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RNA/DNA RATIOS FOR THE ESTIMATION OF METABOLIC AND FUNCTIONAL TRAITS IN DIATOM SPECIES FROM THE NORTHWESTERN ADRIATIC SEA, A POSSIBLE IMPACT ON QUALITY OF MUSSEL POPULATIONS

RAPPORTI RNA/DNA PER LA STIMA DEI CARATTERI METABOLICI E FUNZIONALI NELLE SPECIE DI DIATOMEE DEL MAR ADRIATICO NORD-OCCIDENTALE, UN POSSIBILE IMPATTO SULLA QUALITÀ DELLE POPOLAZIONI DI MITILI

Abstract - *The aim of this study was to evaluate if the RNA/DNA and taxon-specific 18S rRNA/rDNA ratios could be used to assess the metabolic activity of marine phytoplankton. Indeed, traditional biomass indicators such as abundance and chlorophyll* a *are able to measure population size without reflecting phytoplankton functional activity. Two diatom species, namely* Skeletonema marinoi *and* Chaetoceros socialis*, commonly found in the northwestern Adriatic Sea were selected to perform individual and co-culturing growth experiments. The RNA/DNA and 18S rRNA/rDNA ratios were calculated and were found to peak in the early stages of growth followed by a sharp decline. These evidence suggested that these ratios could be useful indicators of phytoplankton metabolic dynamics and, since phytoplankton represent an important source of food for filter feeding animals, they may be useful to understand the potential impact on mussels growth.*

Keywords: *Adriatic Sea, biomass, RNA/DNA ratio, 18S rRNA/rDNA ratio, metabolic dynamics*

Introduction - Phytoplankton, the primary producers in pelagic and coastal ecosystems, play a critical role in transferring energy through the food web by converting sunlight into chemical energy (Falkowski, 2002). Phytoplankton traits like cell size and shape, macromolecule composition in proteins, polysaccharides, lipids, nucleic acids, are essential for understanding their ecological roles, growth, and metabolic activity (Chícharo and Chícharo, 2008). In particular, increased growth rate is linked to higher RNA content and relative protein concentration, and ribosomal RNA (rRNA) content is associated with protein synthesis requirements and ribosome synthesis rate (Flynn *et al*., 2010). In the field, nucleic acids, proteins, carbohydrates, lipids and secondary metabolites that are biochemical components of photosynthetic production can be used to assess actively growing natural assemblages (Finkel *et al.,* 2016). It has previously been demonstrated that the diatom and dinoflagellate 18S rRNA/rDNA ratios were significantly correlated with biomass, and since this parameter is linked to cellular RNA variability, it has the potential to express the growth rate and metabolic dynamics of phytoplankton assemblages (Casabianca *et al.,* 2023). This study investigated whether RNA/DNA and 18S rRNA/rDNA ratios can be useful as indicators of metabolic activity in diatoms, in particular *Chaetoceros socialis* Lauder 1864 and *Skeletonema marinoi* Sarno & Zingone 2005, which dominate seasonal blooms in the northwestern Adriatic Sea. The hypothesis was to test if ratios could be effective indicators of metabolic status during early growth phases, providing insight into phytoplankton dynamics in coastal ecosystems with potential application assessing the health status of mussels that feed on phytoplankton.

Materials and methods - *C. socialis* CBA22 and *S. marinoi* CBA4 were maintained in sterilized f/2 medium (Guillard, 1975) and incubated at 18 ± 1 °C with a light:dark cycle of 12:12 h. Biomass determination was assessed by cell abundance (cell mL^{-1}) using the Sedgewick-Rafter methods and by chlorophyll *a* (Chl-*a*) content estimation. Genomic DNA, RNA, and proteins were extracted from both individual and co-cultured samples of the target diatoms using the RNA/DNA/Protein Purification Plus Kit (Norgen Biotek Corp., Canada). Nucleic acids and proteins were quantified, and cDNA was obtained from the extracted RNAs. The 18S rRNA/rDNA ratio was calculated using primers for *Chaetoceros* spp. (5′- ACTGAAGGGCAAGTCTGGTG-3′; 5′-GAACCCACCAAAAGGTCGGA-3′) and *Skeletonema* spp. (5′-ATTGGAGGGCAAGTCTGGTG-3′; 5′-TTGTGGTCAGTCACTCCTGC-3′). The qPCR reactions were performed with a real-time PCR system, using the cycling conditions described in Casabianca *et al.* (2023). Standard curves for the new primers were generated and accepted if the slopes were between −3.58 and −3.32 (90–100% efficiency) and accuracy (r^2 at least 0.99). Spearman's rank correlation was computed to assess the relationships between the variables considered. All statistical analyses were performed with PAST ver. 4.01.

Results - Significant positive correlations were found between cell abundance, chl *a*, cellular carbon, and protein content for *C. socialis* and *S. marinoi* using Spearman's rank correlations (r_S=0.570 - 0.871, P<0.001). These variables showed a direct proportionality for both species (Fig. 1).

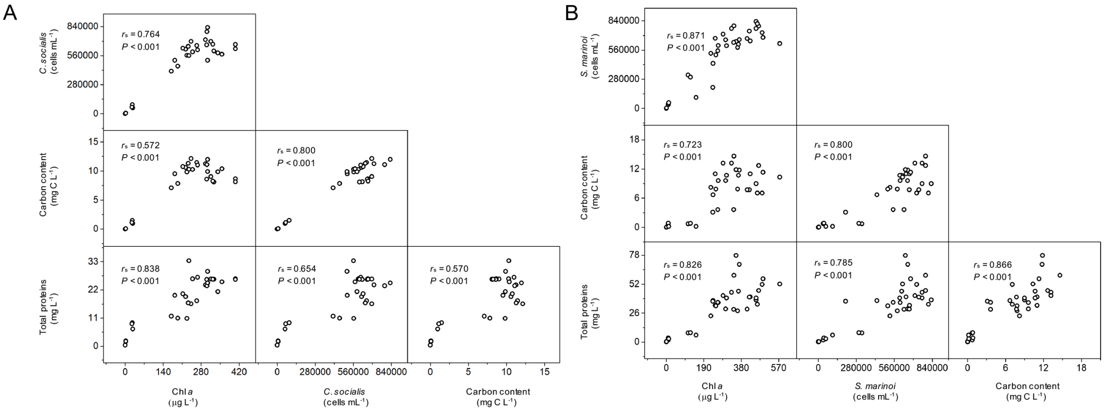

Fig. 1 - Matrix plot of bivariate relationships among abundance, Chl-*a*, carbon content and total proteins for *Chaetoceros socialis* CBA22 (A) and *Skeletonema marinoi* CBA4 (B).
Matrix plot delle relazioni bivariate tra abbondanza, Chl-a, contenuto di carbonio e proteine totali per Chaetoceros socialis *CBA22 (A) e* Skeletonema marinoi *CBA4 (B).*

In the individual growth experiments, the logistic model fitted growth curves for *C. socialis* CBA22 and *S. marinoi* CBA4, based on cell abundance and Chl-*a* estimations. Maximum cell abundances and chl *a* values reached 6.5 x 10^5 cell mL^{-1} and 318.6 µg/L for *C. socialis* and 7.1 x 10^5 cell mL^{-1} and 395.6 µg/L for *S. marinoi*, respectively. The molecular ratios for *C. socialis* and *S. marinoi* peaked at 2-6 days before exponential growth, indicating early growth phase activity. The values for the two species were 23.2 ± 1.5 and 15.3 ± 0.8 for RNA/DNA ratio and 16.2 ± 1.6 and 30.1 ± 5.4 for 18S rRNA/rDNA ratio (Fig. 2). In co-culture experiments, the relationships between cell abundance, Chl-*a*, cellular carbon, and protein content were assessed using Spearman's rank correlations. These variables were all highly correlated with r_s values ranging from 0.678 to 0.997 (data not shown), suggesting either direct proportionality. In the co-culture experiment, the exponential growth phase was delayed compared to individual species cultures. The maximum total abundance reached 4.6 x 10^6 cells mL^{-1}, with 2.1 x 10^3 µg/L of chl *a* at the end of the growth curve. The RNA/DNA and diatom 18S rRNA/rDNA ratios showed a peak at 4 days with values of 13.4 and 9.4, respectively (Fig. 3). *C. socialis* reached a maximum abundance of 4.3 x 10^6 cells mL^{-1} at the end of the growth curve, while *S. marinoi* peaked at 6.6 x 10^5 cells mL^{-1} after 18 days. Molecular ratios, such as *Chaetoceros* and *Skeletonema* 18S rRNA/rDNA, indicated metabolic variation, peaking at 4 days post inoculation with values of 24.4 and 8.2, respectively (Fig. 4).

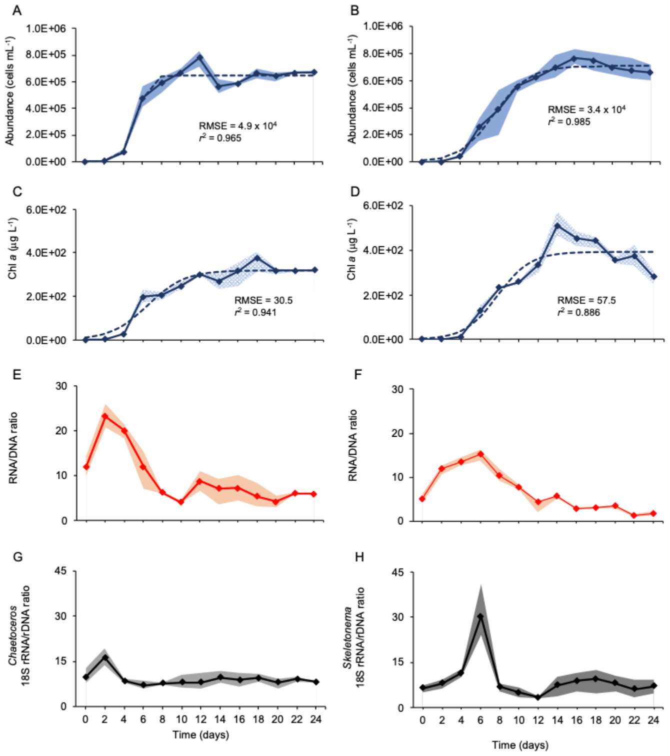

Fig. 2 - Growth curve of *Chaetoceros socialis* CBA22 (left) and *Skeletonema marinoi* CBA4 (right) expressed as abundance (A, B) and Chl-*a* (C, D), and RNA/DNA (E, F) and taxon-specific 18S rRNA/rDNA ratios (G, H). Red dotted line: logistic curve fitted to biomass data. Root mean square error (RMSE) and coefficient of determination (r^2) for abundance and Chl-*a* were shown. For each variable, colored areas are delimited by minimum and maximum values.

Curva di crescita di Chaetoceros socialis *CBA22 (sinistra) e* Skeletonema marinoi *CBA4 (destra) espressa come abbondanza (A, B) e come Chl-a (C, D), e rapporti RNA/DNA (E, F) e taxon-specifici 18S rRNA/rDNA (G, H). Linea tratteggiata rossa: curva logistica adattata ai dati di biomassa. Sono stati mostrati rispettivamente l'errore quadratico medio (RMSE) e il coefficiente di determinazione* (r^2) *per abbondanza e Chl-a. Per ciascuna variabile, le aree colorate sono delimitate dai valori minimo e massimo.*

Conclusions - This study analyzed RNA/DNA and taxon-specific 18S rRNA/rDNA ratios during the growth phases of two diatom species in both individual and co-culture systems.

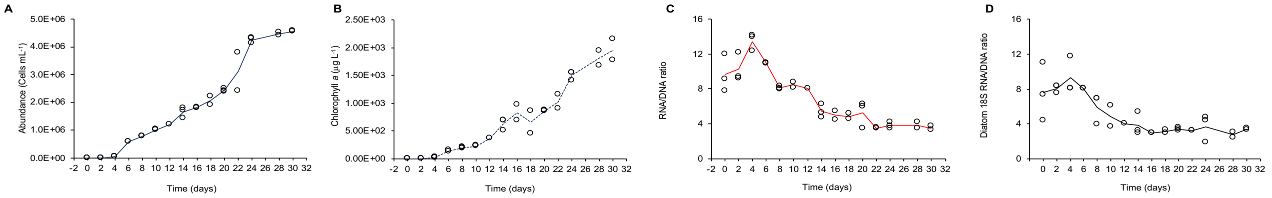

Fig. 3 – Growth curve of *Chaetoceros socialis* CBA22 and *Skeletonema marinoi* CBA4 in co-culture condition as total biomass by abundance (cells mL^{-1}) (A) and Chl-*a* (µg L^{-1}) (B), total RNA/DNA (C) and diatom 18S rRNA/rDNA (D) ratios. Circles represented minimum and maximum values for each variable.

Curva di crescita di Chaetoceros socialis *CBA22 e* Skeletonema marinoi *CBA4 in condizioni di co-coltura come biomassa totale utilizzando abbondanza (cellule mL^{-1}) (A) e Chl-a (µg L^{-1}) (B), rapporti RNA/DNA totale (C) e 18S rRNA/rDNA di diatomee (D). I cerchi rappresentavano i valori minimo e massimo per ciascuna variabile.*

Molecular dynamics showed that variations were linked to changes in metabolic activity, while biomass (cell abundance, Chl-*a*, or carbon content) described diatom growth trends.

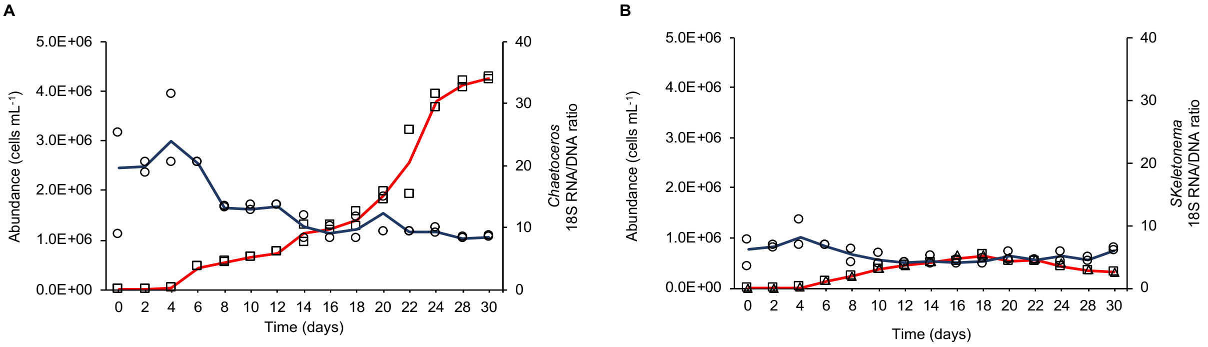

Fig. 4 - Growth curve in mixed cultured conditions analyzed based on abundance (cells mL^{-1}) and molecular ratios of *Chaetoceros socialis* CBA22 (A) and *Skeletonema marinoi* CBA4 (B). Red line represented mean values of abundance (cells mL^{-1}); green line represented mean values of 18S rRNA/rDNA ratio. Triangles and circles represent minimum and maximum values of cell abundance and 18S RNA/DNA ratios, respectively.

Curva di crescita in condizioni di coltura miste analizzata come abbondanza (cellule mL^{-1}) e come rapporti molecolari di Chaetoceros socialis *CBA22 (A) e* Skeletonema marinoi *CBA4 (B). La linea rossa rappresentava i valori medi di abbondanza (cellule mL^{-1}); la linea verde rappresentava i valori medi del rapporto 18S rRNA/rDNA. I triangoli e i cerchi rappresentano rispettivamente i valori minimo e massimo dell'abbondanza cellulare e dei rapporti 18S RNA/DNA.*

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