Journal of Applied Phycology
https://doi.org/10.1007/s10811-026-03989-4

RESEARCH

High-throughput assessment of gametophyte survival and growth in the kelp *Laminaria ochroleuca* through autofluorescence analysis

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Received: 20 May 2026 / Revised: 24 July 2026 / Accepted: 3 August 2026

Abstract
The viability of kelp microscopic gametophytes is currently analyzed using subjective visual methods based on bright-field (BF) microscope images. Fluorescence microscopy (FM) can be employed, but the dyes used can be toxic and blue/UV light intensity may induce gametogenesis. This study aimed to develop a non-invasive and accurate methodology for assessing gametophyte viability over time, using FM observation of chlorophyll autofluorescence emitted by live gametophytes. Six isolated gametophyte strains of *Laminaria ochroleuca* (Italy), maintained at CCMAR Biobank, were cultured in triplicate (female, male, and both sexes combined) in Petri dishes containing 10 mL of half-strength Provasoli's enriched seawater medium (PES), under red light for 1 month. Survival and growth were assessed on days 1, 7 and 14 by photographing 20 fields-of-view, both in BF and FM. Then, cultures were transferred to white light to induce gametogenesis and test whether the autofluorescence analysis (AFA) method affected gametophyte reproduction. Image analysis was performed using FIJI software either by manually counting live gametophytes (survival) and measuring their area (growth), or by implementing a machine-learning (ML) model using FIJI's WEKA segmentation plugin. Results revealed that both methods were correlated, validating our ML model. However, the AFA method was faster and more accurate than BF image analysis, especially for male gametophytes, and did not compromise reproductive capacity. After 9 days under white light, sporophytes were present in mixed cultures. The AFA method provides a reliable technique for assessing gametophyte viability without compromising gametogenesis.

Keywords Phaeophyceae · Chlorophyll · Pigments · Fluorescence · Viability · Machine-learning

Introduction

Marine macroalgae are vital components of coastal ecosystems, providing a wide spectrum of ecological and economic benefits. In recent decades, these photosynthetic organisms have gained attention due to their potential for sustainable industries, including food, pharmaceuticals, cosmetics, biofuels, and bioplastics (Wells et al. 2017; Leandro et al. 2020; Kumar et al. 2021; Hempel et al. 2023; Pandian et al. 2025). Algae production has been increasing continuously, reaching 36.5 million tonnes in 2022 (FAO 2024). This trend, together with the efforts to preserve endangered genetic material (Barrento et al. 2016), has driven the need for algal culture collections that maintain and safeguard a broad range of specimens and regional diversity (Hofmann et al. 2025). In the case of kelps, these biobanks commonly preserve gametophytes, a microscopic and haploid phase of the haplo-diplontic life cycle, which can be maintained in long-term vegetative culture. These algal repositories are essential to preserve biodiversity, as well as research and commercial use, but require repeated time-consuming routines that include phenotyping, strain isolation, frequent media replacement, and regular cell quality assessments (West 2005). Survival and growth are two important quality parameters for culture maintenance, screening, and experimental trials, requiring resources, time, and specialized laboratory work.

In Laminariales (Phaeophyceae), survival is normally assessed by manually counting the number of live gametophytes using bright-field microscopy (Zhang et al. 2008), a

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Published online: 17 August 2026

method that is not only laborious and time-consuming but also prone to inter-observer variability. Besides survival, viability is key for long-term gametophyte development, which can be evaluated through (i) culture recovery, (ii) cell pigmentation index and (iii) cell staining (Yang et al. 2021). The first two of these methods are prone to subjectivity. Culture recovery is generally assessed through survival counts over time, often relying on observational criteria, with no standardized minimum recovery period established. Nevertheless, parameters such gamete germination, gametogenesis rate and sporophyte formation (Wang et al. 2011; Gao et al. 2017) can help improve consistency. Cell pigmentation index is also often qualitative, e.g., relying on an index scored between 0 to 10 according to the appearance of the gametophyte, from completely white (dead) to fully pigmented (live) (Van der Meer and Simpson 1984), or estimated as the proportion of pigmented cells to the total (Visch et al. 2019). To avoid potential biases, some authors prefer to perform chlorophyll measurements (Wang et al. 2011), however it is an endpoint technique as it requires maceration of the biological material.

Alternatively, various cell staining techniques can be used to evaluate viability. Neutral red is the most commonly used stain for macroalgae cells (Jo et al. 2003; Zhuang et al. 2014), but other dyes like erythrosine (Nanba et al. 2009; Lee and Nam 2015) and fluorescein diacetate (FDA) (Zhang et al. 2007a) have also been employed. However, the accuracy of those techniques depends on optimizing and adapting the staining protocol for the biological material in use, and they may overestimate the living cell population, as reported for different species of Laminariales (Kuwano et al. 2004; Zhang et al. 2007b). Moreover, fluorescence-based staining approaches often rely on excitation by blue or UV light, wavelengths known to induce gametogenesis (Lüning and Dring 1975), which represents an obvious drawback for some experimental designs. In addition, some stains, such as FDA, can be toxic for cells, and are therefore more appropriate for endpoint analysis (Invitrogen 2010). Other non-cytotoxic dyes, like carboxyfluorescein diacetate succinimidyl ester (CFSE) (Buhmann et al. 2013), have recently been adapted for tracing generational microalgae viability (Pozzobon et al. 2024), but to our knowledge have not been tested/applied in macroalgal studies.

Despite the variety of methods and protocols available, the research community could benefit from simple and accessible methodologies allowing high throughput, non-invasive, and quantitative assessment of gametophyte viability. This technological gap is particularly important to improve the efficiency of biobank routines and minimize the resources required for culture maintenance, phenotyping and research trials. Fluorescence microscopy (FM) offers a promising alternative by exploiting the intrinsic red autofluorescence of chlorophyll as a natural, label-free indicator of cell viability. This autofluorescence is characteristic of photosynthetically active cells, while its loss is associated with chlorophyll degradation during senescence or cell death, allowing discrimination between live and non-viable material (Schulze et al. 2011). This technique was successfully applied to *Macrocystis pyrifera* gametophytes to select the heat-stress tolerant genotypes (Harden et al. 2024) and to isolate and phenotype several other seaweed species (Alves-Lima et al. 2026). Furthermore, automated image-based systems incorporating fluorescence detection have demonstrated improved accuracy in quantifying live microalgae compared to conventional BF-based methods (Takahashi 2019). Despite this potential, the development and validation of FM-based workflows specifically tailored for kelp gametophytes remain limited. Considering the above, this study aims to develop a non-invasive, accurate, and high throughput methodology, employing FM-based autofluorescence measurements and ML image analysis to monitor *Laminaria ochroleuca* gametophyte viability over time, without compromising gametogenesis. *Laminaria ochroleuca* is a warm-temperate kelp species that plays a critical ecological role, providing habitat and food for many marine organisms (Teagle et al. 2017). Besides, *L. ochroleuca* has great adaptability to various environmental conditions and huge potential for integration into multi-trophic aquaculture systems (Zhu et al. 2025; Cereja et al. 2026), being the most represented in our collection, the CCMAR biobank (www.ccmarbiobank.pt), and a great model species for this experiment.

Materials and methods

Algae stock material

Mature sporophytes of *Laminaria ochroleuca* were sampled from Messina, Italy (38.26356^{o}N, 15.64423^{o}E), at 50 m depth, in November 2019. The collected sori (spore-bearing tissues) were cleaned, and meiospores were released separately in sterile artificial seawater. After spore germination, male and female gametophytes were isolated from each individual to establish unisexual and clonal stock cultures. These gametophyte culture strains are maintained at the CCMAR Biobank in sterile PES medium at 13ºC, under 8 µmol photons m^{-2} s^{-1} of red light and a 16:8 h light:dark (L:D) photoperiod in a climate-controlled chamber (Fitoclima S600, Aralab, Portugal). Culture medium was changed monthly until the beginning of the experiment. Further details on cultures and their maintenance are available on the CCMAR Biobank website (www.ccmarbiobank.pt).

Experimental setup and gametophyte cultivation

Six gametophyte strains of *L. ochroleuca* from CCMAR Biobank [CCMAR-S_LO11.2F_IT (♀1); CCMAR-S_LO5.3M_IT (♂1); CCMAR-S_LO7.1F_IT (♀2); CCMAR-S_LO11.1M_IT (♂2); CCMAR-S_LO10.4F_IT (♀3); and CCMAR-S_LO6.1M_IT (♂3)] were used in triplicate (n = 3), either with female and male strains cultured separately or as mixed crosses (♀1 × ♂2; ♀2 × ♂3; and ♀3 × ♂1). For each strain, a portion of the gametophyte biomass was washed with PES, ground with a sterile pestle and mortar and passed through a 100 µm cell strainer. Gametophyte density was assessed under an inverted microscope (Axio Observer D1, Zeiss, Göttingen, Germany) and adjusted to approximately 500 gametophytes cm^{-2}. Female, male and mixed cultures were cultured in Petri dishes (Ø 5 cm) containing 10 mL of PES, under red light (7 µmol photons m^{-2} s^{-1}, 16:8 LD) at 16ºC. The culture medium was changed every 10 days to ensure optimal culture conditions.

Evaluation of gametophyte survival, growth and reproductive capacity

After 3 days of recovery, gametophyte survival and growth were assessed on days 1, 7 and 14 using images from 20 fields-of-view (fov) per replicate, obtained under both bright-field (BF) and fluorescence microscopy (FM) conditions (40 photographs per replicate, n = 3 replicates). Photographs were taken with a Canon EOS RP camera (Canon, Tokyo, Japan) mounted on an inverted fluorescence microscope (Zeiss Axio Observer D1; 10 × objective; 100 × final magnification) equipped with a red filter (EX BP 560/40 |BS 585| EM BP 630/75). Fluorescence microscopy analyses require the use of a fluorescence microscope equipped with appropriate excitation and emission filters selected according to the spectral properties of the fluorescent compounds present in the sample. In the present study, the selected filter configuration was based on the autofluorescence properties reported for the gametophytes and allowed the detection of the fluorescence emitted by the analysed tissues. The use of this setup enabled the evaluation of gametophyte autofluorescence without the need for additional fluorescent staining. Gametophytes were then transferred to white light (10 µmol photons m^{-2} s^{-1}) to induce gametogenesis and test whether the autofluorescence analysis (AFA) method had any impact on gametophyte reproductive capacity. For that, daily observations were done randomly to evaluate the development of oogonia and antheridia in female and male cultures, respectively. In mixed cultures, once the first eggs appeared in all replicates, counts of vegetative females, females with eggs, vegetative males, males with antheridia, and sporophytes were performed (n = 40 fov per replicate). In gametophytes showing different developmental structures, only the most advanced stage was considered (e.g., a gametophyte showing one or more sporophytes was considered for the counts as a single sporophyte).

Image processing and quantitative analysis

Image analysis was performed using FIJI 2.17.0 software (ImageJ v1.54, Java). Gametophyte survival was quantified by manually counting the number of live gametophytes, while growth was assessed by measuring fragment area. For BF images, preprocessing involved applying the color threshold plugin to convert gametophytes into a red mask, enabling their identification as regions of interest (ROI). These ROIs were subsequently used for manual counting and area measurements. In addition, an automated ML-based image analysis workflow was developed for FM images using the Trainable Weka Segmentation plugin in FIJI, which is based on the WEKA machine-learning framework (Arganda-Carreras et al. 2017). This method combines machine learning algorithms with image features to generate pixel-based segmentation and classification models. The model was trained to detect the red autofluorescence signal emitted by the live gametophyte fragments against a black background. Following model training, the plugin generated a classified image that enabled automated identification of live gametophytes. A custom macro in FIJI was developed to automatically quantify gametophyte density (number of live fragments cm^{-2}) and fragment area (µm^2 cm^{-2}) in the classified images. To avoid background noise, a minimum particle size of 5 $pixel^2$ was used, corresponding approximately to the area of a single gametophyte cell.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics v29 software (IBM Corp., USA), and graphical representations were generated using GraphPad Prism 9 (GraphPad Software, USA). After removing the statistically identified outliers (samples on the 1st or 3rd quartile ± 3 × the interquartile range), the data were checked for normality and homogeneity of variance (Kolgomorov-Smirnov and Levene's tests, respectively). Since each snapshot was analyzed using both methods (manual BF and automatic FM), a paired t-test was applied to identify differences between methods. In addition, to check their correlation, Pearson's analysis was applied per sampling day and considering all data. For density and fragment area, data from the 3 replicates was summed (20 fov from each replicate) and analyzed as n = 60 fov, both for BF and FM images. Significant differences were assumed when $p < 0.05$.

Results

Bright-field (BF) and fluorescence microscopy (FM) image analysis

Differences detected between the two methods

The manual analysis revealed less accurate counts and area measurements, as color thresholding of BF images was more susceptible to background artifacts, contamination, debris, dead gametophytes being incorrectly identified as ROIs, and empty spaces between gametophytes were incorrectly identified as ROIs (Fig. 1A and B). In contrast, the FM image analysis was faster and more accurate since the ML model counted and measured only the gametophyte fragments that emitted autofluorescence (Fig. 1C and D).

Comparison between both methods

Image analysis of gametophyte density revealed significantly higher fragment counts in FM than BF (paired t-test, $p < 0.001$) across all the strains (females, males and females × males) and time points (days 1, 7 and 14; Fig. 2A). In contrast, area measurements obtained from FM were lower than those from BF, with significant differences observed across all strains, but only at specific time points (paired t-test, $p < 0.05$). Overall, more differences were observed in male strains (Fig. 2B). Detailed significant differences and effect sizes are provided in the supplementary material (Table S1).

Correlation between both methods

Linear regression analyses were performed to evaluate the relationships between BF and FM image analysis methods (Fig. 3). The linear regression for density revealed a moderately positive relationship between the two methods ($R^2 = 0.397$, $p < 0.001$: $FMdensity = 3.40 + 1.27 \times BFdensity$). The slope > 1 indicates that FM-derived density estimates are higher than BF-derived density, suggesting that the fluorescence-based method yields higher density estimates as values increase (Fig. 3A). For area measurements, a positive association was also observed between BF and FM with the regression model explaining a larger proportion of variability compared with density measurements ($R^2 = 0.576$, $p < 0.001$). The fitted regression equation with slope < 1 ($FMarea = 0.28 + 0.62 \times BFarea$) indicates that the FM method estimates areas lower than the BF method (Fig. 3B), consistent with observations of incorrect area measurements with the latter method (see Fig. 1).

Pearson's correlation coefficients (r), considering all time points for each strain (FM/BF), indicated a strong correlation between the two methods of image analysis (n = 1451) for both density (r = 0.630; $p < 0.001$) and area (r = 0.759; $p < 0.001$; Table 1). The same strong correlation indices ($p < 0.001$) were achieved when the analysis was performed for each time point separately.

FM impact on gametogenesis

Following transfer to white light, gametophyte reproductive development was observed in both males and females (Fig. 4 and 5). After 9 days, sporophytes were present in cultures containing both sexes (Fig. 4C and 5C). Female gametophytes developed faster, showing eggs (Fig. 5A), in the presence of males than when cultured alone (Fig. 4A and 4C). Males developed faster than females, with 100% showing antheridia (Fig. 4B and 5B).

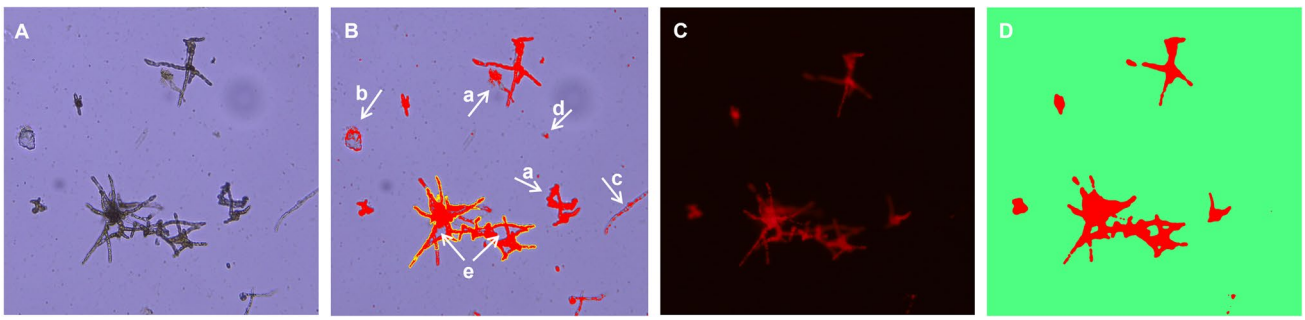

Fig. 1 Representative images of male gametophytes at day 14 under red light: (**A**) BF image, (**B**) manual BF image analysis, (**C**) FM image, and (**D**) machine-learning FM image. Arrows in (B) indicate incorrect identification of (a) contamination, (b) debris, (c) dead gametophytes, (d) background artifacts, and (e) area measurement of empty spaces by the manual BF image analysis

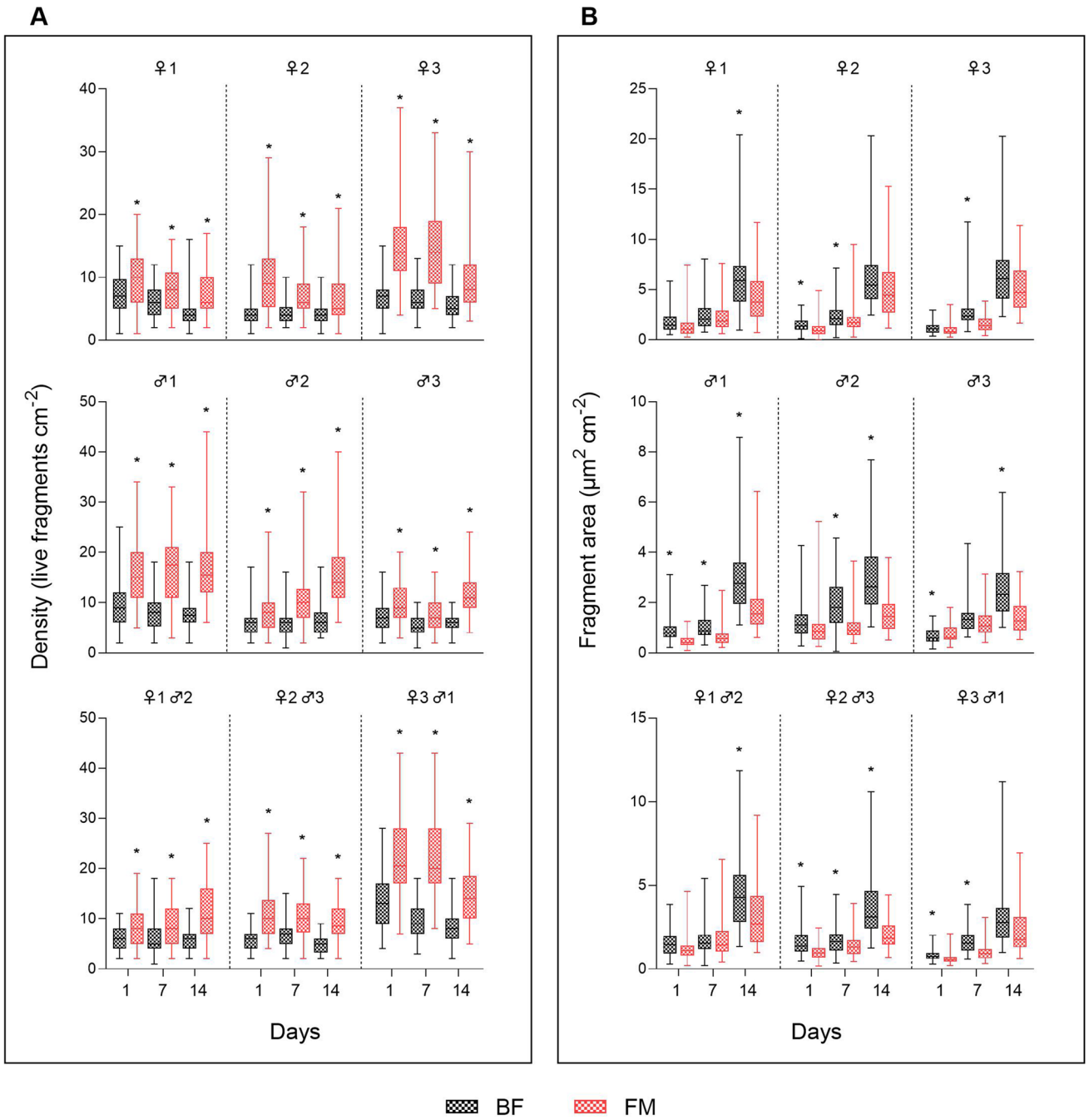

Fig. 2 Bright-field (BF) and fluorescence microscopy (FM) images analysis of *Laminaria ochroleuca* gametophytes. (**A**) Density (number of live fragments cm^{-2}) and (**B**) fragment area (μm^2 cm^{-2}) at days 1, 7, and 14 under red light. Results are represented as interleaved box and whiskers plots (BF – black; FM – red). Boxes represent the interquartile range (25th–75th percentiles), the horizontal line inside each box indicates the median, and whiskers represent the minimum and maximum values (20 fov, 3 replicates, n = 60). Significant differences between BF and FM (paired t-test) are indicated with an asterisk for each day ($p < 0.05$)

Discussion

With the global expansion of algae biobanks, improving cell quality protocols used in maintenance routines and experimental trials has become essential to enhance objectivity, incorporate technological approaches, and reduce hands-on processing time. Our study introduces a new autofluorescence-based analysis (AFA) method for the evaluation of gametophyte survival, growth and viability that was demonstrated to be highly correlated with commonly employed manual analysis, but with improved accuracy and reduced handling time, resulting in less biased observations.

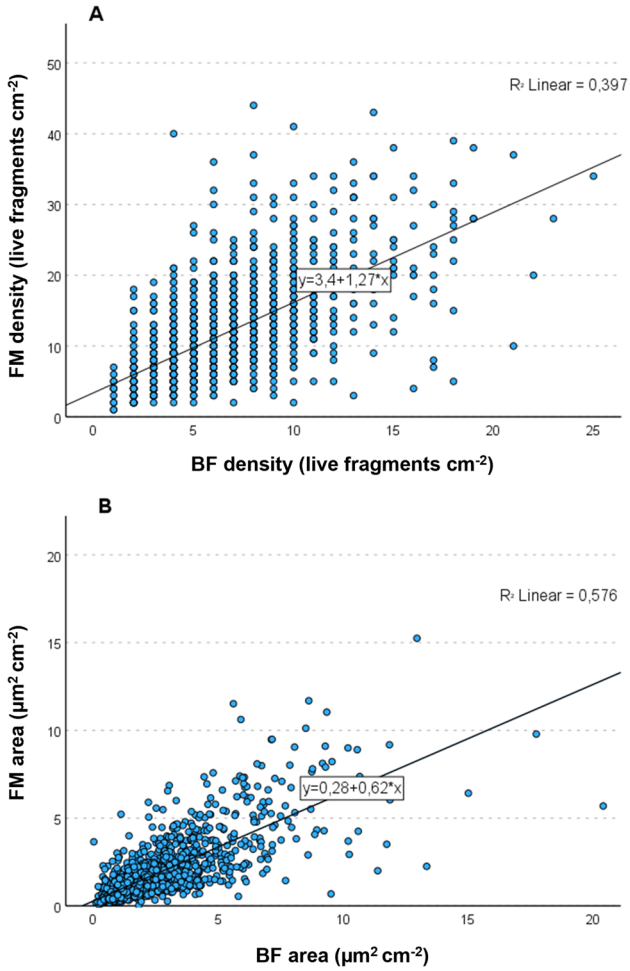

Fig. 3 Relationship between measurements obtained using bright-field (BF) and fluorescence microscopy (FM) image analysis methods. Linear regression between (**A**) BF density and FM density and (**B**) BF area and FM area was measured at the field-of-view (fov) level. Each point represents an individual fov measurement (n = 1451). Solid lines represent fitted linear regression models ($p < 0.001$)

Table 1 Simplified representation of Pearson's correlations between density and area of *Laminaria ochroleuca* gametophytes (3 females, 3 males and 3 females × males) assessed through fluorescence microscopy (FM) and bright-field (BF) image analysis

	FM/BF	Day 1	Day 7	Day 14
Density (live fragments cm^{-2})	0.630**	0.624**	0.660**	0.647**
Area (μm^2 cm^{-2})	0.759**	0.569**	0.588**	0.375**

The correlation was evaluated considering all data (FM/BF; n = 1451) and separately for each time point (day 1, n = 468; day 7, n = 488; and day 14, n = 495). Statistically significant correlations are indicated with asterisks (**$p < 0.001$).

The AFA method is based on the detection of gametophyte chlorophyll autofluorescence, a universal characteristic of photoautotrophic macroalgae. The intensity of red fluorescence emitted is dependent on chlorophyll concentration and type of accessory pigments (phycobiliproteins and xanthophylls: (Juhasz-Dora et al. 2024). In the brown alga *L. ochroleuca*, light is primarily absorbed by chlorophylls *a* and *c* and fucoxanthin (Fernandes et al. 2016). This pigment composition is important when setting up the microscope configuration for fluorescence imaging, to make sure that fluorescence emitted by the photosynthetic apparatus is detected efficiently (Coronado-Parra et al. 2023). To minimize potential light-induced physiological responses, including the induction of gametogenesis, red excitation was deliberately selected instead of UV light excitation. Although the endogenous pigments exhibit stronger excitation in the UV region, their secondary excitation in the red spectral range was sufficient to generate a detectable autofluorescence signal. In our experiment, detected fragments were confirmed as alive based on both chlorophyll fluorescence (FL images) and intact brown coloration under transmission light (BF images), enabling rapid and straightforward cell survival assessment. Contrarily, staining techniques such as FDA may overestimate living cells, since the esterases responsible for converting fluorescein into visible fluorescence can increase not only during growth but also under unfavorable conditions, a reason why some authors have reported that dead cells still emit FDA fluorescence (Markelova et al. 2000).

Using the AFA method, survival is assessed by counting live gametophyte fragments, rather than entire gametophytes that can contain senescence cells. This approach provides results closer to biological reality, as a fragmented multicellular gametophyte has the potential to develop into multiple individuals, depending on the number of live fragments (Destombe and Oppliger 2011). The results of both image analysis methods, BF and FM, showed a decrease in live fragments cm^{-2} in females and mixed cultures over time. This likely reflects two simultaneous effects: higher female mortality and reduced space per fov as remaining live fragments grow. The trend was less evident in males, possibly because of their higher resilience and less pronounced volumetric growth compared to females (Izquierdo et al. 2002). Overall, FM analysis detected a higher number of live fragments at all sampling times and measured smaller fragment area, as confirmed by regression analyses. These findings, together with visual observations, confirm that AFA method is faster and more precise. Manual analysis of survival and growth may consider contamination, unidentified debris, dead gametophytes and background artifacts, either due to observer error or to FIJI's "WandAutoMeasureTool", which automatically sets a threshold to convert detected elements into ROIs. This tool identifies a specimen (it may or may not

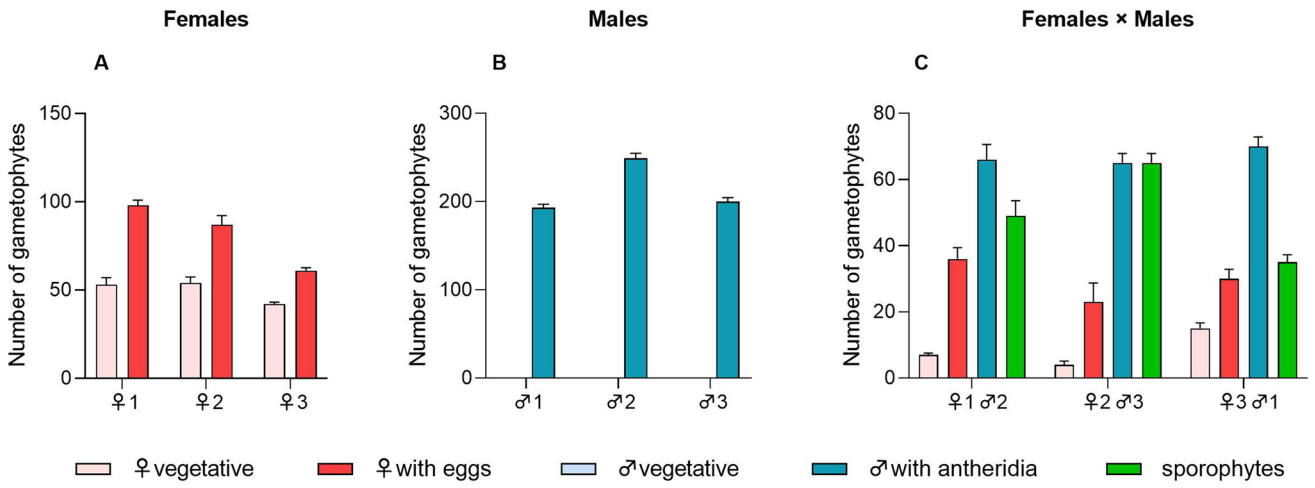

Fig. 4 Ontogenetic development of gametophytes after 9 days under white light, showing (**A**) females, (**B**) males, and (**C**) females × males. Vertical bars represent the mean number of gametophytes at each developmental stage, and error bars indicate the standard deviation of the three replicates analyzed (40 fields-of-view per replicate, n = 3). Vegetative males were not observed. A female gametophyte showing one or more sporophytes was considered as a single sporophyte

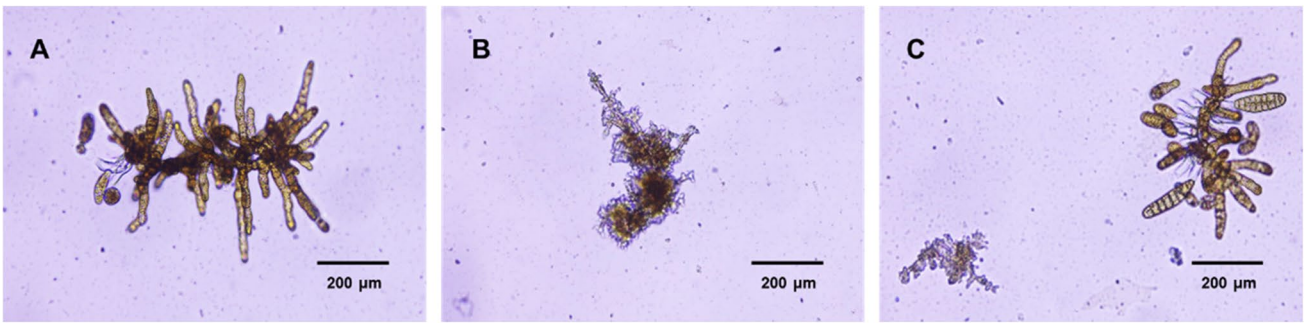

Fig. 5 Representative gametophyte reproductive development after 9 days under white light, shown for (**A**) ♀2, (**B**) ♂3, and (**C**) ♀2 × ♂3

be a gametophyte), converts it to red color and establishes a yellow threshold around it, considering any space inside this boundary and not only the red area (https://imagej.net/ij/docs/tools.html). Although this feature can be used to count and measure *L. ochroleuca* gametophytes, it may overestimate both survival and growth due to the inclusion of area measurements for non-living tissue. This problem could be overcome by using the "watershed" function of FIJI, but it cannot be applied directly to the bright-field image, requiring more time to pre-processing the images, which would convert the image analysis process into a complex workflow. In *Laminaria digitata*, an alternative method for growth determination measured gametophyte length along the main axis (Ratcliff et al. 2017). However, once branching began after four weeks, this approach became unreliable. Branching is expected and does not compromise the AFA method, as all live fragments emit red fluorescence detected by the ML-model and are classified as living area.

As with any other technique, AFA method has its own limitations. In this study, the use of two-dimensional (2D) images may lead to underestimation of counts and area when gametophytes overlap, because the system only differentiates red signal from background. However, this is more likely when gametophytes reach a considerable size, especially the larger females, or when gametophyte densities are very high, which is not a recommended practice (Lawton and Magnusson 2025). FIJI offers a trainable WEKA segmentation 3D plugin, but further research is needed to determine whether this approach can overcome the limitations mentioned. Alternatively, z-stack acquisition followed by extended depth-of-field projection could be used to integrate three-dimensional structural information into a single image. Although this approach may improve segmentation accuracy, it would substantially increase image acquisition and processing times. Additionally, the current model does not distinguish males from females, but given that sexual

dimorphism becomes visible after 1–2 weeks in culture (Redmond et al. 2014), future work should focus on developing and training an ML-model able to differentiate sexual phenotype based on morphological variation.

To test whether this method could be used in reproductive experiments, crosses between female and male gametophytes were performed. Cultures were maintained under red light for one month, considered ideal for maintaining gametophytes in vegetative state (Ebbing et al. 2020), and then transferred to white light. During the red-light period, the light used for FM analysis did not induce gametogenesis, nor was any morphological alteration associated with reproductive development visible. It has been reported that once gametogenesis starts, male *L. ochroleuca* gametophytes aggregate in ball-like shape and develop antheridia, whereas females became more rounded and develop oogonia (Pereira et al. 2011). Thus, only vegetative growth was observed. However, when gametophytes were moved to white light, a key trigger of gametogenesis (Lüning and Dring 1975), antheridia were visible in males and oogonia in females within 5 days. By day 9, sporophytes were present in all mixed cultures. This time course for reproductive development is considered normal for *L. ochroleuca* (Strasser et al. 2022) and is similar to observations in the related *L. digitata* (Martins et al. 2017; Ratcliff et al. 2017). Overall, these results demonstrate that the autofluorescence analysis method does not interfere with gametophyte reproductive development and can therefore be safely applied in reproductive experiments requiring repeated fluorescence observations.

A major advantage of AFA method is the ability to monitor viability over time. Many viability staining techniques are not suitable for repeated measurements due to membrane damage or cumulative toxicity (Riss et al. 2016), increasing the number of required replicates and the complexity of the experimental designs. Moreover, every procedure dependent on the gametophyte color identification by the human eye, which is the case of the commonly seen score indexes, prevents colourblind researchers from performing the analysis (Zhang et al. 2007b; Barrento et al. 2016). The established qualitative indexes also do not allow analytical interpretation of the data, resulting in many publications lacking statistical analysis.

By using open-source machine learning tools, the AFA method not only delivers accurate and reliable data with time- and therefore costs-savings, but most importantly, is broadly accessible to the research community. This promising methodology can be applied to other photosynthetic species with pigments emitting chlorophyll autofluorescence. Exploring algal autofluorescence is not new, but its application to multicellular algae lags far behind achievements with unicellular algae (Enríquez and Borowitzka 2011). With continuous technological developments, it will become useful for many applications, and was already shown to be a rapid diagnostic tool to evaluate the reproductive maturation of macroalgal sori (Juhasz-Dora et al. 2024). Finally, addressing the technological and automation gap in research is critical to support long-term research and for scaling up algae biobanks and cultivation systems, since they are the fundamental backups for research innovation, industrial production, and environmental restoration.

Conclusions

In conclusion, the AFA method represents a reliable, objective, and time-efficient approach for assessing survival and viability of *Laminaria ochroleuca* gametophytes. By leveraging chlorophyll autofluorescence and open-source ML tools, it overcomes the limitations of manual counting and staining-based techniques, reducing observer bias, minimizing overestimation errors, and allowing continuous monitoring of the same biological samples over time. The method proved consistent with conventional analyses while delivering greater precision and speed, without interfering with normal vegetative growth or reproductive development. Moreover, the approach offers strong potential for further refinement, including sex differentiation and 3D segmentation. Overall, AFA method provides a scalable and accessible technological advancement that can enhance experimental standardization, support algae biobanking, and be adapted to a wide range of photosynthetic organisms exhibiting autofluorescence.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-026-03989-4.

Acknowledgements Authors gratefully acknowledge CCMAR Imaging Platform for support & assistance in this work.

Author contribution FF: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. CAL: Formal analysis, Methodology, Data curation. LB: Methodology, Validation. CM: Conceptualization, Methodology, Resources. ES: Project administration, Funding acquisition. GP: Conceptualization, Methodology, Validation, Writing – review & editing. NM: Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Funding CCMAR Biobank was financed by the WP9—Portuguese Blue Biobank under the Blue Economy Pact—Project Nº C644915664-00000026 co-funded by PRR, the Portuguese Republic and the European Union. This study also received Portuguese national funds from the Foundation for Science and Technology (FCT) through contracts UID/04326/2025 (DOIhttps://doi.org/10.54499/UID/04326/2025), UID/PRR/04326/2025 (DOIhttps://doi.org/10.54499/UID/PRR/04326/2025) and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020), through projects AdaptKelp (DOI:https://doi.org/10.54499/2023.16917.ICDT; MPr-2023–12-16551, ALGARVE-FEDER-00772500), POCI/MAR/60883/2004,

PTDC/MAR/105152/2008, MPr-2023–12-16551 (ALGARVE-FEDER-00772500) and from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through contract EMBRC.PT ALG-01–0145-FEDER-022121. NM was also supported by MAR-PLUS—Support for the development of CCMAR's European scientific infrastructure (ALGARVE-FSE + -01177700).

Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Competing interests The authors declare no competing interests.

References

Alves-Lima C, Barreto L, Mónico C, Gouvêa L, Felix F, Varga B, Filipe J, Camacho R, Lymperaki M, Alberto F, Rörig LR, Engelen AH, Serrão EA, Pearson GA, Martins N (2026) SAMMBA is a high-throughput pipeline for isolating and phenotyping macroalgal strains. Algal Res 94:104547

Arganda-Carreras I, Kaynig V, Rueden C, Eliceiri KW, Schindelin J, Cardona A, Sebastian Seung H (2017) Trainable weka segmentation: a machine learning tool for microscopy pixel classification. Bioinformatics 33:2424–2426

Barrento S, Camus C, Sousa-Pinto I, Buschmann AH (2016) Germplasm banking of the giant kelp: our biological insurance in a changing environment. Algal Res 13:134–140

Buhmann MT, Day JG, Kroth PG (2013) Post-cryopreservation viability of the benthic freshwater diatom *Planothidium frequentissimum* depends on light levels. Cryobiology 67:23–29

Cereja R, Pereira A, Brito AC, Pires C, Diniz M, Ferreira I, Mil-Homens M, Brito P, Costa PR, Marques A (2026) *Laminaria ochroleuca* as a natural remediator of copper-based antifouling agents in aquaculture systems. Mar Pollut Bull 223:119023

Coronado-Parra T, Roldan M, Aboal M (2023) Autofluorescence imaging to evaluate red algae physiology. J Vis Exp 192:e64533

Destombe C, Oppliger LV (2011) Male gametophyte fragmentation in *Laminaria digitata*: a life history strategy to enhance reproductive success. Cah Biol Mar 52:385

Ebbing A, Pierik R, Bouma T, Kromkamp JC, Timmermans K (2020) How light and biomass density influence the reproduction of delayed *Saccharina latissima* gametophytes (Phaeophyceae). J Phycol 56:709–718

Enríquez S, Borowitzka MA (2011) The use of the fluorescence signal in studies of seagrasses and macroalgae. In: Suggett DJ, Prásil O, Borowitzka MA (eds) Chlorophyll *a* fluorescence in aquatic sciences: methods and applications. Springer, Dordrecht, pp 187–208

FAO (2024) In brief to the state of world fisheries and aquaculture 2024. Blue transformation in action. FAO, Rome, pp 1–40

Fernandes F, Barbosa M, Oliveira AP, Azevedo IC, Sousa-Pinto I, Valentão P, Andrade PB (2016) The pigments of kelps (Ochrophyta) as part of the flexible response to highly variable marine environments. J Appl Phycol 28:3689–3696

Gao G, Clare AS, Rose C, Caldwell GS (2017) Non-cryogenic preservation of thalli, germlings, and gametes of the green seaweed *Ulva rigida*. Aquaculture 473:246–250

Harden M, Kovalev M, Molano G, Yorke C, Miller R, Reed D, Alberto F, Koos DS, Lansford R, Nuzhdin S (2024) Heat stress analysis suggests a genetic basis for tolerance in *Macrocystis pyrifera* across developmental stages. Commun Biol 7:1147

Hempel MdSS, Colepicolo P, Zambotti-Villela L (2023) Macroalgae biorefinery for the cosmetic industry: basic concept, green technology, and safety guidelines. Phycologia 3:211–241

Hofmann LC, Brakel J, Bartsch I, Montecinos Arismendi G, Bermejo R, Parente MI, Creis E, De Clerck O, Jacquemin B, Knoop J, Lorenz M, Machado LP, Martins N, Orfanidis S, Probert I, Rad Menendez C, Ross M, Rautenberger R, Schiller J, Serrao EA, Steinhagen S, Sulpice R, Valero M, Wichard T (2025) A European biobanking strategy for safeguarding macroalgal genetic material to ensure food security, biosecurity and conservation of biodiversity. Eur J Phycol 60:197–220

Invitrogen (2010) Assays for Cell Viability, Proliferation and Function, Molecular Probes™ Handbook: A Guide to Fluorescent Probes and Labeling Technologies. ThermoFisher Scientific, pp 1–90

Izquierdo J, Pérez-Ruzafa IM, Gallardo T (2002) Effect of temperature and photon fluence rate on gametophytes and young sporophytes of *Laminaria ochroleuca* Pylaie. Helgol Mar Res 55:285–292

Jo Y-H, Kang S-P, Seo T-H, Choi S-J, Kho K-H, Kuwano K, Saga N, Kim M-Y, Shin J-A (2003) Cryopreservation of sporothalli of the genus *Porphyra* (Bangiales, Rhodophyta) from Korea. Algae 18:321–331

Juhasz-Dora T, Lindberg S-K, James P, Wang X (2024) Assessing the potential of fluorescence as a monitoring tool for reproductive tissue in selected macroalgae species. J Appl Phycol 36:2153–2159

Kumar D, Pugazhendi A, Bajhaiya AK, Gugulothu P (2021) Biofuel production from macroalgae: present scenario and future scope. Bioengineered 12:9216

Kuwano K, Kono S, Jo YH, Shin JA, Saga N (2004) Cryopreservation of the gametophytic cells of laminariales (Phaeophyta) in liquid nitrogen. J Phycol 40:606–610

Lawton RJ, Magnusson M (2025) Optimisation of gametophyte culturing and seeding methods for cultivation of the kelp *Ecklonia radiata*. Algal Res 92:104240

Leandro A, Pereira L, Gonçalves AMM (2020) Diverse applications of marine macroalgae. Mar Drugs 18:17

Lee YN, Nam KW (2015) Cryopreservation of gametophytic thalli of *Ulva prolifera* (Ulvales, Chlorophyta) from Korea. J Appl Phycol 28:1207–1213

Lüning K, Dring M (1975) Reproduction, growth and photosynthesis of gametophytes of *Laminaria saccharina* grown in blue and red light. Mar Biol 29:195–200

Markelova A, Vladimirova M, Kuptsova E (2000) A comparison of cytochemical methods for the rapid evaluation of microalgal viability. Russ J Plant Physiol 47:815–819

Martins N, Tanttu H, Pearson GA, Serrão EA, Bartsch I (2017) Interactions of daylength, temperature and nutrients affect thresholds for life stage transitions in the kelp *Laminaria digitata* (Phaeophyceae). Bot Mar 60:109–121

Nanba N, Fujiwara T, Kuwano K, Ishikawa Y, Ogawa H, Kado R (2009) Effect of pre-incubation irradiance on survival of cryopreserved gametophytes of *Undaria pinnatifida* (Phaeophyta) and morphology of sporophytes formed from the gametophytes. Aquat Bot 90:101–104

Pandian A, Rajinikanth V, Narayanan M (2025) Harnessing marine macroalgae for bioplastic materials production: a comprehensive review. Discover Materials 5:172

Pereira TR, Engelen AH, Pearson GA, Serrao EA, Destombe C, Valero M (2011) Temperature effects on the microscopic haploid stage development of *Laminaria ochroleuca* and *Sacchoriza polyschides*, kelps with contrasting life histories. Cah Biol Mar 52:395

Pozzobon V, Lagirarde J, Arnoudts C, Levasseur W (2024) Microalgal cell division tracking using CFSE. Algal Res 80:103501

Ratcliff JJ, Soler-Vila A, Hanniffy D, Johnson MP, Edwards MD (2017) Optimisation of kelp (*Laminaria digitata*) gametophyte growth and gametogenesis: effects of photoperiod and culture media. J Appl Phycol 29:1957–1966

Redmond, S., Green, L., Yarish, C., Kim, J., Neefus, C., 2014. New England Seaweed Culture Handbook-Nursery Systems. Connecticut Sea Grant CTSG-14-01, pp. 92

Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, Minor L (2016) Cell viability assays. Assay guidance manual [Internet]. Eli Lilly & Company and the National Center for Advancing Translational Sciences, Bethesda (MD), pp 1–27. https://www.ncbi.nlm.nih.gov/books/NBK144065/

Schulze K, López DA, Tillich UM, Frohme M (2011) A simple viability analysis for unicellular cyanobacteria using a new autofluorescence assay, automated microscopy, and ImageJ. BMC Biotechnol 11:118

Strasser F-E, Barreto LM, Kaidi S, Sabour B, Serrão EA, Pearson GA, Martins N (2022) Population level variation in reproductive development and output in the golden kelp *Laminaria ochroleuca* under marine heat wave scenarios. Front Mar Sci 9:943511

Takahashi T (2019) Routine management of microalgae using autofluorescence from chlorophyll. Molecules 24:4441

Teagle H, Hawkins SJ, Moore PJ, Smale DA (2017) The role of kelp species as biogenic habitat formers in coastal marine ecosystems. J Exp Mar Biol Ecol 492:81–98

Van der Meer JP, Simpson F (1984) Cryopreservation of *Gracilaria tikvahiae* (Rhodophyta) and other macrophytic marine algae. Phycologia 23:195–202

Visch W, Rad-Menendez C, Nylund GM, Pavia H, Ryan MJ, Day J (2019) Underpinning the development of seaweed biotechnology: cryopreservation of brown algae (*Saccharina latissima*) gametophytes. Biopreserv Biobank 17:378–386

Wang B, Zhang E, Gu Y, Ning S, Wang Q, Zhou J (2011) Cryopreservation of brown algae gametophytes of *Undaria pinnatifida* by encapsulation–vitrification. Aquaculture 317:89–93

Wells ML, Potin P, Craigie JS, Raven JA, Merchant SS, Helliwell KE, Smith AG, Camire ME, Brawley SH (2017) Algae as nutritional and functional food sources: revisiting our understanding. J Appl Phycol 29:949–982

West JA (2005) Long-term macroalgal culture maintenance. In: Andersen R (ed) Algal culturing techniques. Academic Press, New York, pp 157–163

Yang H, Huo Y, Yee JC, Yarish C (2021) Germplasm cryopreservation of macroalgae for aquaculture breeding and natural resource conservation: a review. Aquaculture 544:737037

Zhang Q, Cong Y, Qu S, Luo S, Yang G (2008) Cryopreservation of gametophytes of *Laminaria japonica* (Phaeophyta) using encapsulation-dehydration with two-step cooling method. J Ocean Univ China 7:65–71

Zhang QS, Cong YZ, Qu SC, Luo SJ, Li XJ, Tang XX (2007a) A simple and highly efficient method for the cryopreservation of *Laminaria japonica* (Phaeophyceae) germplasm. Eur J Phycol 42:209–213

Zhang QS, Cong YZ, Qu SC, Luo SJ, Tang XX (2007b) Cryopreservation of gametophytes of *Laminaria japonica* (Phaeophyta) with two-step cooling: interactions between variables related to post-thaw survival. CryoLetters 28:215–222

Zhu Y, Wang N, Wu Z, Chen S, Luo J, Christakos G, Wu J (2025) The role of seaweed cultivation in integrated multi-trophic aquaculture (IMTA): the current status and challenges. Rev Aquac 17:e70042

Zhuang Y, Gong X, Zhang W, Gao W (2014) Cryopreservation of filaments of *Scytosiphon lomentaria* by vitrification. J Appl Phycol 27:1337–1342