Male nest site fidelity and female serial polyandry in lingcod (Ophiodon elongatus, Hexagrammidae)

JACQUELYNNE R. KING and RUTH E. WITHLER
Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, B.C., V9T 6N7, Canada

Abstract
Nest site fidelity and serial polyandry were examined in lingcod, Ophiodon elongatus, a teleost fish in which the nest-guarding male parent invests more heavily in parental care than the elusive female parent. Lingcod parental and progeny genotypes were established for fish spawning on a 200 m² section of Snake Island reef, British Columbia in two successive years to evaluate male and female mate choice (monogamy or polygamy) and nest site reuse by the same parents (nest site fidelity) and/or different parents (nest site affinity). Thirteen nests (egg masses) guarded by nine males and 14 nests guarded by seven males were observed in 2002 and 2003, respectively. No female laid more than one nest per season or spawned in the study area in both years. In contrast, at least six (86%) and possibly all seven (100%) of the 2003 guardian males had been guardian or auxiliary males in 2002. Both nest site affinity and extreme male nest site fidelity were observed, with at least four males reusing the exact same nest site. Serial polyandry resulting from the high male and low female nest site fidelity is consistent with predictions based on a low female parental investment and high rate of progeny loss to predation and cannibalism. Male polygyny, achieved primarily by cuckoldry within seasons, was enhanced by the lack of female fidelity between seasons. Polygamy in both sexes of nest-tending marine fish may minimize reproductive skew and maximize genetic diversity within populations.

Keywords: lingcod, nest site fidelity, polyandry, polygyny

Received 24 June 2004; revision received 4 November 2004; accepted 29 November 2004

Introduction
Among oviparous teleost fish, parental care may be provided to offspring by either, both or neither of the parents, providing incentives for both sexes to maximize their reproductive success through a wide range of mating behaviours ranging from complete monogamy to extensive polygamy. Males are the primary caregivers in many species (Blumer 1979), responsible for establishing a nest or nesting territory, wooing females and defending the fertilized eggs resulting from their courtships. Genetic studies on nest-tending species indicate that guardian males are commonly polygynous within and between breeding seasons, mating with multiple females who deposit eggs in nests guarded by themselves or other males (DeWoody & Avise 2001). Females, whose fecundity is limited by body size and who depend upon their partner(s) to adequately protect their offspring, presumably have the choice between committing to monogamy with an apparently superior male or hedging their bets by breeding with multiple males within a breeding season (polyandry) or between breeding seasons (sequential polyandry). Documented examples of polyandry are few (DeWoody & Avise 2001) and little is known about the occurrence of sequential polyandry in teleost fish.

Lingcod (Ophiodon elongatus) is a demersal marine teleost species of the eastern Pacific Ocean that inhabits nearshore rocky reefs predominantly at depths from 10 to 100 m. It is a large carnivore with maximum sizes of approximately 90 cm for males and 152 cm for females. Males mature at age two and have been known to live up to 14 years, whereas females mature at ages 3–5 and may live to an age of 20 years. Spawning occurs annually in January through March but males arrive in spawning areas and establish territories as early as November. During spawning, which likely occurs at night (Low & Beamish 1978), females lay a single large mass of up to 500 000 eggs (referred to as a nest) in
rocky crevices or under rocks within a male territory. The female leaves the nesting area after spawning but the male remains in close proximity to the nest and actively guards it against predation for 6 weeks until hatching. Two types of guarding behaviors have been observed: (i) males lying directly on or beside the nest and remaining nearly motionless unless touched, and (ii) males lying on a sentry post and defending the nest when other fish swam close (O’Connell 1993). Males typically guard a single nest, but have been reported to guard up to three nests in close proximity (Low & Beamish 1978; King & Winchell 2002).

Lingcod in the Strait of Georgia, British Columbia is considered an overfished population (Cass 1985) with early 1990s estimates of spawning biomass at only 5% of observed historic levels (Martell & Wallace 1998). Recent success in year – class strength has been observed but the stock is still considered to be of conservation concern (King 2001). Nevertheless, a high degree of genetic diversity at microsatellite loci was observed in adult lingcod that is sampled from the region and attributed, at least in part, to a highly polygynous mating structure (Withler et al. 2004). Genetic analyses of tissue samples from lingcod guardian males and fertilized eggs from their 13 nests on Snake Island reef in 2002 revealed that females were monogamous within breeding seasons, with each egg mass laid by a single female and no female spawning more than once. Males guarded an average of fewer than 1.5 nests but achieved a high degree of polygyny through cuckoldry. Thus each nest, consisting of the eggs of a single female, had been fertilized by between one and five males. Cuckolding males included neighbouring male guardians and, likely, non-guarding males. In all, the genotypes of 21 different males were identified among the progeny of the 13 nests attended by nine guardian males.

Lingcod are territorial and have a narrow home range (Matthews 1992), making it possible that lingcod return to the same reef each year to spawn. However, the observed polygyny may serve as a mechanism to maintain diversity in lingcod populations and a further increase in effective population size might occur if either males or females, or both, failed to demonstrate interannual fidelity to nest sites, choosing to spawn on different reefs or different areas of the same reef, in different years. For females, serial polyandry would not only increase the genetic diversity among progeny, but also reduce the costs of a mating with a poor guardian in any one season. Lingcod eggs and juveniles often experience high levels of cannibalism and predation mainly by invertebrates, such as gastropods (Calliostoma ligatum) and sea stars (Pycnopodia helianthoides), and by sculpins (Artedius harringtoni, Jordania zonope), kelp greenling (Hexagrammos decagrammus) and striped seaperch (Embiotoca lateralis) (Low & Beamish 1978; King & Beaith 2001). This leads to the prediction that whereas female are monogamous within a breeding season, they may practice serial polyandry in part to counter the effects of predation and cannibalism. Observations made during nest density surveys suggested that the same locations were being used by lingcod as nesting sites between years (King & Winchell 2002) but provided no information on exact nest location or on male and female site fidelity.

In this study, we have examined nest site fidelity in male and female lingcod by comparing the genotypes of parents that spawned on the Snake Island reef in 2003 with those observed in the 2002 study (Withler et al. 2004). Male and female genotypes for the parental lingcod were again determined by microsatellite analysis of tissue samples from guardian males and egg samples from their nests. The analysis allowed us to distinguish between nest site fidelity (i.e. when an individual selects the same nest site each year) and nest site affinity (i.e. when a nest site, as a result of habitat characteristics, is selected each year by different lingcod). We also tested our prediction that females would practice a bet-hedging strategy of serial polyandry by mating with different males in the second spawning season.

**Study site**

Snake Island reef, situated in the Strait of Georgia (Fig. 1), is the subject of ongoing lingcod nest density surveys (King & Beaith 2001; King & Winchell 2002). The study area, approximately 10 by 20 m with an average depth of 10 m, was noted for recent relatively high nest densities (King & Beaith 2001) and marked with a dive buoy (49°12′43.7″N; 123°53′04.7″W) throughout the study. The study site tended to be flat and open without large flora such as *Agarum* spp. but containing several large rocks and boulders to provide nesting sites.

**Methods**

**Nest site identification**

As part of a genetic study investigating polygamous mating in lingcod (Withler et al. 2004), nests were located by scuba divers in the study site 1–27 February 2002. Each nest was marked with a numbered galvanized spike and the relative orientation (i.e. under a rock or in a crevice) was noted. The nest volume was estimated using length, width and height (nearest cm) measurements made underwater. Given the difficulty in measuring irregularly shaped nests under rocks, measurements were made several times and the average (nearest 0.5 L) was used. At the end of the nesting season, the galvanized spikes were replaced with rocks with plastic numbers attached. Distances between nests were measured to the nearest 0.5 m to develop a map of nest locations.

On 4–21 February 2003, the numbered rocks were located and the locations of the 2003 nests were again marked with numbered galvanized spikes. Nest sites and volumes were calculated as in 2002.

Guardian male identification
Guardian males were attributed to a nest if they were within 2 m of it and exhibited protective or territorial behaviour when a diver approached. We used a coding system for labelling guardian male data and samples: Ny; where N denotes the nest number the male was guarding, and if guarding multiple nests, the male was given only one number from the group of nests; y denotes the year that observations were made. All nests tended by a guardian male were noted and the total length (to the nearest cm) of each guardian estimated by pulling a tape measure alongside the fish as it rested on the bottom. Tissue samples of guardian males were collected in situ by scuba divers using sharp scissors from the terminal tip of the caudal fins. Fin tissue samples were placed in a labelled tube filled with ambient seawater that was later replaced with ethanol.

Egg sample collection and microsatellite analysis
Divers cut egg samples (approximately 1 mL of eggs) from each nest in 2003 and placed in a tube filled with seawater, later with 95% ethanol on shore. Fifteen eggs were removed from each egg sample and subjected to microsatellite analysis.

DNA was obtained from individual eggs or fin tissue (from guardian male) using QIAGEN DNeasy Kits. Five microsatellite loci were assayed: Oel 32, Oel 35, Oel 41, Oel 42 and Oel 45 (Sewall Young, Washington Department of Fish and Wildlife, personal communication). Primers were labelled fluorescently and PCR (polymerase chain reaction) products were run on gels on an ABI 377 automated DNA sequencer. Allele sizes were determined with GENESCAN 3.1 and GENOTYPER 2.5 software (PE Biosystems). These loci possessed between eight and 19 alleles each and heterozygosity values ranging from 83% to 91% in a sample of 80 adult lingcod genotypes from the sample area (Withler et al. 2004). We calculated the probability of identity (P_{ID}) (i.e. the probability of obtaining identical genotypes from two different individuals) for each locus according to equation:

$$P_{ID} = 0.25 + (0.25 \sum p_i^2) + [0.5(\sum p_i^2)^2] - (0.25 p_i^2)$$ (Waits et al. 2001).

This conservative estimate of $P_{ID}$ is actually the estimated likelihood of two full-sib individuals carrying the same genotype and was recommended for use in studies of natural populations in which the possibility of encountering relatives is high (Waits et al. 2001). The $P_{ID}$ across all five loci was calculated as the product of the values obtained at the individual microsatellite loci.

The allelic combinations observed in the progeny at each locus were used to reconstruct parental genotypes. Egg samples from all nests included some eggs fertilized by one or more of the guardian males that were genotyped from fin tissue. This made reconstruction of the maternal genotype apparent by subtraction of paternal alleles and also enabled determination of genotypes for auxiliary males that contributed to fertilization.

Results
Nest site affinity

In 2002, nests 1 through 16 were located, although three of them (numbers 7, 9 and 14) disappeared before genetic
sampling, presumably resulting from predation or becoming dislodged as a result of wave action (Fig. 2, Table 1). Nine male lingcod guarded the 13 nests and fin tissue samples were obtained from six of them (Table 1). In 2003, 14 nests were observed, nine of which were located at the exact same rock or crevice as nests observed in 2002 (Fig. 2, Table 1). The numbers used to identify the locations of these nine nests were retained from the 2002 season and the five nests located at rocks or crevices not used by spawning lingcod in 2002 were assigned nest locations 17 through 21. Each of the five nests in a new site was associated with a guardian male from a nest site that was used in both seasons (Table 1). In total, there were seven males guarding 14 nests in the 2003 spawning season.

Probability of identity

The $P_{ID}$ values for the five lingcod microsatellite loci ranged from 0.311 to 0.351, and provided an overall $P_{ID}$ value of 0.004 across all loci. Thus, these highly polymorphic loci provided the ability to distinguish even closely related individuals in the study area.

Male nest site fidelity

In 2002, six of the nine guardian males were genotyped from tissue samples and putative genotypes for three other unsampled males ($4_{02}$, $6_{02}$ and $12_{02}$) observed guarding nests were generated by extensive analysis of embryo genotypes from their nests (Withler et al. 2004). Genotyping of all seven 2003 guardian males revealed that four of the six guardians genotyped in 2002 returned to the same nest sites (Fig. 3). Thus, the guardian of nest 2 (along with nests 1 and 3 in 2002) was the same male in 2002 and 2003, i.e. male $2_{02}$ was male $2_{03}$ (Fig. 3). Similarly, the 2002 guardian of nest 8 returned to nest site 8 and additionally guarded nests 20 and 21 in 2003. The 2002 nest 10 (and 11) guardian returned to nest site 10 in 2003. Finally, the same male guarded nest 15 in 2002 and 2003, and also guarded nests 16 and 19 in 2003 (Fig. 3).

Of the three 2003 guardian males whose genotypes did not correspond with sampled 2002 guardian males, one ($6_{03}$) matched one of the putative guardian genotypes established in 2002 (whose progeny were detected in 2002 nests 4, 8 and 12). Thus, male $6_{03}$ likely guarded the same or a nearby site in 2002. Another of the 2003 males ($4_{03}$) who did not correspond with a genotyped 2002 male did match a 2002 reconstructed male genotype (auxiliary male A) detected in eggs sampled from nest 1. Although male $2_{02}$ was the guardian of nest 1, male A fertilized half the eggs in the nest and may have been a neighbouring guardian outside the study area (Withler et al. 2004).

Only one of the seven 2003 guardians ($14_{03}$) had a genotype that was not detected among 2002 sampled males or reconstructions of paternal genotypes from 2002 egg sampling. This male guarded nests 7 and 14 in 2003, located in the area from which three nests and a male guardian disappeared in 2002 before sampling. Thus, among the seven 2003 guardian males, at least six returned from 2002 and four are known to have returned to the same nest sites. Two guardian males genotyped in 2002 ($13_{02}$ and $16_{02}$) were not identified in from 2003 guardian male samples or reconstructed from 2003 nest egg samples.

Female nest site fidelity

In 2002, 13 different female genotypes (one for each nest) were identified. In 2003, reconstruction of female genotypes was again possible for all 14 nests from embryonic genotyping.
because all nest egg samples included progeny sired by one or more of the seven guardian males (Table 1). Again, the eggs of each nest were derived from a single female and no female laid eggs in more than one nest. There was no overlap between the 13 females responsible for nests in 2002 and those that produced 14 nests in the same area, and in many of the same nest sites, in 2003. Female lingcod exhibited no interannual site fidelity on spatial scales encompassed by the study area.

**Polygyny, cuckoldry, and serial polyandry**

The limited egg sampling in 2003 revealed progeny attributable to the guardian male in 10 of the 14 nests sampled (Table 1). All seven guardian males contributed to fertilization in at least one of their guarded nests and six guardians fertilized eggs in at least one nest guarded by a neighbour. Progeny fertilized by one or more neighbouring guardian males were observed in samples from seven nests, including the four nests in which the guardian was not detected. In addition, fertilization attributable to at least five auxiliary males was detected (Table 1). These males could not be matched with males observed in 2002.

**Nest volume and guardian male size**

There was a significant relationship between length (cm) of the male lingcod and estimated volume (L) of the guarded nest (or mean volume when more than one nest was guarded): volume = 0.15 length – 6.08 ($F = 5.30; P = 0.036; r^2 = 0.26; n = 17$). Nest volume appears to increase with increasing size of the guardian male (Fig. 4). However, it should be noted that the proportion of variance explained by the regression line is small ($r^2 = 0.26$). Small nests were guarded by either small or relatively large lingcod while there was some indication that large nests were guarded only by relatively large lingcod while there was some indication that large nests were guarded only by relatively large lingcod (Fig. 4). The apparent significance of the regression may be an artefact of the right-hand portion of the scatter plot (Fig. 4). The number of nests guarded by a male lingcod did not vary with size of the guardian male ($F = 1.24; P = 0.32$).

**Discussion**

The interannual fidelity to nest locations exhibited by male lingcod is, to our knowledge, the first record of such behaviour reported for a temperate marine fish.
species. The high rate and extreme precision of nest site fidelity exhibited by guardian males was remarkable. At least six and possibly all seven of the guardian males present in 2003 had established territories on the same section of the Snake Island reef in 2002, indicating a level of nest site fidelity of at least 86%. At least four, and possibly six, of the males used the same territory in successive years and the spatial resolution of nest fidelity was high. In some cases, the orientation of the nest under a boulder or within a crevice was identical between years although the female parents always differed. Nest site fidelity in males but not in females indicates that the males are selecting the nesting sites and undertaking prespawning mate attraction behaviour.

Ridgway et al. (1991) observed that 48% of Lake Opeongo male smallmouth bass returning to spawn in a second year nested within 20 m of the previous year’s site. However, only 81% nested within 200 m of their previous site and some were as far as 1200 m from their previous sites. Among species more closely related to lingcod, kelp greenling (Hexagrammos decagrammus), also exhibits male nest guarding behaviour and the females have been reported to be polyandrous batch spawners, producing at least three egg masses per spawning season (Crow et al. 1997). Male kelp greenling guard an average of four nests, with each nest produced by a different female (Crow et al. 1997). Polygamy has also been reported for painted greenling (Oxylebius pictus) (DeMartini 1987). However, male or female nest site fidelity is yet to be studied for these species.

Nest site affinity was also apparently high, with nine of 14 nests in 2003 located in sites used in 2002. The one potentially new 2003 male guarded a nest site occupied in 2002 by a different guardian. Similarly, the guardian of nest 15 in both spawning seasons took over previous nest locations in the second spawning season when a neighbouring guardian

© 2005 Blackwell Publishing Ltd, Molecular Ecology, 14, 653–660
failed to return. There are likely habitat characteristics that influence nest site choice with the presence of crevices an obvious possibility and other factors such as water movement (Low & Beamish 1978) or the lack of flora coverage, particularly Agarum spp. (King & Winchell 2002), possibly also important.

Fewer males were identified as parents in the 14 nests of 2003 (15 males) than in the 13 nests of 2002 (21 males). Moreover, the guardian male was not detected in eggs sampled from four nests in 2003 compared to only one nest in 2003. The much smaller numbers of eggs analysed in 2003 (15 per nest) likely accounts for both observations. Male contributions to egg fertilization varied greatly in samples collected from locations throughout the egg mass (Withler et al. 2004). However, sampling in both years indicated that guardian males with multiple nests tended to dominate fertilization in only one nest, with auxiliary males achieving more success in the others (Withler et al. 2004). Guardians may spend less time courting and guarding females after they have one nest to protect, providing fertilization prospects for neighbouring guardians and non-territorial males in subsequent nests.

The extreme nest site fidelity coupled with polygynous mating within and between seasons in male lingcod and complete lack of site fidelity at this scale in female lingcod leads to polygamy for both sexes in this species. This is consistent with the suggestion that the advantages of promiscuity increase as the level of parental investment decreases, regardless of parental gender (Knight 2002). Although female lingcod do not appear to be batch spawners as are closely related kelp greenling females, this study indicates that they frequently practice serial polyandry by failing to return to the same nesting areas in successive years. In birds, parents of one or both genders may show nest site fidelity (e.g.

References


