Population genetics of drifting (*Calanus* spp.) and resident (*Acartia clausi*) plankton in Norwegian fjords

A.Bucklin^{1,2}, S.Kaartvedt³, M.Guarnieri¹ and U.Goswami⁴

¹Ocean Process Analysis Laboratory and ²Department of Zoology, University of New Hampshire, Durham, NH 03824, USA, ³Department of Biology, University of Oslo, Oslo, Norway and ⁴National Institute of Oceanography, Goa, India

Abstract. Kaartvedt distinguished between drifting and resident plankton and hypothesized that the latter were distinguished by their ability to maintain their horizontal position in desired habitats (Kaartvedt, 1993). In this study, we examined the population genetic consequences of these two lifestyles for copepods in four fjords of western Norway (Lurefjorden, Masfjorden, Sognefjorden and Sørfjorden) and one fjord in eastern Norway (Oslofjorden). Based on DNA sequence variation of a region of mitochondrial 16S rRNA, we contrasted population genetic diversity and structure in drifting populations of *Calanus* spp. with that of resident populations of *Acartia clausi*. With the exception of Sørfjorden (where Calanus spp. were rare), two or three species of Calanus co-occurred in significantly different proportions in the fjords. Based on a 350 base-pair region of mitochondrial 16S rRNA, Calanus spp. varied in molecular genetic diversity, with the highest values for C.helgolandicus. There was no evidence of significant genetic structure of fjord populations for either C.finmarchicus or C.helgolandicus; the population structure of C.glacialis could not be evaluated as the species was only abundant in Lurefjorden. Acartia clausi was abundant in all five fjords sampled for this study. Molecular genetic diversity of A. clausi, based on a 220 bp region of mt 16S rRNA, was within the range of Calanus spp. values. Populations of A.clausi showed significant genetic structure (i.e. haplotype frequencies differed markedly) among the fjords. The results of this study indicated that little exchange (gene flow) occurs between populations of A.clausi in different fjords, and suggested that the populations are longterm residents of a fjord. In contrast, most Calanus spp. fjord populations may be replaced periodically, as they drift with currents flowing to and from coastal and ford environments.

Introduction

Fjords differ in their degree of exchange with open coastal waters. The biological and physical dynamics of open fjords are thought to be determined largely by advection (Aksnes *et al.*, 1989; Giske *et al.*, 1991), while abundances in more closed fjords may be determined by local production (Aksnes and Magnesen, 1983). Thus, closed and open fjords may differ in zooplankton species' composition and in the degree to which populations of a given species are resident (i.e. self-maintaining).

The numerous zooplankton species characteristically found in Norwegian fjords may exploit these coastal environments in different ways. Some species may be long-term residents of the fjord and use active swimming and positioning behaviors to ensure retention. Other species may drift in and out of the fjord with tidal and coastal currents, or be replaced periodically with basin water exchanges (Lindahl and Hernroth, 1988). Understanding the significance of both short and long time-scale processes is probably essential for evaluating the degree to which species rely on the fjord ecosystem, or are transient residents in fjords but resident in coastal and open ocean waters.

Kaartvedt discussed different behaviors and distributions of planktonic copepods in fjords of western Norway, and differentiated between drifting (e.g. *Calanus finmarchicus*) and resident (e.g. *Acartia clausi*) species (Kaartvedt, 1993). He hypothesized that *A.clausi* may actively control its vertical distribution to ensure retention in desired habitats. The ability of *Acartia* spp. to establish persistent populations in highly advective regions is well documented. Studies of *Acartia* spp. behavior in relation to flow dynamics have revealed remarkable position-keeping abilities by these obligately-estuarine species [see e.g. (Kimmerer and McKinnon, 1987; Kaartvedt and Nordby, 1992; Norrbin, 1992)]. In addition to active swimming behaviors that allow regulation of vertical distribution, *Acartia* spp. have diapause eggs that may reside in benthic sediments and re-populate the estuary after a population crash (Hairston and DeStasio, 1988; Marcus *et al.*, 1994). In general, the life history patterns of *Acartia* spp. would seem to be adapted for life in changeable environments; generation spans are short and reproductive potential is high.

The evidence for species-specific differences in copepod behavioral responses to the physical and biological regime of Norwegian fjords has accumulated from numerous studies (Eilertsen *et al.*, 1981; Tande, 1991; Norrbin, 1992; Kaartvedt and Svendsen, 1995; Falkenhaug *et al.*, 1997). Based on these studies, it is clear that zooplankton species differ markedly in patterns of distribution and abundance within fjords, and that the reasons for these differences are likely to result from both active behaviors and passive responses to current flow (Barthel, 1995), including advective exchange with coastal waters (Aksnes *et al.*, 1989; Falkenhaug *et al.*, 1997).

One approach to the understanding of patterns of dispersal of zooplankton populations is to examine population genetic structure, i.e. the degree to which geographic populations are genetically distinct (Wright, 1969), to infer patterns of gene flow and thus, to estimate the extent to which species are resident in fjords. Resident populations may be expected to show less exchange among fjords—and therefore, more population genetic differentiation—than drifting species, which may be genetically homogenized in coastal waters. An earlier study confirmed that the observed behavior and life history traits of *Acartia* spp. result in genetically differentiated populations. Caudill used population genetic analysis to determine that *A.tonsa* experienced very low levels of gene flow, even between populations in adjacent coastal estuaries of the US east coast (Caudill, 1995). In contrast, previous studies of *Calanus* spp. have demonstrated a lack of population genetic structure at the small- to meso-scale (Bucklin and Kocher, 1996), although populations of *C.finmarchicus* were shown to be structured at the ocean basin scale (Bucklin *et al.*, 1996).

In this study, we examine and compare the population genetic diversity and structure of four species of copepods with distinctive life histories inhabiting Norwegian fjords, *A.clausi* and *Calanus* spp., in order to better understand their ecology and their method of exploitation of fjord environments.

Method

Sample collection

Zooplankton samples were collected by vertical tows in the upper 200–500 m of the four western Norwegian fjords (Sognefjorden, Masfjorden, Lurefjorden and

Table I. Characteristics of the fjords from which *Acartia clausi* and *Calanus* spp. were collected for this study, with collection information for the zooplankton samples. Sill depth and maximum depth (Max. depth) are given for each fjord. Locations, dates (Coll. date), and depths (Coll. depth) of collection for zooplankton samples are given for the samples from which copepod species were sorted

Fjord	Sill depth (m)	Max. depth (m)	Location	Coll. date	Coll. depth (m)
Lurefjorden	20	440	60°41.0′N; 5°10.5′E	21 October 1995	0-250
Masfjorden	75	490	60°52.3'N; 5°24.7'E	22 October 1995	0-250
Oslofjorden	19	160	59°48.0'N; 10°34.0'E	10 November 1995	61-115
Sognefjorden	240	1300	61°7.8'N; 6°4.5'E	19 October 1995	0-500
Sørfjorden	90	380	60°30.0'N; 5°43.2'E	23 October 1995	0-250

Sørfjorden) and in the upper 100–200 m of Oslofjorden in eastern Norway (Table I). Sub-samples of *Calanus* and *A.clausi* females were preserved in 95% ethyl alcohol and shipped to the University of New Hampshire for molecular analysis.

Molecular genetic analysis

Individual adult female copepods were identified under a dissecting microscope at $\times 250$ magnification. They were prepared for molecular analysis by placing them in 0.5 ml microcentrifuge tubes with 17.5 μl distilled water, and submerging the tubes in deionized water kept at a rolling boil for 10 min. The PCR reaction cocktail was then added to the tube.

The oligonucleotide primers used to amplify Calanus spp. were 16SAR and 16SBR (Palumbi et al., 1991), based on the Drosophila vakuba sequence (Clary and Wolstenholme, 1985). The primer sequences are: 16SAR(DROS): 5'-CGCCTGTTTAACAAAAACAT-3' and 16SBR(DROS): 5'-CCGGTTT-GAACTCAGATCACGT-3'. The PCR protocol was 94°C (1 min); 37°C (2 min); 72°C (3 min) for 40 cycles [see (Bucklin et al., 1996) for detailed protocols]. The amplification protocol for A.clausi used the 16SBR(DROS) primer paired with a specifically-designed primer, 16S-167 (Caudill, 1995; Bucklin et al., 1998), with the sequence: 16S-167: 5'-GACGAGAAGACCCTATGAAG-3'. The PCR protocol was 94°C (1 min); 45°C (2 min); 72°C (3 min) for 40 cycles. Amplification products to be sequenced were checked for size and purity by loading 10 μ l on a 2% agarose gel. Approximately 20 µl of product were then loaded into the wells of a 1% SeaPlague gel with ethidium bromide and electrophoresed at 70 V. Product bands were cut from the gel and digested with agarase enzyme in a water-bath incubation. Approximately 250 ng DNA in the digested agarose solution were used for the cycle sequencing step. DNA sequencing was done using an ABI Cycle Sequencing Kit and the 16SBR(DROS) primer for both Calanus spp. and A.clausi. Sequencing was done on an ABI Automated DNA Sequencer, Model 373.

Data analysis

Initially, multiple alignment of the *Calanus* 16S rRNA sequences revealed an exceptional degree of haplotype divergence. Realignment with sequences of known *C.finmarchicus*, *C.glacialis* and *C.helgolandicus* [from (Bucklin *et al.*, 1992, 1995)] indicated that the *Calanus* sequence data comprised individuals of all three species. The specific identification of *A.clausi* was confirmed based on comparison with the 16S rRNA sequences for a number of *Acartia* spp. collected from various locations and sequenced by Caudill (Caudill, 1995).

The numbers of different haplotypes for each species were determined by multiple alignments and cluster analysis using the PileUp program in the Genetic Computer Group (GCG) software package (Devereaux *et al.*, 1984). All population genetic analyses were performed for each species separately. Phylogenetic analysis of mt 16S rRNA sequence variation within *A. clausi* was reconstructed by the UPGMA tree-building algorithm with Tamura-Nei distances (Tamura and Nei, 1993) and bootstrapped ×1000 using the software package MEGA [Molecular Evolutionary Genetics Analysis (Kumar *et al.*, 1993)].

Haplotype frequencies were computed for each species and haplotype diversity, *h*, was calculated by:

$$h = (n/(n-1)) (1 - \sum f_i^2)$$
(1)

where *n* is the number of haplotypes sequenced and f_i is the frequency of the *i*th variant (Nei, 1987). Variance of *h* was estimated according to the method of Nei (Nei, 1987). Nucleotide diversity, π , was calculated according to Nei (Nei, 1987) by:

$$\pi = \sum_{i < j} p_{ij} / n_c \tag{2}$$

where p_{ij} is the proportion of different nucleotides between the *i*th and *j*th haplotypes and n_c is the total number of sequence comparisons [n(n-1)/2]. Variance was calculated according to Tajima (Tajima, 1983).

The geographic distributions of the haplotypes (including the pool of all unique haplotypes) were statistically evaluated by a chi-square test using a Monte Carlo simulation (Roff and Bentzen, 1989). Comparisons among all samples and regions were done to identify significant geographic partitioning. Each comparison used 1000 replicates and generated a P value \pm standard deviation of results from the 1000 simulations. The genetic heterogeneity of *C.finmarchicus*, *C.helgolandicus* and *A.clausi* populations was examined using a hierarchical analysis of variance by Analysis of Molecular Variation [AMOVA (Excoffier *et al.*, 1992)] to compute and statistically evaluate the variance components among populations, V(A).

Results

In the fjords sampled, we confirmed the presence of *Acartia clausi* and three *Calanus* spp.: *C.finmarchicus, C.helgolandicus* and *C.glacialis* (Table II). From

						60
A.clausi	TAATAGGGTC	GAACAGACCC	AATTAGTTTA	ATTTCTGCTT	TAACTAATTC	CGCAATTCAA
SUBS N	G 1	AT.G 37 1		•••••	G 1	•••••
						120
A.clausi	CATCGAGGGT	CGCAAACAGT	TATTTAAATA	AGAGCTTTAT	TAACTATAAC	GCTGTTATCC
SUBS	•••••	G	•••••	G		.G
						180
A.clausi	CTAGAGTACC	TTCTTCACAA	TTTTTAATAA	AGATCAAATT	ATTTAATAGT	TTTAATATAA
SUBS		T		G	.A	GCG
		2		1	6	8 14
		200				
A.clausi	AAATTAATTT	TTTCCCCAAA				
SUBS	AGA					
N	12 1					
SUBS	c	*•••••				
N	1					

Fig. 1. DNA sequence variation of a 200 bp region of the mitochondrial 16S rRNA for 96 individuals of *Acartia clausi* collected from five Norwegian fjords. The most frequent haplotype is shown; variable sites are indicated on the line below (SUBS), with the number of occurrences of each variant below that (N). Non-variable bases indicated by a period (.).

Table II. The relative abundances of three *Calanus* spp. in five fjords based on identification of individuals by mt16 S rRNA sequence variation. *Calanus* spp. were selected from zooplankton subsamples without regard to species; 350 bp sequences were obtained for each individual and compared with sequences from identified individuals collected from regions where only one species occurs. Because sample sizes are small, this analysis is intended only as an approximate index of relative species' abundances of *C.finmarchicus, C.glacialis* and *C.helgolandicus*

Species	Fjord							
	Lurefjorden	Masfjorden	Oslofjorden	Sognefordenj	Sørfjorden			
C.finmarchicus	6 (29%)	16 (72%)	5 (23%)	14 (56%)	2			
C.glacialis	20 (68%)	1 (5%)	- 1	-	-			
<i>C.helgolandicus</i>	4 (13%)	5 (23%)	17 (77%)	12 (44%)	-			
Total	30	22	22	26	2			

our examination of 21–31 individuals of *Calanus* spp. collected from each of four fjords (very few *Calanus* spp. were found in the samples from Sørfjorden), relative species' abundances differed among the fjords (Table II). All three species were present in the sub-samples from two fjords: Lurfjorden and Masfjorden. However, *C.glacialis* predominated in Lurefjorden and *C.finmarchicus* predominated in Masfjorden. In two fjords, *C.finmarchicus* co-occurred with *C.helgolandicus*; in Sognefjorden, the two species were about equal in relative abundance and in Oslofjorden, *C.helgolandicus* predominated (see Table II for relative abundance data).

The DNA sequence of a 220 bp region of 16S rRNA was obtained for 96 individuals of *A.clausi* (Figure 1). The sequence variation resolved 29 different



Fig. 2. Phylogeographic analysis of mitochondrial 16S rRNA sequence variation for *Acartia clausi* from five Norwegian fjords. The gene tree was reconstructed by the UPGMA algorithm (Kumar *et al.*, 1993) using Tamura/Nei distances (Tamura and Nei, 1993) and bootstrapped $\times 1000$. There was no evidence of grouping of haplotypes from the same fjord (i.e. lineage sorting). Numbers along tree branches are branch lengths in units of the distance measure; numbers in bold italic type at branchpoints are bootstrap values (i.e. percentage of trees among 1000 sub-replicates exhibiting that branchpoint).

haplotypes (Table III). Haplotype diversity (h = 0.674) and nucleotide diversity values ($\pi = 0.0026 \pm \text{SD} 0.005$) were low to moderate for *A.clausi*, most of the 220 bp sequences differed by only 1– 2%. Based on a phylogenetic gene tree [reconstructed by UPGMA (Kumar *et al.*, 1993)], there was no evidence of lineage sorting (i.e. geographical grouping of the haplotypes; Figure 2). Haplotype frequencies differed significantly among the samples from the five fjords [$P < 0.001 \pm 0.001$ based on a Monte Carlo chi-square test (Roff and Bentzen, 1989); Figure 3]. Based on AMOVA (Excoffier *et al.*, 1992), 5.99% (P < 0.0099) of the molecular variance was explained by comparison among the five fjord populations.



Fig. 3. Haplotype frequencies of mitochondrial 16S rRNA for *Acartia clausi* collected in five Norwegian fjords: Lurfjorden, Masfjorden, Sognefjorden, and Sørfjorden in western Norway and Oslofjorden in eastern Norway (not shown on figure). Collection locations indicated were the sites of collection for both *A.clausi* and *Calanus* spp. used for molecular population genetic analysis (see Table I). Statistical analysis of the haplotype frequencies indicated significant genetic differentiation among fjord populations (P < 0.001), suggesting very low levels of dispersal (gene flow) between fjords. Numbers of individuals sequenced for 16S rRNA from each fjord are indicated by N. Haplotypes are identified by number, except that all unique haplotypes are grouped into a single haplotype class (Pool; see Table II).

Table III. Mitochondrial 16S rRNA haplotype abundances for *Acartia clausi* for 96 individuals collected from five Norwegian fjords. Haplotypes are numbered 1–9; all unique haplotypes were grouped into a single class (Pool) to reduce the statistical bias of small sample size. The total number of individuals sequenced for a 220 bp region of 16S rRNA from each fjord are shown (Total)

Sample	Haplotype											
	1	2	3	4	5	6	7	8	9	Pool	Total	
Oslofjorden	2	1	1	0	0	0	0	3	7	5	19	
Masfjorden	0	0	0	0	0	1	1	0	13	3	18	
Lurefjorden	0	0	3	4	0	0	2	0	8	5	22	
Sørfjorden	0	1	0	0	0	0	0	0	13	2	16	
Sognefjorden	0	0	0	0	2	1	0	0	13	5	21	
Total											96	

C.finmarchicus SUBS N C.helgolandicus SUBS N C.glacialis SUBS	TTAGTTTCTT TTAGTTTTTT C 1 TTAGTTTCTT	AATTGGAAAA AATTGGAAAAA G 1 AATTGGGAAA	TAGGAATGAAT TGGAATGAAT .A.G 1 1 TGGAATGAAT	GGTTTCACTA C 1 GGCTCCACTA GGTTTCACTA	AAATATAGTT A 1 AAGCATAGTA AAATATGATA	TTTATACTCA A.CC 1 1 9 TTTATACTAA TTTATTCTAA	100
C finnarchicus		መመመል አመርጣል እ	GTCAAATAC	TTACCACTTC	TACTACCACC	AGAAGACCCT	120
SUBS		.A	G				
N		1	1			-	
C.helgolandicus	AAAATGAAAT	TTTAATTTAA	GTGAAAATAC	TTAAATGCTA	TATTTAGACG	AGAAGACCCT	
N	1	G		1 1 10	1		
SUBS	-						
N					1		
C.glacialis	TTTGCGAAAT	TTTAATCTGA	GTGAAAATAC	TCAGAAGATA	TATTTAGACG	AGAAGACCCT	
SUBS	• • • • • • • • • • •	1 14	• • • • • • • • • • •	• • • • • • • • • • •	••••		
		1 14					
							180
C.finmarchicus	ATGAAGCTGG	CAAACTATTA	ATACATATTC	CTATTATTTA	TTAGTTTATT	TTTTGGGGTA	
SUBS		• • • • • • • • • • •	T				
C.helgolandicus	ATGAAGCTGG	TAGACCATAA	GTGTAATTAT	TTCATAGTAG	CAGGTCTATT	TTTTGGGGTA	
SUBS	G		ATA.TC	AA	т		
N	1		2 11 11	1 1	3		
C.glacialis	ATGAAGCTGG	TAGACTTCCA	ATGTAATTAT	ACGATAGTTC	ATGAGTTTAT	TTTTTGGGGT	
N	• • • • • • • • • • • •			•••••		1	
-							
							240
C.finmarchicus	AAA-TTTAAT	AATACTATTA	ACACAATTGT	ACTAAATTAC	ATCCTTTAGG	AATTATGAAG	
N			1		1	1.	
C.helgolandicus	AAA-TTTAAT	AATTATATTA	ATACAGATTT	GTTCAAACTT	ATCCTTCTGG	AATTATGAAT	
SUBS		A	T	$\cdots \cdots $	TA	G.	
N		1	3		11	1	
C.glacialis SUBS	G	AATAGTATTA	ATATTGGCTT	ACTAAATAAT	AICCICITGG	AATTAIGAAA	
N	1			1			
							300
SUBS	AAGCTCCTCT	AGGGATAACA	GCATTAIGCT	TAAAAGAGIT	CTIAICAGAA	TAAGCGTTTG	
N	1 1			1			
C.helgolandicus	AAGCTCCTCT	AGGGATAACA	GCATTATGCT	TAAAAGAGTT	CTTATCAGAA	TAAGCGTTTG	
SUBS							
C.glacialis SUBS	AAGCTCCTCT	AGGGATAACA	GCATTAIGCT	TAAAAGAGTT	CTTATCAGAA	TAAGCGTTTG	
							350
C.finmarchicus	TGACCTCGAT	GTTGAATTAA	ATGCTCTTTT	ATGTGCAGGA	GCTTAGAAGA		
SUBS C. helgolandicus	TGACCTCGAT	GTTGAATTAA	ATACCCCAGT	GTGTGCAGGA	GCTCATTGGA		
SUBS					TGA		
N			2		1 11		
C.glacialis	TGACCTCGAT	GTTGAATTAA	ATACTCTTAT	ATGTGCAGGA	GCTTATAAGA		

Fig. 4. DNA sequence variation of a 350 bp region of mitochondrial 16S rRNA for *Calanus finmarchicus* (42 individuals), *C.helgolandicus* (35 individuals) and *C.glacialis* (21 individuals) collected from Norwegian fjords. The sequence shown is the most frequent haplotype for each species; variable bases are shown on the line below (SUBS), with the number of occurrences of each substitution shown below that (N). Non-variable bases indicated by a period (.).

A 350 bp region of 16S rRNA was sequenced for a total of 98 individuals of *Calanus* spp. collected from four fjords (Figure 4). The sequence variation resolved 11 haplotypes among 42 individuals of *C.finmarchicus*; molecular genetic diversity of the species was low-to-moderate (h = 0.448; $\pi = 0.0022 \pm \text{SD } 0.0034$).

60

Table IV. Mitochondrial 16S rRNA haplotype abundances for: (**A**) 42 individuals of *Calanus finmarchicus*, (**B**) 35 individuals of *C.helgolandicus*, and (**C**) 21 individuals of *C.glacialis* in sub-samples of zooplankton collections from four fjords in Norway. The haplotypes, which are not the same for the two species, are arbitrarily numbered. Unique haplotypes in each species were grouped for the statistical analyses and are indicated by Pool. The number of individuals of each species from each fjord sequenced for a 350 bp region of 16S rRNA is indicated by Total

(A) C.finmarchicus

Sample	Haplotype						
	1	2	Pool	Total			
Masfjorden	2	9	4	15			
Lurfjorden	_	5	1	6			
Oslofiorden	_	5	-	5			
Sognefjorden	_	10	4	14			
Sørfjorden	_	2	-	2			
Totals	2	31	9	42			

(B) C.helgolandicus

Sample	Haplotype							
	1	2	3	4	5	Pool	Total	
Masfjorden	_	_	2	3	_	_	5	
Lurfjorden	1	-	-	3	-	-	4	
Oslofjorden	2	1	3	2	2	4	14	
Sognefjorden	_	1	3	6	_	2	12	
Totals	3	2	8	14	2	6	35	

(C) C.glacialis

Sample	Haplotype						
	1	2	Pool	Total			
Masfjorden	1	-	_	1			
Lurfjorden	15	3	2	20			
Oslofjorden	-	-	-	-			
Sognefjorden	-	-	-	-			
Sørfjorden	-	-	-	-			
Totals	16	3	2	21			

Among 35 individuals of *C.helgolandicus*, 11 haplotypes were resolved (h = 0.769; $\pi = 0.0067 \pm \text{SD } 0.0113$). For *C.glacialis*, there were four haplotypes among 21 individuals (h = 0.395; $\pi = 0.0025 \pm \text{SD } 0.0037$) (Tables IV and V).

Haplotype frequencies did not differ among samples of *C.finmarchicus* ($P < 0.5360 \pm SD \ 0.0158$) or *C.helgolandicus* ($P < 0.4510 \pm SD \ 0.0157$) collected from fjords where they were present. The molecular variance component due to among-population comparisons was not significant for either *C.finmarchicus* (P < 0.861) or *C.helgolandicus* (P < 0.861; Table V); *C.glacialis* were not found in sufficient abundance in any fjord except Lurefjorden to allow statistical examination of population genetic structure (Table II).

Table V. Statistical comparison of values of the molecular population genetic indices of diversity and structure for *Acartia clausi* and *Calanus* spp. Diversity is measured as haplotype diversity, *h*, and nucleotide diversity, π . Population genetic structure was quantified by haplotype frequency differences (significance level of the by χ^2 test) and determination of the variance component, and its statistical significance, due to among-population comparisons, V(A) and significance level. See text for methods of calculation and explanation of meaning

Species	h	$\pi \pm SD$	χ^2 test (<i>P</i> -value)	V(A), <i>P</i> -value
A.clausi C.finmarchicus C.helgolandicus C.glacialis	0.674 0.448 0.769 0.395	$\begin{array}{c} 0.0026 \pm 0.005 \\ 0.0022 + 0.003 \\ 0.0067 + 0.011 \\ 0.0025 + 0.004 \end{array}$	$\begin{array}{l} P < 0.001 \pm 0.001 \\ P < 0.536 + 0.012 \\ P < 0.451 + 0.016 \\ \text{NA} \end{array}$	5.99%, <i>P</i> < 0.0099 -1.92%, <i>P</i> < 0.861 -4.95%, <i>P</i> < 0.861 NA

NA: population genetic structure was not evaluated for *C.glacialis* as it was found in abundance only in Lurefjorden.

Discussion

Morphology of the sampled fjords

The morphology and oceanographic character of a fjord has considerable significance for the make-up and community dynamics of the zooplankton assemblage (Salvanes *et al.*, 1995). Fjords that have deep basins and shallow sills, and that are distant from the coast, are most likely to harbor zooplankton species with selfmaintaining, persistent populations. The fjords sampled in this study differed in depth and sill depth (Table I), degree of openness and distance to open coastal waters, and many other characters (Bagøien, 1999). Lurefjorden was evidently the most enclosed system, despite its close proximity to the coast. In contrast, Masfjorden represented an open environment, where the abundance of zooplankton is regulated by advection rather than production (Aksnes *et al.*, 1989; Giske *et al.*, 1991).

The most important process determining rates and frequencies of exchange between coastal and fjord zooplankton populations is the flushing of waters above sill depth during spring and summer [see e.g. (Kaartvedt, 1993; Barthel, 1995; Lindahl and Hernroth, 1998)]. Populations of copepods may be swept into and out of fjords by regional wind-driven (Aksnes *et al.*, 1989) and coastal currents (Klinck *et al.*, 1981). In addition, advective exchange of basin water below sill depth may contribute to exchange between deeper-dwelling populations, especially in fall and winter (Lindahl and Hernroth, 1988; Bagøien, 1999).

Species' populations of *Acartia* and *Calanus* will be impacted by differences in exchange processes among fjords, in terms of the likelihood of advective loss from the fjords, exchange with coastal waters and exchange among fjords themselves. However, Kaartvedt (Kaartvedt, 1993) suggested that *Calanus* spp. are more subject to transport in surface advective flows than are *Acartia* spp. [see (Sinclair, 1988; Kaartvedt and Nordby, 1992)]. The basis of this study is the related hypothesis that *Acartia* spp. will exhibit greater population genetic structuring among fjords, due to limited gene flow among long-term resident populations in different fjords, than *Calanus* spp., which are more subject to advective exchange among fjords.

Assessment of population genetic structure and diversity

The frequency of exchange of zooplankton between fjord and open coastal populations, and among different fjord populations, may be inferred from population genetic analysis. The differential partitioning of genetic diversity among populations (i.e. population genetic structure) indicates reproductive isolation and restricted gene flow (Wright, 1969). Conversely, genetic homogenization of the species' population reflects high rates of exchange among populations. We have used population genetic analysis to provide some insight into the degree to which zooplankton species depend wholly on fjord environments (i.e. are resident) or instead, invade fjords for some portion of their life or during some seasons of the year (i.e. drift in and out).

By several measures of population genetic structure, *A.clausi* showed marked differentiation in the genetic make-up of populations in the different fjords, including haplotype frequency differences and molecular variance resulting from among-population comparisons (Table V). However, there was no evidence of lineage sorting (i.e. geographical grouping of the haplotypes; Figure 2), which might be expected with isolation of populations on evolutionary time scales. Our conclusion from these results is that *A.clausi* populations may persist for numerous generations in a given fjord, with very little exchange between fjord populations through advective losses and supply. In contrast, *Calanus* spp. showed no significant genetic differentiation between fjord populations, either in haplotype frequencies or analysis of molecular variance (Table V). The simplest explanation for this difference is that *Calanus* spp. populations may be more frequently replenished from coastal waters and intermixed among fjords—by wind events, basin exchanges, and large-scale water mass movements—ensuring their genetic homogenization.

It is informative to compare levels of molecular diversity among the species, based on both haplotype diversity, *h*, and nucleotide diversity, π (i.e. mean pairwise sequence divergence between haplotypes). Calanus and Acartia species populations in Norwegian fjords did not appear to differ markedly in haplotype diversity (Table V), although the different lengths of mt DNA sequences between Calanus spp. (350 bp) and A.clausi (220 bp) makes it difficult to compare the observed values directly. There were differences in haplotype diversity among the three Calanus spp., with highest values for C.helgolandicus and lowest for C.glacialis (Table V). Pairwise sequence differences between haplotypes for A.clausi and all three Calanus spp. were typically 1-2%. Relative values of nucleotide diversity of the species followed the same trend as for haplotype diversities, i.e. low-to-moderate for A. clausi, C. finmarchicus and C. glacialis, and somewhat higher for *C.helgolandicus* (Table V). It is tempting to speculate that latitudinal gradients in genetic diversity of copepods previously observed by Bucklin and Wiebe (Bucklin and Wiebe, 1998) may also pertain to Calanus spp., with diversity decreasing with higher latitudinal distribution. Bucklin and Wiebe hypothesized that differences in nucleotide diversity between copepod species may result from the evolutionary history of the species, including genetic bottlenecks associated with range compression and displacement during glacial periods.

which are more marked for more northern species (Bucklin and Wiebe, 1998). This hypothesis is testable for *C.helgolandicus* (a transition zone species), *C.finmarchicus* (a subarctic species) and *C.glacialis* (an arctic species). Valid examination of the hypothesis will require assay of larger sample sizes representing more of the geographic range of each species.

Molecular genetic diversity values of *C.finmarchicus* examined in this study of fjord populations were higher than those of oceanic populations determined in a previous study of 216 individuals collected across the North Atlantic [h = 0.368; $\pi = 0.0037 \pm \text{SD} 0.003$; see (Bucklin and Wiebe, 1998)]. Estimates of molecular genetic diversity are strongly dependent on sample size, primarily because of sampling error associated with rare alleles; the observed differences will require evaluation by assay of larger sample sizes. The same 16S rRNA haplotype that predominated in the 1992 and 1993 collections from the North Atlantic [see (Bucklin *et al.*, 1996)] was also the most abundant in the 1995 collections from the fjords, providing evidence of lack of genetic divergence between fjord and coastal or open ocean populations.

Nucleotide diversity levels differed markedly between *A.clausi* in Norwegian fjords and *A.tonsa* in estuaries of the USA east coast [$\pi = 0.066$ (Caudill, 1995; Bucklin and Wiebe, 1998)]. Pairwise differences between 16S rRNA haplotypes for *A.tonsa* ranged up to 15–18% (Caudill, 1995), suggesting a very different evolutionary history for the two species [see (Bucklin and Wiebe, 1998)].

Distribution and abundance of Calanus spp.

Considered together, zooplankton samples collected from four fjords of western Norway and one fjord from eastern Norway included *A.clausi* and three species of *Calanus*, although species' abundances and relative proportions differed among the fjords. Oslofjorden was populated primarily by *C.helgolandicus*, as was known from previous studies (Beyer, 1992; Bagøien, 1999; Bucklin *et al.*, 1999). Masfjorden and Sognefjorden had mixtures of *C.finmarchicus* and *C.helgolandicus*. The predominance of *C.glacialis* in Lurefjorden—with the cooccurrence of all three species—is a novel finding that was previously unsuspected. This result was later confirmed by Bagøien (Bagøien, 1999), who examined the zooplankton assemblage in the same five fjords as this study. Molecular analysis of samples collected from Lurefjorden in February 1998, indicated the continued predominance of *C.glacialis* (Bagøien, 1999). A unique, largely resident *Calanus* population may be a permanent feature of this very enclosed fjord.

The biological and physical processes that determine which *Calanus* spp. occur in which fjords are unclear, even from Bagøien's (Bagøien, 1999) more comprehensive examination of hydrography, circulation, and fjord morphology. Which *Calanus* spp. become resident in a particular fjord (and how long they are retained) may have a stochastic component, resulting from the likelihood of the species' presence in coastal waters during advective events.

Population genetic analysis of dispersal

Population genetic analysis suggests that *Calanus* spp. populations do not persist long enough, or become sufficiently isolated in a particular fjord, to accumulate genetic differences. Previous studies have suggested that *Calanus* spp. populations in Masfjorden may be renewed each year by advection from coastal waters (Aksnes *et al.*, 1989). Deep water renewal may displace over-wintering *Calanus* populations in fjords (Lindahl and Hernroth, 1988), while shallow water exchange (i.e. above sill depth) may have a strong impact on species' abundances during spring and summer when *Calanus* abound in upper waters.

In contrast to *Calanus* spp., populations of *A.clausi* were geographically isolated and genetically differentiated, suggesting that exchange (gene flow) among these populations—even those inhabiting geographically contiguous, open fjords—was very rare. The population genetic findings of this study were consistent with previous observations of behavioral responses by *A.clausi* to variable current flow (Sinclair, 1988; Kaartvedt and Nordby, 1992). Further evidence for an active behavioral response by *A.clausi* was that *Calanus* spp. occupying these same fjords showed no evidence of genetic differentiation. The marked differences between *Calanus* spp. and *A.clausi* in geographic patterns of molecular population genetic diversity and structure most probably resulted from both patterns of individual behavior and life history. Our findings suggest that there are fundamental differences in the way *Calanus* spp. and *A.clausi* utilize fjord environments.

Conclusions

Molecular genetic analysis confirmed the presence of the planktonic copepods, *Acartia clausi* and three species of *Calanus* (*C.finmarchicus*, *C.glacialis* and *C.helgolandicus*) in Norwegian fjords. The unexpected presence of the arctic copepod, *C.glacialis*, had not been noticed in previous studies based on morphological species identification. Population genetic analysis based on mitochondrial 16S rRNA sequence variation indicated that fjord populations of *A.clausi* were genetically differentiated, based on haplotype frequencies and hierarchical analysis of molecular variation. In contrast, *Calanus* spp. showed no genetic differentiation among fjord populations. These patterns are consistent with considering *A.clausi* to be 'resident' plankton (i.e. abundances result from local reproduction; populations are self-maintained and persistent), while *Calanus* spp. are 'drifting' plankton (i.e. abundances are advectively controlled; populations are replenished with water renewal).

Acknowledgements

Laboratory technical assistance and advice was provided by S.B.Smolenack, R.S.Hill, J.J.Pierson and A.M.Bentley (University of New Hampshire). The authors would like to thank cruise participants for collection of samples. This collaborative project was partially supported by the Office of Naval Research, through the US–India International Exchange Program. Additional funding was provided to A.B. by the NSF/NOAA US GLOBEC Georges Bank Study (NSF Grant No. OCE-9632840), to S.K. by the Research Council of Norway and to U.G. by the National Institute of Oceanography, Goa, India. Contribution No. 155 from the US GLOBEC Program.

References

- Aksnes, D.L., Aure, J., Kaartvedt, S., Magnesen, T. and Richard, J. (1989) Significance of advection for the carrying capacities of fjord populations. *Mar. Ecol. Prog. Ser.*, **50**, 263–274.
- Aksnes, D.L. and Magnesen, T. (1983) Distribution, development, and production of *Calanus finmarchicus* (Gunnerus) in Lindäspollene, western Norway, 1979. *Sarsia*, **68**, 195–208.
- Bagøien, E. (1999) *Predatory Impact of Invertebrates and Fish on Overwintering* Calanus. Dr Scient thesis, Department of Biology, University of Oslo, Norway.
- Barthel,K.-G. (1995) Zooplankton dynamics in Balsfjorden, northern Norway. In Skjoldal,H.R., Hopkins,C., Erikstad,K.E. and Leinaas,H.P (eds), *Ecology of Fjords and Coastal Waters*. Elsevier, Amsterdam, pp. 113–126.
- Beyer, F. (1992) *Meganyctiphanes norvegica* (M. Sars) (Euphausiacea). A voracious predator on *Calanus*, other copepods, and ctenophores, in Oslofjorden, southern Norway. *Sarsia*, **77**, 189–206.
- Bucklin, A. and Kocher, T.D. (1996) Source regions for recruitment of *Calanus finmarchicus* to Georges Bank: evidence from molecular population genetic analysis of mtDNA. *Deep-Sea Res.*, **43**, 1665–1682.
- Bucklin, A. and Wiebe, P.H. (1998) Low mitochondrial diversity and small effective population sizes of the copepods, *Calanus finmarchicus* and *Nannocalanus minor*: possible impact of climatic variation during recent glaciation. *J. Heredity*, **89**, 383–392.
- Bucklin,A., Frost,B.W. and Kocher,T.D. (1992) Mitochondrial 16S rRNA sequence variation of *Calanus* (Copepoda; Calanoida): intra- and interspecific variation. *Molec. Mar. Biol. Biotech.*, 1, 397–407.
- Bucklin, A., Frost, B.W. and Kocher, T.D. (1995) Molecular systematics of *Calanus* (Copepoda: Crustacea). *Mar. Biol.*, **121**, 655–664.
- Bucklin, A., Sundt, R. and Dahle, G. (1996) Population genetics of *Calanus finmarchicus* in the North Atlantic. *Ophelia*, **44**, 29–45.
- Bucklin, A., Smolenack, S.B., Bentley, A.M. and Wiebe, P.H. (1997) Gene flow patterns of the euphausiid, *Meganyctiphanes norvegica*, in the N. Atlantic based on DNA sequences for mitochondrial cytochrome oxidase I and cytochrome b. *J. Plankton Res.*, **19**, 1763–1781.
- Bucklin,A., Caudill,C.C. and Guarnieri,M. (1998) Population genetics and phylogeny of marine planktonic copepods. In Cooksey,K.C. (ed.), *Molecular Approaches to the Study of the Ocean*, Chapter 14. Chapman & Hall, London, UK, pp. 303–318.
- Bucklin, A., Guarnieri, M., Hill, R.S., Bentley, A.M. and Kaartved, S. (1999) Taxonomic and systematic assessment of planktonic copepods using mitochondrial COI sequence variation and competitive, species-specific PCR. *Hydrobiol.*, **401**, 239–254.
- Caudill,C.C. (1995) *Molecular Evidence of Population Genetic Differentiation and Sibling Species in* Acartia tonsa (*Copepoda: Calanoida*). Master's Thesis. Durham, New Hampshire, University of New Hampshire.
- Clary,D.O. and Wolstenholme,D.R. (1985) The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. *J. Molec. Evol.*, **22**, 252–271.
- Devereaux, J., Haeberli, P. and Smithies, O. (1984) A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.*, **12**, 387–395.
- Eilertsen, H.C., Falk-Petersen, S., Hopkins, C.C.E. and Tande, K.S. (1981) Ecological investigations of the plankton community of Balsfjorden, northern Norway. *Sarsia*, **66**, 25–34.
- Excoffier,L., Smouse,P.E. and Quattro,J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.
- Falkenhaug, T., Tande, K. and Timonin, A. (1997) Spatio-temporal patterns in the copepod community in Malangen, northern Norway. J. Plankton Res., **19**, 449–468.
- Giske, J., Aksnes, D.L., Lie, U. and Wakili, S. (1991) Computer simulation of pelagic production in Masfjorden, western Norway, and its consequences for production of released 0-group cod. *ICES Mar. Sci. Symp.*, **192**, 161–175.

- Hairston, Jr, N.G. and DeStasio, B.T.Jr (1988) Rate of evolution slowed by a dormant propagule pool. *Nature*, **336**, 239–242.
- Kaartvedt, S. (1993) Drifting and resident plankton. Bull. Mar. Sci., 53, 154-159.
- Kaartvedt, S. and Nordby, E. (1992) Impact of a controlled freshwater discharge on zooplankton distribution in a Norwegian fjord. J. Exp. Mar. Biol. Ecol., 162, 279–293.
- Kaartvedt,S. and Svendsen,H. (1995) Effect of freshwater discharge, intrusions of coastal water and bathymetry on zooplankton distribution in a Norwegian fjord system. J. Plankton Res., 17, 493–511.
- Kimmerer, W.J. and McKinnon, A.D. (1987) Zooplankton in a marine bay. 2. Vertical migration to maintain horizontal distributions. *Mar. Ecol. Prog. Ser.*, **41**, 53–60.
- Klinck, J.M., O'Brien, J.J. and Svendsen, H. (1981) A simple model of fjord and coastal circulation interaction. J. Phys. Oceanogr., 11, 1612–1626.
- Kumar, S., Tamura, K. and Nei, M. (1993) *MEGA: Molecular Evolutionary Genetics Analysis*, Version 1.0. Pennsylvania State University, University Park, PA 16802.
- Lindahl,O. and Hernroth,L. (1988) Large-scale and long-term variations in the zooplankton community of the Gullmar fjord, Sweden, in relation to advective processes. *Mar. Ecol. Prog. Ser.*, 43, 161–171.
- Marcus, N.H., Lutz, R.V., Burnett, W. *et al.* (1994) Age, viability, and vertical distribution of zooplankton resting eggs from an anoxic basin: evidence of an egg bank. *Limnol. Oceanogr.*, **39**, 154–158.

Nei, M. (1987) Molecular Evolutionary Genetics. New York, Columbia University Press.

- Norrbin, F. (1992) *Overwintering Strategies of Small Copepods in High Latitude Marine Environments.* PhD dissertation, University of Göteborg, Sweden.
- Palumbi,S., Martin,A., Romano,S., McMillan,W.O., Stice,L. and Grabowski,G. (1991) The Simple Fool's Guide to PCR (Ver. 2). Department of Zoology, University of Hawaii, Honolulu. Unpublished manuscript.
- Roff,D.A. and Bentzen,P. (1989) The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Molec. Biol. Evol.*, **6**, 539–545.
- Salvanes, A.G.V., Aksnes, D., Fossa, J.H. and Giske, J. (1995) Simulated carrying capacities of fish in Norwegian fjords. *Fish. Oceangr.*, **4**, 17–32.
- Sinclair, M. (1988) Marine Populations: An Essay on Population Regulation and Speciation. Washington Sea Grant, Seattle, 252 pp.
- Tajima, F. (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetics*, **105**, 437-460.
- Tamura, K. and Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molec. Biol. Evol.*, **10**, 512–526.
- Tande, K.S. (1991) Calanus in north Norwegian fjords and in the Barents Sea. Polar Res., 10, 389–407.
 Wright, S. (1969) Evolution and the Genetics of Populations. The Theory of Gene Frequencies. University of Chicago Press, Chicago, IL, Vol. 2, pp. 511.

Received on July 7, 1999; accepted on January 10, 2000

Downloaded from http://plankt.oxfordjournals.org/ at NERL on January 21, 2014