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

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

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## 24 Abstract

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

## 37 Ecological and societal position of macroalgae

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

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

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

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

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

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

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

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

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

110 Manipulating the sexual life cycle.

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

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

120 2016).

121 *Promoting parthenogenesis.* 

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

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

In parallel to these improvements for seaweeds cultivated off-shore (Fernand *et al.*, 2017), standardized protocols should also be developed specifically for not-yet cultivated, high-value 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, Himanthalia, Codium, Polysiphonia and Asparagopsis (Abreu et al., 2014), as well as

the red macroalgae *Ochtodes* and *Portieria* cultivated in photobioreactors (Rorrer & Cheney,
2004).

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

# 152 Early and microscopic stages of development

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

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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).

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

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

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

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

#### 208 **Current technological requirements**

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

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

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

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

studies have been reported in both model and exploited macroalgae. In addition, exo-metabolomic 243 profiling in standardized Ulva cultures with a designed microbiome have shown growth phase-244 dependent biomarkers that might be relevant for aquaculture (Alsufyani et al., 2017). Such 245 analyses are assisted by an increasing number of sequenced macroalgal genomes. Currently 18 246 public algal nuclear genomes have been sequenced, including four seaweeds. However, "-OMICS" 247 studies at early developmental stages are hampered by a scarcity of tissue. While proteomics and 248 metabolomics still require a significant biomass, transcriptomics can bypass this handicap through 249 250 RNA amplification. Cell-specific expression patterns were thereby obtained using laser microdissection prior to RNA amplification on the model brown seaweed Ectocarpus (Saint-251 Marcoux *et al.*, 2015), and this technology is easily transferable to larger seaweeds. 252

Finally, transgenesis will be a highly valuable tool to discover how molecular processes are regulated in seaweeds, and to interfere with these processes by knocking down/upregulating endogenous genes. So far, only four multicellular algae, namely *Ulva*, *Pyropia* (*Porphyra*), *Volvox* and *Gonium* are genetically transformable (Schiedlmeier *et al.*, 1994; Oertel *et al.*, 2015; Mikami, 2014; Lerche & Hallmann, 2009), and *Ulva* is the only stably transformable seaweed (Oertel *et al.*, 2015). These first successes must now be replicated in additional, diverse species, *via* investment of time and expertise.

# 260 Conclusion

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

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

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# 283 Author contributions

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**

Seaweed life cycles comprise several (usually 4) multicellular phases, including vegetative and 490 fertile sporophytes and vegetative and fertile gametophytes (grey boxes). On the left, grey arrows 491 indicate the different natural alternatives that seaweeds can use to reproduce (either sexually or 492 asexually). On the right, brown, red and green horizontal lines represent the 3 groups of seaweeds. 493 Transition between two successive phases, and bypassing or maintenance of one phase (either by 494 495 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, 496 induction or inhibition). Dashed arrows indicate asexual looping. A few specific examples are 497 498 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) 499 filaments; horizontal arrow: induction of gametophyte fertility under blue light (Luning & Dring, 500 1975). [2] sporulation maintenance by removal of the basal meristem of *S. latissima* (Pang & 501 Lüning, 2004). [3] maintenance of the vegetative stage of the sporophyte: in *Porphyra* conchocelis 502 by temperature, photoperiod and irradiance (He & Yarish, 2006); of the reproductive stage of the 503

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sporophyte: in *Palmaria* tetrasporophytes by short daylength (Pang & Lüning, 2006). [4] control of 511 the shift to the reproductive phase of the vegetatively propagated *Gracilariopsis* gametophyte by 512 temperature optimisation (Zhou *et al.*, 2013). [5] identification of sporulation-inhibiting factors 513 (Glycoprotein SP-1 and low molecular weight factor SP-2) from Ulva gametophytes and 514 sporophytes (Wichard & Oertel, 2010; Vesty et al., 2015). [6] parthenogenesis in brown algae 515 (Nakahara, 1984) and red algae (*Undaria* female spore seeding; Shan *et al.*, 2013). [7] production 516 of gametophytes from gametes of the Ectocarpus siliculosus mutant ouroboros (Coelho et al., 517 518 2011). [8] production of Ulva gametophytes from the germination of its own gametes when separated from another mating type (Wichard & Oertel, 2010). [9] germination of 519 parthenosporophytes (instead of gametophytes in this strain) from *Ectocarpus* zoospores by an 520 inhibiting factor produced by the parthenosporophyte (Arun *et al.*, 2013). 521

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

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

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

Production of kelp (large brown macroalga) sporophyte juveniles takes place in hatcheries under 535 controlled growth conditions. Cultures of microscopic male and female gametophytes are produced 536 from spores of macroscopic, mature plants collected from the sea. Gametophyte cultures are grown 537 to fertility under controlled temperature and light conditions (see Box 1 for details). Microscopic, 538 fertile, recently fertilised gametophytes, or (in turn) juvenile sporophytes are spread onto 539 cultivation support materials (ropes or 2D substrates), which are subsequently deployed into the 540 sea. Photos kindly provided by Teis Boderskov (Aarhus University, Denmark) and Eric 541 Tamigneaux (Merinov, Canada). 542

# 543 **Figure 3: Scope of beneficial outflow from basic research to seaweed aquaculture.**

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

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



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