

Stable isotopic analysis of Barnacle larvae and their faecal pellets to evaluate the ingested food

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A B S T R A C T

Stable isotopes of carbon and nitrogen are useful in the evaluation of food web dynamics and have been used extensively in the feeding studies. Barnacles are dominant component of the intertidal and bio-fouling communities. Life cycle of barnacles include planktonic naupliar stages followed by a non-feeding pre-settling cyprid stage. Development success of the nauplii depends on the food available for them in the natural environment. However, the food preference of the nauplii in the natural environment is still not well understood. In this study experiments were carried out initially to assess the ^{13}C and ^{15}N fractionation between the food and the *Balanus amphitrite* nauplii that were fed with *Chaetoceros calcitrans*. Laboratory reared larvae fed with *Chaetoceros calcitrans* diet pointed out an enrichment in ^{13}C by 0.95‰ and ^{15}N by 1.42‰. The enrichment level in the faecal pellets was comparatively higher than that obtained from the larvae either fed or starved indicating the role of heterotrophic organisms. Expanding on these observations, efforts were made to evaluate the food of the acorn barnacle larvae collected from the wild during different seasons. The $\delta^{13}\text{C}$ ratios of the larvae and their faecal pellets obtained during the post-monsoon season were heavier compared to pre-monsoon season indicating the feeding of larvae on isotopically heavier food sources during the post-monsoon season. However, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of the larvae and their faecal pellets did not differ significantly between the seasons and this could possibly be due to the broad range of isotopic signatures observed at the base of the food web.

Keywords: Barnacle larvae, Faecal pellets, Stable isotopes, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios, Feeding, Tropical environment.

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1. Introduction

Food availability for the barnacle nauplii during their development governs the success of its population in an area. In spite of this, studies pertaining to feeding of the larvae from the natural environment are scarce (Turner et al., 2001; Vargas et al., 2006; Gaonkar and Anil, 2010). Most of the studies are based on laboratory reared larvae using cultured food (Anil and Kurian, 1996; Qiu and Qian, 1997; Anil et al., 2001; Desai and Anil, 2004; Desai et al., 2006). Traditional approaches such as gut content analysis and laboratory feeding experiments provide a broad perspective of ingested food, but have drawbacks (Michener and Schell, 1994; Schmidt et al., 2003). Analysis of the gut contents provides only snapshots of the diet based on what has been ingested. Whereas, analysis of natural isotopic composition can provide useful information on the food source and provide a measure of carbon assimilation over a period of time (Fry and Sherr, 1984). Naturally occurring stable isotopes can reflect the diet integrated over longer periods in a way that depends on rates of feeding, assimilation, tissue turnover and growth (Peterson and Fry, 1987; Post, 2002). The average $^{13}\text{C}/^{12}\text{C}$ ratio of an organism is closely related to that of its food (DeNiro and Epstein, 1978). However, it should be noted that, although $\delta^{13}\text{C}$ of an organism is closely related to that of its food, there is always a slight difference due to isotopic fractionation, the organisms being generally 1‰ heavier than its diet (Rau et al., 1983). The $\delta^{15}\text{N}$ of an organism is a product of diet as well as trophic fractionation (Minagawa and Wada, 1984). The use of nitrogen stable isotopes ($\delta^{15}\text{N}$) to study the trophic position is based on the fact that they display a constant level of enrichment (~3-4‰) per trophic level (Minagawa and Wada, 1984; Michener and Schell, 1994).

In this study experiments were initially conducted with the laboratory reared larvae fed with mono-algal diet *Chaetoceros calcitrans*, to evaluate the isotopic fractionation and the feasibility of stable isotopic approach in the feeding studies of barnacle larvae. Larval samples were also collected from the wild to study their food preference and the seasonal variations. To our knowledge, this study represents the first attempt to use stable isotopic approach to investigate feeding in the case of barnacle larvae.

2. Materials and methods

2.1. Larval rearing in the laboratory

Larval development in barnacles consists of six naupliar instars and a pre-settling cyprid instar. The I instar nauplius is non-feeding and moults into II instar within a short interval. The II to VI instars are planktivorous. Nauplii obtained from broods of the barnacle *Balanus amphitrite* were mass reared in 2 L glass beakers (larva ml⁻¹) on a diet of *Chaetoceros calcitrans*, a unicellular diatom at a cell concentration of 2x10⁵ cells ml⁻¹. The *Chaetoceros calcitrans* culture was grown in filtered separator enriched with f/2 (Guillard and Ryther, 1962) and maintained temperature in an incubator at 20°C (±1°C) (12h:12h, Light:Dark cycle). Larval density and food concentration was maintained by monitoring mortality of the larvae every day prior to changing the food. The larval naupliar development was completed in 6-7 days.

2.2. Stable isotopic analysis of the laboratory reared larvae

Approximately 100 larvae were sorted from the mass rearing beaker (2 L glass beaker) by working under dissecting microscope with minimal exposure to light and also by minimizing the picking time into a 50ml beaker filled with 0.22µm filtered seawater. Before analyzing the larvae for stable isotopes they were rinsed 5-6 times with 0.22µm filtered sea water to remove the adhering *Chaetoceros calcitrans* cells provided as food. The larvae were filtered onto GF/F (25mm diameter) pre-combusted Whatman filter paper (pre-combusted at 400°C oven for 3 hours). The filter was flushed with MiliQ water 4-5 times to remove the adhering salts. The filter papers containing the larvae were carefully removed from the filtration unit and stored in -20°C until analysis. Before the analysis, filter papers were dried overnight at 60°C and packed into tin capsules (3.2x6mm cup size, Thermo Electron Corporation). The δ¹³C and δ¹⁵N signatures of the samples were determined using a Thermo Finnigan Flash 1112 elemental analyzer, linked with a Thermo Finnigan Delta V plus Isotope Ratio Mass Spectrometer (IRMS). The δ¹³C and δ¹⁵N ratios are expressed as per mil (‰) relative to standards Sucrose and Ammonium Sulphate (International Atomic Energy Agency, Austria), which were calibrated against International Atomic Energy reference materials (PeeDee Belemnite and air for δ¹³C and δ¹⁵N respectively). Five replicate samples were analysed for each of the cases.

2.3. Stable isotopic analysis of faecal pellets defecated from the laboratory reared larvae

The V-VI instars were sorted from the mass rearing beaker. The sorted larvae were transferred in 50 ml beaker (5 replicates) containing 0.22 μ m filtered seawater and were incubated for 24 hours for the complete gut evacuation and to obtain faecal pellets. After incubation, the larvae were separated out and the incubation water was filtered on to 0.22 μ m pre-combusted filter paper by carefully rinsing the beaker. The pellets were washed with MiliQ water for about 5 times to remove the adhering salts and the filters were stored in -20°C refrigerator until analysis. Prior to analysis, the filters were dried in an oven (60°C) overnight and were analyzed for stable isotopes following the procedures mentioned above.

2.4. Field collection of larvae and their stable isotopic analysis

The larval samples were collected from the Dona Paula bay, Goa, west-coast of India, a tropical estuarine environment influenced by monsoons. Horizontal tows of plankton net of mesh size 100 μ m were used to collect the larvae. Immediately after collection they were brought back to the laboratory and kept in 5 litre containers in seawater collected from the study area. The containers were supplied with moderate aeration and maintained at normal room temperature and natural light. Barnacle larvae (V-VI instars) were immediately sorted from rest of the taxa using dissecting microscope into a 50ml beaker containing 0.22 μ m filtered seawater. These larvae were then washed 4-5 times with 0.22 μ m filtered seawater to clean and remove the adhering phytoplankton cells and the dirt from the surface of the larvae and were used for the analysis of stable isotopes following the above mentioned protocol for laboratory reared larvae. To obtain the faecal pellets from the field collected larvae, sorted larvae were incubated for 24 hours and the defecated pellets were collected and processed for the analysis of stable isotopes (five replicates each for larval and faecal pellets).

Larval samples were collected during 16th October and 14th December 2009 (post-monsoon) and 15th March and 17th May 2010 (pre-monsoon). Samples were not collected during the monsoon season (June-September) due to unfavorable weather conditions.

2.5. Data analysis

Normality and homogeneity of variances were determined using Shapiro-Wilk's W test. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of the laboratory reared larvae and field collected larvae fulfilled the assumptions of normality and were therefore analyzed for significant differences between (a) diet (*Chaetoceros*

calcitrans), fed larvae and their faecal pellets using one-way ANOVA, and (b) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of the larvae and their faecal pellets from field collected larvae during different seasons using paired t-test. All these analyses were carried out using STATISTICA 8 software.

3. Results

3.1. Stable isotopic ratios of the laboratory reared larvae

Chaetoceros calcitrans had a $\delta^{13}\text{C}$ ratio of -24.77‰ and $\delta^{15}\text{N}$ ratio of 0.34‰ (Fig. 1 and 2). After feeding on *Chaetoceros calcitrans* diet, the larvae were 0.95‰ enriched in ^{13}C relative to the diet (Fig. 1). The mean $\delta^{13}\text{C}$ ratio of the faecal pellets was -24.42‰, and was enriched in ^{13}C relative to the diet. The mean $\delta^{15}\text{N}$ ratio of the larvae was 1.76‰, and was enriched in ^{15}N by 1.42‰ relative to the diet and the mean $\delta^{15}\text{N}$ ratio of the faecal pellets was 2.82‰, and was enriched in ^{15}N by 2.48‰ relative to the diet and 1.06‰ relative to the fed larvae. The mean $\delta^{13}\text{C}$ ratio of the starved larvae which were provided with *Chaetoceros calcitrans* diet was -23.61‰, and was enriched in ^{13}C by 1.16‰ than the fed diet and the mean $\delta^{15}\text{N}$ ratio was 1.79‰, and was enriched in ^{15}N by 1.45‰ than the fed diet (Fig. 1 and 2).

The differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of the diet versus fed larvae and their faecal pellets did not vary significantly (ANOVA, $p>0.05$). This indicates that any variation shown in isotopic ratios of the growing larvae and their faecal pellets during the experiment can be considered as the influence of ingested food.

3.2. Stable isotopic ratios of the field collected larvae

The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of the larvae collected during post-monsoon season were -21.69‰ (range: -22.32‰ to -20.60‰) and 2.77‰ (range: 2.30‰ to 3.66‰) respectively, whereas the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of the faecal pellets defecated from the field collected larvae during post-monsoon season were -23.21‰ (range: -24.26‰ to -21.45‰) and 2.74‰ (range: 2.2‰ to 3.1‰) respectively (Fig. 3 and 4).

The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of the larvae collected during pre-monsoon season were -22.68‰ (range: -23.64‰ to -21.89‰) and 2.55‰ (range: 1.71‰ to 3.56‰) respectively, whereas the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of the faecal pellets defecated from the field collected larvae during pre-monsoon season

were -24.19‰ (range: -22.51‰ to -25.35‰) and 2.90‰ (range: 2.15‰ to 3.30‰) respectively (Fig. 3 and 4).

The $\delta^{13}\text{C}$ ratios of the larvae and their faecal pellets collected during post-monsoon season were about 1‰ heavier than the larvae and their faecal pellets collected during pre-monsoon season. The $\delta^{15}\text{N}$ ratios of the larvae and their faecal pellets collected during post-monsoon season did not differ much whereas the faecal pellets from the pre-monsoon season were slightly heavier in ^{15}N compared to the larvae. However, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of the field collected larvae and their faecal pellets obtained during post-monsoon and pre-monsoon seasons did not differ significantly (t-test, $p>0.05$).

4. Discussion

4.1. Stable isotopic ratios of the laboratory reared larvae

Laboratory reared larvae fed with *Chaetoceros calcitrans* diet showed an enrichment of ^{13}C by about 0.95‰ and the enrichment level matches well with the theoretical assumptions (DeNiro and Epstein, 1978; Michener and Schell, 1994). ^{15}N enrichment in the laboratory reared larvae fed with *Chaetoceros calcitrans* diet was about 1.42‰ which was slightly lower. Though the $\delta^{15}\text{N}$ ratios of the consumers are known to be heavier by about 3-4‰ than their presumed diet (Michener and Schell, 1994), the laboratory experiments have shown lower trophic enrichment values for ^{15}N , with mean differences in $\delta^{15}\text{N}$ between diet and their consumers being lower in marine (1.48‰) than in freshwater (2.82‰) organisms (Vanderklift and Ponsard, 2003). Accordingly the enrichment levels in our studies lie within the observed range.

Faecal pellets from the laboratory reared larvae fed with *Chaetoceros calcitrans* was enriched in ^{13}C and ^{15}N compared to the ingested food. Studies on faecal pellets from organisms such as copepods and mysids have shown an enrichment of ^{13}C and ^{15}N compared to the ingested food (Checkley and Entzeroth, 1985; Altabet and Small, 1990; Gorokhova and Hansson, 1999), though some studies have reported depleted values (Breteler et al., 2002; Schmidt et al., 2003; Tamelander et al., 2006). Isotopic ratios of the faecal pellets can depend on the extent to which the ingested food is assimilated. Egestion of undigested food material can give a variable fractionation results (Aberle et al., 2005; Tamelander et al., 2006). Studies on copepods have shown that up to 90% of the total fatty acids in the diet are assimilated during gut passage, leaving little lipids in the faecal pellets (Harvey et al., 1987). Pellets

being enriched compared to the diet in our studies, it might be deduced that lipids were assimilated by the larvae.

Bacteria can also play a role in isotopic fractionation of faecal pellets. Bacteria are known to be enriched in ^{13}C relative to their substrate, which can cause a depletion of ^{13}C in the residual material (Macko and Estep, 1984; Coffin et al., 1989). Starved larvae fed earlier with *Chaetoceros calcitrans* until late instars were also enriched in ^{13}C and ^{15}N compared to the ingested food. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of the larvae fed with *Chaetoceros calcitrans* and the larvae which starved later remained the same. This shows that the ratios obtained are not just of the freshly ingested food by the larvae but is a product of ingested as well as assimilated food. However, the consequence of prolonged periods of starvation on isotopic composition of the larvae needs further investigations.

4.2. Stable isotopic ratios of the field collected larvae

^{13}C enrichment patterns in the field collected larvae and their faecal pellets obtained during post-monsoon and pre-monsoon season were similar to that of the laboratory reared larvae. The $\delta^{13}\text{C}$ ratios of the larvae and their faecal pellets collected during post-monsoon season were heavier compared to pre-monsoon season. The $\delta^{13}\text{C}$ ratios of the suspended particulate organic matter (SPOM) in the area were also found to be heavier during the post-monsoon season compared to pre-monsoon season (Maya et al., 2011). ^{15}N enrichment pattern in the field collected larvae and their faecal pellets collected during post-monsoon and pre-monsoon season did not reflect the pattern seen in the case of laboratory reared larvae. The $\delta^{15}\text{N}$ ratios of the larvae and their faecal pellets remained more or less similar in both the seasons. The $\delta^{15}\text{N}$ ratios of the suspended particulate organic matter (SPOM) in the area were also found to be similar in both the seasons (Maya et al., 2011).

The slightly heavier ratios of $\delta^{13}\text{C}$ obtained during the post-monsoon season compared to pre-monsoon season gives an indication that the diet of the larvae during the post-monsoon season must have been derived from isotopically heavier food sources compared to the diet of the larvae during the pre-monsoon season. Earlier studies carried out in the area on faecal pellet contents of the larvae showed the presence of diatom frustules during the post-monsoon season, whereas no frustules were observed in the faecal pellets of larvae obtained during pre-monsoon season (Gaonkar and Anil, 2010). Gut fluorescence analysis of larvae also showed the dominance of autotrophic forms in the larval gut during post-monsoon season when compared to pre-monsoon season (Gaonkar and Anil, 2012).

Phytoplankton abundance and composition in the study area is reported to be influenced by the seasons (Patil and Anil, 2008, 2011). The pre-monsoon season is known to be characterized by lower chlorophyll *a* concentration in the water column. The phytoplankton community is generally dominated by diatoms during the post-monsoon season, whereas during the pre-monsoon season dinoflagellates also contribute substantially to the population along with the diatoms. Microzooplankton are also abundant during the pre-monsoon season along with bacteria in this region (Thakur and Anil, 2000; Smith and Madhupratap, 2005), which can serve as alternate food source for the larvae during the pre-monsoon season when there are less of autotrophic forms. The fact that the $\delta^{13}\text{C}$ ratios of the larvae and their faecal pellets obtained during the post-monsoon season were heavier compared to pre-monsoon season gives an indication that the larval food during the post-monsoon season must have been derived from isotopically heavier food sources such as diatoms, whereas the larval food during the pre-monsoon season must have been derived from isotopically lighter food sources such as flagellates, microzooplakton and bacteria. Since diatoms can utilize isotopically-heavy bicarbonate ion as a carbon source, diatoms are often known to be heavier in $\delta^{13}\text{C}$ than flagellates (Fry and Wainright, 1991).

However, the ratios of larvae and their faecal pellets obtained between the seasons did not differ significantly. The changes in the proportion of food components of the larvae may not have been large enough to induce a significant change in the isotopic ratios. As most of the ratios of lower food chain organisms overlap each other, it is difficult to obtain a clear-cut distinction. Consequently one should be cautious of interpreting the isotopic data, especially while studying the lower food web dynamics and the feeding patterns of primary consumers such as larval forms of various planktotrophic organisms which are directly dependent on primary producers for their food requirements. Further laboratory experiments need to be carried out especially with the larval forms to study the fractionation of different phytoplankton by these larvae and their signatures in faecal pellets.

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References

- Aberle, N., Hillebrand, H., Grey, J., Wiltshire, K.H., 2005. Selectivity and competitive interactions between two benthic invertebrate grazers (*Asellus aquaticus* and *Potamopyrgis antipodarum*): an experimental study using ^{13}C and ^{15}N labelled diatoms. *Freshw. Biol.* 50(2), 369–379.
- Altabet, M.A., Small, L.F., 1990. Nitrogen isotopic ratios in faecal pellets produced by marine zooplankton. *Geochim. Cosmochim. Acta* 54, 155–163.
- Anil, A.C., Kurian, J., 1996. Influence of food concentration, temperature and salinity on the larval development of *Balanus amphitrite*. *Marine Biology* 127, 115–124.
- Anil, A.C., Desai, D.V., Khandeparker, L., 2001. Larval development and metamorphosis in *Balanus amphitrite* Darwin (Cirripedia: Thoracica): significance of food concentration, temperature and nucleic acids. *J. Exp. Mar. Biol. Ecol.* 263, 125–141.
- Breteler, W.C.M.K., Grice, K., Schouten, S., Kloosterhuis, H.T., Damsté, J.S.S., 2002. Stable carbon isotope fractionation in the marine copepod *Temora longicornis*: unexpectedly low $\delta^{13}\text{C}$ value of faecal pellets. *Mar. Ecol. Prog. Ser.* 240, 195–204.
- Checkley, D.M.J., Entzeroth, L.C., 1985. Elemental and isotopic fractionation of carbon and nitrogen by marine, planktonic copepods and implications to the marine nitrogen cycle. *J. Plankton Res.* 7, 553–568.
- Coffin, R.B., Fry, B., Wright, R.T., 1989. Carbon isotopic compositions of estuarine bacteria. *Limnol. Oceanogr.* 34(7), 1305–1310.
- DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42, 495–506.
- Desai, D.V., Anil, A.C., 2004. The impact of food type, temperature and starvation on larval development of *Balanus amphitrite* Darwin (Cirripedia: Thoracica). *J. Exp. Mar. Biol. Ecol.* 306, 113–137.
- Desai, D.V., Khandeparker, L., Shirayama, Y., 2006. Larval development and metamorphosis of *Balanus albicostatus* (Cirripedia: Thoracica): implications of temperature, food concentration and energetics. *J. Mar. Biol. Assoc. U.K.* 86, 335–343.
- Fry, B., Sherr, E.B., 1984. $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib. Mar. Sci.* 27, 13–47.
- Fry, B., Wainright, S.C., 1991. Diatom sources of ^{13}C -rich carbon in marine food webs. *Mar. Ecol. Prog. Ser.* 76, 149–157.
- Gaonkar, C.A., Anil, A.C., 2010. What do barnacle larvae feed on? Implications in biofouling ecology. *J. Mar. Biol. Assoc. U.K.* 90(6), 1241–1247.

- Gaonkar, C.A., Anil, A.C., 2012. Gut fluorescence analysis of barnacle larvae: An approach to quantify the ingested food. *Estuar. Coast. Shel. Sci.* 111, 147-150.
- Gorokhova, E., Hansson, S., 1999. An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. *Can. J. Fish. Aquat. Sci.* 56(11), 2203–2210.
- Guillard, R.L., Ryther, J., 1962. Studies of marine planktonic diatoms. *Can. J. Microbiol.* 8, 229–239.
- Harvey, H.R., Eglinton, G., O'Hara, S.C.M., Corner, E.D.S., 1987. Biotransformation and assimilation of dietary lipids by *Calanus* feeding on a dinoflagellate. *Geochim. Cosmochim. Acta* 51(11), 3031–3040.
- Macko, S.A., Estep, M.L.F., 1984. Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter. *Org. Geochem.* 6, 787–790.
- Maya, M.V., Soares, M.A., Agnihotri, R., Pratihary, A.K., Karapurkar, S., Naik, H., Naqvi, S.W.A., 2011. Variations in some environmental characteristics including C and N stable isotopic composition of suspended organic matter in the Mandovi estuary. *Environ. Monitor. Assess.* 175, 501–517.
- Michener, R.H., Schell, D.M., 1994. Stable isotope ratios as tracers in marine and aquatic food webs. In: Lajtha, K., Michener, R.H. (Eds.), *Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific Publications, Oxford, pp. 138–157.
- Minagawa, M., Wada, T., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim. Cosmochim. Acta* 48, 1135–1140.
- Patil, J.S., Anil, A.C., 2008. Temporal variation of diatom benthic propagules in a monsoon-influenced tropical estuary. *Conti. Shelf Res.* 28, 2404–2416.
- Patil, J.S., Anil, A.C., 2011. Variations in phytoplankton community in a monsoon-influenced tropical estuary. *Environ. Monitor. Assess.* 182, 291-300.
- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* 18, 293–320.
- Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703–718.
- Qiu, J.W., Qian, P.Y., 1997. Effects of food availability, larval source and culture method on larval development of *Balanus amphitrite amphitrite* Darwin: implications for experimental design. *J. Exp. Mar. Biol. Ecol.* 217, 47–61.
- Rau, G.H., Mearns, A.J., Young, D.R., Olson, R.J., Schafer, H.A., Kaplan, I.R., 1983. Animal $^{13}\text{C}/^{12}\text{C}$ correlates with trophic level in pelagic food webs. *Ecology* 64, 1314-1318.

- Schmidt, K., Atkinson, A., Stübing, D., McClelland, J.W., Montoya, J.P., Voss, M., 2003. Trophic relationships among Southern Ocean copepods and krill: some uses and limitations of a stable isotope approach. *Limnol. Oceanogr.* 48(1), 277–289.
- Smith, S.L., Madhupratap, M., 2005. Mesozooplankton of the Arabian Sea: Patterns influenced by seasons, upwelling and oxygen concentrations. *Progre. Oceanogra.* 65, 214–223.
- Tamelander, T., Søreide, J.E., Hop, H., Carroll, M.L., 2006. Fractionation of stable isotopes in the Arctic marine copepod *Calanus glacialis*: effects on the isotopic composition of marine particulate organic matter. *J. Exp. Mar. Biol. Ecol.* 333, 231–240.
- Thakur, N.L., Anil, A.C., 2000. Antibacterial activity of the sponge *Ircinia ramosa*: Importance of its surface-associated bacteria. *J. Chemi. Ecol.* 26(1), 57–73.
- Turner, J.T., Levinsen, H., Nielsen, T.G., Hansen, B.W., 2001. Zooplankton feeding ecology: grazing on phytoplankton and predation on protozoans by copepod and barnacle nauplii in Disko Bay, West Greenland. *Mar. Ecol. Prog. Ser.* 221, 209–219.
- Vanderklift, M.A., Ponsard, S., 2003. Sources of variation in consumer diet $\delta^{15}\text{N}$ enrichment: a meta-analysis. *Oecologia* 136(2), 169–182.
- Vargas, C.A., Manriquez, P.H., Navarrete, S.A., 2006. Feeding by larvae of intertidal invertebrates: assessing their position in pelagic food webs. *Ecology* 87, 444–457.

Legends to figures

Fig. 1. $\delta^{13}\text{C}$ ratios of the laboratory reared larvae fed with *Chaetoceros calcitrans* diet, their faecal pellets and starved larvae (Error bar indicates standard deviation from the mean).

Fig. 2. $\delta^{15}\text{N}$ ratios of the laboratory reared larvae fed with *Chaetoceros calcitrans* diet, their faecal pellets and starved larvae (Error bar indicates standard deviation from the mean).

Fig. 3. $\delta^{13}\text{C}$ ratios of the larvae and their faecal pellets collected from the field during post-monsoon and pre-monsoon seasons [$\delta^{13}\text{C}$ ratios of the SPOM are taken from the literature (Maya et al., 2011) for comparison].

Fig. 4. $\delta^{15}\text{N}$ ratios of the larvae and their faecal pellets collected from the field during post-monsoon and pre-monsoon seasons [$\delta^{15}\text{N}$ ratios of the SPOM are taken from the literature (Maya et al., 2011) for comparison].

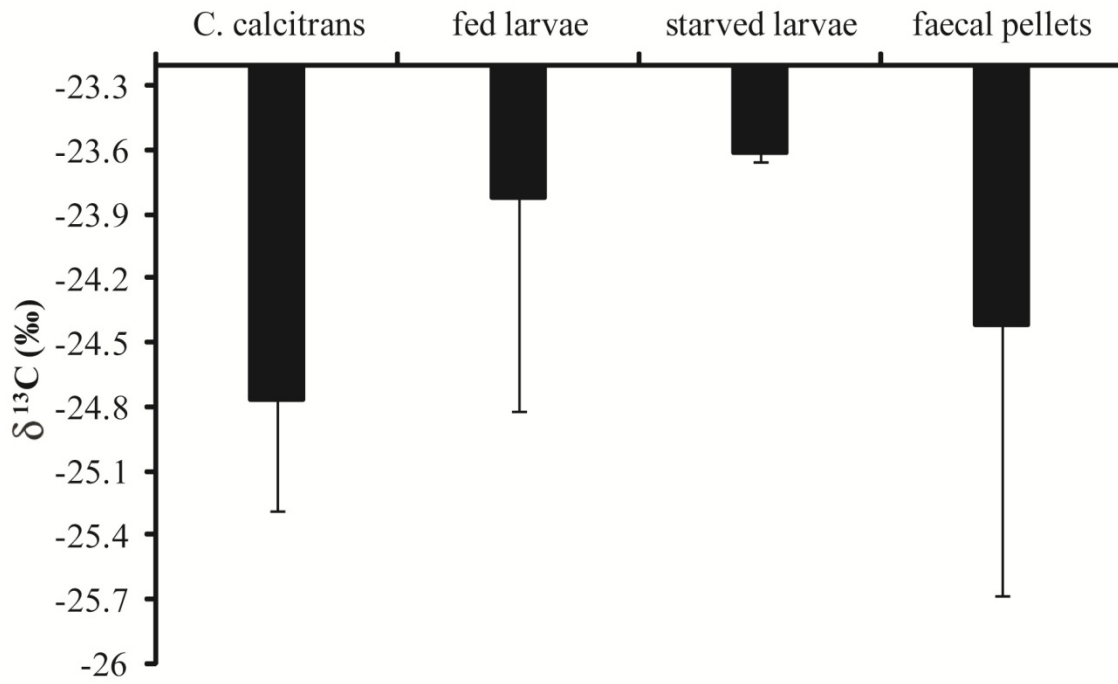


Fig. 1.

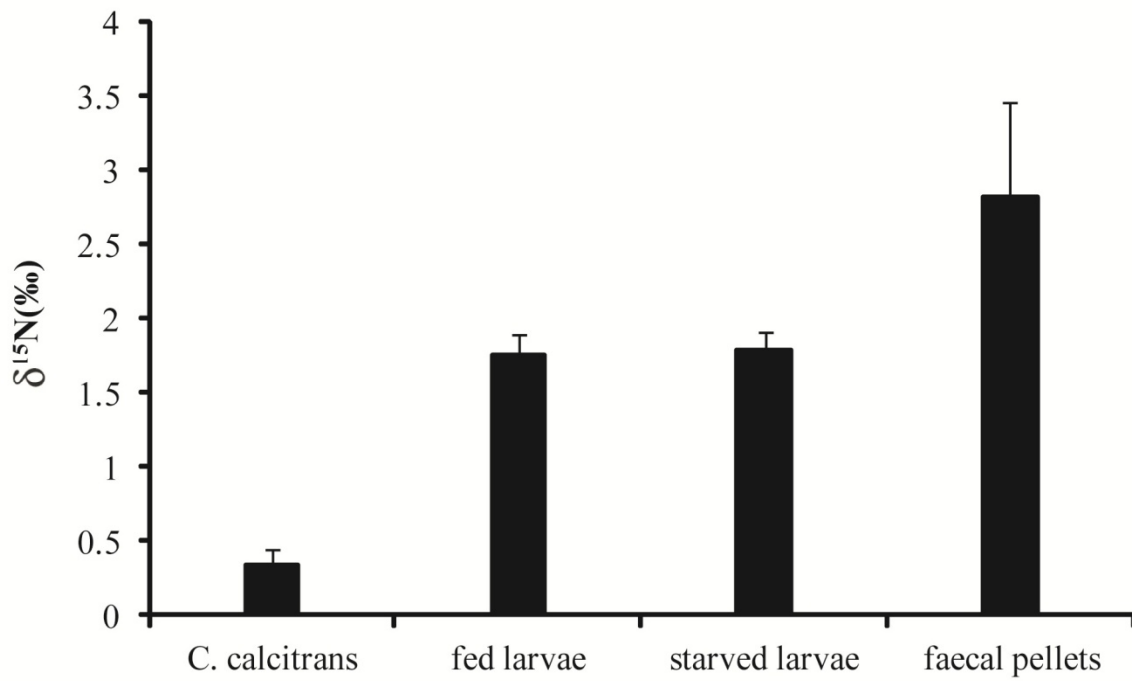


Fig. 2.

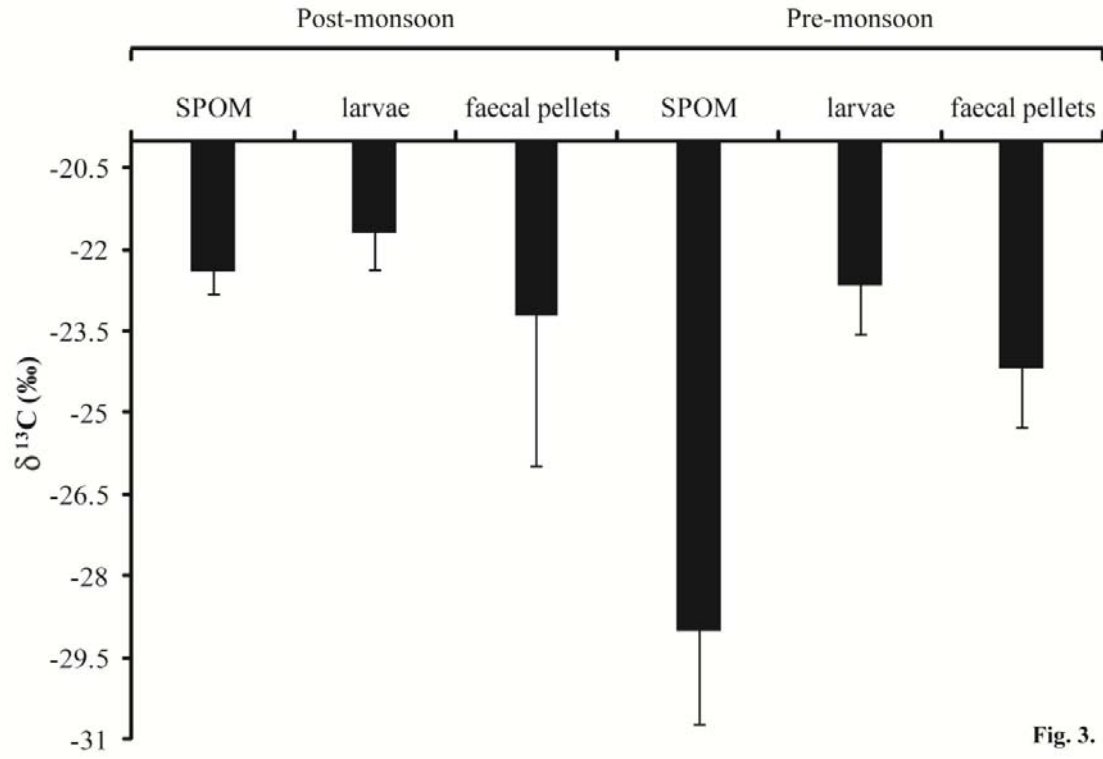


Fig. 3.

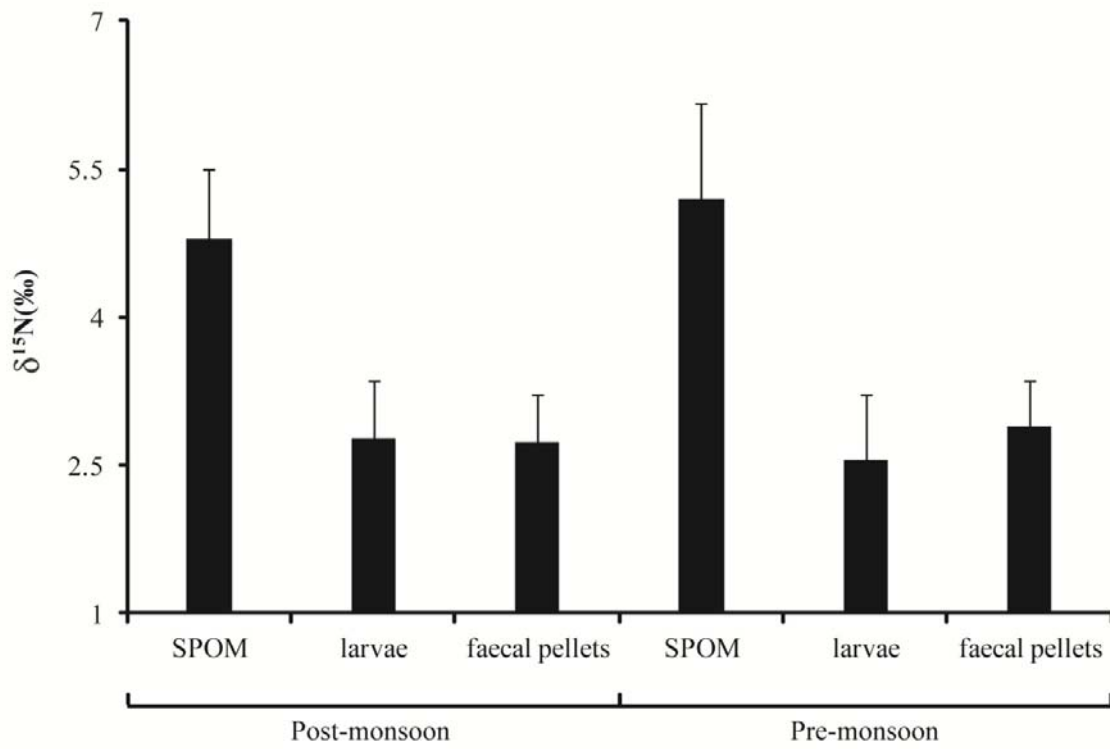


Fig. 4.