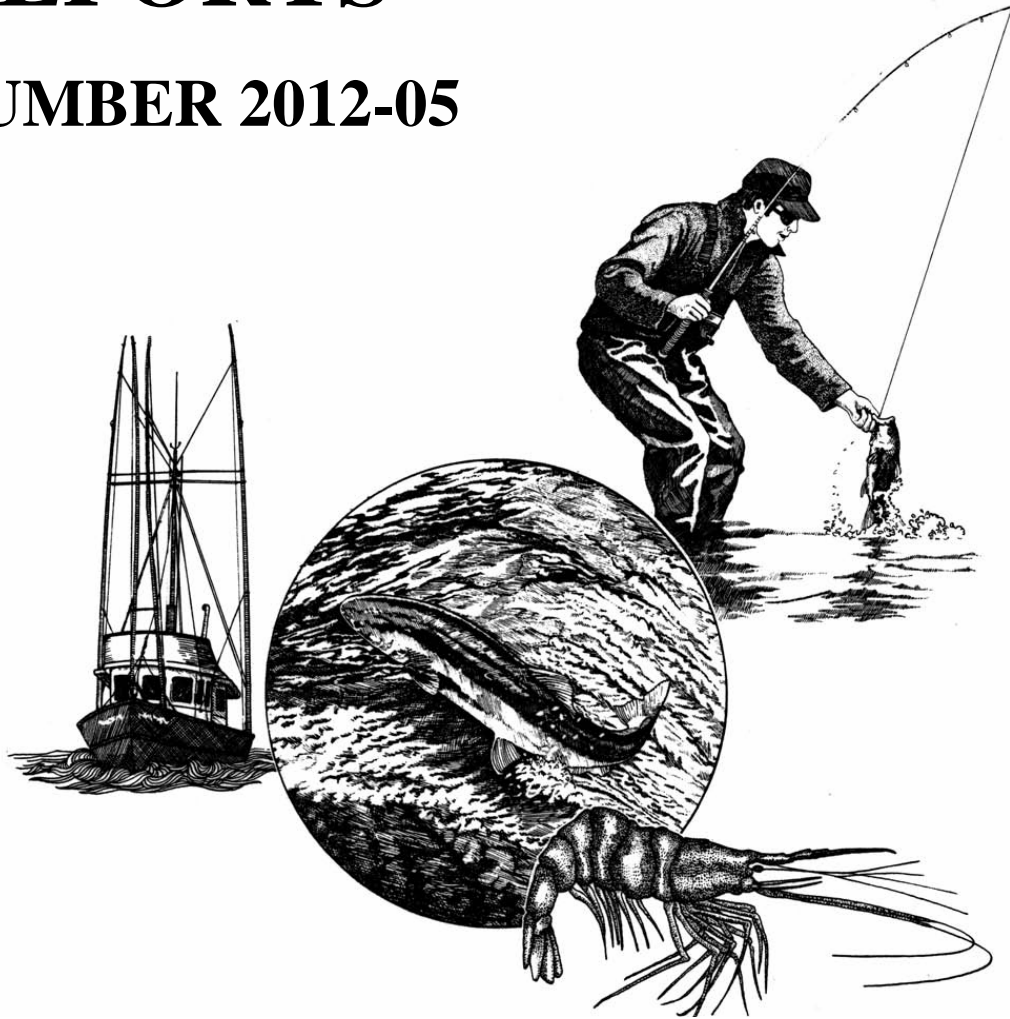


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Age, growth and female maturity of vermillion rockfish (*Sebastes miniatus*) from Oregon waters

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Introduction

Recent changes in U.S. fishery management standards have placed increased emphasis on implementing precautionary harvest levels for all stocks, even those that are only minor components of commercial or recreational fisheries. Precautionary harvest levels are especially critical for long-lived, late-maturing, unassessed stocks that have been harvested for many years in mixed-stock fisheries, such as several nearshore Pacific rockfishes (*Sebastes*) on the U.S west coast. For these typically data-poor stocks, qualitative evaluations of stock vulnerability to overfishing are a useful starting point in developing precautionary management measures (e.g. Patrick et al. 2010, Ormseth and Spencer 2011). Vulnerability analyses in turn depend on accurate basic life history information, such as age, growth and female maturity as a function of length or age.

In Oregon marine waters, the vermilion rockfish (*Sebastes miniatus*) is an example of an unassessed, but potentially vulnerable rockfish stock. Vermilion rockfish live to at least age 60 (Munk 2001) and have been a minor component of both the hook and line commercial and recreational fisheries in Oregon for many decades. They are found in rocky habitats that are not easily surveyed with trawls (Love et al. 2002). Vermilion rockfish are believed to have high site fidelity and a small range of movements (Lea et al. 1999, Hannah and Rankin 2011), making them potentially vulnerable to localized depletion.

The information that is currently available on age, growth and female maturity of vermilion rockfish on the U.S. west coast is not directly applicable to this stock in Oregon waters for several reasons. Findings from recent California studies suggest that fish previously referred to as vermilion rockfish are actually two closely-related cryptic species (Hyde et al. 2008, Hyde and Vetter 2009), one form that is found in deeper waters south of Point Conception, California (proposed name “sunset rockfish”) and one that inhabits waters north of Point Conception, at shallower depths (vermilion rockfish). This new information indicates that some of the published life history information for vermilion rockfish (Phillips 1964, Love et al. 1990) may be based on samples of sunset rockfish or a mixture of these two species. Other available data are based on samples of what are probably all vermilion rockfish collected in northern and central California (Lea et al. 1999, Wyllie Echeverria 1987). However, in these studies, ages were derived from surface reads of otoliths rather than the more accurate “break and burn” technique (Chilton and Beamish 1982). The available maturity data for female vermilion rockfish is also almost exclusively based on visual rather than histological evaluation of ovaries (Lea et al. 1999, Wyllie Echeverria 1987), an approach that has been demonstrated to be inaccurate for several other rockfish species (Hannah and Blume 2011, Hannah et al. 2009). The objective of our study was to develop new information on age, growth and female maturity for vermilion rockfish, specific to Oregon marine waters, using more modern and reliable methods for both age determination and evaluation of female maturity.

Methods

Vermilion rockfish used for this study were sampled from Oregon's recreational and nearshore commercial fishery landings at the major ports from Depoe Bay, south to Brookings, Oregon. All fish were measured (cm FL) and sexed and otoliths were collected for age determination. Maturity stages of female fish were evaluated visually using the criteria shown in Table 1. Whenever possible, a small section of ovary (mostly from fish in stages 1, 2, 3, 6 and 7, Table 1) was collected for histological preparation and microscopic determination of maturity status. These samples were preserved in 10% buffered formalin and later transferred to 70% ethanol for storage. We evaluated vermilion rockfish maturity in this study as a function of both length and age. Ages of vermilion rockfish were determined using the break and burn technique applied to sagittal otoliths (Chilton and Beamish 1982). Using a dissecting microscope with reflected light, age was approximated by counting the translucent zones on the surface of the otolith, then for a more accurate read, cut transversely through the nucleus and the cut face burned over an alcohol flame. The standard von Bertalanffy growth equation was used to fit length to age data using non-linear least squares regression in JMP statistical software (Ver. 6.0.2).

Table 1. Visual (macroscopic) maturity stages and descriptions for rockfish ovaries, adapted from Westheim (1975) and Barss and Wyllie Echeverria (1987).

Stage	Condition	Description of gonad
Females		
1	Immature	Small, translucent
2	Maturing	Small, yellow, translucent or opaque
3	Mature	Large, yellow, granular, 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, grayish red. A few larvae may be present
7	Resting	Moderate size, firm, red to gray, some with black blotches

Female maturity was determined using a multi-step process. First, macroscopic maturity stages and histology slides were reviewed to determine the optimal seasonal “window” for accurately evaluating maturity. Our review of macroscopic stage data focused on evaluating synchrony in seasonal development and identifying the seasonal peak in parturition as shown by the prevalence of ovaries in stages 4-6 (Table 1). Our initial review of histology slides focused on two objectives: determining the accuracy of macroscopic staging, and determining the months in which vitellogenic oocytes or other clear signs that a fish was mature, such as post-ovulatory follicles, were most frequently encountered. Then, for samples collected within the best seasonal “window”, the maturity status of individual specimens was determined using a combination of macroscopic maturity stages and microscopic examination of stained ovary sections. For vermilion rockfish, only females with ovaries in stages 4-5 (Table 1), were treated as unambiguously mature based on macroscopic staging alone. The primary difficulty with

determining maturity status based solely on the macroscopic evaluation of ovaries is that "maturing" and "resting" ovaries cannot be reliably separated (Wallace and Selman 1981, Wyllie Echeverria 1987). 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). To attain the most accurate maturity classification, we microscopically evaluated all of the stage 1, 2, 3, 6 and 7 ovaries from the selected seasonal "window" for which we had histology samples.

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 observed 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, as were fish with obvious signs of post-release reorganization, such as post-ovulatory follicles (Wyllie Echeverria 1987) or residual larvae or larval eye pigment. Fish with non-vitellogenic oocytes that appeared well organized were classified as immature. Fish with ovaries showing some signs of reorganization but without post-ovulatory follicles or other clear signs of prior parturition 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 calculating final curves for 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 (Hannah and Parker 2007). Fish with ovaries showing abortive maturation were treated following the approach of Thompson and Hannah (2102) for aurora rockfish. That is, they 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.

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

$$p_{x_i} = e^{(b_0 + b_1 x_i)} / (1 + e^{(b_0 + b_1 x_i)}) \text{ where,}$$

p is the probability that a fish is mature in a given length (cm) or age 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 or age at 50% maturity was calculated as,

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

Results

Age and growth

Ages were estimated for 258 male and 316 female vermilion rockfish collected between the years 2000 and 2011. Early annuli were diffuse and more difficult to determine but as the fish reached maturity patterns became clearer. Ages of males ranged from 4 to 47 while females ranged from 3 to 68 (Table 2, Figure 1). The Von Bertalanffy growth equation fit the data for both sexes well (Table 2 and Figure 2). Male vermilion rockfish grew faster than females and reached a smaller maximum size (Figure 2, likelihood ratio test, $\chi^2 = 35.7$, $df=3$, $P < 0.0001$).

Table 2. Parameter estimates (\pm standard error) for the standard von Bertalanffy growth formula fitting fork length (cm) against age for male and female vermilion rockfish (*Sebastes miniatus*). L_∞ = asymptotic length; k = growth coefficient; t_0 = hypothetical age at length zero; N = sample size. Age range observed, by sex, is also shown.

Parameter	Females	Males
L_∞	67.11 (2.14)	56.55 (1.28)
K	0.075 (0.009)	0.114 (0.014)
t_0	-4.58 (0.88)	-3.83 (0.94)
N	316	258
Age range	3–68	4–47

Maturity

Maturity was evaluated by visual inspection of ovaries for 335 female vermilion rockfish and histology slides were reviewed for 237 of these (Table 3). Visual staging showed that female ovarian development is seasonally asynchronous (Figure 3). Fish with fertilized (stage 4) and ripe (stage 5) ovaries were encountered from May through September and April through October, respectively (Figure 3), indicating a very broad seasonal peak in development. Based on the sharp increase in the percentage of fish with resting (stage 7) ovaries in August (Figure 3), we chose February through July as the optimum seasonal “window” for evaluation of female maturity.

Microscopic evaluation of ovarian thin sections from the months of February to July showed that visual assessment of maturity is not highly accurate in vermilion rockfish (Table 4). Both immature and mature fish were misclassified visually, even during this optimal seasonal “window” (Table 4). Of 193 fish from this time period that were classified by both methods, 2 females could not be confidently identified as either mature or immature and 26 of the remaining 191 (13.6%) were misclassified visually (Table 4). All of the 7 fish that were reclassified from “immature” to “mature” based on histology, had ovaries with microscopic evidence of early vitellogenesis, and one also showed

residual larval eye pigment. Of the 19 females that were reclassified from “mature” to “immature”, 6 had ovaries with mass atresia of the developing class of oocytes, indicative of abortive maturation. The remaining 13 showed no evidence of vitellogenesis or signs of a previous parturition event.

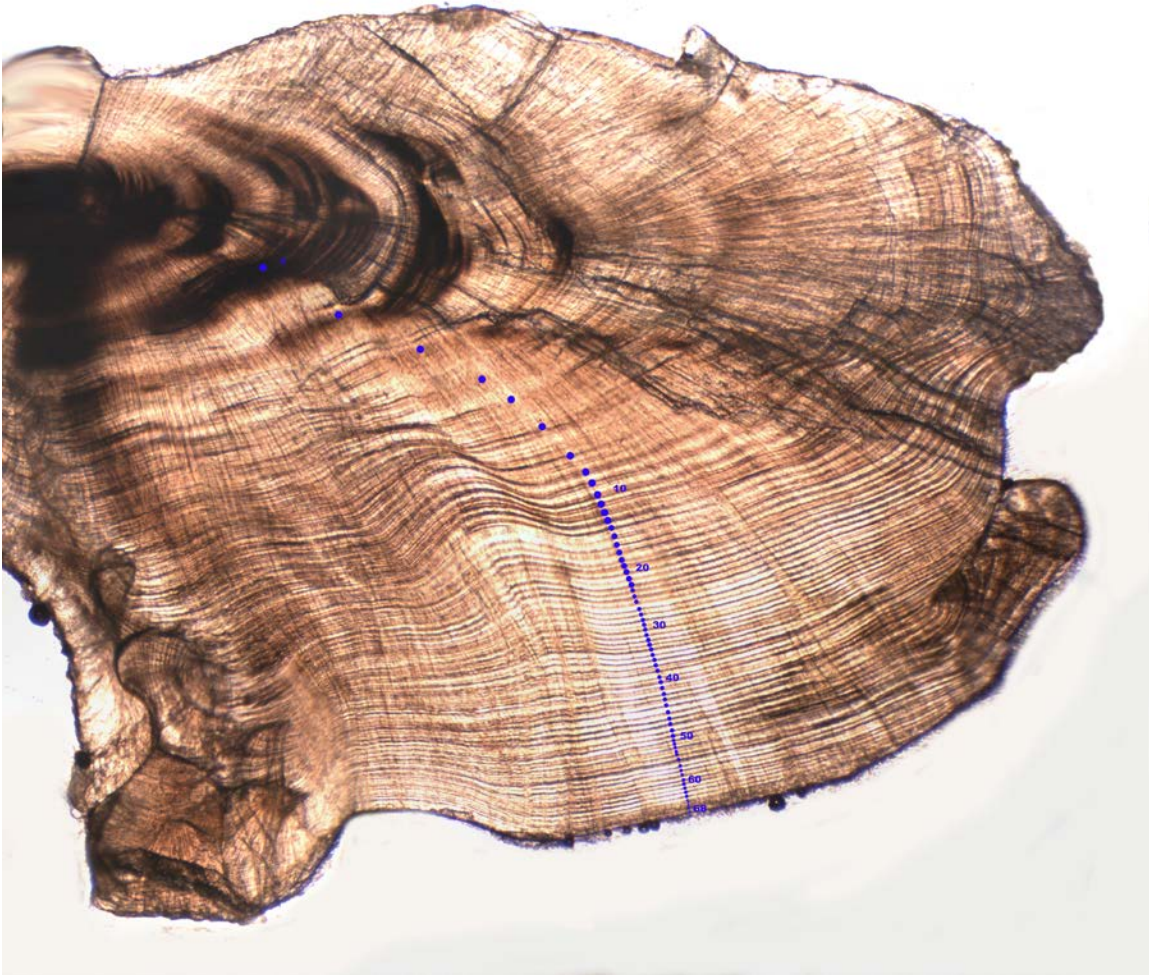


Figure 1. Thin-section of the otolith from a 68-year old female vermilion rockfish showing the annuli (dots).

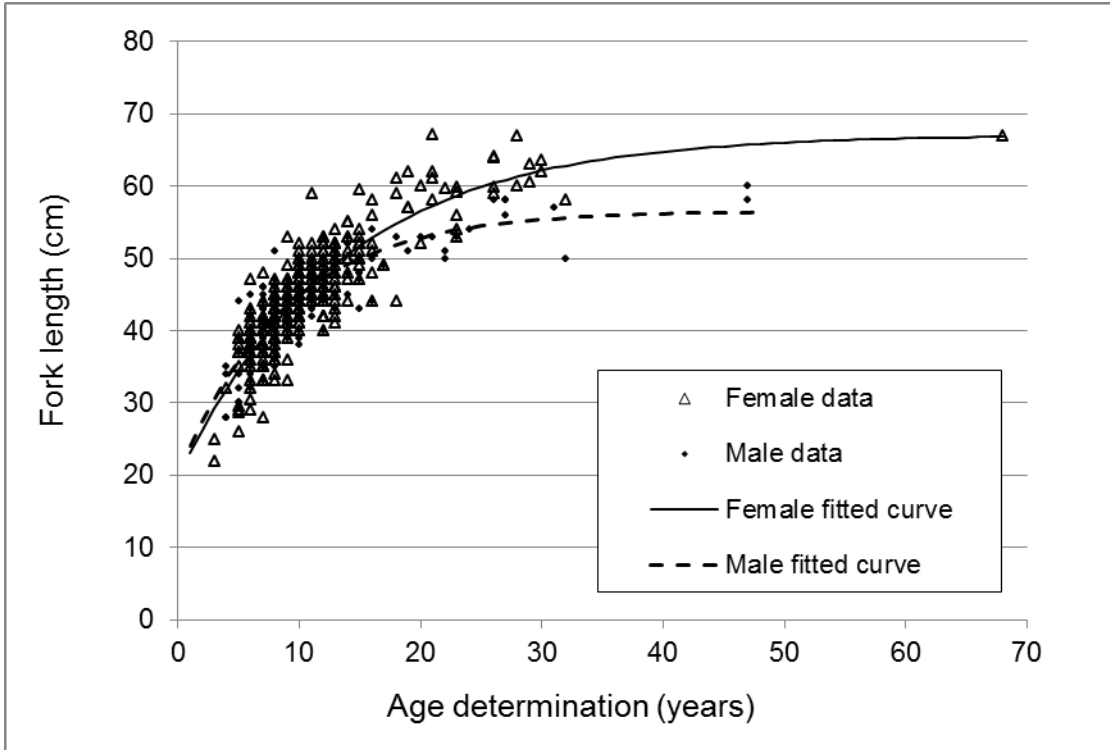


Figure 2. Length-at-age data for male and female vermilion rockfish (*Sebastes miniatus*) and fitted growth curves using the standard von Bertalanffy growth function.

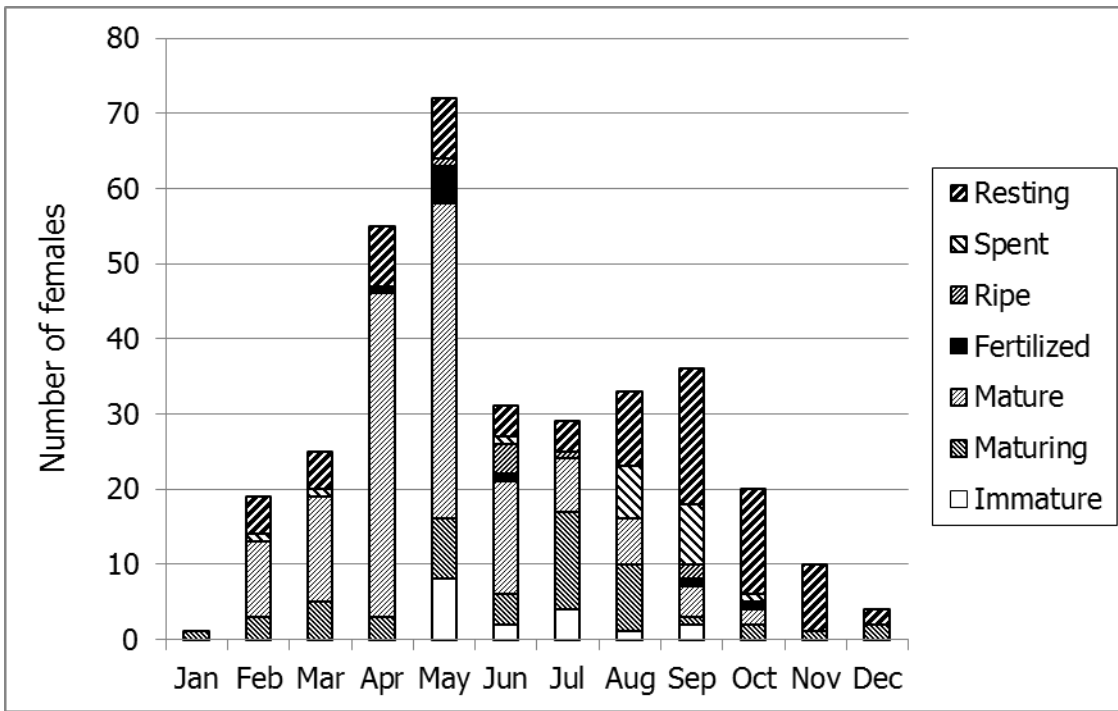


Figure 3. Number of female vermilion rockfish sampled, by visual maturity stage (Table 1) and month.

Table 3. Numbers of female vermilion rockfish maturity (M) and histology (H) samples collected and processed, by month and macroscopic maturity stage (Table 1), 2000-2011.

Maturity stage Month	Immature		Maturing		Mature		Fertilized		Ripe		Spent		Resting		Total	
	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H
January	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
February	0	0	3	3	10	8	0	0	0	0	1	1	5	5	19	17
March	0	0	5	5	14	13	0	0	0	0	1	1	5	5	25	24
April	0	0	3	1	43	37	1	0	0	0	0	0	8	5	55	43
May	8	7	8	6	42	41	5	0	1	1	0	0	8	8	72	63
June	2	2	4	4	15	14	1	1	4	0	1	0	4	2	31	23
July	4	4	13	12	7	5	0	0	1	0	7	0	4	3	29	24
August	1	0	9	5	6	3	0	0	0	0	8	2	10	6	33	16
September	2	2	1	0	4	0	1	0	2	0	1	0	18	6	36	8
October	0	0	2	1	2	2	1	0	0	0	0	0	14	7	20	10
November	0	0	1	0	0	0	0	0	0	0	0	0	9	8	10	8
December	0	0	2	0	0	0	0	0	0	0	0	0	2	1	4	1
Total	17	15	52	37	143	123	9	1	8	1	19	4	87	56	335	237

Table 4. Comparison of macroscopic and microscopic determinations of maturity in female vermilion rockfish collected from Oregon waters for the months of February through July, 2000-2011. Fish in macroscopic stages 4-5 (Table 1) are excluded.

Month	Macroscopic classification		Microscopic classification		
	Condition	Number	Confirmed	Reclassified	Unknown
February	Immature	3	3	0	0
	Mature	14	14	0	0
March	Immature	5	3	2	0
	Mature	19	19	0	0
April	Immature	1	1	0	0
	Mature	42	35	7	0
May	Immature	13	11	2	0
	Mature	49	47	2	0
June	Immature	6	5	1	0
	Mature	16	9	7	0
July	Immature	16	12	2	2
	Mature	9	6	3	0
Total		193	165	26	2

Based on the final sample size of 210 fish with lengths and maturity determinations, female vermilion rockfish were 50% mature at a length of 39.4 cm (Table 5–6 and Figure 4) and were 100% mature at a length of 49.0 cm (Figure 4). Based on 208 of these that were successfully aged, female fish were 50% mature at an age of 7.5 y and 100% mature at an age of 13 y (Table 5–6 and Figure 4). The length range of the 6 females with ovaries undergoing abortive maturation was 38-45 cm and they ranged in age from 5 to 10, both consistent with female fish in the adolescent stage for this species (Figure 4).

Table 5. Number of female vermilion 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
22	1	0.00	3	2	0.00
23	0		4	1	0.00
24	0		5	9	0.33
25	1	0.00	6	14	0.14
26	1	0.00	7	22	0.36
27	0		8	23	0.52
28	1	0.00	9	22	0.77
29	1	0.00	10	25	0.92
30	0		11	12	1.00
31	0		12	21	0.95
32	2	0.00	13	18	1.00
33	5	0.20	14	10	1.00
34	1	0.00	15	8	1.00
35	4	0.50	16	4	1.00
36	5	0.20	17	2	1.00
37	8	0.25	18	2	1.00
38	6	0.17	19	3	1.00
39	8	0.38	20	1	1.00
40	11	0.45	21	3	1.00
41	10	0.70	22	0	
42	9	0.78	23	0	
43	13	0.85	24	0	
44	17	0.88	25	0	
45	14	0.86	26	2	1.00
46	14	0.93	27	0	
47	13	0.92	28	1	1.00
48	10	0.9	29	1	1.00
49	9	1.00	30	1	1.00
50	8	1.00	31	0	
51	4	1.00	32	1	1.00
52	11	1.00			
53	4	1.00			
54	2	1.00			
55	2	1.00			
56	2	1.00			
57	2	1.00			
58	2	1.00			
59	1	1.00			
60	1	1.00			
61	2	1.00			

Table 5 (continued). Number of female vermilion 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
62	3	1.00			
63	1	1.00			
64	1	1.00			
Total	210			208	

Table 6. Results of logistic regression analysis of maturity status of female vermilion rockfish versus length (cm) and age (y).

Independent Variable	Coefficients	Standard error	P-value	L ₅₀ or A ₅₀	95% Confidence Limits
Length				39.4 cm	±0.086
Constant	-14.672	2.344	0.0001		
Length	0.372	0.057	0.0001		
Age				7.54yrs	±0.037
Constant	-6.282	1.069	0.0001		
Age	0.833	0.130	0.0001		

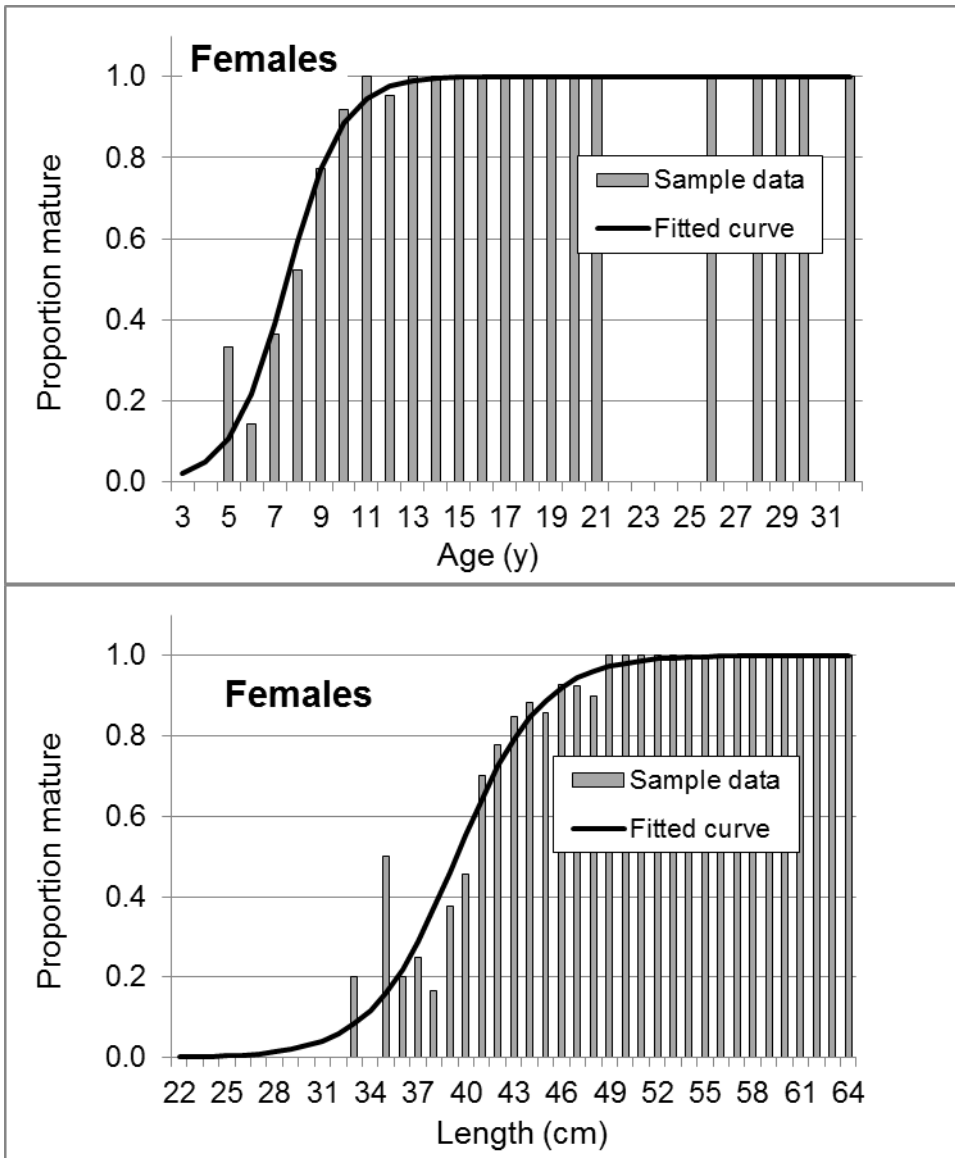


Figure 4. Proportion mature by age (upper panel) and length (lower panel) showing fitted maturity curves (Table 6).

Discussion

Vermilion rockfish in Oregon waters illustrate one of the difficulties of developing assessment models, sustainable catch limits or even basic life history information for species that are only a minor component of commercial and recreational fisheries. Due to the low frequency of vermilion rockfish in fishery catches, our development of new age, growth and female maturity data in this study required combining data from over a decade of age, length and maturity sampling (2000-2011) of fish landed in ports ranging from Brookings, Oregon all the way to Depoe Bay, Oregon.

The age and growth data presented here for vermilion rockfish may be the first to be developed from age estimates generated by the break and burn technique for this species. As a result, it is problematic to compare our Von Bertalanffy curves with those provided by Lea et al. (1999, whole otoliths) and Phillips (1964, scales) for central California. Ages from surface reads of whole otoliths and those from scales will typically underestimate the age of rockfishes (Chilton and Beamish 1982), biasing the estimation of growth curve parameters. The oldest vermilion rockfish encountered in this study, a 68 year old female, is 8 years older than the maximum age of 60 previously reported by Munk (2001).

The lack of strong seasonal synchrony in reproductive development of Oregon female vermilion rockfish is similar to findings for other rockfish species encountered in Oregon's nearshore waters that release larvae in the spring and summer. These include quillback (*Sebastes maliger*), china (*S. nebulosus*, Hannah and Blume 2011) and yelloweye (*S. ruberrimus*, Hannah et al. 2009) rockfish. Conversely, nearshore Oregon rockfish species like black rockfish (*S. melanops*), that release larvae in winter, tend to be more synchronous (Bobko and Berkeley 2004). Asynchronous development, in turn, is associated with difficulties in picking a narrow, optimal seasonal "window" for evaluating female maturity, as well as with poor accuracy in the visual staging of ovaries (Hannah et al. 2009, Hannah and Blume 2011). In species with poor seasonal synchronization of ovary development, samples from nearly any month will include some female fish that have just barely begun vitellogenesis, as well as some fish that have already entered the resting stage. A narrow seasonal window centered on synchronous fertilization can minimize the frequency of both of these types of fish, thus increasing the accuracy of visual staging.

Our data show that female vermilion rockfish in Oregon waters reach 50% maturity at about 39 cm FL and an age of 7.5 y. This is larger and older than specimens collected from northern and central California that were 50% mature at 37 cm (total length, TL) and an age (surface otolith reads) of 5 y (Wyllie Echeverria 1987).

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