Communal spawning leads to high potential for inbreeding in gadoid aquaculture

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ABSTRACT

The potential for relatedness to play a role in haddock (Melanogrammus aeglefinus) mate selection was tested in order to examine the potential for inbreeding in the culturing of this species. A total of 14 egg batches were produced in a communal spawning tank containing three full-sibling families over a 22 day period. Three females and seven males participated in these matings among the five females and eight males present. Based on genotyping results of fertilized eggs, there was no preference for mating with unrelated individuals (i.e. no avoidance of mating with siblings). The maximum number of males contributing to the fertilization success of an egg batch was three but with a single dominant male fertilizing a majority of the eggs for each batch. Only three of the egg batches produced appeared to have been fertilized entirely by a single male.

The degree of relatedness among F_1 commercial haddock broodstock was investigated relative to that of wild Bay of Fundy haddock. This comparison was intended to estimate how rapidly inbreeding could accumulate in a haddock population under cultivation when limited control was exerted over the actual communal mating. Allele richness (standardized numbers of alleles) was markedly lower in the aquaculture samples relative to the wild population (by approximately 25%). The size-selected F_1 haddock sample exhibited slightly lower levels of observed heterozygosity and gene diversity than a random sample of F_1 haddock, but marginally higher allele richness. The reduced family diversity coupled with the fact that haddock do not appear to avoid mating with kin could potentially lead to a high degree of inbreeding in the absence of the infusion of additional wild broodstock.

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1. Introduction

In fishes, inbreeding depression has been manifested as reduced progeny survival, growth, food conversion efficiency and increased frequency of deformities (Mrakovcic and Haley, 1979; Winemiller and Taylor, 1982; Kincaid, 1983; Gjerde et al., 1983; Bondari and Dunham, 1987), suggesting a high cost of mating with relatives. Mate selection in fishes is not well understood but appears to be primarily under female control, with males being selected based on visual and acoustic displays as well as phenotypic traits such as body size, fin size and color pattern (Farr, 1980; Berglund et al., 1986; Myrberg et al., 1986; Basolo, 1990; Reynolds and Gross, 1992; Hutchings et al., 1999; Couldridge and Alexander, 2001; Bremner et al., 2002; Suk and Choe, 2002). In some cases, females prefer rare phenotypes. For example, female guppies, Poecilia reticulata, are more likely to mate with males having novel color patterns (Hughes et al., 1999). Because color pattern in guppies has a genetic basis (Winge and Ditlevsen, 1947; Haskins et al., 1961), the selection of novel-colored mates might be considered a means of reducing the likelihood of inbreeding since females are choosing males with whom they probably have the fewest genes in common (Farr, 1980).

A growing body of evidence suggests that some fishes have the capacity to identify related individuals. Research in this area can be divided into two life history stages: juvenile and adult. Work on juvenile fishes indicates that individuals of some species prefer to associate with relatives and demonstrate reduced aggression levels when in the presence of relatives. Juvenile kin recognition has been mostly reported in salmonids (reviewed by Brown and Brown, 1996; Ward and Hart, 2003) but has also been noted in threespine stickleback, Gasterosteus aculeatus (Fitzgerald and Morrissette, 1992). The reported behavioral changes among juveniles in the presence of kin can lead to enhanced survival and improved growth (Seppä et al., 2001).

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As adults, kin recognition may be important in reducing association among relatives, thereby reducing the likelihood of inbreeding and its associated fitness costs. Limited research has been carried out on this topic. For small-sized aquarium fish Arnold (2000) reported that female rainbowfish, *Melanotaenia eachamensis*, preferred to associate with related females but during breeding preferred non-related over related males. Viken et al. (2006), however, demonstrated that virgin female guppies showed no such preference for unrelated males. To our knowledge, the possibility of mate selection based on relatedness has only been tested once on reproductive adult fishes through genetic analyses of progeny (Landry et al., 2001). These investigators tested the 'good genes as heterozygosity' hypothesis using spawning wild Atlantic salmon, *Salmo salar* in a Quebec river and examined the heterozygosity of their offspring as fry.

Whether or not adult fish recognize and avoid mating with kin is of interest to managers responsible for small isolated wild populations, and has also been of concern in breeding programs (Keller and Waller, 2002). In cultured populations, failure to maintain genetic variability among successive generations of broodstock can result in highly inbred progeny (Wang et al., 2002; Romana-Eguia et al., 2005). For species where families are generated by communal mating, the ability of reproductive individuals to identify and avoid mating with related conspecifics, when a range of potential partners with varying degree of relatedness exists, might alleviate some of the concerns over potential inbreeding. On the other hand, failure to avoid mating with kin coupled with closely related broodstock would result in a high potential for inbreeding.

Previous reviews of allozyme (Ward et al., 1994) and microsatellite (DeWoody and Avise, 2000) molecular genetic studies have reported increased levels of genetic variation within natural populations of marine relative to anadromous and freshwater fishes. These findings may, at least in part, reflect greater effective population size and the paucity of recent population bottlenecks for marine fishes inhabiting larger more stable environments. A possible consequence of these different evolutionary histories may be increased genetic load (deleterious recessive alleles) in marine fishes. Under certain conditions, deleterious alleles of both large (sub lethal and lethal) (Hedrick, 1994) and small (Fu, 1999) effect can be purged by drift. Most research on the magnitude of the effects of inbreeding at a given level on fishes have been carried out on freshwater populations (Kincaid, 1976a,b, 1983) of smaller evolutionarily effective population size, where historic or recent drift may have purged some of the genetic load. Given the possible increased prevalence of deleterious recessive alleles in marine fishes, research on the potential for and the impacts of inbreeding in this understudied group of fishes may be crucial to the understanding of fish populations at low population size as well as the continued development of finfish mariculture.

In the early 2000s, haddock, *Melanogrammus aeglefinus*, a marine broadcast spawner, was considered an emerging aquaculture species in Atlantic Canada (Litvak, 1998). This species does not respond well to manual stripping of gametes so selective breeding would have to be accomplished through the use of paired mating (Trippel and Neil, 2004; Rideout et al., 2005). However, during the early development of aquaculture for this species in Atlantic Canada, the industry relied heavily on communal mating, and communal spawning is used in aquaculture programs involving other marine finfish species (Brown et al., 2005; Herlin et al., 2008), thus creating the need to assess criteria used in mate selection and the potential for inbreeding. Here the potential for relatedness (i.e., kin recognition) to play a role in mate selection and act as a method of inbreeding avoidance were tested. The likelihood of spawning between related individuals was tested where both siblings and non-siblings were available as potential mates. In addition, levels of within population genetic variation were compared between (i) F$_1$ broodstock selected randomly, (ii) F$_1$ broodstock selected on the basis of fish size, and (iii) haddock sampled from the wild.

2. Materials and methods

2.1. Mating experiments (kin vs. non-kin)

Haddock produced from communal mating of wild Bay of Fundy broodstock at the St. Andrews Biological Station were maintained on a diet of frozen raw herring, *Clupea harengus*, northern shortfin squid, *Illex illecebrosus* and Aesop shrimp, *Pandalus montagui*. Fish became sexually mature after two years, at which time they were fin clipped for genotyping and parentage determination analysis (see below). To test if haddock avoided mating with related (full siblings) individuals a spawning tank (15 m^3) was established containing three full-sibling families; 1A consisting of 3 females and 2 males, 3A consisting of 1 female and 4 males and 5A consisting of 1 female and 2 males (Table 1).

The communal spawning tank was equipped with a surface egg collector that was examined daily and any eggs removed (Thorsen et al., 2003). Spawning occurred from April 19 to May 9, 2002 (Table 2). Genotyping of embryos indicated the individual fish that took part in each spawning event permitting an evaluation of the potential for mate selection based on relatedness. Embryos were incubated at 5 °C until 3 days post-fertilization (dpf) and then a sample was stored in 95% ethanol, at which time a sufficient number of cell divisions had presumably occurred to permit genotyping at the microsatellite loci analyzed here. Embryos were also sampled at 10 dpf and stored in 95% ethanol to compare genotyping results with 3 dpf embryos to ensure that a sufficient number of template copies were indeed present at 3 dpf to permit accurate microsatellite analyses.

To determine if the observed proportion of inbred and non-inbred matings were consistent with the hypothesis that relatedness does not influence mate selection, a 1-sided test for 1 proportion was computed (Minitab 15.1, 2007). The null hypothesis was that the true proportion of non-inbred matings was equal to the expected proportion of non-inbred matings, simply calculated as the total number of possible non-inbred matings divided by the total number of possible matings. The alternative hypothesis was that the true proportion of non-inbred matings was greater than the expected proportion, hence that there was active avoidance of inbred matings. Exact tests were computed to avoid problems with small sample size, and power calculations for different alternative proportions were also carried out in Minitab. Here the definition of a mating episode is not necessarily the same as a spawning episode. The number of spawnings would be defined as the number of egg batches produced by individual females on distinct days. However, the number of matings is defined as the number of crosses resulting from those egg batches, such that if eggs from one spawning episode (i.e. one egg batch) were fertilized by three males it was treated as three matings.

Table 1
Information on pre-spawning, individual haddock involved in the communal spawning experiment.

Individual ID	Sex	Family	Length (cm)	Weight (g)	K
28	F	1A	45.5	1529	1.62
20	F	1A	46.9	1616	1.57
4	F	1A	47.6	1742	1.62
12	F	3A	45.5	1493	1.58
52	F	5A	44.7	1370	1.53
25	M	1A	40.5	1004	1.51
42	M	1A	45.0	1412	1.55
29	M	3A	40.1	844	1.31
61	M	3A	43.9	978	1.16
10	M	3A	44.0	1266	1.49
14	M	3A	44.6	1225	1.38
23	M	5A	40.0	850	1.33
45	M	5A	42.7	962	1.24

Condition factor $(K) = (\text{Weight}/\text{Length}^3)$ 100.