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Furthering knowledge on seaweed growth and development to facilitate sustainable aquaculture

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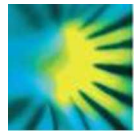
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Furthering knowledge on seaweed growth and development to facilitate sustainable aquaculture.

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24 **Abstract**

25 Macroalgae (seaweeds) are the subject of increasing interest for their potential as a source of
26 valuable, sustainable biomass in the food, feed, chemical and pharmaceutical industries. Compared
27 to microalgae, the pace of knowledge acquisition in seaweeds is slower despite the availability of
28 whole-genome sequences and model organisms for the major seaweed groups. This is partly due to
29 specific hurdles related to the large size of these organisms and their slow growth. As a result, this
30 basic scientific field is falling behind, despite the societal and economic importance of these
31 organisms. Here, we argue that sustainable management of seaweed aquaculture requires
32 fundamental understanding of the underlying biological mechanisms controlling macroalgal life
33 cycles - from the production of germ cells to the growth and fertility of the adult organisms - using
34 diverse approaches requiring a broad range of technological tools. This viewpoint highlights
35 several examples of basic research on macroalgal developmental biology that could enable the
36 step-changes which are required to adequately meet the demands of the aquaculture sector.

37 **Ecological and societal position of macroalgae**

38 Macroalgae are macroscopic aquatic organisms belonging to three distinct and distantly-related
39 eukaryotic lineages (commonly named green, red, and brown algae). Their unicellular ancestors
40 diverged more than 1.6 billion years ago (Parfrey *et al.*, 2011) implying independent acquisitions of
41 multicellularity, and leading to a bewildering diversity of life cycles, fertilization processes and
42 morphogenetic strategies. At the ecological level, macroalgae fulfil important roles as key habitat-
43 structuring agents and primary producers in coastal ecosystems. The goods and services seaweeds
44 (marine macroalgae) support are varied (Figure 1), and include elevated secondary production,

45 nutrient cycling, energy capture and flow, and coastal defence (Steneck *et al.*, 2002). They can also
46 significantly contribute to carbon sequestration at a level exceeding that of angiosperm marine
47 coastal vegetation (up to 1.5 times as much as seagrass meadows, salt marshes and mangroves and
48 up to 2% of the annual anthropogenic emission; Krause-Jensen & Duarte, 2016 and references
49 therein). In addition, macroalgae support complex food webs in coastal zones and provide habitats
50 and food for associated organisms, from apex predators to invertebrates (Reisewitz *et al.*, 2006).
51 Macroalgal communities also enable transfer of biomass between ecosystems (Krumhansl &
52 Scheibling, 2012), removal of dissolved nutrients from coastal waters and coastal protection from
53 erosion (Arkema *et al.*, 2013). De Groot *et al.* (2012) estimated the value of coastal ecosystem
54 services provided by macroalgae to be over 28,000 intl.\$·ha⁻¹·year⁻¹.

55 Seaweeds are also an alternative/additional source of food, feed, fuel, biomolecules and livelihood
56 for humans. Over 80% of macroalgal production and harvesting is at present destined for human
57 consumption directly (Abreu *et al.*, 2014) or as hydrocolloids (thickeners, gelling agents, etc)
58 (Rebours *et al.*, 2014). Macroalgae are also used as fertilizers and animal feed (Makkar *et al.*,
59 2016). In addition, the industrial sector uses seaweed biomass for nutraceuticals, cosmetics,
60 biotechnological and pharmaceutical applications, thus propelling the growth of seaweed
61 biotechnology (Mazarrasa *et al.*, 2013). Currently, ~28 million tonnes of seaweeds per year (wet
62 weight) are produced and, as a proxy for the growth of the biotechnology-market of seaweed-
63 derived products, seaweed-related patent applications increased at a rate of 11% per year since
64 1990 (Mazarrasa *et al.*, 2014).

65 While in Asia 99% of seaweed production is sourced from cultivation (accounting for 93% of the
66 global production in 2013) (FAO, 2016), the dominant practice of non-Asian countries is still
67 harvesting natural stocks. However, the availability of wild stocks under the current scenario of
68 global change needs to be assessed, while management plans for seaweed exploitation must be
69 adapted to the natural population dynamics of commercially important species. Increasing demands
70 for high-quality seaweed biomass may therefore affect the long-term sustainability of seaweed

71 exploitation. Seaweed cultivation is the alternative to cope with industry's demand for biomass,
72 concomitantly protecting natural resources (Fig. 1). Unlike terrestrial crops, they do not compete
73 for arable land, fertilizer and freshwater resources. Furthermore, the development of Integrated
74 Multi-Trophic Aquaculture (IMTA: co-cultivation of seaweeds with fin/shell fishes) enables
75 recapture of excessive inorganic nutrients released in coastal areas by fish farms, thereby
76 improving their sustainability (Holdt & Edwards, 2014). Beyond aquaculture proper, seaweed
77 cultivation could also function as a general instrument for circular resource management (Seghetta
78 *et al.*, 2016), treatment of waste-water produced by land-based farming and municipal treatment
79 plants (Neveux *et al.*, 2016), heavy metal biosorption (He & Chen, 2014) and recolonisation of
80 artificial reefs (Fig. 1). As a response to this assessment, the European seaweed aquaculture sector
81 has progressively expanded, accounting for 12% of total European biomass production in 2013
82 (FAO, 2016). Further expansion calls for advances in seaweed production technology, which rely
83 on a better knowledge of both the environmental and the intrinsic factors controlling the
84 development of macroalgae.

85 **How could developmental biology help solve bottlenecks in seaweed aquaculture?**

86 *Mastering genetics through the control of the life cycle*

87 Most seaweeds have complex, biphasic life cycles, involving free-living haploid gametophyte and
88 diploid sporophyte generations (Coelho *et al.*, 2007) (Box 1). Either phase of the life cycle can be
89 exploited, depending on the seaweed species. The harvestable biomass of kelps consists of
90 sporophytes up to several meters long (45 m in *Macrocystis*), while in nori (*Pyropia and*
91 *Porphyra*), the life stage of interest is the haploid gametophyte. Other exploited seaweeds e.g.
92 *Gracilaria* and *Chondrus* (red algae) have isomorphic life-cycles, with both sporophyte and
93 gametophyte developing macroscopic exploitable thalli. Currently, clonal propagation (e.g. red alga

94 *Kappaphycus*) and recourse to a limited number of parent genotypes (kelp) account for the
95 production of most commonly cultivated seaweeds. The resulting impoverishment of genetic
96 diversity increases seaweed susceptibility to diseases and decreases their fitness within their
97 cultivation environment (Loureiro *et al.*, 2015). For example, the continuous vegetative
98 propagation of the carrageenophyte *Kappaphycus* in intensively cultivated areas has increased its
99 vulnerability to diseases (e.g. bacterial mediated “ice-ice” disease), thereby dramatically impacting
100 the production in various countries (Largo *et al.*, 1995). This problem requires counteraction by the
101 selection of new breeding strains, potentially through artificial hybrids (Gupta *et al.*, 2015), but
102 more optimally through crossings, as somatic hybridisation usually results in severe and unstable
103 phenotypic alteration (Charrier *et al.*, 2015). However, whilst in some seaweeds the promotion of
104 sexual reproduction still requires development (e.g. *Gracilariopsis*; Zhou *et al.*, 2013), the loss of
105 the genetic patrimony resulting from cross-fertilisation might be detrimental to maintaining specific
106 and valuable genotypes resulting from decades of selection. Therefore, manipulating the different
107 steps of the seaweed life cycles would allow a balance between the maintenance of given
108 genotypes of interest and controlled breeding. Progress in basic research opens possible paths to
109 bypass steps of the life cycle, thereby allowing to reach this goal (Box 1).

110 *Manipulating the sexual life cycle.*

111 Most cultivated seaweeds reproduce sexually (kelps, red algae *Porphyra* ssp.), placing both time
112 and genetic constraints on seaweed farmers. Physiological studies have long been establishing
113 protocols for maintaining seaweeds in a vegetative stage or shifting them to the next phase using
114 specific temperature and light conditions, or even by tissue ablation. This allows year-round
115 production of juveniles and increases the cultivated net biomass (Pang & Lüning, 2004). Several
116 illustrations of these practices applied to exploited seaweeds are displayed in Box 1. Recent
117 fundamental studies propose potential alternatives. Treatments with algal phytohormones could be
118 used to control the vegetative-to-reproductive transition and speed up reproduction, as illustrated in

119 the red alga *Grateloupia imbricata* upon addition of methyl jasmonate (García-Jiménez *et al.*,
120 2016).

121 *Promoting parthenogenesis.*

122 Other seaweeds propagate vegetatively from a single life phase through parthenogenesis, mainly by
123 apogamy but also by apomeiosis. The flexibility is high and is a valuable feature for aquaculture, as
124 it allows the maintenance of a specific genotype in potentially morphologically different organisms
125 (Box 1, left side). Parthenogenesis can be induced by hybridisation (e.g. *Caloglossa*
126 tetrasporophytes; Kamiya & West, 2008) or through chemical treatments preventing gamete
127 motility (e.g. formaldehyde in brown algae Ectocarpales; Gwo & Chen, 1999). The lab-based
128 identification of endogenous factors controlling seaweed parthenogenesis might provide more
129 natural alternatives to regulate or manipulate parthenogenesis in aquaculture. Recently, Han *et al.*
130 (2014) identified three mitochondrial proteins involved in the control of parthenogenesis in
131 *Scytosiphon lomentaria* (brown alga Ectocarpales). In parallel, Arun *et al.* (2013) showed that algal
132 chemical factors (so far unidentified) secreted by the parthenosporophyte of *Ectocarpus siliculosus*
133 (brown alga Ectocarpales) control the fate of the released zoospores (Box 1). Coelho *et al.* (2011)
134 showed that the whole parthenosporophytic stage itself was controlled by a single genetic locus.
135 The characterisation of these factors could lead to the development of additional strategies to
136 control parthenogenesis.

137 Finally, Li *et al.*, (2014) produced *Undaria pinnatifida* (brown alga) gametophytes that made only
138 male gametes from both oogonia and antheridia (Shan *et al.*, 2015). These gametes are able to self-
139 cross and to produce homozygous male diploid sporophytes. This example illustrates that crosses
140 are controlled by the morphological identity of the reproductive organs rather than by their
141 genotypes, emphasizing the importance of a control over morphogenesis.

142 In parallel to these improvements for seaweeds cultivated off-shore (Fernand *et al.*, 2017),
143 standardized protocols should also be developed specifically for not-yet cultivated, high-value
144 seaweeds amenable to on-shore cultivation. This includes seaweeds producing high-value

145 chemicals, or seaweeds in high demand on the food market, such as *Ulva*, *Palmaria*, *Porphyra*,
146 *Cystoseira*, *Himantalia*, *Codium*, *Polysiphonia* and *Asparagopsis* (Abreu *et al.*, 2014), as well as
147 the red macroalgae *Ochtodes* and *Portieria* cultivated in photobioreactors (Rorrer & Cheney,
148 2004).

149 Altogether, basic research into the development and reproduction of macroalgae will likely provide
150 alternative means of manipulating seaweed reproduction, which will be very valuable for future
151 breeding programmes and aquaculture practices (Cottier-Cook *et al.*, 2016).

152 *Early and microscopic stages of development*

153 Seaweed growth starts with the formation and development of juveniles, which originate from the
154 release and germination of single cells (zygotes or spores). They subsequently attach to marine
155 substrata to initiate their sessile development (bloom-forming algae are usually free-living).
156 Deciphering the early and microscopic developmental stages of seaweeds is an important
157 requirement for future integrative management of their cultivation (Fig. 2). Exploitation of seaweed
158 biomass concentrates on the macroscopic life-cycle stage, which is the sporophyte in the most
159 predominantly exploited brown algae (*Ecklonia*, *Laminaria*, *Saccharina*, *Undaria*), together with
160 the gametophyte in red seaweeds (*Gracilaria*, *Kappaphycus*, *Eucheama*) and in some isomorphic
161 green (*Ulva*) seaweeds. Optimizing fertilisation success could help control the rate of production of
162 seaweed embryos in hatcheries, which, when too high, impedes the quality of sporophyte juveniles
163 (Fig. 2 and 3). Environmental cues inducing fertility and spore/gamete release have been
164 determined for tens of seaweed species (photoperiod, irradiance, temperature and nutrient
165 concentration; previous section and Box 1). However, the paucity of molecular studies regarding
166 e.g. the periodicity of gamete release, attraction of gametes to opposite sex or mating type, and
167 cell-cell recognition (Fig. 3) stands in a stark contrast to the wealth of eco-physiological and
168 biochemical studies that predate the molecular era. As an illustration, in certain *Ulva* species,

169 gametogenesis and subsequent gamete release can be artificially induced by removal of sporulation
170 and swarming inhibitors (Vesty *et al.*, 2015 and references therein), but so far, neither these
171 inhibitors nor the signalling pathways inducing gametogenesis have been characterised. Similar
172 cases could be made for pheromone signalling in brown seaweeds (Boland, 1995) and glycoprotein
173 recognition between opposite-sex gametes (Schmid *et al.*, 1994).

174 Many macroalgal zygotes experience polarisation prior to the growth and development of the
175 embryo (Fig. 3), similarly to land plants and metazoans. Whether polarisation is necessary for
176 proper development, and the identity of polarisation cues and regulatory factors, are unknown for
177 most macroalgae: only Fucales and Dictyotales (brown algae) zygotes have allowed the
178 identification of detailed polarisation cues (light direction and location of sperm entry; Brownlee *et*
179 *al.*, 2001; Bogaert *et al.*, 2017) and of specific cell cycle checkpoints (Bothwell *et al.*, 2008).
180 Bogaert *et al.* (2017) recently described in *Dictyota* a unique two-phase polarisation mechanism,
181 thereby illustrating the importance of seaweeds to decipher fundamental developmental processes
182 in the tree of life.

183 *Controlled growth and organogenesis factors: towards biomass production monitoring,*

184 Production of large seaweed biomass with specific features of industrial interest (polysaccharides,
185 proteins and pigments) depends both on seaweed net growth and seaweed capacity to grow organs
186 and tissues with specific structures and compositions. Indeed, the quantity and quality of key
187 compounds vary within the algal body (beta-glucan in *Durvillaea*: Bobadilla *et al.*, 2013;
188 phytohormones in *Sargassum*: Li *et al.*, 2016), and cells with thicker walls, storage organelles and
189 vacuoles might be more resistant to dehydration, chemical exposure, eutrophication, and pathogen
190 attacks, and hence be of high interest. Unfortunately, macroalgal cell fate specification is one of the
191 least-understood areas of macroalgal biology. Undoubtedly, both endogenous (e.g. bacteria:
192 Spoerner *et al.*, 2012; circadian rhythm: Cunningham & Guiry, 1989) and abiotic environmental

193 factors (light, temperature, sea currents) are required (Fig. 3), but the intrinsic signalling pathways
194 are largely unknown. To understand how to manipulate hatchery culture conditions to give
195 juveniles the best start in life in tune with aquaculture demands, additional studies assessing the
196 molecular impact of the surrounding physical and chemical environment (light, nutrients, salinity,
197 water movement) are required. In some seaweeds, complex interactions with bacteria are a
198 prerequisite for proper cell growth and differentiation into specific tissues (Goecke *et al.*, 2010).
199 This has been well-illustrated in green seaweeds (*Ulva* and *Monostroma* - Matsuo *et al.*, 2005;
200 Spoerner *et al.*, 2012), as well as in brown algal species where bacteria might control their life
201 cycle (Tapia *et al.*, 2016) and their morphology in waters with different salinities (Dittami *et al.*,
202 2014). It is tempting to hypothesize that controlling macroalgal development with bacteria will
203 direct the chemical composition of the macroalga and its value as cash crop. This is mainly relevant
204 for land-based aquaculture starting with a defined seed-stock (axenic germlings) and a synthetic
205 microbiome, which could influence the production of primary and secondary metabolites.
206 However, further work determining macroalgal-bacterial interactions throughout algal life-cycles is
207 necessary to discriminate between mutualistic, beneficial or pathogenic interactions.

208 **Current technological requirements**

209 Reliable, cost-effective and long-term maintenance of genetic resources is a major requirement to
210 ensure the sustainability of the quality of the exploited traits (biomass yield, quality of extracted
211 polysaccharides, texture and taste of species for human consumption; Chapman *et al.*, 2015). Both
212 sub-culturing of macroalgal explants and cryopreservation of macroalgal omnipotent cells are
213 current techniques to vegetatively propagate macroalgae over time. However, sub-cultivation is
214 time-consuming and re-iteration of the protocol over years is a source of bacterial or fungal
215 contamination. Long-term preservation (through refrigeration or liquid-nitrogen freezing) of
216 commercially important seaweed explants has therefore received increasing attention and several

217 protocols are now available. Techniques depend on the species (e.g. gametophytic filaments of
218 *Macrocystis*; Barrento *et al.*, 2016; pieces of *Ulva* thalli; Lee & Nam, 2016; and apical meristems
219 of *Gracilaria*: Lalrinsanga *et al.*, 2009) and a better knowledge of both the mitotic activities within
220 the thallus and the underlying molecular mechanisms governing cell proliferation *versus* cell
221 differentiation would accelerate the assessment of the regenerative potential of these seaweeds and
222 the necessary development of adequate protocols (Stacey & Day, 2014) (Fig. 3). Basic research has
223 revealed specificities in brown seaweeds, specifically in the *Fucus* embryo, where cell division is
224 subject to distinct control mechanisms compared to other eukaryotes (Corellou *et al.*, 2001). As
225 bacteria play a crucial role in many algal developmental processes (Goecke *et al.*, 2010),
226 macroalgal preservation should also consider cryopreservation of algae with their natural
227 microbiome rather than axenic explants. Therefore, development of seaweed biobanking
228 procedures may be pivotal to meet future aquaculture demands.

229 Beyond cryopreservation, while some techniques are easily transferable from land plants to
230 macroalgae, others require species-specific optimization. The impact of the sea water medium on
231 the ionic concentration of buffers used in standard lab protocols and the different polysaccharide
232 compositions of red and brown algal cell walls (Deniaud-Bouët *et al.*, 2014; Popper *et al.*, 2011)
233 require different cell wall enzymolytic treatments in cytology protocols (Joubert & Fleurence,
234 2008). At the genetic level, the sequence of reporter genes commonly used in other organisms
235 require modification for transgene expression, because of differing codon usages, as shown in red
236 and green seaweeds (Uji *et al.*, 2014; Oertel *et al.*, 2015). The growing interest of the evolutionary
237 developmental biology (“evo-devo”) community in macroalgae would help phycologists develop
238 these techniques further.

239 In addition to the requirement for cell biology and genetic adjustments, ‘OMICS’ technology must
240 be adapted to the level of analysis required to tackle developmental mechanisms taking place at the
241 microscopic and early developmental stages (Fig. 2 and 3). Several transcriptomic (Wang *et al.*,
242 2015), proteomic (Qian *et al.*, 2016) and metabolomic (Kumar *et al.*, 2016 and references therein)

243 studies have been reported in both model and exploited macroalgae. In addition, exo-metabolomic
244 profiling in standardized *Ulva* cultures with a designed microbiome have shown growth phase-
245 dependent biomarkers that might be relevant for aquaculture (Alsufyani *et al.*, 2017). Such
246 analyses are assisted by an increasing number of sequenced macroalgal genomes. Currently 18
247 public algal nuclear genomes have been sequenced, including four seaweeds. However, “-OMICS”
248 studies at early developmental stages are hampered by a scarcity of tissue. While proteomics and
249 metabolomics still require a significant biomass, transcriptomics can bypass this handicap through
250 RNA amplification. Cell-specific expression patterns were thereby obtained using laser
251 microdissection prior to RNA amplification on the model brown seaweed *Ectocarpus* (Saint-
252 Marcoux *et al.*, 2015), and this technology is easily transferable to larger seaweeds.
253 Finally, transgenesis will be a highly valuable tool to discover how molecular processes are
254 regulated in seaweeds, and to interfere with these processes by knocking down/upregulating
255 endogenous genes. So far, only four multicellular algae, namely *Ulva*, *Pyropia* (*Porphyra*), *Volvox*
256 and *Gonium* are genetically transformable (Schiedlmeier *et al.*, 1994; Oertel *et al.*, 2015; Mikami,
257 2014; Lerche & Hallmann, 2009), and *Ulva* is the only stably transformable seaweed (Oertel *et al.*,
258 2015). These first successes must now be replicated in additional, diverse species, *via* investment
259 of time and expertise.

260 **Conclusion**

261 A range of protocols are available to cultivate seaweeds, thanks to previous physiological studies
262 carried out in an applied phycological context. Building on this key achievement, practices must be
263 refined and developed with a more focused and on-demand approach. Indeed, demand from end-
264 users is rising for new, high-commercial potential (mainly for food) seaweeds. However, because
265 of their low production level, these seaweeds have not received high investment so far, and as a
266 result, no standardised cultivation and preservation protocols exist. This second big step is much

267 more delicate, because of the greater number of species and of their reluctance to respond to the
268 simplest, classical protocols. The time has come, now that the first empirical studies have been
269 carried out, to engage the community in an in-depth study of the biological processes driving the
270 whole macroalgal life-cycle, from fertilization to the production of organisms. This must respond
271 to end-users' expectations of robustness against environmental constraints (e.g. climate, infection,
272 mechanical strain), biochemical composition and also natural and nature-friendly production
273 increasingly favoured by the consumers. This is even more necessary since, despite the benefit that
274 the development of cutting-edge technologies in animals and plants can bring to the sector, many of
275 these technologies need to be adapted to macroalgae because of their specific ecological niche
276 (highly saline) and their biology (in part due to their phylogenetic distance from better-known
277 organisms). Therefore, efforts must be intensified to fill the gaps in our fundamental knowledge of
278 macroalgal developmental mechanisms. We also believe that the scientific community of land plant
279 researchers will benefit from a deeper understanding of seaweed developmental biology.

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283 **Author contributions**

284 All authors contributed to the writing of the manuscript.

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488 **Box and Figure legends**

489 **Box 1: Life cycle stages in seaweeds and possible manipulations**

490 Seaweed life cycles comprise several (usually 4) multicellular phases, including vegetative and
491 fertile sporophytes and vegetative and fertile gametophytes (grey boxes). On the left, grey arrows
492 indicate the different natural alternatives that seaweeds can use to reproduce (either sexually or
493 asexually). On the right, brown, red and green horizontal lines represent the 3 groups of seaweeds.
494 Transition between two successive phases, and bypassing or maintenance of one phase (either by
495 delaying the maturation of the organism or by asexual looping) are ways to exert a tight control on
496 the life cycle. Straight arrows indicate controls over a given phase of the life cycle (maintenance,
497 induction or inhibition). Dashed arrows indicate asexual looping. A few specific examples are
498 represented by the numbers that follow. [1] vertical arrow: maintaining vegetative growth of the
499 brown seaweed *Saccharina latissima* gametophytes under red light or by sub-culturing (grinding)
500 filaments; horizontal arrow: induction of gametophyte fertility under blue light (Luning & Dring,
501 1975). [2] sporulation maintenance by removal of the basal meristem of *S. latissima* (Pang &
502 Lüning, 2004). [3] maintenance of the vegetative stage of the sporophyte: in *Porphyra conchocelis*
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511 sporophyte: in *Palmaria* tetrasporophytes by short daylength (Pang & Lüning, 2006). [4] control of
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513 temperature optimisation (Zhou *et al.*, 2013). [5] identification of sporulation-inhibiting factors
514 (Glycoprotein SP-1 and low molecular weight factor SP-2) from *Ulva* gametophytes and
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517 of gametophytes from gametes of the *Ectocarpus siliculosus* mutant *ouroburos* (Coelho *et al.*,
518 2011). [8] production of *Ulva* gametophytes from the germination of its own gametes when
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521 inhibiting factor produced by the parthenosporophyte (Arun *et al.*, 2013).

522 **Figure 1: Position of macroalgae in the scientific and societal landscapes.**

523 Macroalgae grow rapidly in a wide range of temperatures, using only sunlight, atmospheric carbon
524 and naturally nutritious coastal waters. They are therefore valuable feedstock for the production of
525 food, feed, biofuel, hydrocolloids, fertilisers, cosmetics, probiotics, biodegradable packaging
526 through aquaculture and IMTA (see text for details). They provide curative ecological roles
527 necessitated by human activities (waste-water treatments and seabed recolonisation). Ecology also
528 benefits from a knowledge of macroalgal reproductive mechanisms *via* a better understanding of
529 dispersion and persistence of both natural and exotic populations. This also contributes to the
530 development of conservation protocols for threatened or susceptible populations. Because their life
531 histories differ from land plants, macroalgae also inspire molecular evo-devo studies involving the
532 whole green lineage.

533 **Figure 2: Importance of the microscopic early developmental stages in the life cycle of**
534 **exploited seaweeds: Example of the kelp *Saccharina latissima*.**

535 Production of kelp (large brown macroalga) sporophyte juveniles takes place in hatcheries under
536 controlled growth conditions. Cultures of microscopic male and female gametophytes are produced
537 from spores of macroscopic, mature plants collected from the sea. Gametophyte cultures are grown
538 to fertility under controlled temperature and light conditions (see Box 1 for details). Microscopic,
539 fertile, recently fertilised gametophytes, or (in turn) juvenile sporophytes are spread onto
540 cultivation support materials (ropes or 2D substrates), which are subsequently deployed into the
541 sea. Photos kindly provided by Teis Boderskov (Aarhus University, Denmark) and Eric
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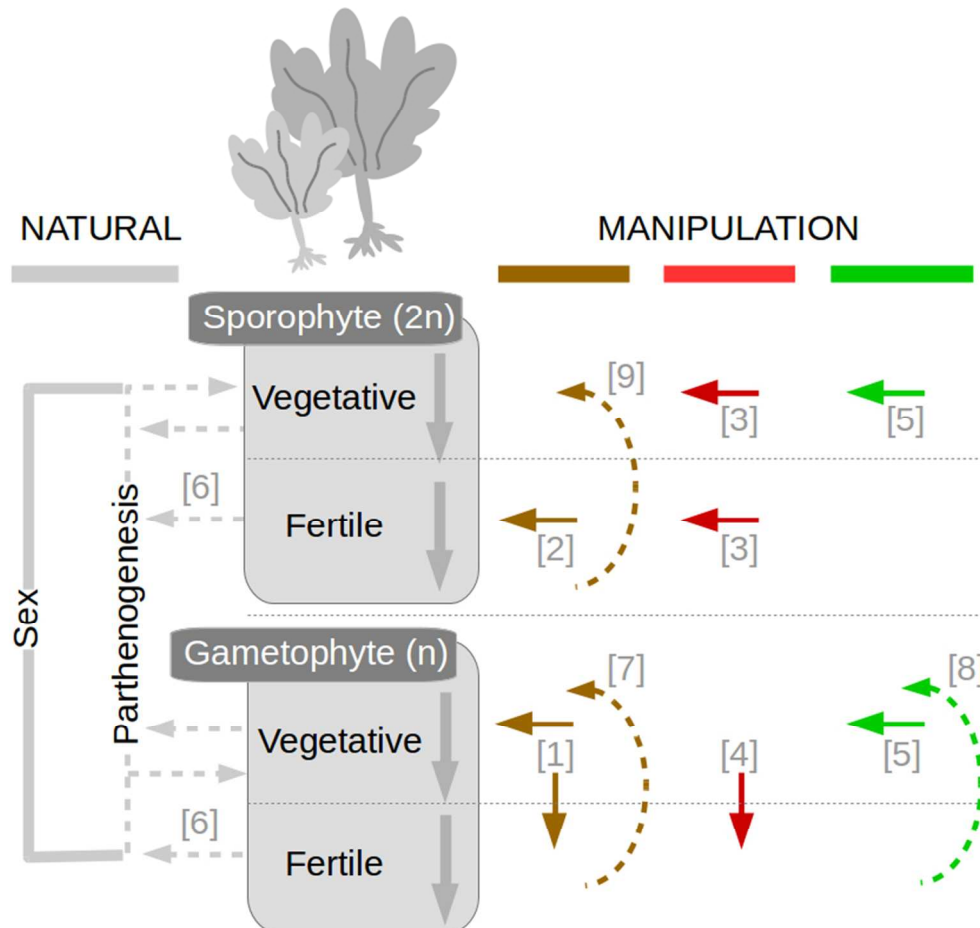
543 **Figure 3: Scope of beneficial outflow from basic research to seaweed aquaculture.**

544 Sexual reproduction (top right) gives rise to polarised embryos (left), which progressively grow
545 and differentiate, giving tissues and organs with specific shape and cellular functions (e.g. blade,
546 stipe, holdfast, reproductive organs). The study of the different steps of the life cycle (here
547 simplified, with adult representing either the sporophyte or the gametophyte) at the basic level (in
548 blue) can lead to the control and improvement of key processes in seaweed aquaculture (in green).
549 In hatcheries, density of juveniles on the cultivation support material depends on both the
550 fertilisation rate and the adhesive potential of the embryos. Fertilisation rate itself depends on the
551 physical interactions between the two gametes (taxis, specific recognition and membrane fusion).
552 Better knowledge of the cell cycle and characterisation of the pluripotent cells (zygotes, meristems)
553 will both contribute to develop cryopreservation protocols. Metabolic patterning of seaweed organs
554 and tissues, mediated by molecular, biochemical or cellular markers, will assist farmers in

555 monitoring seaweed growth and fitness both in hatcheries and in the field. All these processes are
556 under the control of abiotic and biotic factors (see text and Box 1 for references).

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Box 1



Box 1: Life cycle stages in seaweeds and possible manipulations

Seaweed life cycles comprise several (usually 4) multicellular phases, including vegetative and fertile sporophytes and vegetative and fertile gametophytes (grey boxes). On the left, grey arrows indicate the different natural alternatives that seaweeds can use to reproduce (either sexually or asexually). On the right, brown, red and green horizontal lines represent the 3 groups of seaweeds. Transition between two successive phases, and bypassing or maintenance of one phase (either by delaying the maturation of the organism or by asexual looping) are ways to exert a tight control on the life cycle. Straight arrows indicate controls over a given phase of the life cycle (maintenance, induction or inhibition). Dashed arrows indicate asexual looping. A few specific examples are represented by the numbers that follow. [1] vertical arrow: maintaining vegetative growth of the brown seaweed *Saccharina latissima* gametophytes under red light or by sub-culturing (grinding) filaments; horizontal arrow: induction of gametophyte fertility under blue light (Luning & Dring, 1975). [2] sporulation maintenance by removal of the basal meristem of *S. latissima* (Pang & Lüning, 2004). [3] maintenance of the vegetative stage of the sporophyte: in *Porphyra conchocelis* by temperature, photoperiod and irradiance (He & Yarish, 2006); of the reproductive stage of the sporophyte: in *Palmaria tetrasporophytes* by short daylength (Pang & Lüning, 2006). [4] control of the shift to the reproductive phase of the vegetatively propagated *Gracilariopsis* gametophyte by temperature optimisation (Zhou et al., 2013). [5] identification of sporulation-inhibiting factors (Glycoprotein SP-1 and low molecular weight factor SP-2) from *Ulva* gametophytes and sporophytes (Wichard & Oertel, 2010; Vesty et al., 2015). [6] parthenogenesis in brown algae (Nakahara, 1984) and red algae (*Undaria* female spore seeding; Shan et

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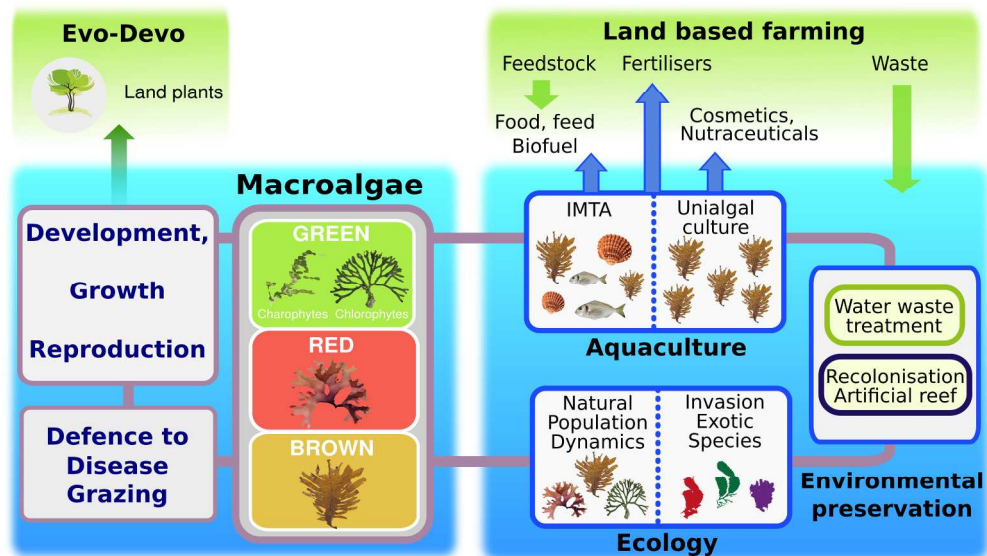


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Figure 2

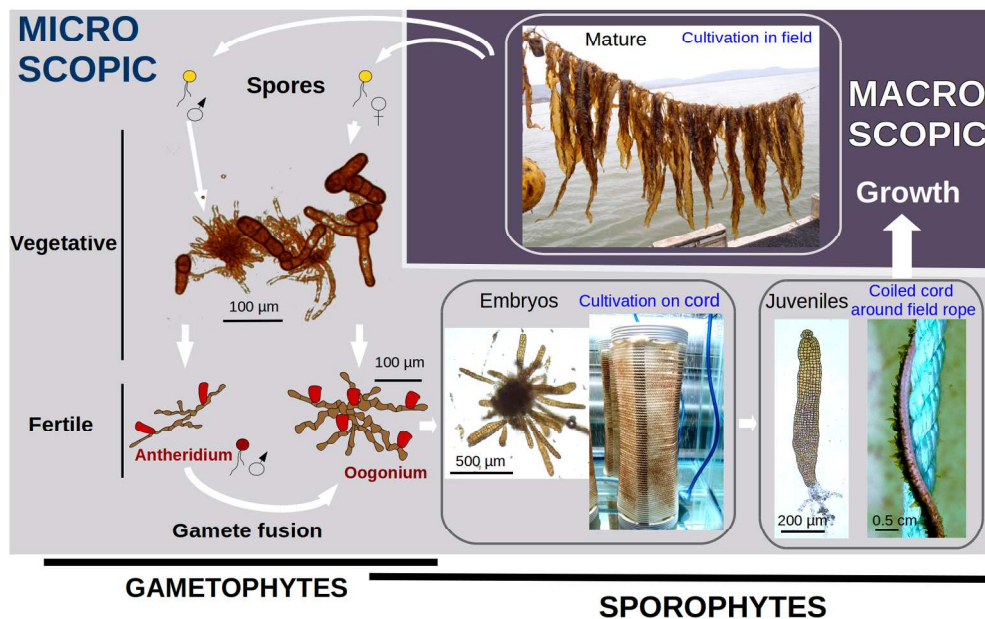


Figure 2: Importance of the microscopic early developmental stages in the life cycle of exploited seaweeds: Example of the kelp *Saccharina latissima*.

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Figure 3

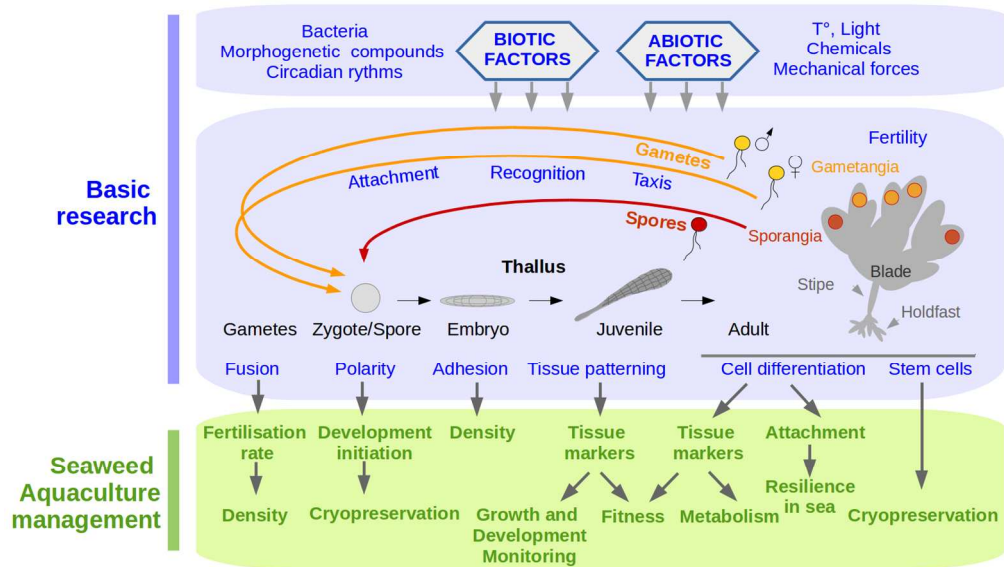


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