

Growth of a red alga species (*Vertebrata lanosa*) in lab culture

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Research Article

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Abstract

Vertebrata lanosa is an intertidal red algal species that grows epiphytically on *Ascophyllum nodosum*, a brown furoid alga. *V. lanosa* received culinary interest in the recent years due to its truffle-like taste and it is currently only harvested from natural populations. This study has focused on the growth of the species in lab cultures and investigated the temperature and salinity leading to higher specific growth rate. *V. lanosa* showed higher growth rate in 10 °C and 30‰. Overall, the study identified optimal temperature and salinity conditions for indoor controlled cultivation of the species and proved that *Vertebrata lanosa* can be cultivated in absence of its host, *A. nodosum*. Furthermore, a complete life cycle of *V. lanosa* has been carried out in culture where all life history phases and stages were observed. Though, to move from experimental culture to a larger scale production, further research is needed both on the cultivation of the species and the biochemical interactions with its host.

Acknowledgements

Introduction

Macroalgae have been part of human diet for centuries, while the earliest documented traces of this habit lead to China during the fourth century AD (Yang & Brodie, 2017). Much later, consumption of algae began to increase in western countries, mainly because Asian cuisine, especially sushi, has been imported (Rioux et al., 2017), but still the use of macroalgae in diet is much lower in Europe. In 2014, the seaweed (farming) industry was led by Asian countries like China, Indonesia, Japan etc. producing from 100 000 to 1 million tonnes wet biomass each. For the same year European countries, such as France, Spain, Ireland etc. produced less than 1 000 tonnes wet biomass each (Buschmann et al., 2017).

Vertebrata lanosa (Linnaeus) T.A.Christensen is a small obligate epiphyte of the furoid brown alga *Ascophyllum nodosum* (Linnaeus) Le Jolis, and so the two species are commonly associated. However, *V. lanosa* appears to be able to photosynthesize independently, but it has been proved that photosynthetical performance of the species is better when it grows on *A. nodosum* (Garbary et al., 2014). *V. lanosa* is limited to places that its host, *A. nodosum*, is present but the distributions on the two species are not similar, which indicates that other environmental factors affect and limit the epiphyte's occurrence (Fralick & Mathieson, 1975; Garbary & Deckert, 2001). The absence of the species in Skagerrak, where its host is very common, is thought to be caused by the low salinity levels in the area due to the Baltic Current (Åberg, 1992). This is also supported by a study indicating that *V. lanosa* demands a salinity range between 25 and 40‰ to photosynthesize (Fralick & Mathieson, 1975).

The life cycle of *Vertebrata lanosa* is characterized as triphasic diplohaplontic which means there are 3 distinct life history stages, the tetrasporophytes, the gametophytes and the carposporophytes, where some of the stages are diploid (2n) and some are haploid (n). The meiosis (transition from diploid to haploid) in *V. lanosa* happens in the tetrasporangia thus it is called sporic meiosis. This life cycle is also isomorphic as the haploid plants are morphologically similar to the diploid plants (Fig. 1).

Vertebrata lanosa has recently received gastronomical attention in several North Atlantic countries due to its truffle-like taste and Nordic chefs refer to *V. lanosa* as “the truffle of the sea”. As the common truffle mushroom, this alga has a strong taste and aroma which make it perfect for flavoring. Currently, apart from Garbary et al.’s (2014) experiments where the species was shortly held in culture conditions, there are no attempts on cultivating *V. lanosa* as it is thought to be difficult due to the nature of its relationship with *Ascophyllum nodosum*, and hence the species is only harvested from natural populations (Bjorndal et al., 2019).

More and more studies examine the effects of harvesting algal populations. These studies suggest that the harvesting tools, frequency, magnitude and seasonality have an effect on the regrowth of the populations and the harvesting can influence the ecological stability and the survivability of a harvested population (Vasquez, 1995; Foster and Barilotti, 1990; Pringle and Mathieson, 1987). Specifically, on *Vertebrata lanosa* and *Ascophyllum nodosum*, Garbary (2017) supports that the harvesting of *A. nodosum* reduces the abundance of *V. lanosa*. In addition, concerns have been rising globally in regards of the impact of climate change on seaweed abundance, distribution and quality (Straub, Thomsen & Wernberg, 2016), and while some algal groups (e.g. kelps) appear to be more resilient, their biomass availability can vary greatly (Bell et al., 2015; Krumhansl et al., 2016).

As *Vertebrata lanosa* could be in an unfavorable condition if its populations decrease as a result of the harvesting of the species, but also the harvesting of its host species and climate change, it is crucial to find ways to cultivate it. Therefore, the main goal of this study is to investigate the possibility of cultivating *Vertebrata lanosa* in absence of *Ascophyllum nodosum*, and to examine the growth rates and identify which temperature and salinity conditions are the most suitable for cultivating *Vertebrata lanosa*.

Materials And Methods

Stock cultures of *Vertebrata lanosa* were initiated by small branched fragments of tetrasporophytes that were collected and isolated prior to this study from Runde, an island south of Ålesund, Norway (62.4006° N, 5.6242° E) (Fig. 2) and an unialgal culture was established. For the experiments, fragments were taken from the upper parts of the alga and had the same number of branches, meaning the same number of apical areas (approximately 5 per fragment).

The growth medium used was IMR ½ (Eppley et al. 1967) which is a half defined medium based on natural seawater collected from about 40 meters depth and filtered through GF/F filter.

After filtration, the salinity was adjusted by adding distilled water and the new salinity was measured with a refractometer. If the salinity was not at the desired level further adjustments were made, by adding small volumes of filtered seawater and/or distilled water and measured again. When the salinity reached the desired level, nutrient, trace elements and vitamins were added according to the medium’s recipe. The medium was changed every 15 days to ensure that the cultures will not become nutrient depleted.

Small aquariums with air pumps providing air bubbles through silicone tubes and plastic straws, kept the cultures constantly rotating. The opening of the culturing flasks was closed with aluminum foil with a small hole for the straw. The flasks were placed in a crescent in front of a light source in such a distance that the light intensity reaching each flask was around $60 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ and followed a light and dark period of 14 hours light and 10 hours darkness. The culturing system described here is illustrated in Fig. 3.

Growth experiments were conducted in culture rooms with temperature conditions of 6 °C, 10 °C, 12 °C, 16 °C and 19 °C. Initially, for all the five temperature conditions, the salinity level was 30‰ and full nutrients, meaning nutrients were added according to the IMR $\frac{1}{2}$ recipe. The results from this experiment were used to highlight the temperatures that gave the higher growth rates in order to narrow down the temperature range for the salinity experiments. Thus, other salinity levels were tested in only three different temperature levels. In more detail, salinity levels of 20‰ and 10‰ were tested in temperature levels of 10 °C, 12 °C, 16 °C in full nutrients medium.

The specific growth rate (as % FW d^{-1} , SGR) was calculated using the biomass (weight in grams, g) measurements of each fragment at the beginning (W_0) and the end (W_1) of the experiments and the time interval between the measurements (in days, t , approximately 45 days), according to the following equation, as described by Kim et al. (2007):

$$SGR = \frac{\ln W_1 - \ln W_0}{t} * 100$$

For the measurement of the biomass, each fragment was individually pat dried with a lint-free paper towel to remove excess water from the surface of the algae, and then weighed.

For the statistical analysis and the production of illustrations based on the results, R studio was used (R Core, ggplot2 from tidyverse library). For the statistical analysis, Welch two sample t-tests (Alfa: 0.05) were performed between the maximum growth rate and the rest growth rates calculated for each experiment.

Results

During the year-long cumulative time of the experiments and the multiple years of maintaining the stock culture, some of the stock culture individuals, kept in 10 °C, as well as some of the individuals growing in 12 °C and in a medium with a salinity of 30‰, were observed to form tetraspores (Fig. 4a). Later on, the tetraspores had undergone mitotic division and germinated to grow gametophytes. The gametophytes became fertile, which means that the male gametophyte produced clusters of spermatangia and spermatia (Fig. 4b). The spermatia fertilized the female plants which developed carposporophytes and enclosed them in pericarps, eg. They formed cystocarps (Fig. 4c).

In relation to temperature, the highest mean SGR for *Vertebrata lanosa* was found at 10 °C and it was 3.10% FW d⁻¹ (± 0.05, s.e.). In 12 °C the mean SGR was 2.35% FW d⁻¹ (± 0.29, s.e.). For the rest of the temperatures tested this species showed very similar responses in terms of growth rate as the mean SGR found for 6 °C, 16 °C and 19 °C were 1.76% FW d⁻¹ (± 0.47, s.e.), 1.87% FW d⁻¹ (± 0.33, s.e.) and 1.82% FW d⁻¹ (± 0.11, s.e.) respectively (Fig. 5). The difference of SGR between the temperature conditions was significant only between the higher SGR and the lower SGR (p = 0.0026).

In the salinity experiment, the higher mean SGR was found to be 3.10% FW d⁻¹ (± 0.05, s.e.) at 10 °C and with 30‰ salinity. Though, in all temperatures the species had similar SGR when it was growing in medium with close to full-strength salinity (12 °C: meanSGR = 2.90% FW d⁻¹, ± 0.15, s.e. – 16 °C: meanSGR = 2.47% FW d⁻¹, ± 0.49, s.e.). In the lowest salinity tested, 10‰, the species performed better when growing in 12 °C giving a mean SGR equal to 1.28% FW d⁻¹ (± 0.16, s.e.) while in 10 °C and 16 °C the mean SGR was 0.83% FW d⁻¹ (± 0.05, s.e.) and 0.71% FW d⁻¹ (± 0.07, s.e.) respectively (Fig. 6). The difference between the higher SGR and each of the rest was significant in most cases.

Discussion

Vertebrata lanosa received attention in the recent years as a new local product with an appealing taste. The alga is an obligate epiphyte on *Ascophyllum nodosum*, and so it has been thought that it is difficult to cultivate (Bjorndal et al. 2019). As the species is getting more and more gastronomical attention, the demand for it grows. Currently, the species is harvested from natural populations putting a potential pressure to these populations. At the same time, the main host of the species, *A. nodosum*, is also harvested further reducing the abundance of *V. lanosa* (Garbary, 2017). Therefore, exploring the cultivation potentials of *V. lanosa* is crucial. This study, as one of the first attempts of culturing *V. lanosa*, shows that not only the cultivation of the species is possible, but it is also possible to complete the life cycle in culture without the presence of *A. nodosum*.

V. lanosa plants have shown a full life cycle with all three life history stages (tetrasporophyte, gametophytes, carposporophyte) being able to grow and survive in lab culture conditions. All the life history stages observed in this study are consistent with the *Polysiphonia*-type life cycle.

The highest growth rate of the species recorded in this study (3.10% FW d⁻¹, ± 0.05) was found in 10 °C. Though, this growth rate is not significantly different (p > 0.05) than most of the growth rates found in other temperatures (1.76–2.35% FW d⁻¹). The general similarity of the growth rates in the different temperatures is not a surprise as the species is naturally found in the lower intertidal zone with its host *A. nodosum*, where it is exposed to, and tolerates, a large range of different temperatures. The intertidal zone in general is characterized by daily variations in temperature as the water of the intertidal zone is subject to the changes of the air temperature throughout the day-night cycles leading to higher temperatures during the sunlit hours of the day and lower temperatures during the night or the cloudy

hours of the day. Therefore, the general similarity of the growth rates in the different temperatures can be explained, as *V. lanosa* must endure all the variability of this environment when growing in the nature.

Fralick and Mathieson (1975) suggested that *V. lanosa*, shows an optimal temperature range of 22–24 °C for photosynthetic production. Even though experiments were not conducted in such high temperatures, the findings of the present study disagree with this suggestion, as the growth rate ($1.82\% \text{ FW d}^{-1}$, ± 0.11 in 19 °C) recorded in the temperature closer to the suggested range, was significantly lower ($p < 0.05$) than the maximum observed. This disagreement might be caused by the fact that the 22–24 °C suggested range was found and described from a local natural population of *V. lanosa* in the Great Bay Estuary System of New Hampshire (USA). On the other hand, the results of the present study were extracted from lab culture conditions of starting material coming from west coast of Norway, which is further north and on other side of Atlantic Ocean. Thus, any genetic difference between the populations might affect their temperature tolerances and optimal regimes.

In respect of salinity, the species performed better in 30‰ regardless of temperature. The highest growth rate ($3.10\% \text{ FW d}^{-1}$, ± 0.05) was recorded from the combination of this salinity with a temperature of 10 °C. This growth rate was significantly higher ($p < 0.05$) than the growth rates observed in other salinities. This is also supported by the findings of Fralick and Mathieson (1975) who showed that *V. lanosa* has higher photosynthetic rates (measured as apparent photosynthesis) in salinities between 25 and 40‰. Also, in the present study, no significant difference ($p > 0.05$) was found between the highest growth rate and the growth rates found in the other temperatures tested with the same salinity (30‰). Moreover, in salinities lower than 30‰, the species showed better growth when growing in 12 °C rather than 10 °C (where highest growth rate was found for all temperatures in 30‰). This might suggest that salinity plays a stronger role in the growth of the species than temperature.

Despite the achievement of cultivating *Vertebrata lanosa* separately from its host, the growth rates measured during this study are relatively low. Even though it has been suggested that long term photosynthetic activity of *V. lanosa* requires its attachment to *A. nodosum* (Garbary et al., 2014), here the species was successfully maintained in stock cultures for more than a year and continued to be able to supply starting material for the experiments. Thus, it is evident that the species can both grow and reproduce without its host plant. Further studies to investigate the exact biochemical interactions, between the species and its host, and provide an insight as to which *Ascophyllum* metabolites, if any, result in higher growth rates of *V. lanosa*, and how they can be used in tank and indoor cultures of the species, will be of interest.

Declarations

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Competing interests

The authors report there are no competing interests to declare.

Author Contribution

Newt Petride wrote the manuscript and prepared the figures. Stein Fredriksen supervised during the whole process and reviewed the manuscript.

Data Availability

The data are available upon request, along with the R code used to analyze them.

References

1. Aberg, P. (1992). A demographic study of two populations of the seaweed *Ascophyllum nodosum*. *Ecology*, 73(4), 1473–1487.
2. Bell, T. W., Cavanaugh, K. C., Reed, D. C., & Siegel, D. A. (2015). Geographical variability in the controls of giant kelp biomass dynamics. *Journal of Biogeography*, 42(10), 2010–2021.
3. Bjordal, M. V., Jensen, K. H., & Sjøtun, K. (2019). A field study of the edible red alga *Vertebrata lanosa* (Rhodophyta). *Journal of Applied Phycology*, 1–11.
4. Buschmann, A. H., Camus, C., Infante, J., Neori, A., Israel, Á., Hernández-González, M. C., ... Critchley, A. T. (2017). Seaweed production: overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology*, 52(4), 391–406.
5. Eppley, R. W., Holmes, R. W., & Strickland, J. D. (1967). Sinking rates of marine phytoplankton measured with a fluorometer. *Journal of experimental marine biology and ecology*, 1(2), 191–208.
6. Foster, M. S., & Barilotti, D. C. (1990). An approach to determining the ecological effects of seaweed harvesting: a summary. In *Thirteenth International Seaweed Symposium* (pp. 15–16). Springer, Dordrecht.
7. Fralick, R. A., & Mathieson, A. C. (1975). Physiological ecology of four *Polysiphonia* species (Rhodophyta, Ceramiales). *Marine Biology*, 29(1), 29–36.

8. Garbary, D. J. (2017). Harvesting *Ascophyllum nodosum* (Phaeophyceae) reduces the abundance of its host-specific epiphyte *Vertebrata lanosa* (Rhodophyta). *Botanica Marina*, 60(3), 297–301.
9. Garbary, D. J., & Deckert, R. J. (2001). Three part harmony—*Ascophyllum* and its symbionts. In *Symbiosis* (pp. 309–321). Springer, Dordrecht.
10. Garbary, D. J., Miller, A. G., & Scrosati, R. A. (2014). *Ascophyllum nodosum* and its symbionts: XI. The epiphyte *Vertebrata lanosa* performs better photosynthetically when attached to *Ascophyllum* than when alone. *Algae*, 29(4), 321–331.
11. Graham, L. E., Graham, J.M., Wilcox, M. E., & Cook, M. E. (2016) *Algae* (third ed.), LJLM Press, (pp. 15.17–15.21) ISBN: 978-0-9863935-3-2
12. Kim, J. K., Kraemer, G. P., Neefus, C. D., Chung, I. K., & Yarish, C. (2007). Effects of temperature and ammonium on growth, pigment production and nitrogen uptake by four species of *Porphyra* (Bangiales, Rhodophyta) native to the New England coast. *Journal of Applied Phycology*, 19(5), 431.
13. Krumhansl, K. A., Okamoto, D. K., Rassweiler, A., Novak, M., Bolton, J. J., Cavanaugh, K. C., ... Micheli, F. (2016). Global patterns of kelp forest change over the past half-century. *Proceedings of the National Academy of Sciences*, 113(48), 13785–13790.
14. Pringle, J. D., & Mathieson, A. C. (1987). *Chondrus crispus* Stackhouse. Case study of seven seaweed resources. Rome, FAO Fisheries Technical Report, 281, 49–122.
15. Radmer, R. J. (1996). Algal diversity and commercial algal products. *Bioscience*, 46(4), 263–270.
16. Rioux, L. E., Beaulieu, L., & Turgeon, S. L. (2017). Seaweeds: A traditional ingredients for new gastronomic sensation. *Food hydrocolloids*, 68, 255–265.
17. Straub, S. C., Thomsen, M. S., & Wernberg, T. (2016). The dynamic biogeography of the Anthropocene: The speed of recent range shifts in seaweeds. In *Seaweed Phylogeography* (pp. 63–93). Springer, Dordrecht.
18. Vásquez, J. A. (1995). Ecological effects of brown seaweed harvesting. *Botanica Marina*, 38(3), 251–257.
19. White, W. L., & Wilson, P. (2015). World seaweed utilization. In *Seaweed Sustainability* (pp. 7–25). Academic Press.
20. Yang, L. E., Lu, Q. Q., & Brodie, J. (2017). A review of the bladed Bangiales (Rhodophyta) in China: history, culture and taxonomy. *European Journal of Phycology*, 52(3), 251–263.

Figures

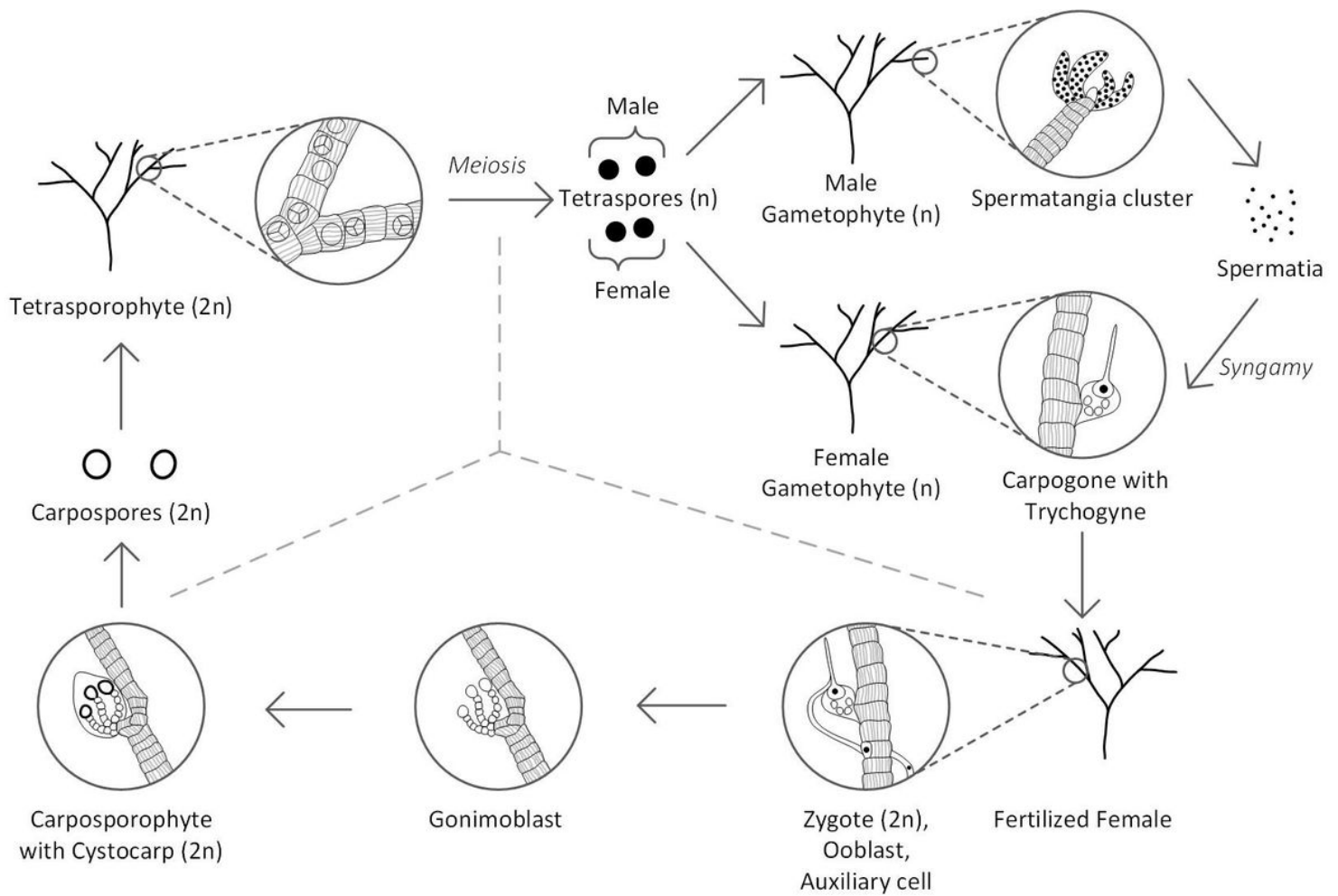


Figure 1

Life history of *Vertebrata lanosa*. The central dashed lines separate the 3 phases on the life cycle (top left: diplontic Tetrasporophytic stage, top right: haplontic gametophytic stage, bottom: developments of diplontic carposporophytic stage on the haplontic female gametophyte)(created by Petride N. using Microsoft Visio).

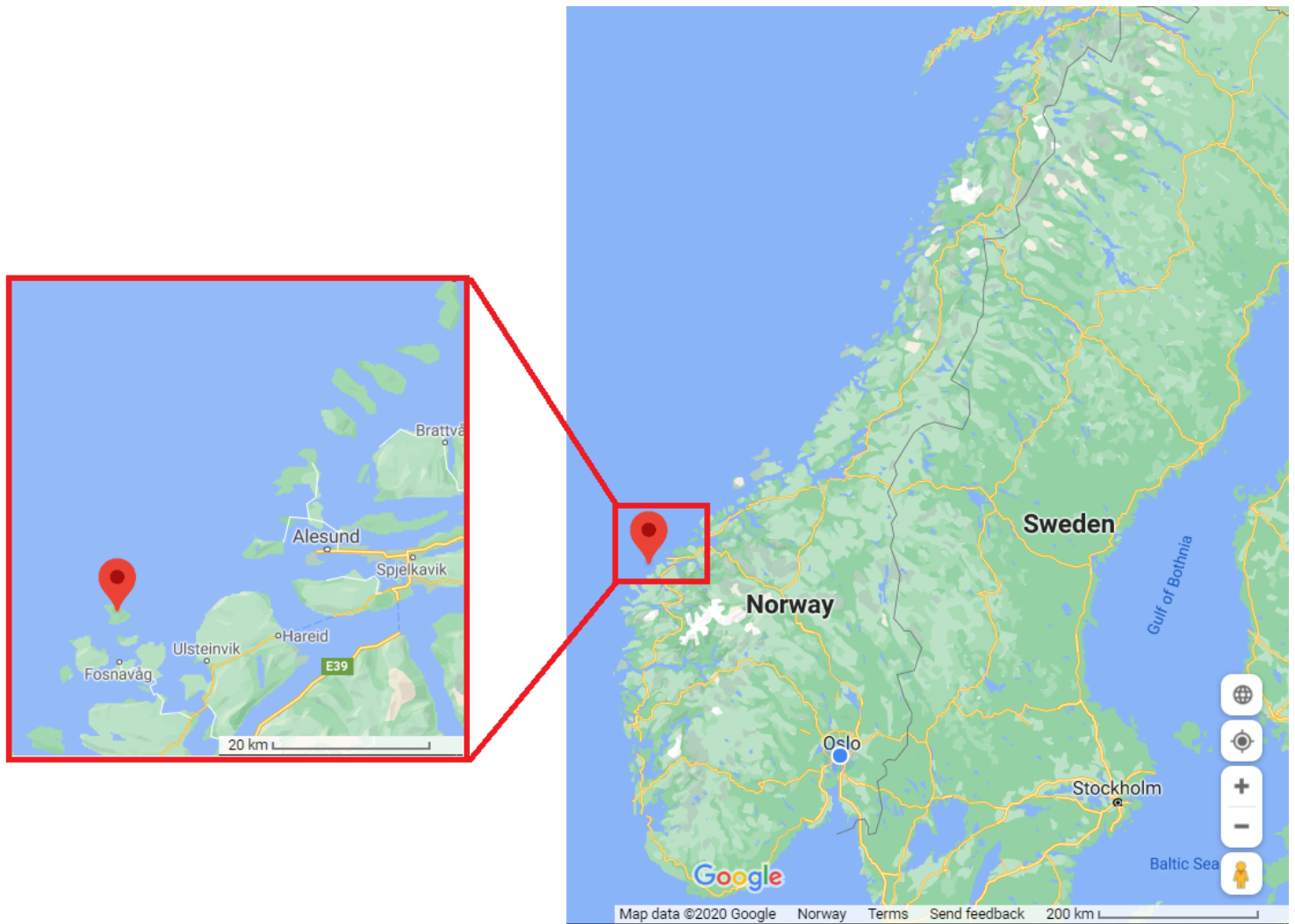


Figure 2

Map of the area where the initial samples of *Vertebrata lanosa* for culture establishment were collected from, Runde, Norway with coordinates 62.4006° N, 5.6242° E (From Google Maps).

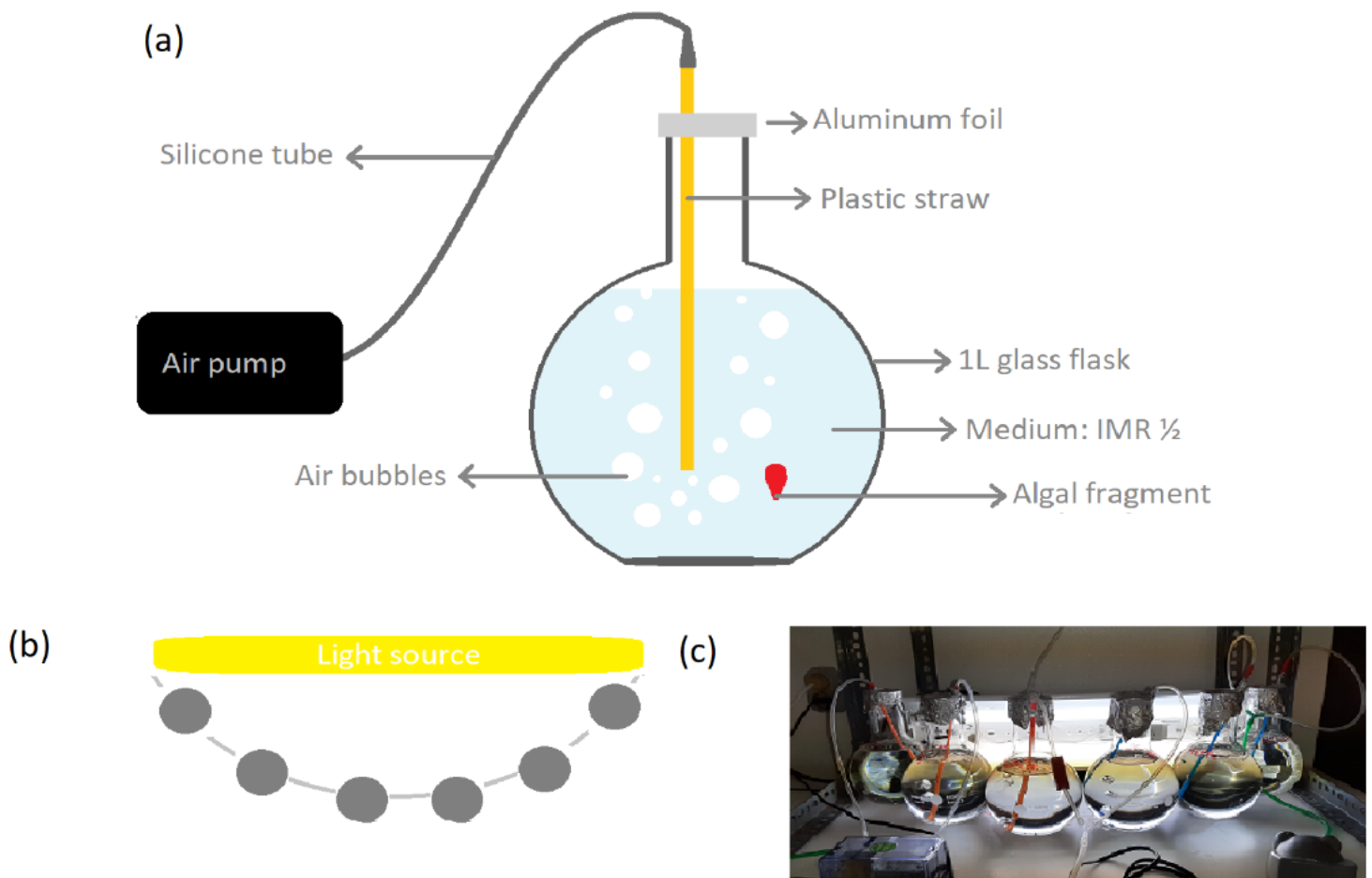


Figure 3

Culturing system: schematic illustration of (a) in-flask culturing system, (b) placement of culture-flasks in front of the light source and (c) photo of culturing system.

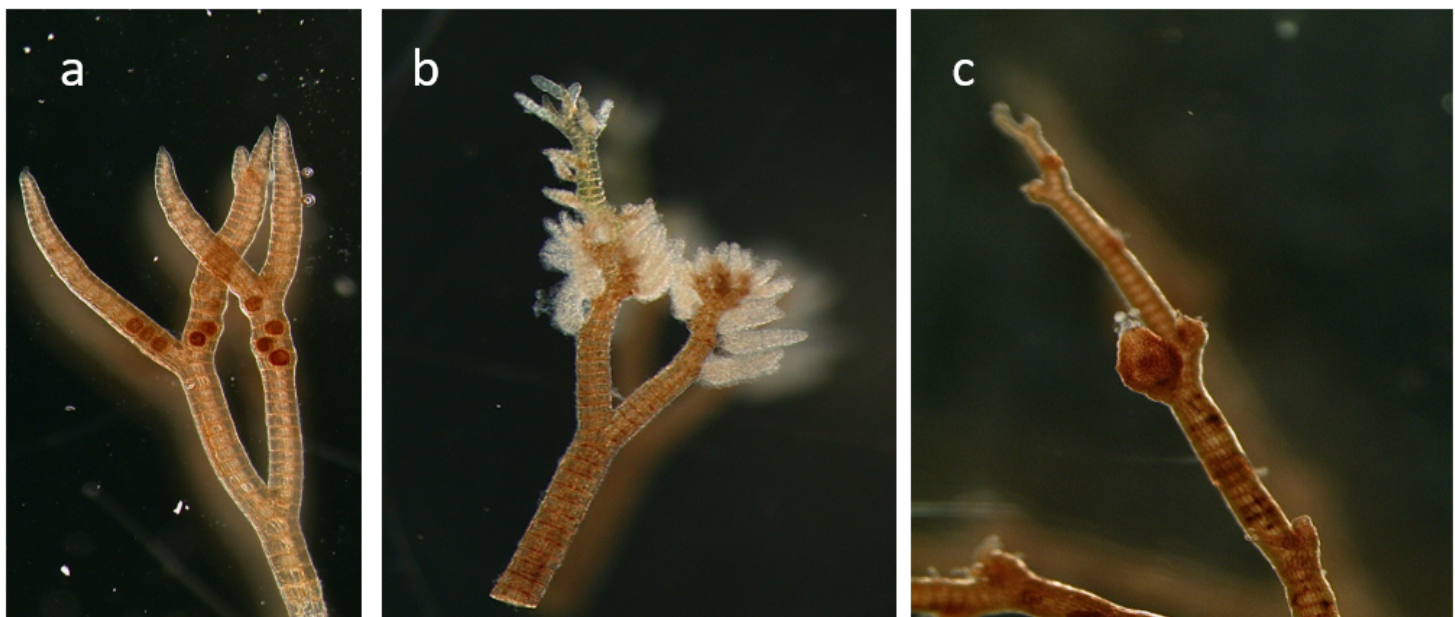


Figure 4

Fertile structures in *Vertebrata lanosa* from lab culture. (a) Tetrasporophyte with tetrasporangia, (b) Male plant with spermatia, (c) Female plant with cystocarp.

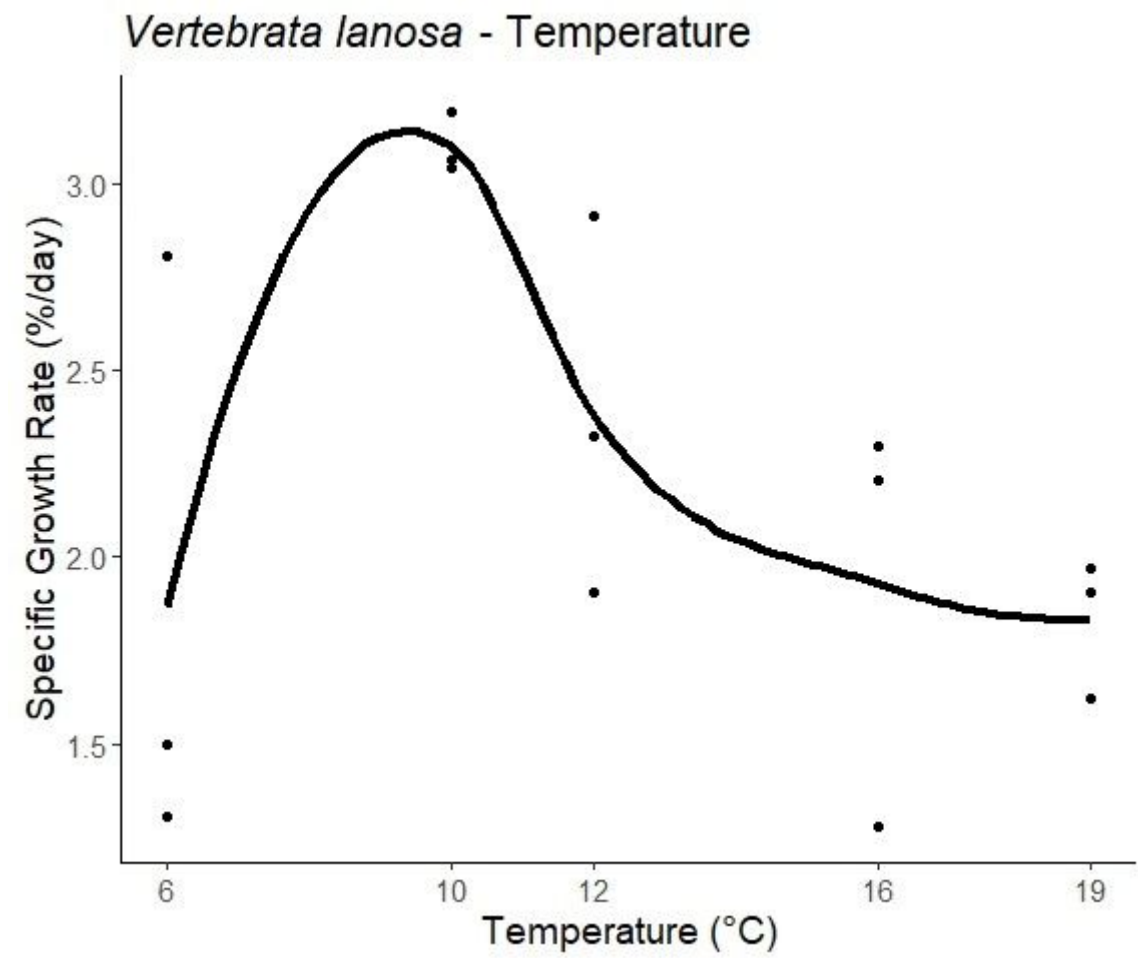


Figure 5

Specific growth rate (SGR) in *Vertebrata lanosa* when growing in different temperatures (6 °C, 10 °C, 12 °C, 16 °C and 19 °C) with a salinity of 30‰. Marked with grey shading is the standard deviation

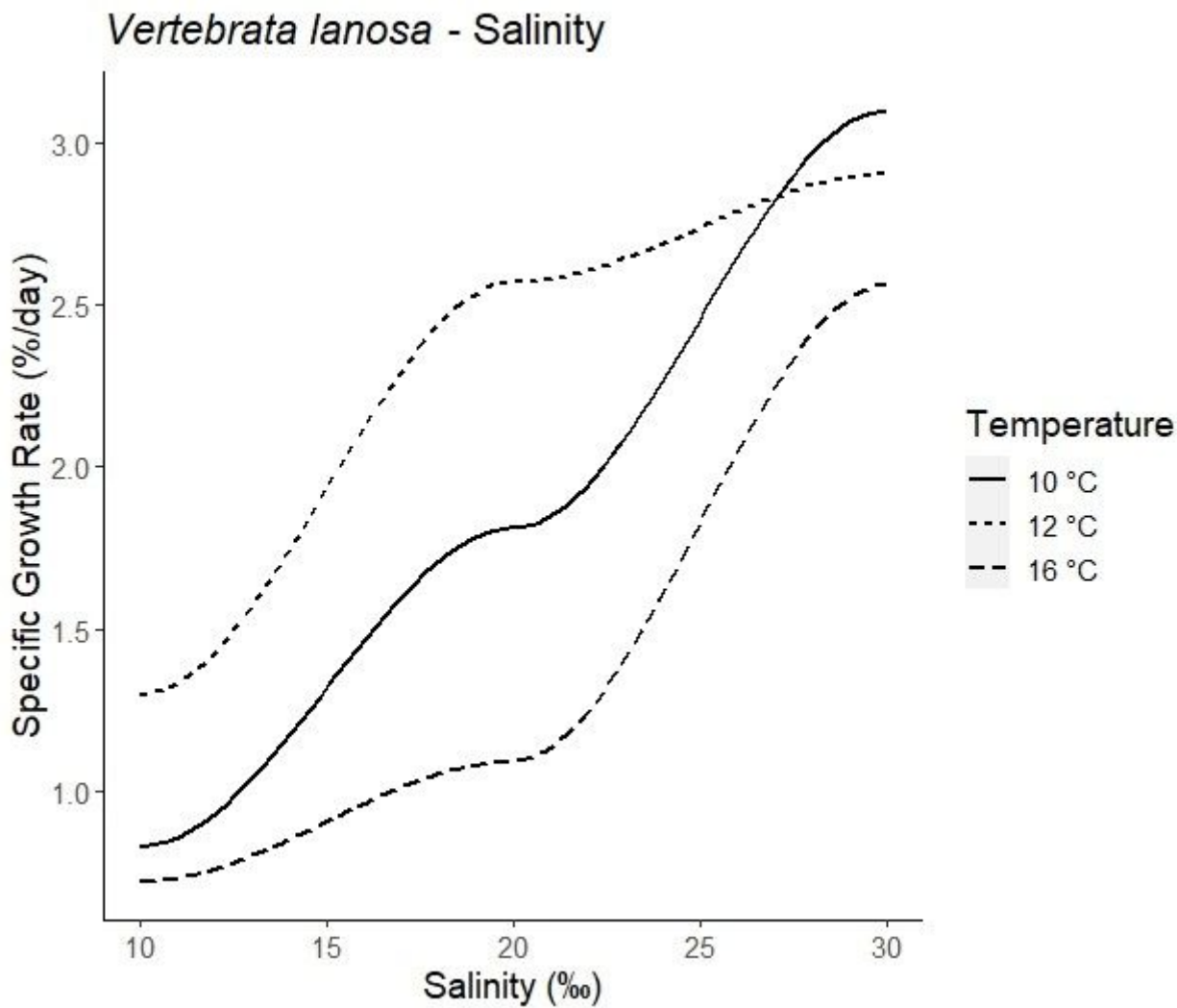


Figure 6

Specific growth rate (SGR) in *Vertebrata lanosa* when growing in different salinity levels, 10‰, 20‰ and 30‰, all tested in three different temperatures (10 °C, 12 °C and 16 °C).