Image analysis approach to estimate fecundity of livebearer rockfishes (*Sebastes* spp.) along the California Coast

Hayley Mapes[1,2*], Sabrina Beyer[1,2], Jessica Choi[1,2], Emma Saas[2], Suzanne Alonzo[1], John Field[2]

[1] University of California Santa Cruz, Department of Ecology and Evolutionary Biology, 1156 High Street, Santa Cruz, California 95064

[2] Fisheries Ecology Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 110 McAllister Way, Santa Cruz, California 95060

*Corresponding Author

Email: hayleymapes@gmail.com

ORCID

Hayley Mapes: 0000-0002-9824-055X

Sabrina Beyer: 0000-0002-4912-5467

Jessica Choi: 0000-0001-6504-6235

Emma Saas: 0000-0002-3801-9298

John Field: 0000-0002-7178-600X

Abstract

Current gravimetric methods, involving manual counts of oocytes or embryos to estimate fecundity for live-bearing rockfishes (*Sebastes* spp.) of the California Current Ecosystem are time-consuming, hindering the rapid assessment of reproductive potential. To improve efficiency in data collection we adapted the autodiametric method which uses image analysis to relate mean oocyte diameter to a weight-based oocyte density to rapidly estimate fecundity. We developed autodiametric calibration curves for unfertilized oocytes of five California Current rockfishes; chilipepper (*Sebastes goodei*), rosy rockfish (*S. rosaceus*), widow rockfish (*S. entomelas*), yellowtail rockfish (*S. flavidus*), and vermilion rockfish (*S. miniatus*). The autodiametric method was nearly five times faster than the gravimetric method

while maintaining precision and unbiased estimates. Autodiametric calibration curves were species-specific due to significant differences in oocyte density for a given mean diameter. The difference in densities was attributed to differences in mean oocyte weight, standardized by diameter, likely due to different stages of pre-fertilized development and/or energy investment among species. The autodiametric method was unable to detect and measure fertilized stages due to poor edge detection and non-circularity of embryos, however, gravimetric counts of embryos were more rapidly made compared to unfertilized samples. Most rockfishes exhibit strong maternal size effects on the number of offspring produced so the ability to rapidly assess maternal effects and to explore spatio-temporal trends in fecundity will provide valuable reproductive information to fisheries managers. This is particularly true given the need to evaluate the impacts of exploitation and climate change on reproductive life history parameters in this diverse genus.

Keywords: fecundity, *Sebastes*, gravimetric, autodiametric, image analysis

Acknowledgements

The authors would like to thank B. Bales, J. Lin, D. Stafford, N. Kashef, R. Miller, L. Lefebvre, K. Bartlett, K. Hanson, A. Del Colletti, T. Ernst, and K. Di Massa for support of laboratory and field collections. We thank N. Kashef, M. Kustra and D. Weiler for assistance with manuscript review and data analysis. Funding provided by the NOAA Saltonstall-Kennedy Grant Program (award # NA18NMF4270216) and the California Sea Grant College Program Project (award #NA18OAR4170323). The statements, findings, conclusions, and recommendations are those of the author(s) and do not necessarily reflect the views of California Sea Grant, NOAA, or the US Department of Commerce.

57 Introduction

58 Estimates of fecundity and other related data to inform reproductive ecology parameters are essential for fisheries
59 stock assessment models. This information allows researchers to estimate the reproductive potential of a stock,
60 which provides the basis for the reference points used by fisheries managers to ensure sustainability (Murua et al.
61 2003; Lambert 2008; Methot and Wetzel 2013; He et al. 2015). Unfortunately, fecundity information is often sparse
62 or lacking due to the relatively intense effort and time required to obtain robust sample sizes (Lambert 2008).
63 Because of this, spawning biomass was historically used as a proxy when fecundity information was lacking
64 (Beverton and Holt 1957), even though spawning biomass is often poorly correlated with total egg production
65 (Marshall et al. 1998; Trippel 1999; Marteinsdottir and Begg 2002). Although fecundity data is lacking for a large
66 fraction of marine fishes, a recent meta-analysis of species for which reproductive data does exist found that larger
67 females commonly invest disproportionately more energy into reproduction, in terms of eggs per gram of body
68 weight, compared with their smaller counterparts (Barneche et al. 2018). Therefore, size-specific fecundity
69 information is critical for population models used to determine stock reproductive potential and stock status (He et
70 al. 2015; Barneche et al. 2018; Marshall et al. 2021). Obtaining fecundity data for multiple species is costly and
71 time-consuming in the collection and processing of samples, the cost of supplies, and labor investment in counting
72 fecundity samples. This represent a major challenge to biologists, often resulting in a paucity of fecundity data for
73 many species (Thorsen and Kjesbu 2001; Witthames et al. 2009).

74 The weight-based gravimetric method is currently one of the most widely-used methods to determine
75 fecundity (Murua et al. 2003). The gravimetric method involves dissecting and weighing a small subsample
76 (typically 0.5 – 1 g) of the ovary, then preserving and counting the oocytes or embryos manually under a
77 microscope. Fecundity is estimated based on the density of oocytes in the subsample (oocytes * g^{-1}) multiplied by
78 the total weight of the ovary (Kjesbu and Holm 1994). The number of oocytes in a half-gram to one-gram subsample
79 can vary from hundreds to over ten-thousand depending on the species, reproductive strategy, size, and stage of
80 development. A range of approximately 5,000 to 40,000 oocytes g^{-1} was reported for Atlantic cod, *Gadus morhua*
81 (Thorsen and Kjesbu 2001). In the live-bearing rockfishes, the number of oocytes per gram ranges from
82 approximately 1,000 for chilipepper (*Sebastes goodei*), which have large oocytes to over 25,000 for rosy rockfish,
83 (*S. rosaceus*), which have smaller oocytes. The number of fertilized embryos in a one-gram sample is less and
84 ranges from 500 embryos per gram in speckled rockfish, which have larger embryos (*S. ovalis*) to over 7,000 for

85 squarespot (*S. hopkinsi*), which have smaller embryos (Beyer et al. unpublished). The time commitment and labor
86 necessary to manually enumerate a large number of oocytes in each sample hinders the rapid collection of fecundity
87 data but is less restrictive for the larger, fertilized stages.
88 The autodiametric fecundity method has been proposed and implemented as a time-efficient alternative to
89 gravimetric counts (Thorsen and Kjesbu 2001; Klibansky and Juanes 2008; Flores et al. 2017; Domínguez-Petit et
90 al. 2018). The autodiametric method uses image analysis software to analyze and measure oocyte diameters. A
91 calibration curve is then developed, which relate the mean oocyte diameter of a sample to an oocyte density. The
92 oocyte density is usually derived from a weight-based sampling and counting method. Similar to the gravimetric
93 method, the oocyte density obtained from the calibration curve is then multiplied by the weight of the ovary to
94 estimate fecundity. Previous studies estimating fecundity of marine fishes with the autodiametric method used a
95 video camera connected to a stereomicroscope (Thorsen and Kjesbu 2001; Witthames et al. 2009), or a flatbed
96 scanner to capture images of oocytes (Friedland et al. 2005; Klibansky and Juanes 2008). Once a calibration curve is
97 developed, weighing, and counting subsamples of oocytes is no longer needed as fecundity is estimated by
98 automated measurements of oocyte diameters and then the calibration curve is used to obtain oocyte density. The
99 oocyte density of the sample from the calibration curve is then multiplied by the gonad weight to estimate fecundity.
100 This method eliminates the need to weigh and count subsamples once the calibration curve is established, making
101 the sampling process more efficient (Thorsen and Kjesbu 2001). An additional benefit of the autodiametric method
102 is the ability to document and archive images of the distribution of oocyte sizes and spawning status (Murua et al.
103 2003).
104 Drawbacks of the autodiametric method include the need for start-up costs to purchase electronic
105 equipment (Murua et al. 2003), the requirement of both gravimetric counts and autodiametric measurements to first
106 develop a calibration curve, and the sensitivity of tissues to different preservation techniques (Friedland et al. 2005).
107 However, the recent increase in the use of this method has shown that the autodiametric method can be cost
108 effective by using a dissecting microscope and camera or standard flatbed scanner and open-access image analysis
109 software (Klibansky and Juanes 2008). A commonly used solution of 10% neutral-buffered formalin (3.7%
110 formaldehyde) maintains oocyte size and shape after preservation, which makes it possible to develop methods for
111 preserved oocytes (Witthames et al. 2009; Alonso-Fernández et al. 2009; McElroy et al. 2013, 2016). Several groups
112 studying European pelagic and benthic fish species with various reproductive systems successfully used the

autodiametric method for rapid fecundity estimation (Thorsen and Kjesbu 2001; Klibansky and Juanes 2008; Witthames et al. 2009; Alonso-Fernández et al. 2009; McElroy et al. 2013, 2016; Flores et al. 2017; Domínguez-Petit et al. 2018; Mion et al. 2018; Sullivan et al. 2019; Lefebvre et al. 2019).

Here, we attempt to adapt the method for the species-rich genus of the Northeast Pacific rockfishes (*Sebastes* spp.) in the California Current Ecosystem. Developing more rapid methods of fecundity assessment will increase much needed fecundity data to use in stock assessments for this diverse genus. There are over one-hundred species in the genus *Sebastes* world-wide, with a hot-spot of diversity of over 65 species observed along the coast of California, U.S.A. (Love et al. 2002; Hyde and Vetter 2007). Although all species in the genus are live-bearers, each has a unique life history and reproductive pattern (Love et al. 2002). Fecundity studies on Northeast Pacific rockfishes traditionally used the gravimetric method to develop size-dependent fecundity relationships for population models to estimate reproductive potential and stock status (Stafford et al. 2014; Beyer et al. 2015; Dick et al. 2017). However, the gravimetric method is labor-intensive and time-consuming and therefore fecundity data is often lacking or scarce for many species. Additionally, recent studies found spatial and temporal variability in fecundity and reproductive strategies both within and among species (Beyer et al. 2015; Lefebvre et al. 2018). These differences are not well understood, but are important to better understand to inform management and to improve understanding of the vulnerability of rockfishes to novel climate change environmental conditions (Hare et al. 2016). Rockfishes are a cornerstone of both commercial and recreational fisheries throughout the Northeast Pacific. Most stocks are actively managed, which include many dozens of species-specific stock assessments developed to inform management (Parker et al. 2000; Yamanaka and Logan 2010; Conrath 2019; Thorson et al. 2019). Therefore, the development and adoption of the autodiametric method for Northeast Pacific rockfish species will allow researchers to quantify fecundity with greater efficiency for each stock. Furthermore, more rapid fecundity estimation greatly improves the ability to explore more complex impacts on reproductive output for this speciose genus, where most species are known to exhibit strong maternal effects on larval production (Berkeley et al. 2004; Sogard et al. 2008; Stafford et al. 2014; Beyer et al. 2015, 2021; Lefevre et al. 2017; Lefebvre et al. 2018).

Sebastes species are unique from most other non-live bearing species where the autodiametric method has been applied because they are viviparous. Rockfish have internal fertilization, gestate embryos for a period of weeks to months (depending on species and temperature) and release small, fully developed, and free-swimming larvae at parturition. Here, we describe the development of oocyte maturation, fertilization, gestation, and larval release to

141 highlight differences from other, more common broadcast species. Similar to other marine fishes, oocytes for a
142 brood are recruited from a standing stock of primary stage oocytes. Recruitment and development of primary stage
143 oocytes through vitellogenesis and oocyte maturation prior to fertilization can be asynchronous, especially in the
144 early stages of vitellogenesis. Asynchronous development in early vitellogenesis is likely a mode of up- or down-
145 regulation of potential fecundity in these species (Lefebvre et al. 2018). Oocytes that are fully recruited for the brood
146 are similar sized after undergoing the final stages of vitellogenesis (Shaw et al. 2012). Following oocyte maturation
147 and fertilization, the development of embryos is group synchronous. Once fully developed, the brood of larvae is
148 released in a single parturition event.

149 Even though all *Sebastes* are live-bearers, the reproductive ecology of rockfishes varies substantially
150 among species. Oocyte sizes throughout development and larval size at parturition differ among species, in addition
151 to overall maternal size. This results in unique size-dependent fecundity relationships for each species and varying
152 degrees of maternal effects (Phillips 1964; Wyllie Echeverria 1987; Love et al. 1990; Sogard et al. 2008; Stafford et
153 al. 2014; Dick et al. 2017). Therefore, it is possible that the development of calibration curves among rockfish
154 species, which relate the mean oocyte diameter of a sample to an oocyte density, may differ even though the species
155 are closely related. Furthermore, the frequency of reproduction differs among species. Rockfishes are iteroparous,
156 meaning they are capable of reproduction annually over a long-life span (Love et al. 2002). Mature females
157 generally develop and extrude a single brood of larvae once per year. However, at least fifteen species are capable of
158 producing multiple broods during the annual reproductive season (Moser 1967; MacGregor 1970; Love et al. 2002;
159 Lefebvre et al. 2018). Multiple brooding is a reproductive strategy in rockfishes that is not fully understood but
160 occurs more often in rockfishes with more southern distributions (Love et al. 2002). Multiple brooding is likely an
161 important adaptation to different environmental conditions in the southern range of the *Sebastes* genus in the
162 California Current (Love et al. 2002; Holder and Field 2019; Lefebvre et al. 2019). The differences in the strength of
163 maternal size effects on fecundity and the frequency of reproduction in this species-rich complex necessitates a need
164 for more efficient methods to estimate fecundity to better understand these different reproductive strategies and to
165 assess the reproductive potential of each stock more rapidly and cost-effectively for management.

166 In this study, we developed autodiametric calibration curves for unfertilized oocyte stages of five rockfish
167 species and explored the possibility of adapting established autodiametric protocols to embryonic stages, unique to
168 live-bearing species. We then assessed the need to develop individual calibration curves for each of these closely-

related species by statistically comparing the slope and intercept parameters of the mean diameter – oocyte density calibration curves. Next, we conducted a time analysis comparison between the new autodiametric and historically-used gravimetric methods to show the value of the autodiametric method for improving the efficiency of fecundity data collection for unfertilized and embryo stages. We also assessed error and bias in the autodiametric method through cross-validation to ensure that fecundity estimates from either method are robust and comparable for future work. Last, we explored differences in individual oocyte weight among the five species at a given diameter as a biological explanation for differences in autodiametric curve parameters among species. Improving the efficiency of fecundity data collection by use of the autodiametric method will allow researchers and managers to expand the available fecundity information and to monitor changes in reproductive potential of stocks in this diverse species complex under current environmental variability and future novel conditions in the highly dynamic California Current Ecosystem.

Methods

Study species

We developed autodiametric calibration curves for chilipepper (*Sebastes goodei*), rosy rockfish (*S. rosaceus*), widow rockfish (*S. entomelas*), yellowtail rockfish (*S. flavidus*), and vermilion rockfish (*S. miniatus*). These species represent a range of reproductive patterns including differences in maximum maternal size, strength of maternal size effects, oocyte size, brood fecundity, and reproductive strategy (Table 1). The five species were chosen based on the availability of fecundity samples, differences in reproductive strategies spanning the genus, and general economic importance. Yellowtail rockfish have small oocytes and high brood-fecundity relative to the larger oocytes and lower brood-fecundity of chilipepper (Beyer et al. 2015). Female chilipepper and rosy rockfish are capable of producing multiple broods per year; whereas yellowtail, widow and vermilion rockfish are limited to a single brood annually (Love et al. 2002; Beyer et al. 2015). All five species are broadly distributed in shelf waters of the California Current along the continental U.S. West Coast. Even though all are broadly distributed, rosy rockfish, vermilion rockfish, and chilipepper have centers of biomass in southern and central California, whereas yellowtail and widow rockfish have more northern (Oregon and Washington coastal waters) centers of biomass (Holder and Field 2019). Early studies collected fecundity information for some rockfish species in the 1950s and 1960s (Phillips 1964; Moser 1967; MacGregor 1970) and since then, fecundity information has been collected sporadically to

benefit stock assessment models, improve the estimation of biological reference points used to guide management decisions, and to better understand the reproductive ecology of these species.

Samples

Rockfish were caught along the central and southern coast of California by hook-and-line fishing in ocean depths ranging from 30-m to 100-m from 2009 to 2019 (Beyer et al. 2015; Lefebvre et al. 2018; Beyer et al. unpublished). Collected fish were euthanized, stored on crushed ice to maintain freshness of tissues, and dissected within 24-48 hours of capture. Fish were measured for total wet weight (nearest gram) and fork length (nearest millimeter) before dissecting the gonads. Gonads were weighed to the nearest 0.01 grams and two weighed subsamples (~0.5 grams) of oocytes or embryos were collected from fresh ovaries. Subsamples were collected from the center of the ovary for standardization. Previous histological study of rockfish ovarian development and fine scale staging of embryos confirm that oocyte maturation and fertilization and the development of embryos is synchronous throughout the ovary (Yamada and Kusakari 1991; Shaw et al. 2012). For the purposes of our study, we defined two sub-stages post-fertilization due to a difference in shape with gestation and development. Embryos were sub-categorized into: early development, where embryos were spherical and late development following eye pigmentation, where pre-hatch eyed-larvae were more oblong in shape. All samples were preserved in a 10% neutral buffered formalin solution in 20-ml plastic scintillation vials for later processing to estimate fecundity. Samples used for the calibration curve remained in formalin for preservation between 6 months to 10 years, although most samples processed for autodiametric imaging had been collected between 2018 through 2020. A previous study has shown that preservation in formalin does not influence oocyte size or shape (Alonso-Fernández et al. 2009). All fecundity counts and imaging used preserved samples. Because the weights of ovaries and subsamples were collected from fresh tissues, the oocyte counts used to determine oocyte density could be directly multiplied by the fresh ovary weight to estimate fecundity without a need for a conversion factor of fresh to preserved weight when preserved ovaries are subsampled.

Oocyte density

To develop the species-specific calibration curves, we first needed samples with both gravimetric counts to calculate oocyte density and corresponding image analysis to measure a mean oocyte diameter. To calculate oocyte

density (oocytes * gram^{-1}), we counted the total number of oocytes or embryos in each weighed subsample with the traditional gravimetric method (Murua et al., 2003; Beyer et al., 2015). Each preserved sample was transferred to a petri dish where excess formalin was removed using a pipette and the sample rinsed with deionized (DI) water. Excess DI water was removed by a pipette. Preparation of samples was completed in a ventilated hood to reduce formalin exposure. Once samples were rinsed with DI water, oocytes or embryos were arranged manually in rows in the petri dish and counted under a light dissection microscope. Oocyte density was obtained by dividing the count of the oocytes or embryos in the subsample by the fresh weight of the subsample.

Mean oocyte diameter

The second part of developing the calibration curves was obtaining a mean oocyte diameter for each subsample. A dissecting microscope (AmScope, Irvine, CA) was used for the autodiametric imaging procedure to measure oocyte diameters with an attached camera (MU800 8MP 2.0 USB CMOS C-mount camera). Oocytes or embryos were arranged in a single layer on a two-by-two tray on the stage plate following methods in Thorsen and Kjesbu (2001) and Lefebvre et al. (2019). The microscope's ocular micrometer was calibrated ahead of time using a micrometer calibration slide. All images were taken at 0.9x magnification. We maintained a consistent microscope calibration of 0.206 pixels per micrometer (μm) for all images in the study.

We used the imaging software ImageJ (version 1.50i, ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, available at https://imagej.nih.gov/ij/), along with the ObjectJ plugin (version 1.04r, University of Amsterdam, available at https://sils.fnwi.uva.nl/bcb/objectj/index.html) to automatically measure oocyte diameters. All images were set in 8-bit form, 3264 x 2448 resolution, with an exposure target of 62, and a high contrast (100%). During ImageJ processing, images were converted to 32-bit form. The macro followed imaging protocols outlined by Lefebvre et al. (2019) and was set to detect diameters in the size range between 300 μm to 1500μm and with a circularity index over 80%. The circularity restriction excluded abnormally shaped oocytes that could be atretic and would affect the estimate of the mean diameter. However, by following these protocols we were unable to accurately measure mean diameter of post-fertilization embryo stages as they did not satisfy the circulatory requirement of > 80% circularity. Additionally, the program often failed to accurately detect the edge of imaged embryos due to transparency of the chorion surrounding the embryos (Fig. 1B). As a result of the challenges of applying our

252 autodiametric protocols to accurately measure mean diameters of the post-fertilization embryo stages, calibration
253 curves were only developed for the unfertilized oocyte stage for each of the five species.
254 Furthermore, to avoid mismeasurements of unfertilized oocytes, any auto-measured overlapping oocytes
255 were manually removed, along with any obviously distorted or atretic oocytes that may have been included in the
256 automated measurements (Fig. 1A). A minimum of 100 oocytes were measured per sample to estimate a mean
257 oocyte diameter. A preliminary sensitivity analysis determined that measuring 100 oocytes was sufficient to
258 determine a mean oocyte diameter at an asymptotic coefficient of variation (CV) (i.e., fewer oocytes measured in a
259 sample resulted in a higher CV, but a greater number of oocytes measured in a sample did not reduce the CV of the
260 mean). For unfertilized oocytes, we only used samples where the oocytes were assumed to be fully recruited to the
261 brood and were generally of similar size within a sample to avoid an underestimate of fecundity when oocytes were
262 not yet fully recruited. To determine which samples met these criteria, we plotted the CV of oocyte diameters across
263 samples of a given species against the mean oocyte diameter of the sample to look for any trend (see Online
264 Resource 1). Based on the plots, we excluded samples with a CV greater than 10% of the mean oocyte diameter,
265 since high variation in oocyte diameter indicated varying sized oocytes within a sample and higher CVs tended to
266 occur among the samples with the smallest mean oocyte diameters (i.e., oocytes in the earliest stages of
267 vitellogenesis). The variation in oocyte diameter declined as oocytes increased in size toward maturation (as
268 expected) and generally had a CV less than 10% (Online Resource 1). This method of exclusion was similar to
269 methods described by Mion et al. (2018) to exclude samples where oocytes were not yet fully recruited, in order to
270 avoid underestimating potential fecundity.
271 We aimed for 50 samples per species from available preserved inventory to develop the calibration curves
272 and attempted to image oocytes spanning a range of mean oocyte size for each of the five species. This was within
273 the restrictions mentioned above for fully recruited broods (Thorsen and Kjesbu 2001; Klibansky and Juanes 2008).
274 For rosy rockfish, widow rockfish and vermilion rockfish, we analyzed all available unfertilized oocyte samples,
275 whereas yellowtail rockfish and chilipepper had over 50 samples available to develop the calibration curves (Table
276 2). When more than 50 samples were available, we selected samples that covered the range in diameter sizes of
277 unfertilized oocytes. Oocytes were first measured for a mean diameter prior to gravimetric counts to establish the
278 range in diameter size and to determine which samples needed to be counted to develop the calibration curves.
279

Autodiametric calibration curves

Once the gravimetric counts and mean oocyte diameter measurements were complete, we developed the species-specific calibration curves by plotting oocyte density ($O_{Density}$, oocytes * gram^{-1}) and mean oocyte diameter ($O_{MeanDiameter}$, µm) for each sample and fit a negative power function to the data (Murua et al., 2003):

$$O_{Density} = a\, O_{MeanDiameter}{}^{b} \quad (1)$$

The coefficients *a* (intercept) and *b* (exponent/slope) were found by fitting a least-squares linear regression of the natural logarithmic transformed data:

$$log(O_{Density}) = log(a) + b\, log(O_{MeanDiameter}) \quad (2)$$

Before finalization of the autodiametric calibration curves we visually assessed the plotted data and statistically tested for outliers. We removed statistical outliers using the Bonferroni Outlier Test (outlierTest function, package "car", in R), which identified outlying values based on studentized residuals and Bonferroni adjusted p-values (Fox and Weisberg 2019). One yellowtail, one rosy, and five widow rockfish (mostly from a single January 2018 sampling date) samples were determined to be outliers and were removed from the final analysis of the species-specific calibration curves. No chilipepper or vermilion rockfish samples were identified as outliers.

Estimating fecundity

Once the autodiametric calibration curve was finalized for each species, fecundity (*F*) was quickly determined by measuring the mean oocyte diameter of a sample through image analysis and then using the calibration curve parameters to calculate the expected oocyte density ($O_{Density}$). The expected oocyte density was multiplied by the total fresh weight of the ovaries (W_{ovary}, g) with the following equation:

$$F = O_{Density} W_{ovary} \quad (3)$$

Efficiency Analysis

To compare the efficiency of the autodiametric method to the gravimetric method, we compared the time required to complete each sample by each method. The following procedures were performed to record time for the gravimetric method in order from start to finish: placing the sample on the petri dish, teasing apart oocytes in unfertilized samples, organizing oocytes or embryos in rows to countable standards, counting, and recording the counts on a datasheet. The following procedures were followed to assess timing of the autodiametric method: placing the sample in the petri dish, pipetting oocytes into a two-by-two tray under a microscope, sorting oocytes into a single layer to minimize the overlap of oocytes, capturing images, manually removing any mismeasured oocytes, and exporting measurements to a spreadsheet.

We statistically tested the difference in timing between methods (gravimetric or autodiametric) and developmental stage (unfertilized oocytes, early development embryos, or late development embryos) with a linear model similar to ANOVA (lm function in R), where the fecundity method and development stage were categorical variables. This was followed by a post-hoc Tukey's Honest Significant Difference (HSD) test to identify pairwise differences in the time to process samples between groups (pairs function with Tukey adjustment, lsmeans, emmeans, and multcompView package in R). All statistical tests were run in R (version 3.5.2) and considered significant at $p < 0.05$. All graphs were made using graphing package for R (ggplot2 version 3.2.0; RColorBrewer version 1.1-2).

Error assessment and cross-validation

To assess precision and possible reader bias in the autodiametric method we had two readers independently process a subset of the same samples to obtain the mean oocyte diameter. The mean oocyte diameter for this subset of samples was compared between reader 1 and reader 2 to look for deviations from a 1:1 estimate. To assess the precision in fecundity estimates obtained from both methods, we used a leave-one-out-cross-validation approach to compare the fecundity estimate from the same sample using either the autodiametric or gravimetric method. The same approach was used to determine the sensitivity of the calibration curves to each datapoint. We assumed that the gravimetric count was the true fecundity estimate presumed to be without error. The predicted fecundity data from the autodiametric method was plotted against the observed gravimetric fecundity data along with a 1:1 line for comparison to assess precision and look for bias for each species.

Individual oocyte weight

Last, we estimated individual oocyte weight, standardized by the mean oocyte diameter (oocyte size), to explore biological explanations for differences in oocyte density, at a given size, among species resulting in differences in the parameters estimates of the species-specific calibration curves. Mean individual oocyte weight (microgram) was calculated for each sample by taking the fecundity subsample weight and dividing by the number of oocytes in the subsample. Differences in mean oocyte weight among species was assessed by a linear model similar to ANCOVA (lm function in R), where the mean oocyte weight of a sample was the continuous response variable, species was a categorical predictor, and the mean oocyte diameter of the sample was a continuous covariate to account for size. Oocyte weight increased as an allometric power function of diameter. We used a linear analysis of the logarithmic-transformed weight and diameter data to test for differences in slope and intercept. This was similar to the statistical methods described above for estimating the parameters of the autodiametric curve (equations 1 & 2). Next, we used a post-hoc Tukey analysis to test for pairwise differences in size-standardized oocyte weight among species. This was essentially the same analysis as for the calibration curves but using predicted oocyte weight rather than oocyte density, which was standardized by the mean oocyte diameter of the sample. The data are visually presented as the expected species-specific oocyte weight at the pooled mean oocyte diameter common to all five species.

Results

Autodiametric calibration curves

Calibration curves were developed for the unfertilized oocyte stage of five rockfish species: rosy rockfish, yellowtail rockfish, chilipepper, widow rockfish, and vermilion rockfish (Fig. 2, Table 2). As described in the methods, we attempted to use the autodiametric method for embryos, unique to live-bearers, but were ultimately unsuccessful. Both early and late development embryos were larger and more translucent compared to unfertilized oocytes leading to inconsistent edge detection by the imaging program (Fig. 1A and 1B). As the embryos developed, their shape became increasingly non-circular, which prevented accurate measurement of a mean diameter. Current preservation techniques of late development embryos often result in breakage of the chorion and thus irregular shapes of the larvae. This also contributed to inaccurate automated measurement of diameter using the current imaging protocols. Because of this, the autodiametric method was only feasible for unfertilized oocyte stages in

rockfishes using the current protocols. Therefore, only autodiametric calibration curves for unfertilized oocytes were developed.

The range in the mean oocyte diameters of unfertilized oocytes differed among species, as did the overall mean size. The following mean diameters of imaged unfertilized oocytes from smallest to largest sized oocytes were: rosy rockfish at 567 µm, widow rockfish at 603 µm, vermilion rockfish at 674 µm, yellowtail rockfish at 681 µm, and chilipepper at 837 µm (Table 3). Using a negative-power-function, oocyte density decreased with mean oocyte diameter in all five species. The exponent parameter, *b*, did not differ among species (i.e., the species by mean diameter interaction was not significant, $p = 0.3219$) and therefore the interaction term was removed from the model and differences in the intercept tested. The intercept parameter, *a*, was significantly different among species (linear model, $F_{(5, 225)} = 1440$, $p < 0.001$, $R^2 = 0.97$). Most post-hoc species pairwise comparisons were significantly different (HSD, $p < 0.05$) except for the vermilion-widow ($p = 0.5623$), vermilion-yellowtail ($p = 0.9661$), and widow-yellowtail ($p = 0.1365$) post-hoc pairwise comparison that did not statistically differ. Widow, vermilion, and yellowtail rockfish had the highest density of oocytes per mean oocyte diameter followed by rosy rockfish and chilipepper.

Efficiency comparison

The autodiametric method was more efficient at estimating fecundity of unfertilized rosy rockfish oocytes in comparison to the traditional, gravimetric method (linear model, $p < 0.001$, Fig. 3). This was in terms of the amount of time needed to process a sample. Most post-hoc pairwise comparisons between oocyte stages and methods showed significant difference in time to process a sample (Tukey HSD, $p < 0.05$). The exceptions were the gravimetric counts for the early and late development embryos comparison, which did not differ ($p = 0.066$) and the time required to process unfertilized oocytes using the autodiametric method compared to gravimetric counts of late development embryos ($p = 0.225$). The average time to count a typical rosy rockfish unfertilized oocyte sample using the gravimetric method was 77 minutes (range 45 to 150 minutes) compared to 16 minutes (range 13 to 18 minutes) using the autodiametric method. Thus, the autodiametric method was nearly five times faster than the gravimetric method for processing unfertilized oocyte samples.

All autodiametric samples were imaged after gravimetric counts to develop the calibration curves. Because of this the oocytes had already been teased apart from the ovarian tissue in the sample and were easier to prepare for

imaging. After the development of calibration curves is completed, gravimetric counts are no longer needed. Because samples are no longer counted ahead of imaging, the time required to image samples for the autodiametric method may be greater than reported because the oocytes are still embedded in the ovarian tissues and need to be teased apart before imaging. To estimate the additional time required, we timed the processing of samples that had already been counted by the gravimetric method and compared that to the time to process samples that had not yet been counted. We found a mean increase of 1.9 minutes to image samples not previously counted, but the increase in processing time was not statistically different (t-test, $n = 20$, $F_{(1,18)} = 2.359$, $p = 0.142$).

The autodiametric method was unsuccessful for fertilized stages so a comparison of efficiency between the two methods for the fertilized stages was not possible. However, the average time to count an early development embryo sample using the gravimetric method was 30 minutes (range 15 to 65 minutes) and only 22 minutes (range 15 to 50 minutes) for late development embryos. Timewise, the manual counts of embryo stages were comparable to using the autodiametric method for unfertilized oocytes.

Time-comparisons are presented for only the rosy rockfish. We were able to collect processing time data for rosy rockfish at all developmental stages and for both methods before laboratory access restrictions were enacted in response to the global COVID-19 pandemic. Following COVID-19 laboratory access restrictions, our protocols changed slightly. Samples continued to be gravimetrically counted and imaged in the laboratory, but the processing of images in ImageJ was done remotely to reduce in-person laboratory work. Due to these circumstances, we were unable to obtain comparable time estimates of timing for the autodiametric method for yellowtail rockfish, chilipepper, widow, and vermilion rockfish since images were taken in the laboratory but processed at a different time and location.

Error assessment and cross-validation

The leave-one-out cross validation method showed a tight correlation between predicted fecundity from the autodiametric method and fecundity estimated by the traditional, gravimetric method for unfertilized oocytes (Fig. 4). The linear regression slope (and correlation coefficient of determination) was 1.04 ($R^2 = 0.93$) for rosy rockfish, 1.01 ($R^2 = 0.95$) for chilipepper, 1.01 ($R^2 = 0.94$) for widow rockfish, 0.99 ($R^2 = 0.95$) for vermilion rockfish, and 0.98 ($R^2 = 0.87$) for yellowtail rockfish (Table 4). A slope of 1.0 indicated a direct 1:1 relationship in fecundity estimates between the two methods.

The calculation of a mean diameter for each sample was consistently reproduced between readers. Again, this comparison was only available for rosy rockfish samples. The independent calculations of mean oocyte diameter by the two readers were closely correlated along a 1:1 line with little evidence of reader bias (linear regression, adjusted $R^2 = 0.98$, $n = 29$, slope = 0.943, Online Resource 3). Two influential data points in samples with the smallest oocyte diameters could be interpreted as a slightly higher diameter bias by reader 2 for samples with the smallest-sized oocytes. Slight differences in the estimate of mean oocyte diameters among readers are not unexpected due to sample preparation methods and removal determination of improperly measured oocytes by the imaging program, which could differ slightly among readers.

Oocyte weights

A power-function fit to the data demonstrated that oocyte weight increased as an allometric function of increasing oocyte diameter. We found no evidence for an interactive effect of oocyte diameter by species (linear model, slope by species interaction, $p = 0.3219$) and therefore removed the interaction term from the model and assumed a similar slope/exponent of $b = 2.90$ for all five species. We found a significant difference in the intercept, indicating that oocyte weight, at a given diameter, differed by species (linear model, $F_{(5,225)} = 1440$, $p < 0.001$). As with the calibration curve analysis, most post-hoc pairwise comparisons between species showed significant difference in oocyte weight (Tukey HSD, $p < 0.05$), except for the vermilion-widow ($p = 0.5623$), vermilion-yellowtail ($p = 0.9661$), and widow-yellowtail post-hoc pairwise comparisons ($p = 0.1365$), in which there was no difference in oocyte weight at a given diameter size. Rosy rockfish had the heaviest oocytes at a given diameter, followed by chilipepper and yellowtail. Vermilion and widow rockfish had comparatively lighter-weight oocytes at a given diameter (Fig. 5).

Discussion

We developed autodiametric calibration curves of unfertilized oocytes for five economically important Northeast Pacific rockfish species: rosy rockfish, yellowtail rockfish, widow rockfish, chilipepper, and vermilion rockfish. In doing so, we compared the efficiency of the autodiametric method and the gravimetric method to estimate fecundity and assessed the precision of each method to replicate results. We found that species-specific calibration curves were necessary for two of the five species in our study, even though the fish were closely related, resided in similar

448 habitats, and had similar live-bearing reproductive strategies. The apparent cause of the difference in oocyte density
449 was due to differences in oocyte weight for a given oocyte diameter among the species, rather than a physical
450 packing property of the oocytes. This is likely due to the weight-based method to obtain oocyte density (more
451 commonly used), rather than a volumetric approach. Rosy rockfish and chilipepper had heavier oocytes for a given
452 size, which resulted in a lower density of oocytes per gram. Widow, vermilion, and yellowtail rockfish had lighter
453 oocytes at a given diameter but were not different from each other. This resulted in relatively higher densities of
454 oocytes per gram for these three species.
455 The differences in oocyte weight at a standardized oocyte diameter size is likely due to different stages of
456 oocyte development at a given size among the species but could also suggest differential investment of energy
457 resources by species. For example, at the pooled mean oocyte diameter of 681 um shown in Fig. 5, oocytes were
458 likely in the mid-range of pre-fertilization development in vermilion, widow, and yellowtail rockfish, at an earlier
459 stage of development in chilipepper (a species that ultimately obtains much larger oocyte sizes), and at a late stage
460 of development nearing oocyte maturation for rosy rockfish (a species with much smaller oocyte size at maturation
461 compared to the other species). The comparison of oocyte weight among species was not standardized by fine scale
462 staging of developing unfertilized oocytes. We were unable to sub-classify the unfertilized stages of oocyte
463 development using only macroscopic methods, but future studies could explore possible differences in energetic
464 investment in individual oocytes among species using histological methods for fine-scale staging of early, mid-, and
465 late- vitellogenesis. Another explanation for the difference in size-standardized oocyte weight among species is
466 potentially related to the two reproductive sub-strategies within the genus. Both rosy rockfish and chilipepper are
467 capable of producing multiple larval broods annually and have heavier oocytes for a given size. Yellowtail,
468 vermilion and widow rockfish are limited to a single larval brood per year (Love et al. 2002; Beyer et al. 2015;
469 Lefebvre et al. 2018; Holder and Field 2019). Other possible explanations could include differences in the amount of
470 ovarian tissue among species influencing the weight of the subsamples and thus estimates of oocyte density.
471 Qualitatively, the amount of ovarian tissue in the fecundity samples was negligible and not likely to have a large
472 influence on subsample weight at the 0.01 g measurement scale. However, consistently greater amounts of ovarian
473 tissue enough to influence the weight of the subsample would bias the estimate of oocyte density higher and oocyte
474 weight lower. Further investigation is warranted to better understand the biological differences among species
475 resulting in different calibration curves. The differences in brooding patterns and other aspects of species-specific

476 ecology, reproductive biology, and evolutionary history likely contributed to differences in oocyte sizes and weights.
477 In our study, these differences resulted in the need to develop unique autodiametric calibration curves, even among
478 closely-related species.

479 Our sample sizes mainly reflected the number of preserved samples available, but we found that a sample
480 size of 40 to 55 was generally adequate to develop an autodiametric calibration curve. This was because of the
481 consistent close correlation of oocyte diameter and oocyte density (i.e., $R^2 > 0.9$ for all study species) and the
482 availability of samples spanning the range of oocyte size for each species. We had fewer samples available for
483 vermilion rockfish (n = 34), but with a high correlation of oocyte diameter and density ($R^2 = 0.96$) over a range of
484 oocyte sizes. Other studies used a greater number of samples to develop calibration curves unique to their species of
485 interest (Thorsen and Kjesbu 2001; Witthames et al. 2009; McElroy et al. 2013, 2016; Flores et al. 2017; Mion et al.
486 2018; Sullivan et al. 2019). However, since both the gravimetric counts and autodiametric imaging is needed to
487 construct the calibration curves, larger sample sizes require more time to develop the curve prior to implementation
488 of the method. Ensuring a range of oocyte sizes helped to minimize the number of samples required when there were
489 abundant samples to choose from.

490 We were unable to develop calibration curves for fertilized stages with the current protocols due to
491 difficulties of the software to accurate detect the edge of semi-transparent eggs and developing embryos, and later
492 stage embryos not meeting the requirements for circularity and breaking free of egg cases during the collection
493 process. Future investigations could explore different methods, such as staining embryos or using a flatbed scanner
494 to backlight images of fertilized eggs for better edge detection (Friedland et al. 2005; Klibansky and Juanes 2008;
495 Anderson et al. 2020). Alternative protocols could enable the development of the autodiametric method for fertilized
496 stages; however, we did not further explore other options because fertilized stages are already faster to process
497 compared to unfertilized stages. The embryos are larger than unfertilized oocytes meaning that there are fewer to
498 count in each sample and have already been ovulated from the follicle meaning embryos are easy to manually
499 separate and line up for counting compared to unfertilized oocytes that must be manually teased apart prior to
500 counting or imaging. We found no statistical difference in the time to process a late-stage embryo sample by
501 manually counting compared to the imaging autodiametric method for an unfertilized sample, but a substantial
502 increase in efficiency using the autodiametric method compared to the gravimetric method for unfertilized stages.

503 The shorter processing time of unfertilized oocyte samples using the autodiametric method greatly
504 improves efficiency in the collection of fecundity data for rockfish. Once the calibration curve is developed for each
505 species, the time-consuming gravimetric method is no longer needed, and an unweighed subsample of preserved
506 oocytes can be imaged quickly to measure the mean oocyte diameter. The measurement of mean diameter is then
507 converted to an oocyte density using the parameters of the calibration curve and multiplied by the total weight of the
508 ovaries to estimate fecundity. Processing time will slightly increase for unfertilized samples not previously counted
509 because oocytes will need to be teased apart; however, the time requirement will still be significantly less than the
510 gravimetric method.
511 The ability to process images remotely proved an added and unexpected benefit of the autodiametric
512 method due to reduced access to laboratory facilities during the COVID-19 pandemic. This additional benefit of the
513 autodiametric method not only supports the time efficiency in processing samples but was useful in reducing the
514 number of samples that were processed in the laboratory during limited in-person work. The autodiametric method
515 maximized laboratory time for researchers to collect images and allowed for the processing of images remotely.
516 Along with previous studies using the autodiametric method, consistency of estimated fecundity results via
517 cross-validation from each method showed high correlation (Thorsen and Kjesbu 2001; Klibansky and Juanes 2008).
518 Maintaining consistent results for estimated fecundity from both methods was important in this study to adopt the
519 autodiametric method for unfertilized stages moving forward and to ensure that future fecundity estimates are
520 comparable to historic estimates that traditionally used the gravimetric method. Conducting a cross-validation
521 analysis directly compared fecundity estimates from each method to assess variability, sensitivity of the curves to
522 any one datapoint, and any evidence of bias. The results were consistent by species and indicated that there were no
523 influential data points affecting the parameter estimates for each calibration curve. The consistency in the results
524 support the use of the autodiametric method for future rockfish fecundity studies involving unfertilized oocytes and
525 allow for comparison to previous work. Future exploration into alternative methods to improve imaging and
526 measurement analysis of early-stage embryos will further improve efficiency. Rockfishes are important to West
527 Coast fisheries and establishing autodiametric calibration curves for these five species is the first step to improving
528 the efficiency of fecundity data collection for West Coast rockfish fisheries management. Given our results showing
529 the autodiametric calibration curves for rockfish being species-specific, further creation of autodiametric calibrations

curves for other rockfish species in this speciose and diverse genus will continue to improve the efficiency and availability of fecundity data.

Conclusion

Size-dependent fecundity information is critically important to stock assessments that are used to assess the reproductive potential of a population in order to accurately estimate biological reference points for management. Fecundity information is also important to understanding the unique reproductive ecology of rockfishes, and to evaluate how reproductive potential varies in response to both environmental variability, across the spatial range of the species being evaluated and potentially in response to exploitation. To gather these valuable data at a large scale in an expedited manner to inform management, efficient methods for rapidly assessing fecundity are essential as traditional methods were time-consuming and labor intensive. The development of calibration curves for the rapid fecundity assessment of chilipepper, yellowtail, vermilion, widow, and rosy rockfish using the autodiametric method will increase the efficiency of data collection in these five species and provide a model moving forward for other closely-related, economically important *Sebastes* spp. in the California Current Ecosystem. With increased efficiency of data collection, rapid fecundity estimation will allow for continued improvements to the size-dependent fecundity relationships used in stock assessments to infer biological reference points for management. Additionally, this method will increase the data available to understand patterns in the spatiotemporal dynamics of reproductive potential within the genus and how to the reproductive potential of these populations will be influenced by novel environmental conditions, such as increased warming and changes in ecosystem productivity with climate change. Monitoring and understanding the reproductive ecology of these diverse, economically important, live-bearing species is crucial for sustainable management and assessment of the vulnerability of these species to climate change.

Tables

Table 1: A comparison of life history information for five rockfish (*Sebastes*) species in this study. Information from Love et al. 2002 (and references therein) and the most recent stock assessments, exceptions noted by superscript.

	Rosy rockfish (*Sebastes rosaceus*)	Yellowtail rockfish (*Sebastes flavidus*)	Chilipepper (*Sebastes goodei*)	Widow rockfish (*Sebastes entomelas*)	Vermilion rockfish (*Sebastes miniatus*)
Asymptotic length (cm)	22.5[a]	52.1[c]	48.1[d]	50.3[e]	55.8[f]
Maximum lifespan (years)	30+ [a]	64+	35	60+	60+
Region	Strait of Juan de Fuca (Washington) to Bahia Tortugas (South Baja California)	East Aleutian Islands to North Baja, California	Queen Charlotte Sound (B.C.) to Bahia Magdalena (South Baja California)	Middle Albatross Bank to North Baja, California	Prince William Sound, Alaska to Central Baja, California
Depth range (m)	7 – 262	90 – 180	75 – 325	140 – 210	6 – 436*
Length at 50% maturity (females)(cm)	16.68 (Central California)[a]	42.5[c]	25.7[d]	31.0[e]	38.4[f]
Months of larval release in Central and Northern California	December – September	January – July	November – June	December – April	September, December, April – June
Oocytes per brood	12,600 – 95,000	56,900 – 1,993,000	18,000 – 538,000	95,000 – 1,113,000	63,000 – 2,600,000*
Broods per year	1+[b]	1	1+	1	1
Fishing pressure	Somewhat commercially important; regularly caught recreationally	Somewhat commercially important	Extremely commercially important	Extremely commercially important	Extremely commercially and recreationally important

[a]Fields 2016, [b]Beyer et al. 2021, [c]Stephens and Taylor 2018, [d]Field et al. 2015, [e]Adams et al. 2019, [f]Monk et al. 2021

*Hyde and Vetter 2007 describe vermilion rockfish residing at shallower depths (<100m) than their cryptic-species-pair, sunset rockfish (*S. crocotulus*)

564 Table 2. Autodiametric calibration curve equations for each rockfish species. The values include the sample size (n),
565 intercept (a), exponential function of intercept [exp(a)], slope (b), the coefficient of determination (R^2) and p-value.
566 Values within parentheses are the 95% confidence interval.

Species	n	a	exp(a)	b	R^2	p-value
Sebastes flavidus	55	26.93	2.89e11	-2.78	0.91	<0.001
		(25.37, 28.49)	(1.04e11, 2.36e12)	(-3.02, -2.54)		
Sebastes goodei	53	28.50	2.38e12	-3.03	0.95	<0.001
		(27.25, 29.75)	(6.83e11, 8.32e12)	(-3.21, -2.84)		
Sebastes rosaceus	48	26.75	4.14e11	-2.78	0.92	<0.001
		(25.22, 28.29)	(8.97e10, 1.93e12)	(-3.02, -2.53)		
Sebastes entomelas	41	27.81	1.20e12	-2.91	0.95	<0.001
		(26.47, 29.15)	(3.13e11, 4.57e12)	(-3.12, -2.70)		
Sebastes miniatus	34	28.37	4.96e11	-3.00	0.96	<0.001
		(27.01, 29.74)	(1.04e11, 2.36e12)	(-3.21, -2.79)		

567
568
569
570
571
572
573
574
575
576
577
578
579

Table 3: Mean diameters of unfertilized oocytes and 25th and 75th quantiles for each species for all samples used to develop the calibration curves. Standard deviation denoted as SD.

Species	25th Quartile ∓ SD (µm)	Mean Diameter ∓ SD (µm)	75th Quartile ∓ SD (µm)
Sebastes flavidus	638.5 ∓ 17.69	680.6 ∓ 20.06	737 ∓ 22.21
Sebastes goodei	746.2 ∓ 21.41	837.2 ∓ 25.23	916 ∓ 27.81
Sebastes rosaceus	526.8 ∓ 16.07	568.9 ∓ 19.12	620.2 ∓ 20.58
Sebastes entomelas	543 ∓ 18.63	602.5 ∓ 20.95	685.4 ∓ 23.62
Sebastes miniatus	601.4 ∓ 18.26	673.7 ∓ 20.49	744.3 ∓ 22.4

599 Table 4: Cross validation values for each species, refer to Fig. 4.

Species	Slope	Adjusted R^2	p-value
Sebastes flavidus	0.98	0.87	<0.001
Sebastes goodei	1.01	0.95	<0.001
Sebastes rosaceus	1.04	0.93	<0.001
Sebastes entomelas	1.01	0.94	<0.001
Sebastes miniatus	0.99	0.95	<0.001

600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617

Fig. 1 Image from ImageJ (version 1.50i) of rosy rockfish (*Sebastes rosaceus*) samples. Fig. 1A shows measured unfertilized oocytes. Oocytes were not measured if they were touching, atretic, lacked definition around the edge, or did not meet the requirements of circularity (>80%). Fig. 1B shows the early development embryonic stage. Embryos at this stage develop oil globules, become translucent, and oblong, making edge detection and measurement through ImageJ unsuccessful. Fig. 1C shows the late development embryonic stage. Embryos at this stage develop eye pigmentation, become more oblong, and develop tail structure, making edge detection and measurement through ImageJ unsuccessful

Fig. 2 Comparison of autodiametric calibration curves for unfertilized oocytes of rosy rockfish (*Sebastes rosaceus*), yellowtail rockfish (*S. flavidus*), chilipepper (*S. goodei*), widow rockfish (*S. entomelas*), and vermilion rockfish (*S. miniatus*). The plot shows differences in the range of unfertilized oocyte size for each species. Chilipepper and rosy rockfish had significantly lower oocyte density at a given oocyte diameter compared with yellowtail rockfish, widow rockfish and vermilion rockfish, which did not statistically differ

Fig. 3 The time required to process fecundity samples for each method and at different stages of unfertilized oocyte, early development embryos, or late development embryos of rosy rockfish (*Sebastes rosaceus*). The gravimetric method to manually count rosy rockfish unfertilized oocytes required an average of 75 minutes for each sample compared to the autodiametric method with an average of only 16 minutes for each sample. Embryonic stages were more similar to the autodiametric method in the time required to manually count a sample, with an average 30 minutes for samples with early development embryos and 22 minutes for samples with late development embryos. The boxplot shows the range in data of the 25th to 75th quantile within the box, the median time required for each method and stage (horizontal dark bar) and outliers indicated by points. Lower case letters indicate significant differences between groups (post-hoc Tukey HSD, $p < 0.05$)

Fig. 4 Comparison between oocyte density determined from the gravimetric method and the predicted oocyte density from the autodiametric method generated by a leave-one-out, cross-validation analysis. The black, dashed line represents a slope of 1, while the colored lines represent the linear regression relationship for each species. The shaded regions are the 95% confidence interval

Fig. 5 The estimated mean weight of an individual oocyte, standardized by diameter, varied by species resulting in different oocyte densities affecting the intercept parameter of the species-specific autodiametric calibration curves. The heavier oocytes of the rosy rockfish (*Sebastes rosaceus*) at a given diameter resulted in decreased oocyte densities. Oocytes of the rosy rockfish were the heaviest at a given size, followed by chilipepper (*S. goodei*), and yellowtail rockfish (*S. flavidus*), vermilion rockfish (*S. miniatus*), and widow rockfish (*S. entomelas*) that were the lightest and did not statistically differ. Weight information is presented at an oocyte diameter of 681 um, the pooled mean diameter across all samples for all five species. The graph shows means by species with 95% confidence intervals. Different lowercase letters indicate significantly different mean oocyte weights between species (post-hoc Tukey HSD, $p < 0.05$)

Statement and Declarations

This research was financially supported by the National Oceanographic and Atmospheric Administration (NOAA) Saltonstall-Kennedy Grant Program (award # NA18NMF4270216) and the California Sea Grant College Program Project (award #NA18OAR4170323) as well as the Cooperative Institute for Marine, Earth, and Atmospheric Systems (CIMEAS), a partnership between the Southwest Fisheries Science Center and University of California Santa Cruz #(NA20OAR4320278-13). The statements, findings, conclusions, and recommendations are those of the author(s) and do not necessarily reflect the views of California Sea Grant, NOAA, or the US Department of Commerce.

Author contributions

All authors contributed to data collection and analysis, Hayley Mapes and Sabrina Beyer developed the initial study design. The submitted draft of the manuscript was written, edited, and approved by all coauthors.

Statement of Significance

Our study improves the efficiency in the collection of important fecundity data for the viviparous Northeastern Pacific rockfishes by developing calibration curves to implement the autodiametric fecundity method for rosy rockfish (*Sebastes rosaceus*), yellowtail rockfish (*S. flavidus*), widow rockfish (*S. entomelas*), vermilion rockfish (*S. miniatus*), and chilipepper (*S. goodei*). We show that calibration curves are species-specific, even in closely related species due to the unique reproductive biology and ecology of each species. The adaptation of the autodiametric method will expedite fecundity quantification efficiency, which is important for estimating and monitoring stock reproductive potential in these economically and ecologically important species.

Data availability

Datasets and analyses code are available upon request of the corresponding author.

Compliance with Ethical Standards

All authors declare no conflicts of interest in this study. The statements, findings, conclusions, and recommendations are those of the author(s) and do not necessarily reflect the views of California Sea Grant, National Oceanographic and Atmospheric Administration (NOAA), or the US Department of Commerce. Fish were collected under the NOAA collecting permit #NOAA-SRP-22-(2010 to 2020) and a scientific collecting permit issued to the NOAA Southwest Fisheries Science Center by the California Department of Fish and Wildlife (#SC-13886). Protocols for the collection of fish were approved by the University of California Santa Cruz, Institutional Animal Care and Use Committee, IACUC (Alons1808).

730 References

731 Adams GD, Kapur MS, McQuaw K, Thurner S, Hamel OS, Stephens A, Wetzel CR (2019) Stock assessment
732 update: Status of Widow Rockfish (*Sebastes entomelas*) along the U.S. West Coast in 2019

733 Alonso-Fernández A, Vallejo AC, Saborido-Rey F, Murua H, Trippel EA (2009) Fecundity estimation of Atlantic
734 cod (*Gadus morhua*) and Haddock (*Melanogrammus aeglefinus*) of Georges Bank: Application of the
735 autodiametric method. Fisheries Research 99:47–54. https://doi.org/10.1016/j.fishres.2009.04.011

736 Anderson KC, Alix M, Charitonidou K, Thorsen A, Thorsheim G, Ganias, K, Schmidt TCdS, Kjebu OS (2020)
737 Development of a new 'ultrametric' method for assessing spawning progression in female teleost serial
738 spawners. Scientific Reports 10:9677. https://doi.org/10.1038%2Fs41598-020-66601-w

739 Barneche DR, Robertson DR, White CR, Marshall DJ (2018) Fish reproductive-energy output increases
740 disproportionately with body size. Science 360:642–645. https://doi.org/10.1126/science.aao6868

741 Berkeley SA, Chapman C, Sogard SM (2004) Maternal age as a determinant of larval growth and survival in a
742 marine fish, *Sebastes melanops*. Ecology 85:1258–1264. https://doi.org/10.1890/03-0706

743 Beverton RJH, Holt SJ (1957) On the dynamics of exploited fish populations. Springer Science & Business Media

744 Beyer SG, Alonzo SH, Sogard SM (2021) Zero, one or more broods: reproductive plasticity in response to
745 temperature, food, and body size in the live-bearing rosy rockfish *Sebastes rosaceus*. Marine Ecology
746 Progress Series 669:151–173. https://doi.org/10.3354/meps13718

747 Beyer SG, Sogard SM, Harvey CJ, Field JC (2015) Variability in Rockfish (*Sebastes* spp.) fecundity: species
748 contrasts, maternal size effects, and spatial differences. Environmental Biology of Fishes 98:81–100.
749 https://doi.org/10.1007/s10641-014-0238-7

750 Conrath CL (2019) Reproductive potential of light Dusky Rockfish (*Sebastes variabilis*) and Northern Rockfish (*S.*
751 *polyspinis*) in the Gulf of Alaska. Fishery Bulletin 117:16–26. https://doi.org/10.7755/FB.117.3.2

752 Dick EJ, Beyer S, Mangel M, Ralston S (2017) A meta-analysis of fecundity in Rockfishes (genus *Sebastes*).
753 Fisheries Research 187:73–85. https://doi.org/10.1016/j.fishres.2016.11.009

754 Domínguez-Petit R, Rideout R, Garabana D, Lambert Y, Hermida M, Morgan MJ (2018) Evaluating the use of the
755 autodiametric method for estimating fecundity of *Reinhardtius hippoglossoides*, a species with an unusual
756 oocyte development strategy. ICES Journal of Marine Science 75: 831–839. https://doi-
757 org.ezproxy.lib.calpoly.edu/10.1093/icesjms/fsx162

758 Field J, Beyer S, He X (2015) Status of the Chilipepper Rockfish, *Sebastes goodei*, in the California Current for
759 2015. Pacific Fisheries Management Council 186

760 Fields R (2016) Spatial and temporal variation in Rosy Rockfish (*Sebastes rosaceus*) Life History Traits. Capstone
761 Projects and Master's Theses

762 Flores A, Queirolo D, Tomasoni M (2017) Estimating fecundity using image analysis in *Cervimunida johni* and
763 *Pleuroncodes monodon*. Fisheries Research 190:34–42. https://doi.org/10.1016/j.fishres.2017.01.018

764 Fox J, Weisberg S (2019) An R companion to applied regression, Third. Sage, Thousand Oaks, CA

765 Friedland KD, Ama-Abasi D, Manning M, Clarke L, Kligys G, Chambers RC (2005) Automated egg counting and
766 sizing from scanned images: Rapid sample processing and large data volumes for fecundity estimates.
767 Journal of Sea Research 54:307–316. https://doi.org/10.1016/j.seares.2005.06.002

768 Hare JA, Morrison WE, Nelson MW, Stachura MM, Teeters EJ, Griffis RB, Alexander MA, Scott JD, Alade L, Bell
769 RJ, Chute AS, Curti KL, Curtis TH, Kircheis D, Kocik JF, Lucey SM, McCandless CT, Milke LM,
770 Richardson DE, Robillard E, Walsh HJ, McManus MC, Marancik KE, Griswold CA (2016) A vulnerability
771 assessment of fish and invertebrates to climate change on the Northeast U.S. Continental shelf. PLOS ONE
772 11:e0146756. https://doi.org/10.1371/journal.pone.0146756

773 He X, Field JC, Beyer SG, Sogard SM (2015) Effects of size-dependent relative fecundity specifications in fishery
774 stock assessments. Fisheries Research 165:54–62. https://doi.org/10.1016/j.fishres.2014.12.023

775 Holder AM, Field JC (2019) An exploration of factors that relate to the occurrence of multiple brooding in
776 rockfishes (*Sebastes* spp.). Fishery Bulletin 117:56–64. https://doi.org/10.7755/FB.117.3.5

777 Hyde JR, Vetter RD (2007) The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier).
778 Molecular Phylogenetics and Evolution 44:790–811. https://doi.org/10.1016/j.ympev.2006.12.026

779 Kjesbu OS, Holm JC (1994) Oocyte recruitment in first-time spawning Atlantic cod (*Gadus morhua*) in relation to
780 feeding regime. Canadian Journal of Fisheries and Aquatic Sciences 51:1893–1898.
781 https://doi.org/10.1139/f94-189

782 Klibansky N, Juanes F (2008) Procedures for efficiently producing high-quality fecundity data on a small budget.
783 Fisheries Research 89:84–89. https://doi.org/10.1016/j.fishres.2007.09.005

784 Lambert Y (2008) Why should we closely monitor fecundity in marine fish populations? Journal of Northwest
785 Atlantic Fishery Science 41:93–106. https://doi.org/10.2960/j.v41.m628

786 Lefebvre LS, Beyer SG, Stafford DM, Kashef NS, Dick EJ, Sogard SM, Field JC (2018) Double or nothing:
787 Plasticity in reproductive output in the chilipepper rockfish (*Sebastes goodei*). Fisheries Research 204:258–
788 268. https://doi.org/10.1016/j.fishres.2018.03.002

789 Lefebvre LS, Friedlander CL, Field JC (2019) Reproductive ecology and size-dependent fecundity in the petrale sole
790 (*Eopsetta jordani*) in waters of California, Oregon, and Washington. Fishery Bulletin 117:291–302.
791 https://doi.org/10.7755/FB.117.4.2

792 Lefevre S, McKenzie DJ, Nilsson GE (2017) Models projecting the fate of fish populations under climate change
793 need to be based on valid physiological mechanisms. Global Change Biology 23:3449–3459.
794 https://doi.org/10.1111/gcb.13652

795 Love MS, Morris P, McCrae M, Collins R (1990) Life history aspects of 19 Rockfish species (*Scorpaenidae*:
796 *Sebastes*) from the Southern California bight. NOAA Tech Rep NMFS 83:1–38

797 Love MS, Yoklavich M, Thorsteinson L (2002) The Rockfishes of the Northeast Pacific. University of California
798 Press, Berkeley

799 MacGregor JS (1970) Fecundity, multiple spawning, and description of the gonads in *Sebastodes*. U.S. Department
800 of the Interior, Bureau of Commercial Fisheries

801 Marshall CT, Kjesbu OS, Yaragina NA, Solemdal P, Ultang Ø (1998) Is spawner biomass a sensitive measure of the
802 reproductive and recruitment potential of Northeast Arctic cod? Canadian Journal of Fishery and Aquatic
803 Science 55:1766–1783. https://doi.org/10.1139/f98-062

804 Marshall DJ, Bode M, Mangel M, Arlinghaus R, Dick EJ (2021) Reproductive hyperallometry and managing the
805 world's fisheries. PNAS 118: https://doi.org/10.1073/pnas.2100695118

Marteinsdottir G, Begg GA (2002) Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. Marine Ecology Progress Series 235:235–256. https://doi.org/10.3354/meps235235

McElroy WD, Wuenschel MJ, Press YK, Towle EK, McBride RS (2013) Differences in female individual reproductive potential among three stocks of winter flounder, *Pseudopleuronectes americanus*. Journal of Sea Research 75:52–61. https://doi.org/10.1016/j.seares.2012.05.018

McElroy WD, Wuenschel MJ, Towle EK, McBride RS (2016) Spatial and annual variation in fecundity and oocyte atresia of yellowtail flounder, *Limanda ferruginea*, in U.S. waters. Journal of Sea Research 107:76–89. https://doi.org/10.1016/j.seares.2015.06.015

Methot RD, Wetzel CR (2013) Stock synthesis: A biological and statistical framework for fish stock assessment and fishery management. Fisheries Research 142:86–99. https://doi.org/10.1016/j.fishres.2012.10.012

Mion M, Thorsen A, Vitale F, Dierking J, Herrmann JP, Huwer B, von Dewitz B, Casini M (2018) Effect of fish length and nutritional condition on the fecundity of distressed Atlantic cod *Gadus morhua* from the Baltic Sea. Journal of Fish Biology 92:1016–1034. https://doi.org/10.1111/jfb.13563

Monk M, Dick E, Field J, et al (2021) The status of Vermilion Rockfish (*Sebastes miniatus*) and Sunset Rockfish (*Sebastes crocotulus*) in U.S. waters off the coast of California north of Point Conception in 2021. Pacific Fisheries Management Council

Moser HG (1967) Reproduction and development of *Sebastodes paucispinis* and comparison with other Rockfishes off Southern California. Copeia 1967:773–797. https://doi.org/10.2307/1441888

Murua H, Kraus G, Saborido-Rey F, Witthames PR, Thorsen A, Junquera S (2003) Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. Journal of Northwest Atlantic Fishery Science 33:33–54

Parker SJ, Berkeley SA, Golden JT, Gunderson DR, Heifetz J, Hixon MA, Larson R, Leaman BM, Love MS, Musick JA, O'Connell VM, Ralston S, Weeks HJ, Yoklavich MM (2000) Management of Pacific Rockfish. Fisheries 25:22–30. https://doi.org/10.1577/1548-8446(2000)025<0022:MOPR>2.0.CO;2

Phillips JB (1964) Life History Studies on Ten Species of Rockfish (Genus *Sebastodes*). California Department of Fish and Game, Fishery Bulletin 126:1–70

Shaw FR, Morado JF, Lowe VC, McDermott SF (2012) An atlas of reproductive development in Rockfishes, genus *Sebastes*. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Scientific Publications Office, Seattle, Washington

Sogard S, Berkeley S, Fisher R (2008) Maternal effects in Rockfishes *Sebastes* spp.: a comparison among species. Marine Ecology Progress Series 360:227–236. https://doi.org/10.3354/meps07468

Stafford DM, Sogard SM, Berkeley SA (2014) Maternal influence on timing of parturition, fecundity, and larval quality in three shelf Rockfishes (*Sebastes* spp.). Aquatic Biology 21:11–24. https://doi.org/10.3354/ab00564

Stephens A, Taylor I (2018) Status of Yellowtail Rockfish (*Sebastes flavidus*) along the U.S. Pacific Coast in 2017. Pacific Fisheries Management Council

Sullivan KM, Bailey MM, Berlinsky DL (2019) Digital image analysis as a technique for Alewife fecundity estimation in a New Hampshire river. North American Journal of Fisheries Management 39:353–361. https://doi.org/10.1002/nafm.10273

Thorsen A, Kjesbu OS (2001) A rapid method for estimation of oocyte size and potential fecundity in Atlantic cod using a computer-aided particle analysis system. Journal of Sea Research 46:295–308. https://doi.org/10.1016/S1385-1101(01)00090-9

Thorson JT, Dorn MW, Hamel OS (2019) Steepness for West Coast Rockfishes: Results from a twelve-year experiment in iterative regional meta-analysis. Fisheries Research 217:11–20. https://doi.org/10.1016/j.fishres.2018.03.014

Trippel EA (1999) Estimation of stock reproductive potential: History and challenges for Canadian Atlantic Gadoid stock assessments. Journal of Northwest Atlantic Fishery Science, 25, 61-81. https://doi.org/10.2960/J.V25.A6

Witthames PR, Thorsen A, Murua H, Saborido-Rey F, Greenwood LN, Dominguez R, Korta M, Kjesbu OS (2009) Advances in methods for determining fecundity: application of the new methods to some marine fishes. Fishery Bulletin 107:148–164

Wyllie Echeverria T (1987) Thirty-four species of California Rockfishes: maturity and seasonality of reproduction. Fishery Bulletin 85:229–250

Yamada J, Kusakari M (1991) Staging and the time course of embryonic development in kurosoi, *Sebastes schlegeli*. Environ Biol Fish 30:103–110. https://doi.org/10.1007/BF02296881

Yamanaka KL, Logan G (2010) Developing British Columbia's inshore rockfish conservation strategy. Marine and Coastal Fisheries 2:28–46. https://doi.org/10.1577/C08-036.1