The Evolutionary Ecology of Multicellularity: Using the Volvocine Green Algae as a Case Study

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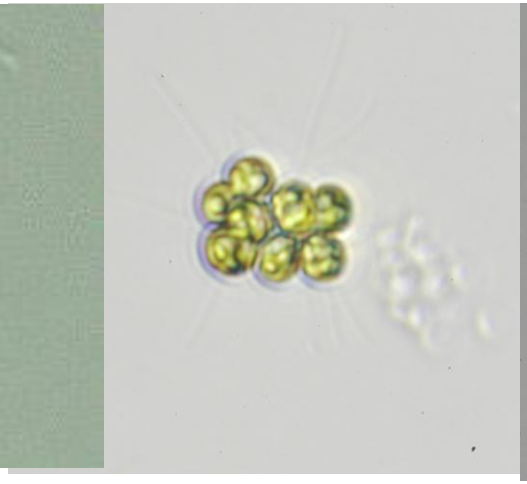
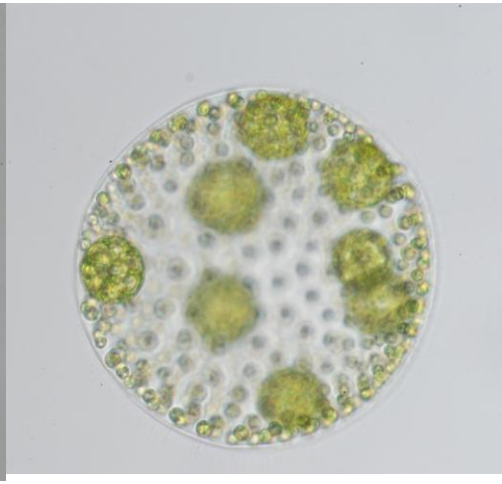
Chlamydomonas *Gonium* *Eudorina* *Volvox*



Unicell

First cell groups

The formation of a coherent extra-cellular matrix

Cellular differentiation and new level of individuality

1- Introduction of the volvocine green algae

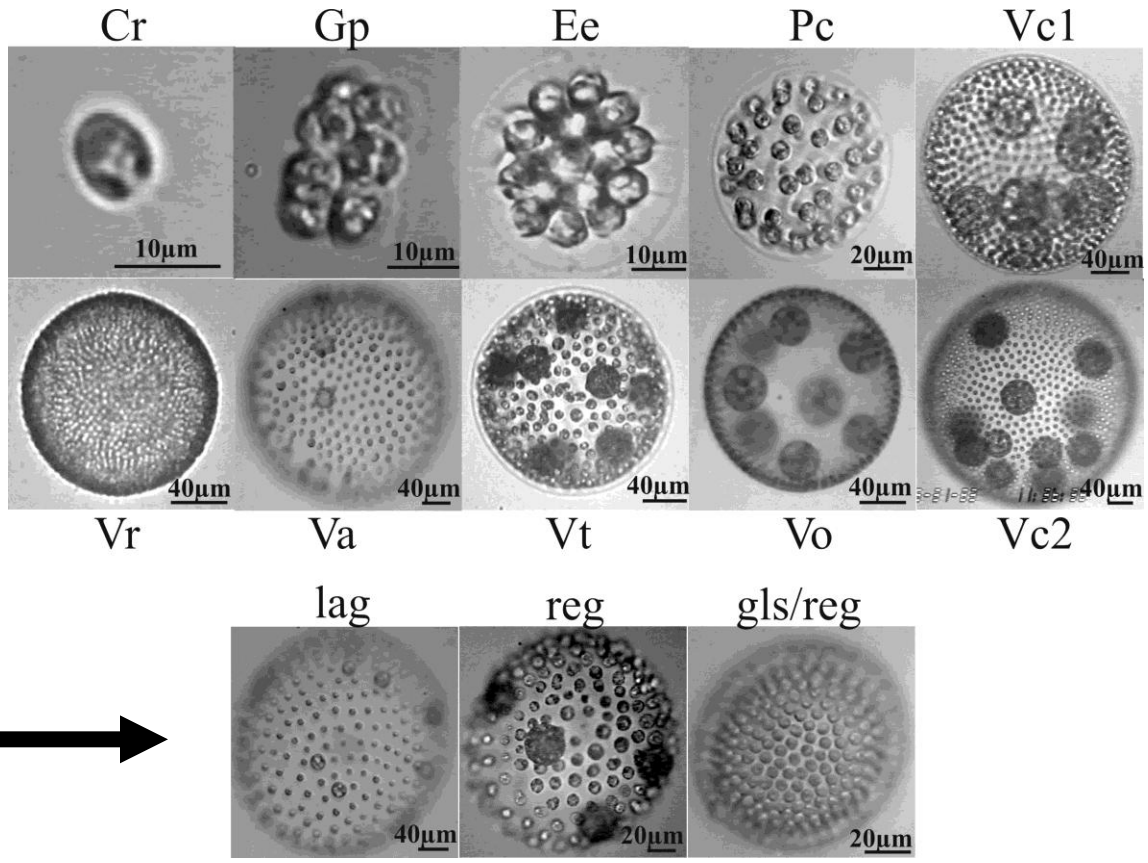
2- The evolution of multicellularity

3- Volvocales as a model system for the evolution of multicellularity

In Volvocales Increased Cellular Differentiation Correlates With Size

• Volvocalean green algae are specially suited to study the *unicellular-multicellular transition* since they range from unicells to undifferentiated colonies, to multicellular individuals with complete germ-soma separation.

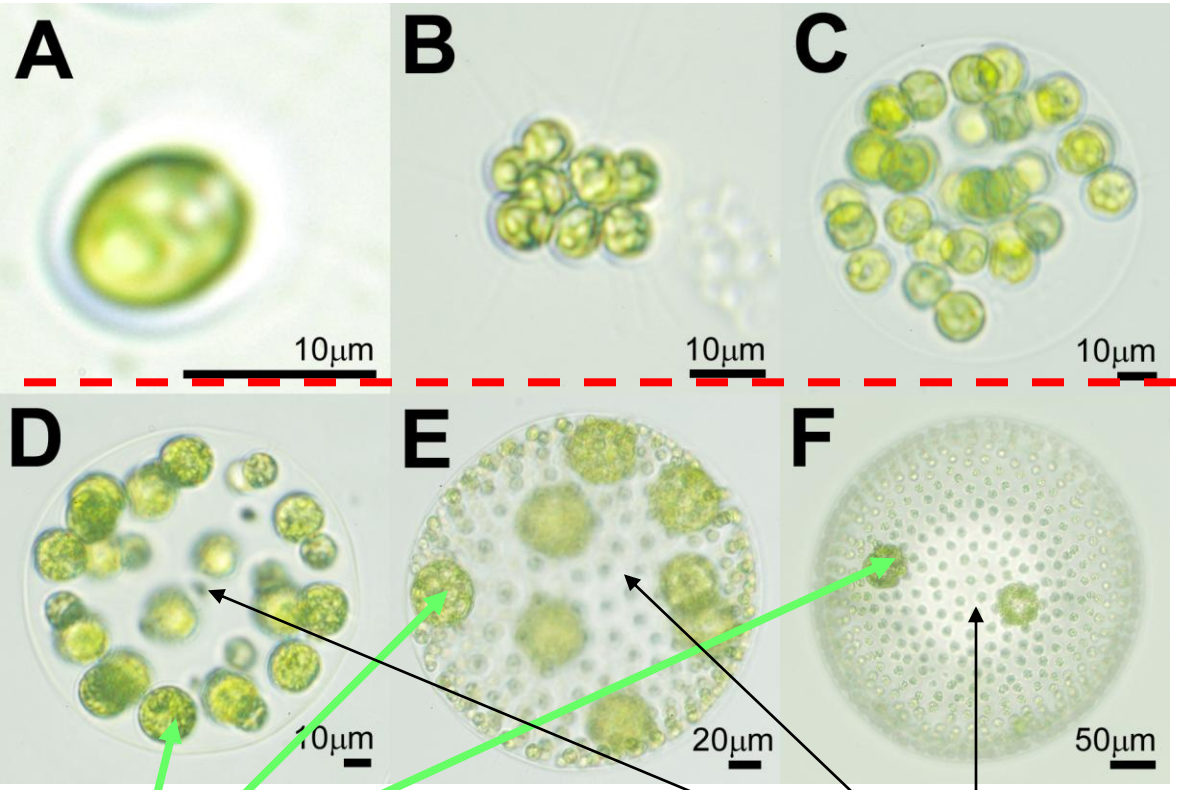
Cr: *Chlamydomonas reinhardtii*
Gp: *Gonium pectorale (8 cells)*
Ee: *Eudorina elegans (16-64 cells)*
Pc: *Pleodorina californica (64-128 cells)*
Vc1: *Volvox carteri 600fc (1000 cells)*
Vc2: *Volvox carteri 1000fc (2000 cells)*
Vr: *Volvox rousseletii (4000 cells)*
Va: *Volvox aureus (2000 cells)*
Vt: *Volvox tertius (1000 cells)*
Vo: *Volvox obversus (500-1000 cells)*



Mutants derived from *V. carteri* that disrupt germ-soma separation

Specialization in reproductive and vegetative functions (i.e., germ-soma separation) characterizes the large members of this lineage.

***Volvocales* are freshwater green algae that comprise a group of closely related lineages with different degrees of cell specialization which seem to represent *"alternative stable states"* (Larson et al 1992)**



A: *Chlamydomonas reinhardtii*
B: *Gonium pectorale (1-16 cells)*
C: *Eudorina elegans (16-64 cells)*
D: *Pleodorina californica (32-256 cells)*
E: *Volvox carteri (500-4000 cells)*
F: *Volvox aureus (500-4000 cells)*

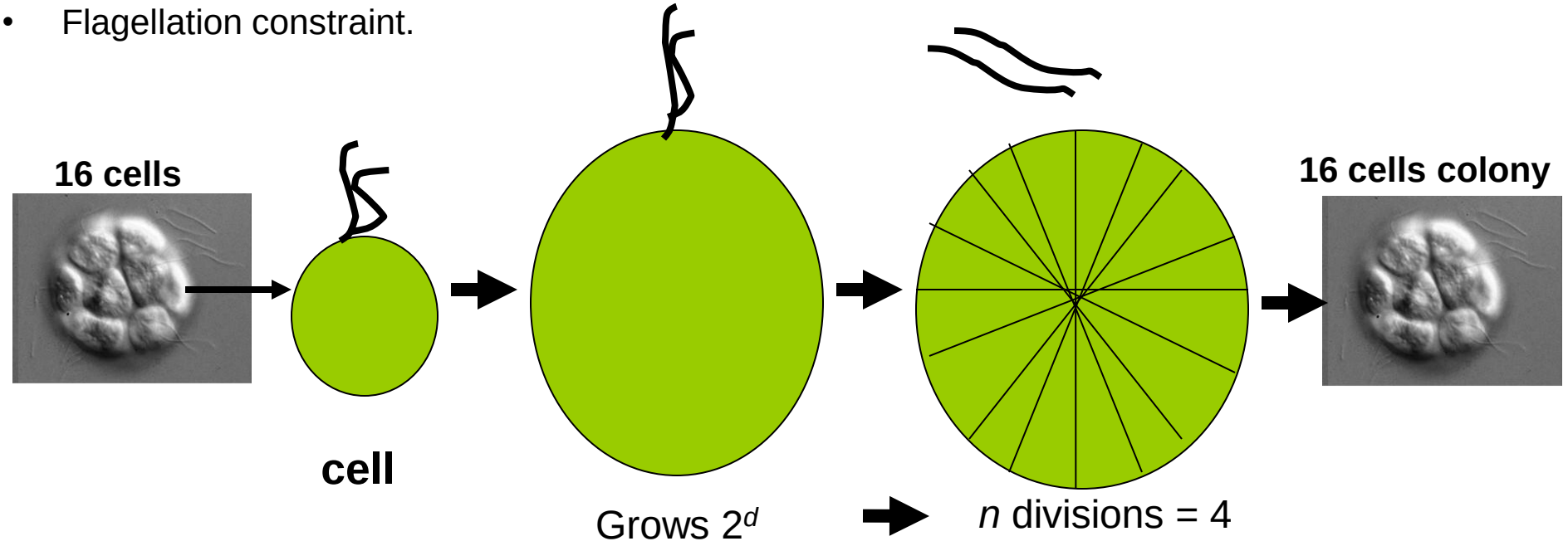
germ-soma differentiation

Unicellular and multicellular forms of Volvocales coexist in transient, quiet bodies of water or in large permanent eutrophic lakes (during spring, summer, or autumn blooms).

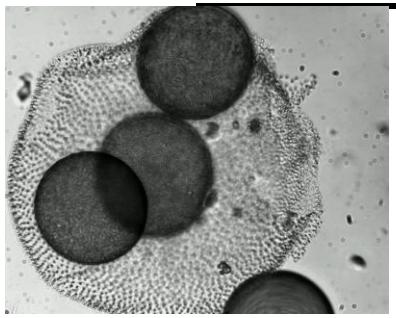
Volvocales have two contrasting developmental programs

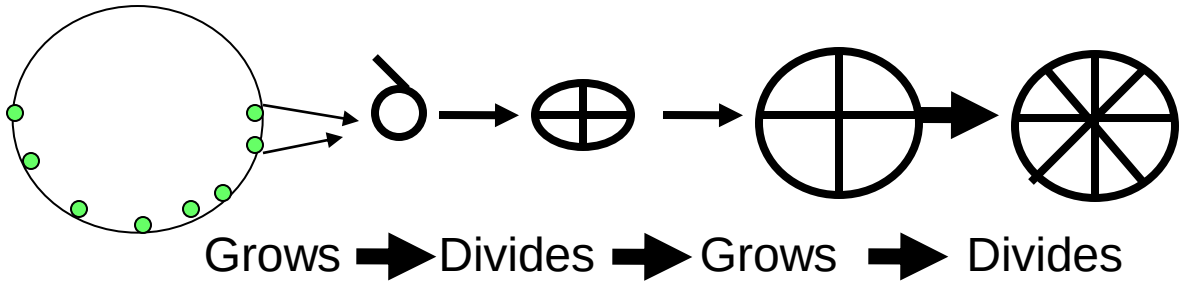
Ancestral developmental program

- **Palintomy:** reproductive cells first grow and then divide by multiple fission.
- Flagellation constraint.



Derived developmental program in some *Volvox* species: binary fission

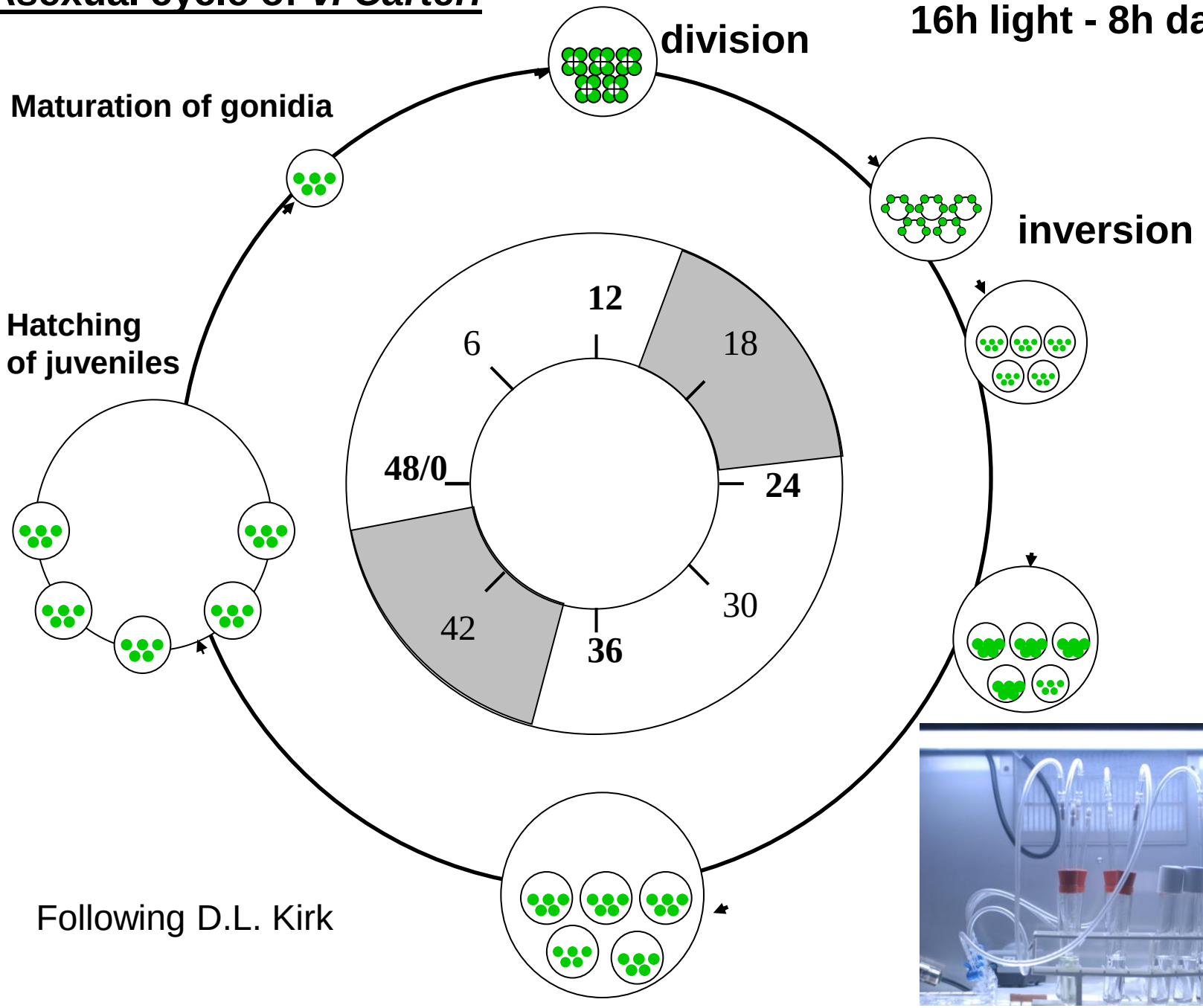




In both cases the reproductive cell or embryo grows inside the mother colony to produce the daughter colony

Asexual cycle of *V. Carteri*

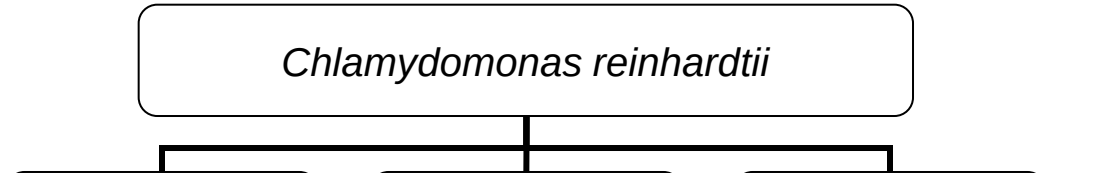
16h light - 8h dark cycle

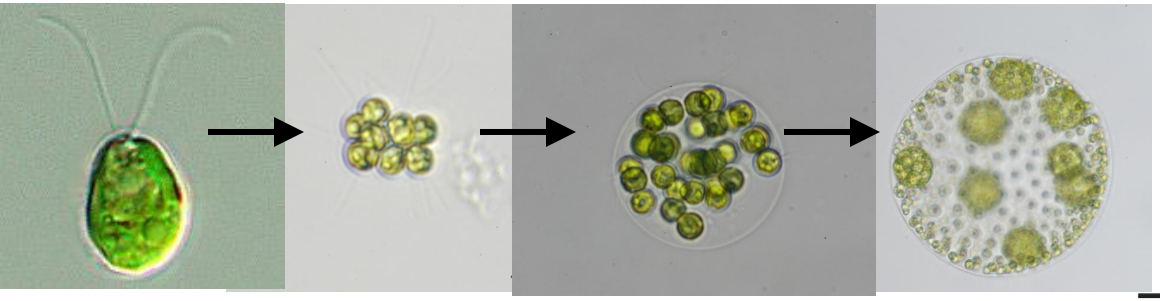


Following D.L. Kirk

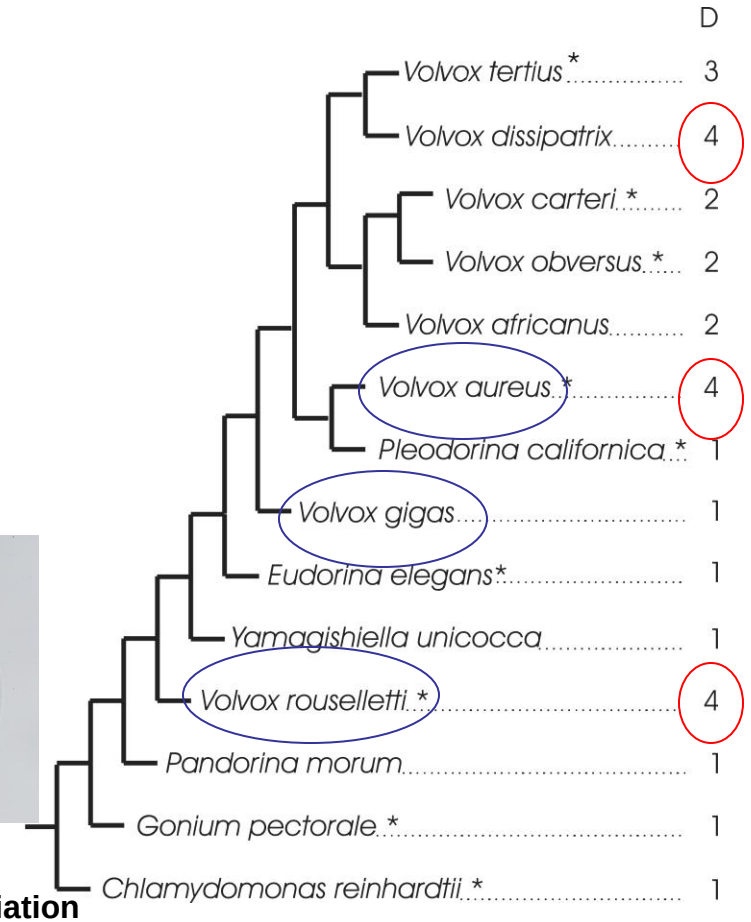
- **The transition from less complex forms to more complex forms such as *Volvox* occurred more than once.**

- **Lineages exhibiting different developmental programs are interspersed with each other and with non-*Volvox* species.**

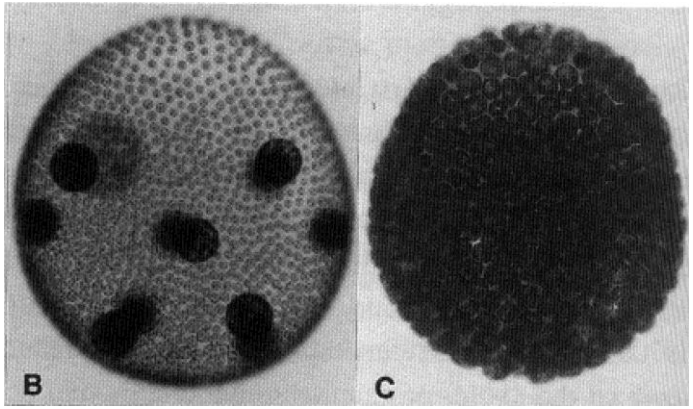




Unicell → First cell groups → The formation of a coherent extra-cellular matrix → Cellular differentiation



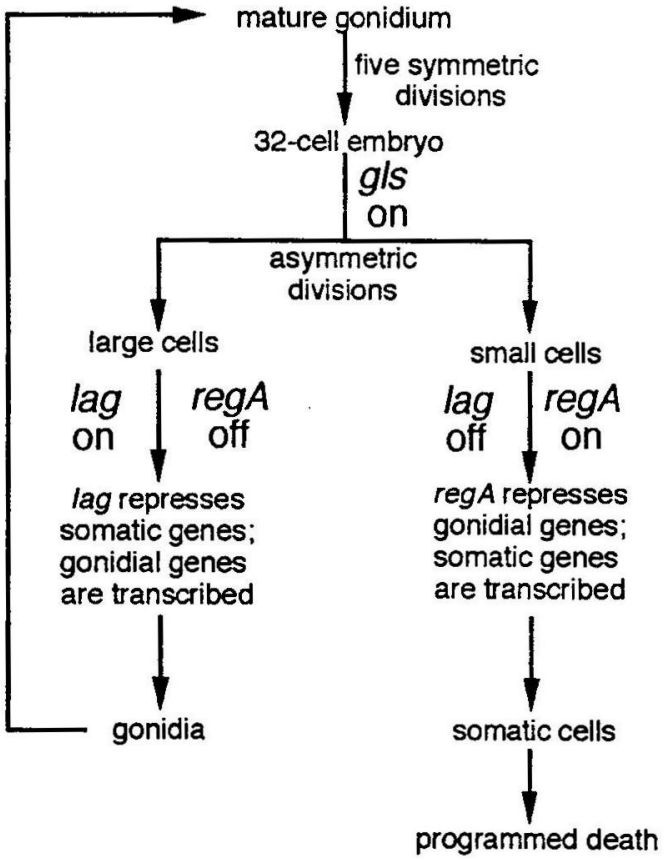
In Volvocales the transition to multicellularity involves few genetic steps



***RegA* mutant**

With only one mutation somatic cells become reproductive in *V. carteri*

Working hypothesis of how cellular differentiation works in *V. Carteri* (Kirk)



Why use Volvocales?

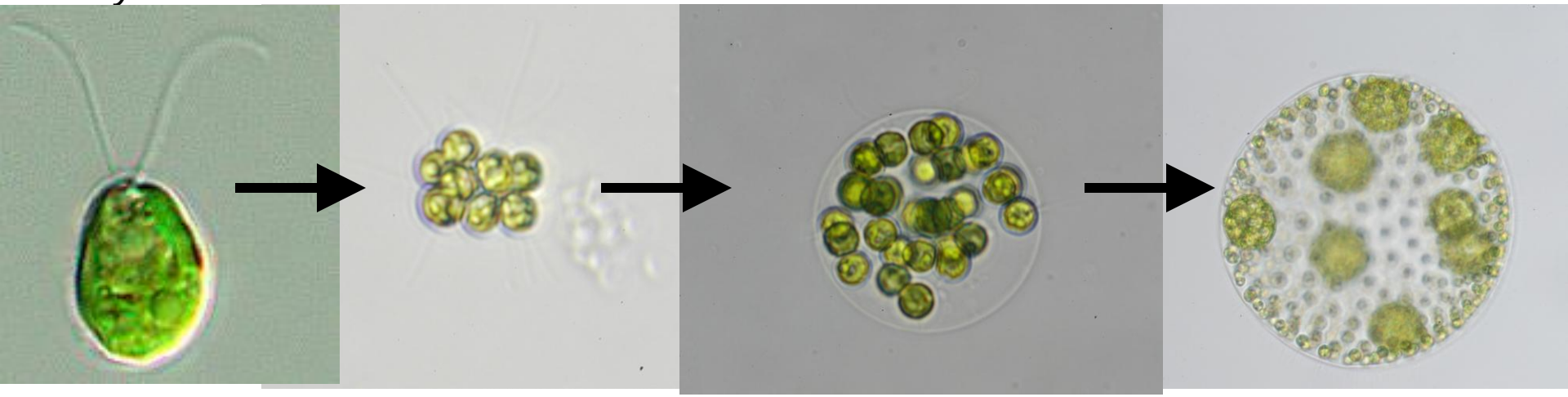
- Asexual, monoclonal populations are easily obtained.
- Populations are easily grown in the lab in well defined media (e.g., SVM).
- Strains range from unicellular to 10,000 cell colonies, including mutants derived from *V. carteri* with germ-soma differentiation disruption.
- Cell, colony, population, and community size and growth rates are easily measurable.
- Many aspects of their biology have been studied (cytology, biochemistry, development, genetics, physiology, natural history, ecology and life-history).
- Due to their range of sizes, they enable the study of scaling laws: 10^0 to ~10^4 cells
- The genome of *C. reinhardtii* and *V. carteri* have been sequenced; others are in the process.
- Different Volvocales constitute a natural competitive guild, competing primarily for light and mineral resources.
- All kinds of communities can be assembled with organisms of different sizes and complexity, but with similar cell biology.

The evolution of multicellularity

General Theme: *To investigate the emergence of new levels of individuality and complexity as size increases.*

Specific Interest: *Understanding the unicellular-multicellular transition.*

Chlamydomonas *Gonium* *Eudorina* *Volvox*



Unicell **First cell groups** **The formation of a coherent extra-cellular matrix** **Cellular differentiation and new level of individuality**

How did a larger individual with two cell types evolved from a smaller colony of undifferentiated cells?

<u>Volvocalean green algae</u> are specially suited to study the <u>unicellular-multicellular transition</u>.

General Life-History Model for the Unicellular-Multicellular Transition:

Within colony variation is negligible.
Variation exists only at the group level

- Asexual reproduction.
- Discrete generation time.
- Extra-cellular material needed is not taken into account.
- Only one somatic cell type.
- Cell number is fixed throughout development.
- Intrinsic growth rate of a unicell is the maximum rate attainable by cells that form groups.
- Initial cell size is the same for both somatic and reproductive cells.

Most of the model assumptions fit the Volvocales life-history

Solari et al (2013) in press AmNat

Trade-offs of germ-soma differentiation

The fitness (*W*) of any evolutionary unit can be understood in terms of its two basic components: fecundity (*L*) and viability (*V*).

W* = *L* x *V
Fitness* = *reproduction rate* x *survival probability

Somatic cells specialize in vegetative functions
Germ cells specialize in reproductive functions

Somatic cells: ↑ Viability ↑↓ Fecundity

Specialized germ cells: ↑ Fecundity ↓ Viability

***Reproduction costs**: Larger size can be beneficial for the fitness of the colony, but can become costly, both in terms of survival and fecundity.*

General Life-History Model for the Unicellular-Multicellular Transition: Fecundity

If we use a standard exponential growth model for the growth of cells in a colony and assume discrete generation time :

$$m = m_o e^{r t}$$

***r* =** cell growth rate
***t* =** generation time
***m* and *mo* =** final and initial mass

If mother colonies produce daughter colonies of the same type:

$$m = n\, m_o \text{ or } n = m / m_o$$

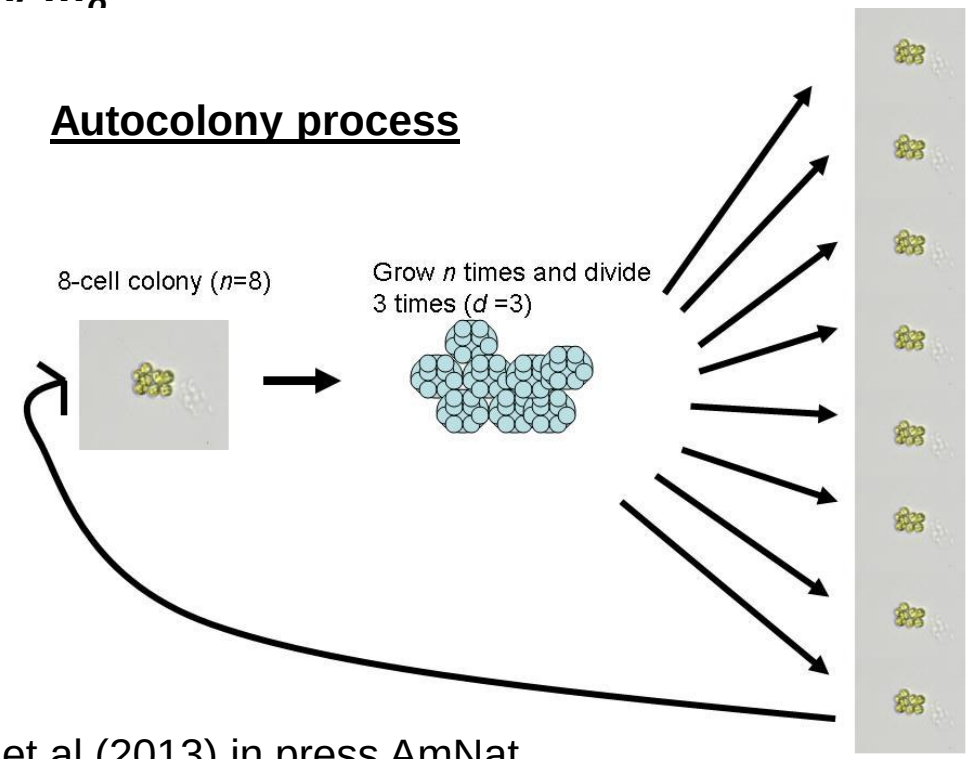
***n* =** colony cell number

$$n = e^{r t} \text{ or } t = Ln\,(n) / r$$

-Increasing colony cell number (n) increases generation time (t).

- Increasing the growth rate (r) decreases generation time (t).

Autocolony process



Solari et al (2013) in press AmNat

Assuming colonies have discrete generation time, the per-generation fecundity of colonies composed of undifferentiated cells:

$$Ro = n$$

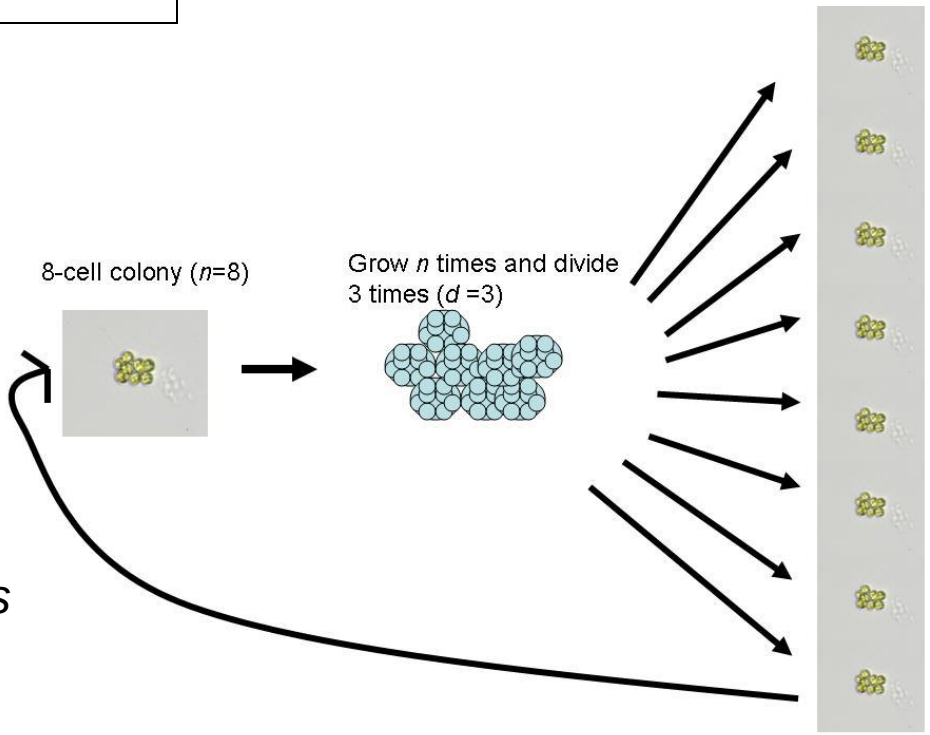
***Ro* can be also written as,**

$$Ro = \lambda^t$$

λ = fecundity rate

Since $n = e^{r\,t}$ and $Ro = n$, then

$$\lambda^t = e^{rt}, \text{ or } \lambda = e^r$$



If r is constant and not size dependant, the fecundity rate for colonies composed of undifferentiated cells is the same regardless of size.

In an ideal world with no size constraints or benefits, size does not matter.

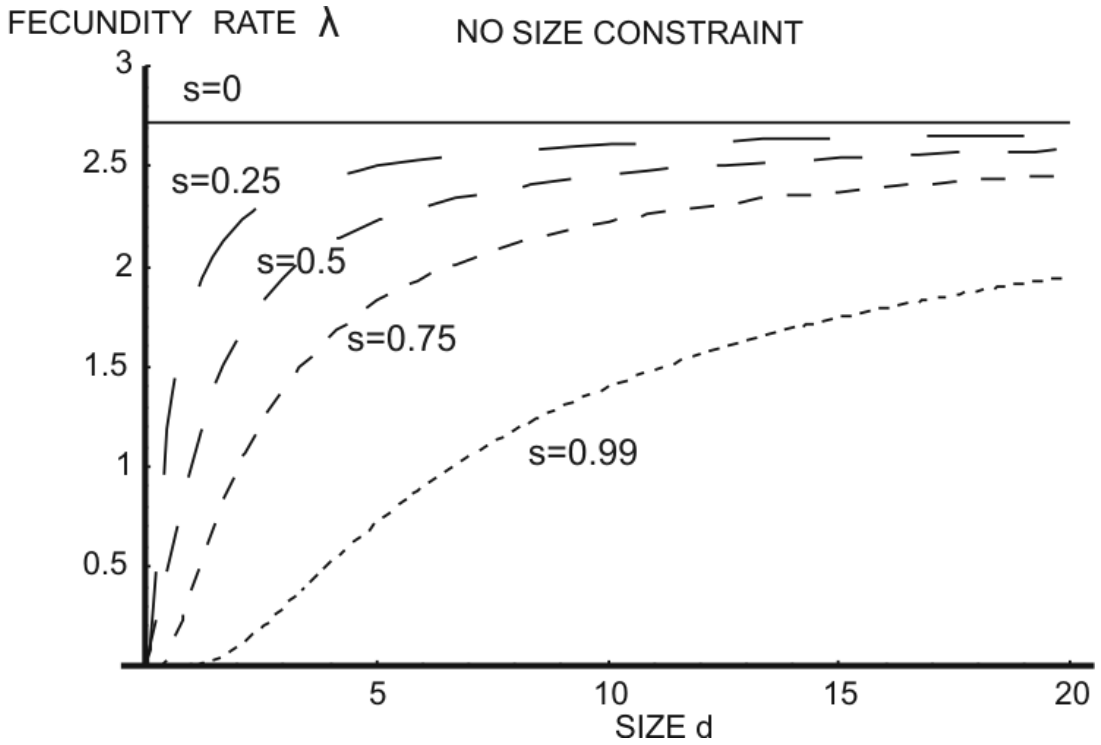
Solari et al (2013) in press AmNat

If colonies invest in soma and a proportion *s* of cells become sterile and do not reproduce:

$$Ro = n(1-s)$$

since $1/t = r / \mathrm{Ln}(n)$

$$\lambda^t = e^{rt}(1-s) \text{ or } \lambda = e^{r}(1-s)^{r/\mathrm{Ln}\, n}$$



$r = 1$

$n = 2^d$

Investing in a proportion of somatic cells decreases the fecundity rate since somatic cells do not reproduce, but this negative effect dilutes as colony size (d) increases regardless of a cost or benefit of size to the fecundity rate.

Solari et al (2013) in press AmNat

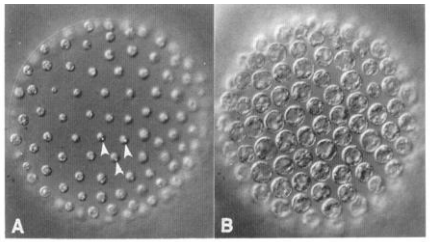
Fecundity rate as size increases

$$\lambda = e^{r}(1-s)^{r\,/\mathrm{Ln}\,n}$$

r depends on the Supply B and Demand C of resources to the colony, which depend on size and cellular differentiation

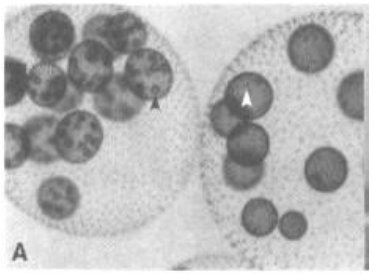
The demand ***C*** depends on the total number of cells (***n***) and to the proportion of somatic cells (***s***) which determines how much a colony has to grow to produce daughter colonies of the same type.

E.g.:**128-cell colony** has to grow enough to produce 128 daughter colonies with 128 cells each before hatching



Total Cost of Reproduction $C = n^2 = 16{,}384$ cells

if the same **128-cell colony** sequesters 3/4 of its cells for somatic functions (***s* = 0.75**):



Total Cost of Reproduction $C = n^2 (1-s) = 4{,}096$ cells.

The cost of reproduction of the soma-differentiated colony is lower, in this case 4 times lower than the cost of reproduction of the undifferentiated colony.

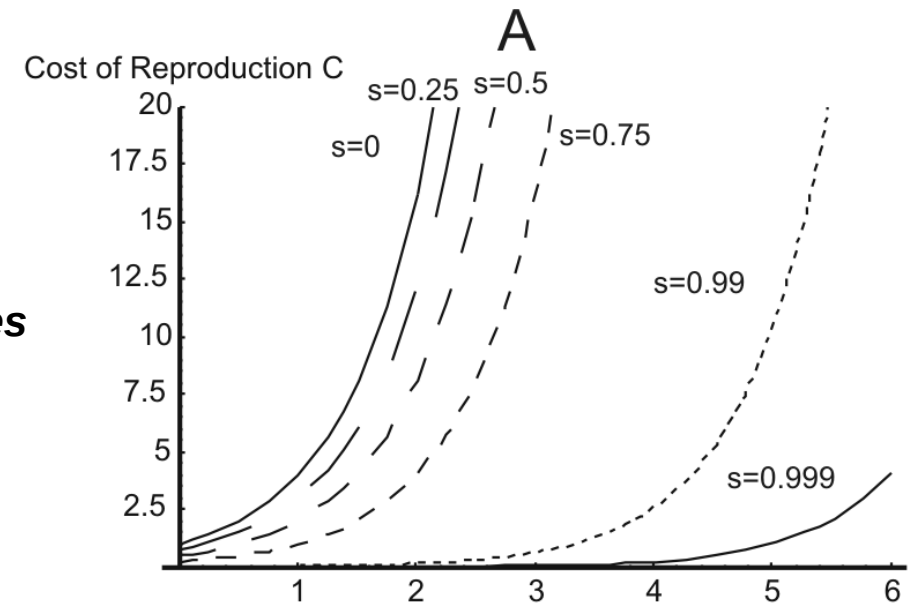
Solari et al (2013) in press AmNat

Fecundity rate as size increases

Demand

Total Cost of Reproduction $C \sim n^2 (1-s)$

Production costs are lowered as colonies invest in soma

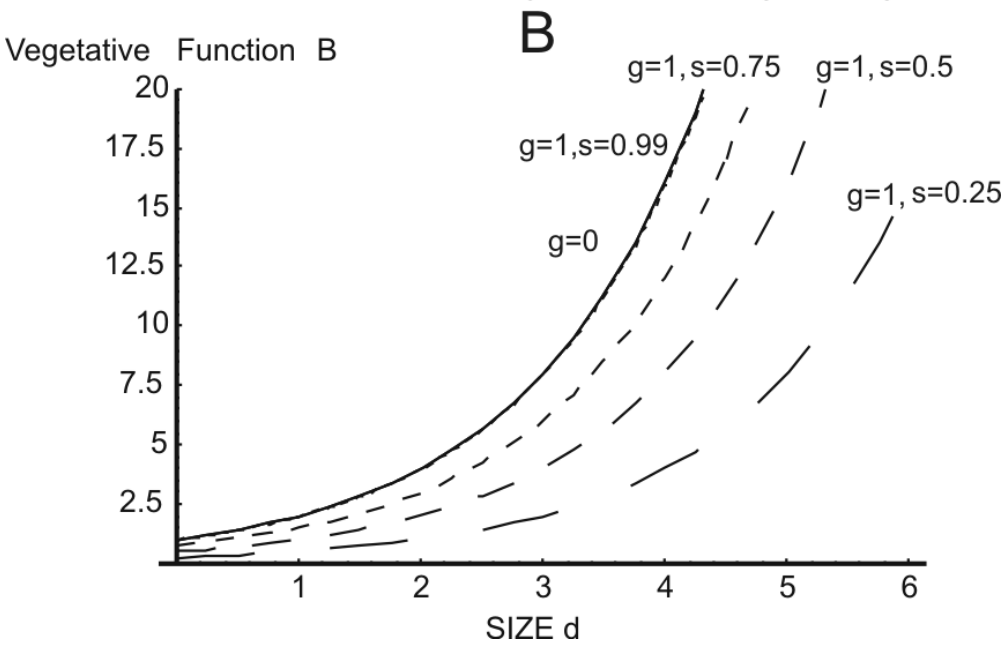


Supply

The vegetative functions ***B*** needed to acquire resources to grow and reproduce are performed by the undifferentiated reproductive cells that retain those functions and soma:

$B \sim ns + n(1-s)(1-g)$

g **=** germ specialization parameter
(we assume an additive contribution between the two cell types)



Solari et al (2013) in press AmNat

Fecundity rate as size increases

We use the ratio between the supply *B* and demand *C* of resources as the factor that may limit the intrinsic growth rate *r* :

$$B/C_r = bB^{\beta} / cC^{\alpha}$$

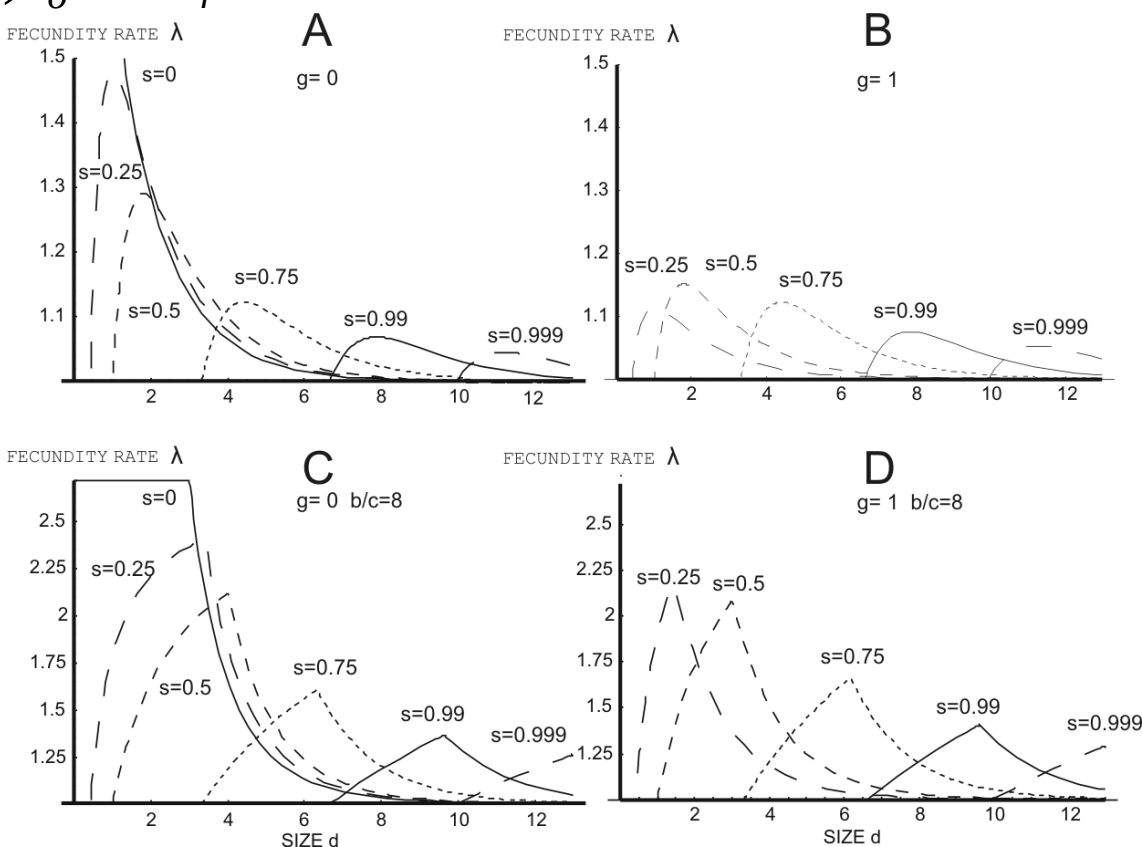
b= supply of resources of the unicell basal to the lineage
c= demand of resources of the unicell basal to the lineage
α and ***β*** = scaling exponents for the demand and supply

if $B/C_r \geq 1 \longrightarrow r = (1+u_g g)r_o \longrightarrow$ **Supply meets Demand**

if $B/C_r < 1 \longrightarrow r = (1+u_g g)r_o B/C_r$

r_o = growth rate of the unicell
u_g = germ specialization benefit

$$\lambda = e^{r}(1-s)^{r/\mathrm{Ln}\, n}$$



Solari et al (2013) in press AmNat

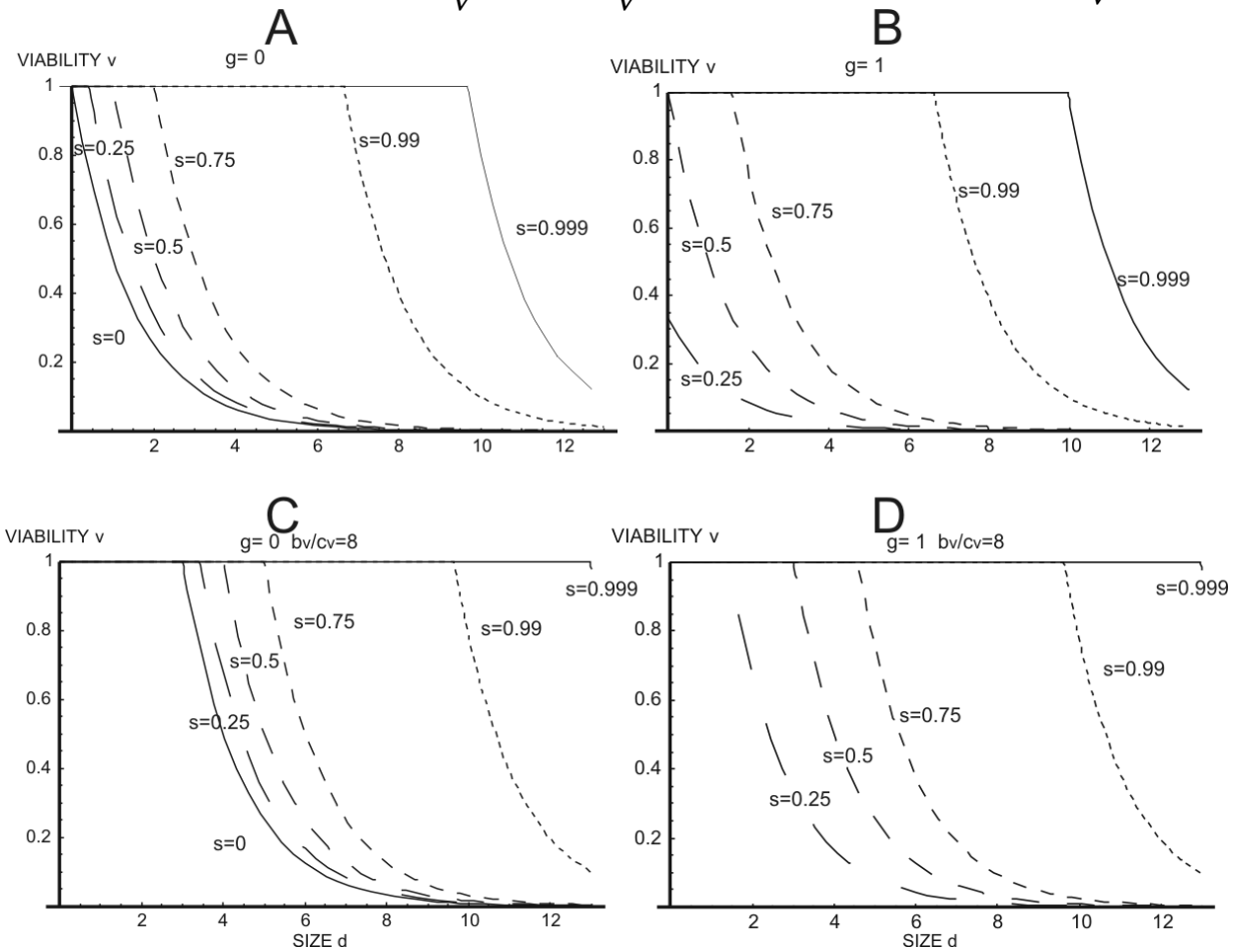
Viability as size increases

Viability ***v*** gives the proportion of colonies that will survive to reproduce the next generation of colonies.

For the sake of argument, we also model viability as the ratio between the contribution ***B*** and the cost ***C*** given by the cells in the colony to viability:

if $b_v B^\gamma / c_v C^\delta \geq 1 \longrightarrow v = 1 \longrightarrow$ **Viability needs are met.**

if $b_v B^\gamma / c_v C^\delta < 1 \longrightarrow v = b_v B^\gamma / c_v C^\delta$



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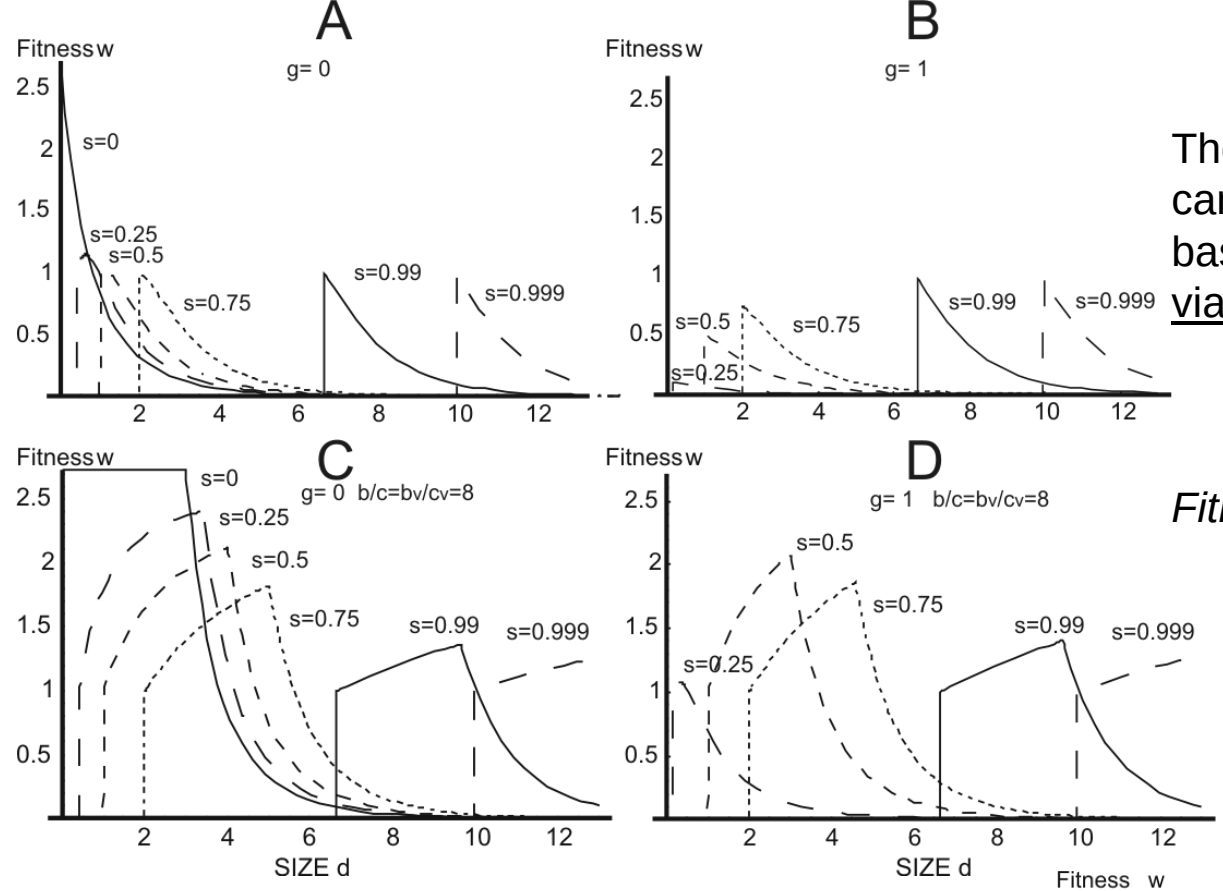
Fitness

The fitness (*W*) of any evolutionary unit can be understood in terms of its two basic components: fecundity (λ) and viability (*V*).

$$W = \lambda \times V$$

Fitness = reproduction rate x *survival*

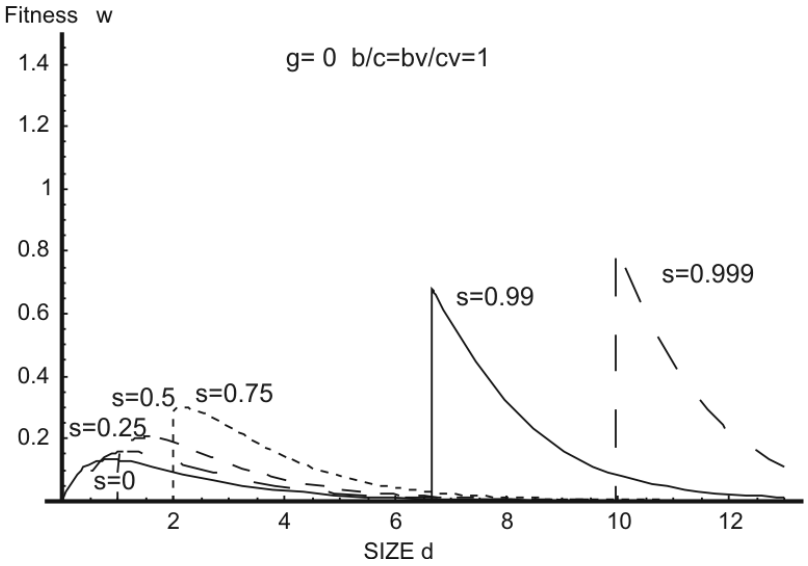
- All scaling exponents equal 1
- u_g =0.1



Fitness with size dependant mortality added: $w = \lambda v p$

$$p = 1 - z\,(n)^{-\mu}$$

$$z = 1,\ \mu = 0.25$$



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Specific Trade-off Investigated in Volvocales

In Volvocales, motility by flagellar beating is a major component of fecundity and viability, since volvocalean green algae need motility to reach light and nutrients, to avoid sinking, and enhance nutrient uptake.

Reproduction (Fecundity) vs Motility (Viability) as Size Increases

Flagellated Sterile Somatic Cells:	↑ Motiltiy	↑↓ Fecundity
Non-flagellated Reproductive cells:	↑ Fecundity	↑↓ Motiltiy

We argue that cell specialization evolved as a means to deal with the costs associated with the production of large multicellular colonies and their metabolic requirements.

Working Hypothesis

The increase in cell specialization observed in extant species as size increases can be explained in terms of the need for increased motility capabilities for self-propulsion and increased nutrient uptake.

Research Approach

1. To investigate this hypothesis we developed a model based on standard hydrodynamics that describes the physical factors involved in motility in these organisms (in collaboration Dr. Kessler).

2. We then measured the motility (self-propulsion) of the different colony types as well as the other variables used in the model.

3. To test the importance of collective flagellar beating on nutrient uptake, we designed experiments and theory that quantify the effect of the advective dynamics on colonies (in collaboration with Dr. Goldstein lab)

Motility as a proxy for Viability

Hydrodynamics Model

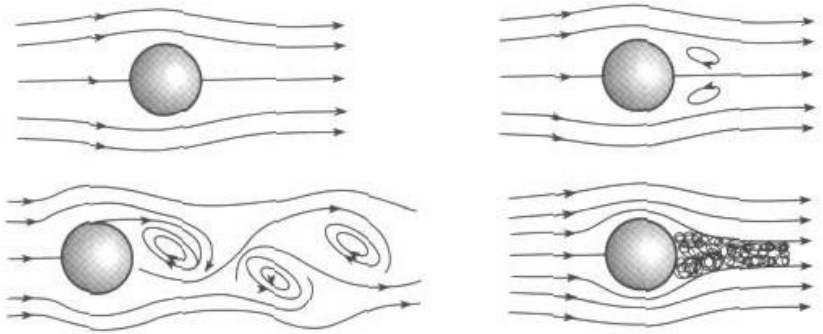
- Volvocalean algae colonies form small-diameter spheroids that swim at low velocities.
- Thus, colonies can be modeled as moving spheres in the low Reynolds number regime.

Re = $RV\rho / \eta < 1$
R = radius of colony
V = velocity
ρ = density of water
η = viscosity of water

e.g. **Large Volvox → Re = .25**
R = .05 cm
V = .05 cm/sec
ρ = 1 g/cm^3
η = .01 g/sec cm

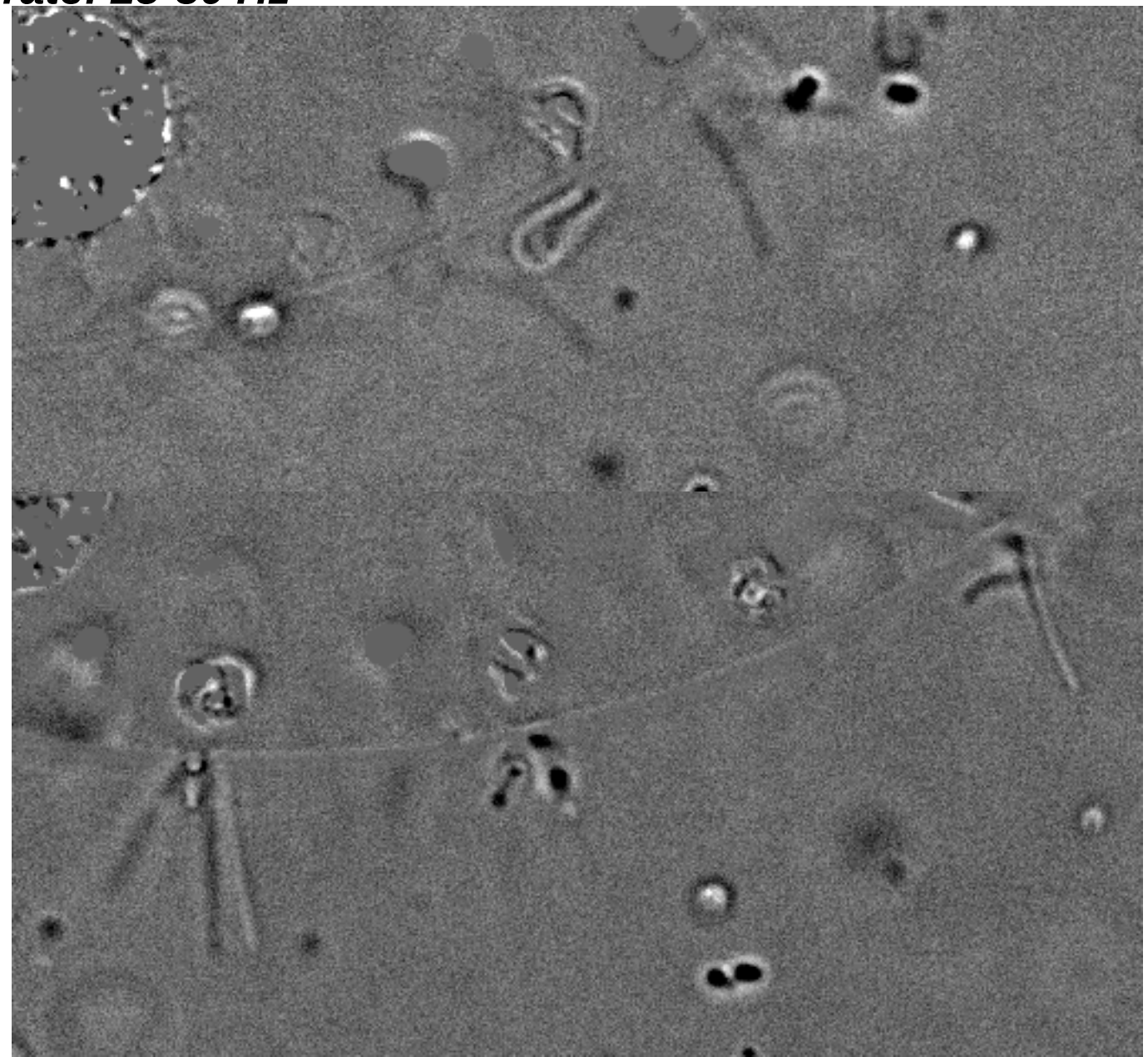
Nonlinearities can be neglected
The relationship between force and velocity is linear

Low Re



High Re

Flagellar beating of V. carteri somatic cells in slow motion.
Beating rate: 25-30 Hz



• At a low Reynolds number, a sedimenting sphere reaches a terminal velocity given by an equilibrium between the Stokes' law drag force and the effect of gravity.

R = radius of colony
V = velocity
η = viscosity of water
g = acceleration of gravity
ΔM = the difference in mass between the colony and the displaced water

$$6\pi\eta R V_{sed} = \Delta M g$$

• Within this framework, the force used by a colony that swims vertically upwards at a specific velocity is the sum of the force overcoming drag and the force of gravity.

Force used by a colony that swims vertically upwards →

$$Nf = 6\pi\eta R V_{up} + \Delta M g$$

Solving for V_{up} →

$$V_{up} = \frac{Nf - \Delta M g}{6\pi\eta R}$$

N = number of cells
f = average force/ flagellated cells

Solari et al (2006) AmNat

$$V_{up} = \frac{Nqf - g\Delta M}{6\pi\eta R}$$

N = number of cells
f = average force/ flagellated cells
q = proportion of flagellated cells

• Total swimming force is produced by the biflagellated cells distributed over the surface of the colony.

$$V_{up} = \frac{Nqf - g\Delta M}{6\pi\eta R}$$

• ***ΔM*** = the difference in mass between the cells and the displaced water.
• We assume that the extra cellular material does not contribute significantly to ***ΔM***.

$$\Delta M \approx \frac{4}{3}\pi\left[(1-s)r^3 + sr_S{}^3\right]\Delta\rho_C N$$

$\Delta\rho_C$ = difference in density between cells and water
r = radius of reproductive cells
r_s = radius of somatic cells
s = proportion of somatic cells

$$V_{up} = \frac{Nqf - g\Delta M}{6\pi\eta R}$$

• To calculate ***R***, we model flagellated cells as circles arrayed on the sphere surface, *A* being a cell concentration term that corrects for the intercellular surface area.

$$R \approx \frac{1}{2}\left[\left(1-\frac{s}{q}\right)r^2 + \frac{s}{q}r_S{}^2 + A\right]^{1/2} q^{1/2}N^{1/2}$$

A = intercellular space term

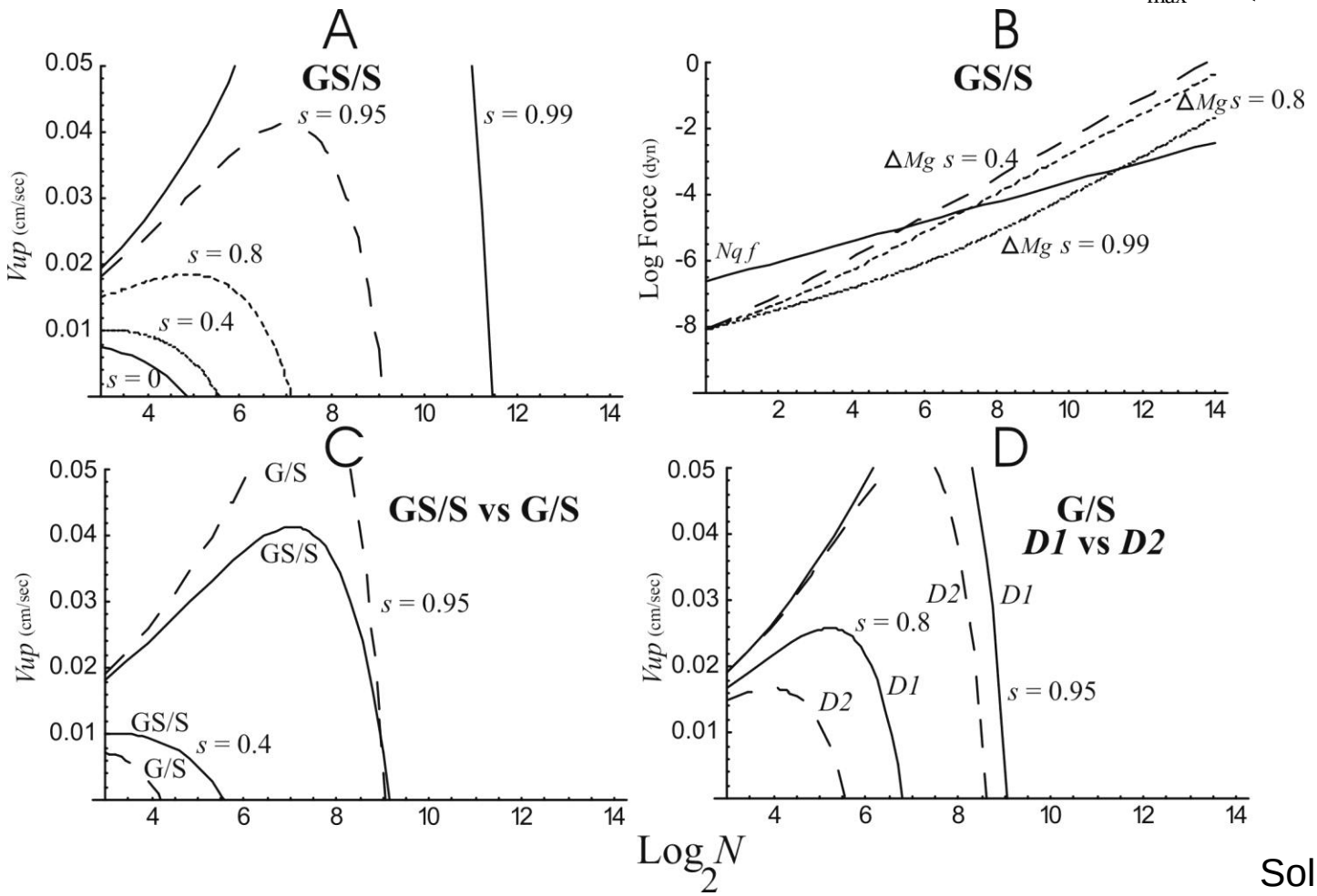
Solari et al (2006) AmNat

Four colony types

GS Undifferentiated colonies
GS/S Soma-differentiated colonies
GS/G Germ-differentiated colonies
G/S Germ-soma colonies

$$V_{up} = \frac{Nf - \Delta Mg}{6\pi\eta R} \longrightarrow V_{up} \approx \left(\frac{qf - g\frac{4}{3}\pi\left[(1-s)r_{\max}{}^3 + sr_S{}^3\right]\Delta\rho_C}{3\pi\eta\left[\left(1-\frac{s}{q}\right)r_{\max}{}^2 + \frac{s}{q}r_S{}^2\right]^{1/2} q^{1/2}} \right) N^{1/2}$$

To analyze the model we calculated the colony's swimming speed when the reproductive cells reach the size necessary to produce daughter colonies $\longrightarrow$ $r_{\max} \approx [(1-s)r_{in}{}^3 + sr_{Sin}{}^3]^{1/3} N^{1/3}$



***C.reinhardtii* data**

$\Delta\rho_C = 0.04$ g / cm^3
$r_{ch} = 3 \times 10^{-4}$ cm
$f = 2 \times 10^{-7}$ dyn
$r_s = r_{ch}$

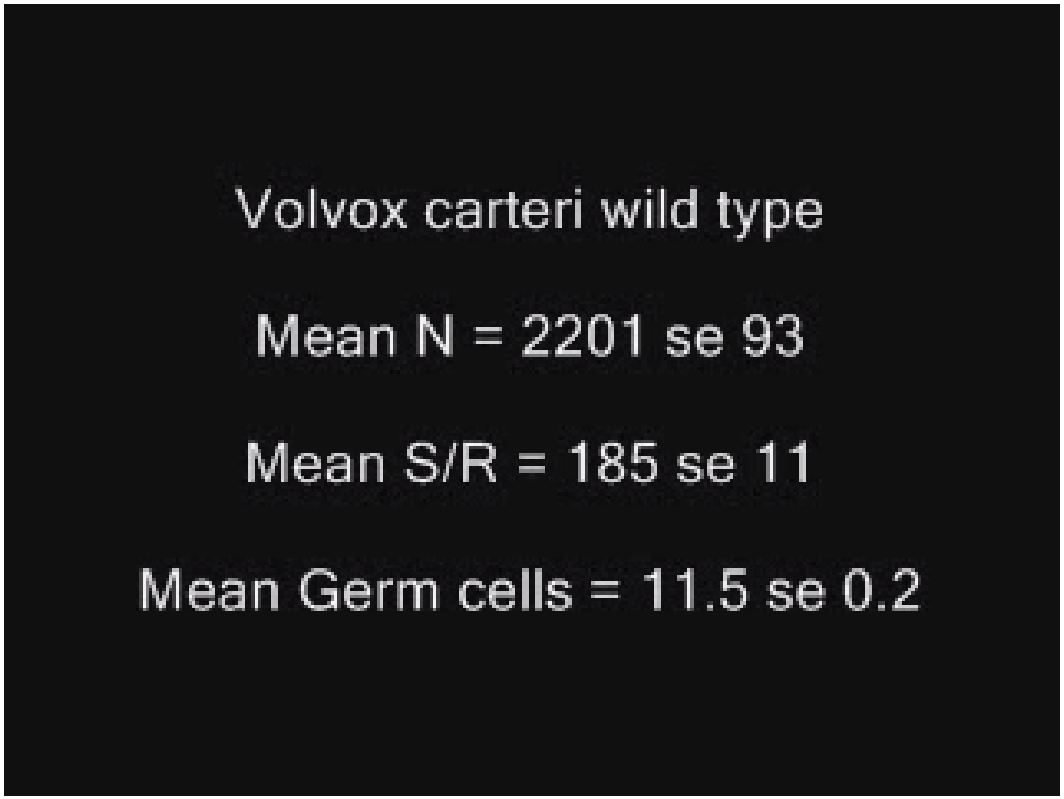
Solari et al (2006) AmNat

Algae were synchronized under standard laboratory conditions to measure swimming and sedimentation speeds

$$6\pi\eta RV_{sed} = \Delta Mg$$

$$Nf = 6\pi\eta RV_{up} + \Delta Mg$$

$$Nf = 6\pi\eta R(V_{sed} + V_{up})$$

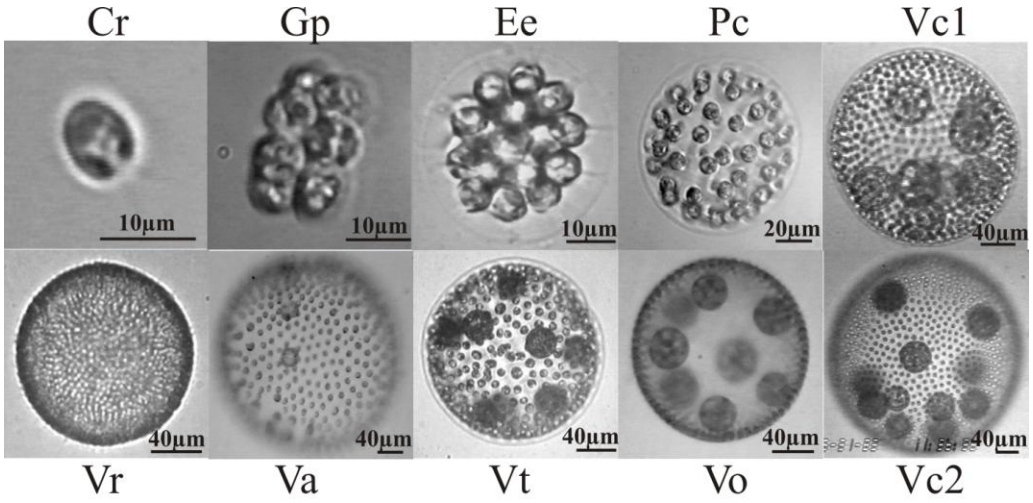


•Algae always swim upward in the dark (gravitaxis). They were videotaped in the dark using a light with an infrared filter.

•An optical bench was used for videotaping. Velocities and direction were calculated using Motion Analysis software.

Solari et al (2006) AmNat

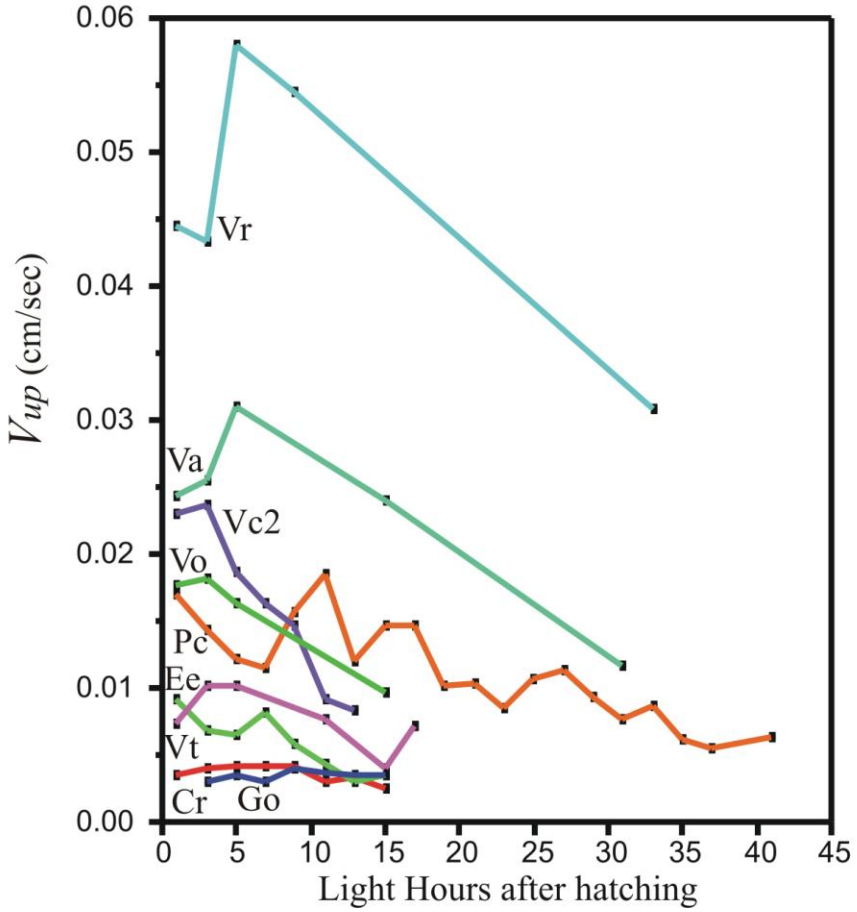
Swimming Speeds as Colonies Develop



- Just after hatching, the colonies of larger species (e.g. *Volvox*) tend to have higher swimming speeds than the colonies of smaller species (e.g. *Chlamydomonas* and *Gonium*).

- As species develop and their components enlarge, their swimming speeds decrease since their mass and radius increase.

- *V. rousseletii* is the fastest swimmer; it swims ten times faster than *C. reinhardtii*.

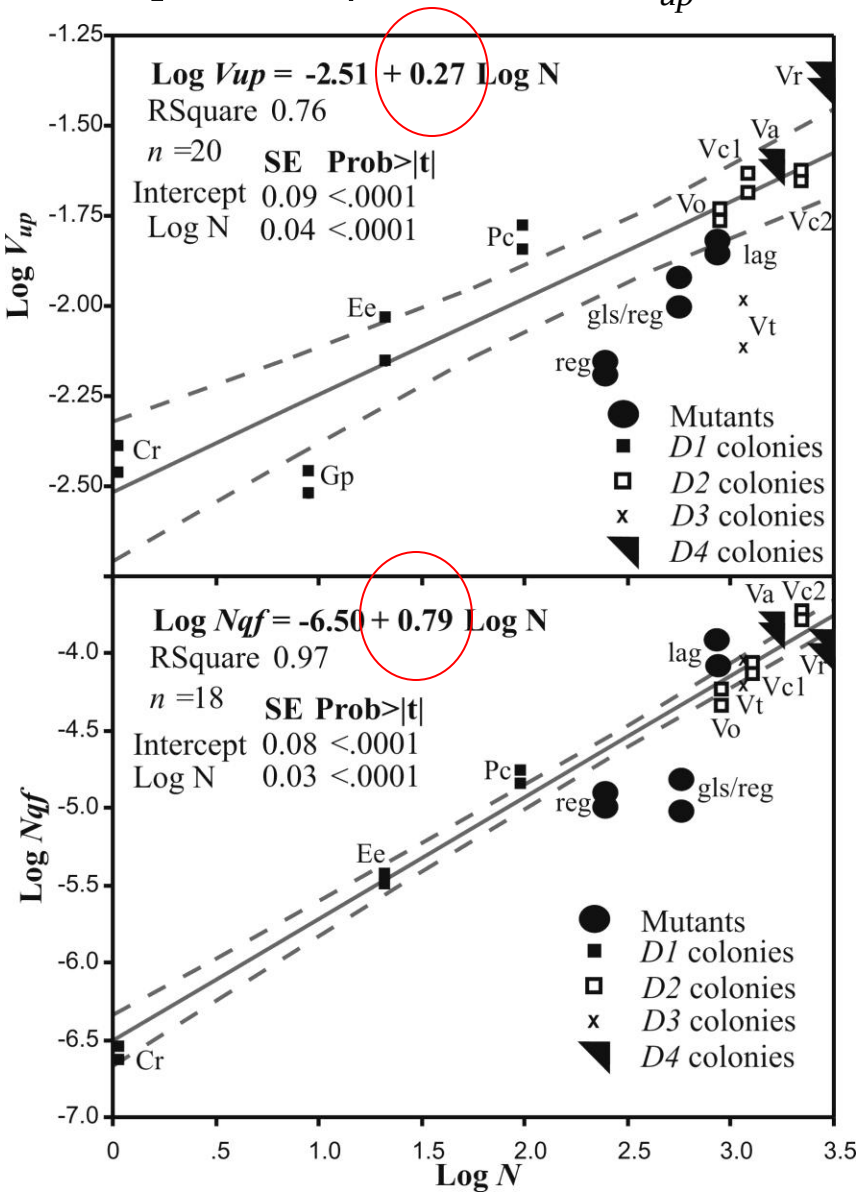


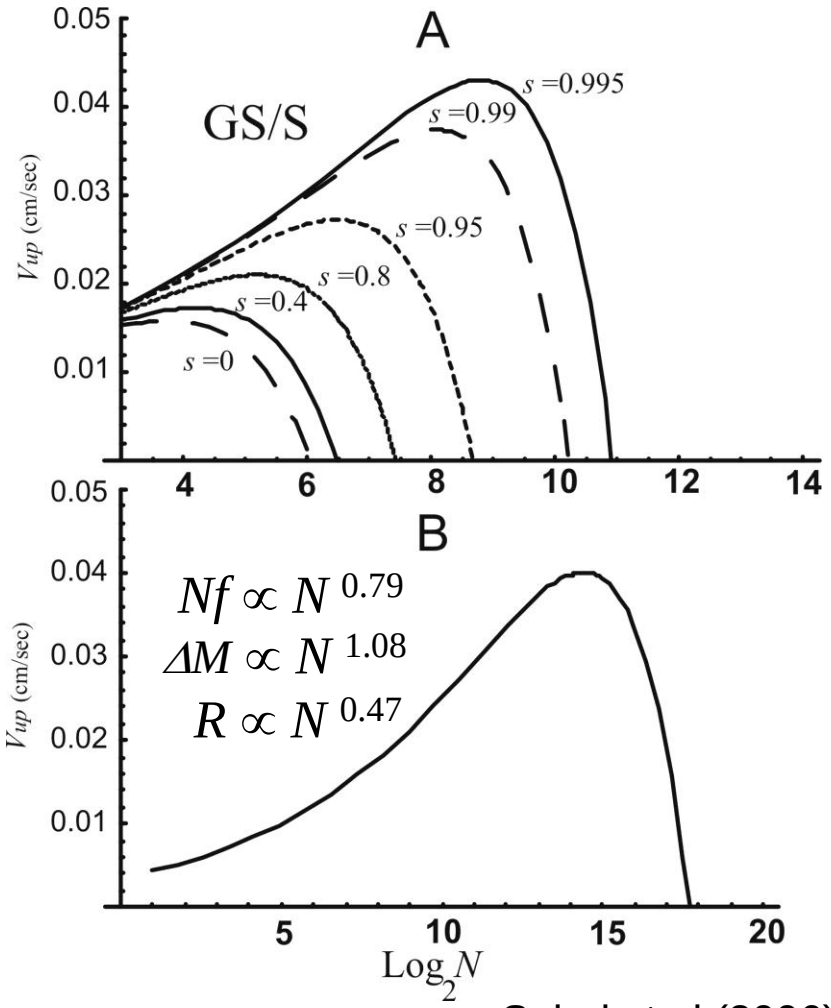
The main assumption of the model is that f is constant

Expected if f is constant: $V_{up} \propto N^{1/2}$

$$V_{up} = \frac{Nf - \Delta Mg}{6\pi\eta R}$$

$$f \propto N^{-0.21}$$





Solari et al (2006) AmNat

Back to the model: Motility as a proxy for Viability

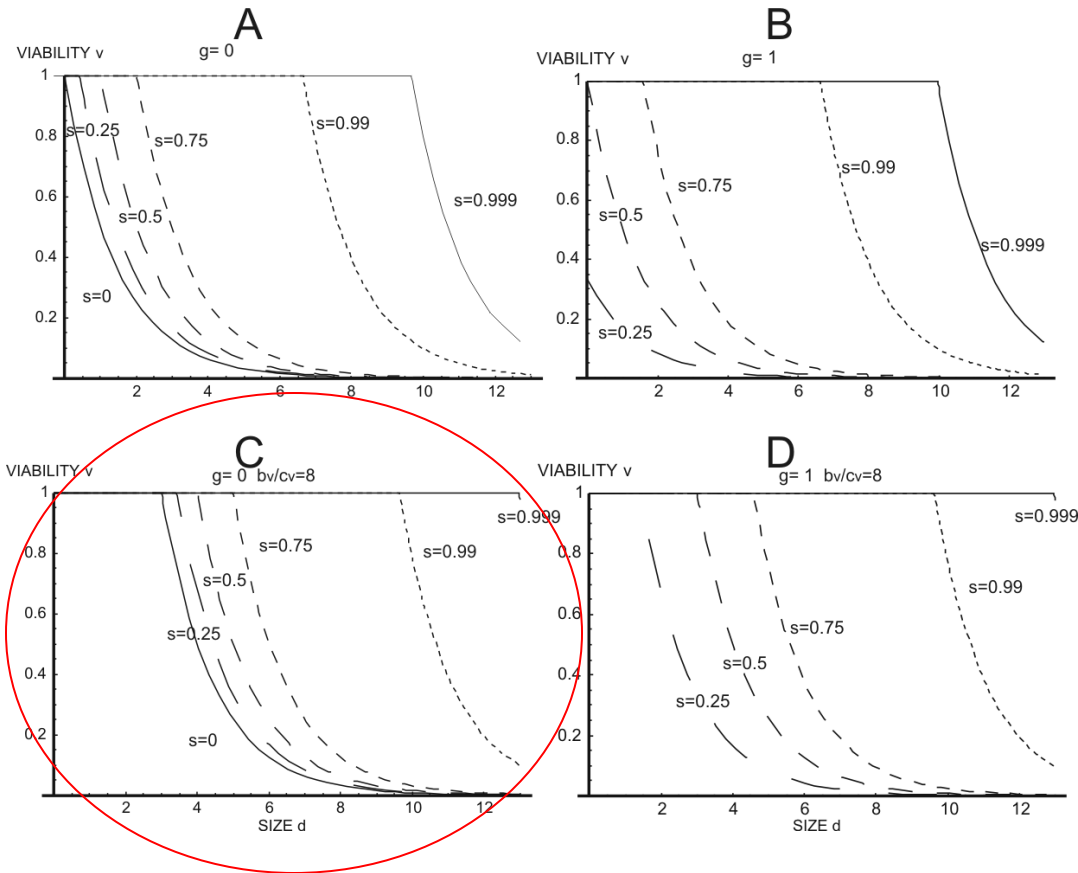
if $b_v B^\gamma / c_v C^\delta \geq 1 \longrightarrow v = 1 \longrightarrow$ **Viability needs are met.**

if $b_v B^\gamma / c_v C^\delta < 1 \longrightarrow v = b_v B^\gamma / c_v C^\delta$

Total Cost of Reproduction: $C \sim n^2 (1\text{-}s)$ **The negative gravitational force exerted by all cells**

Contribution to motility: $B \sim ns + n(1\text{-}s)(1\text{-}g)$ **The total flagellar force**

$$v = f_{ch} B^{3/4} / g_{ch} C$$



Solari et al (2013) in press AmNat

Growth rate *r* as size increases: Flagellar Mixing and Molecular Transport

$$\lambda = e^{r}(1-s)^{r/\mathrm{Ln}\, n}$$

In low Reynolds number regime transport is dominated by diffusion.

• But, vigorous boundary layer stirring and the flow associated with swimming can greatly increase transport rates by advection and mixing of molecules.

• The relative importance of these processes can be evaluated by a ratio of time constants for diffusion and advection called the Peclet number (*P*).

$$t_{diff} = \frac{L^2}{D}$$

$$t_{adv} = \frac{L}{V}$$

$$P = \frac{t_{diff}}{t_{adv}} = \frac{LV}{D}$$

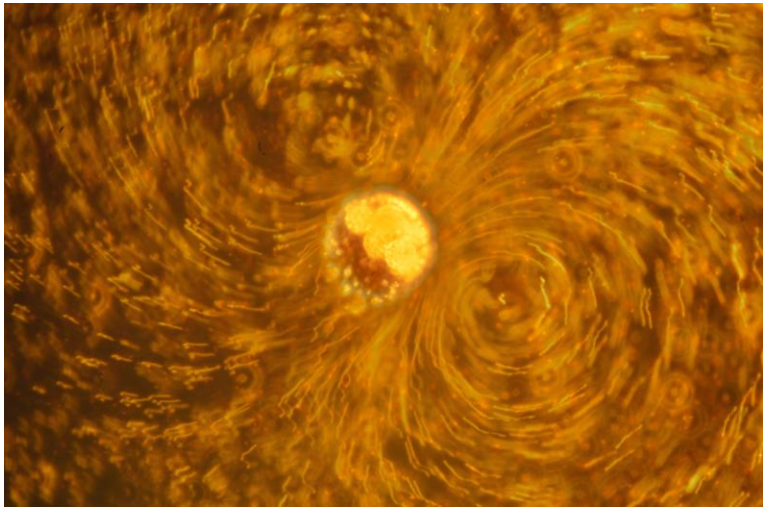
Chlamydomonas	***Volvox***
L = 0.0005 cm	L = 0.02 cm
V = 0.005 cm/sec	V = 0.02 cm/sec
***P* = 0.25**	***P* = 40**
Diffussion dominates	***Advection dominates***

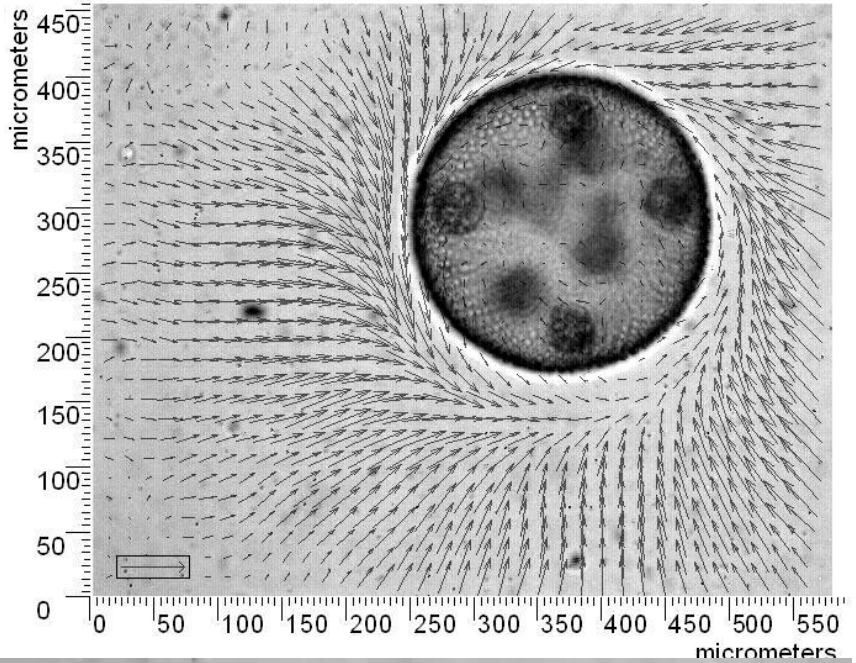
L = characteristic length (colony radius)
V = characteristic velocity (swimming speed)
D = diffusion coefficient ~ 10^{-5} cm^2/sec for O_2

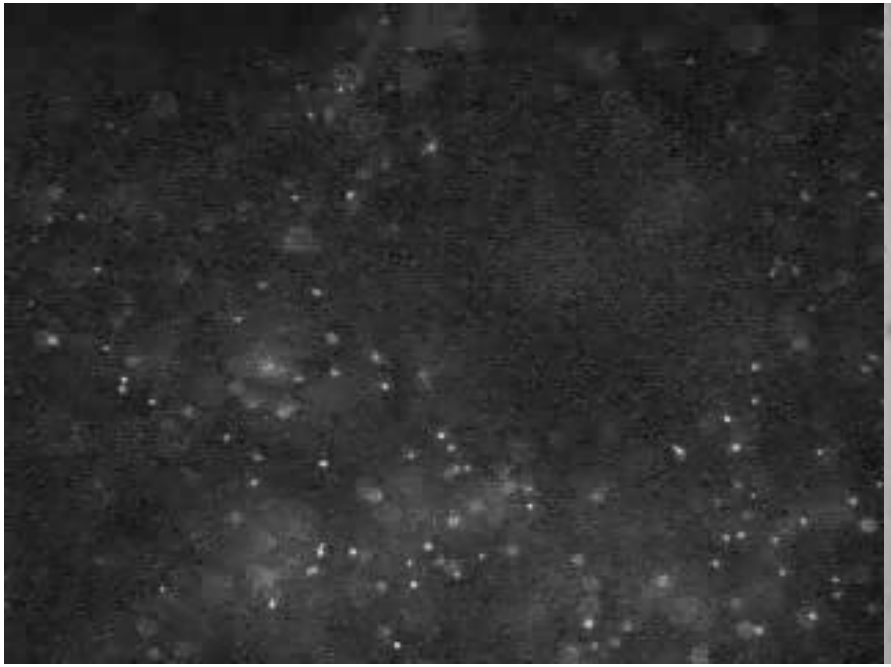
Solari et al (2006) PNAS
Short et al (2006) PNAS

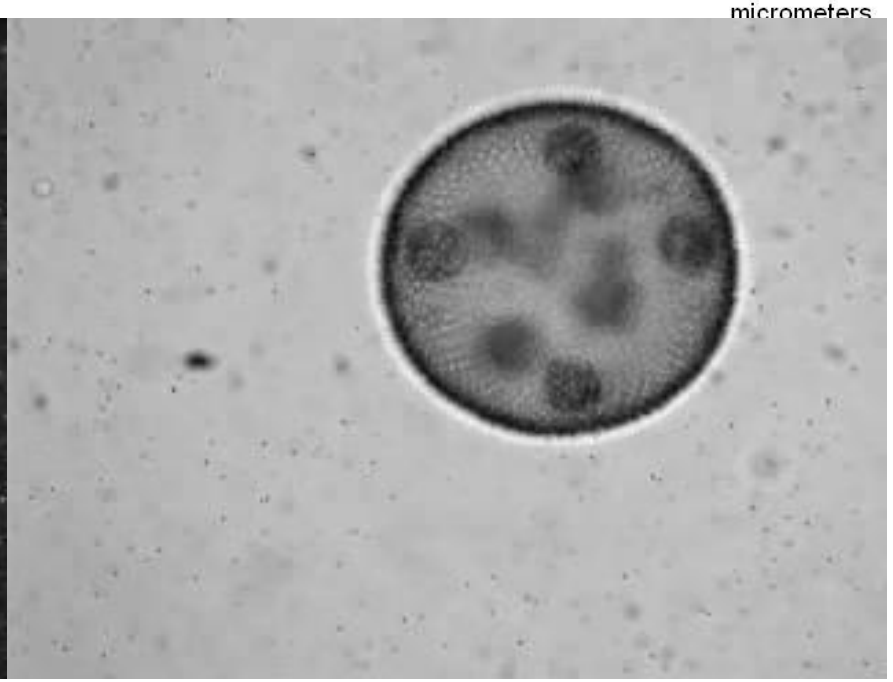
Collective flagellar beating is also important for nutrient uptake

Vector field calculated with Particle Image Velocimetry software





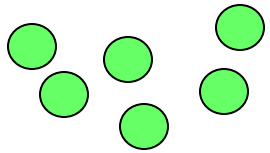




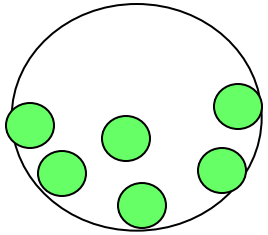
Does advection affects productivity?

• We compared the growth rate of *V. carteri* germ cells liberated by breaking the colonies apart, in deflagellated colonies, and in normal colonies in still and stirred medium, with and without the presence of a flagellar regeneration inhibitor (Colchicine).

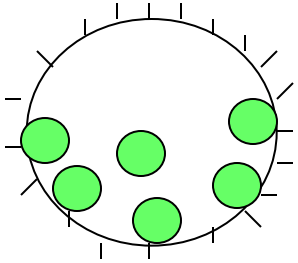
Broken Colonies



Deflagellated Colonies



Flagellated Colonies



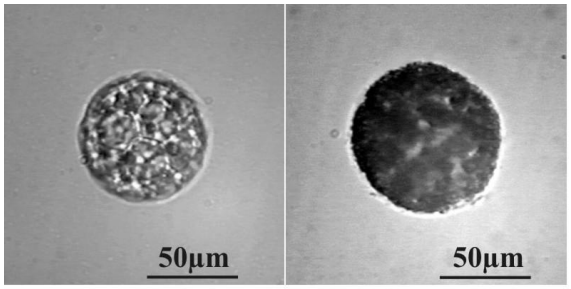
• Colchicine binds to tubulin and prevents polymerization of the microtubules.

• When colonies are deflagellated and flagellar regeneration is inhibited (flagellar mixing prevented) we expected a decrease in the growth rate of the germ cells of these colonies placed in still medium.

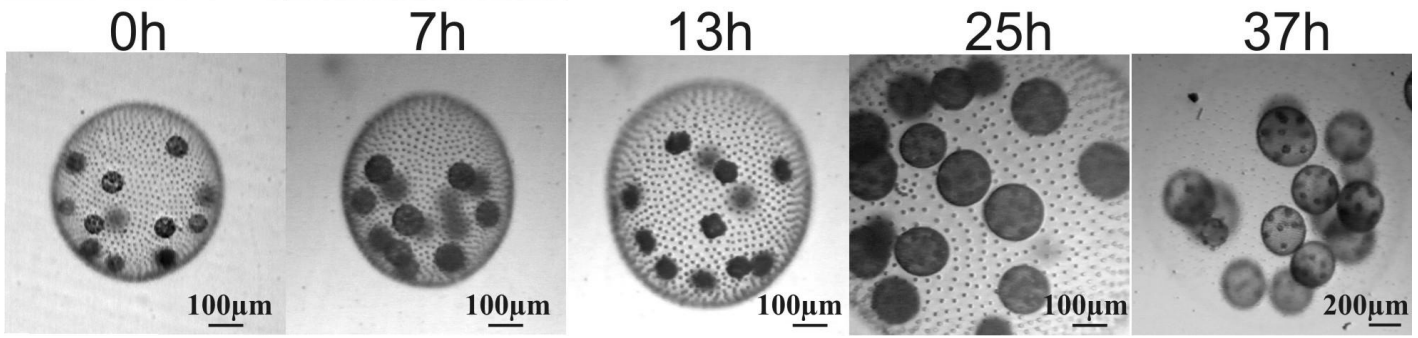
• We expected this negative effect to disappear when placing those permanently deflagellated colonies in a turbulently mixed medium (artificially mixed by bubbling).

Solari et al (2006) PNAS

Experiment



Measured the diameter increase of germ cells



48h Generation Time (16h light/ 8h dark cycle)

	Still medium (S)			Bubbling medium (B)		
	Flagellated (F)	Deflagellated (D)	Broken (L)	Flagellated (F)	Deflagellated (D)	Broken (L)
Without Inhibitor	*FS*	*DS*	*LS*	*FB*	*DB*	*LB*
With inhibitor (I)	*FIS*	*DIS*	*LIS*	*FIB*	*DIB*	*LIB*

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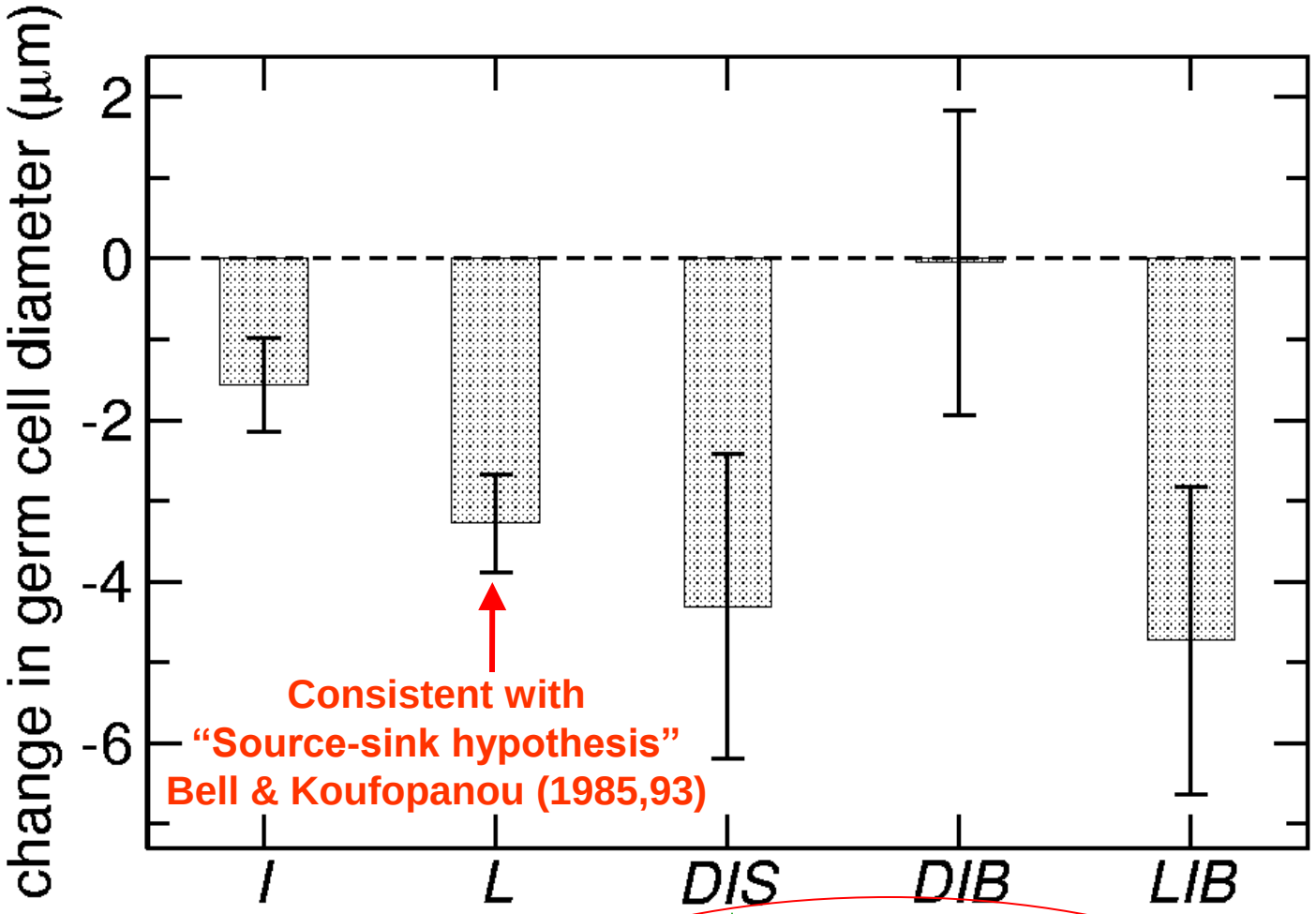
Effects of Forced Stirring



Inhibitor of flagella regeneration

Liberated germ cells

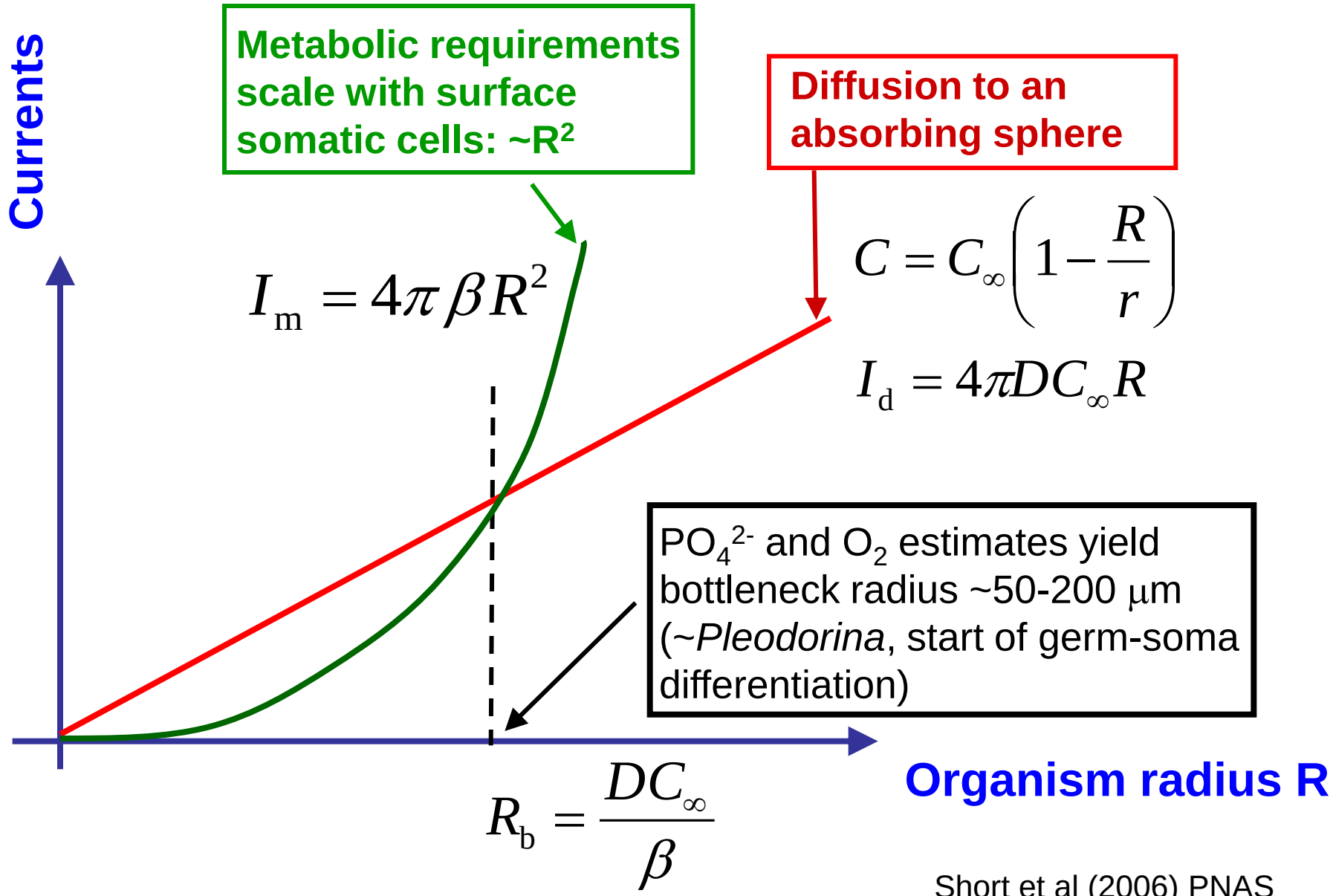
Deflagellation **+I**nhibitor **S**till medium

Deflagellation **+I**nhibitor **B**ubbled medium

Liberated **+I**nhibitor **B**ubbled

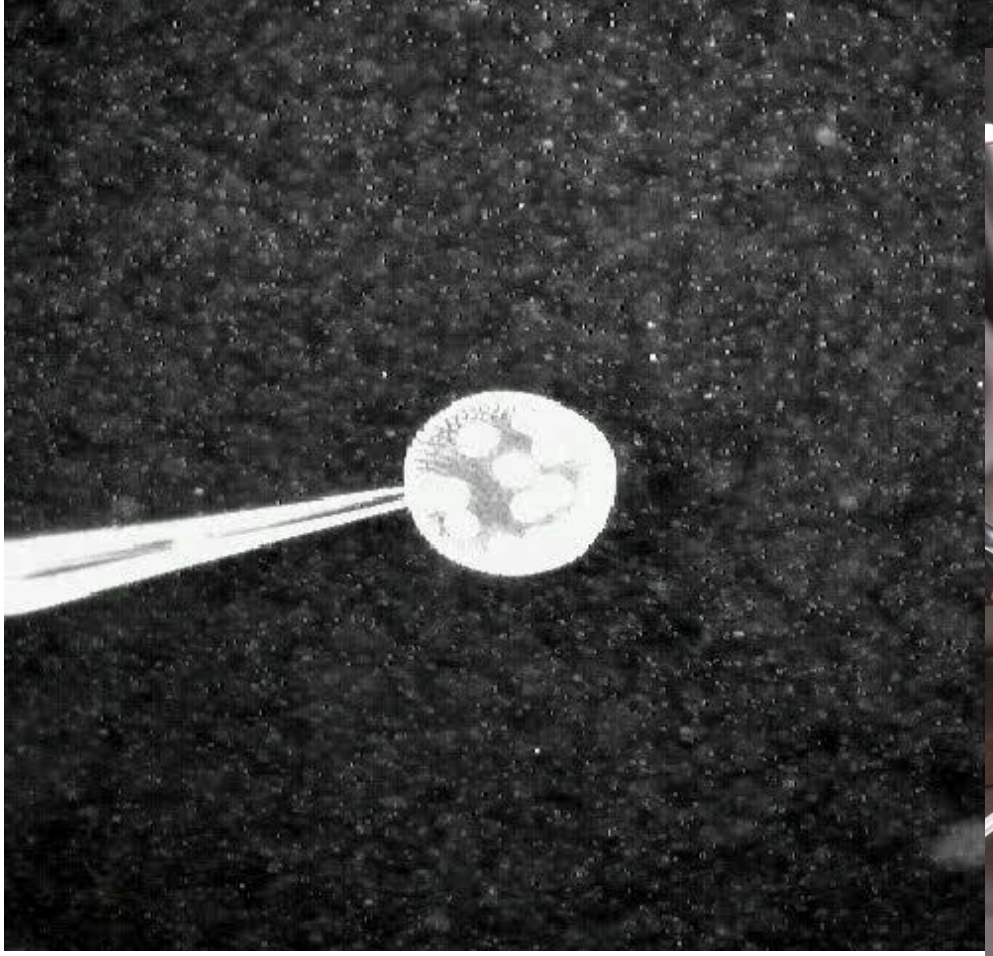
Solari et al (2006) PNAS

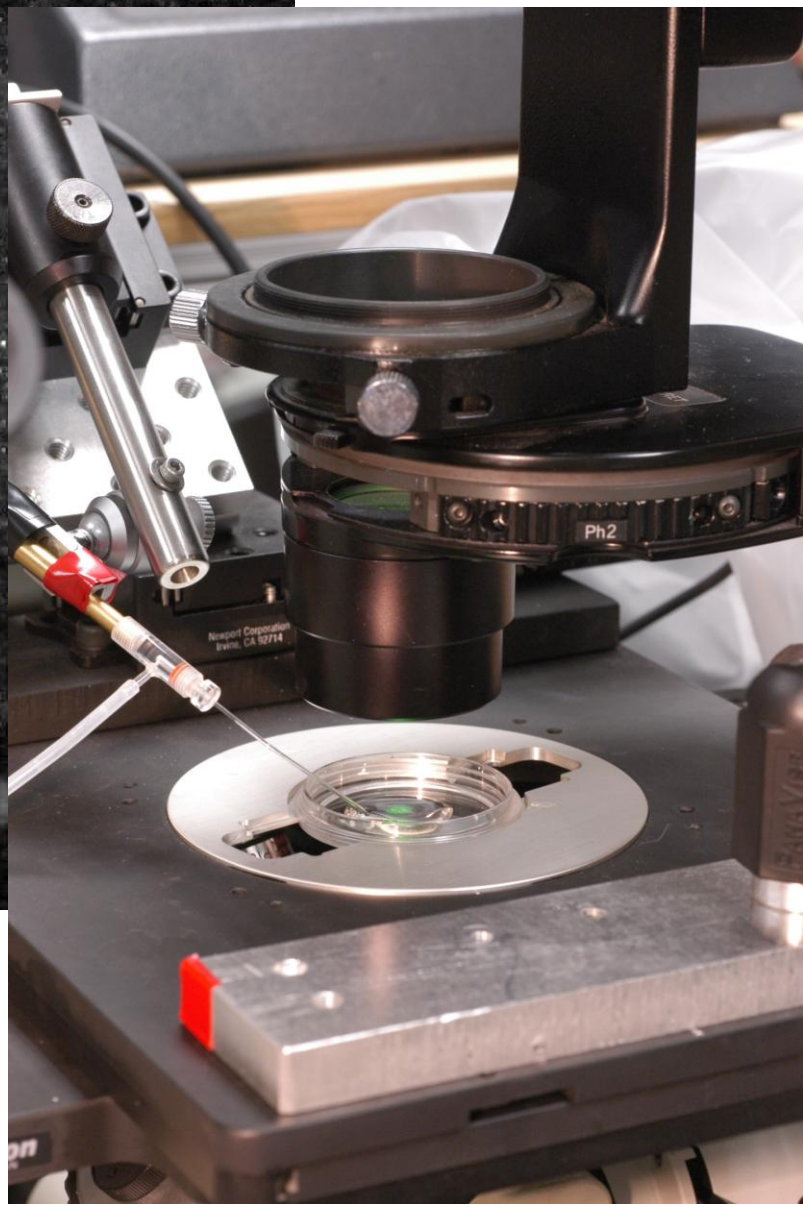
Advection Currents are Important to Circumvent the Diffusional Bottleneck



Short et al (2006) PNAS

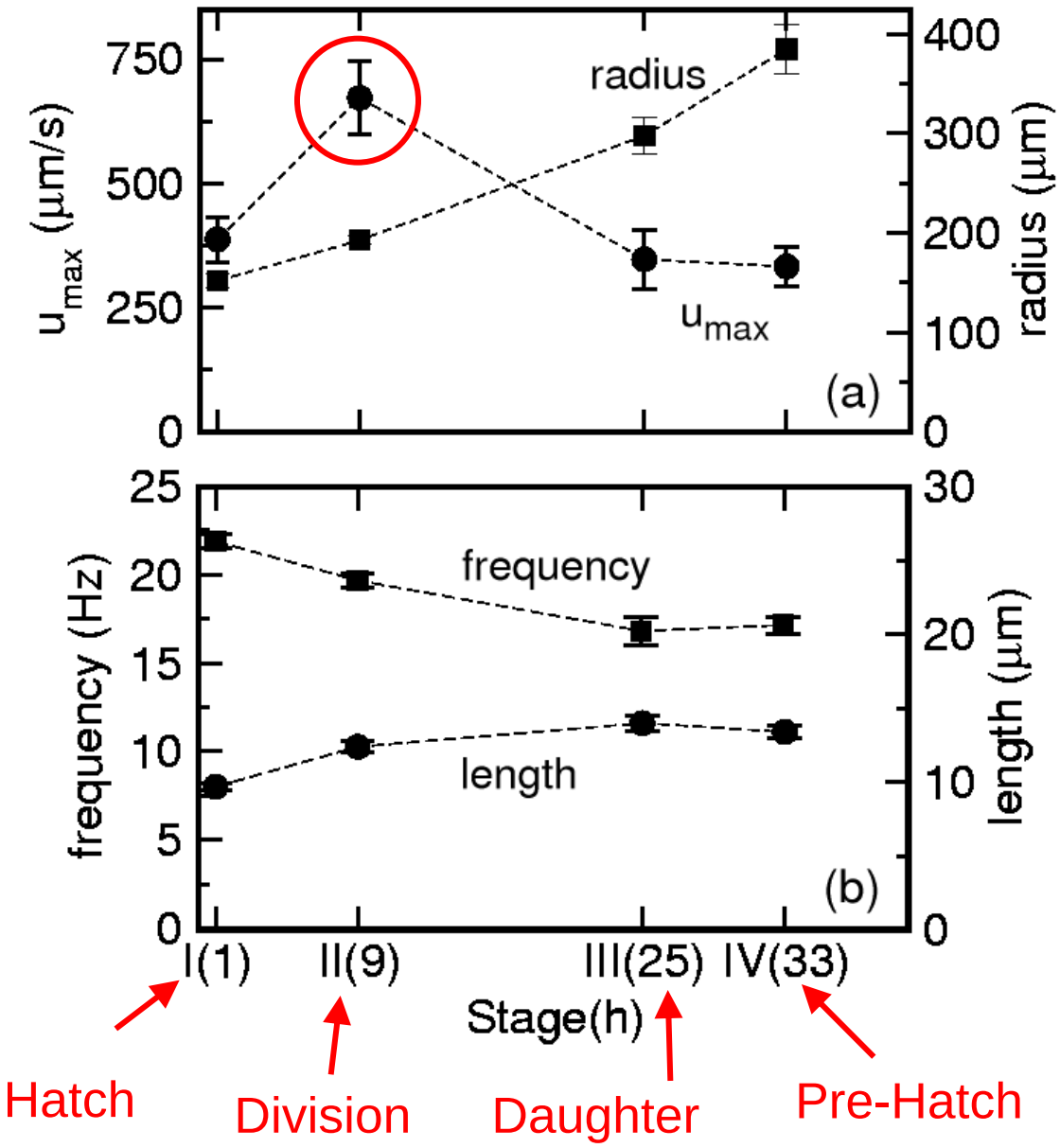
Pseudo-darkfield (4x objective, Ph4 ring)



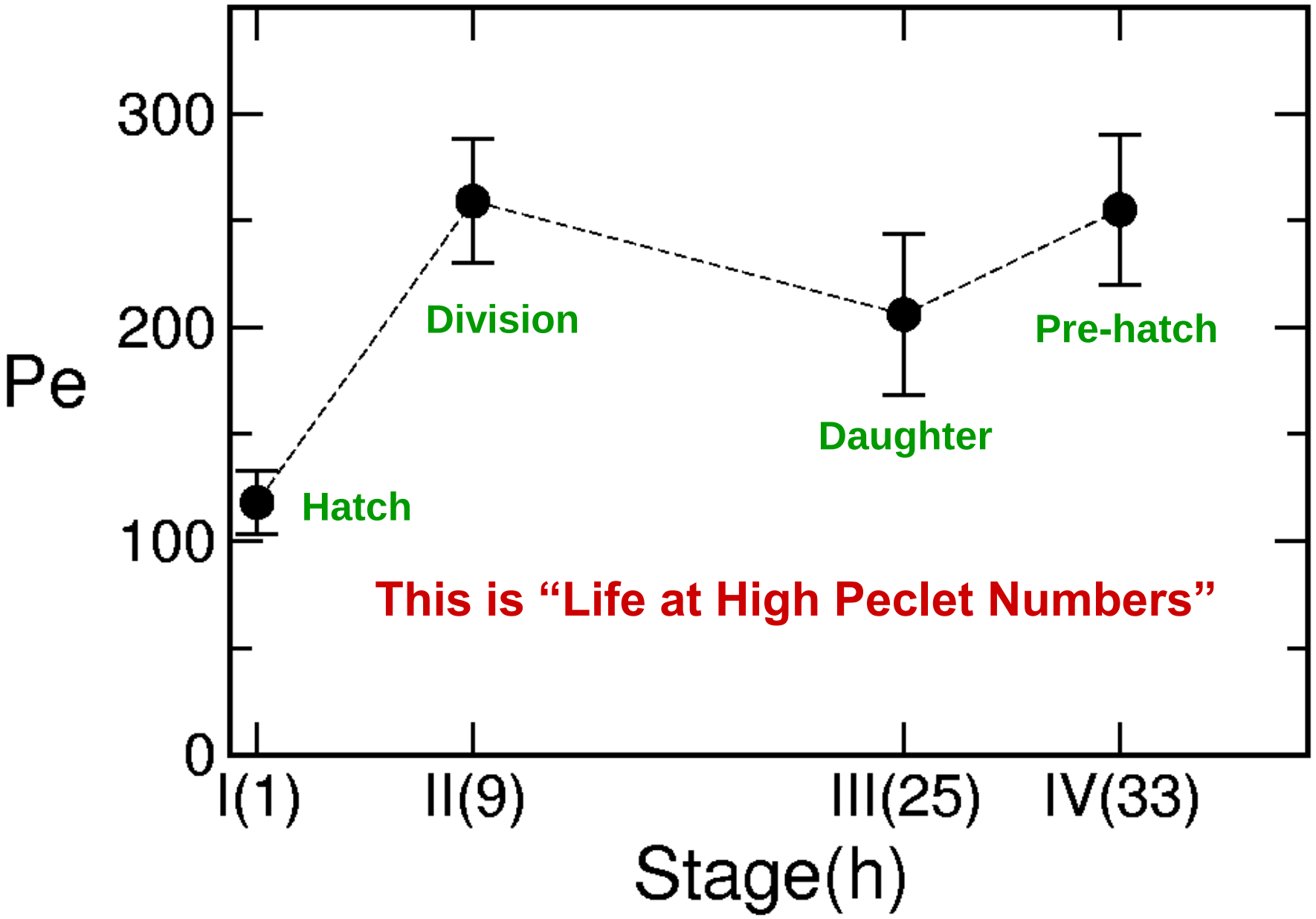


micropipette

Fluid flow rates during life cycle



Peclet Number During Life Cycle



Back to the Model:Fecundity rate as size increases

$B \sim ns + n(1-s)(1-g)$

$I_a \sim R\, Pe^{1/2}$ (Short et al 2006)

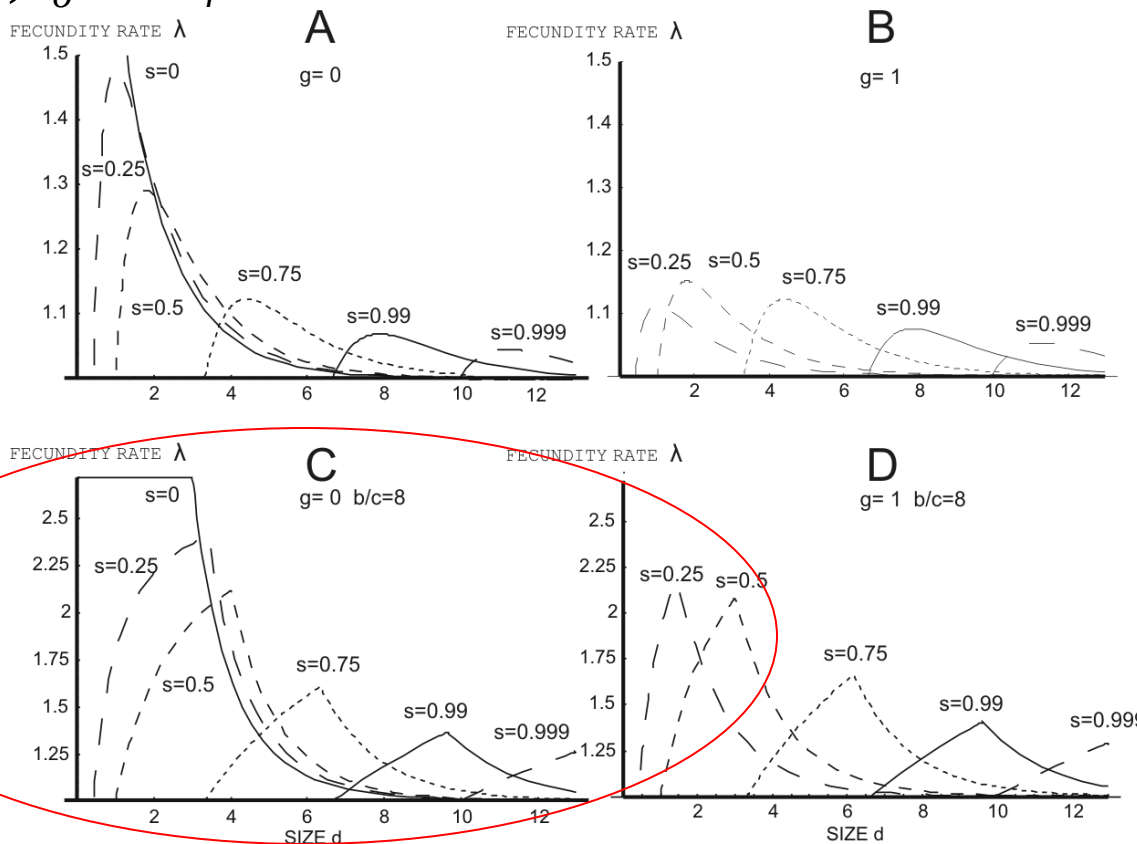
$I_a \sim B^{1/2} (B^{1/2} B^{1/2})^{1/2} \sim B$

$$B/C_r = bB/cC$$

if $B/C_r \geq 1 \longrightarrow r = (1+u_g g) r_o \longrightarrow$ **Supply meets Demand**

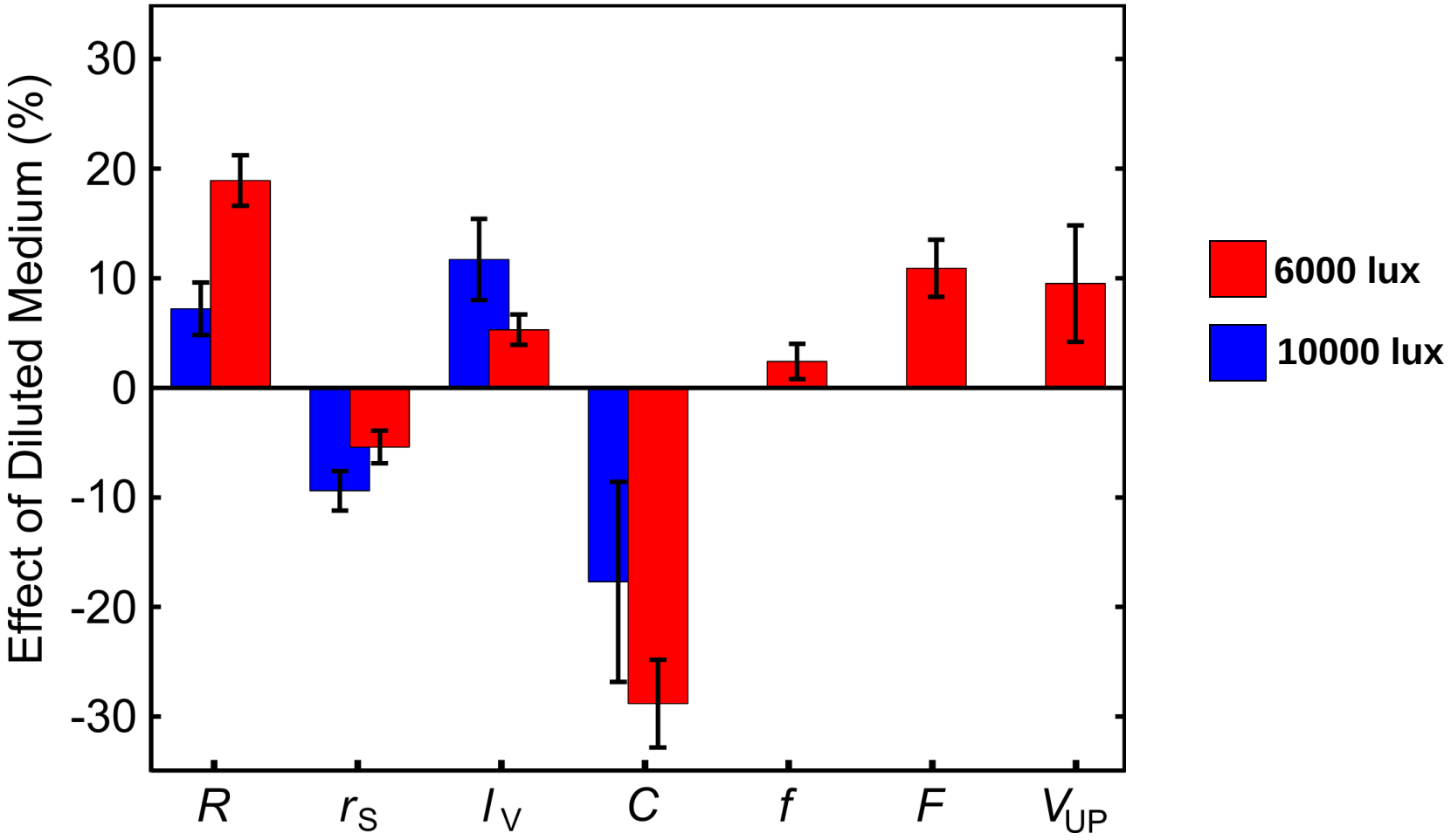
if $B/C_r < 1 \longrightarrow r = (1+u_g g) r_o B/C_r$

$$\lambda = e^{r}(1-s)^{r/\mathrm{Ln}\, n}$$



Solari et al (2013) in press AmNat

Phenotipic plastic response to the deprivation of nutrients in *V. carteri*



No phenotipic plastic response was found in unicellular *C. reinhardtii* or in 1-16 celled colonial *G. pectorale*

Solari et al (2011) Royal Society Interface

Conclusions

- Using life-history theory and allometry, we have produced a model inspired by the volvocine green algae that describes the dynamics of the emergence of germ-soma differentiation as size increases in multicellular organisms.

- The results of the model show that the cost of reproducing an increasingly larger group has likely played an important role in the evolution of complexity and individuality in the transition to multicellularity.

- The trade-offs between fecundity, viability, and size recently studied in Volvocales show in detail how metabolic and viability constraints as colonies increase in size might be strong enough to push the organism design to cellular specialization and higher complexity.

Conclusions (cont.)

- Flagellar motility constraints and opportunities were important driving forces for the evolution of germ-soma separation in this group.

- When colony size exceeds a threshold, a specialized and sterile soma must evolve to keep colonies buoyant and motile and enhance nutrient uptake.

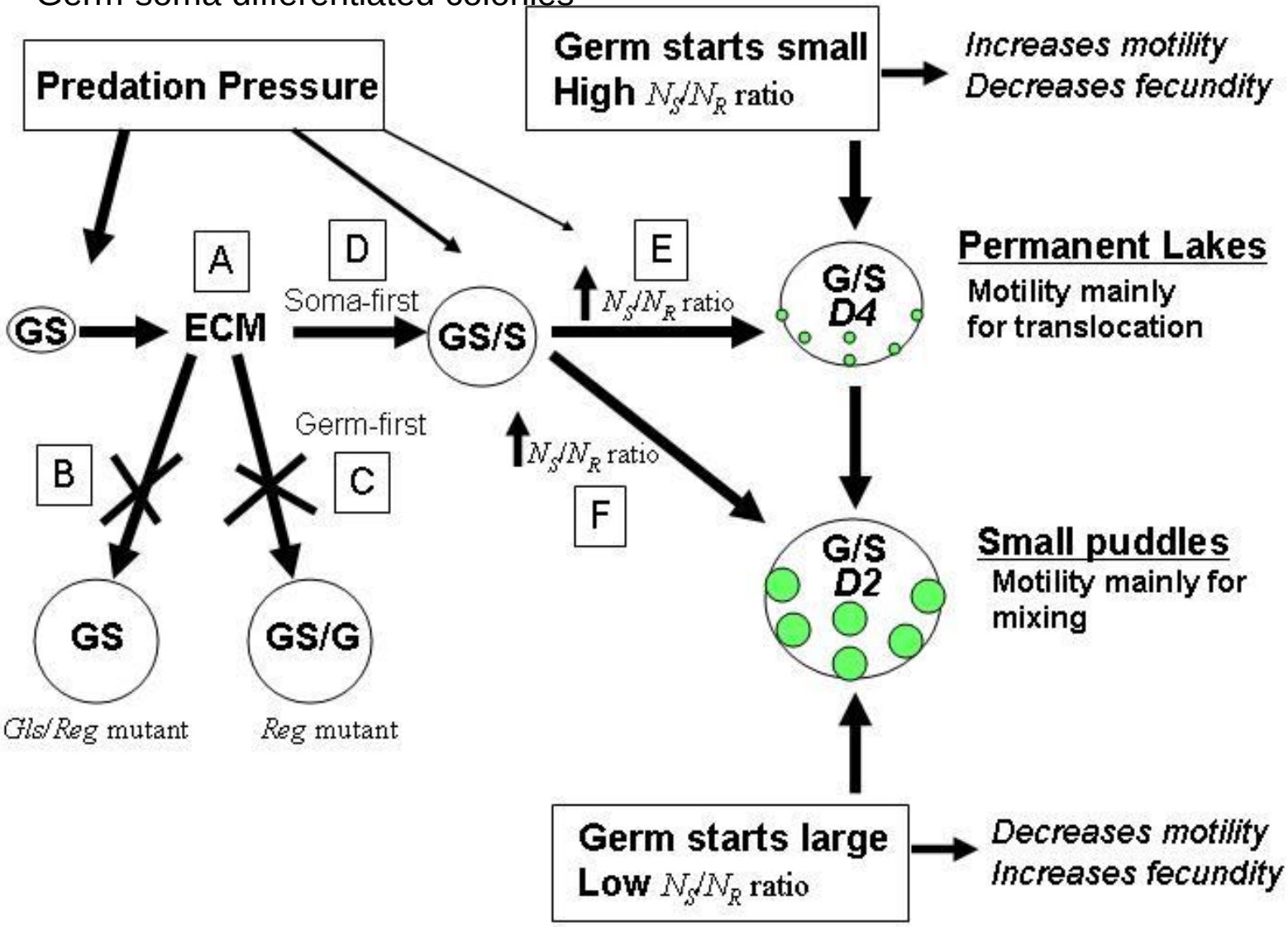
- As colony size increases further, the somatic to reproductive cell ratio must increase to circumvent the motility and nutrient constraints.

- A high proportion of somatic cells allows the germ cell to specialize in reproductive functions.

Four colony types

- **GS** Undifferentiated colonies
- **GS/S** Soma-differentiated colonies
- **GS/G** Germ-differentiated colonies
- **G/S** Germ-soma differentiated colonies

Hypothetical Scenario



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Goldstein Lab in Arizona

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Luis Cisneros

Relevant Papers

Michod R.E., Viossat Y., **Solari C.A**., Nedelcu A.M. and Hurrand M. (2006). Life history evolution and the origin of multicellularity**.** *Journal of Theoretical Biology*, 239: 257-272.

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