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**Origins of two recruitment pulses of 0-group Atlantic cod (*Gadus morhua*)
in Bonavista Bay, Newfoundland during 1999,
determined from genetic variation**

Terry D. Beacham¹, Robert S. Gregory², and John Bratley³

¹Department of Fisheries and Oceans,
Pacific Biological Station,
Nanaimo, BC V9R 5K6

²Memorial University of Newfoundland,
Ocean Sciences Centre,
St. John's, Newfoundland A1C 5S7

³Department of Fisheries and Oceans,
Science Branch, P.O. Box 5667,
St. John's, NF A1C 5X1

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Abstract

Recruitment of 0-group Atlantic cod (*Gadus morhua*) to the nearshore of coastal Newfoundland occurs in two or more pulses. The two largest of these recruitment pulses occur in August and in late September to October. We investigated the origin (parentage) of these two recruitment pulses appearing in Newman Sound (Bonavista Bay), Newfoundland using genetic variation. Variation at seven microsatellite loci (*Gmo3*, *Gmo8*, *Gmo19*, *Gmo34*, *Gmo35*, *Gmo36*, and *Gmo37*) and the synaptophysin locus (*Sypl*) was examined in a sample of 200 0-group cod from each pulse collected by seine during August 16-18 and October 12-13, 1999. Genetic variation within pulses was compared with that of four offshore and five inshore populations of adult cod. For both pulses, variation was most similar to locally spawning adults from within Bonavista Bay in over 50% of 0-group cod. Of those individuals most similar to adults outside Bonavista Bay, the majority were most closely related to those collected north (i.e., "upstream") of the bay. Comparatively few 0-group were most similar to aggregations of adults sampled to the south, and then only in 0-group sampled in October. Offshore populations (primarily Funk Island Bank) were estimated to contribute 49% of the August pulse, but only 30% of the October pulse.

Résumé

Le recrutement des morues de l'Atlantique du groupe 0 (*Gadus morhua*) à proximité de la côte de Terre-Neuve s'opère en au moins deux événements, les deux plus importants étant en août, et fin septembre - octobre. En étudiant la variation génétique, nous avons cherché à établir l'origine (l'ascendance) de ces deux événements de recrutement survenant à Newman Sound, dans la baie de Bonavista (Terre-Neuve). On a examiné la variation à sept locus de microsatellites (*Gmo 3*, *Gmo 8*, *Gmo 19*, *Gmo 34*, *Gmo 35*, *Gmo 36* et *Gmo 37*) et au locus de la synaptophysine (*Sypl*) dans un échantillon de 200 morues du groupe 0, provenant de chacun des événements, et capturées à la senne du 16 au 18 août, ainsi que les 12 et 13 octobre 1999. La variation génétique à l'intérieur des événements a été comparée à celle de quatre populations océaniques et de cinq populations côtières de morues adultes. La variation dans plus de 50 % des morues du groupe 0, pour les deux événements, ressemblait à celle observée chez des adultes de la baie de Bonavista qui fraient dans les environs. La plupart des morues s'apparentant le plus aux adultes à l'extérieur de la baie de Bonavista étaient plus étroitement liées à celles capturées au nord (c.-à-d. «en amont») de la baie. Relativement peu de morues du groupe 0 ressemblaient surtout aux adultes des concentrations échantillonnées au sud, et encore là, elles se trouvaient exclusivement dans le groupe 0 échantillonné en octobre. On a estimé que les populations océaniques (surtout celle du banc de l'île Funk) représentaient 49 % de la poussée d'août, mais seulement 30 % de la poussée d'octobre.

Introduction

The question of whether the northern population of Atlantic cod is a single randomly-breeding aggregate or a complex of subpopulations with little genetic exchange between them has been hotly debated. The genetic structure of the northern population of Atlantic cod (NAFO Divisions 2J3KL) has been investigated since the 1970's (Cross & Payne 1978). The debate has intensified in the past decade with the failure of the offshore components of the stock to rebuild concomitant with the presence of aggregations of adult cod in the inshore. Mitochondrial DNA analysis suggested little variation among cod in North America (Carr & Marshall 1991, Pepin & Carr 1993, Carr et al. 1995). However, higher levels of genetic variation have been found using microsatellite loci (Bentzen et al 1996, Ruzzante et al. 1998).

It is now known that analysis of genetic variation within northern Atlantic cod using microsatellite loci indicates some differentiation among population components. Cod from inshore bays may be distinct from those in the offshore (Ruzzante et al. 1996, 1997, Beacham et al. 1999, 2000). Tagging data have indicated that there is substantial fidelity of offshore cod to specific spawning areas (Lear 1984, Taggart et al. 1995, Taggart 1997) suggesting differentiation between inshore and offshore groups despite extensive mixing in the summer. To date, there has been little evidence of genetically distinct subpopulations within specific bays, except in Gilberts Bay, Labrador (Beacham 1999).

In the past decade, demersal 0-group Atlantic cod in northeast Newfoundland have been distributed predominantly in inshore waters (Dalley & Anderson 1997), mainly close to shore in <10 m depth (Methven & Schneider 1998, Gregory et al. submitted). Pelagic 0-group Atlantic cod settle and become demersal in two temporally discrete recruitment pulses in late summer and early fall in the nearshore (Methven & Bajdik 1994, Grant & Brown 1998, Gregory et al. 2000). This annual pattern appears to occur at several locations in the inshore. The repeatable occurrence of multiple recruitment pulses of 0-group at single locations in the nearshore suggests discrete spawning events occur at separate times, physical locations, or both. The occurrence of discrete recruitment pulses also suggest that their parental origins may be different.

In this study, we investigated the origins of the two recruitment pulses of 0-group Atlantic cod in Newman Sound, Bonavista Bay, Newfoundland in 1999 using genetic variation. We examined variation at seven microsatellite loci and the synaptophysin (Sypl) locus (Beacham et al. 2000). Variation was compared with four offshore and five inshore aggregations of adult cod sampled during 1998 and 1999.

Methods

DNA sample collection

We collected two samples of 0-group cod in Newman Sound in Bonavista Bay by seine (Gregory et al. 2000). The first sample was collected during August 16-18, 1999, and the second sample was collected during October 12-13, 1999 (Fig. 1). Juvenile cod were preserved in 70% ethanol, and tissue samples sent to the molecular genetics laboratory at the Pacific Biological Station for DNA analysis. Methods of DNA extraction and analysis of specific loci to determine the origin of juvenile cod have been described in Beacham et al. (2000). Details of the exact location and timing of collection of samples from adult cod are given in Table 1 in Beacham et al. (2000).

Estimation of stock composition

The analysis was conducted by determining allele frequencies for each locus in each baseline stock (Beacham et al. 2000), and then the model of Fournier et al. (1984) was used to estimate stock composition by the conditional maximum likelihood method. Eleven alleles were scored at Gmo3 but were subsequently condensed by binning into 4 alleles (providing 10 genotypes). The 24 alleles scored at each of Gmo8 and Gmo19 were condensed to 7 alleles (28 genotypes at each locus) by binning adjacent alleles. There was no binning of the 9 alleles scored at Gmo34 (45 genotypes) or 13 alleles at Gmo35 (91 genotypes). The 13 alleles detected at Gmo36 were condensed to 9 alleles (45 genotypes) and the 15 alleles at Gmo37 were condensed to 7 alleles (28 genotypes). No binning of the two alleles was done at Sypl. Combining low frequency adjacent alleles reduced the number of genotypic frequencies to be estimated using the available samples, with little or no loss in the ability to discriminate among samples. For the microsatellite loci, baseline genotypic frequencies for each of the stocks were calculated from the observed allele frequencies under the assumption of Hardy-Weinberg equilibrium. Observed genotypic frequencies were used for the Sypl locus as genotypic frequencies in each of these loci were not those expected under Hardy-Weinberg equilibrium .

Each baseline population was resampled with replacement in order to simulate the random variation involved in the collection of the baseline samples during the estimation of stock composition of each mixture. Populations included in the baseline were limited to those where juveniles could have been reasonably expected to have originated. Therefore, the following offshore populations were included in the baseline used to estimate stock composition: Hawke Channel, Funk Island Bank, northern Grand Bank, and southern Grand Bank. Inshore populations included in the baseline included Gilberts Bay, Notre Dame Bay, Bonavista Bay, Trinity Bay, and Conception Bay. Estimated stock composition of the fishery samples was determined as a point estimate of the period sample, with standard deviations of individual stock estimates derived from 100 bootstrap resamplings of both the baseline populations and the period sample.

Results

Estimation of stock composition

Genetic variation in over 50% of 0-group cod sampled for each of the two recruitment pulses caught in the nearshore was most similar to adults from spawning aggregations sampled in Bonavista Bay. However, the total contribution by inshore and offshore adult components to the juveniles differed between the pulses.

First pulse juveniles caught during August 16-18, 1999 appeared to have originated from both inshore (51%) and offshore (49%) aggregations of adult Atlantic cod. Juveniles from the inshore component were most similar to spawning adults from Bonavista Bay, and the offshore juveniles were most similar to adults obtained on Funk Island Bank (Table 1).

Inshore spawning aggregations contributed 70% of second pulse juveniles sampled during October 12-13, 1999. Again, just over half (54%) of the sample was most similar to adult spawning aggregations sampled in Bonavista Bay. However, ~15% of juveniles were most similar to spawning aggregations sampled in other bays (10% Notre Dame Bay and 5% Conception Bay). A total of 30% of the juveniles were most similar to offshore populations. Again, Funk Island Bank was identified as the major source of the offshore component (Table 1).

Discussion

Our observations are consistent with a higher proportion of spawning by local aggregations of adult fish within Bonavista Bay, and with the prospect that "upstream" spawning adults are important contributors to more southerly juvenile nursery areas within the inshore. We found that over half of the juveniles sampled in the nearshore in both pulses were genetically most similar to aggregations of adult individuals which spawned locally (i.e., in Bonavista Bay). For the first recruitment pulse which settled as demersal juveniles in August, most of the individuals not related to Bonavista Bay spawners were most genetically similar to adult cod caught offshore in the vicinity of Funk Island Bank. For the second recruitment pulse, which settled in the nearshore in late September and October (and as late as November), 70% of juveniles were most genetically similar to inshore aggregations of spawners (54% Bonavista, 11% Notre Dame, and 5% Conception bays). The remaining juveniles were most similar to offshore populations from Funk Island Bank the northern Grand Bank.

Genetic variation in 0-group cod was not consistent with their having been spawned by individuals in the Hawke Channel area or Gilbert's Bay. Hawke Channel may have been too distant to have effectively seeded our collection site. Alternately, 0-group pelagic stages originating from spawning in the Hawke Channel area may have encountered ocean currents not favourable to their dispersal into the inner reaches of Bonavista Bay. Given the prevailing oceanographic currents - primarily

the Labrador Current - the second explanation would seem the most likely (Davidson & deYoung 1995, Pepin & Helbig 1996).

We did not see any 0-group which were most similar to spawners in the Gilbert's Bay or southern Grand Bank populations. Given the small size of its population, distant location in Labrador and comparative isolation, it is unlikely that spawners in Gilbert's Bay could have contributed significantly to the 0-group in Bonavista Bay. Eggs and larvae spawned on the southern Grand Bank could not have drifted "upstream" into Bonavista Bay against the Labrador current (Davidson & deYoung 1995, Pepin & Helbig 1996). It is also unlikely that adults from this area migrated into or "upstream" of Bonavista Bay prior to spawning. Given adult cod migration patterns in this area, albeit based on tagging studies conducted several decades ago (Taggart et al. 1995), it is unlikely that spawning individuals from this area would have contributed genetically to the Bonavista Bay 0-group sample. Our observations are therefore consistent with our prior expectation.

The first pulse of 0-group cod showed a significant proportion of individuals with genetic variation similar to adult cod sampled offshore in the vicinity of Funk Island Bank. This suggests that offshore and inshore adults both contribute to the first recruitment pulse in the nearshore in Bonavista Bay. Given that the adult collections were made as part of the fall groundfish survey, we cannot determine where these "Funk Island Bank" fish spawned, but we do know that they were distinct from Bonavista Bay or other inshore components (Beacham et al. 2000).

The contribution of inshore spawning components relative to that of offshore populations increased for the second recruitment pulse of 0-group cod sampled in Bonavista Bay. However, there was a significant percentage of 0-group in this pulse which were most similar to other inshore bays, primarily Notre Dame Bay and Conception Bay. Among adults sampled in offshore areas in the fall, both the northern Grand Bank and Funk Island Bank appear to have contributed significantly to 0-group in the second recruitment pulse. These observations suggest that spawning which produces the second recruitment pulse (probably in June), may be regionally more complex, involving more population components compared to the first pulse.

The first pulse of 0-group cod in Newman Sound appears to settle in a short time window in August compared to the second pulse which settles over two months (Gregory et al. 2000). This pattern suggests a comparatively short spawning period for the first pulse and a more protracted period for the second one. Contribution of fewer spawning components in the first pulse compared with to the second would be consistent with such a pattern of recruitment.

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Table 1. Estimated percentage composition of two recruitment pulses of 0-group cod sampled by seine in Newman Sound, Bonavista Bay Newfoundland in 1999 (Variation at seven microsatellite loci and Sypl were used to estimate stock composition. Each temporal sample contained 200 fish. Standard deviations are in parentheses.).

Population	August 16-18	October 12-13
Gilbert's Bay	0.0 (0.5)	0.0 (0.0)
Notre Dame Bay	0.0 (4.7)	10.9 (11.4)
Bonavista Bay	50.7 (12.4)	54.2 (14.5)
Trinity Bay	0.0 (0.6)	0.0 (3.5)
Conception Bay	0.0 (9.4)	4.7 (6.2)
Total Inshore	50.7 (11.9)	69.9 (11.6)
Hawke Channel	1.1 (9.7)	0.0 (5.9)
Funk Island Bank	47.3 (12.5)	17.1 (6.1)
Northern Grand Bank	0.9 (5.1)	13.0 (12.2)
Southern Grand Bank	0.0 (1.7)	0.0 (6.2)
Total Offshore	49.3 (11.9)	30.1 (11.6)

Figures

Figure 1. Sizes of 0-group Atlantic cod captured by beach seine in Newman Sound, Bonavista Bay, July - November, 1999 (dates and size range of individuals sampled for genetic variation indicated by boxes).

