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Genetic Assessment of the Mating System and Patterns of Egg Cannibalism in Atka Mackerel

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Abstract.—The mating system and patterns of gender-specific egg cannibalism in Atka mackerel *Pleurogrammus monopterygius* were examined through genetic parentage analysis of embryos in egg clutches produced in captive and wild populations. Like other hexagrammid fishes, Atka mackerel exhibit polygynandry, which is characterized by serial matings by both genders within a breeding season. Most matings in captivity were pairings of females with nest-attendant males, although parentage analysis of clutches produced in a small tank with limited nesting substrate revealed that 31% contained contributions by non–nest-guarding males. In contrast, all egg clutches produced in a large exhibit tank were sired by guardian males. Multiple parentage, sometimes involving both genders, was detected in 35% of egg clutches collected in the field or retrieved from the guts of adult male and female cannibals. Half-sib and unrelated full-sib embryos were found in several putative clutches, indicating that the reproductive output from multiple males and females may be combined sequentially and fused into a single clutch. These results suggested that nest takeovers, combined with alloparental care of existing broods, represent common reproductive tactics in males. Egg cannibalism is a significant seasonal factor in the diets of male and female Atka mackerel. Analysis of loose eggs and partial egg clutches ingested by 4 female and 15 male cannibals showed that nearly all conspecific predation represented heterocannibalism. One instance of partial filial cannibalism was documented in a male that was one of multiple sires for the clutch.

Actinopteryiian fishes exhibit the most diverse range of reproductive behaviors found in any vertebrate group (Breder and Rosen 1966; Sadovy de Mitcheson and Liu 2008). Individual species may be unisexual, gonochoristic (separate sexes), or hermaphroditic (sequential or simultaneous), and mating systems range from broadcast group spawning to self and internal fertilization, including monogamy through various degrees of polygamy by either or both genders (Mank and Avise 2006). Courtship and spawning behaviors in fishes, including male alternative reproductive tactics (MARTs), have formed the basis for theoretical and empirical studies examining sexual selection. A significant component of the observed variety in reproductive modes is the level of parental care of offspring. Broadcast spawners typically provide no parental care, whereas nest builders, brooders, and live bearers often expend considerable energy in defending eggs and developing young. Approximately 21% of the 422 recognized taxonomic families of bony fishes contain species that provide some degree of parental care of offspring (Avise et al. 2002), including 15.5% to 22.5% of families in California and Pacific Canada (DeMartini and Sikkel 2006). Unlike in other vertebrates, about 70% of the parental care is provided by males in fish species that exhibit uniparental care (Blumer 1982; Avise et al. 2002).

In recent years, parentage analysis of offspring using highly polymorphic microsatellite DNA markers has provided unprecedented resolution of fish mating systems (DeWoody and Avise 2001; see review by Avise et al. 2002; Mackiewicz et al. 2005). These behaviors include monogamous or multiple matings, male cuckoldry, and a variety of MARTs to attain

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surrogate parentage, such as egg mimicry, nest piracy and egg-thievery, multiple paternity in female-pregnant species, and sex role reversal in male-pregnant species (e.g., pipefish, seahorses). Parentage analysis has also revealed the presence of MARTs that would probably go unobserved or unrecognized in many field studies and provides a means to quantify fitness consequences, in terms of reproductive output, in natural populations. Other behaviors in complex mating systems, such as patterns of egg or offspring cannibalism, can be readily assessed using parentage analysis.

The hexagrammid Atka mackerel *Pleurogrammus monopterygius* is a semidemersal, schooling species distributed along continental shelves from Asia to North America. Atka mackerel are obligate demersal spawners that attach egg clutches to hard substrates (often in crevices) that are protected from cannibals and other egg predators by territorial males. Males aggregate and acquire nest sites before spawning at depths from 32 to 144 m (Lauth et al. 2007). Nesting typically occurs on rocky substrates in areas exposed to moderate currents, and there is considerable variation among locations with respect to relief, slope, and continuity (Lauth et al. 2006). Nest sites average about 2 m^2 (Fritz and Lowe 1998) and tend to be adjacent to one another (Lauth et al., 2006). Atka mackerel develop conspicuous seasonal sexual dichromatism, and both sexes display ephemeral color changes while engaged in seasonal courtship and territorial defense behaviors (Lauth et al., in press). Females can potentially spawn up to six adhesive egg clutches during the spawning season, each containing approximately 6,700 eggs (McDermott et al. 2007), and males may brood multiple clutches within their territories. Spawning typically begins in late June, and females in spawning condition have been documented from July through October (McDermott and Lowe 1997). Nesting males have been observed in late October, suggesting that the combined mating and brooding phases may extend from summer well into the fall (Lauth et al. 2007).

Specific details of the mating system in Atka mackerel are unknown, but probably include some of the polygamous strategies found in other greenlings. Sequential polyandry (either within or among spawning seasons) by female hexagrammids has been documented by parentage analysis in several species (Crow et al. 1997; King and Withler 2005). Crow et al. (1997) reported that about 40% of the nests of kelp greenling *Hexagrammos decagrammus* contained clutches from multiple females, and DeMartini (1987) reported similar proportions for painted greenling *Oxylebius pictus*. Less well understood are MARTs, which may include adoption of a "sneaker" strategy by adjacent nest-tending males or nonterritory holding males to engage in parasitic fertilizations. Sneaking has been reported in hexagrammids species (personal communication cited in Crow et al. 1997; Munehara et al. 2000), and the reproductive consequences of this behavior have been quantified using molecular genetic techniques in the fat greenling *H. otakii* (Munehara and Takenaka 2000) and lingcod *Ophiodon elongatus* (Withler et al. 2004; King and Withler 2005).

Nest raiding of eggs by both sexes and male filial cannibalism have been reported in other hexagrammids (DeMartini 1987; Munehara and Miura 1995), and adult Atka mackerel are egg cannibals (Yang 1999; Rand 2007). In species with paternal care, male filial cannibalism parasitizes the fecundity of females as a strategy to maximize lifetime reproductive success at the expense of current broods (Rohwer 1978; Sargent 1992) and may benefit females if it increases the probability of successfully rearing the remaining offspring (Lindström 2000). Male energy reserves have often been considered to be the primary factor driving filial cannibalism (DeMartini 1987; Lindström 2000), and male condition may vary seasonally, depending upon the duration of parental care. The extended breeding and brooding period in Atka mackerel probably imposes significant energy demands upon territorial males, but patterns of cannibalistic behaviors in Atka mackerel have not been investigated.

In this study, we used genetic parentage analyses of embryos produced in both captive and natural populations of Atka mackerel to assess the mating system and patterns of egg cannibalism by adults. Genotype data from polymorphic DNA microsatellite loci were obtained for embryos in egg clutches (defined as an event of egg deposition by females) to document mating behaviors in captive populations through parentage analyses. Additional inferences on aspects of the mating system were derived from analyses of clutches collected in the field or retrieved from the stomachs of adult cannibals, where parental data were unavailable. Results from natural populations were contrasted with those observed in captivity to examine the full range of reproductive behaviors in Atka mackerel and the predominance of these behaviors relative to other members of the family. A second objective was to assess the extent of heterocannibalism and filial cannibalism by both genders. The relatedness of male and female cannibals with the embryos they had consumed was determined using microsatellite DNA markers, providing the first quantitative assessment of gender-specific cannibalism in this species.

Methods

Samples from captive populations.—Parentage was assessed for embryos in clutches produced in captivity

TABLE 1.—Sample data for Atka mackerel embryos from captive and wild populations used in parentage analysis for captive females and males in the small (ST; 10,250-L) and large (LT; 400,000-L) tanks at the Alaska SeaLife Center. Partial and complete clutches were also assayed from cannibal gut contents and trawl samples.

Year	Tank or sample type	Spawning substrate (m^2)	Number of			
			Females	Males	Clutches	Embryos
Captive populations						
2004	ST	4.0	4	2	13	8–96
2005	ST	7.2	5	2	38	4
2006	ST	7.2	4	2	27	3
2005	LT	>50.0	13	8	17	12–96
2006	LT	>50.0	11	6	6	29–32
Cannibal gut contents and trawl samples						
2004	Gut		0	5	5[a]	17–94
2005	Gut		4	0	3[a]	7–48
2007	Gut		0	10	12	8–43
2007	Trawl				6	19–107

[a] Two individuals contained loose eggs that were not counted as intact clutches.

from two separate aquaria: a small tank (about 10,250 L, 1.5 m deep) and a large tank (about 400,000 L, 6.4 m deep) at the Alaska SeaLife Center, where adult Atka mackerel had successfully spawned for several years (Table 1). The small tank contained two males and 4 or 5 females in 2004–2006; the tank had 4.0 m^2 of spawning substrate available in 2004 and 7.2 m^2 in 2005 and 2006. The large tank contained 13 females and 8 males in 2005 and 11 females and 6 males in 2006; this tank had more than 50 m^2 of spawning substrate available in both years (Table 1). Fin clips from all adults were collected for parentage analysis, and the identities of territorial males were recorded based upon natural markings.

Eggs were removed from the small tank soon after first discovery to deter cannibalism, and the time, nest location, and identity of the guardian male were recorded. During 2004 and 2005 eggs were incubated at controlled temperatures ranging from 3.9°C to 9.9°C. Samples for genetic analysis were taken in middle to late stage development when eyes were readily visible: approximately 4–10 weeks following fertilization, depending upon temperature. We subsequently found that we could determine parentage of embryos at much earlier stages of development. Thus, clutches were removed from nesting sites after first discovery in 2006 and incubated at 4–6°C for approximately 2 weeks before preservation in 95% nondenatured ethanol. In 2005 and 2006 only three to six embryos were genotyped per clutch to establish maternity of clutches used in study of fecundity and egg energy content (S. McDermott and colleagues, National Marine Fisheries Service, unpublished).

During the 2005 season, Atka mackerel were allowed to spawn undisturbed in the large tank from the onset of spawning in July until the end of August. Four of the eight resident males in the tank successfully brooded clutches that season. A first group of clutches from two territories, each guarded by a single male throughout the season, were removed on the same date. They were again allowed to spawn undisturbed for 12 d, when a second group of clutches was removed. Spawning continued for an additional 38 d before the final group of clutches was removed. Once removed, all eggs were incubated to the eyed stage as described above. In 2006 we allowed adult Atka mackerel to spawn undisturbed for nearly the entire spawning season to evaluate the effects, if any, of immediate egg removal on reproductive behaviors. Clutches were collected from a single territory held by one of the males sampled during the previous year.

Samples from wild populations.—Adult Atka mackerel were collected by trawling between Unimak Island and Amchitka Island, Alaska, during three cruises during 2004–2007 (Table 1). Egg cannibalism was deduced from the distended appearance of the stomach during routine dissections for sex determination. Fish were immediately frozen whole on the vessel and maintained at –20°C until thawed for analysis in the laboratory. Stomach contents were dissected from putative cannibals, and partial clutches were counted, briefly washed in seawater, and preserved in nondenatured 95% ethanol for parentage analysis. Six intact clutches were collected by bottom trawl aboard a chartered commercial bottom trawler in the Aleutian Islands (52.046°N, 172.025°W) on October 13, 2006 (McDermott, unpublished), immediately frozen at –20°C, and subsequently preserved in ethanol.

Extraction, amplification, and analysis of DNA.—Genomic DNA from samples of eggs and fin clips (from captive adults and field-collected cannibals), was extracted using DNeasy tissue kits (Qiagen Inc., Valencia, California) according to the manufacturer's instructions with one modification for egg samples: owing to the small size of developing embryos, an elution buffer volume of 60 μL was used to provide a more concentrated DNA template. Four polymorphic microsatellite loci (*Pmo70*, *Pmo152*, *Pmo367*, and *Pmo399*) isolated from Atka mackerel (Spies et al. 2005) and two loci isolated from lingcod (*Oel42* and *Oel32*; S. Young, Washington Department of Fish and Wildlife, Olympia, Washington, personal communication) were amplified via the polymerase chain reaction (PCR), as described in Spies et al. (2005). Bovine serum albumin (New England Biolabs, Ipswich, Massachusetts) was added to a final concentration of 0.5 mg/mL in PCR reactions involving embryos. Loci were amplified and scored two at a time (following order: *Pmo152*, *Pmo70*, *Pmo399*, *Oel32*, *Pmo367*, and *Oel42*) on eggs randomly selected from clutches of wild and captive populations until sibling relationships could be accurately assessed or, in the case of cannibalism, the cannibal was excluded as a parent. Thus, all loci were not amplified in all samples if fewer loci were sufficient to meet criteria for unequivocal parental identification (captive samples) or determination of sibship relationships. Genotyping was conducted using a LI-COR 4200 Infrared Automatic DNA Sequencer (LI-COR Biotechnology, Lincoln, Nebraska) and analyzed with LI-COR Saga genotyping software.

Parentage analysis.—Binomial sampling theory predicts that a sample of *n* individuals will detect the proportion (p) of offspring contributed by an individual parent with 95% certainty if

$$n \geq (\log_e[0.05])/(\log_e[1-p])$$

(DeWoody and Avise 2001). Thus, genotyping 96 individuals had the power to detect embryos produced by other parents if their contributions to a clutch exceeded 3% and to detect a contribution of 12% or more by sampling 24 embryos. Based upon these calculations, we initially performed exhaustive sampling of 96 embryos from clutches produced in the large tank and subsequently altered the sampling regime based upon those results. Six of the 17 clutches produced in 2005 were exhaustively sampled ($\geq$90 eggs) and the remaining sample sizes exceeded 20 in all but four cases. Genotypes of all possible parents were known for all loci in captive fish, allowing for unambiguous parental assignments by simple exclusion. Parentage in the small tank in 2004 was determined by genotyping 24–96 embryos per egg mass. In subsequent years (2005 and 2006), only three to six embryos were sampled from each clutch to identify the female parent.

Incompatibility of genotypes from ingested embryos provided conclusive proof of nonparentage for egg cannibals. Microsatellite data from 553 adult Atka mackerel collected during 2004–2006 in the Aleutian Islands (Canino et al. 2010) were used to estimate allele frequencies at the six loci used for parentage analysis. Exclusion probabilities (the expected proportion of offspring for which the cannibal would be excluded as a parent) were determined using a frequency-based method (Jamieson and Taylor 1997). Because multilocus genotpyes of cannibals could be determined, we calculated exclusion probabilities using the general formula for one unknown parent. We also calculated the probability of identity, P_{ID} (i.e., the probability that two different individuals have identical genotypes), for each locus using the formula provided by Waits et al. (2001),

$$P_{\mathrm{ID}} = 0.25 + 0.25\cdot\sum p_i^2 + 0.50\cdot\left(\sum p_i^2\right)^2 - 0.25\cdot\sum p_i^4, \tag{1}$$

where p_i is the frequency of the *i*th allele at a locus. The P_{ID} value over six loci was calculated as the product of probabilities for individual loci.

In general, we attempted to genotype a minimum of 24 individuals at four loci from each clutch to determine sibling relationships and conducted more exhaustive sampling when preliminary results indicated the presence of half-sibs or unrelated individuals. Construction of full-sib and half-sib families detected in cannibalized and field-collected clutches (parental genotypes unknown) was conducted using PEDIGREE 2.2 (available at http://herbinger.biology.dal.ca:5080/Pedigree/) and subsequently verified by visual inspection.

Two regions of mitochondrial DNA were sequenced to screen for variation. A 450 base pair (bp) segment of the mitochondrial D-loop region was amplified via PCR in 20 individuals from across the species range (Gulf of Alaska to northern Japan) via Pro-L and H16498 primers previously used for successful detection of maternal haplotypes in kelp greenling (Crow et al. 1997) and lingcod (Withler et al. 2004). Next, a 790-bp segment of the cytochrome *b* gene was amplified using Cytb-F and Cytb-R primers following (Kimura et al. 2007) in nine individuals. The PCR amplicons were purified and sequenced at the High-Throughput Genomics Unit at the University of Washington (http://www.htseq.org).

Results

No sequence variation was found among 20 individuals in the mitogenomic D-loop region or for cytochrome *b* in nine individuals (data not shown). We

TABLE 2.—Observed number of alleles (A_O), expected (H_e) and observed (H_o) heterozygosities, exclusion probabilities (P), and probabilities of genetic identity (P_{ID}) for six microsatellite loci used in parentage analysis of Atka mackerel.

Locus	A_O	H_e	H_o	P	P_{ID}
Pmo70	31	0.894	0.906	0.647	0.278
Pmo152	50	0.947	0.930	0.805	0.264
Pmo367	74	0.955	0.916	0.834	0.261
Pmo399	19	0.786	0.833	0.408	0.310
Oel42	92	0.966	0.968	0.873	0.258
Oel32	12	0.794	0.712	0.424	0.301
All loci				0.9995	0.0005

concluded that haplotype variation was likely to be at frequencies too low to be informative for maternal identification in clutches, a conclusion that was subsequently verified by additional D-loop sequencing (see Canino et al. 2010). Parentage analysis was thus conducted using only microsatellite DNA loci.

The six microsatellite loci had 12–92 alleles each, resulting in single locus exclusion probabilities ranging from 0.424 to 0.873 and a combined multilocus exclusion probability exceeding 0.999 (Table 2). Even when only two loci were used (*Pmo70* and *Pmo152*), the exclusion probability was greater than 0.97. The P_{ID} values for the six microsatellite loci ranged from 0.258 to 0 0.310, providing an overall value of 0.0005 across all loci. These highly polymorphic loci provided sufficient power to discriminate among closely related individuals, enabling us to determine genetic identities for parental assignment or exclusion.

Parentages in the Small Tank

Parentage was assessed for a total of 79 clutches produced in 2004–2006 (Table 3). A single territory on the available 4.0-m^2 spawning substrate was established by a male at the onset of spawning and nesting in mid-July in 2004. It brooded four clutches of eggs until being displaced by the second male a month later (August). That male brooded nine subsequent clutches for the remainder of the spawning season. Although the initial male was displaced and did not continue to brood eggs, he apparently sired all embryos in 12 of 13 clutches produced by the four resident females. Contributions by both males were detected in only one clutch that was produced coincident with the displacement of the first male by the second. In that clutch, the second male sired 9% of the 22 embryos that were successfully genotyped. Samples from the other 12 clutches (24–96 embryos each, mean = 50) produced that year, both before and after the ousting of the first male, should have detected paternity by the second male if it had averaged more than 6% of the embryos sampled during that period. However, there was no evidence for paternal contributions by this male despite the fact that he continued to guard the nesting site during daylight hours for two additional months.

Two different males and an additional female were introduced to the small tank in 2005 and 2006 and the available spawning substrate was increased to 7.2 m^2 (Table 1). Both established and guarded adjacent territories and brooded multiple clutches for the duration of the 2005 spawning and nesting season. The same territories were reestablished in 2006 and defended until one male died in September, ending collections for that year. Assigning parentage to most clutches produced in 2005 (73.7%) and 2006 (71.4%) indicated that the nest-tending male had sired all of the three to six embryos sampled per clutch, but in approximately 28% of the cases, the guardian male was not identified as the genetic sire. One instance of contributions by both males was confirmed in a single clutch produced in 2006 (Table 3). Females produced between 6 and 12 clutches per season (Table 3). They showed no overall preference for mating with either male during 2005 (sign test, $P = 0.256$) but exhibited significant preferences for one male in 2004 and 2006 ($P \leq 0.001$ in both cases).

Parentages in the Large Tank

All males in the large tank established and guarded territories of 4.0–12.0 m^2 for the duration of the spawning and nesting season in 2005 and 2006. However, only four of eight males in 2005 and three of six males in 2006 brooded eggs within their individual territories. Parentage analysis was conducted on 23 clutches produced in the large exhibit tank during 2005 and 2006 (Table 4). No evidence of

TABLE 3.—Parentage analysis of Atka mackerel clutches produced in the small tank at the Alaska SeaLife Center in 2004–2005.

Year	Number of clutches (embryos genotyped)	Female identity (clutches produced)					Male identity (clutches contributed)		Number of cases of dual paternity
2004	13 (21–96)	F1 (3)	F2 (5)	F3 (2)	F4 (3)		M1 (1)	M2 (13)	1
2005	38 (3–6)	F1 (12)	F2 (7)	F32 (7)	F4 (6)	F33 (6)	M3 (15)	M4 (23)	0
2006	28 (3–6)	F1 (7)	[a]	F32 (6)	F4 (5)	F33 (6)	M3 (4)	M4 (24)	1

[a] Female F2 died and was not replaced.

TABLE 4.—Parentage analysis for Atka mackerel clutches produced in the large tank at the Alaska SeaLife Center in 2005–2006.

Collection date	Male identity	Number of females	Number of clutches	Female identity (clutches deposited)	Mean (range) number of embryos genotyped
Aug 25, 2005	25	7	9	14(2), 23(2), 9(1), 16(1), 18(1), 19(1), 22(1)	38 (12–92)
Sep 6, 2005	25	1	2	14(2)	95 (94–96)
Oct 14, 2005	8	6	6	23(2), 9(1), 13(1), 19(1), 24(1)	75 (12–95)
Oct 24, 2006	8	4	6	30(3), 29(1), 31(1), 32(1)	30 (29–31)

TABLE 5.—Parentage of Atka mackerel embryos collected from adult cannibals or in trawl samples.

	Number of			
Sample type	Individuals	Partially intact clutches	Mean (range) number of embryos per clutch	Number of parents (clutches)
Male cannibals	15	13[a]	30 (8–94)	2(9), 3(1), 4(1),7(1), 9(1)
Female cannibals	4	3[a]	32 (26–58)	3(1), 4(1), 5(1)
Trawl		6	47 (19–107)	2(5), 8(1)

[a] Two individuals contained numerous single eggs not counted as intact clutches.

multiple parentage was found in five clutches that were exhaustively sampled (>90 embryos genotyped), or in remaining clutches that were sampled less intensively. Sample sizes of embryos averaged 58 individuals/clutch in 2005 and 30 in 2006, which would have detected contributions from additional parents if they had produced approximately 6–11% of the offspring in the clutch. Our sampling showed that one female spawned multiple times with a single male in 2005, but females as a group exhibited no significant preference for mating with either of the two sampled territorial males (sign test, $P = 0.332$).

Field Samples of Cannibalized and Noncannibalized Clutches

Genotypes were obtained for embryos in intact, partial clutches and loose individual eggs retrieved from the stomachs of 19 adult Atka mackerel (Table 5). In some cases, embryos were too young or digestion had proceeded far enough to prevent extraction of sufficient DNA for genotyping over all loci. All four female cannibals and 14 of 15 male cannibals were not parents of embryos they had consumed, but a single instance of male filial cannibalism was documented. This male was identified as the sire of 11 of 94 (12%) late-stage eyed embryos distributed in three half-sib families in the partial clutch he had consumed (combined exclusion probability $P > 0.999$). The remaining embryos in the clutch were composed of three more half-sib groups produced by four unrelated parents of unknown sex (but probably one male and three females) and individuals from five additional unrelated full-sib families.

The majority (69%) of clutches collected in the field appeared to result from exclusively monogamous matings. Multiple parentage was detected in 7 of 20 intact partial clutches (i.e., excluding loose eggs) ingested by cannibals, and in 1 of 6 clutches collected in trawls (Table 5). In these cases, embryo genotypes were inconsistent with any single parental genotype, indicating the presence of unrelated families in a single clutch of eggs. Although these clutches often appeared to represent a single batch spawning by one female, we observed one (unanalyzed) egg mass to split apart following preservation (Figure 1), suggesting that female Atka mackerel deposit and cement their eggs on existing clutches in the brood nest.

Discussion

Molecular assessment of the mating system in Atka mackerel enabled documentation of behaviors previously inferred from field studies and confirmed reproductive tactics characteristic of other hexagram-

FIGURE 1.—Portions of two individual Atka mackerel egg clutches that were fused together when collected and separated after preservation in a 95% solution of ethanol.

mid species. The vivid dichromatism of males and ephemeral color and behavior displays by both sexes indicate a highly dynamic mating system probably shaped by strong intrasexual and intersexual selection. Conspicuous nuptial coloration is selected by females as a conditional expression of the genotypic and phenotypic quality of males, as well as their nutritional condition, social rank, and mating motivation and success (Kodric-Brown and Brown 1984; Kodric-Brown 1998). Within a given brood year, approximately 15% of adult males display nuptial coloration, whereas the remaining nonbreeding males make no apparent energetic investment. These seasonal patterns appear to be unrelated to male length and thus are unlikely to represent age-dependent alternative reproductive tactics (e.g., Alonzo et al. 2000), but they may reflect a large energetic cost imposed by sexual selection. Carotenoid-based pigmentation in many fishes must be obtained from the diet and is energetically costly for males to produce and further work should examine patterns of annual and seasonal reproductive status. Ephemeral color displays during courtship and agonistic encounters described for the permanently dichromatic painted greenling (DeMartini 1985) and seasonally dichromatic Atka mackerel (Lauth et al., in press) indicate a complex social environment during spawning determined by strong competition for access to territories and mates in males and by readiness to spawn in females.

Atka mackerel exhibited a polygynandrous mating system, resulting from serial polyandry and polygamy within the breeding season. Male polygyny appeared to be achieved primarily through territorial defense in captive populations, although some cuckoldry of guardian males did occur in the small exhibit tank and probably to a greater degree in natural populations. Polyandry in Atka mackerel results from batch spawning of eggs throughout the breeding season (McDermott et al. 2007) coupled with low nest site fidelity by females. Females in captivity sometimes exhibited male preference for mating, although sample sizes were inadequate to assess this behavior. Most of the clutches produced in natural populations (69%) and nearly all of those produced in captivity (98%) indicated monogamous pairings. Nest-guarding males in the large exhibit tank apparently monopolized all fertilization events within their territories. Two documented instances of parasitized fertilizations occurred in the small tank (Table 3). The average number of embryos genotyped per clutch in partial clutches consumed by cannibals and whole clutches taken in trawls (32.5 individuals) would have only detected contributions from extra-pair matings exceeding 10%, but more exhaustive sampling of several clutches indicated that strictly monogamous spawning events are common in natural populations.

Parentage analysis using microsatellite DNA markers unequivocally documented parasitic fertilizations of clutches by males in captivity, but the responsible behaviors were not directly observed. Male cuckoldry is common among fishes exhibiting paternal care and several alternative reproductive tactics have evolved in response to competition for access to mates or their gametes (Taborsky 1994, 2001; Gross 1996). The most common MARTs documented in nest-tending species by parentage assessment are fertilizations by sneaking or satellite males, resulting in the nest-tending male being the sire of most, but not all of his custodial offspring (DeWoody and Avise 2001: Avise et al. 2002; Mackiewicz et al. 2002; Mackiewicz et al. 2005). Sneaking constitutes potential costs to nest-tending males in terms of lowered reproductive output (Rico et al. 1992; Largiader et al. 2001) and the energetic expense of rearing unrelated young. This strategy is associated with strong sexual dimorphism and active courtship and nest defense behaviors by males (Gross 1996; Taborsky 1998), characteristics common to hexagrammids in general. Sneaking has been observed (Munehara et al. 2000) in three species of *Hexagrammos* and verified using molecular genetic analyses in the fat greenling (Munehara and Takenaka 2000). Sneaking is a likely, but unobserved, source of male cuckoldry in Atka mackerel. The frequency of clutches produced in captivity by monogamous pairings of females with territorial males was lower in the small tank containing limited spawning substrate than in the large tank where larger amounts of spawning substrate were available. Density-dependent sneaking has been reported in the bitterling *Rhodeus sericeus* (Reichard et al. 2004). Our experimental design may have favored territoriality when densities were low in the captive tanks and prompted sneaking or other fertilization strategies when they were higher. Another potential MART that may explain parasitized fertilizations is sperm competition from satellite males. Munehara and Takenaka (2000) observed two patterns of sperm emission in male fat greenling and different rates of fertilization success. When the satellite intruder male emitted a large amount of sperm after ejaculation by the territorial male, the sneaker fertilized more eggs. If both males emitted sperm alternately, the territorial male sired a larger number of offspring in the clutch.

Nest-tending males may tolerate fertilizations by nonterritorial males if they help to attract females or aid in nest defense or maintenance (Taborsky 2001). The mixed paternity observed in two single clutches of eggs from the small exhibit tank in 2004 and 2006 resulted from parasitized fertilizations, although sneaking by males was not directly observed. Discrepancies between the apparent guardian and the genetic sire of at least some embryos occurred in all 3 years, suggesting the possibility of additional MARTs. One tactic may be temporary nest takeovers by the nonterritorial male to acquire matings. This may increase reproductive fitness if females prefer males already brooding clutches in the nest (Marconato and Bisazza 1986; DeMartini 1987; Kraak and Groothuis 1994; Goulet 1998) or if there is mate-choice copying by females (Jamieson 1995). Cuckolded males may later return to the nest to care for unrelated offspring if alloparental care improves the possibility of additional spawnings. This potential MART has been documented genetically in other fishes (DeWoody et al. 2001), including lingcod (Withler et al. 2004). Nest takeovers are consistent with the observation that nest site fidelity for male Atka mackerel may not extend for the entire spawning season. Lauth et al. (2007) inferred duration for male nesting and brooding of 33–141 d, based upon archival tag data for four adults. It is unknown if the cessation of nesting represents expulsions by other males or voluntary abandonment of a site.

The presence of unrelated half-sib and full-sib embryos found within single putative clutches from natural populations (Table 5) was a surprising result that may, in part, be explained by egg deposition patterns by females and MARTs inferred from results from captive populations. Previous studies have considered a clutch to be the reproductive output from an individual female—an assumption (DeMartini 1986) inferred from the physical discreteness, yolk coloration, and stage of embryonic development in kelp greenling and whitespotted greenling *Hexagrammos stelleri* and documented using genetic studies in kelp greenling (Crow et al. 1997) and lingcod (Withler et al. 2004). Although this also appeared to be the general case for Atka mackerel in captivity, some clutches produced in wild populations indicated that females may deposit their eggs on existing clutches, fusing them to each other (Figure 1). In the congeneric Okhotsk Atka mackerel *Pleurogrammus azonus*, females used their pectoral fins or side of their belly to wedge fertilized clutches into gaps between rocks from various directions (Munehara and Markevich 2003), and female captive Atka mackerel have been observed to push freshly fertilized eggs into crevices using their mouths (J. Guthridge, unpublished data). Koya et al. (1995) reported that the adhesive surrounding the eggs of the fat greenling required several hours to harden, and these behaviors may help to retain clutches after deposition until they fully adhere to the spawning substrate. The extreme mitogenomic homogeneity exhibited by Atka mackerel (Canino et al. 2010) precluded using mitochondrial DNA haplotypes to determine individual female contributions to clutches retrieved from cannibals or captured in trawls, but the probable sources of complex assortments of related and unrelated offspring within a single clutch are multiple egg depositions and manipulations by females, accompanied by MARTs, such as nest takeover or parasitized fertilizations.

Egg cannibalism constitutes a significant dietary component of Atka mackerel during the spawning and brooding seasons (Yang 1999; Rand 2007). Both genders engage in cannibalism in roughly equal frequencies, but males consume more than twice as much by weight (K. Rand, National Marine Fisheries Service, personal communication). Unlike territorial males, females can forage during the mating and brooding phases in seasonal reproduction and are generally not in proximity of defended nests unless engaged in spawning. Female fat greenling have been observed pecking at egg clutches already present in nests, causing attendant males to repel them without attempting to mate (Munehara et al. 2000). Nest raiding by female Atka mackerel has been observed (R. Lauth, National Marine Fisheries Service, personal communication), suggesting it is the most likely source for opportunistic heterocannibalism by this gender.

Filial cannibalism represents a range of compromise strategies between current and future reproductive success (Rohwer 1978) and is common for males in species with uniparental care. DeMartini (1987) reported apparent heterocannibalistic raiding of nests and some paternal filial cannibalism by guardian male painted greenling. Filial cannibalism has also been observed in fat greenling, when clutches detached from seaweeds to which they had been fastened and were no longer under direct male control (Munehara and Miura 1995). Males may cannibalize some fraction of their offspring as an investment to maximize future reproductive success by enhancing their own survival and that of the remaining brood, whereas females can only benefit from partial, but not total, consumption of their young by males. This results in a conflict between the sexes over the male's investment in the current brood (Lindström 2000). Empirical studies have shown that small and young broods are more often fully cannibalized than larger and older ones that have greater paternal investment by the male (DeMartini 1987; Lindström and Sargent 1997). That was not the case in the single instance of filial cannibalism documented in this study; all of the embryos in the partial clutch were in a later eyed stage. However, most were unrelated to the cannibal, and his relationship to the clutch (e.g., guardian or opportunistic cannibal) was unknown.

Although the parental energy reserve of the male has often been considered as the primary factor in filial cannibalism, mate availability may also be important (Kondoh and Okuda 2002). Males may receive an energetic surplus of eggs when females are abundant, resulting in an energy reserve that can be reallocated to future reproduction. Conditions favoring mate availability include a female-biased sex ratio, an extended period of male care, and a short refractory period between spawnings by females (Kondoh and Okuda 2002).These conditions may be met seasonally, to some degree, in Atka mackerel. The occurrence of adult nonbreeding males in the population (Lauth et al., in press) biases the operational sex ratio (ratio of ready-to-mate males to ready-to-mate females; Emlen and Oring 1977) towards females. Average realized batch fecundity of females is 4.6 clutches per season (McDermott et al. 2007), resulting in clutch production perhaps every 2–3 weeks. The spawning and brooding period for males can last from late July to January (Lauth et al. 2007), and the sole instance of filial cannibalism detected in our study occurred when the spawning phase of seasonal reproduction was largely over (October) and some of the brooding phase remained. Although more extensive sampling would be required to characterize the extent of filial cannibalism by males, the prevalence of heterocannibalism we observed suggests that densities in communal nesting areas may be sufficiently high to make conspecific nest raiding a more common strategy.

Our results underscore both the power and limitations of genetic parentage assessment alone for making inferences about reproductive behaviors and their consequences in natural populations. The study confirmed some general aspects of the mating system in Atka mackerel (e.g., serial polygamy and polyandry) inferred from existing studies on the family but did not resolve more complex behaviors (e.g., MARTs and egg deposition patterns by females) contributing to the clutch genetic diversity observed in wild populations. Future efforts should include pairing observational and parentage approaches in studies of captive and wild populations, that would more fully elucidate the range of reproductive behaviors in both genders.

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References

Alonzo, S. H., M. Taborsky, and P. Wirtz. 2000. Male alternative reproductive behaviours in a Mediterranean wrasse, *Symphodus ocellatus*: evidence from otoliths for multiple life history pathways. Evolutionary Ecology Research 2(8):997–1007.

Avise, J. C., A. G. Jones, D. J. Walker, and J. A. DeWoody. 2002. Genetic mating systems and reproductive natural histories of fishes: lessons for ecology and evolution. Annual Review of Genetics 36:19–45.

Blumer, L. S. 1982. A bibliography and categorization of bony fishes exhibiting parental care. Zoological Journal of the Linnean Society 76:1–22.

Breder, C. M., and D. E. Rosen. 1966. Modes of reproduction in fishes. Natural History Press, Garden City, New York.

Canino, M. F., I. B. Spies, S. A. Lowe, and W. S. Grant. 2010. Highly discordant nuclear and mitochondrial DNA diversities in Atka mackerel. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science. 2:375–387.

Crow, K. D., D. A. Powers, and G. Bernardi. 1997. Evidence for multiple maternal contributors in nests of kelp greenling (*Hexagrammos decagrammus*, Hexagrammidae). Copeia 1997:9–15.

DeMartini, E. E. 1985. Social behavior and coloration changes in painted greenling, *Oxylebius pictus* (Pisces: Hexagrammidae). Copeia 1985:966–975.

DeMartini, E. E. 1986. Reproductive colorations, paternal behavior, and clutches of kelp greenling, *Hexagrammos decagrammus*, and whitespotted greenling, *H. stelleri*. Northwest Science 60:32–35.

DeMartini, E. E. 1987. Paternal defence, cannibalism, and polygamy: factors influencing the reproductive success of painted greenling (Pisces: Hexagrammidae). Animal Behaviour 35:1145–1158.

DeMartini, E. E., and C. Sikkel. 2006. Reproduction. Pages 483–523 *in* L. G. Allen, D. J. Pondella II, and M. H. Horn, editors. The ecology of marine fishes. University of California Press, Berkeley.

DeWoody, J. A., and J. C. Avise. 2001. Genetic perspectives on the natural history of fish mating systems. Journal of Heredity 92:167–172.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and evolution of mating systems. Science 197:215–223.

Fritz, L.W., and S. A. Lowe. 1998. Seasonal distributions of Atka mackerel (*Pleurogrammus monopterygius*) in commercially fished areas of the Aleutian Islands and Gulf of Alaska. NOAA Technical Memorandum NMFS-AFSC-92.

Goulet, D. 1998. Spawning success in the damselfish *Amblyglyphidodon leucogaster*: the influence of eggs in the nest. Animal Behaviour 55:651–664.

Gross, M. R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. Trends in Ecology and Evolution 11:92–98.

Jamieson, A., and S. C. Taylor. 1997. Comparisons of three probability formulae for parentage exclusion. Animal Genetics 28:397–400.

Jamieson, I. 1995. Do female fish prefer to spawn in nests with eggs for reasons of mate choice copying or egg survival? American Naturalist 145:824–832.

Kimura, M. R., T. Yanagimoto, and H. Munehara. 2007. Maternal identification of hybrid eggs in *Hexagrammos* spp. by means of multiplex amplified product length polymorphism of mitochondrial DNA. Aquatic Biology 1:187–194.

King, J. R., and R. E. Withler. 2005. Male nest site fidelity and female serial polyandry in lingcod (*Ophiodon elongatus*, Hexagrammidae). Molecular Ecology 14:653–660.

Kodric-Brown, A. 1998. Sexual dichromatism and temporary color changes in the reproduction of fishes. American Zoologist 38:70–81.

Kodric-Brown, A., and J. H. Brown. 1984. Truth in advertising: the kinds of traits favored by sexual selection. American Naturalist 124:309–323.

Kondoh, M., and N. Okuda. 2002. Mate availability influences filial cannibalism in fish with paternal care. Animal Behaviour 63:227–233.

Koya, Y., H. Munehara, and K. Takano. 1995. Formation of egg adhesive material in masked greenling, *Hexagrammos octogrammus*. Japanese Journal of Ichthyology 42:45–52.

Kraak, S. B. M., and T. G. G. Groothuis. 1994. Female preference for nests with eggs is based on the presence of eggs themselves. Behaviour 131:189–206.

Largiader, C. R., V. Fries, and T. C. M. Bakker. 2001. Genetic analysis of sneaking and egg thievery in a natural population of the threespine stickleback (*Gasterosteus aculeatus* L.). Heredity 86:459–468.

Lauth, R. R., J. L. Guthridge, D. Cooper, and S. W. McEntire. In press. Behavioral ecology of color patterns in Atka mackerel. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science.

Lauth, R. R., J. Guthridge, D. Nichol, S. W. McEntire, and N. Hillgruber. 2007. Timing and duration of mating and brooding periods of Atka mackerel (*Pleurogrammus monopterygius*) in the North Pacific Ocean. U.S. National Marine Fisheries Service Fishery Bulletin 105:560–570.

Lauth, R. R., S. W. McEntire, and H. H. Zenger, Jr. 2006. Geographic distribution, depth range, and description of Atka mackerel, *Pleurogrammus monopterygius*, nesting habitat in Alaska. Alaska Fishery Research Bulletin 12:164–185.

Lindström, K. 2000. The evolution of filial cannibalism and female mate choice strategies as resolutions to sexual conflict in fishes. Evolution 54:617–627.

Lindström, K., and R. C. Sargent. 1997. Food access, brood size, and filial cannibalism in the fantail darter, *Etheostoma flabellare*. Behavioral Ecology and Sociobiology 40:107–110.

Mackiewicz, M., B. A. Porter, E. E. Dakin, and J. C. Avise. 2005. Cuckoldry rates in the Molly Miller (*Scartella cristata*; Blenniidae), a hole-nesting marine fish with alternative reproductive tactics. Marine Biology 148:213–221.

Mank, J. E., and J. C. Avise. 2006. The evolution of reproductive and genomic diversity in ray-finned fishes: insights from phylogeny and comparative analysis. Journal of Fish Biology 69:1–27.

Marconato, A., and A. Bisazza. 1986. Males whose nests contain eggs are preferred by female *Cottus gobio* L. (Pisces, Cottidae). Animal Behaviour 34:1580–1582.

McDermott, S. F., and S. A. Lowe. 1997. The reproductive cycle of Atka mackerel, *Pleurogrammus monopterygius*, in Alaska waters. U.S. National Marine Fisheries Service Fishery Bulletin 95:321–333.

McDermott, S. F., K. Pearson, and D. Gunderson. 2007. Annual fecundity, batch fecundity, and oocyte atresia of Atka mackerel (*Pleurogrammus monopterygius*) in Alaska waters. U.S. National Marine Fisheries Service Fishery Bulletin 105:19–29.

Munehara, H., Z. Kanamoto, and T. Miura. 2000. Spawning behavior and interspecific breeding in three Japanese greenlings (Hexagrammidae). Ichthyological Research 47:287–292.

Munehara, H., and A. Markevich. 2003. Spawning behavior of Japan Sea greenling, *Pleurogrammus azonus*, off the Bol'shoi Pelis Island, Peter the Great Bay, Russia. Bulletin of Fisheries Science of Hokkaido University 54:67–72.

Munehara, H., and T. Miura. 1995. Nonintentional filial egg cannibalism by the guarding male of *Hexagrammos otakii* (Pisces: Hexagrammidae). Journal of Ethology 13:191–193.

Munehara, H., and O. Takenaka. 2000. Microsatellite markers and multiple paternity in a paternal care fish, *Hexagrammos otakii*. Journal of Ethology 18:101–104.

Rand, K. M. 2007. Longitudinal growth differences in Atka mackerel (*Pleurogrammus monopterygius*): using a bioenergetic model to identify underlying mechanisms. Master's thesis. University of Washington, Seattle.

Reichard, M., C. Smith, and W. C. Jordan. 2004. Genetic evidence reveals density-dependent mediated success of alternative mating behaviours in the European bitterling (*Rhodeus sericeus*). Molecular Ecology 13:1569–1578.

Rico, C., U. Kuhnlein, and G. J. Fitzgerald. 1992. Male reproductive tactics in the threespine stickleback: an evaluation by DNA fingerprinting. Molecular Ecology 1:79–87.

Rohwer, S. 1978. Parent cannibalism of offspring and egg raiding as a courtship strategy. American Naturalist 112:429–440.

Sadovy de Mitcheson, Y., and M. Liu. 2008. Functional hermaphroditism in teleosts. Fish and Fisheries 9:1–43.

Sargent, R. C. 1992. Ecology of filial cannibalism in fish: theoretical perspectives. Oxford University Press, New York.

Spies, I. B., S. A. Lowe, Y. Hong, and M. F. Canino. 2005. Development and characterization of seven novel di-, tri-, and tetranucleotide microsatellite markers in Atka mackerel (*Pleurogrammus monopterygius*). Molecular Ecology Notes 5:469–471.

Taborsky, M. 1994. Sneakers, satellites, and helpers: parasitic and cooperative breeding behavior in fish reproduction. Advances in the Study of Behavior 23:1–100.

Taborsky, M. 1998. Sperm competition in fish: “bourgeois” males and parasitic spawning. Trends in Ecology and Evolution 13:222–227.

Taborsky, M. 2001. The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. Journal of Heredity 92:100–110.

Waits, L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10:249–256.

Withler, R. E., J. R. King, J. B. Marliave, B. Beaith, S. Li, K. J. Supernault, and K. M. Miller. 2004. Polygamous mating and high levels of genetic variation in lingcod, *Ophiodon elongatus*, of the Strait of Georgia, British Columbia. Environmental Biology of Fishes 69:345–367.

Yang, M.-S. 1999. The trophic role of Atka mackerel, *Pleurogrammus monopterygius*, in the Aleutian Islands area. U.S. National Marine Fisheries Service Fishery Bulletin 97:1047–1057.