New insights into the biology of *Calanus* spp. (Copepoda) males in the Arctic

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ABSTRACT: Adult males of *Calanus* copepods in the Arctic are mainly observed between late autumn and late spring, and are seldom recorded during summer. Due to logistical constraints, there are still relatively few studies on zooplankton in high-latitude regions during the winter, and subsequently, little is known about *Calanus* males. Here, we present data on abundance, spatial distribution, prosome length, lipid content, respiration and swimming activity of *Calanus* adults, along with adult sex ratios in *Calanus* populations from 5 Arctic fjords in Svalbard, Norway (78–80° N) during the polar night in January 2015, 2016 and 2017. Adult males and females of *Calanus* were observed at all locations and occurred throughout the entire water column. Morphological examination and molecular identification of *Calanus* males proved that all males encountered belong to *Calanus glacialis*, even in the fjords where overwintering copepodite stage CV of *C. finmarchicus* dominated at the time. Adult sex ratios in *C. glacialis* populations varied from 1 male per 4 females to 2 males per female. From 3 to 18 % of females carried spermatophores attached to the genital segment. Lipid content in males was slightly higher than in females. Shipboard experiments showed that males had higher swimming activity and respiration rates than females. Our observations indicate that adult males of *C. glacialis* stay active and demonstrate active mating behavior in mid-winter, and that the mating phenology of *C. glacialis* is decoupled from that of *C. finmarchicus* in the study area in January.

KEY WORDS: *Calanus glacialis* · Polar night · Svalbard · Mating · Sex ratio · Metabolism

INTRODUCTION

Calanoid copepods of the genus *Calanus* dominate the mesozooplankton communities of Arctic and sub-Arctic seas in terms of biomass (Kosobokova & Hirche 2009). They play a major role in the Arctic marine ecosystem, converting their algal diet into energy-rich lipid storages and thus facilitating the transfer of energy from primary production to higher trophic level organisms such as fishes, sea birds and marine mammals (Falk-Petersen et al. 2009). Due to their importance in the marine ecosystem, *Calanus* species are probably the most studied copepod taxa,

not only in the Arctic but also in sub-Arctic and boreal seas. A number of publications have described the spatial distribution of *Calanus* species in these regions (e.g. Conover 1988, Hirche & Kosobokova 2007, Falk-Petersen et al. 2009, Wassmann et al. 2015, Choquet et al. 2017) and there is a good understanding of different aspects of their life history such as timing of reproduction (Niehoff et al. 2002, Søreide et al. 2010, Daase et al. 2013), vertical migration, juvenile development, and energy requirements for reproduction and growth (e.g. Niehoff 2004, Søreide et al. 2008, 2010, Falk-Petersen et al. 2009).

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Three species of *Calanus* co-occur in the Atlantic-influenced part of the Arctic Ocean: the North Atlantic species *C. finmarchicus*, the arctic shelf species *C. glacialis* and the arctic oceanic species *C. hyperboreus* (Conover 1988, Choquet et al. 2017). The basic life cycle of *Calanus* species in Arctic and Sub-Arctic seas includes a seasonal migration, with the main developmental and growth phase occurring near the surface during spring and summer and an overwintering phase at depth with reduced metabolism (diapause) in winter (Falk-Petersen et al. 2009). The final developmental step towards adulthood takes place sometime between late autumn and spring, with the largest and most lipid-rich CVs moulting to adults first, and males appearing before females (Østvedt 1955, Kosobokova 1999, Bailey 2010). The 3 *Calanus* species have tuned their life-history strategies in relation to the timing and predictability of the spring bloom, ice cover and other factors in their main area of distribution (Falk-Petersen et al. 2009). *C. finmarchicus* is advected to the Arctic mainly with Atlantic water currents. In the northernmost part of its distribution range, *C. finmarchicus* has a 1 yr life cycle and relies on external energy supplied by the spring bloom to fuel reproduction (i.e. income breeding). The ability of *C. finmarchicus* to survive and colonize the Arctic Ocean, however, is hampered by short algae growing seasons and low temperatures (Jaschnov 1970, Tande et al. 1985, Ji et al. 2012), and hence the species largely fails to reproduce in the Arctic Ocean and surrounding shelf seas (Hirche et al. 2006). The larger *C. glacialis* is very productive along the entire shelf break and surrounding shelf seas of the Arctic (Kosobokova & Hirche 2001, Ashjian et al. 2003, Hirche & Kosobokova 2003). *C. glacialis* has a 1–2 yr life cycle (Kosobokova 1999, Søreide et al. 2010, Daase et al. 2013) and is efficient at utilizing the 2 available food sources in seasonal ice-covered seas (ice algae and phytoplankton) for reproduction and growth. The early ice algae bloom is primarily utilized to fuel gonad maturation and egg production (income breeding) while the later phytoplankton bloom supports growth and development of its new generation (Hirche 1989, Tourangeau & Runge 1991, Søreide et al. 2010, Wold et al. 2011). However, egg production can also occur before any algal food is present, being fuelled by internal resources only (i.e. capital breeding). The flexible reproductive strategies observed in *C. glacialis* may explain its wide distribution in seasonally ice-covered Arctic shelf seas (Daase et al. 2013), a region of high inter-annual variability in the timing of ice break-up and bloom phenology. The largest of the 3 species, *C.*

hyperboreus, has its centre of distribution in the Greenland Sea and the Central Arctic Ocean, and is specialized to the highly unpredictable timing of the spring bloom in the Arctic Ocean. It is a pure capital breeder, producing eggs at depth in winter decoupled from the spring bloom (Hirche & Niehoff 1996, Hirche 2013).

Given the key role of *Calanus* spp. in the food web, discussions of their fate in a warming Arctic has become a research priority in recent years (e.g. Ji et al. 2012, Kjellerup et al. 2012, Kwasniewski et al. 2012, Grote et al. 2015, Wilson et al. 2016). However, winter studies are still scarce and knowledge on *Calanus* males and their biology is basically non-existent from the Arctic, hindering a thorough understanding of *Calanus* life-history strategies needed to assess their response to Arctic warming.

Calanus males seem to have a rather short life span, similar to males of many other copepod species (Bogorov 1939, Mednikov 1961). Kosobokova (1999) reported that males of *C. glacialis* have only a 3–4 mo life span in the White Sea, and Marshall & Orr (1955) suggested that the life span of *C. finmarchicus* males does not exceed 7 mo even at high latitudes. In comparison, the life span of females of *C. glacialis* may vary from 9–10 mo up to 1.5 yr, and it has been suggested that *C. glacialis* females may even be iteroparous (Kosobokova 1999).

The absence of *C. glacialis* males is noteworthy during the period of most active biological sampling from late spring to autumn (e.g. Kosobokova 1999, Ashjian et al. 2003, Darnis & Fortier 2014), while females are found year-round (e.g. Kosobokova 1999, Wold et al. 2011, Daase et al. 2013). Males start to appear in northern polar waters in early autumn and can persist until May–June, with most studies observing maximum abundance and highest proportion of *C. glacialis* males between December and February (Madsen et al. 2001, Niehoff et al. 2002, Wold et al. 2011, Estrada et al. 2012, Darnis & Fortier 2014). Periods of peak abundance thus coincide with the polar night, a period that is traditionally understudied due to logistical constraints of conducting fieldwork at high latitudes in darkness, extreme low temperatures and in often ice-covered seas. As a result, male abundance, size range and structure, feeding habits, metabolic rates and lipid content have been poorly documented, and their life span remains uncertain.

Here, in order to fill knowledge gaps on *Calanus* spp. reproductive strategies due to the lack of data on males, we collected zooplankton samples in the middle of winter in the Arctic archipelago of Svalbard, during the supposed peak of *Calanus* male abun-

dance (Bailey 2010). Although it is likely that males of all 3 *Calanus* species are present in the studied area, we focused only on *C. finmarchicus* and *C. glacialis* since abundance of *C. hyperboreus* is generally low in the fjords and on the shelf (Daase & Eiane 2007, Blachowiak-Samolyk et al. 2008, Søreide et al. 2008). The vertical distribution, abundance, morphology, activity and physiology of *Calanus* spp. adults were investigated together with the females' gonad maturation state in order to understand and document for the first time the details of the mating phase of *Calanus* spp. in the Arctic.

MATERIALS AND METHODS

Study area

Zooplankton samples were collected in January 2015, 2016 and 2017 in fjords along the western and northern coast of the Svalbard archipelago (Fig. 1, Table S1 in the Supplement at www.int-res.com/articles/suppl/m607p053_supp.pdf) onboard the R/V 'Helmer Hanssen'. In January 2015, samples were collected at 3 stations in Kongsfjorden: the outer (KF1), the middle (KF3) and the innermost part of the fjord close to the glacier front (KF5). In January 2016,

samples were collected in Kongsfjorden (KF3 and KF5), Isfjorden (IF), Billefjorden (BF), Smeerenburgfjorden (SMF) and Rijpfjorden (RF), and in January 2017, sampling was repeated at IF, KF3, SMF and RF (Fig. 1, Table S1).

Isfjorden, Kongsfjorden and Smeerenburgfjorden are located on the western coast of Svalbard and may be affected by inflow of Atlantic water from the West Spitsbergen Current (Cottier et al. 2005, Nilsen et al. 2008). Billefjorden is a sill fjord in the inner part of Isfjorden and is largely unaffected by inflowing Atlantic water but dominated by locally formed cold water (less than −0.5°C year-round), providing a refuge for Arctic zooplankton species (Arnkværn et al. 2005). Rijpfjorden is a north-facing fjord dominated by cold Arctic water masses, but inflow of Atlantic water may occur (Wallace et al. 2010). All fjords were ice-free during our study in January 2015, 2016 and 2017.

Hydrography

Measurements of temperature and salinity were obtained at all stations by a ship-board conductivity, temperature and depth profiler (SBE911plus, Sea-Bird Electronics).

Zooplankton abundance and vertical distribution

Zooplankton were sampled by vertical net hauls (towing speed 0.5 m s^{-1}) from close (10–20 m) to the seafloor up to the surface using a multiple opening/closing net (Multinet; Hydrobios: mouth opening 0.25 m^2, mesh size 180 µm). Up to 5 depth strata were sampled at each location (Table S1). Samples were preserved in a 4% hexamethylenetetramine-buffered formaldehyde-in-seawater solution and analyzed under a Leica stereomicroscope at institutional home laboratories. Samples were examined by subsampling with aliquots obtained by 5 ml automatic pipette, with the pipette tip cut at 5 mm diameter to allow free collection of mesozooplankton. Prior to taking subsamples, large (total length >5 mm) organisms were picked out using forceps. The
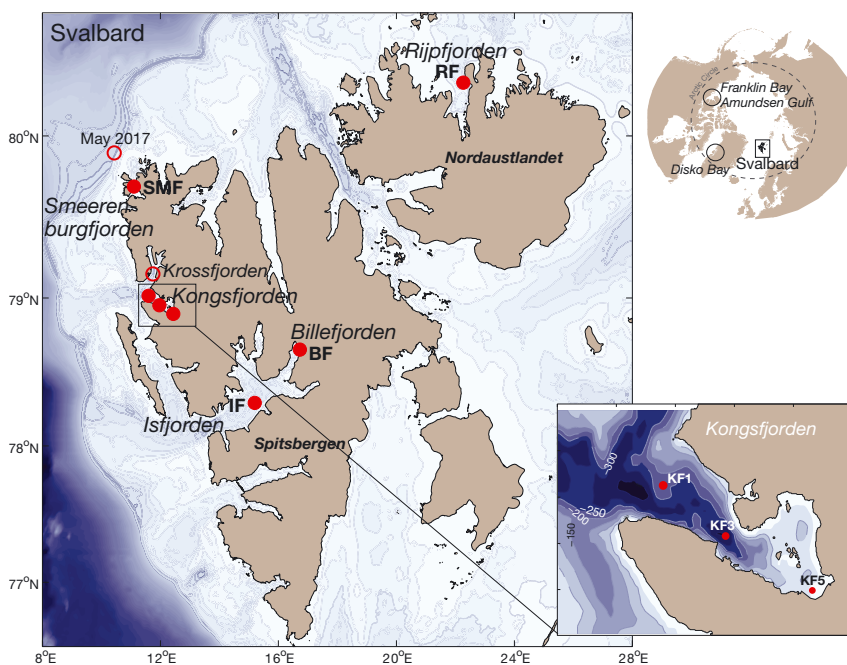

Fig. 1 Map of study area. Red dots show location of main sampling sites in Svalbard fjords. Red circles indicate sampling sites where additional samples were taken. Inset in lower right corner shows locations of stations sampled in Kongsfjorden. Arctic map in upper right corner shows location of Svalbard as well as locations of sampling sites referred to in Fig. 9

number of subsamples analyzed was chosen so that at least 150 individuals of *Calanus* copepodites were counted. Samples with low abundances were examined in their entirety. Adult males (AM) and females (AF) of *Calanus* were counted from the entire samples. The prosome length of all counted individuals of *Calanus* spp. was measured from the tip of cephalosome to the distal lateral end of the last thoracic segment with precision of ±50 μm.

Calanus species identification

To distinguish between the morphologically similar *C. glacialis* and *C. finmarchicus* from formalin-preserved samples, we used size classes derived for each developmental stage (copepodites CIII–CVI) from prosome length frequency analyses for the study region (Daase & Eiane 2007) (Table 1), which were readjusted after considering molecular-based studies (Gabrielsen et al. 2012, Choquet et al. 2018, Renaud et al. 2018). These molecular investigations have indicated a much higher overlap in prosome length between the 2 species than previously assumed, resulting in a regionally variable potential for misidentification. In our study area, misidentifications based on size classes seemed, for the most part, to be unidirectional (Gabrielsen et al. 2012, Choquet et al. 2018) and biased towards an underestimation of *C. glacialis* and a comparative overestimation of *C. finmarchicus.* Since prosome length measurements of genetically identified *Calanus* CV and females indicate a discrepancy between previously published size classes for the study area (e.g. Daase & Eiane 2007, Kwasniewski et al. 2003) and observed prosome length (Gabrielsen et al. 2012, Renaud et al. 2018), we adjusted the size classes and defined CV ≥ 2.9 mm and AF ≥ 2.95 mm as *C. glacialis* (Table 1).

In living *Calanus*, the presence or absence of red pigmentation of the antennules has been genetically

confirmed as useful to distinguish between CIV, CV and AF of *C. finmarchicus* (pale antennules) and *C. glacialis* (antennules with red pigmentation) (Nielsen et al. 2014, Choquet et al. 2018). This characteristic was used to identify *C. glacialis* from digital images taken to estimate lipid content (see below) and when selecting AF for respiration and swimming activity measurements (see below). The pigmentation of antennules is, however, not present in AM of either species.

The morphology of the 5th thoracic leg (swimming leg P5) can also be used to identify *Calanus* to species following descriptions by Jaschnov (1955), Frost (1974) and Brodskii (1967), although the method has recently been proven to be unreliable for CVs and AF (Choquet et al. 2018). For males, the morphological characteristics are more clearly defined than in females, and we used this morphological feature to identify a subset of *Calanus* males (those sampled in Rijpfjorden) to species as described in Choquet et al. (2018) to check the reliability of size classes derived for AM in this study.

Another subset of *Calanus* males was identified to species using molecular tools. A total of 80 *Calanus* males sampled at KF3 in January 2015 using a MIK net (mouth opening 3.14 m^2, mesh size 1500 μm), and 40 males from BF and 74 males from RF sampled in January 2016 using the Multinet were preserved individually in 96% ethanol. Prosome length of each individual was measured from digital images taken prior to preservation. Individuals were genetically identified to species following procedures described in Choquet et al. (2017). To compare the size structure and species composition of *Calanus* males in January with that of *Calanus* males found in spring, we used the same methods on 42 randomly selected *Calanus* males collected with a WP3 net (1 m^2 mouth opening, 1000 μm mesh size) during a cruise in May 2017 north-west of Svalbard (Fig. 1, Table S1).

Estimation of lipid content

In 2015 and 2016, additional Multinet samples were taken at BF, KF3, SMF and RF from which live *Calanus* were sorted out to estimate the lipid content of individuals (see Table S1 for sample depth). Digital images (lateral view) of all specimens in subsamples containing at least 100 *Calanus* were taken following procedures described in Daase et al. (2014) using a Leica stereomicroscope with a camera Leica DFC420 or Sony HDR_HC7 video camera. Copepodite stage of each individual was determined while taking the

Table 1. Size ranges (prosome length, mm) used to differentiate between copepodite stages CIII–CV and adult females (AF) of *Calanus finmarchicus* and *C. glacialis* based on Daase & Eiane (2007). Size classes for CV and AF have been readjusted based on molecular identification (Gabrielsen et al. 2012, Renaud et al. 2018)

	C. finmarchicus	*C. glacialis*
CIII	1.12–1.47	1.47–2.07
CIV	1.6–2.01	2.01–3.63
CV	1.92–2.9	2.9–3.99
AF	2.4–2.95	2.95–4.63

pictures. The digital images were used to measure lipid sac area, prosome length and prosome area of specimens using ImageJ, an open source graphics program (Rasband 1997−2009). Lipid content of individual *Calanus* specimens was calculated from lipid sac area according to Vogedes et al. (2010).

The variance in lipid content and lipid sac area/prosome area ratio (LA/PA; an indication of the fullness of the body) was not homogenous. We therefore applied the non-parametric Kruskal-Wallis test to test for differences in lipid content and LA/PA between copepodite stages, followed by the post hoc test according to Nemenyi for pairwise multiple comparisons of the ranked data. Statistical analyses were done in RStudio v.1.0.143.

Gonad maturation status and spermatophore counts

The gonad maturation stage (GS) of adult *Calanus* females and CVs were examined using formalin-preserved samples. A total of 30 randomly selected females and CVs from each fjord sampled in 2016 were stained with 2% borax carmine solution (Tande & Hopkins 1981), dehydrated and stored in glycerine. The GS of females was assessed according to the classification scheme suggested by Niehoff & Hirche (1996). Four stages of gonad maturation (GS1−GS4) were distinguished. The gonads in CVs were examined to discriminate between sexually undifferentiated specimens and potential females/males, according to Kosobokova (1998, 1999). The number of AF bearing spermatophores was assess in all Multinet samples collected in January 2015 and 2016.

Swimming activity and respiration

Measurements of swimming activity of *Calanus* AM and AF were taken using a modified LAM10 locomotor activity monitor (LAM; Trikinetics) connected to a laptop computer. The LAM monitors use infrared light beam arrays to detect the motion of animals in test chambers (2.5 ml clear acrylic tubes); beam breaks are recorded on the computer. For activity experiments, animals were collected in January 2017 in Krossfjorden (a side-fjord of Kongsfjorden; Fig. 1) and at RF using a Hydrobios WP2 net (mesh size 180 μm, mouth opening 0.25 m^2), vertically hauled from 100 m to the surface. Net contents were immediately transferred to a shipboard temperature-controlled room at 4.5°C, where sorting was undertaken by stereomicroscope under a dim red light.

Sorted animals were individually transferred into the LAM monitor tubes, each containing ~2 ml of 0.5 μm filtered seawater. Animals were left undisturbed and under constant darkness in the shipboard temperature-controlled room at 4.5°C for ~2 d and their activity logged, after which each copepod was photographed to confirm species and stage (see methods described above). Rayleigh's tests were used to determine whether bouts of swimming activity were clustered over the diel cycle, while rank sum tests were used to compare variance of swimming activity between AM and AF at Krossfjorden and RF.

From net collections at RF we also measured weight-specific oxygen consumption rates in individual adult *C. glacialis* males (n = 11) and females (n = 8). Respiration rates were measured in darkness at 4.5°C in 1 min intervals over ~10 h using a 24-well microplate respirometry system (Loligo Instruments). Individual copepods were tested in 200 μl wells, with respiration rates calculated over an interval where partial pressures were 90−80% air saturation in each well, ensuring measurements considered only independent respiration. Copepods were photographed following experiments, from which prosome length was calculated and used to derive dry weight (M. Daase & J. E. Søreide unpubl. data) for correcting respiration by copepod size:

$$DW = e^{2.25}PL^{3.31}$$

where DW is dry weight (mg) and PL is prosome length (mm).

Respiration rates were compared between AM and AF by a rank sum test. While both copepod activity and respiration in the experiments described above could be influenced by tank enclosure effects in these relatively small volumes, we ensured that our methods were consistent between individuals and therefore any differences are very likely due to inherent differences among sexes/sites as opposed to experimental artefacts.

RESULTS

Hydrography

Atlantic and Transformed Atlantic water prevailed in Kongsfjorden and Isfjorden during our studies (Fig. 2). The water column in Kongsfjorden was well-mixed and homogenous in 2015, and stratified, warmer and fresher in 2016 and 2017. Water masses in Isfjorden were similar to Kongsfjorden in 2016 and 2017. Cooler and fresher waters were observed in
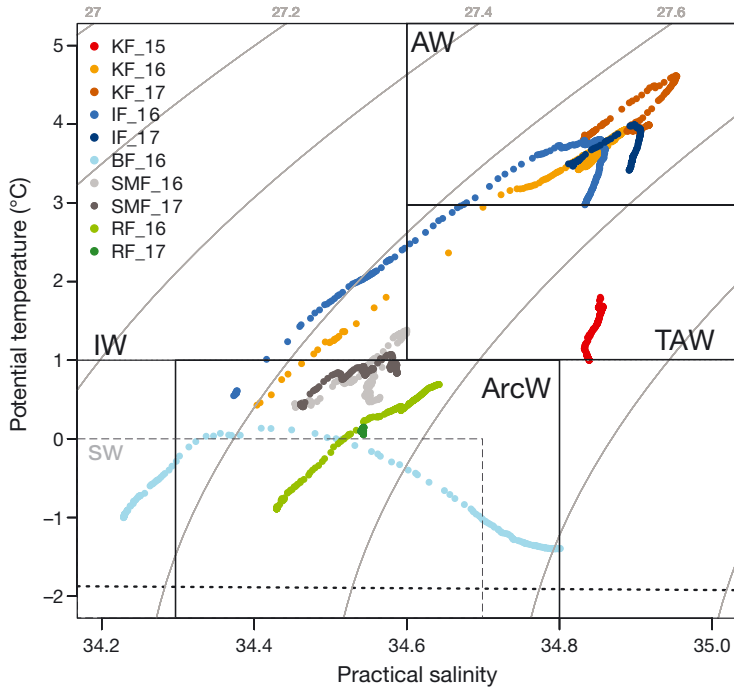
Fig. 2. TS-diagram of water masses in January in Kongsfjorden (KF3): 2015 (KF_15), 2016 (KF_16) and 2017 (KF_17); Isfjorden: 2016 (IF_16) and 2017 (IF_17); Billefjorden: 2016 (BF_16); Smeerenburgfjorden: 2016 (SMF_16) and 2017 (SMF_17); and Rijpfjorden: 2016 (RF_2016) and 2017 (RF_17). AW: Atlantic water; TAW: Transformed Atlantic water; ArcW: Arctic water; IW: intermediate water; SW: surface water (grey dashed box). Black dotted line indicates freezing point. Grey lines show isopycnals at 0.2 intervals. Water mass definitions based on Cottier et al. (2005)

Billefjorden and Rijpfjorden, indicating the presence of Arctic or locally formed water cooled during the winter. Smeerenburgfjorden was warmer than Rijpfjorden and Billefjorden, but not as warm as Kongsfjorden and Isforden.

Calanus stage composition, length frequency and genetics

Abundance of the larger and easily morphologically distinguishable *Calanus hyperboreus* was low in the study area (0.16–2.5 ind. m^{-3}) and we therefore only report data on *C. finmarchicus* and *C. glacialis*, which were abundant in all fjords.

The *Calanus* population in January was dominated by copepodite stages CIV and CV (Fig. 3). CVs dominated at all stations in 2015
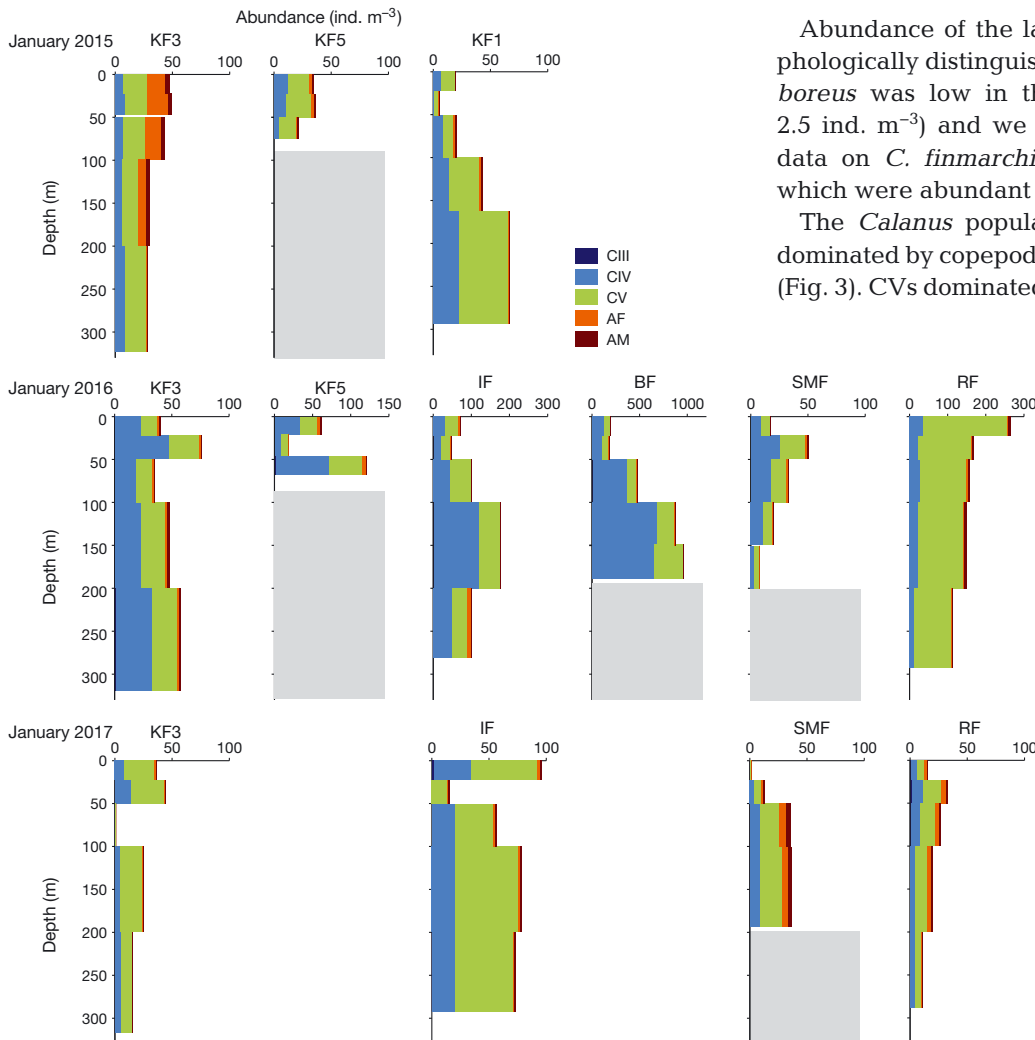

Fig. 3. Vertical distribution, stage composition and abundance of *Calanus* spp. in 5 Svalbard fjords. Note differences in scale of *x*-axis. Gray bars: bottom depth. AF: adult females; AM: adult males
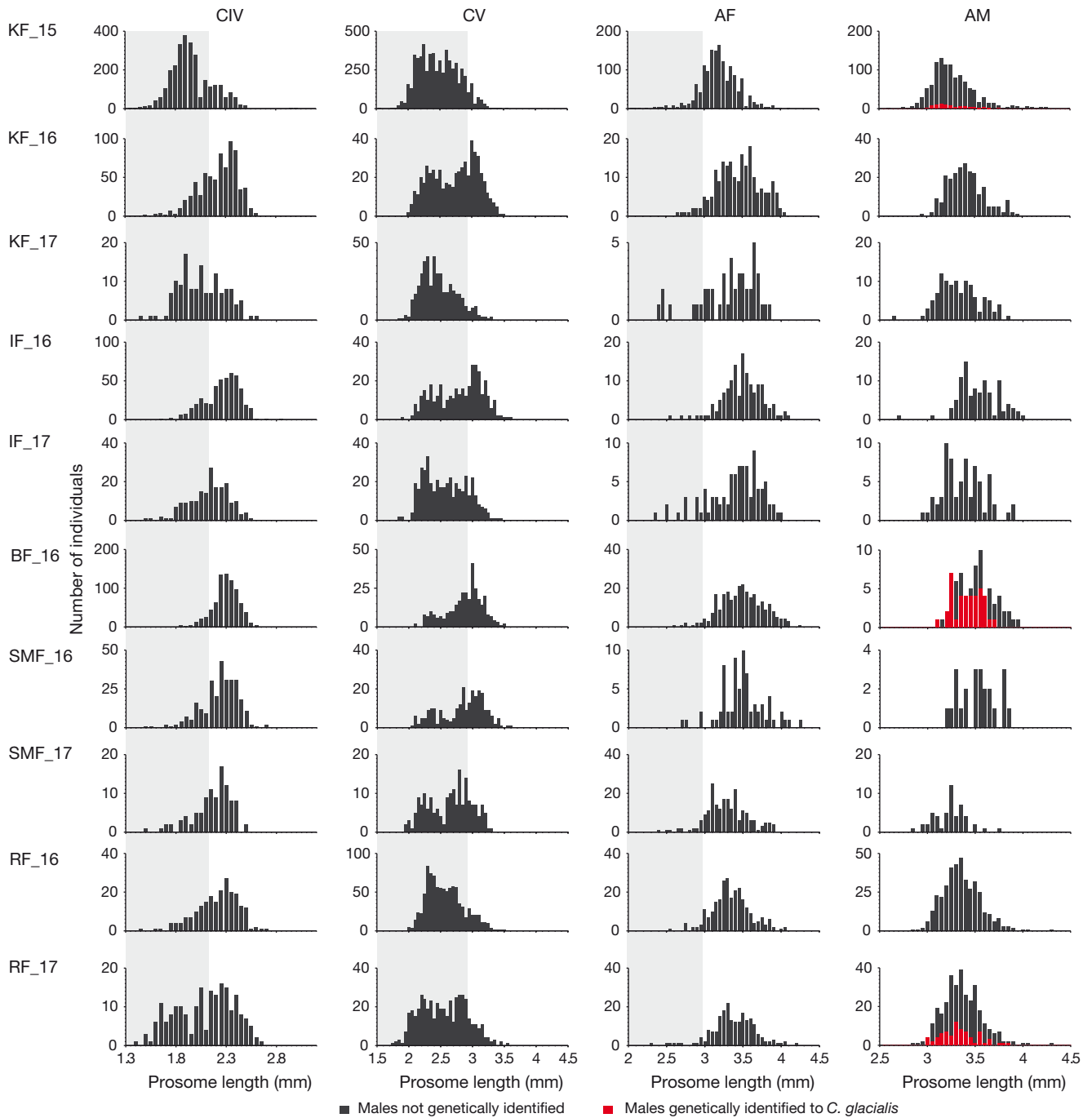
Fig. 4. Station-specific prosome length frequency distributions of *Calanus* spp. copepodite stage CIV and CV, adult females (AF), and adult males (AM) for Kongsfjorden 2015 (KF1, KF3 and KF5 pooled), 2016 (KF3 and KF5 pooled), 2017 (KF3); Isfjorden (IF) 2016, 2017; Billefjorden (BF) 2016; Smeerenburgfjorden (SMF) 2016, 2017; and Rijpfjorden (RF) 2016, 2017. Red bars for AM in KF_15, BF_16 and RF_16 show length frequency distribution of males genetically identified as *C. glacialis*. Gray shaded areas: size range assigned to *C. finmarchicus* (see Table 1)

and 2017, while CIVs were more abundant in 2016, except at RF. The prosome length frequency of copepodite stage IV (CIV) was biomodal but skewed towards larger individuals at almost all locations, indicating a dominance of *C. glacialis* among CIVs

(Fig. 4). Exceptions were Kongsfjorden in 2015 and 2017, where the majority of CIV fell into the size classes assigned to *C. finmarchicus*, and Rijpfjorden in 2017, which showed equal numbers of CIVs for both size classes. In contrast, the length distribution of
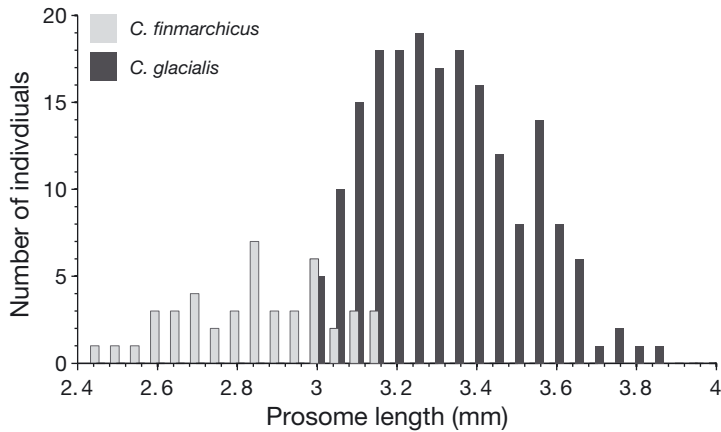
Fig. 5. Prosome length frequency distribution of genetically identified *Calanus* adult males (AM). *C. finmarchicus* AM were sampled in north-west Svalbard in May 2017, and *C. glacialis* AM were collected in Svalbard fjords in January 2015 and 2016 (see also Fig. 3)

CVs was skewed towards smaller sizes, indicating a dominance of *C. finmarchicus* CVs at most locations (Fig. 4), with the exception of BF and SMF in 2016 (Fig. 3). The length distribution of AF was largely unimodal, with only few smaller AF present (Fig. 4). The size range and length frequency distribution pattern of AM closely resembled that of AF, but no AM smaller than 2.7 mm were observed. All 194 AM identified to species using molecular tools (corresponding to 10% of all measured males) were found to be *C. glacialis.* Prosome length of these genetically identified AMs varied from 3.00 to 3.84 mm and the length frequency distribution overlapped entirely with the length frequency distribution of AM not identified genetically (Fig. 4). Morphological examination of the fifth pair of swimming legs (P5) of AM from RF in 2016 indicated that only *C. glacialis* AM were present there, ranging in prosome length from 2.85–4.05 mm. In contrast, *Calanus* AM sampled north-west of Svalbard in May 2017 were smaller than those observed in January. They were all identified as *C. finmarchicus* using molecular tools and displayed a unimodal length frequency distribution with prosome length varying from 2.40–3.14 mm (Fig. 5).
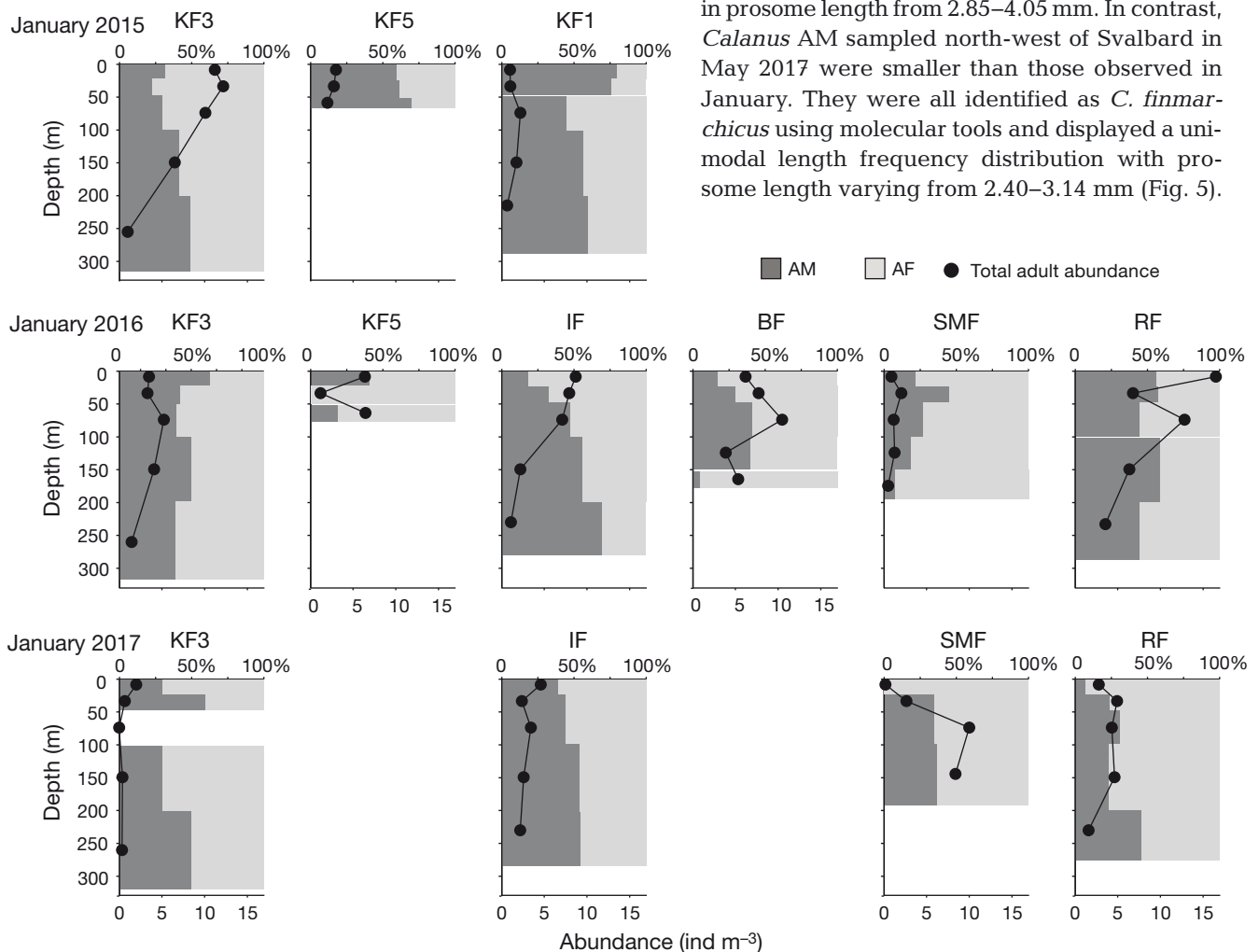

Fig. 6. Vertical distribution of *Calanus glacialis* adult females (AF; light gray) and males (AM; dark gray) at each station in January 2015, 2016 and 2017. Black dots: total adult abundance (ind. m^{-3})

Table 2. Abundance of adult males (AM) and females (AF) of *Calanus glacialis* (ind. m^{-2}), and sex ratios and percentage of *C. glacialis* females observed with spermatophores. nd: not determined

Station	No. AM (ind. m^{-2})	No. AF (ind. m^{-2})	Sex ratio adults males:females	% AF with spermatophores
2015				
KF1	200	276	0.7	13.9
KF3	636	2328	0.3	9.2
KF5	116	128	0.9	17.6
2016				
KF3	384	532	0.7	8.1
KF5	68	176	0.4	nd
IF	356	969	0.4	9.2
BF	256	1048	0.2	3.3
SMF	88	272	0.3	8.3
RF	1240	784	1.6	7.3
2017				
KF3	56	64	0.9	nd
IF	356	328	1.1	nd
SMF	484	784	0.6	nd
RF	260	608	0.4	nd

Calanus vertical distribution and abundance

At almost all stations, the *Calanus* population was distributed throughout the entire water column (Fig. 3). Exceptions were KF1 in 2015 and BF in 2016, where the bulk of the *Calanus* population was concentrated in the deeper layers. Total *Calanus* abundances were highest in RF, BF and IF in 2016 (Fig. 3); low *Calanus* abundance was observed in Kongsfjorden in 2015 (Fig. 4).

Both AM and AF were distributed throughout the entire water column (Fig. 6). The highest abundance of *Calanus* AF was observed in KF3 in 2015 (2328 ind. m^{-2}; Table 2), which was 2–3 times higher than maximum AF abundance recorded in 2016 and 2017. The contribution of *C. glacialis* AM to the total *C. glacialis* population was highest in 2015, at 12–25%. In 2016 and 2017, AM contributed 2–12 and 5–11%, respectively.

Sex ratios and proportion of females with spermatophores

The sex ratio in the *C. glacialis* population varied from 1.6 (ca. 2 AM AF^{-1}) in RF in 2016 to a pronounced prevalence of AF in BF (0.04–0.2 AM AF^{-1}) and SMF (0.1–0.3 AM AF^{-1}) in 2016, especially in the deeper layers (Fig. 6, Table 2).

Between 3 and 18% of the *Calanus* AF carried spermatophores (Table 2). The highest proportion of such females was observed in Kongsfjorden in 2015 (where

we also observed the highest AF abundance), and at IF in 2016. The lowest proportion of AF with spermatophores was found at BF in 2016 (Table 2). AF carrying spermatophores were not counted in 2017. Length measurements of AF with spermatophores showed that the majority fell within the size class of *C. glacialis*, with 18% (24 ind., most of them observed in KF in 2015) being slightly smaller (2.6–2.9 mm) but still within a size range that may include AF of *C. glacialis* (Renaud et al. 2018) (Fig. S1 in the Supplement). There was no relationship between the proportion of AF with spermatophores and the number of AM, AF or the sex ratio, but there was a positive correlation between the proportion of AF with spermatophores and the proportion of *C. glacialis* AM relative to the total *C. glacialis* abundance (Pearson correlation, r = 0.811, p = 0.015).

Gonad status and CV sex ratios

We assessed the gonad stage of AF and the CV sex ratios from samples taken in 2016. The majority of both *C. glacialis* and *C. finmarchicus* AF were immature (>95% with gonad stage GS1) (Table 3). The majority of CVs (75%) in both species were classified as potential females except for CVs from RF and BF, where up to 50% of CV specimens still had sexually undifferentiated gonads (Table 4). Overall, only a small portion (<5%) of CVs were developing male gonads and could be classified as potential males.

Lipids

The adults of *C. glacialis* had higher lipid content and a higher LA/PA compared to CIV and CV in January (Fig. 7). Differences in lipid content and LA/PA

Table 3. Gonad maturation stage (GS) of *Calanus glacialis* (% of adult females [AF] with GS1, GS2 or undifferentiated gonads) in January 2016

	GS1	GS2	Undifferentiated gonads
KF3	99.1	0.9	0.0
IF	100.0	0.0	0.0
BF	98.1	0.0	1.9
SMF	94.0	1.5	4.5
RF	100	0	0

Table 4. Gonad differentiation in *Calanus glacialis* and *C. finmarchicus* CVs in January 2016 (% of CVs with sexually undifferentiated gonads, potential female and potential male gonads). N: number of individuals examined

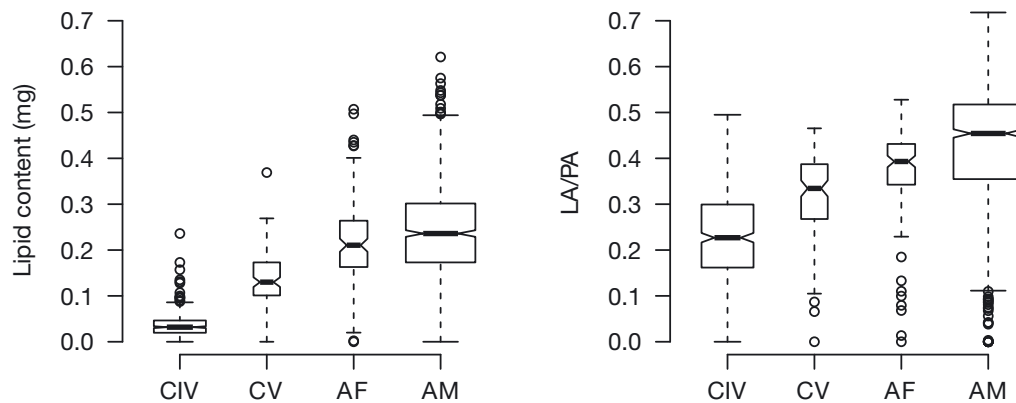| Station		*C. glacialis*					*C. finmarchicus*			
	N	% undiff.	% female	% male	Sex ratio	N	% undiff.	% female	% male	Sex ratio
KF3	137	5.1	91.2	3.6	0.04	15	26.7	73.3	0.0	0.00
IF	132	9.1	88.6	2.3	0.03	15	53.3	46.7	0.0	0.00
BF	97	38.1	59.8	2.1	0.03	52	78.8	21.2	0.0	0.00
SMF	102	9.8	86.3	3.9	0.05	38	39.5	60.5	0.0	0.00
RF	25	16.0	68.0	16.0	0.24	134	78.4	20.9	0.7	0.04

Fig. 7. Box plot of lipid content (mg ind.$^{-1}$) and lipid sac area to prosome area ratio (LA/PA) in copepodite stages CIV, CV, adult females (AF), and males (AM) of *Calanus glacialis* in January 2015 and 2016. Horizontal line: median; bottom and top of the box: 25th and 75th percentiles, respectively; whiskers extend 1.5 times the interquartile range of the sample; values outside this range are marked by circles. The boxes are drawn with widths proportional to the square-roots of the number of observations in the groups. Notches display the variability of the median between samples. If the notches of two boxes do not overlap there is strong evidence that their medians differ at 95% confidence interval (Chambers et al. 1983)

between stages were significant (Kruskal-Wallis, $p < 0.0001$). For lipid content, these differences were due to significant differences between adults and CIV and CV, while there was no significant difference in lipid content between AF and AM (Nemenyi post hoc test, $p = 0.24$). However, LA/PA was significantly different among all stages ($p < 0.001$), i.e. AM had a higher LA/PA ratio than AF. Additionally, there was a higher variability of lipid content in AM than in AF (Fig. 7).

Swimming activity and respiration

Swimming activity in adult *C. glacialis* varied with sex and collection site (Fig. 8). AF from both sites showed little variation in swimming activity over the duration of the experiment. AM from both sites, however, displayed bouts of elevated swimming activity. For Krossfjorden, these activity bouts were clustered at intervals over the diel cycle, while for RF the activity bouts were uniformly distributed (Rayleigh's test, $p < 0.001$ and $p = 0.211$, respectively). The variance

of swimming activity in individuals across time was greater for AM than AF, both in Krossfjorden and RF (rank sum tests, $p < 0.001$ and $p = 0.003$, respectively). This is reflected in bouts of swimming activity up to 381 beam breaks per 30 min in AM from Krossfjorden, and 1118 beam breaks per 30 min for AM from RF. Overall, activity levels were higher at RF than Krossfjorden (19–25 beam versus 5–7 breaks per 30 min). Consistent with activity, weight-specific respiration rates were 2.2-fold higher for AM from RF than for AF (38.6 ± 8.6 SE versus 17.5 ± 4.0 pmol O_2 µg^{-1} DW h^{-1}) ($p = 0.019$, rank sum test).

DISCUSSION

Adult males of *Calanus* were present in all 5 Svalbard fjords during our winter studies in January 2015, 2016 and 2017. Given the unimodal length frequency distribution of males, molecular results and additional examination of the morphology of the 5th swimming leg of individuals from RF in 2016, we conclude that only *C. glacialis* AM were present in the study region
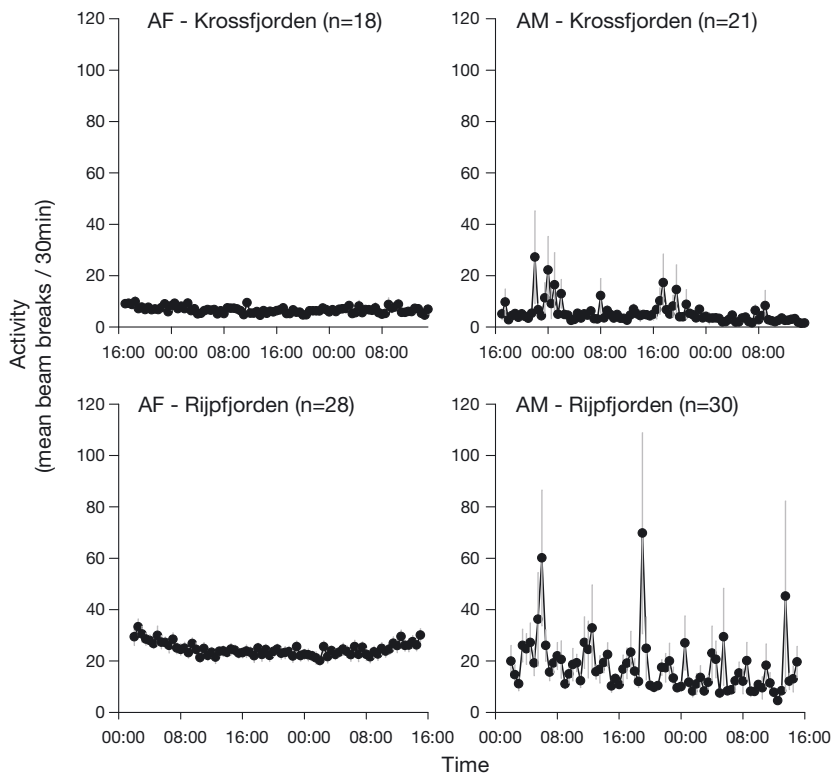
Fig. 8. Swimming activity of *Calanus glacialis* from Krossfjorden and Rijpfjorden in January 2017. Panels are for adult females (AF) and adult males (AM) from each site. Mean (±SE) activity is shown for copepods incubated individually in constant dark conditions over a 2 d period, with activity scored as breaks of infra-red light beam arrays crossing each tube

Male size and species identification

There are very few published data on body size of *Calanus* AM (Table 5), presumably because of their scarcity in historic sampling campaigns. Prosome lengths for *C. glacialis* AM measured during this study are similar to those previously obtained in Billefjorden, but they are smaller than records from the North Atlantic, Arctic Ocean and the White Sea (Table 5). The size structure of *C. finmarchicus* AM sampled in May 2017 differed from those identified as *C. glacialis* in January (Fig. 5, Table 5) confirming that we most likely did not encounter *C. finmarchicus* AM during the January campaigns, and that *C. finmarchicus* moults later into AM than *C. glacialis* in Svalbard waters. Similar observations were made in Disko Bay, were AM of *C. glacialis* were present between September and February with highest proportions in December and January, while *C. finmarchicus* AM were found from February to May, with maximum proportions between March and May (Madsen et al. 2001, Niehoff et al. 2002). In the Norwegian Sea, a similar timing of occurrence of AM as in Disko Bay was observed by Østvedt (1955). However, observations from lower latitudes indicate that moulting of *C. finmarchicus* to adults may have

in January along with a high proportion of AF of the same species. In contrast, *C. finmarchicus* males were absent in January, female abundance was low and late copepodite stages, in particular CV, dominated the overwintering population.

Table 5. Overview of available information on size ranges (mm) for adult males of *Calanus finmarchicus* and *C. glacialis*

Location	*C. finmarchicus* Prosome length (mm)	*C. glacialis*	Reference
Svalbard fjords	–	2.7–4.25 (3–3.84[a])	This study
80°N, western Svalbard	2.43–3.14[a]	–	This study
Greenland Sea, Barents Sea, Norwegian Sea, Central Arctic Ocean	2.34–3.16	3.16–4.1	Frost (1971)
Disko Bay, Greenland	2.16–2.92	2.88–3.62	Swalethorp et al. (2013)
Loch Striven, Clyde area, UK	2.35–2.67	–	Marshall et al. (1934)
White Sea	–	3.5–4.1	Kosobokova (1999)
Billefjorden, Svalbard	–	2.7–3.95	Bailey (2010)
	Total length (mm)		
North Sea (Isle of Man)	2.7–3.2	–	Gunther (1934)
North Sea (L4, UK)	3.01–3.37	–	Russell (1928)
[a]Sizes confirmed by molecular analysis			

already begun in December or January. Adults have
been recorded in January and February in all regions
from the English Channel to East Greenland (Mar-
shall & Orr 1955), and Marshall et al. (1934) observed
highest abundance of AM in January–February in
the North Sea/Scotland, followed by a low constant
presence of AM between April and August. Recently,
Choquet (2017) observed AM of both *C. glacialis* and
C. finmarchicus co-occurring in January and Febru-
ary in 2 northern Norwegian fjords (67° N). This
suggests that *C. finmarchicus* moults earlier into
males at lower latitudes compared to in Svalbard
waters, where this species is at the northern border
of its distributional range (Conover 1988, Choquet
et al. 2017).

Our molecular results from January and May show
that there is an overlap in size between AM of *C. fin-
marchicus* and *C. glacialis*, with maximum length of
3.14 mm of *C. finmarchicus* AM (Table 5, Fig. 5). A
size overlap between these 2 species is common in all
copepodite stages and constitutes a challenge when
identifying these species (Choquet et al. 2017, 2018).
For AM, this problem may be seasonally limited in
our study area, since AM of both species did not
seem to co-occur in January and May. However, this
is likely to differ as soon as *C. finmarchicus* AM start
to appear. From our data on prosome length associ-
ated with molecular identification (Fig. 5), we sug-
gest *C. finmarchicus* AM may be correctly identified
as individuals smaller than 3 mm, and *C. glacialis*
as individuals larger than 3.2 mm, which is similar

to Frost (1971) and Madsen et al. (2001) (Table 5).
A larger data set is needed to improve taxonomic
resolution within the overlapping size range.

Male abundance

The presence of AM in the *C. glacialis* population
in January confirms previous observations from Sval-
bard and other high latitude locations (Fig. 9).
Calanus AM have been observed from September to
June in the White Sea (Kosobokova 1999), the Cana-
dian Arctic (Wold et al. 2011, Estrada et al. 2012, Dar-
nis & Fortier 2014) and western Greenland (Madsen
et al. 2001, Niehoff et al. 2002), with peak abundance
usually observed from November to February (Fig. 9).
In Svalbard, *Calanus* AM have been observed be-
tween October and May in Billefjorden (Bailey 2010)
and in January in Rijpfjorden (Daase et al. 2014),
while Leu et al. (2011) did not observe a single male
of *Calanus* in Rijpfjorden between March and Octo-
ber. Despite Kongsfjorden being one of the most
studied fjords in Svalbard, occurrence of *Calanus*
AM has never been reported (e.g. Kwasniewski et al.
2003, 2013, Daase et al. 2013). In our study, we found
a high variability in AM abundance among the dif-
ferent fjords and years. Peak abundances observed
in Rijpfjorden were comparable to winter abundance
previously observed in Billefjorden, while the lower
abundance estimates were in the same order of mag-
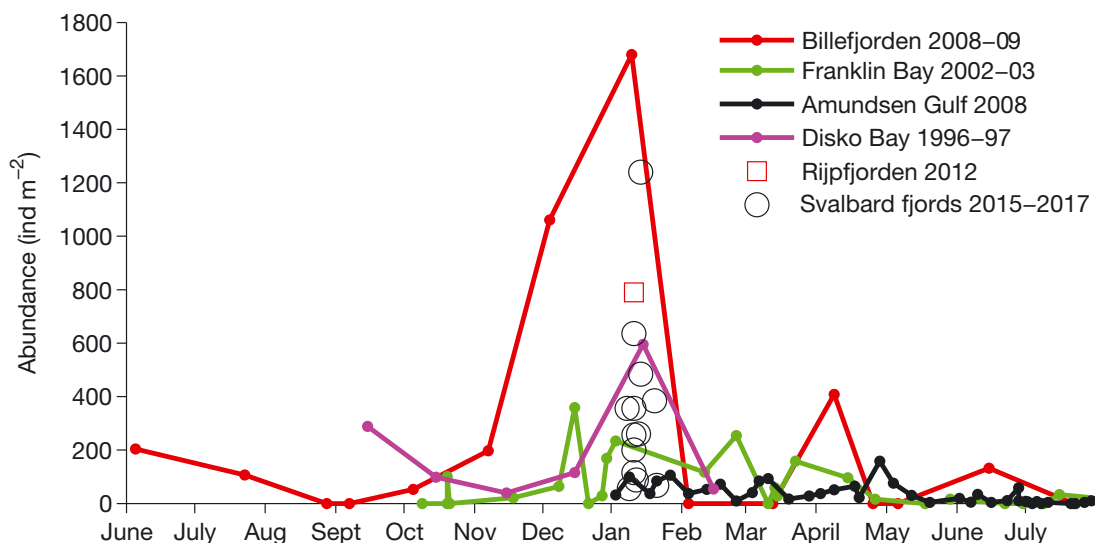nitude as estimates from the Canadian Arctic (Fig. 9).

Fig. 9. Seasonal variability of abundance (ind. m^{-2}) of *Calanus glacialis* males across the Arctic: Billefjorden 2008–2009 (Bailey 2010); Franklin Bay 2002–2003 (Fortier & Darnis 2006); Amundsen Gulf 2007–2008 (Wold et al. 2011); Disko Bay 1996–1997 (Madsen et al. 2001); Rijpfjorden 2012 (Daase et al. 2014); and Svalbard fjords January 2015, 2016 and 2017 (this study). Locations of sampling sites are marked in Fig. 1

Sex ratios

Adult sex ratios observed during this study were relatively consistent, varying from 0.3–0.9 in all years (1–3 females male^{-1}). Exception were in RF in 2016, where AM dominated over AF during our study (sex ratio of 1.6, i.e. 1–2 males female^{-1}), and BF in 2016, where AM were rare (sex ratio of 0.2, i.e. 4–5 females male^{-1}). Such high proportions of males have not been observed in the study area during any other season (Bailey 2010). In other northern geographical locations, *Calanus* AF usually substantially outnumber AM in all other seasons (Marshall & Orr 1955, Conover 1965, Crain & Miller 2000). For *C. glacialis*, the only record of seasonal variability in the sex ratio is from the White Sea, where Kosobokova (1999) reported maximum ratio of 0.5–0.6 males:females in October and November, which is comparable to our observations (Table 3).

The sex ratios of the pre-adult stage CV were clearly skewed towards females during our study. According to published data, moulting of CVs to AM precedes moulting of CVs to AF (Marshall & Orr 1955, Kosobokova 1999), and males seem to generally differentiate from the largest CVs (Grigg et al. 1985, 1987, Miller et al. 1991). This is supported by our observations. The prevalence of potential females in CVs indicates that sex differentiation in males and moulting of potential CV males to adults was largely completed by the time of our January sampling period, with the remaining CVs in the overwintering population presumably developing and moulting to AF later in the season.

The high proportion of sexually undifferentiated CVs in BF indicate that the reproductive phenology may have been delayed in BF compared to the other 4 fjords in 2016. The majority of the sexually undifferentiated CVs from BF were *C. finmarchicus* (Table 4, Fig. S2 in the Supplement). However, 38% of the CVs in the size range of *C. glacialis* were also sexually undifferentiated at the time of sampling. Billefjorden also had the lowest percentage of females with spermatophores and the lowest adult sex ratio. Of all the fjords, the *C. glacialis* population in Billefjorden is the most isolated, since it resides in the inner basin of Billefjorden which experiences reduced water exchange with the outer fjord system and the lowest water temperature (less than −0.5°C). The delay of maturation and moulting of *Calanus* CVs in this fjord may therefore be due to low water temperatures compared to the other locations, as development time is known to increase with decreasing temperatures (Campbell et al. 2001).

In our study, *C. finmarchicus* males were absent in January and the abundance of *C. finmarchicus* females was very low while the proportion of sexually undifferentiated *C. finmarchicus* was high (e.g. in Rijpfjorden). This indicates that the time of moulting into adults and mating does not coincide between *C. finmarchicus* and *C. glacialis*. These elements suggest there is a very low potential for inter-species mating and consequently hybridization is unlikely, which supports results from recent molecular-based studies (Nielsen et al. 2014, Choquet et al. 2017).

Females with spermatophores

In 2015 and 2016, 3–18% of *Calanus* AF had spermatophores attached, indicating that active mating occurred during the studied period. Copulation usually occurs at an early stage in the female's gonad development with a male attaching a spermatophore to the genital segment of a female (Marshall et al. 1934, Marshall & Orr 1955). Our data support these observations, as we found mainly immature *C. glacialis* AF bearing spermatophores in January. The spermatophore is retained by *Calanus* AF only for a short time, while sperm may be stored in spermatheca for a rather long period (i.e. several months), and eggs are presumably fertilized while spawning (Marshall et al. 1934, Marshall & Orr 1955). The highest proportion of *C. glacialis* AF with spermatophores occurred in Kongsfjorden in January 2015, where abundance of AF was also highest (Figs. 4 & 5), as was the relative contribution of AF (28–46%) and AM (12–25%) to the total *C. glacialis* population. In January 2016, AF and AM contributed only 1–7 and 0.3–10%, respectively, to the total *C. glacialis* population, as a much higher proportion of overwintering CIVs was observed (Fig. 4). The high proportion of AF and AM in Kongsfjorden in 2015 likely increased the encounter rate between AM and AF, thus leading to a higher percentage of AF bearing spermatophores that year.

Overall, 18% of females bearing spermatophores fell within the size range defined as *C. finmarchicus* (<2.95 mm). However, the length frequency distribution of females bearing spermatophores was unimodal, with the smallest individuals being 2.6 mm (Fig. S1). Given that *C. glacialis* females may also be of that size (Choquet et al. 2018, Renaud et al. 2018), we find it likely that all females bearing spermatophores were indeed *C. glacialis*.

Vertical distribution and metabolism

It has been proposed that in some calanoid species with extended seasonal migrations AMs concentrate in mesopelagic layers, which ensures high encounter rates and copulation success when AF pass through this layer on their ascent from the overwintering depths (Spiridonov & Kosobokova 1997). Tsuda & Miller (1998) suggested that AF and AM of *Calanus* spp. would benefit from gathering in rather narrow layers of the water column to attract and search for a mate, and that pycnocline or thermohaline stratification would provide favorable conditions for 'painting' pheromone tracks. However, we found that adults of *C. glacialis* did not concentrate at any particular depths in January. They seemed to avoid the very deepest layer but were otherwise present throughout most of the water column. Such a distribution pattern suggests that both sexes are actively swimming in search of a mate for copulation rather than waiting at a certain depth for a mate to swim by. However, males did display higher swimming activity levels and respiration rates than females, indicating that males engage more than females in actively seeking a mate. This fits with observational (Tsuda & Miller 1998, Kiørboe & Bagøien 2005) and theoretical (Kiørboe 2008) studies in other calanoids suggesting that ritualized, directed swimming of males facilitates locating females.

Interestingly, the lipid content of AM was slightly higher than that of AF and much higher than that of the overwintering stages CV and CIV. AM also had significantly higher lipid sac area relative to their body area compared to AF, CVs and CIVs. The relatively high lipid content of AMs observed in January suggests that AMs just recently started to actively mate and thus had not yet depleted their lipid reserves. It may also indicate that AMs may start out with a higher lipid content after moulting to adults compared to AFs, supporting observations from the White Sea that the largest and most lipid-rich CVs are the ones that moult to AMs (Kosobokova 1999). Future studies need to assess the seasonal variability in lipid content between CVs, AM and AF, especially during the moulting period.

Winter abundance and distribution data for *Calanus* from the Arctic are still scarce. Our data on vertical distribution of both *C. glacialis* and *C. finmarchicus* confirm recent observations from the polar night in Svalbard (Daase et al. 2014, Berge et al. 2015, Błachowiak-Samołyk et al. 2015) that overwintering stages and adults of *Calanus* are distributed throughout the entire water column in January instead of being concentrated at depth. These observations were made in the same study area as our study (Rijpfjorden, Kongsfjorden) but also in the off-shelf waters north of Svalbard, indicating that such a distribution pattern is not characteristic to fjord populations only. For adults, this may be an indication of a mid-winter ascent from overwintering depth in order to search for a mate. It also suggests an earlier seasonal ascent of the overwintering population from depths than traditionally believed. There is a marked increase in ambient light from the winter solstice to mid-January that may be sufficient enough to function as a visual cue for *Calanus* to trigger the seasonal ascent (Båtnes et al. 2015, Cohen et al. 2015).

CONCLUSIONS

Males of *Calanus glacialis* were much more abundant in mid-winter in Svalbard fjords compared to all other previously studied seasons. The absence of *C. finmarchicus* AM, low abundance of AF and a high proportion of sexually undifferentiated CVs indicate a distinct reproductive phenology in the 2 species, reducing the likelihood of their interbreeding and hybridization in the study area. The presence of *C. glacialis* AF with spermatophores and elevated swimming activity levels in AM relative to AF revealed that active mate seeking and mating occurs in mid-winter. Furthermore, *Calanus* populations were distributed throughout the water column and not confined to overwintering depths, corroborating recent studies showing that the polar night is a much more biologically active period than previously assumed (Berge et al. 2015, Ludvigsen et al. 2018). It is apparent that for *C. glacialis* the polar night is an important reproductive period. We suggest that further studies with increased seasonal and vertical resolution address gaps in our understanding of the life-history strategies of northern *Calanus* males. Specifically, a better understanding of the timing and energetic costs of the moult to adult, mating and spermatophore production, and the sensitivity of these costs and activities to increased winter temperatures, is now required.

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