NORTH PACIFIC RESEARCH BOARD PROJECT FINAL REPORT

Reproductive Ecology of Atka Mackerel *Pleurogrammus monopterygius* in Alaska

NPRB Project 522 Final Report

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March 2008

CHAPTER 3: ATKA MACKEREL MATING SYSTEM

Genetic assessment of the mating system and patterns of egg cannibalism in Atka mackerel,
Pleurogrammus monopterygius

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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 masses produced in captive and wild populations. Parentage analysis of egg masses produced by a captive population in a small exhibit tank revealed that 38% of the egg masses contained contributions by non-nest attendant males. In contrast, all egg masses produced in a large exhibit tank resulted from monogamous matings of females with resident nest-tending males, suggesting density-dependence of sneaked fertilizations. Females appeared to mate randomly with two nest-tending males in the large exhibit tank at the Alaska SeaLife Center.

Multiple parentage was detected in 35% of the egg masses collected in the field or retrieved from the stomachs of adult cannibals. Half-sib and unrelated full-sib families were found in several fused egg masses, indicating that the reproductive output from multiple males and females may be combined sequentially in a single egg mass and that nest-attendant males may abandon or be excluded from their territory during the spawning season.

Analysis of loose eggs and partial egg masses ingested by four female and 15 male cannibals showed that nearly all conspecific predation represented heterocannibalism. One instance of partial filial cannibalism was detected in a male who was one of multiple sires for that egg mass. The significance of mating and cannibalistic behaviors influenced by sexual selection in fish species with exclusive paternal care is discussed, especially with respect to other greenling species.

Introduction

Fishes exhibit the most diverse range of reproductive behaviors found in any vertebrate group (Breder and Rosen 1996). Individual species may be unisexual, gonochoristic (separate sexes), or hermaphroditic in response to environmental cues. Mating systems in fishes can range from self- and internal fertilization through varying degrees of monogamy and polygamy (with biparental and uniparental care) to broadcast group spawning. Courtship and spawning behaviors in fishes, including alternative reproductive tactics (ARTs), have formed the basis for theoretical and empirical studies assessing sexual selection theories. 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, while 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 provide some degree of parental care of offspring (Avise et al. 2002). In fishes (but in few other

vertebrate species), the most common strategy employed is for males to guard the offspring (Blumer 1982), which has been observed in approximately 70% of species exhibiting uniparental care (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, cuckoldry by both genders, and a variety of ARTs to attain 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 ARTs that would likely 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 filial cannibalism, can be readily assessed using parentage analysis.

The hexagrammid fish Atka mackerel (*Pleurogrammus monopterygius*) is a semi-demersal, schooling species distributed along continental shelves from Asia to North America. Atka mackerel spawn demersal eggs attached to hard substrates (often in crevices) that are protected from conspecific cannibalism and other predation sources by territorial males. Males aggregate and acquire nest sites 1-2 months prior to spawning at depths from 15 – 144 m (Lauth et al. 2007b). Nesting typically occurs on rocky substrates in areas with currents, but there is considerable variation among locations with respect to relief, slope, and continuity (Lauth et al. 2007b).Nests are typically about 2 m^2 in area (Fritz and Lowe 1998) and nest territories tend to be adjacent to one another (Lauth et al. 2007b). Males engage in some degree of nest clearing to expose hard substrate, as well as both active and passive nest defense (R. Lauth, AFSC, pers. comm.). Spawning typically begins in late June and females in spawning condition have been documented from July through October, a period of four months (McDermott and Lowe, 1997). Nesting males have been observed in late October, suggesting that the brooding phase may extend into the winter months (Lauth et al. 2007b). Females can potentially spawn up to six adhesive egg masses during the spawning season, each containing approximately 6700 eggs, (McDermott et al. 2007).

Males develop conspicuous sexual dichromatism and engage in active courtship displays during the spawning season. Specific details of the mating system are unknown but probably include some of the polygamous strategies found in other greenlings. Crow et al. (1997) reported that ~40% of kelp greenling, *Hexagrammos decagrammus*, nests contained egg masses from multiple females and DeMartini (1987) reported similar proportions for painted greenling (*Oxylebius pictus*). Females mate and distribute multiple egg masses with nesting males, potentially increasing their reproductive potential by partitioning egg masses to dilute the risk of egg predation and by selecting guardian male (*e.g.*, color, size) or nest

characteristics that reduce mortality. Less well understood are ARTs employed by male hexagrammids. These may include adoption of a 'sneaker' strategy, where a guardian male may be cuckolded either by adjacent nest-tending males or males who do not defend a territory but engage in parasitic fertilization with females. Sneaking has been reported in kelp greenling and other hexagrammid species (pers. communication cited in Crow et al. 1997; Munehara et al. 2000) but the reproductive consequences of this behavior have only recently been quantified using molecular genetic techniques in the fat greenling, *Hexagrammos otakii* (Munehara and Takenaka 2000) and lingcod, *Ophiodon elongatus*, (Withler et al. 2004). An examination of 13 lingcod egg masses found that each had been produced by a single female and five had been sired entirely by the attendant male. Eight egg masses had been sired by multiple males, often involving neighboring guardian males, but non-territorial males also appear to have participated in spawning. One guardian male was found to be unrelated to any eggs in his clutch, suggesting that nest takeover may represent another ART in lingcod.

Adult Atka mackerel engage in egg cannibalism (Yang 1999), males more so than females (Kim Rand, Alaska Fisheries Science Center, Seattle, WA, pers. comm.). In species with paternal care, filial cannibalism by males parasitizes the fecundity of females as a strategy to maximize their lifetime reproductive success at the expense of current broods (Rowher 1978; Sargent 1992), either by enhancing male survivorship or survivorship of the remaining offspring. Male energy reserves have often been considered as the primary factor driving filial cannibalism (DeMartini 1987; Lindström 2000) and these reserves vary seasonally in response to the duration of parental care. In addition, high population densities, female-biased sex ratios, and short refractory periods for egg clutch production increase mate availability and favor the evolution of filial cannibalism (Kondoh and Okuda 2002). Filial cannibalism by males may also benefit females if it increases the probability of rearing the remaining offspring successfully (Lindström 2000). DeMartini (1985) reported instances of both heterocannibalistic raiding of nests by guardian males and some paternal filial cannibalism in the painted greenling. Filial cannibalism has also been observed in the fat greenling, *Hexagrammos otakii*, when egg masses detached from roots of seaweeds to which they had been fastened and were no longer under direct male control (Munehara and Miura 1995).

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 from developing embryos to test two null hypotheses listed in the research proposal. The first null hypothesis tested was that mating is random in Atka mackerel and that egg mass production involves one fish of each gender (*i.e.* monogamy). It was primarily addressed through parentage analyses of captive populations where genetic data were

available for all potential parents. Additional inferences on aspects of the mating system were derived from parentage analyses of egg masses 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. The second null hypothesis tested was that egg cannibalism is random in Atka mackerel for both genders. Genotypic data were used to determine relatedness of male and female cannibals with the embryos they had consumed, providing the first analysis of cannibalism patterns using molecular markers in this species.

Objectives

The primary objectives for this portion of the project was to examine the mating system of Atka mackerel through genetic parentage analysis of egg batches produced in both captive and wild populations and to evaluate gender-specific patterns of egg cannibalism.

Methods

Samples from captive populations

Parentage was assessed for embryos in egg masses produced in captivity from two separate aquariums: a **small tank** (~ 10,250 L, 1.5 m depth) exhibit and a **large tank** (~ 400,000 L, 6.4 m depth) exhibit at the Alaska SeaLife Center where adult Atka mackerel had been successfully spawning for several years. The small tank contained two males and four or five females in 2004-2006 with 4.0 m^2 of spawning substrate available in 2004 that was increased to 7.2 m^2 in 2005 and 2006. The large tank contained 13 females and eight males in 2005 and 11 females and six males in 2006 with > 50 m^2 of spawning substrate available in both years (Table 3). Fin clips from all adults were collected for parentage analysis. Natural markings from each male in the small tank and six known males in the large tank were documented for subsequent visual identification of males guarding territories.

Eggs were removed from the small tank soon after first discovery to deter cannibalism by adults and the time, location, and the guarding male were documented. During 2004 and 2005 eggs were incubated at controlled temperatures used in an embryonic development study (see Guthridge and Hillgruber, this report), then preserved in 95% nondenatured ethanol (ETOH) for genetic analysis when eyes were readily visible. We subsequently found that we could determine parentage of embryos at much earlier stages of development. Thus, egg masses were removed from nesting sites after first discovery in 2006 and incubated at controlled temperature regimes for approximately two weeks before preservation.

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, when the first group of egg masses was removed from the tank by divers. Egg masses 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 days, when a second group was removed. They continued spawning for an additional 38 days before the final group of egg masses 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 and collected egg masses from a single territory guarded by a male throughout the season.

Samples from natural populations

Cannibalized egg masses: Adult Atka mackerel were collected by trawling between Unimak Island and Amchitka Island, Alaska during three cruises from 2004-2007. Egg cannibalism was inferred from the appearance of the stomach in routine dissections for determining sex. 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 egg masses, if present, were counted, briefly washed in seawater, and preserved in nondenatured 95% ETOH for parentage analysis

Eggs collected by trawl: Six intact egg masses were collected during an Atka mackerel tag recovery cruise aboard a chartered commercial bottom trawler (see Cooper et al., this report) in the Aleutian Islands (52.046 °N, 172.025°W) on October 13, 2006. These egg masses were frozen at -20 °C and subsequently preserved in 95% nondenatured ETOH.

DNA extraction, amplification, and analysis

Genomic DNA from samples of eggs and fin clips (from captive adults and cannibals), was extracted using DNeasy tissue kits (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions with one modification for egg samples: due to the small size of developing embryos, an elution buffer volume of 60 μL was used to provide more concentrated DNA template. Four polymorphic microsatellite loci (*Pmo70, Pmo152, Pmo367, Pmo399*) isolated from Atka mackerel (Spies et al. 2005) and two loci isolated from lingcod, *Ophiodon elongatus*, (*Oel42, Oel32*; Sewall Young, Washington Department of Fish and Wildlife, Olympia, WA, pers. comm.) were amplified via the polymerase chain reaction (PCR) as described in Spies et al. (2005). Bovine serum albumin (New England Biolabs, Ipswich, MA) was added to a final concentration of 0.5mg/ml in PCR reactions involving embryos. Loci

were amplified and scored two at a time in the following order, *Pmo152, Pmo70, Pmo399, Oel32, Pmo367, Oel42* on eggs randomly selected from egg masses from wild and captive populations until sibling relationships could be accurately assessed or, in the case of cannibalism, exclusion of the cannibal 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 groups in samples from wild populations, and exclusion of cannibals as parents of ingested embryos. Genotyping was conducted using a LI-COR 4200 Infrared Automatic DNA Sequencer (LI-COR Biotechnology, Lincoln, NE) and analyzed with LI-COR Saga[GT] genotyping software.

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 $n \geq \ln(0.05)/\ln(1-p)$ (DeWoody and Avise 2001). Thus, genotyping 96 individuals had the power to detect embryos produced by other parents if their contributions to an egg mass 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 egg masses produced in the large tank and subsequently altered the sampling regime based upon the results. Six of the 17 egg masses produced in 2005 were exhaustively sampled ($\geq$ 90 eggs) and all 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), three to six embryos were sampled from each egg mass in order to identify the female parent in a study of fecundity and egg energy content (see Cooper et al., this report) and thus egg masses were not exhaustively sampled. Incompatibility of genotypes from ingested embryos provided conclusive proof of non-parentage for egg cannibals. 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). Since multilocus genotpyes of cannibals could be determined, we calculated exclusion probabilities using the general formula for one unknown parent. Microsatellite data from 553 adult Atka mackerel collected during 2004-2006 (I. Spies, M. Canino, AFSC, unpublished data) were used to generate allele frequencies at the six loci used for parentage analysis. The six loci had 12 - 92 alleles each, resulting in single locus exclusion probabilities ranging from 0.424 - 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. In general, we attempted to genotype a minimum of 24 individuals at four loci from each clutch to determine sibship relationships and conducted more exhaustive sampling when preliminary results indicated the presence of half-sibs or unrelated individuals.Construction of full- and half-sib families detected in cannibalized and field-collected egg

masses, where parental genotypes were 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 (mtDNA) 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) using Pro-L and H16498 primers previously used for successful detection of maternal haplotypes in kelp greenling, *Hexagrammos decagrammus*, (Crow et al. 1987) 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 protocols by (Kimura et al. 2007) in 9 individuals. PCR amplicons were purified and sequenced at the High-Throughput Genomics Unit (HTGU) 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 concluded that haplotypic variation was likely to be at frequencies too low to be informative for maternal identification in egg masses and parentage analysis was conducted using only microsatellite DNA loci.

Parentage – small tank

At the onset of nesting and spawning in mid-July of 2004, one territory was established by male M2 on the available 4.0 m^2 of spawning substrate. Male M2 was displaced by male M1 one month later in August and was the only male observed exhibiting territorial behavior and guarding the nest and eggs for the remainder of the 2004 spawning season. Two different males (M3 and M4) were used in 2005 and 2006 and the available spawning substrate was increased to 7.2 m^2. Both males established and guarded individual adjacent territories, approximately 3.6 m^2 each, and the eggs within for the duration of the 2005 spawning and nesting season. The same territories were reestablished in 2006 and defended until male M3 died in September.

Parentage was assessed for a total of 79 egg masses produced in 2004-2006 (Table 3). Male M2 dominated the matings in 2004, apparently siring all embryos in 12 of 13 egg masses analyzed that year. However, he was the nest-tending male for only three of his 13 individually sired egg masses. There was no evidence that a single egg mass was produced by more than one female. Dual paternity was detected in one egg mass and coincided with the displacement of male M2 by male M1 from the nesting territory. Nine egg masses were sampled (24 – 96 embryos each) following the displacement event and no evidence for paternity by male M1 was found despite the fact that he continued to guard the territory for two additional months after displacing male M2. In 2005 and 2006, new males M3 and M4 were successful in

establishing territories and securing matings with multiple females after additional substrate had been added to the tank. Most egg masses produced in 2005 and 2006, 73.7% and 71.4% respectively, resulted from monogamous matings of females with resident nest-tending males. However, samples from 26.3% of the egg masses produced in 2005 and 23.8% in 2006 showed that the non-attendant male had sired all of the three to six embryos sampled from the egg mass. One instance of dual paternity was confirmed for one egg mass in 2006. Females produced an average of 6.07 egg masses per year, roughly equivalent numbers of egg masses in 2005 and 2006, and showed no overall preference for mating with either male during 2005 (sign test, $P = 0.256$) but a significant preference for male M4 in 2006 ($P \leq 0.001$).

Parentage - large tank

Each male in the large tank established and guarded a territory for the duration of the spawning and nesting season in 2005 and 2006 that ranged from 4.0 m^2 to 12.0 m^2 in size. In 2005 the territories of male 25 and 8 were sampled and each was approximately 6.0 m^2. Male 8 established a new territory in 2006 which was the largest observed at 12.0 m^2. Parentage analysis was conducted on 23 egg masses produced in the large exhibit tank during 2005 and 2006 (Table 4). No evidence of multiple parentage was found in five egg masses that were exhaustively sampled (*i.e.* greater than 90 embryos genotyped) or in remaining egg masses that were sampled less intensively. Our sampling detected that female 14 spawned only with male 25 in 2005, but females as a group exhibited no significant preference for mating with either of the territorial males (sign test, $P = 0.332$).

Field samples

Genotypes were obtained for embryos in intact partial egg masses and loose individual eggs retrieved from the stomachs of 19 adult Atka mackerel (Table 5). In some cases, embryos were too undeveloped and/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 genetically incompatible as parents of the embryos they had consumed. One male (2004-01) was identified as the sire of 11 of 94 (12%) late-stage eyed embryos in the egg mass with a combined exclusion probability of $P >$ 0.999. This result conclusively demonstrated filial cannibalism in male Atka mackerel but it was unknown whether this individual was the custodial parent.

The majority (69%) of intact egg masses collected in the field appeared to result from exclusively monogamous matings. Multiple parentage was detected in seven out of 20 intact egg masses (*i.e.* excluding loose eggs) ingested by cannibals, and in one of six egg masses collected in trawls (Fig. 1). Embryo genotypes were inconsistent with any single parental multilocus genotype across loci, indicating the presence of unrelated families in a single egg mass. In the case of the male filial cannibal (2004-01),

we reconstructed maternal genotypes from half-sib families he had sired (Fig. 1). This male produced three half-sib families representing 12% of the embryos assayed. The remaining embryos in the egg mass contained three more half-sib groups produced by four unrelated parents of unknown sex and individuals from five additional unrelated full-sib families. Other egg masses also exhibited varying levels of multiple parentage (Fig. 1). While these egg masses appeared to represent a single batch spawned by one female, one (unanalyzed) egg mass split apart following preservation (Fig. 2), suggesting that female Atka mackerel may deposit their eggs on top of others in the nest.

Discussion

Molecular assessment of the mating system combined with observations of nest guarding males in captivity and gender-specific egg cannibalism in Atka mackerel revealed patterns consistent with other hexagrammid species, documented behaviors previously inferred from field studies, and provided a novel observation regarding egg mass deposition in this species. Atka mackerel exhibited a polygynandrous mating system, where females deposit egg masses with multiple males during the course of the breeding season. Captive females mated with nest-attendant males in the large exhibit tank who monopolized all fertilization events within their territories. It is noteworthy that the only two documented instances of parasitized fertilizations occurred in the small (10, 250L) tank, which had higher densities at nesting sites than the large exhibit tank. The frequency of egg masses produced in captivity by strictly monogamous pairings of females with nest-attendant males was lowest in the small tank where the spawning substrate was limited and highest (100%) in the large tank where spawning substrate exceeded 50 m^2. Nest-attendant males continued to defend and maintain nests in the small tank even though they were not the sole sire of some egg masses within their territories. Densities in the captive tanks likely affected the frequencies of male ARTs by favoring territoriality when densities were low and shifting toward sneaking when densities were high. Reichard et al. (2004) reported that sneaking behavior increased significantly with male density in the European bitterling, *Rhodeus sericeus*.

Parentage analysis using microsatellite DNA markers unequivocally documented parasitic fertilizations of egg masses by males in captivity and inferred possible instances in natural populations. Male cuckoldry is common among fishes exhibiting paternal care and ARTs have evolved in response to competition for access to mates (Gross 1991, Taborsky 1994). In nest-tending species, the most common ART documented by genetic parentage assessment is sneaked fertilizations, where the attendant male is often the sire of most but not all of his custodial offspring (DeWoody and Avise 2001, Avise et al. 2002, Mackeiwicz et al. 2002; Mackiewicz et al. 2005). Sneaking strategies are associated with large size, strong sexual dimorphism, and active courtship and nest defense behaviors by males (Gross 1996, Taborsky 1998). These characteristics are common in hexagrammid species in general, and sneaking has

been observed in three greenling (*Hexagrammos*) species (Munehara et al 2000), documented using molecular genetic analyses in the fat greenling (Munehara and Takenaka 2000) and lingcod (Withler et al. 2004), and is the most likely source of cuckoldry in Atka mackerel. In addition to sneaking, the amount and timing of sperm release may represent an ART involving sperm competition. Munehara and Takenaka (2000) observed two patterns of sperm emission, with different rates of fertilization success, in male fat greenling, *H. otakii*. When the 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.

Samples produced in natural populations, where parental genetic data were available, were more challenging to analyze and interpret than those from captive matings. The apparent lack of D-Loop and cytochrome b variation in Atka mackerel was surprising given that Crow et al. (1987) detected five D-Loop haplotypes in kelp greenling and Withler et al. (2004) reported 10 unique haplotypes in a sample of 15 lingcod. The mitogenomic homogeneity of Atka mackerel precluded using mtDNA haplotypes to determine individual female identity in egg masses retrieved from cannibals or captured in trawls. However, the presence of both unrelated and half-sib families detected with microsatellite loci indicated that several females and males may contribute to a single egg mass within a nest. Previous studies have considered an egg mass to be the reproductive output from an individual female. This assumption has been inferred from the physical discreteness, yolk coloration, and stage of embryonic development in kelp greenling and whitespotted greenling, *Hexagrammos stelleri* (DeMartini 1986), and documented using genetic studies in kelp greenling (Crow et al. 1997) and lingcod (Withler et al. 2004). This appears to be the general case for Atka mackerel as determined from parentage analysis of egg masses produced in captivity. However, the presence of multiple half-sib families (in the one case of filial cannibalism where maternal genotypes could be reconstructed) and unrelated families within a single fused egg mass indicates that the reproductive output from several females may be physically adhered. It seems unlikely that this result could be an artifact produced by cannibalism. Koya et al. (1995) reported that the adhesive surrounding the eggs of the fat greenling required several hours to harden. All embryos in the partial egg mass retrieved from the filial cannibal were in the eyed stage, indicating that they were not consumed immediately post-spawning and then became adhered in the stomach. One behavior that could plausibly account for this observation is the 'pushing' of fertilized egg masses into crevices by females. In the congeneric Okhostk atka mackerel, *Pleurogrammus azonus*, females were observed to wedge their fertilized egg masses into gaps between rocks from various directions using their pectoral fins or side of their belly (Munehara and Markevich 2003). Female Atka mackerel in captivity have been observed using their mouths to push freshly fertilized eggs into crevices (J. Guthridge, ASLC, unpublished data). This behavior, coupled with multiple (and some unrelated) matings over a short period of time (before the egg

adhesive fully hardens) is suggested by the layering of multiple egg masses (Fig. 2) and could explain the mixed parentage observed in some egg masses.

The presence of unrelated families within an egg mass may indicate nest takeover events resulting from the death, abandonment or expulsion of the resident guardian male. Alloparental care by males has been documented genetically in other fishes (DeWoody et al. 2001) including lingcod (Withler et al. 2004).The selective factors responsible for evolution of this ART are unclear but may provide a usurper male with a territory if there is limited nest-site availability or increase reproductive fitness if females prefer to mate with males when eggs are present in the nest (Marconato and Bisazza 1986, De Martini 1987, Kraak and Groothuis 1994, Goulet 1998). This advantage of nest takeovers may result from mate choice copying by females, because egg masses are visual evidence of mate choice by other females, or possibly represent evidence of the quality of spawning site (Jamieson 1995).

Nest fidelity for male Atka mackerel may not extend for the entire spawning season. Cooper et al. (this report) found that a substantial percentage of males captured during the spawning season was not actively producing sperm, perhaps indicating that some males may not spawn every year or not for the full duration within a given season. Lauth et al. (2007b) inferred duration of nesting based upon archival tag data for three adult male Atka mackerel tagged in late July. Two of the males abandoned nesting locations after one month and a third appeared to leave one nest site and establish another at shallower depth. These behaviors may represent expulsions by other males or voluntary abandonment of a site if males failed to attract mates. If previous egg masses were present and not cannibalized at the time of nest takeover by new guardian males, subsequent egg deposition by females upon existing egg masses could produce unrelated full- and half-sib families within a single nest.

Egg cannibalism constitutes a significant dietary component for both genders of Atka mackerel during the spawning and brooding seasons (Yang 1999, Rand 2007). Females engage in cannibalism less than males (K. Rand, AFSC, personal communication), most likely because of the greater energetic demand on males, who do not forage while brooding, to defend their nests from conspecific predators. Female fat greenling, *H. otakii*, have been observed pecking at egg masses already present in nests, causing attendant males to repulse them without successful matings (Munehara et al. 2000). Filial cannibalism is more common in males in species with uniparental care, and is thought to occur across a range of compromise strategies between current and future reproductive success (Rowher 1978). 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, while 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 compared with larger and older ones

representing greater parenting costs to the male (DeMartini 1987, Lindström and Sargent 1997). While 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 are female-biased sex ratio, an extended period of male care, and a short refractory period between spawnings by females (Kondoh and Okuda 2002). The single incidence of male filial cannibalism documented in this study occurred at a time when the spawning phase of seasonal reproduction was largely over (October) and the brooding phase had commenced. The reproductive status of this male (*e.g.* custodial parent, satellite or sneaker male, etc.) was unknown and thus it is not possible to assess whether it represented voluntary filial cannibalism. The predominance of heterocannibalism in males suggests that the high population densities in nesting areas and communal nesting habits of this species may make conspecific nest raiding a more adaptive strategy than filial cannibalism.

Conclusions

Atka mackerel exhibits a polygynandrous mating system similar to other species within the family Hexagrammidae. Genetic parentage analysis determined that most egg masses produced in captive and wild populations resulted from monogamous matings. Parasitized fertilizations were documented in captivity where nesting habitat was limiting, but absent when nesting densities were lower. Multiple parentage occurred in about 35% of egg masses produced in wild populations. Unfortunately, lack of mtDNA variation did not allow us to fully elucidate parental contributions to egg masses when multiple parents were involved. Parentage analysis excluded all females and nearly all male egg cannibals as possible parents of eggs they had consumed (heterocannibalism). A single instance of male filial cannibalism allowed us to reconstruct full-and half-sib familes sired by that male, plus other unrelated families in a single intact egg mass. Other instances of multiple family groups within a single egg mass were detected and suggest that multiple spawning events over relatively short periods, egg mass manipulation by females, and nest takeover/abandonment by males may lead to a complex assortment of related and unrelated offspring within the egg mass. Our results underscore both the power and limitations of molecular genetic approaches for making inferences about the reproductive behaviors and their consequences in this species. Better resolution of these behaviors and their consequences will likely be obtained if future efforts combine both observational (*e.g.* SCUBA, drop camera) and genetic methods in studies of wild populations.

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Tables

Table 1. Number of females and males resident to the large tank (LT) and small tank (ST) at the Alaska SeaLife Center, the number of egg masses analyzed in each year, and the available spawning substrate (m^2).

Table 2. Locus variability, expected (H_e) and observed (H_o) heterozygosities, and exclusion probabilities (*P*) for six microsatellite loci used in parentage analysis of Atka mackerel.

Table 3. Parentage analysis of Atka mackerel egg masses produced in the small (10,250 L) tank at the Alaska SeaLife Center in 2004-2005. Dam and sire identities (bold) and number of egg masses produced and sired, respectively (in parenthesis). Egg masses with multiple sires are indicated. † Female died and was not replaced.

Table 4. Parentage analysis for Atka mackerel egg masses produced in the large exhibit tank at the Alaska SeaLife Center in 2005-2006. Territories were defended by the associated sires.

Table 5. Samples of Atka mackerel embryos collected from cannibals or in trawls. Cannibal gender, collection dates, standard lengths, numbers of ingested egg masses and embryos minimum number of parents are given.

Table 1.

Year	tank	# females	# males	# egg masses analyzed	spawning substrate (m^2)
2004	ST	4	2	13	4.0
2005	ST	5	2	38	7.2
2006	ST	4	2	27	7.2
2005	LT	13	8	17	> 50.0
2006	LT	11	6	6	> 50.0

Table 2.

Locus	# of alleles	H_e	H_o	P
Pmo70	31	0.894	0.906	0.647
Pmo152	50	0.947	0.930	0.805
Pmo367	74	0.955	0.916	0.834
Pmo399	19	0.786	0.833	0.408
Oel42	92	0.966	0.968	0.873
Oel32	12	0.794	0.712	0.424
All loci				0.99951

Table 3.

year	# egg masses assayed	Dam ID (# egg masses produced)					sire ID (#egg masses sired)		# partial egg masses sired
2004	13	**F1** (6)	**F2** (2)	**F3** (3)	**F4** (3)		**M1** (0)	**M2** (13)	1
2005	38	**F1** (12)	**F2** (7)	**F32** (7)	**F4** (6)	**F33** (6)	**M3** (15)	**M4** (23)	0
2006	28	**F1** (9)	†	**F32** (6)	**F4** (5)	**F33** (6)	**M3** (3)	**M4** (24)	1

Table 4.

Egg mass ID	guarding male	collection date	# embryos assayed	sire ID	dam ID
B	25	8/25/2005	56	25	9
C	25	8/25/2005	92	25	14
J-1	25	8/25/2005	46	25	22
J-2	25	8/25/2005	50	25	23
I-1	25	8/25/2005	30	25	14
I-2	25	8/25/2005	20	25	16
I-3	25	8/25/2005	12	25	18
I-4	25	8/25/2005	12	25	19
I-5	25	8/25/2005	22	25	23
O	25	9/6/2005	94	25	14
Q	25	9/6/2005	96	25	14
R	8	10/14/2005	95	8	23
U	8	10/14/2005	95	8	24
X-1	8	10/14/2005	84	8	9
X-2	8	10/14/2005	12	8	13
Y	8	10/14/2005	95	8	19
Z	8	10/14/2005	71	8	23
1	8	10/24/2006	30	8	11
2	8	10/24/2006	32	8	19
3	8	10/24/2006	31	8	22
4	8	10/24/2006	29	8	19
5	8	10/24/2006	30	8	24
6	8	10/24/2006	30	8	18

Table 5.

sample ID	Type	sex	collection date	length	Number of egg masses	# embryos assayed	Minimum # of parents
2004-03	cannibal	M	10/11/2004	35	Loose eggs	17	5
2004-05	cannibal	M	10/11/2004	39	Loose eggs	94	6
2005-04	cannibal	F	8/28/2005	37	Loose eggs	26	2
2005-05	cannibal	F	8/28/2005	37	Loose eggs	7	2
2004-01	cannibal	M	10/11/2004	39	1	94	9
2004-06	cannibal	M	10/11/2004	43	1	48	2
					2	48	2
2004-08	cannibal	M	10/11/2004	48	1	48	2
					2	48	2
2005-01	cannibal	F	8/28/2005	41	1	48	3
					2	48	5
2005-03	cannibal	F	8/28/2005	42	1	58	4
2007-01	cannibal	M	10/11/2007	42	1	21	2
2007-05	cannibal	M	10/14/2007	39	1	13	3
					2	10	2
2007-06	cannibal	M	10/11/2007	40	1	7	2
2007-08	cannibal	M	10/11/2007	42	1	21	2
2007-14	cannibal	M	10/11/2007	38	1	12	4
					2	8	2
2007-15	cannibal	M	10/11/2007	38	1	17	2
2007-16	cannibal	M	10/11/2007	40	1	43	7
2007-17	cannibal	M	10/10/2007	39	1	9	2
2007-19	cannibal	M	10/11/2007	42	1	9	2
2007-20	cannibal	M	10/11/2007	43	1	9	2
A	field	NA		NA	1	24	2
B	field	NA		NA	1	72	2
D	field	NA		NA	1	107	8
E	field	NA		NA	1	36	2
F	field	NA		NA	1	24	2
G	field	NA		NA	1	19	2

Figures

Figure 1. Family relationships of embryos in eight Atka mackerel egg masses collected from cannibal stomachs or by trawl (Wild egg mass D). Boxes to the right of the sample name represent matings inferred from parentage analyses. Rows within boxes show the minimum number of parents to produce the observed offspring. Boxes with one fish each in upper and lower rows (two parents) represent matings that produced unrelated full-sib families and those with two or more fish in lower rows depict half-sib families. The number of embryos (n) detected in each family and the number of loci used to determine parentage are given. Genders of individual parents are known only for one half-sib group in the top left of figure.

Figure 2. Two separate Atka mackerel egg masses that had fused together and separated after preservation in 95% ETOH.
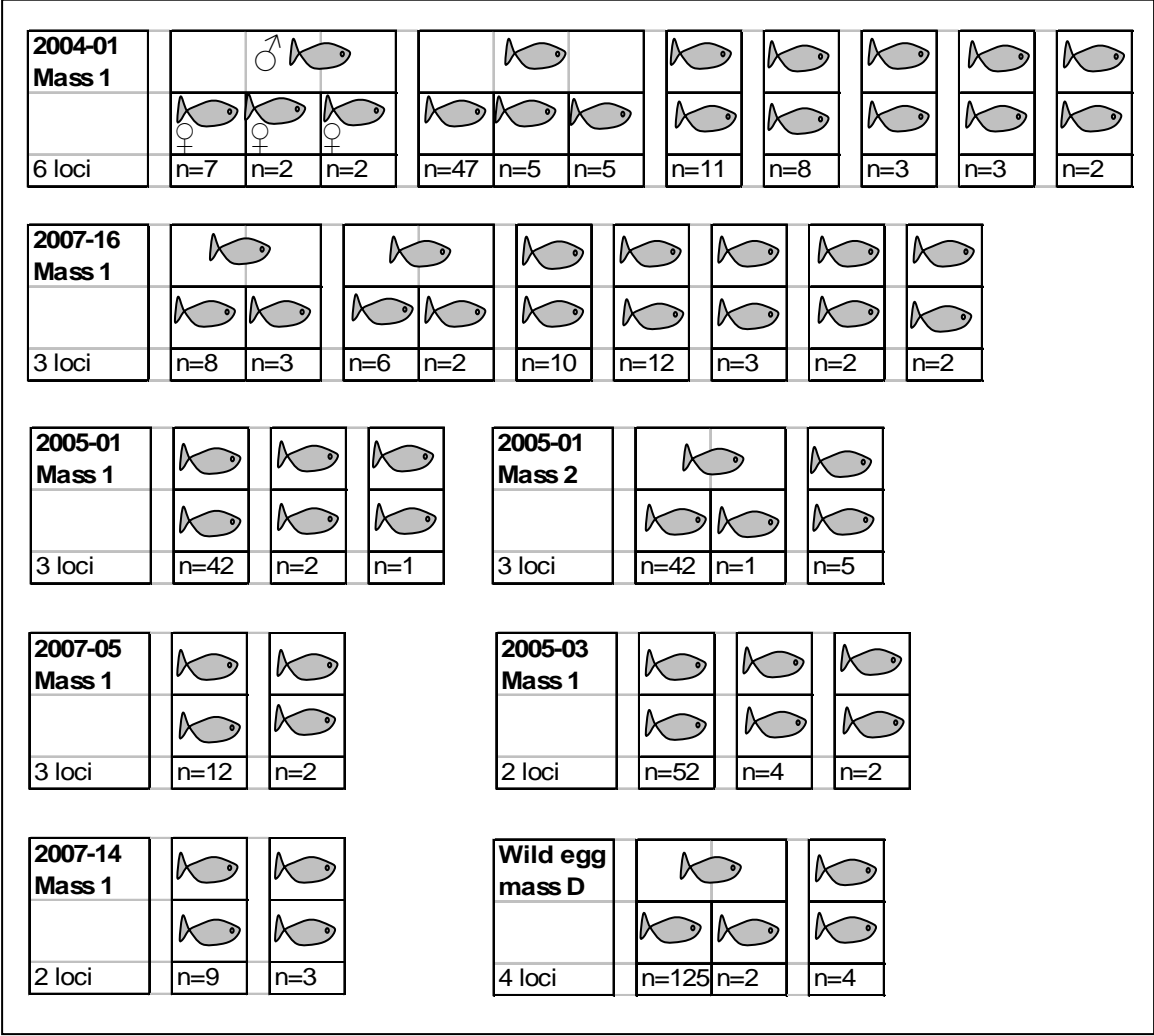
Figure 1.

Figure 2.