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Unravelling Seasonal Shifts: Exploring Carbon and Nitrogen Stable Isotope Signatures in Zooplankton of Kakinada Bay, Andhra Pradesh, India.

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Abstract

The present study focused on the seasonal variability of carbon and nitrogen stable isotopic signatures in zooplankton collected from Kakinada Bay. Physiochemical parameters of the bay, such as temperature, salinity, nitrates, nitrites, dissolved nutrients, phosphates, and chlorophyll a, were evaluated. The isotopic ratios of carbon and nitrogen in zooplankton and POM were determined using an isotope-ratio mass spectrophotometer. The $\delta^{13}C$ values of zooplankton and POM were higher in Kakinada Bay, indicating enrichment of $\delta^{13}C$ in primary production. The $\delta^{15}N$ values of zooplankton ranged from 8.17 to 9.58, with the highest values observed during the monsoon season. The study provides insights into the trophic structure and anthropogenic influences on the marine ecosystem in Kakinada Bay

Keywords: Zooplankton, Stable isotopes, Kakinada Bay, Anthropogenic activities.

INTRODUCTION:

Planktonic organisms in aquatic ecosystems play complex and fundamental roles in mediating the flux of macronutrients, such as carbon (C) and nitrogen (N), from primary producers toward consumers at higher trophic levels (Ji et al., 2010). Our understanding of the flow of energy from producers through zooplankton to edible fish is, however, limited parameterizations are often based on crude estimates of production and assumed subsequent trophic-dynamic relationships (Fan et al., 2023; Hays et al., 2005). The transfer of nutrients (mainly C and N) up the food web is assumed to be highly efficient in these systems because of the repetition of upwelling events and the presence of dense plankton populations leading to large populations of fish and other consumers (Raven and Beardall, 2021). Upwelling productivity is expected to increase with climate change because of an increase in favorable winds (Tweddle et al., 2018) but its effect on different components of the food webs requires further research. For instance, large scale fluctuations in the populations of plankton and planktivorous fish in different upwelling ecosystems were associated mainly with changes in low-frequency atmospheric pressure and oceanic temperature (Henson et al., 2021; Ratnarajah et al., 2023) but currently, there is not a full understanding of the combination of climate forcing, local physical processes, nutrient fluxes, and biology producing such fluctuations mainly because of the complex responses of the plankton communities. The seasonal heterogeneity of primary production changes the diversity of edible food sources for each zooplankton taxon (Abida et al., 2013).

Analysis of the natural abundance of stable nitrogen and carbon isotopes can provide clues on the relevant fluxes and fate of nutrients and organic matter produced by upwelling. Nitrogen isotopes can trace the upwelled nitrate because its relative enrichment in heavy (^{15}N) isotopes compared to ammonium released in surface waters (Relitti et al., 2020). Also, there is a trophic enrichment in ^{15}N isotopes in every trophic step, due to the preferential excretion of light isotopes by consumers (Piscia et al., 2018). This allows for the estimation of trophic positions of consumers relative to a known isotopic baseline by assuming specific values of trophic enrichment. Determinations of appropriate baselines depend on a good knowledge of the N sources used by primary producers (Piscia et al., 2019). The sources of carbon for upwelling food webs can be also traced, because, in contrast with nitrogen, there is almost no enrichment in heavy carbon isotopes through the food web (Eglite et al., 2023; Tunēns et al., 2022). However, diatom carbon is relatively enriched in heavy isotopes compared to other phytoplankton, thus providing a marker that can be used to trace their importance for zooplankton, as diatoms are the dominant phytoplankton in upwelling ecosystems (Schell et al., 1998).

The stable isotope analysis approach requires the different basal resources available to a food web to exhibit distinct and robust isotopic ‘‘signatures.’’ The most frequently used signatures are those derived from the naturally occurring abundance ratios of ^{13}C : ^{12}C and ^{15}N : ^{14}N These signatures can then be traced through the food web because the isotope ratios of organisms reflect those of the diet in a dependable manner (Ho et al., 2021; Kürten et al., 2016). The ^{13}C of an animal generally reflects that of its diet with minor enrichment (,1‰), while the greater enrichment of ;3‰ in ^{15}N reflects the preferential excretion of the lighter isotope during metabolization (Bouillon et al., 2002). Therefore, information on an animal’s putative food sources and trophic level can be deduced by utilizing a combination of carbon and nitrogen isotope signatures. Stable isotope analysis offers advantages over conventional methods such as gut content analysis by representing the assimilated diet, as opposed to ingested material incorporates dietary information over longer time periods as opposed to momentary ‘‘snap-shots’’ and may even identify sources that are not detectable by inspection of ingested material. The latter advantage makes the stable isotope technique particularly useful for studies of planktonic organisms (Heneghan et al., 2023; Piscia et al., 2018).

Studies of seasonality of isotopic signatures in aquatic systems are still relatively rare, especially in freshwater (Ho et al., 2021; Major et al., 2017) but they are crucial when considering organisms of small size with the potential to turn over assimilated isotopes quickly and thus exhibit differing isotopic signatures over a relatively short temporal scale (Piscia et al., 2019).

The present study, focuses on seasonal variability of ^{13}C and ^{15}N stable isotopic signatures of Zooplankton collected from the coastal waters of Kakinada Bay, Andhra Pradesh India to understand the trophic structure, trophic niche and anthropogenic influences on marine ecosystem.

Experimental section:
Study Area and Sampling:

The present study was conducted at 12 stations of Kakinada Bay of Andhra Pradesh lies between geographical coordinates 16° 41.17’ N to 16° 44.07’ N and 82° 15.18’E to 82° 42 26’E during Pre-Monsoon, Monsoon and Post Monsoon seasons and the study period was October 2022 to October 2023.

Approximately 5 Litres of water samples were collected from each station at the depth of 0.5 m below the surface. The *in-situ* measurement of surface sea salinity (SSS) and temperature (SST) were done using multi probe sensor. The water samples were transported to the lab and subjected to evaluation of physiochemical parameters such as nitrates (NO_3^-), Nitrites (NO_2^-), Dissolved Nutrients (DIN) Phosphates (PO_4^{3-}), Particulate Organic Matter (POM) and Chlorophyll a according to the (Ke et al., 2022).

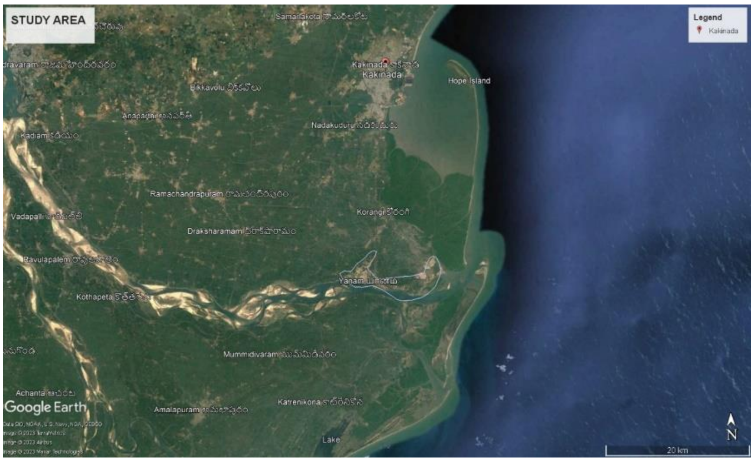

Fig 1: Study area

Collection of Zooplankton and Isotopic determination:

The collection of zooplankton was carried out by using vertical tows method (1-3 m from surface) with a mesh size of 200 µM. 3-5 hauls at each sampling station at Kakinada Bay to obtain enough sample quantity about speed of 0.5 ms^{-1}. The samples transported immediately to the lab and zooplankton was gathered using sieve with mesh size of 200 µM and then placed in a pre-filtered sea water to evacuate gut content. The aggregated samples using nylon sieve were frozen in liquid nitrogen.

The frozen samples were subjected to Isotope-ratio mass spectrophotometer (IRMS): MAT 253 (Thermo Germany) coupled with Elemental analyser Flash 2000HT Gas Bench II. The isotopic ratios of carbon and nitrogen were expressed in δ notations as the deviation in PPM from a standard reference material $\delta^{13}C$ or $\delta^{15}N$ (‰) = (R Sample/R Standard-1) × 1000.
Where R either $^{13}C/^{12}C$ or $^{15}N/^{14}N$
The PeeDee Belemnite and Atmospheric nitrogen (N_2) were used as reference standard for carbon and nitrogen respectively. Casein as working standard.

Statistical analysis:

All statistical analysis was performed with SPSS software package, version 19.0 for windows (SPSS Inc., Chicago, IL, USA).

Result and Discussion:
Physio-chemical parameters:

No broad variation of physiochemical parameters of Kakinada Bay was observed during all three seasons, whereas Chl *a* was found slight increase during monsoon.

Table 1: The seasonal variation of physiochemical parameters (Average) of Kakinada Bay

Parameter	**Pre-monsoon**	**Monsoon**	**Post-monsoon**
Temperature °C	19.42 ± 0.19	17.54 ± 0.54	18.01 ± 0.47
Salinity	33.10 ± 0.94	34.12 ± 0.18	33.87 ± 0.18
NO_3^- (µM)	4.17 ± 0.80	4.01 ± 0.34	3.91 ± 0.96
NO_2^- (µM)	1.99 ± 0.47	2.14 ± 0.19	2.27 ± 0.47
DIN (µM)	9.01 ± 2.29	9.17 ± 0.97	8.67 ± 0.97
PO_4^{3-} (µM)	0.51 ± 0.92	0.55 ± 0.53	0.53 ± 0.08
Chl *a* (µg L^{-1})	6.01 ± 0.56	6.97 ± 0.80	6.12 ± 0.18

Isotopic determination of Zooplankton:
The seasonal variation of $\delta^{13}C$ and $\delta^{15}N$ of zooplankton and POM in the present study are represented in table 2 and the Pearson coefficient are represented in fig.2. Generally, the terrestrial organic matter usually produced by C_3 pathway which exhibits lower $\delta^{13}C$ values ranges from -30 ‰ to -26 ‰, whereas organic matter in marine

environment usually exhibits δ^{13}C value between -22 ‰ to -18 ‰ (Major et al., 2017). The present study suggested that the δ^{13}C values of zooplankton and POM are higher in Kakinada Bay which attributes to the enrichment of δ^{13}C in primary production. Previous studies reported that the δ^{13}C values of organic matter must be high when primary production increases. The river discharge/terrestrial organic matter is one of the key parameters influence the zooplankton δ^{13}C which can be obscured by local or regional variability in primary production (Schell et al., 1998).

Stable δ^{15}N isotopes can be used to evaluate the aquatic ecosystem pollution by anthropogenic activities. Livestock waste and human waste are nitrogen sources usually enriched in N^{15} and values vary between 10 ‰ to 22 ‰ (Kim et al., 2022). The elevated N^{15} in aquatic ecosystem generally result in loading of nitrogen by anthropogenic activities (Francis et al., 2011). However, in this study the δ^{15}N of zooplankton ranges from 8.17-9.58. Most abundant δ^{15}N was found during the Monsoon season, In the case of POM the lower δ^{15}N was found than the zooplankton δ^{15}N.

Table 2: The seasonal variation of stable δ^{13}C and δ^{15}N isotopes and POM (Average) of Kakinada Bay.

	Pre-monsoon	**Monsoon**	**Post-monsoon**
Zooplankton δ^{13}C	-19.95 ± 0.47	-20.71 ± 0.18	-19.87 ± 0.53
Zooplankton δ^{15}N	8.17 ± 0.92	9.58 ± 0.80	8.34 ± 0.47
POM δ^{13}C	-21.87 ± 0.80	-22.57 ± 0.34	-21.69 ± 0.96
POM δ^{15}N	6.12 ± 0.56	6.7 ± 0.19	5.42 ± 0.54

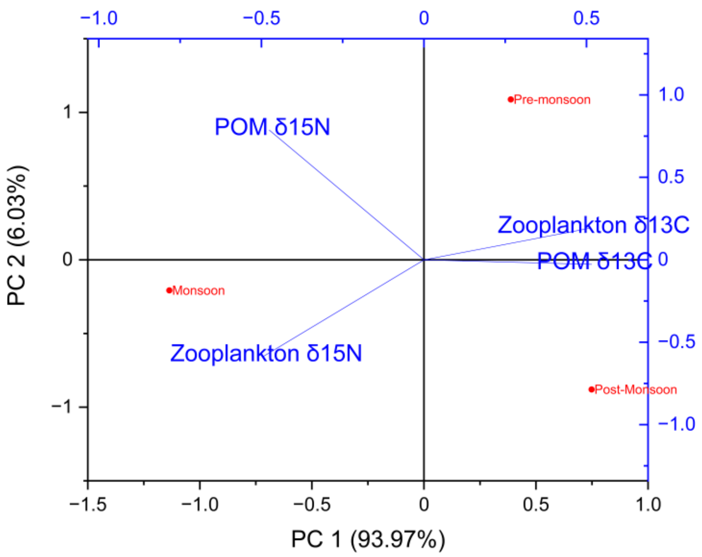

Figure 2: Pearson coefficient of isotopic ratios of Zooplankton and POM

Conclusion:

The study focused on the seasonal variability of stable isotopic signatures of zooplankton in the coastal waters of Kakinada Bay, Andhra Pradesh, India. The analysis of δ^{13}C and δ^{15}N isotopes provided insights into the trophic structure, trophic niche, and anthropogenic influences on the marine ecosystem. The results showed higher δ^{13}C values in zooplankton and particulate organic matter (POM), indicating enrichment of δ^{13}C in primary production. The δ^{15}N values of zooplankton suggested minimal pollution from anthropogenic activities. The study highlights the importance of considering seasonal variations in isotopic signatures when studying small-sized organisms with rapid turnover of assimilated isotopes. Further research is needed to understand the complex responses of plankton communities to climate forcing and anthropogenic activities.

Abbreviations:

Surface sea salinity (SSS)
Surface sea temperature (SST)
Particulate Organic Matter (POM)
Isotope-ratio mass spectrophotometer (IRMS)

Author Contribution:

TVR: Methodology, Investigation, Writing the original manuscript
AM: Data curation, validation
PVN: Editing, Reviewing the manuscript
BC: Validation, Editing
NS: Conceptualization, Methodology, Reviewing the manuscript

Declarations

Competing interest: The authors declare no competing interests.
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