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Spring evolution of *Pseudocalanus* spp. abundance on Georges Bank based on molecular discrimination of *P. moultoni* and *P. newmani*☆

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Abstract

The planktonic copepod sibling species *Pseudocalanus moultoni* and *P. newmani* (Crustacea, Copepoda) are abundant in waters over Georges Bank from late winter until mid-summer and are thought to reproduce throughout this period. The two species cannot be reliably distinguished using morphological characters, but are readily identified and distinguished by simple, rapid, and inexpensive molecular protocols based on sequence variation of mitochondrial DNA (mtDNA). DNA sequence variation of a portion of the mitochondrial cytochrome oxidase I (mtCOI) confirmed the presence of *P. moultoni* and *P. newmani* on Georges Bank; the mtCOI sequences were used to design species-specific oligonucleotide primers for use in a competitive multiplexed species-specific polymerase chain reaction (PCR). Species-specific PCR was used to determine the relative abundances of the two species in sub-samples of zooplankton collections from US GLOBEC Georges Bank Study Broadscale Surveys from February to June, 1997. Based on monthly visualizations, we inferred the spring evolution of the two species' distributions and abundances on Georges Bank. Both species' overall abundances increased from February to May or June: maximum abundance of *P. moultoni* was 38,061 m^{-2} in surface waters on the crest of Georges Bank in June; maximum abundance of *P. newmani* was 13,854 m^{-2} in subsurface waters on the Northeast Peak in April. The Peak in distribution of *P. moultoni* shifted from Georges Basin in April, to the northern edge of the Bank in May, to the center of the Bank in June. In contrast, *P. newmani* was more abundant to the south and east of the Bank. Beginning in April, *P. newmani* occurred on the Bank but was less abundant and less widely-distributed than *P. moultoni*; *P. newmani* abundance peaked in May and declined somewhat in June. Females of the species differed in their patterns of distribution and abundance, with *P. moultoni* always the more abundant species on the crest of the Bank. The spring increase of *P. moultoni* may result from the persistence of reproducing individuals

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over the Bank and/or from advective transport from adjacent regions. In contrast, *P. newmani* may be transported to Georges Bank from upstream populations on the Scotian Shelf and Browns Bank. The processes responsible for the observed patterns cannot be determined from this series of monthly snap-shots alone; ongoing studies use numerical models to examine the biological and physical dynamics causing these distributions.

1. Introduction

Pseudocalanus comprises a suite of sibling species that exhibit exceptional morphological similarity; they lack even the differences in secondary sexual characteristics that discriminate many sibling species of copepods (Frost, 1989). Two sibling species of *Pseudocalanus* occur sympatrically over Georges Bank, in the NW Atlantic Ocean: *P. moultoni* and *P. newmani* differ in size distribution (*P. moultoni* is larger), but individual size alone is not a diagnostic character for the two species (Frost, 1989; Bucklin et al., 1998). Due to the taxonomic confusion of the two species, fundamental aspects of their geographic distribution, population ecology, and life history are incompletely known.

Our current understanding is that *P. newmani* is circumboreal and is abundant in both the N. Atlantic and N. Pacific coastal waters of the US and Asia (Frost, 1989). *Pseudocalanus moultoni* is more coastally distributed and is absent from Asian waters (Frost, 1989). Although *P. moultoni* occurs on both US coasts, the distribution is apparently disjunct (Frost, 1989) and these populations may be geographically isolated (Sevigny et al., 1989). The latitudinal range of the species is also more restricted; on the Atlantic coast, *P. moultoni* is restricted to waters between the New York Bight and Nova Scotia (Frost, 1989).

Life histories of *Pseudocalanus* spp. have been studied in detail. Based on a number of studies, it is clear that there is considerable taxonomic and geographic variation in the life histories of *Pseudocalanus* species (Corkett and McLaren, 1978). In general, however, the species share the characteristics that females brood masses of attached eggs and may produce numerous broods of young; the productive season varies, but the copepods suspend development during the winter in high latitudes and may enter resting stages (disappearing from the water column) during the summer (Corkett and McLaren, 1978). Corkett and McLaren (1978) reviewed life cycle variability of the genus in semi-enclosed bodies of water, including fjords of the Canadian Arctic, Labrador, and Scotland, and coastal regions off the UK and Scandinavia.

The life cycle of *P. newmani* over Browns Bank on the Scotian Shelf was described by McLaren et al. (1989): adults matured in March and by April had produced young, which in turn matured and reproduced before becoming less abundant in June. There was no evidence of resting copepodites stages during the year studied; but the synchrony of population structure through the year suggested that the species either enters a resting stage during the winter or is maturationally delayed by cold temperatures (McLaren et al., 1989). The species may not have been self-sustaining on Browns Bank, since its occurrence was irregular and sparse in spring and summer, when the species was common offshore (McLaren et al., 1989). The description of the life cycle of *P. newmani* differed from that of *P. moultoni* only in that the latter species was relatively less abundant during summer months over Browns Bank.

Pseudocalanus spp. show seasonal variation in their abundance on Georges Bank [Davis, 1984, 1987; E.G. Durbin (University of Rhode Island) and C.J. Meise (National Marine Fisheries Service, Narragansett, RI), personal communications]: the pooled species are most abundant during summer (especially May to July), and least abundant during September to December. The species may predominate the zooplankton assemblage of the Bank during the winter, in the absence of other copepod species (Davis, 1987).

In a study that grouped the two species, Davis (1984) demonstrated that *Pseudocalanus* spp. are adapted to the Georges Bank ecosystem: the life history features of the combined species interact with the mean circulation on the Bank (Butman et al., 1987) to aid retention of the species. Davis (1984) used evidence of stage-specific distributions on the Bank and biophysical models to describe the interaction of copepod population dynamics with the mean circulation of water around the Bank. During December to February, *Pseudocalanus* spp. increased in abundance and were concentrated within the 100-m isobath (Davis, 1982, in Davis, 1984). Davis (1984) hypothesized that the stage-specific patterns resulted from transport of adult females onto the western edge of the Bank from the Gulf of Maine in December. In response to higher phytoplankton levels on the Bank, the females produced eggs that hatched and continued development through copepodid stages as they were transported in the anti-cyclonic circulation over the Bank. Losses from the Bank populations were due primarily to biological causes and secondarily to advective transport off the Bank.

McGillicuddy et al. (1998a) revisited the question of population dynamics of *Pseudocalanus* spp. on Georges Bank using data assimilation into numerical models; they inferred that Georges Bank populations are self-sustaining, with recruitment resulting from reproduction of resident individuals. Since both Davis (1984) and McGillicuddy et al. (1998a) based their conclusions on pooled distributions of both *Pseudocalanus* spp., it is unclear whether the same conclusions would result from separate analyses of the dynamics of *P. moultoni* and *P. newmani*. These conclusions need to be revisited in light of Frost's (1989) revision of the genus and the confirmation that two sibling species co-occur on the Bank (Bucklin et al., 1997, 1998).

Retention of copepods, including *Pseudocalanus* spp., on Georges Bank has been evaluated using biological–physical models of transport (i.e., particle seeding) in realistic flow fields. Lynch et al. (1998) and Miller et al. (1998) simulated population dynamics and behavior of the copepod, *Calanus finmarchicus*, based on realistic starting conditions, including: climatological patterns of distribution and abundance (Meise and O'Reilly, 1996), species' biology (Hirche, 1996), and physical transport from climatological flow fields (Brown and Irish, 1992; Flagg, 1987). They demonstrated that seeding of populations to Georges Bank is a complex, time-variable process that may depend upon many factors.

1.1. Species identification using allele-specific primers and probes

DNA sequence variation can be used to design rapid, molecularly based protocols to discriminate individuals of different species, based on species-specific oligonucleotide primers and probes. This approach has been used to identify a wide variety of marine species, including yeast (Fell, 1995), phytoplankton (DeLong et al., 1989), and the larvae (Olson et al., 1991; Dixon et al., 1995; Medeiros-Bergen et al., 1995) and adults (Banks et al., 1993) of marine invertebrates. For recently diverged or older species that have not differentiated morphologically, mitochondrial

DNA (mtDNA) is an appropriate molecular systematic character by virtue of the characteristic patterns of inter- and intraspecific variability (see Avise, 1994).

A possible molecular systematic method, and the one we have selected for discrimination of planktonic copepods, is allele-specific amplification by the polymerase chain reaction (PCR; see Charlieu, 1994), which has advantages in cost and efficiency. For marine zooplankton species, which are frequently numerous and geographically widespread, hundreds to thousands of individuals must be assayed to document a species' distribution and abundance pattern at high spatial and temporal resolution. Thus, it is essential that molecular protocols for species' identification be rapid, simple, inexpensive, and reliable to be useful for ecological or oceanographic studies.

Individuals of any size and life stage may be identified by a suite of PCR reactions, each using a common primer and a species-specific primer. Alternatively, the individual species-specific reactions may be "multiplexed" (i.e., carried out simultaneously and competitively in a single tube; Gibbs et al., 1989) by designing primers that amplify DNA regions of different size (Fig. 1). Competitive species-specific PCR has been used for a variety of marine organisms, including copepods (Bucklin et al., 1998, 1999, 2000); in some cases, the same PCR primers may yield

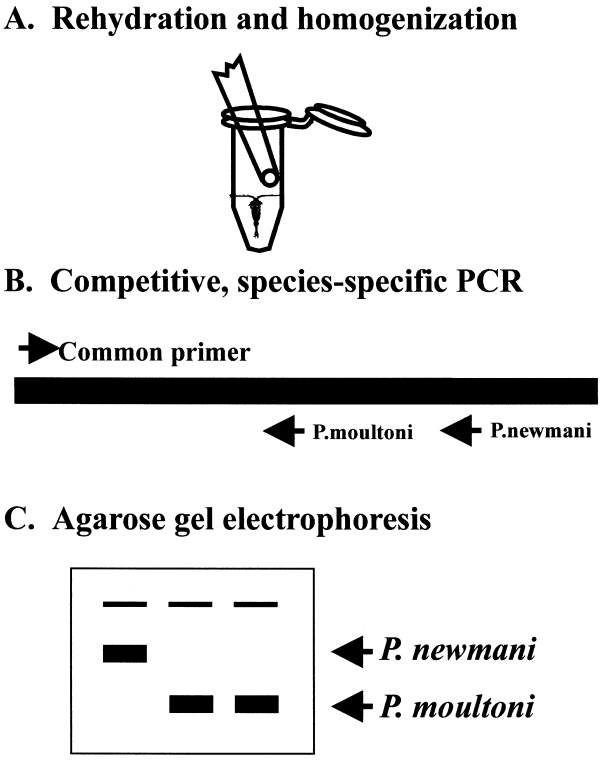

Fig. 1. Diagram of the molecular protocol for competitive, species-specific PCR of *Pseudocalanus* spp. (A) Copepods were rehydrated in distilled water and homogenized in the PCR buffer. (B) The PCR reaction was done without purification of the DNA. Arrows indicate relative positions and directions of the PCR primers. (C) The results of the competitive species-specific PCR reactions and the species' identity of the copepods were determined by agarose gel electrophoresis.

different-sized amplification products in different species (Dixon et al., 1995). Species-specific PCR may be readily adapted for use with tiny organisms, such as copepods, for which little tissue preparation and no DNA purification is needed.

In this study, multiplexed, competitive species-specific PCR was used to identify individual copepods, to describe geographic patterns of distribution, and to the infer the spring evolution of abundance of two sibling species of *Pseudocalanus* on Georges Bank in the NW Atlantic Ocean.

2. Materials and methods

2.1. Collection and preservation of zooplankton samples

Pseudocalanus spp. samples were collected by vertically stratified tows of the Multiple Opening and Closing Net and Environmental Sensing System (MOCNESS: Wiebe et al., 1985) during the Broadscale Survey cruises over Georges Bank from February to June, 1997. Samples were collected and sub-sampled for molecular analysis at 18 standard stations each month, except that 10 stations were sampled in February and 17 in June (see Table 1 and Fig. 2 for sample collection locations).

Table 1
Broadscale Survey standard station numbers and coordinates for zooplankton collections used for this study. The samples were split at sea, and an alcohol-preserved portion of the sample was examined for *Pseudocalanus* spp. for molecular analysis. See Fig. 2 for station locations

Station no.	Latitude	Longitude
3	40.53°N	68.45°W
4	41.02°N	68.27°W
7	40.47°N	67.29°W
12	41.42°N	67.53°W
13	41.26°N	67.17°W
16	40.91°N	66.42°W
17	41.21°N	66.46°W
18	41.41°N	66.70°W
20	41.71°N	66.52°W
23	41.78°N	66.18°W
25	42.31°N	65.84°W
29	42.30°N	66.89°W
30	41.94°N	67.24°W
34	41.86°N	68.33°W
35	41.59°N	68.44°W
36	41.41°N	68.32°W
38	41.48°N	68.94°W
39	42.14°N	66.01°W
40	42.17°N	67.66°W

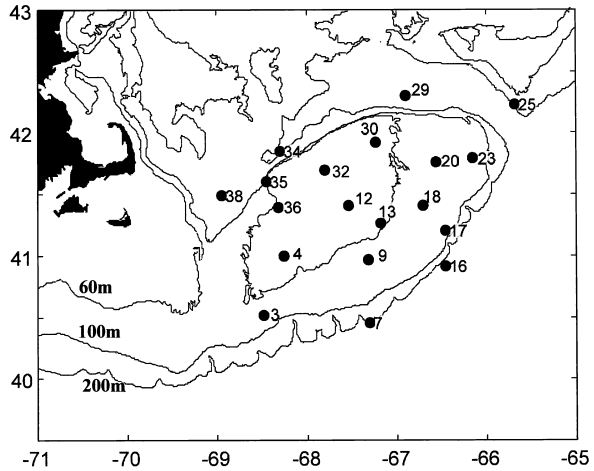

Fig. 2. Standard station numbers and locations for the US GLOBEC Georges Bank Study Broadscale Surveys during February to June, 1997. The numbers are not consecutive because samples from only a subset of the standard stations were split at sea, with a portion preserved in 95% ethanol for molecular analysis. See Table 1 for exact station locations.

A 180 ml sub-sample was taken from the samples collected at two depth intervals (0–15 and 15–40 m) at these stations. At two stations, Stations 4 and 12, water column depth was insufficient for collection of the deeper (15–40 m) sample. In addition, the deeper sample was not collected for some cruises at three other stations, Stations 13, 36, and 38. The sub-samples were preserved in 95% ethanol for molecular analysis; the balance of each sample was preserved in 10% formalin for taxonomic evaluation at the US GLOBEC Zooplankton Sorting Group at the University of Rhode Island.

An aliquot of each sub-sample was removed to a petri dish using a wide-mouth plastic pipette. Individual copepods were identified and selected for molecular analysis by scanning the petri dish under a dissecting microscope. The first 30 adult female *Pseudocalanus* spp. (regardless of size) were removed to another container in preparation for molecular analysis.

2.2. DNA sequence analysis

The copepods were prepared for molecular analysis by boiling in distilled water for 10–15 min to evaporate the alcohol. A $\sim$ 700 bp region of the mitochondrial COI (mtCOI) gene was amplified using consensus primers made according to published sequences (Folmer et al., 1994):

LCO-1490 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′,

HCO-2198 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′.

The primers were named based on position numbers of the *Drosophila yakuba* sequence (Clary and Wolstenholme, 1985). The amplification protocol was: 94°C (1 min), 37°C (2 min), 72°C (3 min) carried out for 40 cycles. The sequencing primer was LCO-1490. Sequencing was carried out on an American Biotechnology, Inc., Model 373, automated DNA sequencer (see Bucklin et al., 1998, 1999, for sequence data and specific protocols).

A 300 base-pair (bp) sequence of mtCOI gene was obtained for individuals of both species collected from Georges Bank. In order to determine whether the Georges Bank populations were the same species described by Frost (1989) from Puget Sound, WA, a 200 bp region of the mtCOI gene was sequenced for individuals of both species collected from Puget Sound, WA and identified by B.W. Frost (University of Washington). A 200 bp region was used in a multiple sequence alignment for two abundant sequence types from Georges Bank and one sequence type from Puget Sound for each species, as well as the homologous sequence for three *Calanus* sibling species: *C. helgolandicus, C. pacificus*, and *C. sinicus* (Bucklin et al., 1999; unpublished data). The sequences were analyzed using the Molecular Evolutionary Genetics Analysis (MEGA), Ver. 1.0, software package (Kumar et al., 1993); a phylogenetic gene tree was reconstructed to show the intraspecific and interspecific relationships by Neighbor Joining (Saitou and Nei, 1987) using Tamura Nei distances (Tamura and Nei, 1993) with an alpha parameter of 0.2. The analysis considered all substitutions; the tree was bootstrapped 1000 times.

2.3. Species-specific PCR

Species-specific oligonucleotide primers were designed for *Pseudocalanus* spp. from regions of the mtCOI gene that were conserved within each species, but variable between them. Primer sequences were evaluated for suitable base composition, temperature of dissociation, and self-compatibility using the software programs OLIGO (Rychlik, 1992) and *Amplify* (Engels, 1992). In order to allow multiplexing of the PCR reactions (i.e., simultaneous and competitive PCR using multiple species-specific primers and one common primer; Gibbs et al., 1989), the primers for the species were selected at different sites along the 300 bp sequence. The resultant amplification products were thus of different lengths and were distinguishable by agarose gel electrophoresis.

The species-specific primers were used with the common primer LCO-1490 (Folmer et al., 1994). The *Pseudocalanus* species-specific primer names, numbers, and sequences were:

P. moultoni (COI-1615) 5′-CCC GAT TAG TGA TCC AGC-3′,

P. newmani (COI-1736) 5′-CCG CAC CCA GTA TTA GAG GT-3′.

The amplification protocol was: 94°C (30 s); 40°C (30 s); 72°C (1 min); for 30 cycles. PCR results were determined by electrophoresing the amplification products on a 2% agarose gel.

2.4. Determination of species' distribution and abundance

A total of 2935 *Pseudocalanus* spp. collected during the February to June, 1997 Broadscale Surveys was assayed by competitive multiplexed PCR to distinguish between *P. moultoni* and *P. newmani*. Approximately 20 female *Pseudocalanus* spp. in each sub-sample were identified to species. Failed PCRs ranged from zero (usually) to 3 for a given sample; additional PCRs were done as necessary to meet the target size of 20 per sample. The relative abundance of each species among the 20 individuals was used to estimate the abundance of each species by multiplying by the total number per square meter of female *Pseudocalanus* spp. in the sample, as determined by the US GLOBEC Zooplankton Sorting Group at the University of Rhode Island (E.G. Durbin and P. Garrahan, University of Rhode Island, unpublished data; also see http://globec.whoi.edu).

2.5. Objective analysis of species' distributions

Numbers per square meter for each depth interval (0–15 and 15–40 m) for each species were determined and the data were $\log_{10}$ transformed for objective analysis. These data were objectively analyzed using the OAX software package (He et al., 1997). The same technique has been used by Lynch et al. (1998), McGillicuddy et al. (1998a, b) to objectively map copepod distributions in this area. Each data point was weighted according to its space/time distance from the analysis point and a four-dimensional correlation function. Patterns of distribution and abundance of each species were visualized in surface (0–15 m) and sub-surface (15–40 m) strata for each monthly Broadscale Survey from February to June, 1997. The geographic coverage of the maps was confined to that area where the mean expected error (averaged over all time periods and both species) is less than approximately 70%.

3. Results

3.1. DNA sequence variation of Pseudocalanus spp.

The most frequent DNA sequence types for a 200 base-pair region of the mtCOI gene were aligned for individuals of *P. moultoni* and *P. newmani* from Georges Bank and Puget Sound, WA and with three sibling species of *Calanus* for comparison (for data and analysis, see Bucklin et al., 1997, 1999). The MtCOI sequence clearly discriminated *P. moultoni* and *P. newmani*. Distances between the *Pseudocalanus* spp. were of the same order as those between sibling species of *Calanus* (see Bucklin et al., 1999). MtCOI sequences for conspecific individuals collected from Georges Bank and Puget Sound, WA differed by 2% (for *P. newmani*) to 3% (for *P. moultoni*); this level of difference was typical of mtCOI sequence divergence within *Calanus* and other species (Bucklin et al., 1998, 1999). Individuals of each species collected in Atlantic and Pacific waters clustered with conspecific individuals in the mtCOI gene tree, indicating that both species are genetically cohesive between N. Atlantic and N. Pacific populations (Fig. 3), despite geographic distance and (in the case of *P. moultoni*) discontinuous distributions.

3.2. Identification and determination of distribution and abundance of Pseudocalanus spp.

The competitive, multiplexed PCR reaction using species-specific primers successfully discriminated *P. moultoni* and *P. newmani* based on simple agarose gel electrophoresis of the PCR products; size differences in the amplified products (246 bp for *P. newmani*; 125 bp for *P. moultoni*) allowed accurate and reliable identification of the two species (Fig. 4). The relative abundances of the two sibling *Pseudocalanus* spp. in 1997 Broadscale Survey samples ranged from 100% *P. moultoni* to 100% *P. newmani*. Maximum abundance of *P. moultoni* was 38,061 m^{-2} (in the 0–15 m stratum in June); maximum abundance of *P. newmani* was 13,854 m^{-2} (in the 15–40 m stratum in April; Table 2).

Bank-wide assessment of the spatial patterns of the distribution and abundance of *P. moultoni* and *P. newmani* revealed differences between the two species. The monthly Broadscale Surveys yielded a series of snapshots through the spring and summer, 1997 (Figs. 5 and 6). During most

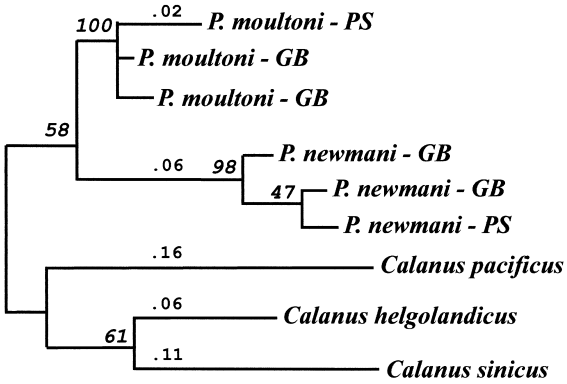

Fig. 3. Gene tree of calanoid copepod species of *Pseudocalanus* and *Calanus* based on a 200 base-pair sequence of the mitochondrial cytochrome oxidase I (COI) gene. The difference between *P. moultoni* and *P. newmani* was similar to that between sibling species of *Calanus* (see Bucklin et al., 1999). Also, sequences of *P. moultoni* and *P. newmani* individuals collected from the N. Atlantic (GB) and N. Pacific Oceans (PS) differed by only 2–3% (see Bucklin et al., 1997), suggesting that the species populations are genetically cohesive despite geographic distance. Numbers on branches are Tamura–Nei distances (Tamura and Nei, 1992); numbers at branchpoints are bootstrap values.

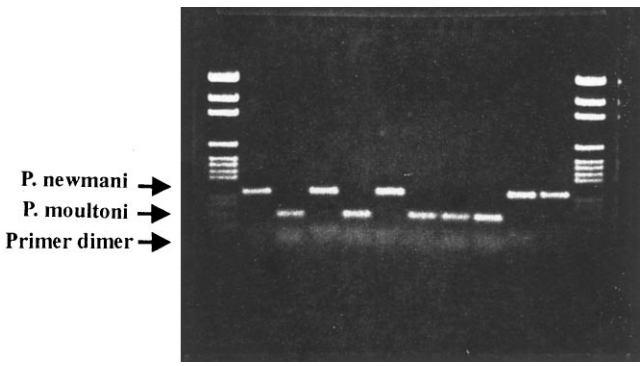

Fig. 4. Gel photo showing species-specific PCR products for individual *Pseudocalanus* spp. First (#1) and last (#12; left to right) lanes are molecular size markers; lanes #2–#11 include PCR products for individual copepods of both species. PCR products for *P. newmani* are larger (246 bp) and migrate less far; products for *P. moultoni* are smaller (125 bp) and migrate farther.

months, the patterns of population abundance were largely concordant in the upper (0–15 m) and lower (15–40 m) strata sampled. The patterns are summarized here month-by-month:

February: Both species were present over the Bank in low abundances, based on a smaller-than-usual number of samples. In two of the three surface samples collected within the 60-m isobath, *P. moultoni* was relatively abundant while *P. newmani* was relatively rare. In contrast, *P. newmani* predominated — albeit in low abundance — in a sample collected on the northern edge of the Northeast Channel (at the southern tip of Browns Bank).

March: Samples on the western end of the Bank and near the Great South Channel contained mostly *P. moultoni*, although abundances varied from low to moderate. Five of six surface samples

Table 2
Numbers per square meter of female *P. moultoni* and *P. newmani* in surface (0–15 m) and sub-surface (15–40 m) samples collected at standard stations during February–June, 1997 US GLOBEC Broadscale Surveys[a, b]

	P. moultoni		*P. newmani*	
STN #	0–15 m	15–40 m	0–15 m	15–40 m
February, 1997				
3	18	384	18	0
4	3887	3025	194	0
7	2	22	7	410
9	0	161	0	209
12	299	—	432	—
13	0	60	0	53
16	0	10	163	65
18	70	295	225	331
20	163	583	74	69
25	14	0	156	816
March, 1997				
3	24	618	27	450
4	368	—	41	—
7	0	10	1072	204
9	51	0	7	0
12	0	—	0	—
13	1078	1874	0	0
16	0	3	5687	26
17	0	21	0	60
18	0	149	0	28
20	41	87	29	36
23	0	194	59	119
25	0	0	1126	185
29	9	6	173	95
30	16	0	3	0
34	335	98	99	68
36	1100	—	324	—
38	45	309	22	55
39	89	0	537	206
April, 1997				
3	482	2025	1204	1246
4	669	0	0	0
7	3	0	56	0
9	0	96	2501	240
12	383	—	0	—
13	356	—	1008	—
17	139	0	7	379
18	112	0	48	1317
20	135	630	1212	13854
23	0	249	807	1060
25	0	0	859	3500
27	80	254	121	367
29	470	1044	138	174
30	35	0	0	0

Table 2 (*continued*)

	P. moultoni		*P. newmani*	
STN #	0–15 m	15–40 m	0–15 m	15–40 m
34	5849	8833	279	0
36	3065	—	0	—
38	42	4083	2	0
39	911	0	580	445
May, 1997				
3	1379	984	13096	1181
4	984	—	895	—
7	0	0	81	0
9	0	252	5751	5532
12	1663	—	594	—
13	589	3553	589	789
16	12	0	242	0
17	106	562	2216	2388
18	93	801	1961	1870
23	869	280	41	16
25	7	109	138	254
27	2438	4856	232	767
29	203	216	1013	68
30	2013	10413	671	2893
35	14330	—	2388	—
36	26489	—	9349	—
39	18222	2031	1822	3047
40	24965	4922	1189	0
June, 1997				
3	83	1947	250	885
4	395	—	362	—
7	0	0	0	0
12	8729	—	2910	—
13	38061	8788	200	0
16	2	117	13	39
17	7	0	61	0
18	5844	4907	2192	0
20	5810	25047	612	0
23	4212	7564	527	688
25	0	0	170	25
27	16369	—	7015	—
29	77	4	385	77
30	4949	9601	1979	3000
34	715	—	168	—
36	8806	—	1554	—
38	785	536	2669	536
39	60	2157	255	0

[a] *Pseudocalanus* spp. abundances were estimated from relative abundances of the two species in sub-samples of the original sample determined by species-specific PCR, and the summed concentration per meter squared of both species in the samples counted by the Georges Bank zooplankton group (unpublished data provided by P. Garrahan and E.G. Durbin, University of Rhode Island). Locations of standard stations are given in Table 1.

[b] Sample not collected.

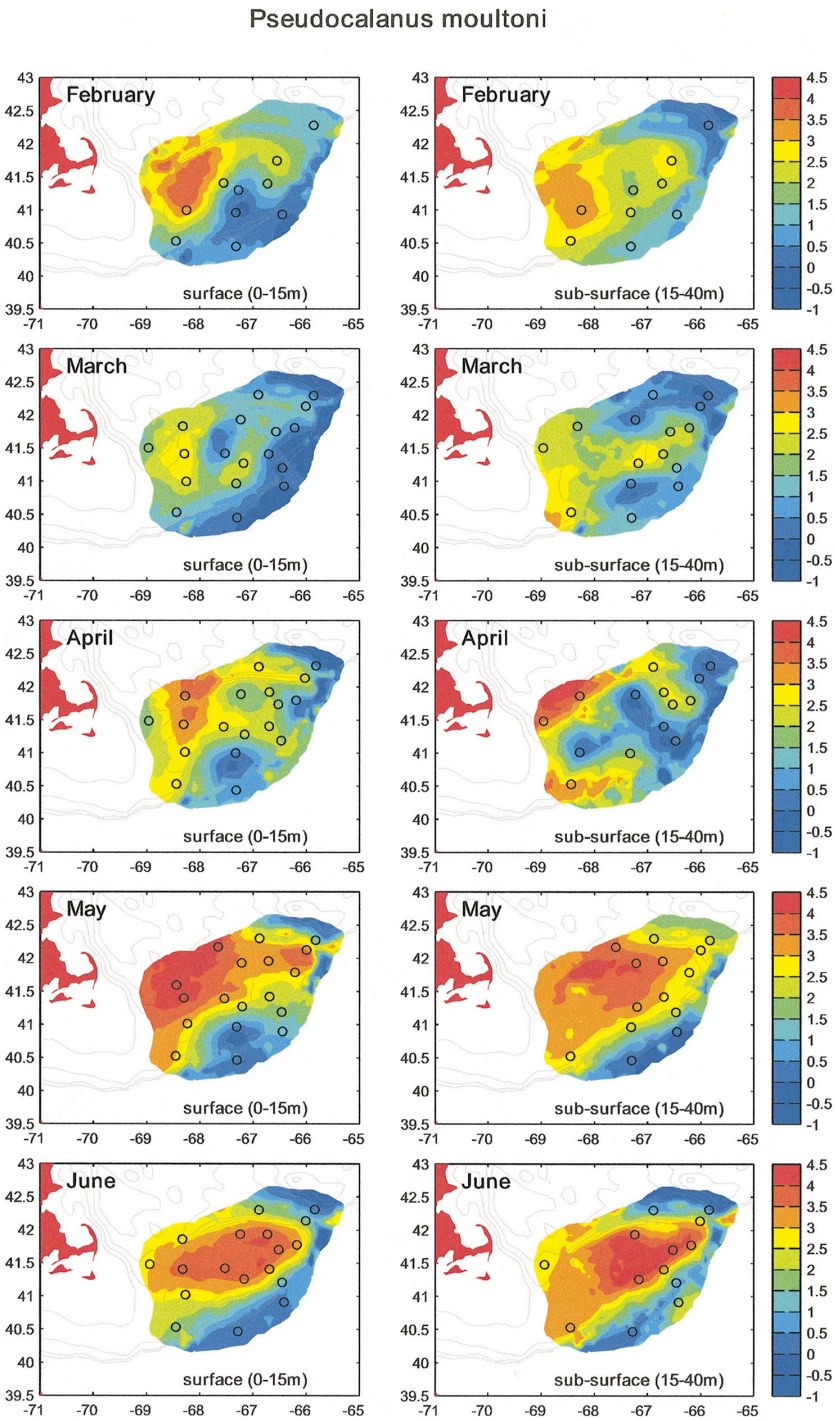

Fig. 5. Seasonal evolution of the distribution and abundance of female *P. moultoni* in surface (0–15 m; left panels) and sub-surface (15–40 m; right panels) depth strata over Georges Bank from February to June, 1997. Abundance data (see Table 2) were $\log_{10}$ transformed and objectively analyzed to produce the visualizations shown. The geographic coverage of the maps was confined to that area where the mean expected error (averaged over all time periods and both species) is less than approximately 70%.

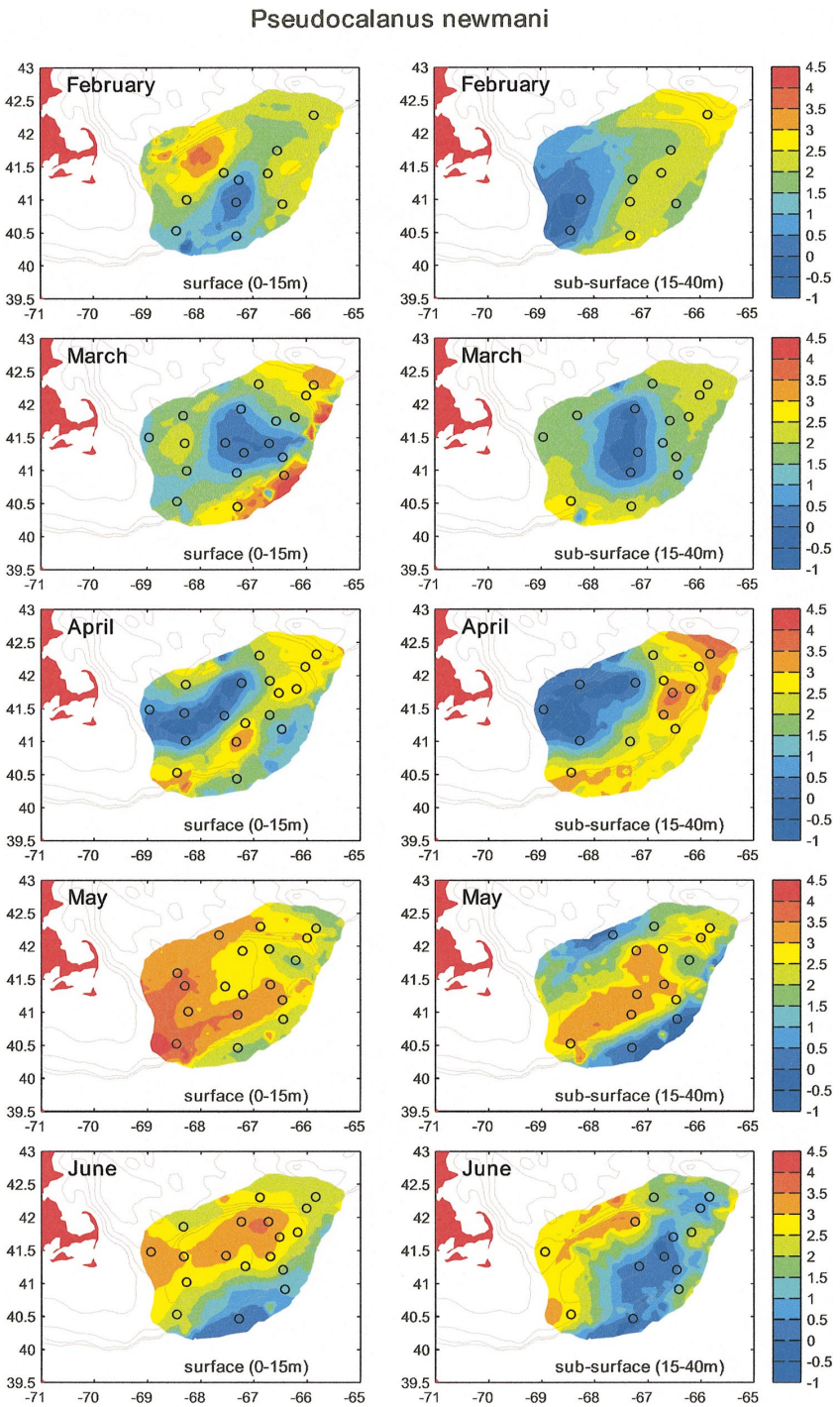

Fig. 6. Seasonal evolution of the distribution and abundance of female *P. newmani* in surface (0–15 m; left panels) and sub-surface (15–40 m; right panels) depth strata over Georges Bank from February to June, 1997. Abundance data (see Table 2) were $\log_{10}$ transformed and objectively analyzed to produce the visualizations shown. The geographic coverage of the maps was confined to that area where the mean expected error (averaged over all time periods and both species) is less than approximately 70%.

collected outside the 100-m isobath predominated in *P. newmani* with moderate abundances, but the species was rare in sub-surface samples from the same region. Samples collected to the north and east of the Bank contained only *P. newmani* in moderate (surface) to low (sub-surface) abundances.

April: The spatial separation of the two species was evident for both surface and deep samples, with samples from the Great South Channel and northern flank of the Bank yielding high abundances of *Pseudocalanus* spp. consisting almost exclusively of *P. moultoni*. Samples outside of the 60-m isobath on the southern flank predominated in *P. newmani*; only *P. newmani* was found in samples collected from the Northeast Channel.

May: The April pattern of spatial separation of the two species recurred in surface samples collected during the May survey, although the distributions were less distinct. Samples collected to the north and east of the Bank contained abundant *P. moultoni*, while *P. newmani* was more abundant at the southern end of the Great South Channel. However, both species were abundant in deeper waters on the crest of the Bank.

June: Across the Bank within the 100-m isobath, samples yielded large numbers of *P. moultoni*, with smaller numbers of *P. newmani* in most samples, especially in sub-surface samples.

4. Discussion

4.1. Molecular systematics and species-specific PCR

The mtCOI gene showed sufficient variation in DNA sequence between species to easily resolve differences between sibling species of *Pseudocalanus* and *Calanus* (Fig. 3). This finding is consistent with previous studies using mtCOI to examine variation within a harpactacoid copepod (Burton and Lee, 1994) and between species of insects (Brown et al., 1994; Juan et al., 1995; Pedersen, 1996; Stauffer et al., 1997), and a diverse group of invertebrates (Folmer et al., 1994), although amounts of sequence differences associated with species comparisons differed among the taxa studied.

The percentage sequence difference between the sibling species of *Pseudocalanus* for the 300 bp region of mtCOI was 18%: which was typical of other calanoid sibling species (Bucklin et al., 1997, 1998, 1999). Despite sequence variation of 2–3% within each species (Bucklin et al., 1997), the mtCOI sequence proved to be a useful molecular systematic character for identification and discrimination of species that lacked diagnostic morphological characters. The sequences differed sufficiently between species to allow identification of regions that were desirable for primer design (in terms of base composition and self-compatibility, see Rychlik, 1992) and were located at sufficiently distant positions along the sequence to allow multiplexing of the reaction. The sequenced mtCOI gene portion included several short regions of sequence that differed between species by $\sim 30\%$ and exhibited little or no variation within each species.

Carefully designed, species-specific oligonucleotide primers are valuable taxonomic tools for ecologists and oceanographers. Using species-specific PCR, hundreds to thousands of individuals can be identified to accurately characterize stage-specific patterns of distribution and abundance at high spatial and temporal resolution.

4.2. Objective analysis of species' distributions

The visualization of species' abundances was done based on $\log_{10}$ transformation of the estimated abundance of each species/m^{-2} in sub-samples of Broadscale Survey zooplankton collections. The $\log_{10}$ transformation of abundance data provided a better fit to theoretical expectations of the functional relationship between a mapped parameter and distance (see Kitanidis, 1997). The $\log_{10}$ transformation also ensured that inaccuracies in the estimation of species' abundances are insignificant in terms of the spatial patterns observed.

Construction of property distribution maps from irregularly spaced observations generally requires some form of interpolation onto a regular grid. Objective analysis is a technique for doing so that minimizes the expected error (in a least-squares sense) at each analysis point via a linear combination of the neighboring observations. Each data point is weighted according to its space/time distance from the analysis point and a four-dimensional correlation function derived from the ensemble of prior data. Thus, statistical information about the measured field is used to optimize the resulting map. This procedure has been used routinely in oceanography for some time (Bretherton et al., 1976; Freeland and Gould, 1976). Open-ocean applications are particularly amenable to such an approach because the statistics of those areas are fairly homogeneous, well-behaved and relatively well characterized.

Objective analysis in coastal domains is a much more difficult problem for essentially two reasons. First, the presence of a coastline imposes geometrical constraints which are not easily handled analytically. Second, coastal processes are in general strongly impacted by topography which results in highly non-uniform statistics. For example, Petrie and Dean-Moore (1986) demonstrated that correlation scales of physical properties on the Scotian shelf were generally larger in the along-isobath direction than across isobaths. He et al. (1997) have implemented these aspects into an objective analysis procedure based on the original algorithm developed by Bretherton et al. (1976). The basic idea is that a set of domain-wide correlation scales are modulated by the local bathymetric gradients, such that the anisotropy with respect to along versus across isobath correlations is explicit. For the purposes of this study, the same correlation scales were used to map *Pseudocalanus* spp. distributions as were used in an objective analysis of hydrographic measurements in the region described by Lynch et al. (1996). Sensitivity analysis showed that the resulting maps of *Pseudocalanus* spp. distributions on Georges Bank were generally robust over a reasonable range of choices for the global correlation scales (20–60 km). However, rigorous assessment of the spatial and temporal covariance statistics of *Pseudocalanus* spp., as well as other zooplankton, is clearly needed in future research.

4.3. Spring evolution of species' distribution and abundance

Although the two sympatric sibling species are nearly indistinguishable based on morphological characters, the patterns of distribution and abundance of *P. moultoni* and *P. newmani* on Georges Bank suggested that the species are ecologically distinct. Although both species' abundances increased from February to May/June, the spring evolution of *P. newmani* abundance differed significantly from that of *P. moultoni*. From April to June, the population center of *P. moultoni* shifted from Georges Basin, to the northern edge of the Bank, to the center of the Bank (Fig. 5). In contrast, *P. newmani* persisted in off-Bank waters (especially to the northeast) during

March and April. A dense population of *P. newmani* occurred over the Bank during May, but had declined in number and spatial extent by June.

Current flow in 1997 followed the climatological mean conditions expected over the Bank to a large extent (Limeburner and Beardsley, 1996; Beardsley et al., 1997); drifter trajectories indicated that retention times were significantly higher for drifters launched inside the 60-m isobath on the crest of the Bank, while drifters launched on the southern flank were rapidly transported to the southwest and off the Bank. A northeast wind event immediately preceding the April Broadscale Survey swept drifters launched on the southern flank — outside of the 60-m isobath — off the Bank (Limeburner and Beardsley, 1998), and may also have swept *P. newmani* into this same area from Browns Bank and regions to the north. If *Pseudocalanus* spp. are passively transported in ocean currents, drifter trajectories suggest that only those populations that occur within the 60-m isobath may persist on Georges Bank to have any possibility of becoming self-sustaining through in situ reproduction.

We may speculate that the spring increase of *P. moultoni* on the crest of the Bank may result both from increased transport of copepods onto the Bank from surrounding regions and from reproduction of individuals that persist within the anticyclonic gyre over the Bank. We cannot discriminate between these processes based on the observed distributions. We may further speculate that the Northeast Channel sample (from Station 25; Fig. 2) represents either the southern edge of an extensive Scotian Shelf population of *P. newmani* or the nearshore extension of an offshore population (see McLaren et al., 1989). If so, these populations may be the source of *P. newmani* populations on Georges Bank; the species may be transient along the southern flank of Georges Bank where the populations are unlikely to be retained or self-maintained. The persistence of *P. newmani* in waters to the north and east of the Bank was consistent with an earlier study by McLaren et al. (1989), who found the species on Browns Bank almost throughout the year. These speculations are consistent with the biogeographic distributions described for the two species by Frost (1989), who considered *P. moultoni* to be a coastal species and *P. newmani* to be a cosmopolitan, oceanic species.

Questions about sources of recruitment of *Pseudocalanus* spp. to Georges Bank will require examination of larval and juvenile life stages of both species. Ongoing studies of *Pseudocalanus* spp. life history by E.G. Durbin (University of Rhode Island) indicate that nauplii are present on Georges Bank almost throughout the year, based on distributional data for the pooled species. Without knowledge of the seasonal timing and spatial location of reproduction and recruitment of each species, we cannot know whether species' abundance, patterns of reproduction, and long retention times coincide for either species on Georges Bank.

Other unknowns are the distribution and abundance of *P. moultoni* and *P. newmani* from July to December. Samples were obtained from an October, 1997 cruise to the Gulf of Maine (cruise #EN307 of the R/V *Endeavor*). Based on PCR assay of 213 female *Pseudocalanus* spp. in seven collections from near-surface waters, the two species were nearly equally abundant (Bucklin, unpublished data). Whether both species persist in the water column between October and February is unclear.

The biological and physical dynamics determining the observed patterns of species' distribution and abundance cannot be determined by distributional data alone. We are currently in the process of incorporating these data into coupled physical-biological models which simulate the interaction of the circulation patterns and the species' life history, behavior, and vertical

distribution. These analyses will allow us to determine the extent to which the spring evolution of abundances of both *Pseudocalanus* spp. on Georges Bank result from advective transport of individuals from adjacent regions and/or from persistence of reproducing individuals over the crest of the Bank. This collaborative study will be completed as part of Phase III of the US GLOBEC Georges Bank Study. Our goal for this continued effort is to determine whether the observed differences in distribution and abundance of the two species have significant impact on their retention times, advective transport, and population abundances over Georges Bank.

5. Conclusions

There were consistent differences between the sibling species of *Pseudocalanus*, *P. moultoni* and *P. newmani*, in their spatial (i.e., Bank-wide) and temporal (i.e., seasonal) patterns of distribution and abundance. The observed pattern of distribution and abundance of *P. moultoni* suggested that, as the seasonal population increase proceeded from April to June, the population center shifted from Georges Basin, to the northern edge of the Bank, to the center of the Bank. In contrast, *P. newmani* occurred in off-Bank waters during March and April, and was most abundant on Georges Bank during May. The spring increase of *P. moultoni* and *P. newmani* populations on Georges Bank may have resulted from either or both advective transport of individuals from adjacent regions and persistence of reproducing individuals within the 60-m isobath. In order to determine whether either species is adapted to retention on Georges Bank, we will need to determine the spatial and temporal patterns of reproduction and to assess the distribution, abundance, and survivorship of critical life stages of both species. Ongoing studies by the authors will use numerical models to examine the species' life histories and patterns of distribution and abundance in the context of a realistic ocean flow field.

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