Biochemical indices of zooplankton production

PICES 2012, W2: Secondary production
Lidia Yebra, IEO, Spain
Somatic growth

\[ g = \ln \frac{W_t}{W_0} \times \frac{1}{t} \]

Reproductive growth

\[ g = E \times W_e \times \frac{1}{t} \]

Production

\[ P = g \times B \]
Direct methods

Classical:

• Individual growth: Length or weight (Heinle 1966)

• Reproduction: EPR (Harding et al. 1951, Dagg 1978)

• Moult rate (Peterson et al. 1991)
Advantages

• Individual growth and moult rate: Direct measurements

• EPR: Incubation only 24 h

Disadvantages

• Incubations: time consuming, manipulation artefacts (scale, food, …)

• Individual growth: Errors on length/weight relationships

• EPR: errors on spEPR calculation, egg and female weights are variable

• Moult rate: only useful on crustaceans moulting (not adults, not dormant)
Indirect methods

Models:

• Huntley & Lopez (1972):
  Growth depends on T

• Hirst & Bunker (2003):
  Growth depends on biomass and Chl

• Weight increment (Landry 1978):
  Growth depends on biomass and development time

• Physiologic method (Le Borgne 1978, 1982):
  Growth from respiration and excretion
Disadvantages

• Huntley & Lopez (1972):
  Growth depends on T, but what about food?

• Hirst & Bunker (2003):
  Growth depends on biomass and Chl, but what about T?

• Weight increment:
  Need to assess mean weight and development time of each stage,
  Rey-Rassat et al. 2004: up to 40% error.

• Physiological method:
  Growth from respiration and excretion, need to assess both or to apply models based on Chl and biomass.
Indirect methods

Biochemical indices:

• Growth:
  • Incorporation of isotopes (\(^{3}\text{H-aa}, \, ^{14}\text{C-aa} – \text{protein synthesis})
  • BrdU uptake (Gómez et al. 2001)
  • RNA/DNA ratio (Dagg & Littlepage 1972)

• Enzyme activities:
  • DNA polymerase (Sapienza & Mague 1979)
  • NDPK (Berges 1990)
  • ATC (Bergeron & Buestel 1979)
  • AARS (Yebra & Hernández-León 2004)

• Moult rate: Chitobiase release (Espie & Roff 1995)
Enzyme activities

Advantages:

- Indices of metabolic rates
- The chemical base of all metabolic processes
- Dimensionally correct, rates (MT$^{-1}$ or T$^{-1}$)
- Quick and economic to measure
- Assays can be run under controlled conditions
- High precision, good repeatability
Enzyme activities

Limitations:

• Specific relationships for species, even stages, usually not useful for the whole community

• Most biochemical indices are correlated with biomass. Is very important to standardize the rates

• As most of them depend on T, rates must be corrected for the in situ field T

• Calibration in vitro may not reflect the real flux of an in vivo process (i.e. addition of substrates to obtain maximum potential activities)
Enzyme activities

Net growth = synthesis rate – degradation rate

• A biochemical marker is NOT a measure of growth, is a correlative

• E.g. Protein synthesis rate is NOT a measure of growth itself. You must take in account the protein turnover rate (recycling rate)

• Indices MUST be calibrated against direct growth rate before its application in the field or laboratory
Disadvantages

• Incorporation of labelled isotopes: radioactive
• BrdU uptake (Gómez et al. 2001): NOT calibrated against growth
• RNA/DNA ratio (e.g. Wagner et al. 2001): Calibrated but stage-specific and taxonomic group dependent
• DNA polymerase (Sapienza & Mague 1979): NOT calibrated against growth
• NDPK (Berges 1990): NOT calibrated against growth
• ATC: Hernández-León et al. 1995: Calibration against growth NOT achieved; Biegala et al. 1999: Calibrated against EPR but NOT conclusive
• AARS (e.g. Yebra & Hernández-León 2004): Calibrated against growth, but NOT conclusive for EPR
• Chitobiase (Oosterhuis et al. 2000): Calibrated against growth, but not useful for adults or gelatinous plankton, time consuming (needs assessment of mean length/weight of the community)
Calibration of RNA:DNA ratio

*Oithona davisae*

**Fig. 4.** Relationship of *Oithona davisae* biochemical indices – specific AARS activity (nmol Pi mg protein$^{-1}$ h$^{-1}$) and RNA/DNA ratio – with somatic growth rates (d$^{-1}$). Curves fitted using fed nauplii (filled circles) and copepodites (open squares) data. Dashed lines represent 95% confidence intervals.

Yebras et al. JEMBE, 2011
Calibration of ATC activity


The use of aspartate transcarbamylase activity to estimate growth rates in zooplankton

Santiago Hernández-León, Carlos Almeida, and Irene Montero

Protein-based growth rates (d\(^{-1}\)) in relation to specific ATC activity in *Acartia clausi*. Observe the highest values of ATC activity coinciding with the highest negative values of growth rates (see text). Symbols used to distinguish the different food levels as in Figure 1. C. Results of the relationship between growth rates and ATC activity in the artificial cohort (white dots) and egg production (black dots) experiments. The line represents the upper regression line of Figure 2A.
Calibration of AARS activity

\[ g_{pr} = -0.191 + 1.103 \times \text{Specific AARS activity} \]

\[ (r^2 = 0.69, p < 0.05) \]

Daphnia magna

Yebra & Hernández-León, JPR, 2004
Calibration of AARS activity

\[
growth = 0.1947 + 0.005 \cdot sp\text{AARS}_{\text{situ}}
\]
\[(R^2 = 0.96, p < 0.001)\]

*Paracartia grani* nauplii

Herrera-Rivero et al. JEMBE, 2011
Calibration of AARS activity

*Calanus finmarchicus*

\[
growth (d^{-1}) = -0.09 + 0.009 \cdot spAARS_{situ}
\]

\[(r = 0.74, p<0.001)\]

Yebra et al. 2006
Calibration of chitobiase activity

Release of the enzyme chitobiase by the copepod *Temora longicornis*: characteristics and potential tool for estimating crustacean biomass production in the sea

Swier S. Oosterhuis*, Martien A. Baars, Wim C. M. Klein Breteler

Fig. 6. *Temora longicornis*. Observed daily increase in total biomass of copepod culture (● in Fig. 5c,d) as function of calculated chitobiase activity released in ambient water in Expts I (a) and II (b) over 24 h. $\Delta W = \text{daily increase in total biomass (µg ash-free dry weight $L^{-1}$)}$; $A = \text{total chitobiase activity released over 24 h (nmol MUF liberated $L^{-1} h^{-1}$)}$.
Current biochemical indices in use

<table>
<thead>
<tr>
<th>Index</th>
<th>SCI articles on ZP</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA/DNA</td>
<td>23</td>
<td>1998-2012</td>
</tr>
<tr>
<td>ATC</td>
<td>9</td>
<td>1995-2009</td>
</tr>
<tr>
<td>AARS</td>
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<td>2004-2012</td>
</tr>
<tr>
<td>Chitobiase</td>
<td>11</td>
<td>1995-2012</td>
</tr>
</tbody>
</table>
Choosing the method

It will depend on your:

• Goals
• Time available (in the laboratory or on a cruise)
• Facilities
• Calibration

Advice:

• Know your target species
• Review the literature (that includes reading carefully the Methods section), be aware that is not the same CALIBRATION that COMPARISON.
Example: AARS good index of *Calanus* growth, but not good index of *Calanus* EPR

**Fig. 2** Relationship between copepodites of *C. helgolandicus* spAARS$_{ssu}$ activity (nmol PPI mg protein$^{-1}$ h$^{-1}$) and their protein-specific somatic growth (day$^{-1}$). *Dashed lines* confidence limits at $P < 0.05$

**Fig. 7** Relationship between female spAARS$_{ssu}$ activity (nmol PPI mg protein$^{-1}$ h$^{-1}$) and spEPR$_{ssu}$ (day$^{-1}$) during the reproductive (RS) and non-reproductive (NRS) seasons. *Dashed lines* confidence limits at $P < 0.05$

Comparison growth (=AARS activity) vs. models

Growth (d⁻¹) CV *Calanus helgolandicus*

Comparison AARS (mixed ZP) vs. chitobiase (sea water) vs. EPR (*Temora longicornis*), N.Sea

![Graph showing comparison between AARS, spEPR, and spAARS](image)

<table>
<thead>
<tr>
<th>Stations sampled</th>
<th>chitobiase</th>
<th>spEPR</th>
<th>spAARS</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (x6-8 depths)</td>
<td>5 (x6-33 replicates)</td>
<td>11 (x4 size fractions)</td>
<td></td>
</tr>
</tbody>
</table>

Notice: Lower spatial coverage when incubations are needed

Dutz, Oosterhuis & Yebra, unpubl.
Comparison EPR vs. models & AARS activity

Mixed zooplankton in subtropical waters (W Australia)

Strezelecki et al. in prep.
Combination of methods

Zooplankton sample

\[ \rightarrow \text{Sonication} \]

\[ \rightarrow \text{AARS} \]
Yebra & Hernández-León 2004

\[ \rightarrow \text{RNA/DNA} \]
Berdalet et al. 2005

\[ \rightarrow \text{Protein content} \]
Lowry et al. 1951

\[ \rightarrow \text{New protocol:} \]
Combined analysis of growth, condition and individual biomass

Yebra et al. JEMBE, 2011
Comparison of RNA:DNA ratio and AARS activity

Fig. 4. Relationship of Oithona davisae biochemical indices – specific AARS activity (nmPPI mg protein$^{-1}$ h$^{-1}$) and RNA/DNA ratio – with somatic growth rates (d$^{-1}$). Curves fitted using fed nauplii (filled circles) and copepodites (open squares) data. Dashed lines represent 95% confidence intervals.

Yebra et al. JEMBE, 2011
Calibration of RNA:DNA ratio vs. AARS activity

Yebra et al. JEMBE, 2011

Oithona davisae

nauplii
copepodites
starved nauplii

Fig. 5. Relationship between specific AARS activity (nmPPI mg protein$^{-1}$ h$^{-1}$) and RNA/DNA ratio of Oithona davisae: fed nauplii (filled circles), starved nauplii (crosses) and fed copepodites (open squares).
## Summary

### Advantages
- No incubations
- No radioactive
- "in vivo" rates
- Easy and cheap

Some indices also:
- Deep ocean sampling, e.g. diapausing organisms
- Non-moulting organisms, e.g. gelatinous plankton
- Multiple indices assay per sample

### Inconveniences
- Inhibition or change of metabolic pathways during vitelogenesis?
- EPR differences with copepod growth rates
- Stage/species/taxon specificity?
Some final thoughts

- The use of biochemical indices facilitates filling data gaps:
  - microZP growth rates (nauplii)
  - non-copepod ZP growth rates (gelatinous plankton)
  - non-adult female physiology
  - dormant organisms

- Also allows for basin-scale wide and depth sampling quite fast

- Indices which are rates are dimensionally correct

- Standardization with protein content can be easily translated into Nitrogen units (e.g. basin scale production models)

- Avoid length/weight relationships whenever possible, determine real biomass (specially for crustaceans)

- PLEASE DO NOT FORGET CALIBRATION!!
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