



Zero, one or more broods: reproductive plasticity in response to temperature, food, and body size in the live-bearing rosy rockfish *Sebastes rosaceus*

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ABSTRACT: Patterns of reproduction, such as size–fecundity relationships used in models to assess fish populations, are generally treated as static through time and invariant to environmental change. However, growing evidence suggests that changes in ocean conditions, such as warming water temperatures and reduced primary productivity, affect life history traits, including reproduction. Under controlled experimental conditions, we documented reproductive plasticity in the live-bearing rosy rockfish *Sebastes rosaceus* in response to different temperature and feeding regimes, with maternal size as a covariate. Rosy rockfish occur throughout the California Current, a highly dynamic ecosystem for which increased environmental variability is predicted with climate change. Females produced 0–5 larval broods annually. Larger females had disproportionately higher fecundity than smaller females by producing larger-sized broods and a greater number of annual broods. Warmer water temperature decreased the time interval between brood releases, likely reflecting faster egg and larval development. However, warmer temperature did not increase the total number of broods, potentially reflecting a tradeoff with increased metabolic demand. Well-fed females had better body condition and higher annual fecundity compared to poorly fed females, primarily due to a greater number of broods. Conversely, females with poor body condition at the start of the reproductive season did not reproduce, providing possible evidence of delayed maturation at smaller sizes or skipped spawning at larger sizes. Reproductive plasticity (in terms of whether and how many broods are produced per year) in response to the environment likely contributes to high inter-annual variation in population larval production. Understanding the causes and consequences of reproductive plasticity is critical to developing sustainable management strategies and predicting population response to changing climate conditions.

KEY WORDS: Reproductive plasticity · Maternal size · Body condition · Food availability · Temperature · *Sebastes* · Climate change · Fisheries management

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1. INTRODUCTION

Successful reproduction is critical to the stability and growth of wild fish populations and is therefore a vital component of natural resource management (Roff 1992, Stearns 1992, Hilborn et al. 1995, Lambert 2008, Clutton-Brock & Sheldon 2010, Kindsvater et al. 2016, Lowerre-Barbieri et al. 2017). Climate

change is likely to affect the reproductive success of marine fishes through changes to the biological and physical environments, affecting resource availability, temperature, and bioenergetics (Hilborn et al. 1995, Harvey et al. 2011, Pankhurst & Munday 2011, Lowerre-Barbieri et al. 2017). However, size-dependent fecundity relationships currently used in population models to assess the health and status of fish

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populations are often treated as static through time and unaffected by environmental change (Lambert 2008), even as a growing body of evidence suggests that changes in the ocean environment, such as temperature and food availability, drive variability in life history traits (Narimatsu et al. 2010, Tillotson & Quinn 2018). Classic life history theory states that tradeoffs exist under limiting resources, leading to predictable shifts in energy allocation affecting growth, maturity, and reproductive effort (Stearns 1992). Phenotypic plasticity in life history traits may evolve, allowing populations to rapidly respond to environmental change (Pigliucci 2001, Piersma & Drent 2003, Botero et al. 2015), yet the extent of reproductive plasticity exhibited by individuals, and subsequent effects on population-level productivity, are largely unknown. Understanding the causes and consequences of reproductive plasticity, including changes in size-dependent fecundity relationships, where females of the same size produce more or less eggs depending on prevailing environmental conditions, will allow resource managers to better assess and predict annual reproductive output of marine fishes.

To study phenotypic plasticity in reproductive effort, an understanding of how fecundity increases with maternal size must be well established. Fecundity generally increases in a hyper-allometric relationship with maternal size in marine fishes (Barneche et al. 2018). This is important to fisheries management because the removal of old, large females from a population disproportionately reduces reproductive output (Scott et al. 1999, Berkeley et al. 2004, O'Farrell & Botsford 2006, He et al. 2015, Barnett et al. 2017). Once the size-dependent fecundity relationship is established, then environmental variables may be explored to explain additional variability in reproductive output. Therefore, maternal size is a requisite covariate in any study of reproductive plasticity in marine fishes.

The speciose genus *Sebastes* (rockfishes) is well suited for the study of reproductive plasticity in response to variable environments. The effect of maternal size on fecundity is well documented across these species (Dick et al. 2017), providing a clear baseline for assessing whether and how this relationship changes with the environment. All rockfishes are live bearers with internal fertilization, releasing larvae that have completed embryogenesis and are ready to begin exogenous feeding (Love et al. 2002). During a relatively short mating season, females copulate with one to several males and store sperm in the ovaries (Sogard et al. 2008b). Fertilization may occur months after mating, and gestation time is variable depend-

ing on species and temperature (Moser 1967, Love et al. 2002). For example, mean gestation of yellowtail rockfish *S. flavidus* embryos was 29.2 d for females held in the laboratory at 11.9°C (Eldridge et al. 2002). In comparison, mean gestation was 48 d in *S. schlegeli* held at 9.8°C (Yamada & Kusakari 1991). Both species-specific genetics and environmental factors such as temperature are likely to influence the length of the gestation period, with shorter gestation possible at lower latitudes and warmer temperatures (Moser 1967).

Individual females exhibit a range of phenotypic plasticity within and across species, with 2 distinct reproductive strategies in the genus. In the majority of *Sebastes*, mature females are limited to the production of a single larval brood annually (i.e. a single brood strategy). However, females of at least 15 species are capable of releasing one or more additional broods per year (i.e. a multiple-brooding strategy) (Moser 1967, Love et al. 2002, Lefebvre et al. 2018). Though rockfishes were historically considered determinate spawners, the recruitment of oocytes for secondary broods throughout the reproductive season recategorizes multiple-brooding rockfishes as indeterminate spawners (Murua & Saborido-Rey 2003, Murua et al. 2003, Lefebvre et al. 2018). Thus, potential annual fecundity in multiple-brooding rockfishes, which is the total number of advanced, vitellogenic oocytes produced per female per year (Murua et al. 2003), cannot be determined by counting oocytes at the start of the reproductive season because not all oocytes for all broods have been recruited yet. Instead, annual realized fecundity (AF), which is the total number of larvae released per female in a year, is a function of the total number of broods and brood fecundity (BF) over the entire reproductive season (Murua et al. 2003). This concept is well established for indeterminate, batch-spawning marine fishes that release eggs (Fitzhugh et al. 2012) but is less well-defined for the live-bearing, multiple-brooding rockfishes (Lefebvre et al. 2018).

The number of larvae in a second brood is often comparable to the first brood (MacGregor 1970, Beyer et al. 2015, Lefebvre et al. 2018), meaning the production of a second brood doubles reproductive output. In addition, producing multiple broods per year increases the number of times at which larvae are released into the ocean environment, increasing the likelihood that some larvae will encounter favorable environmental conditions that increase their survival (Cushing 1975). Larger females may also produce more broods annually compared to smaller females, representing an additional effect of mater-

nal size on reproductive output and exponentially increasing the fecundity of larger, multiple-brooding females (Love et al. 1990, Ralston & MacFarlane 2010, Beyer et al. 2015, Lefebvre et al. 2018).

In contrast to multiple brooding, at least some rockfishes have the ability to forego reproduction in any given year but spawn again in subsequent years (Head et al. 2016, Conrath 2017). This behavior is called skipped spawning and also occurs in other marine fishes (Rideout et al. 2005, Rideout & Tomkiewicz 2011, Skjæraasen et al. 2012, 2015). Skip spawning means reproductive failure in the current year but may result in an increase in allocation of energetic reserves to growth, survival, and future reproductive opportunities (Stearns 1992). A variation on skipped spawning that also occurs in rockfishes is delayed or abortive maturation during the first spawning attempt, where oocytes either take longer than 1 yr to develop or oocytes are recruited but later aborted and resorbed through a mass atresia event (Nichol & Pikitch 1994, Lefebvre & Field 2015, Lefebvre et al. 2018). The occurrence of delayed or abortive maturation in a first-time spawner is considered an adolescent or developing phase, where a female may be physiologically mature but has yet to contribute to population larval production and is thus considered functionally immature (Brown-Peterson et al. 2011, Lowerre-Barbieri et al. 2011). Both skipped spawning and delayed or abortive maturation affect population larval production in terms of which females contribute each year. The diversity of reproductive strategies and behaviors observed in the *Sebastes* genus, ranging from less plastic (i.e. 0 or 1 annual broods) to more plastic (i.e. 0, 1 or more annual broods), provides an excellent study system for understanding how individual reproductive output is affected by environmental variability and how reproductive success may respond to climate change in a widespread, diverse, and economically important species complex.

At least 56 rockfish species are found in the California Current Ecosystem along the West Coast of the USA (Love et al. 2002). The California Current is characterized by high spatiotemporal variability in environmental conditions (Checkley & Barth 2009), with a major geographic break near Point Conception (34.449° N, 120.471° W). To the north, seasonal cycles in temperature and biological productivity are the result of strong upwelling events in coastal waters during the spring and early summer that circulate cold, nutrient-rich waters from depth into the photic zone, driving primary and secondary productivity (Parrish et al. 1981, Checkley & Barth 2009). To

the south, ocean temperatures are warmer, and upwelling in coastal areas is less seasonal and more muted throughout the year (Parrish et al. 1981, Legaard & Thomas 2006, Checkley & Barth 2009). For example, a comparison of regional temperature gradients showed a 2–3°C difference between central California and the southern California Bight, with mean temperature of coastal waters at 10 m depth (all months combined) between 12–13°C in the central region and 14–15°C in the south (Lynn 1967). On a temporal scale, warming trends are documented for the California Current (Di Lorenzo et al. 2005), and the frequency of climate warming events such as El Niño and marine heatwaves has increased in recent history (Checkley & Barth 2009, Cai et al. 2014, Di Lorenzo & Mantua 2016). Ocean warming events in the California Current are correlated with decreased primary productivity through disruptions to ocean circulation, nutrient availability, and shifts in prey quantity or quality affecting energy transfer to upper trophic levels (Legaard & Thomas 2006, Piatt et al. 2020).

In rockfishes, the strong 1982–1983 and 1992–1993 El Niño ocean warming events reduced female body condition and gonadosomatic indices in blue rockfish *S. mystinus* (VenTresca et al. 1995). The more recent 2014–2015 marine heatwave and 2015–2016 El Niño had large ecosystem-level effects on biological communities at multiple trophic levels, such as a shift in the composition of copepod communities, the northern range expansion of sub-tropical species, declines in the abundance of forage fishes, and disruptions to the reproductive success of marine mammals and seabirds (Leising et al. 2015, McClathchie et al. 2016, Peterson et al. 2017, Sanford et al. 2019, Piatt et al. 2020). The abundance of pelagic, young-of-the-year rockfishes in 2015–2016 varied spatially in the California Current, with high abundance in the central region but lower than average abundance to the north and south (McClathchie et al. 2016). Spatially, reproductive patterns of adult rockfishes differ between the north and south regions of the California Current, and reproductive success is likely correlated with environmental conditions affecting female body condition (MacGregor 1970, Love et al. 2002, Beyer et al. 2015, Lefebvre et al. 2018).

Elevated water temperatures increase metabolic demands in ectotherms, affecting female body condition (Johnston & Dunn 1987), and may shorten the gestation period of rockfish embryos (Moser 1967). Warmer water temperatures and reduced seasonality, as in the southern range of the California Current, may contribute to the faster production of broods dur-

ing a spawning season if females have adequate energy reserves. The effect of warming water temperatures on metabolic rates, coupled with shifts in primary and secondary productivity affecting the quality and quantity of food supply, likely both affect female energetic reserves and reproductive decisions. Thus, seasonal, annual, and inter-annual changes in temperature and food abundance are expected to drive spatiotemporal patterns in the reproductive output and reproductive plasticity in rockfishes, for which multiple brooding is most commonly observed in the south, less often in the central region, and has yet to be documented farther north in the California Current (Wyllie-Echeverria 1987, Love et al. 1990, 2002).

In rockfishes, we hypothesized that both water temperature and food availability would affect female body condition and energy allocation to reproductive effort, including the production of multiple broods. We expected that females in poor body condition would reduce their AF by producing fewer broods and/or producing broods of fewer larvae. We also expected that warmer temperatures would decrease gestation time, potentially affecting the likelihood of a female producing multiple broods. We tested our hypotheses under controlled, experimental conditions, manipulating both water temperature and food availability to show how the environment affects female body condition and reproductive plasticity in a multiple-brooding rockfish species. Our study aimed to provide a better understanding of the spatiotemporal dynamics in the reproductive ecology of rockfishes in the California Current and to improve knowledge of how reproduction is likely to be affected by climate change.

2. MATERIALS AND METHODS

2.1. Study species

The rosy rockfish *Sebastes rosaceus* was used as a model to study multiple brooding in rockfishes. Rosy rockfish are a tractable species for laboratory study due to their relatively small size. Individuals are distributed throughout the California Current from the Strait of Juan de Fuca, Washington, to central Baja California, Mexico (Love et al. 2002), with a center of biomass in the central and south regions of the California Current (Holder & Field 2019). Rosy rockfish are associated with rocky reef habitats at depths of 40–150 m (Love et al. 2002). They are generalists, feeding on small invertebrates and small fishes associated with the benthos and in the water column

(Love et al. 2002). Historically, rosy rockfish were commercially important to markets in central California (San Francisco) and are commonly encountered in the California recreational hook-and-line fishery, although they are typically not targeted due to their small size (Love et al. 1990, 2002, Mason 1998). There has been no formal assessment of population abundance or stock status, but the rosy rockfish was among the top 6 most abundant species in a 2012–2014 fishery-independent hook-and-line survey in central California (Marks et al. 2015).

Life history information is relatively limited for rosy rockfish in comparison to other rockfishes. A maximum size of 36 cm is often cited but was questioned as a likely misidentification with the larger, similar appearing rosethorn rockfish *S. helvomaculatus* (Chen 1971). For comparison, a maximum length of 31 cm fork length (FL) was observed in central California (Echeverria & Lenarz 1984) and 27 cm FL in southern California (Love et al. 1990). Length-at-maturity is also variable, suggesting spatiotemporal differences in the maturation schedule and/or differences in the identification of immature and mature fish based on methods of either macroscopic or histological examination of the ovaries. In California, 50% of females collected from 1977–1982 were mature at 18.6 cm FL (Wyllie-Echeverria 1987). More recently in central California, 50% of females collected from 2012–2014 were mature at 16.6 cm FL (Fields 2016). A smaller size at maturity was found in females collected from southern California between 1980 and 1987, with 50% mature at 14.7 cm FL (Love et al. 1990). Wyllie-Echeverria (1987) used both macroscopic and histological methods to identify immature and mature fish, Fields (2016) used macroscopic methods, and Love et al. (1990) used temporally restricted macroscopic methods, where maturity status was determined only during the active reproductive season to avoid misidentification of immature, developing, and resting mature females. Note that total lengths were converted to FL to compare across studies (Echeverria & Lenarz 1984).

Gravid females have been observed from December through September in the central and southern regions of the California Current, suggesting nearly year-round reproduction (Love et al. 2002). Male rosy rockfish gonadosomatic indices peak in October, then decline through November and December to low levels over the rest of the year, indicating a fall mating season (Love et al. 1990). Age determination through the identification of growth increments in otoliths is difficult for this species and has not been validated, but it is thought that females mature

around 6 yr of age, with a maximum age of 35 yr (Wyllie-Echeverria 1987, Fields 2016). Because of imprecision and difficulty in aging rosy rockfish, age determination was not attempted in this study.

2.2. Collections

2.2.1. Laboratory collections

A total of 46 female rosy rockfish were collected at 73 m depth by hook-and-line methods near Monterey, California, at the start of the mating season in October through December of 2015 and 2016 with the help of the Monterey Bay Aquarium (MBA) collecting staff. Rockfishes have a physoclistous swim bladder and are susceptible to injury from barotrauma during capture from depth. To alleviate barotrauma symptoms, fish were re-pressurized to 70 PSI in a 2 chamber, flow-through seawater hyperbaric chamber immediately following capture (J. Welsh, MBA). Because of space limitations in the hyperbaric chamber, a limit of 10–15 rosy rockfish could be collected per sampling trip depending on cumulative fish size. Fish in the chamber were monitored for changes in neutral buoyancy, and the pressure was reduced incrementally each day. After 5 d, fish were acclimated to ambient surface pressure, allowing release into standard, unpressurized seawater tanks. Following acclimation, fish were transported to the flow-through seawater facility at the NOAA National Marine Fisheries Service (NOAA NMFS) in Santa Cruz, California.

2.2.2. Wild collections

Laboratory fish were compared to a data set of wild fish to better understand how the resulting body condition and fecundity in the laboratory compared with fish in the wild. Wild rosy rockfish were opportunistically collected by similar hook-and-line methods from rocky reefs at depths of 27–73 m in central California from 2012–2014 as part of a Rockfish Conservation Area monitoring project and ongoing reproductive studies by researchers at NOAA NMFS (Marks et al. 2015). BF data were available from a subset of 17 females with a mean size of 20.6 cm FL (range: 16.7–23.7 cm) to compare with laboratory females. Fecundity of wild females was determined by the gravimetric method of counting weighed subsamples of eggs or larvae from the ovaries, following methods described in Beyer et al. (2015).

The comparison of body condition of laboratory fish to that of wild fish was restricted to August in 2014, a year of moderate ocean conditions prior to an end-of-year warming event (Leising et al. 2015). The fish were collected at the end of the reproductive season when all females were most likely to be in the resting reproductive stage. Wild fish samples came from a single location at the Farallon Islands, approximately 74 nautical miles northwest of the location in Monterey Bay where laboratory fish were collected. A total of 83 wild females, with a mean size of 20.6 cm FL (range: 13.8–25.8 cm), were used for comparison of body condition.

2.3. Laboratory setup

The laboratory setup and experimental design followed established protocols for reproductive studies of single-brooding rockfish species in captivity (Fisher et al. 2007, Tsang et al. 2007, Sogard et al. 2008a,b, Kashef et al. 2014). To provide females the opportunity to mate, males and females were housed in outdoor group tanks in their respective treatments from October through December during the mating season. The group tank setup and methods followed those described for the successful mating of kelp rockfish *S. atrovirens* in captivity (Sogard et al. 2008b). The group tanks measured approximately 2.29 m in diameter by 1.52 m height, with 4165 l of flow-through seawater. Water flow was set to 250 ml s⁻¹. The group tanks were covered with a tarp, which lowered the light levels in the tank to more closely mimic reduced natural light at depth but still allowed for some light to pass, ensuring exposure to a natural photoperiod. Even though females were expected to mate in the laboratory, some females may have already mated, acquired, and stored sperm prior to capture, which has been documented in captive rockfish studies (Tsang et al. 2007, Sogard et al. 2008b).

Females were assigned a combined temperature and feeding regime in a size-stratified, semi-randomized design, ensuring that the size range and mean size of females across treatments was similar. For example, the 4 largest females from one collection date were randomly assigned to each of the 4 combination treatments, followed by randomly assigning the next 4 largest females until all were assigned. This method ensured that the largest females, considered the most likely to produce multiple broods, were evenly distributed across treatments and that a range of sizes occurred in each treatment (Beyer et

al. 2015, Lefebvre et al. 2018). Males were distributed in the same fashion to ensure a size range of males in each treatment.

Following the mating season, females were isolated from males and moved to smaller indoor tanks of dimensions 0.91 m diameter by 1.52 m depth, with approximately 1040 l of flow-through seawater. The setup of smaller indoor tanks closely followed methods described for fecundity and larval collection in other rockfishes in the laboratory (Fisher et al. 2007, Sogard et al. 2008a, Kashef et al. 2014). Successful fertilization, gestation, and parturition of larvae by females isolated from males is common in rockfish rearing studies because of the ability of females to store sperm for extended periods (Fisher et al. 2007, Tsang et al. 2007, Sogard et al. 2008a,b, Kashef et al. 2014). The smaller tanks allowed for individual tracking of female reproductive status through the reproductive season and the collection of larvae at parturition, which was not possible in large group tanks. A 200 μm mesh filter was attached to the outflow pipe of each tank to prevent the escape of free-swimming larvae following a parturition. Natural photoperiod cycles were maintained in the indoor aquarium. Flows were set to 25 ml s^{-1} , and life support systems, including seawater flow, water temperature, and compressed air supply, were checked daily. Tanks were cleaned twice a week by siphoning detrital material and rinsing the mesh outflow filters to prevent clogging of debris and overflow of the tank when larvae were released (Sogard et al. 2008a). The experiment was conducted from October to the following September during the 2015–2016 and 2016–2017 reproductive seasons, referred to as the 2016 and 2017 reproductive seasons.

2.4. Experimental design

Our experimental design examined the interactive effects of water temperature and food availability on reproductive effort. Two temperature levels were tested, ambient (warmer treatment) and chilled (cooler treatment), to mimic natural temperature differences between central and southern California and future temperature increase in central California. Flow-through seawater from intake pipes located in 9 m of water off the coastal NOAA NMFS facility was filtered and delivered directly to ambient treatment tanks. Water for chilled tanks passed through a chiller to lower temperatures by 2–3°C relative to ambient temperatures. Water temperatures thus fluctuated throughout the year to mimic natural cycles

while maintaining a 2–3°C difference between warm and cool treatments. Resulting temperatures cycled annually from 12–15°C in the warmer treatment and from 9–13°C in the cooler treatment. Temperatures in the cool treatment reflected a range in the annual temperature cycle typically encountered at 75 m depth in Monterey Bay, central California, where rosy rockfish were collected (Pennington & Chavez 2000). Temperatures in the warm treatment reflected warmer temperatures encountered by rosy rockfish in southern California (Lynn 1967) and general warming trends predicted for central California (Di Lorenzo et al. 2005).

Food availability was manipulated using the following 3 feeding regimes: fish fed twice wk^{-1} (high ration), once every 2 wk (medium ration), and once mo^{-1} (low ration). Feeding regimes were designed to mimic natural variability in body condition in the wild and were initially based on feeding regimes of captive rockfish in previous studies (Fisher et al. 2007, Sogard et al. 2008a,b, Kashef et al. 2014). In 2016, all fish were fed either a high or medium ration. We found that the body condition of these females at the end of the reproductive season was similar to, or higher than, fish collected from the wild during a similar time period. Thus, in 2017, the feeding levels were altered to a high and a low feeding regime to reflect the greater range in body condition occurring in wild fish. During scheduled feeding days, all fish were fed to satiation with a high protein and lipid diet of market squid *Doryteuthis opalescens* and northern anchovy *Engraulis mordax*. The selection of diet was based on successful captive rearing studies of closely related rockfishes (Fisher et al. 2007, Sogard et al. 2008a,b, Kashef et al. 2014) and expert opinion from the MBA animal husbandry unit experienced with rosy rockfish specifically (J. Welsh, MBA). Sample sizes and starting measurements for females and males in all treatment combinations in each year are listed in Table 1. Protocols for this study were approved by the University of California Santa Cruz Institutional Animal Care and Use Committee (IACUC) in a permit issued to the NOAA NMFS Fisheries Ecology Division, Santa Cruz, California.

2.5. Measurement of size and body condition

When first brought into the laboratory, fish were measured for FL (nearest mm) and total wet weight (nearest 0.1 g) and internally tagged with a passive integrated transponder (PIT) tag in the muscle just below the dorsal spine on the left side of the body. A

Table 1. Starting measurements and sample size of female and male rosy rockfish in 2 temperature treatments (warm, cool) and 3 feeding regimes (low, medium, high) over 2 reproductive seasons (2016, 2017). Note that 2017 experiments included 35 females also used in 2016 experiments, along with 8 new females collected prior to the setup of mating groups in 2017. In total, there were 46 unique females in the study, resulting in 81 observations of female reproductive effort across 2 yr. Female identity was treated as a random effect to account for multiple observations

Treatment (Year)	Females		Males	
	n	Mean \pm SD (range)	n	Mean \pm SD (range)
High ration (2016)				
Cool	10	201 \pm 17 (174–232)	2	203 \pm 10 (196–221)
Warm	8	199 \pm 13 (190–228)	5	202 \pm 16 (184–221)
High ration (2017)				
Cool	10	208 \pm 17 (190–240)	5	200 \pm 15 (190–227)
Warm	11	199 \pm 13 (179–225)	5	218 \pm 13 (195–226)
Medium ration (2016)				
Cool	10	198 \pm 14 (178–219)	3	196 \pm 16 (181–213)
Warm	10	195 \pm 16 (175–237)	3	205 \pm 4 (203–210)
Low ration (2017)				
Cool	13	198 \pm 16 (176–225)	6	201 \pm 9 (189–213)
Warm	9	195 \pm 16 (180–235)	5	210 \pm 7 (203–218)

small fin clip from the upper (females) or lower (males) caudal fin was collected for genetic archive and to visually distinguish males from females in the group mating tanks. All fish in the study were measured in October of both years, providing starting measurements before the mating season. Ending measurements were made in August of both years when the majority of females had stopped reproducing. Body condition provided an index of energy reserves and was approximated by calculating Fulton's condition index (K):

$$K = (W \times L^{-3}) \times 100 \quad (1)$$

where W is total wet weight (g) and L is fork length (cm).

We compared the body condition of laboratory females at the end of each reproductive season to wild females. These comparisons allowed us to evaluate how well the 3 ration treatments mimicked variability in feeding success and body condition in the wild during a year of moderate ocean productivity.

2.6. Reproductive response variables

Reproductive plasticity in response to food and temperature was measured as the following 4 response variables: (1) brood count, (2) brood interval, (3) BF, and (4) AF. Brood count was the total number of broods released per female in a reproductive sea-

son. The brood interval was measured as the number of days between parturition events. BF was estimated by collecting and counting larvae from each brood release. AF was the sum of all larvae in all broods over one reproductive season. During the study, gestating females were identified by a combination of detailed daily notetaking on the visual appearance of individual females, including swelling of the abdomen when eggs were thought to be hydrated, and scanning of the internal PIT tag to confirm individual identification before and after parturition.

All parturition events in the laboratory occurred overnight, with the release of the entire brood. Mesh filters on outflow pipes retained larvae within the tank until collection the following morning. Following a parturition, adult females were carefully netted, rinsed with seawater, and

removed from the tank. Larvae were free-swimming in the tank and gently collected in a 2 step process of netting and siphoning seawater through a 200 μ m bucket sieve. Once collected, larvae were preserved in 95 % ethanol in 250 ml plastic jars.

BF was estimated using the volumetric subsampling method (Bagenal 1957, Murua et al. 2003). Ethanol in the preserved sample was first drained through a 200 μ m sieve and larvae were gently rinsed with deionized (DI) water into a 1000 ml graduated glass beaker. The beaker was then filled to 500 ml with DI water. Next, the sample was slowly stirred in a figure-eight motion to evenly disperse larvae, and four 10 ml subsamples were collected with a graduated, large-aperture pipette, transferred to a 250 ml glass beaker to confirm volume, and lastly pipetted into Petri dishes for counting under a dissection microscope. The count of larvae for each 10 ml subsample was multiplied by the total volume of the whole sample to estimate BF:

$$\text{BF} = (\text{subsample count} \times \text{subsample volume}^{-1}) \times \text{total volume} \quad (2)$$

BF was the mean estimate of the 4 subsamples. The resulting coefficient of variation (CV) in counts from the 4 subsamples averaged 9.7 %, with a range from 3.8–25.4 %. BF was calculated for broods where live larvae were released into the tanks.

Unexpectedly, a portion of the broods released in the laboratory was not larvae, but mature, hydrated,

unfertilized eggs, or a mix of both live larvae and unfertilized eggs (Fig. 1). If present, hydrated, unfertilized eggs were buoyant and clearly visible at the surface of the tank following a brood release. We assumed that such events were an indication of sperm limitation, and that sperm limitation was presumably a laboratory effect of females being isolated from males, which has occasionally been observed in other studies (Tsang et al. 2007). We did not consider the release of hydrated, unfertilized eggs to be evidence of skipped spawning, which is defined as mass atresia and resorption of oocytes (Rideout & Tomkiewicz 2011), but rather a reproductive attempt by the female which would have resulted in a brood of live larvae if sperm were present. Unfertilized eggs could not be collected and counted for fecundity due to the delicate nature and rupturing of the chorion during the collection process. Because we could not collect and count unfertilized eggs, brood releases of 100% unfertilized eggs were excluded from all fecundity analyses. However, unfertilized broods were still recorded in the brood count and assumed to represent a reproductive attempt.

In contrast, an estimate of BF for brood releases with a mix of live larvae and unfertilized eggs was possible. A brood release of both live larvae and unfertilized eggs was considered partial sperm limitation (i.e. a fraction of the brood was larvae and the other fraction mature, hydrated, unfertilized eggs; example Fig. 1). For broods with partial sperm limita-



Fig. 1. Image of a 1 d old rosy rockfish larva (center) and a mature, hydrated, unfertilized egg (upper left) released in the same brood as an example of partial sperm limitation. The hydrated, unfertilized egg contained a large, consolidated oil globule and degrading cellular material. Below the larva is a free-floating oil globule, remnants of an egg burst during the collection process. Scale bar drawn for size

tion, a simple visual estimate of the proportion of unfertilized eggs observed in the tank was incorporated into the BF estimate to account for the additional reproductive effort that would have occurred without sperm limitation. This was done by estimating the percent of unfertilized eggs compared to live larvae in the tank and including that percent in the calculation of BF. Sperm limitation was minimal in such broods in the fecundity analysis, with an average of only 6% of the brood estimated to be unfertilized.

To calculate AF, we summed the total number of larvae from all broods in a reproductive season for each female (Murua et al. 2003):

$$AF = \sum_{i=1}^n BF_i \quad (3)$$

where BF is of the i^{th} brood of n annual broods per female. Females that had any broods comprised entirely of unfertilized eggs were excluded from the fecundity analyses due to the inability to count unfertilized eggs, as noted above.

2.7. Dissections

Fish were dissected at the end of the 2 yr study on 4 October 2017 to assess the final reproductive state following the end of the reproductive season. Fish were euthanized by an overdose of tricaine methanesulfonate (MS-222) in a buffered seawater solution (Neiffer & Stamper 2009) and placed in a cooler of crushed ice for 24 h to maintain freshness of tissues without freezing. Dissections followed the next day. Fish were weighed (nearest 0.1 g) and measured (FL, nearest mm). The ovaries and liver were excised and weighed (nearest 0.01 g) to calculate the gonadosomatic and hepatosomatic indices, proportional gonad and liver weights, respectively.

Macroscopic classification of ovarian development followed the general description for rockfishes (Shaw et al. 2012) and more specific description of multiple brooding in the related chilipepper, *S. goodei* (see Table 2 in Lefebvre et al. 2018). The 'pre-vitellogenic' and 'resting' stages described in Lefebvre et al. (2018) were combined, as these stages represent a continuum from resting to early development of oocytes for the next season, more accurately distinguished by histological methods not used in this study. Ovaries of rosy rockfish were macroscopically classified as (1) 'immature', appearing thin and translucent; (2) 'resting or transitional', appearing thicker and less translucent or progressing to early oocyte development; or (3) 'vitellogenic', containing larger, macroscopically visi-

ble vitellogenic oocytes in development for the next reproductive season. Other ovarian stages, including fertilized embryos, eyed larvae, and recently spent females, were not observed at the time of dissections. It should be noted that macroscopic differentiation of immature and mature females outside of the reproductive season is not as accurate as when identified during the reproductive season due to the similar appearance of immature and resting mature ovaries (Love et al. 1990, Lefebvre et al. 2018).

2.8. Statistical analysis

2.8.1. Body condition

We used a linear regression model to assess how female body condition, measured by Fulton's K , was affected by feeding treatment and in comparison with wild fish ('lm' function in R version 3.6.1; R Core Team 2019). The categorical groupings included the 3 feeding regimes (high, medium, and low) and a wild fish (wild) group. The general expression for the linear model was:

$$K_i = b_0 + b_{X_i} + \varepsilon_i \quad \varepsilon_i \sim \text{Normal}(0, \sigma^2) \quad (4)$$

where b_0 is the intercept, X_i is an integer representing different levels of the categorical variable for the i^{th} fish, b_{X_i} the associated coefficient, and errors (ε_i) normally distributed. A post hoc, pairwise comparison identified significant differences across the feeding categories (Tukey multiple comparisons of means test, 'aov' function in R). A similar linear regression model was used to test for differences in female body condition, prior to the reproductive season, between females that reproduced in the laboratory (reproductively active) and those that did not (reproductively inactive).

2.8.2. Reproductive response

We used generalized linear mixed effects models to test for environmental and maternal size effects on the 4 reproductive response variables of (1) brood count, (2) brood interval, (3) BF, and (4) AF. Generalized linear mixed models are useful for behavioral studies with multiple observations of an individual because they model the combined effects of independent explanatory variables (i.e. fixed effects) while also accounting for within-individual variability (i.e. a random effect; Zuur et al. 2009). We modeled female identity as a random effect where individual females were observed in either Year 1, Year 2, or in

both years of the study depending on when they were collected, how often they reproduced, and whether they successfully fertilized all broods within a year.

Generalized linear mixed effects models were estimated using the 'lme4' package in R (Bates et al. 2015). Count data in the number of annual broods was modeled as a Poisson distribution using a canonical logarithmic link function (GLMM, 'glmer' function in R). Data for the brood interval (measured in days) and fecundity (measured by number of larvae) were modeled as Gaussian distributions using the canonical identity link function (LMM, 'lmer' function in R), as these were large counts for which a normal approximation seemed valid after transformation. In particular, the response variables of brood interval, BF and AF were natural log transformed. The general expression for the generalized linear mixed effects model was:

$$G[E(Y_{ij})] = \alpha_i + \beta_1 X_{1ij} + \dots + \beta_n X_{nij} \quad (5)$$

where G is the appropriate link function, as discussed above, and $E(Y_{ij})$ is the expected value of the response variable.

The response variable Y_{ij} is the reproductive response for the i^{th} fish in the j^{th} observation, which is a function of the fixed effects ($X_{1 \rightarrow n}$), with corresponding coefficients ($\beta_{1 \rightarrow n}$), and with the random effect of female identity modeled as a unique intercept for each female (α_i).

The fixed effects considered in each model were maternal length, water temperature, feeding regime, experimental year (2016 or 2017), and the interaction of temperature and food ration. Brood number was also considered in the models of brood interval and BF. Maternal length was treated as a continuous covariate and natural log transformed. All other fixed effects (water temperature, feeding regime, experimental year, and brood number) were treated as categorical variables. This approach followed standardized methods to model fecundity as a natural-log transformed power function of maternal size (Dick et al. 2017, Barneche et al. 2018), allowing for the comparison of fecundity parameters and the strength of maternal size effects to other species. The general model of fecundity (FEC) is specified as (Dick et al. 2017):

$$\text{FEC} = aL^b \quad (6)$$

where L is maternal length (mm), and the parameters a and b are the coefficients of the natural log-transformed linear regression of fecundity and length, incorporated into the mixed effects models described above:

$$\log(\text{FEC}) = \log(a) + b \times \log(L) \quad (7)$$

We used post hoc, pairwise comparisons (multiple comparisons of means: Tukey contrasts) to identify specific differences across categorical groupings within a fixed effect using the 'glht' function (package 'multcomp') in R (Hothorn et al. 2008). The model assumptions of normality, linearity, and homoscedasticity were assessed visually with $Q-Q$ plots and by plotting the model residuals against the fitted values (Zuur et al. 2009). We used the Shapiro-Wilk statistic to test for normality (Zuur et al. 2009) and the Levene's test for homoscedasticity (Glaser 2006). Coefficients and 95% confidence intervals are presented for the final models.

To select the most appropriate model, we first estimated a full model including all fixed effects and compared it with an intercept-only model to test for overall model significance (Zuur et al. 2009). If the full model was significantly different from the intercept-only model, model selection was initiated. We used backward, stepwise model selection based on a series of F tests to determine the order of testing for fixed effects (Zuur et al. 2009). Significance of the fixed effect was assessed by a likelihood ratio test using the 'anova' function in R, by sequentially removing the variable of interest and comparing with the previous model, which included the effect (Zuur et al. 2009). Non-significant effects were sequentially removed and significant effects retained in the final model (Zuur et al. 2009). All final models included the random effect of female identity.

2.9. Sample sizes

The sample size for each of the reproductive response variables, including the number of unique females contributing and how often they were observed for each analysis, was as follows. The experiment included 35 females observed in both years (2016 and 2017). Three females died at the end of the 2016 reproductive season, and 8 females were added to the study in 2017. This resulted in a total of 81 observations of annual brood count from the 46 unique females over the 2 years. Of these 46 females, 35 were observed twice (Year 1 and Year 2) and 11 were observed in only a single year (Year 1 or Year 2). There were 43 observations of brood intervals from 15 unique females that had successfully fertilized broods and reproduced at least twice in a year. Of these 15 females, 5 were observed in both years and 10 were observed in only a single year. There were

46 observations of BF from 17 unique females successfully fertilizing all annual broods in either Year 1, Year 2, or both years. This resulted in 21 observations of AF. Of these 17 unique females, 4 females were observed twice, meaning they fertilized all broods in both Year 1 and Year 2, and 13 females were observed once, meaning they fertilized all broods in only a single year (Year 1 or Year 2).

3. RESULTS

3.1. Female body condition

Food availability affected female body condition in each of the 3 feeding regimes compared to the body condition of wild fish (linear model, $F_{3,157} = 11.6$, $p < 0.001$; Fig. 2). Females in the high ration treatment were, on average, in better condition than wild females in a year of moderate ocean conditions (post hoc Tukey, $p < 0.001$). The body condition of females fed medium rations was not significantly different than that of wild females (Tukey, $p = 0.494$). Females

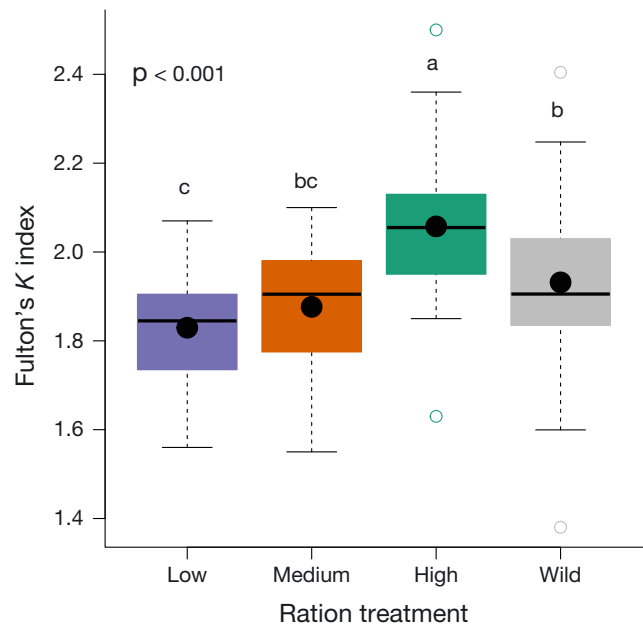


Fig. 2. The 3 feeding regimes (low, medium, and high) successfully manipulated female rosy rockfish body condition, measured by Fulton's K condition index, in relation to natural variation observed in wild fish during a year of moderate ocean conditions (wild). Body condition measurements were made in August, near the end of the reproductive season. Boxplots show median values (horizontal black bar), 25th and 75th quantiles, and the range of body condition values within each category. Open circles: outlying values; solid, black circles: means. Different lowercase letters indicate statistically significant differences ($p \leq 0.05$) across groups determined by a post hoc, pairwise comparison (see Section 2.8)

held on low rations had significantly lower body condition than high ration females (Tukey, $p < 0.001$) and marginally lower condition than wild females in a year of moderate ocean conditions (Tukey, $p = 0.048$). Considering the variability within each treatment and within the wild group, the 3 feeding regimes resulted in a range of female body condition similar to that likely experienced by fish across years of good and poor feeding conditions in the wild. Wild fish were collected in a single year, 2014, from a single location at the Farallon Islands in central California, so the natural variability reported here is likely a conservative estimate and expected to be greater across years of above or below average ocean conditions.

Table 2. Linear mixed effects analyses showing maternal size and environmental effects on reproductive output in rosy rockfish. The table lists all fixed effects considered in the starting models. Non-significant fixed effects were removed sequentially in order from top to bottom through backward, stepwise model selection. Log [] indicates a natural logarithm data transformation. Significance determined by a likelihood ratio test; fixed effects retained in the final models ($p \leq 0.05$) are in **bold**. Coefficients of the final models are presented in Table S1 in the Supplement at www.int-res.com/articles/suppl/m669p151_supp.pdf

Response	Fixed effect	χ^2	df	p
Annual broods per female (n = 81, units = brood count)				
	Ration \times temperature	2.339	2	0.311
	Temperature	0.276	1	0.600
	Food ration	3.028	2	0.220
	Year	1.829	1	0.176
	Log(length)	10.208	1	0.001
Interval between broods ^a (n = 43, units = log[days])				
	Ration \times temperature	0.785	2	0.675
	Food ration	1.686	2	0.430
	Year	2.798	1	0.094
	Brood number	11.653	3	0.009
	Temperature	5.134	1	0.024
	Log(length)	0.719	1	0.396
Brood fecundity ^b (n = 46, units = log[number of larvae])				
	Ration \times temperature	0.175	2	0.916
	Year	2.076	1	0.150
	Temperature	0.197	1	0.657
	Food ration^c	5.898	2	0.053
	Brood number	12.213	4	0.016
	Log(length)	5.750	1	0.017
Annual fecundity ^b (n = 21, units = log[number of larvae])				
	Ration \times temperature	5.159	2	0.076
	Temperature	0.059	1	0.808
	Year	1.607	1	0.205
	Food ration	6.445	2	0.040
	Log(length)	4.121	1	0.042

^aFertilized broods; ^bFemales fertilizing all broods; ^cMarginal significance, retained in final model

3.2. Number of annual broods

Rosy rockfish released 0–5 broods yr^{-1} . At least some females in all treatments reproduced, and maternal length was positively correlated with the number of annual broods (Table 2, Fig. 3). Temperature and feeding regime were not statistically significant predictors of variation in the number of broods per year (Table 2); however, no female in the low ration feeding regime produced more than 3 broods (Fig. 3). Among the reproductively active females (i.e. females that released broods), the mean number of annual broods was 1.6 in the low, 2.7 in the medium, and 3 in the high feeding regime, suggesting a trend of increasing brood numbers with increasing rations. There was no evidence of an effect of experimental year on the number of annual broods per female (Table 2).

In each treatment combination, some females were not reproductively active, meaning they never released a brood. The number of females not reproducing in the warm treatment at high, medium, and low ration was 7 of 19 (37%), 3 of 10 (30%), and 3 of 9

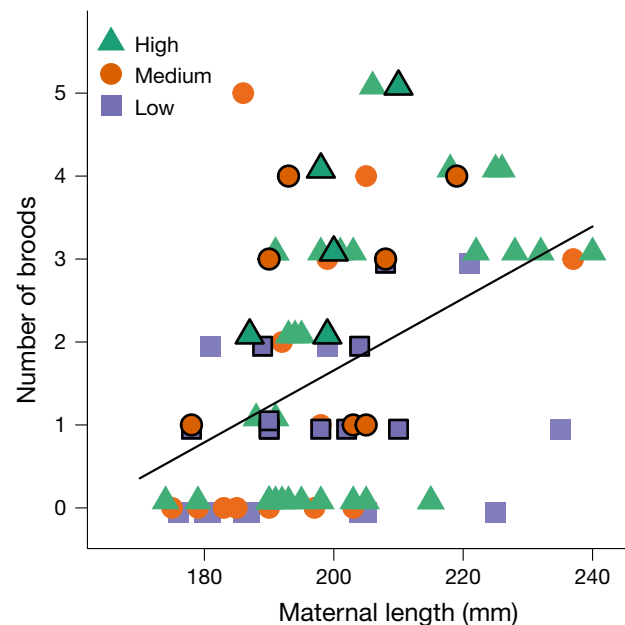


Fig. 3. Rosy rockfish maternal size had a positive effect on the number of annual broods per female. Data shown for all observations (n = 81) in each of the feeding regimes: low, medium, and high. Outlined symbols are females that fertilized all broods in a year and were included in the fecundity analyses (n = 21). Symbols without an outline are either females that released at least one fully unfertilized brood (sperm limitation) and therefore could not be used in the fecundity analyses, or non-reproductively active females (shown by zero broods). Regression line is for all treatments combined (see Table 2)

(33%), respectively. The number of females not reproducing in the cool treatment at high, medium, and low ration was 6 of 20 (30%), 4 of 10 (40%), and 6 of 13 (46%), respectively. The lack of reproduction in some females was potentially due to unknown laboratory effects, but it is possible that the larger females may have been exhibiting skip spawning behavior (i.e. having spawned in the past, but foregoing reproduction in the current year), and smaller females may have been either fully immature or exhibiting delayed maturation (i.e. delaying or aborting a first-time spawning event). Non-reproductively active females were in poorer body condition at the start of the season compared to females that reproduced (linear model, $F_{1,79} = 7.281$, $p = 0.009$; Fig. 4).

3.3. Interval between broods

The brood interval was significantly shorter in the warmer water treatment (Table 2, Fig. 5), with a mean of 55 d compared to 74 d in the cooler treatment. We did not find evidence that food ration or maternal length affected how quickly broods were released (Table 2). The interval between broods decreased over time for multiple brooders (Table 2), but this quicker release could also be explained by warmer water temperatures later in the reproductive

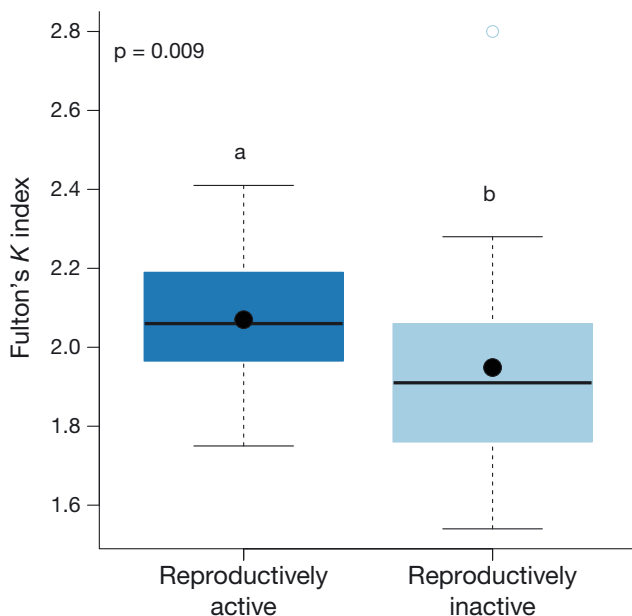


Fig. 4. Female rosy rockfish that released broods (reproductively active) were in better body condition at the start of the reproductive season in comparison with females that did not reproduce (reproductively inactive). Boxplot parameters as in Fig. 2; lowercase letters indicate statistically significant difference ($p \leq 0.05$) between groups

season. There was no evidence of an effect of experimental year on brood interval.

Only intervals between fertilized broods were considered in the interval analysis, but a secondary comparison of the interval between fertilized broods and the interval between non-fertilized broods showed that fertilization did not significantly affect the amount of time between brood releases (linear model, $F_{1,80} = 0.588$, $p = 0.446$). This meant that females not fertilizing broods did not release broods more quickly than females fertilizing broods. Females releasing unfertilized broods appeared to internally retain hydrated eggs for a similar amount of time as gestation of embryos. Hydrated eggs were roughly twice the size of developing, vitellogenic oocytes, and females were noticeably more rotund following maturation and hydration of eggs. Both females that fertilized and did not fertilize broods appeared visually 'pregnant' with swollen abdomens in the weeks prior to a parturition.

3.4. BF

Of the 133 broods released in the laboratory, 68 (roughly half) were fertilized. Of the 68 fertilized broods, we analyzed fecundity of 46 broods from 21 females that had fertilized all broods over the entire

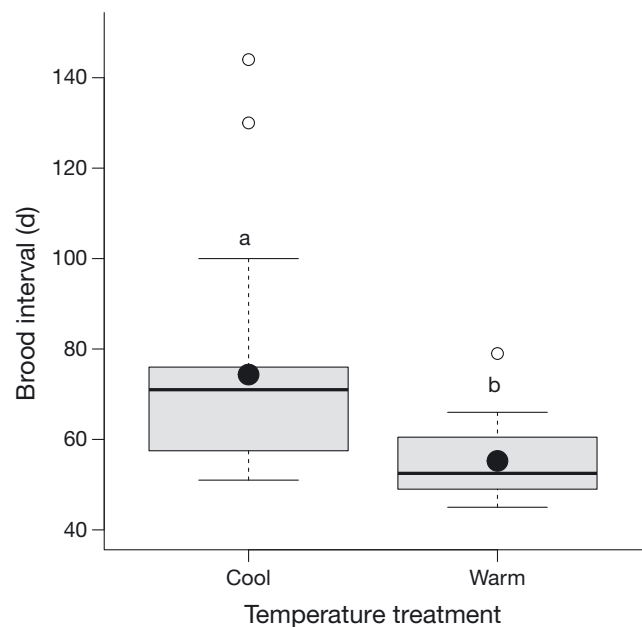


Fig. 5. Female rosy rockfish in the warmer water treatment released broods more quickly compared with females in the cooler water treatment. Boxplot parameters as in Fig. 2. Different lowercase letters indicate statistically significant difference ($p \leq 0.05$) between groups (Table 2)

year, allowing a calculation of AF (high ration, $n = 5$ females; medium ration, $n = 7$ females; low ration, $n = 9$ females). BF varied widely, from 3990–75 340 larvae, depending on brood number, female size, and food rations (Table 2, Fig. 6a,b). For multiple brooders, fecundity tended to decline in late-season broods (Table 2, Fig. 6a). In a few cases, second broods were more fecund than the first, potentially a function of females adjusting to laboratory conditions and feeding regimes. Food ration was marginally significant ($p = 0.052$; Table 2) and retained in the final model, with a trend of females in the low ration treatment producing smaller broods than females in high ration treatment (Tukey, $p = 0.063$; Fig. 6b). Larger females produced disproportionately larger sized broods than small females, with a length–fecundity exponent of

$b = 4.225$, greater than the length–weight exponent of $b = 3.340$ (Table 3). There was no supporting evidence of an effect of temperature or experimental year on BF.

BF in the laboratory was marginally lower than BF of wild fish with respect to maternal size (multiple regression, $F_{2,60} = 12.23$, $p < 0.001$; lab–wild Tukey, $p = 0.048$) when combining all laboratory broods. BF did not statistically differ if the analysis was restricted to first broods released in the laboratory compared to wild broods (multiple regression, $F_{2,35} = 13.52$, $p < 0.001$; lab–wild Tukey, $p = 0.117$; Fig. 6c). Restricting the analysis to first laboratory broods in comparison to wild broods removed the influence of lower fecundity in later broods. Brood number in wild fish was unknown.

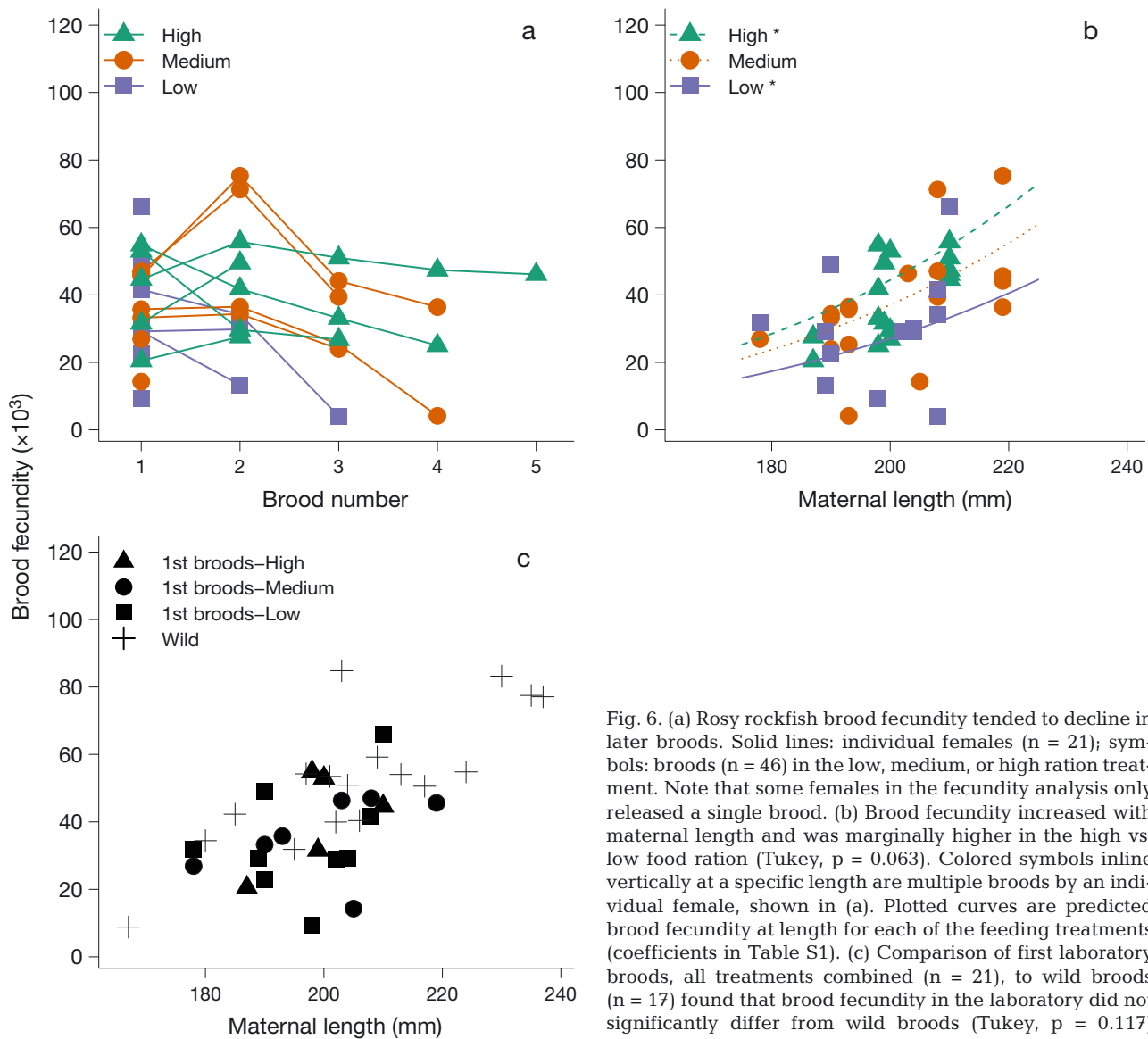


Fig. 6. (a) Rosy rockfish brood fecundity tended to decline in later broods. Solid lines: individual females ($n = 21$); symbols: broods ($n = 46$) in the low, medium, or high ration treatment. Note that some females in the fecundity analysis only released a single brood. (b) Brood fecundity increased with maternal length and was marginally higher in the high vs. low food ration (Tukey, $p = 0.063$). Colored symbols inline vertically at a specific length are multiple broods by an individual female, shown in (a). Plotted curves are predicted brood fecundity at length for each of the feeding treatments (coefficients in Table S1). (c) Comparison of first laboratory broods, all treatments combined ($n = 21$), to wild broods ($n = 17$) found that brood fecundity in the laboratory did not significantly differ from wild broods (Tukey, $p = 0.117$)

Table 3. Comparison of the allometric length–weight relationship of rosy rockfish with the hyper-allometric length–fecundity relationship for brood and annual fecundity (this study) in comparison with length–fecundity parameters reported in Dick et al. (2017). The strength of the maternal size effect on fecundity is indicated by the exponent (b) The intercept is $\log(a)$ (see Eqs. 6 & 7). Note different statistical approaches between studies, where the present study reported mean parameter estimates with 95% confidence intervals from linear mixed effects models and Dick et al. (2017) used a Bayesian hierarchical approach and reported parameter estimates as median values with 95% credible intervals of the posterior distribution

Study	Comparison	Log(a) (95% CI)	(b) (95% CI)
Captive rosy rockfish (present study)	Length–total weight	–12.610 (–12.41, –9.81)	3.340 (2.81, 3.87)
	Length–brood fecundity	–12.179 (–28.97, 1.51)	4.225 (1.63, 7.40)
	Length–annual fecundity	–21.698 (–52.39, 9.19)	6.105 (0.26, 11.92)
Bayesian hierarchical meta-analysis of <i>Sebastes</i> (Dick et al. 2017)	Rosy rockfish Length–fecundity ^a	–11.713 (–13.65, –9.74)	4.195 (3.83, 4.56)
	All <i>Sebastes</i> Length–fecundity ^a	–11.938 (–16.25, –8.30)	4.043 (3.43, 4.71)

^aDoes not account for multiple broods

3.5. AF

AF ranged from 9400–244 877 larvae and was significantly affected by maternal length and food ration (Table 2, Fig. 7). Larger females produced disproportionately more offspring annually compared with smaller females, with a length–AF exponent of $b =$

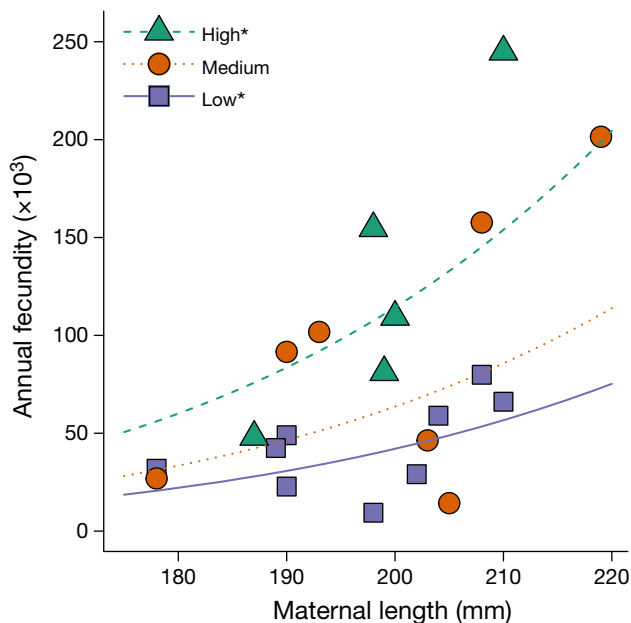


Fig. 7. Larger female rosy rockfish produced disproportionately more offspring than smaller females as a function of a greater number of annual broods and higher brood fecundity (Tables 2 & 3). Females fed high rations produced significantly more offspring annually than females fed low rations ($p < 0.05$, denoted by *) (Table 2, Tukey, $p = 0.044$). Colored symbols are annual fecundity estimates for individual females fertilizing all broods ($n = 21$) and are grouped by low, medium, or high ration treatment. Plotted curves are predicted annual fecundity for each feeding treatment (coefficients in Table S1)

6.105 (Table 3). Females fed low rations produced significantly fewer larvae annually compared with females fed high rations, with an average of 63% fewer larvae in poorly fed females (Tukey, $p = 0.044$). AF in the medium ration treatment was highly variable and not significantly different from the low (Tukey, $p = 0.393$), or high (Tukey, $p = 0.365$) ration groups. Even though broods were released more quickly at warmer temperatures, a greater number of broods were not released, and there was no supporting evidence of a temperature or experimental year effect on AF (Table 2). The higher AF in well-fed females was primarily a function of a greater number of broods, but larger individual broods also contributed to higher fecundity.

3.6. Dissections

Dissections at the end of the study following the reproductive season in Year 2 were possible for 39 of the 46 unique females, with the exception of 3 fish that died after 2016 and 4 that were removed from the study shortly before dissections in 2017. Of the 39 dissected at the end of the study, 33 were in a resting or transitional state or in the early to late stages of vitellogenesis for the next reproductive season (Table 4). Six females were classified as immature; however, macroscopic identification distinguishing immature and the mature resting stage in rockfish outside of the reproductive season is difficult (Lefebvre & Field 2015). Females classified as immature were the smallest in the study in comparison to resting and vitellogenic females (ANOVA, $F_{2,36} = 14.59$, $p < 0.001$; Table 4). Females initiating vitellogenesis of oocytes for the next season had larger proportional gonads, measured by the gonadosomatic index (ANOVA,

Table 4. Summary of rosy rockfish dissections conducted on 4 October 2017 at the termination of the experiment following the end of the second-year reproductive season. All fish were weighed and measured and ovarian stage identified macroscopically (see Section 2.7). The gonadosomatic index (GSI), Fulton's condition index (K), and hepatosomatic index (HSI) were calculated with somatic weight. Sample sizes and means (\pm SD) are summarized by stage. Asterisk (*) indicates statistical difference ($p \leq 0.05$) across groups (ANOVA); lowercase letters show specific groupings (post hoc Tukey). Note fertilized embryos and spent stages were not observed at the time of dissection during the presumed resting and mating season

Ovarian stage	n	Length*	GSI*	K^*	HSI
Immature	6	178 ^a (± 3)	0.23 ^a (± 0.05)	1.74 ^a (± 0.09)	2.37 (± 0.75)
Resting or transitional	20	201 ^b (± 14)	0.44 ^a (± 0.16)	1.90 ^{ab} (± 0.16)	2.33 (± 0.84)
Vitellogenic	13	215 ^c (± 16)	2.54 ^b (± 2.21)	1.99 ^b (± 0.12)	2.11 (± 0.77)

$F_{2,36} = 12.3$, $p < 0.001$; Table 4) and were in better body condition than immature females, measured by Fulton's K (ANOVA, $F_{2,36} = 6.797$, $p = 0.003$; Table 4). There was no difference in the hepatosomatic index across females in different stages of development (ANOVA, $F_{2,36} = 0.348$, $p = 0.709$; Table 4).

The classification of immature and mature females provided additional information for the interpretation of females that did not reproduce in the study. Six of the non-reproductively active females were among the smallest fish in the study and were classified as immature, suggesting a possible adolescent phase with delayed or abortive maturation since most exceeded the reported 100% length at maturity of 175 mm FL for females in central California (Fields 2016, our Fig. 8). The other 7 non-reproductively active females that were larger in size and classified as mature were possible examples of skipped spawning (Fig. 8). The dissections did not find evidence of internal trauma, such as scar tissue due to the collection process, or other abnormalities that would have prevented reproduction, although it should be noted that the dissections were conducted 1–2 yr post capture, and any injuries could have healed entirely if they had occurred.

3.7. Model validation

A visual and statistical evaluation of model assumptions found departures from normality for all 4 mixed effects models describing brood count, brood interval, BF, and AF (Shapiro-Wilk test of normality, $p = 0.001$, $p = 0.024$, $p < 0.001$, and $p = 0.010$, respectively). Visual assessment of the Q - Q plots suggested only minor departures from normality at the tails, and

linear mixed effects models are robust to violations of normality, which is why they are often useful for ecological data sets (Sokal & Rohlf 1995, Zar 1999, Zuur et al. 2009, Schielzeth et al. 2020).

We used the Levene's test for homoscedasticity and found evidence of heterogeneity in the variance for the models of brood count ($p = 0.028$) and AF ($p = 0.013$) but not in the models of brood interval ($p = 0.286$) and BF ($p = 0.356$). While small deviations from homogeneity are not likely to affect model significance, large amounts of heterogeneity will (Zuur et al. 2009, Schielzeth et al. 2020). There were no clear patterns in the residuals for the

model of AF, suggesting that heterogeneity was minor and not likely to affect interpretation of the results (Zuur et al. 2009, Schielzeth et al. 2020).

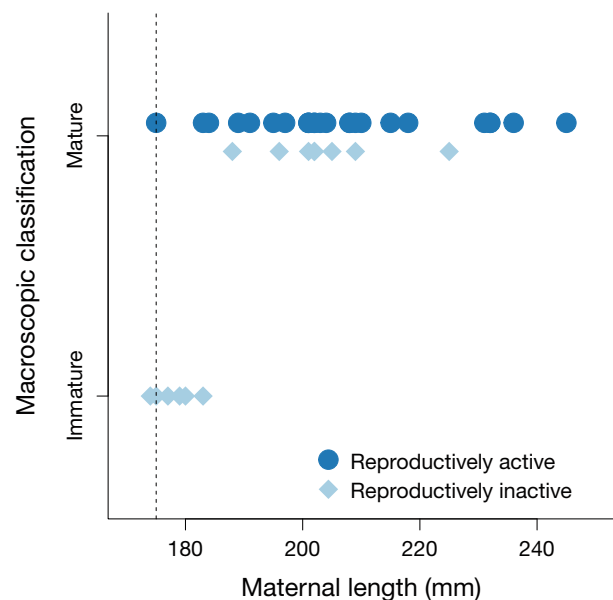


Fig. 8. Maturity status of female rosy rockfish that released broods in the laboratory (reproductively active) and those that did not release broods (reproductively inactive) by size. Maturity status (immature vs. mature) was determined by macroscopic examination of ovaries at the end of the study and outside of the reproductive season. Reproductively inactive females at the smallest sizes were possibly physiologically immature, or more likely in an adolescent phase exhibiting delayed or abortive maturation of a first reproductive attempt. Larger mature females may have skipped spawning. Neither skipped spawning nor delayed or abortive maturation could be distinguished from possible laboratory effects that may have contributed to the failure to reproduce. Length at 100% maturity was most recently reported at 175 mm fork length for females in central California (Fields 2016, vertical dashed line); most females were above that size

Patterns in the residuals of the brood count model were evident. Further data transformation of the explanatory variable, length, was attempted but did not resolve the pattern (Zuur et al. 2009). Large departures from homogeneity in the variance can result in a higher chance of a Type I error (Zuur et al. 2009). However, we feel the results and interpretation of the brood count model are robust since length, the only significant explanatory variable, was well supported ($p = 0.001$, Table 2), and all other possible explanatory variables were highly non-significant (Table 2). It is possible, however, that a non-linear relationship between maternal length and brood count, such as an S-shaped, asymptotic curve, would better describe the relationship between maternal size and the number of broods.

4. DISCUSSION

4.1. Annual brood production

To our knowledge, this was the first study to track individual reproductive effort in a multiple-brooding rockfish over an entire reproductive season in the laboratory, providing valuable basic biological information on the frequency of how often broods are released and the total number of annual broods. Documenting reproductive effort over an entire year enabled estimates of AF for this species. Rosy rockfish females released between 0 and 5 broods yr^{-1} , depending on maternal size. For comparison, up to 3 broods yr^{-1} have been reported in other multiple-brooding rockfishes, such as chilipepper *Sebastes goodei* and bocaccio *S. paucispinis* (Ralston & MacFarlane 2010, Lefebvre et al. 2018), but it was unknown if rockfish were capable of greater than 3. The maximum of 5 broods in rosy rockfish observed in the laboratory may represent an upper physiological limit in a best-case scenario of nearly unlimited food supply, and we are unsure if this would occur in the wild. Previous studies documenting multiple broods from wild collections were limited to a snapshot in time of reproductive effort. The timing of collections during the reproductive season is important for observing multiple broods in wild collections. Because of the lengthy brood interval, it is generally not possible to tell how many broods a female will produce at the beginning of the reproductive season. At the end of the reproductive season, it is generally not possible to tell how many broods a female had released because previous broods in the ovary, evident as residual eyed larvae or post-ovulatory folli-

cles, degrade and are resorbed over time. Therefore, outside of the laboratory, multiple-brooding females can be identified over only a relatively short period annually and may not reflect the entire reproductive effort over the year.

Rosy rockfish have one of the longest parturition seasons within the genus, with nearly year-round reproduction in the central and southern regions of the California Current (Love et al. 1990, 2002). The protracted reproductive season may allow this rockfish species to produce a greater number of annual broods in comparison to other multiple-brooding rockfish that have shorter periods of larval release. In many rockfish species, larvae are released over a shorter 2 or 3 mo period annually, especially at more northern latitudes (Wyllie-Echeverria 1987, Love et al. 1990, 2002). The total number of annual broods possible is likely species-specific.

4.2. Temperature effects on reproductive timing

Warmer water temperatures shortened the time interval between parturition events. We did not measure embryo development directly, but a shorter brood interval suggests that eggs and larvae develop more quickly at warmer water temperatures. Warmer temperatures likely increase metabolic rates in adults, embryos, and offspring. For example, warmer water temperature was shown to increase the rate of yolk depletion of embryos in the closely related rosethorn rockfish *S. helvomaculatus* (formerly *S. rhodochloris*) (Moser 1967). Warmer temperature is also associated with an increased rate of oogenesis and degradation of postovulatory follicles (Kjesbu et al. 2010, Kurita et al. 2011). Therefore, warmer water temperature is likely to affect the timing of reproductive events at multiple stages, including oogenesis, gestation, spawning recovery, and the timing of when larvae are released in the wild.

Broods were released more quickly at warmer water temperatures but females in the warmer water did not release an overall greater number of annual broods compared with females in the cooler water treatment, although there was a non-significant trend in that direction. The quicker release of broods at warm temperatures is likely balanced by increased metabolic demands, which affect female body condition and reproduction. More work is required to understand how spatial patterns and increasing global ocean temperatures will affect the bioenergetics and reproduction in this and other economically and ecologically important marine fishes worldwide.

For example, Somarakis et al. (2019) found similar temperature effects driving reproductive plasticity in small pelagic clupeoids. In those species, warmer water temperatures increased the rate of egg development and the frequency of spawning events in adults but came at a cost of higher metabolism and increased mortality rates of larvae (Somarakis et al. 2019). Qualitatively, adult female rosy rockfish in the warmer treatments were more physically active (i.e. observed swimming more often rather than sedentary) and were more aggressive feeders (i.e. fed more quickly) compared with females in the cool treatment. These qualitative observations suggested higher metabolic rates and increased energetic demands, but specific rates were not measured and should be the focus of future work.

4.3. Maternal size effects on fecundity

Both BF and AF increased in a hyper-allometric relationship with maternal length in rosy rockfish, meaning that spawning biomass will not reliably predict larval output in this species. Larger females produced disproportionately more young per gram of female body mass compared with smaller females. The disproportionate increase is shown by comparison of the allometric length–body weight relationship, where weight increases with length approximately volumetrically to a power of $b = 3$, but fecundity increases at a power greater than $b = 3$ (see Eq. 6, Table 3). The production of multiple broods increased the strength of the maternal size effect on fecundity from a power of $b = 4.335$ when only considering BF, to a power of $b = 6.105$ for AF (Table 3). The more rapid increase in AF with maternal size reflected both larger sized broods and a greater number of annual broods by larger females than smaller females.

Our results suggest that the strength of the effect of maternal size on fecundity has been underestimated in multiple-brooding rockfishes. A Bayesian hierarchical meta-analysis of fecundity–length relationships within *Sebastes*, which considered only single broods due to a lack of available data on multiple brooding, found many of the multiple-brooding species to have smaller maternal size effects on fecundity compared to single-brooding species (Dick et al. 2017). Dick et al. (2017) reported rosy rockfish fecundity to increase with maternal length at a power of $b = 4.195$, similar to our BF estimate and a genus-wide estimate for *Sebastes* at a power of $b = 4.043$ (Table 3). When incorporating the biology of multiple broods, rosy rockfish fecundity increased with mater-

nal length at a power of $b = 6.105$, well above what has been reported for rockfishes. Multiple brooding is yet another example of the effect of maternal size on reproductive effort and reinforces the importance of age-structure and the disproportionate contribution of older, larger females to population larval production. Updating the size-dependent fecundity relationships for multiple-brooding rockfish species will increase the accuracy in calculations of larval output used to assess the health and status of the fishery.

4.4. Environmental effects on fecundity

The large variation in annual reproductive output relative to feeding regime, with 63% fewer larvae produced by poorly fed females in comparison with well-fed females, is an example of phenotypic flexibility in reproductive effort in response to prevailing environmental conditions. The results suggest that, at the population level, larval output is influenced by environmental conditions affecting female body condition. In years of favorable ocean conditions, females are able to rapidly ramp up reproductive effort. The increase in AF in well-fed females was mainly due to the production of additional broods, though BF also increased with higher food availability. In years of poor ocean conditions, females are likely to be in poorer body condition, produce fewer larvae, and may exhibit skip spawning behavior or delayed maturation, further reducing population larval output.

In addition to interannual variability, reproductive patterns differ geographically from north to south in the California Current. Across species, rockfishes are more likely to skip spawn at higher latitudes and produce multiple broods at lower latitudes (Moser 1967, MacGregor 1970, Love et al. 2002, Beyer et al. 2015, Head et al. 2016, Conrath 2017, Lefebvre et al. 2018, Holder & Field 2019). Historically, multiple brooding was considered a uniquely southern occurrence in females south of Point Conception in southern California (Moser 1967, MacGregor 1970, Wyllie-Echeverria 1987). However, recent studies have documented multiple brooding in central California, assumed to be related to interannual variability in ocean conditions favoring the production of multiple broods (Wyllie-Echeverria 1987, Beyer et al. 2015, Lefebvre et al. 2018). In waters off central California, spring and summer upwelling drive an annual pulse of high primary productivity, matched by a corresponding annual peak in female body condition prior to the fall mating and winter reproductive season (Gullemot et al. 1985, Wyllie-Echeverria 1987, Beyer et al. 2015).

In the south, temperatures are warmer and upwelling is weaker but more persistent throughout the year (Parrish et al. 1981, Checkley & Barth 2009). Latitudinal differences in the seasonal patterns of food availability and temperature likely influence the accumulation of energetic reserves and reproductive patterns. Similar to multiple brooding in rockfishes, multiple batch spawning in broadcast spawners is more common at lower latitudes (McBride et al. 2015), suggesting commonality in patterns of spawning frequency across a diverse group of marine fishes. Ultimately, reproductive frequency within a year likely reflects the interplay of physiological capabilities and evolutionary adaptation to the seasonality of larval survival probability.

4.5. Non-reproducing females

While many females in our study reproduced multiple times, some never released broods (29 of 81 observations). We attempted to collect only mature females based on previous studies reporting length at maturity (Fields 2016), but at the smallest sizes it is likely that females were either immature or in an adolescent phase. At the larger sizes it was possible that females were exhibiting skipped spawning behavior. However, we cannot exclude the possibility that the failure of some females to reproduce was due to unknown laboratory effects.

We were unable to differentiate among the different reasons why females in the study did not reproduce. Dissections at the end of the study, well beyond the end of the spawning season, were inconclusive. By then, any evidence of past internal trauma from the collecting process or evidence of recent spawning, including post-ovulatory follicles and residual eyed larvae, or mass atresia of aborted broods were not evident by macroscopic methods. However, skipped spawning and delayed maturation occur in other rockfish species, are important to population dynamics, and should be further investigated in this species (Lefebvre & Field 2015, Head et al. 2016, 2020, Conrath 2017). Detailed histological studies during the reproductive season are able to differentiate between immature, adolescent, and resting mature females. Further investigations involving monthly sampling of wild fish, with histological examination of the ovaries, are warranted to provide better information on the occurrence of delayed maturation and skipped spawning (Lefebvre & Field 2015), since these behaviors have not previously been reported for rosy rockfish in the wild.

Overall, non-reproducing females in our study were in poorer body condition entering the reproductive season than females that reproduced. Foregoing reproduction in iteroparous species due to low energy reserves is a life history adaptation to conserve, or divert, energy to growth, survival, and/or future reproduction (Stearns 1992). The act of reproducing is energetically demanding, especially in live-bearing species because of the increased respiratory demand to provide oxygen to embryos during gestation (Moser 1967, Wourms 1991). The ovaries of a female rockfish weigh up to one-third of the total body weight, and the gestation of fertilized embryos through the eyed-larvae stage and parturition lasts on the order of months depending on the species and water temperature (Love et al. 1990, 2002). With long-lived, iteroparous fish, such as many rockfishes (Mangel et al. 2007), the ability to divert energy to growth or over-winter survival during years of poor conditions and to invest resources into reproduction during favorable years is likely an adaptation to the highly variable environmental conditions of the California Current and low natural mortality rates of adults (Gullemot et al. 1985, Botero et al. 2015).

Nonetheless, it was previously unknown if females were capable of reproducing multiple times in the laboratory, and the number of females successfully reproducing following capture from depth and repressurization in a hyperbaric chamber is remarkable. The reproductive success of captive females suggests that capture and careful, rapid release at depth of regulated species in the fishery, such as with commercially available descending devices (Belquist et al. 2019), may allow for full recovery and normal reproduction.

4.6. Sperm limitation

Sperm limitation was differentiated from skipped spawning or delayed maturation by females that released broods of eggs at a mature, hydrated, unfertilized stage, as opposed to females that never released broods. Tsang et al. (2007) similarly reported sperm limitation in captive grass rockfish *S. rastreliger*, isolated from males, which released broods of mature, hydrated eggs with a degraded appearance. The presence of hydrated, unfertilized eggs is not often observed in rockfish collected from the wild (S. G. Beyer et al. unpubl. data). Oocyte maturation, hydration, ovulation, and fertilization are thought to occur rapidly and near simultaneously when sperm are present in the ovary (Moser 1967, Shaw et al.

2012). Release of unfertilized, hydrated eggs in the wild is likely rare or absent, as females in the wild are able to mate freely and acquire additional sperm for secondary broods throughout the reproductive season. The females in the present study did not appear able to resorb eggs at the hydrated stage.

Sperm storage is not well understood in rockfishes. In general, sperm is thought to be stored within the interstitial tissues of the ovary but no specialized internal storage structures are evident (Shaw et al. 2012). In *S. schlegeli*, a cultivated rockfish of Japan, sperm are released from the male in batches of spermatophores (Feng et al. 2014). Sperm are observed floating within the ovarian fluid shortly after copulation, with eventual migration and long-term storage within the epithelium of the ovigerous tissue (Mori et al. 2003). Mechanisms of sperm storage may differ across the genus and potentially between single- and multiple-brooding rockfish species.

The acquisition of sperm through mating generally occurs in the months prior to vitellogenesis or during early vitellogenesis (Love et al. 1990). In rockfishes, male gonadosomatic indices peak in the months prior to a peak in female gonadosomatic indices, suggesting mating occurs well before fertilization (Gullemot et al. 1985, Wyllie-Echeverria 1987, Love et al. 1990). Before our study, it was unknown if females were able to reserve sperm and maintain viability while fertilizing multiple broods, or if re-mating was required between broods. Females in our study were isolated from males following the mating season and were able to reserve sperm for multiple broods without re-mating, suggesting a mechanism for maintaining viability and storage of sperm across broods and over many months. At least one female stored sperm and successfully fertilized 5 broods over the course of 10 mo in isolation from males. Alternatively, evidence of sperm limitation suggests that females may need to mate multiple times in the wild to acquire enough sperm to fully fertilize multiple broods, or mate over a longer period during the reproductive season.

It was unknown if females mated with the captive males in the laboratory or if females had previously acquired sperm in the wild, as is common in captive rockfishes (Tsang et al. 2007, Sogard et al. 2008b). If females did not mate in the laboratory, then sperm storage was longer than the reported 10 mo. However, males in the laboratory were observed actively courting females in the group mating tanks, similar to courtship behavior described for wild *S. inermis*, a small, benthic rockfish of Japan (Shinomiya & Ezaki 1991). In all 4 treatments, a single male would dis-

play dominant, aggressive behavior toward other males, including biting and chasing. Aggressive behavior of territorial males during the mating season was also described in wild *S. inermis* (Shinomiya & Ezaki 1991). The aggressive behavior in the laboratory resulted in the death of some males, often over a single night, where fins and scales were entirely missing the following morning, reducing the number of males in each treatment. Dominant males courted females by circling and fin display similar to *S. inermis* (Shinomiya & Ezaki 1991), and also displayed a change in head coloration from pale to bright red banding across the mandibles and operculum (S. G. Beyer pers. obs.). While courtship behavior was observed in the laboratory, the act of copulation, which likely occurs at dusk or night (Helvey 1982, Shinomiya & Ezaki 1991), was not observed during routine daytime observations. A better physiological understanding of mating behavior, mate choice, sperm storage, and evidence for post-copulatory, cryptic female choice in sperm selection (Eberhard 1996) would help to explain potential sperm limitation and mating requirements of multiple-brooding females in the wild and provide a broader context for sexual selection in this diverse and species-rich group of fishes.

4.7. Implications for fisheries management

Understanding how size-dependent fecundity relationships change due to reproductive plasticity in response to the environment will improve estimates of population larval production, help to scale estimates of spawning stock biomass from ichthyoplankton surveys (Ralston & MacFarlane 2010), and inform predictions of how climate change will affect reproductive success and fishery productivity (Hare et al. 2016). The increased plasticity of the multiple-brooding strategy likely allows females to take advantage of intermittent, favorable conditions, unlike closely related single-brooding species. Conversely, reduced fecundity and occurrences of delayed maturation or skipped spawning reduce population-level larval production in years of poor conditions. The reduced numbers of females contributing to larval production may be particularly important for management of exploited species during prolonged, multi-year periods of poor environmental conditions as can occur in the California Current. Over such years, larval production will be less than expected based on maturity ogives and length–fecundity relationships derived from fish collected in moderate to good years.

Understanding how the environment affects larval production may help to improve stock–recruitment relationships of marine fishes. Weak correlations in stock–recruitment relationships result in reduced predictive power of future recruitment events important for decision-making in fisheries management (Myers et al. 1995). Incorporating age- and/or size-structure of females, coupled with age- and/or size-specific fecundity, has resulted in a more accurate calculation of total egg production of mature females (Marteinsdottir & Thorarinnsson 1998). Updating size–fecundity relationships in population models for stock assessment has shown how exploitation and the removal of large females disproportionately reduces larval output and is important for determining stock status (He et al. 2015). However, reproductive plasticity in response to environmental conditions is often not modeled due to a lack of data and mechanistic understanding. Efforts to estimate female energetics, such as through measurement of proportional liver weights, were successful in better estimating stock–recruitment relationships in Atlantic cod *Gadus morhua*, and represent a relatively straightforward method of measuring energy reserves that fluctuate with the environment (Marshall et al. 1999). Variability in the reproductive potential of small pelagic clupeoids, related to energetics, was correlated with recruitment patterns in some cases, showing a direct connection between interannual variability in body condition, fecundity, and recruitment (Somarakis et al. 2019). Clearly, bioenergetics affected reproductive plasticity in rosy rockfish and likely affects reproductive output in closely related rockfishes and other marine fishes with similar reproductive strategies.

It is still unknown exactly how wild populations will respond to environmental change, but the prolonged parturition season and frequent occurrence of multiple brooding in the southern region of the California Current suggests that females are encountering an adequate food supply throughout the reproductive season to maintain high enough energy reserves for multiple broods, and that warmer temperatures enable broods to be released more quickly. Fewer annual broods farther north may reflect greater seasonality in food availability and colder temperatures increasing the time interval between brood releases. In addition, the increased plasticity of multiple broods allows for the release of larvae over a longer period annually and is possibly an adaptation within the genus to geographic differences in larval survival from north to south in the California Current. Temporally, it is likely that the number of annual broods, and thus AF, will be lower in years of

reduced food resources, such as during El Niño, or other climate-warming events, which should be considered for management and the focus of future investigation. These patterns are not unique to rockfishes and may also explain spatiotemporal variability in reproductive effort in other species that spawn over a wide latitudinal range and encounter interannual variability in environmental conditions, such as the northeast Arctic stock of Atlantic cod (Opdal & Jørgensen 2015).

Increased efforts to understand the causes and consequences of reproductive plasticity and the important role of maternal size in determining fecundity and total egg production of populations have the potential to improve predictions of strong and weak recruitment events. The capacity for such large variability in fecundity, including the possibility of skipped spawning of mature females and delayed maturation in adolescents, may contribute to the large recruitment variability observed in most rockfishes, where strong year classes supporting fisheries may be followed by up to a decade of poor recruitment, adversely affecting fisheries. Previous work has focused on the important role of the environment encountered by post-extrusion larvae and juveniles in structuring strong and weak year classes (Cushing 1975), but high interannual and spatial variability in the environment experienced by females preparing for and during reproduction is also likely an important, but often overlooked, contributing factor. Even though females in better body condition produced more larvae, it remains unclear if females base current reproductive effort solely on stored energetic reserves, or if they are able to use an environmental cue, which may be related to energetic reserves, to either increase reproductive effort in years when larvae may have a higher chance of survival or to divert energy to other life history traits, such as survival and growth, during poor years.

5. CONCLUSIONS

We demonstrated that annual reproductive output across individuals is not static and varies both with maternal size and environmental conditions. Female body condition and energetic reserves were important determinants of AF. Increased reproductive plasticity, through the production of multiple broods, likely provides a fitness advantage in long-lived, iteroparous species residing in highly dynamic environments, such as the rockfishes in the California Current Ecosystem. The phenotypic flexibility of the

multiple-brooding strategy allows females to rapidly take advantage of productive ocean conditions in a way that single-brooding species are unable and likely contributes to large fluctuations in population-level larval output depending on prevailing oceanographic conditions. Understanding the reproductive ecology and how fisheries productivity will respond to changing climate conditions should be a high priority for the sustainable management of marine fish stocks.

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