Planktonic copepods of the genus *Calanus* play a central role in North Atlantic/Arctic marine food webs. Here, using molecular markers, we redrew the distributional ranges of *Calanus* species inhabiting the North Atlantic and Arctic Oceans and revealed much wider and more broadly overlapping distributions than previously described. The Arctic shelf species, *C. glacialis*, dominated the zooplankton assemblage of many Norwegian fjords, where only *C. finmarchicus* has been reported previously. In these fjords, high occurrences of the Arctic species *C. hyperboreus* were also found. Molecular markers revealed that the most common method of species identification, prosome length, cannot reliably discriminate the species in Norwegian fjords. Differences in degree of genetic differentiation among fjord populations of the two species suggested that *C. glacialis* is a more permanent resident of the fjords than *C. finmarchicus*. We found no evidence of hybridization between the species. Our results indicate a critical need for the wider use of molecular markers to reliably identify and discriminate these morphologically similar copepod species, which serve as important indicators of climate responses.

1. Introduction

Copepods of the genus *Calanus* are central in North Atlantic and Arctic pelagic food webs. Rich in lipids, they form a key link between primary producers and secondary consumers and predators. Four species of the genus *Calanus* occur throughout the North Atlantic and Arctic Oceans (figure 1): *C. helgolandicus* (Chel), *C. hyperboreus* (Chyp), *C. finmarchicus* (Cfin) and *C. glacialis* (Cglia); and there has been considerable effort to document and model their distributional changes [1,2]. Importantly, abundances and dynamics of fish stocks are strongly associated with *Calanus* species composition and abundances [3], and climate-driven changes in their biogeographical distributions (i.e. range shifts) can lead to ecosystem regime shifts and potential collapse of fish stocks such as cod [4]. However, distinguishing *Calanus* species is challenging due to their morphological similarity and lack of diagnostic characters. The
usual method of species identification is body (prosome) length, although this approach has been questioned [5,6]. Misidentification may thus occur, impacting the reliability of our current knowledge of species distributions and preventing accurate assessment of species geographical range shifts in response to climate change.

Here we re-examine the distributional ranges of four co-occurring *Calanus* species in the North Atlantic and Arctic Oceans, using six molecular markers designed to ensure reliable species identification.

2. Material and methods

(a) Sample collection

Zooplankton samples were collected from 83 locations in the North Atlantic and Arctic Oceans (electronic supplementary material, S1) by vertical nets tows with 150–200 μm mesh sizes and preserved in 70–80% ethanol. A Folsom plankton splitter was used to make subsamples containing up to 150 *Calanus* individuals from developmental stage CIV–CVI (electronic supplementary material, S1). No species level morphological identification was performed for any individuals.

(b) Molecular species identification

DNA was extracted from the excised antennae of each specimen using the HotSHOT protocol [7], and molecular species identification of 4434 individuals was achieved using six nuclear markers type InDels (Insertion or Deletion motifs) [8] scored on a 3500xL genetic analyzer (Applied Biosystems). These bi-parentally inherited markers are easy to use and can potentially detect hybridization [9]. Their reliability was confirmed by the traditional, but more cost- and labour-intensive mitochondrial 16S rDNA sequencing (mtDNA) [10,11] of 159 individuals from 53 locations (electronic supplementary material, S2 and S3), following Smolina *et al.* [8]. In addition, 129 individuals from Saltfjord/Skjerstadfjord were measured (prosome length) and sequenced for the 16S (table 1; electronic supplementary material, S4 and S5). Identification of specimens from InDels and 16S rDNA sequences was congruent for all 677 individuals investigated (288 in present study (electronic supplementary material, S2–S4) and 389 in Nielsen *et al.* [9]). InDel markers were also used to test for the presence of hybrids between *Cfin* and *Cgla* [9] (electronic supplementary material, S6).

(c) Population differentiation

Population genetic analysis was carried out to distinguish between fjord resident and drifting (seasonally transient) species [12] (electronic supplementary material, S7). Focusing on *Cfin*...
Some zooplankton species are long-term residents of Norwegian fjords, while others are replaced periodically with basin water exchanges [21]. Resident species are expected to show greater genetic differentiation among fjord populations than drifting species [12]. Our analysis found no significant genetic differentiation among fjord populations of Cfin (FST = 0.0043), but Cgla populations did differ significantly (FST = 0.039), suggesting lower rates of exchange (i.e. gene flow) for Cgla than for Cfin. These results support previous descriptions of Cfin as a drifting species [12] that is advected into and out of fjords seasonally [22]. Less gene flow— together with the absence of offshore populations—suggests that Cgla populations are resident [12]. In both the White Sea [23] and Lurefjord [24], Cgla is known to migrate in early summer from warm surface layers to colder deep water. This may explain the species’ ability to maintain local populations and avoid transport out of fjords.

Hybridization between Cfin and Cgla has been suggested in the Northwest Atlantic [14] based on microsatellite markers developed for C. finmarchicus. Notably, no first-generation hybrids were found in our survey of 4434 individuals from samples collected throughout the Northeast Atlantic and Arctic Oceans (electronic supplementary material, S6). Based on the nature of the molecular characters (nuclear, co-dominant InDels) used for species identification and careful ground-truthing of our molecular results, we conclude that hybridization between the species, if it occurs at all, is rare or episodic.

4. Conclusion

Marine zooplankton have been regarded as sentinels of climate change [25] due to their short life histories and rapid responses to environmental variation. Development and use of molecular characters that can ensure accurate and reliable identification and discrimination of key indicator species, such as those within the Calanus genus, are critically needed. Only then can these species be used to document past, present and future patterns of biogeographical distributions, and detect and track responses of pelagic communities to climate change.
Figure 2. Calanus species distributional ranges in the North Atlantic and Arctic Oceans based on molecular species identifications. Pie charts represent relative frequencies of *C. glacialis* (blue), *C. finmarchicus* (red), *C. hyperboreus* (green) and *C. helgolandicus* (yellow) in each sample. Stars indicate non-quantitative species records.
References


5. Nielsen TG, Kjellerup S, Smolina I, Hoarau G, M.C. & G.H. designed the study, collected and analysed data, developed the method and wrote the first draft of the manuscript. M.H., W.M., S.S., K.E., A.B., K.K., J.E.S., A.K.S.D., S.K. and C.S. collected and analysed data. I.S. collected data and contributed to the development of the method. M.D. and V.T. analysed data and made the figures. All authors contributed significantly to the manuscript, approved the final version and agreed to be held accountable for the content therein.

Competing interests. We declare we have no competing interests.

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