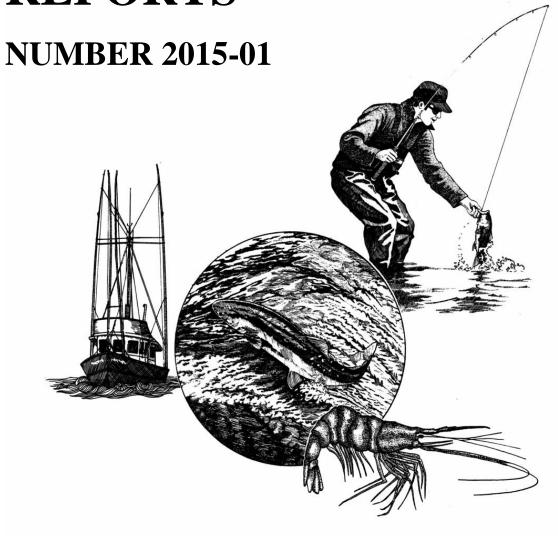
INFORMATION REPORTS



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Cryptic speciation in the blue rockfish (*Sebastes mystinus*): age, growth and female maturity of the blue-sided rockfish, a newly identified species, from Oregon waters

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Cryptic speciation in the blue rockfish (*Sebastes mystinus*): age, growth and female maturity of the blue-sided rockfish, a newly identified species, from Oregon waters

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## Introduction

Several studies of genetic variation in blue rockfish (Sebastes mystinus) from California and Oregon waters have demonstrated that blue rockfish are actually two reproductively isolated, cryptic rockfish species (Cope 2004, Burford and Bernardi 2008, Burford 2009, Burford et al. 2011). As a result, current published life history information for blue rockfish is based on an unknown mixture of the two species, and can no longer be considered accurate. To begin to generate species-specific life history information requires methods to differentiate the two species for sampling purposes. Oregon Department of Fish and Wildlife staff began separating the two species in samples from Newport and Depoe Bay recreational fishery catches based on body shape and pigmentation differences in 2009, specifically for the purpose of gathering speciesspecific information on age, length and female maturity. This effort was part of a longterm project begun in 2000 aimed at developing improved female maturity information for a variety of groundfish species (Hannah 2014). The purpose of this report is to document, and compare, the new life history information generated from 2009-2014 for the two variants of the blue rockfish. We present a summary of the data gathered on age, growth and female maturity as a function of length and age for both variants.

## Methods

In this report, we follow the nomenclature used by Love (2011) to refer to the two variants of blue rockfish as blue-blotched rockfish and blue-sided rockfish. Examination of preserved historical specimens from the Smithsonian Institute in Washington D.C. (specimens USNM-: 26947, 47154, 52914, 104771) suggest that the blue-blotched variant was the species originally described as the blue rockfish *S. mystinus* (personal observation, D.W. Wagman), leaving the blue-sided variant as the "new" species. The blue-blotched variant has a notably deeper body depth, large pigmented blotches on its sides and is presumably the species associated with Burford's (2009) southern genetic lineage, distributed principally in California and north into southern Oregon. The blue-sided variant has more even lateral pigmentation, a body shape that is less deep, a more prominent lower jaw and is presumably Burford's (2009) northern lineage, found primarily off of Oregon, but also south into northern California.

Most of the blue-sided and blue-blotched rockfish used for this study were sampled from Oregon's recreational fishery catches at the ports of Newport and Depoe Bay. In an effort to obtain better data on length at age, a brief effort was also undertaken in October 2014 to capture very small specimens by using herring jigs as the terminal tackle in waters off Depoe Bay, Oregon. In this effort, four very small blue-sided rockfish were collected however no small blue-blotched rockfish were encountered.

All fish sampled were measured (cm, fork length) and sexed and otoliths were collected for age determination. For most female specimens, ovaries were examined and assigned a maturity stage (Table 1) following the criteria of Westrheim (1975, Table 1). When

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 of maturity. Ovary 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             |

Maturity status was evaluated using a multi-step process. First, the optimal months for evaluating maturity were chosen based on the prevalence of females with ovaries in stages 4-6 (Table 1), indicating incipient or recently-completed parturition. In selecting the months to use, the need for adequate numbers of small or young fish in the final sample to help define a maturity curve was also considered. For samples collected during the chosen months, the maturity status of individual specimens was determined using a combination of macroscopic maturity codes and microscopic examination of histology slides. Female rockfish with ovaries in macroscopic stages 4-5 were considered unambiguously mature, as were fish in stage 6 or 7 in which visible black spots in the ovary were noted, indicative of residual larval eye pigment following parturition. Determining maturity status based solely on the macroscopic evaluation of ovaries is problematic in that "maturing" and "resting" ovaries cannot be reliably separated (Wallace and Selman 1981, Wyllie Echeverria 1987). Externally, these stages can appear similar but represent different maturity states. In several rockfish species, adolescent females have 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, we microscopically evaluated all stage 1, 2, 3, 6 and 7 ovaries from the optimal months for which ovary tissue samples were available, and excluded those without either macroscopic evidence of residual larval eye pigment or a tissue sample that could be evaluated microscopically.

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 most advanced stage of oocyte development was noted following Bowers (1992). Maturity status was classified as mature, immature or unknown. Ovaries with large oocytes showing dark-staining

vitellogenin were classified as mature, unless they showed clear indications of mass atresia, typified by a complete lack of cell nuclei. 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. 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 analysis of age or length at maturity. Evidence of abortive maturation 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 microscopic and macroscopic evaluations, considering only the months chosen as best for assessing maturity.

We evaluated maturity for both blue rockfish variants as a function of length and age. Ages 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. For a few fish, ages determined from the surface of the otolith were considered preferable and were used. 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).

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

$$p_{X_1} = e^{\left(b_0 + b_1 x_1\right)} / (1 + e^{\left(b_0 + b_1 x_1\right)}) \ \ \text{where,}$$

p is the probability that a fish is mature in a given length or age interval and  $b_0$  and  $b_1$  are parameters that define the location and shape of the fitted sigmoid curve. The predicted length or age at 50% maturity was calculated as,

L (or A)<sub>50</sub> = 
$$-b_0/b_1$$
.

## **Results and Discussion**

# Blue-sided rockfish

Age and length data were generated for 356 female and 134 male blue-sided rockfish. The data for both sexes fit the Von Bertalanffy curve well and showed that females grow slower, but get larger than males, with asymptotic length ( $\pm$  standard error) estimates of 37.90 ( $\pm$ 0.31) and 29.54 ( $\pm$ 0.23) cm for females and males, respectively (Table 2, Figure 1).

Table 2. Parameter estimates ( $\pm$  standard error) for the standard von Bertalanffy growth formula fitting fork length (cm) against age for male and female blue-sided rockfish.  $L_{\infty}$ = 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         |  |
|--------------|---------------|---------------|--|
| $L_{\infty}$ | 37.90 (0.31)  | 29.54 (0.23)  |  |
| K            | 0.244 (0.016) | 0.413 (0.033) |  |
| $t_0$        | -0.70 (0.34)  | -0.31 (0.26)  |  |
| N            | 356           | 134           |  |
| Age range    | 1-26          | 1-32          |  |

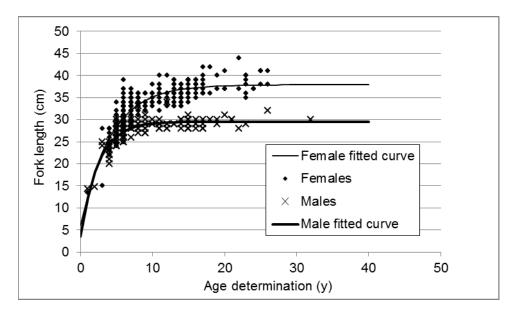


Figure 1. Length (cm) versus age (y) for male and female blue-sided rockfish and fitted von Bertalanffy growth curves.

Maturity sampling resulted in macroscopic maturity stage data for 397 blue-sided rockfish collected between 2009 and 2014 (Table 3). Histology samples collected and processed for microscopic evaluation of female maturity totaled 275 (Table 3). Note that one female sampled in November was not assigned a macroscopic maturity stage, but was found to be mature based on microscopic evaluation of the ovary sample (Table 3). Based on the macroscopic staging of ovaries, parturition in blue-sided rockfish is synchronous, with fertilized and ripe ovaries (stages 4 and 5) observed only in January and February (Figure 2, Table 3). However, stage 3 ovaries were noted as early as August and only 4 females were sampled in the month of December (Figure 2, Table 3). This sample size is too small to accurately indicate whether fertilized or ripe females might be encountered in December. Based on the macroscopic stage data and a review of histology slides from all months, November through March was selected as the best seasonal time period for accurate evaluation of female maturity status in blue-sided rockfish.

Comparison of macroscopic and microscopic evaluation of maturity status for blue-sided rockfish showed that macroscopic classification performed well in January, when fertilized and ripe ovaries were most common, with all 24 of the macroscopic maturity assignments confirmed microscopically (Table 4). By March, 15 of 90 ovary samples could not be classified with confidence even microscopically (Table 4). Note that two older female blue-sided rockfish, one aged at 15 y from November samples and another aged 14 y from January samples, were classified as skip-spawners, based on well-organized ovaries without signs of vitellogenesis or prior parturition. Because these were also judged macroscopically to be immature, they were considered to be "confirmed" in Table 4. However, they were treated as mature for the purpose of estimating maturity as a function of length and age, as recommended by Hannah and Parker (2007). Five other females showed evidence of abortive maturation through mass atresia of the developing oocytes however these were all within an age and size range considered to be consistent with the adolescent period and were treated as immature.

Female length at maturity for blue-sided rockfish was evaluated using a final data set consisting of 193 specimens with definitive maturity determinations from the months of November through March (Table 5). Female blue-sided rockfish matured as small as 27 cm and were 100% mature at 32 cm (Table 5). The fork length of specimens included in the sample ranged from 22 to 44 cm (Table 5). A logistic regression of maturity on length fit the data well (P<0.0001,  $r^2 = 0.642$ ) and indicated a length at 50% maturity (95% confidence interval) of 29.23 ( $\pm 0.04$ ) cm (Figure 3, Table 6). The data set for evaluating age at maturity consisted of 184 specimens with definitive determinations of both maturity and age (Table 5). Blue-sided rockfish females first matured at age 5 and were fully mature at age 8 (Table 5). The ages of specimens included in the final determination of maturity ranged from 3 to 26 y (Table 5). The logistic regression of maturity on age also fit the data well (P<0.0001,  $r^2 = 0.577$ ) and indicated an age of 50% maturity of 5.73 ( $\pm 0.02$ ) y (Table 6, Figure 3).

Table 3. Numbers of female blue-sided rockfish maturity (M) and histology (H) samples collected and processed, by month and macroscopic maturity stage (Table 1), 2009-2014.

| Maturity stage | Imm | ature_ | Matı | ıring | Ma | <u>ture</u> | Ferti | lized | Ri | pe | Sp | ent | Res | ting | To  | <u>tal</u> |
|----------------|-----|--------|------|-------|----|-------------|-------|-------|----|----|----|-----|-----|------|-----|------------|
| Month          | M   | Н      | M    | Н     | M  | Н           | M     | Н     | M  | Н  | M  | Н   | M   | Н    | M   | Н          |
| January        | 2   | 2      | 11   | 11    | 1  | 0           | 8     | 0     | 4  | 0  | 8  | 6   | 6   | 5    | 40  | 24         |
| February       | 5   | 4      | 16   | 16    | 1  | 1           | 0     | 0     | 6  | 0  | 5  | 5   | 27  | 24   | 60  | 50         |
| March          | 2   | 2      | 17   | 14    | 1  | 1           | 0     | 0     | 0  | 0  | 9  | 9   | 82  | 64   | 111 | 90         |
| April          | 1   | 0      | 9    | 8     | 0  | 0           | 0     | 0     | 0  | 0  | 1  | 1   | 23  | 17   | 34  | 26         |
| May            | 0   | 0      | 5    | 4     | 0  | 0           | 0     | 0     | 0  | 0  | 0  | 0   | 10  | 10   | 15  | 14         |
| June           | 0   | 0      | 1    | 0     | 0  | 0           | 0     | 0     | 0  | 0  | 0  | 0   | 21  | 14   | 22  | 14         |
| July           | 0   | 0      | 4    | 0     | 0  | 0           | 0     | 0     | 0  | 0  | 1  | 1   | 31  | 15   | 36  | 16         |
| August         | 0   | 0      | 3    | 2     | 6  | 6           | 0     | 0     | 0  | 0  | 0  | 0   | 2   | 1    | 11  | 9          |
| September      | 0   | 0      | 0    | 0     | 10 | 1           | 0     | 0     | 0  | 0  | 0  | 0   | 6   | 3    | 16  | 4          |
| October        | 1   | 1      | 7    | 3     | 26 | 4           | 0     | 0     | 0  | 0  | 0  | 0   | 0   | 0    | 34  | 9          |
| November       | 0   | 0      | 3    | 3     | 11 | 11          | 0     | 0     | 0  | 0  | 0  | 0   | 0   | 0    | 14  | $15^{1}$   |
| December       | 0   | 0      | 2    | 2     | 2  | 2           | 0     | 0     | 0  | 0  | 0  | 0   | 0   | 0    | 4   | 4          |
| Total          | 11  | 9      | 78   | 63    | 58 | 26          | 8     | 0     | 10 | 0  | 24 | 22  | 208 | 153  | 397 | 275        |

<sup>&</sup>lt;sup>T</sup>Note that one female fish had a histology sample taken, but no macroscopic stage was assigned.

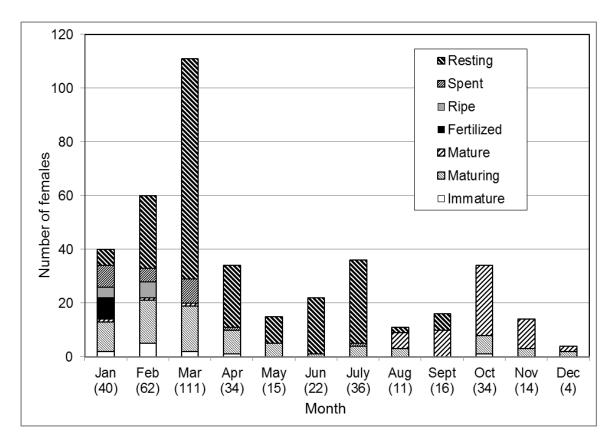


Figure 2. Number of female blue-sided rockfish sampled by macroscopic maturity stage (Table 1) and month, 2009-2014.

Table 4. Comparison of macroscopic and microscopic determinations of maturity in female blue-sided rockfish collected from waters off Newport and Depoe Bay, Oregon, for the months of November through March, 2009-2014.

|          | Macros<br>classifi |        | Microscopic classification |              |         |  |  |  |  |
|----------|--------------------|--------|----------------------------|--------------|---------|--|--|--|--|
| Month    | Condition          | Number | Confirmed                  | Reclassified | Unknown |  |  |  |  |
| November | Immature           | 3      | 2                          | 1            | 0       |  |  |  |  |
|          | Mature             | 11     | 11                         | 0            | 0       |  |  |  |  |
| December | Immature           | 2      | 1                          | 1            | 0       |  |  |  |  |
|          | Mature             | 2      | 2                          | 0            | 0       |  |  |  |  |
| January  | <b>Immature</b>    | 13     | 13                         | 0            | 0       |  |  |  |  |
|          | Mature             | 11     | 11                         | 0            | 0       |  |  |  |  |
| February | <b>Immature</b>    | 20     | 14                         | 2            | 4       |  |  |  |  |
| -        | Mature             | 30     | 29                         | 0            | 1       |  |  |  |  |
| March    | Immature           | 16     | 13                         | 0            | 3       |  |  |  |  |
|          | Mature             | 74     | 56                         | 6            | 12      |  |  |  |  |
| Total    |                    | 182    | 152                        | 10           | 20      |  |  |  |  |

Table 5. Number of female blue-sided rockfish used in determining age and length at maturity and proportion mature, by length (cm) and age (y).

| Length (cm) | Number  | Proportion | Age (y) | Number  | Proportion |
|-------------|---------|------------|---------|---------|------------|
|             | sampled | mature     |         | sampled | mature     |
| 22          | 1       | 0.00       | 3       | 1       | 0.00       |
| 23          | 2       | 0.00       | 4       | 10      | 0.00       |
| 24          | 4       | 0.00       | 5       | 23      | 0.13       |
| 25          | 8       | 0.00       | 6       | 46      | 0.70       |
| 26          | 5       | 0.00       | 7       | 14      | 0.86       |
| 27          | 7       | 0.29       | 8       | 11      | 1.00       |
| 28          | 8       | 0.25       | 9       | 13      | 1.00       |
| 29          | 9       | 0.33       | 10      | 4       | 1.00       |
| 30          | 19      | 0.63       | 11      | 3       | 1.00       |
| 31          | 18      | 0.83       | 12      | 6       | 1.00       |
| 32          | 17      | 1.00       | 13      | 8       | 1.00       |
| 33          | 18      | 1.00       | 14      | 13      | 1.00       |
| 34          | 18      | 1.00       | 15      | 10      | 1.00       |
| 35          | 11      | 1.00       | 16      | 2       | 1.00       |
| 36          | 19      | 1.00       | 17      | 4       | 1.00       |
| 37          | 10      | 1.00       | 18      | 2       | 1.00       |
| 38          | 3       | 1.00       | 19      | 2       | 1.00       |
| 39          | 8       | 1.00       | 20      | 1       | 1.00       |
| 40          | 5       | 1.00       | 21      | 0       |            |
| 41          | 1       | 1.00       | 22      | 2       | 1.00       |
| 42          | 1       | 1.00       | 23      | 4       | 1.00       |
| 43          | 0       |            | 24      | 2       | 1.00       |
| 44          | 1       | 1.00       | 25      | 2       | 1.00       |
|             |         |            | 26      | 1       | 1.00       |
| Total       | 193     |            |         | 184     |            |

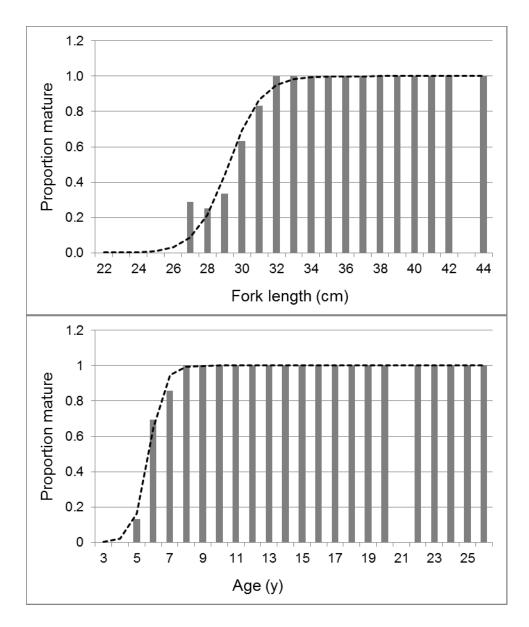


Figure 3. Proportion of mature female blue-sided rockfish as a function of length (upper panel, cm) and age (lower panel, y) with fitted logistic curves.

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

| Indepen  | ndent      | Coefficients | Standard | P-     | L <sub>50</sub> or A <sub>50</sub> | 95% confidence | Coefficient of |
|----------|------------|--------------|----------|--------|------------------------------------|----------------|----------------|
| variable | 2          |              | error    | value  |                                    | limits         | determination  |
| Length   |            |              |          |        | 29.23 cm                           | ±0.04          |                |
|          | Constant   | -31.129      | 5.600    | 0.0001 |                                    |                |                |
|          | Length     | 1.065        | 0.188    | 0.0001 |                                    |                |                |
|          | Full model |              |          | 0.0001 |                                    |                | 0.642          |
| Age      |            |              |          |        | 5.73 y                             | $\pm 0.02$     |                |
|          | Constant   | -12.983      | 2.773    | 0.0001 |                                    |                |                |
|          | Age        | 2.267        | 0.474    | 0.0001 |                                    |                |                |
|          | Full model |              |          | 0.0001 |                                    |                | 0.577          |

# Blue-blotched rockfish

Blue-blotched rockfish were less frequently caught by anglers fishing out of Newport and Depoe Bay than blue-sided rockfish. Consequently, fewer samples for age, length and female maturity were collected for this species. Age and length data were generated for 128 female and 85 male blue-blotched rockfish (Table 7). Although the fit of the age and length data to the Von Bertalanffy equation was adequate, the parameters are not all well estimated, due to a lack of younger and smaller fish of both sexes (Figure 4). The age and length data show that blue-blotched females reach a larger asymptotic length than males (Table 7, Figure 4). The asymptotic length (± standard error) for females and males was estimated at 40.79 (±0.99) cm and 31.21 (±0.76) cm, respectively (Table 7).

Table 7. Parameter estimates ( $\pm$  standard error) for the standard von Bertalanffy growth formula fitting fork length (cm) against age for male and female blue-blotched rockfish.  $L_{\infty}$ = 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         |
|--------------|---------------|---------------|
| $L_{\infty}$ | 40.79 (0.99)  | 31.21 (0.76)  |
| K            | 0.105 (0.017) | 0.102 (0.031) |
| $t_0$        | -6.36 (1.54)  | -12.00 (4.46  |
| N            | 128           | 85            |
| Age range    | 5-34          | 4-29          |

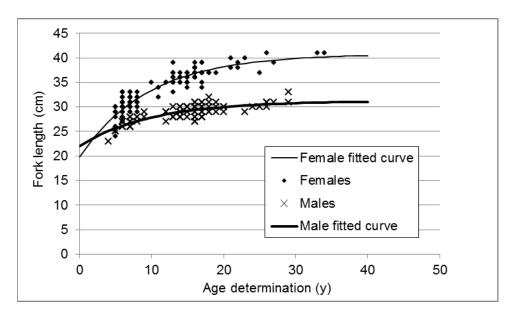


Figure 4. Length (cm) versus age (y) for male and female blue-blotched rockfish and fitted von Bertalanffy growth curves.

Maturity sampling resulted in macroscopic maturity stage data and histology samples for 212 and 147 female blue-blotched rockfish, respectively (Table 8). The macroscopic stage data suggest that ovary development is also synchronous in blue-blotched rockfish (Figure 5). Fish with ovaries in either fertilized or ripe condition were observed only in February however just 7 females were sampled from the months of December through January. To increase total sample size for estimating length and age at 50% maturity for blue-blotched rockfish, a wider seasonal window of October through March was chosen for evaluating female maturity.

Macroscopic evaluation of maturity status for blue-blotched rockfish was mostly accurate in February, when fertilized and ripe ovaries were also most frequently noted (Table 9). In October samples, 4 females classified visually as immature showed microscopic evidence of early vitellogenesis and were reclassified as mature (Table 9). As in blue-sided rockfish, the March samples included some fish for which maturity could not be confidently assigned, even microscopically. No older or larger skip-spawners were noted for blue-blotched rockfish. Abortive maturation via mass atresia was noted in three fish that ranged in size from 25-31 cm and in age from 5-6 y. These fish were considered to be in the adolescent stage and were treated as immature.

Length at maturity for female blue-blotched rockfish was evaluated using a final data set consisting of 120 specimens with definitive maturity determinations from the months of October through March (Table 10). Female blue-blotched rockfish matured as small as 26 cm and were 100% mature at 31 cm (Table 10). The females included in the sample ranged in length from 24 to 41 cm (Table 10). A logistic regression of maturity on length (P<0.0001,  $r^2 = 0.279$ ) indicated a length at 50% maturity (95% confidence interval) of 25.98 ( $\pm 0.22$ ) cm (Figure 6, Table 11). The data set for evaluating age at maturity consisted of 119 specimens with definitive determinations of both maturity and age (Table 10). Blue-blotched rockfish females first matured at age 5 and were fully mature at age 10, however, fish younger than age 5 and fish of age 9 were not sampled (Table 10). The ages of specimens included in the final determination of maturity ranged from 5 to 34 y (Table 10). The logistic regression of maturity on age (P<0.0001,  $r^2 = 0.272$ ) indicated an age of 50% maturity of 4.39 ( $\pm 0.16$ ) y (Table 11, Figure 6).

Table 8. Numbers of female blue-blotched rockfish maturity (M) and histology (H) samples collected and processed, by month and macroscopic maturity stage (Table 1), 2009-2014.

| Maturity stage | Imm | ature | Matu | ıring | Ma | ture | Ferti | lized | Ri | pe | Spe | <u>ent</u> | Res | ting | To  | <u>tal</u> |
|----------------|-----|-------|------|-------|----|------|-------|-------|----|----|-----|------------|-----|------|-----|------------|
| Month          | M   | Н     | M    | Н     | M  | Н    | M     | Η     | M  | Н  | M   | Н          | M   | Н    | M   | Н          |
| January        | 0   | 0     | 1    | 1     | 0  | 0    | 0     | 0     | 0  | 0  | 0   | 0          | 2   | 1    | 3   | 2          |
| February       | 0   | 0     | 6    | 5     | 0  | 0    | 1     | 0     | 2  | 0  | 2   | 2          | 9   | 9    | 20  | 16         |
| March          | 0   | 0     | 3    | 3     | 0  | 0    | 0     | 0     | 0  | 0  | 7   | 7          | 53  | 44   | 63  | 54         |
| April          | 0   | 0     | 3    | 2     | 0  | 0    | 0     | 0     | 0  | 0  | 1   | 0          | 18  | 6    | 22  | 8          |
| May            | 0   | 0     | 0    | 0     | 0  | 0    | 0     | 0     | 0  | 0  | 1   | 0          | 6   | 6    | 7   | 6          |
| June           | 0   | 0     | 0    | 0     | 1  | 1    | 0     | 0     | 0  | 0  | 0   | 0          | 6   | 4    | 7   | 5          |
| July           | 0   | 0     | 0    | 0     | 9  | 0    | 0     | 0     | 0  | 0  | 0   | 0          | 7   | 4    | 16  | 4          |
| August         | 0   | 0     | 0    | 0     | 6  | 2    | 0     | 0     | 0  | 0  | 0   | 0          | 3   | 0    | 9   | 2          |
| September      | 0   | 0     | 2    | 2     | 15 | 1    | 0     | 0     | 0  | 0  | 0   | 0          | 3   | 3    | 20  | 6          |
| October        | 0   | 0     | 4    | 4     | 26 | 25   | 0     | 0     | 0  | 0  | 0   | 0          | 0   | 0    | 30  | 29         |
| November       | 0   | 0     | 1    | 1     | 10 | 10   | 0     | 0     | 0  | 0  | 0   | 0          | 0   | 0    | 11  | 11         |
| December       | 0   | 0     | 0    | 0     | 4  | 4    | 0     | 0     | 0  | 0  | 0   | 0          | 0   | 0    | 4   | 4          |
| Total          | 0   | 0     | 20   | 18    | 71 | 43   | 1     | 0     | 2  | 0  | 11  | 9          | 107 | 77   | 212 | 147        |

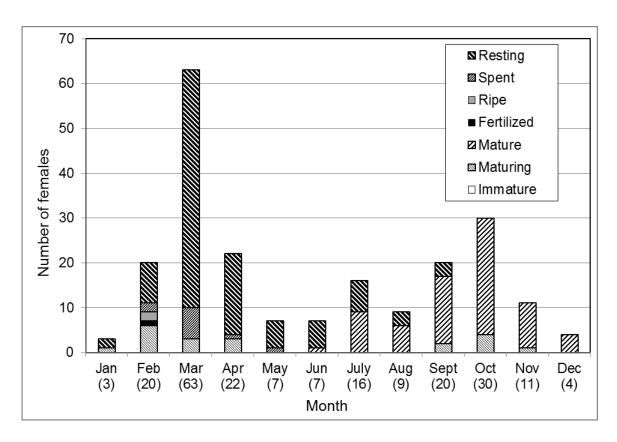


Figure 5. Number of female blue-blotched rockfish sampled by macroscopic maturity stage (Table 1) and month, 2009-2014.

Table 9. Comparison of macroscopic and microscopic determinations of maturity in female blue-blotched rockfish collected from waters off Newport and Depoe Bay, Oregon, for the months of October through March, 2009-2014.

|          | Macros<br>classifi | -      | Microscopic classification |              |         |  |  |  |  |  |
|----------|--------------------|--------|----------------------------|--------------|---------|--|--|--|--|--|
| Month    | Condition          | Number | Confirmed                  | Reclassified | Unknown |  |  |  |  |  |
| October  | Immature           | 4      | 0                          | 4            | 0       |  |  |  |  |  |
|          | Mature             | 25     | 24                         | 1            | 0       |  |  |  |  |  |
| November | <b>Immature</b>    | 1      | 0                          | 1            | 0       |  |  |  |  |  |
|          | Mature             | 10     | 10                         | 0            | 0       |  |  |  |  |  |
| December | Immature           | 0      | 0                          | 0            | 0       |  |  |  |  |  |
|          | Mature             | 4      | 4                          | 0            | 0       |  |  |  |  |  |
| January  | Immature           | 1      | 0                          | 1            | 0       |  |  |  |  |  |
| -        | Mature             | 1      | 1                          | 0            | 0       |  |  |  |  |  |
| February | Immature           | 5      | 4                          | 1            | 0       |  |  |  |  |  |
|          | Mature             | 11     | 11                         | 0            | 0       |  |  |  |  |  |
| March    | Immature           | 3      | 3                          | 0            | 0       |  |  |  |  |  |
|          | Mature             | 51     | 42                         | 2            | 7       |  |  |  |  |  |
| Total    |                    | 116    | 99                         | 10           | 7       |  |  |  |  |  |

Table 10. Number of female blue-blotched rockfish used in determining age and length at maturity and proportion mature, by length (cm) and age (y).

| Length (cm) | Number  | Proportion | Age (y) | Number  | Proportion |
|-------------|---------|------------|---------|---------|------------|
|             | sampled | mature     |         | sampled | mature     |
| 24          | 1       | 0.00       | 5       | 9       | 0.56       |
| 25          | 1       | 0.00       | 6       | 25      | 0.84       |
| 26          | 2       | 0.5        | 7       | 19      | 0.95       |
| 27          | 3       | 1.00       | 8       | 7       | 0.86       |
| 28          | 5       | 0.60       | 9       | 0       |            |
| 29          | 9       | 1.00       | 10      | 1       | 1.00       |
| 30          | 14      | 0.75       | 11      | 2       | 1.00       |
| 31          | 16      | 1.00       | 12      | 2       | 1.00       |
| 32          | 6       | 1.00       | 13      | 9       | 1.00       |
| 33          | 6       | 1.00       | 14      | 5       | 1.00       |
| 34          | 3       | 1.00       | 15      | 8       | 1.00       |
| 35          | 10      | 1.00       | 16      | 12      | 1.00       |
| 36          | 11      | 1.00       | 17      | 7       | 1.00       |
| 37          | 16      | 1.00       | 18      | 2       | 1.00       |
| 38          | 6       | 1.00       | 19      | 1       | 1.00       |
| 39          | 6       | 1.00       | 20      | 0       |            |
| 40          | 2       | 1.00       | 21      | 2       | 1.00       |
| 41          | 3       | 1.00       | 22      | 2       | 1.00       |
|             |         |            | 23      | 1       | 1.00       |
|             |         |            | 24      | 0       |            |
|             |         |            | 25      | 1       | 1.00       |
|             |         |            | 26      | 1       | 1.00       |
|             |         |            | 27      | 1       | 1.00       |
|             |         |            | 28      | 0       |            |
|             |         |            | 29      | 0       |            |
|             |         |            | 30      | 0       |            |
|             |         |            | 31      | 0       |            |
|             |         |            | 32      | 0       |            |
|             |         |            | 33      | 1       | 1.00       |
|             |         |            | 34      | 1       | 1.00       |
| Total       | 120     |            |         | 119     |            |

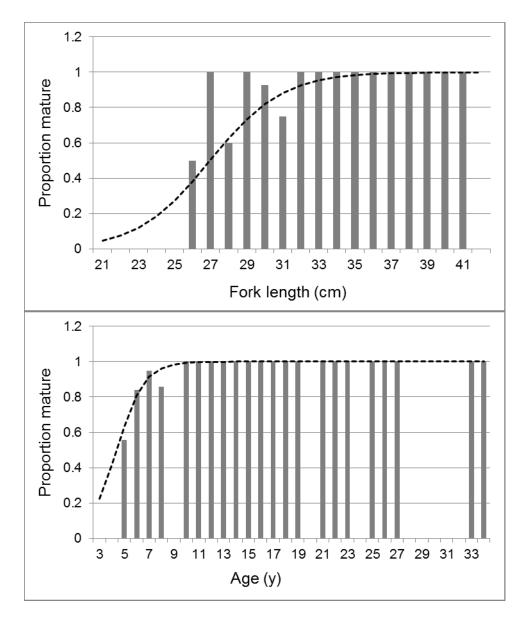


Figure 6. Proportion of mature female blue-blotched rockfish as a function of length (upper panel, cm) and age (lower panel, y) with fitted logistic curves.

Table 11. Results of logistic regression analysis of maturity status of female blue-blotched rockfish versus length (cm) and age (y).

| Indepen<br>variable |            | Coefficients | Standard<br>error | P-<br>value | L <sub>50</sub> or A <sub>50</sub> | 95% confidence limits | Coefficient of determination |
|---------------------|------------|--------------|-------------------|-------------|------------------------------------|-----------------------|------------------------------|
| Length              |            |              |                   |             | 25.98 cm                           | ±0.22                 |                              |
|                     | Constant   | -13.046      | 4.490             | 0.0037      |                                    |                       |                              |
|                     | Length     | 0.502        | 0.153             | 0.0010      |                                    |                       |                              |
|                     | Full model |              |                   | 0.0001      |                                    |                       | 0.279                        |
| Age                 |            |              |                   |             | 4.39 y                             | ±0.16                 |                              |
|                     | Constant   | -3.944       | 2.641             | 0.1353      |                                    |                       |                              |
|                     | Age        | 0.898        | 0.436             | 0.0392      |                                    |                       |                              |
|                     | Full model |              |                   | 0.0001      |                                    |                       | 0.272                        |

# Comparing the two species

The age-length curves for blue-sided and blue-blotched rockfish illustrate one of the many reasons they may have been mistaken for the same species for so long. In both species, females grow larger than males, and reach approximately the same asymptotic sizes, about 40 and 30 cm for females and males, respectively (Figures 1 and 4). The absence of age and length data for very young blue-blotched rockfish in our collections (Figure 4) makes any more detailed comparison of estimated age and growth parameters from these data problematic.

The macroscopic stage data for females also shows a very similar seasonal timing of parturition for the two species, centered on the winter months of January and February (Figures 2 and 5). Two notable differences between the two species are in the length and age at 50% maturity. Blue-blotched rockfish females were 50% mature at 25.98 cm and at an age of 4.39 y, while blue-sided rockfish were 50% mature at a larger size of 29.23 cm and at an older age of 5.73 y (Tables 6 and 11). As in the age and growth data, the lack of small and very young females suggests the need for additional maturity sampling for blue-blotched rockfish from Oregon waters (Table 10).

In addition to the differences in body shape and pigmentation (Figures 7 and 8), another notable difference between the two species was in the color of the ovaries. In blue-sided rockfish, the ovary was always a pink-cream color (Figure 9). In stark contrast, the ovaries of blue-blotched rockfish were always bright yellow in coloration (Figure 9).



Figure 7. Picture of live blue-sided rockfish (upper) and blue-blotched rockfish (lower).



Figure 8. Picture of dead blue-sided rockfish (upper) and blue-blotched rockfish (lower).

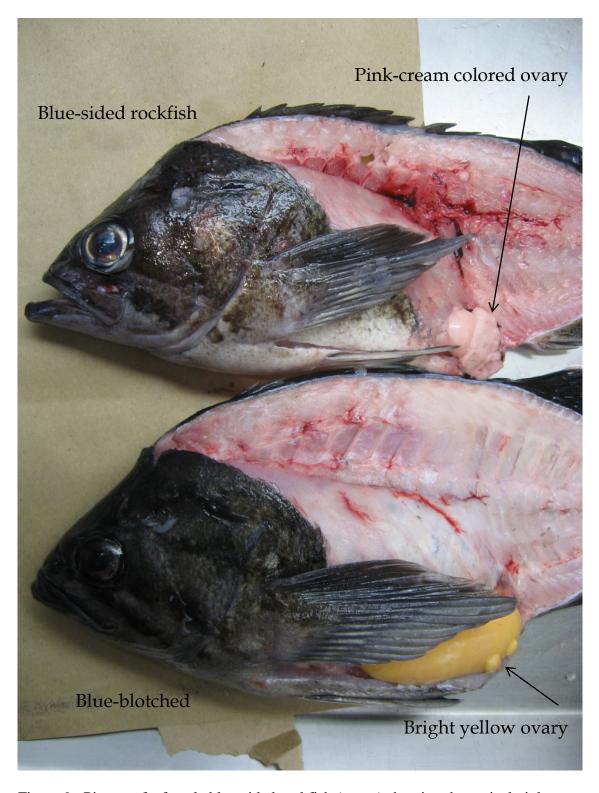


Figure 9. Picture of a female blue-sided rockfish (upper) showing the typical pink-cream color of the ovary and a blue-blotched rockfish (lower), showing the bright yellow ovary color.

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