

Reproduction of two species of *Halichondria* (Demospongiae: Halichondriidae) in the White Sea

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Abstract: Life history investigations of Arctic sponges allow one to observe the reproductive adaptations of these primitive metazoans to an extreme climate environment. The present study focuses on the life histories of two sympatric species *Halichondria panicea* and *Halichondria sitiens* in the White Sea. Both species appeared to be ovoviviparous and are characterized by asynchronism of gametogenesis and embryogenesis within the populations and within individuals, which agrees well with the data on halichondriids from other species and regions. On the contrary there are considerable differences in sexuality, which can be classified as successive hermaphroditism in the White Sea sponges but which varies from contemporaneous hermaphroditism to gonochorism in halichondriids from other regions. Substantial differences between halichondriid species and populations in terms of reproductive stages should also be emphasized. Oogenesis in the White Sea *H. sitiens* began at the same time as *H. panicea* but was of longer duration such that the last stages of gamete maturation and embryogenesis occurred later. This difference may be partially explained by the 2-3 week delay in warming of the deeper water layer inhabited by *H. sitiens* in contrast to *H. panicea*. A more significant reason may be related to the physiological differences between these species. Thus, the differences in sponge reproductive patterns may be caused either by environmental differences between the habitats occupied by the species or by physiological distinctions between the different taxa.

Keywords: *Halichondria panicea*, *Halichondria sitiens*, sympatric species, reproduction

Introduction

Life history investigations of Arctic Porifera increase our knowledge of the reproductive adaptations of these primitive metazoans to an extreme climate. At present, even basic information concerning the reproductive biology of sponges in the Russian Arctic Region is lacking. Only two studies dealing with their life histories have been conducted (Ivanova 1981, Ereskovsky 2000). This is especially surprising since sponges are known to dominate some common benthic communities in this region (Ereskovsky 1995).

The present study focuses on the family Halichondriidae, which contains sponges that are relatively common and abundant in high latitudes, dominating several hard bottom communities of the Barents and White Sea (Propp 1971). Therefore, the first aim of our research was to obtain baseline life history data on the White Sea halichondriids for the purpose of future long-term population monitoring. However, the taxonomy of the family Halichondriidae, one of the pivotal demosponge taxa, still remains difficult and confused (Erpenbeck and van Soest 2002). The use of traditional taxonomic characters in the classification of halichondriids is hampered due to considerable variation in their skeletons, which are made up entirely of smooth monaxonal megascleres. The need for additional

discriminating characters calls for the application of genetic, biochemical and life history data. In particular, a comparative embryological approach has been successfully used for the discrimination of several sympatric halichondriid species (Vethaak *et al.* 1982, Wapstra and van Soest 1987, Hoshino *et al.* 2004). Consequently, the second aim of our research was to look for any differences between the life histories of two sympatric species, *Halichondria* (*Halichondria*) *panicea* (Pallas, 1766) and *Halichondria* (*Eumastia*) *sitiens* (Schmidt, 1870). It should be emphasized that there are many data on the reproduction of the former species (Fell 1974, Ivanova 1981, Vethaak *et al.* 1982, Wapstra and van Soest 1987, Witte and Barthel 1994), whereas no life history studies had been undertaken on the latter species prior the present study.

Material and methods

Study area and its hydrological conditions

This investigation was conducted in the area of Keret Archipelago in the Kandalaksha Bay of the White Sea (Fig. 1). The study area is characterized by a complicated bottom relief including vertical rock cliffs, large stones, sandy plains and silted trenches. The average depth is about 20 m, while the maximum depth can reach 65 m. Brown algae occur from the

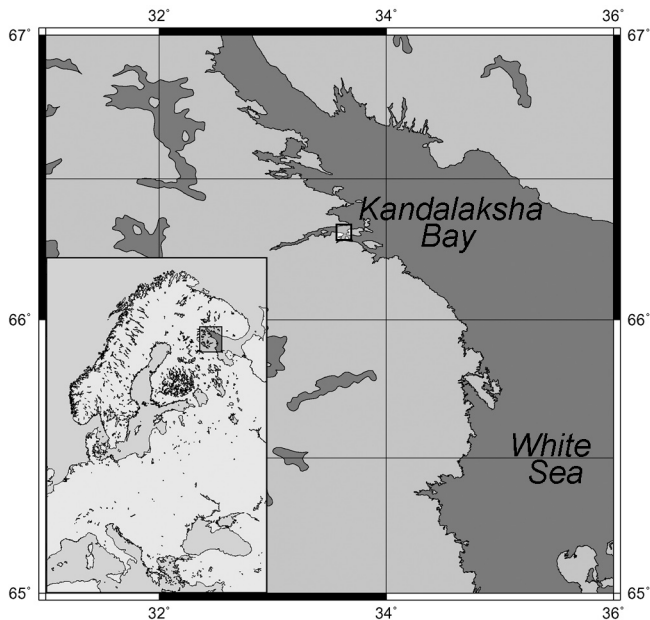


Fig. 1: Map of the study area.

intertidal to 5-8 m, while the depth range from 5-8 to 10-12 m is occupied by red algae. The hydrological conditions of the Kandalaksha Bay are characterized by considerable seasonal fluctuations of water temperature and salinity (Babkov and Golikov 1984). In addition, the water is stratified most of the year, and the amplitude of the seasonal fluctuations decreases from the surface to deeper depths. The temperature regime is determined by a long severe winter and short and relatively warm summer. From December to mid-May the coastal zone of Kandalaksha Bay is covered with ice. According to the data obtained in 2004-2005, the temperature was below zero from the beginning of December to the end of May (annual minimum -1.4 to -1.5°C) in the depth range 5-15 m, which corresponded to hydrological winter (Fig. 2). In June the temperature rose rapidly, which corresponded to hydrological spring. Hydrological summer lasted from the end of June to the beginning of September. The annual temperature peak measured $15-17^{\circ}\text{C}$ depending on depth. According to the 2004-2005 data for the 5-15 m depth range of the Chupa Inlet, a salinity minimum of 22-23‰ was registered at the end of May. In summer the salinity fluctuated between 23-26‰. Hydrological winter was characterized by higher salinity values exceeding 27‰.

Sampling and processing of the material

Sponge sampling was performed by SCUBA diving from mid-June to mid-September of 2004 at approximately two week intervals. At each sampling time 5-8 individuals of each species were collected. Altogether 34 specimens of *H. panicea* and 42 specimens of *H. sitiens* were sampled. Individuals of *H. panicea* were collected between 3-6 m in the brown algae *Laminaria sp.* zone from rock substrata or from the algae thalli. *H. sitiens* sponges were sampled from 8-12 m depth in the red alga zone from rocks or ascidians *Styela rustica*. Fragments

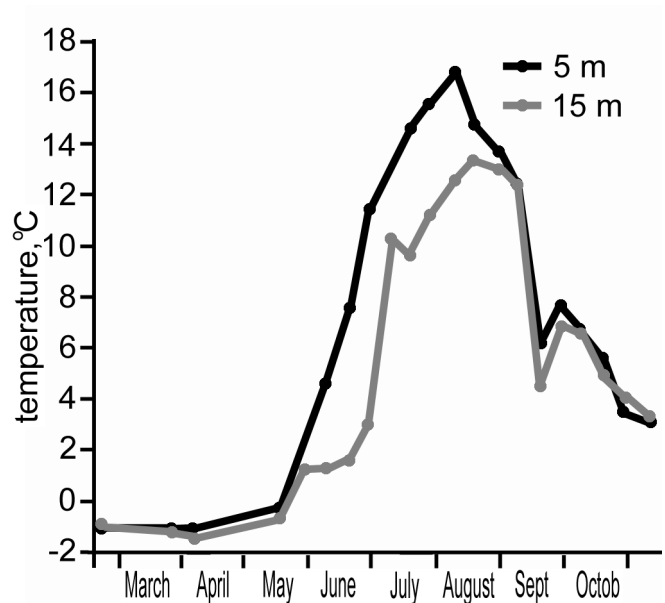


Fig. 2: Water temperatures in the study area in 2004.

of approximately 0.5 cm^3 were cut from different parts of the sponges and fixed in Bouin fluid. Further processing of sponge fragments followed standard histological techniques (Ereskovksy 2000), which included spicule dissolution in 20 % fluoride acid, dehydration in an ethanol series, celloidin-castor oil mixture and chloroform, embedding in paraffin, and sectioning to $6 \mu\text{m}$ thickness. The resulting sections were cleared of paraffin, stained with Mayer's hematoxylin, eosin and mounted on slides. Five sections of each sponge were examined with light microscopy.

Results

Halichondria panicea (Fig. 3)

Halichondria panicea is ovoviviparous. Gametogenesis and embryogenesis appear to be asynchronous both within the population studied and within individuals. Of the eight sponges sampled in June, one contained no reproductive elements, one contained both male and female gametes, and the ratio between females and males was 2:1 for the remainder of the specimens. No males were found from July to September. The ratio between reproductive and non reproductive specimens was 1:1 in July while no reproductive sponges were found in August, and in September only one sponge contained reproductive elements.

Spermatogenesis occurred in June. Spherical spermatocysts of about $70 \mu\text{m}$ in diameter were scattered throughout the choanosome and were most abundant in the middle and basal areas of the sponge body (Fig. 4A). The development of gametes within each spermatocyst was synchronous. All male sponges contained spermatocysts at various stages of maturation, from spermatogonia to mature sperm. The single hermaphroditic sponge possessed both spermatocysts and previtellogenic oocytes.

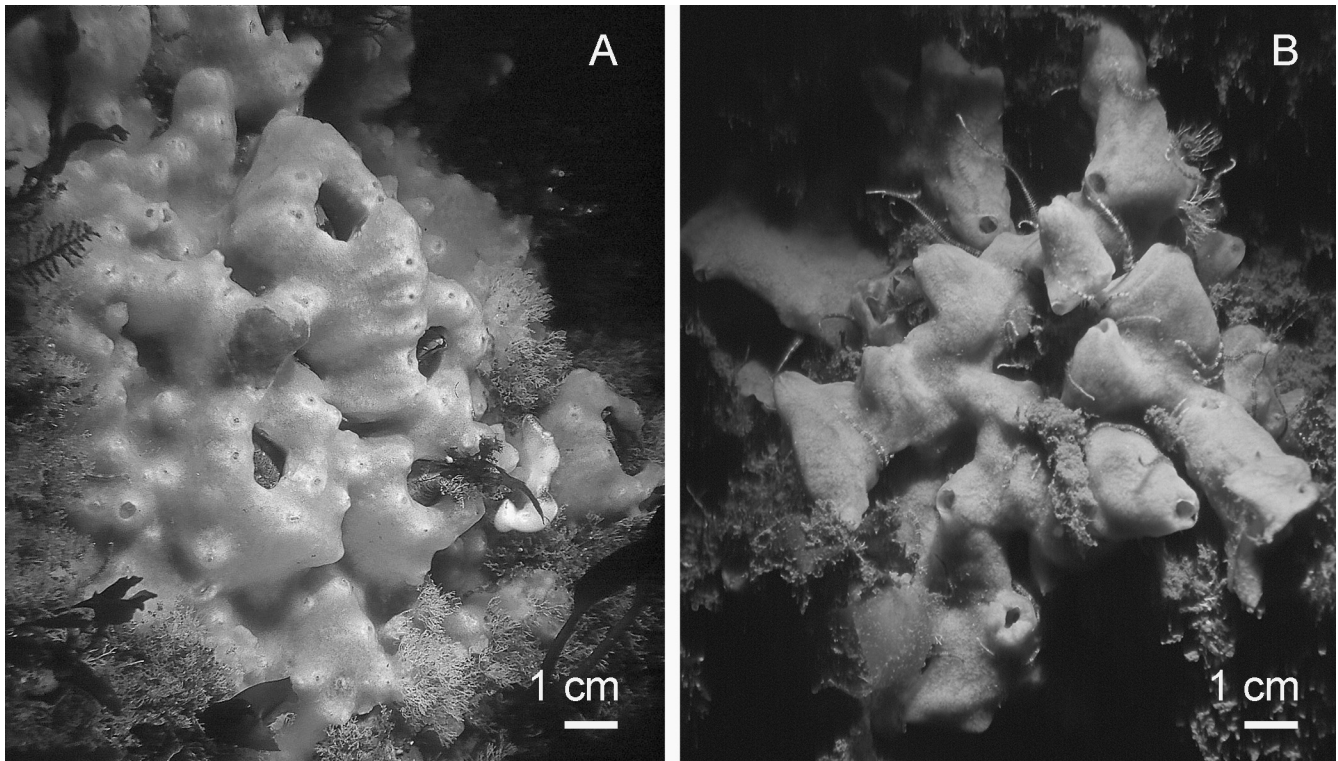


Fig. 3: Growth forms of *Halichondria panicea*, underwater photographs.

Females containing previtellogenic oocytes were found from the middle of June. The youngest oocytes were characterized by an irregular or oval shape, homogenous cytoplasm with rare small inclusions, an homogenous nucleus and prominent nucleolus (Fig. 4B). They measured 15-24 μm in diameter with nuclei of 6-11 μm and nucleoli of 2.8-4.3 μm . Vitellogenesis took place most actively in the second part of June. During vitellogenesis oocytes were gradually being surrounded by aggregates of ameboid cells. (Fig. 4C, D). These aggregates became a single layer of flat pinacocyte-like cells coating the mature oocytes and developing embryos. The mature oocytes had a spherical or slightly oval shape and measured 170-200 μm . By the end of June the oocytes were at all developmental stages within an individual sponge.

Cleavage and larval formation occurred in July. Cleavage was total, equal and chaotic, resulting in a stereoblastula (Fig. 4E). Additionally vitellogenic and previtellogenic oocytes were found among the groups of cleaving embryos within some sponges in the middle of July. At the same time, the first prelarvae were also observed. The prelarvae were typical parenchymellae with an outer layer of ciliated cells and spicules between the internal cell masses. The larvae were elongate and measured up to 225x115 μm (Fig. 4F). Just prior to larval release the larval surface became folded. Release occurred in the last third of July. In the mesohyl of one sponge collected in September were a few early oocytes.

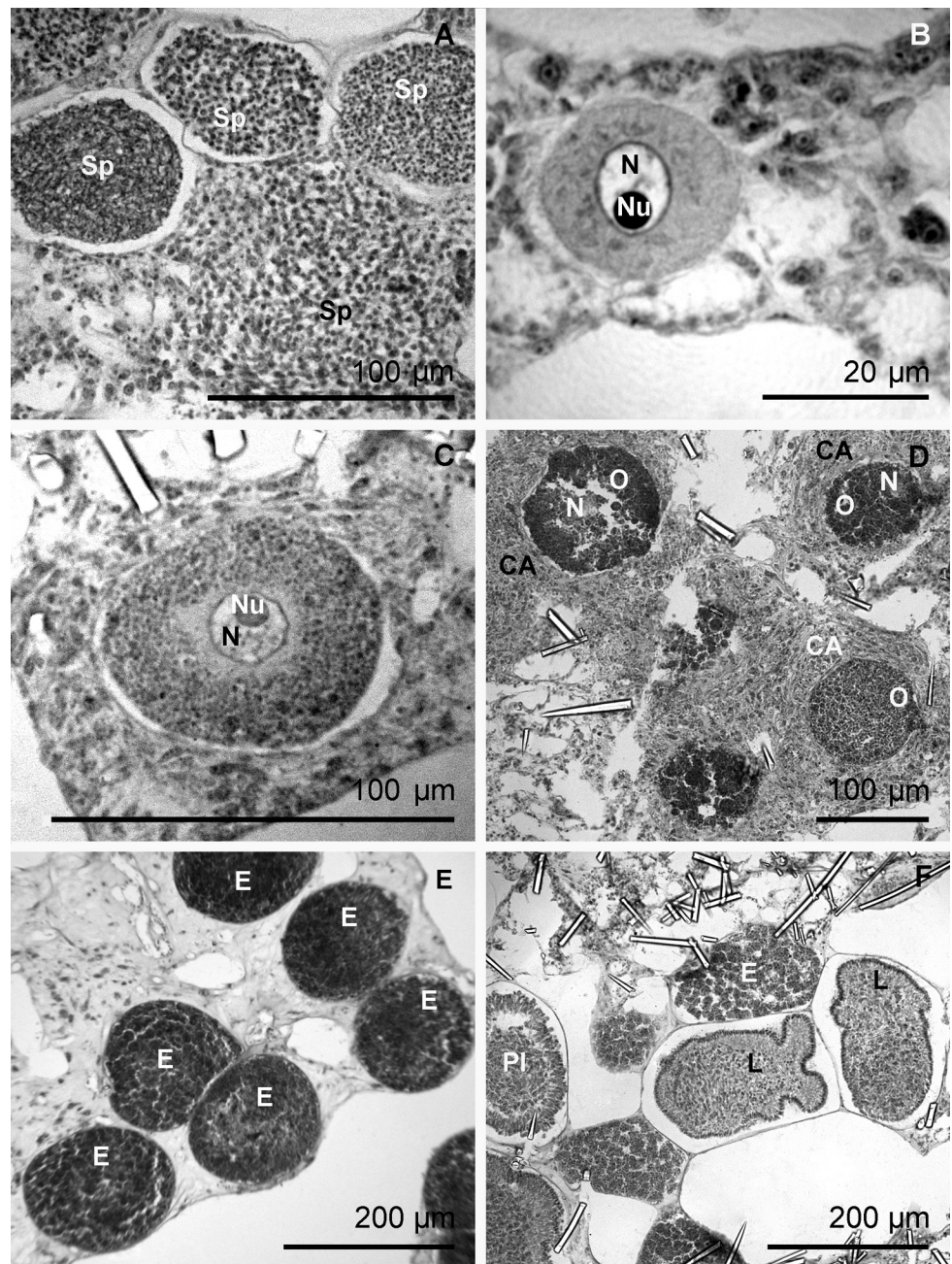
Halichondria sitchensis (Fig. 5)

Halichondria sitchensis is ovoviviparous. Gametogenesis and embryogenesis of this species appears to be asynchronous both within the population studied and within individuals. The ratio between reproductive and non reproductive specimens was 2:3 in June, 8:1 in July and 6:1 in August, while in September all studied sponges contained reproductive elements. In June only females were found and the sex ratio between females and males was respectively 4:1 in July, 1:2 in August and 2:1 in September. Also in August one individual contained both male and female gametes.

Spermatogenesis was found from the middle of July to the middle of September. Spermatocysts were spherical and measured up to 80 μm (Fig. 6A). As recorded in *H. panicea* the development of sperm of *H. sitchensis* was synchronous within a spermatocyst, but each male could possess spermatocysts at different stages of maturation. The single hermaphroditic august sponge contained both spermatocysts and previtellogenic oocytes.

Young previtellogenic oocytes were found from the middle of June. They measured 15-40 μm in diameter with nuclei of 7-24 μm , and nucleoli of 3-8 μm (Fig. 6B). In July and August most of the female specimens contained both previtellogenic and vitellogenic oocytes while some sponges possessed exclusively previtellogenic oocytes. In the middle of August some oocytes reached maturity. They had a spherical or slightly oval shape and measured 180-220 μm (Fig. 6C). In the middle of September all female sponges studied contained cleaving embryos but some also contained

Fig. 4: Reproductive elements of *Halichondria panicea*. **A.** different stages of spermatogenesis within spermatocysts (Sp). **B.** Previtellogenic oocyte. **C.** Oocyte at the early vitellogenic stage. **D.** Oocytes (O) at the late stage of vitellogenesis, surrounded by an aggregate of ameoboid cells (CA). **E.** Cleaving embryos (E). **F.** Embryos on stereoblastula stage (E), prelarvae (Pl) and larvae (L) before release. Abbreviations: N – nucleus; Nu – nucleolus.



vitellogenic and previtellogenic oocytes. Cleavage was total, equal and chaotic, resulting in a stereoblastula (Fig. 6D).

Discussion

Halichondria panicea and *H. sitiens* in the present study appear to be ovoviviparous and are characterized by asynchronous gametogenesis and embryogenesis both within the populations and within individuals. These life history features agree well with previous data on all *Halichondria* species investigated to date (Sarà 1993, Ereskovsky 2005). Gamete morphology and cleavage pattern observed by light

microscopy are also similar to reports of halichondriids from other regions (Ivanova 1981, Barthel and Detmer 1990, Witte and Barthel 1994).

However some differences in sexuality should be emphasized. It is possible to suppose that the sexuality of both species studied is successive hermaphroditism, when the sexes are mainly separated but very a few hermaphrodites exist. The same type of sexuality was registered in the Barents Sea population of *H. panicea* (Ivanova 1981). Meanwhile, *H. panicea* and *H. bowerbanki* from the SW coast of the Netherlands were demonstrating contemporaneous hermaphroditism (Wapstra and van Soest 1987), while *H. panicea* from the Kiel Bight of the Baltic Sea was reported

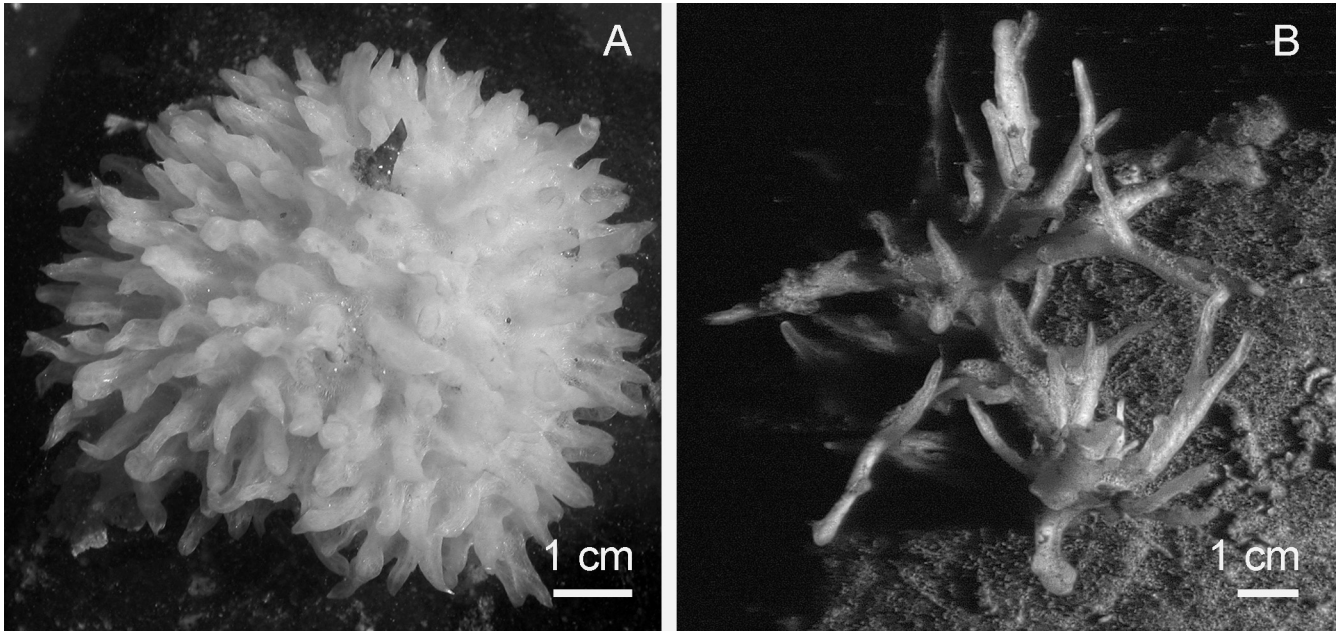
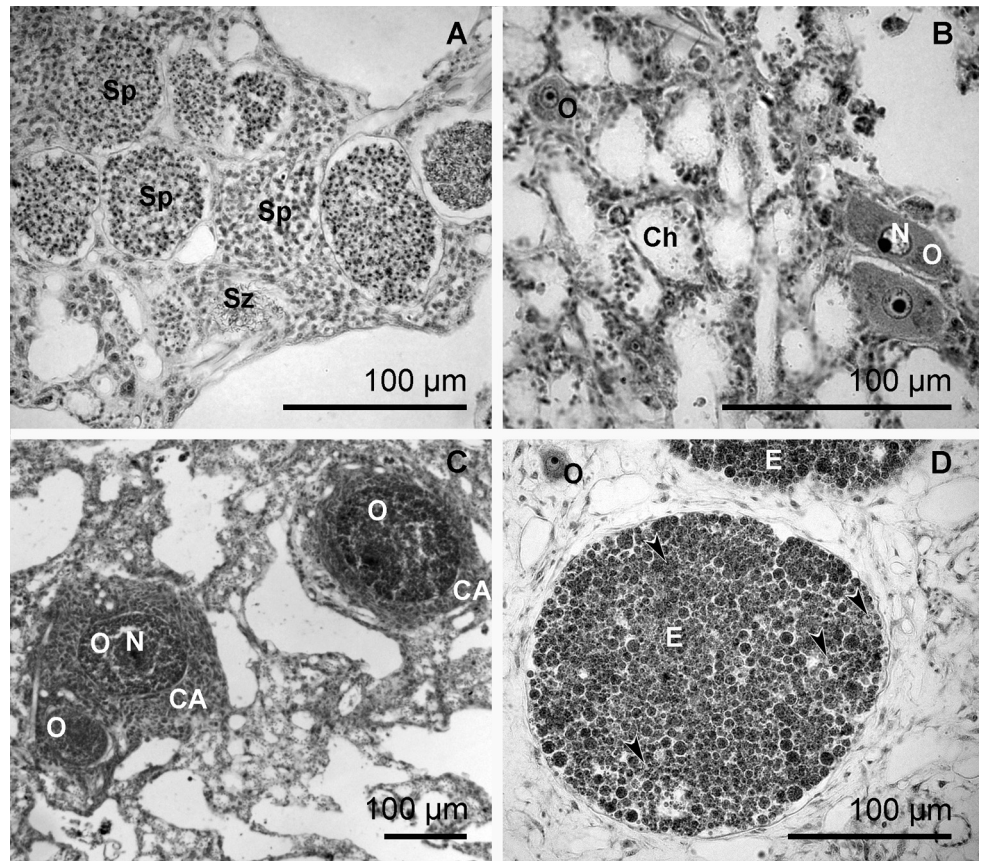


Fig. 5: Growth forms of *Halichondria sitchensis*, underwater photographs.

Fig. 6: Reproductive elements of *Halichondria sitchensis*. **A.** Different stages of spermatogenesis within spermatocysts. **B.** Previtellogenic oocytes. **C.** Vitellogenic oocytes (O) at different developmental stages, surrounded by an aggregate of amoeboid cells (CA). **D.** Cleaving embryos in the mesohyl. Nuclei of blastomeres are indicated by arrows. Abbreviations: N - nucleus; Nu - nucleolus; Sp - spermatocyst; Sz - spermatocyst with the spermatozoa.



to be gonochoristic (Witte and Barthel 1994). Thus, it can be concluded that the species within the genus *Halichondria* as well as the populations within the species *H. panicea* are characterized by a labile sexuality. This finding is a result of differences in environmental conditions rather than to taxonomic differences.

There are also substantial differences between halichondriid species and populations in terms of reproductive stages (Table 1). In the White Sea subtidal population of *H. panicea* studied here gametogenesis went active in June – early July when the local water temperature was rapidly rising from 0°C to 10–13°C, and larvae were released in late July – early August when the temperature reached its annual peak of 15–17°C. In the Baltic Sea population of this species the period of larval release varied in different years from the April to August depending on temperature dynamics (Barthel 1986, 1988, Witte and Barthel 1994). Similar variability in timing of larval release was observed in the Netherlands population of *H. panicea*. Vethaak *et al.* (1982) registered mature oocytes and embryos from mid-May to mid-August whereas Wapstra and van Soest (1987) observed embryos and larvae from May to September. The Barents Sea intertidal population of *H. panicea* examined by Ivanova (1981) was reproductively active mainly in July–August, when the local water temperature reached the annual peak of 7–8°C and larvae were released in September–October, when the temperature fell to 4–5°C.

These differences between the three populations of *H. panicea* in terms of reproduction may be explained by the environmental preferences of this Atlantic boreal-Arctic species. The Kiel Bight of the Baltic Sea where Witte and Barthel (1994) conducted their studies is located approximately in the middle of the biogeographical range inhabited by *H. panicea* and is characterized by a mild climate with relatively high annual temperatures and early spring warming. On the contrary the White and Barents Seas regions are the extreme of the distributional ranges of *H. panicea* and these areas are characterized by severe climates with late spring warming. This may cause the delay in reproductive activity of local sponges.

More significant differences can be found if one compares the reproductive timing of different species of *Halichondria*. In *H. bowerbanki* populations inhabiting the Netherlands waters, mature oocytes and embryos were recorded at either of two times, from early August to mid-October (Vethaak *et al.* 1982) or from June to November (Wapstra and van Soest 1987). The latter authors suppose that in its reaction to water temperatures *H. panicea* exhibits a cooler thermal range for existence and reproduction than *H. bowerbanki*, which requires warmer conditions in the Oosterschelde area. The reduced tolerance to lower temperature causes *H. bowerbanki* to reproduce later in the year. It appears that there is little or no geographical variation in the reproductive periods: *H. panicea* invariably breeds within a rising temperature range, *H. bowerbanki* in a stable or decreasing temperature range (Wapstra and van Soest 1987). In the White Sea population of *H. sitchensis* in this study the last stages of gametogenesis and embryogenesis occurred about 8 weeks later in comparison with *H. panicea* of the same region. It may be partially explained by the 2–3 weeks delay of increasing

Table 1: Reproductive periods of *Halichondria* species at different localities.

Species	Region	Spermatogenesis	Previtellogenesis	Vitellogenesis	Embryogenesis	Larvae	Reference
<i>H. panicea</i>	Murman Coast, Barents sea	June–October	late January–October	July–August	July–August	late August–early October	Ivanova, 1981
<i>H. panicea</i>	Oosterschelde area, North sea	June–November	Year-round		May–September	June–September	Wapstra and van Soest, 1987
<i>H. bowerbanki</i>	Oosterschelde area, North sea	June–November	April–November		June–November	July–November	Wapstra and van Soest, 1987
<i>H. panicea</i>	Kiel Bight, Baltic sea	March–May	August–November	November–May	March–May	April–June	Witte and Barthel, 1994
<i>H. panicea</i>	Kandalaksha Bay, White sea	June–July	June–September	June–July	July	late July–early August	present study
<i>H. sitchensis</i>	Kandalaksha Bay, White sea	June–September	June–September	July–September	late August–September	? late September–October	present study

water temperature at 8-12 m depth from which *H. sitiens* was sampled. In comparison the sampling site of *H. panicea* was 3-6 m depth and increasing temperature occurred earlier. An additional more likely reason for the stated difference in reproductive onset between the two species may be the result of the physiological differences between the species. The similar situation was observed by Ereskovsky (2000) in the White Sea populations of *Halisarca dujardini*, *Myxilla incrustans* and *Iophon piceus*. The release of larvae in the former species sampled from the depth range 1,5-5 m took place in July, whereas in two other species collected from 15-25 m the larvae were released in September-October. We propose two explanations for the later reproductive onset of *H. sitiens* compared to *H. panicea*. The first explanation applies to the low tolerance to low temperatures and subsequent delay of the most energy-consuming stage of gametogenesis, i.e. vitellogenesis, until a warmer period, as emphasized by Wapstra and van Soest (1987) for *H. bowerbanki*. The second explanation may concern the fact that the released larvae of *H. sitiens* are probably adapted for a rather narrow temperature range. In contrast to *H. panicea*, *H. sitiens* is a high boreal-Arctic species known to occur as far north as the Greenland and the Kara Sea (Koltun 1966) and is evidently well adapted to severe climatic conditions. The White Sea is situated in the middle of its range and the local climate allows for the extension of gametogenesis, and the delay of larval release until the autumn temperature decrease.

It can be concluded that the differences in reproductive patterns of halichondriid sponges may be caused either by environmental differences within the geographical range occupied by a species, or by physiological distinctions between different taxa.

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