



Spatio-temporal variation in reproductive strategies of *Porphyra umbilicalis* along the Northeast Atlantic Coast

Jordi Morcillo, Olivier De Clerck, Sofie D'hondt, Frederik Leliaert, Sofie Vranken & Jessica Knoop

To cite this article: Jordi Morcillo, Olivier De Clerck, Sofie D'hondt, Frederik Leliaert, Sofie Vranken & Jessica Knoop (2026) Spatio-temporal variation in reproductive strategies of *Porphyra umbilicalis* along the Northeast Atlantic Coast, European Journal of Phycology, 61:1, 65-78, DOI: [10.1080/09670262.2025.2581105](https://doi.org/10.1080/09670262.2025.2581105)

To link to this article: <https://doi.org/10.1080/09670262.2025.2581105>



Published online: 01 Dec 2025.



Submit your article to this journal [↗](#)



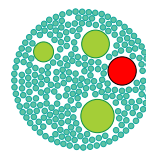
Article views: 153



View related articles [↗](#)



View Crossmark data [↗](#)



Spatio-temporal variation in reproductive strategies of *Porphyra umbilicalis* along the Northeast Atlantic Coast

Jordi Morcillo ^a, Olivier De Clerck ^a, Sofie D'hondt ^a, Frederik Leliaert ^{a,b}, Sofie Vranken ^a and Jessica Knoop ^{a,c}

^aPhycology Research Group, Department of Biology, Ghent University, Ghent 9000, Belgium; ^bMeise Botanic Garden, Meise 1860, Belgium; ^cDepartment of Estuarine and Delta Systems, Royal Netherlands Institute for Sea Research, Yerseke 4401 NT, the Netherlands

ABSTRACT

Since the late 19th century, studies of the genus *Porphyra* sensu lato (Bangiales, Rhodophyta) have revealed a broad spectrum of life history strategies and reproductive modes. These variations are often interpreted as adaptive responses to the selective pressures imposed by spatially and temporally heterogeneous environments. However, the dynamics and prevalence of sexual versus asexual reproduction in natural populations remain poorly understood and insufficiently documented. In this study, we investigated the spatio-temporal variation in reproductive strategies of *Porphyra umbilicalis*, an emerging target species for aquaculture in the Northeast Atlantic, across a broad European range. Through microscopic examination and tracking the germination of spores released from fertile individuals in multiple populations, four main patterns were identified: (1) clear geographic structuring of reproductive strategies, with asexual reproduction prevailing in the Southern Bight of the North Sea and English Channel, and sexual populations more common in the South European Atlantic Shelf and Celtic Seas; (2) persistent dominance of asexual reproduction in populations with mixed reproductive modes, with a seasonal increase in the frequency of sexually reproductive individuals during winter; (3) microhabitat partitioning within the high intertidal zone, with asexual individuals occupying uppermost elevations and sexual individuals concentrated below, indicating ecologically driven niche differentiation; and (4) no consistent link between thallus size and reproductive mode, although sexual individuals were significantly larger during winter and spring. Collectively, these findings illustrate how environmental gradients shape reproductive strategies in *P. umbilicalis*, and suggest that asexuality may confer adaptive advantages in marginal intertidal zones.

HIGHLIGHTS

- Reproductive strategies show clear geographic structuring along the Northeast Atlantic.
- Asexual reproduction predominates year-round in populations with mixed reproductive strategies.
- Ecological niche differentiation occurs between sexual and asexual gametophytes.

ARTICLE HISTORY Received 2 July 2025; Revised 6 October 2025; Accepted 22 October 2025

KEYWORDS Asexuality; life cycle; nori; reproduction; rhodophyta; seaweed

Introduction

The balance between sexual and asexual reproduction, the relative rate of selfing versus outcrossing, and the allocation of male and female functions within and among individuals are among the most influential life history traits (Barrett, 2002, 2011; Krueger-Hadfield, 2024). These traits shape patterns of inheritance and genetic diversity, ultimately influencing population dynamics, ecological functioning and the evolutionary fate of species (Halkett *et al.*, 2005; Orive *et al.*, 2023).

In lineages with complex life cycles – such as algae, fungi, mosses and ferns – reproductive traits are expressed across distinct, alternating free-living life phases. These phases may differ in morphology, physiology, karyology and demography (Hughes & Otto, 1999). Such differentiation often confers phase-specific ecological advantages and differential susceptibilities to

abiotic and biotic pressures (Lubchenco & Cubit, 1980; Mable & Otto, 1998; Coelho *et al.*, 2007). The transition between life cycle phases is often contingent on sexual reproduction (i.e. fertilization and meiosis), which triggers the shift from one phase to the next. Accordingly, the degree of flexibility or constraint between sexual and asexual events can determine whether entire life phases persist, are bypassed or collapse (Yang & Kim, 2016; Orive *et al.*, 2017; Krueger-Hadfield *et al.*, 2024). Elucidating how sexual and asexual reproduction are balanced in natural populations is thus essential for interpreting their ecological and evolutionary dynamics.

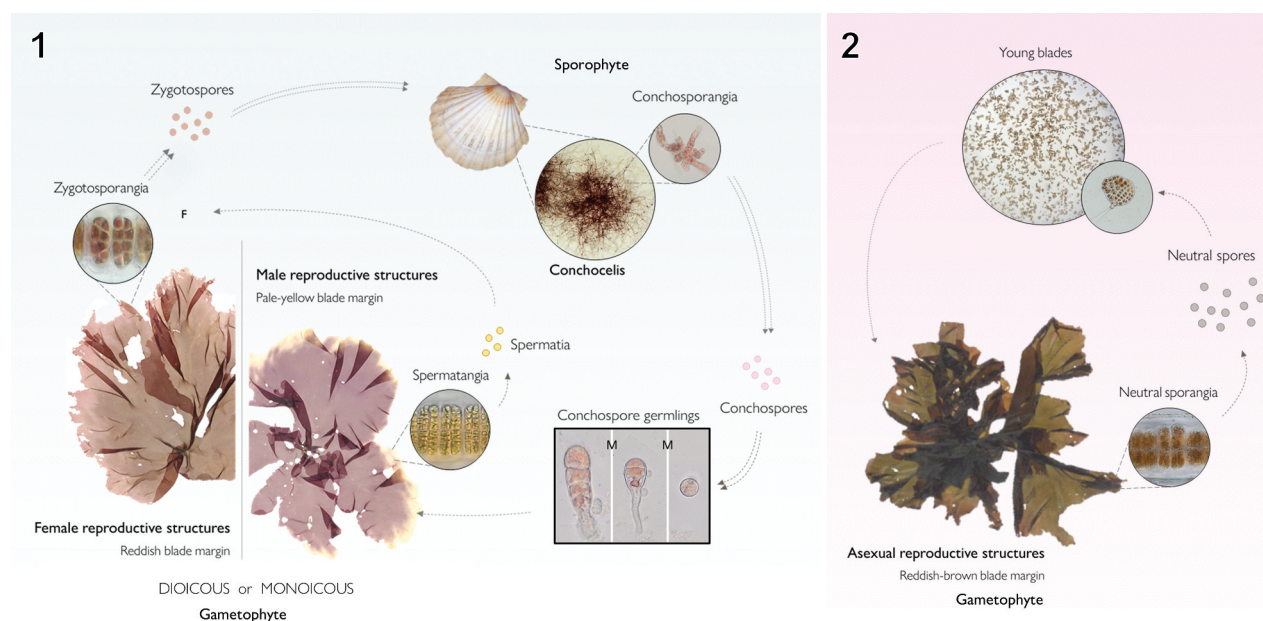
Belonging to the ancient order Bangiales (Rhodophyta), the genera *Porphyra* and *Pyropia* offer valuable opportunities to investigate the ecological and evolutionary dynamics of complex life cycles. These algae exhibit a heteromorphic sexual life cycle, alternating between the foliose, macroscopic gametophyte

(referred to as the blade phase) and the microscopic, filamentous sporophyte (known as the conchocelis phase) (Drew, 1954). Besides this canonical cycle, a variety of asexual reproductive modes have been recognized in both life phases across the order, involving several types of apomictic spores – i.e. spores produced without fertilization, meiosis or recombination, sensu Krueger-Hadfield (2024) – as well as vegetative fragmentation at the sporophytic phase (cf. Notoya, 1997; Nelson *et al.*, 1999).

The maintenance of this alternation of generations likely reflects adaptive functional differentiation, enabling each life phase to exploit distinct ecological niches (Rescan *et al.*, 2016; Krueger-Hadfield, 2020). The conchocelis phase, residing within calcareous shells or rock crevices, benefits from a stable, buffered microenvironment that protects against desiccation, excessive light and herbivory (Lubchenco & Cubit, 1980; Varela-Álvarez *et al.*, 2004). In contrast, the foliose gametophyte endures extremely stressful conditions in the intertidal. Its survival relies on morphological, biochemical and physiological adaptations, including tolerance to desiccation levels exceeding 95% and irradiances of up to 2000 μmol

photons $\text{m}^{-2} \text{s}^{-1}$ during summer exposure (Blouin *et al.*, 2011) – partly mediated by the accumulation of photoprotective compounds such as mycosporine-like amino acids (MAAs) and high antioxidant capacity (van Groenigen *et al.*, 2022).

In the North Atlantic, *Porphyra umbilicalis* Kützinger is one of the most widespread native Bangiales (Brodie *et al.*, 2008), thriving year-round (Conway, 1964; Kornmann & Sahling, 1991) on temperate rocky shores in the high intertidal zone (Teasdale & Klein, 2010; Guiry & Guiry, 2025). In its northeastern native range, both sexual and asexual life histories have been reported (Figs 1–2), with sexual reproduction traditionally regarded as the dominant pathway along European coasts (Brodie & Irvine, 2003; Blouin *et al.*, 2011) (Fig. 1). Conversely, in the northwestern Atlantic, reproduction appears to occur exclusively via asexual neutral spores (Fig. 2), with no evidence of sexual structures or a conchocelis phase (Blouin *et al.*, 2007; Gantt *et al.*, 2010; Blouin & Brawley, 2012). These western populations are hypothesized to have originated from Northeast Atlantic lineages introduced via trans-Atlantic currents after the last glacial maximum (Ingolfsson,



Figs 1–2. Simplified sexual and asexual life cycles of *Porphyra umbilicalis*. **Fig. 1.** Sexual life cycle of *P. umbilicalis*. The heteromorphic sexual life cycle alternates between the macroscopic gametophyte (blade phase) and the microscopic sporophyte (conchocelis phase). Fertilization between male (spermatia) and female gametes, typically occurring near the blade margin, results in the formation of zygotosporangia (sensu Guiry, 1990). Subsequently, zygotospores are released from the zygotosporangia and germinate unipolarly into the filamentous sporophytic phase on calcareous substrata (Brodie & Irvine, 2003; Gantt *et al.*, 2010). When mature, conchocelis produce conchospores which release conchospores that germinate into young gametophytic thalli, giving rise to the new blade generation (Kornmann, 1994). Photographs of female and male blades are scans of herbarium specimens (BR501009727747V and BR5010097292610V) at Meise Botanic Garden (botanicalcollections.be). **Fig. 2.** Asexual life cycle of *P. umbilicalis*. In the asexual life cycle, neutral (apomictic) spores are produced by the gametophyte, bypassing the sporophyte phase to directly regenerate new gametophytic clones (Blouin *et al.*, 2007). Abbreviations: fertilization (F); meiosis (M). Simple arrow = (n); Double arrow = (2n). The traditional haploid–diploid framework in Bangiales is currently under reconsideration (see Varela-Álvarez *et al.*, 2018, 2021); hence, the exact ploidy levels of each phase remain to be clarified.

1992; Teasdale & Klein, 2010), but the apparent loss of sexual reproduction in these populations remains unexplained (Eriksen *et al.*, 2016; Cao, 2018).

The east–west biogeographic asymmetry in the reproductive strategies of *P. umbilicalis* can be framed within the concept of geographic parthenogenesis, a term introduced by Vandel (1928) to describe patterns in which sexual and asexual lineages occupy distinct geographic ranges (Hörandl, 2009). Notably, despite the prevailing assumption that sexual reproduction dominates along the Northeast Atlantic coast (Brodie & Irvine, 2003; Blouin *et al.*, 2011), recent field observations from northern France, Belgium and the Netherlands suggest that asexuality may be more common than previously thought. This raises important questions about the factors shaping reproductive dynamics in *P. umbilicalis* and the broader implications for the development, maintenance and genetic diversity of its populations.

To date, little is known about the triggers of asexual versus sexual reproduction in European species, such as *P. umbilicalis*. By contrast, studies of Asian Bangiales, such as *Pyropia yezoensis* (Ueda) M.S.Hwang & H.G.Choi and species of *Bangia* Lyngbye, have reported a well-documented capacity for intrinsic plasticity in reproductive strategies (Mikami & Takahashi, 2023). These species can shift between vegetative growth and asexual or sexual reproduction in response to environmental and chemical stressors, such as fluctuations in temperature, salinity and nutrient availability. Such modulation enables these species to optimize reproductive success, rapidly accelerating spore release or gametogenesis depending on environmental cues (cf. Mikami & Takahashi, 2023, for a summary table on the flexible promotion of reproduction in Bangiales). Moreover, in *Pyropia*, a clear relationship has been observed between thallus size and reproductive mode, with smaller individuals preferentially engaging in asexual reproduction, while larger individuals allocate more resources to the energetically costly sexual reproduction (Ruangchuay & Notoya, 2007). However, whether analogous mechanisms operate in *P. umbilicalis* remains unknown. To bridge this gap, we investigated the reproductive strategies of *P. umbilicalis* across a broad expanse of its Northeast Atlantic native range, aiming to identify key drivers of life history variation. Specifically, the present study aims to: (1) document broad-scale spatial variation in reproductive modes across multiple European populations; (2) assess temporal variation in mixed populations, where both sexual and asexual individuals co-occur; (3) estimate whether fine-scale patterns – such as those associated with the vertical intertidal gradient – correlate with specific reproductive modes; and (4) evaluate the influence of thallus size on the balance between sexual and asexual reproduction.

Material and methods

Sample collection

To assess spatial and temporal variation in reproductive modes, 30–45 putative gametophytic blades of *P. umbilicalis* – as recognized by the characteristic rosette form – were randomly collected during low tide from multiple populations along the Northeast Atlantic coast. Collections were carried out in areas where the species is known to occur, typically forming dense patches on rocks and rocky dikes in the high intertidal zone. Three complementary approaches were employed: (1) to examine large-scale spatial variation across a broad expanse of the species' Northeast Atlantic range, specimens were collected from coastal sites in the Netherlands, Belgium, France, the UK and Portugal, the latter representing the southernmost distribution limit; (2) to monitor seasonal dynamics, quarterly sampling was conducted at three focal sites – Ambleteuse (France), Ostend (Belgium) and Neeltje-Jans (the Netherlands); and (3) to investigate fine-scale spatial patterns, two subpopulations within the high intertidal zone were sampled at Ostend, comparing the reproductive status of gametophytes collected from the uppermost zone (adjacent to the highest high tide level) with those situated approximately 2 m below (lower zone). Additionally, at this site, individuals were measured (i.e. maximum length, from the holdfast to the margin) to assess the potential relationship between thallus size and reproductive mode. All samples were promptly refrigerated and transported to the laboratory for further processing.

Species identification: DNA extraction, amplification and restriction digests

Given the frequent confusion of *P. umbilicalis* with sympatric and morphologically similar taxa (Knoop *et al.*, 2024), such as *P. dioica* J.Brodie & L.M.Irvine, *P. linearis* Greville or *P. purpurea* (Roth) C.Agardh, the identity of all specimens was confirmed using RFLP analysis of the *rbcL* gene (Teasdale *et al.*, 2002). For this purpose, a fragment from each gametophyte was stored in silica gel for rapid dehydration and to preserve the DNA. Samples of dry tissue (≈ 0.25 g) were pulverized in liquid nitrogen and total DNA was extracted by a standard CTAB method (Doyle & Doyle, 1987). The DNA was stored at -20°C .

A 1481 bp region encompassing the *rbcL-rbcS* genes and their intergenic spacer, was amplified using primers F67 (5'-TACGCTAAAATGGGTTACTG-3') and *rbc-spc* (5'-CACTATTCTATGCTCCTTATTKTTAT-3') (Teasdale *et al.*, 2002). All PCR procedures were

performed in a Biometra TProfessional Basic 96 Gradient Thermocycler (Biometra GmbH, Göttingen, DE), in 25 µl volumes containing 1 µl of DNA, 2.5 µl of 10 mM of GeneAmp dNTP Blend (Fisher Scientific), 1 µl of 10 mg/ml BSA, 1.25 µl of AmpliTaq DNA Polymerase (5 U µl⁻¹), 1.25 µl of 10 µM of each amplification primer, and 2.5 µl of 10 X PCR Buffer I (Fisher Scientific) with 15 mM MgCl₂. Cycling conditions involved an initial denaturation step of 95°C for 3 min, followed by 40 cycles of 30s at 95°C, 30s at 45°C annealing temperature, and 1 min at 72°C, and a final extension at 72°C for 5 min. PCR-amplified products were stored at 4°C and checked by 1% agarose gel electrophoresis. RFLP analysis was conducted using the restriction enzyme *Hae*III, in accordance with the manufacturer's specifications. Fragments of all restriction digests were separated by capillary electrophoresis on a 5300 Fragment Analyser System (Agilent, Santa Clara, California, USA), using a High Sensitivity NGS Fragment Analysis Kit (1 bp–6000 bp) (Agilent, Santa Clara, California, USA), and complying with the brand's specifications. The quality and efficiency of this method was validated by Sanger sequencing on a subset of 30 samples representing different species and collection sites (MacroGen Europe, Amsterdam, the Netherlands).

Assessment of reproductive strategies: microscopic examination and characterization of spore identity

Specimens of *P. umbilicalis* were examined using a stereomicroscope and classified according to reproductive status based on thallus margin pigmentation, cell arrangement and cell size (Figs 3–14) (Mitman & van der Meer, 1994; Brodie & Irvine, 2003; Brodie *et al.*, 2008). Stages of maturity of cells varied across the blade thallus: vegetative cells were found at the centre of the thallus, premature reproductive stages at the submargins, and mature reproductive cells at the blade margins. Thalli lacking reproductive sori were classified as vegetative (Figs 3–4). Male gametophytes were identified by pale-yellow sori composed of small spermatangial cells (3–6 µm wide × 6–9 µm long), typically arranged in packets of 16-cells (Figs 5–6). Unfertilized female gametophytes were characterized by the presence of prototrichogynes in transverse section (Figs 7–8) (Kornmann & Sahling, 1991). Gametophytes bearing both spermatangia and zygosporangia (present on a single blade or on separate blades attached to the same holdfast) were categorized as monoicous. Fertile gametophytes with reddish or reddish-brown sori, consisting of larger cells (9–11 µm wide × 10–15 µm long) arranged in 8- or 4-cell packets, were initially categorized as possessing either zygosporangia (sexual reproductive structure) (Figs 9–12) or neutral sporangia (asexual reproductive structure) (Figs 13–14). Due to the morphological and

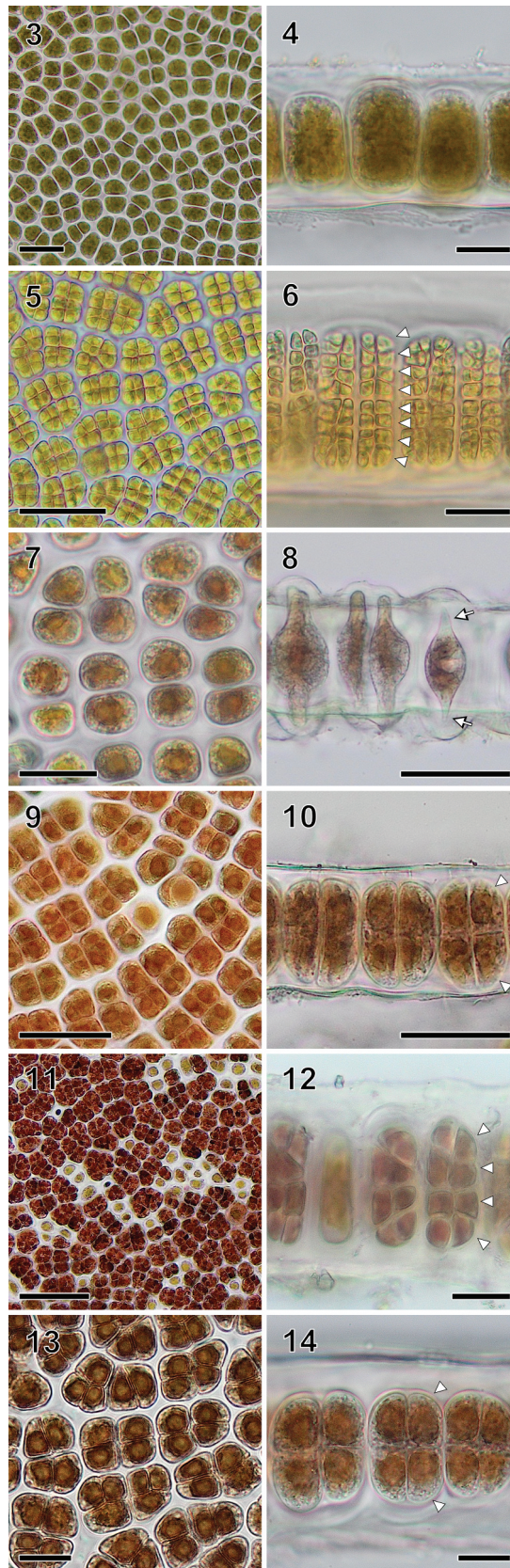
cytological similarity between these reproductive structures (Figs 9, 10 vs. 13, 14), culture-based assessment of spore identity was employed to ensure accurate classification of reproductive modes.

For the extraction and cultivation of spores, a 1–2 cm² section of mature tissue was excised from each female or asexual gametophyte, respectively. Subsequently, the tissue sample was rinsed multiple times with filtered and sterilized seawater, followed by gentle brushing with a paper towel to remove potential epiphytes and other contaminants (Dlaza, 2011). The cleaned fragment was wrapped in a moist paper towel and placed overnight at 4°C in complete darkness (Knoop *et al.*, 2020). Spore release was induced by re-immersing the tissue section in sterile 6-well culture plates containing 7 ml of nutrient- and vitamin-enriched (mPES, 10 ml l⁻¹) sterile seawater (West & McBride, 1999). Germanium dioxide (GeO₂, 1 ml l⁻¹ of a 1 g l⁻¹ solution) was added to inhibit potential diatom proliferation (Markham & Hagmeier, 1982). Once released, the obtained zygotospores/neutral spores were transferred into new 6-well culture plates, filled as described above. Spores were incubated at 15°C, under a photon flux density of 30 µmol photons m⁻² s⁻¹ and a neutral photoperiod (12:12 h L:D). Nutrient medium was refreshed every 2 weeks. Since zygotospores and neutral spores were morphologically identical, their developmental outcomes were used to differentiate between sexual and asexual reproduction (Figs 15–22). Zygotospores (Fig. 15), which germinated unipolarly (Fig. 16), developed into the filamentous conchocelis phase (Fig. 17), indicating sexual reproduction. In contrast, neutral spores (Fig. 18) germinated directly into a new blade generation (Figs 19–22), as expected for asexual reproduction. Terminology used to describe reproduction and life history phases follows Nelson *et al.* (1999).

Photomicrographs were taken with an Olympus SC50 camera attached to an Axio Observer.A1 microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) or an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan).

Statistical analysis

Statistical analyses were performed in R v4.2.2 (R Core Team, 2022). Reproductive mode was treated as a binary response: asexual = 1 and sexual (male, female, monoicous) = 0. To assess spatio-temporal variation in reproductive mode across European populations and to mitigate potential separation issues (i.e. when one category perfectly predicts the response), we fitted bias-reduced generalized linear models (GLMs) using Firth's penalized likelihood logistic regression via the *brglm2* package (Kosmidis, 2023). Model significance was evaluated using Type III Wald chi-square tests from the *car* package (Fox & Weisberg, 2020), and post-hoc



Figs 3–14. Vegetative and reproductive structures of *Porphyra umbilicalis*. **Fig. 3.** Vegetative cells in the central region of the thallus (Neeltje-Jans, April). **Fig. 4.** Cross section through the central region of the thallus showing a monostromatic layer of vegetative cells (Neeltje-Jans, April). **Fig. 5.** Segment of the marginal region of the thallus displaying mature spermatangial packets of 16 cells (4×4) (Ostend, September). **Fig. 6.** Cross section through the marginal region of the thallus displaying mature spermatangia, each having undergone three mitotic divisions, resulting in 8 spermatia (arrowheads) and totalling 128 spermatia. Note that spermatia can be easily distinguished from other reproductive cells as they are smaller and colourless (Ostend, September). **Fig. 7.** Segment of the submarginal region of the thallus displaying female gametangial cells. Prior to fertilization, gametangial cells will differentiate to function as female gametes (Ostend, January). **Fig. 8.** Cross section through the submarginal region of the thallus with female gametes prior to fertilization. Note these cells exhibit a spindle shape due to the presence of two prototrichogynes (arrows), a short receptive extension of the cell onto which male gametes will attach, settle and fertilize the nucleus of the female gamete, resulting in zygotosporangia (Ostend, January). **Fig. 9.** Segment of the submarginal region of the thallus displaying early-

comparisons were performed with the *emmeans* package (Lenth *et al.*, 2018) using Tukey's method. The effect of thallus size on reproductive mode was examined with a two-way ANOVA on maximum thallus length, with reproductive mode, season and their interaction as predictors. Model assumptions were checked using the Shapiro–Wilk test for normality (Shapiro & Wilk, 1965) and Bartlett's test for homogeneity of variances (Bartlett, 1937). Due to minor deviations from normality, Kruskal–Wallis tests were used as a non-parametric validation. Post hoc contrasts were again evaluated using *emmeans*. Significance was set at $\alpha = 0.05$. Graphs were generated either with Excel or using the *ggplot2* v3.3.3 package (Wickham, 2016). Vegetative specimens and species other than *P. umbilicalis* were excluded from the dataset.

Results

Among the 835 specimens collected, 769 were identified as *P. umbilicalis* based on RFLP diagnostic patterns. Notably, *P. dioica* was more prevalent than *P. umbilicalis* at Roscoff, Buarcos, Swansea, Thurlestone Rock and Ambleteuse. Likewise, *P. linearis* was regularly observed at Neeltje-Jans in winter. Individuals collected varied in colour, ranging from olive-green hues in the summer months to darker reddish-brown or brownish tones in winter. Thalli typically grew around a central holdfast, with considerable variation in gross morphology observed among specimens. Blades ranged from orbiculate to elongate, with occasionally pleated margins, and commonly featured multiple blades that overlapped, imparting an umbilicate appearance reminiscent of a rosette. Most field-collected individuals of *P. umbilicalis* were reproductive throughout all months and populations tested, and those possessing zygotosporengia or neutral sporangia released viable spores.

Broad-scale spatial variation in reproductive mode

Across the Northeast Atlantic, *P. umbilicalis* exhibited a pronounced geographic partitioning of reproductive strategies (Fig. 23). Asexual reproduction was geographically constrained to populations in the Southern Bight of the North Sea and the adjacent English Channel – specifically Ambleteuse, Kats, Neeltje-Jans, Ostend and Terschelling – where individuals were predominantly asexual and sexual individuals occurred

only occasionally. In contrast, in populations from the UK (Thurlestone, Swansea, Marloes), Portugal (Buarcos, Leirosa, Vagueira) and France (Roscoff) only sexually reproducing individuals were observed. This spatial structuring was statistically supported by Firth logistic regression, with post-hoc pairwise comparisons confirming significant differences between asexual- and sexually dominated populations (Wald $\chi^2 = 54.99$, $p < 0.0001$).

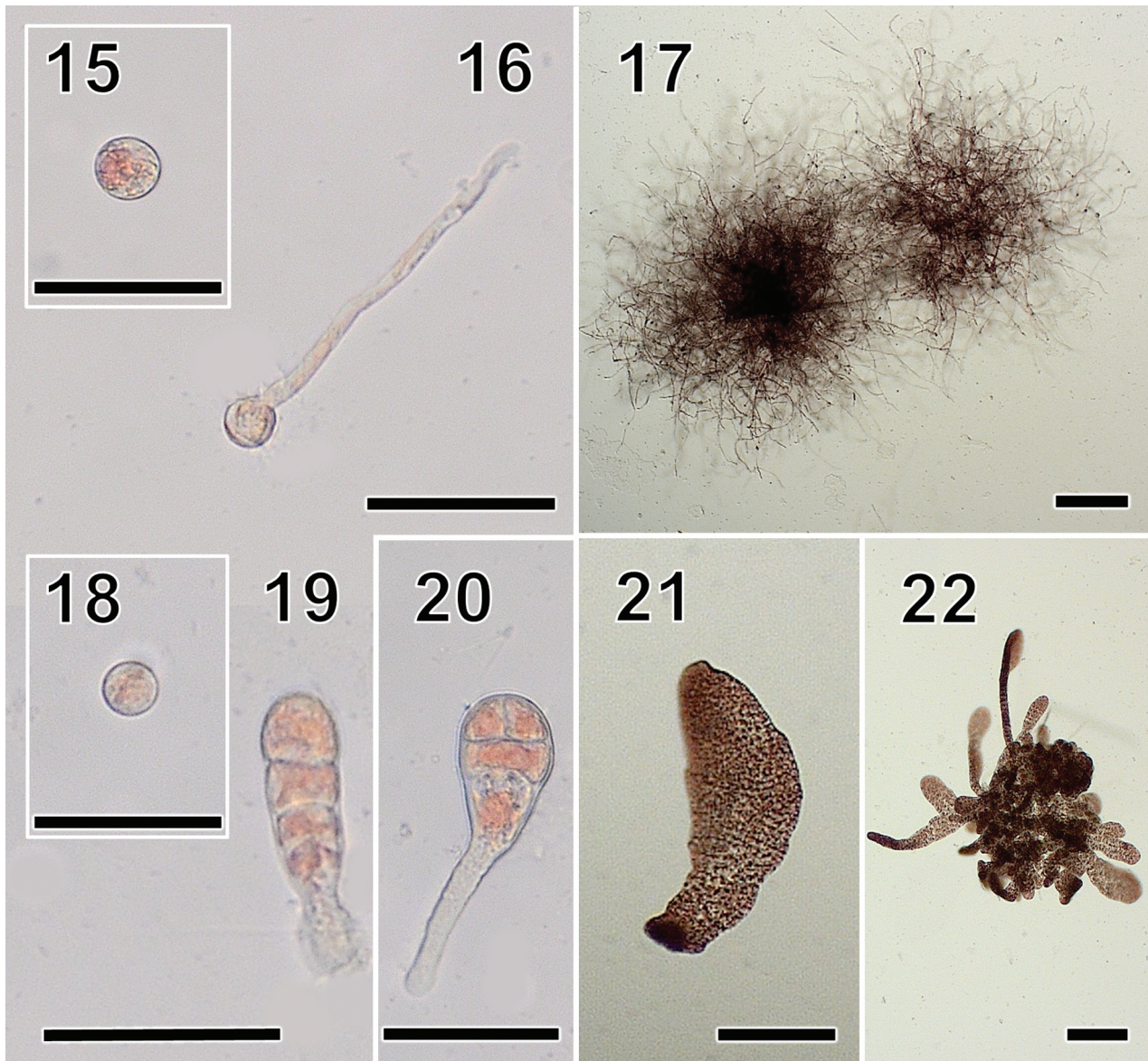
Seasonal dynamics in populations with mixed reproductive modes

In populations where both sexual and asexual individuals co-occurred – specifically Ambleteuse, Ostend and Neeltje-Jans – asexual reproduction was the dominant reproductive mode throughout the year (Fig. 24). Overall, asexual individuals comprised 81.8–100% of samples in autumn, 76.2–77.4% in winter, 73.5–95.2% in spring and 72.0–100% in summer. Bias-reduced logistic regression revealed significant seasonal variation in reproductive mode (Wald $\chi^2 = 12.96$, $p = 0.0047$), primarily driven by a pronounced decline in asexual prevalence during winter compared with autumn (estimate = 1.49 ± 0.47 SE, $z = 3.15$, $p = 0.0088$). Spring and summer did not differ significantly from autumn ($p = 0.3736$ and $p = 0.9121$, respectively), nor did any other seasonal comparisons. Differences among locations were also significant (Wald $\chi^2 = 17.05$, $p = 0.0002$), with Ambleteuse exhibiting a significantly lower proportion of asexual individuals than both Ostend ($p = 0.0037$) and Neeltje-Jans ($p = 0.0020$), which did not differ significantly from each other ($p = 0.9990$).

Fine-scale vertical partitioning within the high intertidal zone

At Ostend, variation in reproductive strategies was observed, with a significant difference in reproductive mode occurring within 2 m of vertical distance in the high intertidal zone (Fig. 25). Asexual individuals dominated the uppermost level of the high intertidal zone, while sexually reproductive individuals were more frequently observed further below. This spatial stratification was statistically supported by generalized linear models (Wald $\chi^2 = 61.21$, $p < 0.0001$) and

postfertilization stages of zygotosporengial packets (Ostend, January). **Fig. 10.** First postfertilization stage where zygotosporengia have undergone the first mitotic division, resulting in two cells (arrowheads) (Neeltje-Jans, March). **Fig. 11.** Segment of the marginal region of the thallus displaying mature zygotosporengia in packets of 8 cells (2×4), giving the surface of the blade a bumpy appearance (Ostend, September). **Fig. 12.** A later stage of Fig. 10, where zygotosporengia have experienced the second mitotic division, resulting in 4 cells (arrowheads) (Ostend, January). **Fig. 13.** Segment of the marginal region of the thallus showing mature neutral sporangia arranged in packets of 4 cells (Terschelling, November). **Fig. 14.** Cross section through the marginal region of the thallus showing neutral sporangia. Note each sporangium has undergone one mitotic division, resulting in 2 cells (arrowheads) (Terschelling, November). All cross sections were handmade. Scale bars: **Figs 3, 5, 7–10,** 50 μm ; **Figs 4, 6, 12–14,** 20 μm ; **Fig. 11,** 100 μm .



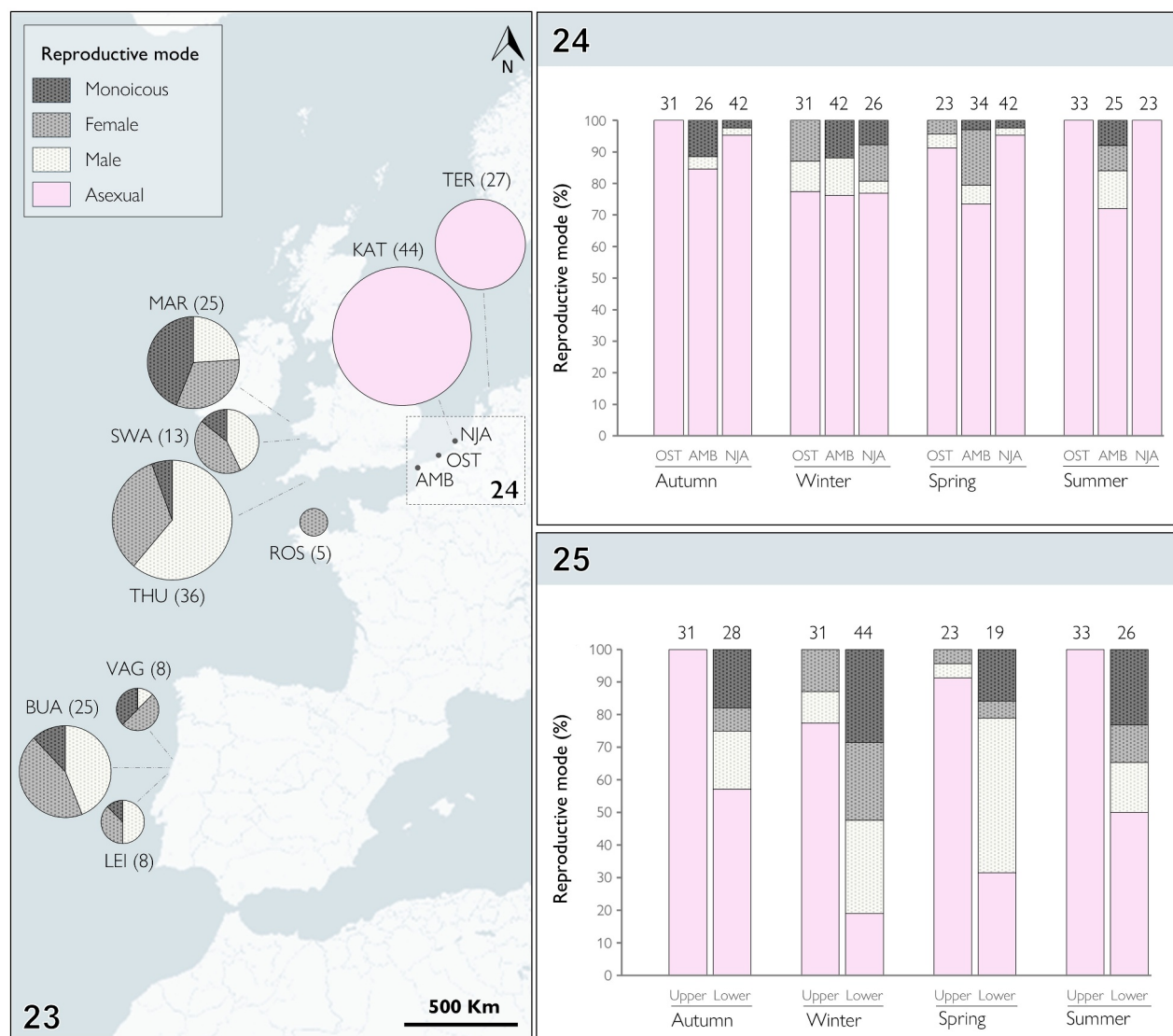
Figs 15–22. Zygospore (Figs 15–17) and neutral spore (Figs 18–22) development of *Porphyra umbilicalis* in culture. **Fig. 15.** A freshly discharged non-motile zygospore. Note its identical morphology to the neutral spore shown in **Fig. 18** (Neeltje-Jans, April). **Fig. 16.** A single-celled zygospore germinating *in situ* (Neeltje-Jans, April). **Fig. 17.** Habit of the filamentous sporophyte (conchocelis phase) after three months of growth (Ostend, March). **Fig. 18.** A freshly discharged non-motile neutral spore (Neeltje-Jans, April). **Figs 19–20.** Most common patterns of early development, with germlings exhibiting either multiple linear divisions (**Fig. 19**) or linear and bilateral divisions (**Fig. 20**) (Neeltje-Jans, April). **Figs 21–22.** Aspect of blades after 1 month of growth. Note some individuals grow as a single blade (**Fig. 21**) while some others (**Fig. 22**) exhibit several blades, probably due to the product of the coalescence of more than one spore (Ostend, March). Scale bars: Figs 15, 16, 18–20, 50 μ m; Figs 17, 21, 22, 200 μ m.

confirmed through Tukey's pairwise comparisons ($p < 0.0001$). In fact, the odds ratio for asexual reproduction in the upper zone was $3.12 (\pm 0.40)$ times higher compared with the lower zone, indicating a stronger prevalence of asexual reproduction in the higher intertidal zone.

Relationship between thallus size and reproductive mode

To further examine potential influences on reproductive strategy, thallus length was assessed in the Ostend population. Individuals varied widely in

length (1 cm to 12 cm), and all reproductive modes were observed across this range (**Fig. 26**). Overall, no significant direct effect was found between reproductive mode and thallus length ($F = 1.61$, $p = 0.2057$). However, when seasonal variation was incorporated into the analysis, a significant interaction between reproductive mode and season emerged ($F = 4.57$, $p = 0.0039$). Post hoc comparisons revealed that sexual individuals were significantly larger than asexual individuals in winter (estimate = 2.21, $p < 0.0001$), with a smaller but still significant size difference also observed in spring (estimate = 1.15, $p = 0.0369$). In contrast,



Figs 23–25. Spatio-temporal variation in reproductive strategies of *Porphyra umbilicalis*. **Fig. 23.** Geographic structuring of reproductive modes along a European gradient. Three populations – Ambleteuse (AMB), Ostend (OST) and Neeltje-Jans (NJA) – were examined in greater detail in the subsequent panel. Pie chart dimensions scale according to sample size. **Fig. 24.** Seasonal variation in the relative abundance (%) of asexual and sexual individuals in mixed populations from the Southern Bight of the North Sea and adjacent English Channel. The numbers above each bar indicate total sample sizes. **Fig. 25.** Fine-scale vertical distribution of reproductive modes in Ostend, Belgium. Relative abundance (%) of asexual and sexual individuals is shown across two vertical levels within the high intertidal zone: the uppermost level (adjacent to the highest high tide mark) and approximately 2 m below. Seasonal patterns are also depicted. The numbers above each bar indicate total sample sizes.

no significant size differences were detected between reproductive modes in autumn ($p = 0.8832$) or summer ($p = 0.6678$).

Discussion

This study presents a comprehensive assessment of the spatial and temporal variation in reproductive strategies of *P. umbilicalis* along the Northeast Atlantic. The main findings are as follows: (1) broad-scale geographic structuring of reproductive modes, with predominantly asexual populations

observed in the Southern Bight of the North Sea and adjacent English Channel; (2) persistent asexual dominance in these populations, accompanied by a higher frequency of sexual individuals during winter months; (3) fine-scale vertical partitioning in the high intertidal zone, where asexual individuals dominate the uppermost levels and sexual individuals occur further down, suggesting microhabitat-driven reproductive patterns and ecological niche differentiation; and (4) no consistent association between thallus size and reproductive strategy, although sexual individuals were larger than asexual individuals during winter and spring.

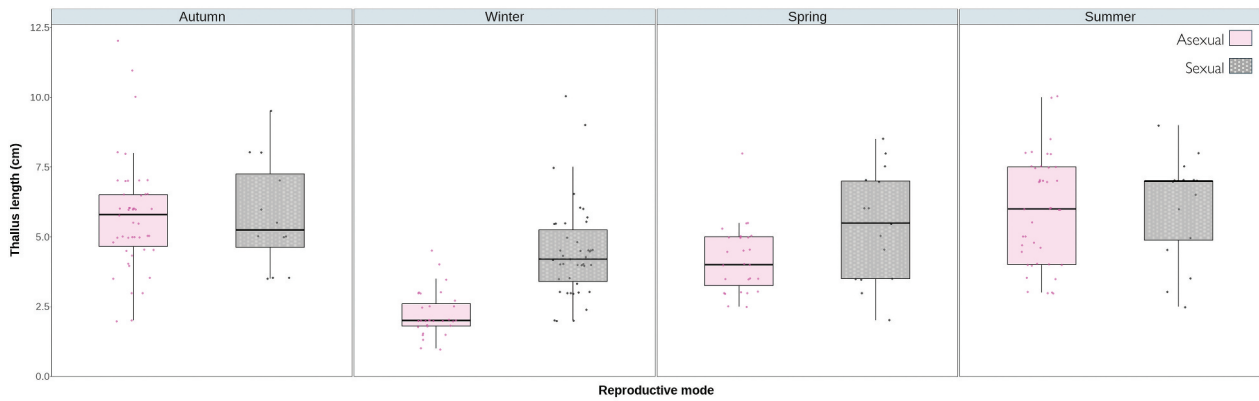


Fig. 26. Box-and-whisker plots comparing thallus length between asexual and sexual individuals of *Porphyra umbilicalis* from Ostend, Belgium. Thallus length was measured as the maximum distance from the holdfast to the blade margin. Boxes represent the interquartile range (IQR), whiskers indicate the range excluding outliers, the horizontal line within each box shows the median, and individual points represent all measured values.

Geographic structuring: common asexual reproduction in Southern Bight of the North Sea and English Channel

Existing literature on *P. umbilicalis* points to a clear example of geographic parthenogenesis, with contrasting reproductive strategies observed between eastern and western North Atlantic populations (Eriksen *et al.*, 2016; Cao, 2018). Our findings extend this framework by revealing a strong geographic structuring in reproductive modes in the native European range of *P. umbilicalis*. Specifically, we found a marked dominance of asexual reproduction in populations from the Southern Bight of the North Sea and adjacent English Channel, while only sexual reproduction occurred in populations from the South European Atlantic Shelf and Celtic Seas. Although asexual reproduction was not observed in our samples from Portugal or Ireland, previous studies have reported consistent asexual propagation of *P. umbilicalis* collected from specific coastal areas in both regions (personal communication, Helena Abreu, Algalink). Notably, the prevalence of asexual reproduction in the Southern North Sea was not as sporadic or rare as previously reported (Conway, 1964; Kornmann & Sahling, 1991; Brodie & Irvine, 2003; Brodie *et al.*, 2008). Instead, our results indicate persistent and dominant asexual reproduction across seasons, suggesting a stable and widespread life history strategy. Sexual individuals conversely appeared at much lower frequency, with a slight but significant increase in winter months. This more constrained window for sexual reproduction likely reflects the synchronization required for successful fertilization, as both male and female gametogenesis must occur concurrently (Fierst *et al.*, 2010). A comparable winter-biased reproductive pattern has been reported in *P. dioica* from Devon (UK), where conchocelis-

producing individuals were most abundant from December to April, albeit no asexual reproduction was observed in that species (Holmes & Brodie, 2004). In Maine (USA), Royer *et al.* (2019) reported clear seasonal differences in successful asexual reproduction by *P. umbilicalis*, which peaked in winter, declined sharply in summer, and may be affected in autumn and spring due to grazing by amphipods. While we did not quantify reproductive output such as number of released and viable spores, we found asexually reproductive individuals year-round.

Adding further nuance, we observed unexpected diversity in sexual systems. While previous reports suggest that monoicous individuals are rare in *P. umbilicalis* (Brodie *et al.*, 2008) and *P. dioica* (around 5%, Holmes & Brodie, 2004), our results revealed an approximately 1:1 ratio of monoicous and dioicous individuals in mixed populations, and a 1:2 ratio in exclusively sexual populations. Two forms of monoicy were identified: individuals bearing both spermatangia and zygotosporangia on the same blade, and individuals with distinct male and female blades arising from the same holdfast. We consider the former to be true monoicous individuals, while the latter may result from the coalescence of more than one spore during germination (Brodie & Irvine, 2003). Theoretical models suggest that flexibility in sex determination and mating systems is critical for population resilience in heterogeneous, resource-limited environments. Trioicous populations – where females, males and monoicous individuals coexist – can be maintained depending on environmental stress dynamics, with dioicy predominating under low or absent stress, monoicy arising under constant high stress, and stable trioicy persisting under intermediate or fluctuating stress conditions (Anderson *et al.*, 2020). Nonetheless, in *P. umbilicalis*, this balance may be disrupted by the potential for asexual reproduction, which might offer a more advantageous strategy

under stressful conditions. Further investigation using population genetic tools is required to understand allele distribution and gene flow within and among populations, uncover potential cryptic sex, and clarify the genetic consequences of possible intragametophytic selfing suggested by the consistent production of zygospores among monoicous individuals.

Earlier records of reproductive patterns in *P. umbilicalis* often relied exclusively on morphological criteria, raising concerns about potential misidentification, especially in light of the historical misapplication of the name *P. umbilicalis* to more than one species (Sutherland *et al.*, 2011). Using molecular identification and extensive spatial and temporal sampling, our study provides robust confirmation of widespread asexuality in Southern North Sea populations. Nevertheless, as these sites are recognized hotspots for non-indigenous species (Knoop *et al.*, 2024; van der Loos *et al.*, 2024), we cannot exclude the possibility of a cryptic introduction of an asexual genotype from the Northwest Atlantic (Blouin & Brawley, 2012; Eriksen *et al.*, 2016). Further elucidation of the origins and evolutionary dynamics underlying the high prevalence of asexual individuals in this region will require high-resolution population genetic/genomic data, broader geographic sampling across the Northeast and Northwest Atlantic, and experimental assessment of life history traits under controlled conditions.

Ecological niche structure between sexual and asexual gametophytes

Throughout the course of a single tidal cycle, intertidal organisms are subjected to profound and rapid fluctuations in both abiotic (e.g. desiccation, temperature, oxygen availability, salinity, pH) and biotic (e.g. herbivory, competition) conditions (Dring, 1982; Horn *et al.*, 2021), which can vary dramatically over spatial scales of just a few metres. Within this dynamic framework, the vertical gradient of the rocky intertidal zone presents a powerful axis of ecological differentiation, along which the reproductive strategies of *P. umbilicalis* appear finely attuned to microhabitat conditions. As demonstrated in this study, the *P. umbilicalis* population in Ostend (Belgium) exhibits a pronounced spatial partitioning of reproductive modes with asexual individuals dominating the upper zone of the high intertidal and sexual reproduction becoming increasingly prevalent at lower elevations. These findings support the hypothesis that microhabitat-driven niche partitioning enables the coexistence of distinct life-history strategies within the same species or population (Rushworth *et al.*, 2018), echoing broader patterns of geographic parthenogenesis in which asexual lineages tend to dominate in marginal habitats – such as high altitudes, recently colonized areas or environments subject to strong

abiotic stressors – rather than their sexual counterparts (Hörandl, 2009; Tilquin & Kokko, 2016).

In the uppermost high intertidal zone – characterized by prolonged aerial exposure, extreme thermal fluctuations and reduced nutrient availability – organisms face intense physiological constraints and limited opportunities for successful gamete fusion. In such environments, asexual reproduction provides a metabolically conservative and ecologically robust alternative to sex, enabling continuous persistence and clonal expansion despite limited mate availability and high energetic costs (Schmidt *et al.*, 2008; Yang & Kim, 2016; Krueger-Hadfield *et al.*, 2021). Mechanistic insights from physiological studies on closely related taxa, such as *Bangia* spp. and *Pyropia* spp., suggest that environmental stress can induce life cycle transitions via calcium-mediated signalling cascades and stress-induced membrane rigidification, thereby promoting asexual reproduction (Monotilla & Notoya, 2004; Blouin *et al.*, 2007; Mikami & Takahashi, 2023). For instance, increased membrane rigidity under thermal stress may impair the development of the prototrichogyne, thus interfering with successful fertilization. Adding a genomic perspective, Eriksen and Klein (2018) found that Northwest Atlantic individuals of *P. umbilicalis* from contrasting environments – a typical open-coast site and an atypical estuarine tidal rapid – exhibit habitat-specific patterns of gene expression, including differential regulation of nitrogen transporters. Their results suggest that asexual populations may fine-tune physiological responses to habitat-specific stressors, reinforcing the view that asexual reproduction can support persistence and ecological versatility under fluctuating or extreme conditions. Conversely, in the lower intertidal – where immersion occurs more frequently, abiotic stress is buffered and ecological interactions such as herbivory and competition intensify – sexual reproduction becomes both viable and advantageous. Here, the transition to the conchocelis phase may function as an adaptive escape strategy against intense grazing pressure and other disturbance events (Lubchenco & Cubit, 1980; Martínez, 1990; Knoop *et al.*, 2020).

Analogous patterns of niche-driven variation in reproductive mode, as observed here in *P. umbilicalis*, have been documented in other intertidal macroalgae. For instance, Engel and Destombe (2002) demonstrated that tidal elevation significantly modulates fertilization success in *Gracilaria gracilis* (Stackhouse) Steentoft, L.M.Irvine & Farnham (Gracilariales, Rhodophyta), underscoring the critical role of spatial positioning in reproductive dynamics. Within the *Fucus spiralis*–*vesiculosus* species complex (Fucales, Ochrophyta), shifts towards selfing and hermaphroditism in upper intertidal populations have been interpreted as adaptive responses to abiotic

stress and reduced mate availability at ecological margins (Billard *et al.*, 2010; Zardi *et al.*, 2011). Further evidence comes from recent investigations into *Chondrus crispus* Stackhouse and *Mastocarpus papillatus* (C.Agardh) Kützinger (Gigartinales, Rhodophyta), which reveal how environmental gradients shape fine-scale genetic structure and reproductive strategy. In *C. crispus*, elevated levels of intergametophytic selfing and reduced genetic diversity in high-intertidal populations suggest adaptation to increased physiological stress and isolation (Krueger-Hadfield *et al.*, 2013). Similarly, in *M. papillatus*, geographic parthenogenesis between genetically different sexual and obligate asexual variants has been described at both latitudinal and tidal height gradients (Fierst *et al.*, 2010; Dudgeon *et al.*, 2017).

Thallus size and reproductive mode in Porphyra umbilicalis

The energetic cost of reproduction is often reflected in the relationship between fecundity and size, highlighting a trade-off between vegetative growth and reproductive investment in both sexual and asexual strategies (Zhang & Zhang, 2007; Liu *et al.*, 2017). For instance, Blouin *et al.* (2007) reported that most purely vegetative *P. umbilicalis* thalli from high-intertidal zones were ≤ 4 cm in length. Similarly, in several species of *Pyropia*, including *Py. yezoensis*, *Py. vietnemensis* (Tanaka & P.H.Hô) J.E.Sutherland & Monotilla and *Py. spiralis* (E.C.Oliveira & Coll) M.C.Oliveira, D. Milstein & E.C.Oliveira, juvenile blades prioritize growth and asexual reproduction until reaching maturity, when gametangia begin to develop (Kapaun & Lemus, 1987; Ruangchuay & Notoya, 2007). However, our findings indicate no direct relationship between thallus size and reproductive mode in *P. umbilicalis*. Although sexual individuals were significantly larger than asexual ones in winter – and to a lesser extent in spring – no overall size differences were observed between male, female, monoicous and asexual individuals. Additionally, culture observations of gametophytes originating from neutral spores showed that juvenile blades – just a few millimetres long – could spontaneously reproduce asexually, echoing early observations by Kurogi (1972) and Brodie & Irvine (2003) in asexual spore-producing species. This suggests that asexuality in *P. umbilicalis* may function as a fixed reproductive strategy rather than a transitional stage preceding sexuality and highlights the strong influence of environmental context and intrinsic regulatory mechanisms in determining reproductive mode.

Altogether, the findings of this study underscore the ecological and evolutionary significance of reproductive diversity in *P. umbilicalis*, shedding light on

how divergent life history strategies facilitate persistence across heterogeneous and dynamic environments. Future research should explore the genetic and environmental mechanisms regulating life cycle transitions, with particular attention to the triggers of sexual versus asexual reproduction and their potential applications in advancing sustainable aquaculture.

Acknowledgements

We thank Luna van der Loos for collecting and providing additional samples of *Porphyra umbilicalis* from Terschelling (the Netherlands).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by Flanders Innovation & Entrepreneurship (VLAIO) under the ICON project BlueMarine3.com [AIOSBO2019001503] and cSBO project GAME [HBC.2023.0503] as well as EMBC Belgium and the Research Foundation Flanders [projects I000825N and I001621N], the Delta Climate Center (funded by the Province of Zeeland and the ministry of OCW) and the Fonds Wetenschappelijk Onderzoek [1148826N].

Data availability statement

Data supporting this article will be shared on reasonable request to the corresponding author.

Author contributions

J. Morcillo: Data curation (equal), Formal analysis (equal), Investigation (equal), Methodology (equal), Project administration (equal), Resources (equal), Validation (equal), Visualization (lead), Writing - original draft (lead), Writing - review & editing (equal); O. De Clerck: Funding acquisition (lead), Validation (equal), Visualization (supporting), Writing - review & editing (supporting); S. D'hondt: Methodology (supporting); F. Leliaert: Writing - review & editing (supporting); S. Vranken: Visualization (supporting), Writing - review & editing (supporting); J. Knoop: Conceptualization (lead), Data curation (equal), Formal Analysis (equal), Funding acquisition (supporting), Investigation (equal), Methodology (equal), Project administration (equal), Resources (equal), Supervision (lead), Validation (equal), Visualization (supporting), Writing - review & editing (equal).

ORCID

Jordi Morcillo  <http://orcid.org/0009-0001-2276-8171>
Olivier De Clerck  <http://orcid.org/0000-0002-3699-8402>
Sofie D'hondt  <http://orcid.org/0000-0002-2128-0553>
Frederik Leliaert  <http://orcid.org/0000-0002-4627-7318>
Sofie Vranken  <http://orcid.org/0000-0002-8459-041X>
Jessica Knoop  <http://orcid.org/0000-0001-9374-0179>

References

- Anderson, G., Bubrig, L. & Fierst, J. (2020). Environmental stress maintains trioecy in nematode worms. *Evolution*, **74**: 518–527.
- Barrett, S.C.H. (2002). The evolution of plant sexual diversity. *Nature Reviews Genetics*, **3**: 274–284.
- Barrett, S.C.H. (2011). Why reproductive systems matter for the invasion biology of plants. In *Fifty years of invasion ecology: The legacy of Charles Elton* (Richardson, D.M., eds.), 195–210. Blackwell Publishing, Oxford.
- Bartlett, M.S. (1937). Properties of sufficiency and statistical tests. *Proceedings of the Royal Society of London. Series A – Mathematical and Physical Sciences*, **160**: 268–282.
- Billard, E., Serrão, E., Pearson, G., Destombe, C. & Valero, M. (2010). *Fucus vesiculosus* and *spiralis* species complex: a nested model of local adaptation at the shore level. *Marine Ecology Progress Series*, **405**: 163–174.
- Blouin, N.A., Brodie, J.A., Grossman, A.C., Xu, P. & Brawley, S.H. (2011). *Porphyra*: a marine crop shaped by stress. *Trends in Plant Science*, **16**: 29–37.
- Blouin, N. & Brawley, S. (2012). An AFLP-based test of clonality in widespread, putatively asexual populations of *Porphyra umbilicalis* (Rhodophyta) in the Northwest Atlantic with an *in silico* analysis for bacterial contamination. *Marine Biology*, **159**: 2723–2729.
- Blouin, N., Xiugeng, F., Peng, J., Yarish, C. & Brawley, S.H. (2007). Seeding nets with neutral spores of the red alga *Porphyra umbilicalis* (L.) Kützinger for use in integrated multi-trophic aquaculture (IMTA). *Aquaculture*, **270**: 77–91.
- Brodie, J.A. & Irvine, L.M. (2003). *Seaweeds of the British Isles, volume 1, Rhodophyta, part 3B: Bangiophycidae*. Intercept, Andover.
- Brodie, J., Irvine, L., Neefus, C.D. & Russell, S. (2008). *Ulva umbilicalis* L. and *Porphyra umbilicalis* Kütz. (Rhodophyta, Bangiaceae): a molecular and morphological redescription of the species, with a typification update. *Taxon*, **57**: 1328–1331.
- Cao, Y. (2018). Population genetics and desiccation stress of *Porphyra umbilicalis* Kützinger in the Gulf of Maine. Doctoral dissertation, University of New Hampshire.
- Coelho, S.M., Peters, A.F., Charrier, B., Roze, D., Destombe, C., Valero, M. & Cock, J.M. (2007). Complex life cycles of multicellular eukaryotes: new approaches based on the use of model organisms. *Gene*, **406**: 152–170.
- Conway, E. (1964). Autecological studies of the genus *Porphyra*: II. *Porphyra umbilicalis* (L.) J. Ag. *British Phycological Bulletin*, **2**: 349–363.
- Dlaza, T.S. (2011). *Development in culture, ecophysiology and nutritional content of three South African Porphyra (Rhodophyta, Bangiales) species*. Doctoral dissertation, University of Cape Town.
- Doyle, J.J. & Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, **19**: 11–15.
- Drew, K.M. (1954). Studies in the Bangioideae III. The life-history of *Porphyra umbilicalis* (L.) Kütz. var. *lociniata* (Lightf.) J. Ag. *Annals of Botany*, **18**: 183–184.
- Dring, M.J. (1982). *The biology of marine plants*. Cambridge University Press, Cambridge.
- Dudgeon, S., Kübler, J.E., West, J.A., Kamiya, M. & Krueger-Hadfield, S.A. (2017). Asexuality and the cryptic species problem. *Perspectives in Phycology*, **4**: 47–59.
- Engel, C.R. & Destombe, C. (2002). Reproductive ecology of an intertidal red seaweed, *Gracilaria gracilis*: influence of high and low tides on fertilization success. *Journal of the Marine Biological Association of the United Kingdom*, **82**: 281–288.
- Eriksen, R.L., Green, L.A. & Klein, A.S. (2016). Genetic variation within and among asexual populations of *Porphyra umbilicalis* Kützinger (Bangiales, Rhodophyta) in the Gulf of Maine, USA. *Botanica Marina*, **59**: 1–12.
- Eriksen, R.L. & Klein, A.S. (2018). Organism-environment interactions and differential gene expression patterns among open-coastal and estuarine populations of *Porphyra umbilicalis* Kützinger (Rhodophyta) in the Northwest Atlantic. *Fisheries and Aquatic Sciences*, **21**: 28.
- Fierst, J.L., Kübler, J.E. & Dudgeon, S.R. (2010). Spatial distribution and reproductive phenology of sexual and asexual *Mastocarpus papillatus* (Rhodophyta). *Phycologia*, **49**: 274–282.
- Fox, J. & Weisberg, S. (2020). Using car and effects functions in other functions. *Cranio*, **3**: 1–5.
- Gantt, E., Berg, G.M., Bhattacharya, D., Blouin, N.A., Brodie, J.A., Chan, C.X., Collén, J., Cunningham, F.X., Jr, Gross, J., Grossman, A.R., Karpowicz, S., Kitade, Y., Klein, A.S., Levine, I.A., Lin, S., Lu, S., Lynch, M., Minocha, S.C., Müller, K., Neefus, C.D., Oliveira, M.C., Rymarquis, L., Smith, A., Stiller, J.W., Wu, W.-K., Yarish, C., Zhuang, Y. & Brawley, S.H. (2010). *Porphyra*: Complex life histories in a harsh environment: *P. umbilicalis*, an intertidal red alga for genomic analysis. In *Red algae in the genomic age. Cellular origin, life in extreme habitats and astrobiology* (Seckbach, J. & Chapman, D., eds.), 129–148. Vol. 13. Springer, Dordrecht.
- Guiry, M.D. (1990). Sporangia and Spores. In *Biology of red algae* (Cole, K.M. & Sheath, R.G., eds.), 347–376. Cambridge University Press, Cambridge.
- Guiry, M.D. & Guiry, G.M. (2025). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <https://www.algaebase.org>; searched on 5 March 2025.
- Halkett, F., Simon, J.C. & Balloux, F. (2005). Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology and Evolution*, **20**: 194–201.
- Holmes, M.J. & Brodie, J. (2004). Morphology, seasonal phenology and observations on some aspects of the life history in culture of *Porphyra dioica* (Bangiales, Rhodophyta) from Devon, UK. *Phycologia*, **43**: 176–188.
- Hörandl, E. (2009). Geographical parthenogenesis: opportunities for asexuality. In *Lost sex: the evolutionary biology of parthenogenesis* (Schön, I., Martens, K. & van Dijk, P., eds.), 161–186. Springer, Dordrecht.
- Horn, K.M., Fournet, M.E.H., Liautaud, K.A., Morton, L. N., Cyr, A.M., Handley, A.L., Dotterweich, M.M., Anderson, K.N., Zippay, M.L. & Hardy, K.M. (2021). Effects of intertidal position on metabolism and behavior in the acorn barnacle, *Balanus glandula*. *Integrative Organismal Biology*, **3**: 1–23.
- Hughes, J.S. & Otto, S.P. (1999). Ecology and the evolution of biphasic life cycles. *The American Naturalist*, **154**: 306–320.
- Ingolfsson, A. (1992). The origin of the rocky shore fauna of Iceland and the Canadian Maritimes. *Journal of Biogeography*, **19**: 705–712.
- Kapraun, D. & Lemus, A. (1987). Field and culture studies of *Porphyra spiralis* var. *amplifolia* Oliveira Filho et Coll

- (Bangiales, Rhodophyta) from Isla de Margarita, Venezuela. *Botanica Marina*, **30**: 483–490.
- Knoop, J., Bouckennooghe, S., van der Loos, L.M., D'hondt, S., Karremans, M., Perk, F., Leliaert, F. & De Clerck, O. (2024). DNA barcoding reveals an extensive number of cryptic introductions of bladed Bangiales (Rhodophyta) in the Southern North Sea. *Frontiers in Marine Science*, **11**: 1415034.
- Knoop, J., Griffin, J. & Barrento, S. (2020). Cultivation of early life history stages of *Porphyra dioica* from the British Isles. *Journal of Applied Phycology*, **32**: 459–471.
- Kornmann, P. (1994). Life histories of monostromatic *Porphyra* species as a basis for taxonomy and classification. *European Journal of Phycology*, **29**: 69–71.
- Kornmann, P. & Sahling, P.-H. (1991). The *Porphyra* species of Helgoland (Bangiales, Rhodophyta). *Helgoländer Meeresuntersuchungen*, **45**: 1–38.
- Kosmidis, I. (2023). *brglm2: bias reduction in generalized linear models*. R package version 0.9.2. <https://CRAN.R-project.org/package=brglm2>.
- Krueger-Hadfield, S.A. (2020). What's ploidy got to do with it? Understanding the evolutionary ecology of macroalgal invasions necessitates incorporating life cycle complexity. *Evolutionary Applications*, **13**: 486–499.
- Krueger-Hadfield, S.A. (2024). Let's talk about sex: why reproductive systems matter for understanding algae. *Journal of Phycology*, **60**: 581–597.
- Krueger-Hadfield, S.A., Guillemin, M.-L., Destombe, C., Valero, M. & Stoeckel, S. (2021). Exploring the genetic consequences of clonality in haplodiplontic taxa. *Journal of Heredity*, **112**: 92–107.
- Krueger-Hadfield, S.A., Roze, D., Mauger, S. & Valero, M. (2013). Intergametophytic selfing and microgeographic genetic structure shape populations of the intertidal red seaweed *Chondrus crispus*. *Molecular Ecology*, **22**: 3242–3260.
- Krueger-Hadfield, S.A., Shainker-Connelly, S.J., Crowell, R.M. & Vis, M.L. (2024). The eco-evolutionary importance of reproductive system variation in the macroalgae: freshwater reds as a case study. *Journal of Phycology*, **60**: 15–25.
- Kurogi, M. (1972). Systematics of *Porphyra* in Japan. In *Contributions to the systematics of benthic marine algae of the North Pacific* (Abbott, I.A. & Kurogi, M., eds.), 167–191. Japanese Society of Phycology, Kobe.
- Lenth, R., Singmann, H., Love, J., Buerkner, P. & Herve, M. (2018). *emmeans: estimated marginal means, aka least-squares means*. R package version 4.0-3. <https://CRAN.R-project.org/package=emmeans>
- Liu, X., Bogaert, K., Engelen, A.H., Leliaert, F., Roleda, M. Y. & De Clerck, O. (2017). Seaweed reproductive biology: environmental and genetic controls. *Botanica Marina*, **60**: 89–108.
- Lubchenco, J. & Cubit, J. (1980). Heteromorphic life histories of certain marine algae as adaptations to variations in herbivory. *Ecology*, **61**: 676–687.
- Mable, B.K. & Otto, S.P. (1998). The evolution of life cycles with haploid and diploid phases. *Bioessays*, **20**: 453–462.
- Markham, J. & Hagmeier, E. (1982). Observations on the effects of germanium dioxide on the growth of macroalgae and diatoms. *Phycologia*, **21**: 125–130.
- Martínez, E. (1990). The conchocelis-phase of *Porphyra* (Rhodophyta) in the intertidal of San Juan Island, Washington, USA. *Phycologia*, **29**: 391–395.
- Mikami, K. & Takahashi, M. (2023). Life cycle and reproduction dynamics of Bangiales in response to environmental stresses. *Seminars in Cell and Developmental Biology*, **134**: 14–26.
- Mitman, G.G. & van der Meer, J.P. (1994). Meiosis, blade development, and sex determination in *Porphyra purpurea* (Rhodophyta). *Journal of Phycology*, **30**: 147–159.
- Monotilla, W.D. & Notoya, M. (2004). Morphological and physiological responses of *Porphyra suborbiculata* Kjellman (Bangiales, Rhodophyta) blades from five localities. *Botanica Marina*, **47**: 323–334.
- Nelson, W.A., Brodie, J. & Guiry, M.D. (1999). Terminology used to describe reproduction and life history stages in the genus *Porphyra* (Bangiales, Rhodophyta). *Journal of Applied Phycology*, **11**: 407–410.
- Notoya, M. (1997). Diversity of life history in the genus *Porphyra*. *Natural History Research*, Special Issue, **3**: 47–56.
- Orive, M.E., Barfield, M., Fernandez, C. & Holt, R.D. (2017). Effects of clonal reproduction on evolutionary lag and evolutionary rescue. *The American Naturalist*, **190**: 469–490.
- Orive, M.E., Barfield, M. & Holt, R.D. (2023). Partial clonality expands the opportunity for spatial adaptation. *The American Naturalist*, **202**: 681–698.
- R Core Team. (2022). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.r-project.org/>.
- Rescan, M., Lenormand, T. & Roze, D. (2016). Interactions between genetic and ecological effects on the evolution of life cycles. *The American Naturalist*, **187**: 19–34.
- Royer, C.J., Redmond, S., Lai, C.S. & Brawley, S.H. (2019). *Porphyra umbilicalis* in applied and basic research: reproductive phenology, development, seed stock culture, and a field trial for aquaculture. *Journal of Applied Phycology*, **31**: 547–560.
- Ruangchuay, R. & Notoya, M. (2007). Reproductive strategy and occurrence of gametophytes of Thai laver *Porphyra vietnamensis* Tanaka et Pham-Hoang Ho (Bangiales, Rhodophyta) from Songkhla Province. *Agriculture and Natural Resources*, **41**: 143–152.
- Rushworth, C.A., Windham, M.D., Keith, R.A. & Mitchell-Olds, T. (2018). Ecological differentiation facilitates fine-scale coexistence of sexual and asexual *Boechera*. *American Journal of Botany*, **105**: 2051–2064.
- Schmidt, P.S., Serrão, E.A., Pearson, G.A., Riginos, C., Rawson, P.D., Hilbish, T.J., Brawley, S.H., Trussell, G. C., Carrington, E. & Wetthey, D.S. (2008). Ecological genetics in the North Atlantic: environmental gradients and adaptation at specific loci. *Ecology*, **89**: 91–107.
- Shapiro, S.S. & Wilk, M.B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, **52**: 591–611.
- Sutherland, J.E., Lindstrom, S.C., Nelson, W.A., Brodie, J., Lynch, M.D., Hwang, M.S., Choi, H.G., Miyata, M., Kikuchi, N. & Oliveira, M.C. (2011). A new look at an ancient order: generic revision of the Bangiales (Rhodophyta) 1. *Journal of Phycology*, **47**: 1131–1151.
- Teasdale, B., West, A., Taylor, H. & Klein, A. (2002). A simple restriction fragment length polymorphism (RFLP) assay to discriminate common *Porphyra* (Bangiophyceae, Rhodophyta) taxa from the Northwest Atlantic. *Journal of Applied Phycology*, **14**: 293–298.
- Teasdale, B.W. & Klein, A.S. (2010). Genetic variation and biogeographical boundaries within the red alga *Porphyra umbilicalis* (Bangiales, Rhodophyta). *Botanica Marina*, **53**: 417–431.

- Tilquin, A. & Kokko, H. (2016). What does the geography of parthenogenesis teach us about sex? *Philosophical Transactions of the Royal Society B: Biological Sciences*, **371**: 20150538.
- Vandel, A. (1928). La parthénogenèse géographique. Contribution à l'étude biologique et cytologique de la parthénogenèse naturelle. *Bulletin Biologique De La France Et De La Belgique*, **62**: 164–281.
- van der Loos, L., Bafort, Q., Bosch, S., Ballesteros, E., Bárbara, I., Berecibar, E., Blanfuné, A., Bogaert, K., Bouckennooghe, S., Boudouresque, C.-F., Brodie, J., Cecere, E., Díaz-Tapia, P., Engelen, A.H., Gunnarson, K., Hamdy Shabaka, S., Hoffman, R., Husa, V., Israel, Á., Karremans, M., Knoop, J., Le Gall, L., Maggs, C.A., Mineur, F., Parente, M., Perk, F., Petrocelli, A., Rodríguez-Prieto, C., Ruitton, S., Sansón, M., Serrão, E.A., Sfriso, A., Sjøtun, K., Stiger-Pouvreau, V., Surget, G., Taskin, E., Thibaut, T., Tsiamis, K., Van De Weghe, L., Verlaque, M., Viard, F., Vranken, S., Leliaert, F. & De Clerck, O. (2024). Non-indigenous seaweeds in the Northeast Atlantic Ocean, the Mediterranean Sea and Macaronesia: a critical synthesis of diversity, spatial and temporal patterns. *European Journal of Phycology*, **59**: 127–156.
- van Groenigen, J., Derksen, G.C.H. & Timmermans, K.R. (2022). Review of presence, induction and isolation of major cellular constituents from *Porphyra* sensu lato (Rhodophyceae), including mycosporine-like amino acids (MAA's). *Journal of Marine Biology and Aquaculture Research*, **4**: 30–46.
- Varela-Álvarez, E., Loureiro, J., Meirmans, P.G., Castro, M. & Serrão, E.A. (2021). Genomes vary in size and spatial patterns within chimeric blades of *Porphyra* spp. *Frontiers In Marine Science*, **8**: 628183.
- Varela-Álvarez, E., Loureiro, J., Paulino, C. & Serrão, E.A. (2018). Polyploid lineages in the genus *Porphyra*. *Scientific Reports*, **8**: 8696.
- Varela-Álvarez, E., Stengel, D.B. & Guiry, M.D. (2004). The use of image processing in assessing conchocelis growth and conchospore production in *Porphyra linearis*. *Phycologia*, **43**: 282–287.
- West, J.A. & McBride, D.L. (1999). Long-term and diurnal carpospore discharge patterns in the Ceramiaceae, Rhodomelaceae and Delesseriaceae (Rhodophyta). *Hydrobiologia*, **398-399**: 101–114.
- Wickham, H. (2016). *ggplot2: elegant graphics for data analysis*. Springer, New York. <https://ggplot2.tidyverse.org>.
- Yang, Y.Y. & Kim, J.G. (2016). The optimal balance between sexual and asexual reproduction in variable environments: a systematic review. *Journal of Ecology and Environment*, **40**: 1–18.
- Zardi, G.I., Nicastro, K.R., Canovas, F., Ferreira Costa, J., Serrao, E.A. & Pearson, G.A. (2011). Adaptive traits are maintained on steep selective gradients despite gene flow and hybridization in the intertidal zone. *PLOS ONE*, **6**: e19402.
- Zhang, Y. & Zhang, D. (2007). Asexual and sexual reproductive strategies in clonal plants. *Frontiers of Biology in China*, **2**: 256–262.