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DOUBLE OR NOTHING: PLASTICITY IN REPRODUCTIVE OUTPUT IN THE CHILIPEPPER ROCKFISH (*SEBASTES GOODEI*)

Lyndsey S. Lefebvre[1,2,A], Sabrina G. Beyer[1,2], David M. Stafford[1,2], Neosha S Kashef[1,2] , Edward J. Dick[2], Susan M. Sogard[2], and John C. Field[2]

[1] Cooperative Institute for Marine Ecosystems and Climate, Institute of Marine Sciences, University of California Santa Cruz
[2] Fisheries Ecology Division
Southwest Fisheries Science Center
National Marine Fisheries Service
National Oceanographic and Atmospheric Administration
110 McAllister Way, Santa Cruz, CA 95060

Lyndsey S. Lefebvre: Lyndsey.Lefebvre@noaa.gov
Sabrina G. Beyer: Sabrina.Beyer@noaa.gov
David M. Stafford: David.Stafford@noaa.gov
Neosha S. Kashef: Neosha.Kashef@noaa.gov
Edward J. Dick: Edward.Dick@noaa.gov
Susan M. Sogard: Susan.Sogard@noaa.gov
John C. Field: John.Field@noaa.gov

Corresponding author:
Lyndsey S. Lefebvre

[A] Current address:
Integrated Statistics
Under contract with:
Northeast Fisheries Science Center, NMFS, NOAA
166 Water St.
Woods Hole, MA 02543

ABSTRACT

An understanding of the reproductive biology of a species is fundamental to successful management of fish stocks. Rockfish (*Sebastes* spp.) have complex reproduction, being viviparous and generally late to mature, often with extended adolescent periods characterized by abortive maturation events. Furthermore, whereas the majority of *Sebastes* species in the California Current region produce one brood of larvae annually, several are known to produce more than one, though no stock assessments have yet considered the impact multiple brooding may have on population spawning potential for these species. We documented abortive maturation and examined the prevalence and size-dependent and regional patterns of multiple brooding using macroscopic evaluation and detailed histological analysis of ovaries from a model species, chilipepper (*S. goodei*), collected off Central and Southern California. We modeled the size-related maternal effect on the probability of multiple brooding, and quantified size-dependent fecundity relationships. Our results indicate that the most robust estimation of reproductive output, as a function of the fecundity-length relationship, is improved for chilipepper when multiple brooding is incorporated, due to the greater probability of additional broods, and thus greater spawning potential, in larger females.

Keywords: Reproductive potential, viviparity, multiple brooding, fecundity, maternal effects

1. INTRODUCTION

An understanding of reproductive potential, as well as the drivers and causes of plasticity (i.e. variability) in reproductive traits, is fundamental to successful management of fish stocks. The historical assumption that total egg production is proportional to the spawning biomass of a stock has repeatedly been shown to be incorrect when species exhibit strong maternal effects, as exemplified in rockfishes (*Sebastes* spp.; Dick 2009; Beyer et al. 2015). Maternal size- and age-dependent traits, such as increased relative fecundity (eggs per g of maternal somatic weight) or spawning frequency (number of spawning events in a season) with increasing size or age, are evident in a variety of species with varying reproductive strategies (see reviews by Fitzhugh et al. 2012 and Hixon et al. 2014). In these cases, accounting for these maternal effects when estimating reproductive output in stock assessments is more appropriate than using spawning stock biomass (Spencer and Dorn 2013; He et al. 2015).

Rockfishes—a speciose genus of great commercial and recreational importance throughout the California Current region—display strong maternal effects, with larger, older females contributing disproportionately to production in terms of both offspring quantity and quality (Sogard et al. 2008; Stafford et al. 2014; Dick et al. 2017). Rockfishes are generally long-lived, slow-growing, and late to mature, with several species showing prolonged adolescent periods during which small, young individuals may experience "false starts" to reproduction (abortive maturation; Nichol and Pikitch 1994; Conrath and Knoth 2013; Lefebvre and Field 2015). All *Sebastes* species are live-bearers, with gestation periods ranging from one to two months (Love et al. 2002). Although the majority of species produce one brood of larvae annually, several have long been known to be capable of producing multiple broods in a given reproductive season (Moser 1967a; Love et al. 1990). The extent to which abortive maturation and multiple brooding occur is not well studied, and the mechanisms driving these two phenomena are not well understood; however, both may affect reproductive reference points used in stock assessments (age- or size-at-maturity and reproductive output, respectively).

Mass atresia events, the arresting of development and subsequent resorption of the leading cohort of vitellogenic oocytes, are known to occur in continental slope and deep dwelling rockfishes, which tend to have the slowest growth and greatest longevity within the *Sebastes* genus (Nichol and Pikitch 1994; Conrath and Knoth 2013; Lefebvre and Field 2015). Most of these mass atresia events appeared to be abortive maturation (as opposed to skip spawning), as most females were below the length or age at 95% maturity (L_{95}). Abortive maturation, as defined here and by Ramsay and Whitthames (1996), is the occurrence of the resorbing type spawning omission (Rideout et al. 2005), in which mass atresia occurs in females that have not previously reproduced. Although these females are physiologically mature, they are functionally immature as they are not yet contributing to production. Alternately, mass atresia in females that have reproduced in previous years represents skip spawning (Rideout et al. 2005). Further complexity in the reproductive traits of some rockfishes is the presence of an extended adolescent period, which is characterized by abortive maturation events or by oocyte development arrested at the oil vacuole stage (first secondary growth stage oocyte) for one or more years (Hannah and Parker 2007; Lefebvre and Field 2015). Maturity ogives, or more appropriately spawning ogives, can better account for the consequences of abortive maturation, extended adolescence, and skip spawning by designating such individuals as functionally immature, which results in shifts of the ogive to larger, older individuals (Hannah and Parker 2007; Lefebvre and Field 2015).

The phenomenon of multiple brooding in rockfishes was initially noted by Moser (1967a) and MacGregor (1970) in bocaccio (*S. paucispinis*), speckled rockfish (*S. ovalis*), and starry rockfish (*S. constellatus*). Multiple broods were macroscopically identified in ovaries containing fertilized eggs (primary brood) by the presence of developing oocytes (the eventual secondary brood), as well as in ovaries with developing oocytes (the eventual secondary brood) with entrained larvae from the primary brood that had failed to be extruded from the ovary during parturition (larval release). Multiple brooding has since been documented in more than a dozen *Sebastes* species (Table 1) with primarily southerly distributions in the Southern California Bight. It was generally believed to be uncommon north of Point Conception, CA (Echeverria 1987; Love et al. 2002). The Southern California Bight, a region extending from Point Conception, California, to Punta Eugenia, Baja California, Mexico, is characterized by warmer, more variable and less productive conditions relative to more northern waters of the California Current, where stronger, more predictable seasonal upwelling occurs (Parrish et al. 1981; Checkley and Barth 2009). The mechanisms driving and allowing for the production of multiple broods are not fully understood, nor are the limits to the number of broods produced by a given species annually, though macroscopic evaluations have suggested as many as three broods in some individuals (Kashef, unpublished data).

Moser (1967a) and all other authors to more recently document multiple brooding have noted the need to account for the phenomenon in any accurate assessment of fecundity in rockfishes. Both Moser (1967b) and MacGregor (1970) noted that secondary broods were recruited from reserve oocytes after the eggs of primary broods had been fertilized, suggesting an indeterminate fecundity pattern rather than the determinate fecundity pattern typical of *Sebastes* (Love et al. 2002). In fish with determinate fecundity, annual fecundity is fixed prior to any spawning activity, whereas in fish with an indeterminate fecundity pattern, fecundity is not set prior to spawning and is the product of the number of reproductive events throughout the season and the fecundity of each batch (Murua and Saborido-Rey 2003). In a comparison between brood fecundity estimates in chilipepper and speckled rockfish, Beyer et al. (2015) found that secondary broods were nearly as fecund as primary broods. If fecundity in chilipepper is indeterminate, then multiple brooding females could be doubling (or more, if more than two broods are produced) their annual reproductive output compared to single brooding females. It is particularly important to account for multiple brooding if the likelihood of producing two (or more) broods is size-dependent. Ralston and MacFarlane (2010) observed a higher frequency of multiple brooding in larger versus smaller female bocaccio; however, they also recognized that the challenges associated with false negatives precluded an effective accounting of the phenomenon in defining a fecundity function. If multiple brooding is size-dependent, the strength of the fecundity-size relationship would increase, resulting in a further departure from the assumption of egg production being proportional to spawning stock biomass (Dick et al. 2017). Fecundity-size relationships, which account for the disproportional reproductive contribution of larger females, have been incorporated in over half (12 of 21) of recent rockfish stock assessments on the U.S. West Coast (He et al. 2015). Additionally, a recent meta-analysis provided the basis to account for size-dependent fecundity for all species, regardless of the available data (Dick et al. 2017). The effect of multiple brooding females, however, has not yet been considered in any rockfish stock assessment, and could substantially alter the relationship between fecundity and size or age.

Chilipepper was chosen as a model species to explore the effect of multiple brooding on fecundity estimates due to the relatively high abundance and healthy status of the stock, and the relative ease of capture. Chilipepper is a continental shelf dwelling species distributed along the west coast of North America and is most common from Cape Mendocino, California, to northern Baja California, Mexico. Multiple brooding has been documented in this species off Southern and, less frequently, Central California (Beyer et al. 2015).

The objectives of this study were to 1) document the occurrence of what might be considered atypical ovarian development, such as abortive maturation, prolonged adolescence, and skip spawning; 2) determine the prevalence of and regional and size-related patterns related to multiple brooding; and 3) model how fecundity estimates and fecundity-length relationships change when accounting for multiple brooding. Furthermore, the implications of the plasticity in reproductive strategy in chilipepper and other multiple brooding rockfish species are considered in terms of potential changes in environmental conditions.

2. MATERIALS AND METHODS

2.1 SAMPLE COLLECTION AND PROCESSING

Female chilipepper were collected via rod-and-reel onboard chartered recreational and commercial fishing vessels in Central (near Cordell Bank and off Half Moon Bay) and Southern (Santa Barbara Channel) California waters (Fig. 1). Samples were collected during the 2013-14, 2014-15, and 2015-16 reproductive seasons (September to March), with samples collected in January, February, and March attributed to the previous calendar year's cohort. Processing protocols followed Beyer et al. (2015) and are briefly summarized here. Fish were stored on ice and processed within 24 hours of capture. Fish were weighed (g) and measured (fork length [FL], mm), the sagittal otoliths were removed for age analysis, and the liver was removed and weighed (g). All ovaries were removed, weighed (g), and assigned a macroscopic development stage according to Beyer et al. 2015 (Table 2), with a "pre-vitellogenic" stage added between the immature and vitellogenic stages to account for a distinct transitional stage (observed in females initiating development for their first reproductive event as well as mature females initiating development for the start of the reproductive season). Ovaries with evidence of multiple brooding were macroscopically identified and recorded as either having evidence of an upcoming brood (vitellogenic oocytes co-occurring with fertilized eggs or eyed-larvae) or a previous brood (evidenced by developing vitellogenic oocytes, fertilized eggs, or eyed-larvae with either the ovary having a loose appearance with excess ovarian fluid and blood or residual, degrading eyed-larvae). Two weighed subsamples (0.5-1.0 g) of eggs or larvae were collected from ovaries with vitellogenic oocytes or developing embryos and fixed in 10% neutral buffered formalin for fecundity analyses.

2.2 HISTOLOGICAL ANALYSIS

For histological analysis, a small portion of ovarian tissue was collected from the middle of one ovarian lobe, placed in a tissue cassette, and fixed in 10% neutral buffered formalin for a minimum of one week before being stored in 70% ethanol (n=581; limited collections from macroscopically staged immature and spent ovaries). Tissue from ovaries with fertilized eggs or eyed-larvae was rinsed prior to placement in tissue cassettes in an effort to reduce the number of loose eggs and larvae present in the section, which obstructed the viewing of ovigerous tissue. Ovarian tissue samples selected for histological analyses were processed according to standard paraffin embedding and hematoxylin-eosin staining techniques (Humason 1972).

Histological sections were examined at 40-250x magnification using a compound microscope and were read blindly (no size, age, or macroscopic stage data provided). Each ovary was assigned an ovarian phase based on the stage of the leading oocyte cohort, incidence and state of atresia, and presence and number of stages of postovulatory follicle complexes (POF; Table 2). Ovarian phases were developed from observations of 565 chilipepper histological sections (all macroscopic stages of development) from this and other studies (Field et al. 2016). Ovarian phases utilized standard terminology suggested by Brown-Peterson et al. (2011) and were based on descriptions of teleost oocyte development in Wallace and Selman (1981) and *Sebastes* oocyte and ovarian development in Shaw et al. (2012) and Lefebvre and Field (2015), with modifications to account for abortive maturation, skip spawning, and multiple brooding. Stages of atresia were based on Hunter and Macewicz (1985). In addition to ovarian phase, all stages of oocytes present were recorded, and diameters (µm) of the largest oocyte in five fields-of-view were measured using an ocular micrometer and the mean recorded (mean maximum oocyte diameter). The incidence of alpha atresia of advancing oocytes was assigned as low (0-5%), moderate (>5-25%), high (>25-50%), or mass (>50%) in ovaries in the developing and spawning capable phases. Ovaries with mass atresia, a thin ovarian wall, and no evidence of a previous parturition were classified into the abortive maturation subphase (Table 2). Qualitative assessment of the ovarian wall thickness (thin or thick based on a threshold of approximately 75 μm) and prominence of blood vessels and late stages of atresia (delta and gamma) throughout ovarian lamellae were also noted.

To examine the prevalence of abortive maturation and adolescent females, ovarian samples from all females macroscopically staged as pre-vitellogenic or resting between October and January (n=66) were histologically analyzed. Previous examination of chilipepper ovaries during this time period indicated a high incidence of abortive maturation in these stages of development and mass atresia has not been previously observed at other stages of development (Field et al. 2016). Temporal restriction of this analysis to the typical peak parturition season for chilipepper avoided confusing abortive maturation with the regression of the ovary typical at the end of the season. Females were classified as "adolescents" when ovaries were in the early developing or regenerating histological subphases and had the following characteristics: thin ovarian wall; beta or delta atresia; and a paucity of prominent blood vessels, muscle bundles, connective tissue, or melanomacrophage centers.

For multiple brooding analyses, samples were restricted to December to January and November to January in the central and southern regions, respectively. Ovarian development during these time periods was expected to be sufficiently advanced to identify females producing multiple broods but early enough to minimize failing to detect multiple brooding due to degradation of spawning markers (POFs). Only reproductively active females (those with ovaries in the vitellogenic, fertilized, and eyed-larvae macroscopic stages) were selected for analyses, as accurate detection of multiple brooders decreases significantly at other stages of development. Fifty percent (or at least 15 if fewer than 30 fish were collected) of the reproductively active ovaries were processed for each month, year, and region combination, and samples were selected to match the observed proportion of macroscopically identified multiple vs. single brooding fish. To ensure sampling across the size range of the collection event, fish were sorted by length, with selection for processing including the smallest, largest, and every second fish.

Multiple brooding females identified histologically had ovaries in the partially spent phase (Table 2), and had evidence of either an upcoming brood or a previous brood in addition to the brood characterized by the leading cohort (Fig. 2). Upcoming broods were indicated by the presence of non-atretic oocytes at the secondary vitellogenic stage or later that co-occurred with fertilized eggs or eyed-larvae (Fig. 2a). Previous broods were indicated by the presence of degrading, “old” POFs co-occurring with vitellogenic oocytes (Fig. 2C) or with “recent” POFs from developing embryos. The distinction between “recent” and “old” POFs was based upon comparison of the two stages within the same ovary (Fig. 3.). The terms are meant to reflect relative age and are not a definition of a particular stage of POF degradation. As oocyte growth rates are unknown for chilipepper and mass atretic events were most common with oocytes at early stages of vitellogenesis (Lefebvre, unpublished data), primary vitellogenic oocytes were deemed to be too early in development to be certain oogenesis and fertilization could be completed within the reproductive season. Therefore, secondary vitellogenic oocytes were set as the minimum oocyte stage necessary to definitively indicate development of an upcoming brood.

To quantify the relationship between the probability of a mature female producing a second brood and maternal size, we modeled the proportion of multiple brooding chilipepper (P_{MB}) as a logistic regression (0=single brooder, 1=multiple brooder), with FL as a continuous variable and region (central or southern) as a categorical covariate. Model selection was determined by the Akaike information criterion (AIC); delta-AIC values (best fit=0.00) and model weights were reported. Only samples examined histologically were included in analyses.

2.3 FECUNDITY ANALYSES

Fecundity of each female was estimated gravimetrically by enumerating preserved oocytes or developing embryos from two weighed subsamples using a stereo microscope (see Beyer et al. 2015 for details). The two estimates of fecundity for each female were averaged, and the coefficient of variation was calculated as a measure of error. Samples were excluded from further analyses when the coefficient of variation was greater than 10%. Fecundity estimates spanned the entire collection time range from September to March of each reproductive season, and years were combined. Absolute fecundity (F, total number of oocytes or embryos) was estimated as the quotient of the subsample count and weight multiplied by the weight of the ovary. Weight-specific, or relative fecundity (F_{rel}), was the quotient of F and maternal somatic weight (g). Samples from ovaries in the vitellogenic macroscopic stage that had multiple sizes of vitellogenic oocytes were excluded from analyses, as it was presumed that either recruitment of oocytes to the spawning cohort was incomplete or oocytes from multiple cohorts (batches) were present in these samples. In samples from ovaries with developing embryos, only the embryos were collected and enumerated. As such, these fecundity estimates represent potential annual fecundity for single brooders and brood (batch) fecundity for multiple brooders.

The relationships of length with F and F_{rel} were examined to determine if there was a significant maternal size effect on egg production. The fecundity-length relationship was best described by the power function:

$$\boldsymbol{F = a \times L^{b}}$$

where L = fork length (mm). The parameters a and b were the intercept and slope, respectively, of the linear regression of natural-log transformations of absolute fecundity and length. The value for a is reported after back-transformation using the bias correction term exp ($\sigma^2/2$). Values for p and R^2 are reported for the log-transformed equation. Geographic effects were examined by including region as a categorical covariate in an analysis of covariance (ANCOVA). Relative fecundity was best described as a linear function of female length as:

$$\boldsymbol{F_{rel} = c + Ld}$$

where L = fork length (mm), and c and d are the intercept and slope, respectively, of the linear regression fit to the data.

2.4 ANALYSIS OF PRODUCTIVITY

To assess the effect of multiple brooding on production, potential annual fecundity (*PAF*) was modeled as:

$$\boldsymbol{PAF = F \times (1 + P_{MB})}$$

which assumed that females produced a maximum of two broods and brood fecundity was equal between broods (Appendix A). The relative potential annual fecundity (PAF_{rel}) at length (L) was estimated as:

$$\boldsymbol{PAF_{rel} = \frac{F \times (1 + P_{MB})}{W}}$$

where W was the somatic weight g at L, as determined using the relationship $W = 4.05e^{-6} * L^{3.2}$ from the 2015 stock assessment (Field et al. 2016). *PAF* and PAF_{rel} are "potential" fecundity estimates as fecundity down-regulation through atresia was not fully accounted for. All data were analyzed using R (Version 3.2.1) with a significance level of $p<0.05$.

3. RESULTS

In total, 794 female chilipepper rockfish were collected in the 2013-14, 2014-15, and 2015-16 reproductive seasons (September to March) from Central (n=417) and Southern (n=377) California waters (Table 3). No collections were made in September or March off Southern California. Fish collected off Central California had higher average (± standard deviation) and maximum lengths (392 ± 69 and 500 mm FL, respectively) than those from Southern California (305 ± 44 and 454 mm FL, respectively; Table 3). Based on macroscopic staging, the period of peak ovarian development and gestation was October through January in both regions, though vitellogenesis initiated prior to the first collections (Fig. 4).

Ovarian histological samples from all females macroscopically staged as pre-vitellogenic or resting between October and January (n=66) were examined to identify adolescent females and those undergoing abortive maturation. The maximum length of these females was 401 mm, and 83% (n=55) of these were below the reported length at 95% maturity (L_{95}; 352 mm; Field et al. 2016). Fifteen (23%) of these ovaries were undergoing abortive maturation (<2.5% of all females and 4% of all fish <L_{95}), and fourteen (21%) were adolescents. These 29 fish, all <340mm FL, were unlikely to have been capable of reproducing during the season of capture All adolescent females appeared to have experienced abortive maturation; however, degeneration within the ovary was too advanced to definitively define abortive maturation. Of the remaining pre-vitellogenic or resting stage ovaries, eight (12%) were immature; 18 (27%) were in the developing or spawning capable subphases and capable of reproducing in the season of capture; and 11 (17%) had likely already reproduced during the season of capture (regressing and regenerating phases). There was no evidence of skip spawning.

Incidence of alpha atresia of the leading oocyte cohort was examined in the 113 ovaries that were placed in the developing and spawning capable histological phases. When atresia was observed, individuals generally displayed low (5% or less; n=88) or moderate (>5-25%; n=21) downregulation.

Four females with gestating eggs/embryos showed mass atresia of the leading oocyte cohort (which was at the primary vitellogenic stage in all ovaries) suggesting they were aborting a secondary brood.

Multiple broods were identified macroscopically in 230 chilipepper ovaries (49% of all fish with ovaries in the vitellogenic and fertilized egg or eyed-larvae macroscopic stages). They were first evident in November in the central region and October in the southern region (Table 4). The smallest multiple brooders were 300 and 252 mm FL in the central and southern regions, respectively. Most fish showed evidence of a maximum of two broods; however, four (two from each region; <3% of all multiple brooders) had evidence of three broods (e.g. macroscopically visible as early eyed-larvae [brood #2] with residual, degrading eyed-larvae [brood #1] and developing vitellogenic oocytes [brood #3], or histologically presenting as two stages of POFs [broods #1 & #2] with developing vitellogenic oocytes [brood #3]).

Overall agreement between macroscopically and histologically designated multiple brooding chilipepper was good (92%) for samples collected between October and (n=229; Table 4). However, histological examination provided the most accurate assessment, as 12 (5%) fish were identified as multiple brooders with histological but not macroscopic examination. These females either had developing vitellogenic oocytes and POFs (n=3, 25%); were gestating a brood and had upcoming vitellogenic oocytes (n=4, 33%); or were gestating a brood and had a second, older POF stage (n=5, 42%). Two fish (2%) identified macroscopically as multiple brooders did not show histological evidence (2 stages of POFs). Anecdotal evidence suggests degradation of residual eyed-larvae takes longer than that of POFs (Kashef and Stafford, unpublished data), and it is possible that the older POFs were fully degraded or sparse enough to preclude histological detection in these cases. Two (2%) additional fish from January in Southern California were macroscopically staged as having upcoming broods but had primary vitellogenic oocytes, below the developmental stage threshold for defining an upcoming brood histologically.

Based on the subset of histology samples selected for multiple brooding analyses (n=97 Central; n=78 Southern), 59% (n=57) and 69% (n=54) of chilipepper ovaries showed evidence of multiple brooding in Central and Southern California, respectively (Table 4). Recruitment from the reserve stock of primary growth and oil vacuole stage oocytes for secondary broods appeared to occur when the leading oocyte cohort was nearing maturation (mean maximum oocyte diameter >650 μm) or during gestation of the primary brood.

In modeling P_{MB}, region was a significant covariate (Table 5). In the best fit model, the slopes of the logistic regressions were the same between regions, but the intercepts were significantly different (Table 5; Fig. 5), possibly reflecting regional differences in life history traits. The resulting logistic regression model showed that P_{MB} increased with maternal size, with a greater proportion of multiple brooding in southern fish (Table 6; Fig. 5). At the maximum observed sizes in each region, >90% of females were predicted and observed to be producing multiple broods (Fig. 5).

Single brood (e.g. batch) fecundity estimates from 358 chilipepper (n=168 Central; n=190 Southern) were included in the analysis of regional and maternal size effects. Region was not a significant covariate (ANCOVA; p=0.07; df=354) and estimates were combined. The relationship between absolute fecundity and length was $F = 3.06e^{-5} * L^{3.73}$ (R^2=0.88; p<0.01; df=356; Fig. 6A). Relative fecundity was also significantly related to maternal size, with $F_{rel} = 46.44 + 0.31L$ (R^2=0.26; p<0.01; df=356; Fig. 6B).

After incorporating P_{MB} into fecundity estimates, both PAF and PAF_{rel} showed substantial increases in both regions compared to estimates of a single brood (Fig. 7).

4. DISCUSSION

All fishes face life-history trade-offs between somatic growth and reproduction. Total fecundity increases with size, so unless survival to the next reproductive season is unlikely, lifetime reproductive value may be increased when a female delays maturity and invests in growth (Charnov et al. 2001; Rideout et al. 2005; Harvey et al. 2011). This is particularly true when annual egg production disproportionately increases with size, as evident in the increase in F_{rel} with female size for most *Sebastes* species (Dick et al. 2017). The trade-off between somatic growth and reproduction continues even after reproductive maturity has been attained, as seen by the shorter spawning seasons and lower spawning frequency of smaller/younger compared to larger/older indeterminate spawners (Ganias et al. 2007; Fitzhugh et al. 2012; Lefebvre et al. 2016). Chilipepper off Central and Southern California appear to demonstrate both of these scenarios.

Chilipepper were shown here to exhibit delayed maturation: some females reached physiological maturity but remained functionally immature, as evidenced by adolescent females and those undergoing abortive maturation, all of which were below the reported L_{95}. The observation of abortive maturation in chilipepper is the first report of this phenomenon occurring in a *Sebastes* species with a relatively shallow distribution (continental shelf) and moderate longevity (max age 35 years; Love et al. 2002). Mass atresia events have been documented in several rockfish species, including blackgill (*S. melanostomus*; Lefebvre and Field 2015), darkblotched (*S. crameri*; Nichol and Pikitch 1994), and Pacific Ocean perch (*S. alutus*; Hannah and Parker 2007), all species with deep distributions and lifespans of 60 to 90 years. As mass atresia events in these species occurred mostly in fish below the length or age at 99% maturity, and given the longevity, low natural mortality rates, and strong maternal effects observed in rockfishes (Berkeley et al. 2004), it seems plausible these females were shunting energy to growth by delaying maturation completely (e.g. abortive maturation) or skipping opportunities early in their reproductive lives in favor of increasing their fitness (in terms of lifetime reproduction; Charnov et al. 2001).

In chilipepper, ovaries in the early stages of mass atresia events were easily identifiable in histological sections. Among all females, the observed rate of abortive maturation was low (<2.5%); however, distinguishing between fish that experienced mass atresia but were in later stages of resorption from fish that were resorbing material left over after successful reproduction was difficult. Histological examination improved the classification of early developing, post-parturition (regressing and regenerating), and abnormally developing ovaries (abortive maturation), but could not resolve with absolute certainty the maturity status of some individuals, an issue common to all reproductive studies (Hunter and Macewicz 2003). Due to asynchrony in the timing of spawning within this population, it is possible that some of the females classified as adolescents had actually reproduced previously or that some of those with ovaries staged as regenerating had undergone abortive maturation. However, histological characteristics of the tissue lend support for those females classified as adolescents or undergoing abortive maturation having not yet successfully reproduced (Lowerre-Barbieri et al. 2011). Documentation of a prolonged adolescent period in chilipepper and other *Sebastes* species emphasizes the necessity for careful consideration of sampling time periods and the distinction between physiological and functional maturity when determining spawning ogives.

Multiple brooding was a common phenomenon in chilipepper in waters off both Central and Southern California during the 2013-14, 2014-15, and 2015-16 reproductive seasons. Multiple brooding in other *Sebastes* species was also observed more frequently than in previous years in both regions (Beyer, unpublished data). Unexpectedly, the observed frequency of multiple brooding in chilipepper was nearly equal between the regions during this time period (Table 4), despite indications from previous studies that multiple brooding by any rockfish species was relatively rare north of Point Conception (Love et al. 1990; Ralston and MacFarlane 2010; Stafford et al. 2014). Interestingly, in collections dating to 2009, multiple brooding (as identified macroscopically) was never observed at Cordell Bank[2], only occasionally observed in Half Moon Bay, and observed at a lower frequency in the south prior to the 2013-14 reproductive season (Beyer et al. 2015). While rates between this and the earlier collections are not directly comparable, because earlier collections relied solely on macroscopic observations of residual larvae in ovaries with developing oocytes to signify multiple brooding, this type of observation did increase in the current study.

The rates of multiple brooding observed in chilipepper in this study, regardless of whether or not they were atypical, should be considered conservative estimates. Females developing a secondary brood have been detected histologically as early as September in both regions (Lefebvre, unpublished data) and macroscopically as late as March (Table 4). However, the protracted reproductive season and observed asynchronicity in ovarian development within the population made it difficult to accurately assess the prevalence of multiple brooding outside of a narrow temporal window. The time period chosen for analyses should have removed any confounding effects of seasonality and size on the propensity to produce multiple broods. Even if small, young females initiated ovarian development later than larger, older females (as is common across taxa; Fitzhugh et al. 2012; Hixon et al. 2014), evidence of an upcoming brood should have been present given the duration of gestation and the fact that the majority of females (of all sizes) had ovaries in a post-parturition stage by February. The restricted sampling period more likely resulted in an underrepresentation of females that were capable of producing or had already produced more than two broods. The conservative definition used to identify an upcoming brood and the inability to

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[2] Although not observed macroscopically, anecdotal evidence of multiple brooding in chilipepper was found in 2004-2008 at Cordell Bank (Stafford et al. 2014), where a bimodal trend in parturition timing was observed. Multiple broods were potentially missed macroscopically due to sampling periodicity.

detect more than two POF classes (due to degradation during the long brood intervals) further contributed to limited evidence of three or more broods.

While the environmental and physiological factors allowing for and driving multiple brooding in rockfishes are unknown, given the typically southerly distribution of multiple brooding within and among rockfish species, temperature could be a significant factor. Metabolic rates, and subsequently rates of oogenesis and embryonic development, increase with increasing temperature (Kjesbu et al. 2010; Lefebvre and Denson 2012). Rockfishes in the warm waters of the Southern California Bight may be better positioned in "normal" years, compared to fishes residing in cooler waters of the California Current, to produce multiple broods due to a shorter brood interval that results from increased metabolic rates. High food availability may also be an important factor enabling females to produce multiple broods, as increased egg production is positively correlated with female condition (Beyer et al. 2015). Regardless of how interannual variability differs between and within these two regions, the significant increase in the prevalence of multiple brooding with female size (P_{MB}) contributes further to the suite of maternal size- and age-related effects that result in a disproportionate contribution of larger, older females to larval production in rockfishes (see Hixon et al. 2014).

We speculate that atypical oceanographic conditions were the major contributing factor to the increased incidence of multiple brooding in the Central California region during this study period. The Northeast Pacific experienced a "marine heatwave" from 2013-2016, during which time the sea surface temperatures were at unusually high levels yet coastal waters in the California Current experienced average to above average upwelling and abundant forage (pelagic micronekton) conditions (Di Lorenzo and Mantua 2016; McClatchie et al. 2016; Sakuma et al. 2016). In particular, the abundance of pelagic juvenile rockfish off of Central California during the time period of this study (2013-14, 2014-15 and 2015-16 spawning seasons, corresponding to pelagic juvenile abundance in the late spring of 2014-2016) were among the highest observed in a 30+ year time series (Sakuma et al. 2016, Wells et al. 2017). Several stock assessments also indicated very strong recruitment for recent year classes such as 2013 and 2014 (Field et al. 2016; He and Field 2018), although data to inform more recent year classes have not yet been incorporated into assessment models. While it is premature to directly connect high pelagic juvenile abundance and strong year classes from assessments to the potential increase in multiple brooding, it is fair to assume that an increase in the frequency of multiple brooding, which presents an increase in larval productivity, should presumably also increase the overall recruitment in chilipepper and other multiple brooding species. These unusual conditions of warm temperatures with potentially favorable foraging opportunities may have allowed more females in the central region to successfully produce a second brood during the study period. We expect the frequency of multiple brooding to decrease in years with more typical oceanographic conditions for the region; however, at present, our time series of the frequency of multiple brooding is too short to formally evaluate any potential relationship to environmental factors.

Reproductive strategy, including fecundity pattern, is generally considered a fixed life-history characteristic; however, chilipepper demonstrated flexibility in this regard. Grier et al. (2005) suggested viviparity represents a special case of indeterminate batch fecundity (Murua and Saborido-Rey 2003). The development of distinct broods, whose oocytes are recruited independently from the reserve stock of primary growth and oil vacuole stage oocytes, observed in chilipepper in this study and in other multiple brooding rockfishes (Moser 1967b; MacGregor 1970), supports this theory. Furthermore, the development of discrete batches, as opposed to overlapping batches typical of indeterminate spawners (Lefebvre et al. 2016), has been observed outside of *Sebastes* (McBride et al. 2017). Historically, chilipepper in the central region (and all single brooding rockfishes), appeared to be determinate total spawners (Murua and Saborido-Rey 2003), synchronously maturing all oocytes for production of a single brood annually. The observation of multiple brooding off Central California may not represent a change in fecundity pattern per se, as these fish are likely always biologically capable of indeterminate batch spawning behavior, but may instead be a response to favorable environmental and physiological factors. In particular, larger females, whose somatic growth has slowed, may be better positioned to exploit favorable conditions and "switch" to multiple brooding. This switch results in a near doubling of *PA*F, though the relationship between brood fecundity and brood number requires further exploration and refinement.

Most species of *Sebastes* have never been observed to produce multiple broods. The results of Beyer et al. (2015) and Dick et al. (2017) suggest that the maternal effects on brood fecundity are greater in single brooding species (such as yellowtail [*S.* flavidus] and blackgill rockfish), based on the steepness of the fecundity-size relationships for single broods. However, when multiple broods were accounted for in the present study, the slope of the relative fecundity relationships became considerably steeper for chilipepper, indicating that maternal effects are as important in multiple brooders as they are in single brooding species. In population assessment models, changes in the slope of fecundity–size relationships can result in significant changes to estimates of stock depletion, which in turn, may alter perception of stock status (Spencer and Dorn 2013; He et al. 2015).

For five decades, researchers have recognized the need to account for multiple brooding in estimates of egg production in multiple brooding rockfishes (Moser 1967a; Love et al. 1990; Beyer et al. 2015). We provided a basic approach to account for the relationship between multiple brooding and maternal length that could easily be incorporated into age-structured stock assessment models such as Stock Synthesis (Methot Jr. and Wetzel 2013). The model could be refined to account for the production of more than two broods and differences in fecundity among sequential broods (Appendix A). Accurately accounting for multiple brooding remains difficult, as sampling needs to be timed appropriately and ovaries need to be examined microscopically (fresh or histologically) to be able to best detect the phenomenon. However, an understanding of oocyte growth rates could enable earlier detection of upcoming broods and allow for estimating the maximum number of broods possible within the reproductive season. The observed maternal effect and the propensity to produce multiple broods may strengthen further if there is also a size effect on the duration of the reproductive season, such that, of multiple brooding individuals, larger females may be more likely to produce three or more broods. Understanding the biological and environmental drivers of multiple brooding, how the rates compare among species, and how its prevalence varies interannually will be essential to future management of multiple brooding *Sebastes* species.

The plasticity in reproductive effort demonstrated by chilipepper may help them and other multiple brooding *Sebastes* species buffer the effects of future climate change in the California Current. While fisheries productivity is forecasted to increase in temperate regions due to expansion of habitat and increased primary productivity (Britten et al. 2016), within the California Current region, patterns of circulation and wind forcing are expected to change such that seasonal upwelling will become less predictable (Checkley and Barth 2009; Rykaczewski et al. 2015). Given the relationship of both P_{MB} and F_{rel} with maternal size, the ability to forego reproduction through abortive maturation when environmental or individual physiological conditions are unfavorable may increase lifetime fitness by allowing excess energy to be devoted to somatic growth (Harvey et al. 2011). Furthermore, the flexibility to change the frequency of reproduction and double (or more) annual fecundity may allow these species to take advantage of favorable conditions. Given that the frequency of abortive maturation and multiple brooding likely vary both spatially and temporally, monitoring the prevalence and modeling the significance of these phenomena will be key to accurately assessing the reproductive potential of these stocks as oceanographic conditions in the California Current change.

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FIGURE LEGEND

Figure 1. Map of sampling locations. Sampling locations of chilipepper rockfish along the coast of California. Collections from Cordell Bank and Half Moon Bay were combined into the Central region, while collections from the Santa Barbara Channel in the Southern California Bight were considered the Southern region.

Figure 2. Histological micrographs of single and multiple brooding rockfish. Histological comparison of multiple brooding (A, C) and single brooding (B, D) female chilipepper. A and B show postovulatory follicle complexes (POF) from the current, gestating brood. The secondary vitellogenic oocytes (Vtg2) in A are evidence of an upcoming brood, whereas oil vacuole (OV) and primary growth (PG) stage oocytes in B indicate that female would not produce another brood. C) Multiple brooding female in the partially spent histological subphase with evidence of a previous brood (POF). D) Single brooding female in the late developing subphase with the leading oocyte cohort at a similar stage of development to C. Primary vitellogenic oocytes (Vtg1) in D were below the stage of development required to define them as an upcoming brood. E=Embryo; Vtg3=tertiary vitellogenic oocytes. Scale bars=250 μm.

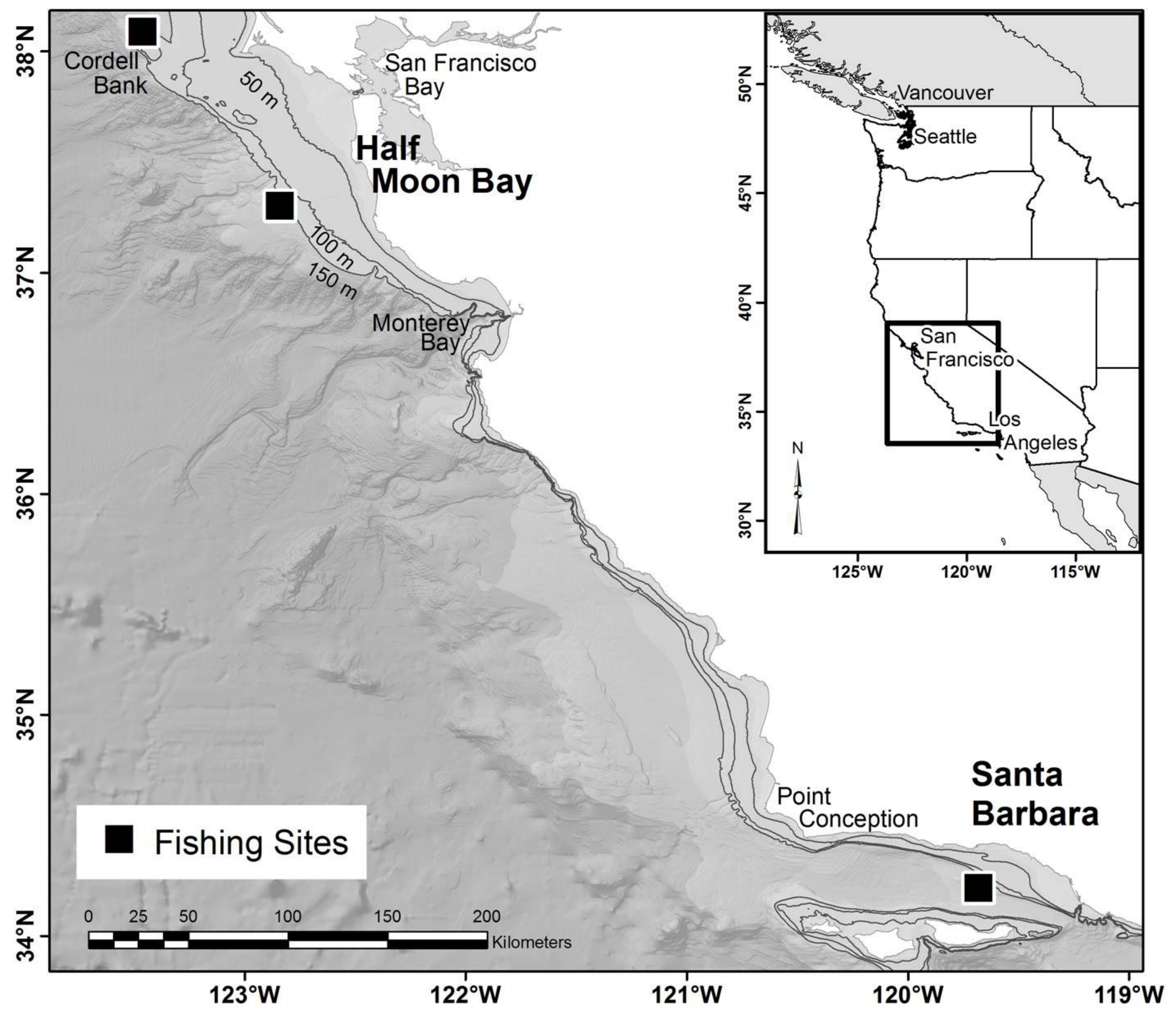
Figure 3. Histological micrographs of POF A) Micrograph from a histological ovarian section from a chilipepper collected off central California in December 2103, showing fertilized eggs (FE) and two stages of postovulatory follicle complexes (POF). B) Recent POFs (POF-R) had open lumens (L), clusters of red blood cells (RBC) in the thecal layer, and thecal and granulosa cells that were mostly intact. C) Older POFs (POF-O) lacked a distinct lumen, were more vacuolated compared to POF-R, and showed degeneration of thecal and granulosa cells. The arrow in (C) points to the POF-O. Scale bar equals 250 μm in A and 50 μm in B and C.

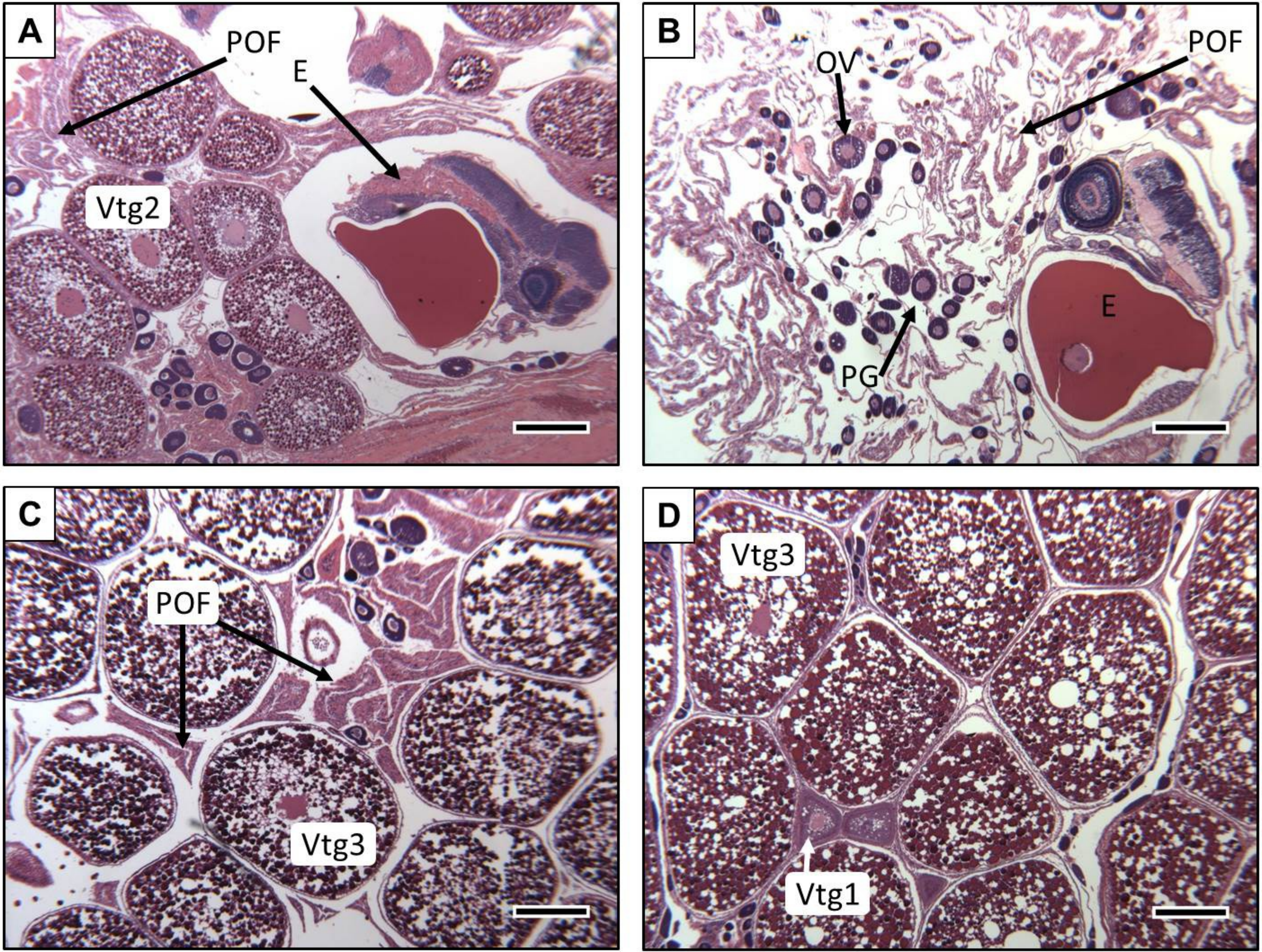
Figure 4. Seasonality of ovarian development. Seasonal pattern of ovarian development from macroscopically staged ovaries (n=794) of chilipepper in (A) Central and (B) Southern California. Years were combined, and monthly sample sizes are indicated above the bars.

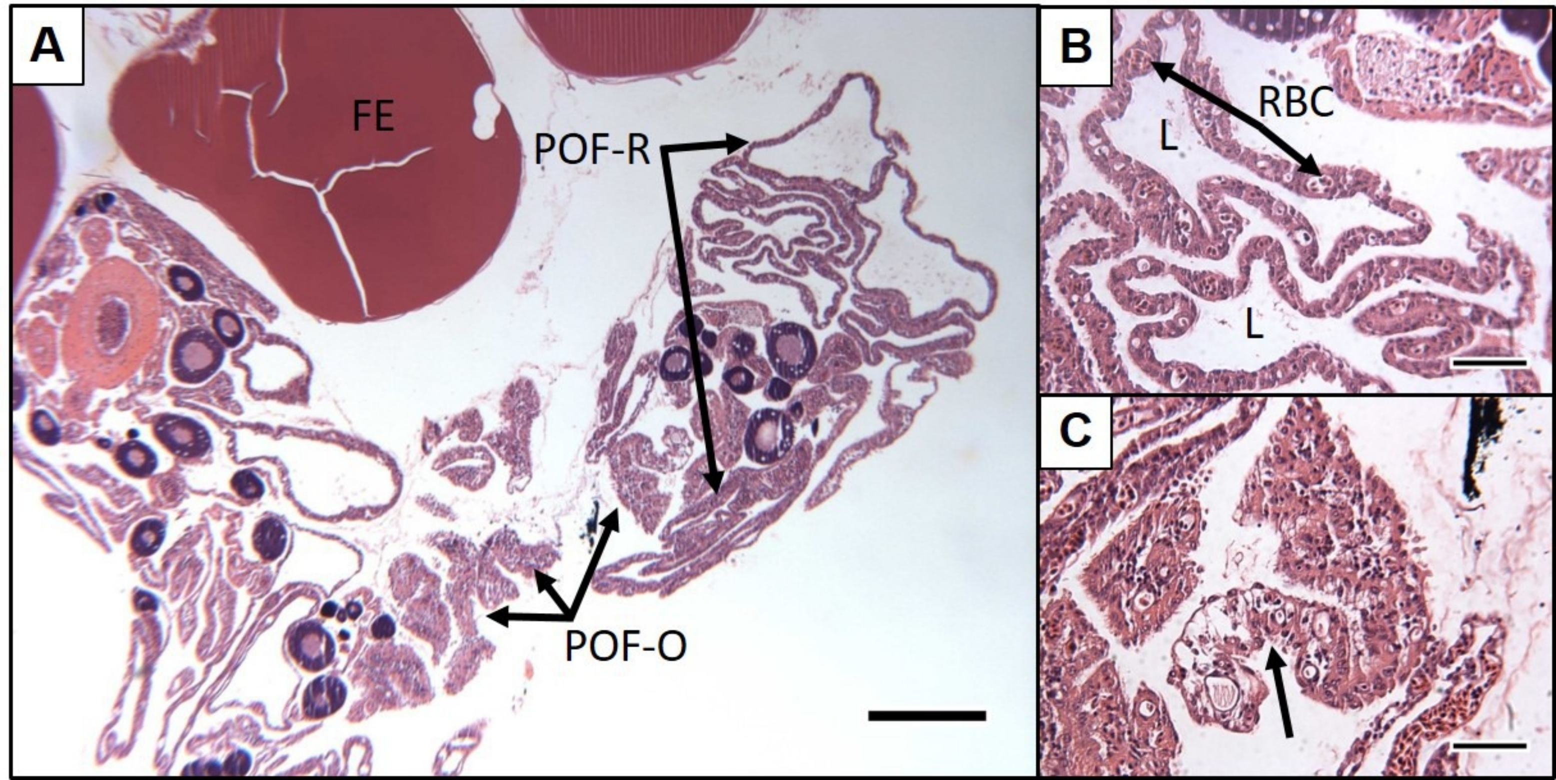
Figure 5. Prevalence of multiple brooding. The proportion of female chilipepper multiple brooding at length off Central and Southern California. Curves of the logistic regression (model 3) reflect minimum and maximum collected lengths by region. The symbols (square and circle) represent observed proportions at 20 mm length-bins in each region (central and southern, respectively) and were excluded when there were less than five observations in a given bin.

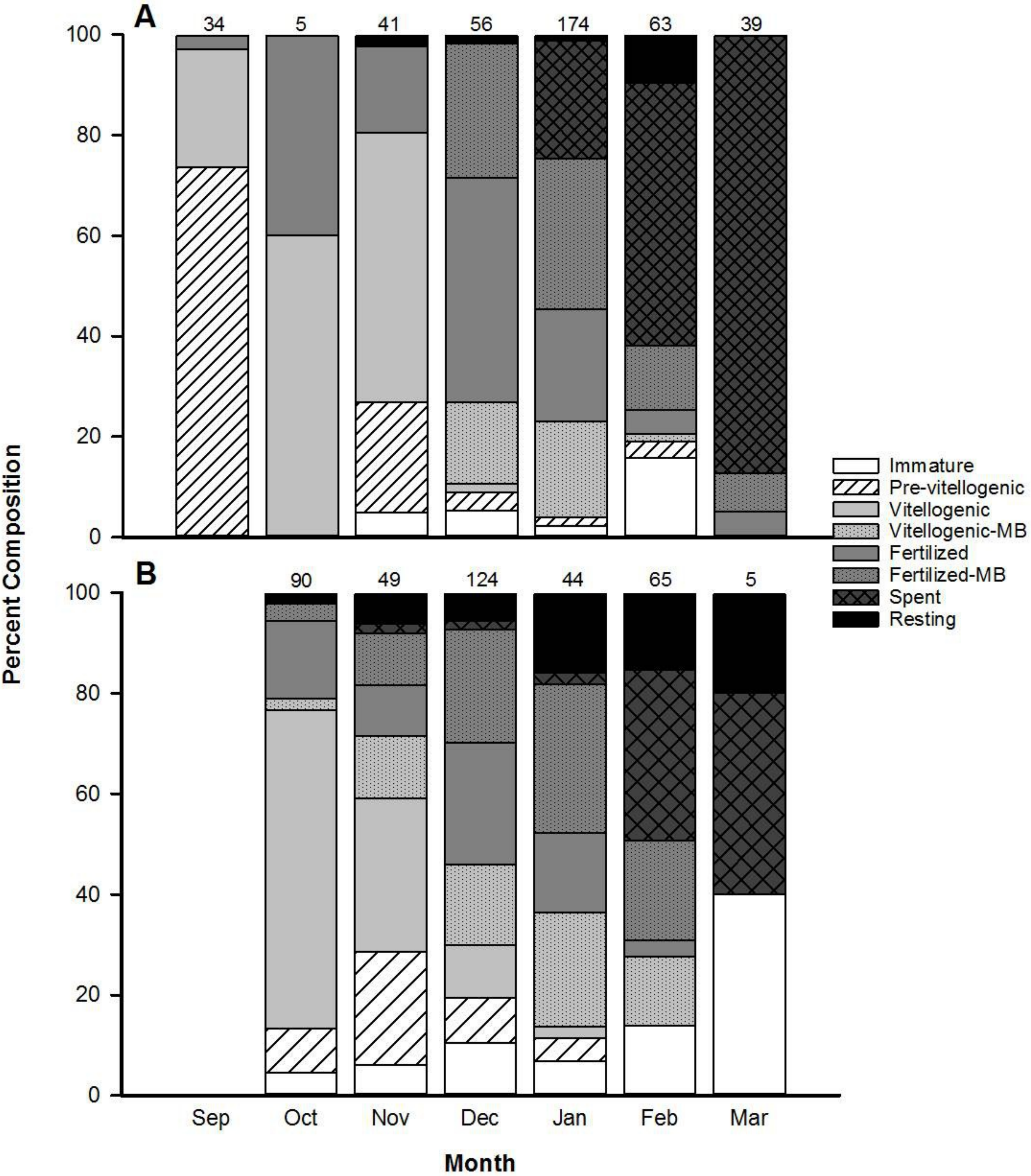
Figure 6. Basic fecundity relationships. A) Total and B) relative fecundity in chilipepper rockfish with ovaries in the developing or fertilized/eyed-larvae stages. Regions were combined for analyses.

Figure 7. Potential annual fecundity relationships.A) Potential annual fecundity (PAF) and B) relative potential annual fecundity (PAF_{rel}) in chilipepper, incorporating the probability of multiple brooding by maternal size, with separate functions for each region. Fecundity of single broods at length (F and F_{rel}) is plotted for comparison. The plots reflect the minimum and maximum sizes collected in each region.


Cordell Bank
San Francisco Bay
Half Moon Bay
50 m
100 m
150 m
Monterey Bay
Point Conception
Santa Barbara
Fishing Sites
0
25
50
100
150
200
Kilometers
38°N
37°N
36°N
35°N
34°N
123°W
122°W
121°W
120°W
119°W
Vancouver
Seattle
San Francisco
Los Angeles
N
50°N
45°N
40°N
35°N
30°N
125°W
120°W
115°W


A
POF
E
Vtg2
B
OV
POF
E
PG
C
POF
Vtg3
D
Vtg3
Vtg1


A
FE
POF-R
POF-O
B
RBC
L
L
C

A
B
34
5
41
56
174
63
39
90
49
124
44
65
5
100
80
60
40
20
0
Percent Composition
Sep
Oct
Nov
Dec
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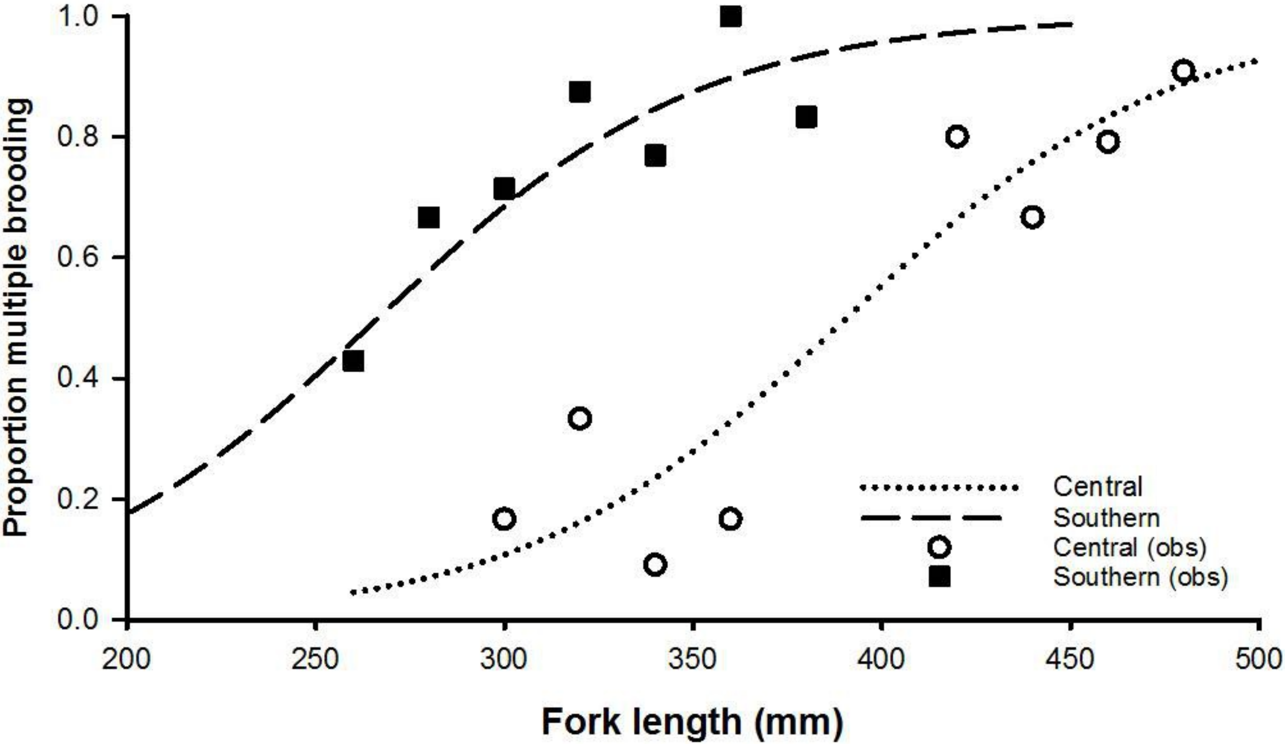
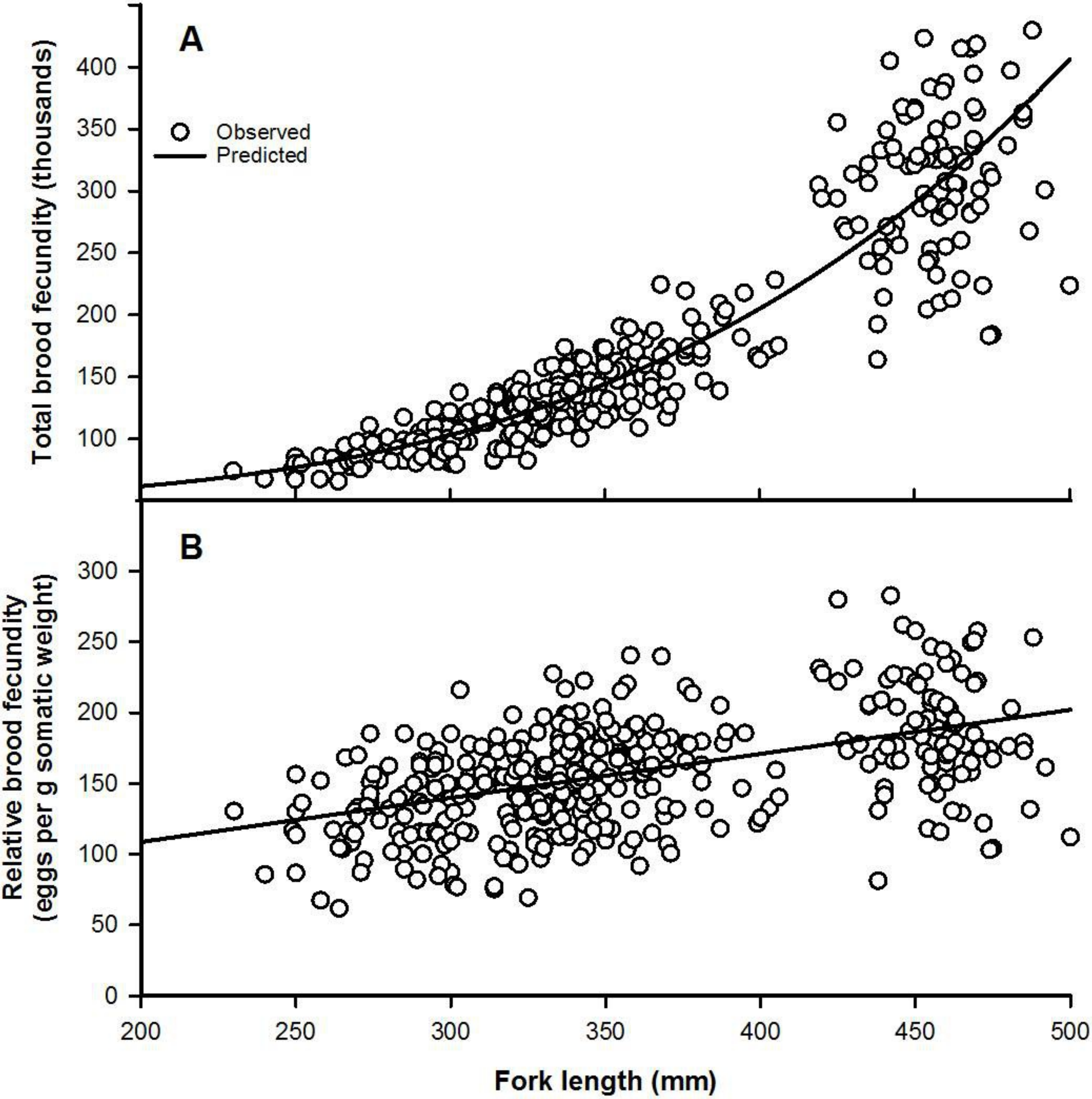
Fertilized
Fertilized-MB
Spent
Resting


Proportion multiple brooding
1.0
0.8
0.6
0.4
0.2
0.0
200
250
300
350
400
450
500
Fork length (mm)
Central
Southern
Central (obs)
Southern (obs)


A
Observed
Predicted
Total brood fecundity (thousands)
100
150
200
250
300
350
400
B
Relative brood fecundity (eggs per g somatic weight)
0
50
100
150
200
250
300
200
250
300
350
400
450
500
Fork length (mm)

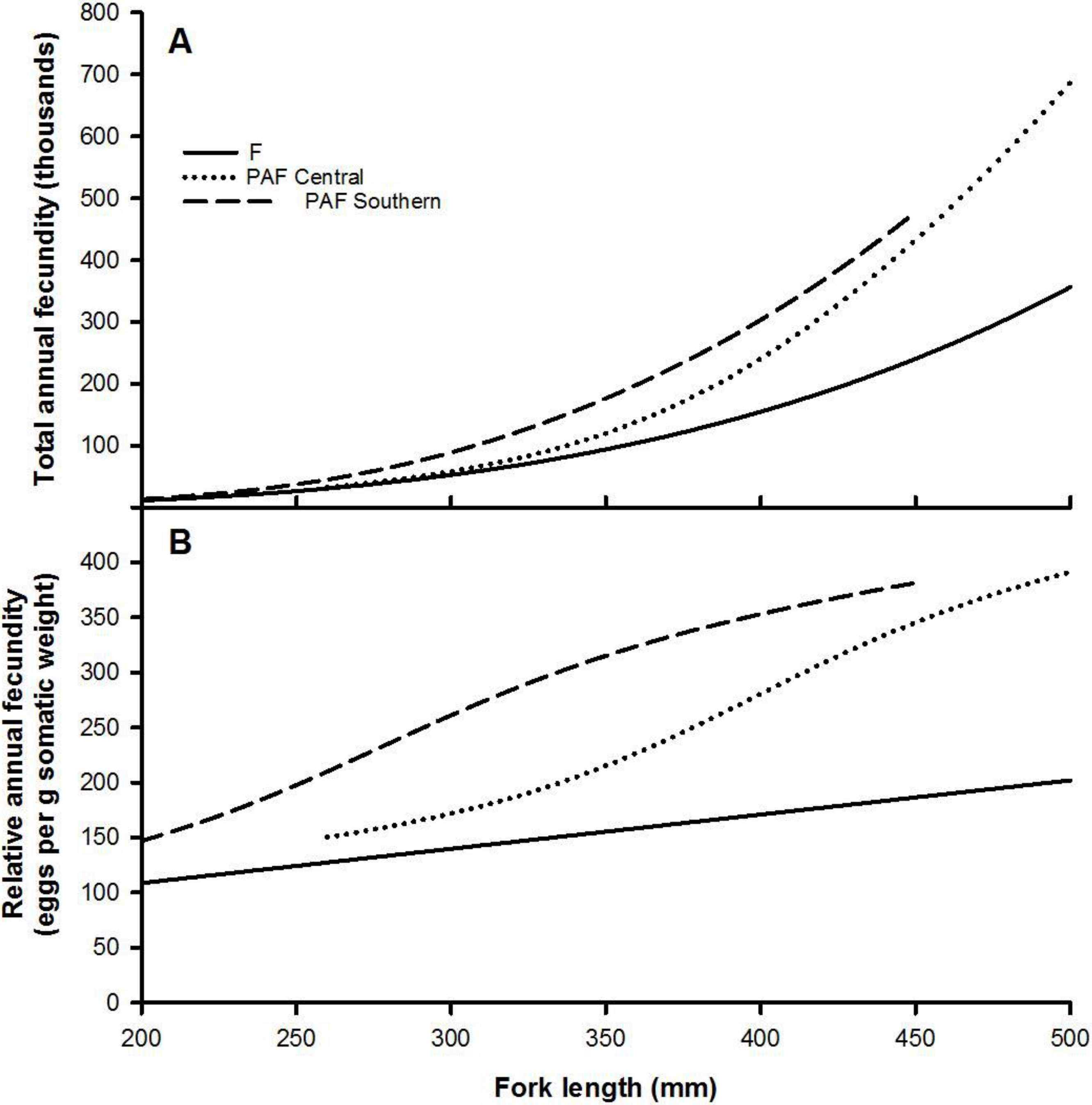

A
B
F
PAF Central
PAF Southern
Total annual fecundity (thousands)
800
700
600
500
400
300
200
100
Relative annual fecundity (eggs per g somatic weight)
400
350
300
250
200
150
100
50
0
200
250
300
350
400
450
500
Fork length (mm)

Table 1. *Sebastes* species in which multiple brooding has been macroscopically observed. All central California (CCA) observations are from unpublished data. SCB=Southern California Bight. Sources: A=Moser 1967a; B=MacGregor 1970; C=Echeverria 1987; D=Love et al. 1990; E=Love et al. 2002; F=Ralston and MacFarlane 2010; G=Beyer et al. 2015; H=Stafford and Kashef, unpublished data; I=Beyer, unpublished data.

Scientific name	**Common name**	**Region**	**Source(s)**
S. auriculatus	Brown rockfish	CCA	E, H
S. chlorostictus	Greenspotted rockfish	SCB	D, E, I
S. constellatus	Starry rockfish	SCB, CCA	B, D, E, I
S. elongatus	Greenstriped rockfish	SCB	D, E
S. ensifer	Swordspine	SCB	D, E, I
S. goodei	Chilipepper rockfish	SCB	D, E, G, I
S. hopkinsi	Squarespot rockfish	SCB	D, E, I
S. jordani	Shortbelly rockfish	SCB	E, I
S. levis	Cowcod	SCB	D, E, I
S. ovalis	Speckled rockfish	SCB, CCA	B, D, E, G, I
S. paucispinus	Bocaccio	SCB, CCA	A, B, C, D, E, F, I
S. rosaceus	Rosy rockfish	SCB, CCA	D, E, I
S. rosenblatti	Greenblotched rockfish	SCB	D, E
S. rufus	Bank rockfish	SCB	D, E

Table 2. Macroscopic stages and corresponding histological phases of ovarian development in chilipepper. GVB=germinal vesicle breakdown oocyte; GVM=germinal vesicle migration oocyte; HO=hydrated oocyte; LC=leading oocyte cohort; PG=primary growth oocyte; POF=post ovulatory follicle complex; OV=oil vacuole oocyte; Vtg1=primary vitellogenic oocyte; Vtg2=secondary vitellogenic oocyte; Vtg3=tertiary vitellogenic oocyte.

MACROSCOPIC	**HISTOLOGICAL**		
Stage	**Phase**	**Subphase**	**Description**
Immature	Immature		Only PG oocytes present. Lamellae are well organized with little to no atresia and no prominent blood vessels, muscular bundles, or connective tissue. Ovarian wall is thin.
Pre-vitellogenic	Developing	Early developing	The LC is OV or Vtg1 (<25% cytoplasm is vitellogenin). Low to moderate alpha atresia may be present. Prominent blood vessels, muscular bundles, connective tissue, and late stages of atresia; tissue disorganization; and a thick ovarian wall are indicative of females that have spawned in previous years.
Vitellogenic		Developing	The LC is Vtg2 (25-50% cytoplasm is vitellogenin). Low to moderate alpha atresia may be present.
	Spawning Capable	Late developing	The LC is Vtg3 (vitellogenin occupying >50% cytoplasm), GVM, GVB, and unovulated HO.

			There is no evidence of recent spawning. Low to moderate alpha atresia may be present.
Fertilized Eggs or Eyed-Larvae		Gestating	Ovulated HO, fertilized eggs, or eyed-larvae present in ovarian lumen. One class of POF present and abundant. The LC is Vtg1 or earlier.
		Partially spent	Upcoming brood: resembles gestating subphase but with LC Vtg2 or later. Previous brood: resembles developing or late developing subphase with POFs present; or gestating subphase with recent (from developing embryos) and old POFs present.
Spent	Regressing	Regressing	The non-atretic LC is oogonia, PG, or OV; other oocyte stages present are undergoing atresia (mostly alpha and beta). POFs may be present. Lamellae appear loose and disorganized. Blood vessels, muscular bundles, and connective tissue may be prominent and abundant.
		*Abortive Maturation	>50% of LC are undergoing alpha atresia. Ovarian wall is thin. There is no evidence of recent reproduction (POFs).
**Note: no corresponding macroscopic stage*		*Skipped Spawning	>50% of LC are undergoing alpha atresia. Ovarian wall is thick. Blood vessels, muscular bundles, and

		connective tissue are prominent and abundant throughout lamellae.
Resting	Regenerating	The LC is oogonia, PG, or OV. Oogonial nests are proliferating. Lamellae appear more organized compared to regressing subphase, though atresia (mostly delta and gamma) is common. Blood vessels, muscular bundles, and connective tissue maybe prominent and abundant. Oogonial nests are proliferating.

Table 3. Collections of chilipepper rockfish by year and region, with the number available for abortive maturation (AM), multiple brooding (MB, with the number examined histologically in parentheses), and fecundity analyses.

Year	**Region**	**Total**	**Length range (average lengths ± standard deviation)**	**No. AM subset**	**No. MB subset**	**No. Fecundity**
2013	Central	179	217-506 (405 ± 63)	11	86 (48)	84
	Southern	79	229-454 (322 ± 41)	5	30 (15)	57
2014	Central	124	240-500 (349 ± 81)	5	54 (31)	47
	Southern	176	175-376 (294 ±43)	26	58 (31)	74
2015	Central	114	247-487 (377 ±53)	1	35 (18)	37
	Southern	122	204-403 (303 ± 46)	18	63 (32)	59
OVERALL	Central	417	217-506 (381 ±71)	17	175 (97)	168
	Southern	377	175-454 (303 ±45)	49	151 (78)	190

Table 4. Total number of female chilipepper collected (total no. females) and available for assessing prevalence of multiple brooding (No. vtg+) throughout the reproductive season. The number and percentages of all multiple brooders (No. MB) are noted as well as the subset selected for analysis of P_{MB} (No. in analysis). Bold text = totals by region; vtg+ = number of ovaries in the vitellogenic and fertilized egg/eyed-larvae macroscopic stages. MMB=macroscopically identified MB; HMB = histologically identified MB.

Region	**Month**	**Total no. females**	**No. vtg+**	**No. MB**	**% MB**	**No. in analysis**	**No. MMB**	**% MMB**	**No. HMB**	**% HMB**
Central	Sep	34								
	Oct	5	5	0	0%					
	Nov	46	34	2	6%					
	Dec	56	50	22	44%	32	16	50%	16	50%
	Jan	179	125	90	72%	65	44	68%	41	63%
	Feb	53	6	4	67%					
	Mar	44	5	3	60%					
	TOTAL	**417**	**225**	**121**	**54%**	**97**	**60**	**62%**	**57**	**59%**
Southern	Oct	95	77	5	6%					
	Nov	44	30	11	37%	15	7	47%	7	47%
	Dec	124	95	52	55%	48	29	60%	33	69%
	Jan	39	26	19	73%	15	10	67%	14	93%
	Feb	75	25	22	88%					
	TOTAL	**377**	**253**	**109**	**43%**	**78**	**46**	**59%**	**54**	**69%**

Table 5. Selection criteria and parameters of the best-fit model for the logistic regression modeling the proportion of multiple brooding chilipepper at length (*P_{MB}*): colons represent interactions between variables and bold text indicates the best-fit model.

Model	**# of Parameters**	**Delta-AIC**	**AIC-weights**
1) Intercept only	1	49.28	0.00
2) FL	2	33.37	0.00
3) FL + region	**3**	**0.00**	**0.69**
4) FL + region + FL:region	4	1.62	0.31

Table 6. Results of the logistic regression model 3 of the proportion of multiple brooding chilipepper at length (0=single brooder, 1=multiple brooder; fork length was a continuous variable and region [central, southern] was a categorical variable).

Coefficient	**Coefficient Estimate**	**Standard Error**
Intercept-Central	-9.0801	1.5719
Intercept-Southern	-6.1951	0.5598
Slope	0.0232	0.0038