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Microsatellite pedigree analysis reveals high variance in reproductive success and reduced genetic diversity in hatchery-spawned northern abalone

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ABSTRACT

The northern (or pinto) abalone, *Haliotis kamtschatkana*, is a broadcast-spawning marine gastropod that was recently listed as endangered in Canada. To aid in species recovery, a captive-breeding and supplementation program is underway in Barkley Sound, British Columbia. We genotyped first generation progeny for five microsatellite loci and used a pedigree reconstruction program (PEDIGREE 2.2) to identify their genealogical relationships in the absence of information on parental genotypes. We analyzed progeny from three separate group-spawning events and inferred considerable variation in the number of offspring produced by each parent; in the most severe case a single male sired all the progeny produced during one spawning event. After only one generation of captive-breeding we found a 55–60% reduction in allelic richness and a 17–18% reduction in heterozygosity relative to the diverse wild source population. This study illustrates the difficulty of managing genetic diversity in hatchery populations of a broadcast-spawning species, even when gametes are collected separately from each individual broodstock.

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1. Introduction

The northern (or pinto) abalone, *Haliotis kamtschatkana*, is a broadcast-spawning marine mollusk that has a patchy distribution within the rocky subtidal zone from Baja California to Alaska (Campbell, 2000). Historically, the northern abalone was a commercially important marine shellfish. However, over-fishing resulted in a 75–80% decline in its abundance during the 1980s, and consequently by 1990 the fishery in British Columbia was closed (Sloan and Breen, 1988; Campbell, 2000). Recent studies suggest that wild northern abalone stocks are not returning to historical levels of abundance despite the complete ban on harvesting (Campbell, 2000).

The status of *H. kamtschatkana* in Canada was first listed as threatened in 1999 then upgraded to endangered in 2009 by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC; www.cosewic.gc.ca) and under Schedule 1 of the Species at Risk Act (SARA). The COSEWIC report mentions illegal fishing and reduced fertilization success at low densities as being responsible for the continuing decline of this species.

To mitigate the continued decline of *H. kamtschatkana*, the Bamfield Huu-ay-aht Community Abalone Project (BHCAP) was created with a primary goal of producing northern abalone for supplementing wild populations and a secondary goal of producing abalone for commercial sale to local restaurants. BHCAP manages a hatchery and reintroduction program and has been artificially spawning abalone since 2001. First generation captive-bred (hereafter f1) larvae and juveniles produced by the hatchery have been used by Fisheries and Oceans Canada to supplement the threatened wild abalone population. In addition, abalone produced at this hatchery constitutes the only legal source of northern abalone currently being sold for human consumption in Canada. Recently, the scarcity of mature wild abalone has resulted in severe restrictions on the continued collection of wild broodstock by BHCAP. These regulations make it likely that future abalone sold for human consumption will have to be produced using only hatchery-reared broodstock.

Organisms, such as the northern abalone, that reproduce by broadcast-spawning present a unique challenge for selective or captive-breeding programs that must use closed populations. During broadcast-spawning, all individuals in an aggregation eject their gametes into the water column, and fertilization is dependant on the coordinated timing of a large number of individuals in close proximity to each other (Hedgecock, 1994). The resulting offspring may form a highly complex pedigree involving all possible crosses between a large number of males and females (Levitan, 2005). In addition, broadcast-spawning has the potential to produce a very large population from only a limited number of parents (Smith and Conroy, 1992; Boudry et al., 2002). This is problematic because the effective population size may be very small despite there being a large census population size. Hedgecock (1994) describes this phenomenon as the 'sweepstakes hypothesis' in which high fecundity and high early mortality of broadcast-spawning animals with planktonic larvae may lead to substantial variance in reproductive success. In this situation, a small

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number of breeders can replace the entire population in each generation, causing substantial reduction in effective population size and imposing genetic structure between generations (Li and Hedgecock, 1998), especially if related individuals are likely to settle next to one another (reviewed by Lee and Boulding, 2007).

To slow the loss of genetic variation and the accumulation of inbreeding in captive populations, it is helpful to have information about the relatedness of the wild founding animals prior to captive-breeding (Selvamani et al., 2001; Jones et al., 2006). Several software programs are available that use data from neutral genetic markers to reconstruct pedigrees (reviewed by Blouin, 2003; Butler et al., 2004). Reconstructed pedigrees can then be used to prevent the mating of closely related individuals, and to reduce the variance in reproductive success among members of the breeding population. This method is especially useful when studying animals where the parentage cannot be easily tracked or controlled. For example, observation-based pedigree assignment is very difficult among organisms that lack parental care, have polygamous mating systems, or reproduce by broadcast-spawning (Boudry et al., 2002; Wilson and Ferguson, 2002; Herbinger et al., 2006).

The purpose of our study was to determine the relative family sizes produced by different parents during three independent spawning events carried out by technicians at the BHCAP hatchery. This pedigree analysis would have been more straightforward if we had been able to genotype all the broodstock used in each spawning event (Herbinger et al., 2006). Unfortunately, most of the broodstock had been returned to the wild before the onset of our study, which made it impossible to collect DNA samples from all of the potential parents. However we did have records listing the plastic tag number for all of the male and female broodstock used in each of the spawning events. To carry out this analysis, we employed a pedigree reconstruction algorithm that infers genealogical relationships using information from microsatellite markers in the absence of parental information (PEDIGREE 2.2, Smith et al., 2001; Herbinger, 2005). We also tested whether the BHCAP captive-breeding program has had a detectable impact on microsatellite genetic diversity relative to the high diversity of the wild abalone population (Withler et al. 2003).

Given that captive-breeding programs for threatened or endangered broadcast-spawning invertebrates are becoming more frequent (for examples see: Davis, 2000; Gruenthal and Burton, 2005; Jones et al., 2006; Preston et al., 2007), we feel that the northern abalone provides a useful case study for similar captive-breeding initiatives. In such programs it may be desirable to determine the genealogical relationships of progeny many years after the inception of the captive-breeding program. If record keeping has been poor there may be little information about the number of parents or genotypes of the parents used.

2. Material and methods

2.1. Sample collection and analysis

The northern abalone reaches reproductive maturity at approximately 3 years of age or a shell length between 50 and 65 mm (Sloan and Breen, 1988). Before each spawning at the BHCAP facility, hatchery managers visually inspected individual wild broodstock to assess the ripeness of its gonads and to determine its sex (D. Renfrew, pers. comm.). Three to five mature abalone of each sex were then chemically induced to spawn into separate individual containers; this system allowed hatchery managers to be certain that gametes were contributed from all broodstock. The sperm from each male was diluted separately to prevent polyspermy (D. Renfrew, pers. com.). Diluted gametes from the three to five males and three to five females were then mixed together in the same tank so that all possible male–female crosses were possible (i.e. fully factorial mating design). The resulting fertilized eggs were kept in hatchery tanks while they developed into lecithotrophic (non-feeding) larvae. Approximately one week after spawning the larvae were chemically induced to metamorphose into benthic juveniles.

Between May 2005 and August 2006 we sampled offspring that were the product of three hatchery-spawning events. These groups were spawned in spring 2002, summer 2002, and fall 2003 and will be referred to as the R-group, the B-group, and the T-group respectively (see Table 1 for the exact mating design). All of the broodstock used in these hatchery-spawnings were from the wild, which made these offspring the first generation of captive-bred abalone at the BHCAP facility. Many of the parents used in these spawning groups had already been returned to the wild, and unfortunately their archived DNA samples had been accidentally destroyed. We were able to non-invasively collect DNA from the 32 broodstock that were still available for sampling.

In addition to the hatchery-spawned samples, we also obtained DNA from a large number of wild northern abalone. DNA templates for all wild *H. kamtschatkana* used in this study was supplied by Fisheries and Oceans Canada, Nanaimo British Columbia, using a similar protocol to that described here. These DNA samples were from abalone collected in Trevor Channel near Bamfield British Columbia in 2001 (for complete sampling and DNA extraction protocol see Withler et al., 2003), and are from the same geographical location as the wild broodstock initially used by BHCAP for captive-breeding.

2.2. DNA extraction, microsatellites, and genotyping

DNA was obtained from live abalone by removing a single epipodal tentacle with forceps and storing it in 95% ethanol at −20 °C. Removal of a single epipodal tentacle is considered non-destructive sampling because it does not impact the survival of the abalone (Withler et al., 2003). DNA was then extracted from the ethanol-preserved tentacle samples using a Qiagen DNEasy 96-well extraction kit^{TM} according to the supplier's protocol.

We screened only microsatellite loci that appeared not to have null alleles as manifested by their observed heterozygosity being close to their expected heterozygosity. This resulted in us optimizing seven out of the 12 microsatellite loci developed by Miller et al. (2001) (Appendix A). We later also discarded *Hka12* and *Hka48* because of the high failure rates of their polymerase chain reactions (PCR) and because the peaks corresponding to their alleles were difficult to score.

PCR was carried out using a RoboCycler® 96 Gradient Cycler with Hot Top PCR machine (Stratagene, Canada); all PCR reagents were purchased from Invitrogen unless otherwise indicated. PCR amplification was carried out in a 10 µl reaction mixture that contained 1.0 µl of undiluted DNA template with 1 µl of 10X PCR buffer, 80 µM of dNTPs, 0.25 units of Taq DNA polymerase, either 2.7 mM $MgCl_2$ (*Hka40* and *Hka43*) or 0.3 mM $MgCl_2$ (*Hka37*, *Hka56*, and *Hka65*), 1.25 µM of each

Table 1
The four sample groups of northern abalone analyzed in this study consist of one wild population and three hatchery-spawned groups.

Group	Year of spawn	Potential fathers	Potential mothers	Number of offspring genotyped
Wild	–	–	–	106
R-group	Spring 2002	6 (1)	7 (1)	179
T-group	Fall 2003	5 (3)	3 (2)	154
B-group[a]	Summer 2002	3 (1) + 3 (1)	4 (3) + 3 (1)	215

Hatchery records were used to determine the minimum number of wild males and females that could have contributed gametes to each spawning group. Values in parentheses indicate the number of parents for which DNA samples were still available at the time of our study. Information about the age or relatedness of the wild abalone broodstock is unknown.

[a] The B-group was composed of two small group-spawnings whose offspring were pooled before the onset of our study. The first used three males and four females, the second used three males and three females. The result is 21 possible full-sib families in this group.

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