The calcification process and measurement techniques
What is calcification?

- The accumulation of calcium salts into body tissue, such as bones, shells, and carapaces.
- 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.
What is calcification?

Ca$^{2+}$ + 2HCO$_3^-$ $\leftrightarrow$ CaCO$_3$ + CO$_2$ + H$_2$O

**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

$$\Omega = \frac{[\text{Ca}^{2+}][\text{CO}_3^{2-}]}{K_{sp}'}$$

$\Omega > 1$ Supersaturated with respect to CaCO$_3$

$\Omega < 1$ Undersaturated with respect to CaCO$_3$ (dissolution)

Steinacher et al. 2009
Who calcifies, and how?

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

Other organism types:
- Formaminifera
- 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 CaCO₃ 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,

- 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.

*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.
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
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?

- 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

Pane & Barry 2007; Photo MBARI (2006)
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

Vad et al. in review

Hennige et al. 2015
Why should ocean acidification impact calcification?

3. Additional energy requirements needed for maintaining and producing calcium carbonate material in unfavourable conditions, e.g. trade-offs between physiological processes... brittlestars, mussels, many others...

Wood et al. 2008

Bibby et al. 2008
Some definitions

- **Gross calcification** $\text{CaCO}_3$ precipitated by an organism or community

- **Net calcification** $\text{CaCO}_3$ precipitated by an organism or community minus dissolution of $\text{CaCO}_3$ from the organism or community.

- **Potential calcification** Gross calcification, assuming that the organisms considered cover 100% of the area

- **Net accumulation** Amount of $\text{CaCO}_3$ precipitated locally plus the amount of material imported minus dissolution and export
Summary of techniques

- Geological approach
- Sedimentalological 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
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.
Alkalinity Anomaly Technique

Alkalinity is lowered by two equivalents for each mole of CaCO3 precipitated. Net calcification is calculated by measuring the TA before and after an incubation period, and the ΔTA is scaled to ΔCaCO3 (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.
pH-O₂

Relationships exist between ΔO₂ 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 O₂. 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

![Graphs showing pH-O₂ relationship](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)

---

![Graph A](image1.png)

![Graph B](image2.png)

*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
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
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 organism's 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
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
Changes in the content of the particulate carbon content of an organism reflect its accumulation 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

![Graph showing the relationship between [CO₂] and Calcite/POC](attachment:image.png)

Riebesell et al. 2000
Molecular

Genetics controls the calcification process, by measuring the activity of genes involved in the calcification 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.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Full name</th>
<th>Protein ID/GenBank accession number</th>
<th>Potentiometer function</th>
<th>Primer name</th>
<th>Primer sequence 5'-3'</th>
<th>Amplification size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPE1</td>
<td>Sparcalin Acceptor 1</td>
<td>DQ44577</td>
<td>sparcalciton receptor gene</td>
<td>SPE1P-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>A17H</td>
<td>Actin1</td>
<td>AK019500</td>
<td>1</td>
<td>A17H-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>A15H</td>
<td>Actin1</td>
<td>AK019500</td>
<td>1</td>
<td>A15H-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>P2</td>
<td>Polymorphonuclear granulocyte</td>
<td>E42096.1</td>
<td>mmp2</td>
<td>P2F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>A17H</td>
<td>Actin1</td>
<td>AK019500</td>
<td>1</td>
<td>A17H-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>EOA</td>
<td>Acidic alpha-1 antitrypsin 2</td>
<td>AK019500</td>
<td>1</td>
<td>EOA-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>CCA</td>
<td>Carbonic anhydrase</td>
<td>AK019500</td>
<td>1</td>
<td>CCA-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>SCLA</td>
<td>Somatic cell adhesion molecule</td>
<td>AK019500</td>
<td>1</td>
<td>SCLA-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>CAMS</td>
<td>Cadherin</td>
<td>AK019500</td>
<td>1</td>
<td>CAMS-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>ATP2C2</td>
<td>Mitochondrial ATP synthase</td>
<td>AK019500</td>
<td>1</td>
<td>ATP2C2-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>P1H3</td>
<td>Protein 1H3/1H3</td>
<td>AK019500</td>
<td>1</td>
<td>P1H3-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>BDH</td>
<td>Beta-dystroglycan</td>
<td>AK019500</td>
<td>1</td>
<td>BDH-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>LCA</td>
<td>Leukocyte chemokine</td>
<td>AK019500</td>
<td>1</td>
<td>LCA-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
<tr>
<td>CCA</td>
<td>Carbonic anhydrase</td>
<td>AK019500</td>
<td>1</td>
<td>CCA-F</td>
<td>ATGCGGAAGAAGGCCTCTTCT70</td>
<td>151</td>
<td>Kothari et al. 2003</td>
</tr>
</tbody>
</table>

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
Summary

- Calcification ability has a **connection to energy** budgets

- **Feeding rates** may overcome some of the costs – will food supply change?

- **Dissolution rates** will increase as saturation state decreases – important for exposed material

- **Bio-erosion** may also further impact of OA

- **Adaptation** potential?

- **Interactions** between organisms

- Complexity of **multiple stressors**