PHYCOMORPH

Advancing Knowledge On Seaweed Growth And Development

WG2
Reproduction and initiation of new generations

Olivier De Clerck

UNIVERSITEIT GENT

UGent Phycology

Deliverables

- **DL2-1*- Identify the parameters triggering the release of reproductive cells into seawater.**

- DL2-2*- Characterize the physiological parameters of germ cells' motility within the seawater: velocity, survival, phototaxis.

- DL2-3*- Characterize the chemo-attraction of female and male germ cells and identify the molecular factors enabling physical recognition and contact between the female and male germ cells. Describe their fusion mechanisms at the cellular and molecular levels.

- DL2-4- Identify the processes of cytoplasmic heredity in the first steps of zygotic cell division (contributing to improved seaweed selection for algoculture).

- **DL2-5*- Characterize the establishment of the cell polarity axis at the sub-cellular level prior to and after the first cell division (cytoskeleton, cell nucleus position and orientation of the division plan). This will impact on the further developmental pattern of the seaweed.**

Brown algae – reproduction – gamete release

- Evolution - diversity in life cycles
- Developmental biology
- Fertilisation ecology

The evolution of the life cycle of brown seaweeds

GRAHAM BELL

seminars in CELL & DEVELOPMENTAL BIOLOGY, Vol 9, 1998: pp. 179–185

Polarity determination in *Fucus*: From zygote to multicellular embryo

*Colin Brownlee† and Francois-Yves Bouget**

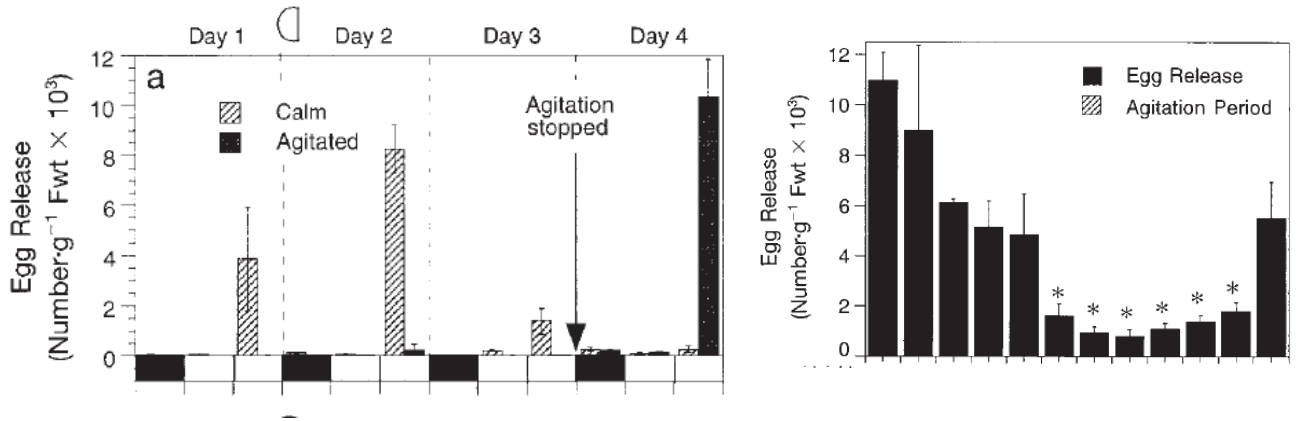
Successful external fertilization in turbulent environments

(Fucales/gamete release/reproductive ecology/spawning/water motion)

ESTER A. SERRÃO*†‡, GARETH PEARSON*, LENA KAUTSKY§, AND SUSAN H. BRAWLEY*

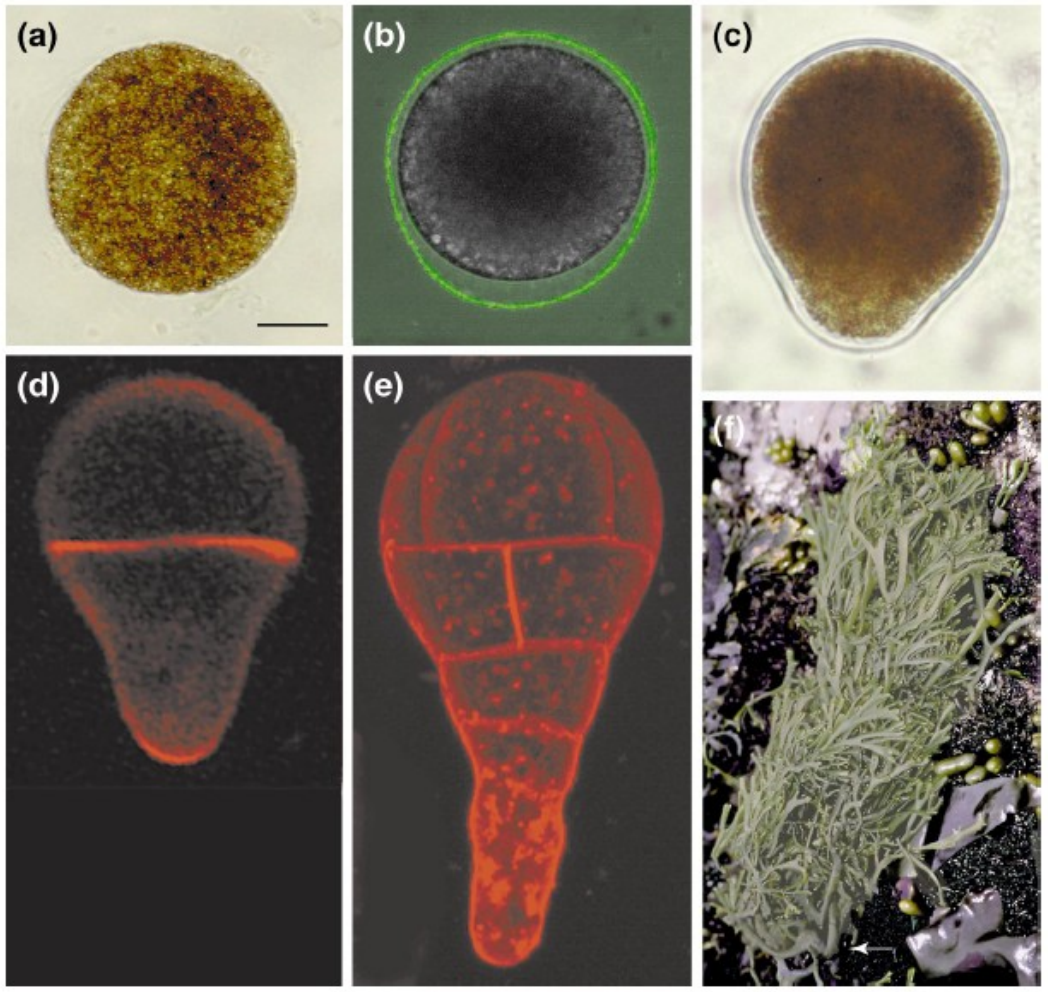
Pre-Phycomorph

Fertilisation ecology



Serrao et al. 1996

Polarity establishment



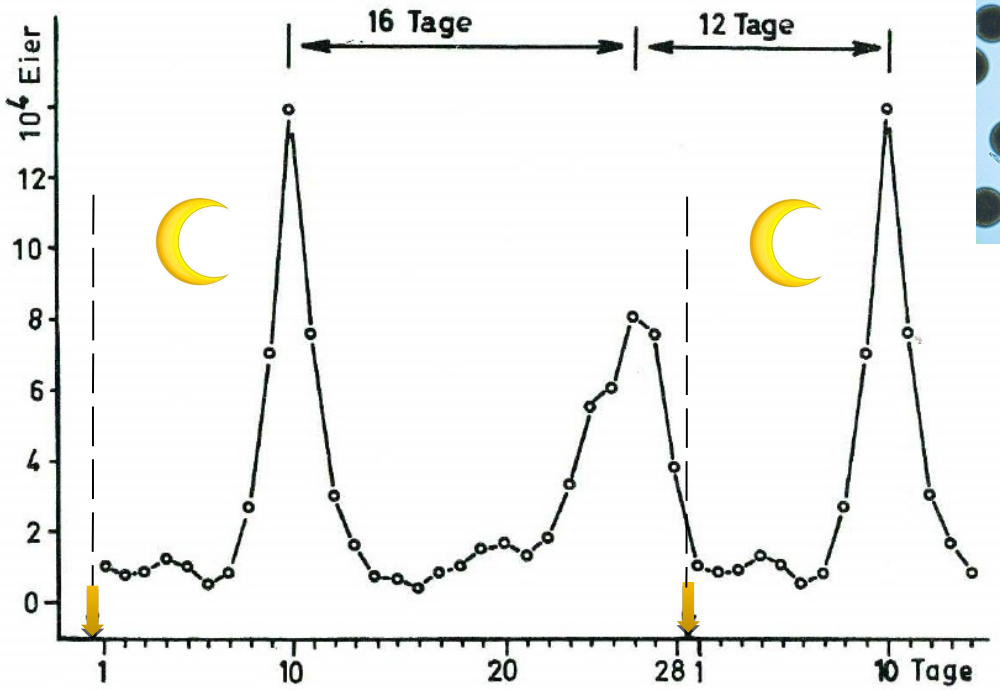
Kropf et al. 1999

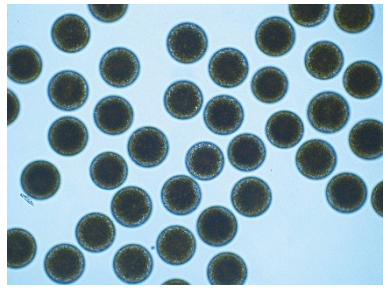
Brown algae – release of reproductive cells

Lunar Periodicity
Dictyota

Über jahres- und lunarperiodische Erscheinungen bei einigen Braunalgen

DIETER MÜLLER
Botanisches Institut, Tübingen





Dictyota gamete release ~ lunar periodicity

[Muller 1962]

Dictyota – release of reproductive cells

Studies in the Dictyotaceae.

III. The Periodicity of the Sexual Cells in Dictyota dichotoma.

BY

J. LLOYD WILLIAMS,

Assistant Lecturer in Botany, University College, Bangor.

1905

THE PERIODIC FRUITING OF DICTYOTA AND ITS RELATION TO THE ENVIRONMENT [1]

W. D. Hoyt

1927

PERIODICITY IN DICTYOTA AT NAPLES

I. F. Lewis

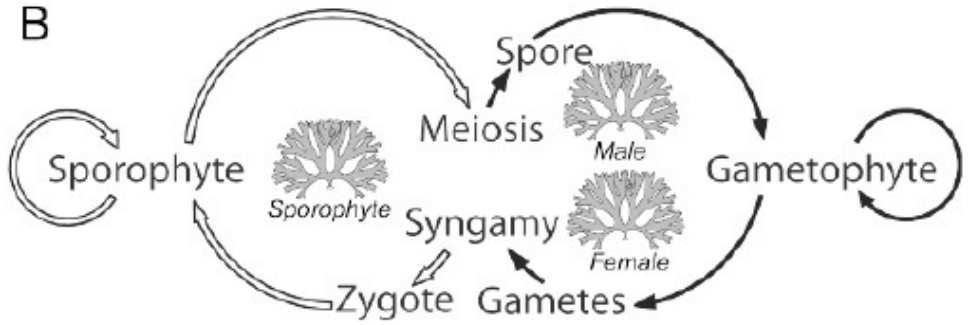
1910

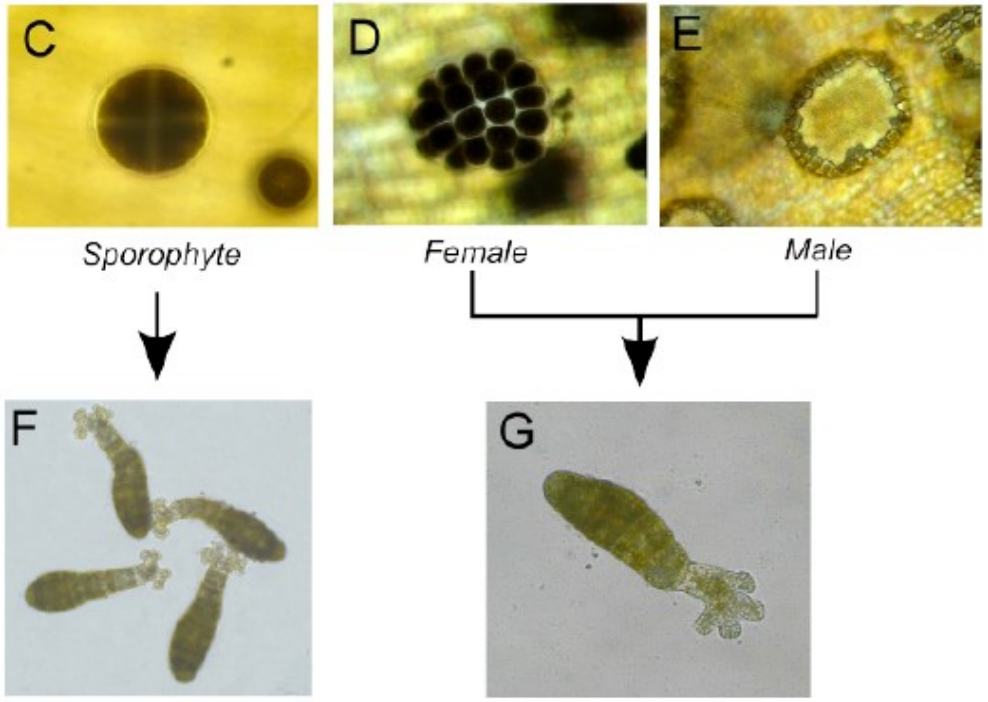
Personal observations
[Roscoff, Wimereux, Goes]

Release windows differ between localities

Dictyota – release of reproductive cells

- Isomorphic
- Oogamous
- Meiospores 4, non-flagellate





[Bogaert et al. 2016]

Dictyota – release of reproductive cells

Abiotic regulation of growth and fertility in the sporophyte of *Dictyota dichotoma* (Hudson) J.V. Lamouroux (Dictyotales, Phaeophyceae)

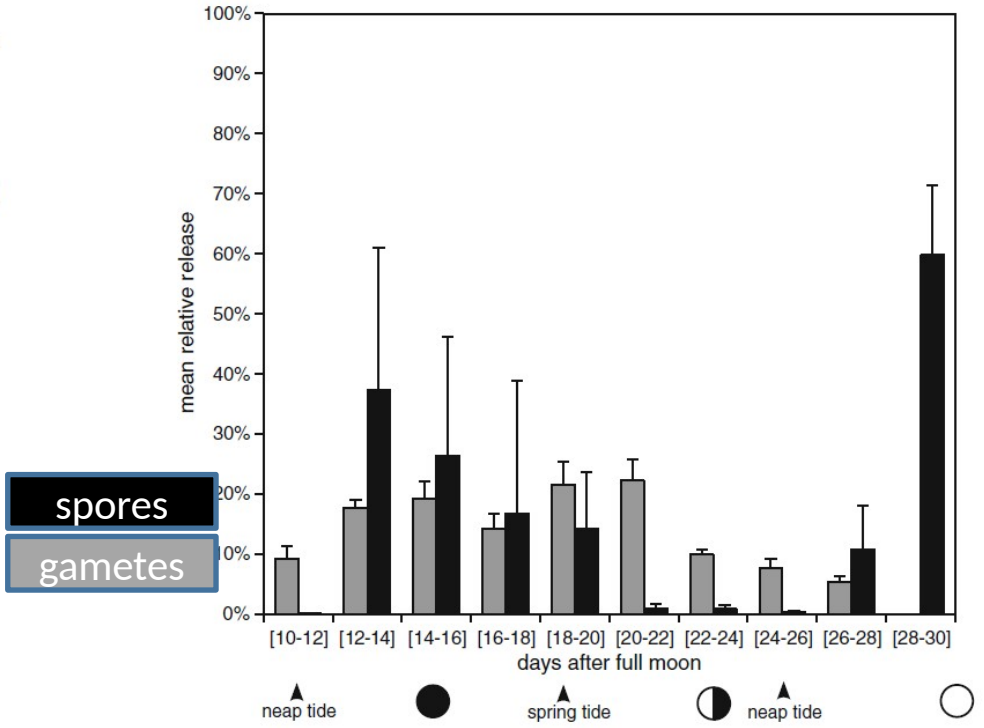
Kenny Bogaert[1] · Tom Beeckman[2,3] · Olivier De Clerck[1]

Periodicity - monthly

Gametes show semi-lunar periodicity,

Spores not

Fig. 5 Fortnightly release periodicity of *Dictyota dichotoma* sporophytes (*grey bars*) using gametophytes as a control (*black bars*) near l'Ancient Fort Croix (Wimereux, France). The histogram shows the percentage of total release of eggs (*black*) and spores (*grey*) for the hatched part of the lunar cycle (*error bars* denote standard errors). Approximate positions of spring and neap tides in Wimereux are marked on the lunar cycle with *arrowheads*. *Black circle* new moon, *half darkened circle* second quarter, *white circle* full moon



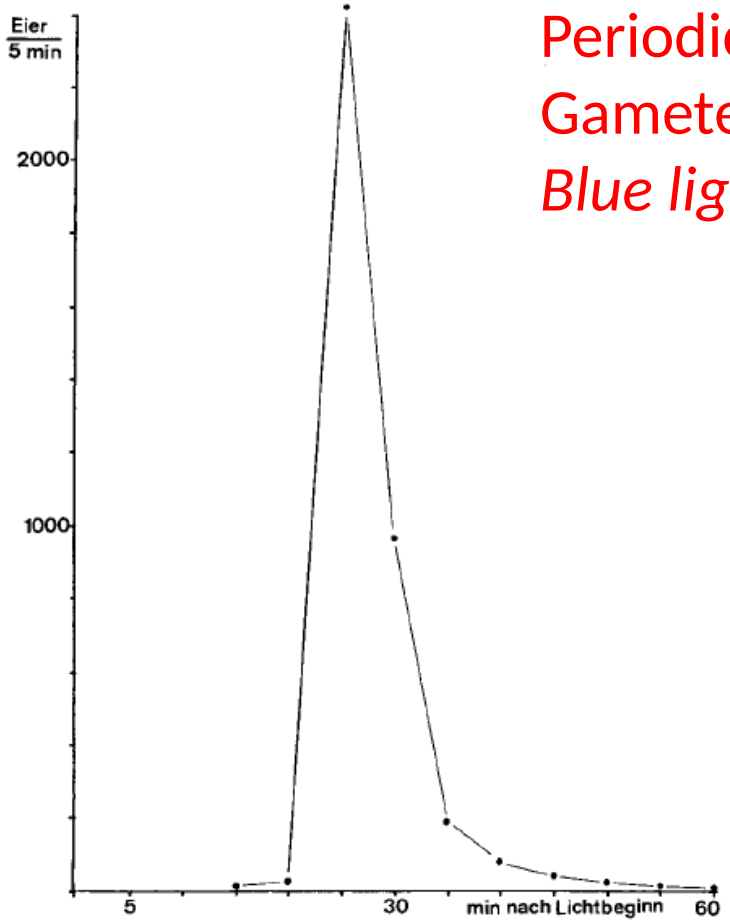
[Bogaert et al. J.Apl.Phycol., 2016]

Dictyota – release of reproductive cells

Botanisches Institut der Universität Erlangen

Beiträge zur Periodizität der Oogon-Entleerung bei *Dictyota dichotoma* (Phaeophyta)

JENS KUMKE



Periodicity - day

Gamete release ~ light induced

Blue light response

[Kumke, Zeitschr. Pflanzenphysiologie 1973]

Dictyota – release of reproductive cells

Abiotic regulation of growth and fertility in the sporophyte of *Dictyota dichotoma* (Hudson) J.V. Lamouroux (Dictyotales, Phaeophyceae)

Kenny Bogaert[1] · Tom Beeckman[2,3] · Olivier De Clerck[1]

Periodicity - day

Spore release ~ light induced

Wave lengths not tested

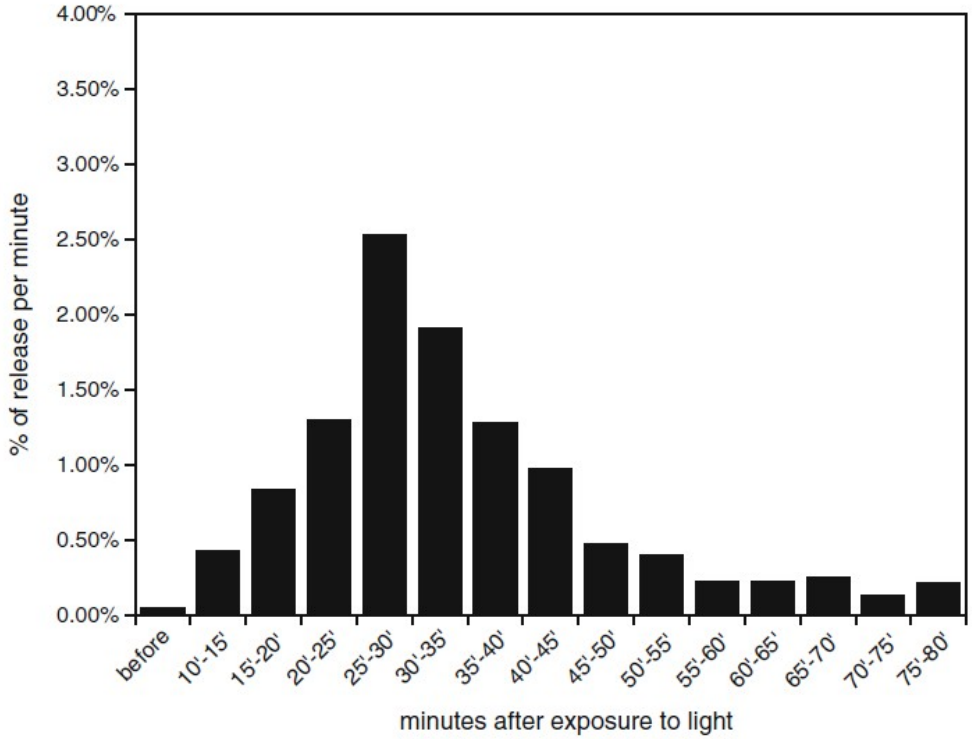


Fig. 4 Diurnal release periodicity of *Dictyota dichotoma* spores by field-sampled sporophytes (near l'Ancient Fort Croix, Wimereux). Fraction of total observed spores per time unit of 1 min. The first interval consists of release during the 15 h of incubation in the dark and subsequent 10 min of rinsing. The other intervals depict the fraction released at a 5-min interval. *Error bars* indicate standard errors ($n = 3$)

[Bogaert et al. J.Apl.Phycol., 2016]

Rhythmicity

The genomic basis of circadian and circalunar timing adaptations in a midge

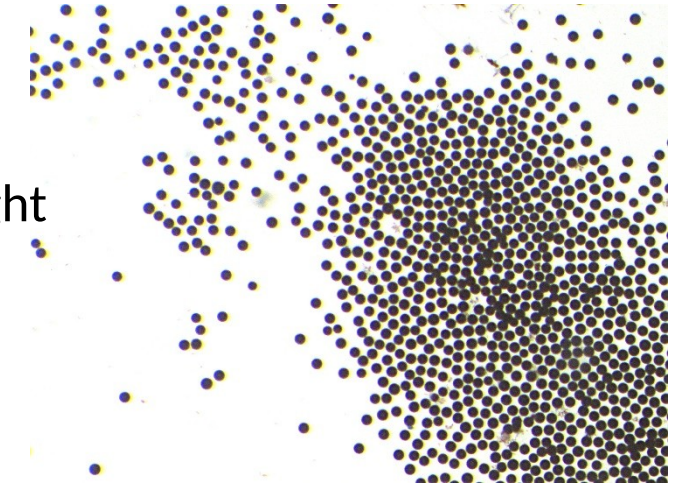
[Kaiser et al., Nature 2016]

Clunio marinus

- Mating occurs around the new or full moon during a few specific hours surrounding low tide
- Timing of low tide differs between localities ~ local adaptation between populations
- The differences in circadian and circalunar timing are genetically determined

---

- Gamete release occurs twice a month but invariably at dawn (in lab – blue light)
- Release windows can be easily reset in the lab by simulating night light
- A second cue involved. Tides?



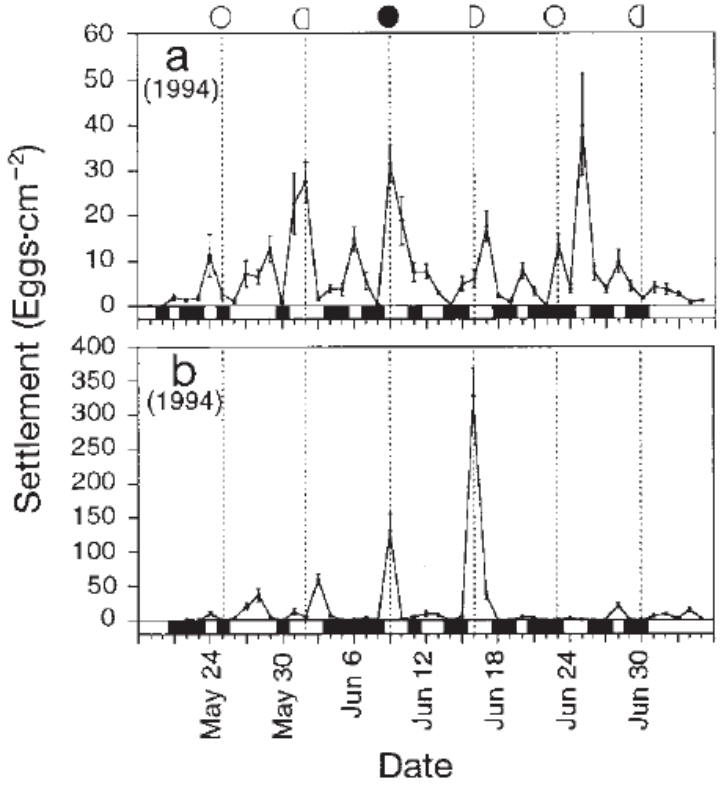
Fucus – release of reproductive cells

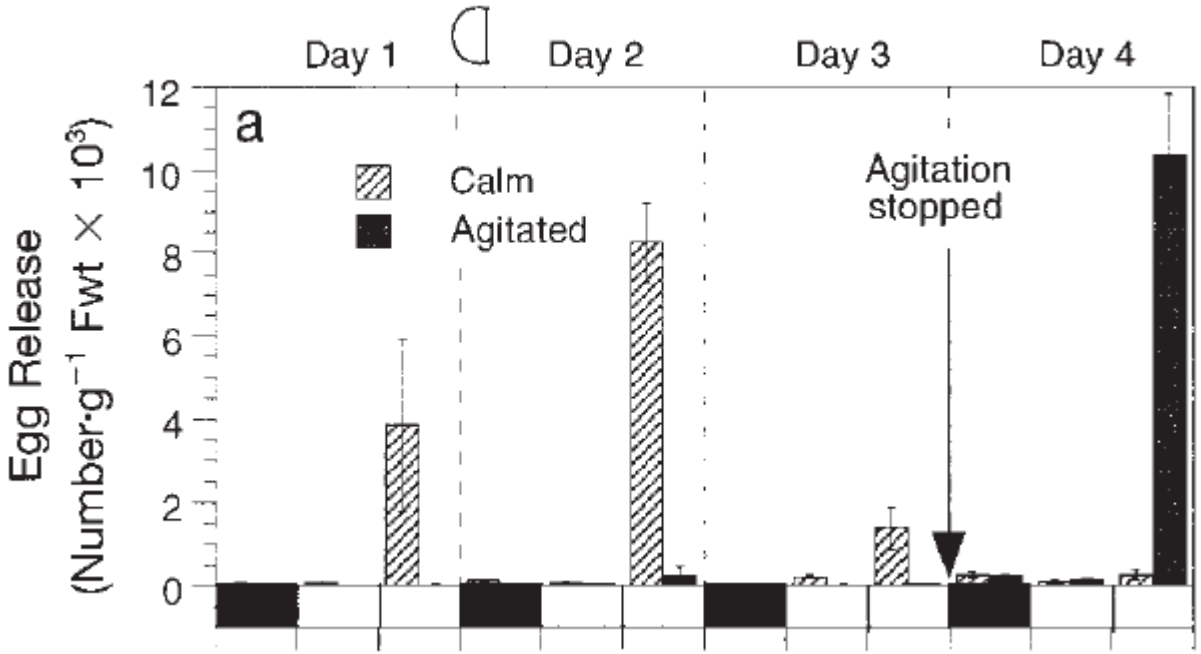
Successful external fertilization in turbulent environments

(Fucales/gamete release/reproductive ecology/spawning/water motion)

ESTER A. SERRÃO*†‡, GARETH PEARSON*, LENA KAUTSKY§, AND SUSAN H. BRAWLEY*

Egg release ~ semidiurnal
Release ~ calm conditions





[Serrao et al. PNAS, 1996]

Fucus – release of reproductive cells

Temporal windows of reproductive opportunity reinforce species barriers in a marine broadcast spawning assemblage

Carla A. Monteiro, Cristina Paulino, Rita Jacinto, Ester A. Serrão & Gareth A. Pearson

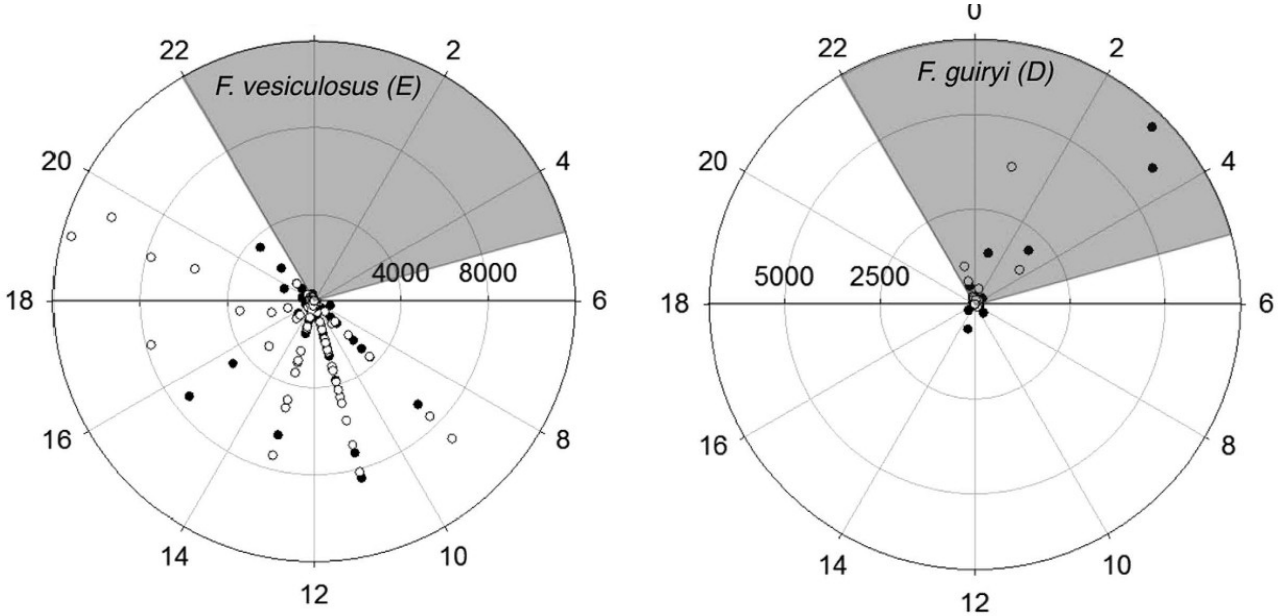
Gareth A. Pearson · Ester A. Serrão · Matthew Dring · Rainer Schmid

Blue- and green-light signals for gamete release in the brown alga, *Silvetia compressa*

Confirmation of the semi-lunar cycle

But including a

- tidal factor
- species factor



[Monteira et al. Sci.Rep., 2016]

[Pearson et al. Oecologia, 2004]

Fucus – release of reproductive cells

Temporal windows of reproductive opportunity reinforce species barriers in a marine broadcast spawning assemblage

Carla A. Monteiro, Cristina Paulino, Rita Jacinto, Ester A. Serrão & Gareth A. Pearson

[Monteira et al. Sci.Rep., 2016]

Gareth A. Pearson · Ester A. Serrão · Matthew Dring · Rainer Schmid

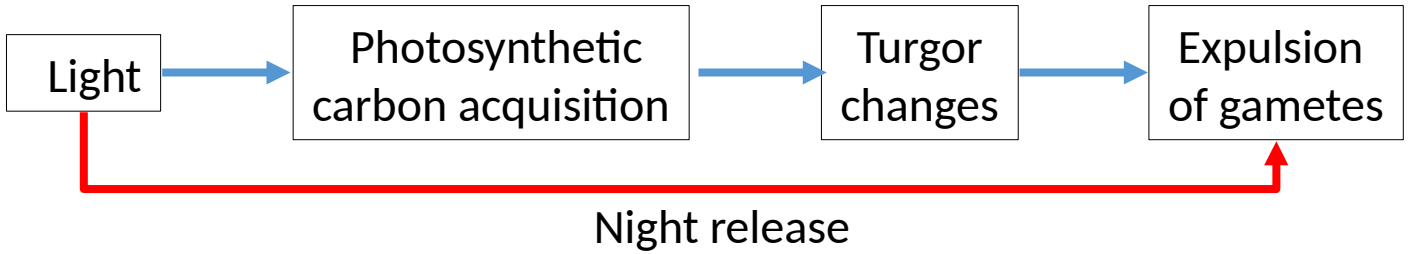
Blue- and green-light signals for gamete release in the brown alga, *Silvetia compressa*

[Pearson et al. Oecologia, 2004]

Confirmation of the semi-lunar cycle

But including a

- tidal factor
- species factor

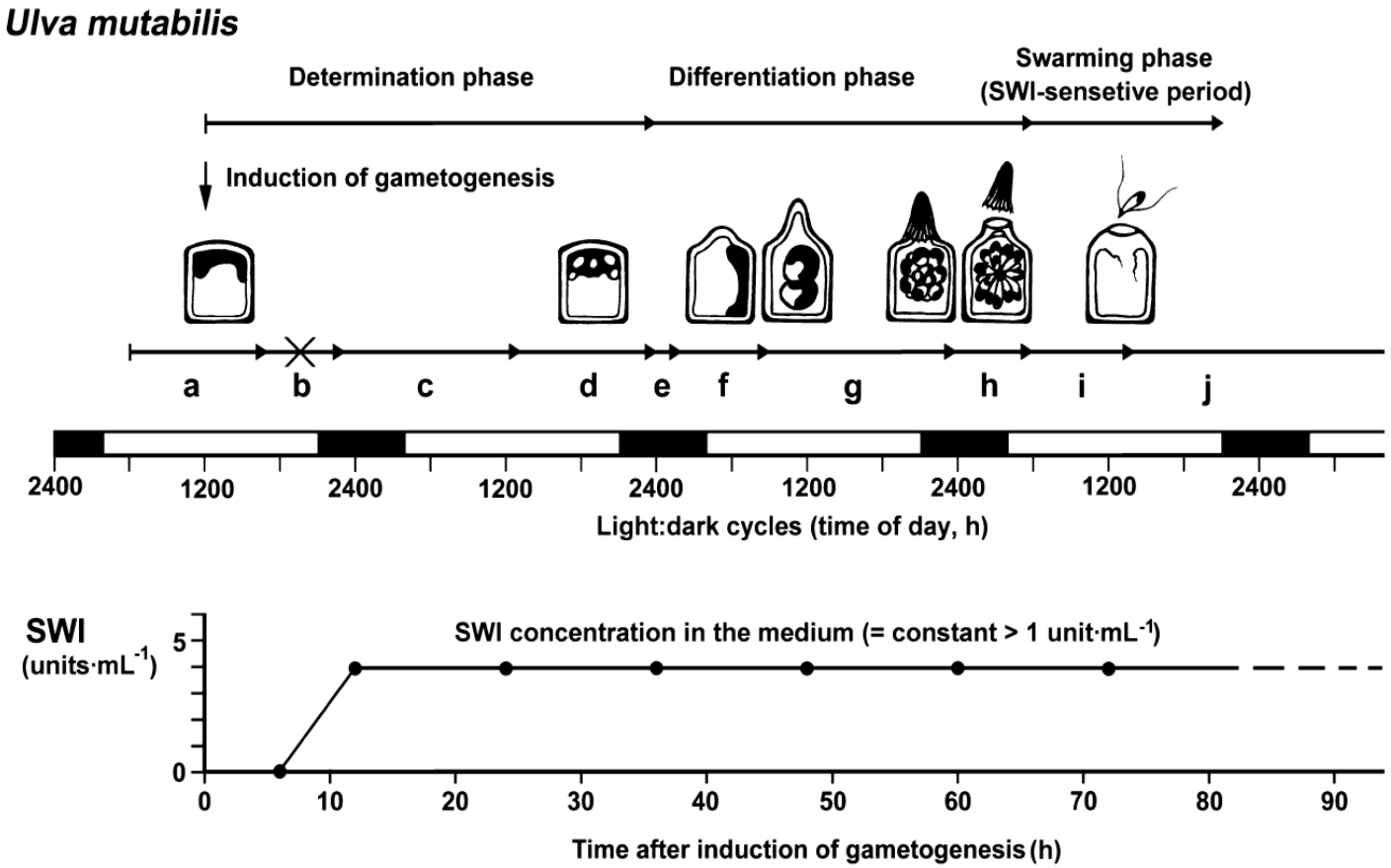


Ulva – release of reproductive cells

GAMETOGENESIS AND GAMETE RELEASE OF *ULVA MUTABILIS* AND *ULVA LACTUCA* (CHLOROPHYTA): REGULATORY EFFECTS AND CHEMICAL CHARACTERIZATION OF THE ''SWARMING INHIBITOR''[1]

Classic paper

- Sporulation inhibitors SI-1 and SI-2
- Swarming inhibitor SWI



[Wichard & Oertel, J.Phycol 2010]

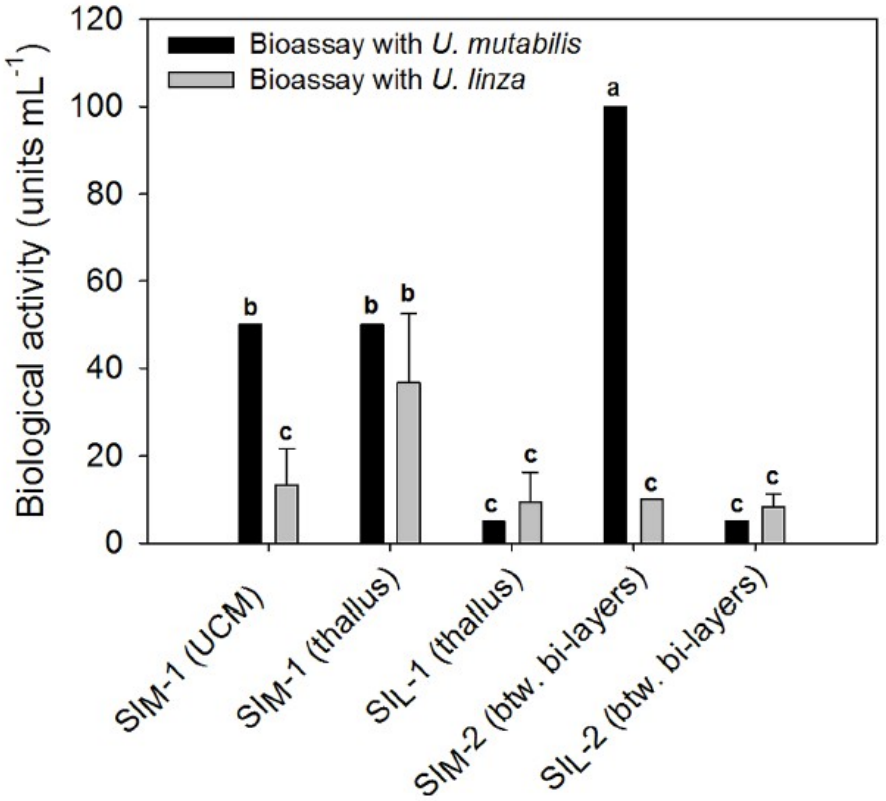
Ulva – release of reproductive cells

Regulation of gametogenesis and zoosporogenesis in *Ulva linza* (Chlorophyta): comparison with *Ulva mutabilis* and potential for laboratory culture

Eleanor F. Vesty[1], Ralf W. Kessler[2], Thomas Wichard[2] * and Juliet C. Coates[1] *

Classic paper

- Sporulation inhibitors SI-1 and SI-2
- Swarming inhibitor SWI



[Vesty et al. Frontiers Plant Sci, 2015]

Ulva – release of reproductive cells

Purification of sporulation and swarming inhibitors from Ulva

Application in algal life-cycle controlling

Ralf W. Kessler, Taghreed Alsufyani, and Thomas Wichard

Protocol paper

8.3.5 *Sporulation inhibitor 1: Extraction*

- *Growth stage of* Ulva: *Noninducible gametogenesis (N.I.G.)* or axenic cultures (Table 8.1).
- Liquid nitrogen.
- Pestle and mortar.
- Dounce tissue grinder set (Sigma Aldrich, München, Germany).
- *Buffer*: 10 and 50 mM Tris–HCl (pH 8.0) at room temperature.
- *Extraction solution*: Phenol saturated with 100 mM Tris–HCl and 1 mM EDTA adjusted to pH 7.5 in a brown glass bottle at room temperature.
- Three-necked flask.
- Acetone.
- Absolute ethanol at –20°C.
- Bench top refrigerated centrifuge (at 0°C).
- Camaprene® (Honeywell, USA) or equivalent security gloves.

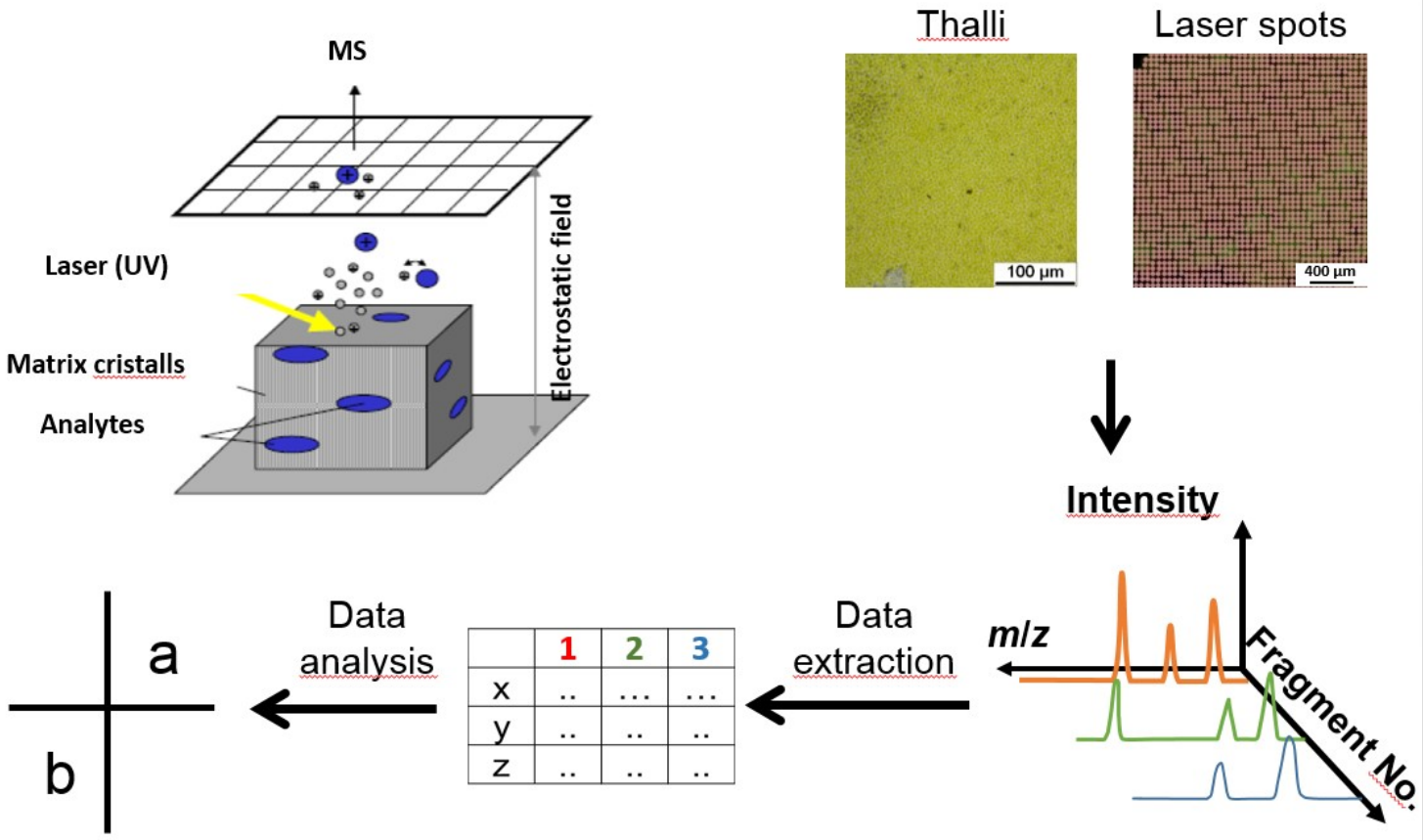
[Kessler et al. 2018, Protocols …]

Ulva – release of reproductive cells

In situ monitoring of molecular changes during cell differentiation processes in marine macroalgae through mass spectrometric imaging

Ralf W. Kessler[1] · Anna C. Crecelius[2,3] · Ulrich S. Schubert[2,3] · Thomas Wichard[1]

Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI)paper



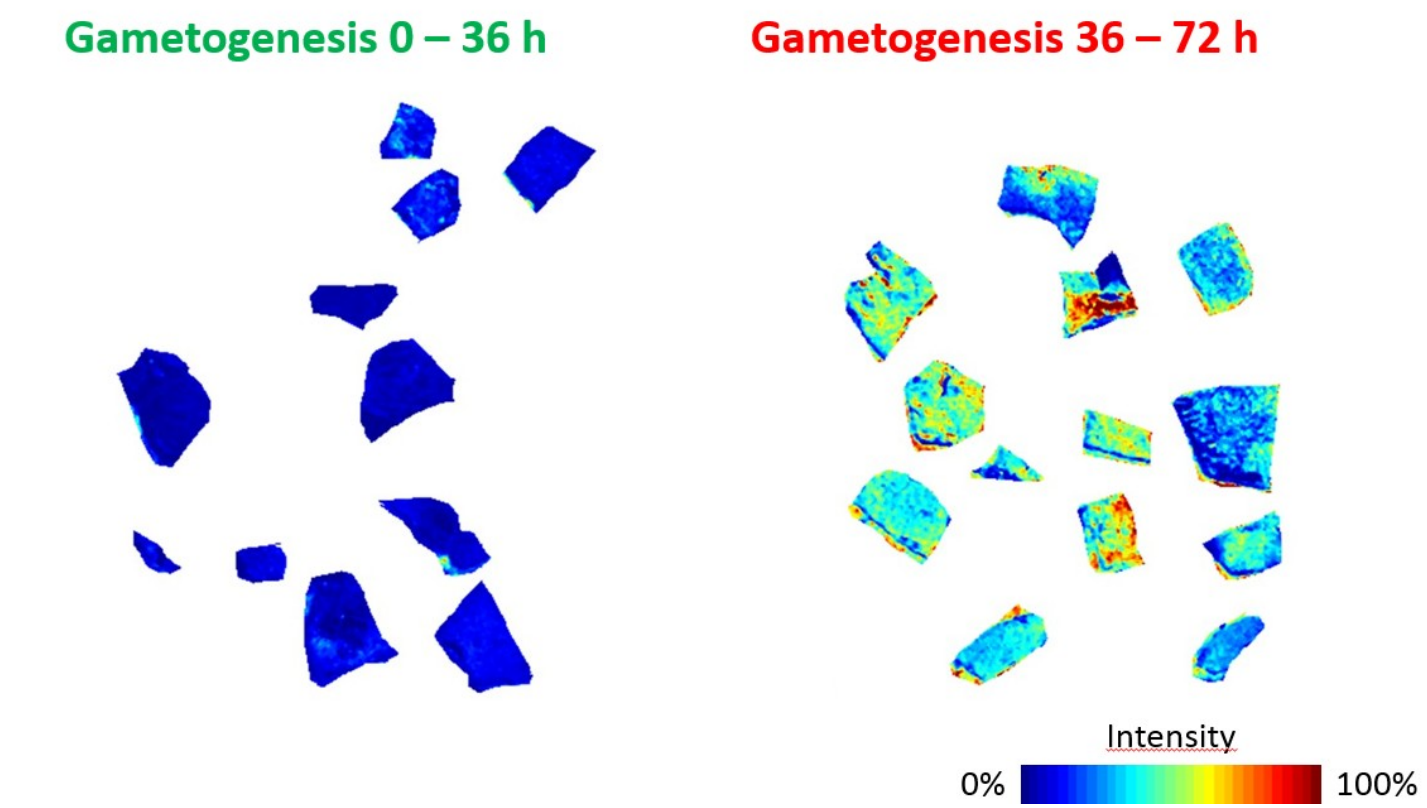
[Kessler et al. Anal Bioanal Chem, 2017]

Ulva – release of reproductive cells

In situ monitoring of molecular changes during cell differentiation processes in marine macroalgae through mass spectrometric imaging

Ralf W. Kessler[1] · Anna C. Crecelius[2,3] · Ulrich S. Schubert[2,3] · Thomas Wichard[1]

Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI)paper

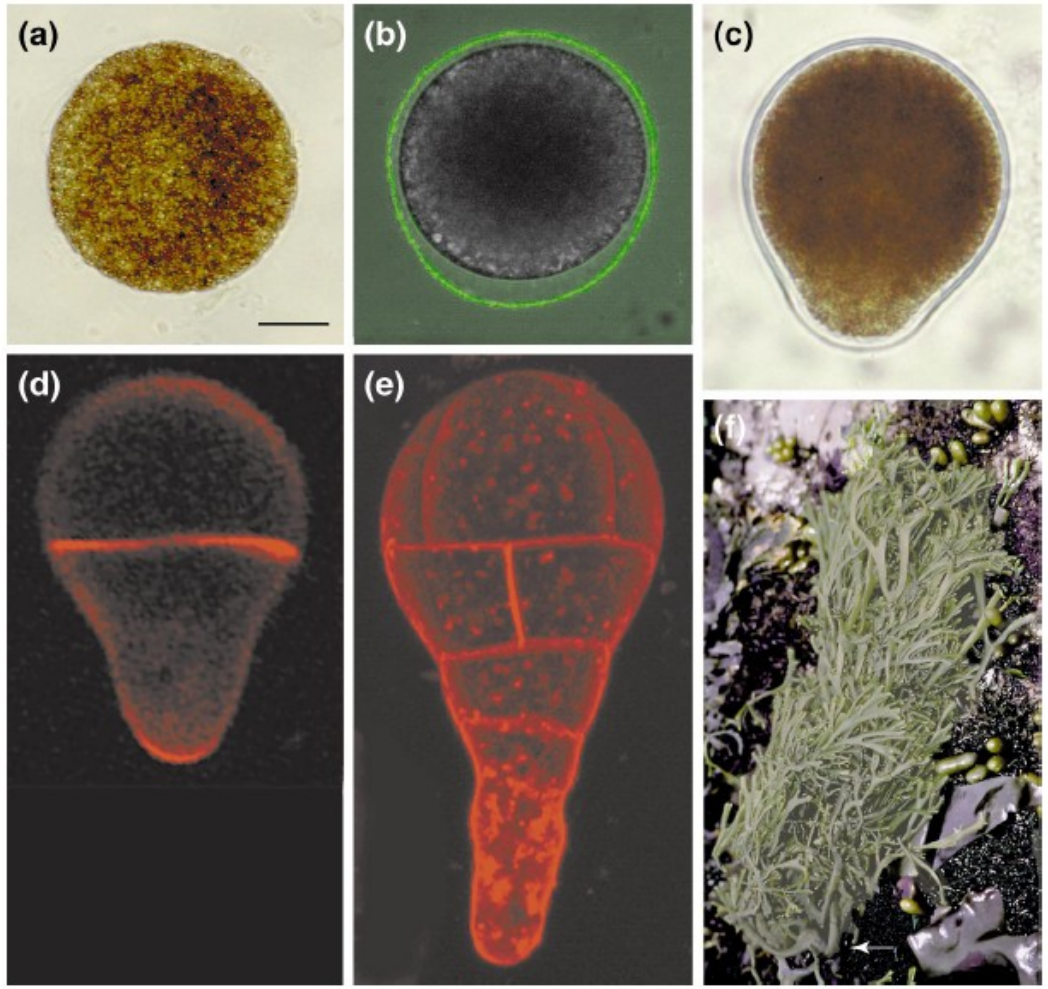


Imaging of the distribution of *m/z* values during gametogenesis

[Kessler et al. Anal Bioanal Chem, 2017]

Brown algal – cell polarity

Polarity establishment



Kropf et al. 1999

A long history of using fucoid algae for cell polarity studies.
Toooooo long to list.

Brown algal – cell polarity

Photopolarization of *Fucus* zygotes is determined by time sensitive vectorial addition of environmental cues during axis amplification

Kenny A. Bogaert[1], Tom Beeckman[2,3] and Olivier De Clerck[1]*

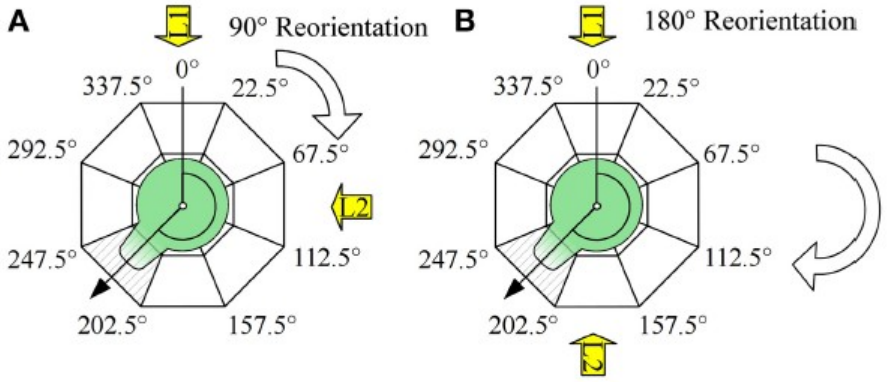
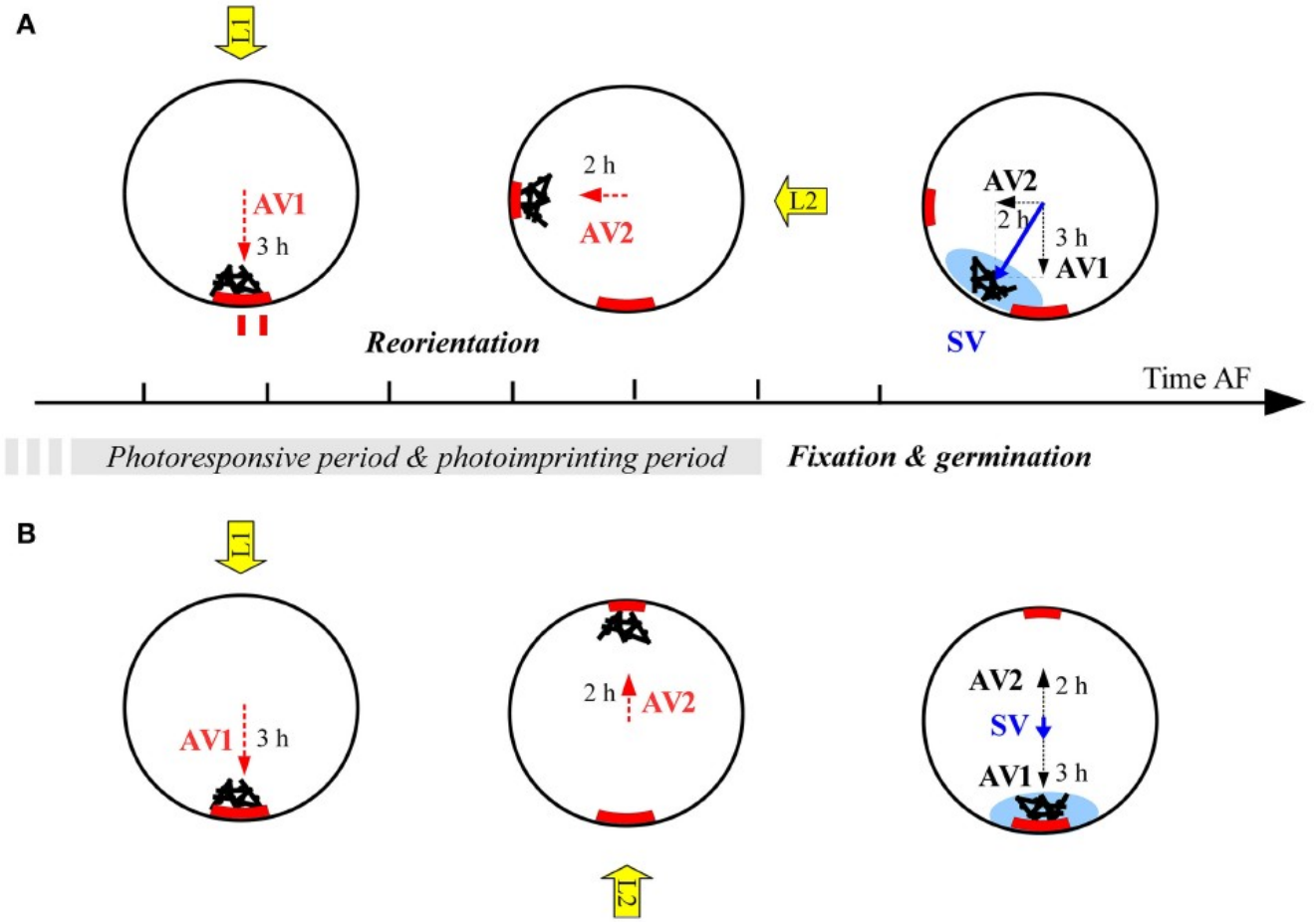


FIGURE 1 | Experimental design and scoring method and (A) 90° reorientation, (B) 180° reorientations. Angle between the vector pointing

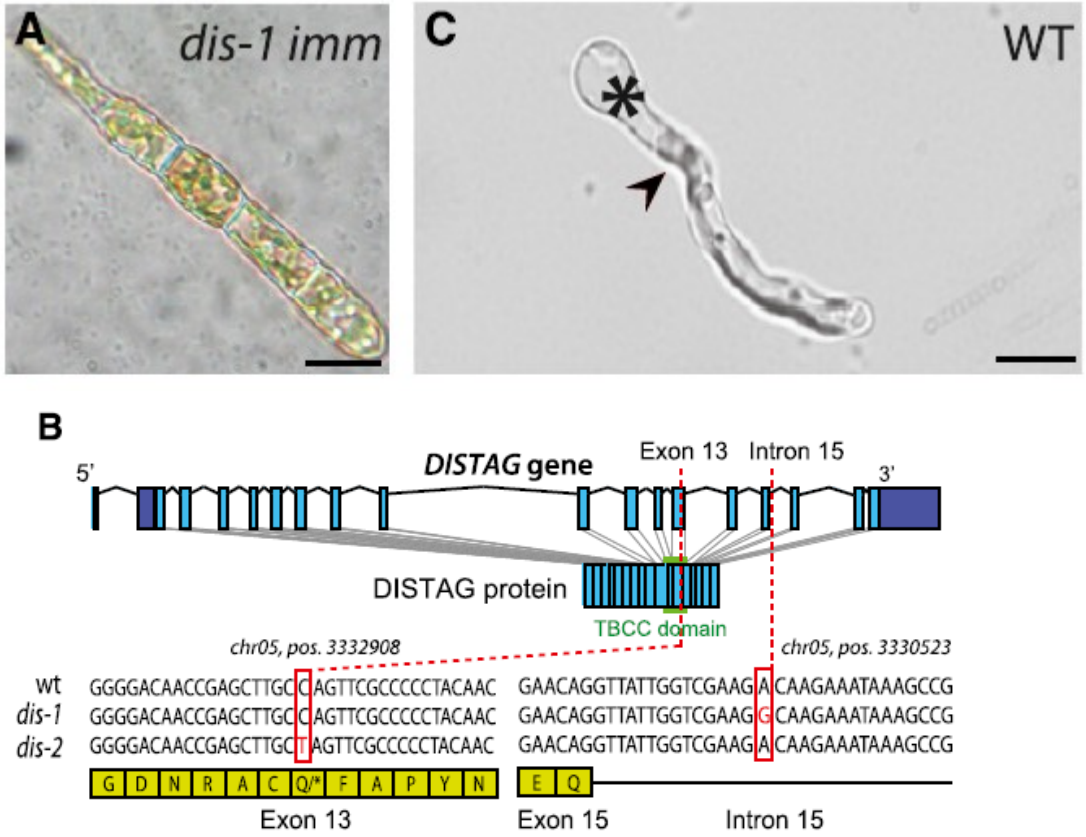


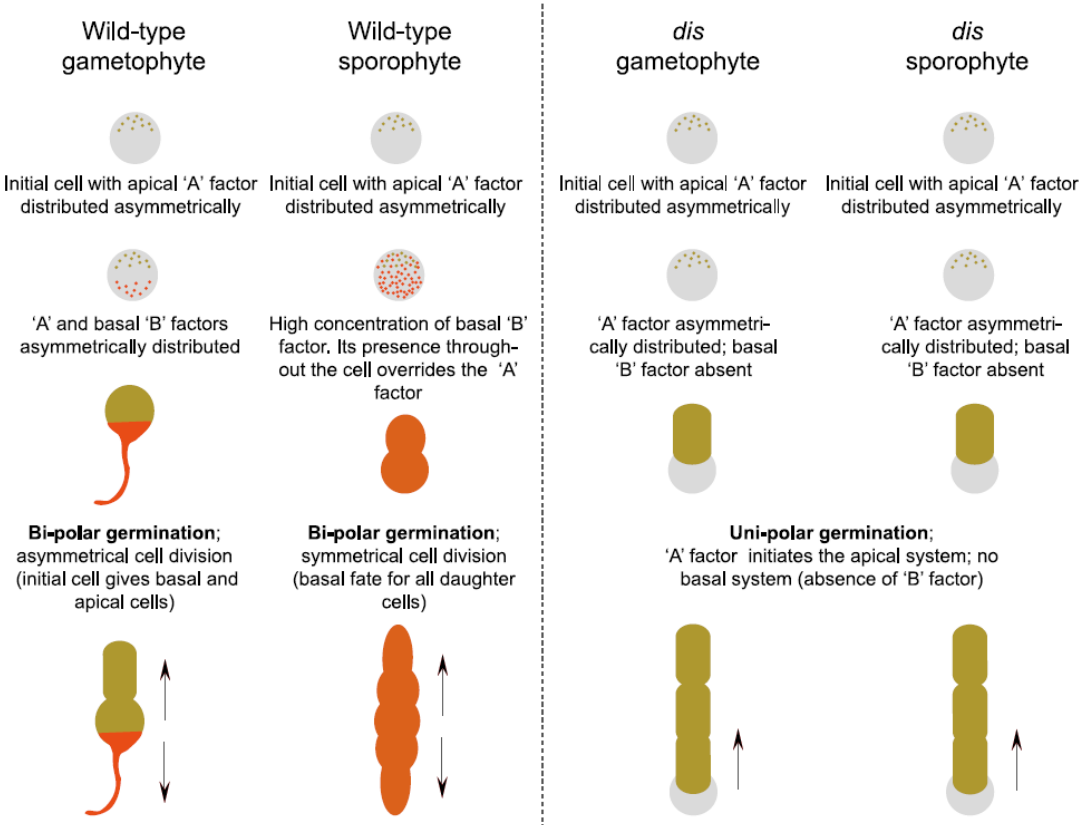
[Bogaert et al. Front. Plant Sci, 2015]

Brown algal – cell polarity

DISTAG/TBCCd1 Is Required for Basal Cell Fate Determination in *Ectocarpus*

Olivier Godfroy,[a,1] Toshiki Uji,[a,1] Chikako Nagasato,[b] Agnieszka P. Lipinska,[a] Delphine Scornet,[a] Akira F. Peters,[c] Komlan Avia,[a,d] Sebastien Colin,[e] Laure Mignerot,[a] Taizo Motomura,[b] J. Mark Cock,[a] and Susana M. Coelho[a,2]



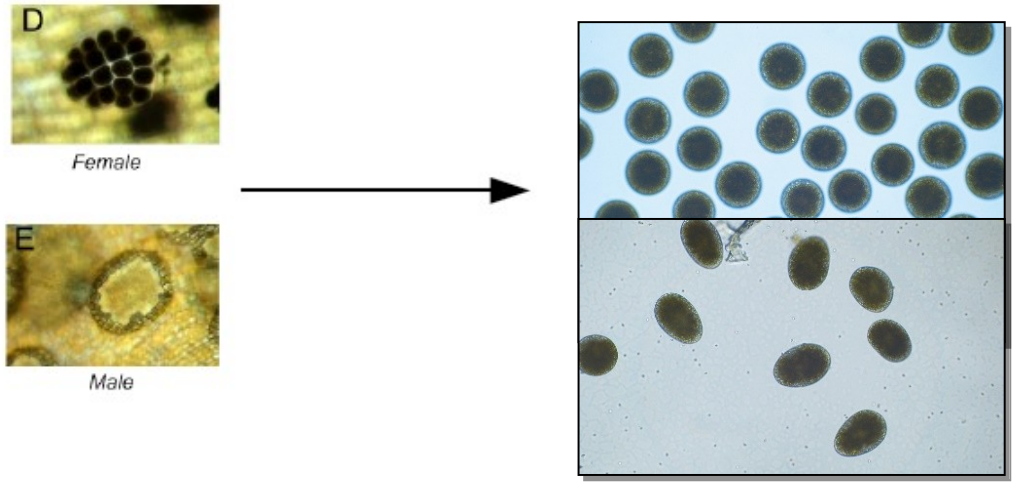


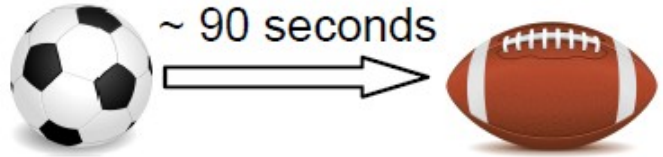
[Godfroy et al. Plant Cell, 2017]

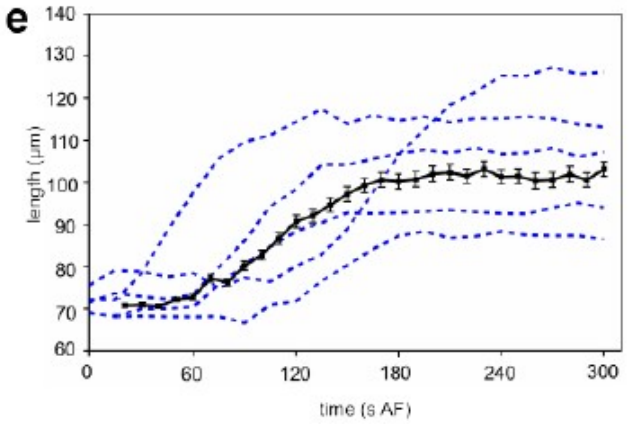
Dictyota – cell

polarisation

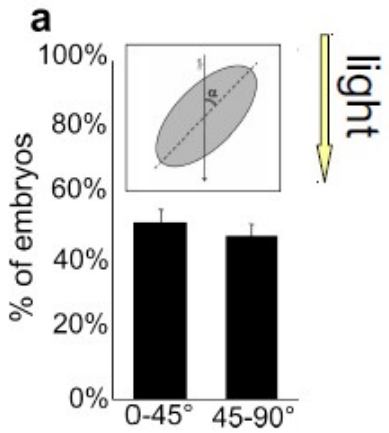
Fast elongation upon egg activation (90')

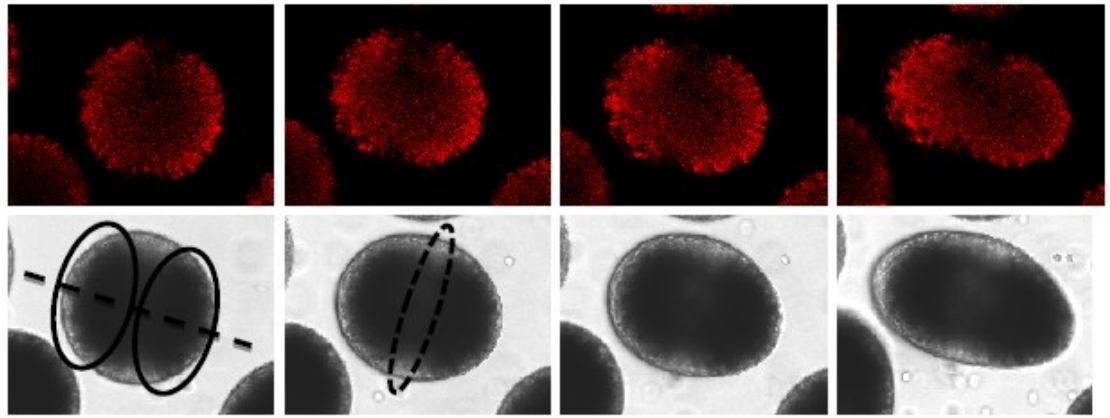






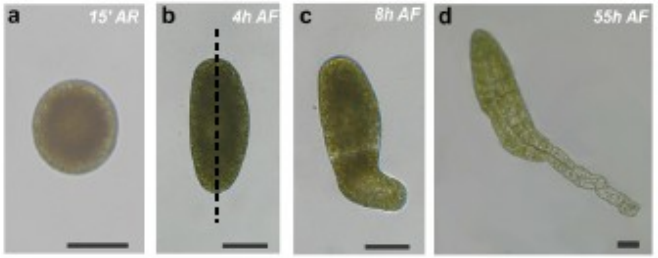
Elongation direction is independent of unilateral light, but maternally determined



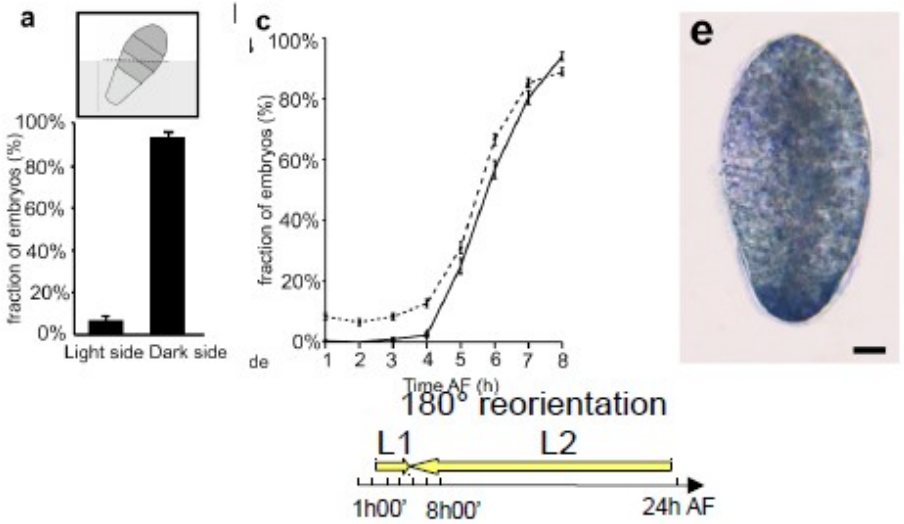


[Bogaert et al. Nature Plants, 2017a]

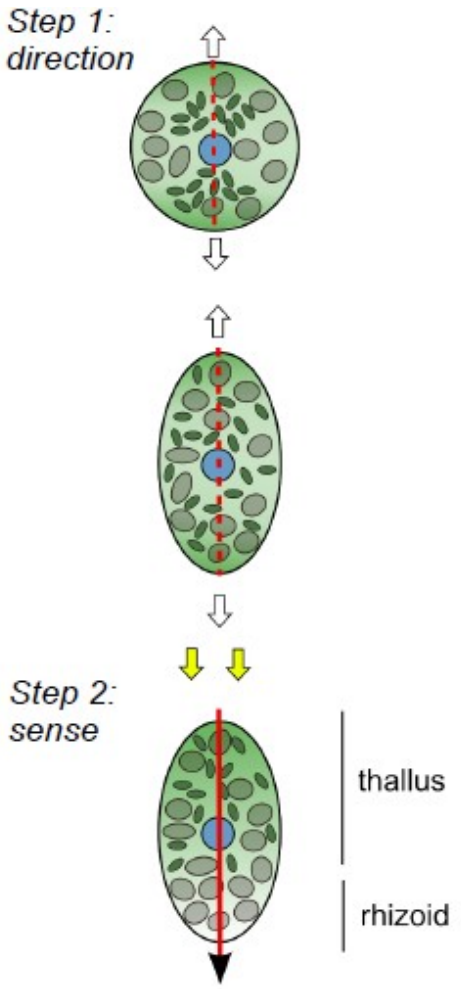
Elongation direction determines ***direction*** of polarisation vector

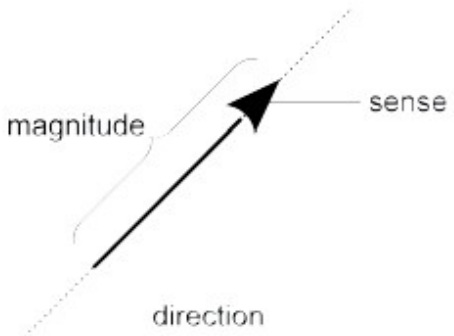


The ***sense*** of the polarisation vector is determined by the light



→ Determination of direction and sense are:
- distinct in timing
- distinct in mechanism





[Bogaert et al. Nature Plants, 2017a]