Models of embryogenesis and biomineralization **II**

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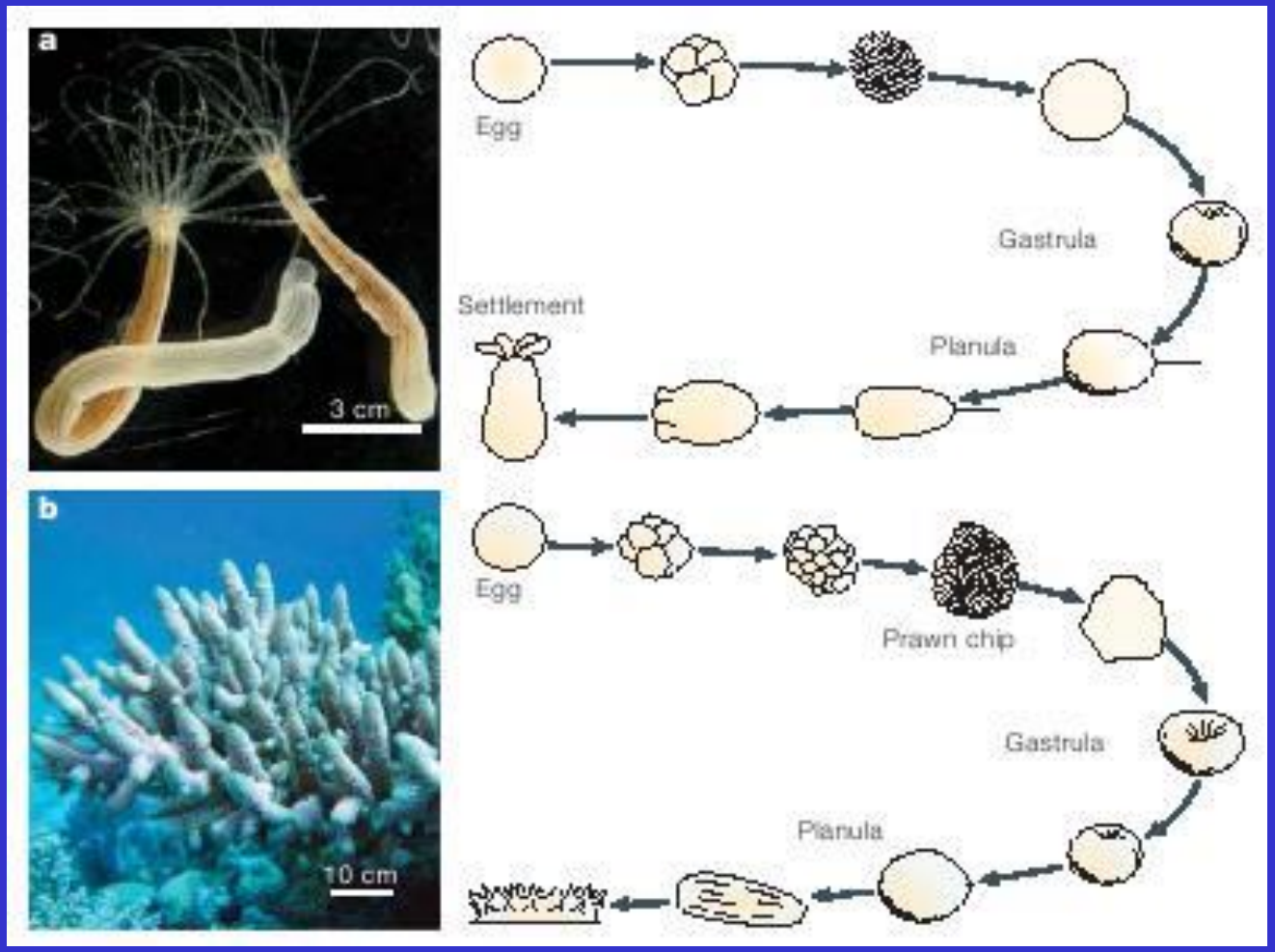
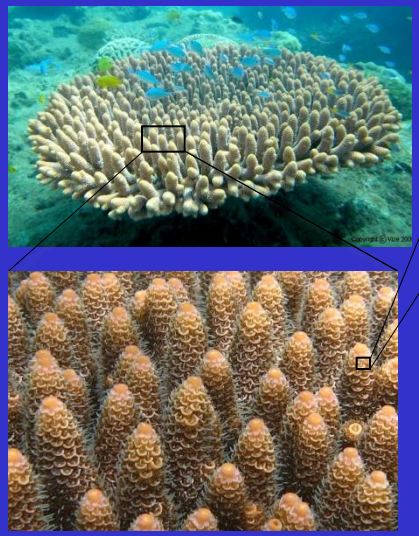
Overview

- Modelling gene regulation
- The sea anemone *Nematostella vectensis* case study modelling gene regulation
- Cell-based modelling of gastrulation in *Nematostella* and the hydrozoan *Clytia hemisphaerica*
- Biomineralization in the scleractinian coral *Acropora millepora*
- Modelling calcification physiology in corals
- Morpological plasticity and the physical environment in corals
- Modelling growth and form of corals (e.g *Madracis sp.*) and the impact of the physical environment

Nematostella vectensis (top) / *Acropora millepora* (bottom) (Ball et al., Nature Reviews Genetics: 567, 2004)

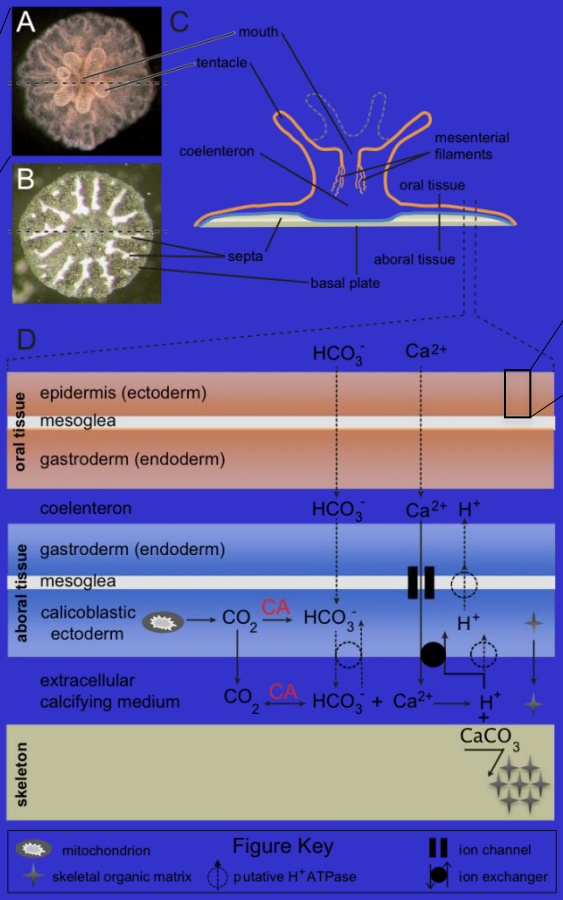


Growth of corals: a multiscale problem



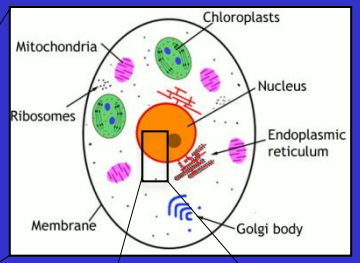
Macromorphology
cm – m
months – years

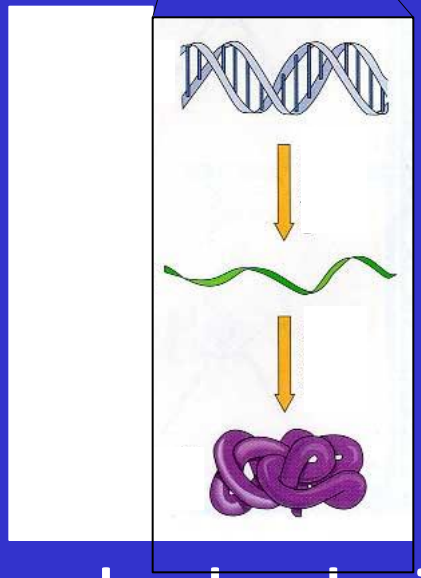
Micromorphology
µm – mm; days - weeks



tissue physiology
1 – 100 µm; ns -µs

cell physiology
1 – 10 µm; ns - hours

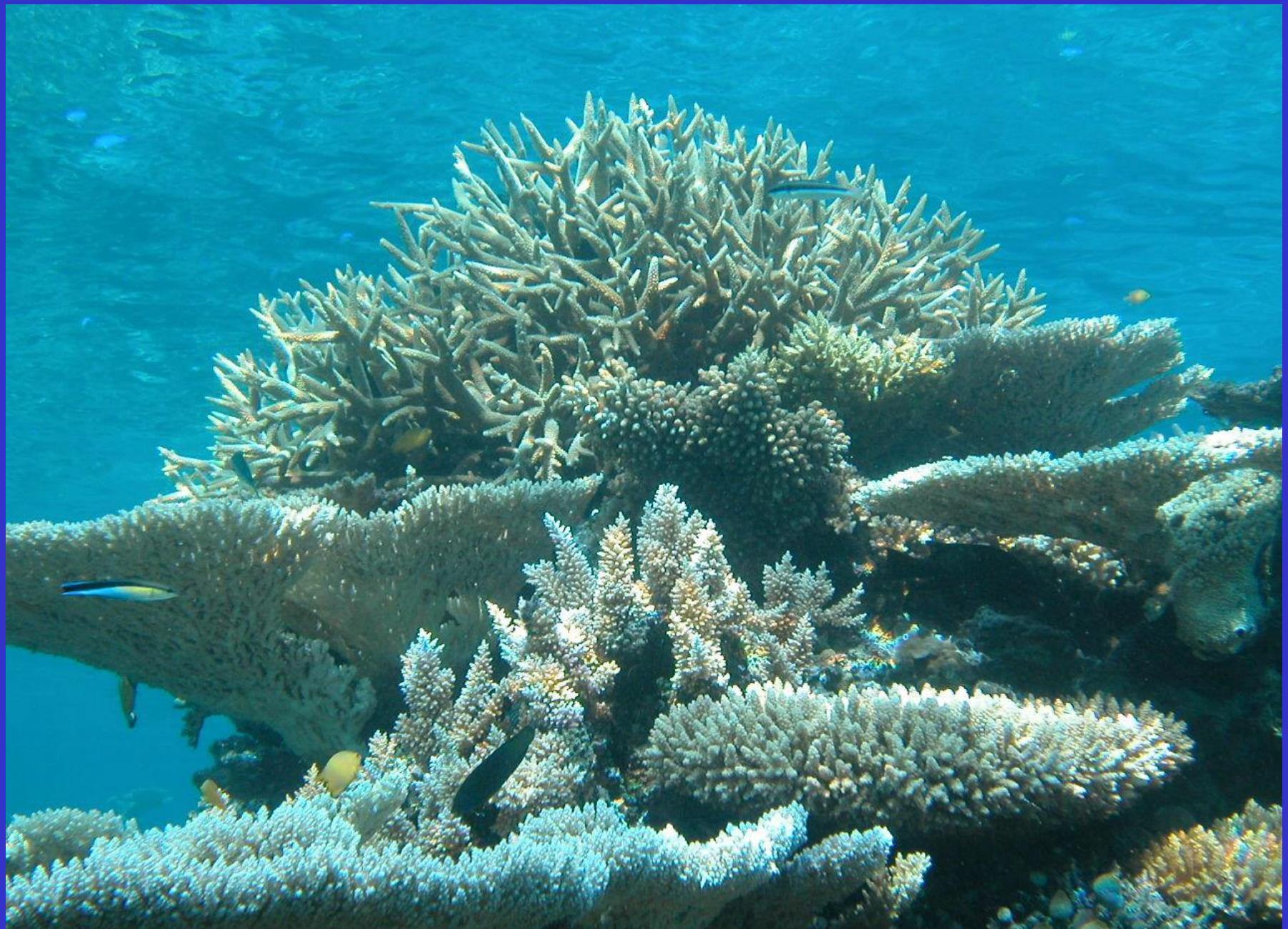




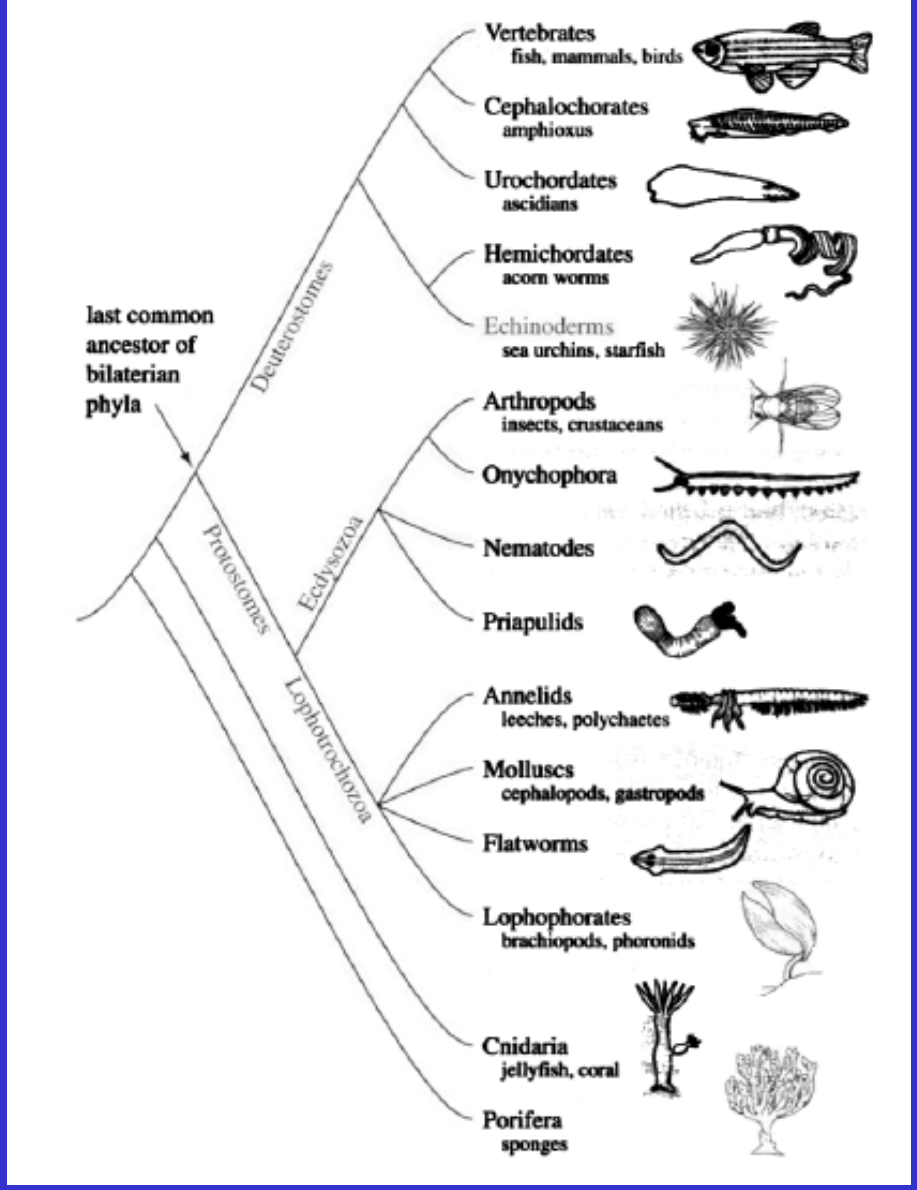
molecular physiology
Å – nm; s - mins

Great Barrier Reef Australia I

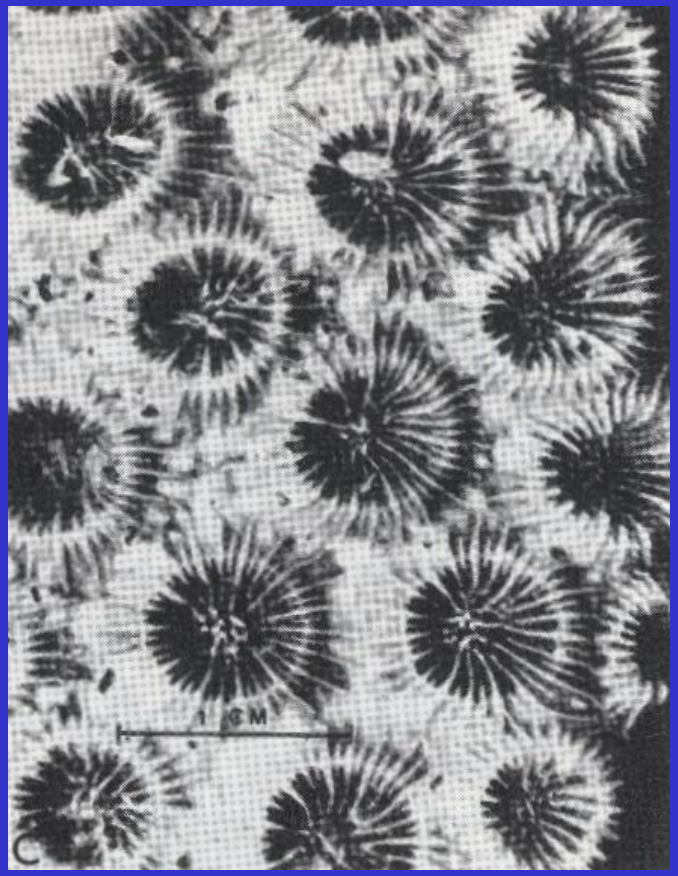
Great Barrier Reef Australia II

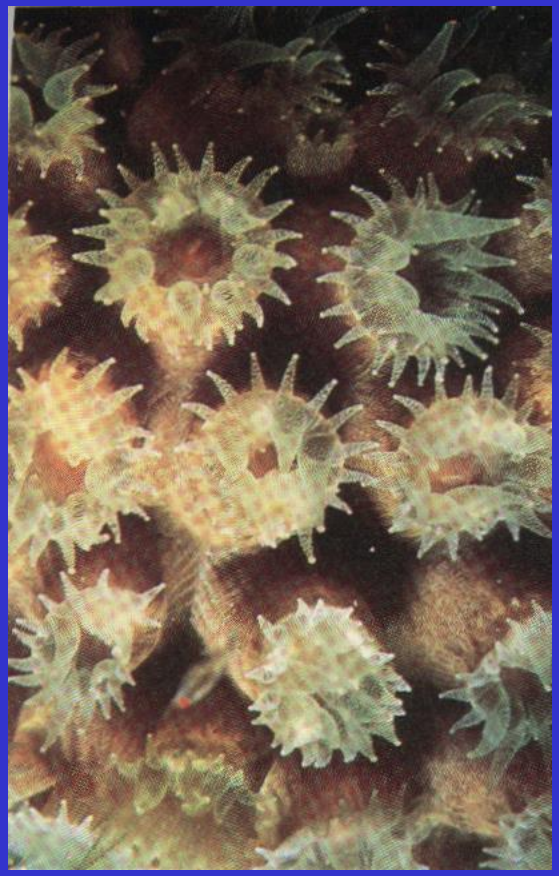


Scleractinian corals

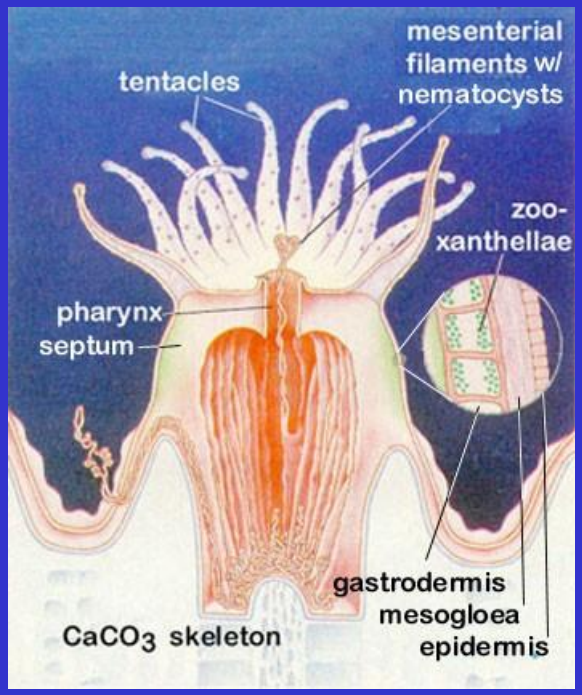


Coral polyps

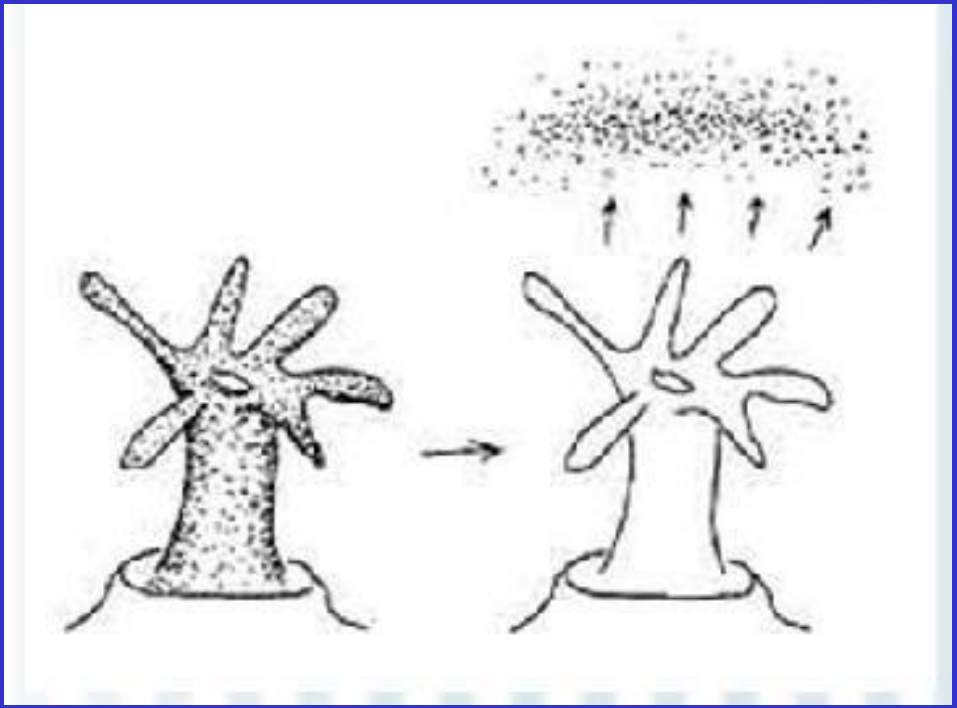




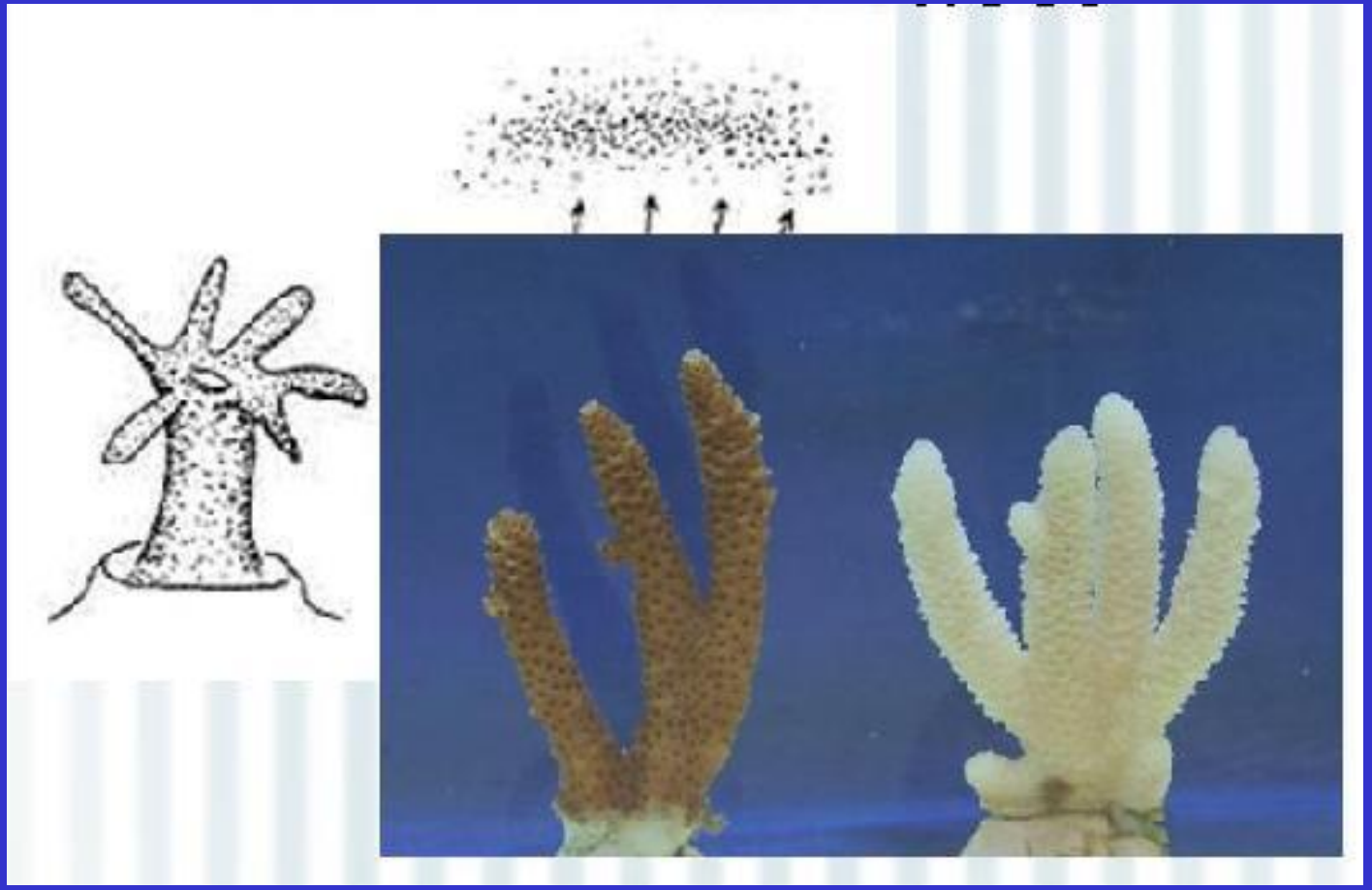
Coral polyp with symbiotic algae (zooxanthellae)



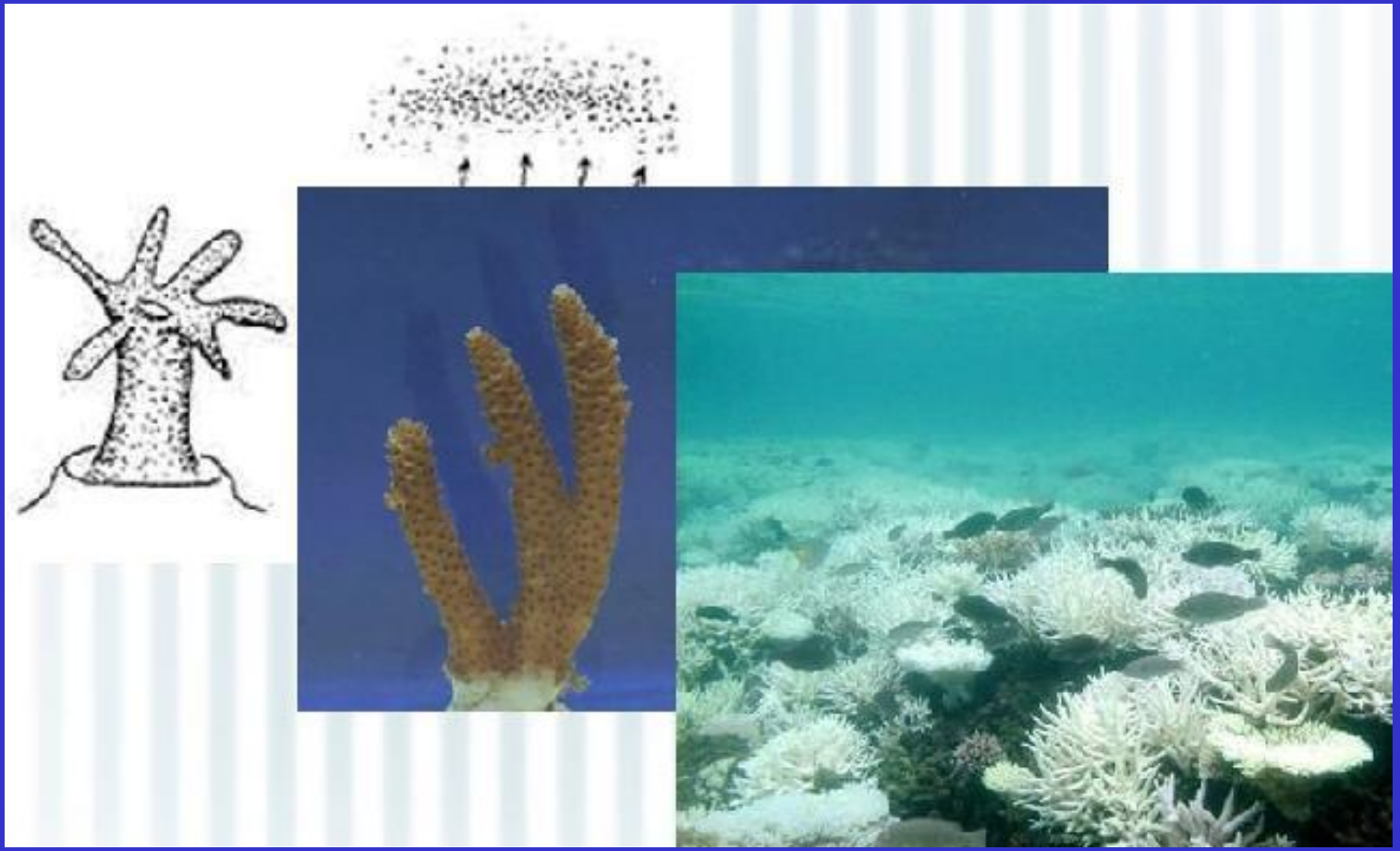
Coral bleaching I (van Oppen, 2005)



Coral bleaching II



Coral bleaching III



Calcification and $C0_2$

photosynthesis

$$CO_2 + H_2O \rightarrow CH_2O + O_2 \qquad (1)$$

respiration

$$CH_2O + O_2 \rightarrow CO_2 + H_2O \qquad (2)$$

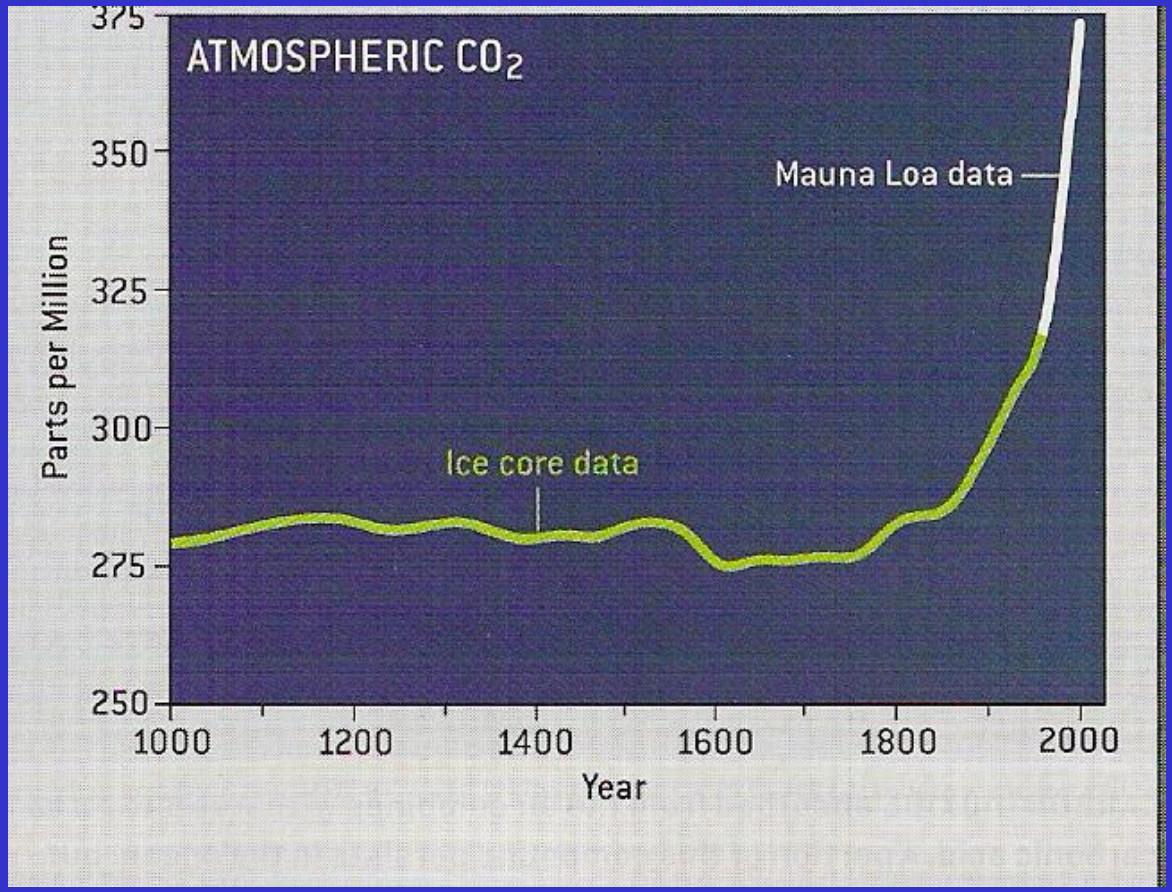
calcification

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O \qquad (3)$$

equilibrium reaction

$$CO_2 + H_2O \overleftrightarrow{} H^+ + HCO_3^- \qquad (4)$$

Acidification of oceans I



On concretions, spicules, and specular skeletons (D.W Thompson, 1942)

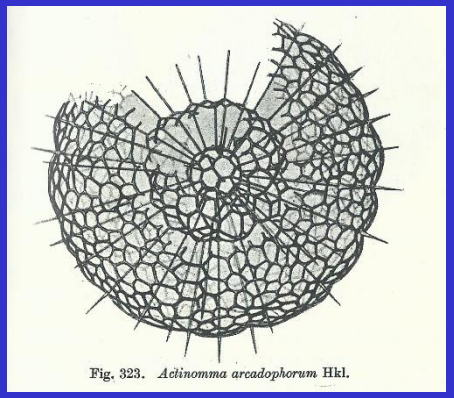


Fig. 323. *Actinomma arcadophorum* Hkl.

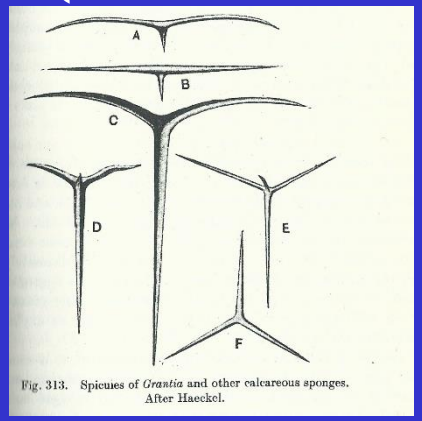


Fig. 313. Spicules of *Grantia* and other calcareous sponges. After Haeckel.

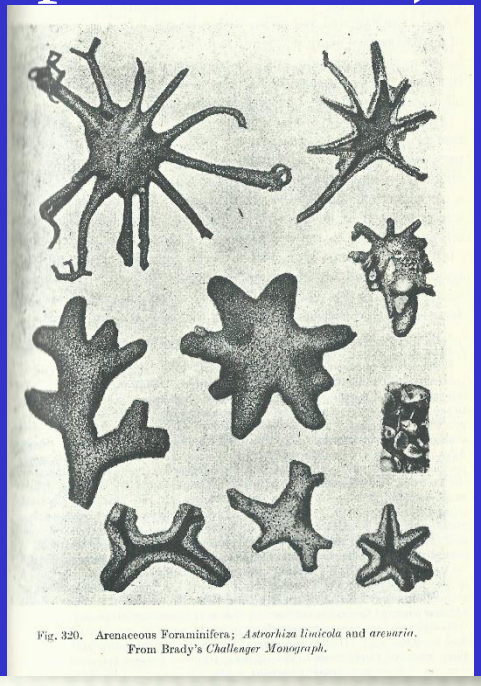


Fig. 320. Arenaceous Foraminifera; *Astrorhiza limicola* and *arenaria*. From Brady's *Challenger Monograph*.

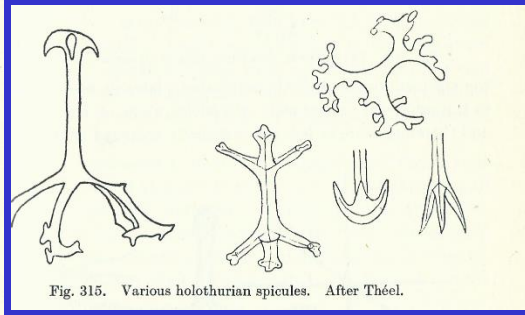


Fig. 315. Various holothurian spicules. After Théel.

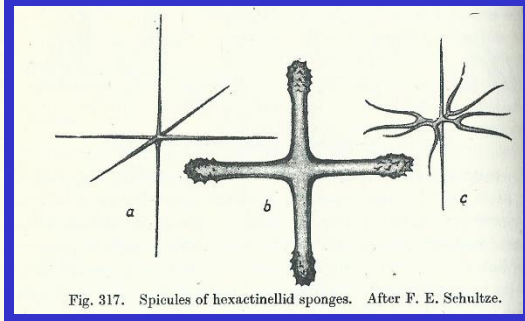


Fig. 317. Spicules of hexactinellid sponges. After F. E. Schultze.

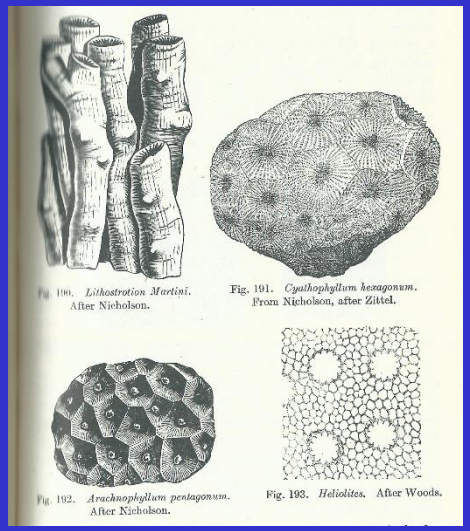


Fig. 190. *Lithostrotion Martini*. After Nicholson.

Fig. 191. *Cyathophyllum hexagonum*. From Nicholson, after Zittel.

Fig. 192. *Arachnophyllum pentagonum*. After Nicholson.

Fig. 193. *Heliolites*. After Woods.

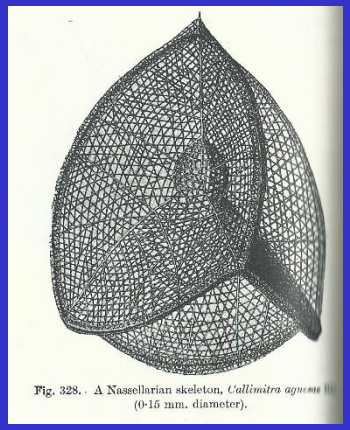


Fig. 328. A Nassellarian skeleton, *Callimitra agnesa* (0·15 mm. diameter).

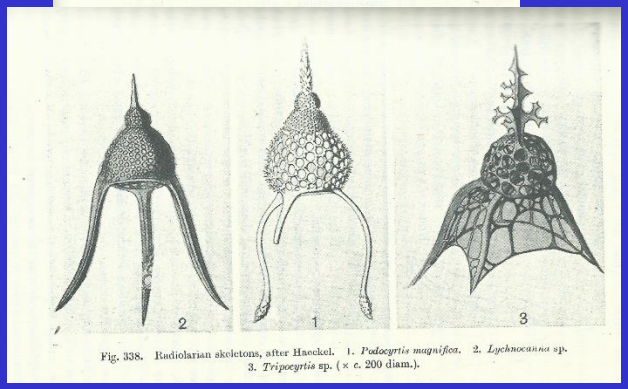


Fig. 338. Radiolarian skeletons, after Haeckel. 1. *Podocyrtis magnifica*. 2. *Lychnocanna* sp. 3. *Tripocyrtis* sp. (× *c.* 200 diam.).

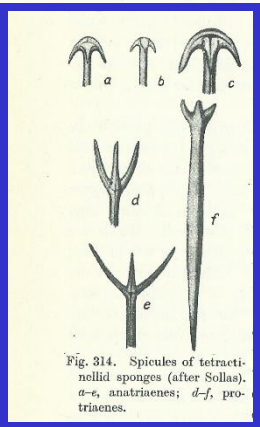
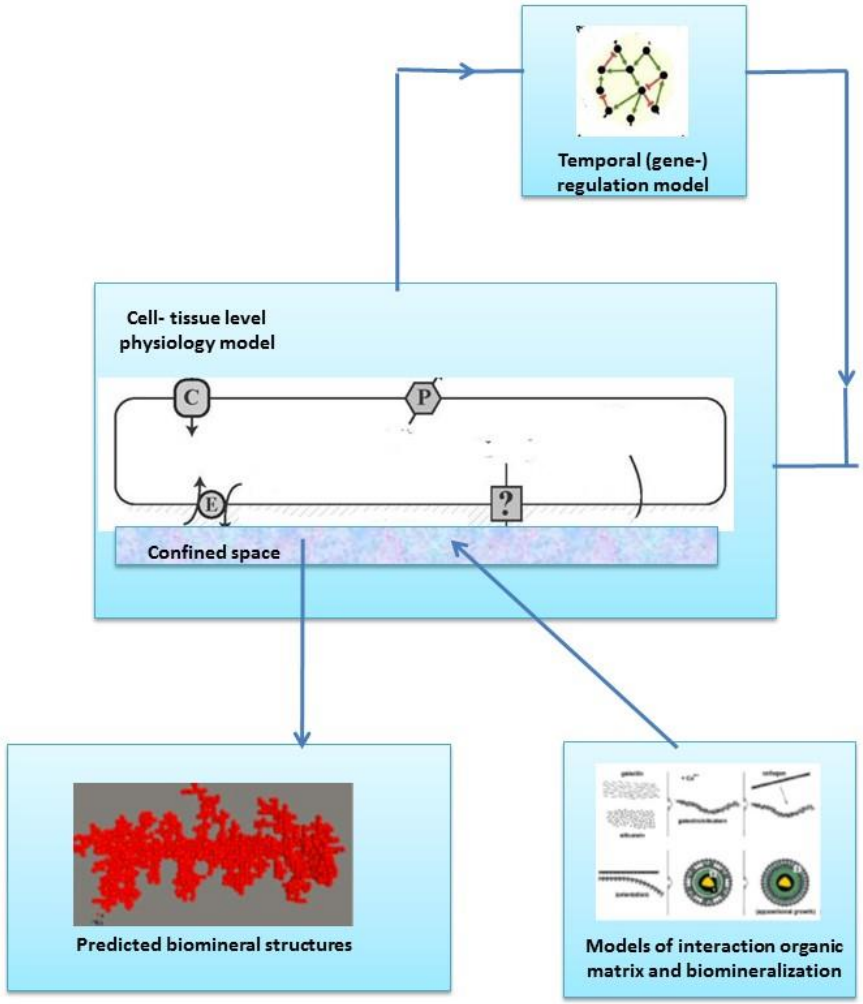


Fig. 314. Spicules of tetractinellid sponges (after Sollas). *a–e*, anatriaenes; *d–f*, protriaenes.

Central concept of a model of biomineralization in a confined space



The cell physiology is controlling the concentrations of the inorganic components by channels, exchangers and pumps. Gene regulation controls the release of organic components in the system. The actual place of the biomineralization is confined (located in vesicles in unicellular organisms or outside the cell in multicellular organisms). In the confined space the biomineralization is controlled by the concentrations of inorganic agents and the interaction with the organic matrix

What do *Acropora* skeletal proteins tell us about coral biocalcification ?

- P. Ramos-Silva, F. Marin, J.A. Kaandorp, and B. Marie, PNAS, 3–5. 2013
- P. Ramos-Silva J.A, Kaandorp, L. Huisman, B. Marie, I. Zanella-Cléon, N. Guichard, D.J. Miller and F. Marin, Molecular Biology and Evolution, 2013

Organic matrix extraction in the scleractinian coral *Acropora millepora* (Ramos-Silva et al., Molec. Biol. Evol., 2013)

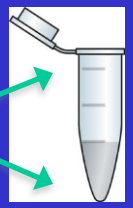
NaOCl (5%, v/v) 72 h

Powder < 200 µm

NaOCl (1%, v/v) 5 h

The organic matrix extraction:

- Decalcification (Acid)
- Centrifugation
- Filtration
- Ultra-filtration
- Dialysis
- Lyophilization



ASM

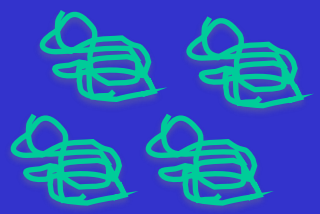
AIM

Organic Matrix (OM)

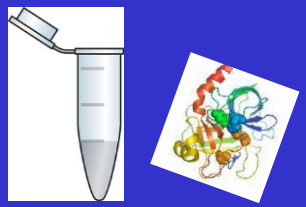
- ✓ Acid-soluble matrix (ASM)
- ✓ Acid insoluble matrix (AIM)

Mass spectrometry analysis of the Organic matrix

Protein samples: ASM AIM



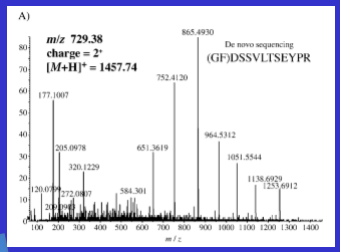
Proteins are denatured, reducedand alkylated



Trypsin digestion

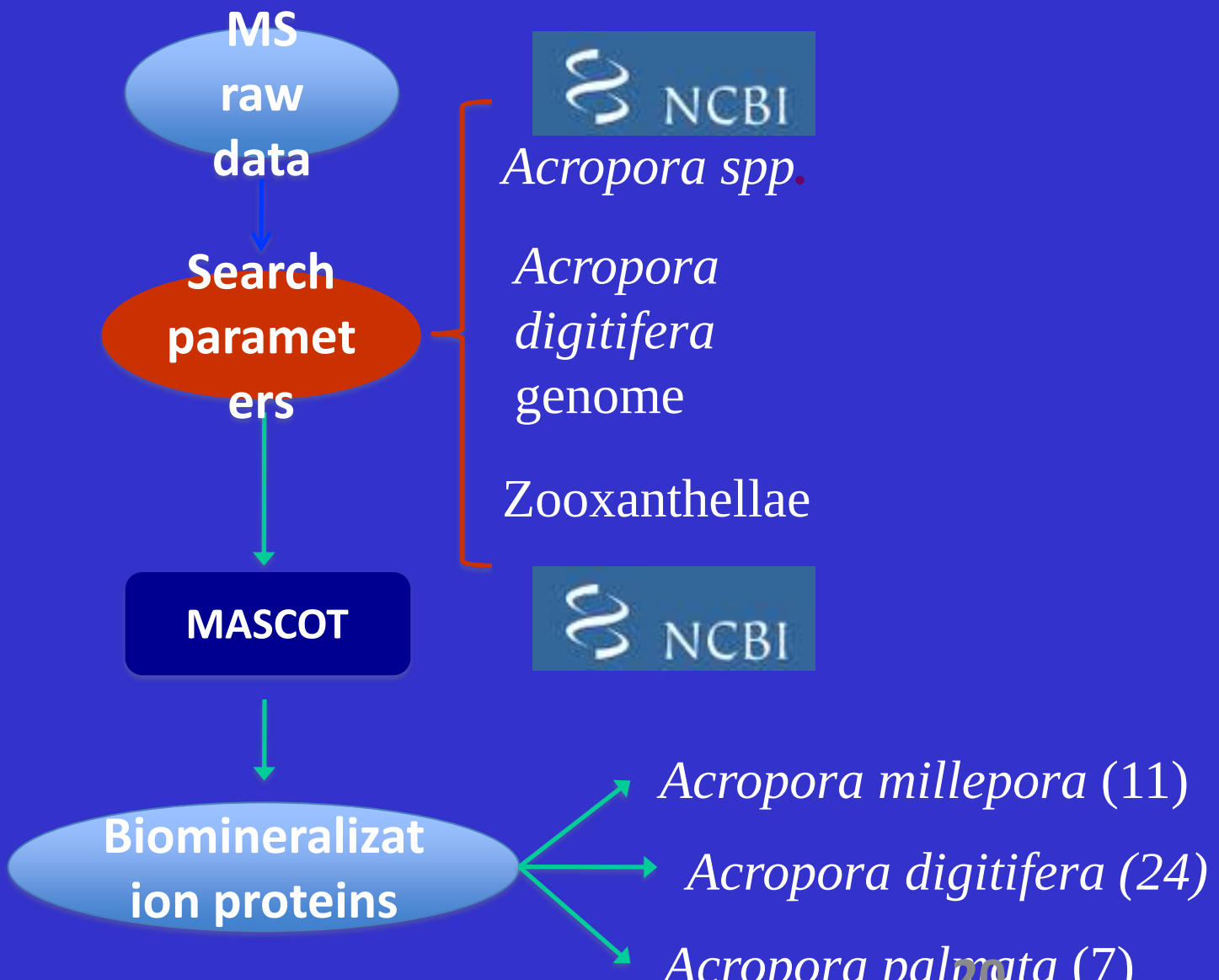
Peptides

Mass Spectrometry

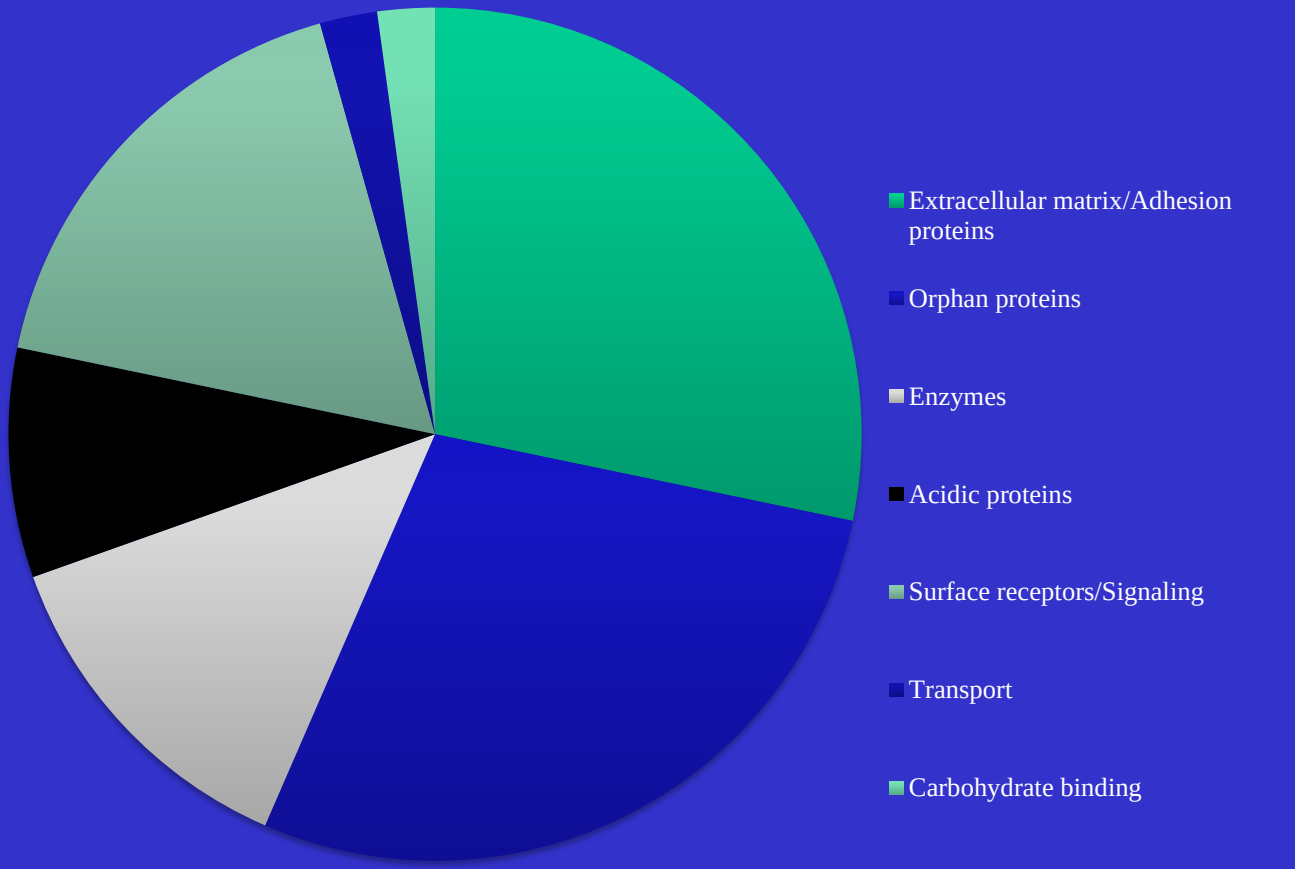


Protein identification

Protein identification



Protein analysis: distribution of functions from proteins of the Acidic Soluble Matrix



G-coupled receptor

Surface receptors/
Signaling
Transport

Extracellular/Adhesion
Proteases
Orphan proteins

Copper oxidase
Acidic proteins

•ECM-like,-2, -3

• Galaxin

•Ig-like

• Contactin-like

• LDL/Fibronectin

• Receptor for REJ-like

• ZP-like

•CUB (2)

•Lectin

• SCP-like extracellular protein

• ECM-4

• Ras/GTP binding protein

• Collagen

• Proteases (3)

•Cupredoxin

Hydra

• Uncharacterized Protein (1)

• Thr-rich/TSP

•Thr-rich

•G-coupled receptor

Nematostella

•Uncharacterized proteins (6)

•Asprich-1

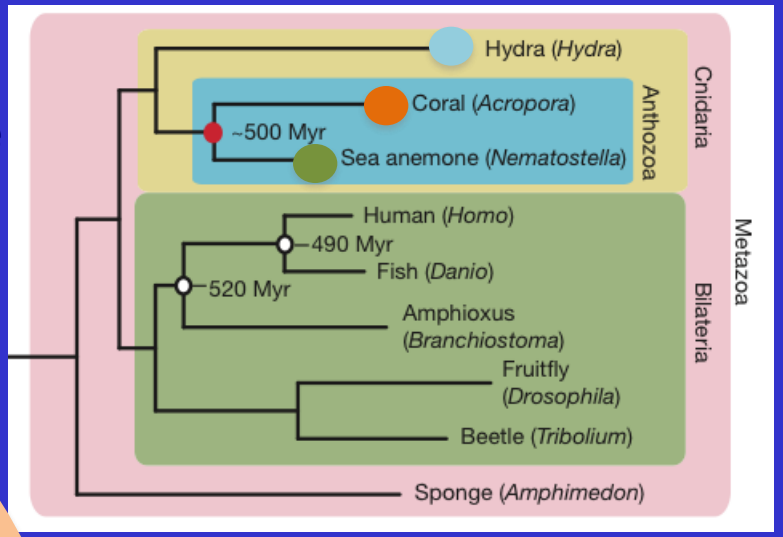
•sap1-like

•sap1

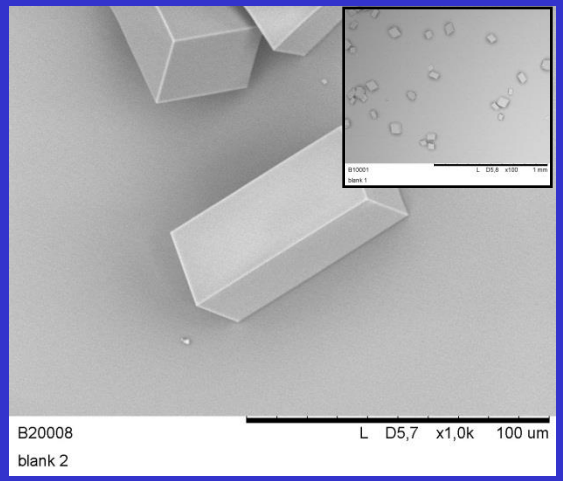
•Asprich-2

•Glu-rich

Acropora



In vitro interaction of Acid Soluble Matrix with $CaCO_3$ (Ramos-Silva et al., Molec. Biol. Evol., 2013)



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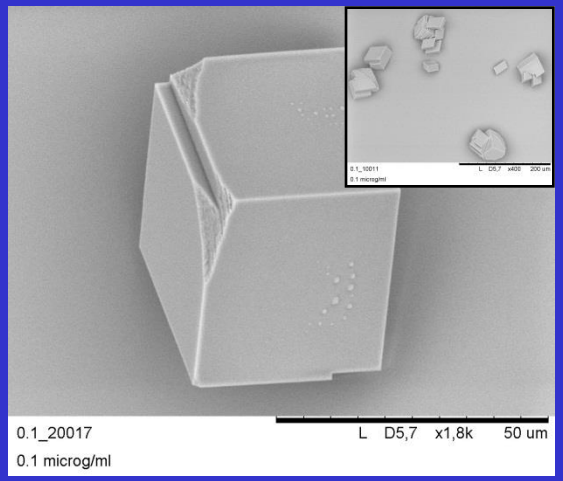

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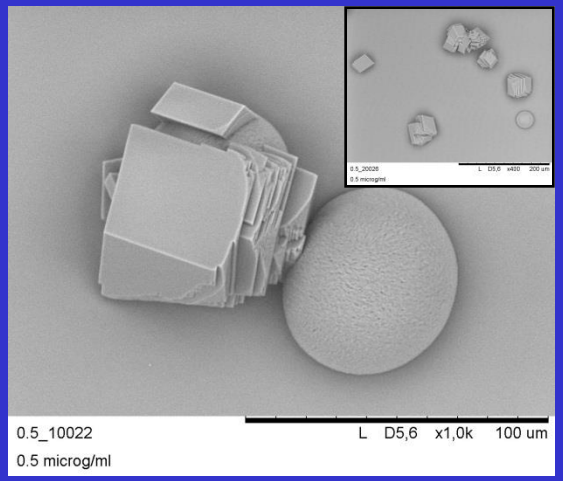

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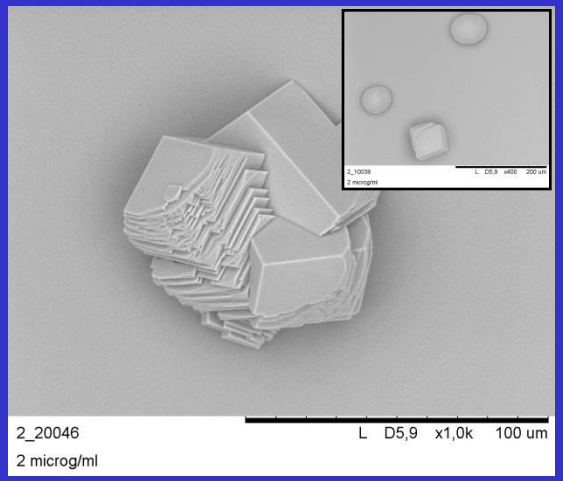

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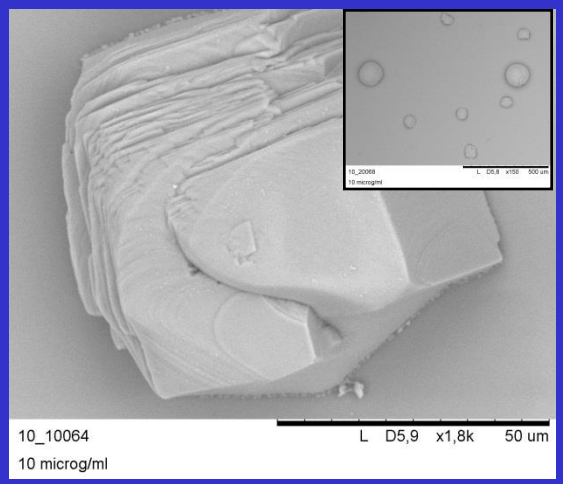

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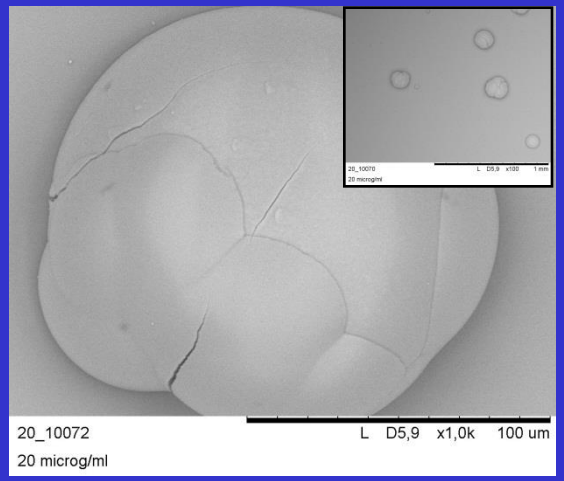

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Conclusions

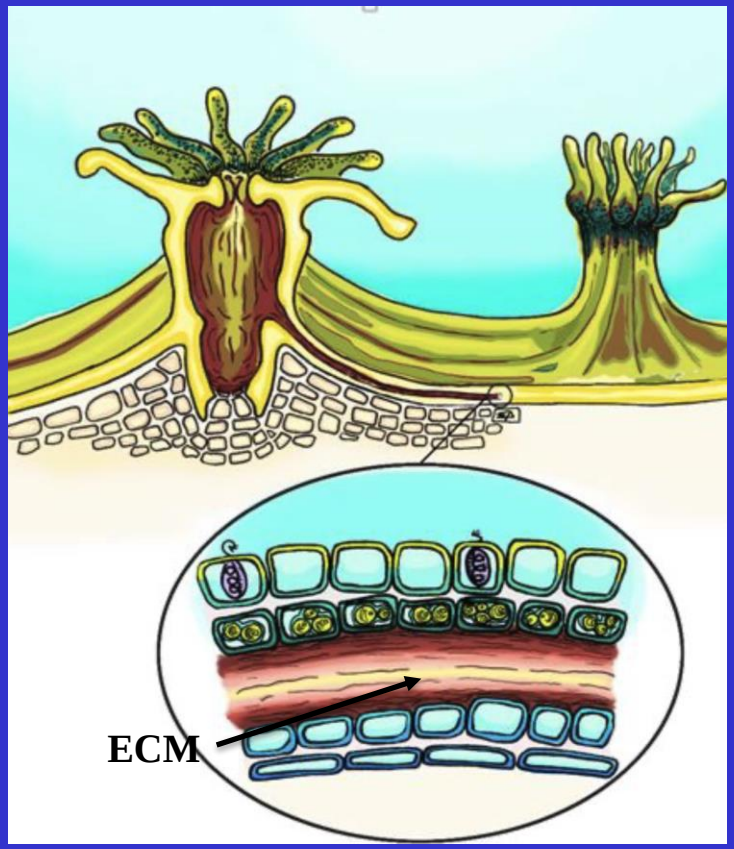
- First attempt to fully characterize the organic matrix of a reef coral using proteomics together with the available genomic resources
- *In vitro* interaction of Acid Soluble Matrix with $CaCO_3$ shows that the crystallization process is strongly influenced

Modelling calcification physiology in a confined space

- H.F. Willard, E.S. Deutekom, D. Allemand, S. Tambutté, J. A. Kaandorp, Testing hypotheses on the calcification in scleractinian corals using a spatio-temporal model that shows a high degree of robustness, Journal of Theoretical Biology, Volume 561, 21 March 2023

Coral calcification

- Enhanced Calcifying Medium (ECM)
  - Located between tissue and skeleton
  - Biomineralization
  - Strong biological control
  - High calcification
  - Increased pH and Ca^{2+}-concentration
- Light-enhanced calcification (LEC)



Many hypotheses!

- Hypotheses ion-transport, reviewed by Allemand et al. (2011):
  - Only paracellular (passive) transport
  - Only transcellular (active) transport
  - Combination of paracellular and transcellular transport

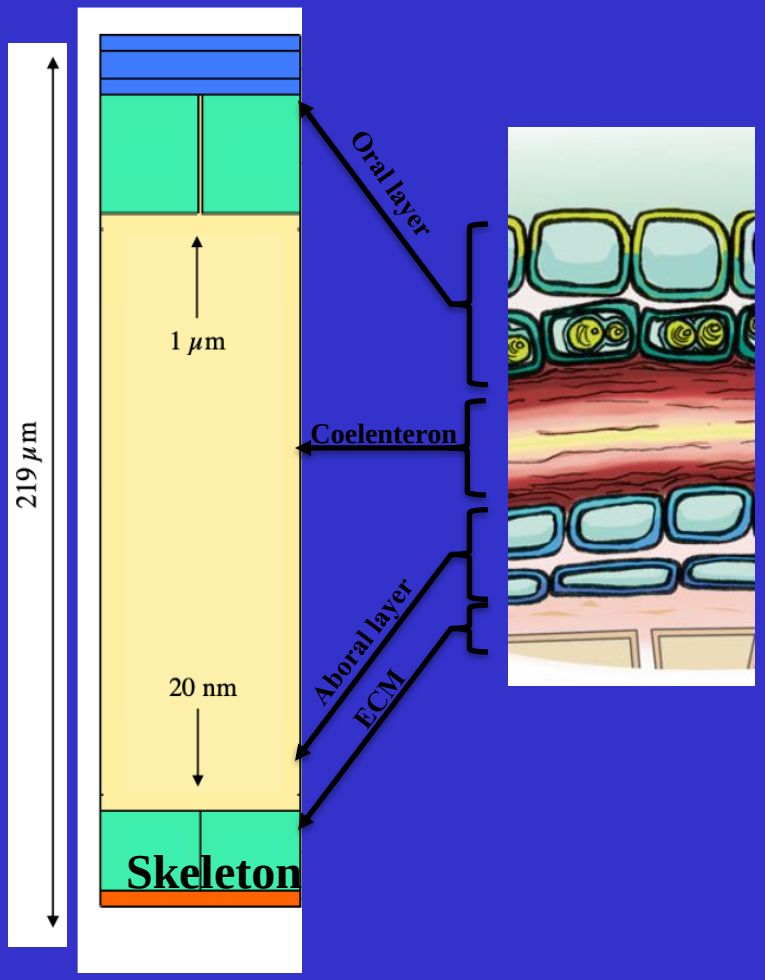
- Hypotheses on Light-Enhanced Calcification
  - Modification of the CO_2 -chemistry within coral tissues caused by CO_2 uptake for photosynthesis (Goreau, 1959, McConnaughey and Whelan, 1997)
  - Increased available energy, simulating ion-transport. (Fukuda et al., 2003, Colombo-Pallotta et al., 2010)
  - Stimulation respiration by more available O_2 (Rinkevich and Loya, 1984)
  - Removal of inhibiting substances (Simkiss, 1964)
  - Synthesis by symbionts of organic matrix moleculesor precursors (Muscatine and Cernichiari, 1969)
  - Stimulation Ca^{2+}-ATPase in light conditions (Al-Horani et al., 2003, Taubner et al., 2019)

Spatio-temporal modelling

- Modelling technique using both time and space
- Used to test and create hypotheses
- Spatio-temporal experimental data
  - Microscopy in combination with pH-sensitive dye (Venn et al. 2011)
  - Measurements with microsensors (Al-Horani et al. 2003)
  - More spatio-temporal data (e.g. Ca 2+ concentrations)

Calcification model

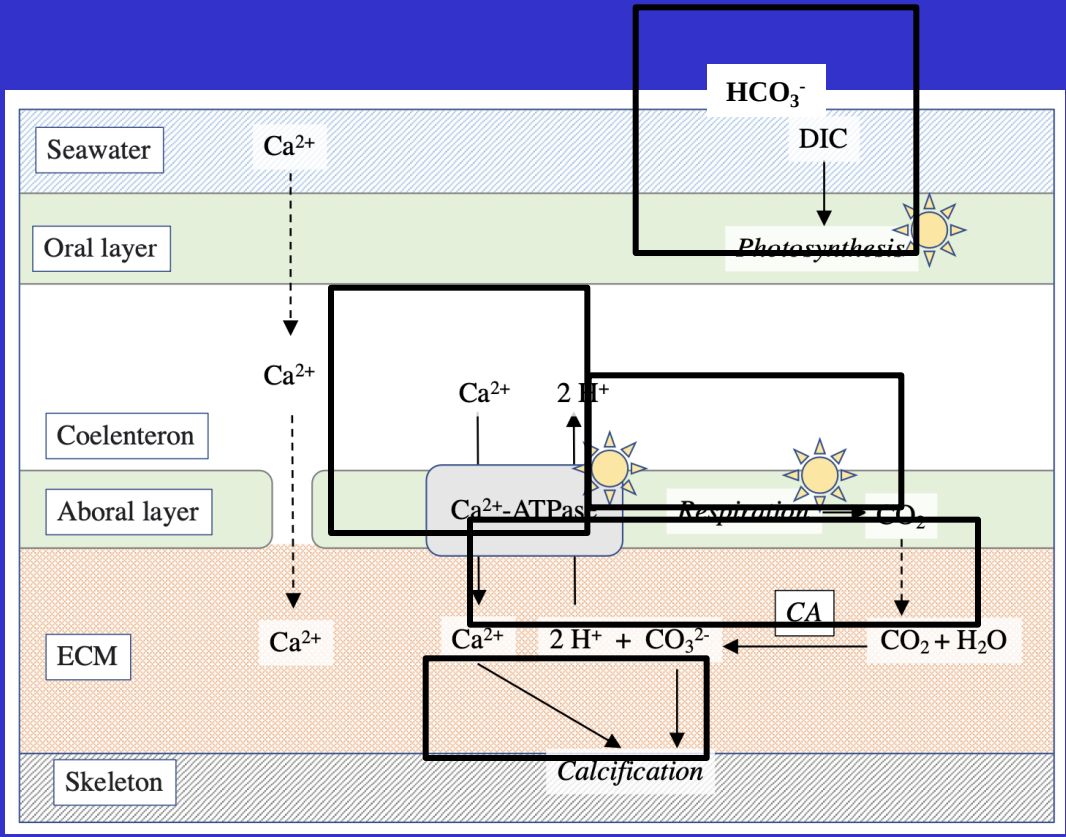
- Topology is as simple as possible!
  - Cell layers are combined
- Reaction-diffusion model:
  CO_2-chemistry (Zeebe et al. 2001)
  - H^+/OH^-
  - $CO_2/HCO_3^-/CO_3^{2-}$
  - $B(OH)_3/B(OH)_4^-$
- Only CO_2 diffuses over cell membrane



Calcification model

- Photosynthesis
- Respiration
- Active ion-transport
- Carbonic Anhydrase (CA)
- Calcification

Light dependent processes

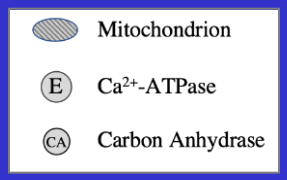


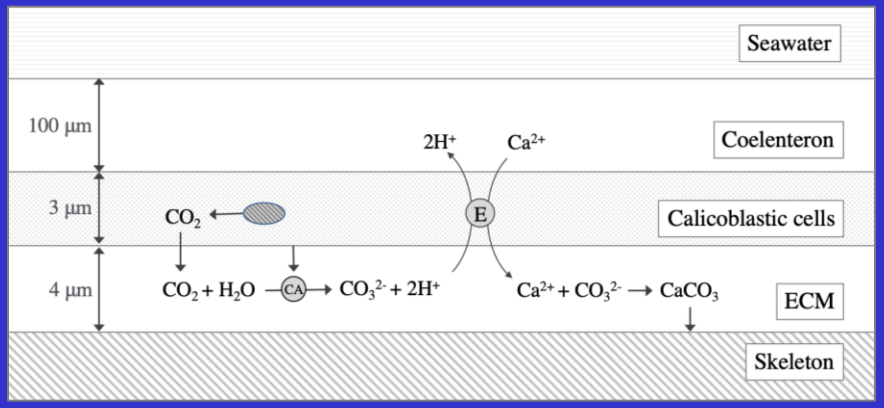
Modelling approach I

- Spatial model considering chemical processes controlling the chemical composition of the ECM.
- Both diffusion (spatial information) and chemical reactions.
- Can we create a simple spatial model that reproduces data from in vivo measured data (e. g. Al Horani 2003) of the ECM in reef building corals?
- Can we, using this model, gain a better understanding on how the coral keeps the ECM's chemical composition favorable for calcification?
- Can we, using this model, reproduce the light-dark dynamics that are the effect of LEC?

Modelling approach II

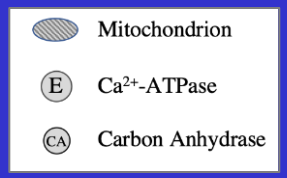
- Spatial Reaction-Diffusion model
  - Including CO_2-chemistry based on the system of Zeebe and Gladrow (2001)
- Simple topology
  - Cell membranes only permeable by CO_2
  - Calcification at skeleton boundary
  - Seawater constant concentrations

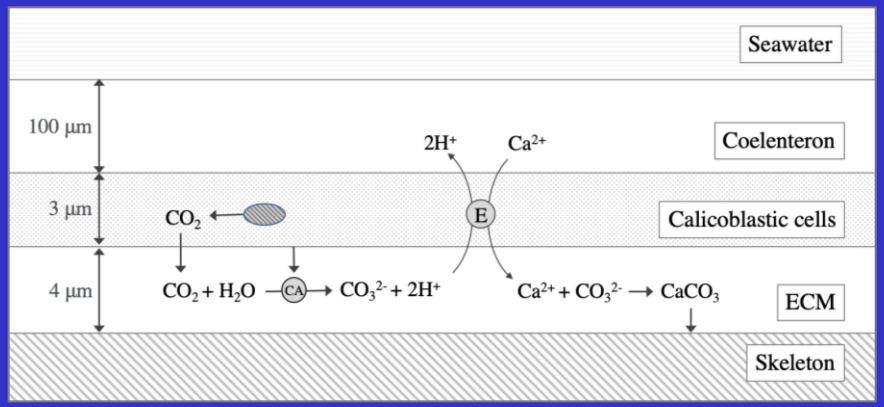




Modelling approach III

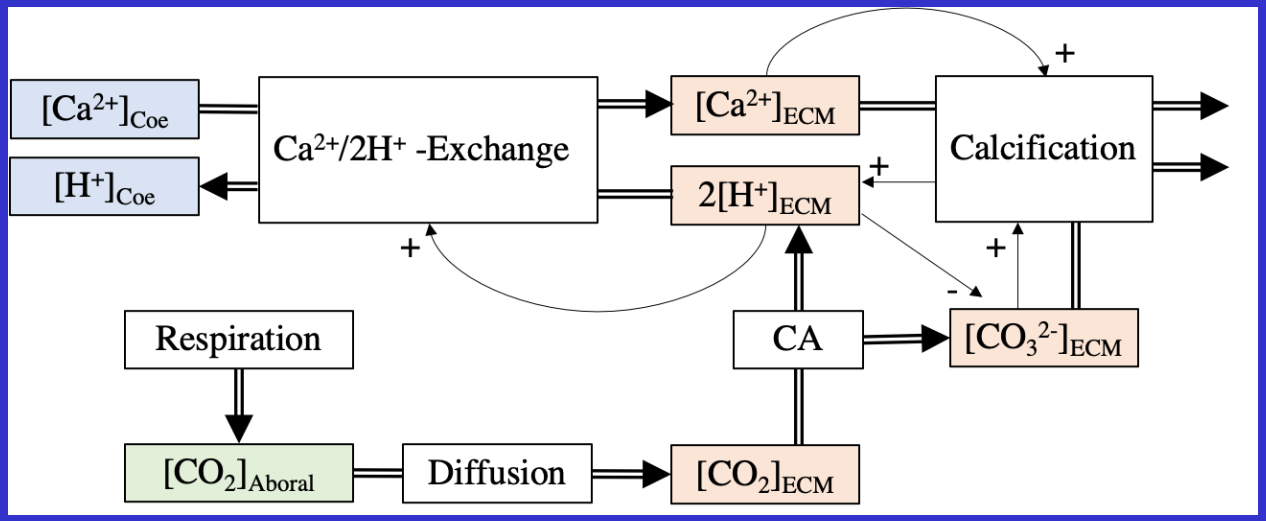
- Chemical composition ECM is controlled by
  - Respiration in Calicoblastic cells
  - Ion transport of Ca^{2+}-ATPase
    - Modelled as flux over Calicoblastic cells
    - $J_{ex} = J_{ex}^{max} \frac{[H^+]^2[Ca^{2+}]_{cell}}{K_{ex} + [H^+]^2[Ca^{2+}]_{cell}}$
  - Carbonic Anhydrase
    - $V_{CA} = E(CA)_{tot}\, k_{cat} \frac{[CO_2]}{K_{CA} + [CO_2]}$





Modelling approach IV

- System reaches steady state
  - Flux of Ca^{2+}-ATPase equals calcification rate
  - CO_2 – diffusion equals calcification rate



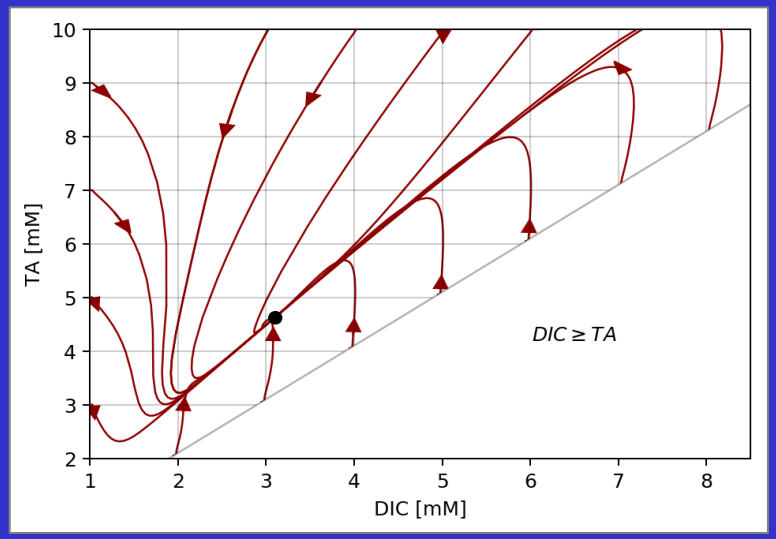
Modelling approach V

- We assume that, for every biologically relevant set of parameters, there exists at least one *stable* equilibrium point for which the concentrations in the ECM are constant. The latter also for biologically relevant ranges.
- The *chemical concentrations* corresponding to this equilibrium point are assumed to be controlled by the Calicoblastic cells.
- By understanding how this stable point is controlled, we might be able to simulate *the light-dark dynamics* as observed by, among others, Al-Horani (2003).

Steady state of the model

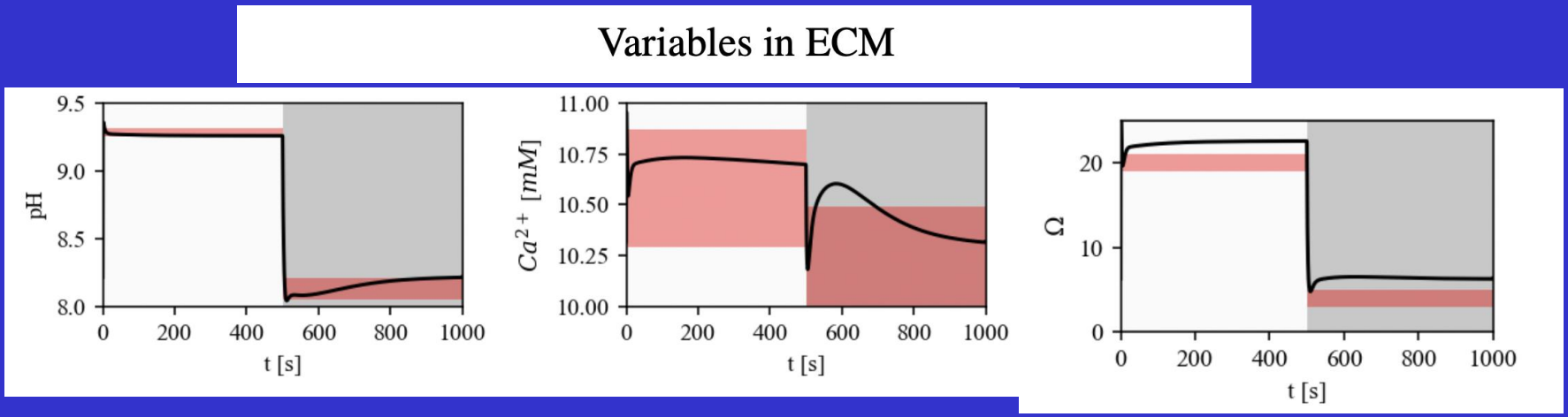
- System shows one stable point
  - {DIC*, TA*} = {3.1 mM, 4.6 mM}
    - These values correspond with literature [10] [14]
  - $\Omega^* = 24.1$
- Stable points seems global
  - not formally proven
- Steady state is analyzed by changing
  - J_{ex}^{max}
  - R_{resp}
  - $E(CA)_{tot}$

$E(CA)_{tot} = 0.05 \mu mol$
$J_{max}^{ex} = 10 \mu mol/(m^2 s)$
$R_{resp} = 2 mM/s$

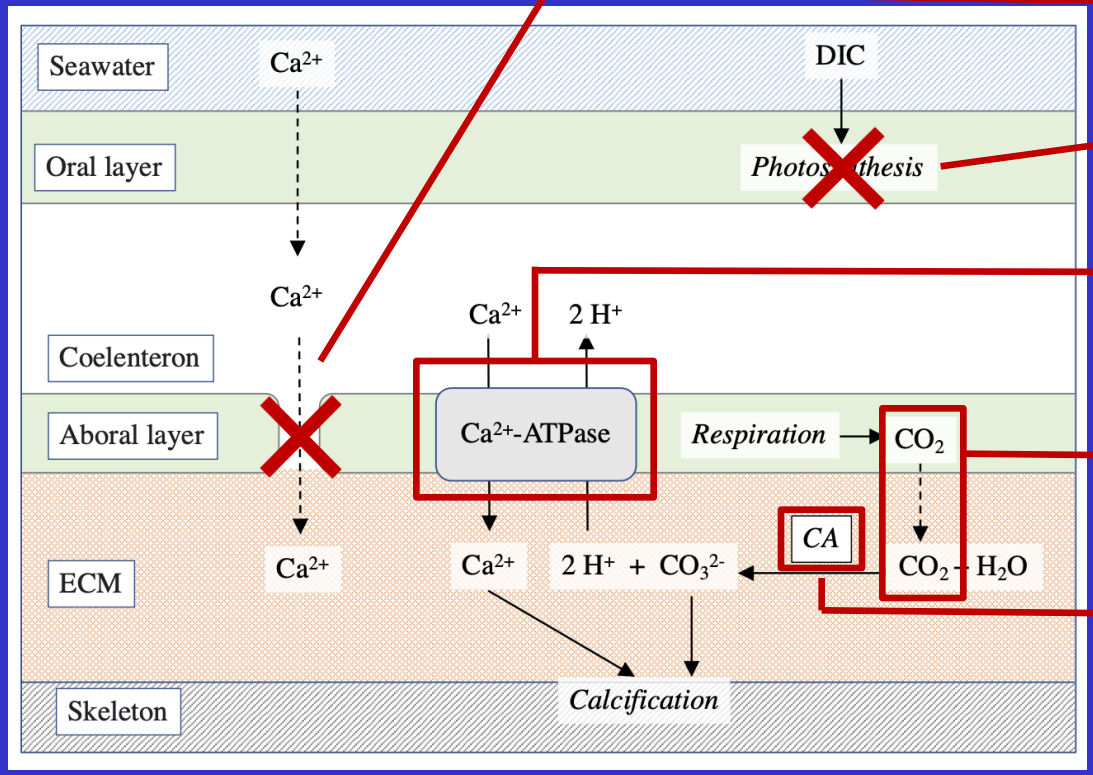


Calcification model results

- Model (solid black line)can reproduce experimental data (red areas) (Al-Horani et al. 2003) for light and dark (grey shade) conditions
- Model is robust, essential for biological models (Kitano et al. 2002)



Testing hypotheses using calcification model



Only paracellular transport insufficient for calcification

Effect of photosynthesis on $[DIC]_{ECM}$ is limited

Controls pH_{ECM}
Limited effect on Calcification rate

Influx CO_2 rate-determining step

CA is essential for CO_2 supply to ECM

Conclusions

- The effect of paracellular transport was limited in the model. Only paracellular transport does not sufficiently supply ions to the ECM for calcification.
- Carbonic Anhydrase is essential for the CO_2-supply into the model's ECM.
- Light-enhanced calcification was the result of two processes: more available respirational CO_2 and increased activity of Ca^{2+}-ATPase.

Morphological plasticity in scleractinian corals: examples

The scleractinian coral *Montastrea annularis*

1m

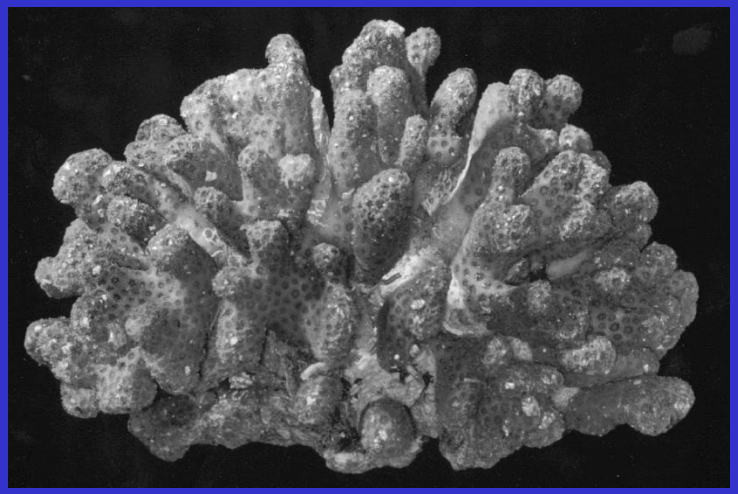
22m

25m

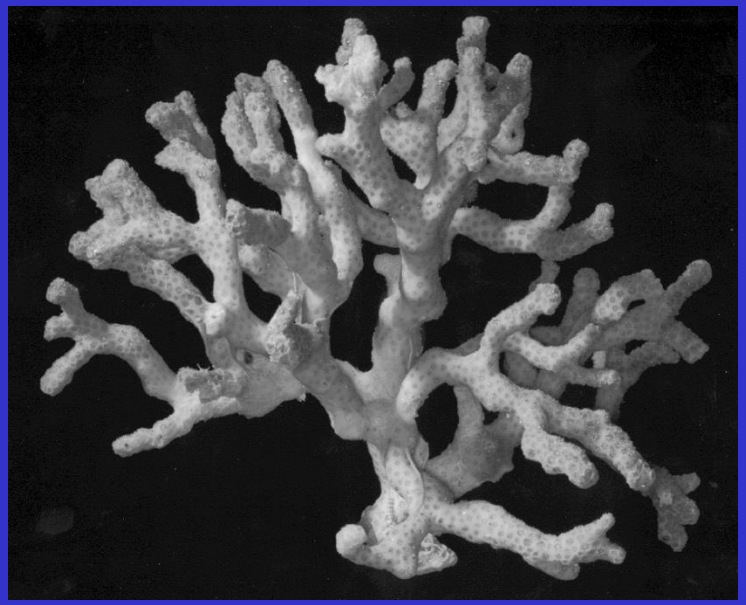
30m

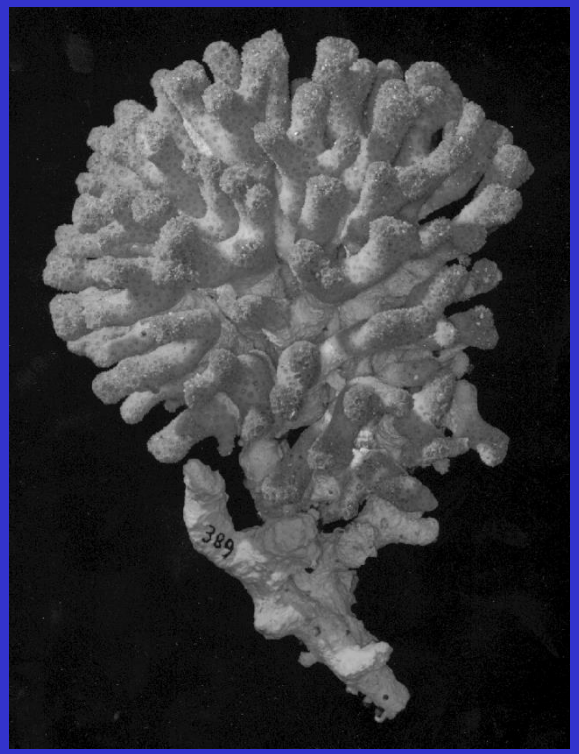
The stony coral *Madracis mirabilis*

6m



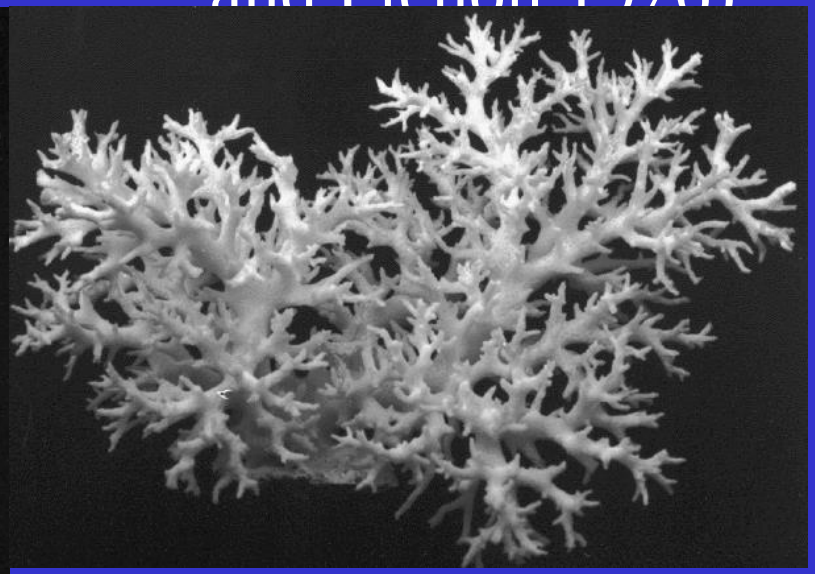
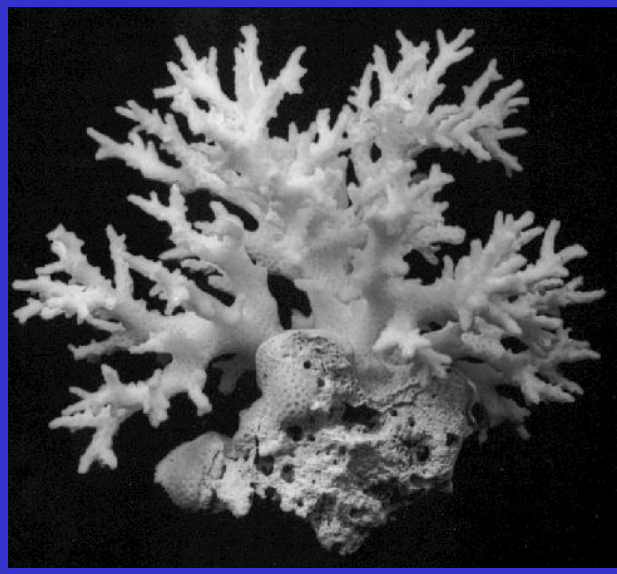
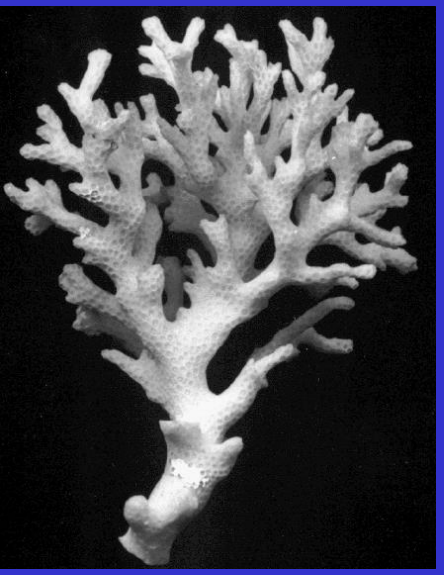
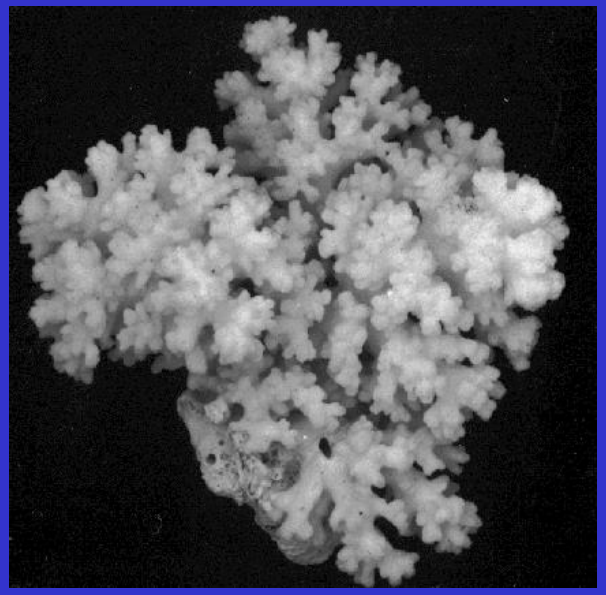
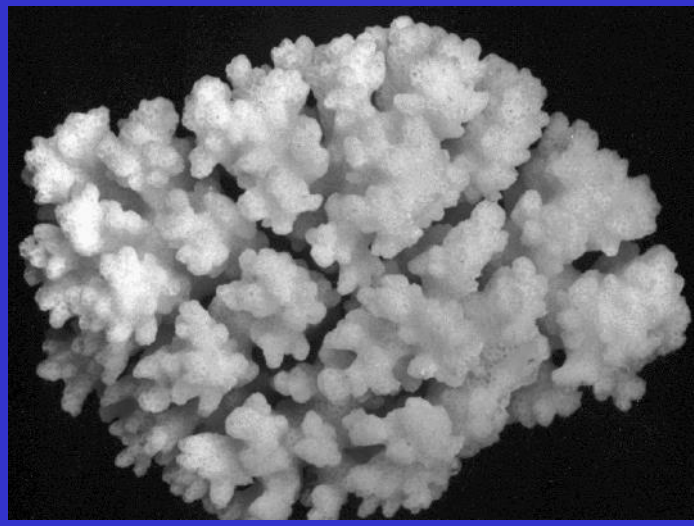
20m



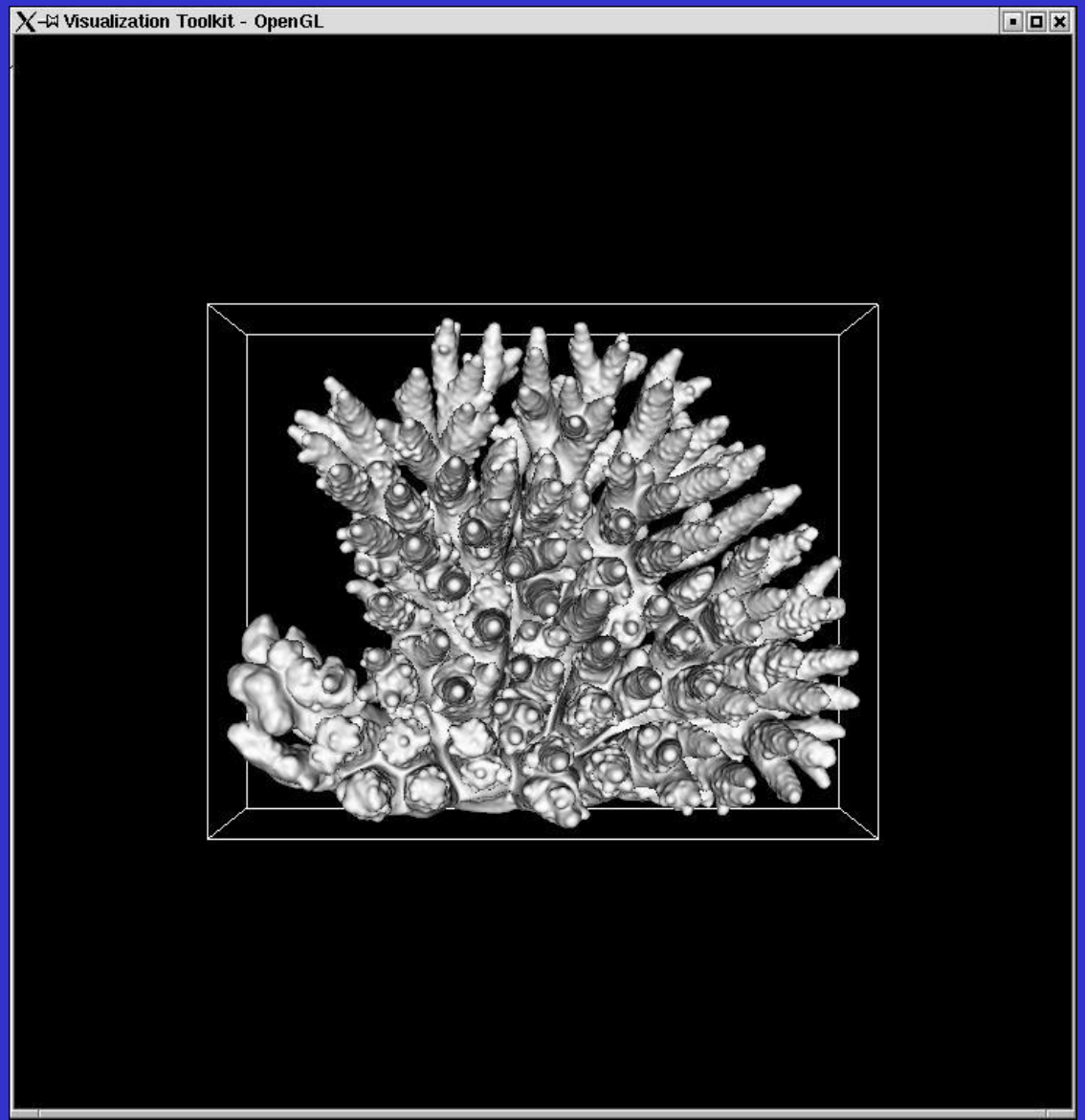


15m

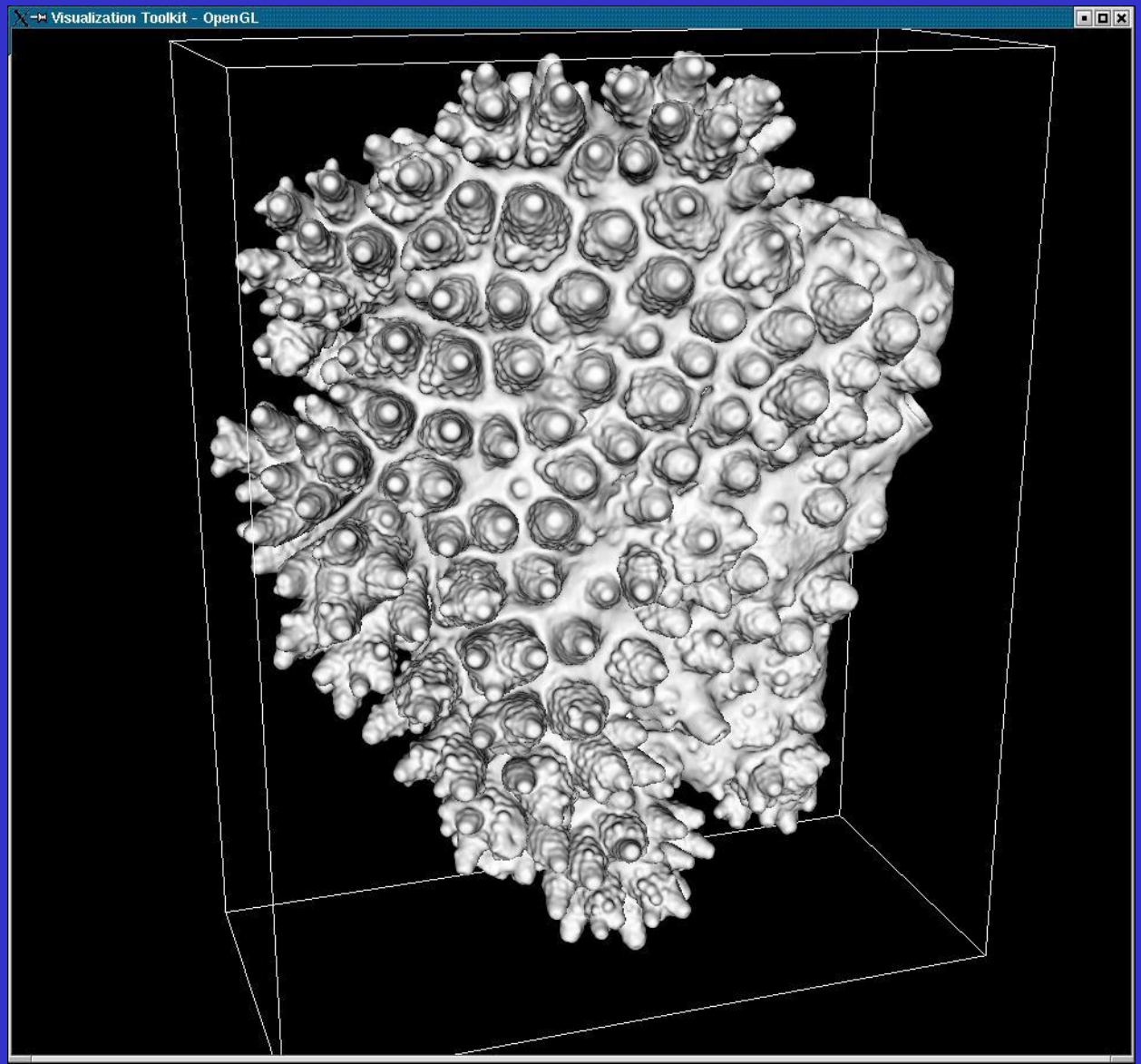
The stony coral *Pocillopora damicornis;* a range from very sheltered – very exposed (top left to right bottom) (after Veron and Pichon 1976)

Acropora digitifera low flow morphology



Acropora digitifera high flow morphology



Survival of *Acropora digitifera* for high and low flow velocities (Nakamura & van Woesik, 2001)

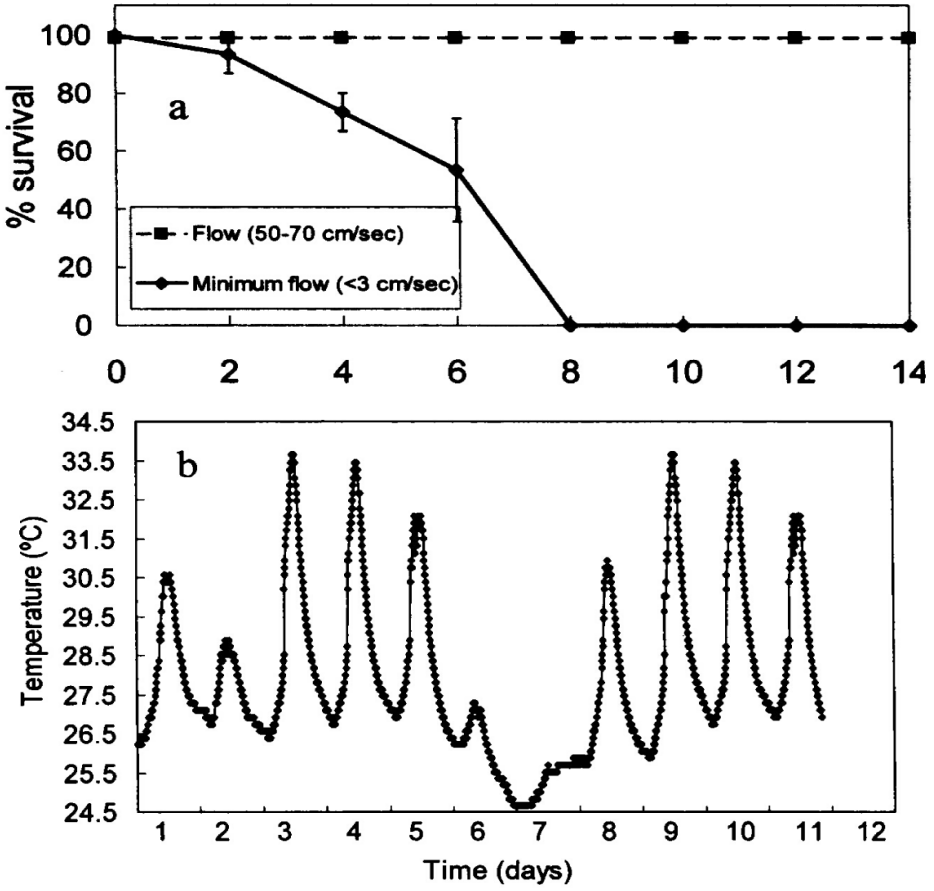


Figure 2. Percent survival (a)of *Acropora digitifera* at elevated temperatures(b) while maintained under high (50-70 cm/sec) and low (<3 cm/sec) flow (Nakamura & Van Woesik 2001).

Symmetry in the colony shape of the scleractinian coral *Pocillopora verrucosa* (experiment by Mass & Genin, 2008)

Symmetry of colony shape

- Is morphological plasticity in corals genetically controlled or influenced by external factors? Growth of the coral *Pocillopora verrucosa* under the influence of ***uni-directional*** current.

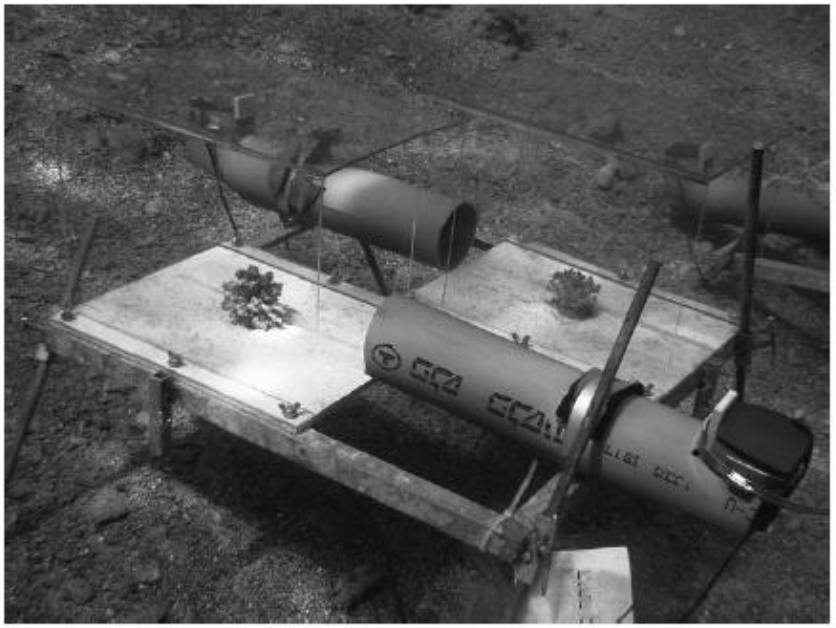
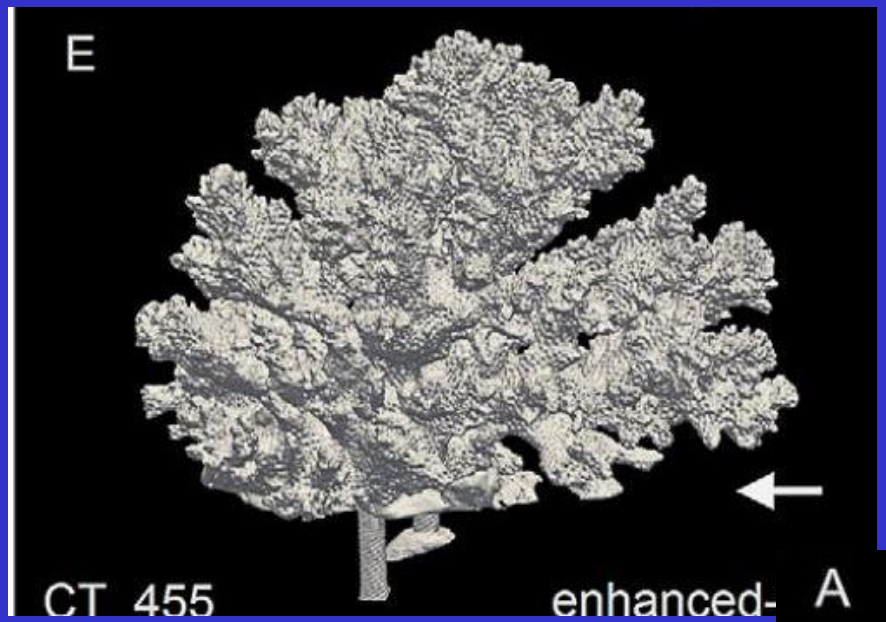


Fig. 1. Underwater setup of flow-manipulation experiment showing 2 units each consisting of a transparent, upside down U-shape box (40 × 40 × 30 cm) and a pump attached to a 50 cm long, 10.1 cm diameter pipe directed at the coral

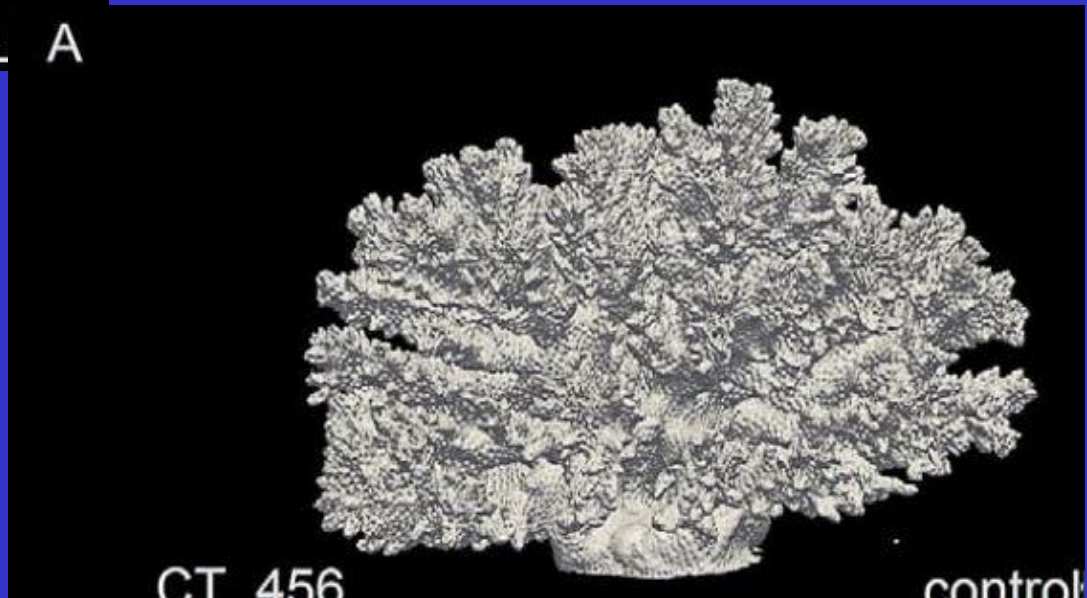
Mass et al., 2010

Symmetry in *Pocillopora verrucosa* (experiment by Mass & Genin 2008)



A-symmetrical form (uni-directional flow

Symmetrical form



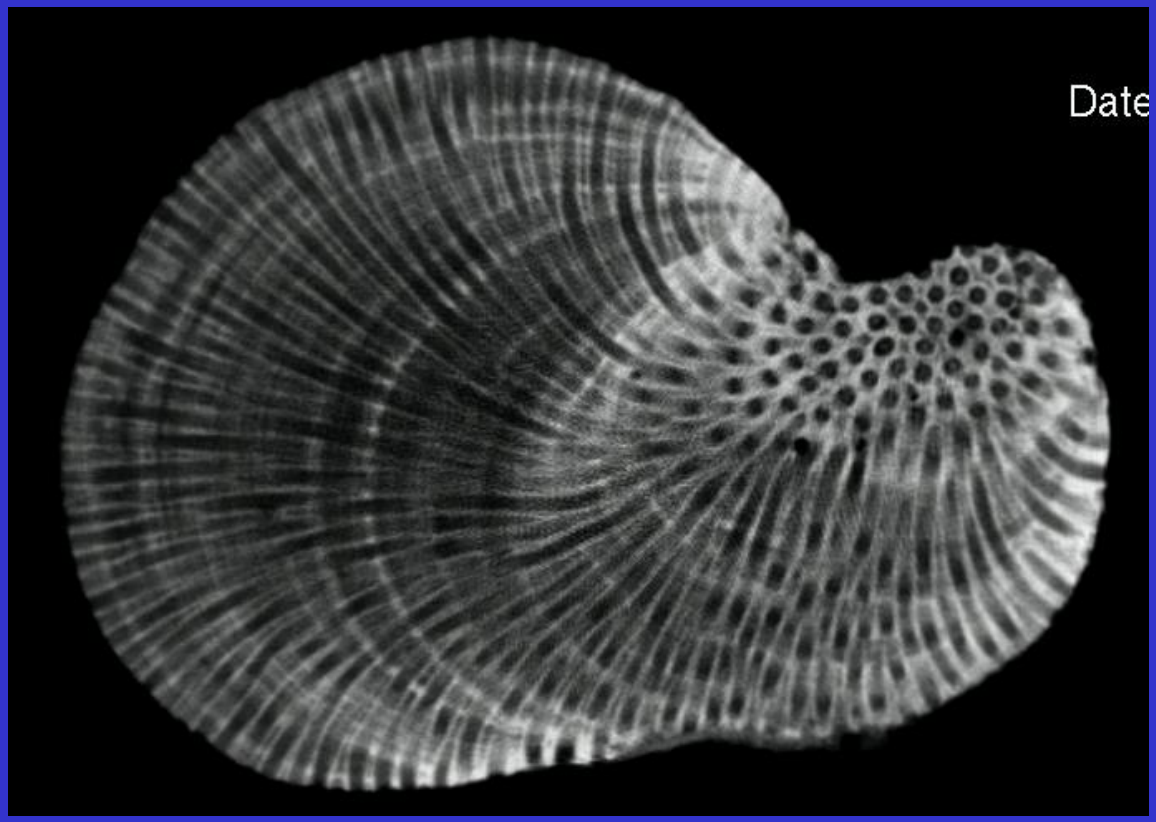
Research Questions

CT scan *Pocillopora verrucosa* (from experiment by Mass & Genin 2008)

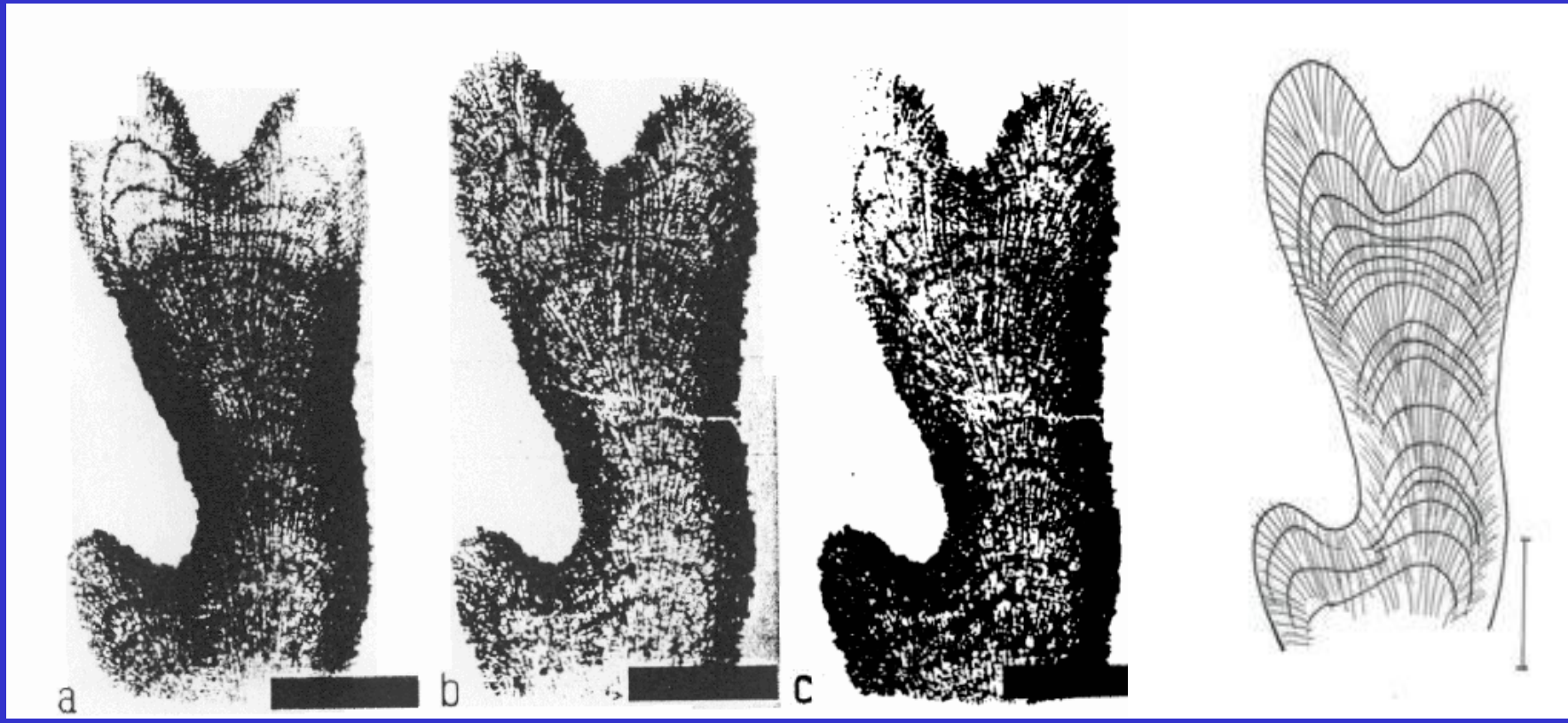
- **Research question 1**: Is the symmetry found in coral colony determined by symmetry in the flow rather than intrinsic control by the coral?
- **(Additional) Research question 2**: Is a local increase of O2 concentrations produced by photosynthesis the cause of bleaching under low flow conditions?

Radiate accretive growth in scleractinian corals and sponges: growth layers and surface structure of a growth layer

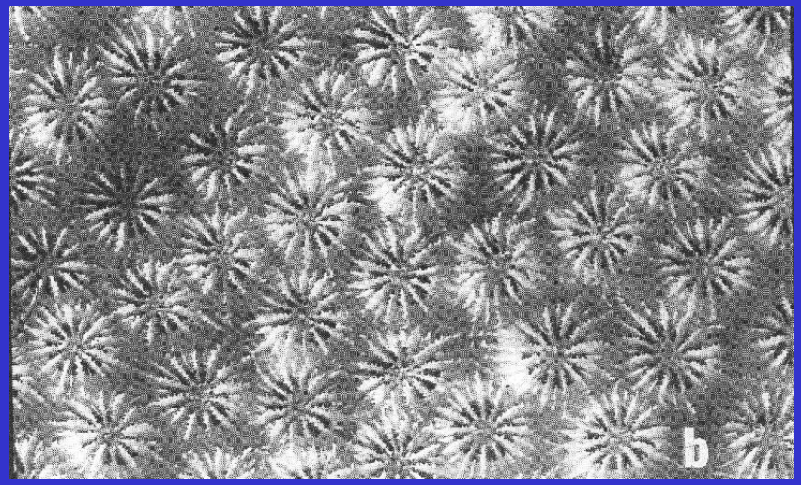
Growth layers in 3D images of corals *(Montastrea annularis)*

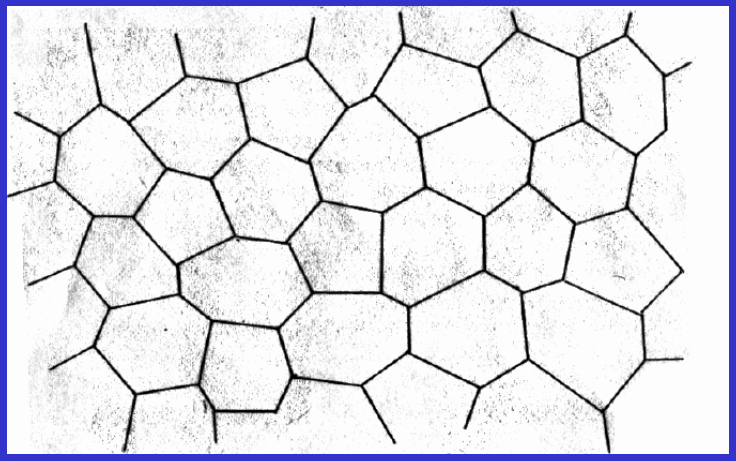


Radiographs of slices through the scleractinian *Porites porites* (after Tissier et al., 1994)



Surface view of the scleractinian *Montastrea annularis*

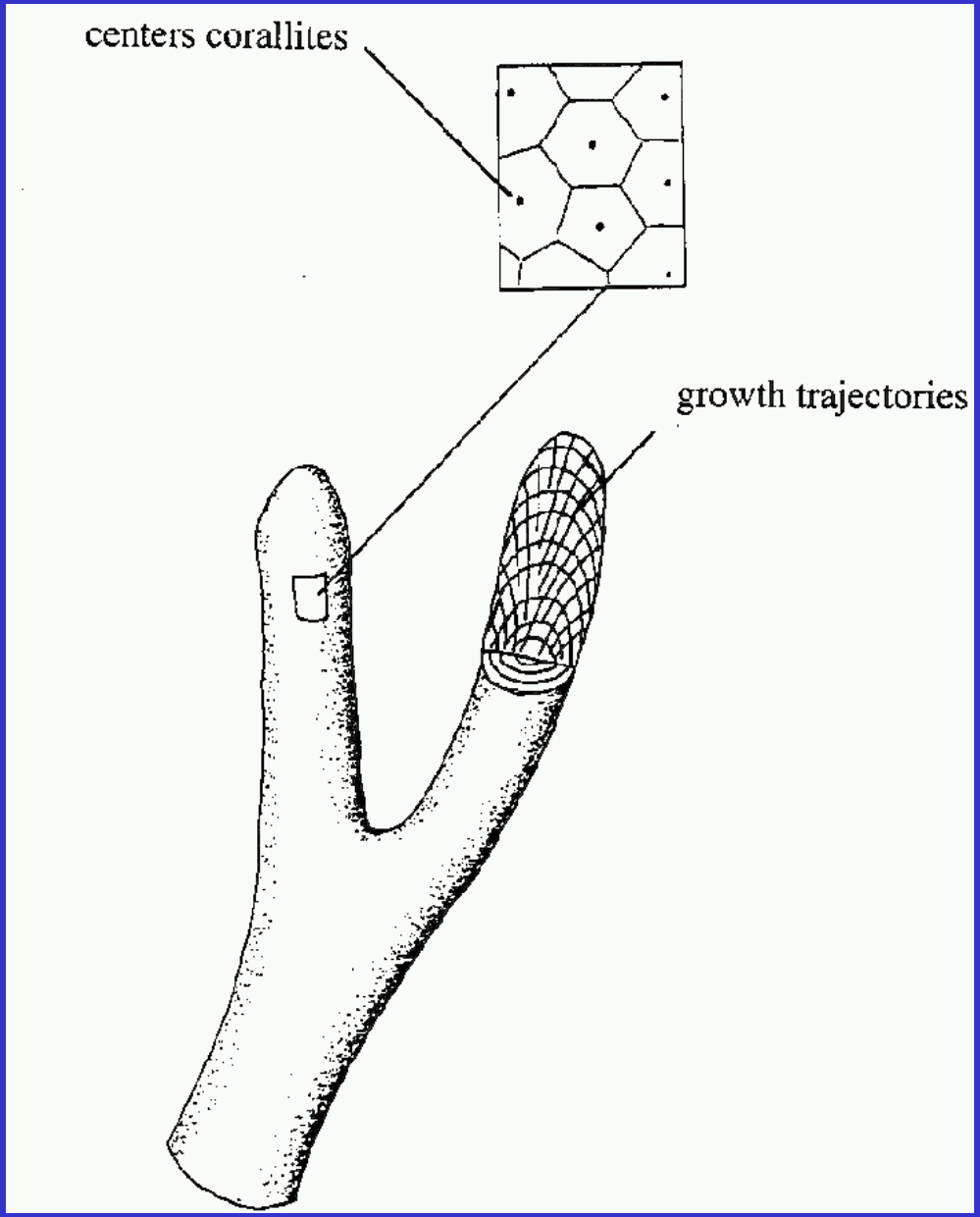




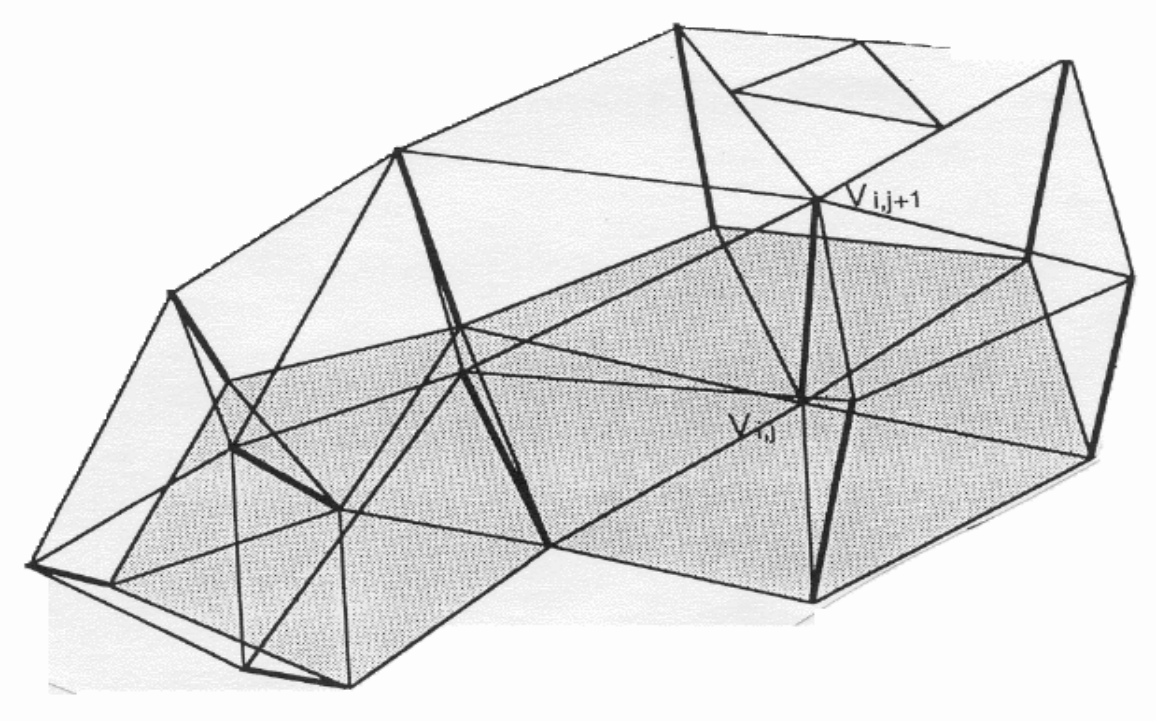
Modelling the influence of the physical environment (diffusion, light, hydrodynamics) on calcification

Central concept of accretive growth

- Layers of material are deposited on top of the previous ones. The previous layers remain unchanged
- The local thickness of a new growth layer is determined by the local absorption of nutrients / local light intensity along the surface normal vector

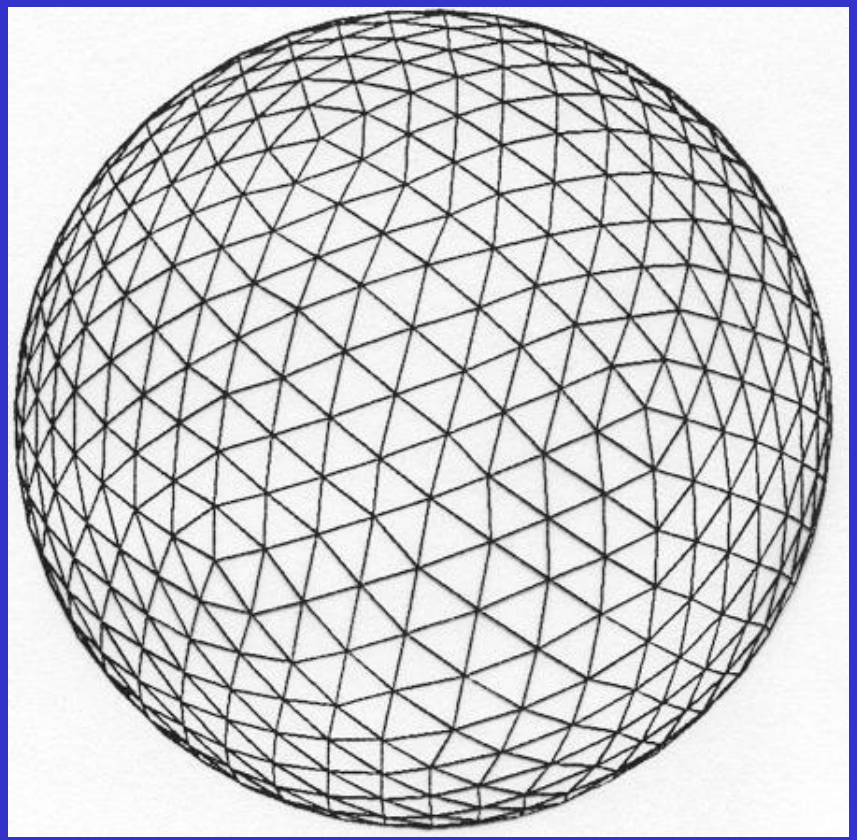


Simulation of radiate accretive growth:



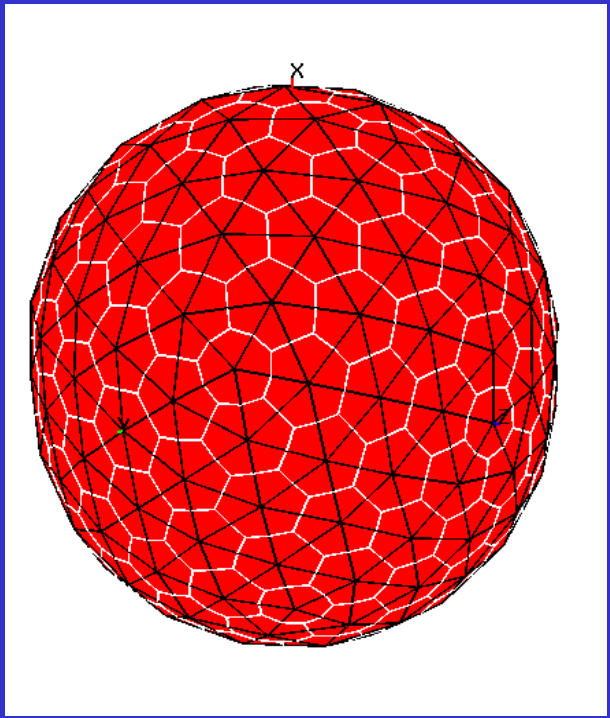
1. a new layer of triangles is constructed on top of a previous one
2. The previous deposited layers remain unchangend
3. the triangles are organized in polygons
4. The thickness *l* of a new layer is determined by the local amount of absorption of nutrient / light along the mean surface normal vector

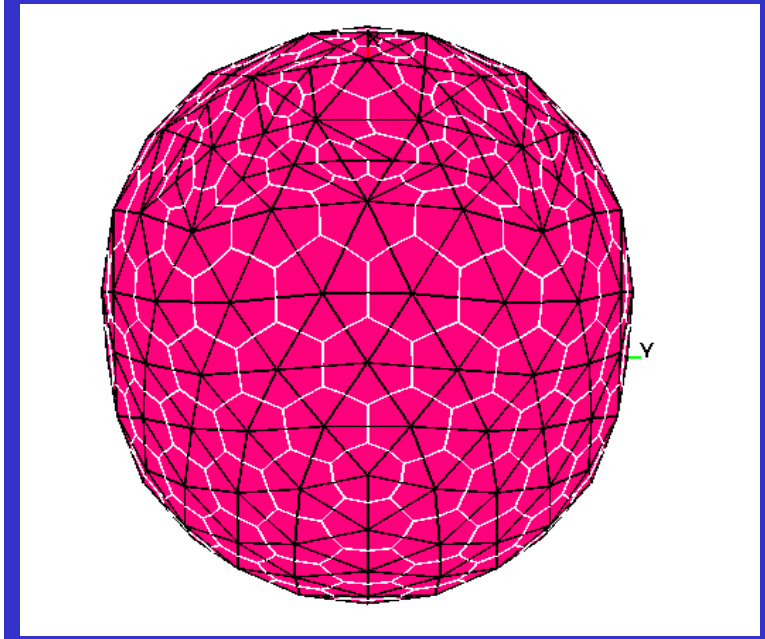
Accretive growth: initial object



Insertion/fusion rules

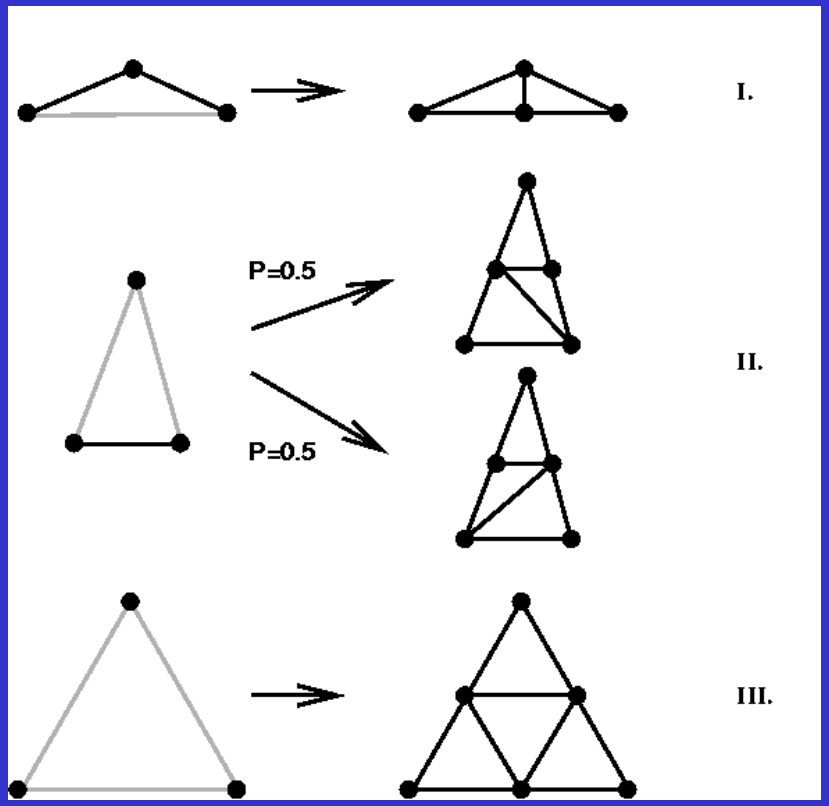
Two subsequent meshes



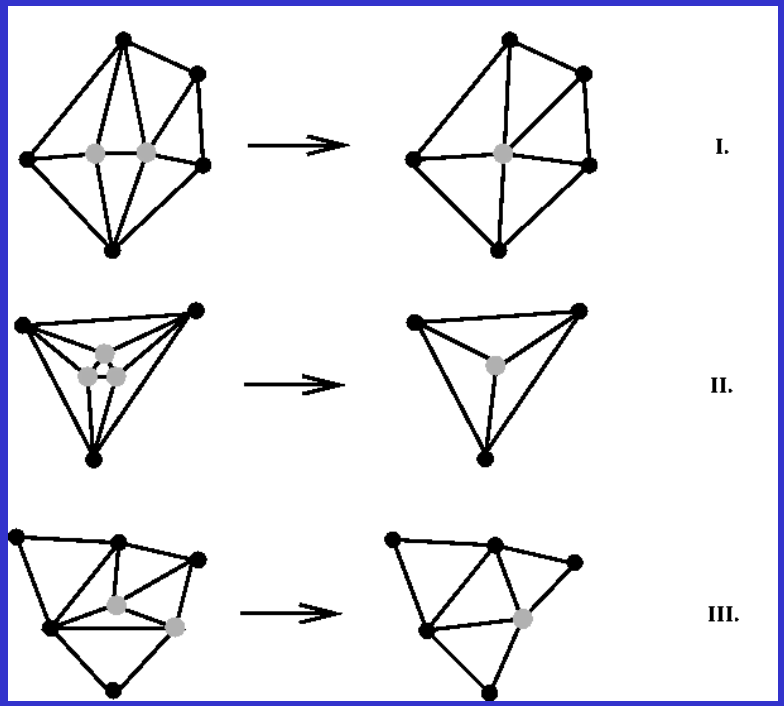


Insertion and fusion rules

Consequences for triangle mesh

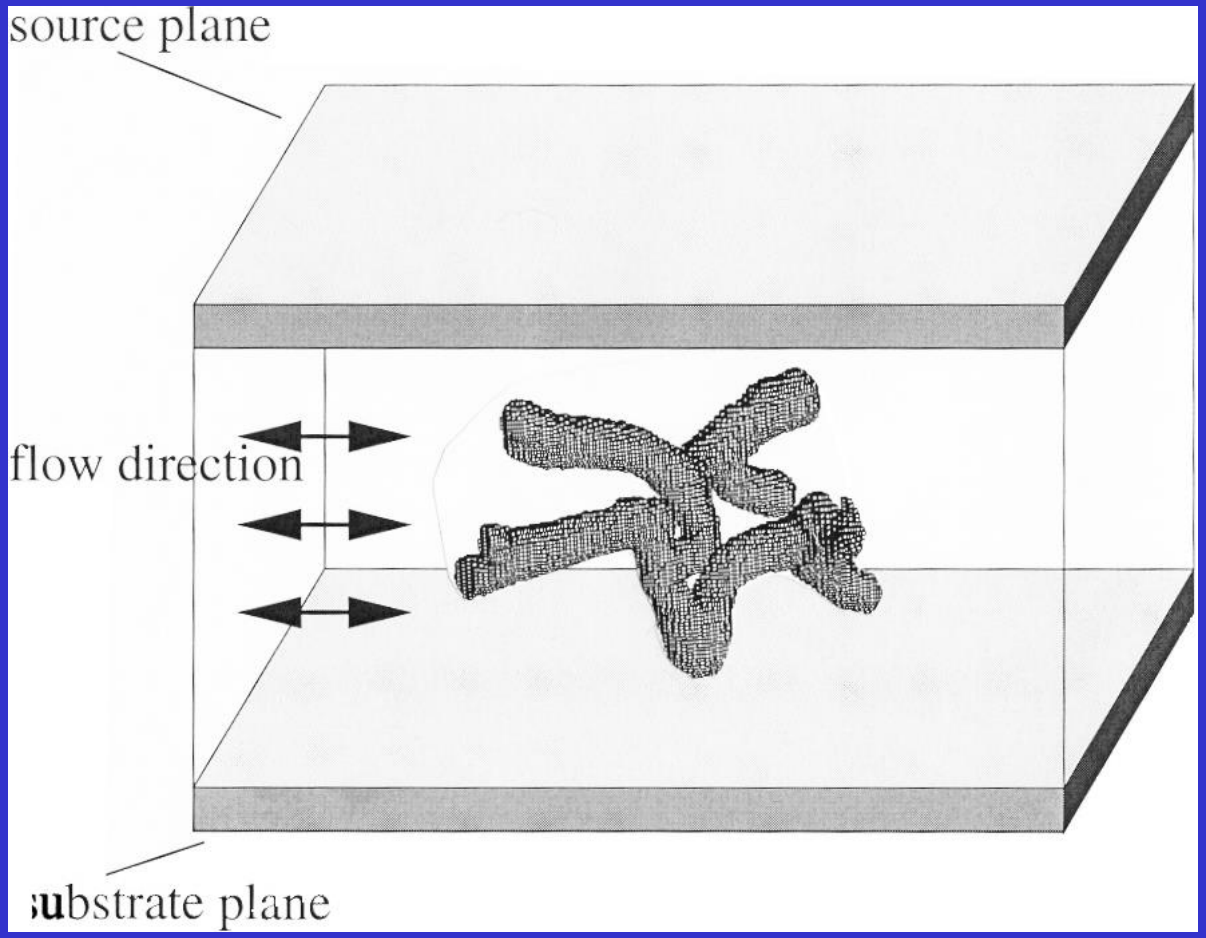


insertion



fusion

Coupling accretive growth model and diffusion / light model



Light direction Corresponds to vertical

Modelling diffusion-limited growth

$$\frac{dc}{dt} = \mathcal{D} \nabla^2 c$$

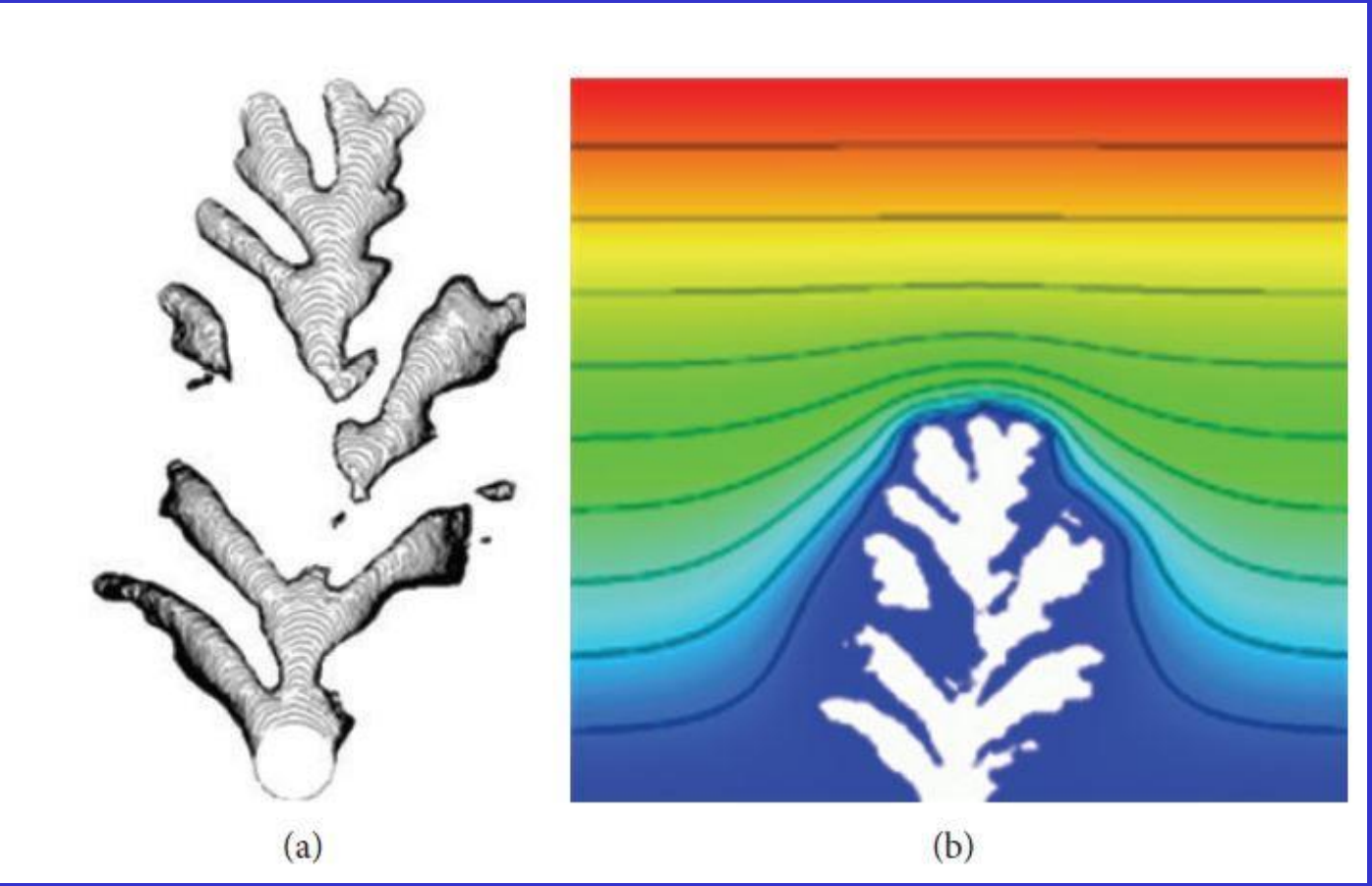
where c is the concentration, t time and $\mathcal{D}$ the diffusion coefficient.

•The thickness of a new layer l, the distance between two successive vertices Vi and Vi+1, is computed by using the growth function:

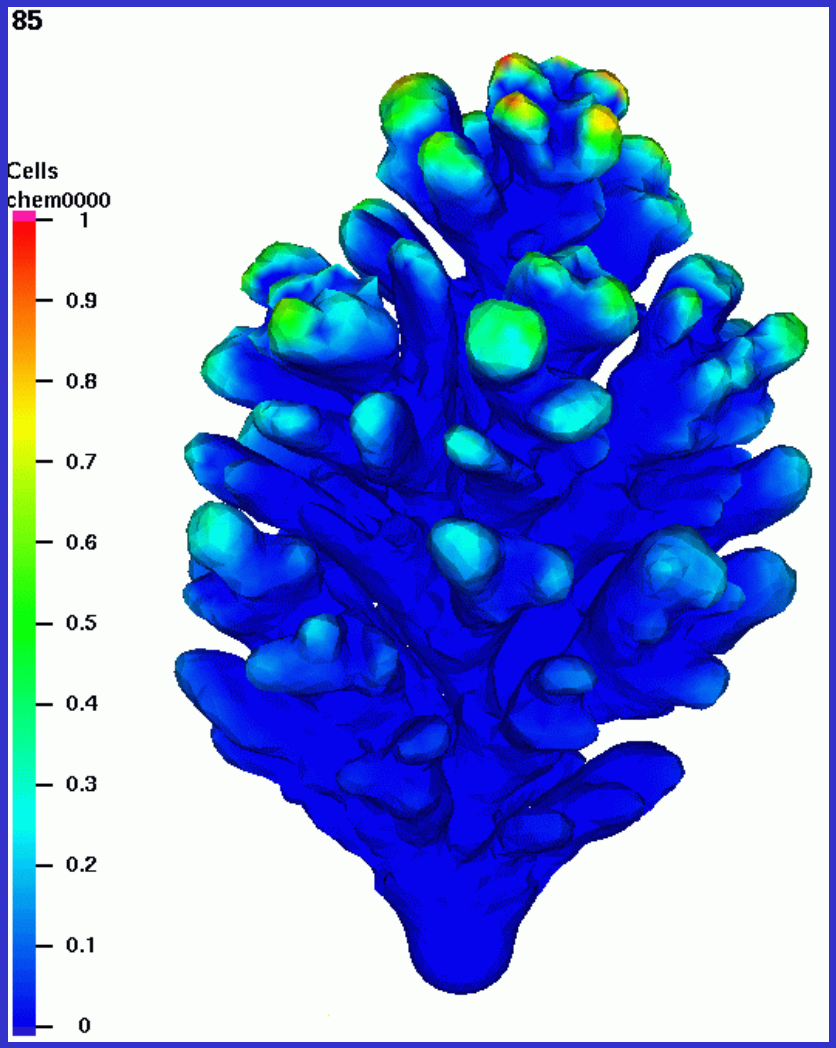
$$l = \vec{n} c_i^{\text{total}} s,$$

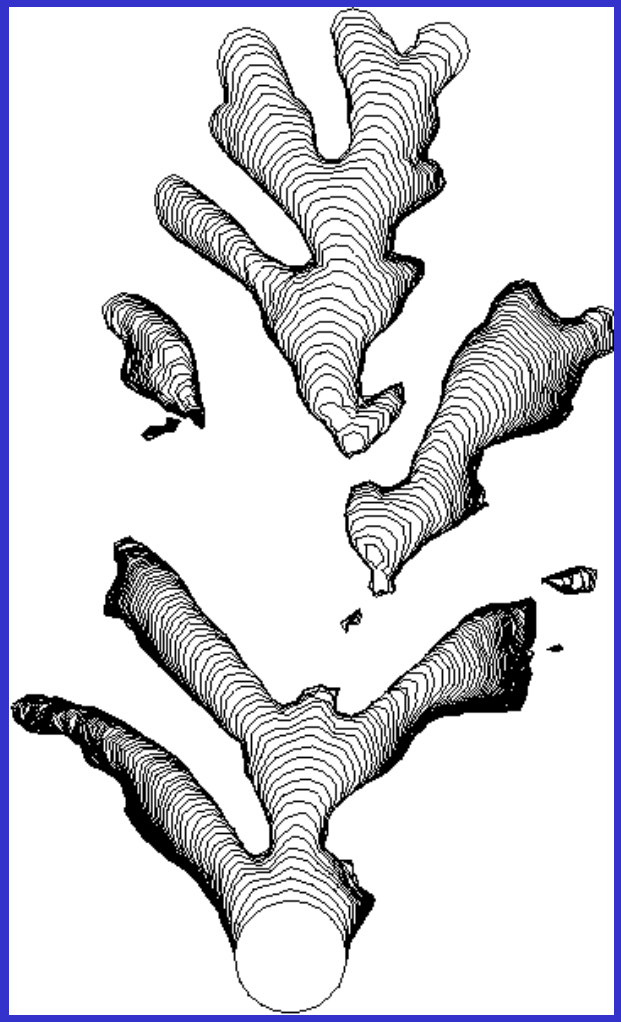
•where $\vec{n}$ is the average normal vector in vertex V and c_i^{total} the amount of absorbed simulated nutrients and s is the maximal thickness of the growth layer.

Accretive growth: layered deposition of material (diffusion limited growth)



Accretive growth: layered deposition of material (diffusion limited growth)



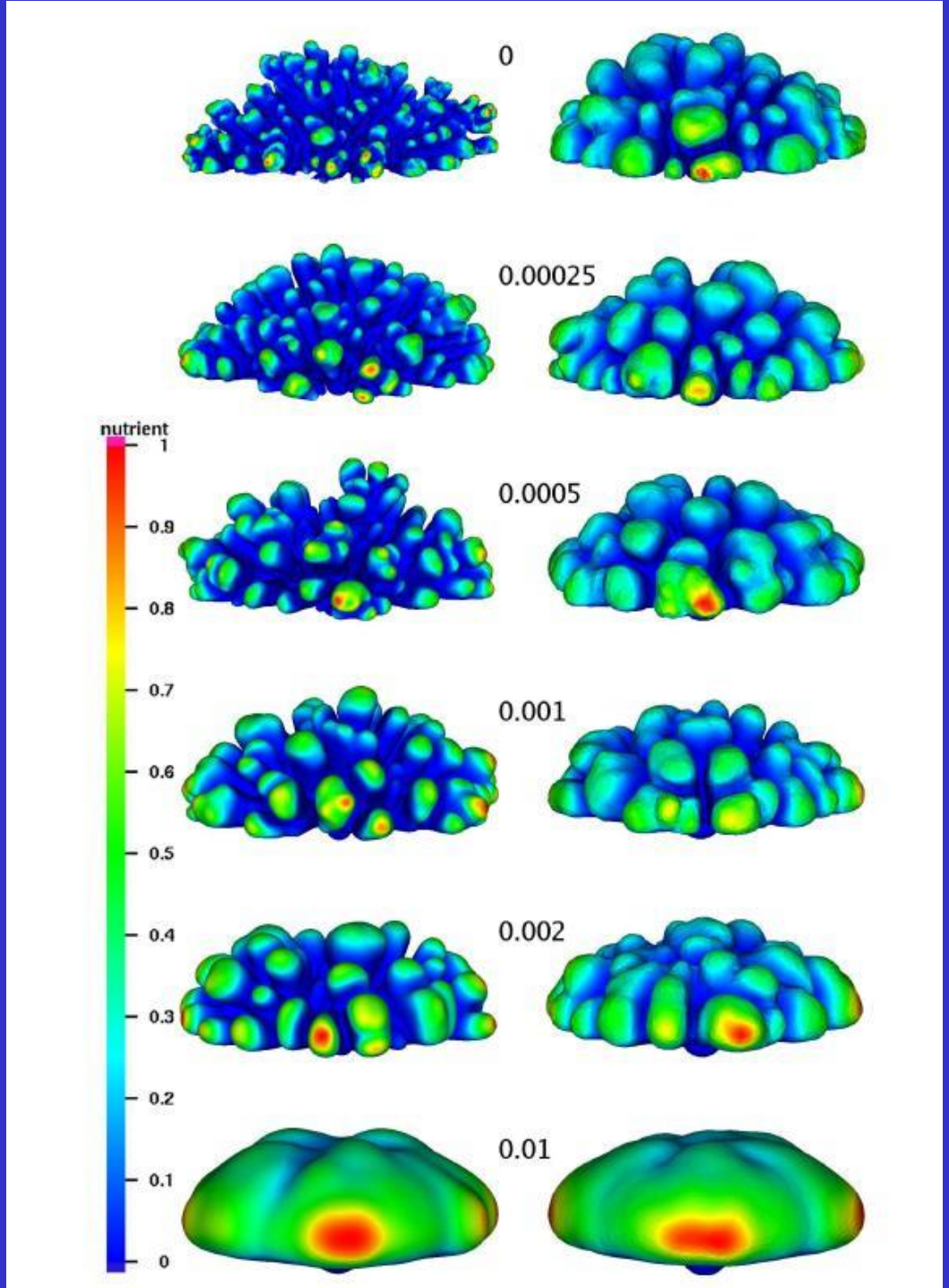


Modelling diffusion-limited growth and tranlocation of absorbed nutrients by surface diffusion

•The translocation of absorbed nutrients by surface diffusion is modelled by:

$$\frac{\partial c(x,t)}{\partial t} = D_{\text{surf}} \nabla^2 c(x,t)$$

Diffusion limited growth + surface diffusion: from top to bottom amount of surface diffusion (Dsurf) is increased



Modelling diffusion-limited growth and the influence of light intensities

•The thickness of a new layer l, the distance between two successive vertices Vi and Vi+1, is computed by using the growth function:

$$c_i^{\text{total}} = (1-\alpha)\, c_i^{\text{nutrient}} + \alpha c_i^{\text{light}}, \quad 0 \le \alpha \le 1$$

•Where c_i^{nutrient} are local absorbed nutrient and c_i^{light} are the local abosorbed light intensity, and alpha is a weight factor .

Computing local light intensity

$$c_i^{\text{light}} = (1 - \text{ambient}) \cos\theta + \text{ambient},$$
$$0 \leq \text{ambient} \leq 1.$$

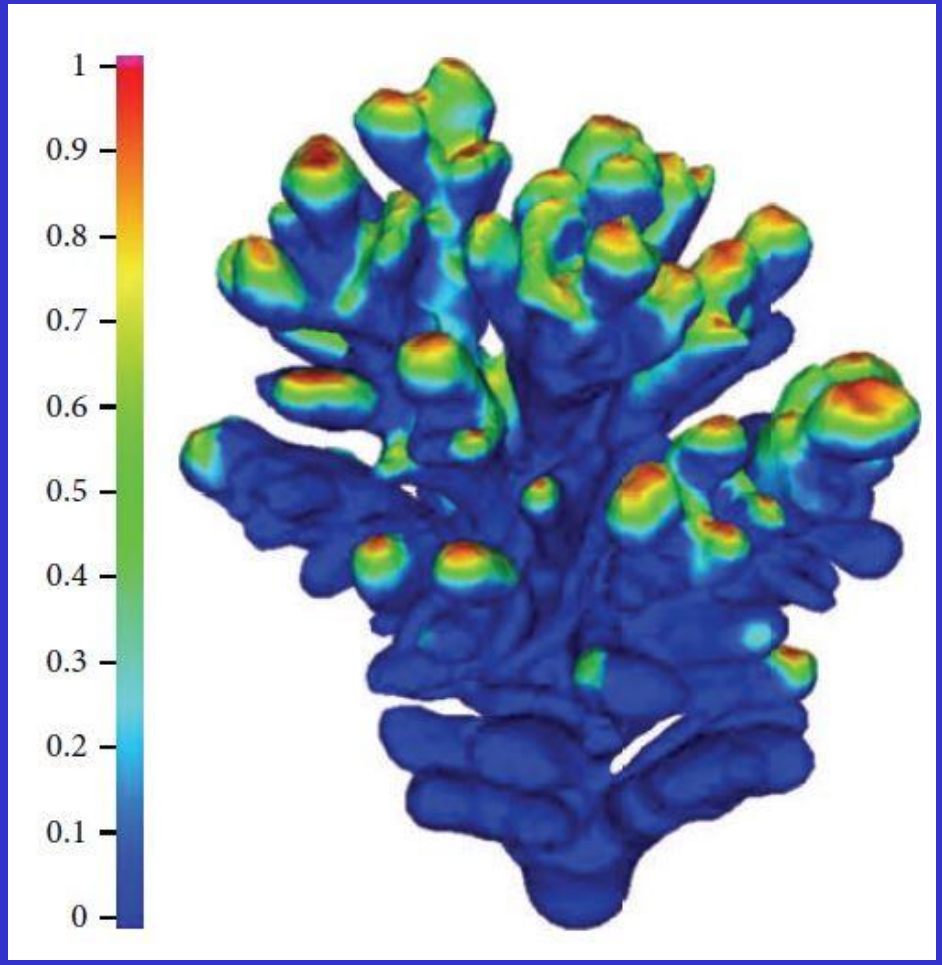
•Where θ is the angle between the mean normal vector in vertex V*i* and the direction to the light source (the vertical in the simulation box). The parameter *ambient* is included to capture ambient light by refections from the environment.

Computing local light intensity, correctiong for shading effects

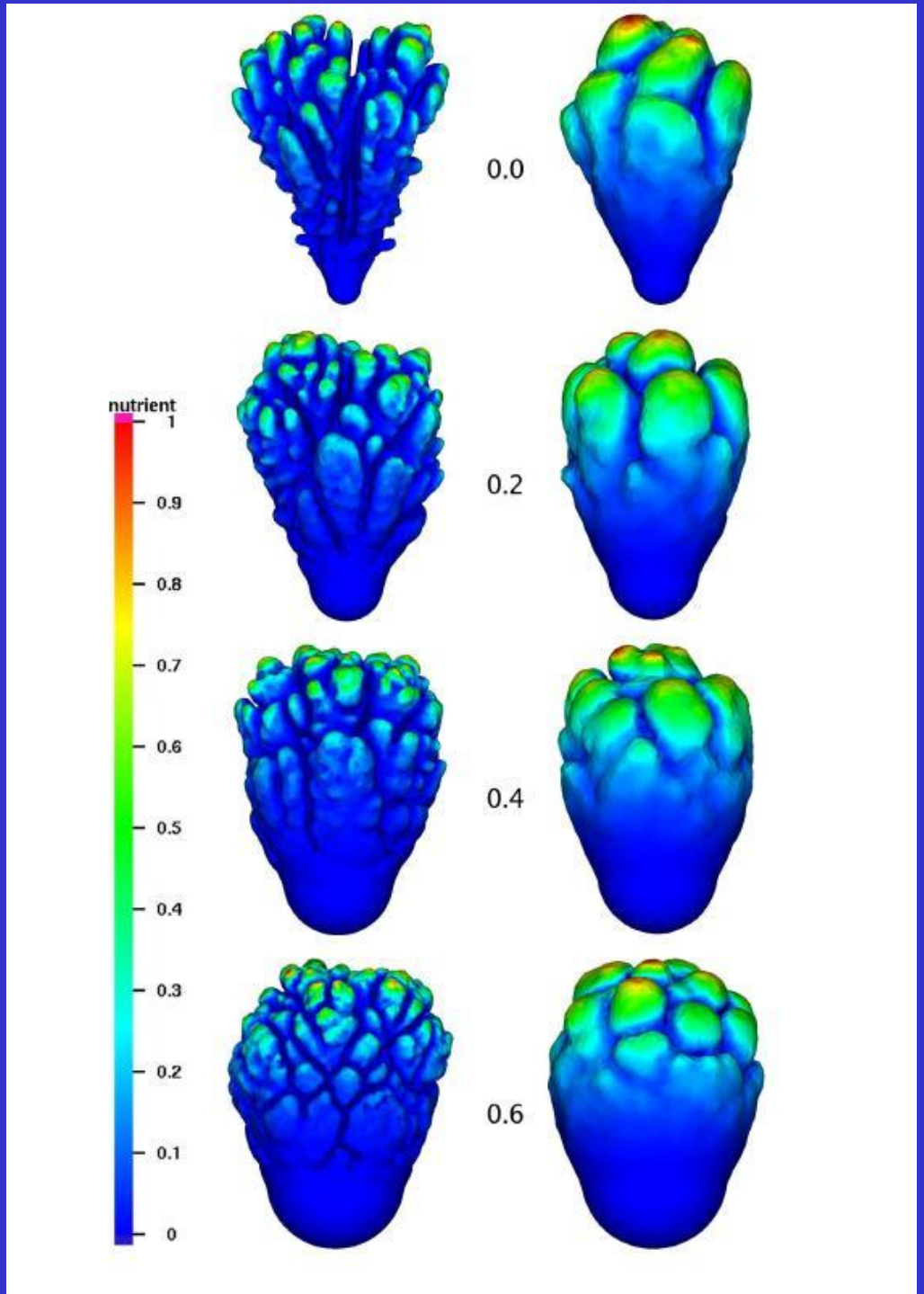
$$c_i^{\text{light}} = c_i^{\text{light}} \times \frac{\#\text{voxels}-\text{illum}-\text{in}-\text{triangles}-\text{surrounding}-i}{\#\text{voxels}-\text{total}-\text{in}-\text{triangles}-\text{surrounding}-i}.$$

- In the previous equation there is no shading of triangles included; to correct for shading effects by occlusion of other parts of the simulated object we have used an algorithm based on volume rendering techniques in which the object is represented in a three dimensional lattice

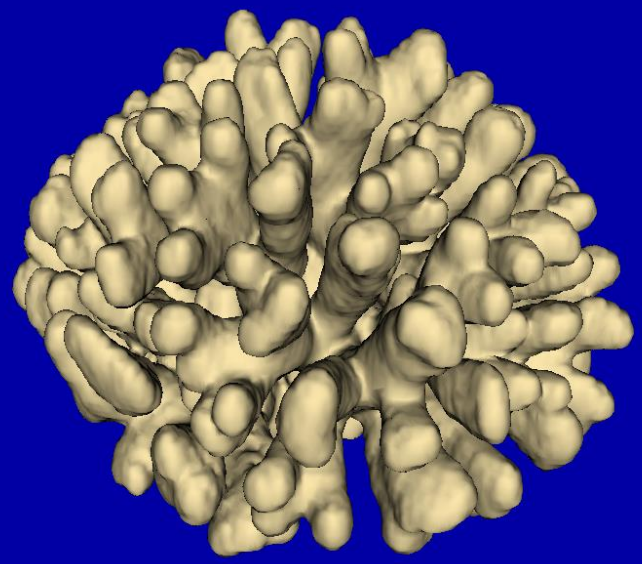
Computing light intensities in complex-shaped branching object

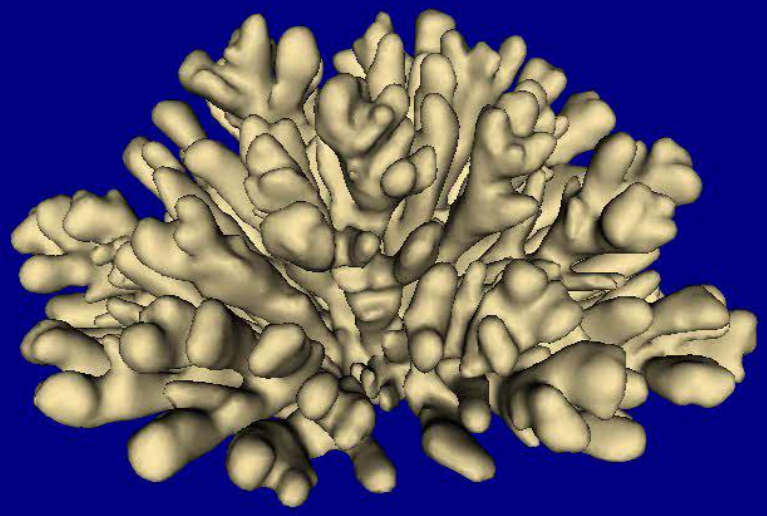


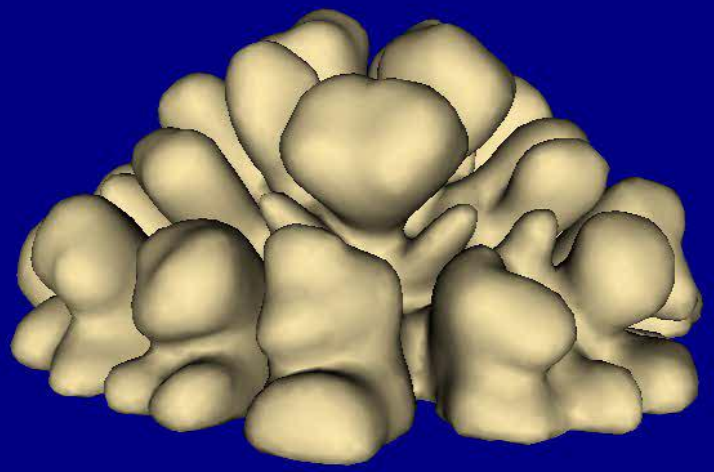
Diffusion limited growth + influence local light intensity: from top to bottom influence of light (alpha parameter) is increased



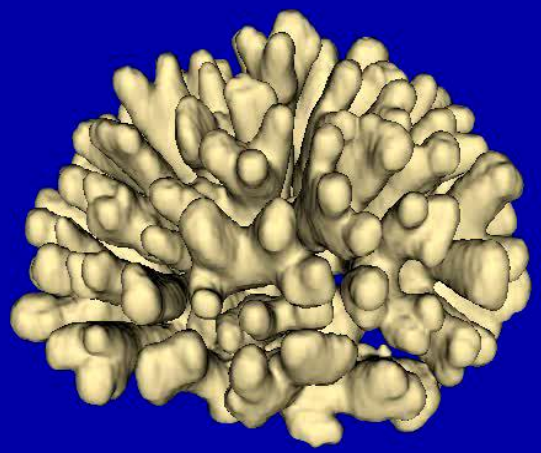
Turing test
Accretive growth model (diffusion limited conditions)
(Kaandorp et al., Proc. Roy. Soc. Lond. B, 2005)

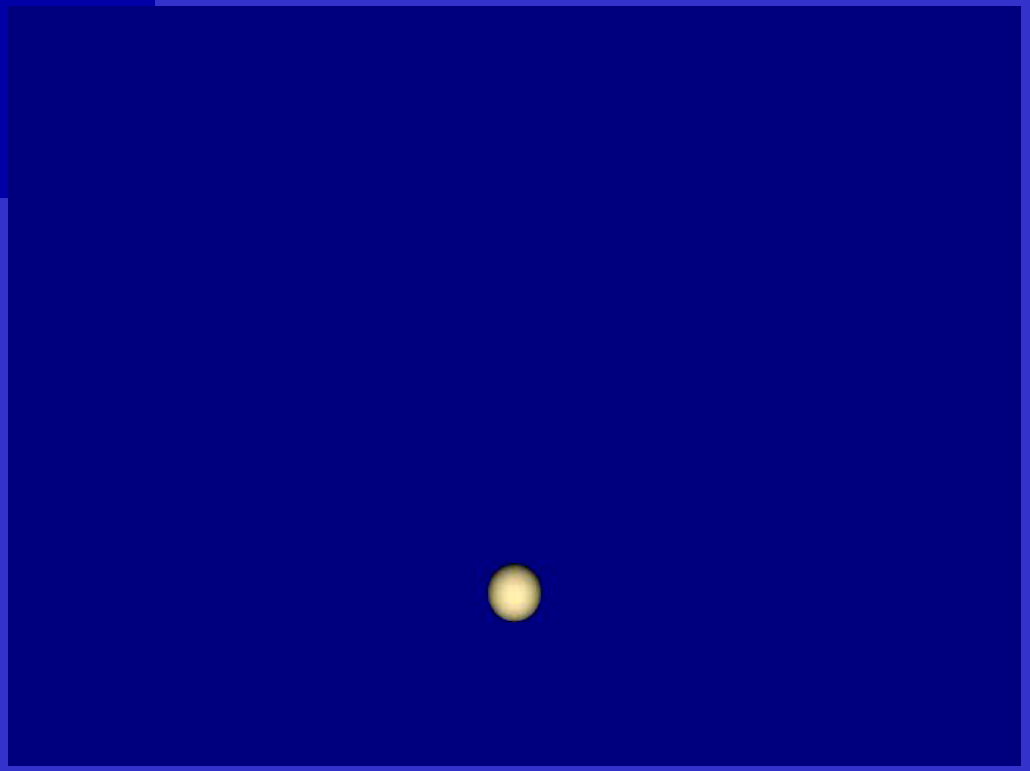




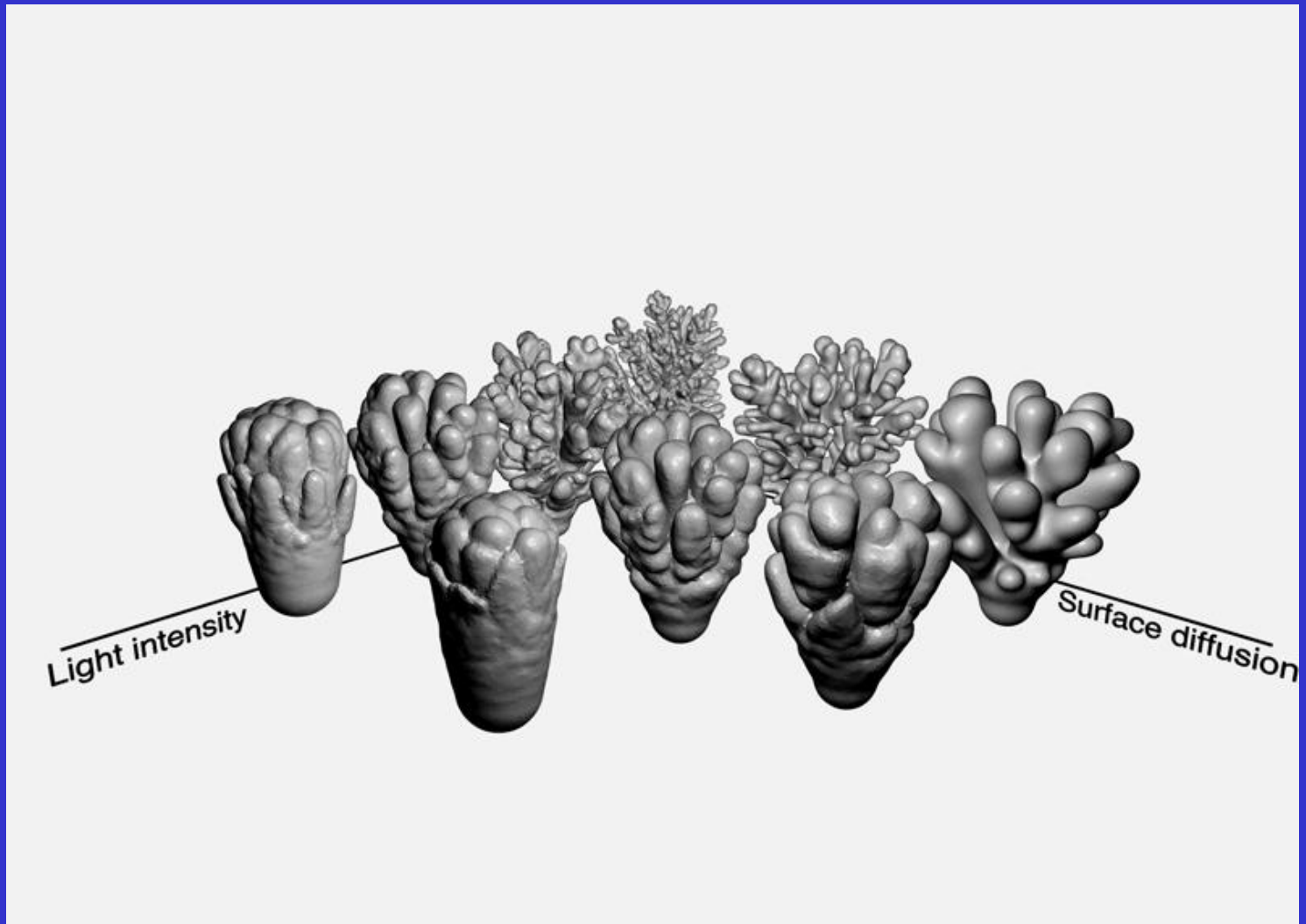


Turing test Accretive growth model

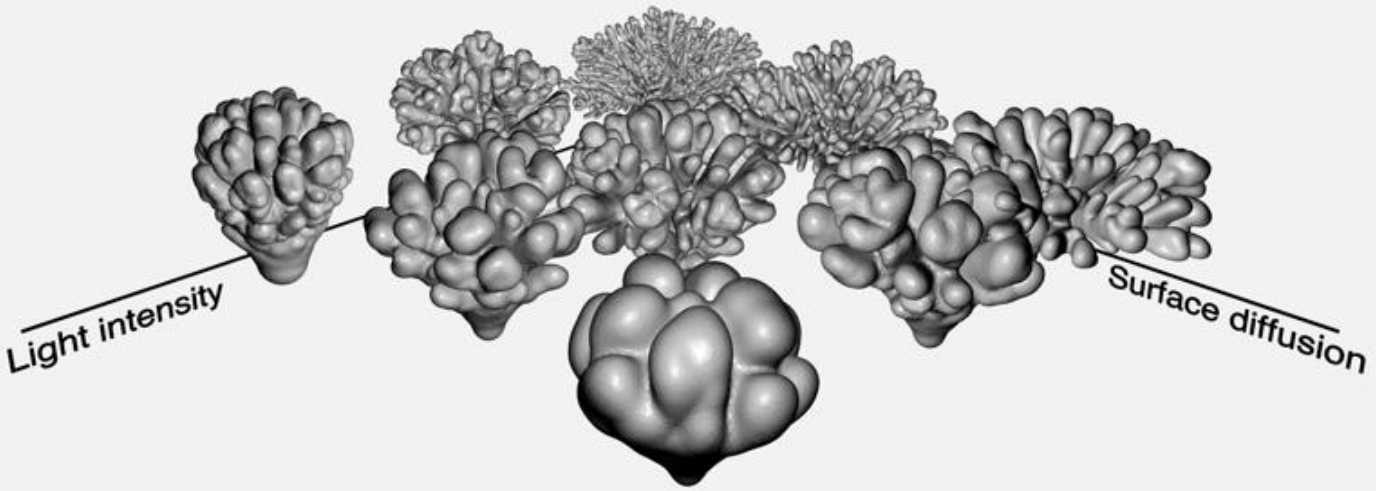




Simulated Morphospace (Filatov et al, Proc Roy Soc B, 2010)



Simulated Morphospace II



Modelling flow

II Hydrodynamics

$$\frac{\partial \rho}{\partial t} + \nabla \cdot \rho U = 0$$

$$\frac{\partial U}{\partial t} = -(U \cdot \nabla)U - \frac{1}{\rho}\nabla P + \nu \nabla^2 U$$

The first equation expresses the conservation of mass and the second one the conservation of momentum, where ρ represents the mass density, t the time, U the flow velocity, P the pressure, and ν the kinematic viscosity.

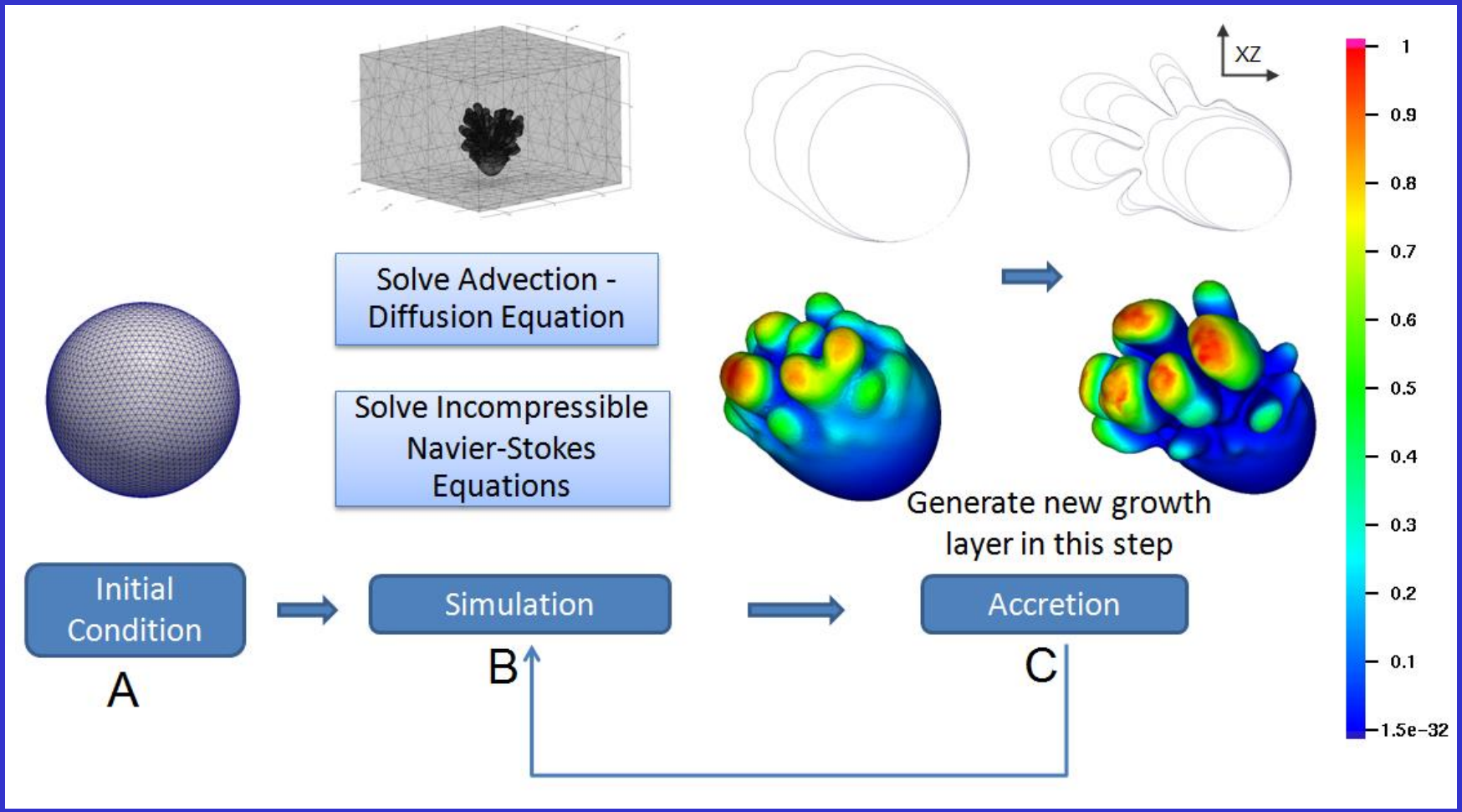
Modelling Advection- diffusion-limited growth

•The thickness of a new layer l, the distance between two successive vertices Vi and Vi+1, is computed by using the growth function:

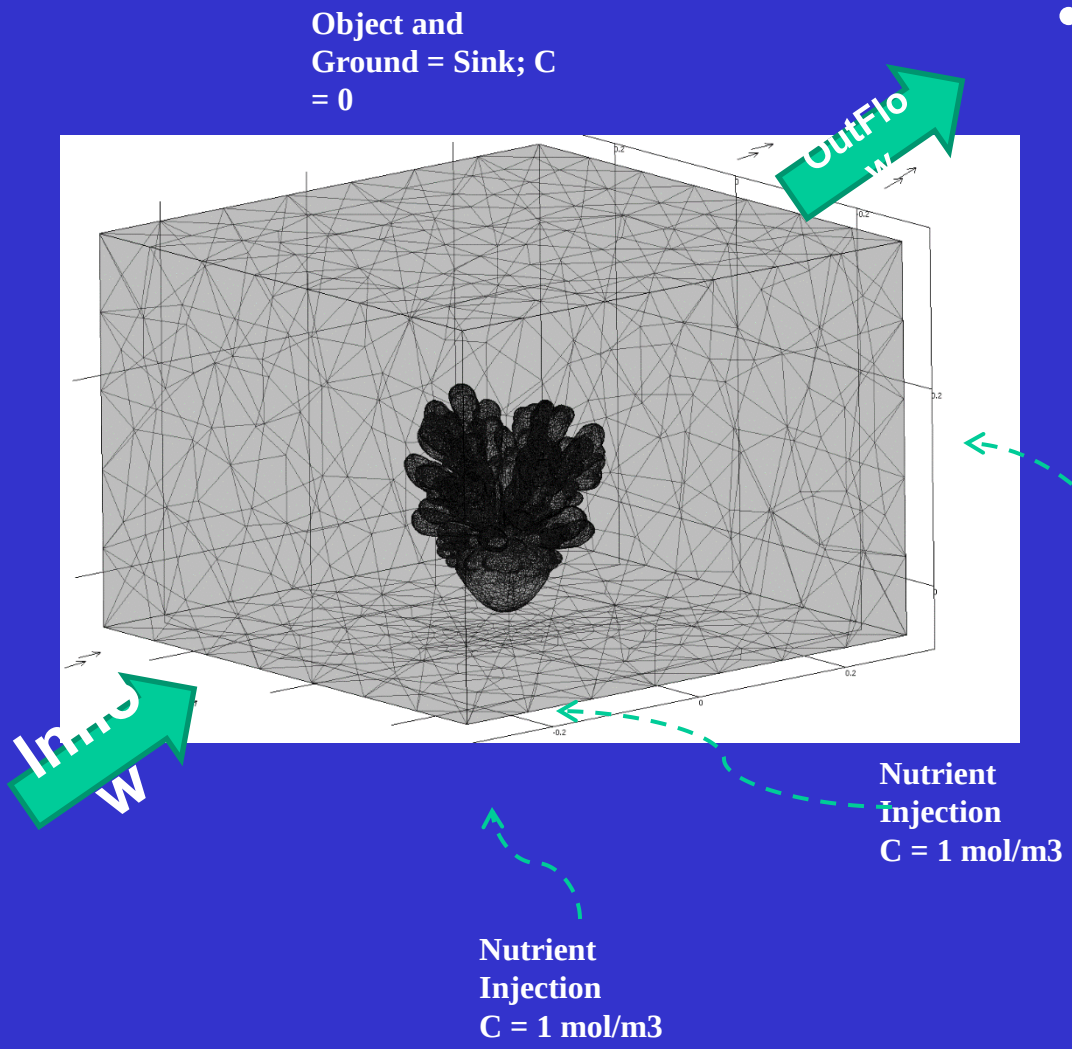
$$l = \vec{n} c_i^{\text{total}} s,$$

•where $\vec{n}$ is the average normal vector in vertex V and c_i^{total} the amount of absorbed simulated nutrients and s is the maximal thickness of the growth layer.

Modelling Coral Growth



Simulation Domain



- A finite-element mesh was constructed by generating a simulation box with dimensions 60 cm in *x* and *y* direction and 40 cm in *z* direction (the height of the simulation box) . The spherical object with an initial diameter of 6 cm was then imported to the simulation box.

- The flow fields around the simulated coral was obtained by solving the incompressible Navier-Stokes (NVS) equations:

$$\rho\frac{\partial \mathbf{u}}{\partial t} - \nabla \cdot [\eta(\nabla \mathbf{u} + (\nabla \mathbf{u})^T)] + \rho(\mathbf{u} \cdot \nabla)\mathbf{u} + \nabla p = \mathbf{F}$$

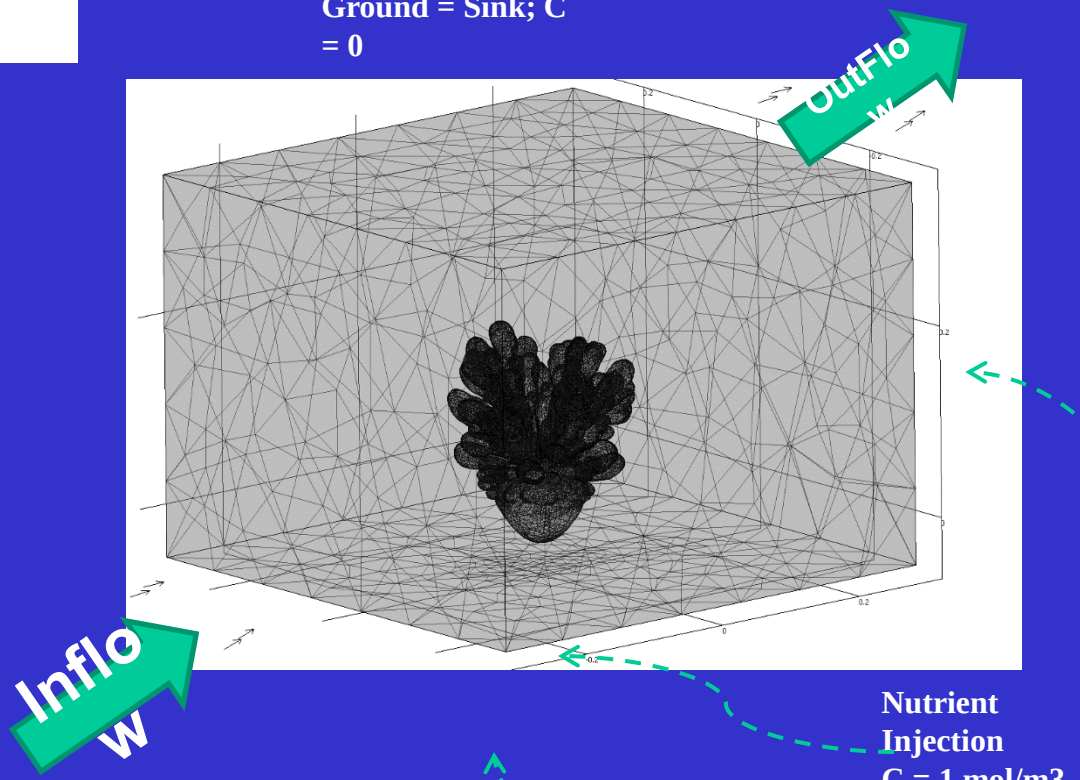
$$\nabla \cdot \mathbf{u} = 0$$

u is the velocity field,
p is the pressure,
ρ is the density,
η is the dynamic viscosity
F is a volume force field such as Gravity

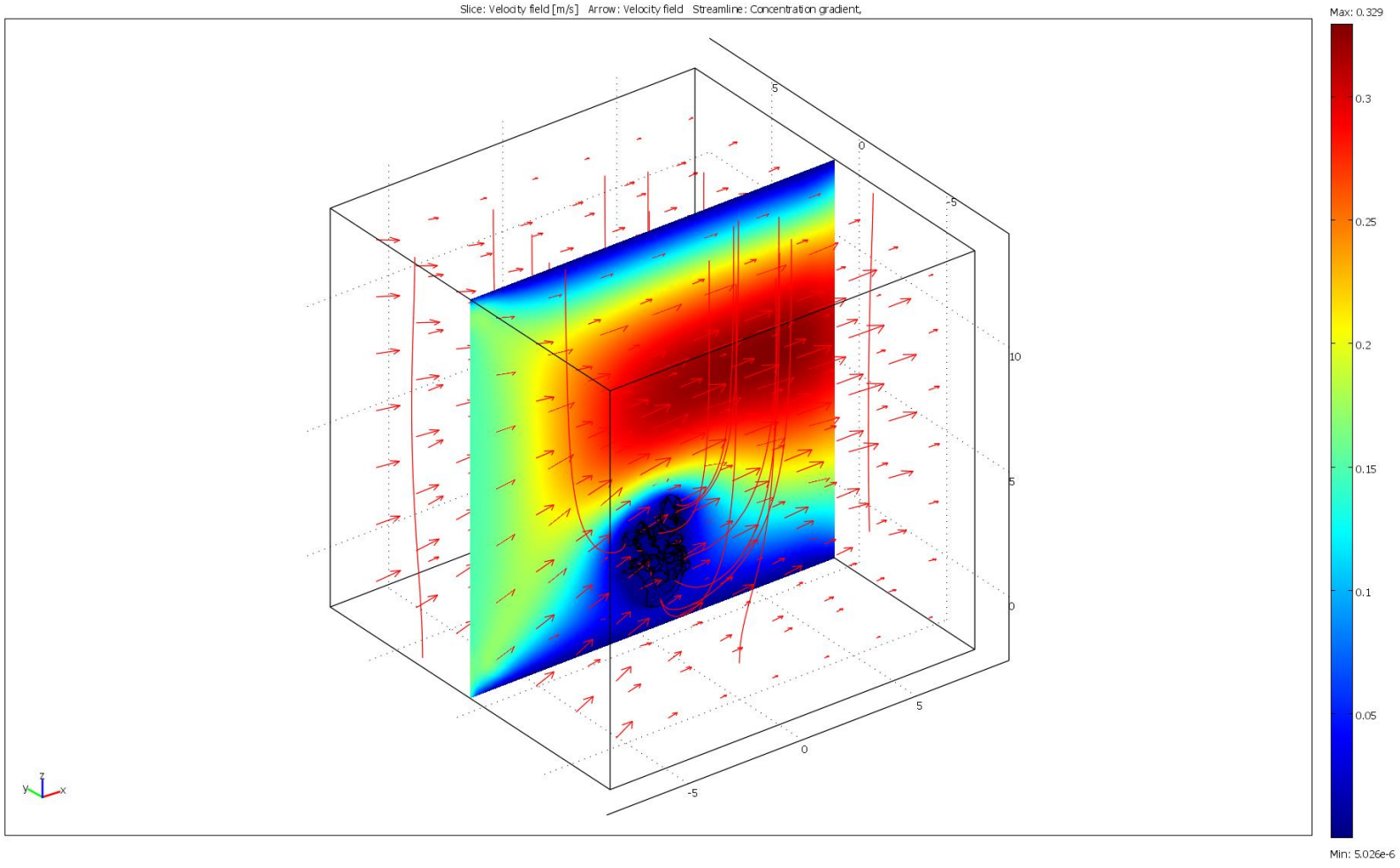
- **The transport of nutrients was obtained by solving Advection Diffusion Equation**

$$\frac{\partial C}{\partial t} + \vec{u} \cdot \nabla C = D\nabla^2 C,$$

u is the advection velocity vector,
D is the diffusion coefficient,
C is a concentration

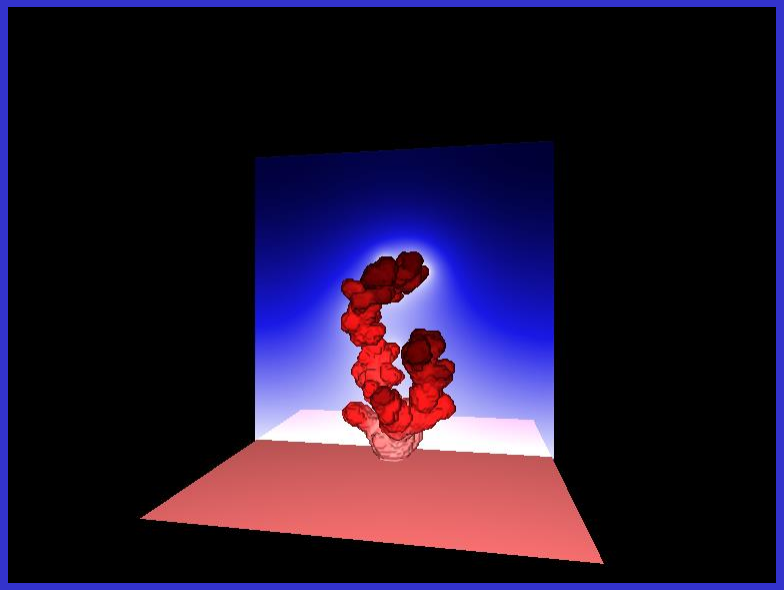


Advection-diffusion simulations (arrow indicates local flow velocity, colour indicated local nutrient concentration)

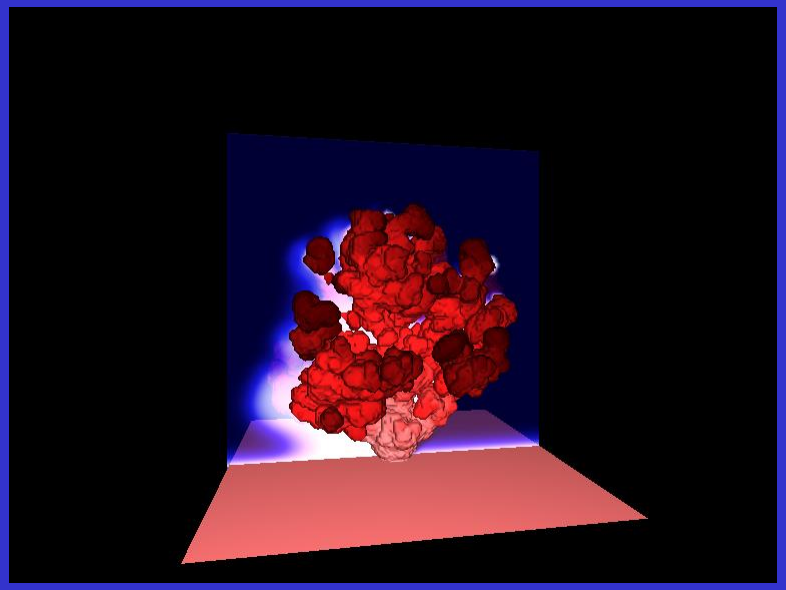


Accretive growth model nutrient distributions

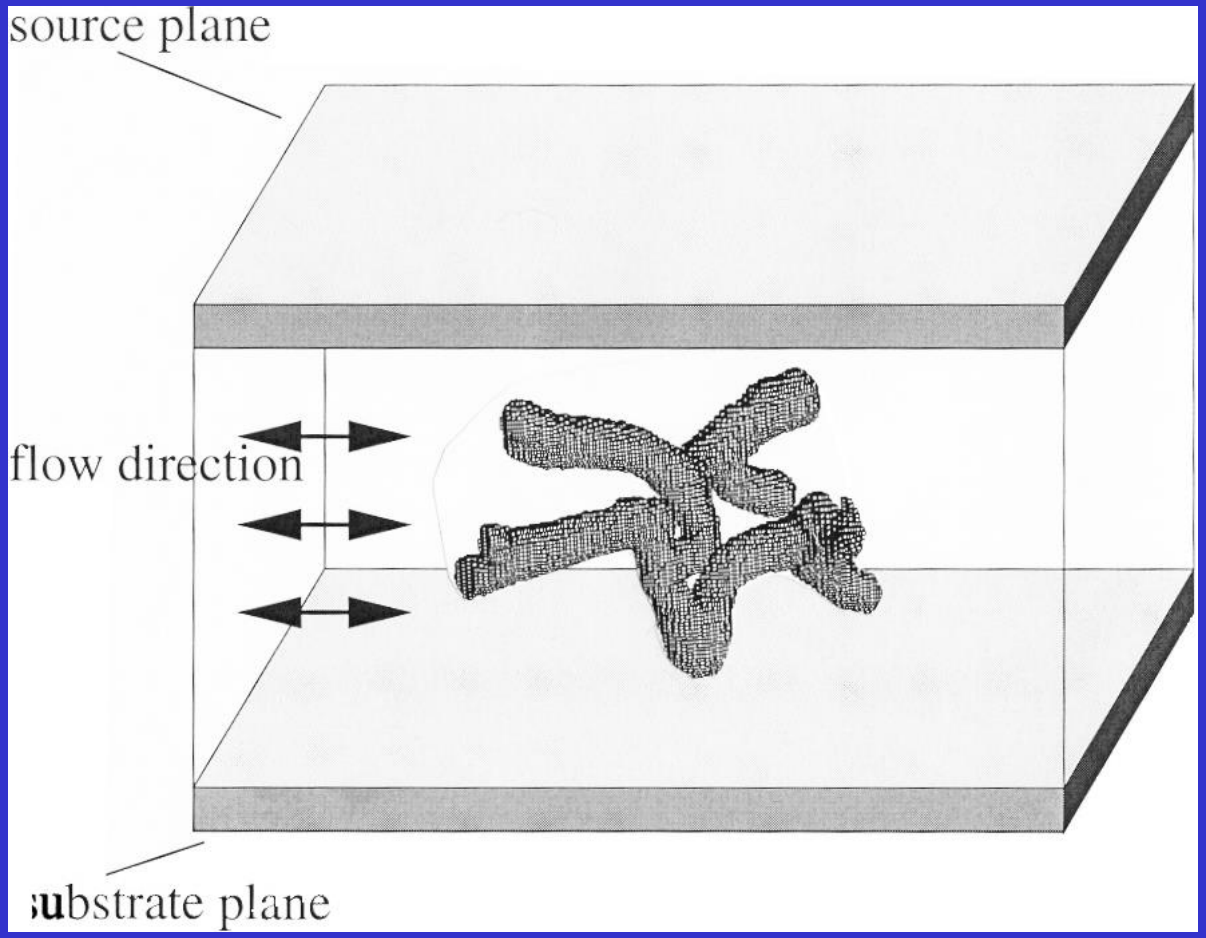
Diffusion limited



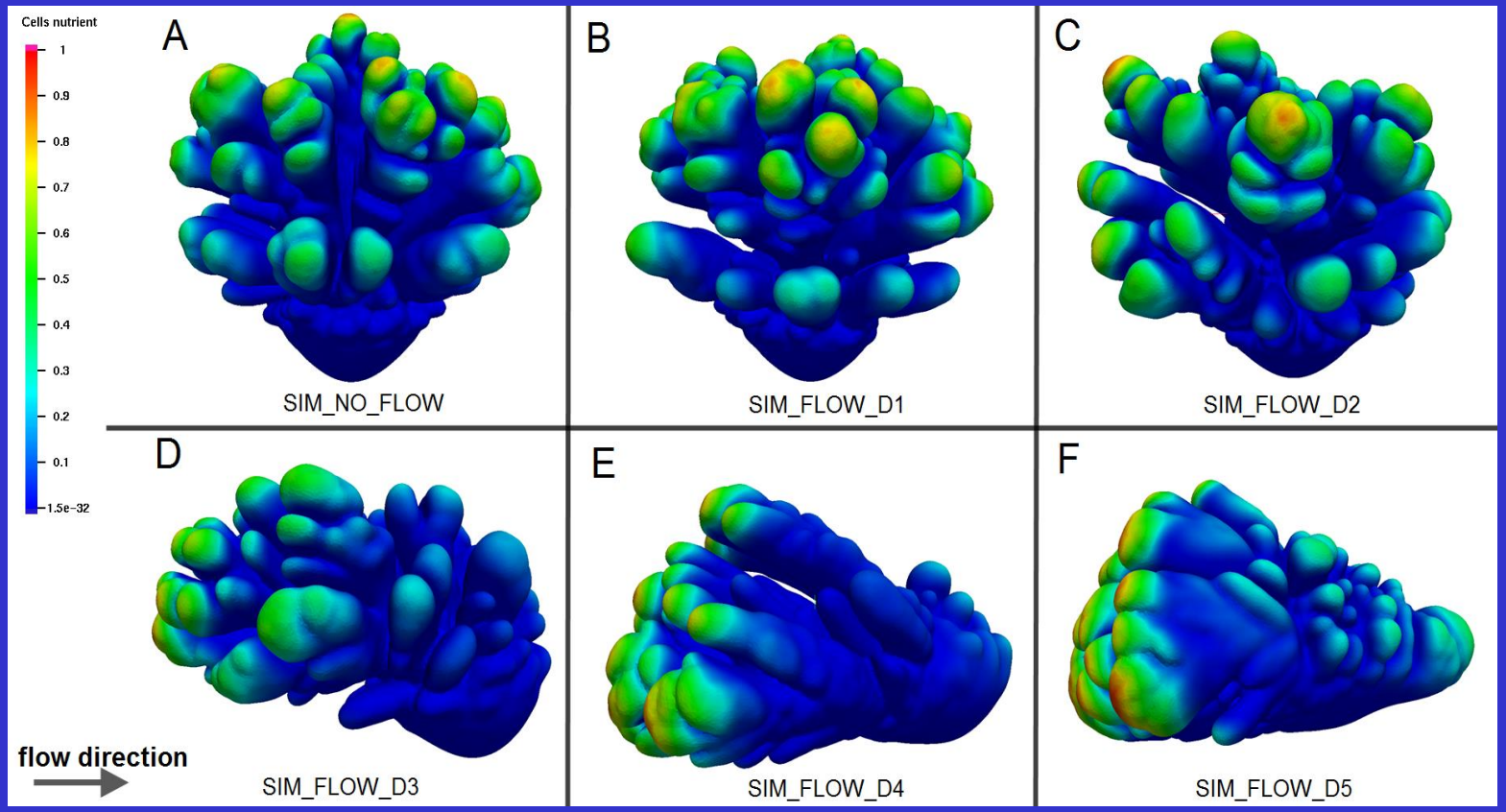
Flow limited



Coupling accretive growth model and advection-diffusion



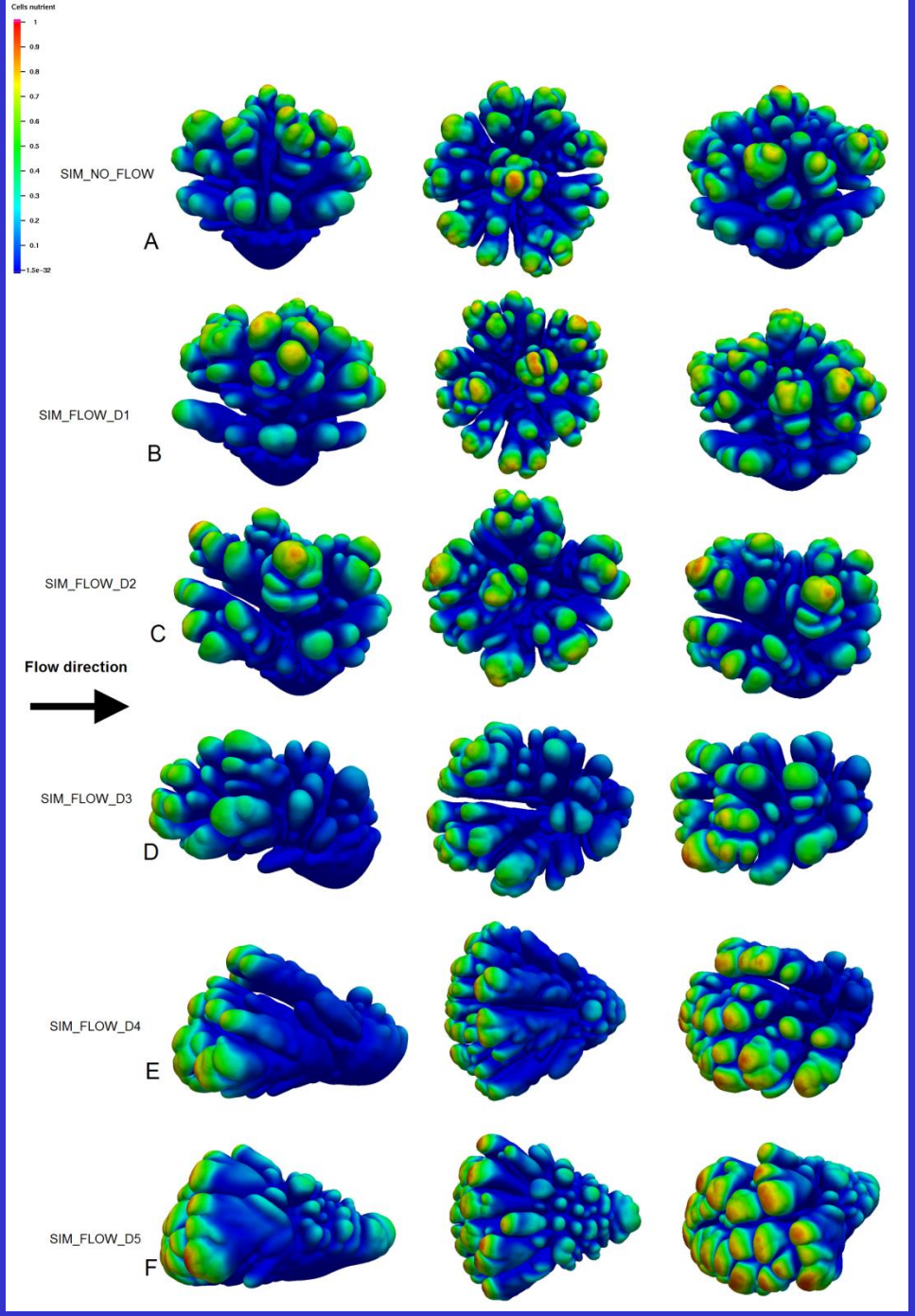
Results (Chindapol et al., Plos Comp. Biol., 2013)

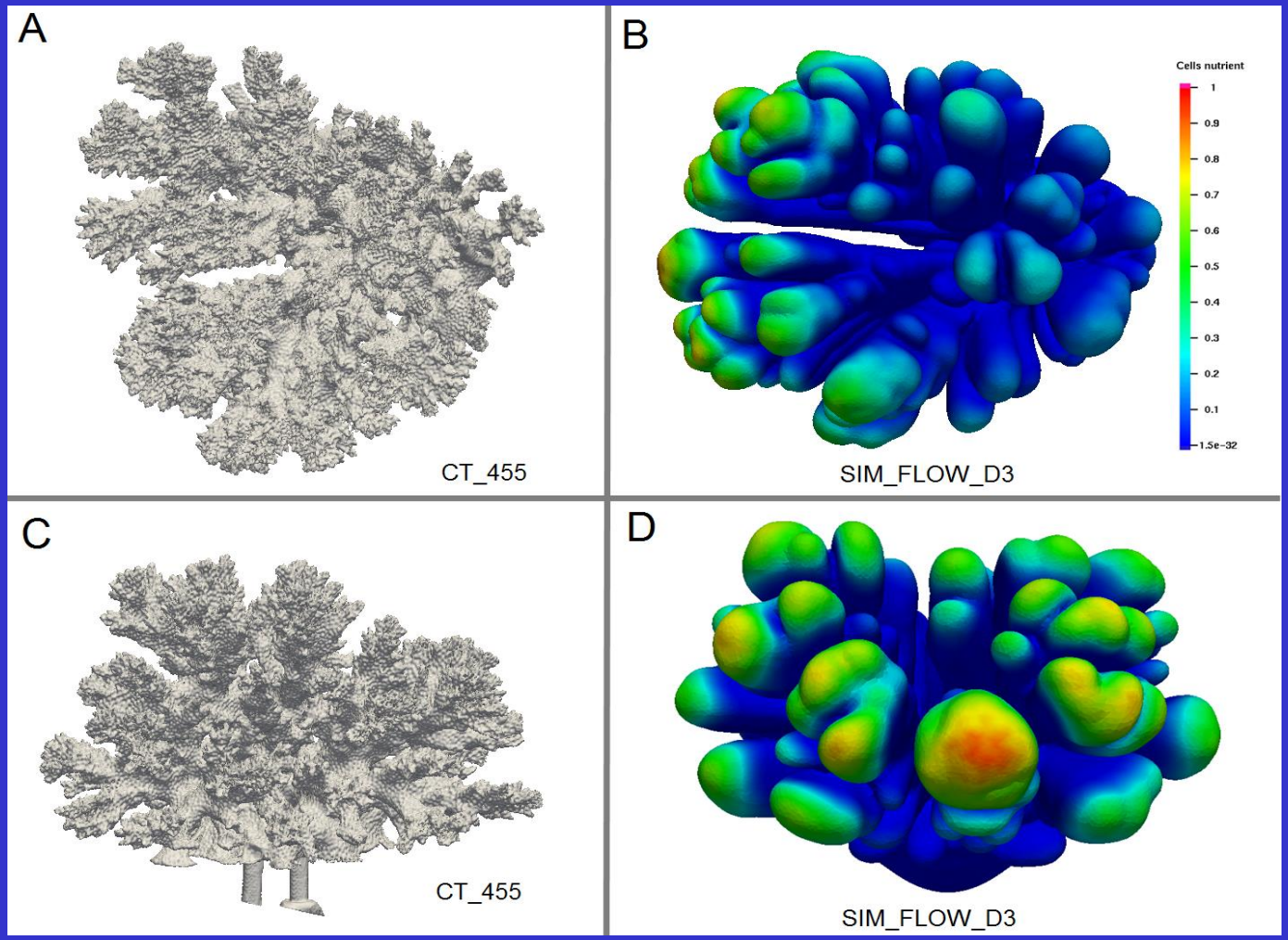


- (A) Simulated coral in a no-flow condition. (B-F) Simulated growth forms from different flow simulations (B) *Pe* =0.34, (C) *Pe* = 3.45, (D) *Pe* = 33.5, (E) *Pe* = 302.89, (F) *Pe* ~ 3000, Arrow indicates flow direction. The labels of the simulated corals are located on the bottom of each figure (See Table 1 for labels).

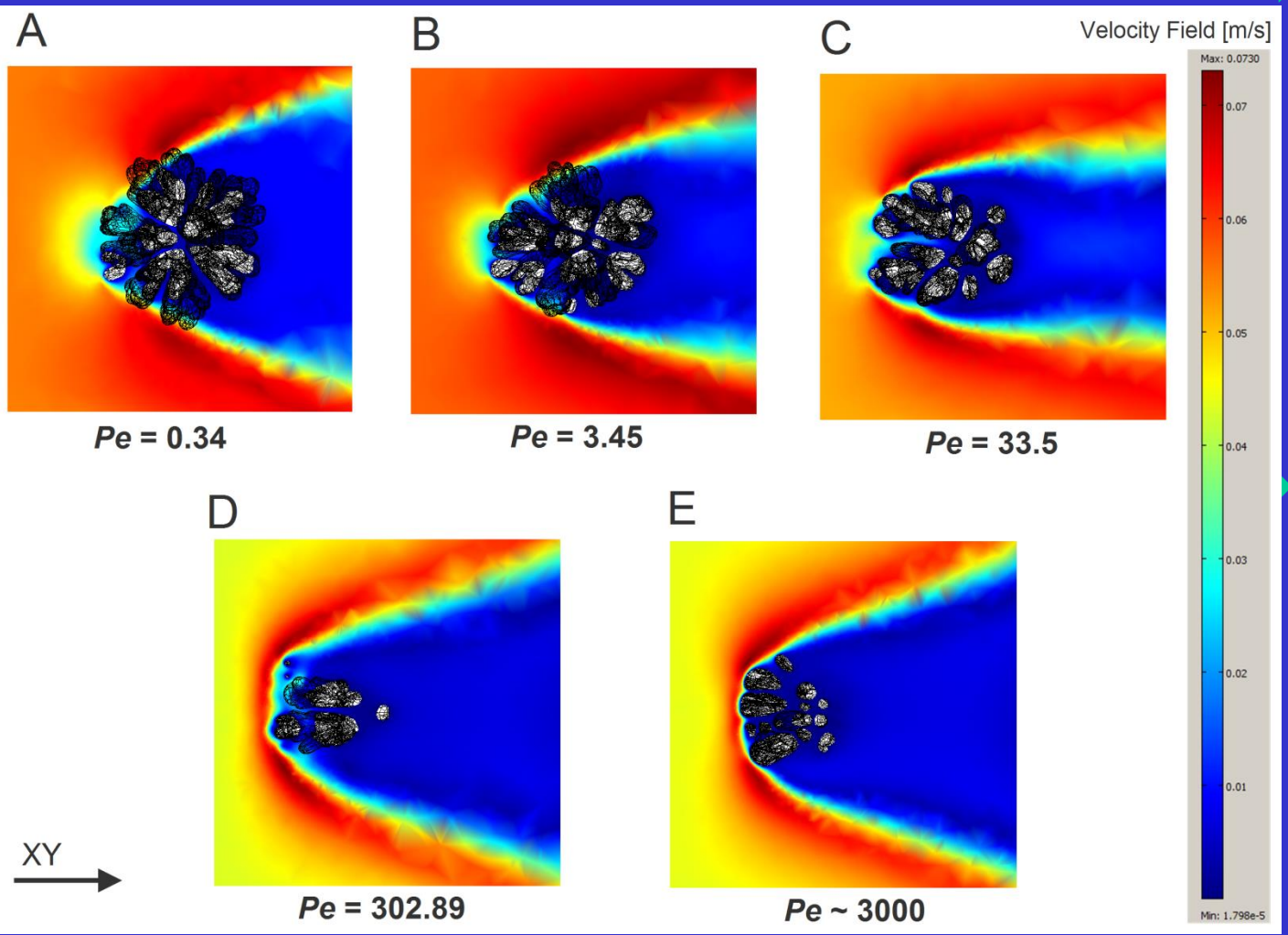
Influence of flow on morphology

We observed that in a range of increasing *Pe* numbers the degree of asymmetry of the branching objects becomes larger. In this range branches tend to be formed in the stream upward direction. While branch formation on the downstream sides is gradually suppressed





- Qualitative comparison between the real (A,C) and simulated coral (B,D *Pe* = 33.5)



z-plane Slices (z=0.07) of the flow pattern around the simulated corals, the slices were taken from the middle part of the colony.

- At higher *Pe* values the simulated growth forms show an asymmetrical branching pattern with a high degree of compactification.
- The gradient in flow velocities in the upstream part of the simulated object becomes steeper for higher *Pe* numbers, leading to a higher degree of absorption o simulated nutrient in the upstream part of the object.

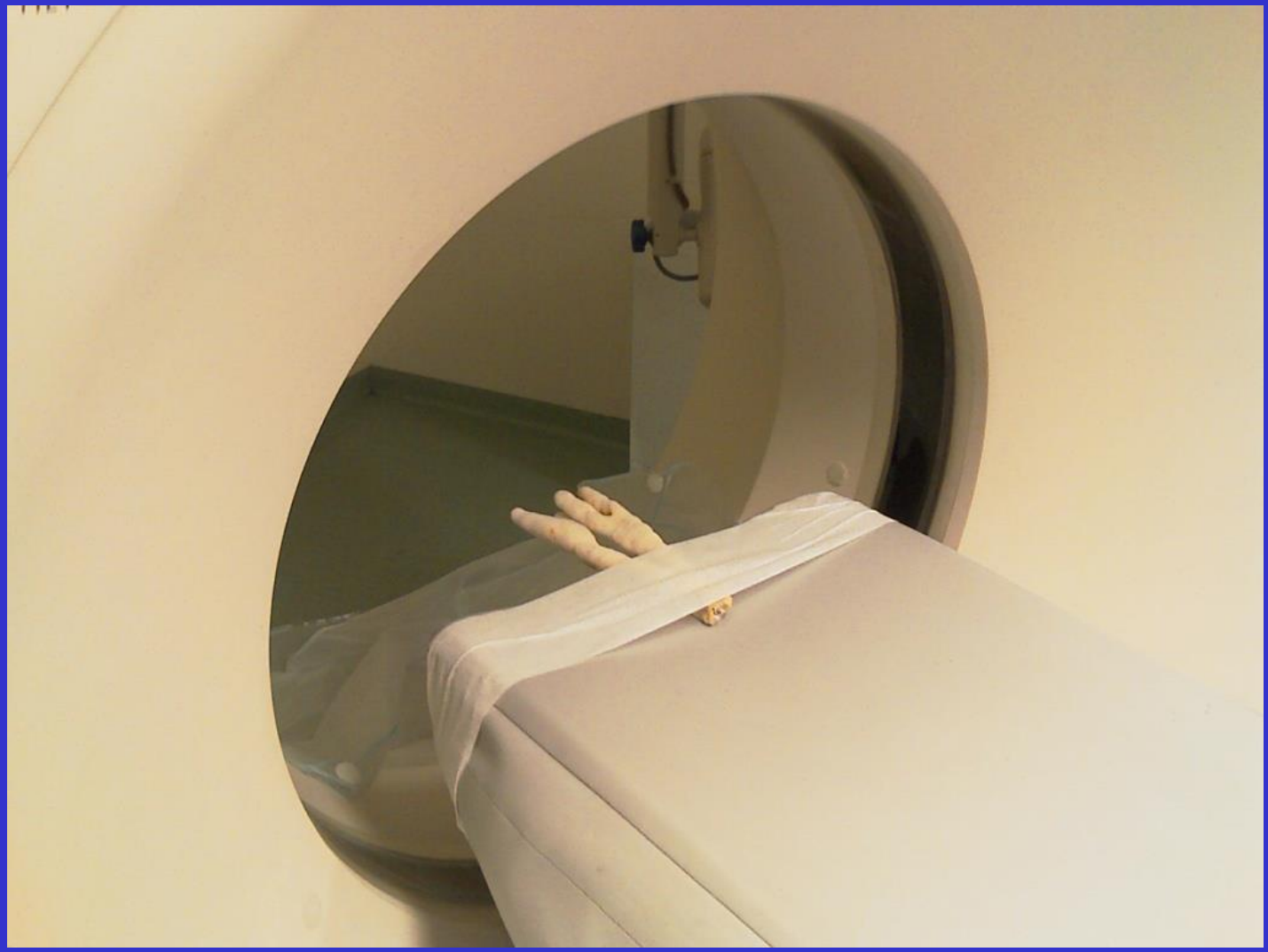
Conclusion

- Increasing the *Pe* number induces the formation of asymmetrical branching growth forms
- In the flow simulations, we have found a decreasing trend of the surface/volume ratio and increased skewedness which is the same as real corals
- Our model shows that in this case there is no gene regulation needed to explain the formation of asymmetrical branching forms
- In reality most scleractinian corals will not be growing under uni-directional flow conditions but will be exposed to a two-phase flow where the flow direction is reversing twice a day because of the tidal movements.

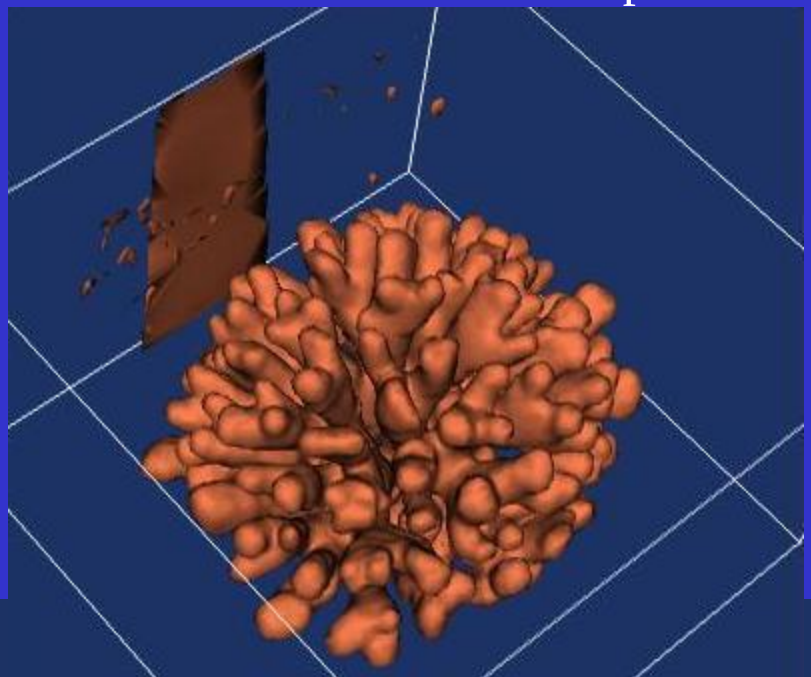
Morphometrics of 3D indeterminate complex-shaped (branching) growth forms

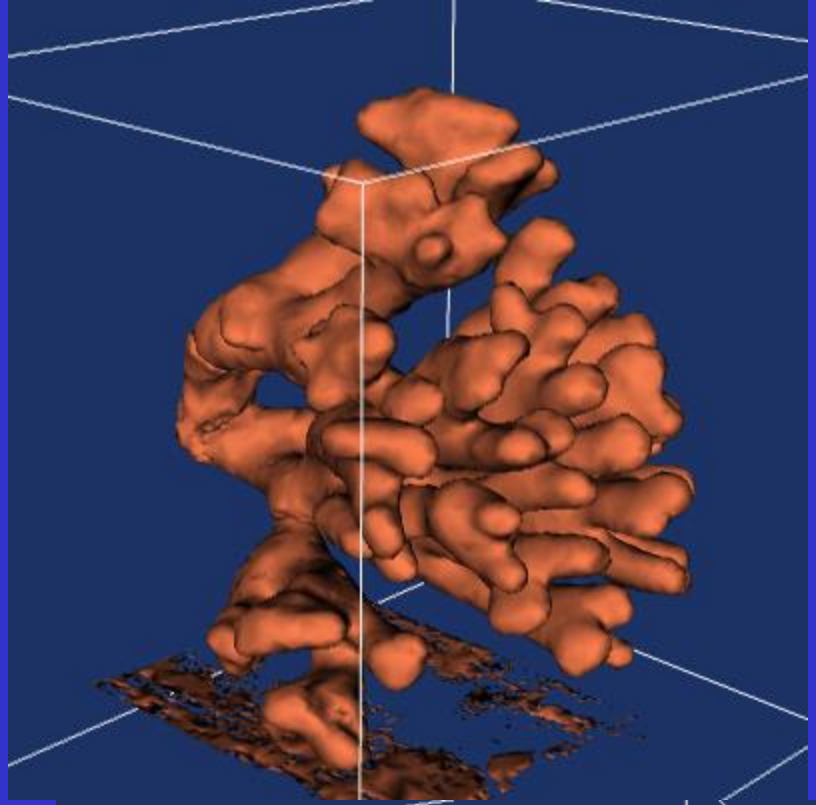
- Global measurements: fractal dimensions,branch ordening, compactness, surface, volume, surface/volume
- Local measurements: local curvature and morphological skeletons in 3D
- Quantifying absorption of nutrient and light in a growth form in 3D simulations

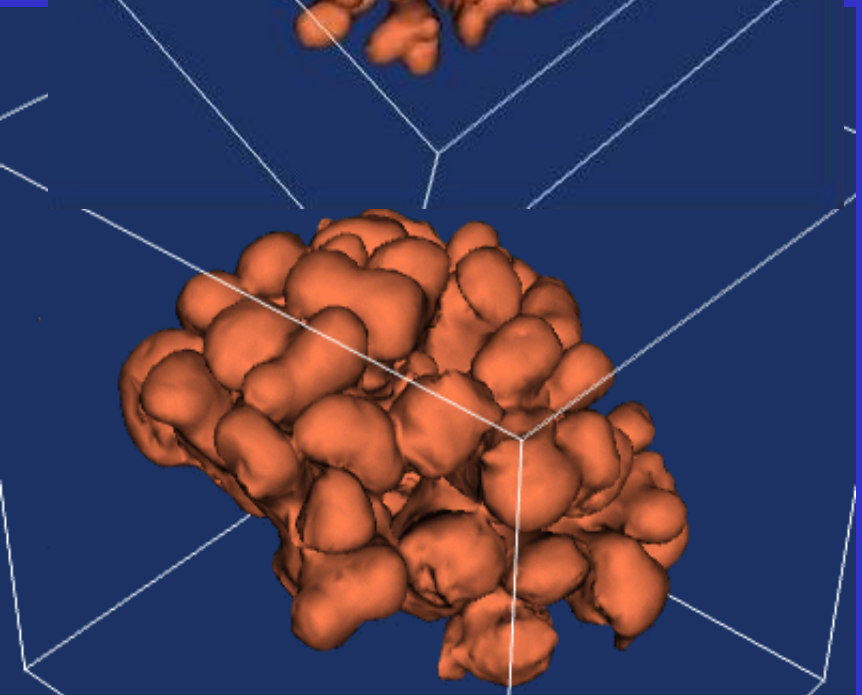
Data acquisition

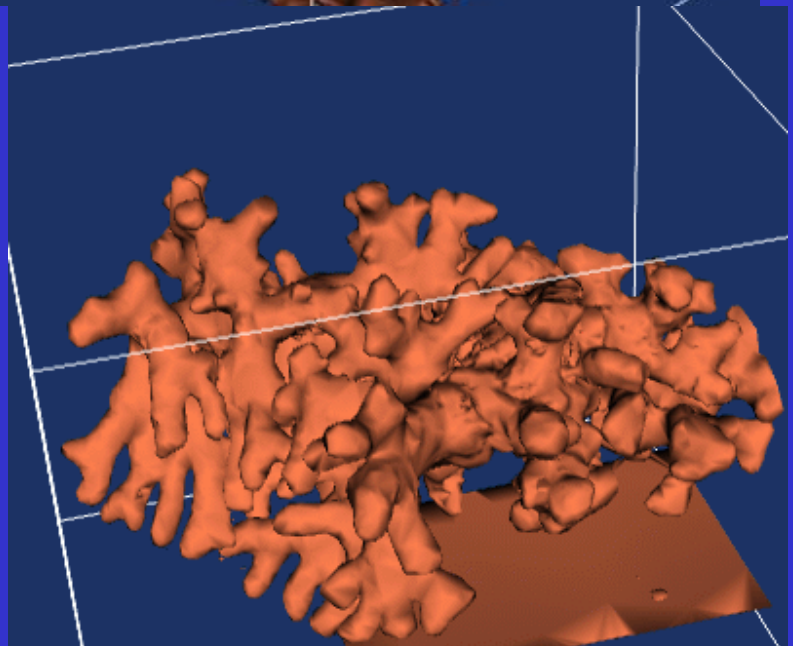


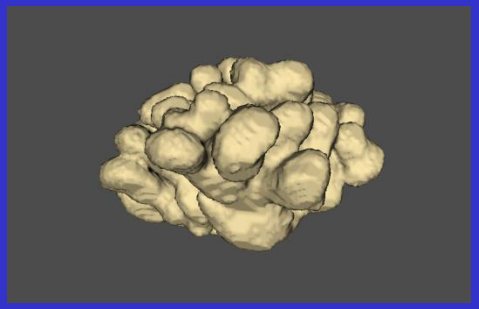
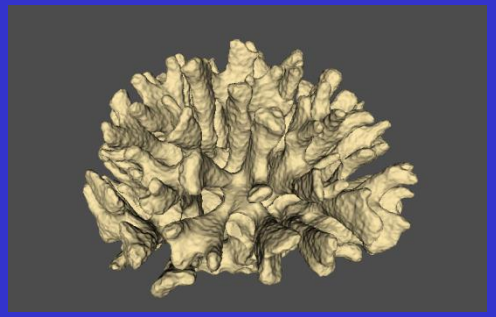
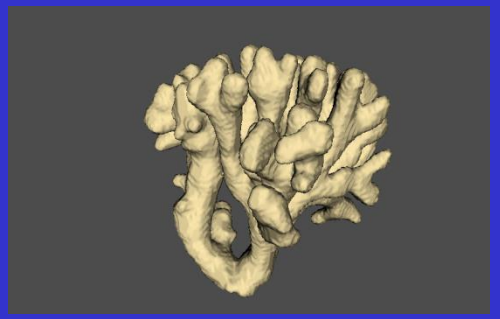
Comparison morphologies in different *Madracis* species



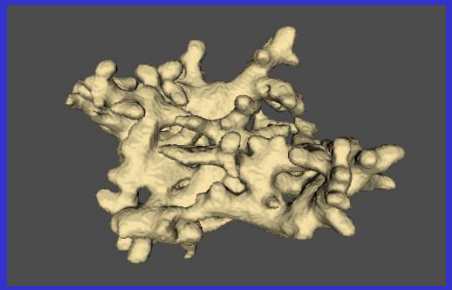






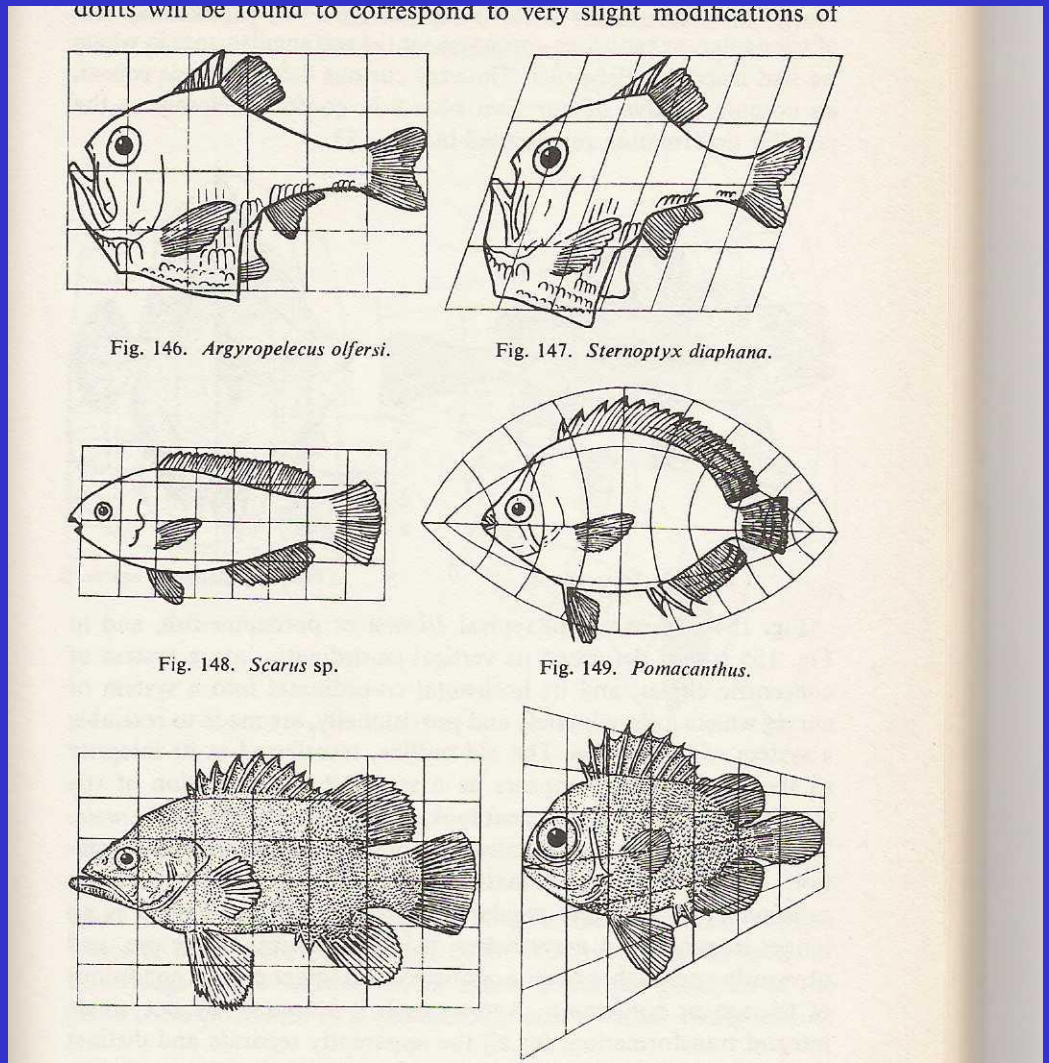
- Samples (CT-scans) of 4 coral species
  - *Madracis decactis*
  - *Madracis mirabilis*
  - *Madracis carmabi*
  - *Madracis formosa*



Data processing

- DICOM image processing (e.g. filtering)
- Iso-surface extraction
- 3D-volume construction

Landmark-based methods in unitary organisms (D'Arcy Thompson, 1917)



points will be found to correspond to very slight modifications of

Fig. 146. *Argyropelecus olfersi.*

Fig. 147. *Sternoptyx diaphana.*

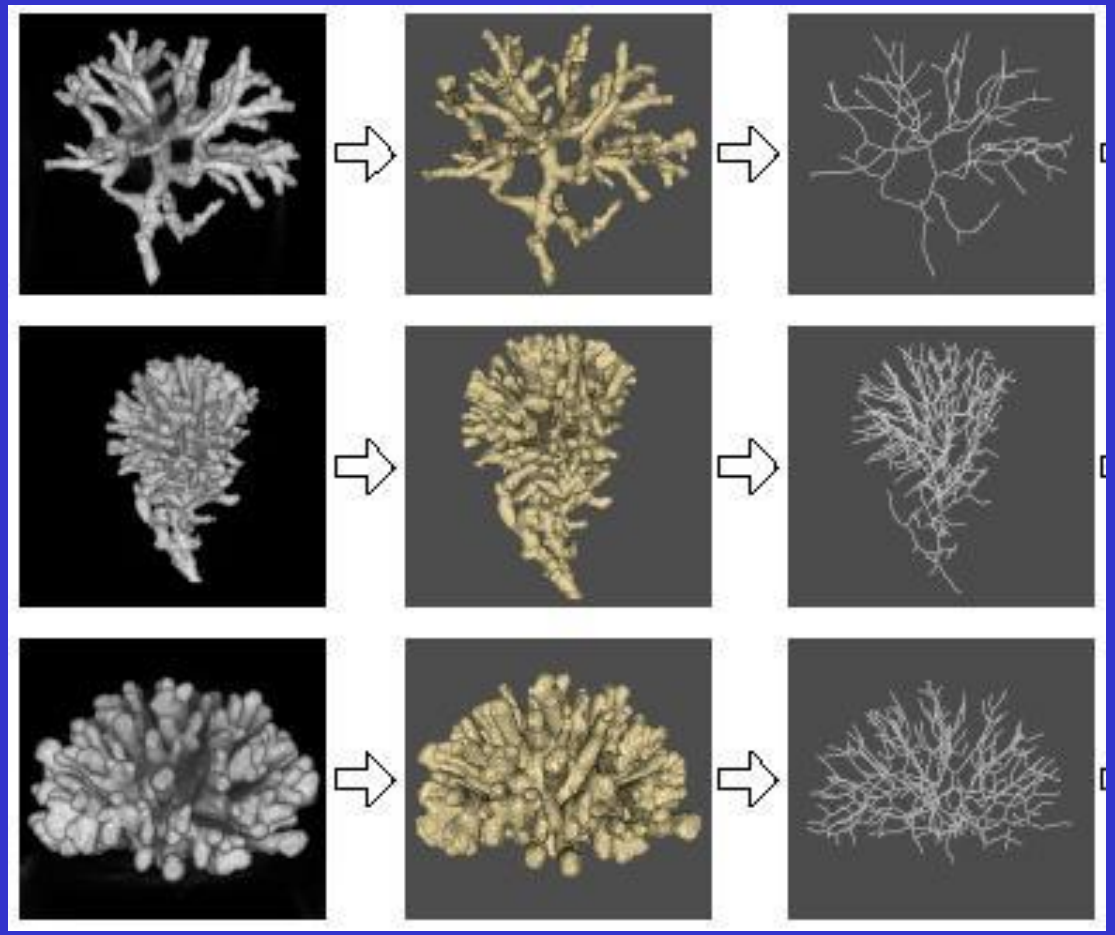
Fig. 148. *Scarus* sp.

Fig. 149. *Pomacanthus.*

Morphometrics of 3D indeterminate (branching) growth forms

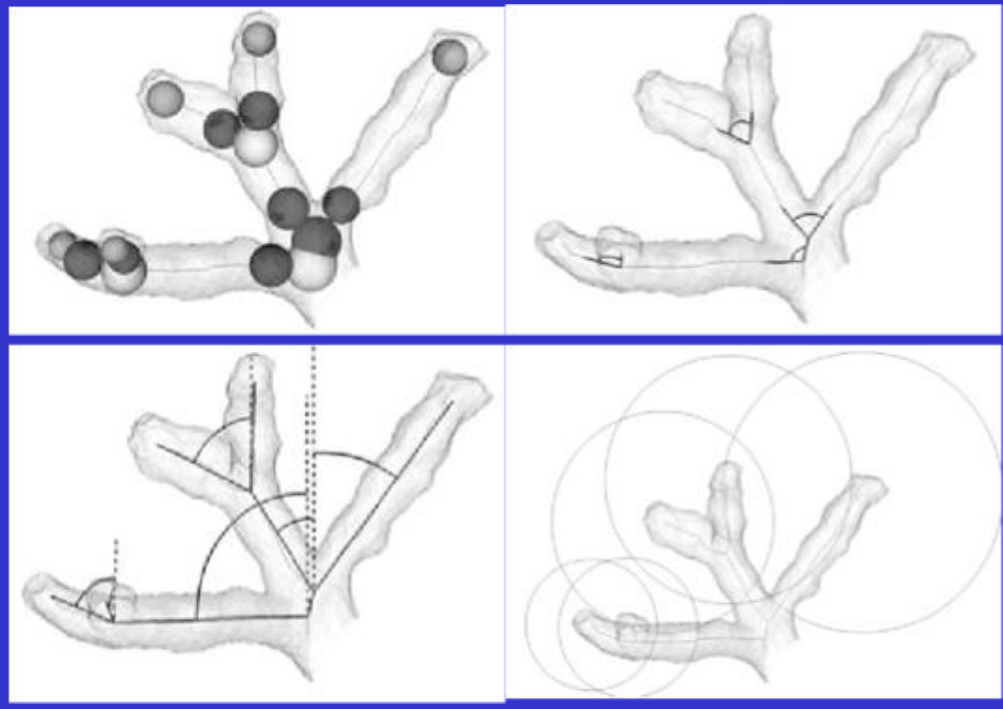
- Global measurements: fractal dimensions,branch ordening, compactness, surface, volume, surface/volume
- Local measurements: local curvature and morphological skeletons in 3D

Morphological Skeleton in a 3D images of the coral *Madracis mirabilis* (K. Kruszynski, J.A. Kaandorp, R. van Liere, Coral Reefs 2007)

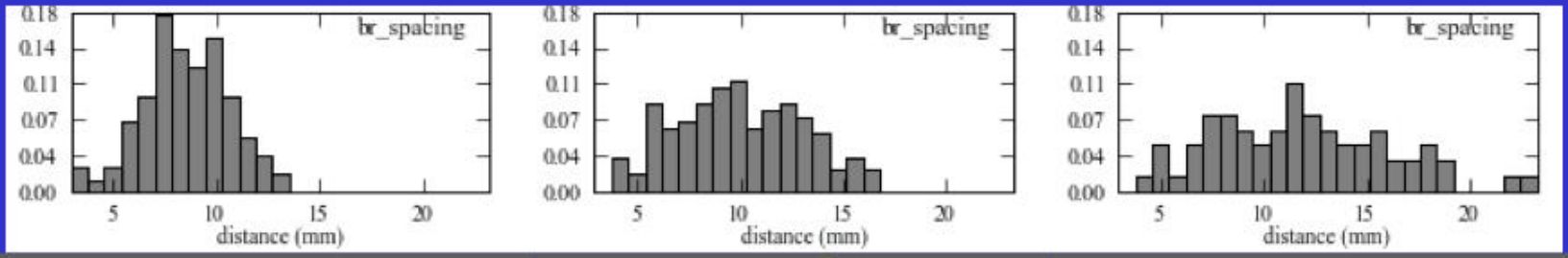


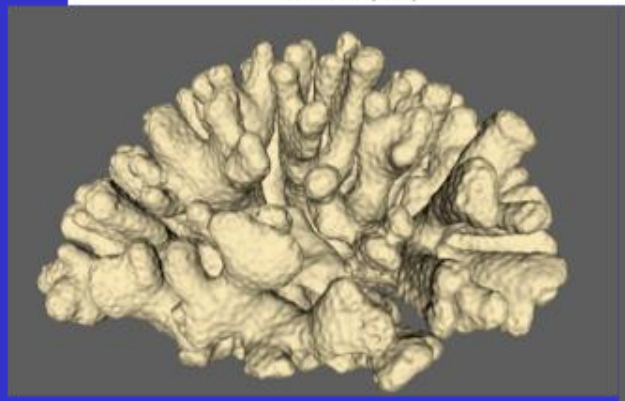
Coral Morphometrics

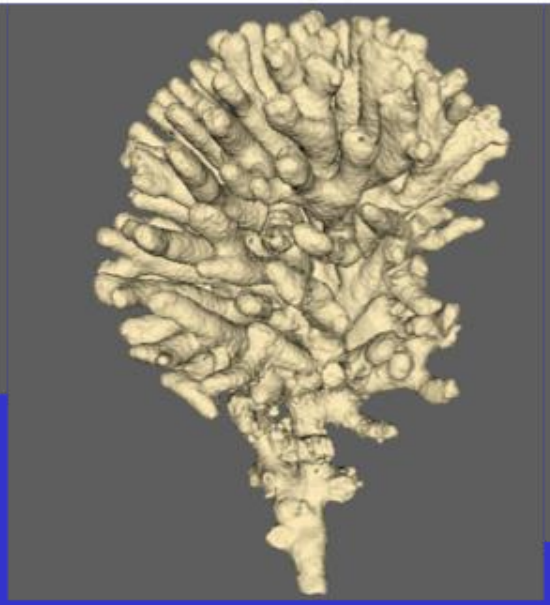
- Branch thickness
- Branching angle
- Branch spacing

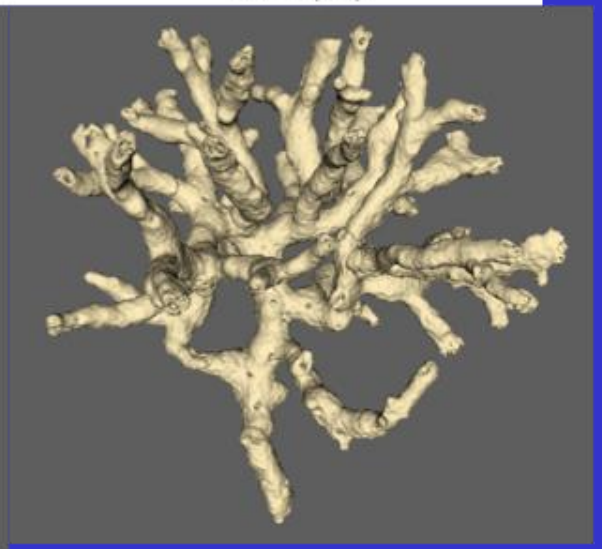


Coral Morphometrics II

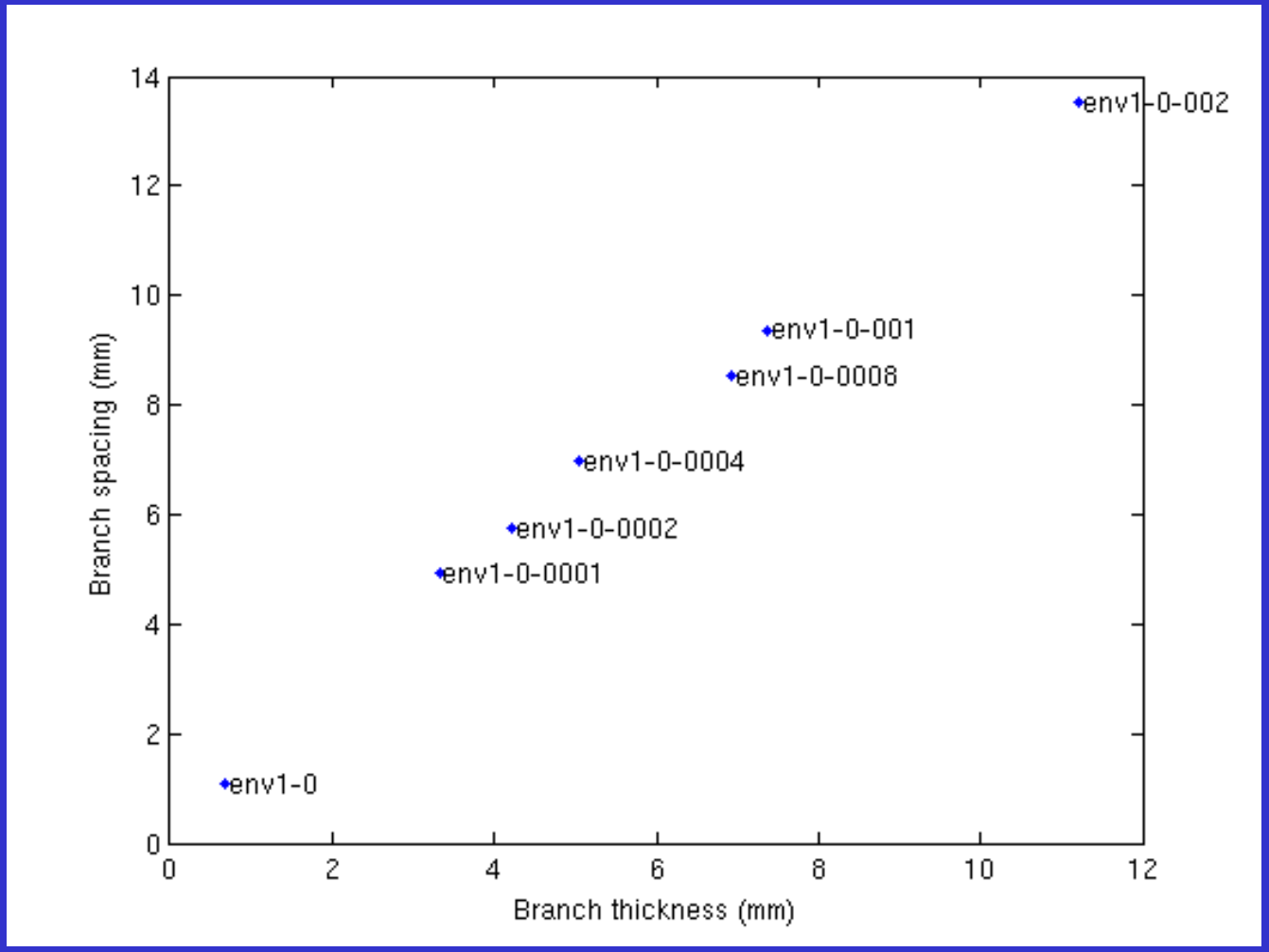




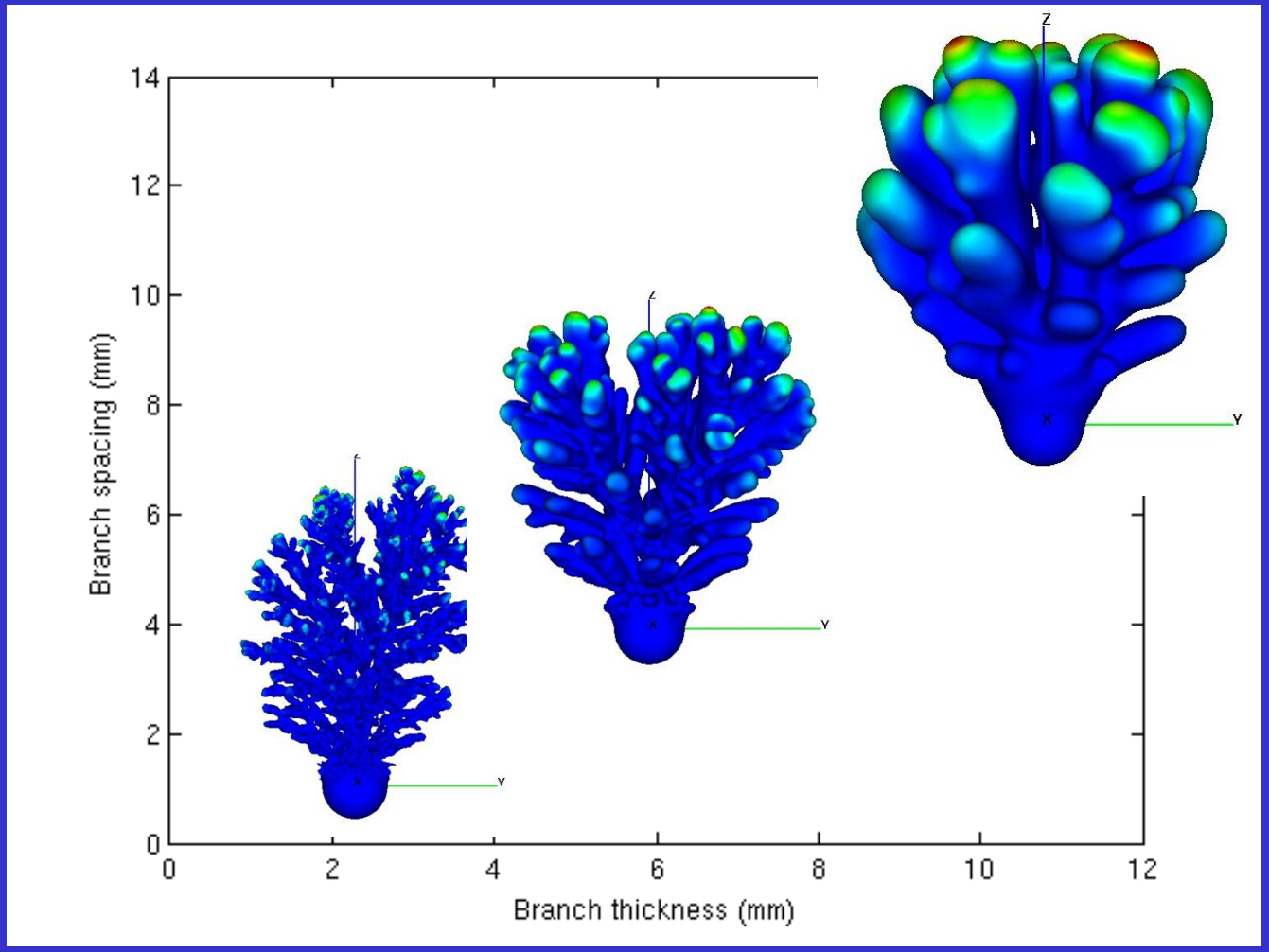




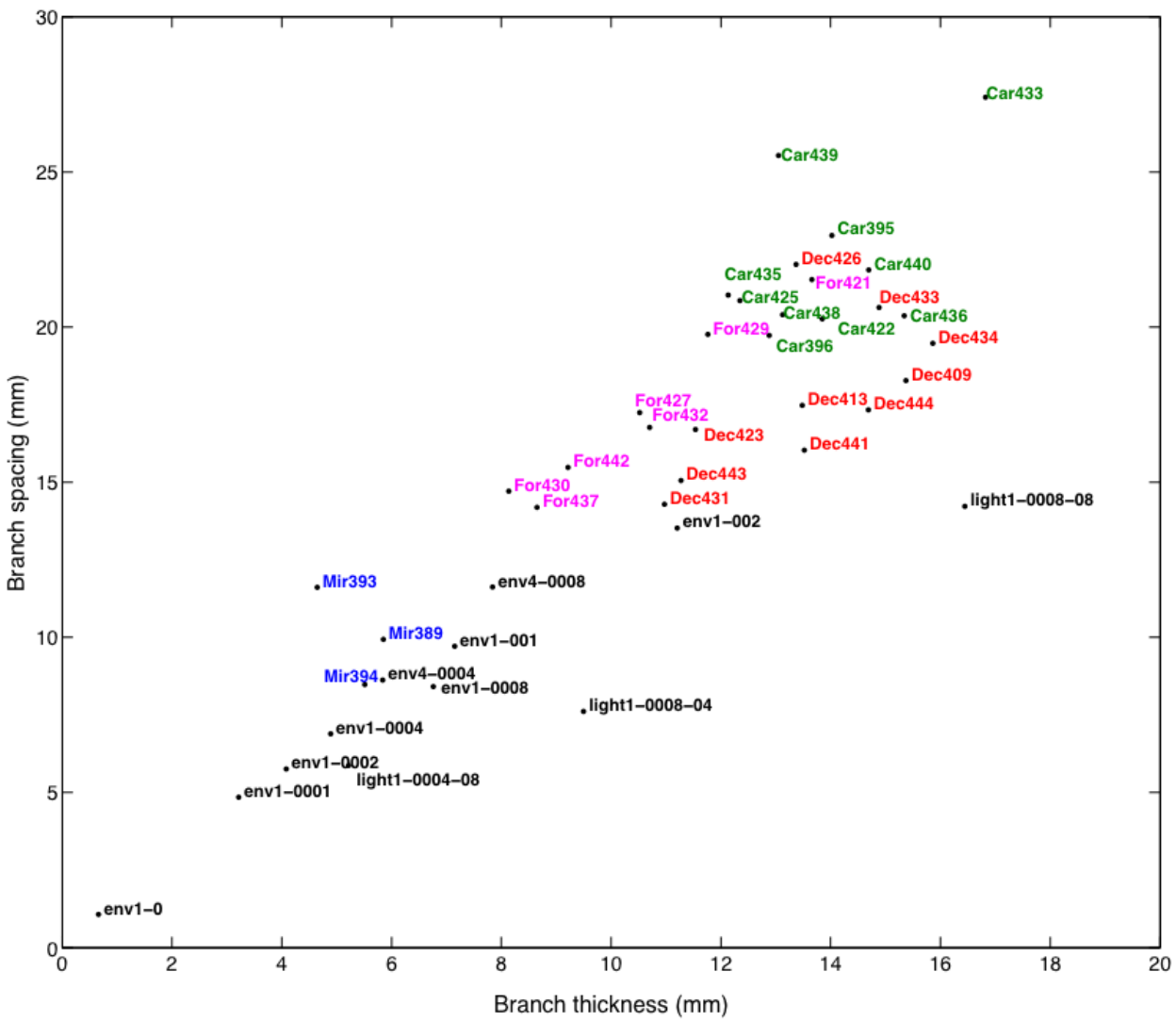
Results I


Branch spacing (mm)
Branch thickness (mm)
0
2
4
6
8
10
12
14
env1-0-002
env1-0-001
env1-0-0008
env1-0-0004
env1-0-0002
env1-0-0001
env1-0

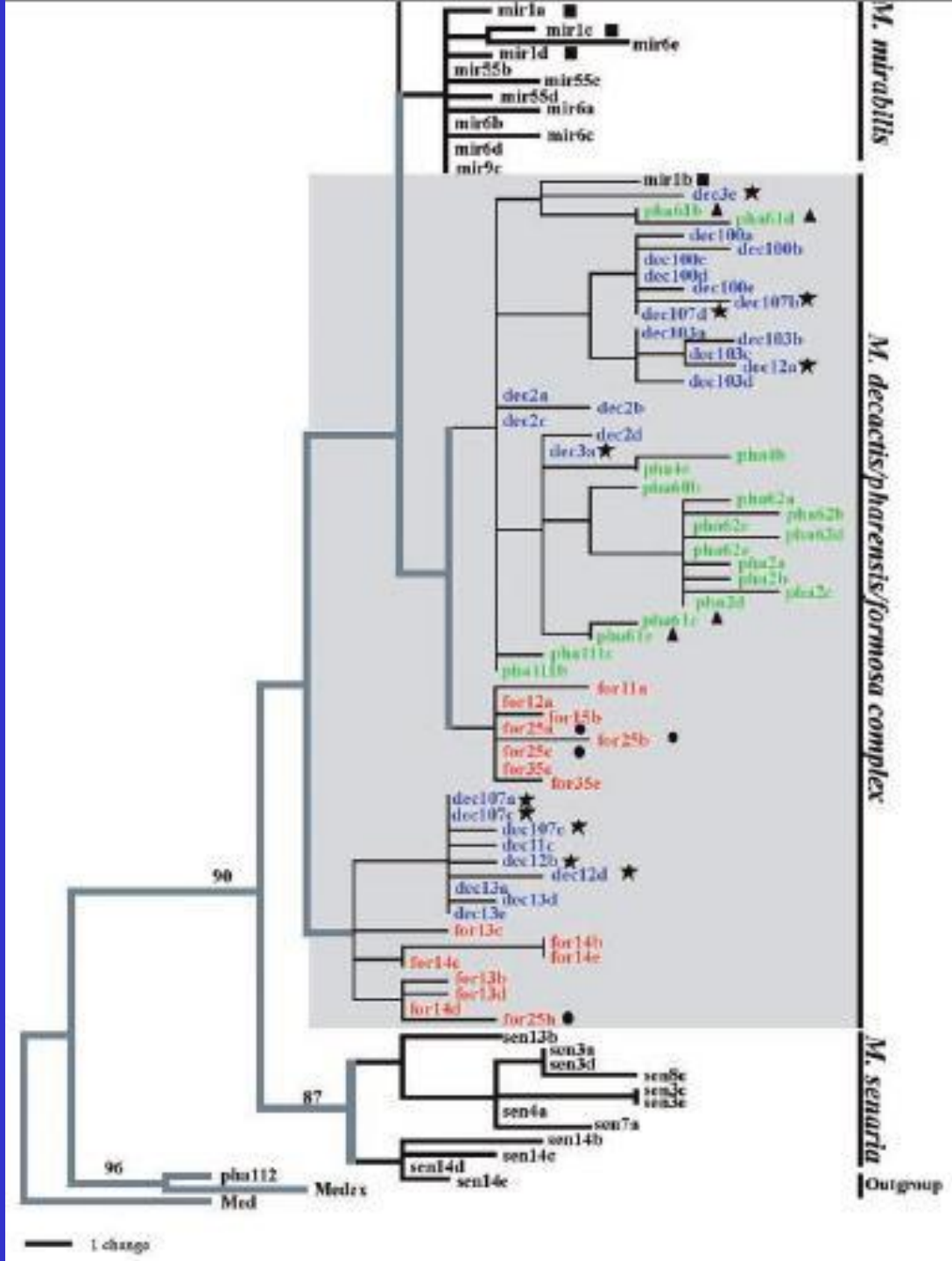
Results I



Quantitative comparison simulated and actual growth forms *Madracis sp.*



Genetic comparison Madracis species (Diekmann et al., 2001; Frade et al., 2009);
M. V. Filatov, P. R. Frade, , R. P.M. Bak, M. J.A. Vermeij, and J. A. Kaandorp,, Plos One, 2013



Quantitative three-dimensional morphological analysis supports species discrimination in complex-shaped and taxonomically challenging corals,

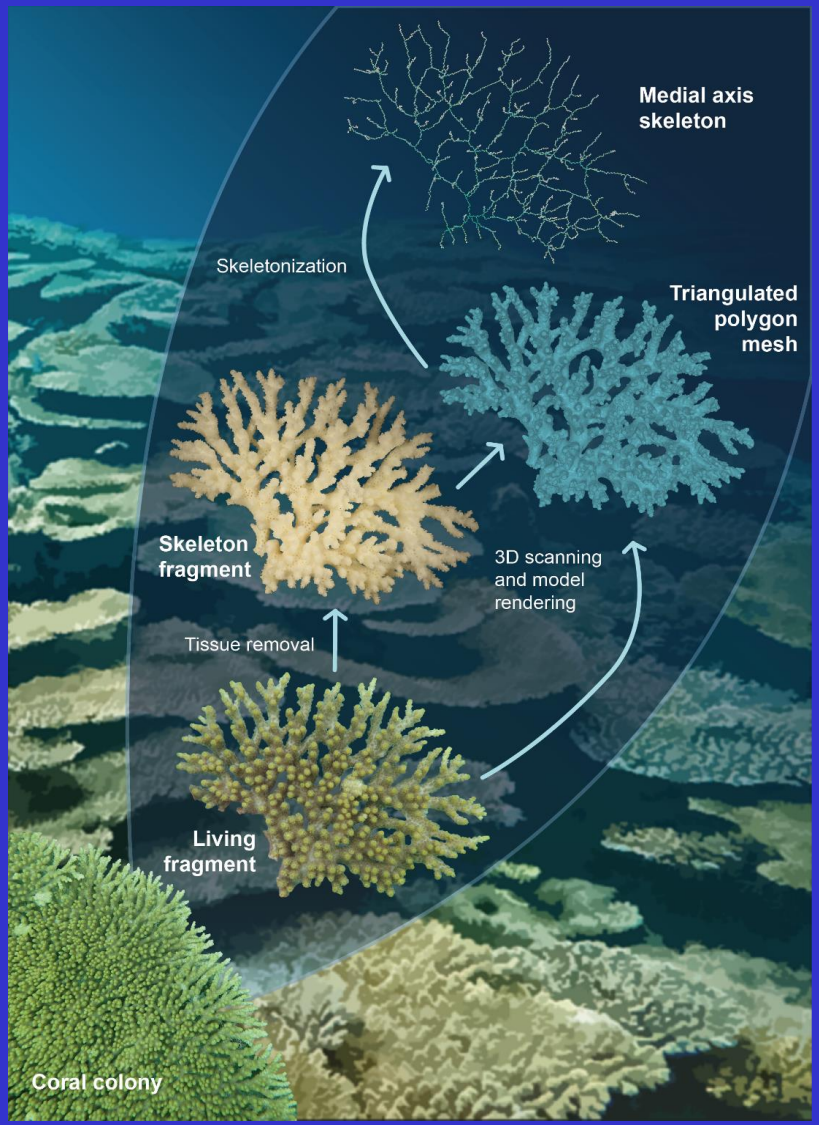
C. Ramirez-Portilla , I. M. Bieger ,R.t G. Belleman , T. Wilke, Jean-Francois Flot ,A. H. Baird , S. Harii , F. Sinniger and J. A. Kaandorp

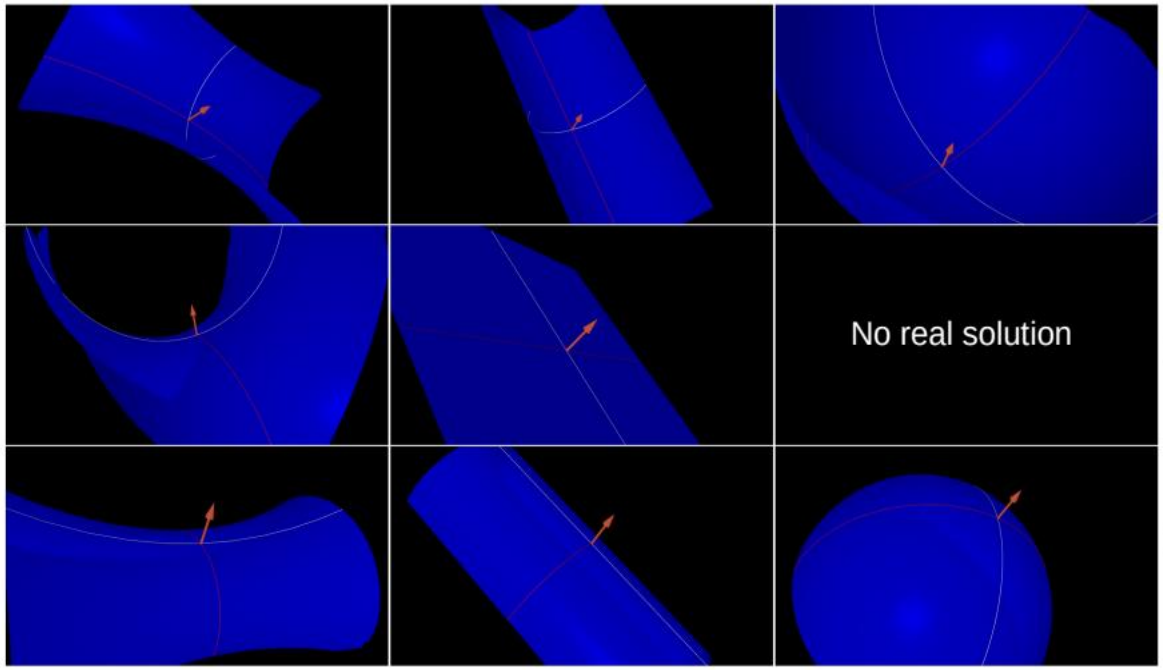
September 2022. Frontiers in Marine Science 9:955582, DOI: 10.3389/fmars.2022.955582

Develop quantitative measures to characterize morphology using high resolution polygon meshes from three closely related *Acropora (A.)* species *(C. Ramirez-Portilla, I Bieger et al., submitted)*:

- *A.* cf. *bifurcata*
- *A.* cf. *cytherea*
- *A.* aff. *hyacinthus*

Which 3D-derived metrics can aid to delineate these species?





Curvature of a surface

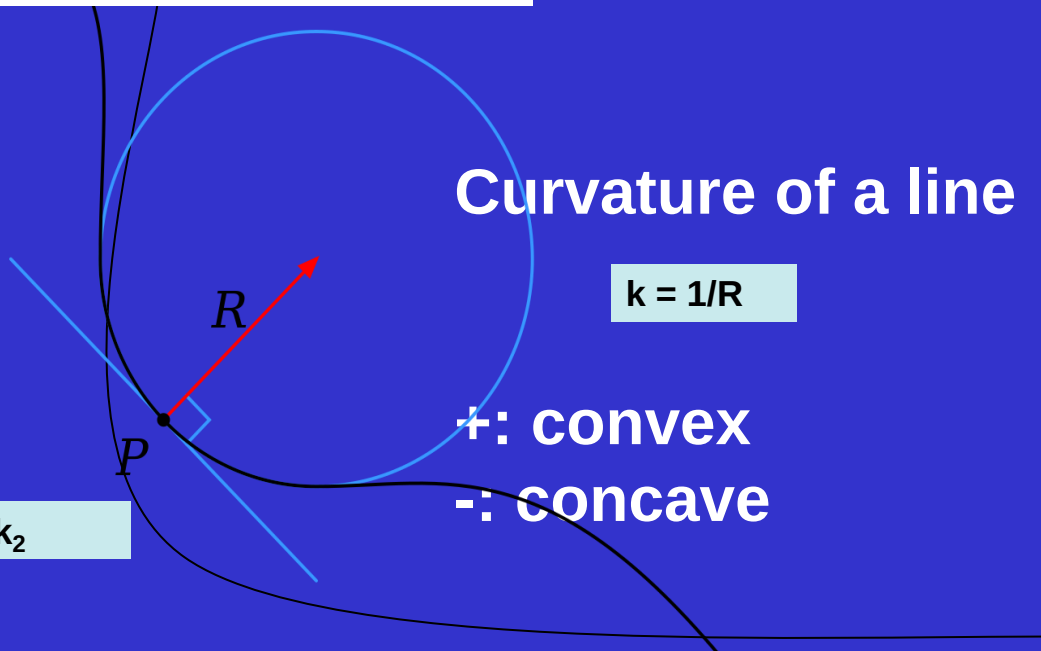
k_1 : maximum curvature

k_2 : minimum curvature

Mean (H) curvature:

$H = (k_1 + k_2)/2$

Gaussian (K) curvatur $K = k_1 * k_2$

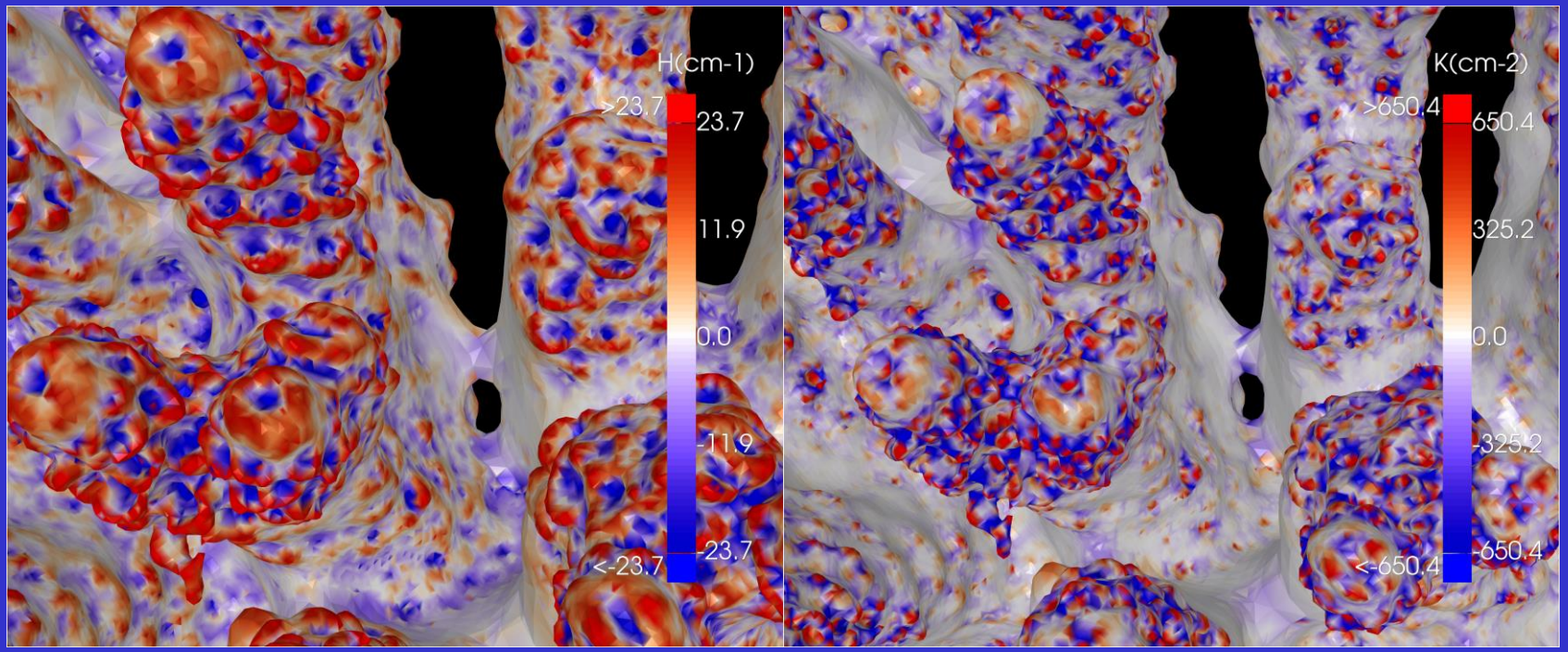


Curvature of a line

$k = 1/R$

+: convex

-: concave

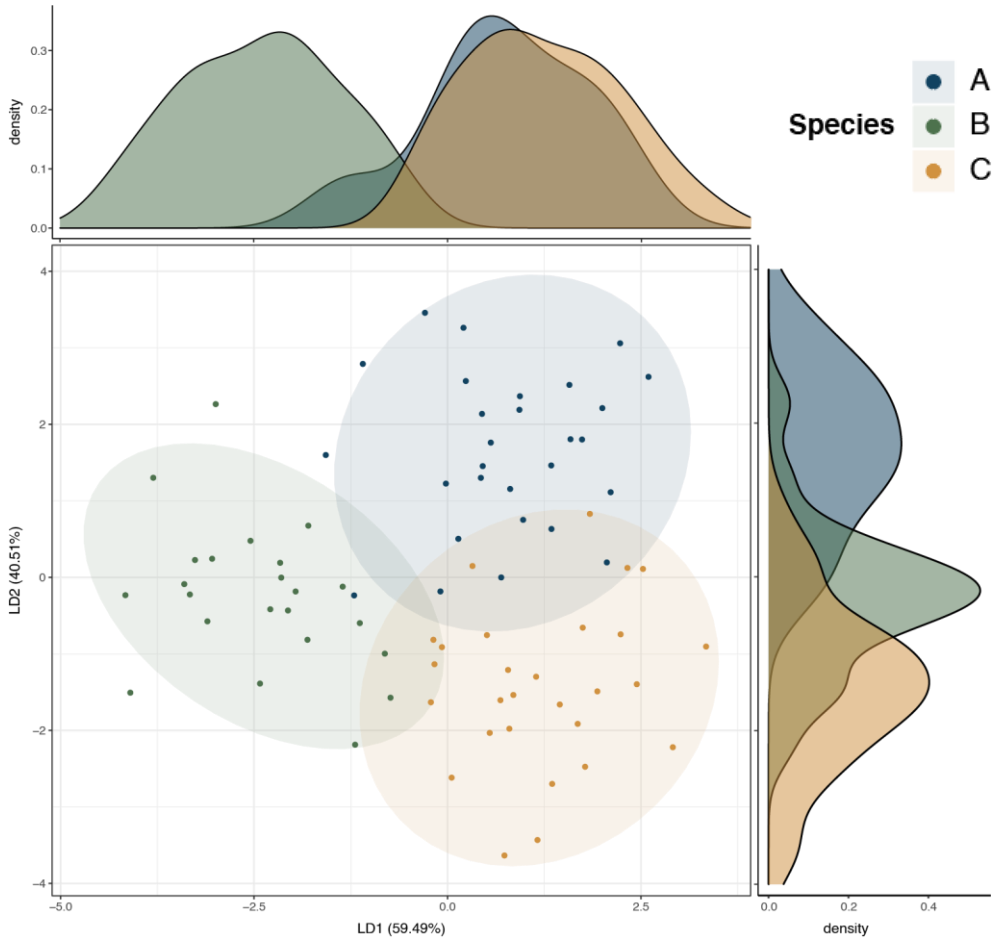

H(cm-1)
>23.7
23.7
11.9
0.0
-11.9
-23.7
<-23.7
K(cm-2)
>650.4
650.4
325.2
0.0
-325.2
-650.4
<-650.4

Species A

Species B

Species C

Pictures by Andrew H. Baird



Linear discriminant analysis (LDA)

LDA	Allocated to A	Allocated to B	Allocated to C
A	23	2	3
B	0	22	1
C	2	0	26

89.87% of discriminative power (71/79 specimens)

Conclusions and Open Questions

- Many crucial parts of information are still missing, there are many gaps in the knowledge of molecular biology and the physiology of calcification
- Many (Most) details about gene regulation of calcification are missing
- The genome of a scleractinian coral and its symbiont are available, important for research on gene regulation of calcification
- How are gene expression in the host (the coral) and symbiont related is not very well known
- Models can be used to infer regulatory networks from gene expression data, understand the fluxes in complicated metabolic pathways, study the influence of the physical environment in detail, to study how the different processes at very different scales in time and space are coupled
- Models can be used to organize data and data collection and to detect the missing pieces of knowledge in a systematic way

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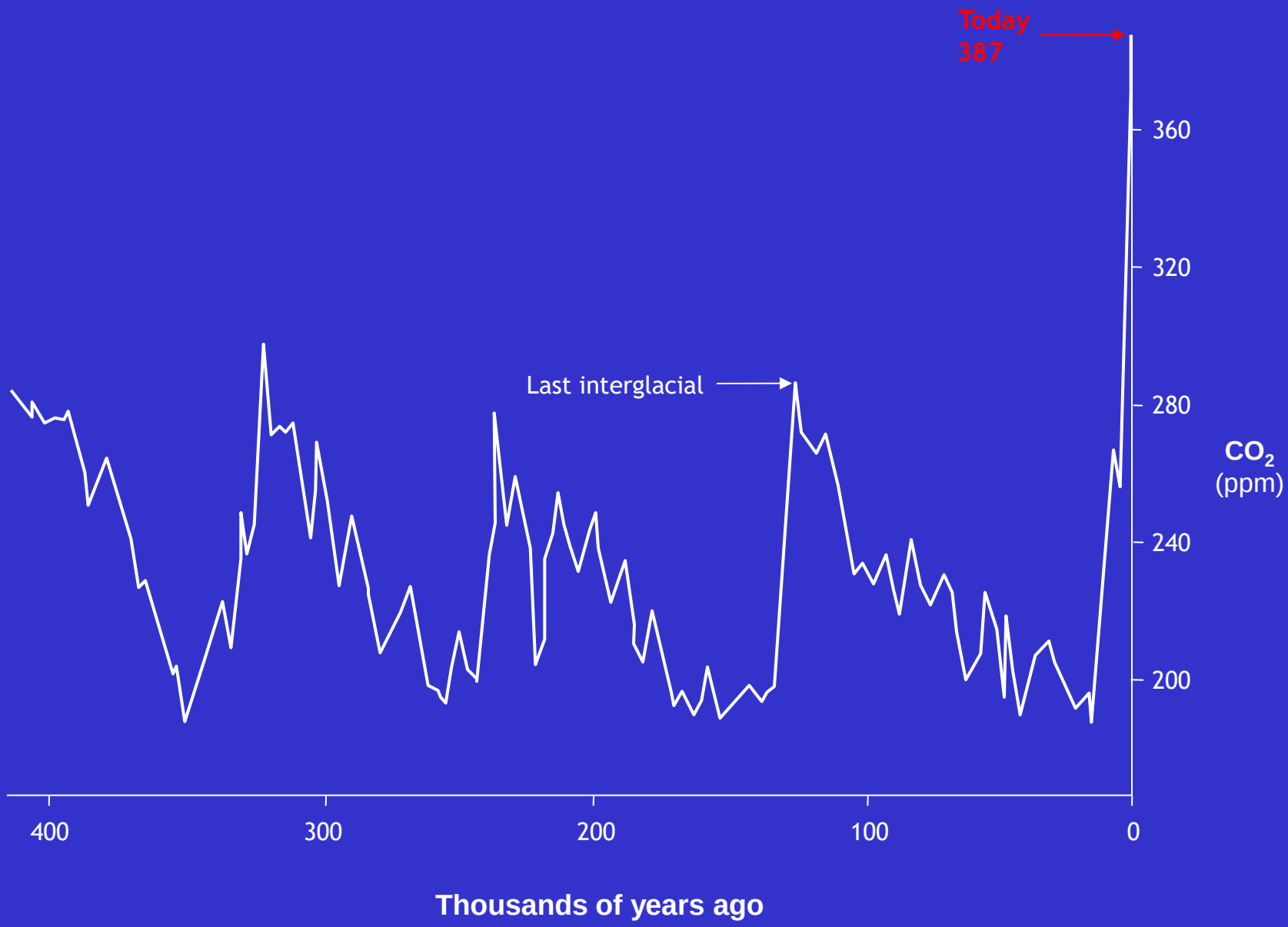
(Veron, 2009)


Today
387
Last interglacial
360
320
280
240
200
CO_2
(ppm)
400
300
200
100
0
Thousands of years ago

In summary

- Dissolving CO_2 in seawater increases the hydrogen ion ion (H+) concentration in the ocean and decreases ocean pH. Since the industrial revolution began, it is estimated that surface ocean pH has dropped by slightly less than 0.1 units (on the logarithmic scale of pH; approximately a 25% increase in H+), and it is estimated that it will drop by a further 0.3 to 0.5 units by 2100 as the oceans absorb more anthropogenic CO_2 (see also wikipedia, ocean acidification) "

$$CO_2 + CO_3^{-2} + H_2O \rightarrow 2HCO_3^-$$

- Dissolving CO_2 decreases carbonate ion(CO_3^{2-}) concentration in the ocean and lowers the saturation state of carbonate minerals (see also wikipedia, ocean acidification)

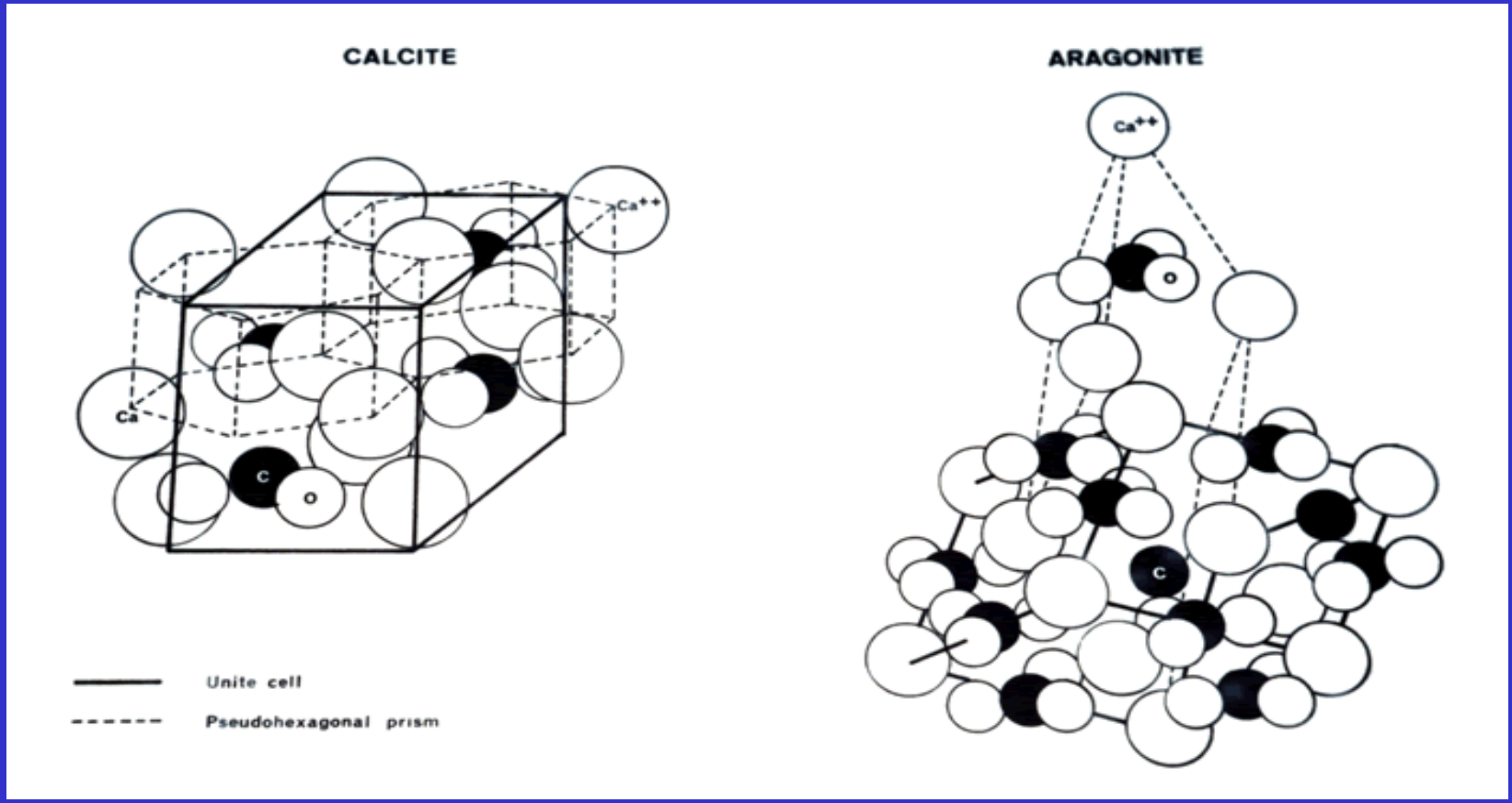
Saturation state of aragonite in corals (wikipedia)

- "The saturation state of seawater for a mineral(Ω) is a measure of the thermodynamic potential for the mineral to form or to dissolve; specifically it is the product of the concentrations (or activities) of the reacting ions that form the mineral (Ca^{2+} and CO_3^{2-}), divided by the product of the concentrations of those ions when the mineral is at equilibrium (K_{sp}), that is, when the mineral is neither forming nor dissolving.

$$\Omega_{sp} = \frac{[Ca^{2+}][CO_3^{-2}]}{K_{sp}}$$

- In seawater, a natural boundary is formed as a result of temperature, pressure, and depth, and is known as the saturation horizon. It is above this saturation horizon that calcifying organisms live, as CaCO3 does not readily dissolve there
- Calcium carbonate exists in 2 commonly occurring forms: aragonite and calcite. The aragonite form is much more soluble than the calcite form which means that the aragonite saturation horizon is always nearer to the surface than the calcite saturation horizon. This means that those organisms that produce calcite may possibly be less vulnerable to changes in ocean acidity than those which produce aragonite (the scleractinian corals).

$CaC0_3$ Crystals



carbonate minerals

Aragonite

Calcite

Aragonite saturation state vs calcification rate (Leclerq et al., 2000)