The calcification process and measurement techniques

Photo from JC073 Changing Oceans Research Cruise

What is calcification?

- The accumulation of calcium salts into body tissue, such as bones, shells, tubes and carapaces. A process by which organisms precipitate calcium carbonate.
- A biologically-mediated process
- In marine calcifiers, calcification predominantly results in calcium carbonate structures that are made of either calcite, aragonite or high-Mg calcite.

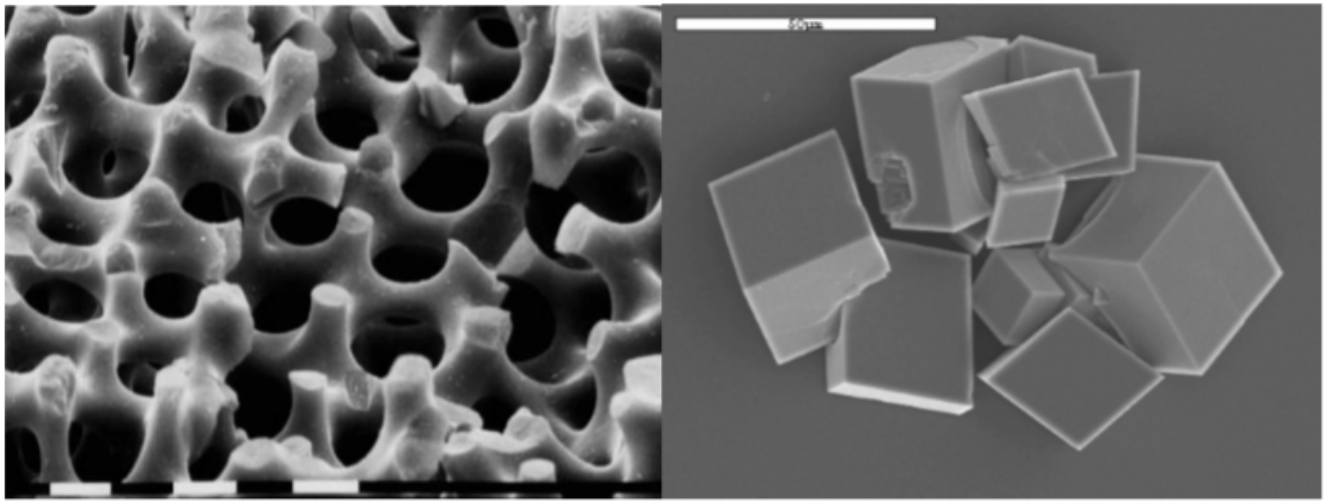


Figure 1. Comparison of calcite single crystals: (*left*) stereom of echinoderm and (*right*) synthetically produced rhombohedral forms.

What is calcification?

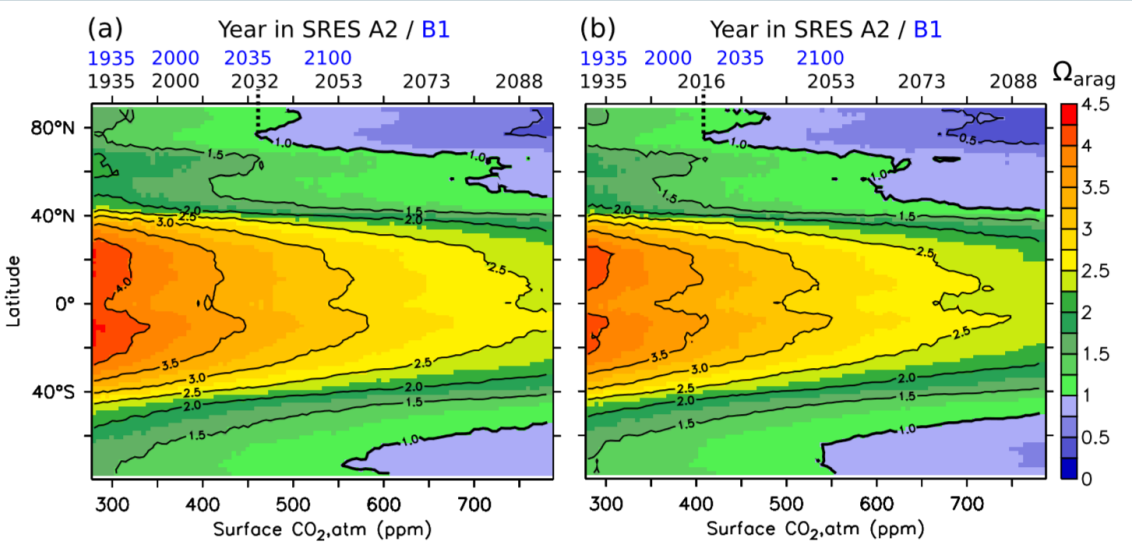
$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + CO_2 + H_2O$$

Saturation State – degree to which seawater is saturated (or not) with relevant ions; provides a measure of the thermodynamic potential for the mineral to form or to dissolve

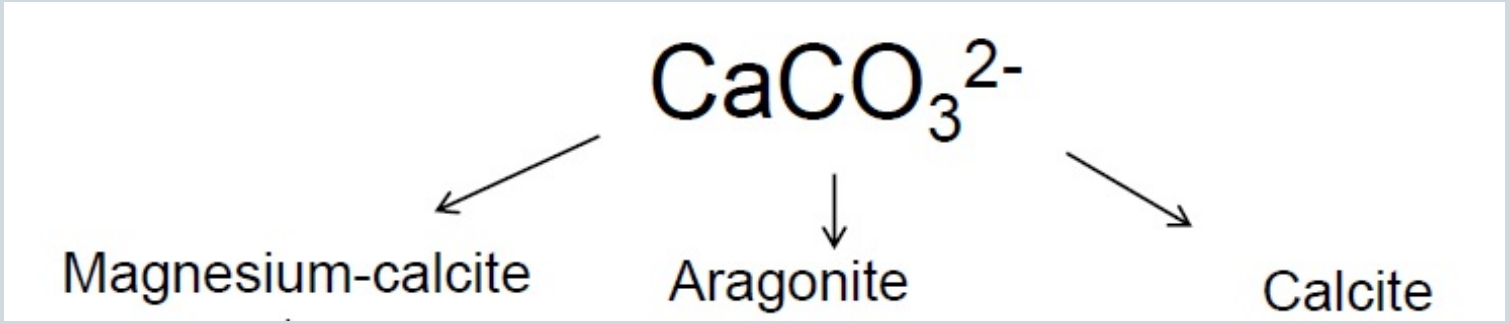
$$\Omega = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp'}}$$

$\Omega > 1$ Supersaturated with respect to $CaCO_3$

$\Omega < 1$ Undersaturated with respect to $CaCO_3$ (dissolution)



Steinacher et al. 2009



Understand how pH at the site of calcification changes in response to changes in the external seawater environment

How marine calcifying organisms will respond

Who calcifies, and how?

Major invertebrate calcifying groups:
- Molluscs
- Cnidarians
- Echinoderms
- Crustaceans
- Polychaetes

Other organism types:
- Foraminifera
- Phytoplankton: Haptophytes (coccolithophores)
- Algae: Rhodophytes (coralline algae)

In most biological systems, the **site of mineral deposition is isolated** from the environment, the extent of isolation is variable.

Biologically induced mineralisation – organism uses cellular activities to direct the nucleation, growth, morphology, and final location of the mineral that is deposited. Several types, but most $CaCO_3$ forming marine organisms either use an **extracellular** biologically-controlled process or an **intracellular** strategy.

Who calcifies, and how?

Extracellular biologically-controlled process e.g. Molluscs, Corals,

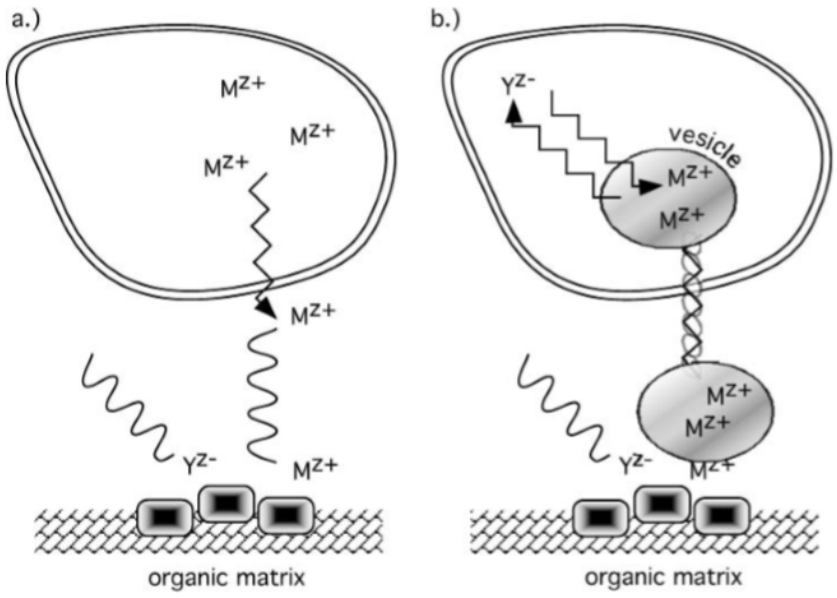
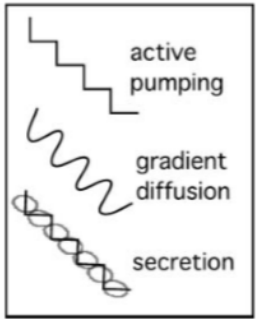


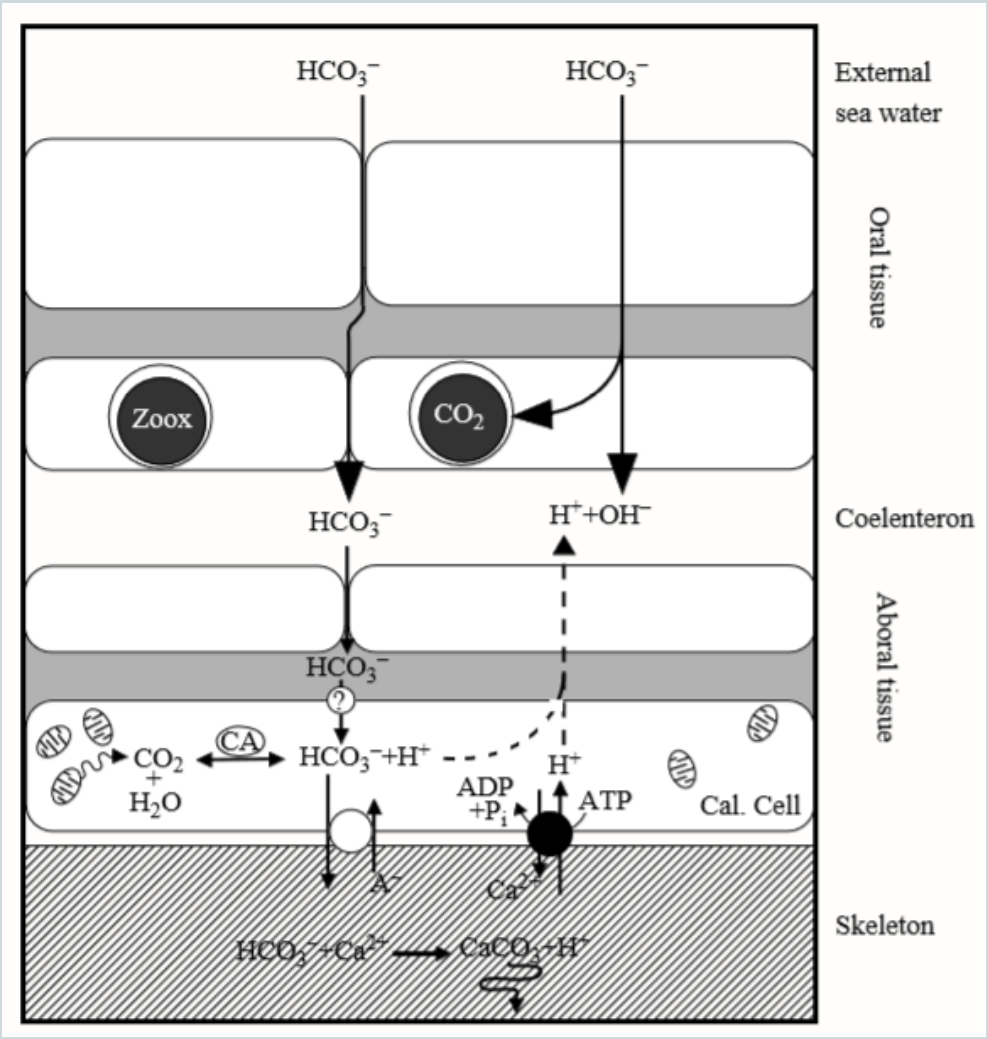
Figure 5. Illustrations of biologically controlled extracellular mineralization showing that this process is distinguished by nucleation outside of the cell. a.) Cations are pumped across the cell membrane and move by passive diffusion through extracellular fluids to the site of mineralization. b.) Cations are concentrated intracellularly as aqueous ions into a vesicle that is subsequently secreted. Compartment breakdown at site of mineralization releases cations for biomineral formation.



- Basic form of calcification
- Organic matrix important for defining structure
- Ions can be actively pumped out of the cell *or* pumped into a vesicle within the cell which is then secreted outside.

Who calcifies, and how?

e.g. Corals

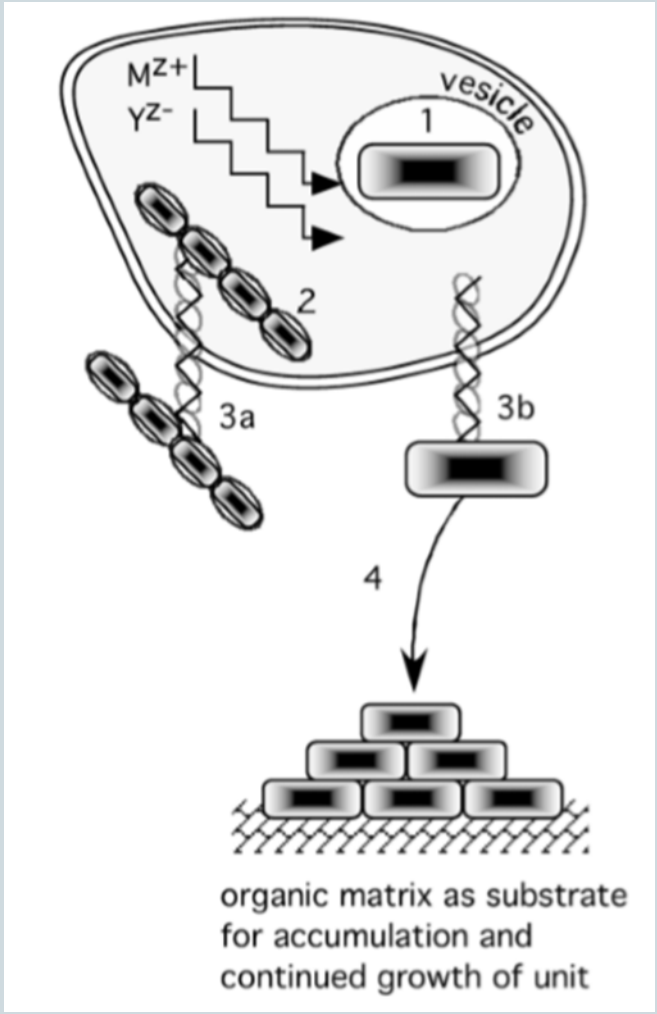


- Model of dissolved inorganic carbon (DIC) absorption for coral calcification and photosynthesis.
- Extracellular space has controlled pH environment
- Anion exchange pumps are utilised for control

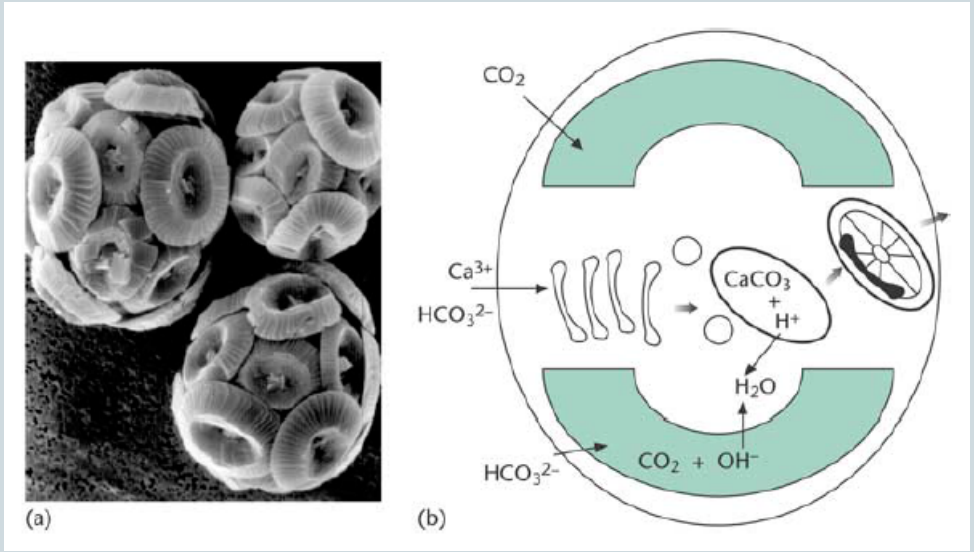
Furla et al. 2000

Who calcifies, and how?

Intracellular strategy. E.g. Echinoderms (urchins), coccolithophores…

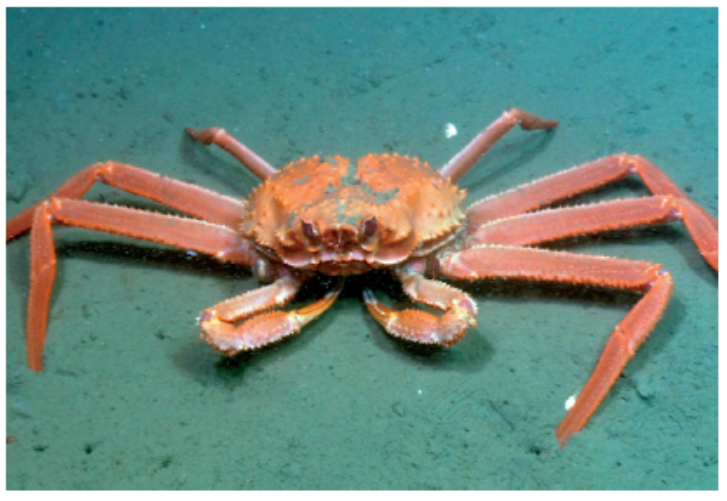


- Can form huge mineralised products within a vesicle that is the product of many cells fusing their membranes.
- Mineral is exposed to the environment only when the membrane is degraded.



Browlee & Taylor 2002

Who calcifies, and how?



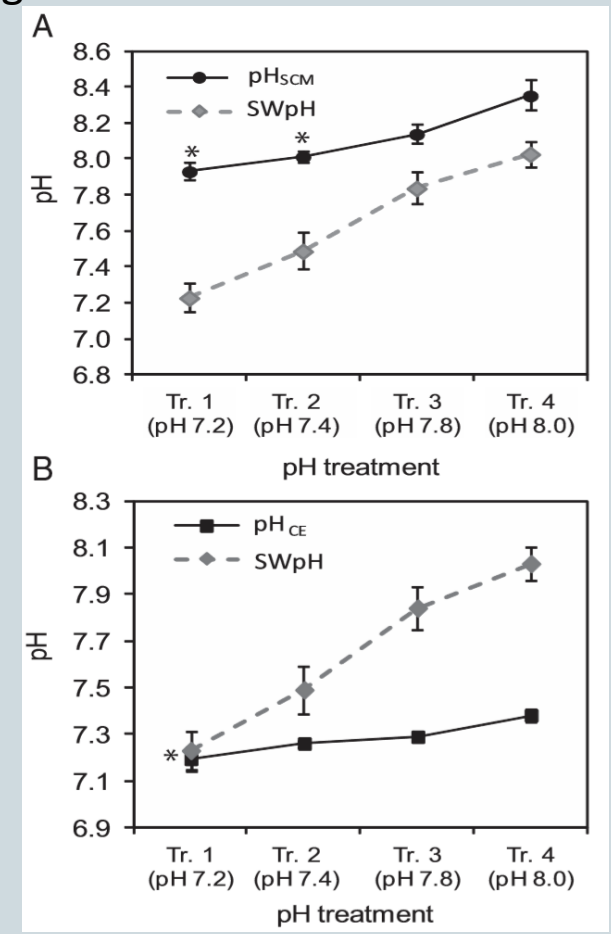
Pane & Barry 2007; Photo MBARI (2006)

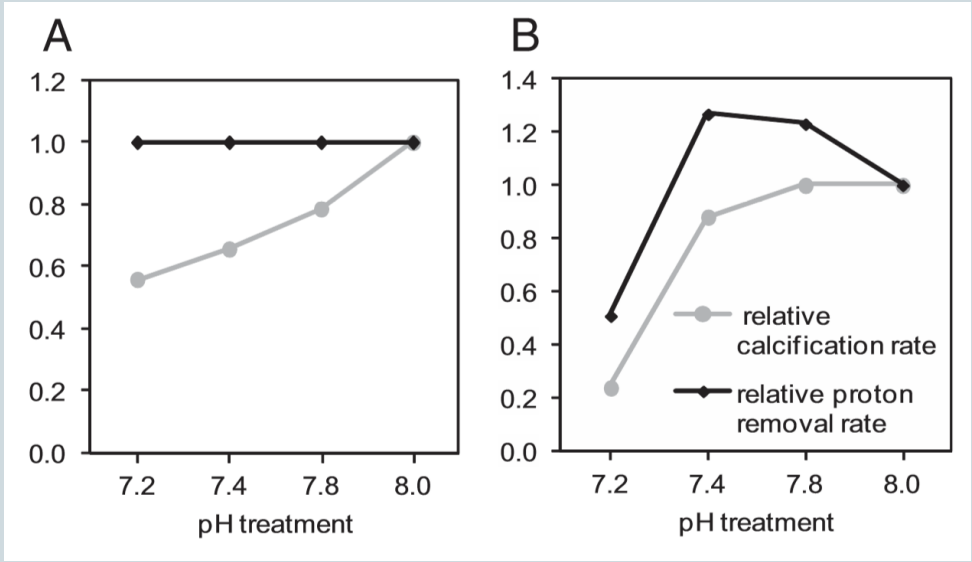
- Crustaceans have complex moult cycles
- Able to reabsorb minerals from ‘old’ shell to incorporate into ‘new’ shell
- High organic component, as well as chitin
- Organic matrix important for structuring mineral formation
- Different parts of crustaceans (e.g. claws, carapace, legs) have different mineral content which determines ‘hardness’ and strength

Why should ocean acidification impact calcification?

1. Direct shifts in acid-base balance (pH, ionic composition) of intracellular fluids that compromise calcification process

e.g. Corals

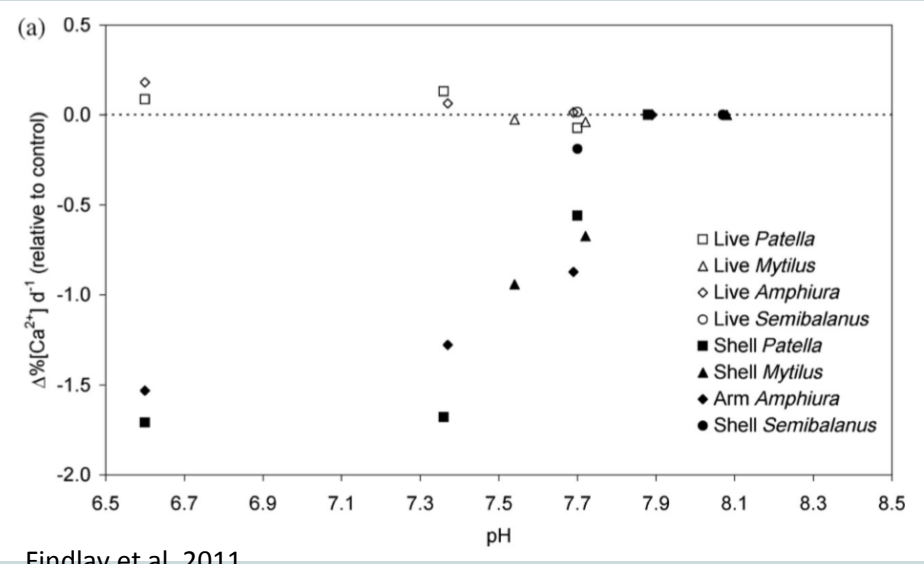




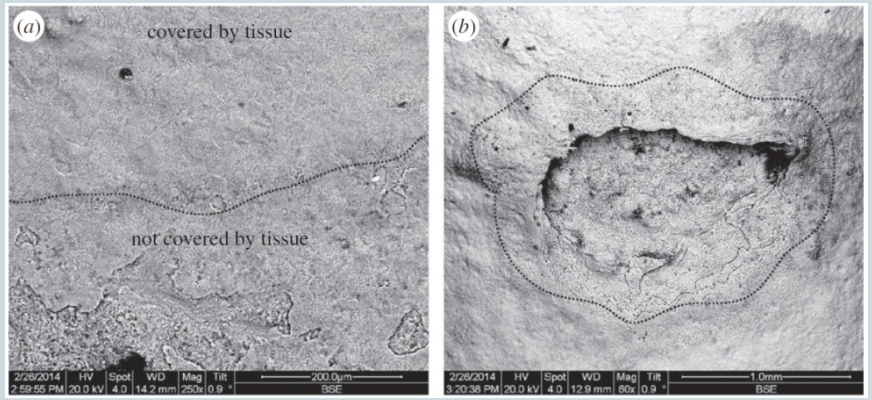
Venn et al. 2013

Why should ocean acidification impact calcification?

2. Enhanced dissolution in undersaturated conditions
e.g. dissolution of "dead" structures compared to "live"

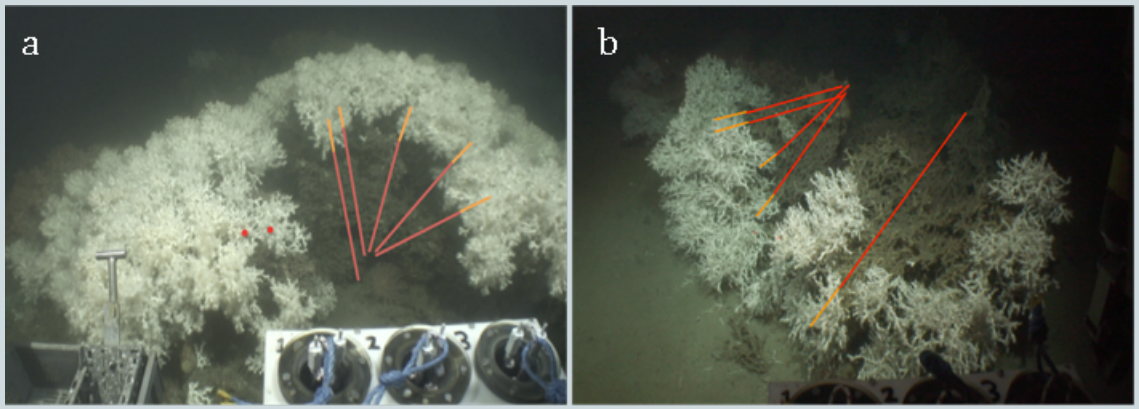


Findlay et al. 2011



Hennige et al. 2015

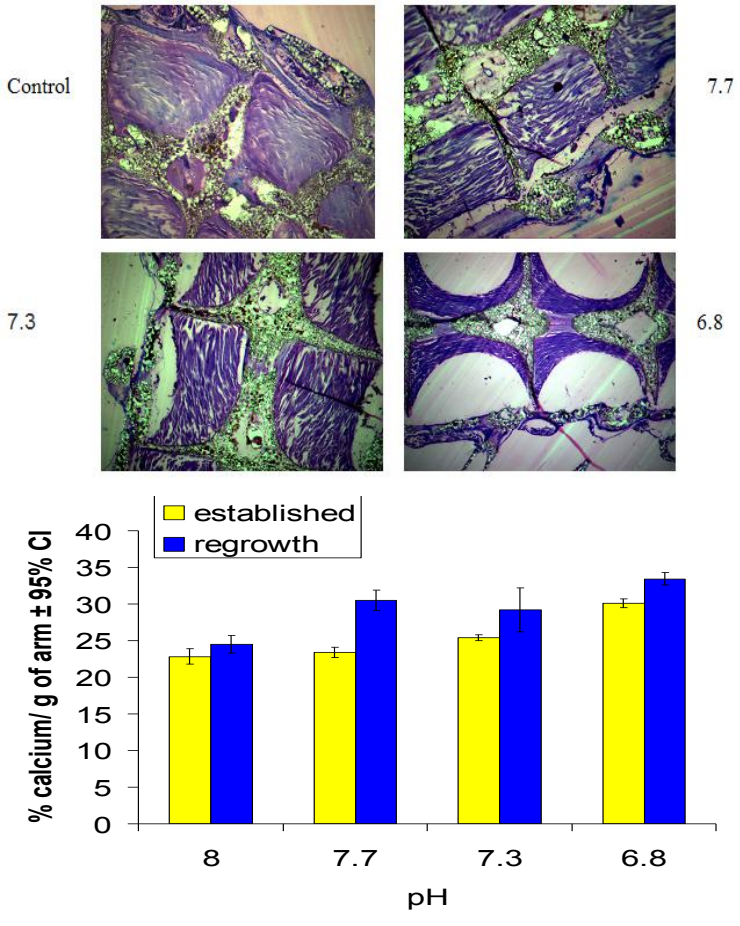
Vad et al. in review



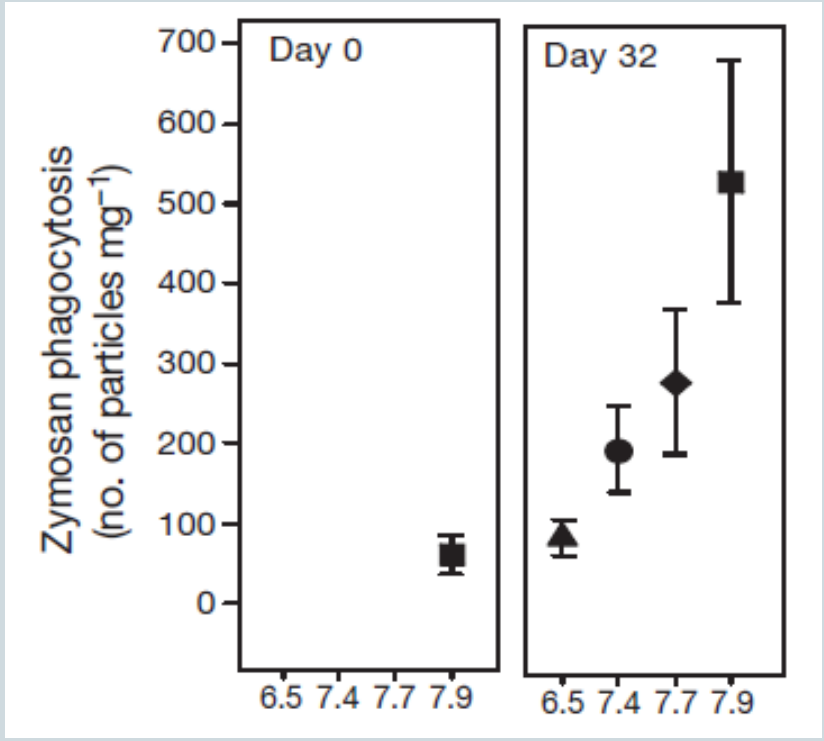
Why should ocean acidification impact calcification?

Additional energy requirements needed for maintaining and producing calcium carbonate material in unfavourable conditions

e.g. trade-offs between physiological process… brittlestars, mussels, many others…



Wood et al. 2008



Bibby et al. 2008

Some definitions

- ***Gross calcification*** $CaCO_3$ precipitated by an organism or community
- ***Net calcification*** $CaCO_3$ precipitated by an organism or community minus dissolution of $CaCO_3$ from the organism or community.
- ***Potential calcification*** Gross calcification, assuming that the organisms considered cover 100% of the area
- ***Net accumulation*** Amount of $CaCO_3$ precipitated locally plus the amount of material imported minus dissolution and export

Summary of techniques

- Geological approach
- Sedimentological approach
- Alkalinity Anomaly Technique
- pH-O_2
- Change in calcium concentration
- Radioisotopes (^{45}Ca, ^{14}C, ^{3}H-tetracycline)
- Changes in particulate calcium content
- X-ray analysis
- Buoyant weight
- “Biological” approach
- Changes in Particulate Inorganic Carbon content
- Molecular tools

Geological

$CaCO_3$ accumulates in sediment over long time periods giving an indication of rates of calcification.

Net accumulation of $CaCO_3$ is calculated by the thickness of the layer multiplied by the density, divided by the time increment (measured by radiocarbon dating)

Level: Community

Timescale: 1000-20000 years

Examples: Chave et al. (1972)

Pros: Provides integrated, long-term estimates

Cons: Numerous uncertainties and assumptions. Highly constrained by sea level

Turley et al. 2009

Sedimentological

Calcified organisms accumulate within sediments. **Net calcification (?)** is measured using the percentage weight contribution in sedimentary skeletal components

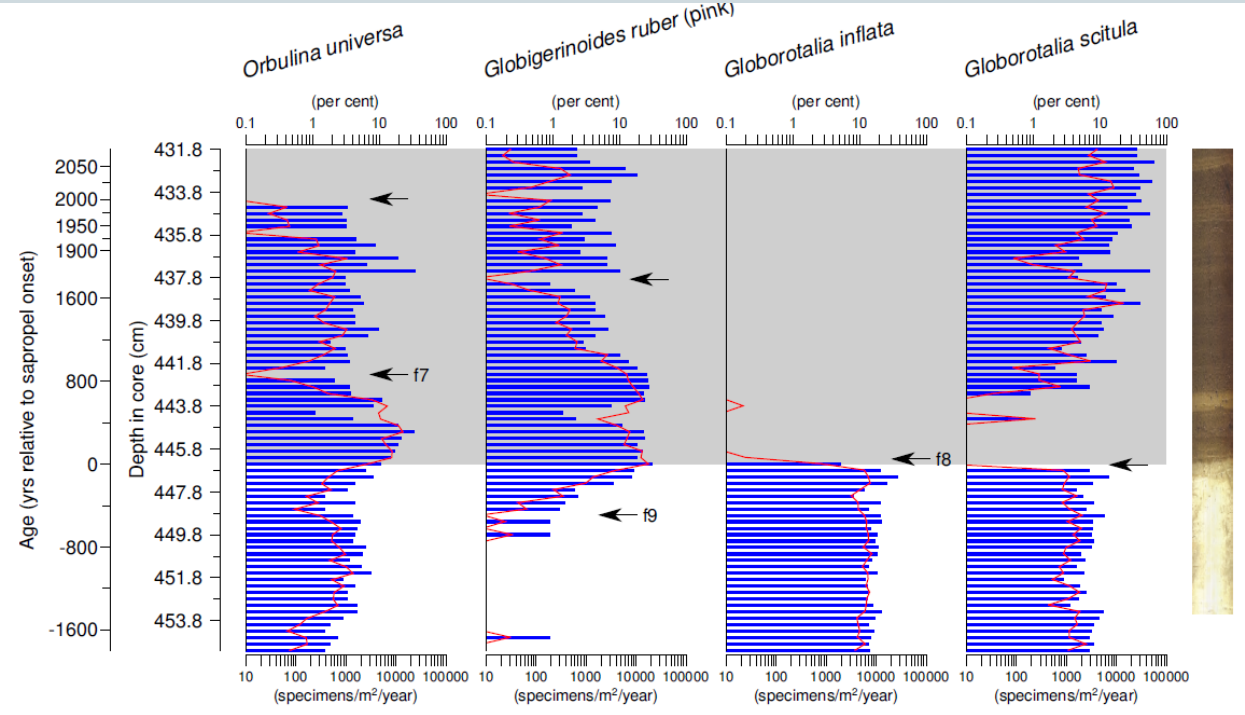
Level: Community

Timescale: Months

Examples: Langer et al. (1997), Wienkauf et al. 2013

Pros: Only needs sediment samples.

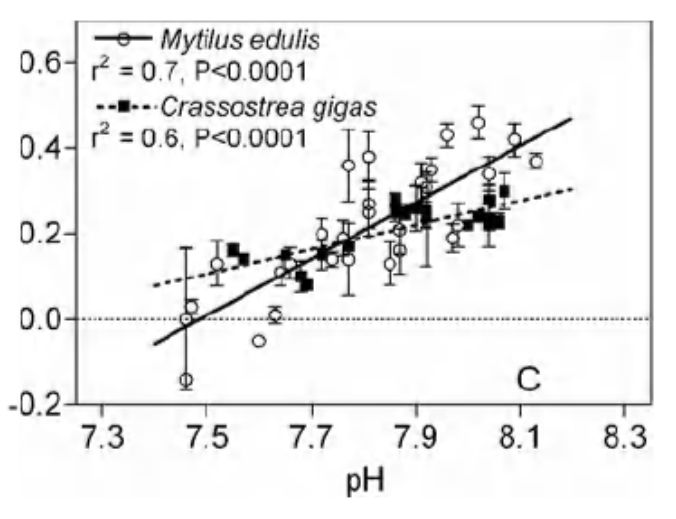
Cons: It is not clear what this approach measures, it does not account for advection terms



Weinkof et al. 2013

Alkalinity Anomaly Technique

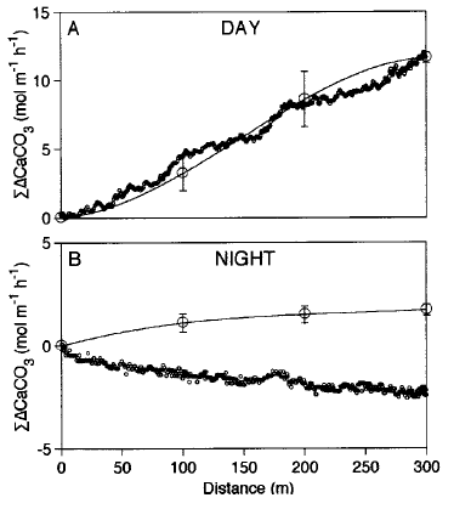
Alkalinity is lowered by two equivalents for each mole of $CaCO_3$ precipitated.
Net calcification is calculated by measuring the TA before and after an incubation period, and the ΔTA is scaled to $\Delta CaCO_3$ (i.e. calcification = 0.5xΔTA)
Level: Organisms and communities
Timescale: Hours to weeks
Examples: Smith & Key (1975), Gazeau et al. (2007), Martin et al. (2013), Inoue et al. (2013)
Pros: Very precise (1 SD = 3 µmol/kg or about 0.2%)
Cons: Needs discrete samples (but see Watanabe et al., 2004). A correction for changes in nutrients may be needed. Need to enclose or know residence time.



Gazeau et al. 2007

pH-O_2

Relationships exist between ΔO_2 and ΔDIC_{org}, the metabolic quotients.
Net calcification can be measured by estimating net community production and respiration from changes in the concentration of dissolved O_2. ΔDIC_{calc} is then calculated by subtracting ΔDIC_{org} from the upstream DIC value. ΔDIC_{calc} can be converted to ΔTA and consequently calcification.
Level: Organisms and communities
Timescale: Hours
Examples: Chisholm & Barnes (1998), Barnes (1983)
Pros: It does not require TA monitor (which is timely)
Cons: Needs DIC (hence TA) upstream. Assumes metabolic quotients



Chrisholm & Barnes 1998

Calcium concentration

Calcium concentration can directly be measured within internal fluids of organisms. **Net calcification** can be estimated from calcium removal measured using chemical titrations or sensors

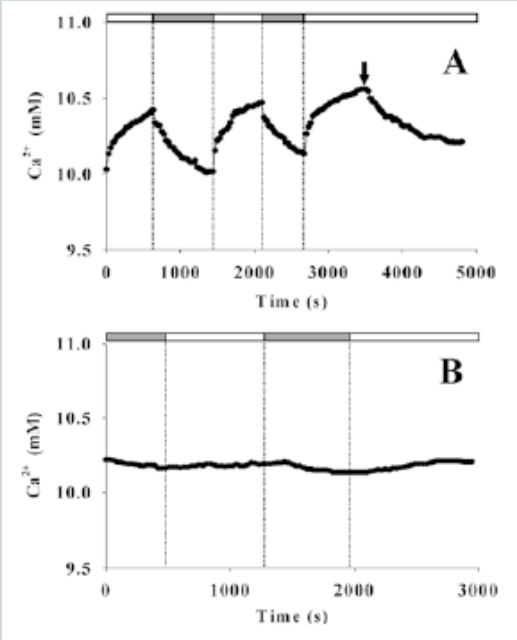
Level: Organisms and communities

Timescale: Minutes to weeks

Examples: Chisholm & Gattuso (1991), Al-Horani et al. (2003)

Pros: Direct measurement of calcium uptake; no major assumptions

Cons: Low detection limit, high background concentration (10 mmol/l)



Al-Horni et al. 2003

Radio isotopes

Calcium is taken up into the organisms skeletal components, the calcium uptake can be measured using radiolabelled elements (^{45}Ca, ^{14}C and ^{3}H) to estimate **net calcification**

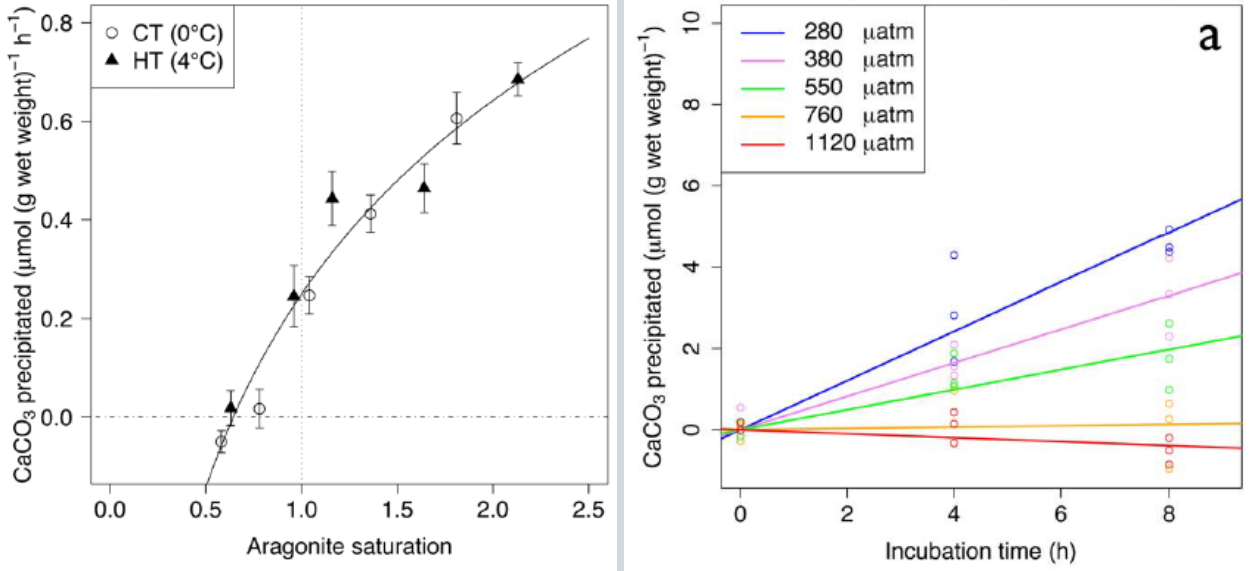
Level: Organisms

Timescale: Minutes to hours

Examples: Fabry et al. (1989), Comeau et al. 2010

Pros: Extremely sensitive, Short-term incubations

Cons: Destructive, Non-biological adsorption, Use of radioisotopes restricted



Comeau et al. 2010

Changes in particulate calcium

Calcium is taken up into the organisms skeletal components, the calcium concentration can be measured by flame atomic absorption spectroscopy to give an estimate of **net calcification**.

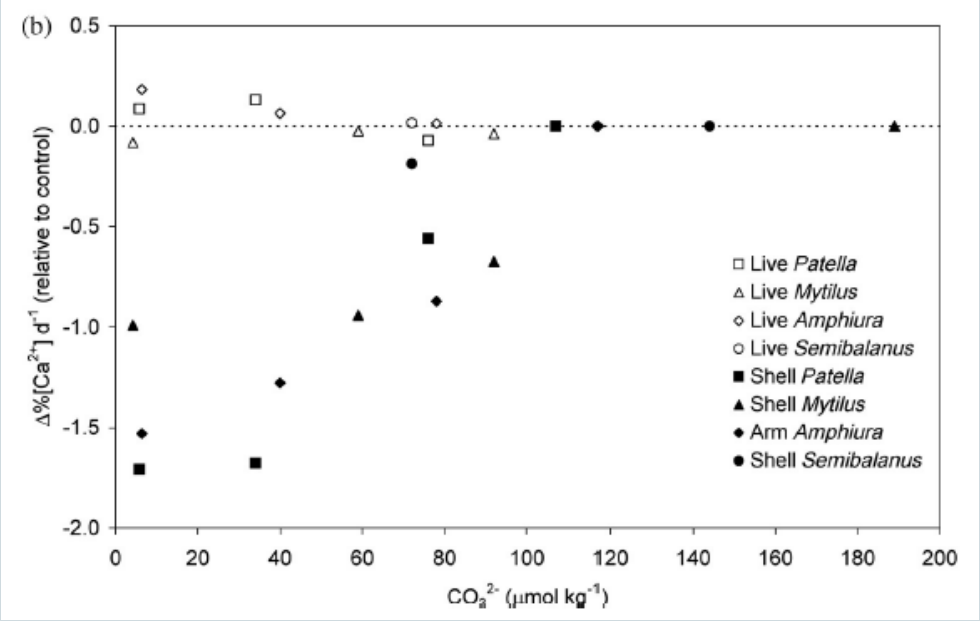
Level: Organisms

Timescale: Hours to days

Examples: (Stoll et al., 2002); (Findlay et al. 2011)

Pros: Precision is adequate when growth rates are high (cultures)

Cons: Analytical care Instrumentation



Findlay et al. 2011

X-rays

X-rays (and Computerised tomography (CT) scanning) measure the density and mass of skeleton, providing a direct measure of **net calcification**, particularly through time (using long-lived coral structures).

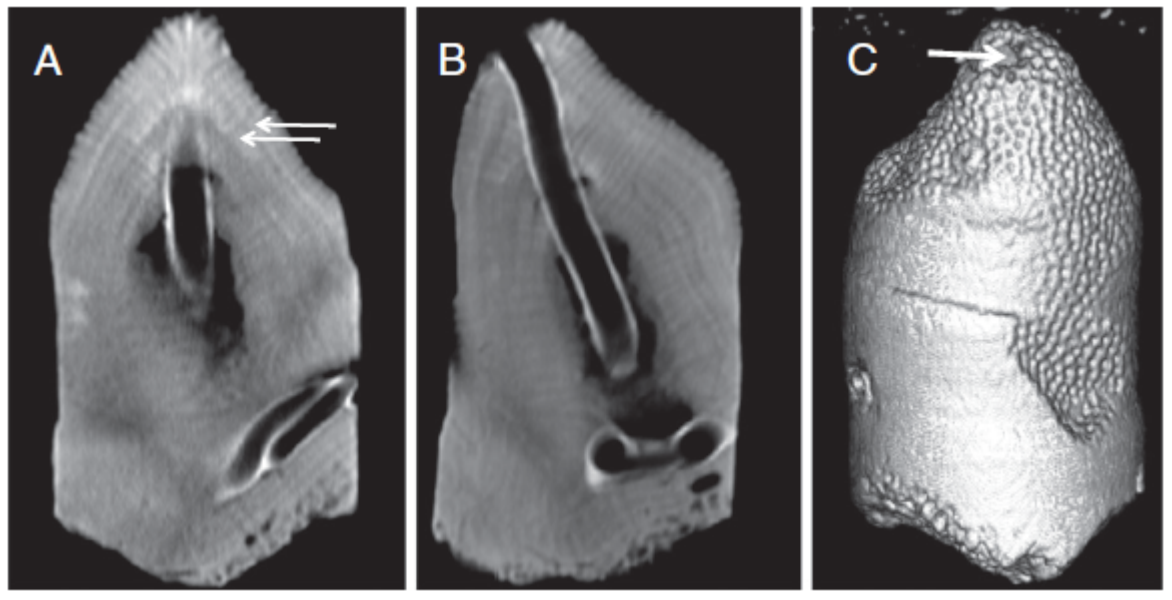
Level: Organisms

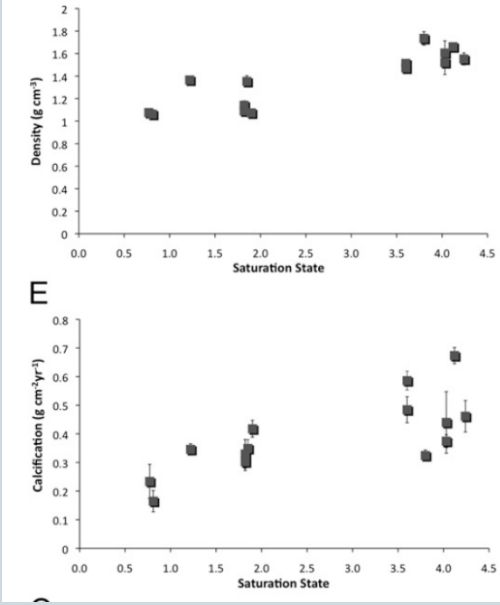
Timescale: days, months, to 100s years

Examples: Lough & Barnes (2000), Crook et al. (2013)

Pros: Enables retrospective analysis, provides an assessment of erosion

Cons: Requires substantial equipment & instrumentation





Crook et al. 2013

Buoyant weight

Increases in mass of an organisms skeleton directly correspond to increases in **net calcification**.
Level: Organisms
Timescale: Sub-daily to months/years
Examples: Dodge et al. 1984, Jokiel et al. 2008
Pros: Quite sensitive, Not destructive, No incubation required
Cons: Serious problem of normalization for comparative analysis

Dodge et al. 1984

Biological approaches

Growth measurements or turnover rates (for populations) are associated with an increase in mass of calcifed structure and can be used to estimate **net calcification**. Techniques can include using flurouscent dyes (e.g. calcein staining) to observe specific growth areas.

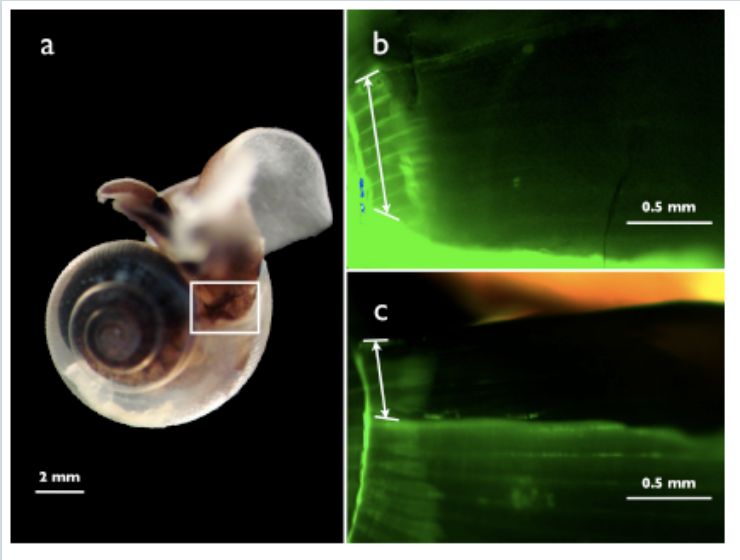
Level: Organisms

Timescale: Days, months to years

Examples: Fabry (1990), Smith (1972), Migné et al. (1998), Comeau et al. (2009)

Pros: Simple, individual level

Cons: Short term growth not always significant, lots of variability



Comeau et al. 2009

Changes in PIC

Changes in the content of the particulate carbon content of an organism reflect its accumlation or loss of carbon and provide an estimate of **net calcification**.

Total particulate carbon (TPC) and particulate organic carbon (POC) are measured (CHN analyzer, mass spectrophotometry). PIC = TPC - POC.

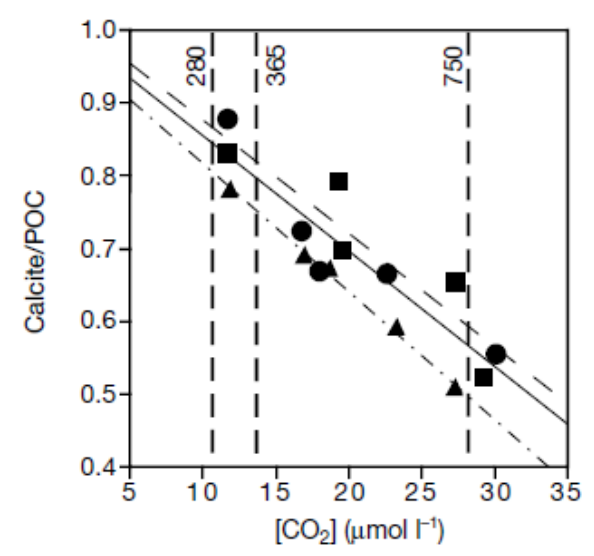
Level: Organisms

Timescale: Hours to days

Examples: Riebesell et al. (2000), Sciandra et al. (2003)

Pros: Adequate with cultures and field samples (?)

Cons: Instrumentation, Not amenable to automation



Riebesell et al. 2000

Molecular

Genetics controls the calcification process, by measuring the activity of genes involved in the calcifcation process (measure mRNA) gives an idea of the **gross calcification (?)**

Level: Organisms, perhaps communities?

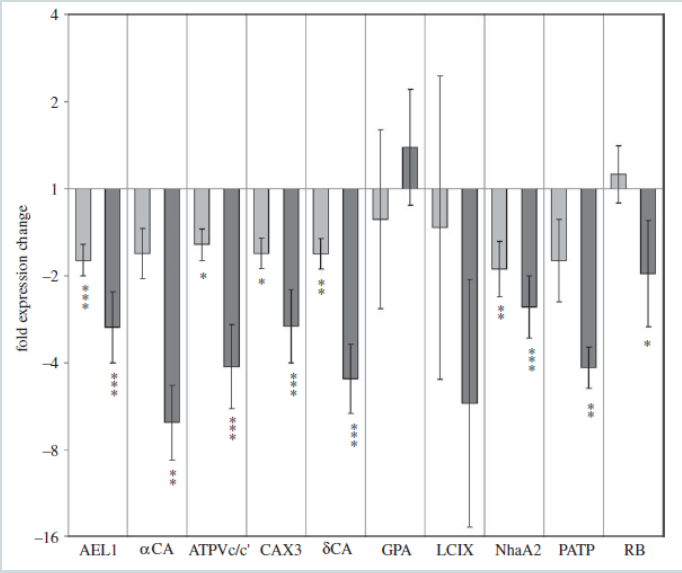
Timescale: Hours (to days?)

Examples: Lohbeck et al. 2014

Pros: High sampling rate because no incubation required

Cons: Post-translational regulation, Poor precision (semi-quantitative), Reliance on instrumentation (quantitative real-time PCR), not clearly related to actual production of calcium carbonate skeleton.

Gene name	Full name	Protein ID/*GenBank accession number	Putative function	Primer name	Primer sequence 5'-3'	Amplicon size	Reference
EFG1	Elongation Factor 1	462457	endogenous reference gene	EFG1_F	GCT GGA AGA AGG ACT TTG TTG	101	Mackinder et al. 2011
				EFG1_R	TCC ACC AGT CCA TGT TCT TC		
Actin	Actin	564188.1*, 564193.1*, 564192.1*, 564191.1*, 564190.1*, 564189.1*	endogenous reference gene	Actin_F	GAC CGA CTG GAT GGT CAA G	96	Mackinder et al. 2011
				Actin_R	GCC AGC TTC TCC TTG ATG TC		
αTUB	α Tubulin	multiple copy	endogenous reference gene	αTUB_F	GCA TCG CCG AGA TCT ACT C	84	Bach et al. 2013
				αTUB_R	TCG CCG ACG TAC CAG TG		
RB	Rubisco	D45845.1	Gene coding for large subunit of RUBISCO	RB_F	CAA TCG GTC ACC CAG ATG GTA	100	Bruhn et al. 2010
				RB_R	GCG ATA TAA TCA CGG CCT TCG		
AEL1	Anion Exchanger Like 1	99943	Bicarbonate transporter, SLC4 family	AEL1_F	TTC ACG CTC TTC CAG TTC TC	102	Mackinder et al. 2011
				AEL1_R	GAG GAA GGC GAT GAA GAA TG		
αCA	α Carbonic Anhydrase 2	456048	Alpha carbonic anhydrase	αCA2_F	AGA GCA GAG CCC TAT CAA CA	134	Richier et al. 2011
				αCA2_R	TCG TCT CGA AGA GCT GGA A		
δCA	δ Carbonic Anhydrase	436031	Delta carbonic anhydase	δCA_F	ACG AGC ACG AGA TGT TCA AG	87	Bach et al. 2013
				δCA_R	TCT CGC CAA CCA TCA TCT C		
CAX3	Ca^{2+}/H^{+} exchanger 3	416800	Ca2+/H+ exchangers, similar to CAX family	CAX3_F2	CTC CTC TGC GTC TTT GCA T	90	Mackinder et al. 2011
				CAX3_R2	GAG GGC GGT GAT GAG GTA		
ATPVc'/c	Vacuolar-type H^{+} pump	359783	Vacuolar H+-ATPase, V0, subunit c/c'	ATPV_F	TAC GGC ACT GCA AAG TCT G	83	Mackinder et al. 2011
				ATPV_R	ACG GGG ATG AAG GAC TTC		
PATP	Plasma membrane type H^{+} pump	67081	P type H+-ATPase	PATP_F	GAG CAC AAG TTC CTC ATC GTC	105	Bach et al. 2013
				PATP_R	CAC GTC GGC CTT CTT GAG		
NhaA2	Na^{+}/H^{+} exchanger 2	447659	Na+/H+ antiporter	NhaA2_F	CTC GTC TGC TAT GGC ATC TC	80	Bach et al. 2013
				NhaA2_R	GTT GCT CGC GTC CAT TC		
LCIX	Low CO_2 induced gene	457739	Protein in Emiliania huxleyi 457793	LCIX_F	CAG CAG TCG TGG CTC AAG	94	Bach et al. 2013
				LCIX_R	CGT AAG CGA CGT GGA TCA G		
GPA	Ca^{2+} binding protein	431830	Calcium-binding protein in *Emiliania huxleyi*	gpaBR_F	AGG CCT TCT CCA GCA TCA T	70	Richier et al. 2009
				gpaBR_R	GTT CAG CGT GCT CTC CGA G		



Lohbeck et al. 2014

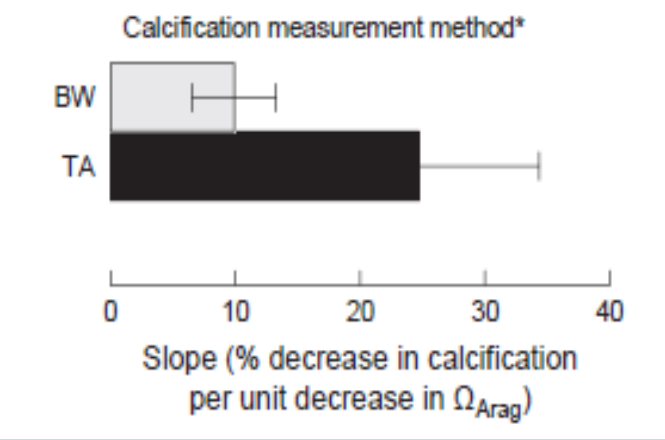
Generic measuring issues

- Considerably **different units** across the different techniques

- Measurements tend to **need to be normalised**
- organism: surface area, skeletal weight, body mass, biomass…
- communities: volumetric, surface area…

- **Not trivial to compare**!

- Most measure **NET** calcification – difficult to disentangle the impacts on the organisms ability to calcify with dissolution.



Chan & Connolly, 2013