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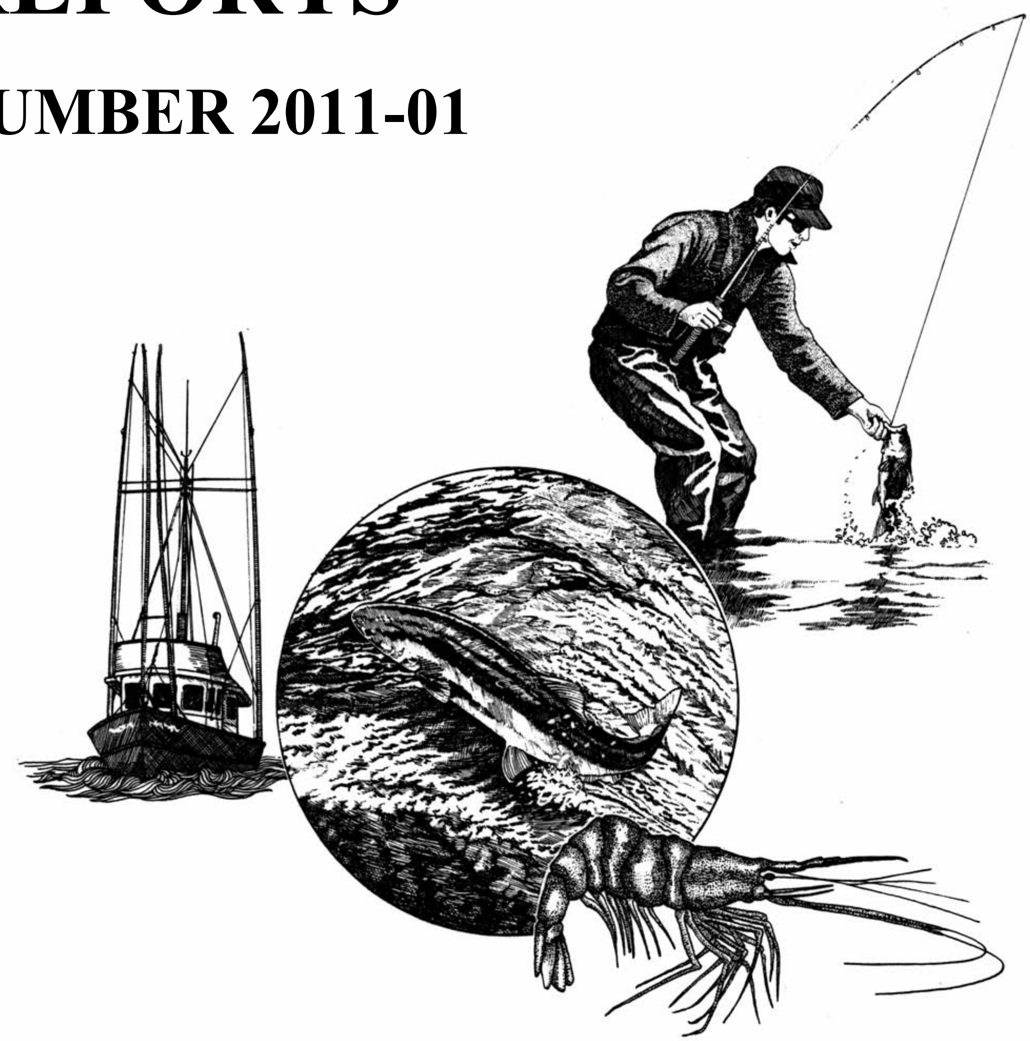

FISH DIVISION
Oregon Department of Fish and Wildlife

Maturity of female quillback (*Sebastes maliger*) and china rockfish (*S. nebulosus*) from Oregon waters based on histological evaluation of ovaries

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Introduction

Since 2000, ODFW's Marine Resources Program has been collecting female maturity data on a variety of groundfish species in an effort to improve the maturity data available for use in stock assessments (e.g. Hannah et al. 2002, Hannah and Parker 2007). For many U.S. west coast groundfish stocks, data on female age and length at maturity is of questionable quality. The maturity curves currently used in some stock assessments are based on macroscopic (visual) assessment of ovary condition. However, there is strong evidence 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). We report here on the development of histologically-based length and age at maturity data for female quillback rockfish (*Sebastes maliger*) and length at maturity data for female china rockfish (*S. nebulosus*).

Methods

Quillback and china rockfish used for this study were sampled from Oregon's recreational fishery landings at the ports of Newport and Depoe Bay. All fish were measured (cm TL) and sexed and otoliths were collected for age determination. Maturity data were collected only from female fish. Each ovary was removed 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.

We evaluated quillback rockfish maturity in this study as a function of both length and age. For china rockfish, maturity was evaluated with regard to length only because the ages generated from china rockfish otoliths were not considered reliable. Ages of quillback rockfish were determined using the break and burn technique applied to sagittal otoliths (Chilton and Beamish 1982).

Female maturity for both species 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 approaching 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 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 4-6, were considered unambiguously mature. 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 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 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 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 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 calculating final age or length at maturity curves. 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 4-6), was evaluated by comparing the maturity status determined from the macroscopic and microscopic evaluations.

The timing of ovarian development of large or old, as opposed to small or young females of both species was evaluated by graphically and statistically (Kruskal-Wallis test) comparing the mean macroscopic stage, by month, for fish in macroscopic stages 3-6. For this analysis, the females sampled during the optimal seasonal "window" for determining maturity, that were also in macroscopic stages 3-6, were simply split into two groups, identified as either old or young or large or small, based on being above or below the median length or age of the samples.

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,

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

Results

Quillback rockfish

Sample collections resulted in a total of 666 maturity samples for female quillback rockfish, with most of the samples obtained from the months of February to October (Table 2). The seasonal distribution of females with ovaries in fertilized, ripe or spent condition (stages 4-6, Table 1) showed that ovarian development in quillback rockfish is not strongly synchronous. Fish with fertilized ovaries (stage 4) were collected in 5 different months, as were fish with ripe ovaries (stage 5). Females with spent ovaries (stage 6) were collected in 7 different months (Table 2, Figure 1). The distribution of these macroscopic stages suggests a broad seasonal peak in ovarian development and subsequent parturition, lasting from about February through at least August. We chose February through June as the "window" for examining length and age at maturity in this species because it bracketed the peak in stage 5 (ripe) ovaries in April and May (Figure 1) and also because the frequency of fish with stage 7 (resting) ovaries increased sharply in July (Figure 2). A total of 128 (70%) of the 184 histology samples prepared for quillback rockfish were from these 5 months (Table 2).

The accuracy of macroscopic staging for identifying maturity in female quillback rockfish was low (Table 3). Histology samples from females with stage 1, 2, 3 and 7 ovaries (Table 1), showed that even for the 5 months when macroscopic evaluations should have been most accurate, only 91 (72%) out of 128 were correctly identified as mature or immature, while 28 (22%) were reclassified after microscopic evaluation (Table 3). Most (24) of the females that were reclassified were fish identified macroscopically as immature that showed post-ovulatory follicles or residual larvae or larval eye pigment upon microscopic evaluation.

Six specimens showed evidence of abortive maturation via mass atresia of the developing class of oocytes. Five of these fish ranged in size from 30-33 cm and were considered to be adolescent fish. These specimens were treated as "immature". One 40 cm female with evidence of mass atresia was treated as "mature", equivalent to assuming it was a mature fish that was skip-spawning in the year of collection.

Nine of the 128 samples evaluated histologically from the February to June "window" surrounding the peak of parturition could not be confidently identified as mature or immature (Table 3). These specimens typically showed some evidence of reorganization and resorption within the ovary. However, they lacked clear evidence of either mass atresia of vitellogenic oocytes that would suggest an adolescent immature fish or post-ovulatory follicles or residual larvae or larval eye pigment that would confirm maturity. These specimens were excluded from further analysis of age and length at maturity, leaving a final sample size for female length and age at maturity of 221 and 217, respectively (Table 4).

The logistic function fit the proportion mature as a function of both length and age well (Figure 2, Table 5). Female quillback rockfish were 50% mature at 29.2 cm and 5.3 y of age, and were 100% mature at a length of about 34 cm and an age of about 12 y. Females classified in this study as either large (≥ 38 cm), or old (>12 y), consistently developed their ovaries earlier in the year than small or young fish (Figure 3), however, for both length and age, the difference was only statistically significant in the months of April and May ($P<0.01$).

China rockfish

Sample collections resulted in a total of 622 maturity samples for female china rockfish, with most of the samples obtained from the months of February to October (Table 6). The seasonal distribution of females with ovaries in fertilized, ripe or spent condition (stages 4-6, Table 6) showed that, similarly to quillback rockfish, ovarian development in china rockfish was not strongly synchronous. Fish with fertilized ovaries (stage 4) were collected in 6 different months and fish with ripe ovaries (stage 5) in 3 different months. Females with spent ovaries (stage 6) were collected in 7 different months (Table 6, Figure 4). The distribution of these macroscopic stages suggests a broad seasonal peak in ovarian development and subsequent parturition, lasting from about February through August. We chose March through July as the "window" for examining length at maturity in this species. July was included despite the high frequency of fish with stage 7 (resting) ovaries because small (<30 cm) china rockfish were not common in our samples and July samples included several small fish. A total of 182 (68%) of the 266 histology samples prepared for china rockfish were from these 5 months (Table 6).

The accuracy of macroscopic staging for identifying maturity in female china rockfish was also low (Table 7). Histology samples from females with stage 1, 2, 3 and 7 ovaries (Table 1), showed that even for the 5 months we chose as "optimum", when macroscopic evaluations should have been most accurate, only 120 (76%) out of 157 were correctly identified as mature or immature, while 19 (12%) were reclassified after microscopic evaluation and 18 (11%) could not be classified as mature or immature (Table 7). Ten of the eighteen fish that could not be classified were from July samples, showing the difficulty of accurately evaluating maturity, even using histology, once many females have entered the resting stage (Table 7). These "unknown" specimens were excluded from further analysis of length at maturity, leaving a final sample size for female length and age at maturity of 239 females (Table 8). As in quillback rockfish, most (18 out of 19) of the females that were reclassified were fish identified macroscopically as immature that showed post-ovulatory follicles or other definitive signs of maturity upon microscopic evaluation. Three specimens showed evidence of abortive maturation via mass atresia of the developing class of oocytes. These 3 fish ranged in size from 27-33 cm and were considered to be adolescent fish and treated as "immature". Noteworthy also for china rockfish was the high prevalence of black larval eye pigment as a residual marker of maturity in the ovarian thin-sections. Out of 138 females that were classified as mature based on histology, 83 (60%) were noted to show larval eye pigment.

The logistic function fit the proportion mature as a function of length fairly well (Figure 5, Table 5). Female china rockfish were 50% mature at 28.5 cm. and 100% mature at 36 cm. Females classified in this study as large (> 36 cm), did not consistently develop their ovaries earlier in the year than small fish (Figure 6, P>0.05).

Discussion

The relative inaccuracy of macroscopic staging for female quillback and china rockfish in stages 1-3 and 7 reported here is consistent with results for yelloweye rockfish, another species with weakly synchronized ovarian development (Hannah et al. 2009). This contrasts with the greater accuracy of macroscopic staging reported for Pacific ocean perch (*Sebastes alutus*, Hannah and Parker 2007) and aurora rockfish (*Sebastes aurora*, Thompson and Hannah 2010), species with more synchronous development. The difference in accuracy most likely results from greater variability in the size and color of both resting and developing ovaries at any single sampling time in species with asynchronous development. For rockfish with synchronous development that are sampled near the time of parturition, most females are in an unambiguous maturity state, either with large, well-developed ovaries or with fertilized eggs, visible larvae or an obviously spent ovary. Fish outside of these categories will then generally have a broad or possibly bimodal size distribution, some large fish in stage 7 and some smaller fish in stages 1 and 2. Under these circumstances, differences between resting and immature ovaries, in terms of size and color will be more obvious. One other factor that may have contributed to the low accuracy of macroscopic staging for china rockfish in our study was our choice of seasonal "window" for evaluating maturity. By including July samples in our analysis despite a high proportion of stage 7 (resting) ovaries, we may have reduced the apparent accuracy of macroscopic staging.

The length at 50% maturity determined for quillback rockfish in this study, 29.2 cm, is larger than reported for central California waters (26 cm, Wyllie Echeverria 1987). The age at 50% maturity however, 5.3 y, is younger than reported for central California waters (6 y, Wyllie Echeverria 1987). Haldorson and Love (1991) report a value from Rosenthal et al. (1982) of 36 cm for length at 50% maturity for quillback rockfish from southeastern Alaska waters. Love et al. 2002 report a length and age at 50% maturity from British Columbia waters of 29 cm and 11 y of age, about the same size, but much older than values determined for Oregon waters in our study. The length at 50% maturity of 28.5 cm determined here for china rockfish is slightly larger than the estimate for northern and central California of 27 cm from Wyllie Echeverria (1987).

The data presented here on the seasonal timing of ovarian development show that older or larger female quillback rockfish develop and release larvae earlier in the season than younger or smaller females (Figure 3). This is consistent with the findings of Sogard et al. (2008) that parturition date in black rockfish (*Sebastes melanops,* Bobko and Berkeley 2004), blue rockfish (*S. mystinus*), kelp rockfish (*S. atrovirens*) and yellowtail rockfish (*S. flavidus*) is earlier in larger females. It is also consistent with the findings of Nichol and Pikitch (1994) that larger darkblotched rockfish (*S. crameri*) females spawn earlier in the season than smaller fish. Conversely, a maternal size effect on the seasonal timing of parturition was not demonstrated for china rockfish in this study, consistent with findings for Pacific ocean perch (Hannah and Parker 2007), and gopher rockfish and olive rockfish (Sogard et al. 2008).

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Josie Thompson and William Barss provided ages for quillback rockfish. Field staff from ODFW's black rockfish PIT-tagging and ORBS projects asssisted with field sampling of quillback and china rockfish.

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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. Numbers of quillback rockfish maturity (M) and histology (H) samples collected and processed, by month and macroscopic maturity stage (Table 1), 2000-2009.

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	3	0	0	0	0	0	0	0	0	0	4	1
February	0	0	3	3	12	0	11	0	0	0	0	0	0	0	26	3
March	2	1	4	2	30	20	11	0	2	0	0	0	0	0	49	23
April	2	2	4	4	12	12	8	0	12	0	3	0	3	2	44	22
May	2	1	11	10	5	4	6	0	5	0	19	0	22	17	70	32
June	9	9	16	13	5	5	2	0	1	0	32	0	25	20	90	48
July	6	2	7	6	0	0	0	0	0	0	39	0	69	20	121	29
August	7	1	18	4	4	2	0	0	1	0	17	0	94	13	141	20
September	6	0	7	1	1	0	0	0	0	0	7	0	48	3	69	4
October	0	0	4	1	3	0	0	0	0	0	3	0	37	1	47	2
November	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
December	0	0	1	0	4	0	0	0	0	0	0	0	0	0	5	0
Total	34	16	76	45	79	43	38	0	21	0	120	4	298	76	666	184

Table 3. Comparison of macroscopic and microscopic determinations of maturity in female quillback rockfish collected from waters off Newport and Depoe Bay, Oregon, for the months of February through June, 2000-2009. Fish in macroscopic stages 4-6 (Table 1) are excluded.

	Macroscopic classification		Microscopic classification		
Month	Condition	Number	Confirmed	Reclassified	Unknown
February	Immature	3	0	3	0
	Mature	0	0	0	0
March	Immature	3	1	1	1
	Mature	20	20	0	0
April	Immature	6	1	5	0
	Mature	16	16	0	0
May	Immature	11	3	6	2
	Mature	21	17	3	1
June	Immature	22	8	9	5
	Mature	26	25	1	0
Total		128	91	28	9

Table 4. Number of female quillback 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
23	1	0.00	4	2	0.00
24	0	--	5	3	0.67
25	1	0.00	6	12	0.58
26	1	0.00	7	20	0.85
27	4	0.20	8	21	0.90
28	0	0.00	9	29	0.93
29	7	0.71	10	13	0.92
30	5	0.60	11	26	0.92
31	6	0.86	12	13	1.00
32	15	0.83	13	23	1.00
33	18	0.95	14	11	1.00
34	15	1.00	15	11	1.00
35	16	1.00	16	8	1.00
36	12	1.00	17	7	1.00
37	23	1.00	18	5	1.00
38	20	1.00	19	4	1.00
39	15	1.00	20	0	--
40	15	1.00	21	3	1.00
41	12	1.00	22	3	1.00
42	11	1.00	23	1	1.00
43	3	1.00	24	0	--
44	4	1.00	25	0	--
45	8	1.00	26	3	1.00
46	6	1.00	27	2	1.00
47	1	1.00	28	0	--
48	0	--	29	0	--
49	1	1.00	30	0	--
50	1	1.00	31	0	--
			32	1	1.00
			33	1	1.00
			34	0	--
			35+	2	1.00
Total	221			217	

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

Independent Variable		Coefficients	Standard error	P-value	L_{50} or A_{50}	95% Confidence Limits
Quillback rockfish						
Length					29.23 cm	±0.067
	Constant	-23.394	5.352	0.0001		
	Length	0.800	0.173	0.0001		
Age					5.27 yrs	±0.095
	Constant	-3.671	1.345	0.0063		
	Age	0.696	0.174	0.0001		
China rockfish						
Length	Constant	-13.320	2.917	0.0001	28.52 cm	±0.110
	Length	0.467	0.091	0.0001		

Table 6. Numbers of china rockfish maturity (M) and histology (H) samples collected and processed, by month and macroscopic maturity stage (Table 1), 2002-2010.

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	1	1	0	0	0	0	0	0	0	0	1	1
February	0	0	5	0	19	4	3	0	0	0	0	0	0	0	27	10
March	2	1	2	1	15	0	8	0	4	0	0	0	0	0	31	6
April	1	1	8	6	0	3	3	0	10	0	5	0	5	4	32	11
May	2	2	14	9	3	1	1	0	1	0	21	8	30	23	72	46 [a]
June	7	7	14	10	2	0	2	0	0	0	25	15	36	25	86	59 [a]
July	7	3	21	14	4	0	0	0	0	0	26	5	65	43	123	66 [a]
August	7	0	33	9	5	0	1	0	0	0	16	11	71	20	133	40
September	5	1	11	0	7	1	0	0	0	0	8	7	35	10	66	19
October	2	0	8	3	6	0	0	0	0	0	9	8	20	7	45	18
November	0	0	0	0	6	0	0	0	0	0	0	0	0	0	6	0
December	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	33	15	116	52	68	10	18	0	15	0	110	54	262	132	622	266[a]

[a] Total includes fish were that sampled histologically but not staged macroscopically.

Table 7. Comparison of macroscopic and microscopic determinations of maturity in female china rockfish collected from waters off Newport and Depoe Bay, Oregon, 2002-2010. Fish in macroscopic stages 4-6 (Table 1) are excluded.

	Macroscopic classification		Microscopic classification		
Month	Condition	Number	Confirmed	Reclassified	Unknown
March	Immature	2	2	0	0
	Mature	4	4	0	0
April	Immature	7	7	0	0
	Mature	4	3	0	1
May	Immature	11	3	7	1
	Mature	26	24	1	1
June	Immature	17	10	6	1
	Mature	26	22	0	4
July	Immature	17	8	5	4
	Mature	43	37	0	6
Total		157	120	19	18

Table 8. Number of female china rockfish used in determining length at maturity and proportion mature, by length (cm).

Length (cm)	Number sampled	Proportion mature
25	1	0.00
26	0	--
27	2	0.50
28	2	0.50
29	8	0.50
30	12	0.83
31	10	0.80
32	13	0.69
33	28	0.86
34	22	0.95
35	25	0.88
36	35	1.00
37	25	1.00
38	23	1.00
39	15	1.00
40	10	1.00
41	6	1.00
42	2	1.00
Total	239	

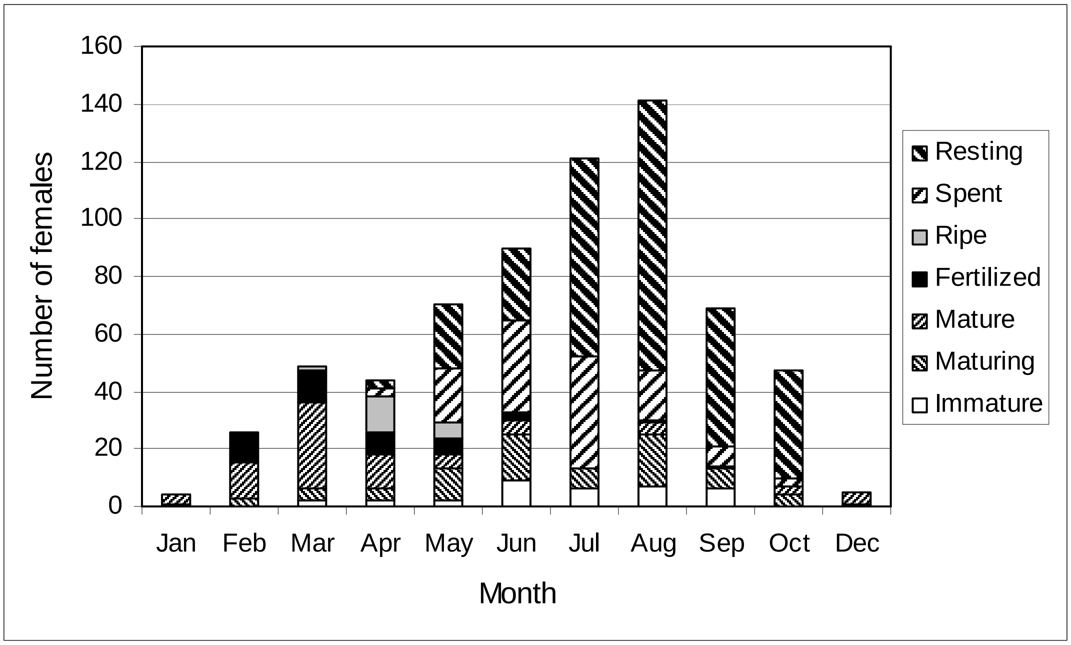

Figure 1. Number of female quillback rockfish sampled for maturity, by macroscopic (visual) maturity stage and month, 2000-2009.

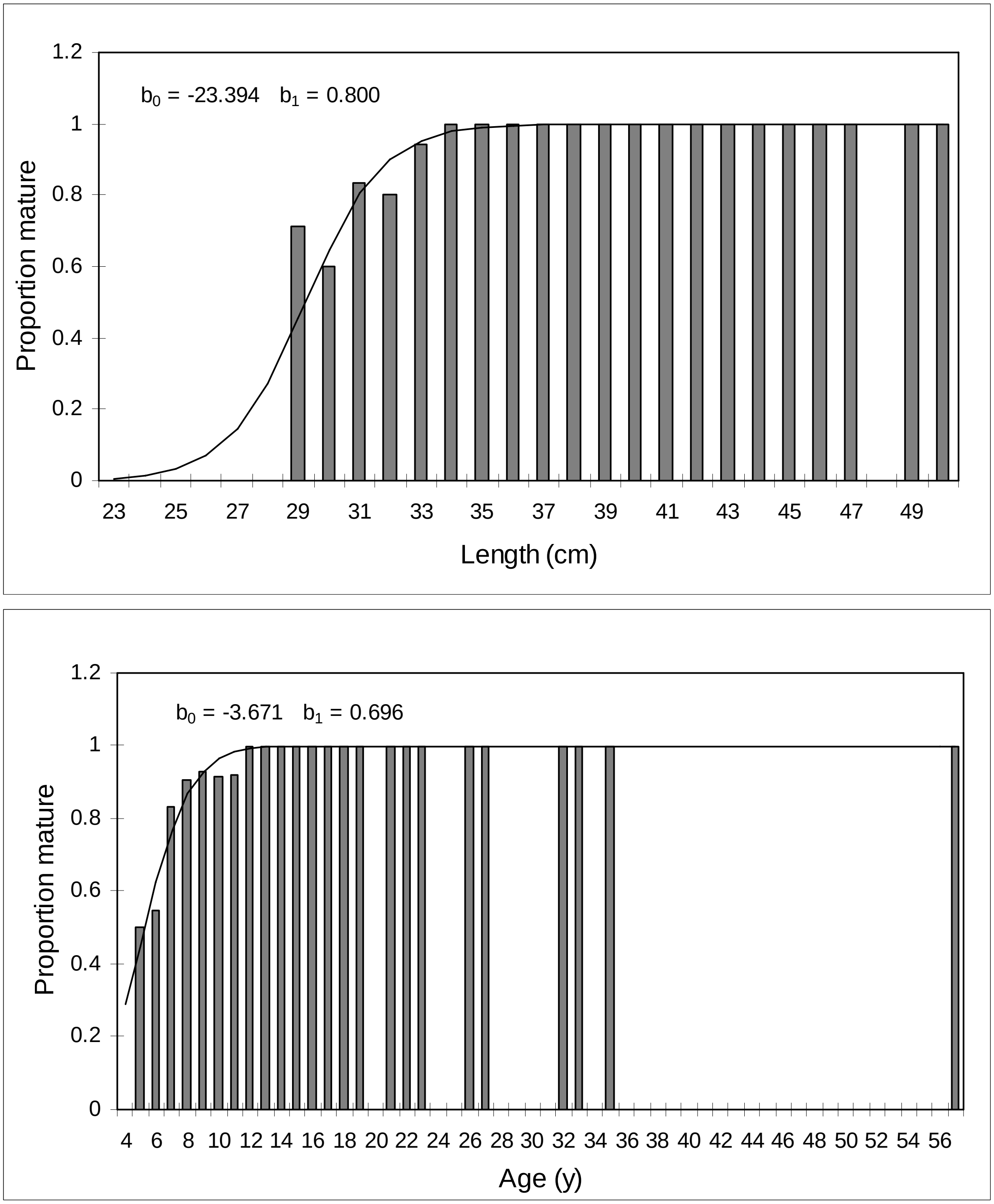

Figure 2. Proportion of female quillback rockfish that were mature, as a function of length (cm, upper panel) and age (y, lower panel) showing fitted logistic curves.

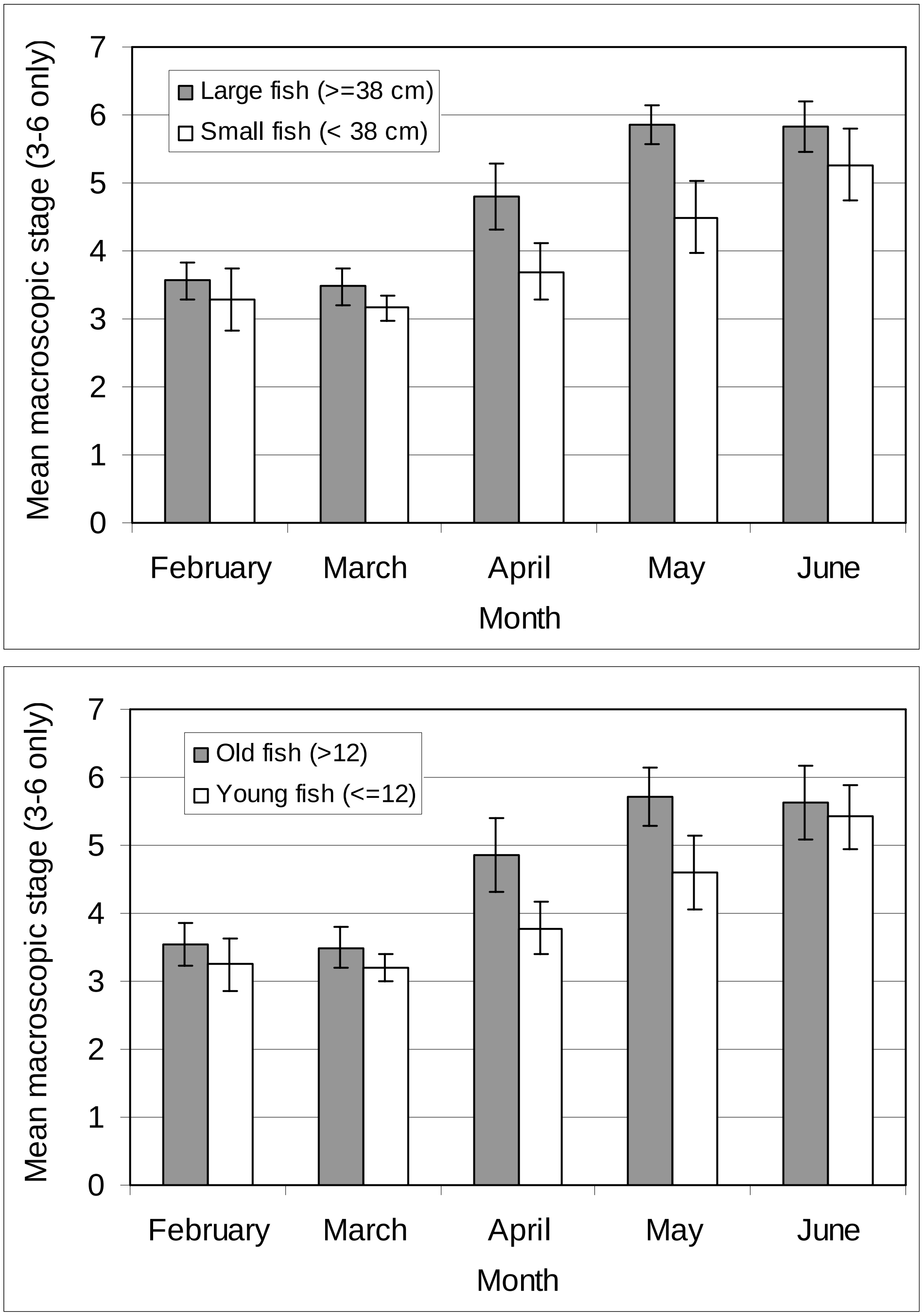

Figure 3. Comparison of mean maturity stage (includes only fish in macroscopic stages 3-6), by month, for large (≥38 cm) and small (upper panel), and old(>12) and young, female quillback rockfish.

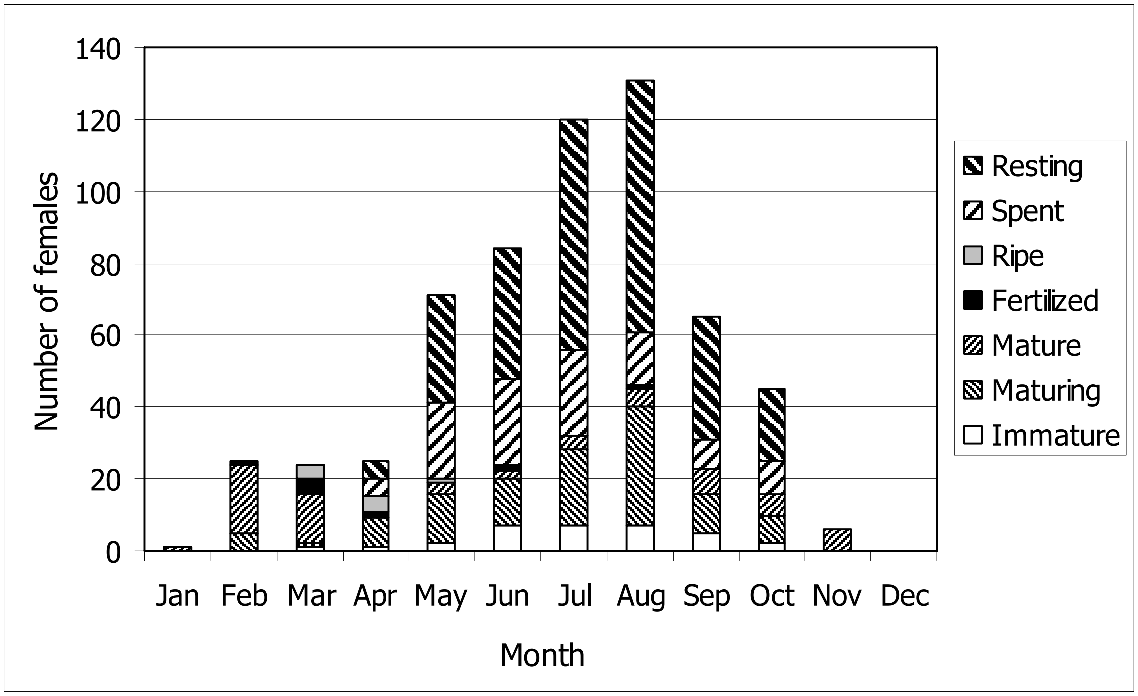

Figure 4. Number of female china rockfish sampled for maturity, by macroscopic (visual) maturity stage and month, 2002-2010.

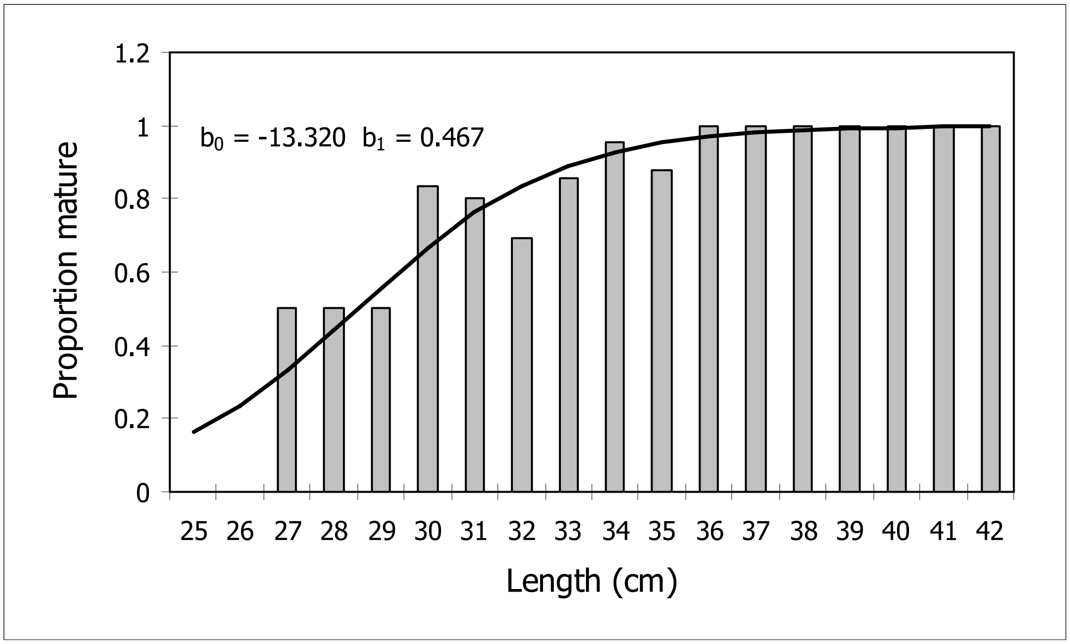

Figure 5. Proportion of female china rockfish that were mature, as a function of length (cm), including a fitted logistic curve.

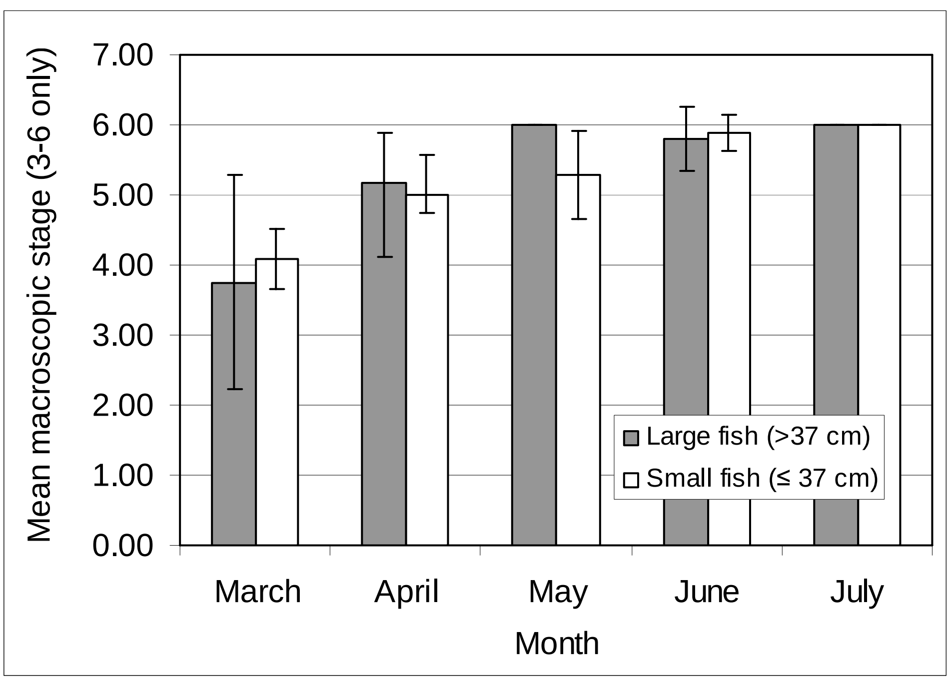

Figure 6. Comparison of mean maturity stage (includes only fish in macroscopic stages 3-6), by month, for large (>37 cm) and small female china rockfish.