DEPARTMENT OF FUNCTIONAL ECOLOGY
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Interspecific competition of sympatric Artic kelps under environmental influence

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No data can be taken out of this work without prior approval of the Thesis Promoter and Supervisor Dr. Inka Bartsch (Inka.Barstch@awi.de).

I hereby confirm that I have independently composed this Master Thesis and that no other than the indicated aid and sources have been used. This work has not been presented to any other examination board.

Bremerhaven, 5th June 2015 Alberto Daniel Moreno

Executive Summary

Brown kelps of the order Laminariales are the most important habitat structuring macroalgae species along temperate to polar rocky coastal ecosystems, growing in dense forests and supporting different marine communities. At most sites several kelp species co-occur but often one species is dominant. Latitudinal biographic distribution of seaweeds depends on temperature requirements and temperature tolerance for growth and reproduction, while the major factor for determination of depth zonation was found to be the susceptibility of kelp spores to irradiance, especially UV radiation. However, the character and intensity of interspecific competitive interactions, either by using more effectively the available resources or by direct interactions with allelochemicals, are also very important in the process of formation and functioning of any seaweed community.

This study focused on competitive interactions between gametophytes and sporophytes of two cold-temperate kelp species from Kongsfjorden (Spitsbergen): *Alaria esculenta* and *Laminaria digitata*. Two laboratory experiments were performed at three different temperatures (5, 10 and 15°C). Gametophytic and sporophytic development was followed during two months by determining developmental stages (vegetative gametophytes, oogonia, egg cells and sporophytes) under the microscope, measuring sporophytic size and weighing fresh and dry sporophytic biomass.

During this study, it was quantitatively documented a clear interspecific sporophytic resource competition at 5°C, in which *A. esculenta* displaced *L. digitata* when these two kelp species were cultured together under low light condition (12±1 μmol photons/m^2s). In addition, intraspecific sporophytic resource competition was also verified at 5°C, whereas interspecific gametophytic interference competition, probably through allelochemicals, was qualitatively observed.

A. esculenta gametophytes developed faster at 10°C than 5°C, while its gametogenesis was drastically retarded at 15ºC. *L. digitata* gametophytes developed the fastest at 10°C, then 5°C and then 15°C, while its sporophytes developed faster at 15°C than 5°C. Since these kelp species grow differently depending on the temperature, the rising of the sea water temperature may change their distribution, causing new interspecific interactions and competition with other seaweeds that could also influence the marine environment. Moreover, other factors such as life cycle stage, nutrient concentration, temperature and light intensity can regulate the intensity of interspecific competition.

These results demonstrate that interspecific kelp interactions are complex and variable, while not much is known about this topic. Thereby, future multifactorial and field studies are needed to draw more accurately final conclusions.

Abstract

The effects of interspecific competition on *Alaria esculenta* and *Laminaria digitata*, two cold-temperate kelp species from Kongsfjorden (Spitsbergen), have been investigated at three different temperatures. Sporophytic resource competition between these species was documented at 5°C, in which *A. esculenta* displaced *L. digitata* when the two kelp species were culture together under low light condition (12±1 μmol photons/m^2s). In addition, intraspecific sporophytic resource competition was also verified at 5°C when the species were cultured singly, whereas interspecific gametophytic competition, probably through allelochemicals, was qualitatively observed. *A. esculenta* gametophytes developed faster at 10°C than 5°C, while the development was retarded at 15°C. *L. digitata* gametophytes developed the fastest at 10°C, while 5°C was faster than 15°C.

Key words: *Alaria esculenta*, *Laminaria digitata*, interspecific competition, gametophytes, sporophytes, temperature effects, Kongsfjord.

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2. Introduction

2.1 Investigated organism

Brown kelps of the order Laminariales are major habitat structuring macroalgae species along temperate to polar rocky coastal ecosystems, especially in the northern hemisphere (Kain 1962; Bold & Wynne, 1985; Lüning 1990; Müller *et al.,* 2008). They grow in dense forests and represent a carbon sink through huge production of biomass up to 10kg fresh weight/m^2 (Wiencke *et al.*, 2011).

A shift or loss of kelp forests would severely affect coastal ecosystems, as they support different marine communities being nurseries for many animals, providing food for herbivores, a physical structure for shelter and protection from predators (Schultze *et al.,* 1990; Roleda *et al.*, 2007). Furthermore, they are also economically important, since they are consumed as food and cultivated for cosmetic products and fertilizers, especially in western pacific regions (Bold & Wynne, 1985). To enhance the economic success of kelp aquaculture, *Laminaria* could be cultivated in areas near coastal sewage outfalls, increasing its growth and acting as nutrient sinks (Yarish *et al.*, 1990). Nevertheless, eutrophication, sedimentation, invasion of foreign species and the global warming are threatening the abundance of kelp beds in artic and temperate waters worldwide (Givernaud *et al.*, 1991; Cosson, 1999; Moy *et al.*, 2003).

Latitudinal biographic distribution of seaweeds depends on temperature requirements for growth and reproduction, as well as on the temperature tolerance of life cycle stages (Breeman, 1988; tom Dieck, 1993; Wiencke *et al.*, 1994), especially in reproductive cells as they are more vulnerable to changes compared to other stages (van den Hoek, 1982; Coelho *et al.*, 2000). However, the main factor that determines depth zonation of seaweeds was found to be the susceptibility of kelp spores to irradiance, especially UV radiation (Hanelt, 1998; Roleda *et al.*, 2005; Wiencke *et al.*, 2006; Müller *et al.*, 2008).

At most sites several kelp species co-occur but often one species is dominant and there is a clear depth zonation. In the cold-temperate North Atlantic we find 4 species of Laminariales: *Alaria esculenta, Laminaria hyperborea*, *Laminaria digitata* and *Saccharina latissima*. The endemic

Laminaria solidungula can also be found in the Arctic and along southern European shorelines we additionally find the warm-temperate kelp *Laminaria ochroleuca* (Kain, 1979).

In this thesis, two cold-temperate kelp species from the Kongsfjord (western Spitsbergen) were utilized: *Alaria esculenta* and *Laminaria digitata* (Fig. 1). They are not restricted to Arctic regions, but have a broad latitudinal distribution range, being Brittany (France) their Southern boundary (Lüning & tom Dieck, 1990; Lüning, 1990; www.seaweed.ie).

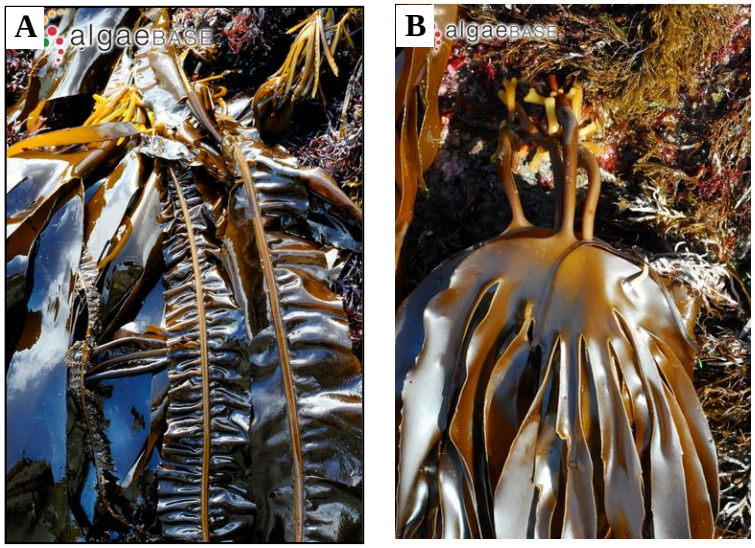

Figure 1. Mature sporophytes of the kelps used in this study.
A: *Alaria esculenta*, B: *Laminaria digitata.* (Source: algaebase.org)

The Laminariales have a complex heteromorphic, diplohaplontic life cycle (Fig. 2), consisting of diploid sporophytes up to several meters length and microscopic haploid gametophytes. Sporangia are formed in areas called sori on the blades of the diploid sporophytes (Kain, 1979; Bold & Wynne, 1985). Meiosis takes place within these sporangia and leads to the formation of microscopic, haploid zoospores of 4-8µm size (Bartsch *et al.*, 2008) which are released and dispersed by currents (Dayton, 1985). Male and female gametophytes develop out of the spores and during their gametogenesis produce antheridia and oogonia, respectively. Antheridia release spermatozoids and the oogonia release egg cells. After fertilization, the diploid zygote is formed and develops into a macroscopic sporophyte (Dayton, 1985). Furthermore, unfertilized egg cells may develop into haploid parthenosporophytes (Bartsch *et al.*, 2008).

Parthenogenesis in kelps was first described by Schreiber (1930), but he only reported abnormal morphologies of small parthenosporophytes. Later studies revealed that in some cases adult,

fertile parthenosporophytes with normal morphology may develop (e.g.: *Laminaria japonica*: Fang *et al.*, 1978; Lewis *et al.*, 1993; Bai & Qin, 1998; *L. saccharina*: Ar Gall *et al.*, 1996).

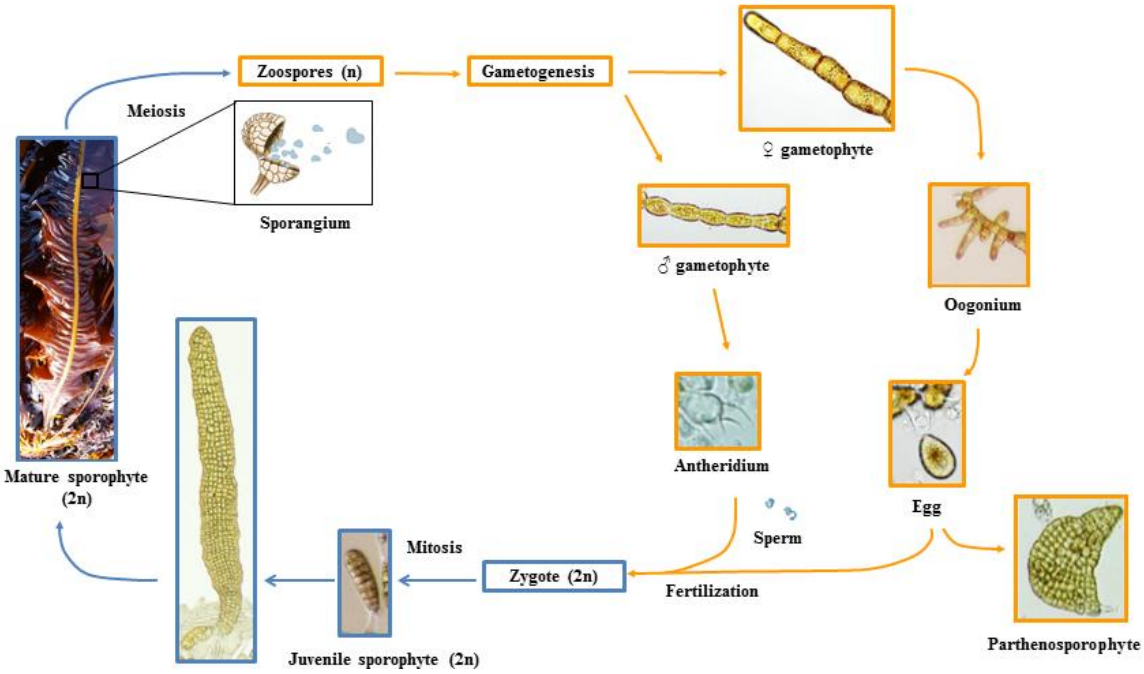

Figure 2. Life cycle of *Laminaria*. Blue: diploid phase, Orange: haploid phase. Pictures were taken with the inverse and Axiophot microscopes throughout the experiments. Source mature sporophyte picture: algaebase.org

Just after their release the egg cells of the female gametophytes secrete pheromones that induce sperm release from antheridia and the chemotactic orientation of sperm towards the egg (Maier *et al*., 2001). The main pheromone in egg secretions which plays an important role for fertilization of all Laminariales species is lamoxirene (cis-2-cyclohepta-2', 5'-dienyl-3-vinyloxirane, Fig. 3) (Lüning, 1981; Boland, 1987; Hertweck & Boland, 1997).

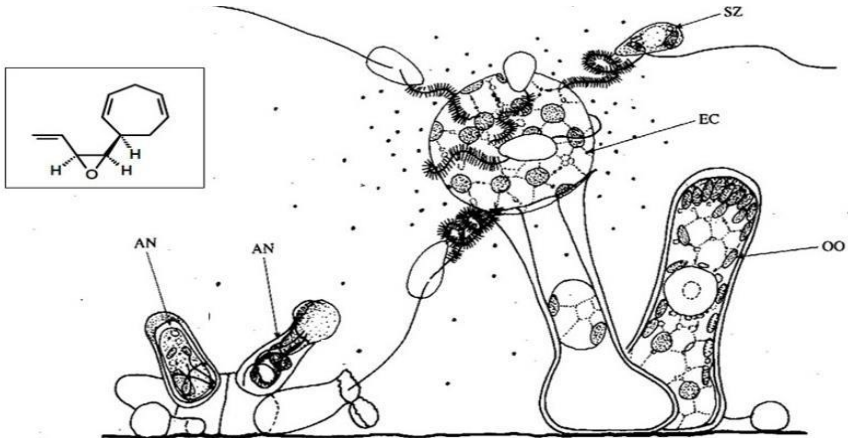

Figure 3. Pheromonal interaction during sexual reproduction. Chemical formula of lamoxirene is shown in the box on the left. AN= antheridium, EC = egg cell, OO = oogonium, SZ = spermatozoid. Source: van den Hoek (1995).

2.2 Study site

The species used for this thesis were sampled at the Kongsfjord on Spitsbergen, Svalbard (Fig. 4). The glacial fjord is located on the west coast of Spitsbergen between 78°40'-77°30'N and 11°3'-13°6'E. It is 20km long and 4-10km wide (Svendsen *et al.*, 2002). It is influenced by the warm Atlantic current that brings warm and salty water masses into the West Spitsbergen current in different amounts, depending on climatic variability. It is expected that global warming will increase the influence of Atlantic water masses (Hop *et al.*, 2002). Furthermore, the Kongsfjord is influenced by melting glaciers. Both effects make this a suitable site for observing the impacts of climatic change.

A total of 62 macroalgae species were present at Kongsfjorden between 1996 and 1998 until a maximum depth of 30m (Hop *et al*., 2012). According to Hop *et al*. (2012) and Bartsch *et al*. (2015), *A. esculenta* and *L. digitata* co-occur in the shallow sublittoral at a depth of 2.5m and 5m. *L. digitata* is more abundant than *A. esculenta* at 2.5m, whereas the leaf area of the former is considerably reduced at 5m (Bartsch *et al*., 2015). At 10m *A. esculenta* is more abundant as only *L. digitata* juvenile recruit stages are present at this depth.

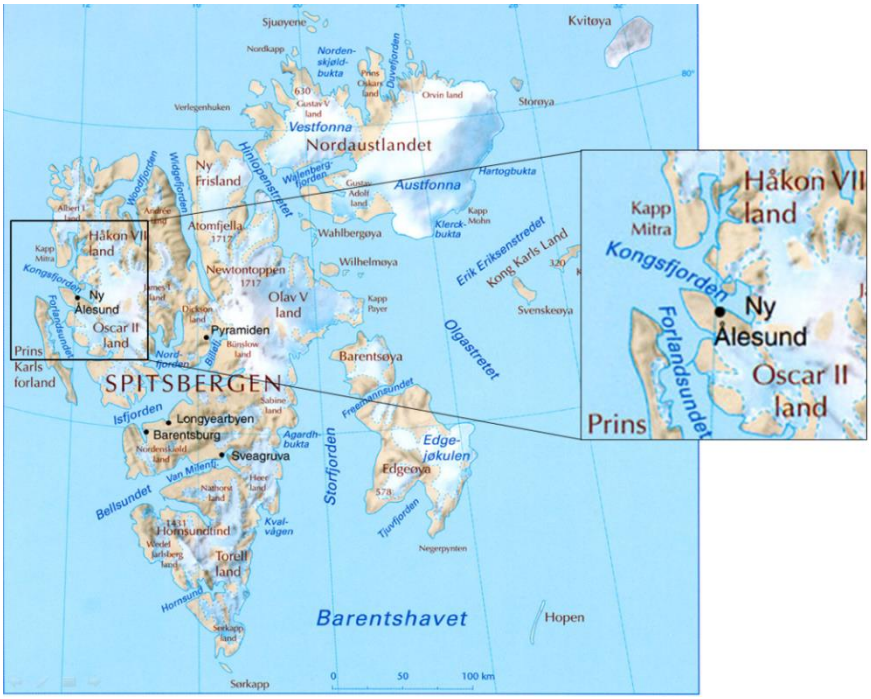

Figure 4. Svalbard archipelago and indication of Kongsfjorden. (Source: www.arctic05.org, magnification added).

2.3 Climate change

Changes in species composition of flora and fauna around the Spitsbergen due to climate change, concretely owing to temperature increase, are already reported (e.g. Beuchel *et al*., 2006; Weslawski *et al*., 2010; Kortsch *et al*., 2012). Currently, the sea ice is thinning and disappearing, resulting in new habitats available for seaweed colonization (Gerland and Renner, 2007).

For instance, as presented by Bartsch *et al.* (2015), the overall seaweed biomass in the Kongsfjorden has almost doubled (1.7x fold) between 1996/98 and 2012/14, while the seaweed biomass at a shallow depth of 2.5m has multiplied by 4.7x. Furthermore, a decrease in the depth limit by 2 to 5 m of most kelp has been documented, except *Alaria esculenta* which extended its depth range by 3 m (Bartsch *et al*., 2015).

Other models predict a retreat of the arctic-endemic species *L. solidungula* further north owing to the rise in sea water temperature. The southern distribution limit of this species is limited by a sea-surface summer temperature of 5-6°C (Müller *et al.*, 2009), which might turn sites like Kongsfjorden into inadequate locations for its growth by the end of this century.

Generally, the community structure of species within the fjord is strongly influenced by environmental factors and is very likely to change which may have consequences for the whole polar food-web. These changes in the community structure, with retreat and arrival of different algae species, may cause new interspecific interactions and competition that could also influence the marine environment of the Kongsfjorden.

2.4 Algae competition

As explain before (see 2.1), abiotic factors such as temperature and UV radiation, shape latitudinal and depth distribution of seaweeds. However, this might not explain the full situation in the Kongsfjorden since the two studied kelp species co-occur in the shallow sublittoral although they have different susceptibility towards UV radiation (Wiencke *et al*., 2006) and temperature (Lüning, 1980). Thus, besides the primary abiotic factors, also biotic factors such as interspecific competition may act on different levels (spores, gametophytes and/or sporophytes).

Competition is described as one of the primary biotic factors that contour patterns of distribution, abundance and diversity in ecological communities (Begon *et al*., 2006). It is already known a lot about marine animal-plant interactions (e.g. Toth & Pavia, 2007; Jormalainen & Honkanen, 2008; Molis *et al*., 2008) but not very much about plant-plant interactions in the marine environment.

According to Pain (1990) and Melville & Connel (2001), the character and intensity of interspecific competitive interactions are very important in the process of formation and functioning of any seaweed community, which are generally dominated by species that have the highest competitive ability under given conditions (Carpeter, 1990).

Kelp species occupying the same habitat, although with different abundances and depth distributions, have similar life cycle characteristics (Kain, 1979) and their time of spore release may overlap, revealing competition processes among the microscopic and macroscopic life cycle stages (Nabivailo *et al*., 2014). Thus, it is possible that the two investigated kelp species affect each other since they are both fertile between July and September (Olischläger & Wiencke, 2013) (Table 1).

Table 1. Period of zoospore release in the two investigated kelp species occurring in the Kongsfjord. Source: Olischläger & Wiencke (2013).

Species	**Time of fertility**
A. esculenta	June - September
L. digitata	Late July - September

As shown by Bartsch *et al*. (2008) and Nabivailo *et al*., (2014), interspecific competition may be divided into ‘resource competition’ and ‘interference competition’:

Resource competition between photosynthetic organisms is considered to occur mainly for space, light and nutrients (Bartsch *et al.*, 2008). Those species with the ability to use the scarce resources quickly and effectively would make them unavailable for their competitors (Nabivailo *et al*., 2014). For example, sporophytes from the faster species could shade the competitor and therefore reduce the amount of light available for the latter.

Alternatively, interference competition tackles more direct interactions, such as effects of allelochemicals on other species, influencing the competitor's physiological processes via allelopathy (Olson & Lubchenco, 1990; Xu *et al.*, 2013). As an example, male kelp gametophytes belonging to the Laminariales, react on the pheromone lamoxirene (Fig. 3), secreted by the released eggs as attractant for male sperm (van den Hoek *et al.*, 1995; Bartsch *et al.*, 2008; Müller *et al.*, 2009). Theoretically, since male gametophytes ripen faster than female oogonia, the extruded eggs of the fastest species may induce the release of male sperm from the same but also from the sympatric species, thereby reducing the fertilization success of the competitor.

2.5 Experiments

In order to clarify possible positive or negative interactions between sympatric kelp species, we performed two laboratory experiments in which gametophyte and young sporophyte development was monitored at different temperatures to reveal competition between the sexual stages of gametophytes and between juvenile sporophytes of two kelp species isolated from Spitsbergen.

Besides some recent studies performed with seaweeds (e.g. Xu *et al*., 2013; Nabivailo *et al*., 2014; Bernard, 2014; Bollen *et al*., 2015), not much experimental work has been conducted on this subject, especially not on the interspecific competition and interactions between kelp species. Nevertheless, there is a lot of information available about the tolerance limits of single kelp species, especially to temperature or irradiance that can be applied to this research (Lüning, 1990; Bartsch *et al*., 2008; Müller *et al.*, 2009).

In similar previous studies, positive interactions though allelopathic substances were observed by Xu *et al*. (2013) between the green macroalgae *Monostroma arctium* and the red macroalgae *Porphyra yezoensis*, in which *M. arctium* facilitated nutrient uptake of *P. yezoensis*. Furthermore, Bollen *et al*. (2015) documented an enhancement in oogonia formation by 5.8% in the kelp species *Undaria pinnatifida* when co-cultured with *Macrocystis pyrifera*, indicating a competitive advantage for *U. pinnatifada*. Alternatively, Nabivailo *et al*. (2014) described

positive (stimulation of growth and photosynthesis) but also negative (resource competition and allelopathic inhibition) interactions between common species of an *Ahnfeltia* bed community.

Thus, this is one of the first studies on interspecific competition between sympatric kelp species at different temperatures. The temperatures tested were 5°C (current average summer temperature in the Kongsfjord; Svendsen *et al*., 2002), 10°C (optimum temperature for *A. esculenta* and *L. digitata*; Lüning, 1980) and 15°C (approximate mean summer sea-surface temperature at southern distribution limit of *L. digitata* and *A. esculenta* in Brittany; Lüning & tom Dieck, 1990; Müller *et al.* 2009).

Two experiments were performed during this study. The first one aimed to examine interspecific and sex interactions during gametogenis mainly via allelopathy (e.g. pheromone lamoxinere, see 2.4: interference competition) at 10°C, while the second experiment was directed to general interspecific competition at two temperatures (5 and 15°C). In both experiments *A. esculenta* and *L. digitata* were cultured singly (single species treatments) and together (mixed treatment), being in the latter where competition may possibly take place while the former is used as control. Interspecific interactions were tested by comparing the kelp development in the single species and mixed cultures. Thus, if there is no interaction, the developmental stage percentages of the mixed treatment should be an average of both single species treatments. If the mixed treatment is significantly different to the mean percentage of both single treatments, an interspecific interaction has taken place.

Therefore, the specific objectives on this thesis are: (a) to investigate interspecific and sex interactions of the two kelp species *Laminaria digitata* and *Alaria esculenta* from an Arctic site, monitoring their gametophyte fertility and sporophyte growth at three different temperatures and (b) to explore temperature responses of these processes for the two species and get some ideas of how changing temperatures may influence the interaction between polar kelp communities in future.

We hypothesized that (a) there is an interaction during the fertilization process and/or sporophyte development when both species are cultured together and (b) species gametophyte and sporophyte development and their interaction is exhibited differently at each temperature.

3. Materials and methods

3.1 Algal material

The experiments were performed with gametophytes and sporophytes of two different sympatric North Atlantic kelp species: *Alaria esculenta* and *Laminaria digitata*. These kelp species were originally sampled at Kongsfjorden, Spitsbergen, and are available in the seaweed culture collection at the AWI. The stock cultures were maintained in an unfertile vegetative stage, in Provasoli enriched seawater (PES) (Provasoli, 1968; see annexes Fig. 38), at a constant temperature of 10ºC and under low light conditions with a daily cycle of 16h of light, followed by 8h of darkness, while sea water was changed once per month. Stock cultures of each species, sex and strain, were cultured separately in different beakers (Table 2). During the experiments, the stock solutions used for the male and female *Alaria esculenta* treatments were a mixture of 4 strains, taken approximately the same amount of gametophytes (¼) from each one.

Table 2. Culture numbers of the stock cultures from the AWI seaweed collection. Both species were originally sampled in Kongsfjorden, Spitzbergen.

Species	Sex	Culture number (strain)
A. esculenta	♂	3405, 3413, 3415, 3417
	♀	3406, 3414, 3416, 3418
L. digitata	♂	3200
	♀	3199

3.2 Experimental conditions

Sea water was sterile filtered through a 0.22µm filter (Millipore Durapore Cartridge, Jaffrey, NH USA) and additionally cooked at 100ºC for 4 hours. For all the experiments performed, sterile Provasoli enriched seawater (PES) was used, diluting 200 ml of Provasoli nutrient solution in 10L of filtered seawater.

The temperature and light intensity conditions for the experiments were performed in two walk-in culture rooms. These rooms included cooling systems, fluorescent lamps, light cycle controllers and air supply. The temperatures selected for the experiments were 5±0.75°C,

10±0.25°C and 15±0.25ºC. The lower temperature represents the sea-surface summer temperature in the Konfjorden (Svendsen *et al.*, 2002), 10°C is the optimum temperature for *A. esculenta* and *L. digitata* (Lüning, 1980) and 15ºC the southern distribution limit of *Alaria esculenta* along the coast of Britany, France (Lüning & tom Dieck, 1990; Müller *et al.*, 2009).

In both experiments the light intensity was 12±1 μmol photons/m^2s, measured with a LI-COR LI-185B Photometer (LI-COR, Inc., Lincoln, USA) and generated by fluorescent lamps of the type OSRAM L36W/965 (Biolux, München, Germany), with a daily cycle of 20h of light, followed by 4h of darkness. This 12±1 μmol photons/m^2s intensity and the 20/4h daily cycle, represents respectively the light intensity and day length that kelp species hold during the artic summer below the kelp canopy. Certainly, the day length in arctic summer is 24/0h, but since it has very low values overnight, it was represented as darkness in this study.

3.3 Microscopes and scaling

Three types of microscopes were used during this investigation, all holding a net micrometre. Before start of the experiments the objectives were scaled for each magnification utilizing a measuring slide (See annexes Tables 4, 5, 6). The microscopes used were: an inverted microscope Olympus CKX41 (Japan), a microscope Axiophot Ilford FP4 135 DX 36 (Germany) and a stereo microscope Olympus SZX10 (Japan).

3.4 Experimental gametophyte stock cultures and initial control

Before starting the experiment, with the purpose to avoid future misleading results and guarantee equal initial characteristics and abundance of gametophyte fragments in every treatment of the experiments, the preliminary condition in the stock cultures and treatments (cell size, fragment length, number of cells per fragment and density) was controlled.

3.4.1 Preliminary condition

First of all, the initial condition of the fragments was observed using the inverted microscope. Their developmental stage was qualitatively recorded to ensure that the majority of the fragments from the different species, sexes and strains were in a vegetative phase.

3.4.2 Cell Size

Secondly, the cell size was measured in mounted slides from the stock cultures using the Axiophot microscope with a scaled objective (Pl 10x/20, Carl Zeiss Microscopy GMBH, Germany) and 40x fold magnification. Thus, 50 cells from each species and gender were measured, making a total of 200 measurements.

3.4.3 Experimental Stock Solution and Control of Fragment Length and Cell Numbers

Male (♂) and female (♀) vegetative gametophyte clusters of *Alaria esculenta* and *Laminaria digitata* (Table 2) were separately taken with an Eppendorf Pipette and placed in a sterile mortar with a tad of sterile seawater. Then, they were carefully ground into few-celled fragments with a pestle. The resulting suspension was sieved through a sterile 100µm sieve. The filtrated material was sieved again through a sterile 63µm filter. The remains on the 63µm filter were then rinsed and diluted with 150ml of PES to gain an experimental stock solution for the experiments. The length of 40 fragments from each replicate of every treatment was measured using the Axiophot microscope with a scaled objective to ensure similarity among fragments. Consequently, the number of cells per fragment was calculated dividing the fragment length by the cell size.

3.4.4 Density

Finally, to determine the density of the 4 stock solutions, 1ml of each suspended solution was poured using an Eppendorf (1000) pipette into small petri dishes to quantify the number of fragments per cm^2. This was carried out using the inverted microscope with a net micrometer objective (No.464027, Carl Zeiss Microscopy GMBH, Germany) and a counter (Assistent 345 Counter AC-8). Thus, 300 fragments and their respective grids (nets) were counted for each of the sexes within each species. This allowed calculating the volume needed from each single sex

stock solution necessary to get the same gametophyte concentration in each treatment replicate of the experiments.

3.5 Sowing procedure

Densities from each single-sex stock culture were calculated. Thus, the precise volume needed to achieve the same density (300-400 fragments/cm^2) in every treatment was determined for each single-sex stock: *Alaria esculenta* ♂ *(Aesc♂)*, *Alaria esculenta* ♀ *(Aesc♀)*, *Laminaria digitata* ♂ *(Ldig♂)* and *Laminaria digitata* ♀ *(Ldig♀)*. Utilizing a Color Squid IKAMAG magnetic stirrer (Germany) the gametophytes were homogeneously suspended. Afterwards, the calculated volume needed was added to replicate petri dishes which had already been filled with a defined volume of PES.

In experiment 1, each replicate consisted of a small plastic petri dish (∅=5.3cm), pre-filled with 12ml PES and holding 3 crystal cover slips. In experiment 2, each replicate consisted of a small plastic petri dish (∅=5.3cm) pre-filled with 12ml PES and, simultaneously, a big crystal petri dish (∅=7.5cm) prefilled with 50ml PES and holding 5 glass slides (2.1x2.6 cm) to serve as a substratum. The big crystal petri dish was exactly twice as big as the small plastic petri dish, and therefore the double volume of suspended gametophytes was used.

Table 3. Abbreviations used for the treatments in experiments 1 and 2.

Treatments	Experiment	Coding
Single-sex	1	*Aesc♂*
	1	*Aesc♀*
	1	*Ldig♂*
	1	*Ldig♀*
Same-sex-two-species	1	*Aesc♂ x Ldig♂*
	1	*Aesc♀ x Ldig♀*
Different sex-two-species	1	*Aesc♂ x Ldig♀*
	1	*Aesc♀ x Ldig♂.*
Different-sex-one-species	1 and 2	*Aesc*
	1 and 2	*Ldig*
Fully-mixed	1 and 2	*Aesc x Ldig*

3.6 Experiment 1: Interactions between sexes and species

Three replicates in each treatment with kelp gametophytes of *Alaria esculenta* and *Laminaria digitata* were cultured at 10°C±0.25°C (optimum temperature for both species) in a one-factorial design (fixed factor 'Species') to observe interactions between the female and male sexes of single species or between the sexes within or between two species. Unisex treatments served as control. Thus, gametogenesis of 11 different treatments was observed with all possible combinations of sex and species, singly or combined (Fig.5), forming the following set of treatments:

- 4 single-sex treatments (*Aesc♂, Aesc♀, Ldig♂* and *Ldig♀)*.
- 2 same-sex-two-species treatments (*Aesc♂ x Ldig♂* and *Aesc♀ x Ldig♀)*.
- 2 different sex-two-species treatments (*Aesc♂ x Ldig♀* and *Ldig♂ x Aesc♀).*
- 2 different-sex-one-species treatments (*Aesc♂ x Aesc♀* and *Ldig♂ x Ldig♀)*.
- 1 fully-mixed treatment (*Aesc♂ x Aesc♀ x Ldig♂ x Ldig♀)*.

The abbreviations utilized for the treatments are summarized in Table 3.

Each replicate hold 3 cover slips for later microscopic examination after fragments had attached. All Petri dishes were sealed with Menasha parafilm (USA) and exposed to the experimental conditions directly after preparation.

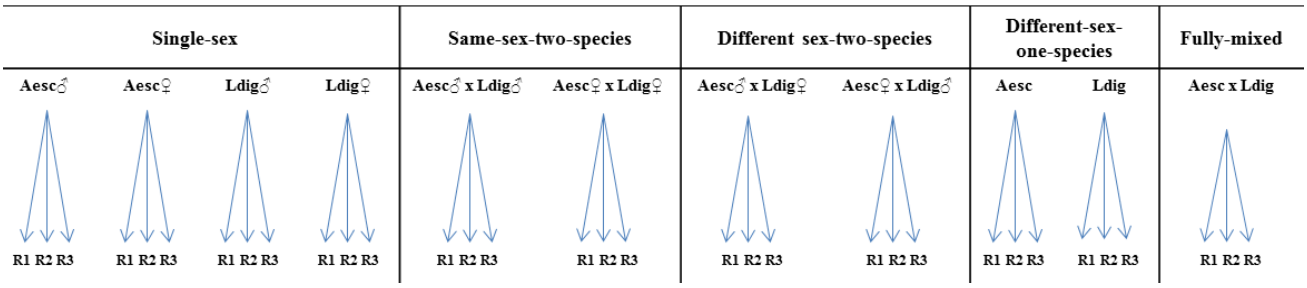

Figure 5. Scheme of the interaction experiment between sexes and species of *A. esculenta (Aesc)* and *L. digitata (Ldig).*

Development of all the cultures was followed by checking the petri dishes using the inverted microscope and documenting with a Canon EOS 550D reflex camera (Canon, Tokyo, Japan)

attached to the microscope. The following developmental stages were counted at day 0, 5, 9 and 14 (Fig. 6):

- Vegetative gametophytes with or without oogonia.
- Released eggs.
- Juvenile microscopic sporophytes.

Each time 300 fragments were counted in each replicate of every treatment using a counter. Male developmental stages (vegetative, antheridium) (Fig. 7) were quantified only on Day 0. For each fragment the furthest developed stage was recorded. Female fragments were counted as sporophytes as soon as the first egg-cell division was visible. Eggs and sporophytes had to be attached to a fragment, otherwise they were not taken into account. A net micrometre objective (No.464027, Carl Zeiss Microscopy GMBH, Germany) was used for counting. The number of fragments within each grid (net) was recorded, moving the grid each time, until 300 fragments were recorded. The number of counted nets was documented to calculate density (fragments/cm²).

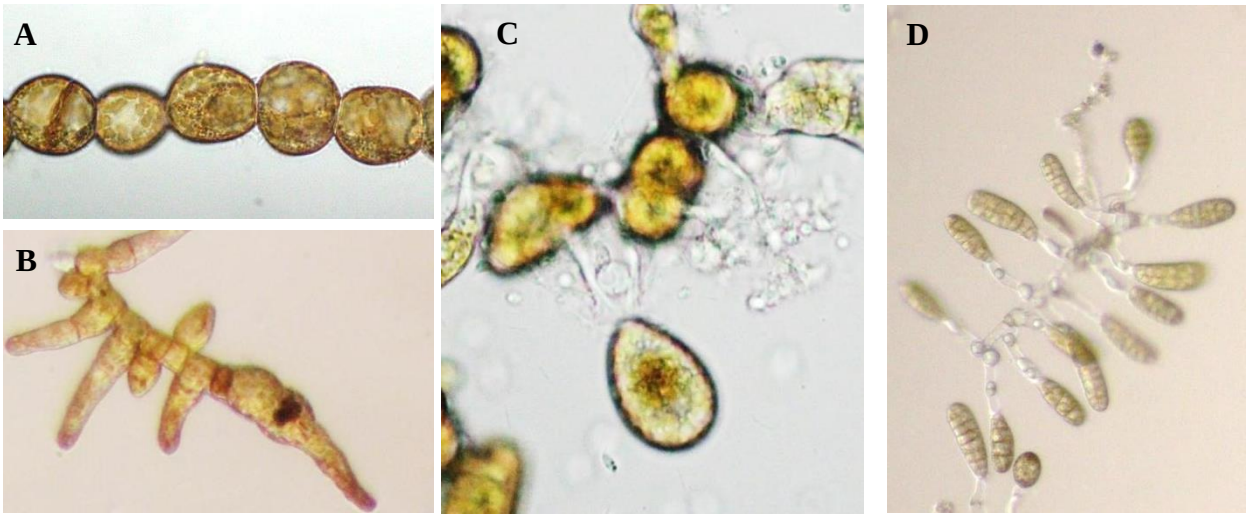

Figure 6. Female developmental stages studied during experiment 1 and 2. A: Vegetative gametophyte fragment (♀); B: Gametophyte fragment with Oogonia; C: Released egg; D: Juvenile macroscopic sporophyte. Note that A and B are considered as a single developmental stage in experiment 1.

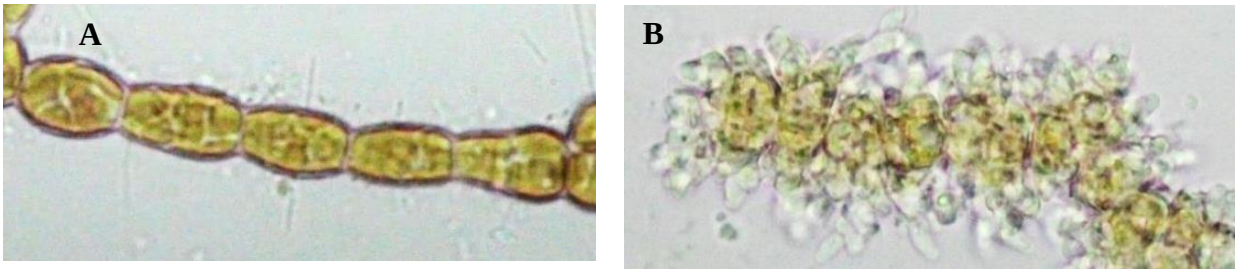

Figure 7. Male developmental stages studied during experiment 1. A: Vegetative gametophyte fragment. B: Gametophyte fragment with antheridia.

The day 0 counting, right after the preparation, was carried out to observe and control whether or not all the fragments were in a vegetative stage. The experiment was stopped on day 14 since there was at least one treatment with all the fragments in a sporophyte stage and thereby with a saturated response.

On day 6, male treatments were observed under the Axiophot microscope and photographed. Their fragment stages were only evaluated qualitatively since fertility was not readily quantifiable.

On day 15, once the experiment was terminated, the cover slips from each petri dish were semi fixed with corn syrup onto slides (Fig. 8). The length and width of 20 fragments from each replicate of the fertile treatments were measured using the Axiophot microscope with a scaled objective. Furthermore, male fragments were again qualitatively analysed.

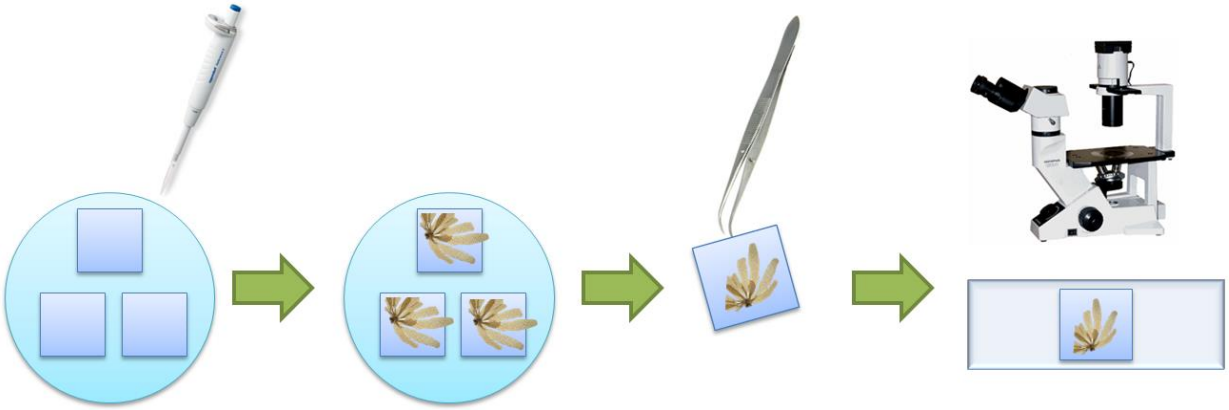

Figure 8. Semi-permanent slide preparation. Three cover slips, with fragments attached to them, are transferred on day 15 from the Petri dishes and mounted into slides for better later microscope examinations.

3.7 Experiment 2: Interspecific competition at different temperatures

The two kelp species *Alaria esculenta* and *Laminaria digitata* were cultured in a two-factorial design (fixed factors 'Temperature' and 'Species') to observe possible interactions between the species during gametogenesis, sporophyte formation and sporophyte growth at 5 and 15°C. In this case, 3 different treatments were prepared: *A. esculenta* ♂ x ♀ treatment, *L. digitata* ♂ x ♀ treatment and 1 mixed treatment with both species and sexes. The abbreviations utilized for the treatments are summarized in Table 3. This experiment consisted of two phases: (1) a microscopic phase following gametogenesis and early sporophyte development until day 24, and (2) a macroscopic phase to observe further sporophyte development until day 67.

Each of the 3 treatments consisted of 5 replicates per treatment (Fig. 9). The total duration of the experiment was 9.5 weeks.

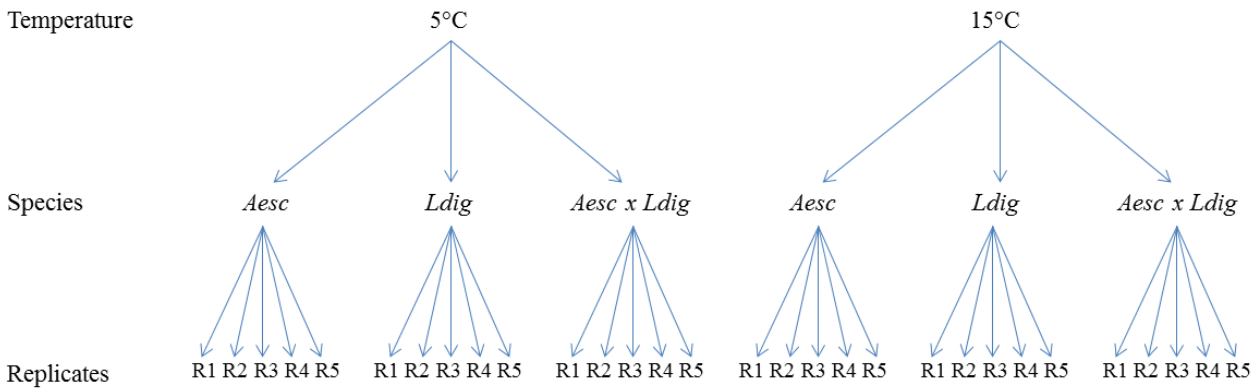

Figure 9. Set-up of the interspecific competition experiment between *A. esculenta (Aesc)* and *L. digitata (Ldig)* at different temperatures (5 and 15°C).

3.7.1 Microscopic phase

The microscopic phase was performed in small plastic Petri dishes and big crystal Petri dishes. After sowing simultaneously in both type of dishes with their respectively volumes, the small petri dishes were used to follow kelp gametogenesis under the inverted microscope, whilst the big crystal petri dishes were not moved during the whole process. The duration of this microscopic phase was 24 days.

Thus, 15 small plastic Petri dishes and 15 big crystal Petri dishes were randomly placed in each culture room under the experimental light conditions (Fig. 10).

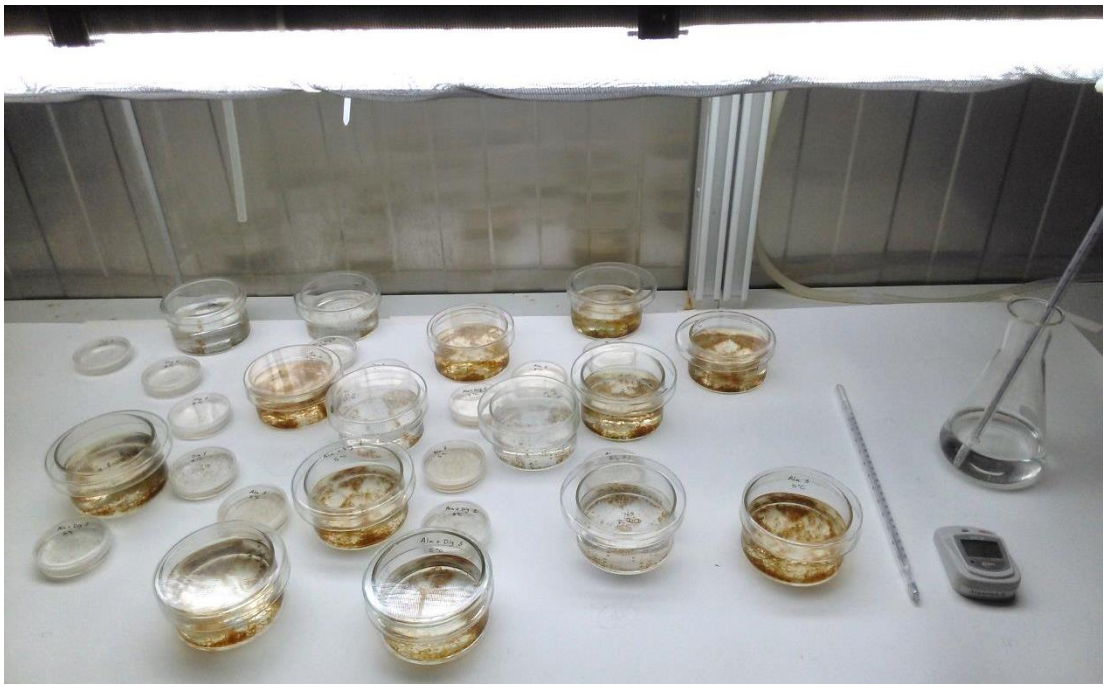

Figure 10. Set-up of experiment 2. Big crystal petri dishes filled with small glass tiles and small plastic petri dishes randomly placed in the culture room for the competition experiment between *A. esculenta* and *L. digitata* at 5°C and 15°C.

Once the gametophytes were settled, gametogenesis was followed using the small plastic petri dishes. On day 0, the number of male and female gametophytes and the female developmental stages was counted separately in 3 out of 5 replicates, to record the developmental stage (see Fig. 6) of all fragments. Moreover, the developmental stages found in all 5 replicates of every treatment were counted on days 4, 8, 12, 16, 20 and 24. For this experiment 4 categories were taken into account: vegetative gametophytes, oogonia, eggs and sporophytes. The PES was changed in the small petri dishes on day 18 to avoid possible nutrient limitation that could affect the development rate. 5 ml of the old water were removed and substituted by 5ml of new PES.

On day 19 the slides with the fragments attached were transferred from the crystal Petri dishes into bigger slightly aerated plastic containers (10x10x10cm) prefilled with 600ml of PES (Fig. 11). The big crystal Petri dishes were kept for another week and their water was changed on day

20 since they still had fragments with sporophytes attached to the bottom. On day 28, these crystal Petri dishes were utilized to measure the largest sporophyte of 20 fragments in every replicate from all treatments. These measurements were performed using a microscope (Axiophot) with a scaled objective.

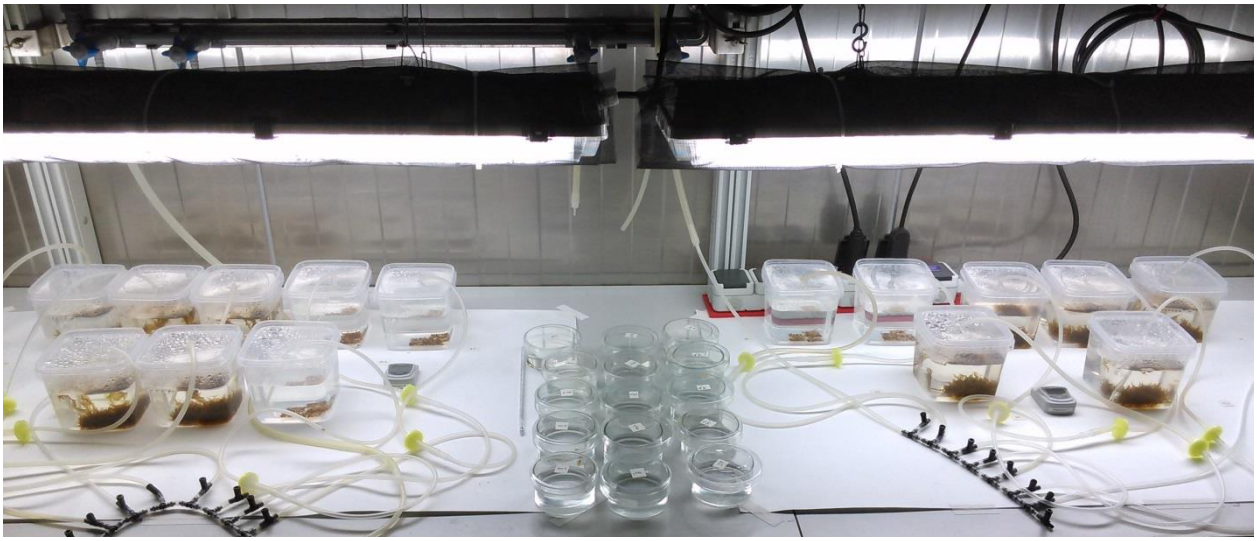

Figure 11. Plastic containers of the competition experiment between *A. esculenta* and *L. digitata* at 5 and 15°C. Compressed air ventilation was established to build up a proper environment for the growing sporophytes.

On day 32, one of the five slides from each plastic container was transferred into a Petri dish to determine the fragment with sporophytes and sporophyte density per cm^2 under the stereo microscope with a net micrometer objective. Thus, the number of fragments and number of sporophytes confined in two randomly chosen parts of the slide were counted in each treatment. Finally, the amount of sporophytes per fragment was calculated dividing the total number of sporophytes by the total number of fragments with sporophytes.

3.7.2 Macroscopic phase

After day 32, the kelp sporophytes were too big to be observed through a microscope. Thus, the macroscopic phase started. Once a week, on days 35, 42 and 49, each plastic container was placed on a light table (Kaiser slimlite LED base, Germany) and a picture was taken at the same height to qualitatively document the growth with time (Canon EOS 550D reflex camera, Tokyo, Japan). PES (600 ml) was changed weekly to avoid nutrient depletion. Some fragments dropped

off from the slides during this process and thereby developed faster which was considered in the data analysis.

On day 49, slides were transferred to even bigger plastic beakers of 3L prefilled with 2L of PES (Fig. 12) to obtain proper growing conditions for the developing sporophytes. Both pictures and changes of water were carried out on days 56 and 63.

Figure 12. Bigger plastic beakers of the competition experiment between *A. esculenta* and *L. digitata* at 5 and 15°C. Compressed air ventilation was established to build up a proper environment for the growing sporophytes.

3.7.3 Analysis of sporophyte density and biomass

Kelps were cultivated until it was unequivocally possible to morphologically distinguish the two species from each other and determine the proportion of sporophytes of each species in the mixed culture. Thus, after approximately ten weeks (67 days) the experiment was stopped. Each of the 5 slides per replicate was scraped off with a scraper. The 10 biggest sporophytes from each treatment were measured in length. All sporophytes of ≥ 0.5 cm size were counted per replicate in order to calculate sporophyte densities (sporophytes/cm²).

The two species were differentiated according to the following criteria (Fig. 13):

- *Alaria esculenta*: reaches bigger size, light brown, lanceolate blade.
- *Laminaria digitata*: dark brown, rounded blade.

The fresh and dry weight of every group of sporophytes from each slide per replicate, and separately the loose sporophytes of each replicate, were determined (Sartorious microbalance;

Göttingen, Germany). For the dry weight algae were dried at 60°C in a Heraeus Kelvitron t oven (Hanau, Germany) over night.

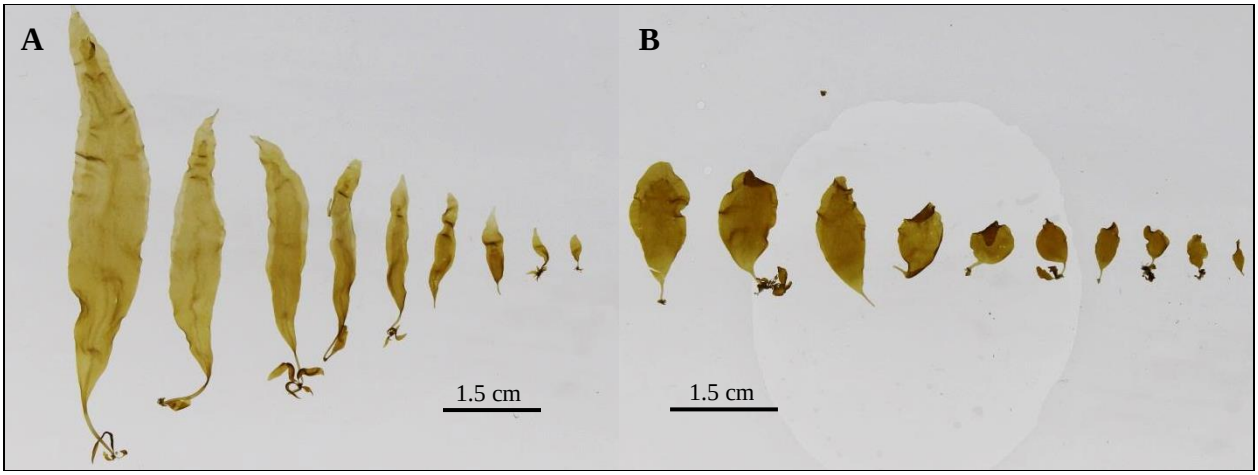

Figure 13. Sporophytic size scale. Intra- and interspecies differences can be observed. A: *A. esculenta* sporophytes. B: *L. Digitata* sporophytes.

3.8 Statistical analysis

Statistical analyses were performed with STATISTICA 6 (StatSoft Inc., Tulsa, OK, USA). According to Underwood (1996), normal distribution is not important when the sample size is small, thereby the data were not tested for normality. However, data sets were tested for homogeneity of variances with the Levene's test. If homogeneity of data did not comply, percentage data sets were Arcsine-square root or logarithmically transformed. When homogeneity of data was still not given after transformation, the p-value indicating significant results was lowered to $p < 0.01$ to avoid type-I-errors.

For both experiments, single sample t-tests were executed to test the interaction among the single species and the mixed treatments. Thus, the mixed treatment mean value was tested against the average of both single species treatments. Furthermore, the t-test independent by groups was also utilized in both experiments to test relations between two treatments.

Generally, Analysis of variance (ANOVA) was carried out with one factor (species or temperature) in experiment 1 and 2, although some repeated Anovas were performed with two

factors (species and temperature) in experiment 2. Moreover, the Tukey-Posthoc Test was used to evaluate further interactions.

Calculations and transformations were done with Excel 2010 (Microsoft Office, Microsoft Corporation, Redmond, USA).

4. Results

4.1 Experimental gametophyte stock cultures and initial controls

4.1.1 Preliminary state of gametophyte stock cultures

Before the starting of the experiment, three out of four non-sieved stock cultures (*Alaria esculenta* ♀, *Alaria esculenta* ♂ and *Laminaria digitata* ♂) were in a vegetative stage (Fig. 14 A, C, D), while *Laminaria digitata* ♀ stock culture contained some oogonia (Fig. 14 B). However, all the cultures looked healthy, although *L. digitata* ♀ and ♂ presented dinoflagellates.

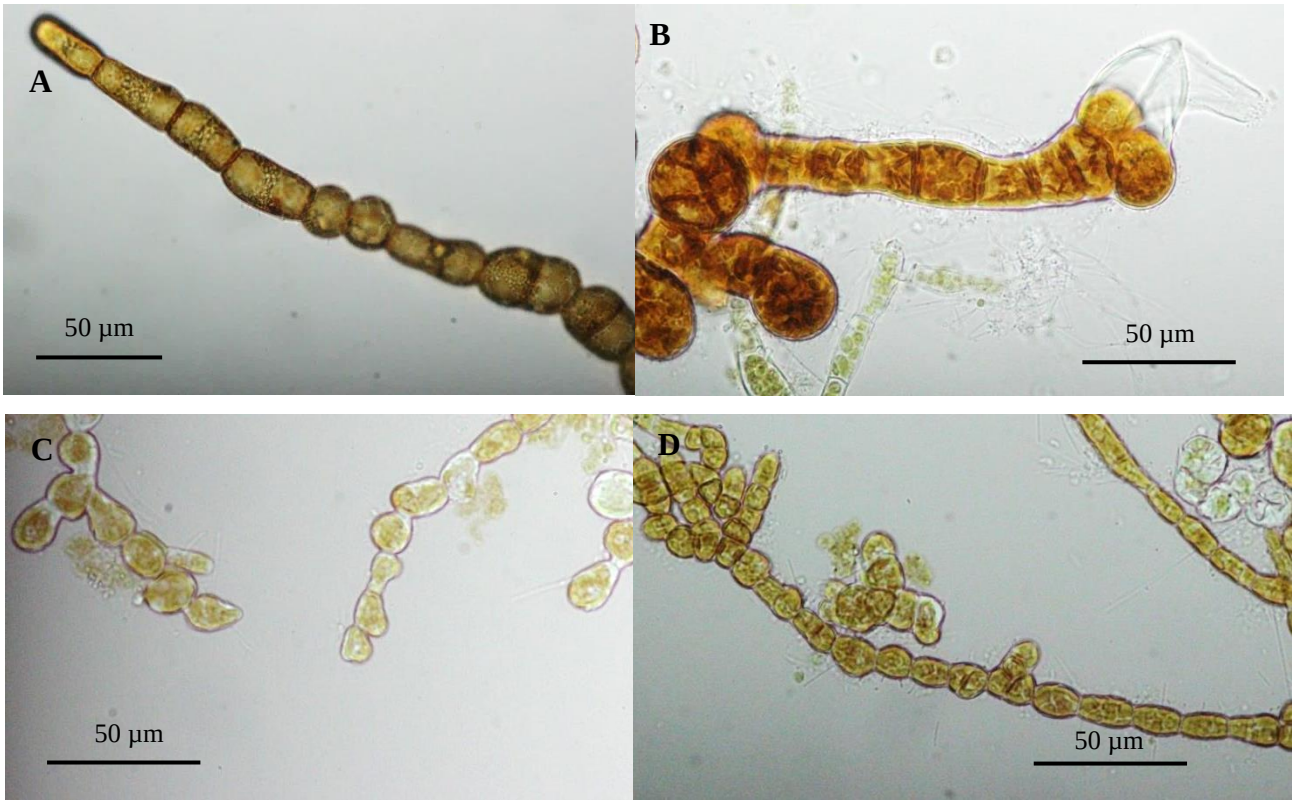

Figure 14. Non-sieved vegetative gametophyte fragments from the 10°C stock cultures. Pictures were taken with the Axiophot microscope (40x). A: *A. esculenta* ♀. B: *L. digitata* ♀. C: *A. esculenta* ♂. D: *L. digitata* ♂.

4.1.2 Cell size

At the beginning of experiment 1 and 2, *A. esculenta* ♀ and *L. digitata* ♀ cells were significantly different within each experiment, being *A. esculenta* significantly ($p < 0.001$) larger (24 µm) than *L. digitata* (17 µm), whereas the cell size of *A. esculenta* ♂ (24 µm) and *L. digitata* ♂ (24 µm) was not significantly different within each experiment. Between experiments, the female cell size from the same species was not significantly different while all males from both species were not significantly different in experiments 1 and 2, being females always significantly larger ($p < 0.001$) than males (Fig. 15, see annexes Table 7, 8).

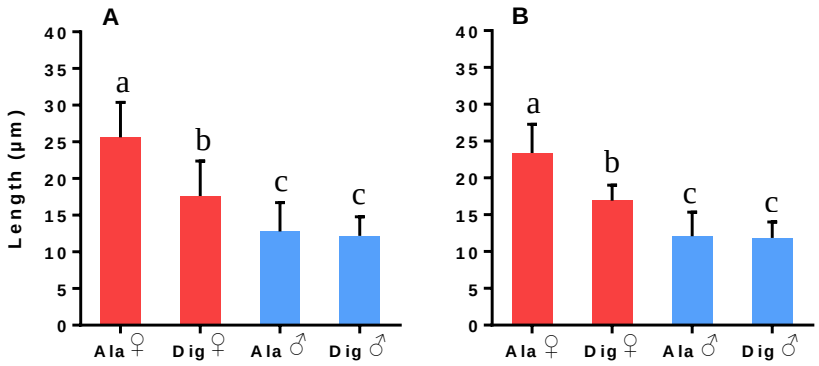

Figure 15. Cell length of gametophyte stock cultures used for seeding of experiment 1 and 2 (mean + SD; N=50). A: measurements before experiment 1. B: measurements before experiment 2. Lower case letters denote significant differences ($p < 0.05$).

4.1.3 Fragment Length of gametophytes

The mean lengths of the sieved fragments utilized in experiment 1 and 2 were not significantly different within and between each experiment, respectively (Fig. 16; see annexes Table 9, 10). All fragments varied between 49 and 441 µm, being 196µm the mean length in experiment 1 and 172µm in experiment 2.

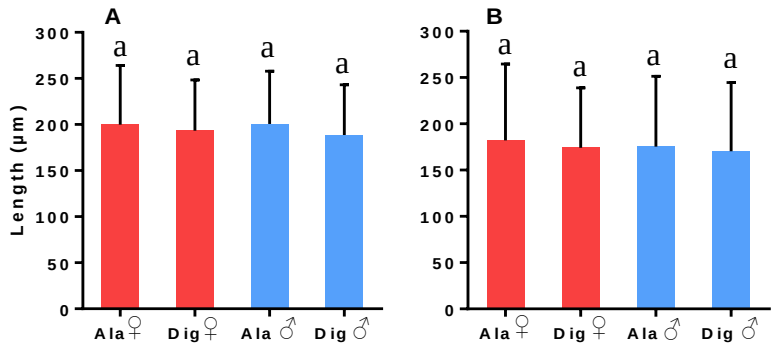

Figure 16. Sieved fragment length of gametophyte stock cultures used for seeding of experiment 1 and 2 (mean + SD; N=40). A: Length before experiment 1. B: Length before experiment 2. 2. Lower case letters denote significant differences ($p < 0.05$).

4.1.4 Number of cells per fragment

Female gametophytes had fewer cells per fragment than males, *A. esculenta* ♀ presenting less cells per fragment (8) than *L. digitata* ♂ (11). On the other hand, *A. esculenta* ♂ and *L digitata* ♂ were not different from each other (15 cells per fragment). The same species and sex fragments in experiments 1 and 2 had no different amount of cells per fragment (Fig. 17).

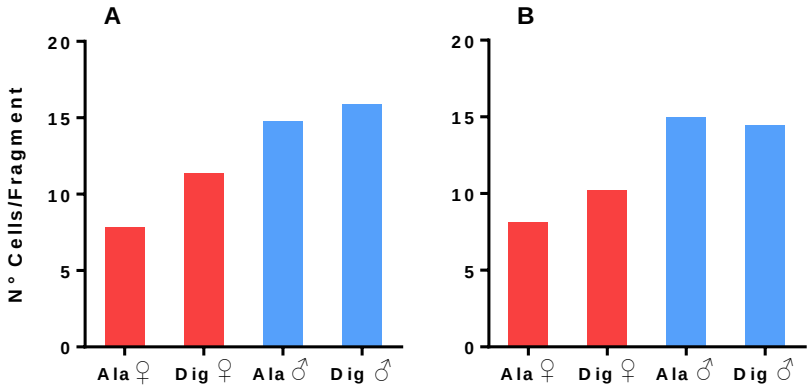

Figure 17. Amount of cells per sieved fragment. A: before experiment 1. B: before experiment 2. No standard deviation is provided since this graph depicts the result of dividing the mean fragment length of each treatment by the correspondent mean size length. Note that no SD is shown since this values are the result of dividing the mean fragment length by the mean number of cells for each sex and species.

4.1.5 Density

In experiment 1, treatment densities were not significantly different, ranging from 260 to 366 fragments/cm². In experiment 2, treatment densities were not significantly different either ranging from 351 to 400. Densities of both experiments were significantly different ($p < 0.001$) between them, ranging from 260 to 400 fragments/cm² (Fig. 18; annexes Table 11, 12, 13).

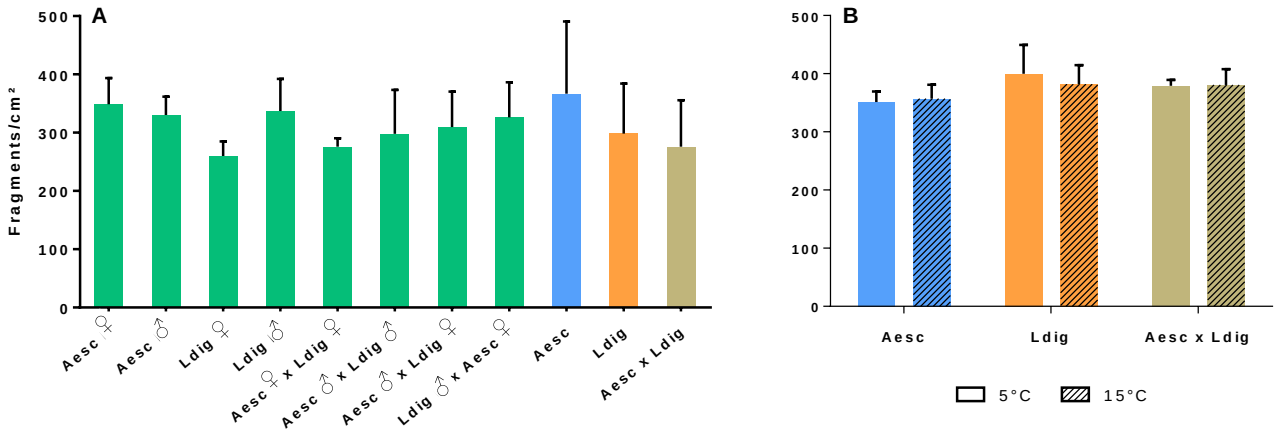

Figure 18. Initial fragment densities measured on day 0. mean + SD). A: experiment 1 density (N=4); B: experiment 2 density (N=3).

4.2 Experiment 1: Competition between sexes and species

On day 0, *A. esculenta* ♂ and *L. digitata* ♂ did not have significant development differences right before starting the experiment. Likewise, *A. esculenta* ♀ and *L. digitata* ♀ developments were not significantly different either (Fig. 19B, annexes Table 14). Fragments had already started to develop into a fertile stage at Day 0. Most of *A. esculenta* ♂ and *L. digitata* ♂ had developed some antheridia (80%) (Fig. 19A, annexes Table 15). Moreover, even though it is not reflected in the graph, oogonia stages were already observed in both female cultures of the two species.

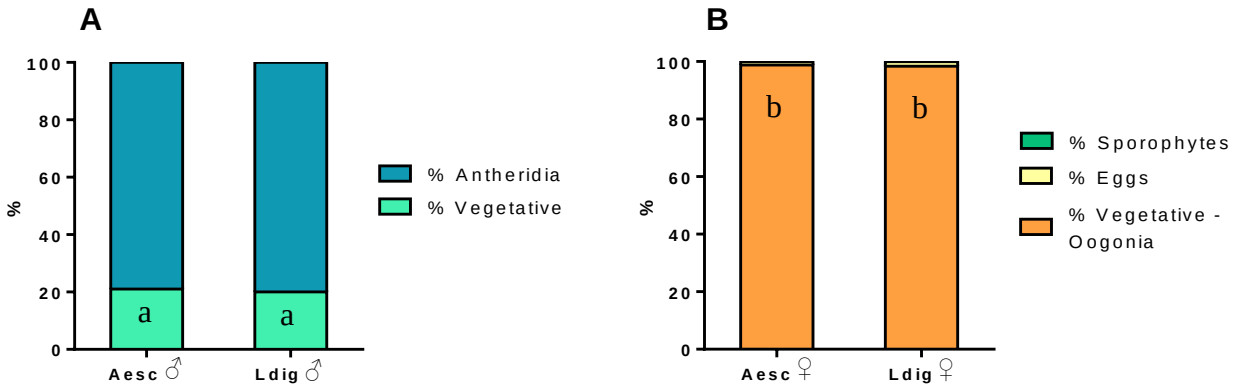

Figure 19. A. esculenta and *L. digitata* developmental stage percentages on Day 0 (mean values; N=4). A: Male developmental stages. B: Female developmental stages. No SD are given for clarity. Lower case letters denote significant differences ($p < 0.05$).

4.2.1 Development of male gametophytes

On day 6, it was attempted to quantitatively assess the formation of antheridia and their release in male gametophytes throughout the experiment, but due to the minuscule size of antheridia it was not possible to quantify these differences. Nonetheless, the situation was qualitatively estimated. When there was no female gametophyte present, antheridia of both species seemed closed and sperms unreleased (Fig. 20, 21), whereas if a female from the same species is present, antheridia were empty, sperm was released and fecundation took place, so that sporophytes thrived. If the female present was not from the same species as the male gametophytes, their antheridia seemed to be emptied as well in males close to the female (Fig. 22). However, in some cases this assumption was not complied.

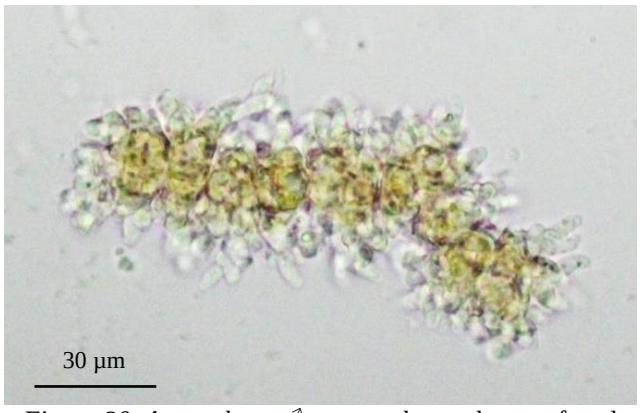

Figure 20. *A. esculenta* ♂ gametophyte when no female is present. Full antheridia. Picture taken on day 6.

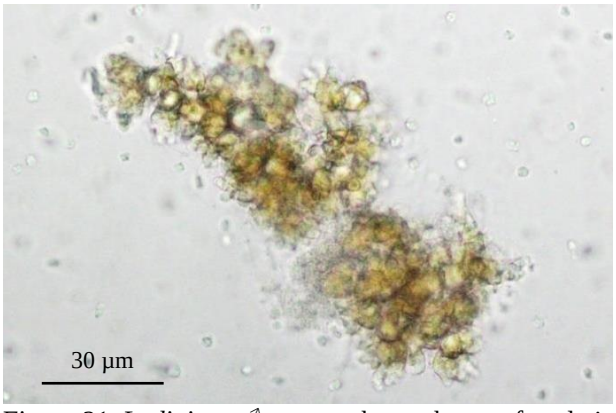

Figure 21. *L. digitata* ♂ gametophyte when no female is present. Full antheridia. Picture taken on day 6.

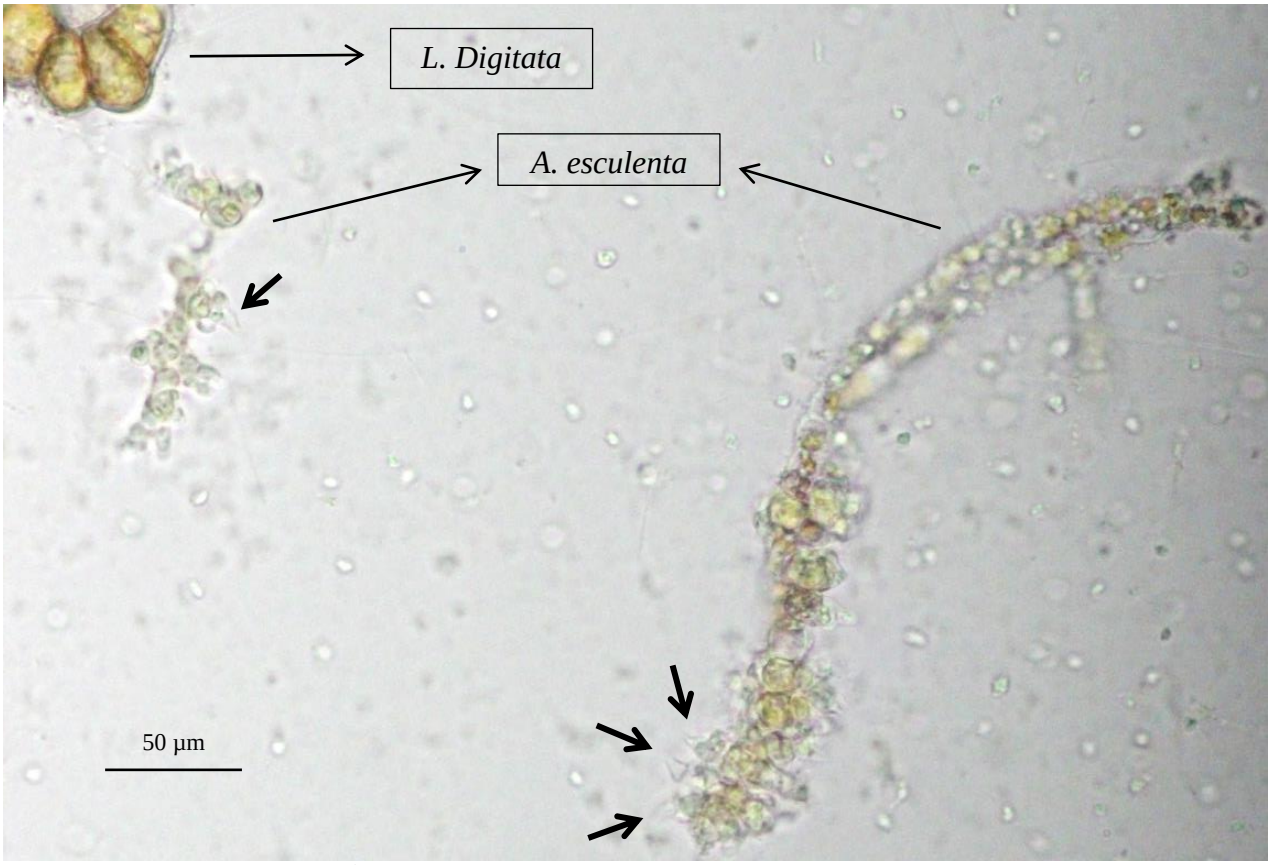

Figure 22. Two *A. esculenta* ♂ with empty antheridia in presence of *L. digitata* ♀ gametophytes. Empty antheridia marked with black bold arrows. Picture taken on day 6.

On day 15, the material fixed with corn syrup in semi-permanent slides was qualitatively judged. The image quality was lower and all male treatments had open antheridia, a situation which was also caused by the fixation process itself. Thus treatment effects cannot be established in fixed material.

4.2.2 Development of female gametophytes

In the following, the different developmental stages on days 5, 9 and 14 of all treatments containing females were described during their microscopic stages and an overview over the pattern is given in Fig. 23. Statistical differences within one stage were only evaluated until the ontogenetic process was saturated in one treatment. For example, on day 9, *A. esculenta* sporophytes were already saturated and therefore this stage cannot be evaluated any longer.

At day 5 there were significantly more fragments with the stage 'vegetative-oogonia' in the single species *L. digitata* (*Ldig*) and the fully mixed (*Aesc x Ldig)* treatments than in the single species *A. esculenta* (*Aesc*) treatment, meaning that the latter produced significantly faster juvenile sporophyte ($p < 0.01$) recruits if not in combination with *L. digitata* (Fig. 23A; see annexes Table 18).

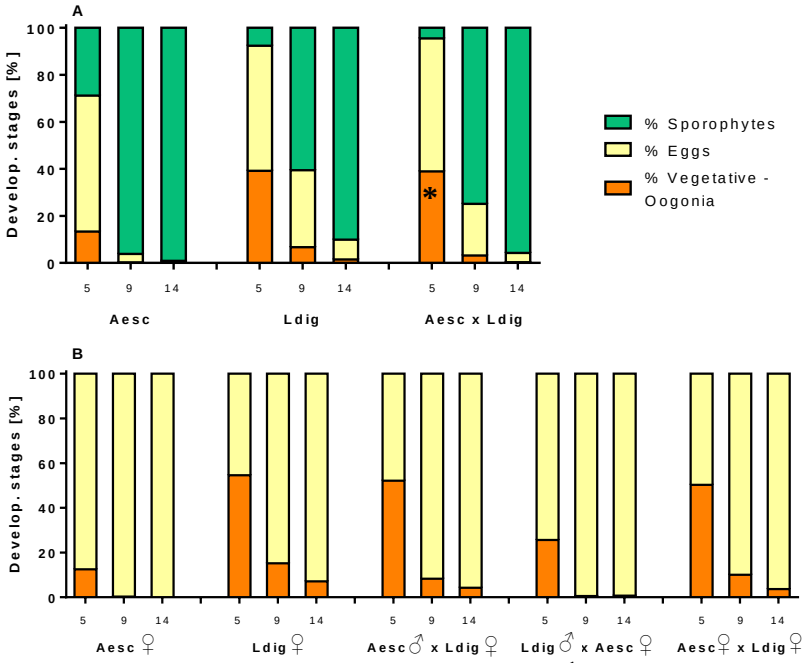

Figure 23. Relative number of developmental stages of all treatments containing females on days 5, 9 and 14 (mean values; N=3). A: Treatments with sporophytes. B: Treatments in which sporophytes were not estimated. No SD are given for clarity. Note that documented interspecific interactions are indicated by '*'.

Similarly, when only comparing the vegetative-oogonium stages of treatments, where either *A. esculenta* ♀ or *L. digitata* ♀ was present at day 5 (Fig. 24), it turned out to be apparent that *L. digitata* ♀ became fertile at a significantly lower rate ($p < 0.05$), either alone or in any combination with males from *L. digitata* or *A. esculenta*, than *A. esculenta* ♀. Interestingly, the presence of *L. digitata* ♂ significantly retarded ($p < 0.01$) the development of *A. esculenta* ♀ (25.6% vs. 12.5% of fragments with the stage 'vegetative-oogonium') (Fig. 24; annexes Tables 19, 20, 21, 22).

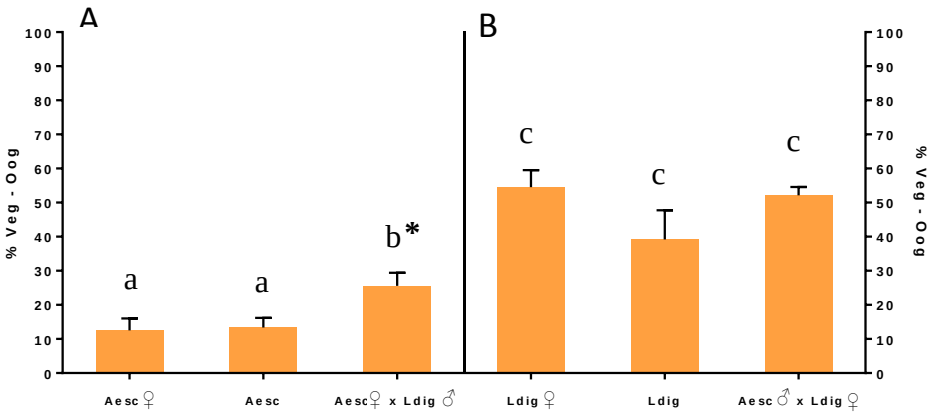

Figure 24. Interaction of female kelp gametophytes with intra- and interspecific male gametophyte. Relative number of 'vegetative-oogonia' developmental stage on day 5 (mean + SD; N=3). A: *A. esculenta* Treatments. B: *L. digitata* treatments. Low case letters denote significant differences ($p < 0.05$). Note that documented interspecific interactions are indicated by '*'.

At day 5, there were deviations from the expectation ($p < 0.01$) in the mixed treatment for 'vegetative-oogonia' and 'sporophytes', meaning that there was an interaction between the species in the mixed treatment, while this situation was not further processed at day 5 and 9 (Fig. 25; see Annexes Table 16, 18).

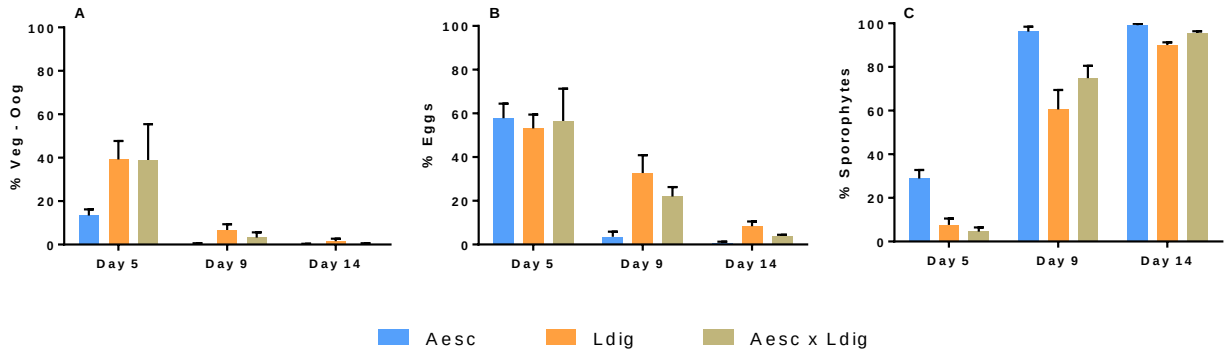

Figure 25. Development of different developmental stages over time in *A. esculenta* (*Aesc*), *L. digitata* (*Ldig*) and mixed *A. esculenta/L. digitata* (*Aesc x Ldig*) cultures (means + SD, N=3). A: vegetative to oogonium stage (Veg-Oog). B: Egg stage. C: Juvenile sporophyte stage.

4.2.3 Size of microscopic sporophytes

At the end of the experiment, the size of microscopical sporophytes was determined at day 15 with two major results. Firstly, there was a deviation from the expectation in the mixed treatment sporophyte length (479µm) and width (103µm), being significantly higher ($p < 0.001$) than the combine mean length (314µm) and width (66µm) of both single species treatment (see annexes, Table 23). It means that there was an interaction between the species in the mixed treatment. Secondly, *A. esculenta* was significantly larger ($p < 0.001$) than *L. digitata* (427µm vs. 200µm), whereas they are not significantly different in width (66µm vs. 66µm) (Fig. 26; see annexes Table 24).

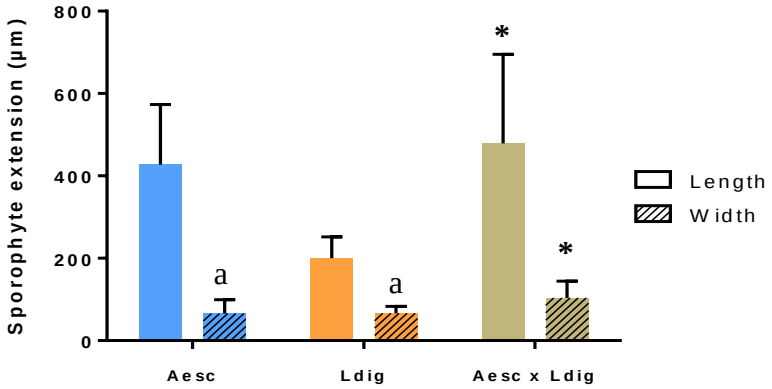

Figure 26. Length and width of 20 biggest sporophytes per fragment of the fixed material on day 15 (mean + SD; N=60). Low case letters denote significant differences ($p < 0.05$). Note that documented interspecific interactions are indicated by '*'.

4.3 Experiment 2: Competition between species at different temperatures

4.3.1 Microscopic phase

In the following, the different developmental stages over time (day 4, 8, 12, 16, 20, 24) in the single species and mixed treatments at 5 and 15°C is described during their microscopic stages and an overview over the pattern is given in Fig. 27. Statistical differences within one stage were only evaluated until the ontogenetic process was saturated in one treatment. For instance, on day 20, *A. esculenta* sporophytes at 5°C were already saturated and therefore this stage cannot be evaluated any longer at this temperature (Fig. 28).

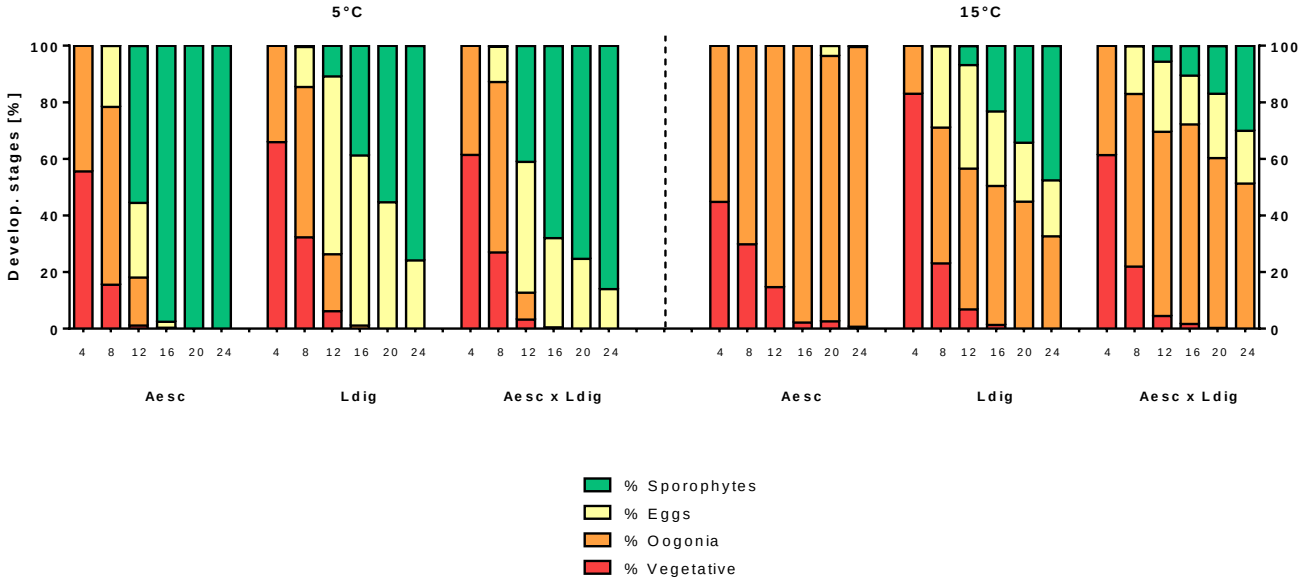

Figure 27. Development of ontogenetic stages over time in *A. esculenta*, *L. digitata* and the mixed treatment (*A. esculenta* + *L. digitata*) at 5°C and 15°C (mean values; N=5). Counting was done on days 4, 8, 12, 16, 20 and 24. No SD are given for clarity.

4.3.1.1 Vegetative gametophytes and formation of oogonia:

There was not apparent interaction between the species in the mixed treatment when regarding vegetative gametophytes and the formation of oogonia at 5°C and 15°C. Thus, there was no deviation from the expectation in the mixed treatments (Fig. 28A, B).

4.3.1.2 Egg release

At 5°C, the relative amount of eggs in the mixed treatment was only significantly lower ($p < 0.001$) than the expectation on day 8, meaning that there was an interaction between the species in the mixed treatment, while this was not the case on day 12 and 16 (Fig. 28; see annexes Table 25).

At 15°C just a minuscule relative amount of *A. esculenta* eggs was present on day 20 but they did not develop further on and decayed (Fig. 27, 28). However, the relative amount of eggs in the mixed treatment became significantly higher ($p < 0.05$) than expected on days 16 onwards, which implies interspecific interactions (Fig. 28; see annexes Table 27).

4.3.1.3 Sporophyte formation

Regarding the sporophyte development at 5°C, the relative amount of sporophytes in the mixed treatment was significantly higher ($p < 0.05$) than the expectation on day 12 but not on day 16, whereas at 15°C was never significantly different than the expectation (Fig. 28; see annexes Table 26). It means that there might be a temporal interspecific interaction in the mixed treatment on day 12 at 5°C.

4.3.1.4 Temperature comparison

The relative amount of *A. esculenta* sporophytes was significantly higher at 5°C than 15°C throughout the whole process since no sporophytes were recorded during the 24 first days (Fig. 28) On the other hand, the relative amount of *L. digitata* sporophytes was not significantly different between 5°C and 15°C on day 12, whereas it turned out to have significantly higher relative amount of sporophytes on day 16, 20 and 24 ($p < 0.001$) (Fig. 28).

Generally, *A. esculenta* developed higher relative amount of sporophytes than *L. digitata* at 5°C, while *L. digitata* developed higher relative amount of sporophytes at 15°C. During these first days of the experiment, densities decreased with time in most of the treatments (see annexes Fig. 40).

However, on day 24, it became obvious that the treatments containing *L. digitata* at 15°C were contaminated with a small undetermined brown algal species (Ectocarpales) (Fig. 29).

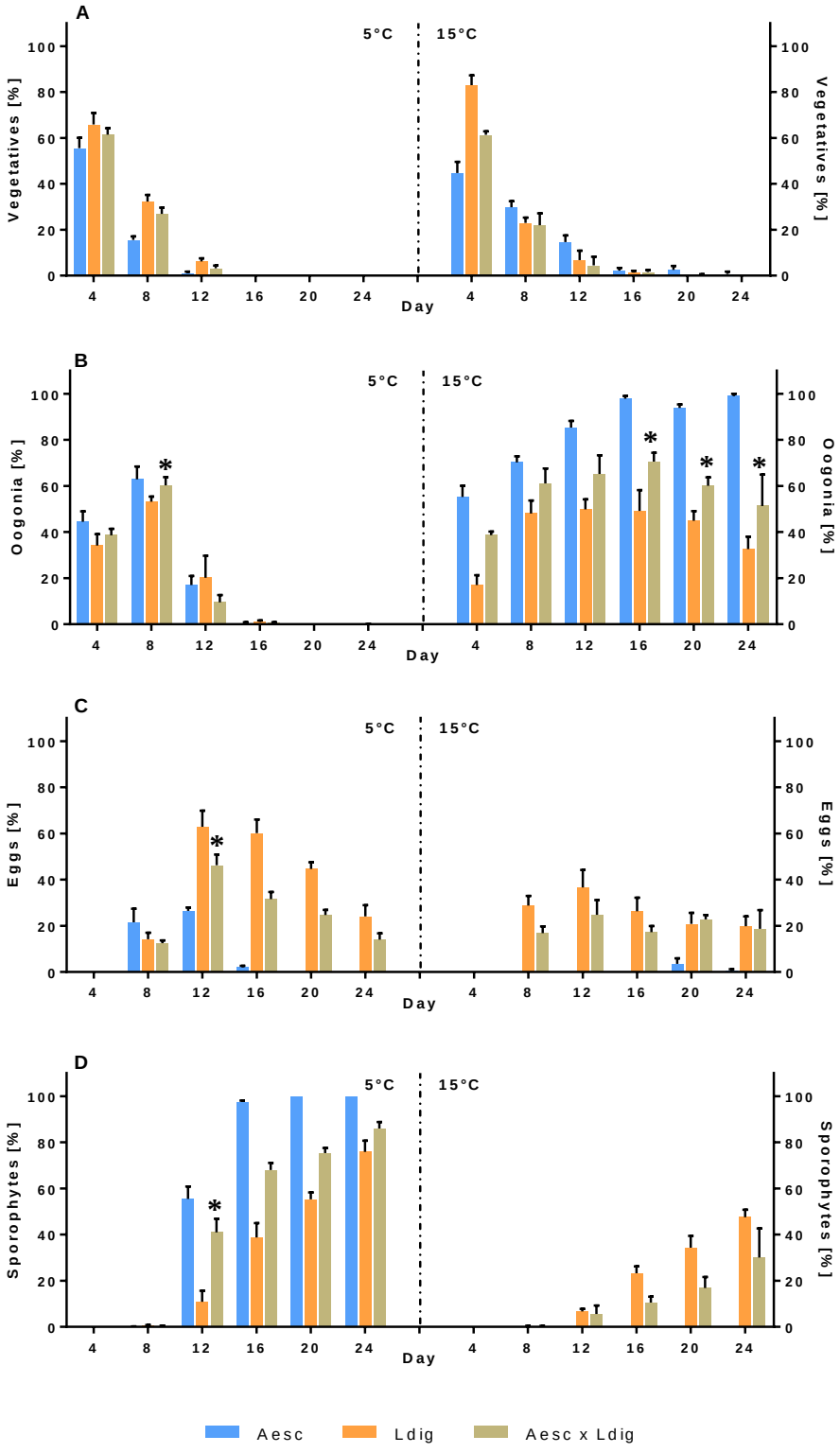

Figure 28. Development of the different developmental stages over time in *A. esculenta* (*Aesc*), *L. digitata* (*Ldig*) and mixed *A. esculenta/L. digitata* (*Aesc x Ldig*) cultures at 5°C and 15°C. Counting was done on day 4, 8, 12, 16, 20 and 24 (means + SD, N=5). A: vegetative stage; B: Oogonia stage. C: Egg stage. D: Juvenile sporophyte stage. Note that documented interspecific interactions are indicated by '*'.

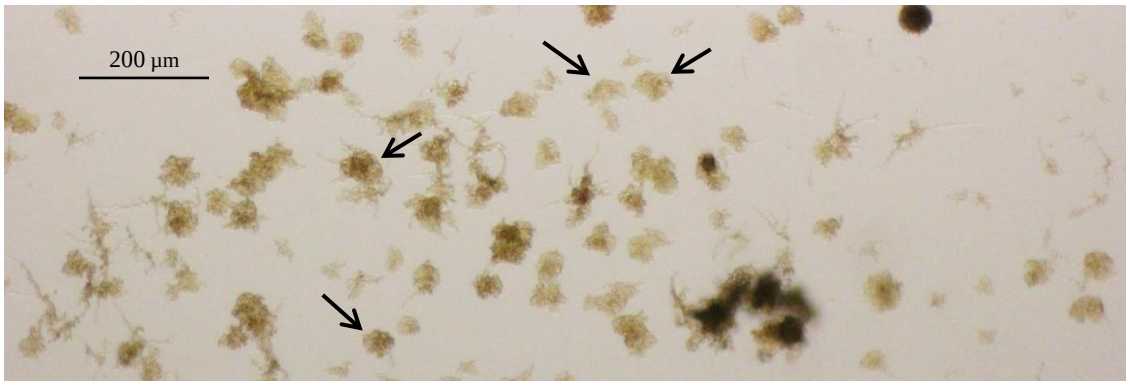

Figure 29. Aspect of brown algae contamination developing at 15°C in *L. digitata* treatments. Picture was taken on day 24. Black arrows show brown algae.

In the following, the length of the sporophytes measured on day 28 at 5°C and 15°C, is described. At 5°C, sporophytes in the mixed treatment (*Aesc x Ldig*) were not significantly different to the expectation on day 28, whereas at 15°C they were significantly longer ($p < 0.001$) (see annexes Table 30). Furthermore, the sporophytes in the mixed treatment at 15°C were significantly larger ($p < 0.05$) to *L. digitata* (Fig. 30; see annexes Table 31). *A. esculenta* at 5°C developed the longest sporophytes on day 28, while no sporophytes were recorded for this species at 15°C. In *L. digitata* sporophytes were significantly larger ($p < 0.001$) at 15°C than at 5°C (Fig. 30).

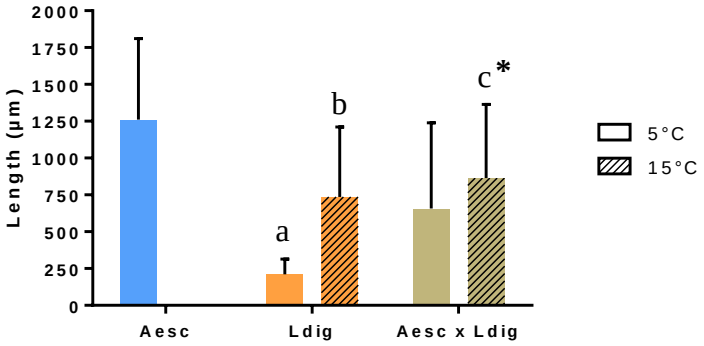

Figure 30. Mean sporophyte lengths in µm of *A. esculenta* (*Aesc*), *L. digitata* (*Ldig*) and the mixed treatment *A. esculenta*/*L. digitata* (*Aesc x L dig*) at 5°C and 15°C (means + SD, N=100). Lengths were measured on day 28. Low case letters denote significant differences ($p < 0.05$). Note that documented interspecific interactions are indicated by '*'.

On day 34, the fragments with sporophyte(s) density, sporophyte density and number of sporophytes per fragment measured at 5°C and 15°C, are described (Fig. 31).

At 5°C, there were significantly less fragments with sporophyte(s) in the mixed treatment (14 frag./cm²) to the expectation (21 frag./cm²; $p < 0.05$), and significantly lower density of sporophytes in the mixed (84 spor./cm²) to the expected value (130 spor./cm²; $p < 0.05$). This means that there might be an interspecific interaction. However, there was no difference in the number of sporophytes per fragment in the mixed (6 spor./frag.) to the expectation (6 spor./frag.), neither between the mixed and the *L. digitata* treatment (4 sporophytes/frag.) (Fig. 31; Annexes Table 32).

At 15°C, there were more fragments with sporophyte(s) per cm² in the mixed treatment (9 frag./cm²) than the expected value (5 frag./cm²; $p < 0.01$). Likewise, there were more sporophytes per cm² in the mixed than the expectation (19 vs. 12 spor./cm²; $p < 0.01$) and more sporophytes per fragment in the mixed than expected (2 vs. 1 sporophytes/frag.; $p < 0.001$). The sporophytes per fragment were not significantly different between *L. digitata* and the mixed treatments.

Regarding differences and similarities between the two different temperatures (*A. esculenta* 15°C was not taken into account for the statistics since no sporophytes were recorded) there were two major results. Firstly, there were no significantly different densities of fragments with sporophyte(s) per cm² among all treatments (Fig. 31; Annexes Table 33).
Moreover, there were significantly more fragments with sporophyte(s) per cm² in *L. Digitata* treatment at 5°C (50 frag./cm²) than at 15°C (30 frag./cm²; $p < 0.05$) (Fig. 31).

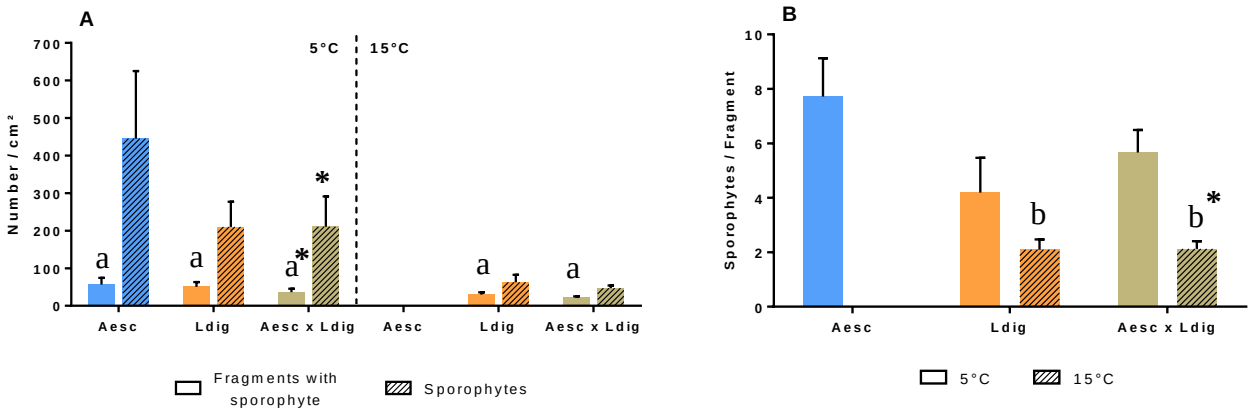

Figure 31. Fragment density, fragments with sporophyte(s) density and number of sporophytes per fragment at 5°C and 15°C, measured on day 32 in *A. esculenta* (*Aesc*), *L. digitata* (*Ldig*) and the mixed *A. esculenta*/*L. digitata* (*Aesc x Ldig)* cultures (mean + SD; N=5). A: Sporophyte and fragments with sporophyte(s) densities per cm². B: Number of sporophytes per fragment. Lengths were measured on day 28. Low case letters denote significant differences ($p < 0.05$). Note that documented interspecific interactions are indicated by '*'.

4.3.2 Macroscopic phase

In Fig. 32 and 33 it can be observed a series of pictures taken on days 35, 42 and 49, showing the relative growth evolution of the three different treatments during two weeks while disposed in small plastic containers (1L) at 5°C and 15°C.

Visually, it can be perceived that *A. esculenta* developed faster and denser than *L. digitata* at 5°C, while the mixed treatment had patches of each species on day 35 and gradually *A. esculenta* ended up covering most of the slide surface by day 49 (Fig. 32).

On the other hand, *L. digitata* developed sporophytes at 15°C, whereas *A. esculenta* only developed one visually perceptible sporophyte on day 35. Nonetheless, on day 49, some other small sporophytes can be observed in *A. esculenta* treatment. The mixed treatment seemed to be composed mainly by *L. digitata*. Nevertheless, at 15°C *L. digitata* and mixed treatments were contaminated by brown algae (ectocarpales) which can be already observed on day 35 (Fig. 33).

When comparing both temperatures, *A. esculenta* developed more effectively at 5°C than 15°C, while *L. digitata* gave the impression to develop faster and bigger sporophytes at 15°C on day 35, but the contamination became very aggravated with time, which made difficult further evaluations.

In Fig. 34 and 35 it can be observed another series of pictures taken on days 56 and 63. These photographs show the relative growth evolution of the three treatments during one week while disposed in bigger plastic beakers (3L) at 5°C and 15°C.

Visually, it can be perceived that *A. esculenta* has developed faster, bigger and denser than *L. digitata* at 5°C, while the mixed treatment looks almost as the *A. esculenta* treatment or even look denser, so that only this species can be appreciated.
At 15°C, *L. digitata* and the mixed treatments cannot be evaluated owing to the massive brown algae contamination. However, *A. esculenta* treatment kept developing sporophytes on day 56 and 63.

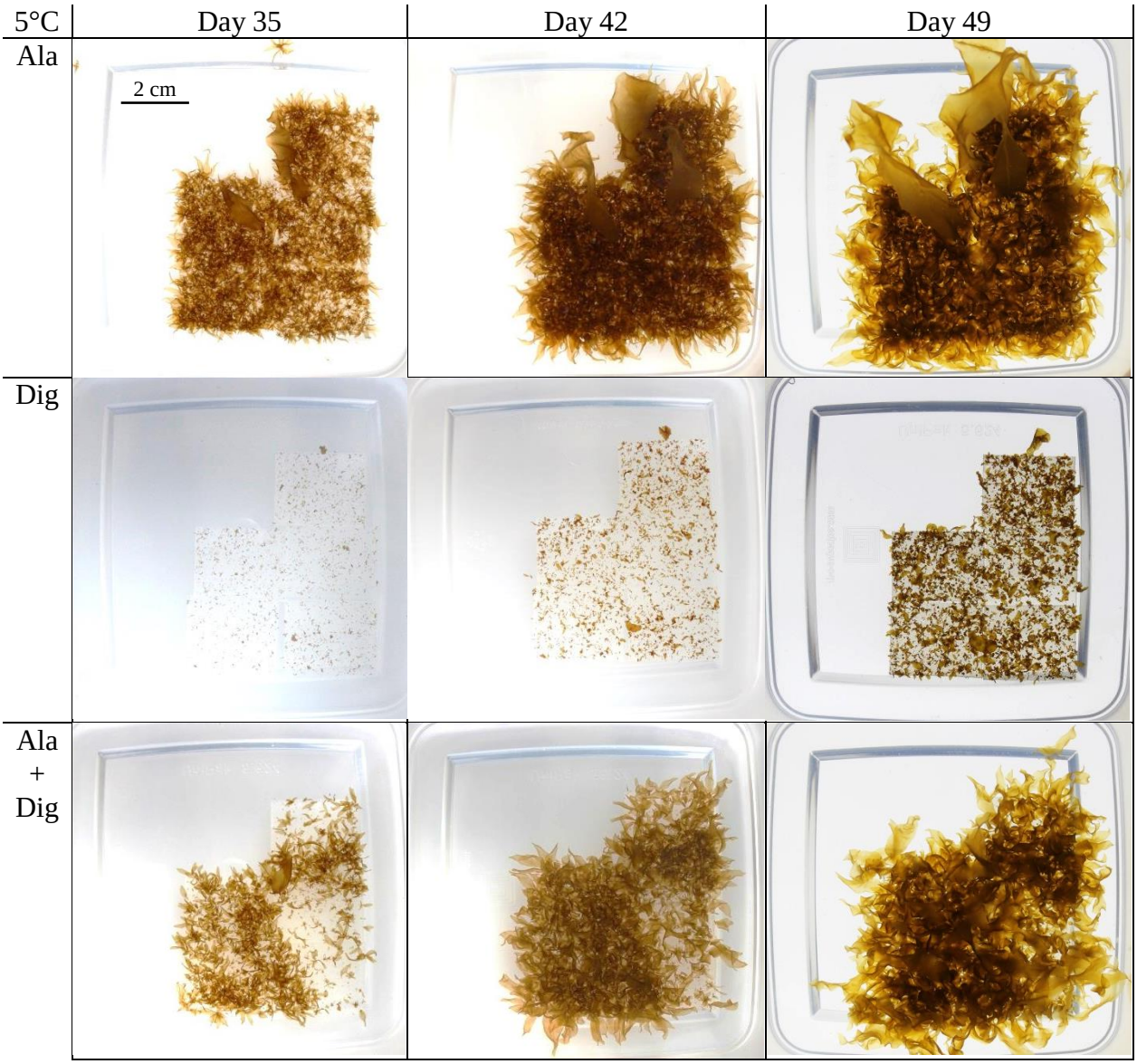

Figure 32. Relative growth evolution of all three 5ºC treatments in the small containers. Pictures were taken on days 35, 42 and 49. All pictures to the same scale.

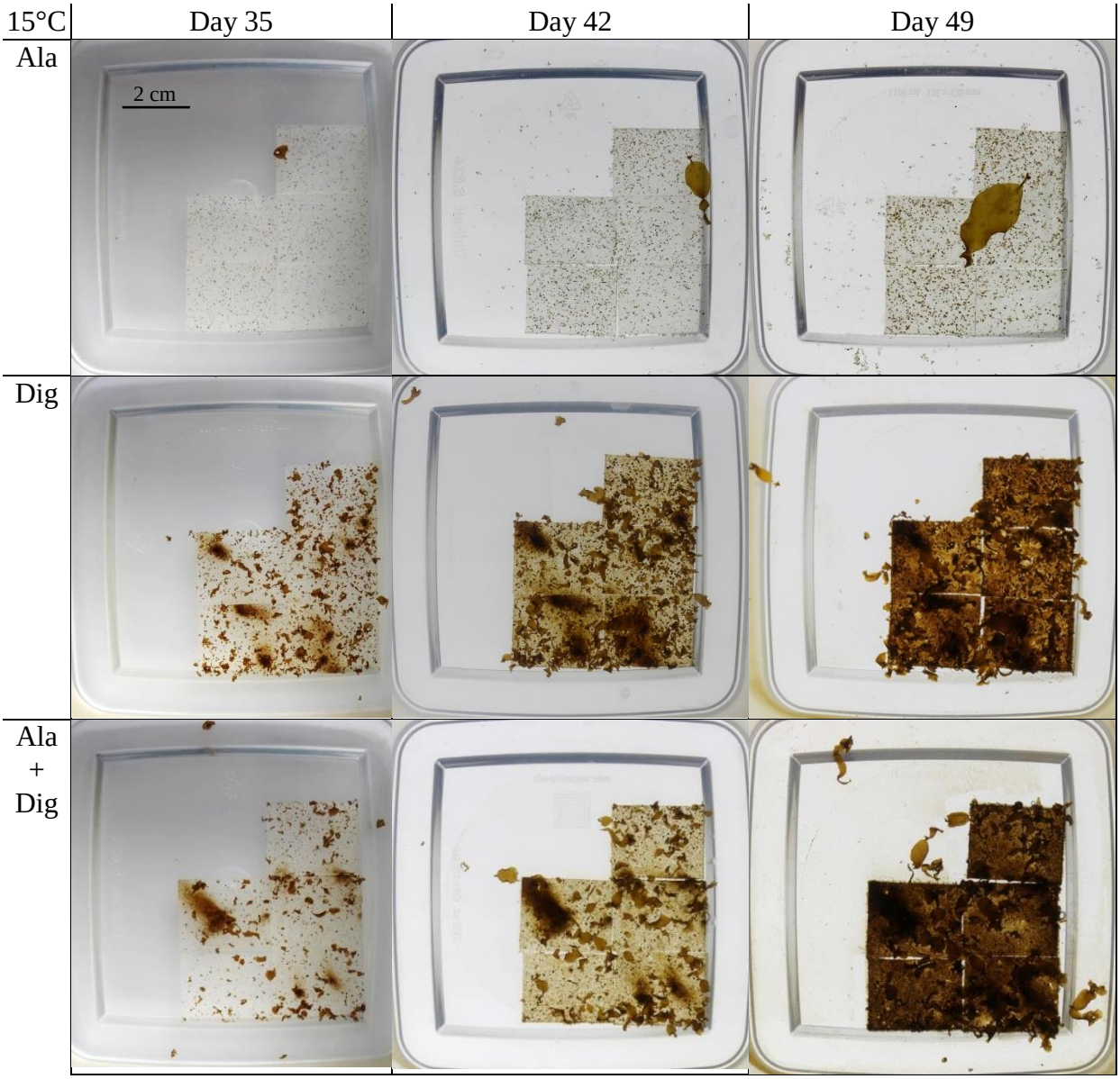

Figure 33. Relative growth evolution of all 15ºC treatments in the small containers. Pictures were taken on days 35, 42 and 49. All pictures to the same scale. Note that from day 42 onwards, the contamination in the treatments containing *L. digitata* became overwhelming. Nevertheless the general pattern is visible.

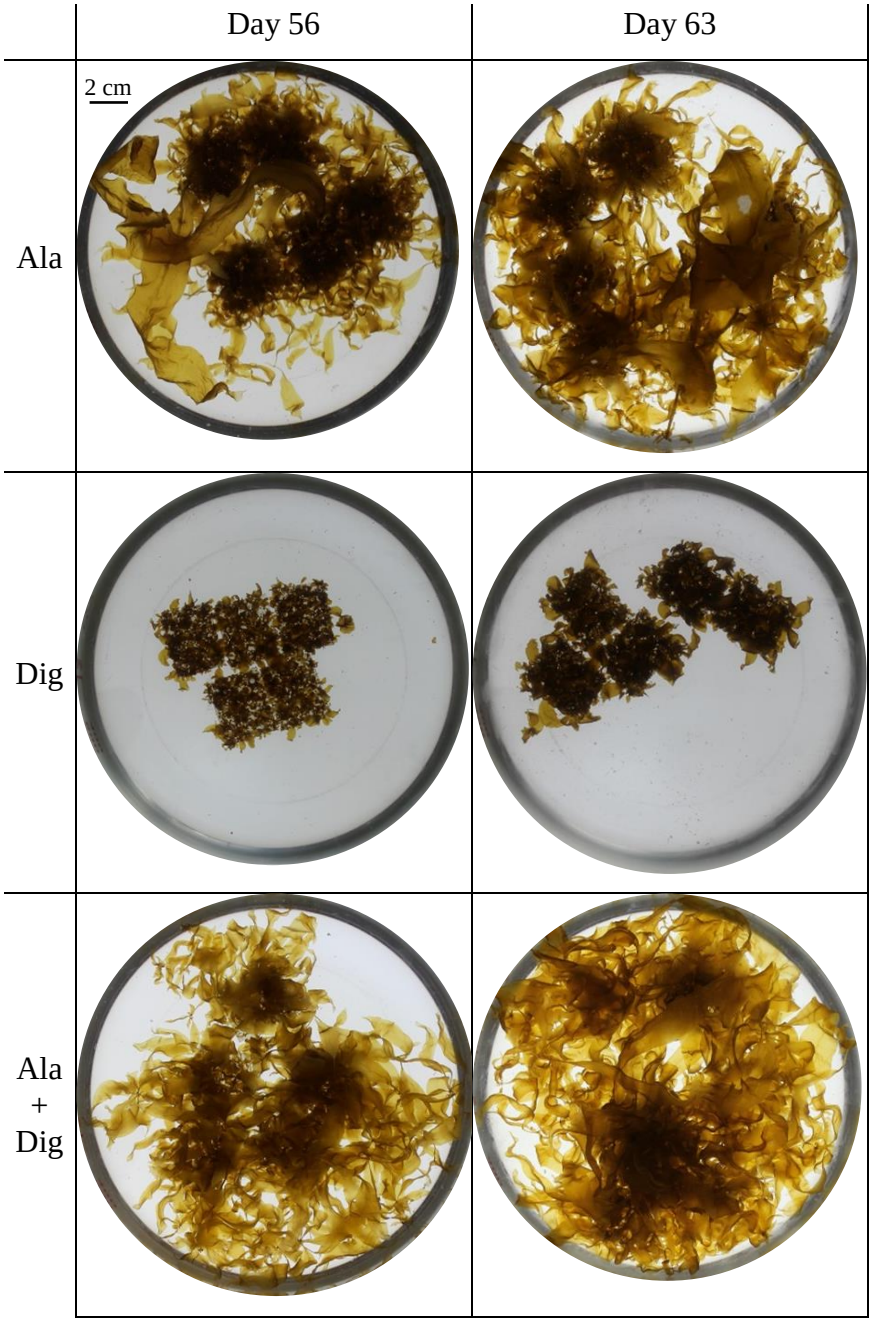

Figure 34. Relative growth evolution of all three 5ºC treatments in the big beakers. Pictures were taken on days 56 and 63. All pictures to the same scale.

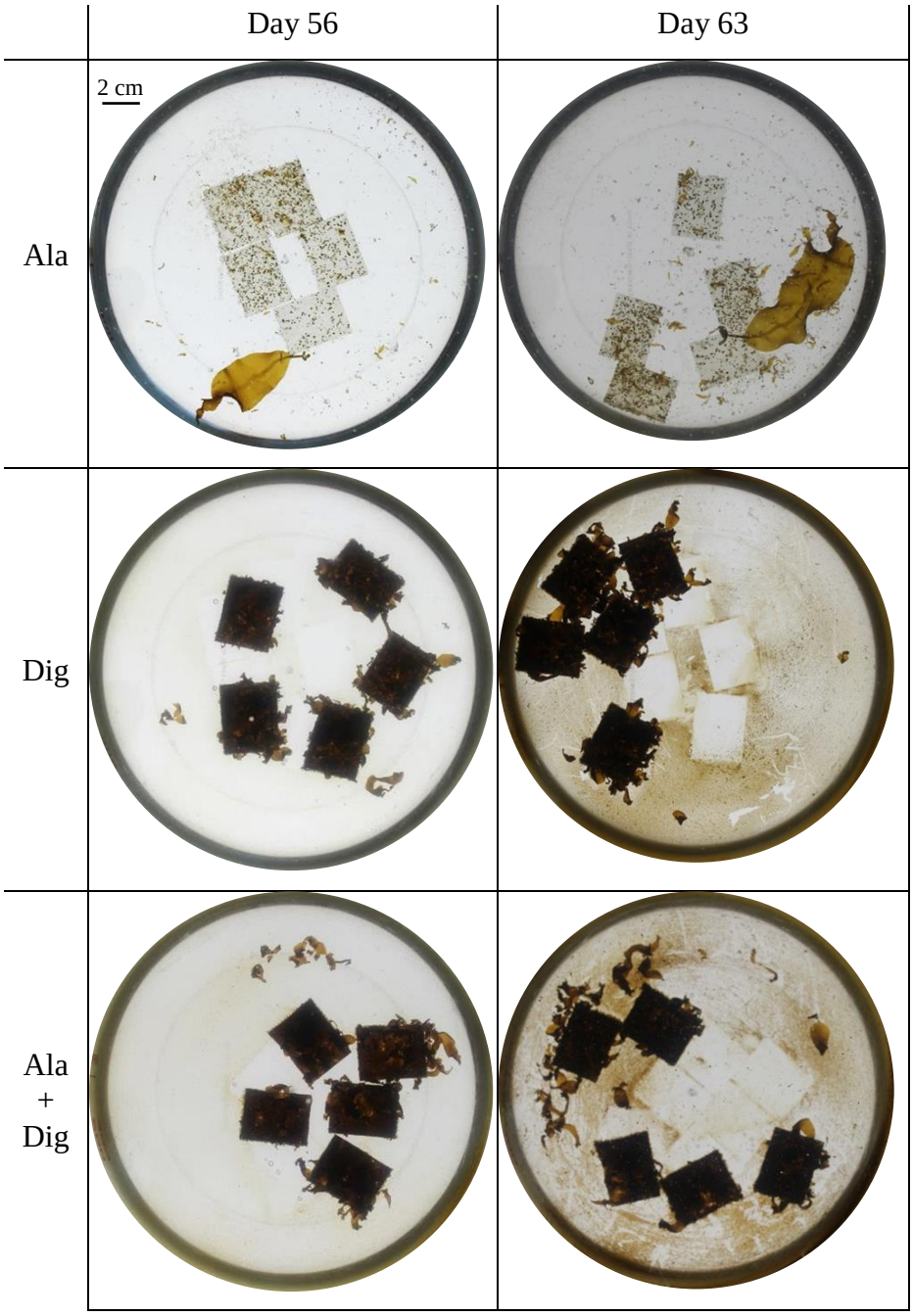

Figure 35. Relative growth evolution of all 15ºC treatments in the big beakers. Pictures were taken on days 56 and 63. All pictures to the same scale. The contamination in the treatments containing *L. digitata* was devastating, and the general pattern is not visible. Nonetheless, note the delayed evolution of several small sporophytes in Alaria from day 56 onwards.

On the day 67 the length, density and biomass of the sporophytes from the treatments are described (Fig. 36, 37). Note that *L. digitata* and the mixed treatments at 15°C are not being considered since the contamination was severe from day 42 onwards.

4.3.3 Length and Density of gametophytes

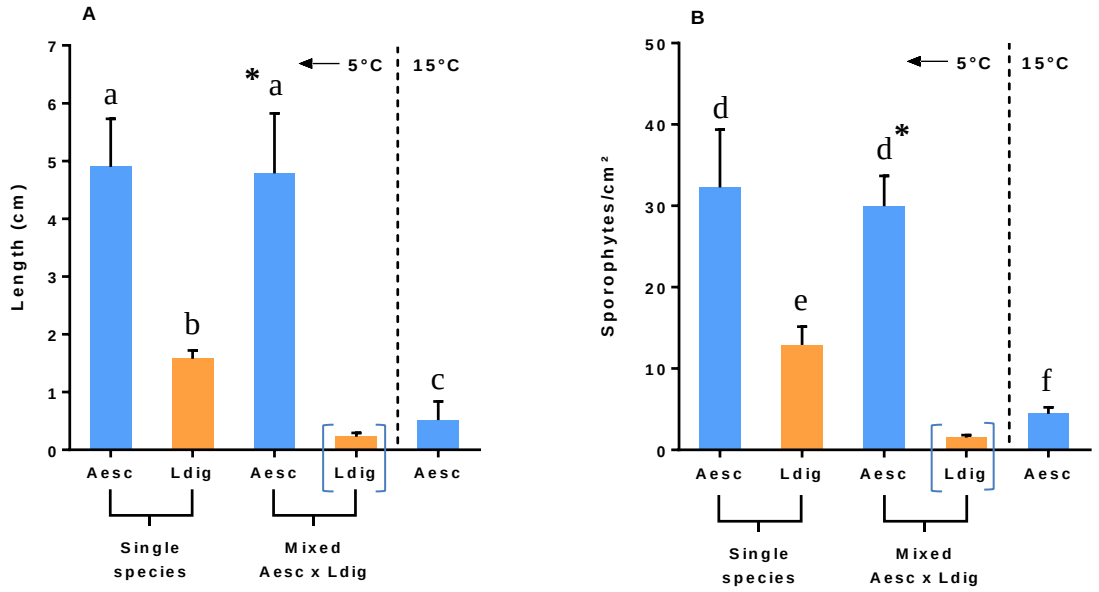

Figure 36. Mean density and length of *A. esculenta* (*Aesc*), *L. digitata* (*Ldig*) and the mixed treatment *A. esculenta/L. digitata* (*Aesc x Ldig*) sporophytes larger than 0.5 cm, measured on day 67 at 5°C and 15°C (means + SD, N=5). A: Mean length of sporophytes per treatment. B: Mean density per treatment. Note that *L. digitata* and mixed treatments at 15°C are not included owing to contamination. Moreover, *L. digitata* sporophytes found in the mixed treatment are included between brackets, even though they were all under 0.5 cm, to qualitatively assess the circumstances. Low case letters denote significant differences ($p < 0.05$). Note that documented interspecific interactions are indicated by '*'.

At 5°C, the mean length of the sporophytes in the mixed treatment (4.8 cm) was significantly higher ($p < 0.05$; Annexes Table 38) than the expectation (3.2 cm). In addition, the mean length of the sporophytes in *A. esculenta* treatment was significantly smaller ($p < 0.01$) at 15°C (0.5 cm) than at 5°C (4.8 cm) (Fig. 36A; Annexes Table 39). Furthermore, the mean length of the sporophytes in *A.esculenta*, *L. digitata* and the mixed treatments were significantly different among them ($p < 0.001$; Annexes Table 40), whereas *A. esculenta* and the mixed treatment were not significantly different in length (Fig. 36A).

There were no *L. digitata* sporophytes bigger than 0.5 cm in the mixed treatment on day 67 at 5°C (bar between brackets in Fig. 36A). When taking into account the tiny amount of *L. digitata* sporophytes measuring between 0.1 and 0.4 in the mixed treatment, there was indeed a significant difference among the treatments.

At 5°C, the number of sporophytes per cm² in the mixed treatment (30 spor./cm²) was significantly higher ($p < 0.05$; Annexes Table 42) than the expectation (23 spor./cm²), whereas *A. esculenta* and mixed treatments were not significantly different in density (32 vs. 30 spor./cm²; Fig. 36B; Annexes Tables 44, 45). On the other hand, *A. esculenta* density at 15°C (4 spor./cm²) was significantly smaller ($p < 0.001$) than *A. esculenta* density at 5°C (32 sporophytes/cm²) (Fig. 36B; Annexes Table 43).

4.3.4 Biomass of gametophytes

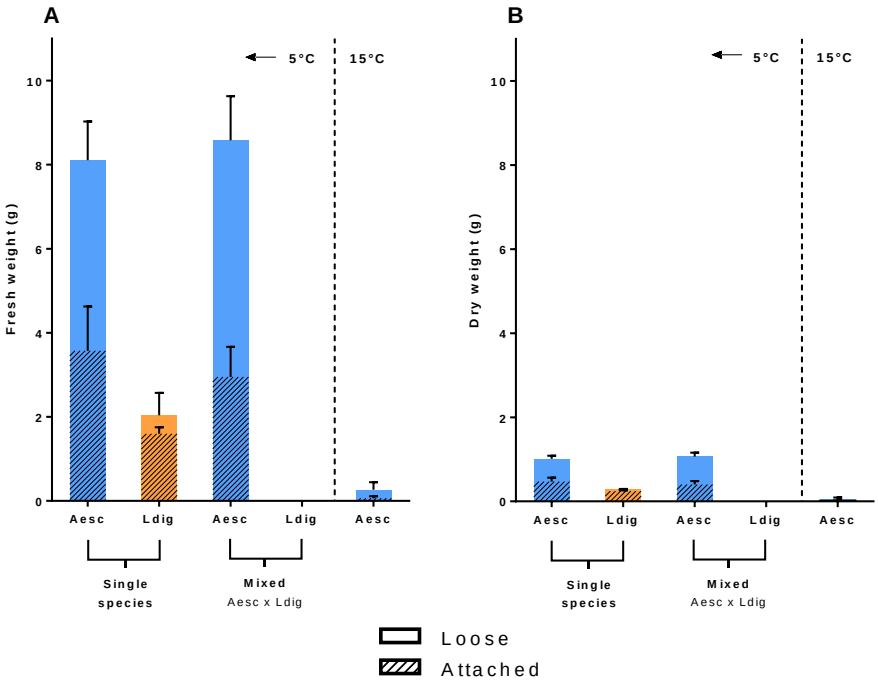

Figure 37. Mean loose and attached weight of *A. esculenta* (*Aesc*), *L. digitata* (*Ldig*) and the mixed treatment *A. esculenta/L. digitata* (*Aesc x Ldig*) sporophytes larger than 0.5 cm at 5°C and 15°C (means + SD, N=5). Sporophytes were weighed on day 67. A: Fresh weight of sporophytes. B: Dry weight of sporophytes. Note that *L. digitata* and mixed treatments at 15°C are not included owing to contamination. Moreover, *L. digitata* sporophytes in the mixed treatment are considered in the graph to qualitatively assess the circumstances.

At 5°C, the fresh and dry weight of the attached sporophytes in the mixed treatment (2.9g; 0.4g) was not significantly different to the expectation (2.6g; 0.36g) (Fig. 37). When the loose material is taken into account at 5°C, the total fresh weight of the sporophytes in the mixed treatment (8.6g) was significantly greater ($p < 0.01$) than the expectation (5.1g) (Fig. 37A). Likewise, the total dry weight of the sporophytes in the mixed treatment (1.1 g) is significantly larger ($p < 0.001$) than the expectation (0.65g) (Fig. 37B; Annexes Table 36).

The fresh and dry weight of the attached sporophytes in *A. esculenta* treatment was significantly smaller at 15°C (0.06g; 0.01g) than 5°C (3.6g; 0.5g) (Fig. 37). In addition, the total fresh and dry weight of the sporophytes in the *A. esculenta* treatment is significantly smaller at 15°C (0.27g; 0.05g) than 5°C (8.1g; 1.0g) (Fig. 37).

5. Discussion

5.1 Methodological discussion

Several methodological aspects have to be taken into account when evaluating the results. In earlier experiments carried out (Bernard 2014), the initial conditions and fragment densities were not thoroughly controlled, leading to confounding or doubtful results that showed possible interspecific interactions between *A. esculenta* and *L. digitata*. Thanks to the initial control of gametophytes during the present investigation (see 3.4, 4.1), the starting conditions were ideally similar at the beginning of both experiments, assuring reliability on the obtained results.

5.1.1 Initial control of gametophytes

Before starting the experiments, *Alaria esculenta* (♂, ♀) and *Laminaria digitata* ♂ stock cultures, were in a vegetative condition, while *Laminaria digitata* ♀ stock culture presented already some oogonia. The genetic heterogeneity of *A. esculenta* stock cultures (8 strains: 4 ♂ and 4 ♀) in contrast to two older stock cultures of *L. digitata* (2 strains: 1 ♂ and 1 ♀), might not be representative for the whole population of the Kongsfjorden (Spitsbergen). Therefore, in future experiments it would be desirable to use recently isolated *L. digitata* gametophytes from several strains.

Gametophyte fragments in both experiments were sieved to attain similar mean lengths, assuring theoretically the same number of cells and therefore the same possibilities to develop sporophytes, since every female gametophyte cell is potentially able to develop a sporophyte (Lüning & Dring, 1972; Lüning, 1981). However, although both female sieved stocks solutions had the same fragment lengths, the cell length measurements determined that *A. esculenta* ♀ was significantly longer than *L. digitata* ♀, what means that the latter had more number of cells per fragments and consequently a higher chance to develop eggs and sporophytes than the former. Nevertheless, on day 32 became evident that *A. esculenta* developed a higher number of sporophytes per fragment even though *L. digitata* had an initial advantage (see Fig. 30B).

On the other hand, female gametophytes of both species had significantly bigger cells than the males as is a general characteristic of the Laminariales (e.g. Lüning & Dring, 1972; Kain, 1979). The cell length of *A. esculenta* ♂ and *L. digitata* ♂ was not significantly different to one another, which would imply same number of antheridia per fragment, and therefore similar chances to form sperm for egg fertilization (Bartsch *et al*., 2008).

Since *A. esculenta* ♀ cells were bigger, perhaps in future designs it would be better to sieve *L. digitata* with another set of filters with a smaller pore size, so that the mean length of its fragments was lesser than those of *A. esculenta*, hence being able to better control for the same female cell numbers in every treatment. Thereby, the two female species can be compared more accurately.

The applied sowing procedure accurately produced the same initial densities which were not significantly different among the treatments in experiment 1 and 2. This was a precondition for the interpretation of results. If any interaction was detected, most probably will not be produced by different initial amount of gametophytes and hence it will not be an artefact, but refers to a true interaction between gametophyte sexes or kelp species, which was the main focus of this thesis.

5.1.2 Experiments

In experiment 1, the formation of antheridia and their release in male gametophytes could not be quantitatively assessed due to the minuscule size of antheridia. Besides, syrup fixed material deteriorated the antheridia condition and hence it was no longer reliable material. In future studies, it should be measured qualitatively the male-female interspecific interaction, perhaps by finding a less destructive fixation process or studying them live.

Moreover, parthenosporophytes that developed in the single female (*Aesc*♀, *Ldig*♀), same-sex-two-species (*Aesc*♂ *x Ldig*♂ and *Aesc*♀ *x Ldig*♀*)* and the two different sex-two-species (*Aesc*♂ *x Ldig*♀ and *Ldig*♂ *x Aesc*♀*)* treatments were included in this study, since they were not considered fertile sporophytes due to their irregular small morphologies. However, as explained by Bartsch *et al*. (2008), adult fertile parthenosporophytes with normal morphology may develop in some species but normally not in *L. digitata* (tom Dieck, 1992). The main problem in no

counting parthenosporophytes for this study was that no comparative statistics among all treatments could be done any longer, since one class suddenly is omitted.

Finally, there was a one-day delay in between the sowing process and the 'Day 0' counting in experiment 1, in which all treatments were kept in darkness. However, gametophytes were sown under low light conditions and therefore, when the 'Day 0' counting was carried out, most of the fragments were not in a vegetative stage anymore, even though they were immediately placed in darkness condition. According to Lüning & Dring (1972) and Lüning (1981), the reproductive activity of gametophytes is induced by just 6 hours or less of blue light irradiation. For future studies, there must not be a time gap between sowing and the starting of the experiment since this could have caused a mismatch in the schedule.

There were also a few methodological problems during the sporophytic macroscopic phase of experiment 2. Firstly, there was a postponement when transferring the slides with the attached sporophytes into bigger containers. *A. esculenta* treatment at 5°C, was transfer two days before the other treatments, which could have made *A. esculenta* to grow faster during this two days due to the higher amount of nutrients. However, this is a long-term experiment that was running for more than two months, hence this delay probably would not affect significantly the final results.

Furthermore, since it was necessary to change the water on a weekly basis as well as the container size when sporophytes were too big, slides were transferred to already filled clean containers. This relocation caused a significant amount of sporophytes to loosen from the slide on which they were attached. For similar upcoming experiments, it should be used another technique to refill the containers and transfer the slides, or maybe the loose material could be taken out and cultivate further in separate container.

Finally, a brown algae contamination came into view in the single *L. digitata* and mixed treatments. Consequently, these treatments were discarded and the results of the 15°C experiment could only be evaluated until day 24 (microscopic phase). The further development was only observed qualitatively.

Regarding a general methodological discussion for both experiments, using Provasoli solution (Provasoli, 1968) means always high concentration of nutrients (eutrophication) which does not take place in the artic. As explained by Svendsen *et al*. (2002), the concentrations of particulate inorganic matter (PIM) and particulate organic matter (POM) in Kongsfjorden, depends on the activity of glaciers, depth and distance from the glacier. Thus, a different enriching solution or an intermittent variation in the amount of nutrients, could have been more similar to artic conditions and may have revealed different results. Besides, the more available the resources are, the lower the negative impact of a dominant species would be. A lower concentration of nutrients (e.g. half or a quarter Provasoli solution), could have enhanced the negative interactions between species. Nevertheless, it is important to emphasize that in those containers without air supply, there was no movement of the water and therefore a shortage of nutrient may have taken place. In contrast, the PES in bigger containers was changed weekly to avoid nutrient depletion and the air supply was moving constantly the water.

Secondly, the stock cultures that were utilized in experiment 2 had been previously kept under very low light after being used in experiment 1. During this time they had already developed a few oogonia and antheridia, hence the preconditions in experiment 1 and 2 were slightly different.

Furthermore, the counting should have been carried out the same days in experiment 1 and 2, as well as taking into account the same developmental stage categories. This would have enabled a combined statistical analysis and better comparison among the three different temperatures. As both experiments were designed for a different purpose, the high amount of counting would not have been achievable in experiment 1, but was needed for experiment 2 in order not to miss pre-saturation stages (Bernard, 2014) which are highly important for showing possible competition effects in gametophytes.

Finally, three types of contamination came into sight: bacterial, dinoflagellates and brown algae. This has to be avoided as much as possible, since results can be confounded. Recently isolated healthier stock cultures and less concentrated nutrient solution are some of the possible solutions to resolve this issue.

5.2 Interspecific competition in kelp development

A. esculenta and *L. digitata* occur in the Artic and consequently their distributions overlap (Lüning, 1990). Moreover, in Kongfjorden (Spitsbergen) both species co-occur in the shallow sublittoral at a depth of 2.5m and 5m (Hop *et al*., 2012; Bartsch *et al*., 2015), causing different types of interactions between them. Besides some recent studies performed with seaweeds (e.g. Xu *et al*., 2013; Bernard 2014; Nabivailo *et al*., 2014; Bollen *et al*., 2015), not much experimental work has been conducted on this subject, especially not on the interspecific competition and interactions between these two artic kelp species. Furthermore, kelps were cultured more than two months during this study, reaching sporophyte stages of several centimeters long. In contrast, most previous investigations were carried out on microsporophytes up to a few mm in length owing to the logistical problems of culturing large algae in the laboratory (Bolton & Lüning, 1982).

The competition experiments performed with *A. esculenta* and *L. digitata* in the Alfred Wegener Institute (AWI) in Bremerhaven differed in two aspects: Firstly, experiment 1 lasted for 15 days, while experiment 2 was running for more than 2 months reaching macroscopic stages. Secondly, in experiment 1 it was attempted to study interactions between sexes and species at a particular temperature (10°C), whereas the interspecific competition at two different temperatures (5°C and 15°C), was the main focus in experiment 2.

Interspecific competition can be divided into ‘interference competition’ and ‘resource competition’ (Bartsch *et al*., 2008; Nabivailo *et al*., 2014). Both type of competitions were studied and observed during the experiments.

5.2.1 Interference competition

Interference competition is the ability to influence competitor’s physiological processes via allelopathy. Organisms produce alkaloids, cyclic peptides, terpens, volatile organic compounds (Leflaive & Ten-Hage, 2007) and pheromones, which may act as allelopathic substances and influence the growth, survival, and reproduction of other organisms (Olson & Lubchenco, 1990; Bartsch *et al*., 2008; Nabivailo *et al*., 2014). There is not much known about this topic in algae (Xu et al., 2013; Bollen *et al*., 2015).

Results of experiment 1 show that the different sexes of kelp gametophytes may exert a negative or positive effect on the development of the respective sympatric or same species.

A qualitative observation revealed that single cultured *A. Laminaria* and *L. digitata* males had closed antheridia full of sperm if no female was present, whereas in two-sex-single-species treatments males had empty antheridia. According to Lüning (1981) and Bartsch *et al*. (2008), males develop antheridia and remain unchanged until females of the same species extrude eggs and produce the pheromone lamoxirene which induces the release and attracts spermatozoids. Interestingly, in those treatments where the males were cultured only with females from the other species (*Aesc*♂ x *Ldig*♀ and *Aesc*♀ x *Ldig*♂), empty antheridia were also observed. This supports the hypothesis that the common sperm-releasing pheromone lamoxirene, shared by Laminariaceae, Alariaceae and Lessoniaceae (Maier *et al*., 2001), may act as an allelochemical compound producing an effect on gametophytes from other species. Lamoxirene could affect negatively one of the species co-existing in the same space. The extruded eggs from the faster species would release this pheromone (Maier, 1995), which would cause antheridia from both species to discharge the sperm. As explained by Bartsch *et al*. (2008), spermatozoids within 1mm range (Müller, 1981), swim directly towards the pheromone source. After the fecundation of the eggs, the sperm of both species decays, preventing fertilization of the competitor (Müller, 1981).

With the purpose to clarify the interference competition of male gametophytes with the other gametophytes in any combination, the material was fixed with syrup in semi-permanent slides on day 15 and observed afterwards. However, the image quality was low and unfortunately all males had open antheridia probably due to the fixation process. Thus, it was no longer possible to tackle visually the interaction between males and females from different species.

Quantitative analysis of gametogenesis stages in experiment 1 revealed that *L. digitata* ♂ exerts a negative effect on *A. esculenta* ♀ and a supporting effect on *L. digitata* ♀. As a consequence, less *A. esculenta* ♀ sporophytes will develop. On the other hand, the presence of *A. esculenta* ♂ did not exert any positive or negative effect on the ripening of gametophytes in any combination of sexes.

As this phenomenon was only observed at day 5, it might be a temporal negative unidirectional allelopathic interaction between the two species which only acts during oogenesis. A similar phenomenon was observed between the two kelps *Undaria* and *Macrociystis* (Bollen *et al*., 2015).

Another possible interference competition became apparent during experiment 2. On day 32 at 5°C, the density of fragments with sporophytes was significantly lower in the mixed treatment than expected. Therefore, less fragments developed sporophytes when both species were cultured together. As I could not differentiate between species at that stage, the direction of the interaction remains unknown as it was also transient. However, there is the possibility that this interaction was an unknown experimental artefact since at all other dates in both temperatures no deviations from expectation occurred in the mixed treatments.

In similar previous studies, positive interactions though allelopathic substances were also observed by Xu *et al*. (2013) between the green macroalgae *Monostroma arctium* and the red macroalgae *Porphyra yezoensis*, since the former facilitated nutrient uptake of the later. Furthermore, Bollen *et al*. (2015) documented an enhancement in oogonia formation in the kelp species *Undaria pinnatifida* when co-cultured with *Macrocystis pyrifera*, indicating a competitive advantage for *U. pinnatifada*. Alternatively, Nabivailo *et al*. (2014) indicated positive but also negative (allelopathic inhibition) interactions between common species of an *Ahnfeltia* bed community.

In any case, further studies on this area are necessary to examine mechanisms of competition between sexes and species in order to find out for instance if other allelochemical compounds such as self-produced metabolites, as presented by Nabivailo *et al*. (2014), are also involved in this type of competition.

5.2.2 Resource competition

Resource competition is the capacity to use scarce resources effectively, making them unavailable for the competitors (Carpenter, 1990). It is considered to occur mainly for space, light and nutrients (Bartsch *et al*., 2008). There is not much known about this competition in algae (e.g. Dayton *et al*., 1984; Carpenter, 1990; Nabivailo *et al*., 2014).

The results obtained in experiment 1 and 2 show that *A. esculenta* and *L. digitata* probably competed for resources. *A. esculenta* had the highest competitive ability at 5° and 10°C, while the latter was fastest at 15°C. This has been concluded analysing the development of gametophytes and sporophytes.

Both experiments were performed in the laboratory, hence space was always confined to the dimensions of the containers (plastic petri dishes, crystal petri dishes, plastic containers and plastic beakers). Moreover, in the long-term experiment 2, there was an extra space limitation since fragments were attached to 5.46 cm² slides during two months.

The light intensities used for the experiments were very low (12±1 μmol photons/m^2s) but strong enough to enable photosynthetic processes in the investigated species. As reported by Han & Kain (1996), 1-2 μmol photons/m^2s are sufficient for the growth of young sporophytes of *A. esculenta* and *L. digitata*.

5.2.2.1 Development of Gametophytes

When following gametogenesis in experiment 1 (during 15 days at 10°C) and experiment 2 (during 24 days at 5 and 15°C), *A. esculenta* developed oogonia, eggs and finally sporophytes faster than *L. digitata* at 5°C and 10°C but not at 15°C. Gametogenesis of *A. esculenta* was drastically retarded at 15°C. *L. digitata* gametogenesis developed the fastest at 10°C, while 5°C was faster than 15°C. During this time there was no general interspecific interaction, hence no resource competition, since the mixed treatment showed the expected average developmental percentages compared to the two single species treatments. Therefore, light, space and nutrients were probably not a problem during the first weeks of the kelp development under the given experimental conditions.

The possible negative impacts of one species on the other changes with the concentration of nutrients in water (Steen, 2004). As presented by Fong *et al*. (1996), the addition of nitrogen to the water suppressed the negative influence of *Enteromorpha interstinalis* on *Ulva expansa*. Thus, it can be possible that, by adding fewer nutrients to the cultures, *A. esculenta* and *L. digitata* competitive interactions during gametogenesis might change.

5.2.2.2 Sporophyte development

One main result of the present study is the clear sporophytic resource competition at 5°C, in which *A. esculenta* almost completely displaced *L. digitata* and did not let it grow further in the mixed species treatment. After 67 days, no *L. digitata* sporophytes >0.5 cm were present in the mixed cultures. After a careful examination just a few tiny sporophytes measuring 0.1-0.4 cm were present, whereas in the single species treatment *L. digitata* sporophytes grew densely but much slower than *A. esculenta* and reached lengths up to1.6 cm. Furthermore, since there is a total suppression, also allelophatic interaction might be possible.

In this case, it seems to be more a matter of space and light shortage than competition for nutrients, since the length of the sessile *A. esculenta* sporophytes in the mixed treatment was not significantly different to the *A. esculenta* single species treatment and the biomass was as expected. Besides, the air supply was mixing the water and distributing the nutrients. If it were a problem of nutrients, *A. esculenta* would have grown larger in the mixed than in the single species treatment, since there were less individuals and hence probably more nutrients available (Steen, 2004). As it was explained before, by adding fewer nutrients to all the treatments, the differences between *A. esculenta* and *L. digitata* in the mixed and single species treatments probably had been even more pronounced.

As shown in the results of experiment 1 and 2, *A. esculenta* developed faster at 5 and 10°C than *L. digitata*, which enabled the former to grow larger, occupying more space and benefitting from the light. Thereby, *A. esculenta* was probably able to act as a canopy, shading and preventing *L. digitata* to develop normally. Similarly, Melville & Connel (2001) and Dayton (1985) explain that kelp canopies have implication for the distribution and abundances of coralline algae. In addition, in experiment 2 there were loose floating sporophytes that enhanced the canopy effect on both algae.

Consequently, it can be speculated that *A. esculenta* at 5°C has a more active consumption and more efficient use of the nutrients, light and space when high nutrient concentration, low light and very limited space are given, which makes this algae very competitive and dominant. Probably, if this experiment had lasted longer, *L. digitata* would have been completely displaced and not even tiny individuals had been found.

Unfortunately due to the developing brown algal contamination sporophytic resource competition at 15°C could not be followed, as all the data would have been confounded. However, microscopic measurements done on day 28, when the contamination was just beginning, showed that the length of sporophytes in the mixed treatment was significantly larger than expected. As nearly no *A.esculenta* sporophytes had developed in the single species treatment, the sporophytes in the mixed treatment probably were mostly *L. digitata*. Thus, the growth enhancement of *L. digitata* sporophytes in the mixed treatment at 15°C probably was due to more space, less shading and more nutrients available due to the absence of normal *A. esculenta* sporophytes.

Nonetheless, *A. esculenta* developed retarded sporophytes at 15°C, especially on day 42 and 49. The parthenosporophytes development cannot be fully excluded as development was not followed microscopically after day 24. However, at day 67 the mean sporophyte length of *A. esculenta* treatment was around eight times smaller at 15°C than at 5°C.

5.3 Intraspecific competition in kelp development

Apart from the already discussed interspecific competition, negative interactions within one species were also observed during this study, especially in the sporophytic stage. These interactions can be considered intraspecific resource competition, in which space, nutrients and light may have played an important role (Carpenter, 1990).

In *A. esculenta* treatment in experiment 2 at 5°C, there were around 1020 female gametophyte fragments in each 5.46cm² slide on day 0, whereas only 305 of those fragments developed a total of 2402 sporophytes by day 32. Finally, only 170 sporophytes were bigger than 0.5 cm on day 67. Likewise, in *L. digitata* treatment in experiment 2 at 5°C, there were around 1040 female gametophyte fragments in each 5.46 cm² slide on day 0, whereas only 273 of those fragments developed a total of 1146 sporophytes by day 32. Finally, only 71 sporophytes were bigger than 0.5cm on day 67 (data is not shown in the results).

According to Steen & Scrosati (2004), high settlement densities are likely to lead to intraspecific competition for resources. Thus, densities decreased drastically with time, due to intraspecific resource competition among sporophytes that ended up hindering the smaller individuals.

5.4 Non-competitive positive interactions

Some positive interspecific interactions may have taken place during experiment 2. At 5°C, the egg and sporophyte development on day 8 and 12 respectively, were significantly higher than expected in the mixed treatment. Likewise, it was also found some temporal interactions on day 16, 20 and 24 at 15°C, as the egg development was again higher than expected in the mixed treatment. This could mean that, when both species are co-cultured together at these temperatures, there is an enhancement in the formation of eggs and subsequently sporophytes at certain time points only. One of the possible mechanisms for these positive interspecific interactions, as suggested by Nabivailo *et al*. (2014), is the use of exometabolites from neighboring gametophytes or sporophytes as an additional source of nitrogen.

5.5 Temperature tolerance and biogeographical considerations in an era of climate change

The present study was carried out at three different temperatures, so that it could be explored temperature responses of *A. esculenta* and *L. digitata* and get some ideas of how changing temperatures may influence the interaction between polar kelp communities in future.

As presented by Cosson (1973) and Lüning (1980), the growth of *L. digitata* gametophytes is retarded at 5°C while above 10°C there is a broad optimum and an optimal egg production between 10°C and 17°C (Yarish *et al*., 1990; tom Dieck, 1992). This conclusion partially matches with the results obtained in experiment 2, since *L. digitata* developed the fastest at 10°C, while 5°C was faster than 15°C. Thus, it can be concluded that *L. digitata* has a fertility optimum at 10°C while 5°C and 15°C are still within its lower and upper fertility limits. The growth at 5°C was not retarded and even faster than at 15°C, therefore this can be a genetic adaptation of the Spitzbergen strain.

Despite the fact that *L. digitata* developed eggs and sporophytes faster at 5°C than at 15°C, the longest sporophytes were measured at 15°C. Thus, there is a difference between the temperature optimum of the gametophyte development velocity and the sporophyte growth speed. It can be then concluded that 5°C is a more appropriated temperature for *L. digitata* gametogenesis, while 15°C is better for the sporophyte growth of this species.

A. esculenta gametophytes developed faster at 10°C than 5°C. On the other hand, it presented oogonia at 15°C, but it did not extrude eggs except from day 20 onwards. However, these eggs did not develop further on and decayed, so that they were not visible any longer on day 24. Probably more eggs were released later as a retarded sporophyte development became apparent after 49 days. According to tom Dieck (1993), the upper survival temperature of *A. esculenta* gametophytes is 19-20°C and 20-21°C in male and female gametophytes, respectively. Nevertheless, as it can be observed in the experiments, the temperature limits for gametophyte growth does not match with the fertility limits, which supports the theory that reproduction in kelps has a smaller range than survival (Bartsch *et al*., 2013). Thus, it can be concluded that *A. esculenta* has a fertility optimum at 10°C while 5°C is still within its fertility limits and 15°C is just at its upper fertility limit.

Nevertheless, it should be taken into account that southern strains of *A. esculenta* and *L. digitata* may behave differently than the arctic ones and therefore they might not have the same fertility limit as the strains utilized for this study.

Undoubtedly, Artic and temperate marine environments will change in the future, rising the sea water temperature an average of 1.5-2.5°C by 2100 in response to enhanced concentrations of atmospheric greenhouse gases (Bijlsma *et al*., 1995; Hoegh-Guldberg, 1999; Schmittner *et al*., 2005; IPCC, 2007). With the rising of the sea water temperature, polar seaweeds will retreat while progressing temperature seaweeds will occupy northern habitats. These changes in the community structure, with retreat and arrival of different algae species, may cause new interspecific interactions and competition that could also influence the marine environment in the Kongsfjorden.

5.6 Outlook

The results would suggest a dominance of *Alaria esculenta* over *Lamianria digitata* in Kongsfjorden, with *A. esculenta* outcompeting *L. digitata* at 5°C, through its massive growth in low light conditions. However, this result matches with the density and biomass information documented by Bartsch *et al*. (2015) at 10 m but not at 2.5 and 5 m depth. Thus, the situation changes according to depth gradient since at shallow depth (2.5 m) *L. digitata* is extremely dominant compared to *A. esculenta*. At this depth the light intensity and UV, even under the canopy, is much higher in summer than during our experiments. As a consequence, the competition might change if higher irradiances are included.

Temperature may also play an important role in the interspecific competition between these two kelp species. In this study, *A. esculenta* was dominant and competitively more efficient at 5ºC while *L. digitata* was dominating at 15ºC.

Therefore, interspecific competition varies depending on the life cycle stage, nutrient concentration, temperature and light intensity. However, further multifactorial and field experiments are urgently needed to draw more accurately final conclusion about interspecific competition in kelp communities and extrapolate them to other species and locations.

6. Conclusions

The character and intensity of interspecific competitive interactions are very important in the process of formation and functioning of any seaweed community. During this study, a clear interspecific sporophytic resource competition at 5°C was quantitatively documented, in which *A. esculenta* displaced *L. digitata* when these two kelp species were cultured together under low light condition. In addition, intraspecific sporophytic resource competition also took place in *A. esculenta* and *L. digitata* single species cultures at 5°C. Alternatively, interspecific gametophytic interference competition, probably through allelochemicals, was qualitatively observed, indicating that kelp gametophytes may exert a negative effect on the development of the sympatric species.

A. esculenta gametophytes developed faster at 10°C than 5°C, while its gametogenesis was drastically retarded at 15ªC. *L. digitata* gametophytes developed the fastest at 10°C, whereas 5°C was faster than 15°C. In contrast, the sporophytes of *L. digitata* developed faster at 15°C than 5°C. Since these kelp species develop differently depending on the temperature, the rising of the sea water temperature may change their distribution, retreating artic species while temperate seaweeds progress further north. These changes in the community structure may cause new interspecific interactions and competition that could also influence the marine environment.

Other factors, such as life cycle stage, nutrient concentration, temperature and light irradiance can regulate the intensity of interspecific competition. Thus, further multifactorial and field studies are needed to draw more accurate final conclusions about interspecific competition in kelp communities.

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Other sources

www.algaebase.org

www.arctic05.org

www.seatemperature.org

www.seaweed.ie

9. Annexes

9.1 Microscope scaling

Table 4. Microscope scaling for the inverted microscope Olympus CKX41 (Japan).

Magnification	Length of the side (mm)		Grid area (mm²)
	Grid	Square	
x4	2,5	0,25	6,25
x10	1	0,1	1
x20	0,5	0,05	0,0025
x40	0,25	0,025	0,00625

Magnification	Length (mm)	
	Whole Ruler	Smallest division
x4	2,5	0,025
x10	1	0,01
x20	0,5	0,005
x40	0,25	0,0025

Table 5. Microscope scaling for the microscope Axiophot Ilford FP4 135 DX 36 (Germany).

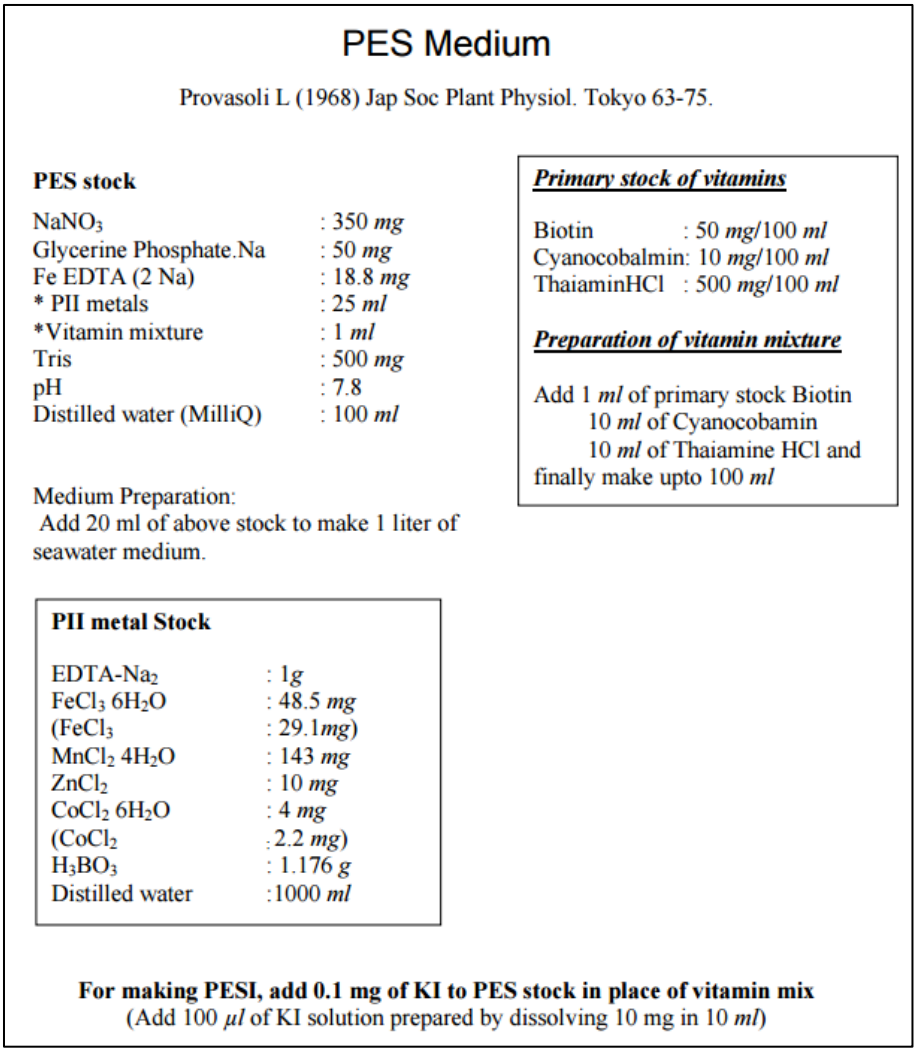
Magnification	Length of the side (mm)		Grid area (mm²)
	Grid	Square	
x4	2,5	0,25	6,25
x10	1	0,1	1
x20	0,5	0,05	0,0025
x40	0,25	0,025	0,00625

Magnification	Length (mm)	
	Whole Ruler	Smallest division
x5	2	0,02
x10	1	0,01
x20	0,5	0,005
x40	0,25	0,0025

Table 6. Microscope scaling for the stereo microscope Olympus SZX10 (Japan).

Magnification	Length of the side (mm)		Grid area (mm²)
	Grid	Square	
X1,6	6,3	0,63	39,69
X3,2	3,2	0,32	10,24
X6,3	0,16	0,16	0,0256

9.2 Provasoli enriched sea water (PES)



PES Medium

Provasoli L (1968) Jap Soc Plant Physiol. Tokyo 63-75.

PES stock

$NaNO_3$	: 350 *mg*
Glycerine Phosphate.Na	: 50 *mg*
Fe EDTA (2 Na)	: 18.8 *mg*
* PII metals	: 25 *ml*
*Vitamin mixture	: 1 *ml*
Tris	: 500 *mg*
pH	: 7.8
Distilled water (MilliQ)	: 100 *ml*

Medium Preparation:
Add 20 ml of above stock to make 1 liter of seawater medium.

Primary stock of vitamins

Biotin	: 50 *mg*/100 *ml*
Cyanocobalmin	: 10 *mg*/100 *ml*
ThaiaminHCl	: 500 *mg*/100 *ml*

Preparation of vitamin mixture

Add 1 *ml* of primary stock Biotin
10 *ml* of Cyanocobamin
10 *ml* of Thaiamine HCl and
finally make upto 100 *ml*

PII metal Stock

$EDTA\text{-}Na_2$	: 1*g*
$FeCl_3\ 6H_2O$	: 48.5 *mg*
($FeCl_3$	: 29.1*mg*)
$MnCl_2\ 4H_2O$	: 143 *mg*
$ZnCl_2$	: 10 *mg*
$CoCl_2\ 6H_2O$	: 4 *mg*
($CoCl_2$	: 2.2 *mg*)
H_3BO_3	: 1.176 *g*
Distilled water	:1000 *ml*

For making PESI, add 0.1 mg of KI to PES stock in place of vitamin mix
(Add 100 *µl* of KI solution prepared by dissolving 10 mg in 10 *ml*)

Fig 38. Ingredients of Provasoli nutrient solution utilized to enrich the sea water during the experiments. Source: Provasoli (1968).

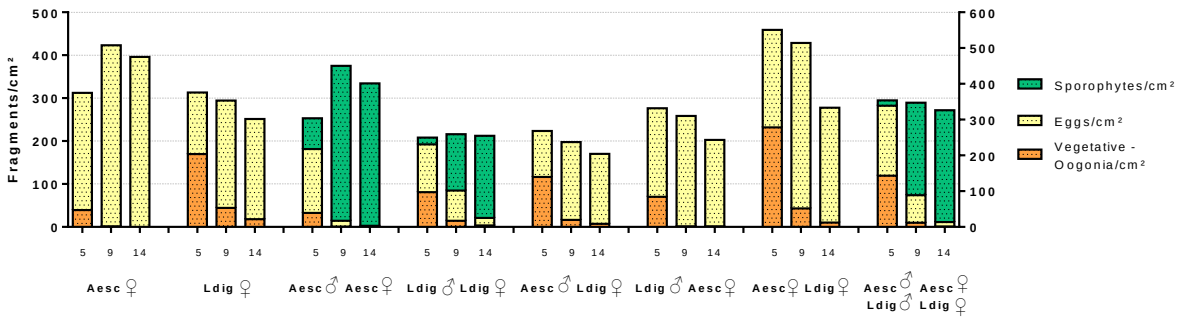


Fig 39. Fragment densities of the treatments containing female gametophytes in experiment 1 on days 5, 9 and 14. No SD are given for clarity.

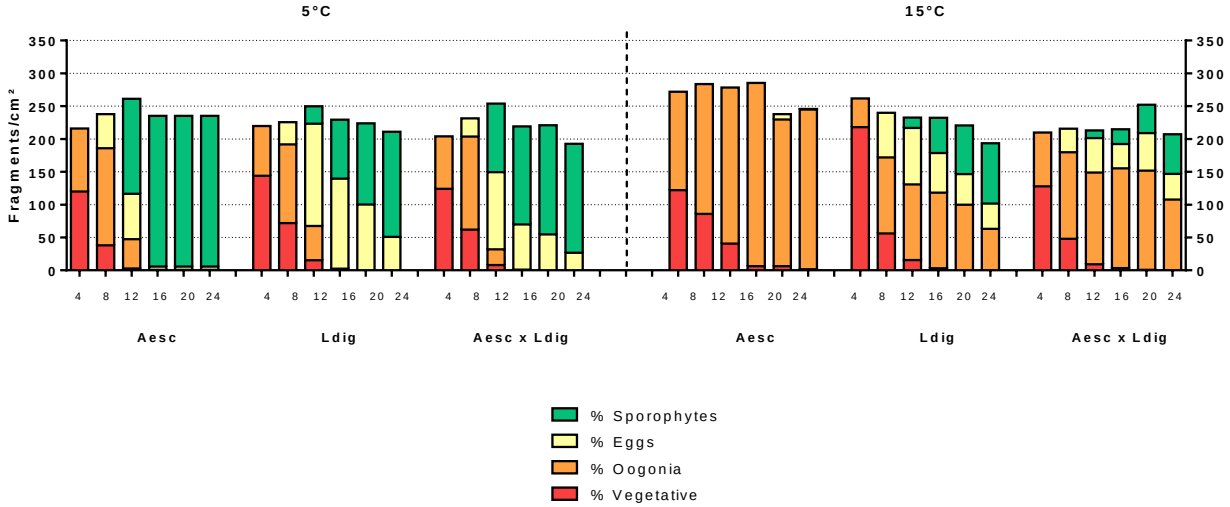


Fig 40. Fragment densities of the three treatments (single species and mixed) in experiment 2 on days 4, 8, 12, 16, 20 and 24 at two temperatures (5 and 15°C). No SD are given for clarity.

STATISTICAL ANALYSIS

9.3 Experimental gametophyte stock cultures and initial control

9.3.1 Cell Size

Table 7. Results from analyses of variance (three-factorial ANOVA) comparing cell size on sexes (♀/♂) and species (*A. esculenta, L. digitata*). p-values are set to 0,05. Significant values in red.

Source	SS	df	MS	F	p
Intercept	90216.79	1	90216.79	6603.768	0.000000
Sex	6158.15	1	6158.15	450.769	0.000000
Exp	79.79	1	79.79	5.841	0.016199
Species	1201.69	1	1201.69	87.962	0.000000
Sex*Exp	18.20	1	18.20	1.332	0.249246
Sex*Species	954.56	1	954.56	69.873	0.000000
Exp*Species	19.58	1	19.58	1.433	0.232136
Sex*Exp*Species	6.83	1	6.83	0.500	0.480111
Error	4535.59	332	13.66		

Table 8. Tukey HSD test for effects of cell size on sexes (♀/♂) and species (*A. esculenta, L. digitata*). p-values are set to 0,05. Significant values in red.

	Sex	Expe	Species	{1}25.600	{2}17.600	{3}23.370	{4}16.921	{5}12.790	{6}12.175	{7}12.076	{8}11.860
1	♀	1	*Aesc*		0.000032	0.111474	0.000032	0.000032	0.000032	0.000032	0.000032
2	♀	1	*Ldig*	0.000032		0.000032	0.991235	0.000032	0.000032	0.000032	0.000032
3	♀	2	*Aesc*	0.111474	0.000032		0.000032	0.000032	0.000032	0.000032	0.000032
4	♀	2	*Ldig*	0.000032	0.991235	0.000032		0.000042	0.000032	0.000033	0.000032
5	♂	1	*Aesc*	0.000032	0.000032	0.000032	0.000042		0.991331	0.988167	0.947744
6	♂	1	*Ldig*	0.000032	0.000032	0.000032	0.000032	0.991331		1.000000	0.999941
7	♂	2	*Aesc*	0.000032	0.000032	0.000032	0.000033	0.988167	1.000000		0.999997
8	♂	2	*Ldig*	0.000032	0.000032	0.000032	0.000032	0.947744	0.999941	0.999997	

9.3.2 Fragment Length

Table 9. Results from analyses of variance (three-factorial ANOVA) comparing fragment length on sexes (♀/♂) and species (*A. esculenta, L. digitata*). p-values are set to 0,05. Significant values in red.

	SS	Df	MS	F	p
Intercept	15814535	1	15814535	4227.773	0.000000
Sex	1453	1	1453	0.388	0.533307
Exp	47128	1	47128	12.599	0.000409
Species	7150	1	7150	1.912	0.167190
Sex*Exp	316	1	316	0.084	0.771506
Sex*Species	45	1	45	0.012	0.912380
Exp*Species	222	1	222	0.059	0.807660
Sex*Exp*Species	520	1	520	0.139	0.709300
Error	2887766	772	3741		

Table 10. Tukey HSD test for effects of fragment length on sexes (♀/♂) and species (*A. esculenta, L. digitata*). p-values are set to 0,05. Significant values in red.

	Sex	Exp	Species	{1}199.86	{2}193.34	{3}182.00	{4}174.00	{5}200.72	{6}188.68	{7}175.29	{8}170.29
1	♀	1	*Aesc*		0.980564	0.771478	0.312701	1.000000	0.729203	0.380661	0.158955
2	♀	1	*Ldig*	0.980564		0.975535	0.691162	0.961202	0.997500	0.761623	0.468390
3	♀	2	*Aesc*	0.771478	0.975535		0.999391	0.725942	0.999054	0.999810	0.993101
4	♀	2	*Ldig*	0.312701	0.691162	0.999391		0.271289	0.904222	1.000000	0.999997
5	♂	1	*Aesc*	1.000000	0.961202	0.725942	0.271289		0.647281	0.334597	0.132974
6	♂	1	*Ldig*	0.729203	0.997500	0.999054	0.904222	0.647281		0.939502	0.743395
7	♂	2	*Aesc*	0.380661	0.761623	0.999810	1.000000	0.334597	0.939502		0.999974
8	♂	2	*Ldig*	0.158955	0.468390	0.993101	0.999997	0.132974	0.743395	0.999974	

9.3.3 Density

Experiment 1

Table 11. Results from analyses of variance (one-factorial ANOVA) compering fragment density on treatments. p-values are set to 0,05. Significant values in red.

	SS	Df	MS	F	p
Intercept	4269785	1	4269785	958.3316	0.000000
Treatment	44894	10	4489	1.0076	0.457629
Error	147029	33	4455		

Experiment 2

Table 12. Results from analyses of variance (two-factorial ANOVA) comparing fragment density on temperature (5/15°C) and treatments. p-values are set to 0,05. Significant values in red.

	SS	Df	MS	F	p
Intercept	2530849	1	2530849	2867.576	0.000000
Temp	53	1	53	0.060	0.810596
Treatment	4234	2	2117	2.399	0.132924
Temp*Treatment	462	2	231	0.262	0.773794
Error	10591	12	883		

Experiment 1 vs. experiment 2

Table 13. Results from t-test (independent by groups) analyses comparing fragment densities in experiment 1 and 2. p-values are set to 0,05. Significant values in red.

	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	P Variances
Dens 2 vs. Dens 1	374.9703	311.5134	3.858870	60	0.000281	18	44	30.03977	66.80812	4.946126	0.000814

9.4 Experiment 1

Table 14. Results from t-test (independent by groups) analyses comparing female developmental stages (Vegetative-oogonia and eggs) on day 0. p-values are set to 0,05. Significant values in red.

Variable	Mean *Aesc* ♀	Mean *Ldig* ♀	t-value	df	p	Valid N *Aesc* ♀	Valid N *Ldig* ♀	Std. Dev. *Aesc* ♀	Std. Dev. *Ldig* ♀	F-ratio Variances	p Variances
Veg-Oog	98.7965	98.4066	0.3952	6.0000	0.7063	4.0000	4.0000	1.1586	1.5971	1.9003	0.6112
Eggs	1.2035	1.5919	-0.3942	6.0000	0.7071	4.0000	4.0000	1.1586	1.5942	1.8933	0.6132

Table 15. Results from t-test (independent by groups) analyses comparing male developmental stages (Vegetative and antheridia) on day 0. p-values are set to 0,05. Significant values in red.

Variable	Mean *Aesc* ♀	Mean *Ldig* ♀	t-value	df	p	Valid N *Aesc* ♀	Valid N *Ldig* ♀	Std. Dev. *Aesc* ♀	Std. Dev. *Ldig* ♀	F-ratio Variances	p Variances
Veget.	21.0558	20.0589	0.2543	6.0000	0.8078	4.0000	4.0000	6.8228	3.8633	3.1189	0.3752
Anther	78.9442	79.9411	-0.2543	6.0000	0.8078	4.0000	4.0000	6.8228	3.8633	3.1189	0.3752

Table 16. Results from t-test (single sample) analyses comparing 'Vegetative-Oogonia' developmental stage percentage of the mixed treatment against the average of the 2 single treatment percentages (Reference constant column) on day 5. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dv.	N	Std. Err.	Reference Constant	t-value	df	p
Day 9	21.93631	4.344235	3	2.508145	18.16000	1.505620	2	0.271114

Table 17. Results from t-test (single sample) analyses comparing 'Eggs' developmental stage percentage of the mixed treatment against the average of the 2 single treatment percentages (Reference constant column) on day 5. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dv.	N	Std. Err.	Reference Constant	t-value	df	p
Day 9	21.93631	4.344235	3	2.508145	18.16000	1.505620	2	0.271114

Table 18. Results from t-test (single sample) analyses comparing 'Sporophytes' developmental stage percentage of the mixed treatment against the average of the 2 single treatment percentages (Reference constant column) on day 5 and 9. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dv.	N	Std. Err.	Reference Constant	t-value	df	p
Day 9	74.88512	5.697216	3	3.289289	78.38682	-1.06457	2	0.398586

Table 19. Results from analyses of variance (one-factorial ANOVA) for differences of 'Vegetative-Oogonia' developmental stages among 3 treatments with *Aesc♀ (Aesc♀, Aesc♂ x Aesc♀, Dig♂ x Ala♀)* on day 5. p-values are set to 0,05. Significant values in red. (Levene test > 0.05).

Day 5	SS	Df	MS	F	p
Intercept	2649.909	1	2649.909	227.0377	0.000005
Species	321.415	2	160.707	13.7690	0.005726
Error	70.030	6	11.672		

Table 20. Tukey HSD test for differences of 'Vegetative-Oogonia' developmental stages among 3 treatments with *Aesc♀ (Aesc♀, Aesc♂ x Aesc♀, Ldig♂ x Aesc♀)* on day 5. p-values are set to 0,05. Significant values in red.

	Species	{1} 13.375	{2} 12.507	{3} 25.596
1	*Aesc*		0.948527	0.011202
2	*Aesc ♀*	0.948527		0.008141
3	*Ldig ♂ x Aesc ♀*	0.011202	0.008141	

Table 21. Results from analyses of variance (one-factorial ANOVA) for differences of 'Vegetative-Oogonia' developmental stages among 3 treatments with *Ldig♀ (Ldig♀, Ldig♂ x Ldig♀, Aesc♂ x Ldig♀)* on day 5. p-values are set to 0,05. Significant values in red. (Levene test > 0.05).

Day 5	SS	Df	MS	F	p
Intercept	21289.51	1	21289.51	621.3298	0.000000
Species	407.77	2	203.89	5.9504	0.037657
Error	205.59	6	34.26		

Table 22. Tukey HSD test for differences of 'Vegetative-Oogonia' developmental stages among 3 treatments with *Ldig♀ (Ldig♀, Ldig♂ x Ldig♀, Aesc♂ x Ldig♀*) on day 5. p-values are set to 0,05. Significant values in red.

	Species	{1} 39.220	{2} 54.552	{3} 52.137
1	*Ldig*		0.042280	0.079044
2	*Ldig ♀*	0.042280		0.871666
3	*Aesc ♂ x Ldig ♀*	0.079044	0.871666	

9.4.3 Day 15 – Semi-permanent slides

Table 23. Results from t-test (single sample) analyses comparing 'Length' and 'Width' of the mixed treatment against the average of the 2 single treatment (Reference constant column) on day 15. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Length	478.9167	216.3156	60	27.92623	314.0000	5.905440	59	0.000000
Width	103.4167	41.24638	60	5.324885	66.08333	7.011106	59	0.000000

Table 24. Results from t-test (independent by groups) analyses comparing 'Length' and 'Width' of Aesc vs. Ldig on day 15. p-values are set to 0,05. Significant values in red.

	Mean *Aesc*	Mean *Ldig*	t-value	df	p
Length	427.5000	200.5000	11.35237	118	0.000000
Width	66.00000	66.16667	-0.034243	118	0.972741

9.5 Experiment 2

9.5.1 Microscopic phase

Table 25. Results from t-test (single sample) analyses comparing 'Eggs' developmental stage percentage of the mixed treatment against the average of the 2 single treatment percentages (Reference constant column) on day 8, 12 and 16 at 5°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Day 8	12.52000	1.156287	5	0.517107	17.88000	-10.3654	4	0.000489
Day 12	46.30000	4.548626	5	2.034207	44.67000	0.801295	4	0.467857
Day 16	31.56000	3.138152	5	1.403424	31.12000	0.313519	4	0.769555

Table 26. Results from t-test (single sample) analyses comparing 'Sporophytes' developmental stage percentage of the mixed treatment against the average of the 2 single treatment percentages (Reference constant column) on day 12 and 16 at 5°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Day 12	41.00000	5.887274	5	2.632869	33.16000	2.977740	4	0.040830
Day 16	67.98000	3.101129	5	1.386867	68.16000	-0.129789	4	0.902998

Table 27. Results from t-test (single sample) analyses comparing 'Eggs' developmental stage percentage of the mixed treatment against the average of the 2 single treatment percentages (Reference constant column) on day 8, 12 and 16 at 15°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Day 8	16.82000	2.892577	5	1.293600	14.38000	1.886210	4	0.132328
Day 12	24.78000	6.367653	5	2.847701	18.31000	2.272008	4	0.085538
Day 16	17.28000	2.601346	5	1.163357	13.16000	3.541475	4	0.023982

Table 28. Results from t-test (single sample) analyses comparing 'Sporophytes' developmental stage percentage of the mixed treatment against the average of the 2 single treatment percentages (Reference constant column) on day 12, 16, 20 and 24 at 15°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Day 12	5.600000	3.644859	5	1.630031	3.350000	1.380342	4	0.239604
Day 16	10.52000	2.533180	5	1.132872	11.62000	-0.970983	4	0.386540
Day 20	16.88000	4.769906	5	2.133167	17.14000	-0.121885	4	0.908868
Day 24	30.06000	12.60726	5	5.638138	23.77000	1.115617	4	0.327084

Table 29. Results from t-test (single sample) analyses comparing 'Length' of the mixed treatment against the average length of the 2 single treatments (Reference Constant) on day 28 at 5°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Length	658.2000	581.3818	100	58.13818	736.0000	-1.33819	99	0.183900

Table 30. Results from t-test (single sample) analyses comparing 'Length' of the mixed treatment against the average length of the 2 single treatments (Reference Constant) on day 28 at 15°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Length	863.4000	500.4003	100	50.04003	367.6000	9.908068	99	0.000000

Table 31. Results from t-test (single sample) analyses comparing 'Length' of the mixed treatment against *L. digitata* treatment (Reference Constant) on day 28 at 15°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Length	863.4000	500.4003	100	50.04003	735.2000	2.561949	99	0.011916

Table 32. Results from t-test (single sample) analyses comparing 'Fragments with sporophyte(s)', 'Sporophytes' and 'Sporophytes per fragment' of the mixed treatment against the average of the 2 single treatment (Reference constant column) on day 32 at 5°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Frag	14.40000	3.748333	5	1.676305	21.30000	-4.11619	4	0.014658
Spor	83.60000	32.04177	5	14.32951	130.1000	-3.24505	4	0.031523
Sp/Fr	5.662577	0.829581	5	0.371000	5.951969	-0.780032	4	0.478950

Table 33. Results from analyses of variance (one-factorial ANOVA) comparing 'Fragments with sporophyte(s)' in the mixed, *L. digitata* and *A. esculenta* treatments on day 32 at 5°C. p-values are set to 0,05. Significant values in red.

	SS	Df	MS	F	p
Intercept	5415.000	1	5415.000	181.5591	0.000000
Species	173.100	2	86.550	2.9019	0.093757
Error	357.900	12	29.825		

Table 34. Results from t-test (single sample) analyses comparing 'Fragments with sporophyte(s)', 'Sporophytes' and 'Sporophytes per fragment' of the mixed treatment against the average of the 2 single treatments (Reference constant column) on day 32 at 15°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Frag	8.800000	1.151086	5	0.514782	5.850000	5.730587	4	0.004592
Spor	18.60000	2.902585	5	1.298075	12.45000	4.737783	4	0.009052
Sp/Fr	2.124755	0.279366	5	0.124936	1.046001	8.634416	4	0.000989

Table 35. Results from t-test (independent by groups) analyses comparing number of 'Sporophytes per fragment' in the mixed and *L. digitata* treatments on day 32 at 15°C. p-values are set to 0,05. Significant values in red.

	Mean *Ldig*	Mean *Aesc x Ldig*	t-value	df	p
Sp/Fr	2.092003	2.124755	-0.155199	8	0.880508

9.5.2 Macroscopic phase

Table 36. Results from t-test (single sample) analyses comparing 'Attached/Total Dry/Fresh Weight' of the mixed treatment against the average of the 2 single treatments (Reference constant column) on day 67 at 5°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Attached Fresh Weight	2.949320	0.715472	5	0.319969	2.584070	1.141517	4	0.317351
Attached Dry Weight	0.400620	0.083164	5	0.037192	0.358280	1.138408	4	0.318505
Total Fresh Weight	8.579800	1.143411	5	0.511349	5.072180	6.859541	4	0.002365
Total Dry Weight	1.066340	0.104719	5	0.046832	0.646650	8.961641	4	0.000858

Table 37. Results from t-test (independent by groups) analyses comparing 'Fresh weight' in *A. esculenta* and the mixed treatments on day 67 at 5°C. p-values are set to 0,05. Significant values in red.

	Mean *Aesc*	Mean *Aesc x Ldig*	t-value	df	p	Valid N *Aesc*	Valid N *Aesc x Ldig*	Std. Dev. *Aesc*	Std. Dev. *Aesc x Ldig*	F-ratio Variances	p Variances
Sum FW	3.5710	2.9493	1.0889	8	0.3078	5	5	1.0572	0.7154	2.1837	0.4679

Table 38. Results from t-test (single sample) analyses comparing 'Length' of the mixed treatment against the average of the 2 single treatments (Reference constant column) on day 67 at 5°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Length	4.786000	1.039485	5	0.464872	3.238000	3.329949	4	0.029107

Table 39. Results from t-test (independent by groups) analyses comparing 'Length' in *A. esculenta* at 5°C and 15°C on day 67. p-values are set to 0,05. Significant values in red.

	Mean Dig	Mean *Ala x Dig*	t-value	df	p
Length	4.786000	0.515000	21.58354	53	0.000000

Table 40. Results from analyses of variance (one-factorial ANOVA) comparing 'Length' in the mixed, *L. digitata* and *A. esculenta* treatments on day 67 at 5°C. p-values are set to 0,05. Significant values in red. (Levene > 0.05)

	SS	Df	MS	F	p
Intercept	211.3877	1	211.3877	354.1881	0.000000
Species	35.5437	2	17.7718	29.7774	0.000022
Error	7.1619	12	0.5968		

Table 41. Tukey HSD test comparing 'Length' in the mixed, *L. digitata* and *A. esculenta* treatments on day 67 at 5°C. p-values are set to 0,05. Significant values in red.

	Species	{1} 4.8980	{2} 1.5780	{3} 4.7860
1	*Aesc*		0.000234	0.971596
2	*Ldig*	0.000234		0.000251
3	*Aesc x Ldig*	0.971596	0.000251	

Table 42. Results from t-test (single sample) analyses comparing 'Density of sporophytes' of the mixed treatment against the average of the 2 single treatments (Reference constant column) on day 67 at 5°C. p-values are set to 0,05. Significant values in red.

	Mean	Std. Dev.	N	Std. Err.	Reference Constant	t-value	df	p
Length	29.96337	3.705341	5	1.657079	22.58242	4.454197	4	0.011210

Table 43. Results from t-test (independent by groups) analyses comparing 'Density of sporophytes' in *A. esculenta* at 5°C and 15°C on day 67. p-values are set to 0,05. Significant values in red.

	Mean Aesc5	Mean Aesc15	t-value	df	p
Length	32.27106	4.432234	8.723826	8	0.000023

Table 44. Results from analyses of variance (one-factorial ANOVA) comparing 'Density of sporophytes' in the mixed, *L. digitata* and *A. esculenta* treatments on day 67 at 5°C. p-values are set to 0,05. Significant values in red. (Levene > 0.05)

	SS	Df	MS	F	p
Intercept	9407.078	1	9407.078	408.4475	0.000000
Species	1120.293	2	560.147	24.3211	0.000060
Error	276.376	12	23.031		

Table 45. Tukey HSD test comparing 'Density of sporophytes' in the mixed, *L. digitata* and *A. esculenta* treatments on day 67 at 5°C. p-values are set to 0,05. Significant values in red.

	Species	{1} 4.8980	{2} 1.5780	{3} 4.7860
1	*Aesc*		0.000270	0.733451
2	*Ldig*	0.000270		0.000462
3	*Aesc x Ldig*	0.733451	0.000462	