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Atlantic observation of *Calanus marshallae* (Copepoda; Calanoida)

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ABSTRACT: An observation of *Calanus marshallae* Frost, 1974, on the Atlantic side of the Polar Ocean is reported. Copepods were identified by comparing nucleotide sequences of mitochondrial 16S ribosomal RNA with a previously reported sequence from the Pacific region. Presence of the species in the Barents Sea may explain multi-modal length distributions previously obtained in the region. Potential implications for future identification of *Calanus* spp. in this region are discussed.

KEY WORDS: *Calanus marshallae* · *Calanus finmarchicus* · *Calanus glacialis* · Mitochondrial DNA · 16S ribosomal RNA · Molecular species identification

INTRODUCTION

Calanus spp. are considered to be the most important secondary producers in the marine food-web in Atlantic and Arctic water masses in the north-east Atlantic. The copepods have a key role as a link between primary production and many species of commercially exploited fish (e.g. Pavshtiks 1956, Hassel et al. 1991). Besides *C. hyperboreus* with its population center in the Greenland Sea (Conover 1988, Hirche 1991), the genus is represented by 2 other important species in the region. *C. glacialis* is an Arctic species with its centre of distribution over the shallow shelf surrounding the Arctic Ocean (Hirche 1991). *C. finmarchicus* is an Atlantic species with 2 centres of distribution, in the Subpolar Gyre south of Greenland and in the southern Norwegian Sea (e.g. Aksnes & Blindheim 1996). *C. hyperboreus*, *C. glacialis* and *C. finmarchicus* have overlapping distributions at the front between Atlantic and Arctic water masses in the north-east Atlantic (Hassel 1986, Hirche et al. 1994).

During identification of copepods for a population genetic study of *Calanus finmarchicus*, 3 out of 7 adult females of *Calanus* spp. smaller than 3 mm were identified as *C. marshallae*. The sample was collected 8 August 1994 in Arctic water in Isfjorden, Spitsbergen at 50 to 250 m (Fig. 1). The reported main distribution of *C. marshallae* is over the shelves of the north Pacific and in the Bering Sea (Frost 1974). Previous records of the species north of the Bering Strait, near Banks Island (Frost 1974), are some 2300 km from the present observation (Fig. 1).

METHODS

Copepods were preserved in 95% ethanol for 3 yr before DNA was PCR (polymerase chain reaction) amplified using the primers 16SAR: 5'-CGCCTGTTT-AACAAAAACAT-3' and 16SBR: 5'-CCGGTTTGAA-CTCAGATCACGT-3' (Palumbi et al. 1991). Amplification products were checked for size and purity using electrophoresis in 2% Metaphore™ gel. Product bands were cut from the gel and the DNA was extracted and purified using Sephaglas™ BandPrep Kit (Pharmasia Biotech). Templates were sequenced using the 16SBR primer. Cycle sequencing was run using fluorescently labelled di-deoxy terminators according to the manufacturer's recommended conditions (Applied Biosystems, Inc., ABI). For details see Bucklin & Kocher (1996). Unincorporated primers and dNTPs (deoxyribonucleotide triphosphates) were removed using Autoseq™ G-50 columns (Pharmacia Biotech). Nucleo-

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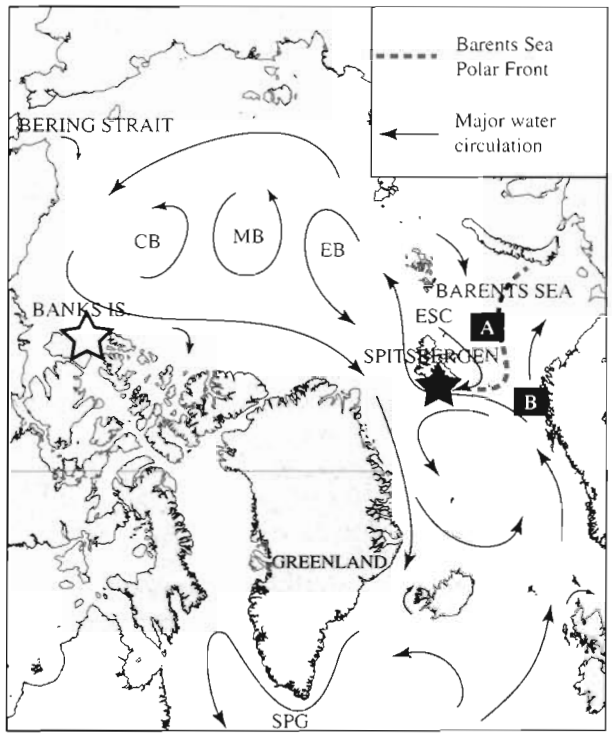

Fig. 1 Major circulation in the Atlantic and Arctic Oceans [after Grotefent et al. (in press); coastlines from GMT (Generic Mapping Tools), Wessel & Smith (1995)]. (★) Present observation of *Calanus marshallae*; (☆) observations from Frost (1974). CB: Canadian Basin; MB: Makarov Basin; EB: Eurasian Basin; ESC: East Spitsbergen Current; SPG: Subpolar Gyre. Rectangles A and B denote sampling areas for stations presented in Fig. 3

tide sequencing was carried out in an ABI Automated DNA Sequencer, Model 377. The sequences were further compiled using the program Sequencing Analysis 3.0 and checked thoroughly for accurate machine reading. Specimens were identified by comparing nucleotide sequences of a 387 base pair portion of the mitochondrial 16S ribosomal RNA gene with a previous reported sequence of *Calanus marshallae* collected in Puget Sound, Washington, USA (Bucklin et al. 1995).

RESULTS

The haplotype from the present study (same for all 3 individuals) differed with one transition (one pyrimidine base has been substituted for the other) from the 387 base pair sequence of *Calanus marshallae* from the Pacific region (Fig. 2). The sequence difference between the closest related species *C. marshallae* and *C. glacialis* is 7.3%, whereas it is approximately 1 to 2% within individuals of *C. finmarchicus* (Bucklin et al. 1995). The most common *C. finmarchicus* haplotype in the Spitsbergen sample and a *C. glacialis* haplotype from Gulf of St. Lawrence are given in Fig. 2.

DISCUSSION

Calanus marshallae could have been advected to Spitsbergen from the Arctic Ocean (Fig. 1), but it is also possible that the species maintains a local population and co-occurs with *C. finmarchicus* and *C.*

Fig. 2. *Calanus* spp. Sequence data for a 387 base pair region of the mitochondrial 16S rRNA gene for *C. marshallae* and *C. finmarchicus* (most common haplotype) from the Spitsbergen sample. *C. marshallae** and *C. glacialis* from Bucklin et al. (1995). (.) Base same as that of reference sequence; (–) alignment gap

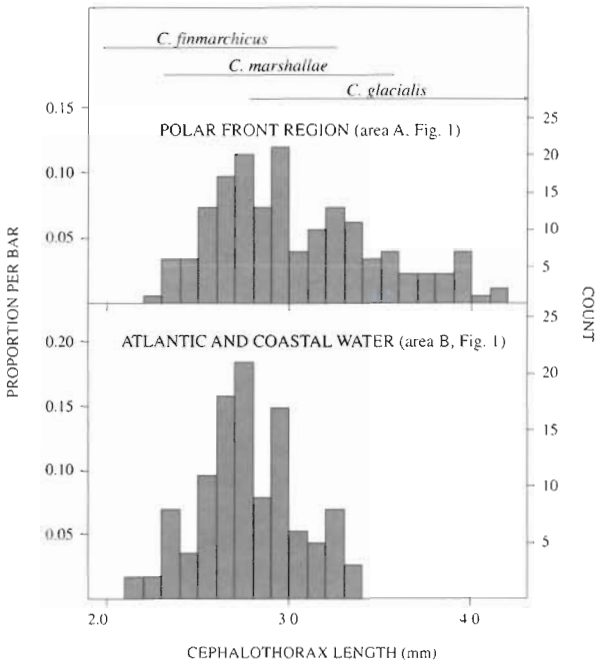

Fig. 3. *Calanus* spp. Example of distribution of cephalothorax length of adult females. Data obtained at 14 stations in Atlantic and Coastal water masses (lower panel) and 9 stations in the Polar front region of the Barents Sea (upper panel, see Fig. 1 for sampling areas). Range of cephalothorax lengths, including extremes, given in upper panel (Frost 1974)

glacialis in the Barents Sea region. Length measurements of the *Calanus* spp. stocks of cold water regions, for example the Barents Sea, have revealed that length distributions of *C. finmarchicus* and *C. glacialis* overlap to a great extent (Jaschnov 1972, Frost 1974). In length distributions of *Calanus* spp. obtained from the Polar front region of the Barents Sea, intermediate-sized specimens are abundant (Fig. 3). In copepods it is known that individuals of a given developmental stage tend to be larger at low temperatures (Deevey 1960, Grainger 1961, McLaren 1965, Carlotti et al. 1993), and intermediate-sized specimens could be individuals of *C. finmarchicus* or *C. glacialis* brought into colder or warmer water, respectively. Intermediate-sized specimens have also been suggested to be hybrids of *C. finmarchicus* and *C. glacialis* (Jaschnov 1972), although this was rejected by Frost (1974) because intermediate-sized specimens are found in areas where the species exist alone as well. Thus, an intermediate-sized sibling species of *C. finmarchicus* and *C. glacialis* may be common in the Polar front region of the Barents Sea. The observation of *C. marshallae* in Arctic water west of Spitsbergen suggests that identification with special focus on this species should be undertaken in the Barents Sea and the north-east Atlantic as a whole. Because of differences in length of life cycle among species (Tande et al. 1985), correct identification is important for studies of production and system ecology.

Large-scale identification based on morphological characters is laborious and to some extent uncertain. The species can also be identified based on differences in proteins (Sevigny & McLaren 1988). However, this technique has major drawbacks like the need for continuos cryogenic preservation to avoid degradation of samples. Nucleotide sequences from PCR products separate the species unambiguously and are very sensitive with respect to the amount of sample needed, but it is too expensive for screening of large numbers of individuals. We are currently making attempts to develop methods based on RAPD (random amplified polymorphic DNA) and restriction analysis of PCR amplified DNA that eventually will allow unambiguous identification of ethanol preserved samples at lower costs.

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