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Abstract

Multiple paternity, also termed polyandry, is a reproductive strategy that can increase the genetic variation within a brood to help preserve diversity within a population. A paternity analysis using multilocus microsatellites revealed that 8 of 25 (32%) quillback rockfish (*Sebastes maliger*) females mated with more than one male. Of the eight broods sired by multiple males, three were sired by two males and five were sired by three males. Polyandry is likely an important consideration when managing rockfish stocks for genetic diversity and productivity. The probability of polyandry increased with increasing weight and condition factor of the female, but not with increasing age or length. These results suggest polyandry is a common mating strategy in quillback rockfish in Alaska and may be related to female size.

Contents

Introduction

Rockfish, *Sebastes* spp., are a diverse genera of over 60 species distributed along the northeast Pacific Rim. They are viviparous with internal fertilization and larval development (Love et. al. 2002), and are able to hold sperm after mating for later fertilization (Gunderson 1971, Sogard et al. 2008). Recently, molecular techniques have identified multiple maternity within a brood, also termed polyandry, in rockfishes (Ng et al. 2003, Hyde, et al. 2008, Sogard et al. 2008, Van Doornik et al. 2008). Polyandry is a reproductive strategy that can increase the genetic variation within a brood and help preserve gene diversity within a population.

Some rockfish species, but not all, have shown to be polyandrous (e.g., Hyde et al. 2008, 10 of 17 species). Although quillback rockfish have not been examined, polyandry has been found in rockfish species closely related to quillback rockfish, including captive grass rockfish (*S. rastrelliger*) and wild kelp rockfish (*S. atrovirens*), while polyandry was not detected in the closely related wild brown rockfish (*S. auriculatus*). These findings suggest that polyandry may vary between closely related rockfish species.

Maternal age and size is related to the rate of multiple maternity in Pacific ocean perch (*S. alutus*); monogamous females were significantly smaller and younger than polyandrous females. Besides polyandry, larval growth and survival has also been shown to be related to a higher maternal age (Berkeley et al. 2004, Sogard et al. 2008). These types of correlations are important to understand in rockfish because, if they exist, population viability and productivity may be influenced by the number of older, larger females in the population. This may have effects on the modeling of fish populations as most population models assume equal reproductive success for all sizes and ages of mature fish (Spencer et al. 2007).

Quillback rockfish are exploited by commercial and recreational fishing and, in certain areas, they are subject to high exploitation rates (DFO 2012). (In fact, the Washington Department of Fish and Wildlife listed quillback rockfish as depleted in south Puget Sound and vulnerable in north Puget Sound (Palsson et al. 2009), the Department of Fisheries and Oceans, Canada, initiated a rockfish conservation strategy that included increased monitoring and research of quillback rockfish, and a review of quillback rockfish along the Pacific coast of British Columbia (Yamanaka et al. 2006) prompted the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) to list British Columbia quillback rockfish as a threatened in 2009 (COSEWIC 2009, DFO 2012).) Because they are caught by hook and line, which selects for larger fish, high exploitation can result in age or size truncation and potentially a decrease in productivity (Gunderson 1977, Love et al. 1998, Berkeley and Markle 1999). If polyandry is linked to maternal size and age, like in Pacific ocean perch (Van Doornik et al. 2008), over-exploitation of larger, older females could result in a loss of genetic diversity. Additionally, quillback are long-lived (up to 90 years, Munk 2001) and late to mature (between the ages of 5 and 22 years, Love et al. 2002), which makes them slower to rebound from population declines than fish that have faster growth rates and earlier onset of reproduction.

Our objectives were to 1) use microsatellite markers to determine if quillback rockfish are polyandrous, 2) determine the number of males that contributed to each brood, and 3) to correlate female size and age with the practice of polyandry.

Materials and Methods

Sampling

In April 2007, gravid female quillback rockfish were collected by hook-and-line in nearshore waters adjacent to Alaska Fisheries Science Center's Little Port Walter marine research station located on southeastern Baranof Island in Southeast Alaska (Fig. 1). Length (mm) and weight (g) were recorded and age was later determined by break and burn otolith analyses (Chilton et al. 1982). Ovaries containing embryos and larvae from each female were collected. To ensure detection of sires that produce low proportions of offspring, ovaries were opened and all larvae were thoroughly mixed before a subsample of each brood was fixed in 95% EtOH. A fin clip was also taken from each female and stored in 95% EtOH. To test the selected microsatellite loci for parentage analysis, we initially analyzed two females and 48 individual offspring from each of their broods. After the initial test was completed, we analyzed an additional 23 females and 24 individual offspring from each of their broods.

Genotyping

DNA was isolated from female fin tissue and whole larvae using a CAS-1820 X-tractor Gene Instrument (Corbett[1]), following the manufactures instructions. Microsatellite genotyping was performed for 14 genetic markers (Table 1). Samples were analyzed by transferring 1 uL of a 1:4 dilution of purified DNA (final DNA concentration estimated to be 10-25 ng/uL) to wells of a 96 well plate. All liquid handling steps were performed using a Perkin-Elmer Janus AJL8M01 Robot[1]. Each multiplexed Polymerase Chain Reaction (PCR) was conducted in a 10 uL volume containing the template DNA, QIAGEN Multiplex PCR Mastermix[1], 0.2 uM of each forward fluorescently labeled primer and unlabeled reverse primer, and RNase free water. Primer designs for the 14 loci have been described in the following publications: *Sma10* (Wimberger et al. 1999); *Spi4*, *Spi6*, *Spi10*, *Spi12* ; *Sra.5-9* (Gomez-Uchida et al., 2003); *Sra.7-2*, *Sra.7-7*, *Sra.7-25*, *Sra15-8*, *Sra.16-5* (Westerman et al., 2005); *Sth3B*, *Sth37*, *Sth56* (Sekino et al. 2000). Thermal cycling was performed on a dual 96-well GeneAmp PCR System 9700 (Applied Biosystems, Inc.[1]) using the following protocol: an initial denaturation of 15 min. at 95ºC and then 33 cycles of 94ºC for 30 sec. and 60ºC for 1.5 min., and polymerization of 1 min. at 72ºC. After cycling, a final polymerization step for 30 min. at 60ºC was employed and the reaction stored at 15ºC until removal from the thermocycler.

For analysis on a ABI 3130xl Genetic Analyzer (Applied Biosystems, Inc.), samples were diluted in 10 uL total buffer as follows: 1 uL undiluted PCR reaction, 4.4 ul Hi-Di formamide, 4.4 uL ddH2O, 0.2 uL LIZ 600 size standard (Applied Biosystems,

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[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Inc.). Samples were denatured for 3 min. at 95ºC, then cooled to 4ºC and stored until loaded on the ABI 3130xl. Samples were analyzed on a 16-capillary 36 cm array and scored using GeneMapper software (Applied Biosystems, Inc.).

Data Analysis

Initial analysis was performed by confirming that each larva contained 1 allele at each locus from its mother. The program GenAlEx 6 (Peak all and Smouse 2006) was used to calculate the number and frequency of alleles at each locus, the probability of exclusion given one known parent (P2), and the probability of identity (PI) which estimates the average probability that two unrelated individuals will by chance have the same multilocus genotype (Table 1). For this experiment, known maternal and progeny genotypes for a given brood were used to reconstruct all possible paternal genotypes for each brood using the computer program GERUD 2.0 (Jones 2005). GERUD 2.0 uses an exhaustive search algorithm to reconstruct all parental genotypes from half-sib progeny arrays with known or unknown parents. When multiple minimum father solutions were found for the proportion of offspring produced by each sire, GERUD 2.0 was used to calculate their relative probabilities (based on Mendelian segregation and genotypic frequencies in the population) and rank them by likelihood. The most likely solution was used to estimate the sire contribution for each brood. Because GERUD 2.0 is computer intensive, 7 of the 14 most informative loci based on P2 scores were used for paternity analysis. Also, GERUD 2.0 does not take into account mutational events and null alleles, each of which can manifest itself in the results as an overestimate of the number of sires contributing to a brood; therefore, each parent pair and perspective offspring were considered carefully by hand for these anomalies.

Maternal weight, length, condition (weight relative to length), and age were examined for their relationship with the presence of polyandry. Condition was calculated as,

$$\frac{weight}{length^3} \mathrm{x}100{,}000 \quad . \qquad (1)$$

A logistic model, where the binary response for each mother was either multiple paternity detected or not detected, was evaluated with four separate models, one for each maternal factor.

Results

For all 14 loci for 25 females observed heterozygosity ranged from 0.33 to 0.91, observed P2 scores ranged from 0.05 to 0.64, with a total exclusion probability of 0.999. Observed PI scores ranged from 0.02 to 0.48, with a total PI of 2.31×10^{-13} across all 14 loci. The observed heterozygosity for 7 loci chosen for parentage analysis ranged from 0.67 = to 0.87, P2 scores ranged from 0.37 to 0.64, with a total exclusion probability of 0.99, and observed PI scores ranged from 0.02 to 0.09, with a total PI of 1.23×10^{-09} (Table 1).

Polyandry was discovered in 8 of 25 broods, with up to three sires per female (Table 2). Of the 8 broods, 3 were sired by two males and 5 were sired by three males

(Table 2). Within broods with multiple paternity, the proportion of offspring produced by each sire ranged from skewed (52:32:16) to highly skewed (92:8) (Fig. 2).

Parentage analysis also revealed a single developed larva, from a monogamous brood, which was homozygous across all 14 loci for paternal alleles only. This indicates the larva developed from an egg which lacked a nucleus or maternal chromosomes.

The logistic regression of weight and the presence or absence of multiple paternity was significant ($P = 0.025$) (Table 3). This indicates that with increasing maternal weight there is an increase in the probability of polyandry. The range in weights was greater for polyandrous females (single sire range = 0.70 to 1.50 kg; polyandrous range = 0.58 to 1.66 kg). The logistic regression with condition factor also had a low *P*-value (0.057) (Table 3). The range in condition factors was also greater for polyandrous females (single sire range = 1.68 to 2.73; polyandrous range = 1.52 to 2.92). Length and age logistic regressions were not statistically significant (Table 3).

Discussion

We found that 32% of quillback rockfish examined were polyandrous. Previous studies of *Sebastes* have found a high incidence of polyandry for Korean rockfish (88%) (*S. schelegeli*) (Yoshida and Wada 2001), kelp rockfish (100%) (Sogard et al. 2008), and Pacific ocean perch (71.2%) (Van Doornik et al. 2008). In contrast, polyandry was evident for only 1 of 31 broods of *S. marmoratus* (Ng et al. 2003). It is presumed that females of species with internal fertilization have some control over how many males they mate with (DeWoody and Avise 2001). Given this assumption, the variable degree of multiple paternity found among these studies may indicate rockfish exhibit a wide range of mate selection strategies across species.

Gravid quillback rockfish that were heavier or had higher condition factors had a greater chance of being polyandrous than females that were lighter or had lower condition factors; however, we did not find a similar relationship with length or age. Pacific ocean perch with larger lengths had a greater chance of being polyandrous, however, weight and condition were not examined (Van Doornik et al. 2008). A greater chance of polyandry with increasing female size or condition could be related to mating preferences. For example, heavier females may mate with more males or out-compete other females for mates. Both Sogard et al. (2007) and Van Doornik et al. (2008) hypothesized that at higher fecundities, greater quantities of sperm may be required for complete fertilization of a brood; therefore, higher fecund females may prefer to mate with multiple males. Likewise, males may prefer to mate with heavier females who appear to have more energy reserves and are more likely to produce larger numbers of offspring. However, it is also possible that polyandry may be an indicator of population density for species that have little movement, where high densities increase the chance that females will mate with multiple males. Such a theory could be tested in the future by combining catch per unit effort data and genetic techniques.

Most population models used for fisheries management assume equal reproductive success for all sizes and ages of mature fish. For fisheries managers, knowledge of life history traits such as polyandry, mating behavior based on size, age, and timing, are important to understand because these traits may have profound effects on population productivity and genetic diversity that are not being addressed by current fish population models. For slow growing, late to mature nearshore rockfish, such as quillback rockfish, understanding these traits are particularly important because these fish are exploited both commercially and by sport fisheries, which tend to harvest large individuals (Gunderson 1977, Love et al. 1998, Berkeley and Markle 1999). In the case of quillback rockfish, hook and line gear and high exploitation can result in size truncation in a population. Because heavier and higher condition fish have a higher chance of being polyandrous, size truncation could reduce genetic diversity, making it difficult to recover from overfishing events. Also, a decrease in genetic diversity could make the population vulnerable to fluctuating annual environmental conditions, where higher diversity may make it more probable that a portion of the population would succeed a wider variety of conditions (Hyde et al. 2008). In this case, a more conservative harvest would be needed to avoid population declines.

For polyandrous rockfish species, previous studies have demonstrated that dominance of a single sire in a brood decreases as the number of sires per brood increase, termed “skewed paternity” (Sogard et al. 2008, Van Doornik et al. 2008). We also found skewed paternity in wild quillback rockfish. Given the high fecundity of rockfish and their ability to store sperm (Gunderson 1971, Van Doornik et al. 2008), skewed paternity in rockfish may be a product of the quantity of sperm donated by each male and not an effect of the order of mating, where the male inseminating a female at peak fertility sires the most offspring (Birkhead and Pizzari 2002). However, other possibilities such as sexual selection in the form of sperm competition, found in insects (Parker 1970, Simmons 2001) and fishes (Gharrett and Shirley 1985), and cryptic female choice (postcopulatory female sperm choice) found in a number of organisms (Thornhill 1983, Eberhard 1996, Simmons 2001) should be considered and investigated in rockfish. These processes could exert strong postcopulatory sexual selection, as seen in crickets (Simmons 2010), and play a role in population dynamics.

As an interesting aside, one larva that we analyzed lacked maternal alleles but was homozygous for paternal alleles across all 14 loci, suggesting androgenic reproduction. Natural reproduction by androgenesis, in which diploid offspring carry only nuclear chromosomes from the male parent, is documented in hermaphrodite breeding systems such as the freshwater clam (*Corbicula* species) and the Saharan cypress (*Cupressus dupreziana*), but is rare in dioecious breeding systems, documented only in stick insects (*Bacillus rossius* and *Bacillius grandii* hybrids) (McKone and Halpern 2003). The conception that produced the homozygous larva in this study is most likely a rare and spontaneous event. Human conceptions produced by androgenesis are termed "Hydatidiform moles" (HM) (Kajii and Ohama 1977). Hydatidiform moles are separated into two types: complete (CHM) and partial moles (PHM) (Lawler et al. 1982). Complete hydatidiform moles almost always contain paternal-only genomes and arise from either an ovum lacking a nucleus that is fertilized by a single sperm, followed by duplication (homozygous, 90% of CHM cases), or by fertilization by two sperm simultaneously (heterozygous, 10% of CHM cases). Partial hydatidiform moles are like CHMs, but are triploid: arising from a normal ovum being fertilized either by a sperm with a duplicated genome (homozygous, 10% of PHM cases), or two normal sperm simultaneously (heterozygous, 90%) (Bifulco et al. 2008). DNA genotyping techniques used in this study are similar to DNA-based techniques used in humans to distinguish between CHMs and PHMs (Bifulco et al. 2008). Most likely, the homozygous larva that we analyzed is similar to a CHM found in humans, arising from a sperm fertilizing an ovum lacking a nucleus.

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Table 1. -- Number of alleles (Na), probability of identity (PI), and probability of exclusion where only one parent is known (P2), and observed heterozygosity (Ho) of microsatellite loci.

Loci	Na	PI	P2	Ho
Sra.5-9	3	0.20	0.21	0.74
Sra.7-2	2	0.31	0.12	0.91
Sra.7-7*	7	0.09	0.38	0.79
Sra.7-25	5	0.12	0.31	0.62
Sra15-8*	7	0.09	0.37	0.71
Sra.16-5*	12	0.05	0.51	0.87
Sth3B	8	0.41	0.07	0.33
Sth37	2	0.40	0.10	0.71
Sth56*	16	0.06	0.46	0.67
Sma10*	8	0.08	0.36	0.71
Spi4*	13	0.02	0.64	0.78
Spi6*	13	0.03	0.57	0.86
Spi10	4	0.26	0.16	0.56
Spi12	4	0.48	0.05	0.37

* Loci used for paternity analysis.

Table 2. -- Number (*n*) of larvae analyzed per brood, number (*n*) of sires that contributed to a single brood, and the age in years, weight, and length of the gravid female.

Female Id	Larvae (*n*)	Sires (*n*)	Age	Weight (kg)	Length (cm)
1	24	1	14	1.06	36.60
2	48	3	25	1.48	41.70
3	48	1	17	0.80	32.30
4	24	1	13	0.82	35.40
5	24	2	28	1.48	39.60
6	24	1	17	1.14	37.20
7	24	1	19	0.92	35.60
8	24	1	30	1.28	38.40
9	24	3	31	1.66	40.10
10	24	1	23	1.30	41.40
11	24	1	74	1.08	39.30
12	24	1	17	0.84	35.80
13	24	1	41	1.50	41.00
14	24	1	15	1.12	37.50
15	24	1	12	0.72	35.00
16	24	1	15	0.92	36.40
17	24	1	20	1.04	37.10
18	24	3	10	0.66	32.80
19	24	1	11	0.76	32.30
20	24	2	37	1.60	39.00
21	24	2	39	1.54	41.10
22	24	1	22	1.20	39.00
23	24	1	13	0.70	34.10
24	24	3	11	0.58	33.70
25	24	3	17	1.54	37.50

Table 3. -- Chi-square significance tests for logistic regressions of maternal factors (age, length, weight, and condition factor) and the binary response of the presence or absence of polyandry. The p-value (*P*) associated with the Chi-square (ChiSq) test for the significance of the model and the parameter estimates are presented as well and the standard error (SE) of the parameter estimate.

	Model ChiSq (*P*)	Parameter estimate	Estimate SE	Parameter ChiSq (*P*)
Age	0.638	-0.014	0.030	0.636
Length	0.218	-0.200	0.166	0.237
Weight	0.025	-3.230	1.600	0.043
Condition	0.057	-2.760	1.600	0.084

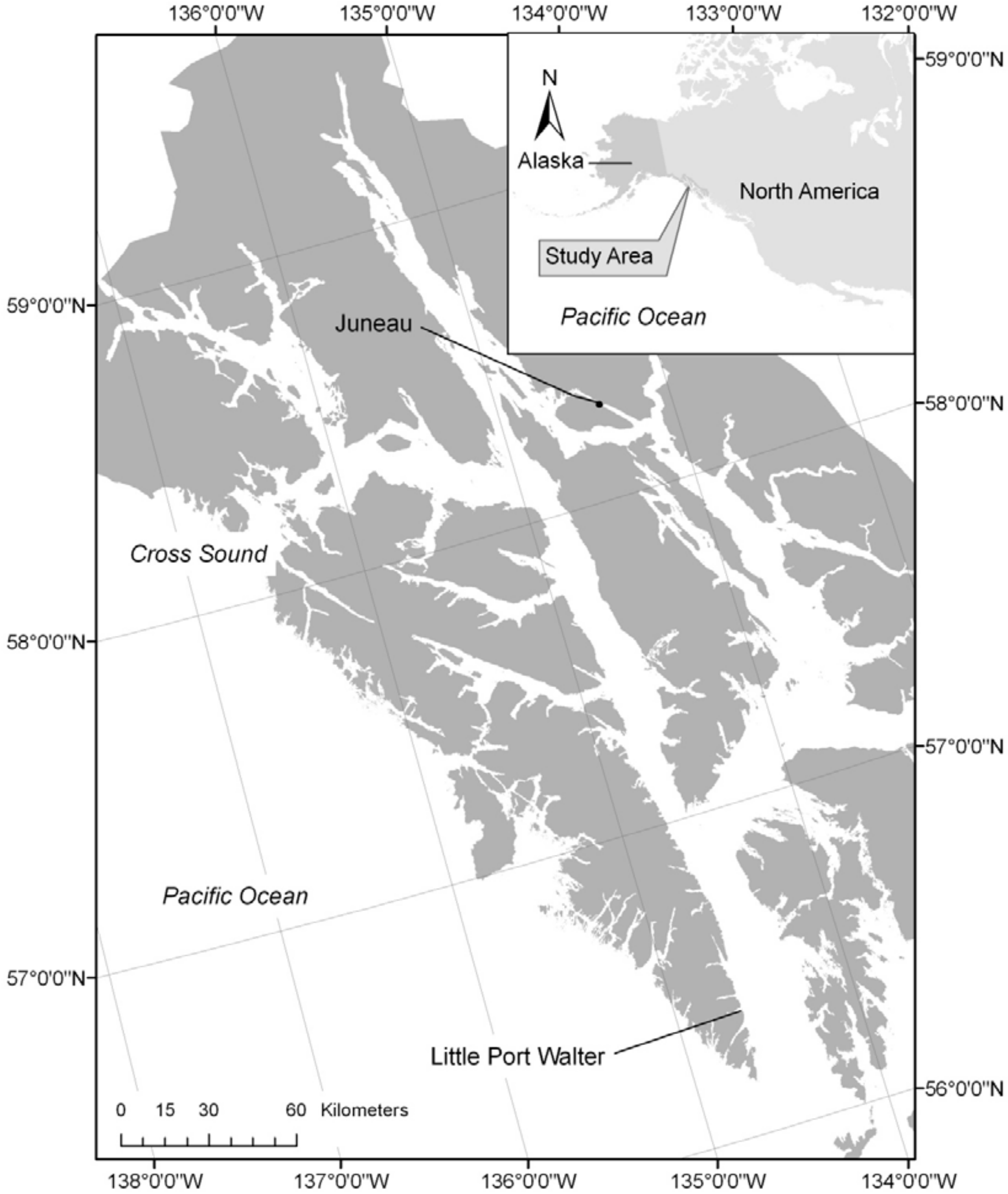

Figure 1. -- Map of study location at NOAA's Alaska Fisheries Science Center's Little Port Walter (LPW) marine research station located on southeastern Baranof Island in Southeast Alaska.

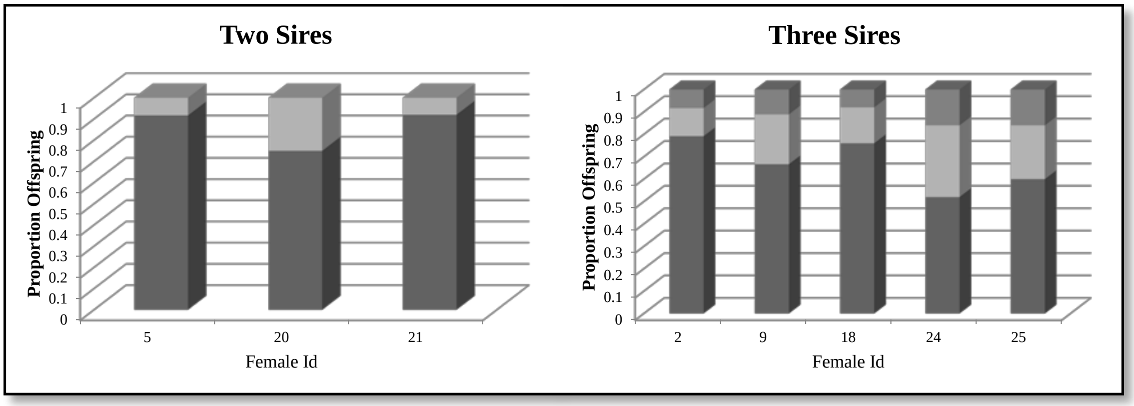

Figure 2. -- Average proportions of offspring per sire for broods sired by two (*n* = 3) or three males (*n* = 5).

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