Biochemical indices of zooplankton production

PICES 2012, W2: Secondary production

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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 asses 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 asses both or to apply models based on Chl and biomass.

Indirect methods

Biochemical indices:

- Growth:
  - Incorporation of isotopes (^{3}H-aa, ^{14}C-aa – 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

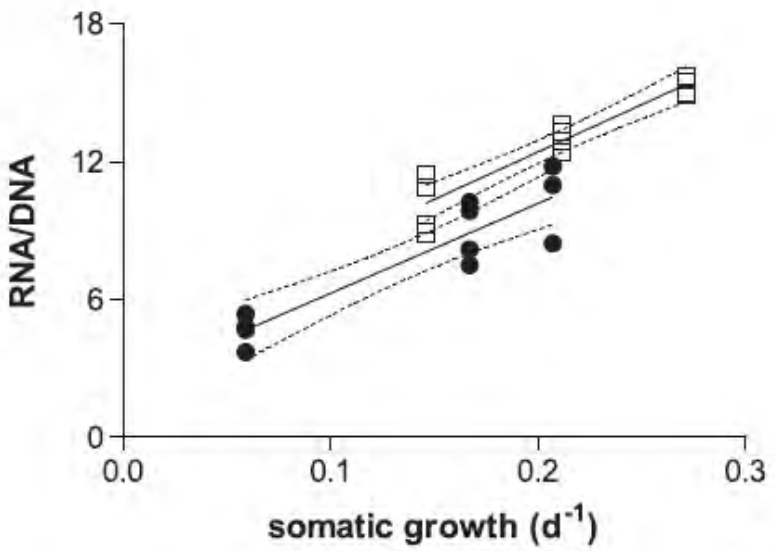


Fig. 4. Relationship of *Oithona davisae* biochemical indices – specific AARS activity (nmPPi mg protein^{-1} h^{-1}) and RNA/DNA ratio – with somatic growth rates (d^{-1}). Curves fitted using fed nauplii (filled circles) and copepodites (open squares) data. Dashed lines represent 95% confidence intervals.

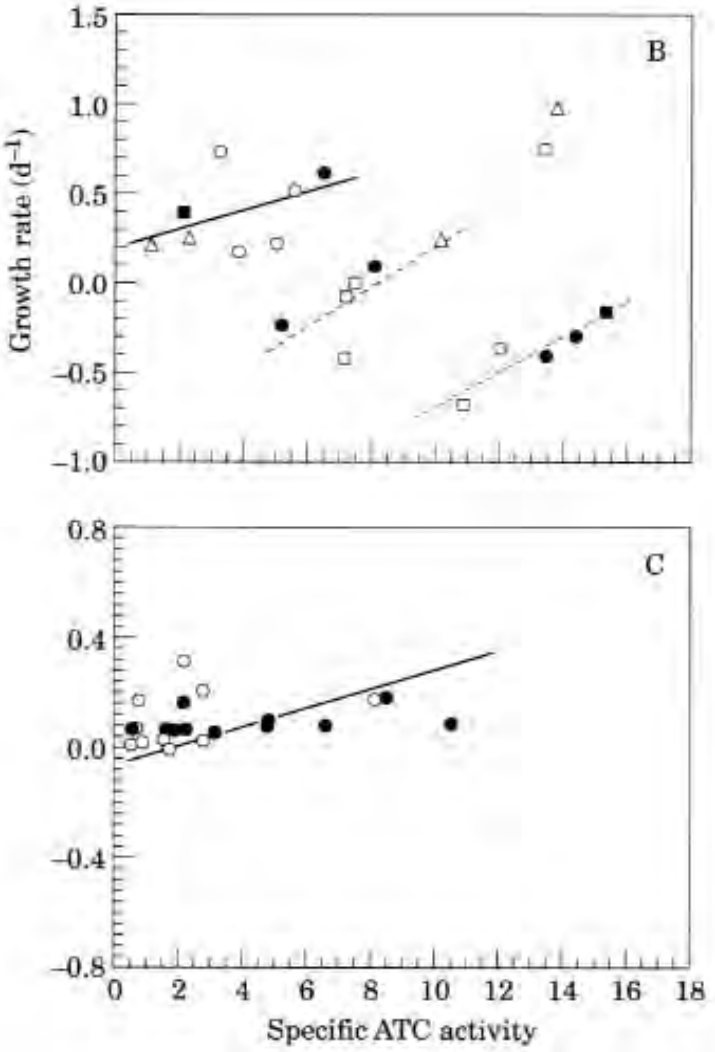
Yebra et al. JEMBE, 2011

Calibration of ATC activity

ICES J. mar. Sci., 52: 377–383. 1995

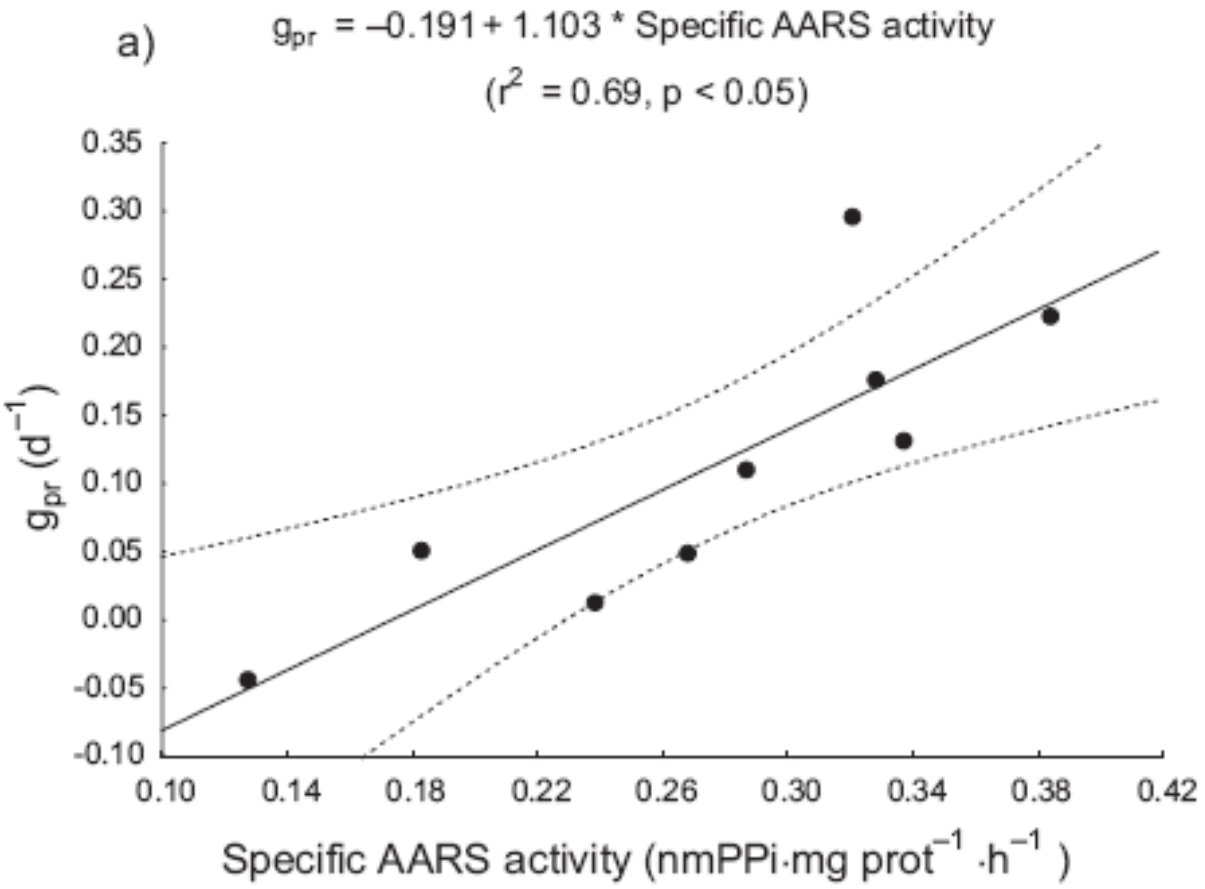
The use of aspartate transcarbamylase activity to estimate growth rates in zooplankton

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



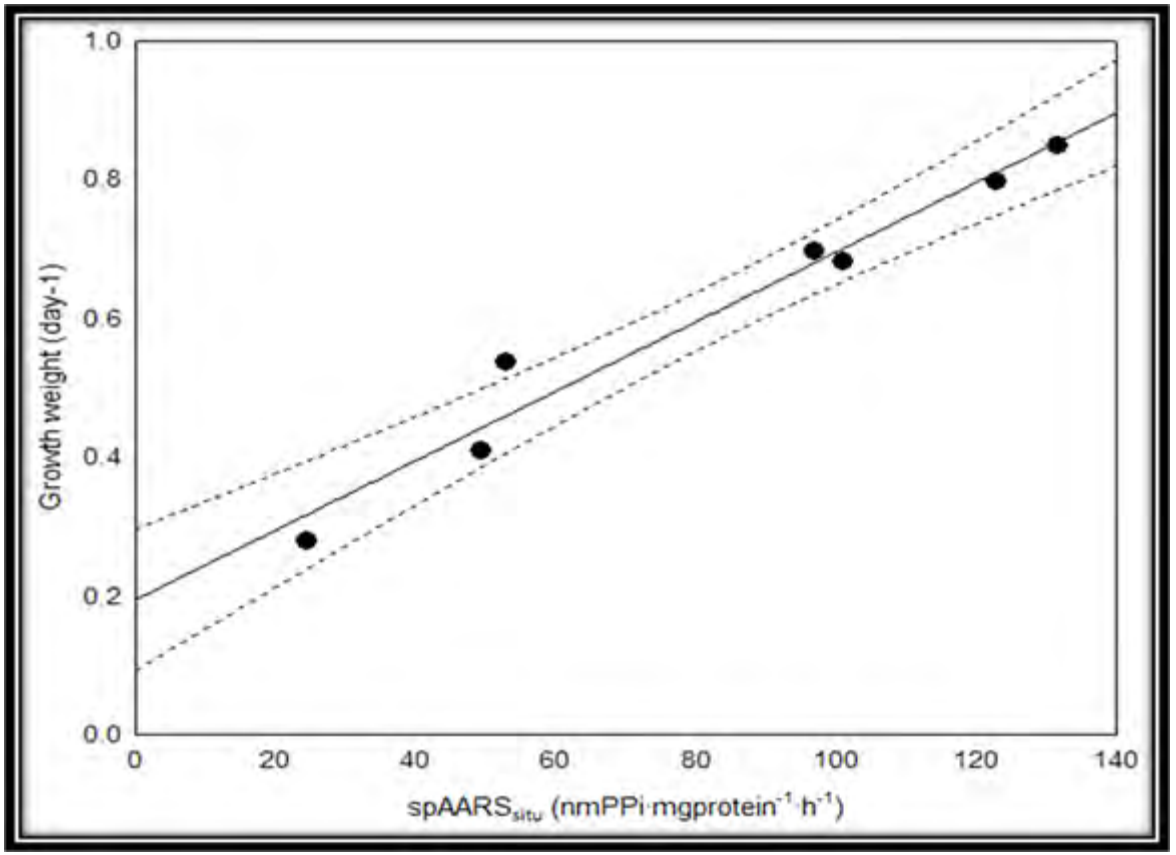
Daphnia magna

Yebra & Hernández-León, JPR, 2004

Calibration of AARS activity

$$growth = 0.1947 + 0.005 \cdot spAARS_{situ}$$
$$(R^2 = 0.96,\ p < 0.001)$$

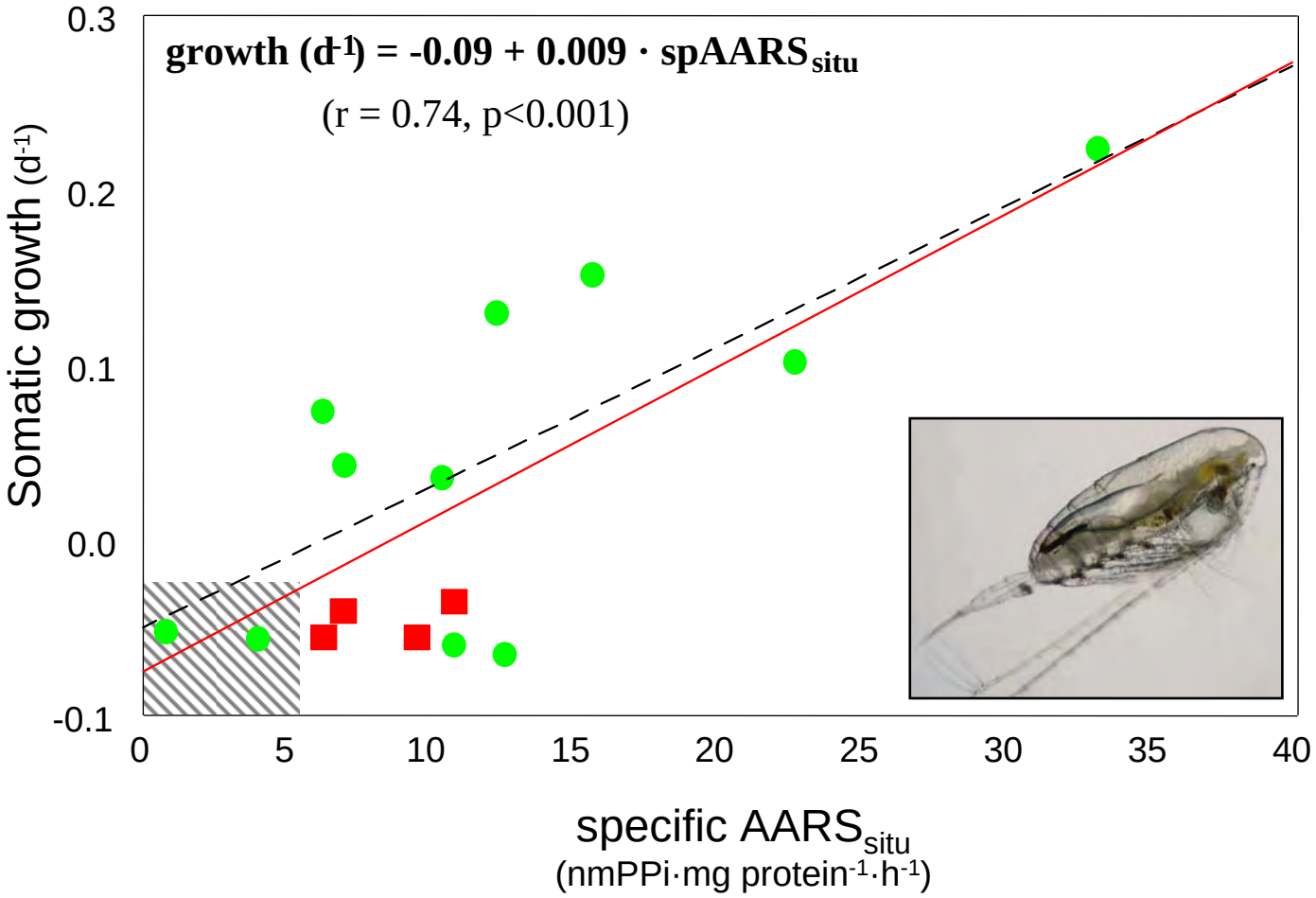
Paracartia grani
nauplii



Herrera-Rivero et al. JEMBE, 2011

Calibration of AARS activity

Calanus finmarchicus



Yebra et al. 2006

Calibration of chitobiase activity

Vol. 196: 195–206, 2000 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published April 18

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

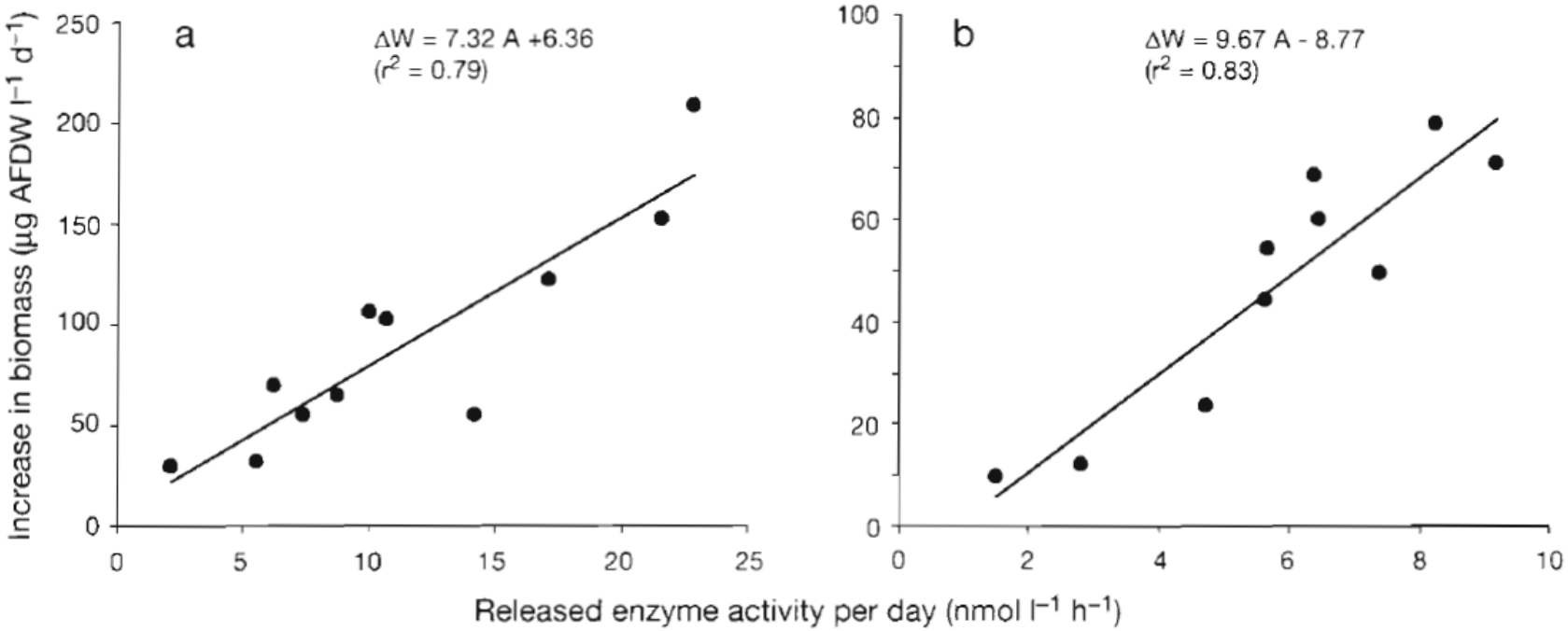


Fig. 6. *Temora longicornis*. Observed daily increase in total biomass of copepod culture (● in Fig. 5c,d) as function of calculated chitobiase activity released in ambient water in Expts I (a) and II (b) over 24 h. ΔW = daily increase in total biomass (µg ash-free dry weight l^{-1}), A = total chitobiase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

Current biochemical indices in use

Index	SCI articles on ZP	Period
RNA/DNA	23	1998-2012
ATC	9	1995-2009
AARS	12	2004-2012
Chitobiase	11	1995-2012

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

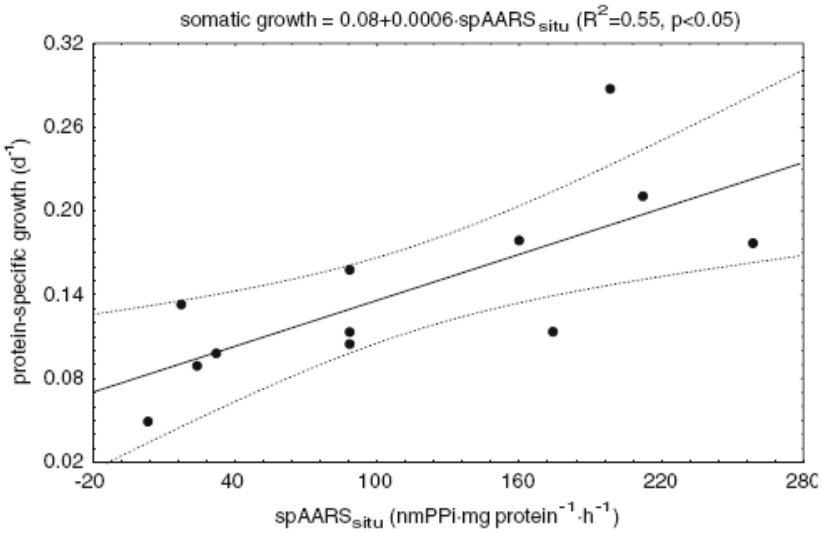


Fig. 2 Relationship between copepodites of *C. helgolandicus* $spAARS_{situ}$ activity (nmol PPi mg protein^{-1} h^{-1}) and their protein-specific somatic growth (day^{-1}). *Dashed lines* confidence limits at $P < 0.05$

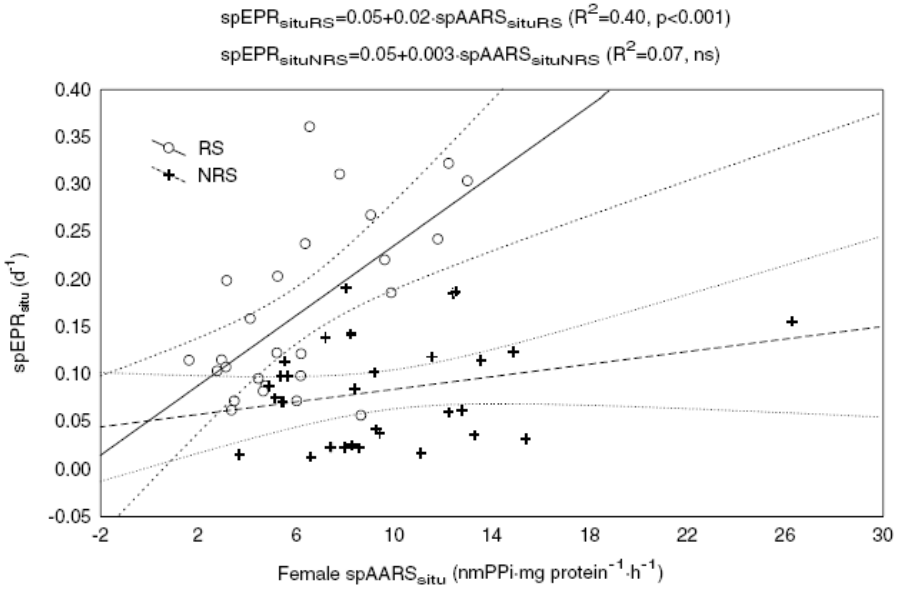
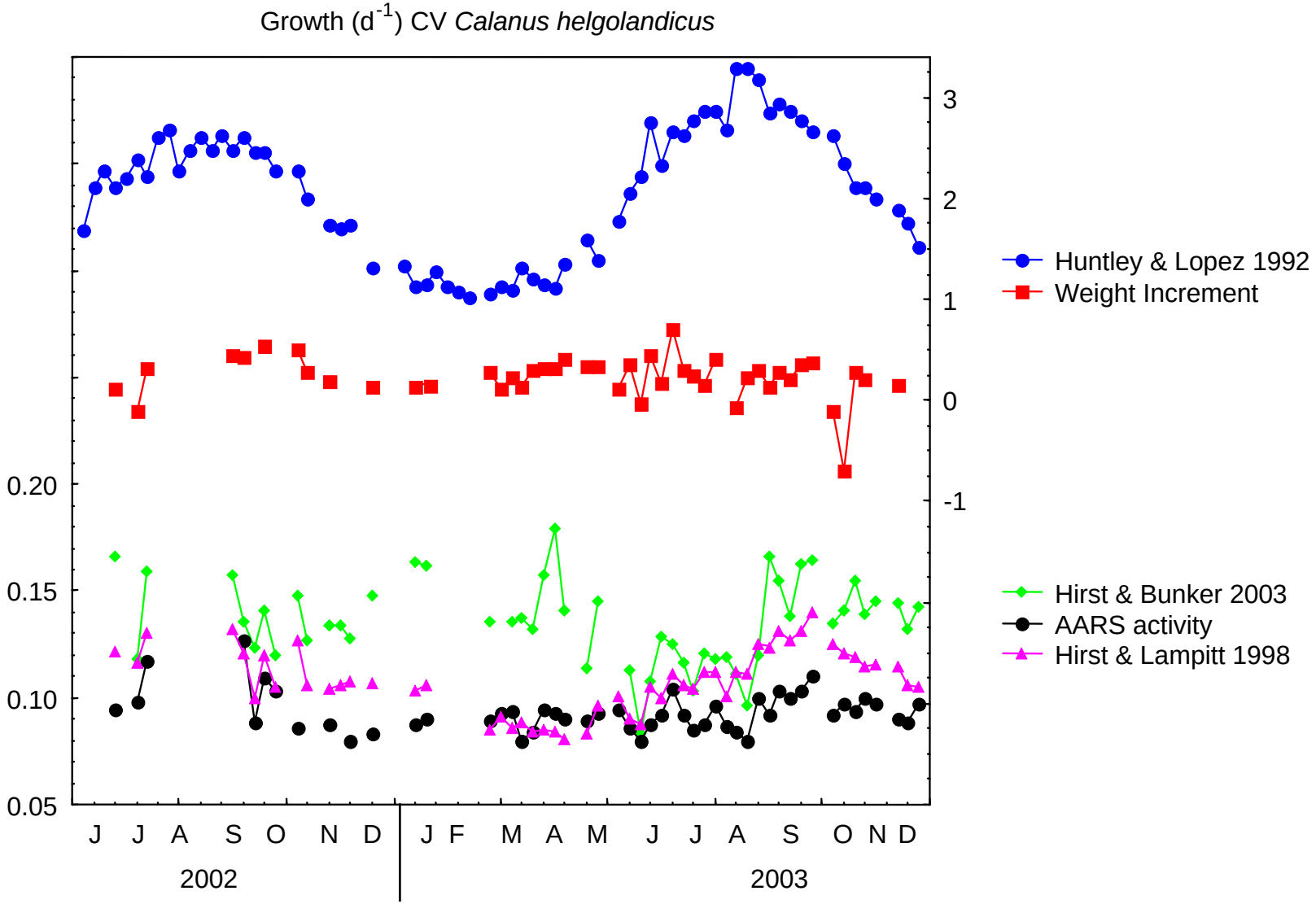


Fig. 7 Relationship between female $spAARS_{situ}$ activity (nmol PPi mg protein^{-1} h^{-1}) and $spEPR_{situ}$ (day^{-1}) during the reproductive (*RS*) and non-reproductive (*NRS*) seasons. *Dashed lines* confidence limits at $P < 0.05$

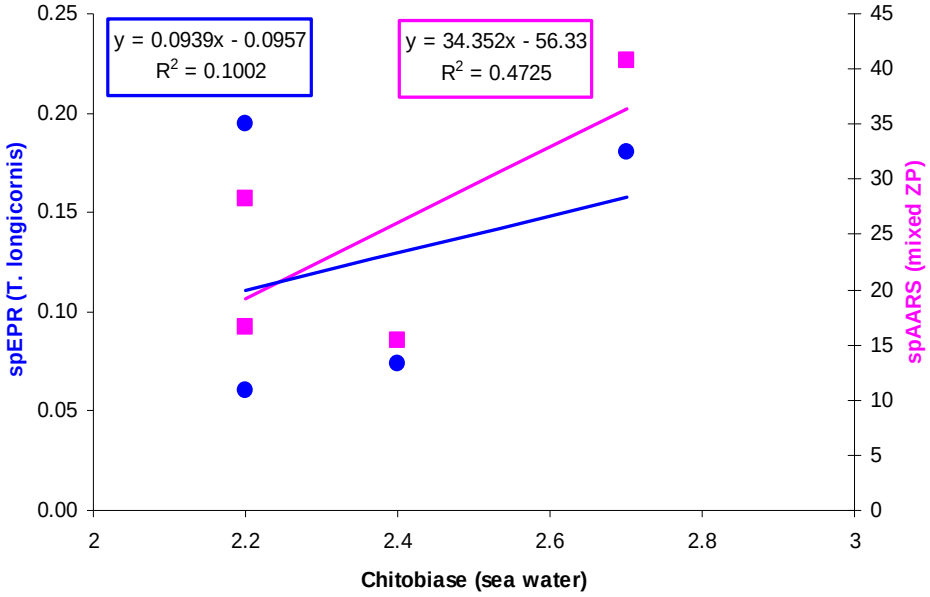
Yebra et al. Mar. Biol. 2005

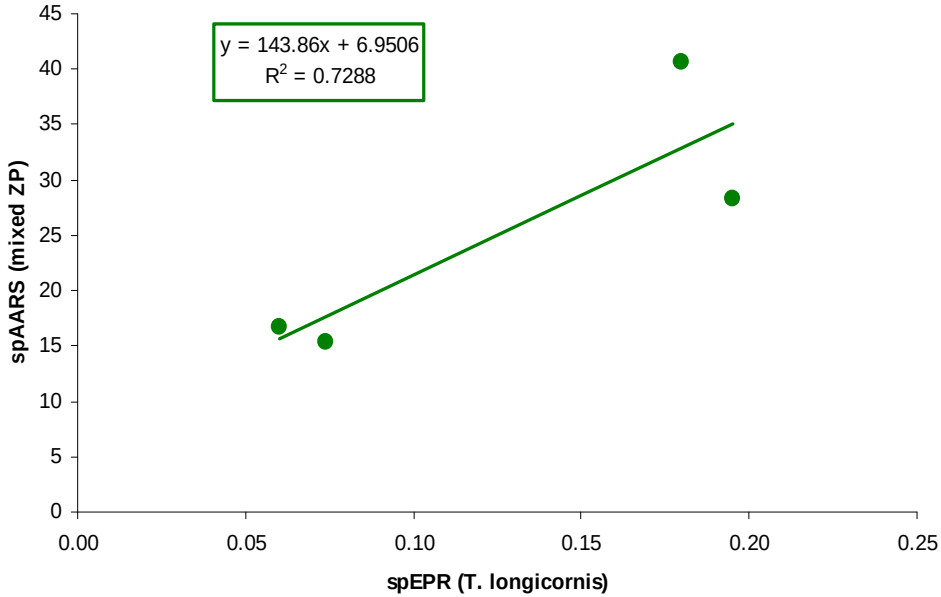
Comparison growth (=AARS activity) vs. models



Modified from Yebra et al. Mar. Biol. 2005

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





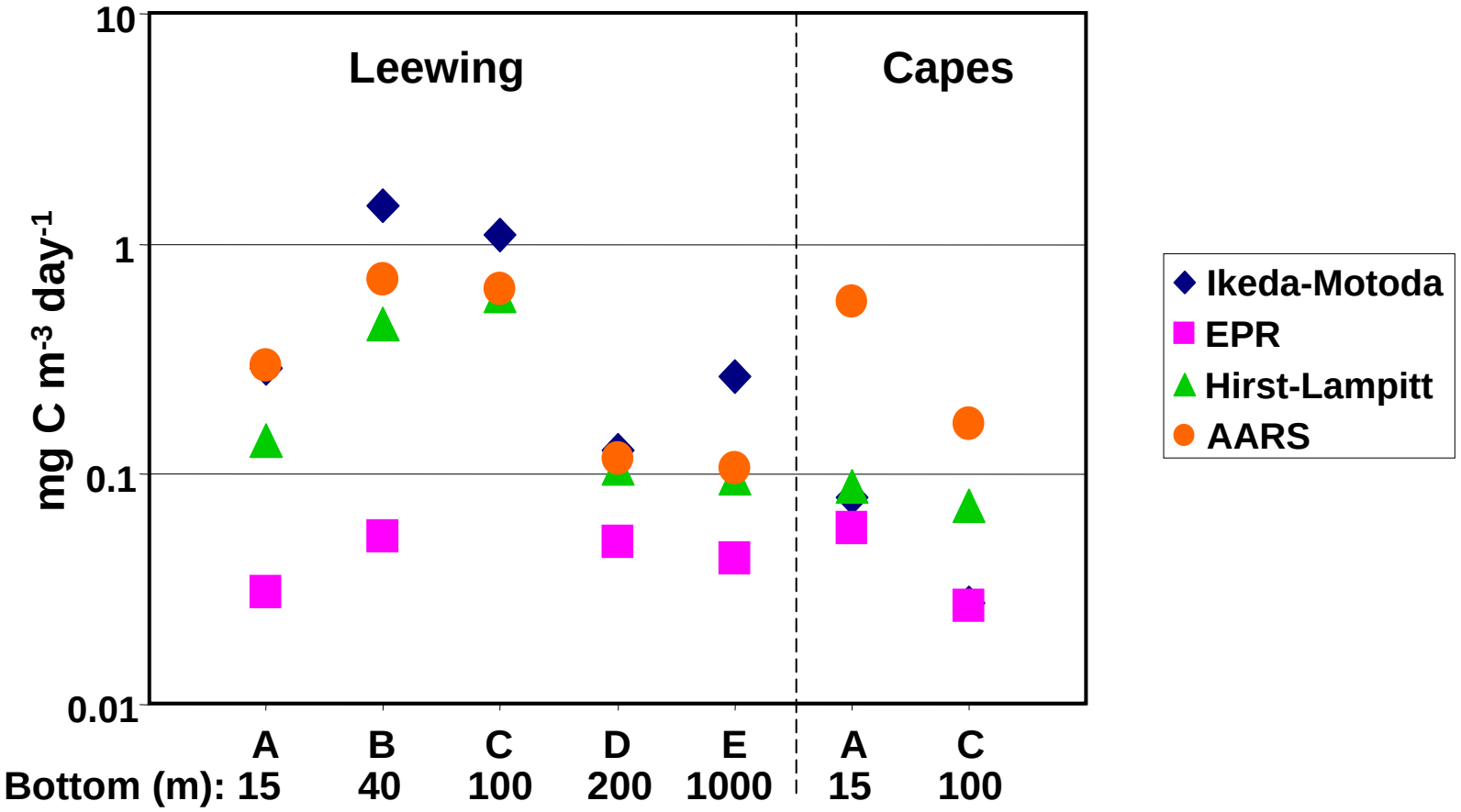
	chitobiase	spEPR	spAARS
Stations sampled	5 (x6-8 depths)	5 (x6-33 replicates)	11 (x4 size fractions)

Notice: Lower spatial coverage when incubations are needed

Dutz, Oosterhuis & Yebra, unpubl.

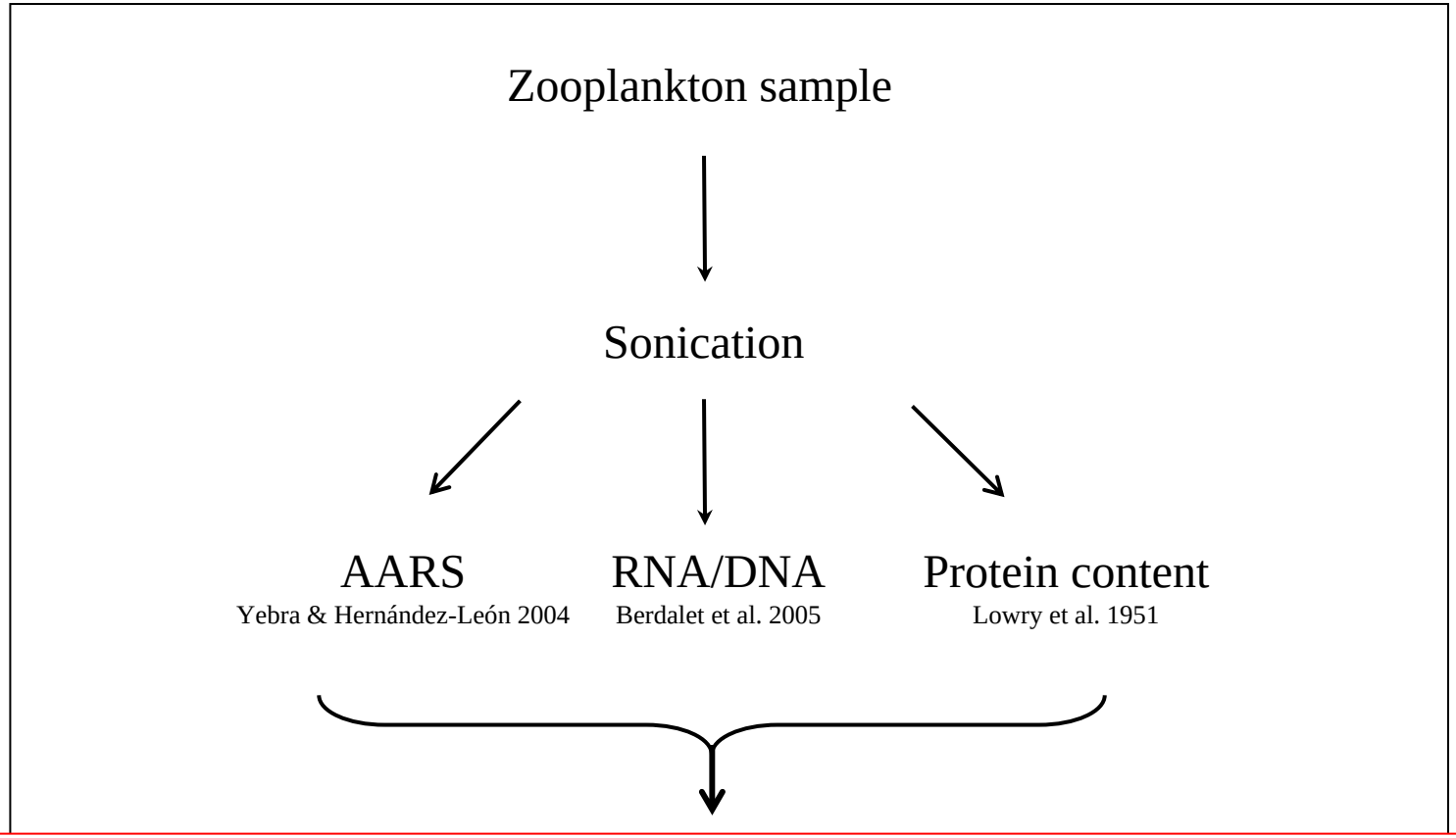
Comparison EPR vs. models & AARS activity

Mixed zooplankton in subtropical waters (W Australia)



Strezelecki et al. in prep.

Combination of methods



New protocol:
Combined analysis of growth, condition and individual biomass

Yebra et al. JEMBE, 2011

Comparison of RNA:DNA ratio and AARS activity

Oithona davisae

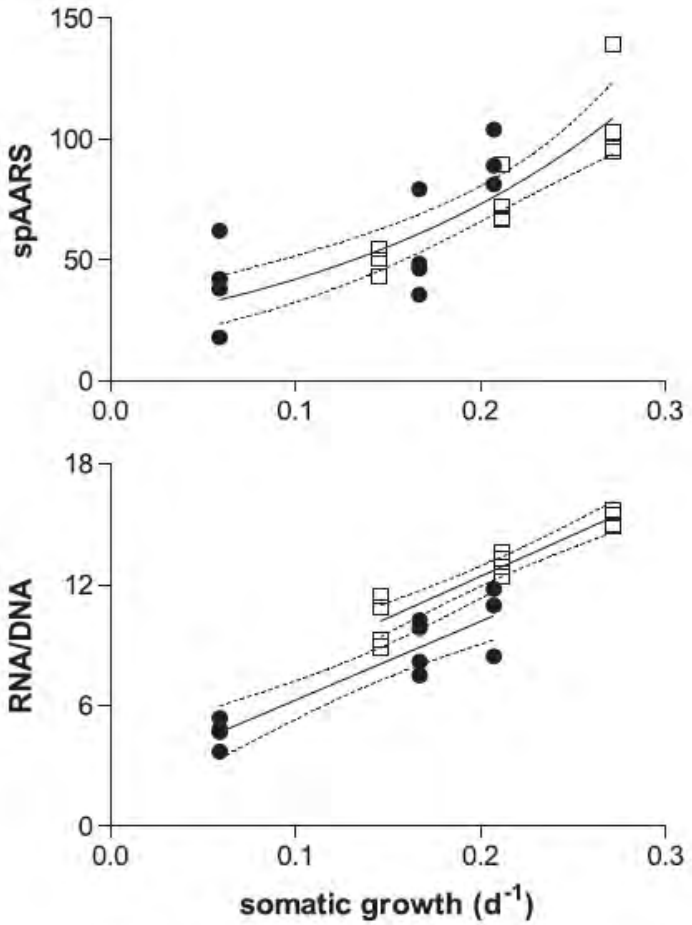
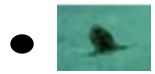


Fig. 4. Relationship of *Oithona davisae* biochemical indices – specific AARS activity (nmPPi mg protein^{-1} h^{-1}) and RNA/DNA ratio – with somatic growth rates (d^{-1}). Curves fitted using fed nauplii (filled circles) and copepodites (open squares) data. Dashed lines represent 95% confidence intervals.


nauplii


copepodites

Yebra et al. JEMBE, 2011

Calibration of RNA:DNA ratio vs. AARS activity

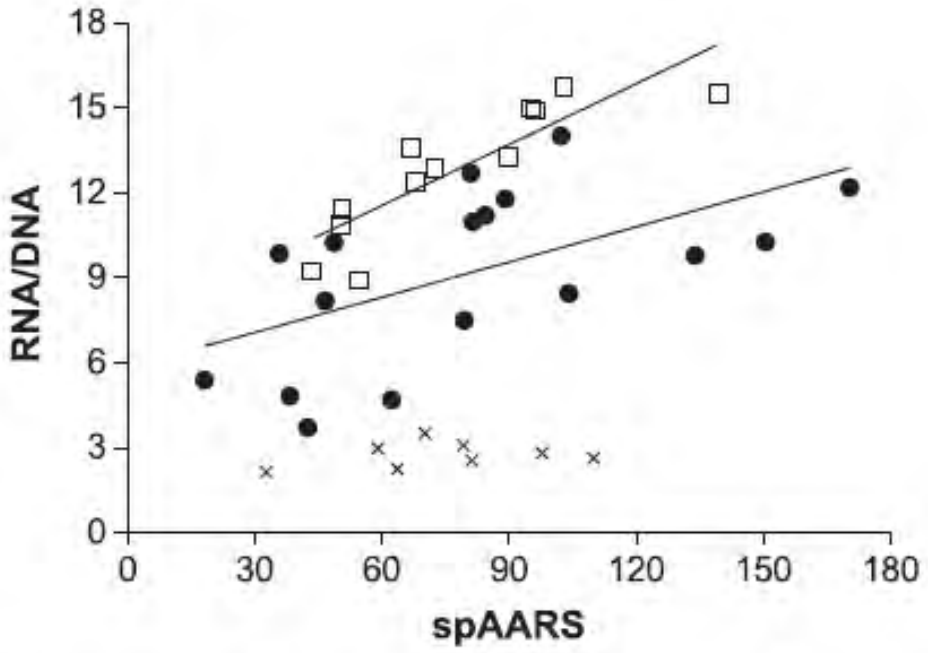


Fig. 5. Relationship between specific AARS activity (nmPPi mg protein^{-1} h^{-1}) and RNA/DNA ratio of *Oithona davisae*: fed nauplii (filled circles), starved nauplii (crosses) and fed copepodites (open squares).

Oithona davisae

Yebra et al. JEMBE, 2011

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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Thank you!

どうもありがとう