Gametogenesis of an intertidal population of *Mytilus trossulus* in NW Greenland: not a limitation for potential Arctic range expansion

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ABSTRACT: The drivers determining species’ northern distribution limits remain elusive and, combined with inadequate knowledge of past and current distribution ranges, this prevents accurate determination of potential changes in the Arctic. The northernmost population of the bivalve *Mytilus trossulus* is found in north Greenland, an area characterized by low temperature, prolonged winters and a short productive period. This population, therefore, provides a unique opportunity to study whether a temperate bivalve species can sustain a population near its poleward distribution limit through reproduction. We assessed gametogenesis and biochemical composition in intertidal *M. trossulus* specimens during the ice-free period (July–September). Results showed that the qualitative traits of gametogenesis and oocyte production in this population were comparable to *M. trossulus* found in temperate regions. In addition, we found that *M. trossulus* larvae settle annually in the lowest intertidal zone, indicating successful fertilization of eggs and larval survival. Our combined results suggest that gonadal development or larval survival do not limit the northern distribution of this species, even in a region characterized by limited food supply and sub-zero water temperatures for 9 mo of the year. Instead, for this marine invertebrate with a larval life-stage, oceanographic conditions and dispersal barriers, rather than physiological constraints, could be more important in determining the northern range limit.

KEY WORDS: Arctic benthos · Biochemical composition · Blue mussels · Glycogen · Gametogenesis · Spawning · Temperature · Oogenesis

INTRODUCTION

Making accurate observations and predictions of how climate change is redistributing species is a key task in ecology. However, this task remains challenging because of inadequate knowledge on current distribution ranges, and intraspecific species responses to environmental changes that depend on interactions among traits, such as physiological tolerance, dispersal capacity, abiotic parameters and life-stages (Robinson et al. 2011, Przeslawski et al. 2015, Kroecker et al. 2016). Predictions are further complicated by local adaptation/acclimatization (Sanford & Kelly 2011) and biotic interactions (Van der Putten et al. 2010), which are known to affect species distributional patterns through trophic cascades, habitat provision and host–parasite interactions. Understanding the complex processes determining current distribution ranges is therefore essential to understanding the impacts of climate change on distribution. A widely used method to improve biogeographic knowledge is to investigate the mechanisms shaping the distribution of a well-described model species and then extrapolate to other less-studied species. 

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Although this approach is valuable, it remains difficult to apply in the Arctic, where ecological and biogeographic knowledge of most species remains limited (Wassmann et al. 2011).

One way to advance knowledge on the distribution of marine species is to study intertidal species, as they are often used as harbingers for climate change (Helmuth et al. 2002). Since limited knowledge is available from the Arctic Greenland intertidal zone (Blicher et al. 2013, Høgslund et al. 2014), research efforts should focus on abundant keystone species because of their facilitating ecosystem services (Zippar & Helmuth 2012). One example is shell-forming species (e.g. gastropods, bivalves), as they may expedite diversity of intertidal and sessile communities (Gutiérrez et al. 2003, Thyrring et al. 2013). In west Greenland, the bivalve genus *Mytilus* is widely distributed in the intertidal zone (Blicher et al. 2013), and has an important ecological role (Gosling 2015). *Mytilus* mussels are also used as monitoring organisms to detect environmental pollution (Thyrring et al. 2015a). However, even though the biogeographic patterns of *Mytilus* have been studied for years, factors governing their northern distribution limits remain elusive. Until recently, only *M. edulis* was registered from the Arctic. However, genetic markers have recently shown that 3 *Mytilus* spp. occur in the Arctic region (Mathiesen et al. 2017). For example, an isolated and sparse intertidal *M. trossulus* population has been found near Qaanaaq (77° N; see Fig. 1) in northwest Greenland (Mathiesen et al. 2017). Individuals here are restricted to the lower intertidal zone and found only between boulders or under macroalgal fronds, where they are protected from abiotic stress (Watt & Scrosati 2013). This *M. trossulus* population constitutes the northernmost population described (Mathiesen et al. 2017), and since adult *M. trossulus* display no signs of impaired physiological performance at this High Arctic location, the drivers determining their pole-ward distribution limit are unknown (Thyrring et al. 2015b, 2017a).

Temperature, metabolism and energy availability have been identified as important factors in shaping the southern distribution edge of marine ectotherms (Sunday et al. 2012, Deutsch et al. 2015), yet these factors do not always apply to the northern distribution limits (Fly & Hilbish 2013). Water temperature and food availability are major factors affecting gametogenesis and spawning in bivalves (Giese 1959, Gosling 2015). For example, for the Pacific oyster *Crassostrea gigas*, spawning occurs at temperatures above 18°C (Miossec et al. 2009), and starvation has resulted in failed oocyte maturation in the bay scallop *Aequipecten irradians* (Sastry 1968). Thus, a feasible mechanism limiting the northern distribution of ectotherms could be suppressed gonadal maturation and subsequent failed spawning, as low temperatures and a short period of primary production characterize the High Arctic region of Greenland (Sejr et al. 2009). In temperate regions, gonads mature by early spring (Bayne et al. 1976), whereas in subarctic environments, rapid maturation coincides with spring blooms as food becomes abundant (Thorarinsdottir & Gunnarsson 2003). Consequently, Arctic *M. trossulus* only have a narrow time window for gonad maturation and spawning, in addition to energy accumulation to survive the following winter, during which *Mytilus* spp. utilize stored energy reserves to meet metabolic demands. Thus, leading-edge populations in Arctic regions may have partly or completely suppressed gametogenesis.

Therefore, considering that *M. trossulus* from Qaanaaq is the northernmost population discovered, this study aims to test the hypothesis that their northern distribution is limited by failure of gametogenesis, given the short ice-free period and low water temperature. Specifically, we examined gonad histology and whole-animal biochemical composition of mussels collected during the ice-free period to investigate if *M. trossulus* has the potential to reproduce, or if the population is a result of larval spin-off from more southern or sublittoraly located populations.

**MATERIALS AND METHODS**

**Study site**

The Qaanaaq fjord (77°28’00.32” N, 69°13’58.90” W; Fig. 1) is characterized by ice cover from November to June. In 2014, the sea ice broke up during June and July, followed by a fully open-water period in August and September before ice formation initiated in October. Thus, the productive open-water period was limited to approximately 90 days because sea ice hampers light penetration in spring, while day length limits productivity in autumn (Sejr et al. 2009).

The Qaanaaq fjord is located <30 km from the ice-free polynya, the North Water Polynya (NOW), situated at −76–79° N and −70–80° W (Heide-Jørgensen et al. 2013). In the NOW, sea ice is absent during spring and the phytoplankton bloom starts as early as April, with the highest estimated primary production from the High Arctic (Klein et al. 2002). Extending from the NOW, the open-water area gradually expands into the Qaanaaq fjord (e.g. Fig. 1; ice cover
in June 2014) with productive ice-edge-associated plankton blooms (Perrette et al. 2011).

From September 2009 to August 2010, water temperatures at 10 m depth were measured near Qaanaaq (77° 27' 44.2" N, 69° 15' 17.5" W) every 30 min using a HOBO temperature logger (ONSET).

**Animal collection**

In 2014, the study site became sea ice-free on July 4, making animal collection possible. Ten adult *Mytilus trossulus* with a shell length of 40−73 mm were hand-collected during low tide in the lowest intertidal zone on 5 dates (July 6, July 27, August 11, August 27, September 9) throughout the ice-free period. Mussels were frozen immediately after collection and transported frozen to laboratory facilities at Aarhus University, Denmark and kept frozen until further analysis.

To investigate if larvae settle annually at the study site, we collected all mussels within 4 m² at the collection site on September 9. All collected animals were age-estimated by counting winter growth increment as described in Blicher et al. (2013).

**Histology**

After thawing the samples in the laboratory, mantle tissue was preserved in a freshly prepared Davidson fixative containing glycerol, glacial acetic acid, formaldehyde (37%), ethanol (95%), and filtrated seawater with a salinity of 27 (1:1:2:3 v/v/v/v/v). Tissue samples were kept in fixative at room temperature for 48 h, dehydrated through a graded ethanol series (70%, 2× 90%, 2× 96% and 2× 99.9%) and embedded in plastic resin (Technovit, EMS). Thin cross-sections (3 μm) were obtained using a microtome (Leica Biosystems), placed on glass slides and stained with hematoxylin and 1% eosin yellow (VWR − Bie & Berntsen) before being sealed with Eukitt mounting media (Fluka). Final sections were observed using a light microscope (Zeiss) at 200× magnification, and the shortest diameter of 300 oocytes was measured from 4 randomly selected females collected in July.

**Gonad developmental stages**

The different developmental stages were identified following Seed (1969) and Dei Tos et al. (2016). Gonads were classified into 4 main phases: developing (Phase I), mature (Phase II), spawning (Phase III) and reabsorption (Phase IV), characterized as follows

- Developing (Phase I): All individuals can be sexed in this stage. In female follicles, oogonia rest on the follicle membrane and developing oocytes have started vitellogenesis. Mature oocytes can be found in follicles with a nucleus and are detached from the membrane. In males, immature spermatogonia and spermatocytes dominate the follicles.
• Mature (Phase II): The mantle tissue is closely packed with germinal epithelium. In females, the majority of oocytes are mature with a developed nucleus and yolk granulate and are detached from the follicle membrane. Developing oocytes attached to the follicular wall are also present. In males, immature spermatogonia rest on the follicle membrane. Spermatocytes and spermatids are visible toward the lumen and mature spermatozoa are found accumulated closest to the lumen.

• Spawning (Phase III): The connective mantle epithelium between follicles increases as gametes are released. The density of oocytes decreases in the follicles, and in males, follicles became thinner and empty in the middle as gametes are released.

• Reabsorption (Phase IV): This is the post-spawning stage. Sperm and oocytes at various developmental stages can be found in this phase. In both sexes, follicles are deformed and germinal epithelium undergoes cytolysis as a part of the reabsorption process.

Biochemical composition

To determine total content of lipids, glycogen and protein, 5 individuals from each of the 5 collections (a total of 25 individuals) were dried at 60°C for 36 h, milled and kept dry in a desiccator.

Lipid content was determined gravimetrically after Soxhlet extraction following Fisker et al. (2014): petroleum ether was used to wash ~1.5 g of dry tissue repeatedly before being re-dried. The difference in dry weight (DW) before and after extraction was assumed to represent total lipid content.

Glycogen content was measured using spectrophotometry following protocols described previously by Fisker et al. (2014): dried tissue (40–45 mg) was transferred to 1 ml 1 M NaOH, and heated for 3 h at 75°C, which extracted all glycogen while degrading free glucose. Nine hundred µl acetate buffer (0.25 M, pH 4.75) containing 400 mg l−1 amyloglucosidase (EC 3.2.1.3, Sigma-Aldrich) was then transferred to 100 µl of the glycogen extract. The sample was left for 90 min at 25°C to allow the amyloglucosidase to cleave all glycogen to glucose. The glucose was measured spectrophotometrically at 340 nm using a glucose kit (Glucose Gluc-DH, Dianostic Systems), and the glucose was calculated relative to glucose standards subject to identical extraction procedures.

Nitrogen content was determined after the Kjeldahl method (Kjeldahl 1883), and protein content was estimated by multiplying nitrogen content by a nitrogen-to-protein conversion factor of 6.25 (N × 6.25).

Data analysis

All data analyses were conducted using R (R Core Team 2017). To analyze the difference in glycogen, lipid and protein content between sampling dates, 1-way ANOVAs followed by Tukey’s HSD analysis were used. Bartlett’s test was used to evaluate variance of homogeneity, and data were log-transformed to ensure homogeneity and normally distributed data. Data are presented as mean ± SD.

RESULTS

Temperature

Water temperature at 10 m depth in Qaanaaq from September 2009 until August 2010 is shown in Fig. 2. The temperature was characterized by persistent sub-zero temperatures from mid-September to late June, followed by a warmer period in July and August. The highest recorded water temperature was 8.8°C (Fig. 2).

Gametogenesis

We collected both male and female Mytilus trossulus at all collection dates, and 40–50% of the collected animals were males, depending on the collection date. The temporal pattern of gametogenesis was similar in both sexes, and mussels were observed in all 4 gonad developmental stages described above in ‘Materials and methods’ (Figs. 3 & 4). In the male developing phase (Phase 1), germinal epithelium con-
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sisted of follicles full of spermatocytes, and there was little mantle epithelium (ME) between the follicles, depending on developmental stage (Fig. 3a). In developing females, immature oogonia rested on the follicle membrane and larger developing oocytes were still attached to the membrane (Fig. 4a). Mature oocytes were found in the germinal epithelium with a clear nucleus and yolked globules (Fig. 4a).

In mature males, large follicles contained immature spermatogonia at the follicle membrane, in addition to spermatocytes and spermatids and mature spermatozoa that were found abundant nearest to the lumen (Fig. 3b). In females, the proportion of germinal epithelium was high compared to mantle epithelium, and mature oocytes with a clear nucleus were abundant (Fig. 4b). During spawning, the mantle epithelium increased between follicles. Spawning males were characterized by having mature spermatozoa released from the follicles (Fig. 3c). Female oocyte density decreased in the follicles during spawning following the release of oocytes (Fig. 4c). A post-spawning reabsorption of germinal epithelium was found in both sexes (Figs. 3d & 4d). Deformed and at times thin follicles were found in this phase, in addition to various stages of oocytes and sperm cells throughout the tissue.

During the sampling period, the highest proportion of mature individuals (44%) was found on July 6
Spawning started after July 6 and continued throughout July and August (Fig. 5). Developing mussels were found from July to September (except on August 11), and post-spawned reabsorbing individuals were only found from August 11 to September 9 (Fig. 5). In September, the reabsorption phase was observed in approximately 55% of mussels, in addition to mature (22%) and developing (23%) individuals (Fig. 5). Oocyte sizes in July ranged from 17.6 to 85.6 µm, with a mean of 42.2 ± 11.4 µm (Fig. 6).

Age of the mussels spanned from 3 to 11 yr (n = 61) (Fig. 7). Though newly settled mussels were also observed, they were restricted to crevasses between boulders and were inaccessible.
The highest glycogen content was observed in *M. trossulus* collected on July 6, after which a significant drop was found between July 6 and July 27 (Tukey’s HSD; *p* < 0.001; Fig. 8a), corresponding with the initiation of main spawning. In the following period, July 27 to September 9, glycogen content remained relatively constant at 85 ± 20 to 138 ± 47 mg g⁻¹ DW (Fig. 8a). Lipid content ranged between 84 ± 8 and 155 ± 38.4 mg g⁻¹ DW and changed significantly over time (ANOVA; *F*<sub>4,20</sub> = 11.35, *p* < 0.001; Fig. 8b), with the highest values found on July 6 and August 11. No significant changes in protein content (553 ± 31 to 617 ± 47 mg g⁻¹ DW) were observed over the season (ANOVA; *F*<sub>4,20</sub> = 11.35, *p* = 0.136; Fig. 8c); thus protein content did not correlate with summer gonad development or spawning in *M. trossulus*.

**DISCUSSION**

Here, we show that the northernmost intertidal population of *Mytilus trossulus* from Qaanaaq, Greenland (77° N) can produce mature gonads and spawn. We had expected that temperate *M. trossulus* mussels would have suppressed or delayed gonad maturation or produced small oocytes to conserve energy in the High Arctic. However, gametogenesis of *M. trossulus*
from Qaanaaq is similar to what has been reported for temperate *M. trossulus* (Toro et al. 2002) and subarctic *M. edulis* (Thorarinsdottir & Gunnarsson 2003). For example, in both Greenland and Canadian populations, spawning occurs scattered from summer to early autumn, and we found that 58% of *M. trossulus* were developing in early July, which is comparable to *M. trossulus* from Canada, where ~60% were developing in July (Toro et al. 2002). Oocyte diameter was (average: 42.2 µm) comparable to sizes reported from Icelandic *M. edulis* populations (average: 41.1 and 46.5 µm), even though oocyte size has been suggested to be larger in *M. edulis* than in *M. trossulus* (Toro et al. 2002). Furthermore, based on the age group composition, it appears that *Mytilus* larvae settle annually in Qaanaaq. Though we did not collect mussels of ages 0 to 2, we did observe small juvenile mussels in deep crevasses. Combined, our results do not support the hypothesis that the northern distribution of *M. trossulus* is limited by gonadal development or spawning. Nonetheless, the biochemical quality and quantity of eggs produced should be studied further to verify the viability of eggs from this High Arctic intertidal population of *M. trossulus*.

The prevailing paradigm about drivers shaping marine species distribution limits is oxygen limitation (Pörtner 2010) and ability to cope with unsuitable temperatures (Sunday et al. 2012). For example, Jones et al. (2010) found that the equator-ward biogeographic limit of *M. edulis* on the east coast of North America was closely correlated to environmental temperatures; because marine ectotherms already fill their thermal range, they are in general expected to contract their equator-ward limits in response to warming (Sunday et al. 2012). However, environmental temperatures may to a lesser extent control intertidal species’ pole-ward distribution. Intertidal species (such as *Littorina* spp. and *Mytilus* spp.) are known to display high levels of local adaptation and phenotypic plasticity on temporal and spatial scales (Riginos & Cunningham 2005, Kuo & Sanford 2009, Thyrring et al. 2017a). In northwest Greenland, for example, *M. trossulus* show no depression in metabolic performance (Thyrring et al. 2015b), as would have been expected following the oxygen limitation hypothesis by Pörtner (2010). Most organisms in the Greenland intertidal zone are found in microhabitats between boulders, in crevices or underneath macroalgae. These microhabitats provide a refuge from extreme temperatures and organisms are rarely exposed to sub-zero temperatures below the thermal limit (Blicher et al. 2013, Thyrring et al. 2015a). Accordingly, the distribution limits of *M. trossulus* cannot necessarily be predicted from atmospheric temperature scenarios.

Considering the sparse intertidal population (Thyrring pers. obs.), it seems unlikely that the population is self-sustained and isolated from other populations. We have not explored if there are sublittoral populations, but we know that abundant sublittoral populations of *Mytilus* spp. occur at other locations in west Greenland (e.g. Disko Bay and Uummannaq). If such populations also occur seawards of our *M. trossulus* population, they might be the source for recruits in the intertidal zone. Regardless of larval origin, it seems that distribution limitations occur at the larval or juvenile life-stage independent of adult fitness. Our results indirectly support the contention that viability of larvae and post-settlement processes determine the northern range limits of littoral invertebrates as suggested by Lewis (1986). This highlights the importance of including larval survival and dispersal when studying large-scale distribution patterns. The importance of larval dispersal in marine biogeography was also illustrated by the recent spread and settlement of *M. edulis* to Svalbard through intrusion of unusually warm Atlantic water (Berge et al. 2005). Another example across the Arctic is that *Mytilus* spp. are only absent in northeast Greenland (Mathiesen et al. 2017), despite similar climatic conditions in northeast and northwest Greenland (Sejr et al. 2009). Such distributional patterns are likely a result of dispersal limitation; while Svalbard and west Greenland are influenced by north-flowing currents, the currents along east Greenland flow from north to south, limiting dispersal from source populations (Mathiesen et al. 2017). Combined, these results support the notion that oceanographic conditions, which include currents and frontal systems, are of central importance in determining distributional patterns and pole-ward limits in marine species, rather than physiological constraints (Fetzer & Arntz 2008, Robinson et al. 2011). Moreover, this points to the large dispersal potential of *M. trossulus* if introduced to novel Arctic regions. We suggest that increased shipping in the Arctic could be a key driver for range expansion for this species, allowing it to transcend current natural dispersal barriers (Eguíluz et al. 2016).

From September to March, pelagic primary production is negligible in the High Arctic region of Greenland (Sejr et al. 2009). We therefore argue that even though mature individuals were found until September, spawning would not occur, as the limited food availability is unsuitable for larval survival and settlement. Instead, gametes could be reabsorbed and
utilized as energy during winter. Consequently, *M. trossulus* in north Greenland may only spawn in some years, and gametogenesis could be independent of the energy accumulated the year before. This strategy has been found in other *Mytilus* populations, where rapid gametogenesis occurs in late spring (i.e., during the spring bloom) and throughout the summer, with plenty of food to support growth and settlement of larvae (Toro et al. 2002, Thorarinsdottir & Gunnarsson 2003). However, based on the present data, we cannot completely exclude the possibility of autumn spawning events and winter settlement as observed in temperate and subarctic regions (Thorarinsdottir & Gunnarsson 2003, Duinker et al. 2008).

Even though the intertidal zone of north Greenland is ice-covered until early July, glycogen content was surprisingly high in July. The high glycogen levels at ice break suggest feeding on phytoplankton from open waters being advected to the sampling site in spring/early summer before ice breakup. The NOW is in this regard of particular interest. The eastern region (<30 km away from our sampling site) of the NOW is characterized by a spring bloom as early as April (Klein et al. 2002), almost 4 mo before the first sampling. Furthermore, the ice-free area gradually expands from April into the Qaanaaq fjord (Fig. 1), with ice-edge-associated plankton blooms (Perrette et al. 2011). Thus, local water currents could feed ice-covered populations with phytoplankton originating from the open water, allowing bivalves to begin gametogenesis and regain nutritional status before intertidal ice breaks. An alternative scenario is that intertidal filter-feeding organisms feed on alternative food sources during winter to sustain nutritional status and survival. The prevailing paradigm is that filter-feeding bivalves feed mainly on pelagic phytoplankton. However, in the Arctic, alternative food sources, including macroalgal detritus, ice algal and re-suspended materials, may be important (Renaud et al. 2015, Kohlbach et al. 2016). Macroalgal detritus constitutes >50% of assimilated carbon in filter-feeding bivalves from Svalbard (Renaud et al. 2015), and *M. trossulus* has been found feeding on ice algae during ice-covered periods in spring and early summer (Thyrring et al. 2017b). However, in subarctic Greenland, *Chlamys islandica* appear not to utilize alternative food sources and display suppressed growth in winter (Blicher et al. 2010), and in the bivalve *Clinocardium ciliatum* and the sea urchin *Strongylocentrotus droebachiensis*, growth rates were strongly correlated to the length of the open-water pelagic primary production period in Greenland (Blicher et al. 2007, Sejr et al. 2009). Thus, the nutritional quality of alternative food sources, and temporal and spatial processes affecting growth, dispersal and fitness in Arctic ectotherms, need to be studied further.

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**LITERATURE CITED**


