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Age, growth and female length and age at maturity of redbanded rockfish (*Sebastes babcocki*) from Oregon waters

Age, growth and female length and age at maturity of redbanded rockfish (*Sebastes babcocki*) from Oregon waters

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Introduction

This is the tenth 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 from Oregon waters. This report describes the development of histologically-based length- and age-at-maturity data for female redbanded rockfish (*Sebastes babcocki*). Age and growth data for male and female redbanded rockfish from Oregon waters are also included.

Methods

Redbanded rockfish sampled for this study were obtained from three sources. These included regular dockside sampling of Oregon's commercial bottom trawl fishery catch in Newport and Astoria, Oregon, opportunistic sampling during chartered research trawl trips and sampling of landed bycatch from the International Pacific Halibut Commission's Pacific halibut (*Hippoglossus stenolepis*) longline surveys.

All fish sampled were measured (cm, fork length) and sexed. 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.

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" we 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 in the selected months, 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 4-6 were considered unambiguously mature, with one exception. One specimen classified as stage 6 that was only 30 cm was excluded from the analysis because all the other females observed in stages 4-6 were 36 cm or larger, sugggesting an error in staging or length measurement of this single specimen.

To attain the most accurate maturity classification, we microscopically evaluated all stage 1, 2, 3 and 7 ovaries from the selected months (Wallace and Selman 1981, Wyllie Echeverria 1987). A few stage 6 ovaries were also evaluated microscopically to verify the accuracy of classifying this macroscopic 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 first 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.

Accurate classification of the maturity status of female rockfishes is complicated by the high prevalence of abortive maturation in adolescent females (Nichol and Pikitch 1994, Hannah and Parker 2007, Thompson and Hannah 2010). We use the term "adolescent females" to refer to those in the length or age classes near the inflection point of the maturity ogive (Hannah and Parker 2007). These fish often develop oocytes to an early vitellogenic stage and then abort the maturation process via mass atresia of the developing oocytes. Older or larger females of some rockfish species also sometimes "skip spawn", however these females are frequently treated as mature for the prupose of estimating age or length at 50% maturity (Hannah and Parker 2007). Most studies of maturity in rockfish have utilized the presence of vitellogenin, detected either directly via histology or indirectly through macroscopic staging, as one conclusive indicator of a mature female. However, if all adolescent females with evidence of some degree of vitellogenisis are treated as mature, while some would have ultimately failed to complete the maturation process, the age or length at 50% maturity can be underestimated. In several histology-based studies of female rockfish maturity, this difficulty was partially addressed by only classifying fish with early vitellogenic oocytes as mature when they also lacked evidence of mass atresia (Hannah and Parker 2007, Hannah and Blume 2011, Hannah 2014, Hannah et al. 2015). Another approach to address this problem was used by Chilton (2010). In that study of female northern rockfish (*Sebastes variabilis*) maturity, fish with oocytes that had reached the migratory nucleus stage were treated as mature, but those with less-developed vitellogenic oocytes were treated as immature. Another approach that has been suggested is to classify rockfish with developing oocytes as mature or immature based on the proportion of vitellogenin in the cytoplasm and the measured frequency of atretic cells (M. Head, personal communication, July 2015). This last approach has been referred to as "functional maturity" as opposed to "biological maturity", which is based on the presence of vitellogenin. In all of these approaches, females with ovaries showing post-ovulatory follicles, other definite indicators of completed parturition or more advanced oocyte development (fertilized oocytes, larvae present) were also treated as mature.

In this study, we present female redbanded rockfish maturity data using two different systems for classifying adolescent females, which we will refer to as "vitellogenin-based" and "functional" maturity (Table 2). In both classification systems, most fish were classified as mature or immature in the same manner. 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 classified as mature. Fish with non-vitellogenic oocytes that appeared well organized were classified as immature. Those 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. Evidence of abortive maturation, characterized by mass atresia of the developing class of oocytes from a vitellogenic stage, was also noted at this time, and these fish were classified as immature, unless they were notably larger or older than the adolescent phase, in which case they were treated as mature (Hannah and Parker 2007). For vitellogenin-based maturity, ovaries of adolescent fish with vitellogenic oocytes, but without mass atresia, were treated as mature. For functional maturity, adolescent fish with oocytes in which at least 50% of the cytoplasm was filled with vitellogenin were treated as mature, while fish with less developed vitellogenic oocytes were treated as immature (Table 2).

We evaluated redbanded rockfish maturity in this study as a function of both length and age. Ages of redbanded rockfish were determined using the break and burn technique applied to sagittal otoliths (Chilton and Beamish 1982). Age and length data for both male and female fish were fitted to the 3-parameter von Bertalanffy growth function using non-linear least squares regression in JMP 6.0:

$$L_t = L_\infty(1-\exp(-K(t-t_0))),$$

where L_t is the mean fork length (cm) at age t, L_∞ the theoretic asymptotic length, K the Brody growth coefficient and t_0 the theoretical age at zero length. Logistic regression was used to fit sigmoid curves to the proportion mature by length and age, in the form,

$$p_{x_1} = e^{(b_0+b_1x_1)}/(1+e^{(b_0+b_1x_1)}) \text{ where,}$$

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,

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

Results

Female maturity

Sample collection from 2004-2014 resulted in macroscopic maturity stage data for 342 female redbanded rockfish (Table 3), with most of the samples from the months of April through August. Histology samples collected and processed for microscopic evaluation of female maturity totaled 212, including 5 females for which a macroscopic maturity code was not assigned (Table 3).

The highest frequency of female redbanded rockfish with fertilized or ripe ovaries (Table 3, Figure 1) was observed in April and May, suggesting mostly late spring synchronous reproduction in Oregon waters. The perisistence of some spent fish through October, along with a few fish with fertilized or ripe ovaries in February, June and August indicate some variability in the timing of reproduction in redbanded rockfish (Figure 1). Based on the monthly distribution of these macroscopic maturity stages and the presence of mature (stage 3) females in February through July (Figure 1), further histological analysis of female maturity was conducted on samples from the months of February through August.

Microscopic examination of histology slides for three females with spent (stage 6) ovaries (Table 3) from June and July showed clearly identifiable post-ovulatory follicles, supporting our treatment of all stage 6 females as mature, except for the one small fish previously noted. No females were classified as "unknown" maturity, and no old or large "skip-spawners" were noted.

The final data set for evaluating female length at maturity consisted of 267 specimens for which maturity determinations were considered definitive (Tables 4 and 5). Vitellogenin-based maturity indicated mature females as small as 30 cm, while the functional maturity criteria indicated first maturity at 36 cm (Tables 4 and 5). Both approaches to classifying maturity showed 100% of females were mature at 49 cm (Tables 4 and 5). Logistic regression of vitellogenin-based maturity on length indicated female redbanded rockfish were 50% mature at 39.6 cm ($P<0.0001$). Using the functional maturity criteria increased the length at 50% maturity to 41.0 cm ($P<0.0001$, Table 6, Figures 3-5). The functional maturity criteria also produced a steeper maturity curve (Figure 4) and a better fit to the proportion mature, with an r-squared of 0.769 as compared to a value of 0.671 for the vitellogenin-based curve (Figure 2, Table 6).

The final data set for evaluating age at maturity consisted of 253 female redbanded rockfish with both acceptable ages and definitive maturity determinations (Tables 4 and 5). Using the vitellogenin-based approach to classifying maturity indicated an age at first maturity of 9 y versus 10 y for the functional maturity approach (Tables 4 and 5). Both approaches indicated an age at 100% maturity of 22 y for female redbanded rockfish (Tables 4 and 5). Logistic regression resulted in an age at 50% maturity of 13.8 y for the vitellogenin-based approach ($P<0.0001$) and an older age at 50% maturity of 14.6 y for the functional maturity approach ($P<0.0001$, Table 6, Figures 2-3). Using the functional maturity approach again resulted in a closer fit of the maturity curve to the proportion mature data (r-squared of 0.545 versus 0.482, Table 6, Figure 3), and also resulted in a slightly steeper ogive (Figure 4). Both approaches suggest a protracted adolescent period in redbanded rockfish of about 10-12 y (Tables 4 and 5).

Age and growth

The final data set for age and growth analysis totalled 100 male and 339 female redbanded rockfish (Table 7). Ages of male fish ranged from 3 y to 86 y, while females ranged in age from 4 y to 85 y (Table 7). Male and female redbanded rockfish grew at a similar rate at young ages, however, female fish reached a larger asytmptotic size (Table 7, Figure 5). Both males and females were slow growing, taking about 60-70 years to approach the asymptotic sizes of 58.9 and 63.8 cm, respectively (Figure 5).

Discussion

The functional maturity criteria produced higher estimates of length and age at 50% maturity for female redbanded rockfish than the criteria based on presence/absence of vitellogenin (Table 6). In both approaches, adolescent females with mass atresia were classified as immature, however the two approaches used different assumptions about the status of females showing only early vitellogenic oocytes. The vitellogenin-based approach assumes all of these females would have completed development through fertilization and parturition that year, while the functional maturity approach assumes that none would have completed development. The functional maturity approach seems to be preferable in that abortive maturation has been observed in adolescent females for quite a number of rockfish species (Nichol and Pikitch 1994, Hannah and Parker 2007, Hannah and Blume 2011, Thompsosn and Hannah 2010, Hannah 2014). Use of the functional maturity criteria also produced steeper maturity ogives and a better fit to the proportion mature as a function of length and age (Table 6, Figures 2-3).

While this is encouraging, the criteria we used for defining functional maturity are unproven, and were simply based on the degree of vitellogenic development at the point the fish was sampled, and the presence or absence of mass atresia. Some of the adolescent fish sampled with just a small amount of vitellogenin would probably have gone on to develop through fertilization and parturition, while some would likely have gone on to develop mass atresia. Additional research is needed to evaluate whether the probability of these adolescent females completing oocyte development can be reliably related to the degree of vitellogenic development seen in the most advanced oocyte.

The estimates of female length and age at 50% maturity generated in our study seem reasonable given the large variation in length and age at maturity that has been noted for this species across its range (Love 2011). In California waters, females were shown to be 50% mature at 34 cm (TL) and 4 y (Wyllie Echeverria 1987). In British Columbia, females were 50% mature at 42 cm and 18.7 y (Love 2011, Haigh and Starr 2006). Our estimates for Oregon waters fall between these values, with 50% maturity (functional) at 41 cm and 14.6 y (Table 6). Our estimates also agree reasonably well with a prior estimate of length at 50% maturity for Oregon waters from Barss (1989) of 43 cm (FL) based on macroscopic staging of ovaries from a sample of just 68 females.

Our estimates of age and growth parameters (Figure 5) and female age at 50% maturity Table 6) confirm that in Oregon waters, redbanded rockfish are a very slow growing and late-maturing species, with a protracted adolescent phase. Our data for functional maturity indicate that the female adolescent phase encompasses 12 y in redbanded rockfish (Table 5), a value comparable to other slow-growing slope rockfishes like aurora rockfish (*Sebastes aurora*), with an adolescent period lasting about 15 y (Thompson and Hannah 2010). The age of 100% maturity in redbanded rockfish is about 22 y, also comparable to the value of about 25 y for aurora rockfish (Thompson and Hannah 2010).

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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

Table 2. Comparison of maturity status determination of ovaries with specific features using the vitellogenin-based and functional maturity criteria (see text).

Microscopic ovary features	Size or age	Status	
		Vitellogenin-based	*Functional maturity*
Well-organized non-vitellogenic oocytes	Adolescent or smaller/younger	Immature	Immature
Mass atresia, no post-ovulatory follicles	Adolescent	Immature	Immature
Vitellogenic oocytes with vitellogenin filling less than 50% of the cytoplasm	Adolescent	Mature	Immature
Vitellogenic oocytes with vitellogenin filling 50% or more of the cytoplasm	Adolescent	Mature	Mature
Mass atresia or small non-vitellogenic oocytes, no post-ovulatory follicles or residual larval eye pigment (skip spawner)	Adult	Mature	Mature
Post ovulatory folicles or residual larval eye pigment	All	Mature	Mature

Table 3. Numbers of female redbanded rockfish maturity (M) and histology (H) samples collected and processed, by month and macroscopic maturity stage (Table 1), 2004-2014.

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	1	1	0	0	0	0	0	0	0	0	0	0	1	1
February	7	7	3	3	3	3	1	0	0	0	0	0	1	0	15	13
March	3	3	6	6	1	1	0	0	0	0	0	0	0	0	10	10
April	4	3	6	6	14	2	11	0	30	0	1	0	3	3	69	14
May	54	50	37	29	2	2	5	1	6	0	16	0	3	3	123	85
June	13	12	3	3	3	2	1	0	1	0	7	2	2	2	30	21
July	3	0	3	3	3	3	0	0	0	0	1	1	3	3	13	10
August	19	14	11	11	0	0	0	0	1	0	16	0	5	5	52	30
September	6	6	1	1	0	0	0	0	0	0	0	0	2	1	9	8
October	0	0	4	2	1	1	0	0	0	0	1	0	8	6	14	9
November	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
December	0	0	3	3	2	2	0	0	0	0	0	0	0	0	5	5
Total	110	96	78	68	29	16	18	1	38	0	42	3	27	23	342	207[1]

[1] Note that histology samples were collected from 5 female redbanded rockfish, not included in this table, for which no macroscopic stage was assigned.

Table 4. Number of female redbanded rockfish used in determining age and length at maturity and proportion mature, by length (cm) and age (y) using vitellogenin-based microscopic maturity determinations (see text).

Length (cm)	Number sampled	Proportion mature	Age (y)	Number sampled	Proportion mature
23	1	0.00	6	1	0.00
24	1	0.00	7	5	0.00
25	0	--	8	20	0.00
26	1	0.00	9	16	0.06
27	4	0.00	10	32	0.09
28	2	0.00	11	35	0.17
29	10	0.00	12	26	0.38
30	17	0.06	13	13	0.54
31	9	0.00	14	7	0.57
32	19	0.00	15	9	0.56
33	16	0.06	16	12	0.83
34	13	0.08	17	4	0.25
35	6	0.00	18	7	1.00
36	14	0.14	19	9	0.90
37	10	0.30	20	6	1.00
38	7	0.14	21	10	0.89
39	9	0.33	22	4	1.00
40	5	0.60	23	3	1.00
41	8	0.63	24	3	1.00
42	9	0.78	25	2	1.00
43	11	0.91	26	8	1.00
44	8	1.00	27	1	1.00
45	7	0.86	28	2	1.00
46	5	1.00	29	2	1.00
47	8	1.00	30	1	1.00
48	12	0.92	31	0	
49	7	1.00	32	0	
50	4	1.00	33	1	1.00
51	10	1.00	34	0	
52	3	1.00	35	0	
53	5	1.00	36	0	
54	5	1.00	37	0	
55	2	1.00	38	1	1.00
56	1	1.00	39	3	1.00
57	2	1.00	40	1	1.00
58	7	1.00	41	1	1.00
59	1	1.00	42	2	1.00
60	1	1.00	43	0	

61	1	1.00	44	0	
62	1	1.00	45+	6	1.00
63	4	1.00			
64		--			
65	1	1.00			
Total	267			253	

Table 5. Number of female redbanded rockfish used in determining age and length at maturity and proportion mature, by length (cm) and age (y) using functional maturity criteria for microscopic classification of maturity (see text).

Length (cm)	Number sampled	Proportion mature	Age (y)	Number sampled	Proportion mature
23	1	0.00	6	1	0.00
24	1	0.00	7	5	0.00
25	0	0.00	8	20	0.00
26	1	0.00	9	16	0.00
27	4	0.00	10	32	0.06
28	2	0.00	11	35	0.09
29	10	0.00	12	26	0.27
30	17	0.00	13	13	0.46
31	9	0.00	14	7	0.57
32	19	0.00	15	9	0.33
33	16	0.00	16	12	0.75
34	13	0.00	17	4	0.25
35	6	0.00	18	7	1.00
36	14	0.07	19	9	0.89
37	10	0.20	20	6	1.00
38	7	0.00	21	10	0.90
39	9	0.11	22	4	1.00
40	5	0.20	23	3	1.00
41	8	0.50	24	3	1.00
42	9	0.78	25	2	1.00
43	11	0.82	26	8	1.00
44	8	1.00	27	1	1.00
45	7	0.86	28	2	1.00
46	5	1.00	29	2	1.00
47	8	1.00	30	1	1.00
48	12	0.92	31	0	--
49	7	1.00	32	0	--
50	4	1.00	33	1	1.00
51	10	1.00	34	0	--
52	3	1.00	35	0	--
53	5	1.00	36	0	--
54	5	1.00	37	0	--
55	2	1.00	38	1	1.00
56	1	1.00	39	3	1.00
57	2	1.00	40	1	1.00
58	7	1.00	41	1	1.00
59	1	1.00	42	2	1.00
60	1	1.00	43	0	--
61	1	1.00	44	0	--
62	1	1.00	45+	6	1.00

63	4	1.00	
64			
65	1	1.00	
Total	267		253

Table 6. Results of logistic regression analysis of maturity status of female redbanded rockfish versus length (cm) and age (y). Results are shown for vitellogenin-based maturity and functional maturity (see text).

Independent variable		Coefficients	Standard error	P-value	L_{50} or A_{50}	95% confidence limits	Coefficient of determination
Vitellogenin-based							
Length							
	Constant	-19.027	2.377	0.0001			
	Length	0.480	0.060	0.0001			
	Full model			0.0001	39.63 cm	±0.06	0.671
Age							
	Constant	-6.917	0.856	0.0001			
	Age	0.500	0.066	0.0001			
	Full model			0.0001	13.82 y	±0.05	0.482
Functional maturity							
Length							
	Constant	-26.596	3.935	0.0001			
	Length	0.649	0.096	0.0001			
	Full model			0.0001	40.96 cm	±0.05	0.769
Age							
	Constant	-7.849	0.939	0.0001			
	Age	0.537	0.069	0.0001			
	Full model			0.0001	14.61 y	±0.05	0.545

Table 7. Parameter estimates (± standard error) for the standard von Bertalanffy growth formula fitting fork length (cm) against age for male and female redbanded rockfish. 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_∞	63.780 (0.946)	58.906 (1.124)
K	0.061 (0.003)	0.062 (0.005)
t_0	-1.997 (0.482)	-2.8291 (0.676)
N	339	100
Age range	4-85	3-86

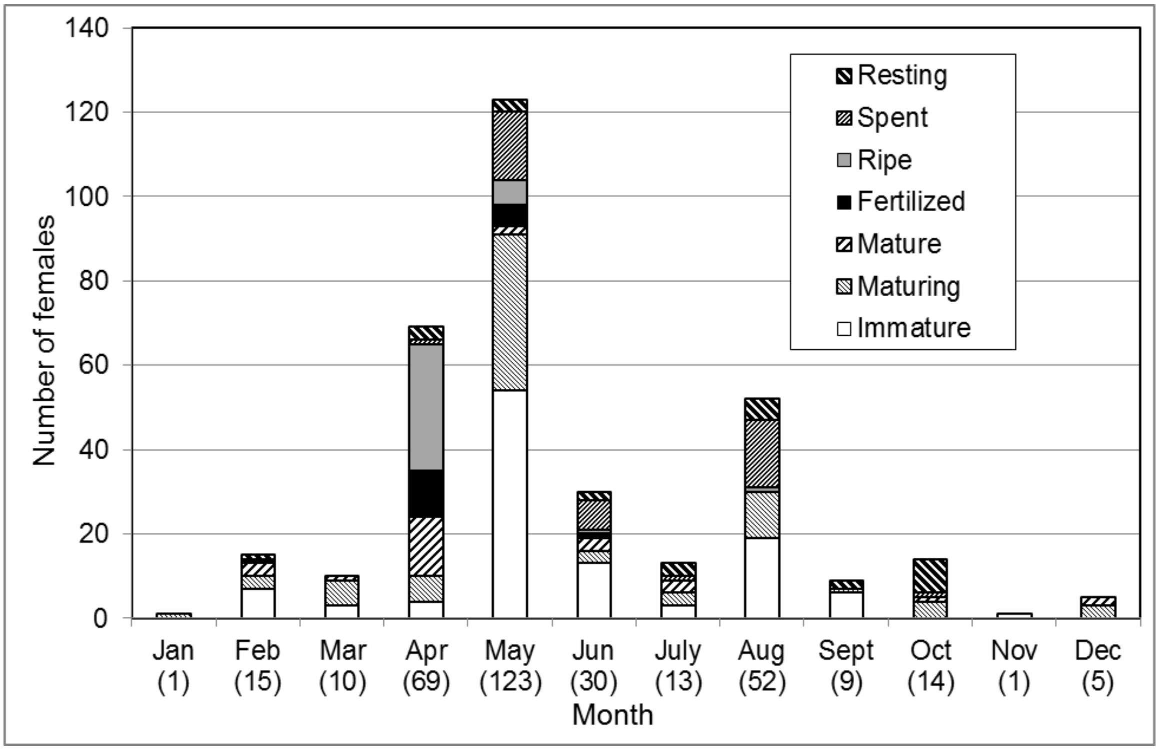

Figure 1. Number of female redbanded rockfish sampled by macroscopic maturity stage (Table 1) and month, 2004-2014.

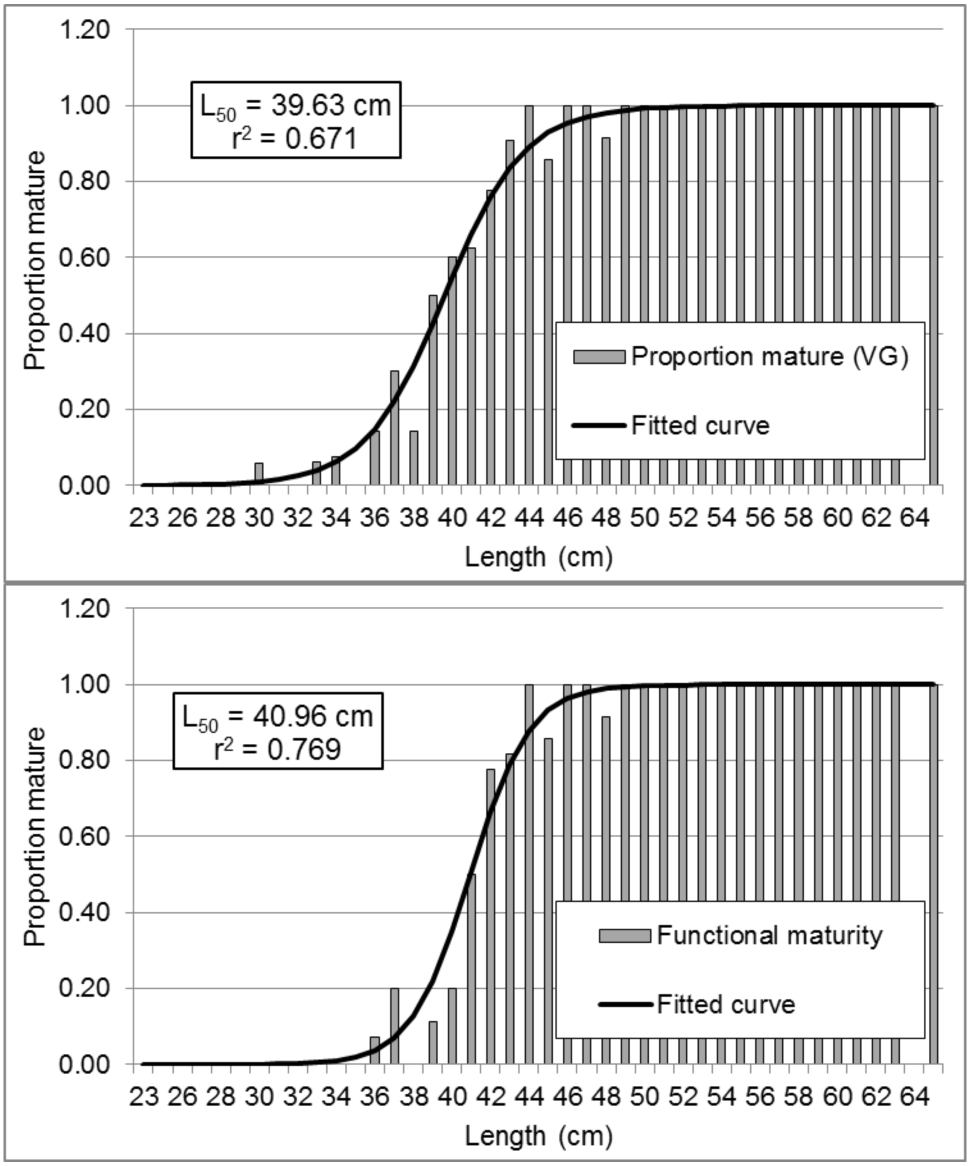

Figure 2. Proportion of mature female redbanded rockfish as a function of length (cm), using vitellogenin-based microscopic maturity criteria (upper panel, see text) and functional maturity criteria (lower panel), with fitted logistic curves.

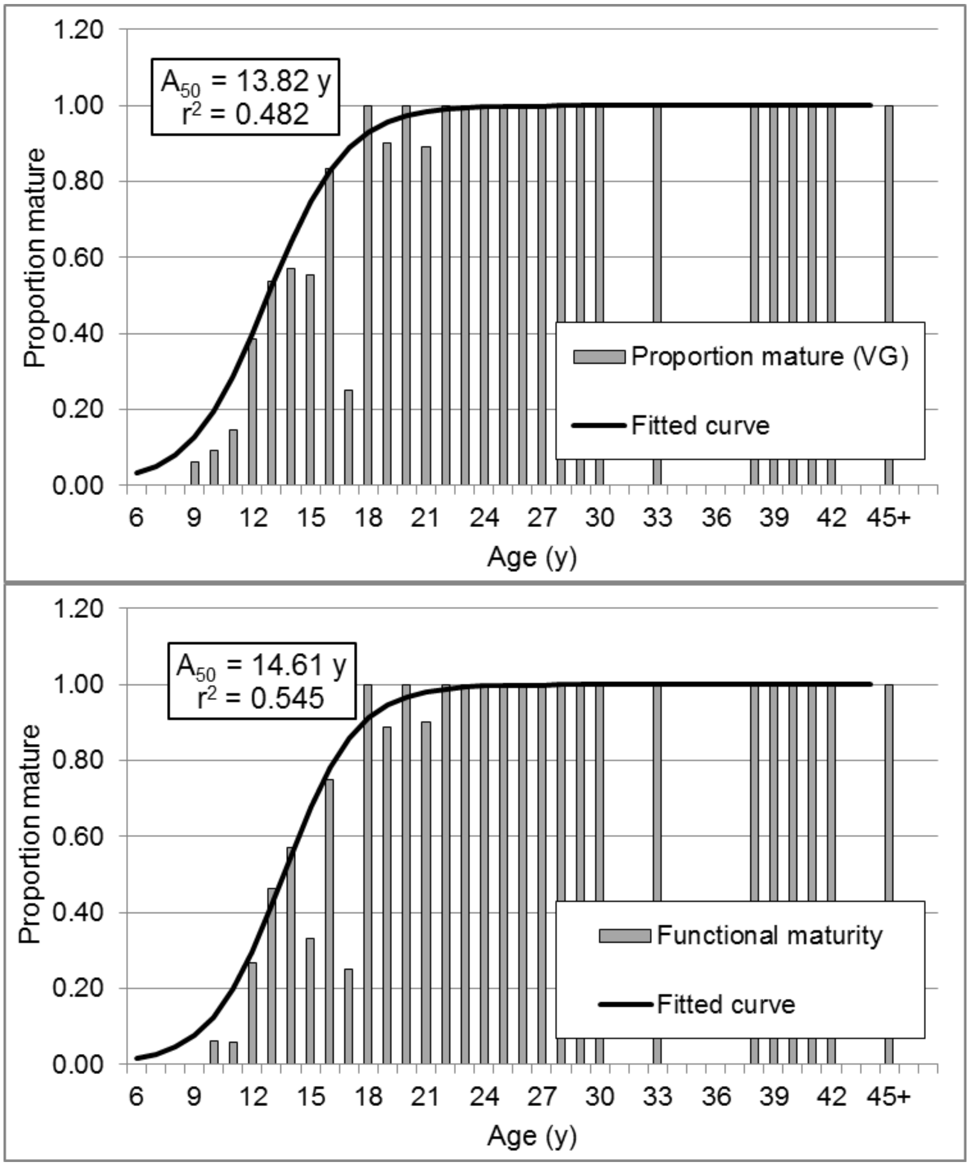

Figure 3. Proportion of mature female redbanded rockfish as a function of age (y), using vitellogenin-based microscopic maturity criteria (upper panel, see text) and functional maturity criteria (lower panel), with fitted logistic curves.

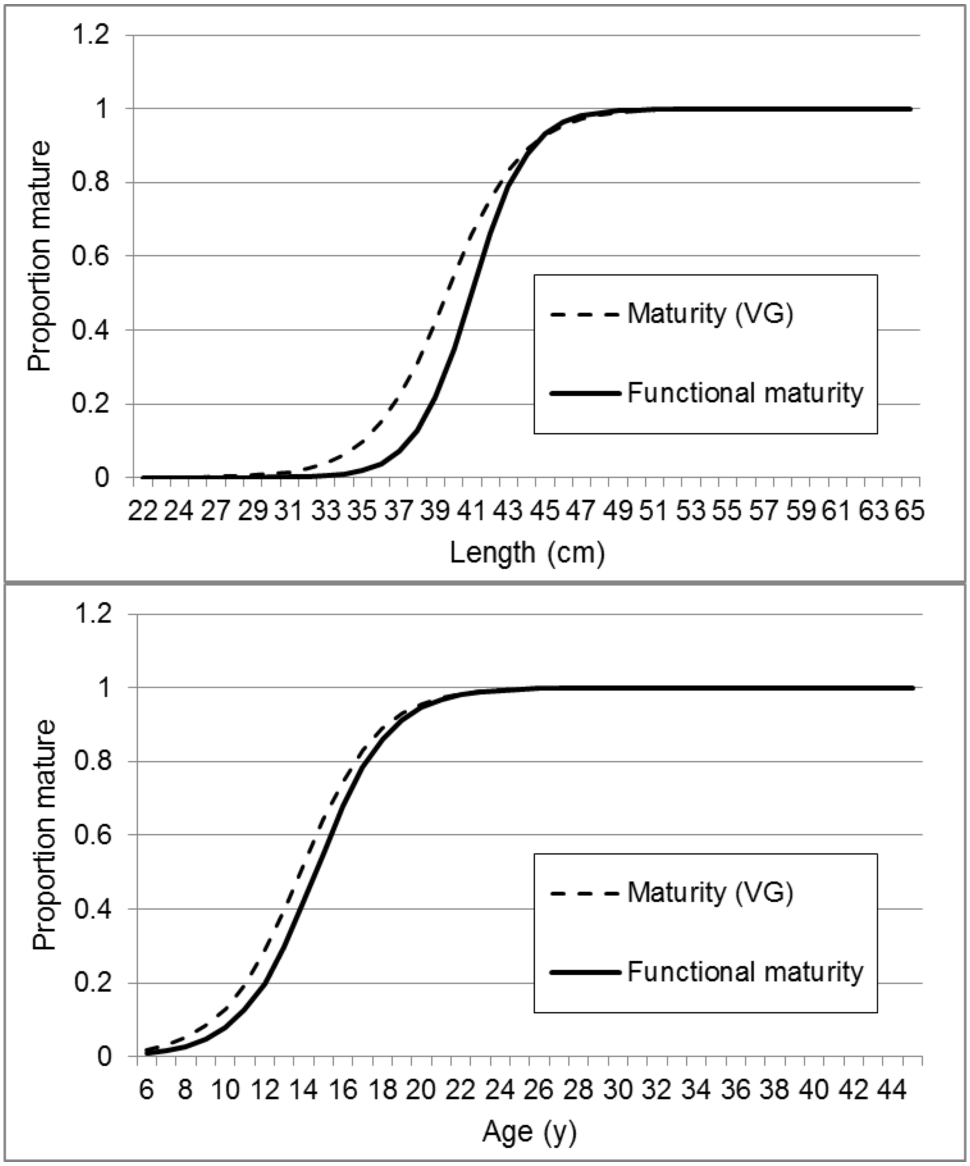

Figure 4. Comparison of length (cm, upper panel) and age (y, lower panel) at maturity curves resulting from using vitellogenin-based (dashed line) and functional maturity criteria (solid line, see text) for classification of adolescent females with incomplete vitellogenesis.

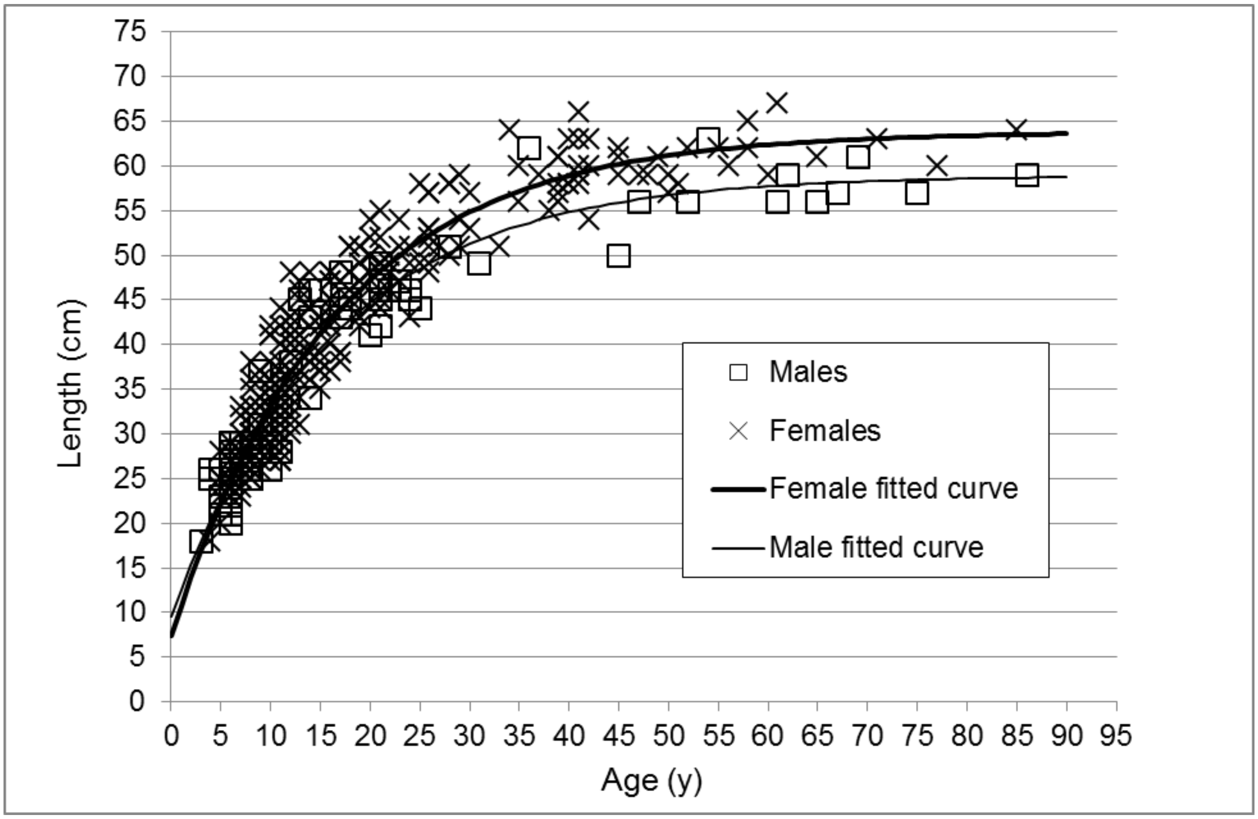

Figure 5. Length (cm) versus age (y) for male and female redbanded rockfish and fitted von Bertalanffy growth curves.

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