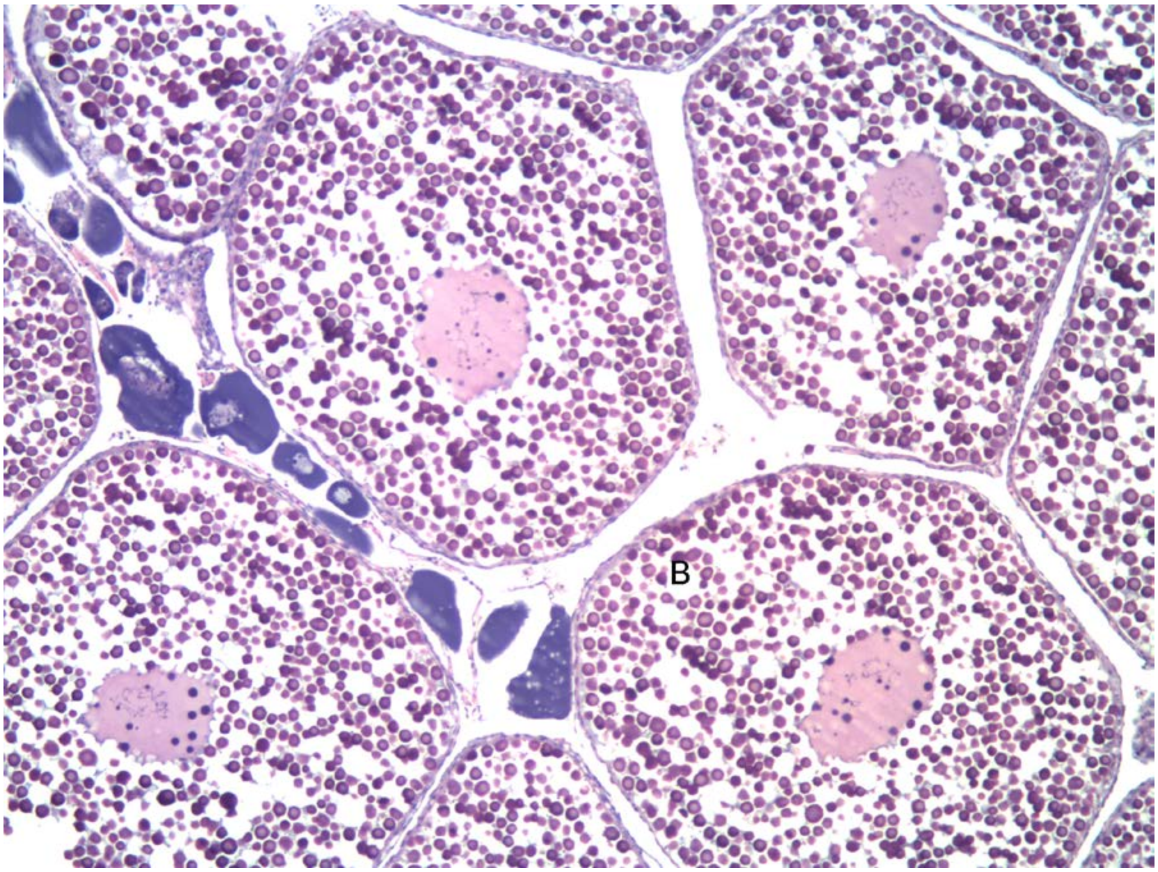

Figure 2. Stained ovary thin-section of a copper rockfish showing the advanced development of magenta-staining vitellogenin (B). Magnification is 100x.

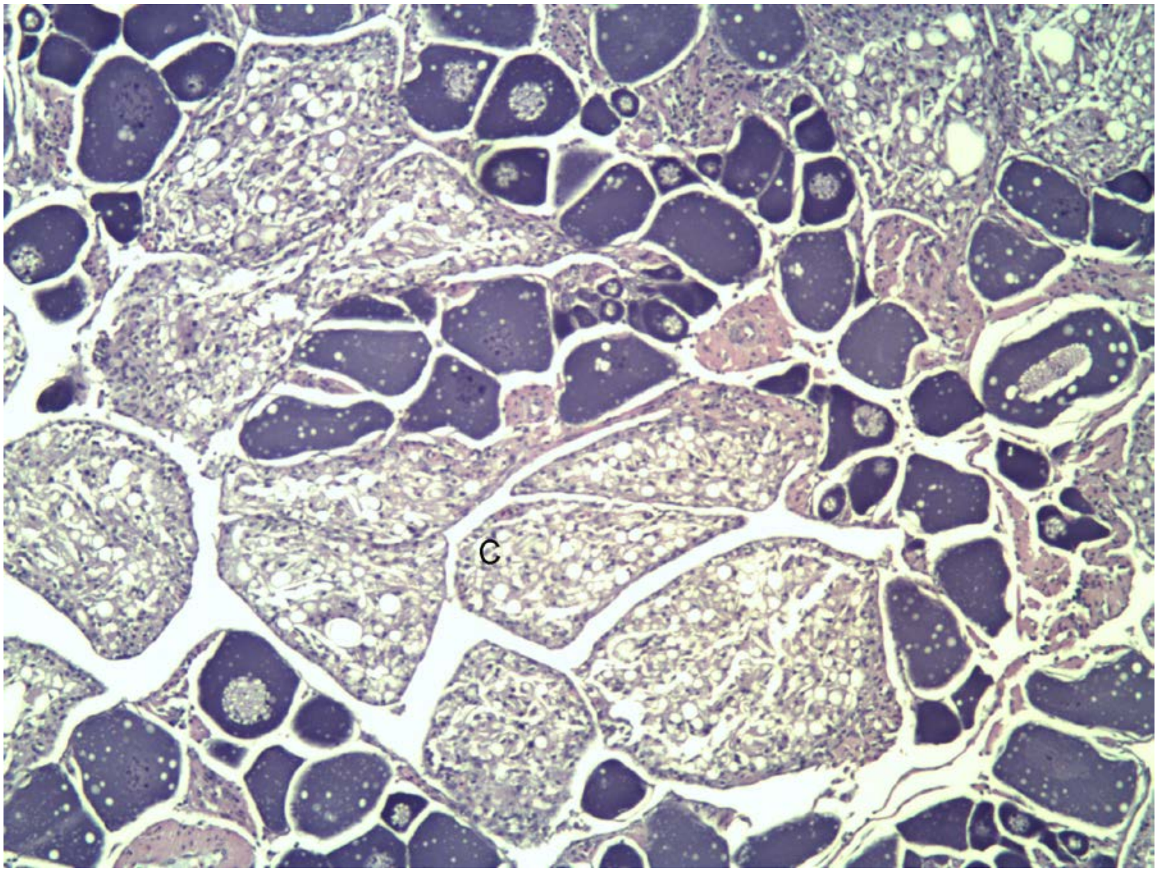

Figure 3. Stained ovary thin-section from a copper rockfish showing mass atresia of the developing class of oocytes (C). Magnification is 100x.

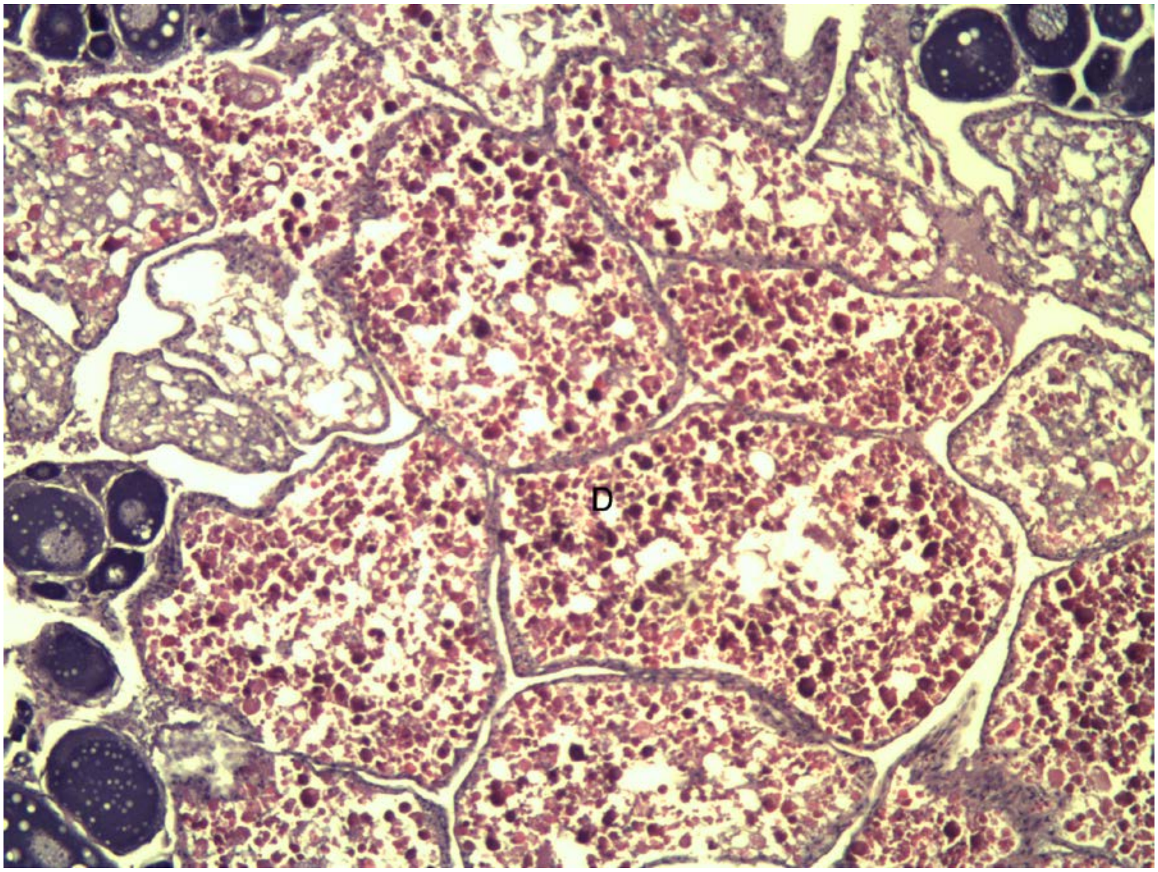

Figure 4. Stained ovary thin-section from a copper rockfish showing mass atresia of the developing oocytes from a heavily-vitellogenic state (D). Note complete lack of nuclei. Magnification is 100x.

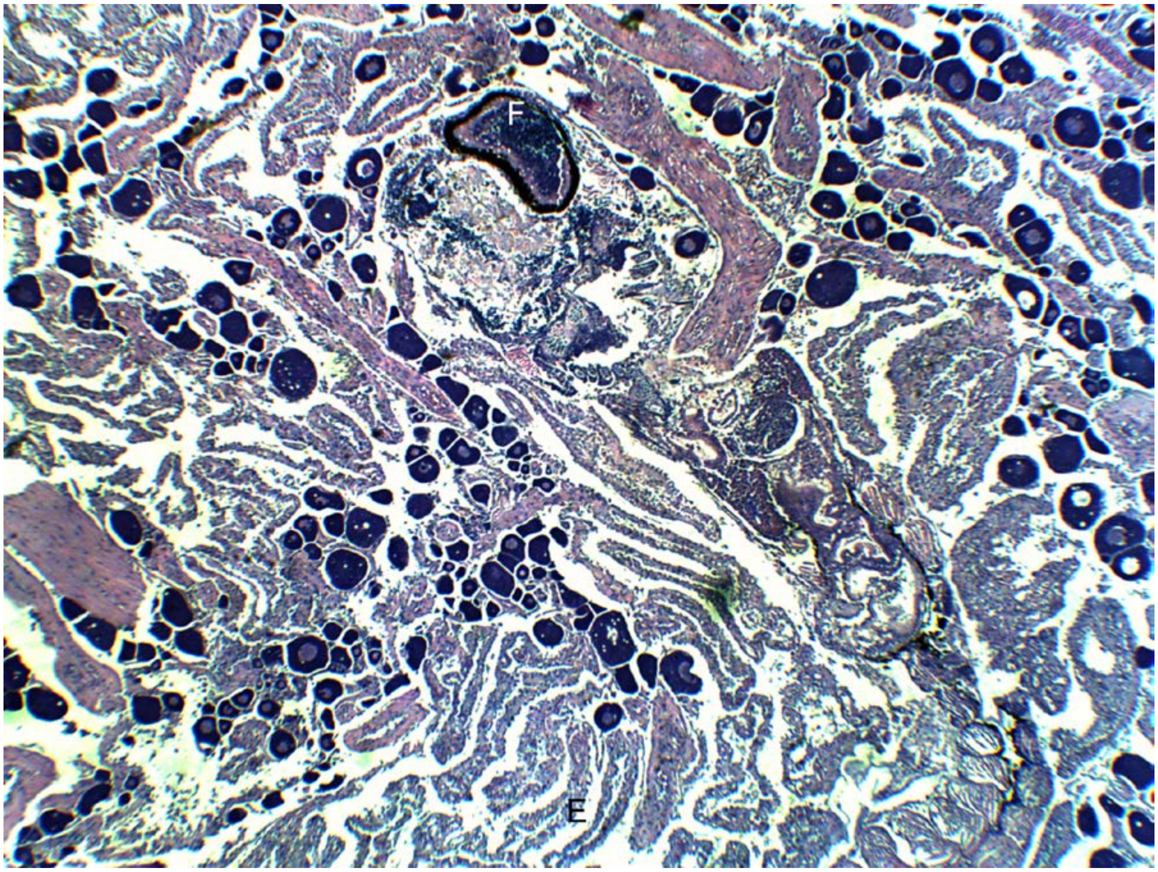

Figure 5. Stained ovary thin-section of a spent copper rockfish showing the classic "deflated balloon" appearance of post ovulatory follicles (E) and a residual larval eye surrounded by black eye pigment (F). Magnification is 40x.

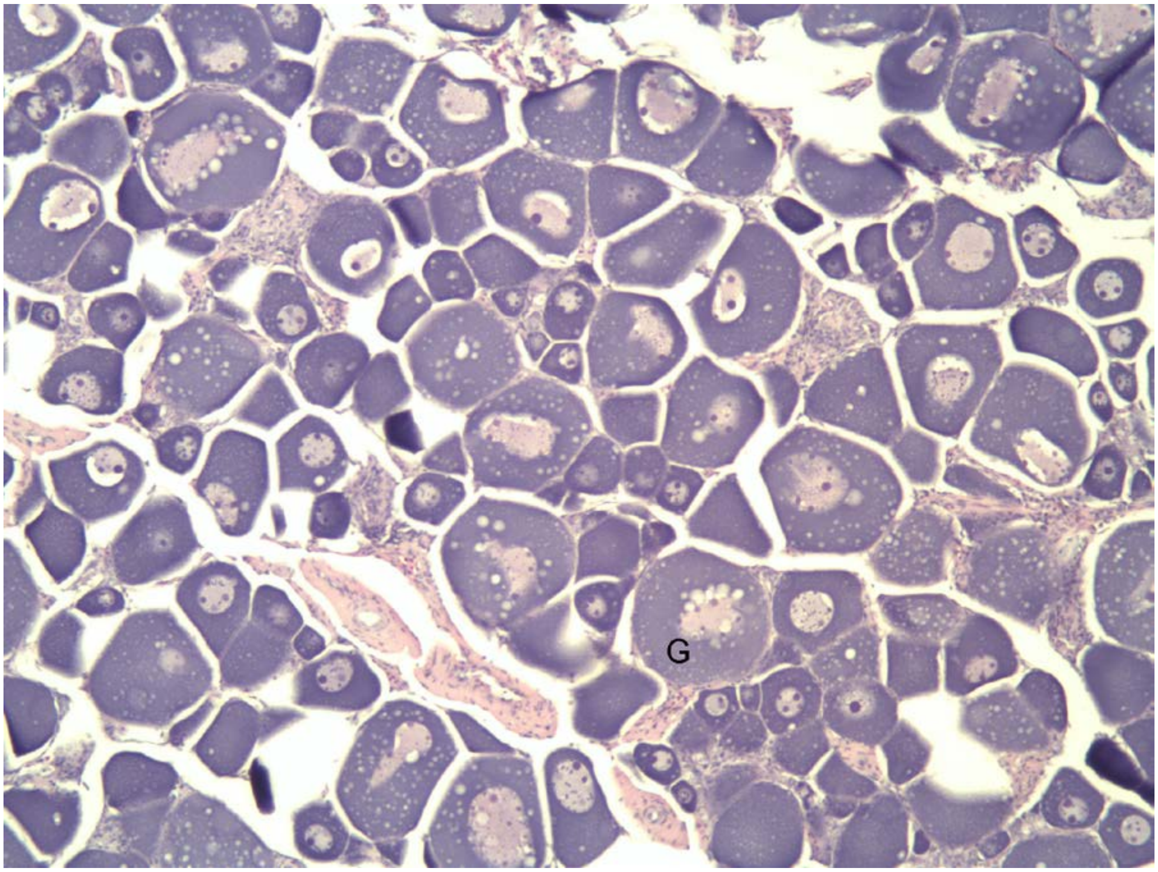

Figure 6. Stained ovary thin-section showing small oocytes of an immature copper rockfish. The largest oocyte shown here (G) is approximately 140µ in diameter and lacks magenta-staining vitellogenin. Magnification is 100X.

Results

Sample collection resulted in macroscopic maturity stage data for 902 copper rockfish collected between 2000 and 2010 (Table 2), with samples most prevalent in the months of April through August. This distribution of sample frequency mirrors the variation in the source of the samples, the fishing effort of the Newport and Depoe Bay recreational charter boat fishery. Histology samples collected and processed for microscopic evaluation of female maturity totaled 441 (Table 2).

Based on the macroscopic staging of ovaries, parturition in copper rockfish is not highly synchronous, with fertilized and ripe ovaries (stages 4 and 5) observed at low frequencies from January through August, peaking in February. The relatively high frequency of spent ovaries from March through August, in comparison with the low frequency at which fertilized or ripe ovaries were observed for the same months, suggests that female copper rockfish in the fertilized or ripe stages may have reduced fishery catchability (Figure 7). Alternatively, copper rockfish ovaries may simply be very recognizable as "spent" for an extended period of time. Based on the monthly distribution of fish with

Table 2. Numbers of female copper rockfish maturity (M) and histology (H) samples collected and processed, by month and macroscopic maturity stage (Table 1), 2000-2010.

Maturity stage	Immature		Maturing		Mature		Fertilized		Ripe		Spent		Resting		Total	
Month	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H
January	0	0	0	0	8	6	1	0	0	0	0	0	0	0	9	6
February	0	0	3	3	8	5	4	0	7	0	3	3	5	4	30	15
March	0	0	3	0	13	9	4	0	3	0	21	19	14	14	58	42
April	2	1	14	13	13	12	1	0	2	0	29	26	30	30	91	82
May[1]	2	2	17	16	2	0	3	0	0	0	21	4	48	36	93	58
June	3	3	22	19	3	3	1	0	0	0	30	4	57	43	116	72
July	0	0	24	22	3	3	0	0	0	0	41	15	92	84	160	124
August	4	0	28	4	1	0	0	0	0	0	25	2	135	17	193	23
September	2	0	16	1	11	0	0	0	0	0	4	0	54	3	87	4
October	1	0	12	3	8	1	0	0	0	0	3	0	23	3	47	7
November	0	0	1	0	5	1	0	0	0	0	0	0	4	0	10	1
December	1	1	0	0	7	6	0	0	0	0	0	0	0	0	8	7
Total	15	7	140	81	82	46	14	0	12	0	177	73	462	234	902	441

[1] Note that histology samples were collected from 2 female copper rockfish in May for which no macroscopic stage was assigned

ovaries in the developing (stage 3), fertilized and ripe stages, I chose a seasonal window of December through July for microscopic evaluation of female maturity (Figure 7).

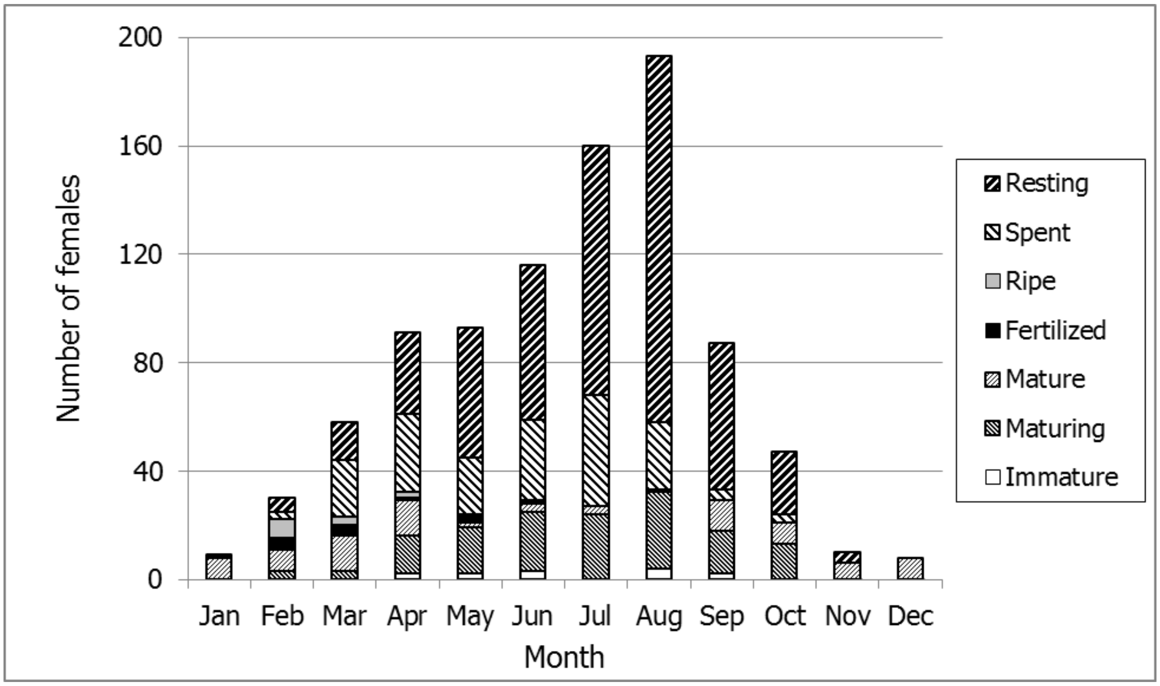

Figure 7. Number of female copper rockfish sampled for maturity, by macroscopic (visual) maturity stage and month, 2000-2010.

Comparison of macroscopic and microscopic evaluations of female maturity showed that macroscopic staging of copper rockfish ovaries was not very accurate (Table 3). Microscopic evaluations of 406 ovaries collected in the months of December through July resulted in the reclassification of maturity status for 26 specimens (6.4%) and "unknown" status for 130 specimens (32%, Table 3). Of the 10 immature fish that were reclassified as mature based on microscopy, 7 showed clear evidence of prior parturition, based on post ovulatory folicles or black larval eye pigment in the ovary section, while 3 showed vitellogenesis that was not evident macroscopically. Of the 16 fish that were reclassified from mature to immature, 9 showed evidence of mass atresia, indicative of abortive maturation, while 7 showed well-organized oocytes with no evidence of vitellogenesis. The 9 females with abortive maturation were all within a size or age range consistent with an adolescent period, and were treated as immature fish for the fitting of logistic regressions of age and length at maturity (Hannah and Parker 2007).

The large number of ovaries classified as "unknown" microscopically (32%) showed that histology was not highly effective in resolving maturity status for female copper rockfish, at least for the seasonal window chosen (Table 3). Ovaries classified as "unknown" were most often females sampled in the months of March through July and classified macroscopically as "resting" (stage 7). Microscopically, these fish showed evidence of reorganization in the ovary, but lacked definitive markers of prior parturition, such as clear post ovulatory follicles or traces of black larval eye pigment, and also lacked clear signs of atresia, indicative of abortive maturation.

Table 3. Comparison of macroscopic and microscopic determinations of maturity in female copper rockfish collected from waters off Newport and Depoe Bay, Oregon, for the months of December through July, 2000-2010.

	Macroscopic classification		Microscopic classification			
Month	Condition	Number	Confirmed	Reclassified	Unknown	Not used
December	Immature	1	1	0	0	0
	Mature	6	6	0	0	0
January	Immature	0	0	0	0	0
	Mature	6	6	0	0	0
February	Immature	3	2	1	0	0
	Mature	12	12	0	0	0
March	Immature	0	0	0	0	0
	Mature	42	30	2	9	1
April	Immature	14	9	3	2	0
	Mature	68	44	3	21	0
May	Immature	18	6	2	10	0
	Mature	40	19	0	19	2
June	Immature	22	14	3	5	0
	Mature	50	26	5	19	0
July	Immature	22	17	1	3	1
	Mature	102	54	6	42	0
Total		406	246	26	130	4

Microscopic examination of stained ovarian thin-sections also showed a high frequency of what appeared to be nematodes within copper rockfish ovaries. It's unknown what effect nematodes in the ovary has on normal ovarian development, fertilization or parturition in copper rockfish.

The final data set for evaluating female length at maturity consisted of 300 specimens collected during the months of December through July for which maturity determinations were considered definitive (Table 4). These data showed that females matured as small as 30 cm and were 100% mature at 44 cm (Table 4). The fork length of specimens included ranged from 26 to 53 cm (Table 4). A logistic regression of maturity on length fit the data well ($P<0.0001$, $r^2 = 0.537$) and indicated a length at 50% maturity of 34.81 cm (Figure 8, Table 5).

Table 4. Number of female copper rockfish used in determining age and length at maturity and proportion mature, by length (cm) and age (y).

Length (cm)	Number sampled	Proportion mature	Age (y)	Number sampled	Proportion mature
26	1	0.00	4	11	0.00
27	0	--	5	33	0.39
28	1	0.00	6	45	0.60
29	2	0.00	7	45	0.80
30	9	0.11	8	36	0.92
31	3	0.00	9	24	0.88
32	12	0.08	10	22	1.00
33	15	0.27	11	15	1.00
34	15	0.33	12	9	1.00
35	14	0.57	13	12	1.00
36	17	0.59	14	2	1.00
37	16	1.00	15	5	1.00
38	17	0.82	16	3	1.00
39	12	0.92	17	0	--
40	15	1.00	18	3	1.00
41	15	1.00	19	1	1.00
42	13	0.92	20	0	--
43	22	0.95	21	4	1.00
44	21	1.00	22	3	1.00
45	18	1.00	23	1	1.00
46	15	1.00	24	0	--
47	15	1.00	25	1	1.00
48	10	1.00	26	0	--
49	6	1.00	27	0	--
50	7	1.00	28	0	--
51	3	1.00	29	0	--
52	5	1.00	30	0	--
53	1	1.00	31	1	1.00
Total	300			276	

Table 5. Results of logistic regression analysis of maturity status of female copper rockfish versus length (cm) and age (y).

Independent variable		Coefficients	Standard error	P-value	L_{50} or A_{50}	95% confidence limits	Coefficient of determination
Length					34.81 cm	±0.041	
	Constant	-20.617	2.852	0.0001			
	Length	0.592	0.080	0.0001			
	Full model			0.0001			0.537
Age					5.65 y	±0.024	
	Constant	-5.372	0.907	0.0001			
	Age	0.951	0.142	0.0001			
	Full model			0.0001			0.340

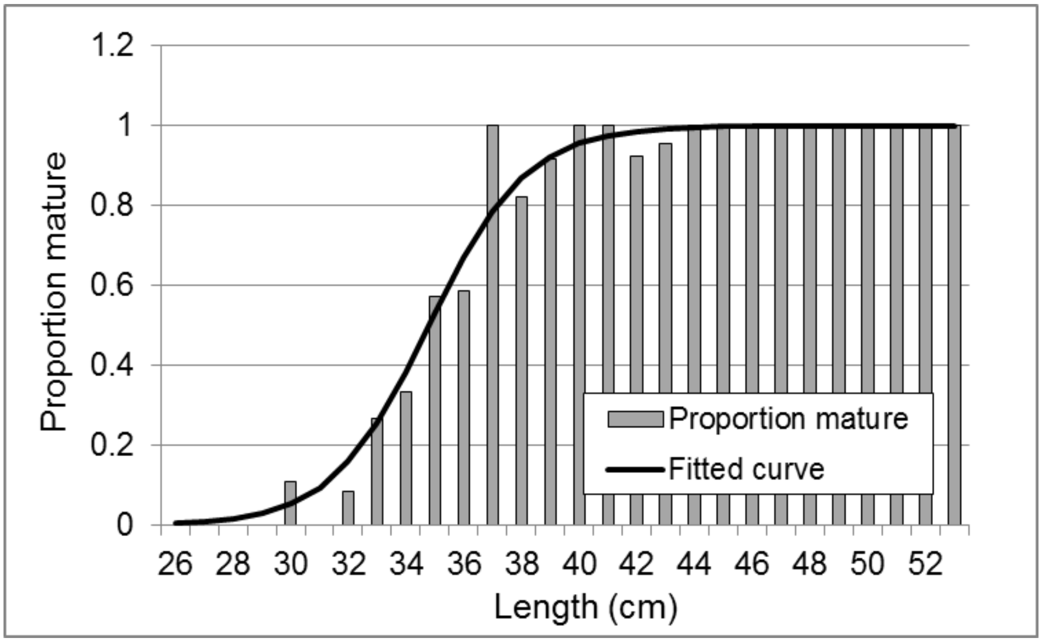

Figure 8. Proportion of female copper rockfish that were mature, as a function of length (cm), showing the fitted logistic curve.

The final data set for evaluating age at maturity consisted of 276 specimens with definitive determinations of both age and maturity (Table 4). Copper rockfish females first matured at age 5 and were fully mature at age 10 (Table 4). The ages of specimens included in the final determination of maturity ranged from 4 to 31 y (Table 4). The logistic regression of maturity on age was highly significant ($P<0.0001$, $r^2 = 0.340$) and indicated an age of 50% maturity of 5.65 y (Table 5, Figure 9).

Discussion

To our knowledge, this is the first study to evaluate female copper rockfish length and age at maturity based on a large sample size of both macroscopic and histology-based microscopic maturity evaluations. This is especially true for Oregon waters. Barss (1989) evaluated age and length at maturity for a wide range of rockfish species from Oregon waters, but sampled only 3 female copper rockfish. Wyllie Echeverria (1987) studied the age and length at maturity of copper rockfish from central California. Based on macroscopic evaluations of 325 copper rockfish (males and females) and histology-based evaluations of just 14 ovaries, Wyllie Echeverria (1987) estimated female length at 50% maturity at 34 cm (total length), close to our estimate of 34.81 cm (fork length) for central Oregon waters. Wyllie Echeverria estimated age at 50% maturity for female copper rockfish at 6 y, again consistent with our estimate of 5.65 y. The peak month of parturition in that study was also February, as in our study. Lea et al. (1999) studied the reproductive development of copper rockfish from central California waters, but did not estimate length or age at 50% maturity. Lea et al. (1999) did note female copper rockfish in gravid condition in February and March and that spent females were found throughout the year, generally consistent with our results for Oregon.

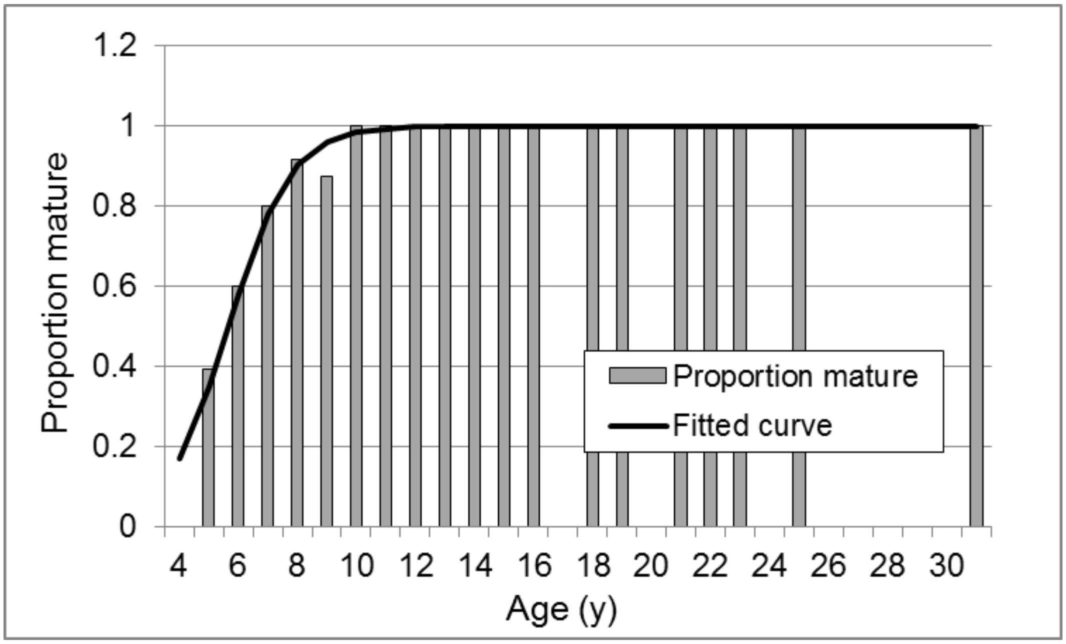

Figure 9. Proportion of female copper rockfish that were mature, as a function of age (y), showing the fitted logistic curve.

Acknowledgements

Lisa Kautzi provided ages for the copper rockfish otoliths. Field staff from ODFW's black rockfish PIT-tagging, Groundfish Monitoring and Oregon Recreational Boat Survey projects assisted with field sampling of copper rockfish.

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